Subcellular Biochemistry 78

Gregory Lloyd Blatch
Adrienne Lesley Edkins *Editors*

The Networking of Chaperones by Co-chaperones

Control of Cellular Protein Homeostasis

Springer

Subcellular Biochemistry

Volume 78

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ISSN 0306-0225
Subcellular Biochemistry
ISBN 978-3-319-11730-0 ISBN 978-3-319-11731-7 (eBook)
DOI 10.1007/978-3-319-11731-7

Library of Congress Control Number: 2014956684

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Cellular protein homeostasis is vital for cellular survival and requires a balance between the integrated processes of protein folding, degradation and translocation. Proteostasis is regulated by a diverse family of proteins known as molecular chaperones. Molecular chaperones act as catalysts for protein homeostasis by preventing protein aggregation, promoting protein folding and mediating appropriate protein degradation under both physiological and stressful conditions. These chaperones rely on a network of accessory proteins, termed co-chaperones, to fine-tune their function. As a consequence, co-chaperones are important mediators of the outcome of chaperone assisted protein homeostasis. Indeed, Hsp70 molecular chaperones cannot participate in productive protein folding without an Hsp40 co-chaperone. Equally, the co-chaperones Hop and CHIP interact with the Hsp70/Hsp90 chaperones to control triage of protein clients towards folding or degradation pathways. A co-chaperone can be defined as a non-client protein that interacts with a protein chaperone and/or its client protein to regulate chaperone function. Co-chaperones are evolutionarily conserved together with their chaperone counterparts (even being identified in the recently sequenced genome and transcriptome of the Coelacanth). Co-chaperones often outnumber their respective chaperones and are hence a way to induce specialisation of a relatively small number of chaperone isoforms. Co-chaperones may fulfil this function in a number of ways; by inducing conformational changes, delivering client proteins or regulating inherent enzymatic activities of chaperones. Many co-chaperones are modular proteins that combine the ability to bind client proteins with the capacity to interact with or modulate the activity of chaperones. Therefore, whilst co-chaperones are structurally diverse, there are conserved structural features within some families (such as the J domain of Hsp40 and the tetratricopepetide repeat (TPR) domain of some Hsp90/Hsp70 co-chaperones). Some co-chaperones (e.g. many Hsp40 isoforms) have chaperone-like activity in that they can bind and prevent aggregation of client proteins. However, most co-chaperones lack the inherent ATPase activity of chaperones and hence cannot actively refold proteins in the absence of chaperones. This second edition is timely since research in recent years has substantially expanded our understanding of co-chaperone function. For some co-chaperones, a number of new isoforms have been discovered, including FKBP immunophilin isoforms, virally encoded GroES

and the first putative co-chaperone for the organelle Hsp90, Gp96. However, the role of many of the numerous Hsp40 co-chaperones remains undefined. Our understanding and integration of the roles of known co-chaperones into cytosolic chaperone pathways has expanded. In particular, the roles of the structurally diverse Hsp90 co-chaperones during the ATP-dependent Hsp90 folding cycle have begun to emerge. We are beginning to appreciate that certain co-chaperones also function independently of chaperones and have features that are not normally associated with co-chaperone function. In particular, the established Hsp90/Hsp70 co-chaperone, Hop, is the first of this group to be shown to have independent ATPase activity; a characteristic not associated with co-chaperones. Does this suggest that it is time to reclassify Hop as a chaperone? Or will future analyses discover similar features of other co-chaperones, necessitating us to redefine the features of a co-chaperone? We have a new understanding of the role played by co-chaperones in human disease. Cell biological studies have demonstrated that some co-chaperones, like Hop and Cdc37, are expressed at higher levels in cancer, where they may contribute to maintenance of the malignant state and as such are now being considered as drug targets. We are starting to recognise that some co-chaperones are collaborative whilst others are mutually exclusive, although we perhaps don't fully appreciate the functional redundancy between co-chaperones yet. However, we still do not have a complete understanding of the spatial and temporal control of co-chaperone function. The mechanisms that control co-chaperone expression and subcellular localisation are poorly understood. Furthermore, the global control of co-chaperone and chaperone function through fluctuations of ATP levels ("energy" levels) in the cell, has not been studied in any detail. This represents a logical area to investigate towards understanding how the co-chaperone-chaperone network is tuned for different cellular states from normal through to stress and disease states. How do chaperones select their co-chaperones, particularly in cases of potential functional redundancy between certain isoforms? Likewise, while many co-chaperone isoforms (e.g. Hop) have been detected in the extracellular environment, we do not know whether these proteins function as co-chaperones outside of the cell. Indeed, many chaperones are now known to have extracellular functions and therefore it is likely that co-chaperones may too. Are there any co-chaperokines waiting to be identified? Are extracellular co-chaperones analogous to their intracellular counterparts? Our recent advances in analysis of co-chaperone function has demonstrated that there is still much to learn, and led to new questions that will ensure that research into our understanding of this important family of proteins continues.

Acknowledgements

We would like to thank all of those who have assisted us with the preparation of this book. In particular, without the commitment of new authors and re-commitment of previous authors, this book would not have been possible. We are very grateful for the essential contributions of the reviewers to the completion of the book. A special mention of thanks is also due to Mike Cheetham (UCL) for his many contributions to this process.

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Gregory Lloyd Blatch is Professor and Dean of the College of Health and Biomedicine, Victoria University (Australia). His personal research interests fall within the broad field of stress biology, and he is internationally recognized for his work on the role of molecular chaperones and co-chaperones in disease (e.g. cancer) and infection (e.g. malaria). He was awarded a Wellcome Trust International Senior Research Fellowship (UK) for biomedical aspects of his research (2002 -2007). He received his PhD from the University of Cape Town (South Africa; 1990) and did his postdoctoral at Harvard University Medical School (USA). He started his university career at the University of the Witwatersrand (South Africa), before moving to Rhodes University (South Africa). While he is now based at Victoria University, he is also a Rhodes University Visiting Professor.

Adrienne L. Edkins is Senior Lecturer in Biochemistry at Rhodes University, Grahamstown, South Africa and Acting Director of the Biomedical Biotechnology Research Unit (BioBRU). Her research interests are in the area of Hsp90 complex in cancer and stem cells, with emphasis on the analysis of extracellular Hsp90 and the TPR-containing co-chaperone Hop. Her group is funded by the NRF, MRC and CANSA and she holds a Y1 rating from the NRF. She received her PhD from the University of Glasgow (Scotland, UK; 2009) and was the recipient of a Wellcome Trust 4 Year PhD Fellowship and Beit International Postgraduate Fellowship for Zimbabwe.

Chapter 1
GrpE, Hsp110/Grp170, HspBP1/Sil1 and BAG Domain Proteins: Nucleotide Exchange Factors for Hsp70 Molecular Chaperones

Andreas Bracher and Jacob Verghese

Abstract Molecular chaperones of the Hsp70 family are key components of the cellular protein folding machinery. Substrate folding is accomplished by iterative cycles of ATP binding, hydrolysis and release. The ATPase activity of Hsp70 is regulated by two main classes of cochaperones: J-domain proteins stimulate ATPase hydrolysis by Hsp70, while nucleotide exchange factors (NEF) facilitate its conversion from the ADP-bound to the ATP-bound state, thus closing the chaperone folding cycle. Beginning with the discovery of the prototypical bacterial NEF GrpE, a large diversity of Hsp70 nucleotide exchange factors has been identified, connecting Hsp70 to a multitude of cellular processes in the eukaryotic cell. Here we review recent advances towards structure and function of nucleotide exchange factors from the Hsp110/Grp170, HspBP1/Sil1 and BAG domain protein families and discuss how these cochaperones connect protein folding with quality control and degradation pathways.

Keywords Disaggregase activity · Proteostasis · Protein structure · Protein quality control

Introduction

Cells are confronted with a variety of adverse environmental conditions such as heat shock, oxidative injury, heavy metals and glucose-depletion and pathologic states such as inflammation, tissue damage, infection, ischemia and reperfusion. To cope with this plethora of stresses, cells induce the expression of cytoprotective genes including heat shock proteins (Hsps). Many Hsps function as molecular chaperones that aid the folding, assembly and targeting of their substrate proteins. Under stress conditions, chaperones shield denatured proteins from aggregation, disassemble protein aggregates and assist protein refolding or targeting to the degradation

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_1

machinery in order to maintain protein homeostasis (proteostasis) in the cell (Hartl et al. 2011; Balch et al. 2008). Hsps can be classified into families based on their molecular mass: Hsp60, Hsp70, Hsp90, Hsp100 and small heat shock proteins. Importantly, these general molecular chaperones do not work by themselves, but are dependent on a plethora of cochaperones, which control their function. As a whole, these factors form an elaborate network that orchestrates protein folding in the cell (Kim et al. 2013; Bukau et al. 2006). Within this proteostasis network, the Hsp70 system forms a central hub at the crossroads between the translation apparatus, specialized downstream chaperones and the cellular degradation machinery. Hsp70 function is regulated by cochaperones which control its ATP hydrolysis activity. In this review we will focus on a specific group of Hsp70 cochaperones, the nucleotide exchange factors (NEF). We will present the structures and molecular function of NEFs, and discuss their role in the cellular protein folding and degradation machinery.

Hsp70 Architecture and Functional Cycle

Hsp70 was initially identified in the bacterium *Escherichia coli*, where it is named DnaK. Later Hsp70 proteins were found to be conserved in eukaryotes as well (Gupta 1998). In eukaryotes, compartment-specific isoforms were identified in cytosol/nucleus, endoplasmic reticulum (ER) lumen, and mitochondria. Human cytosol contains multiple Hsp70 paralogs, including constitutively expressed (Hsc70/HSPA8) and stress-inducible isoforms (Hsp72/HSPA1A/B). The ER-lumenal and mitochondrial forms are named BiP/Grp78/HSPA5 and mortalin/Grp75/HSPA9, respectively.

Hsp70 proteins share a conserved domain architecture containing two major domains (Fig. 1.1): an amino-terminal nucleotide binding domain (NBD) and a carboxy-terminal substrate-binding domain (SBD) (Mayer and Bukau 2005). The NBD is approximately 44 kDa in size and forms a bilobular structure that encloses a cleft with the nucleotide binding pocket at the bottom (Fig. 1.1c) (Flaherty et al. 1990). The structurally homologous lobes (I and II) of the NBD are subdivided into regions A and B. The SBD comprises of a β-sandwich subdomain with a groove that binds hydrophobic polypeptides and a carboxy-terminal α-helical "lid" that folds over the peptide binding site and facilitates high affinity substrate interaction (Zhu et al. 1996). The conserved hydrophobic NBD-SBD inter-domain linker plays an important role in conveying conformational information between the domains (Vogel et al. 2006; Swain et al. 2007).

Studies on DnaK from *E. coli* showed that Hsp70 functions through an ATP-dependent cycle (Fig. 1.1a). When ATP is bound to the NBD, the Hsp70 SBD rearranges to a conformation with low affinity for the substrate (Fig. 1.1b) (Kityk et al. 2012; Qi et al. 2013). ATP hydrolysis induces a conformational rearrangement in the NBD that detaches the SBD to assume a conformation with high affinity for segments with five consecutive hydrophobic amino acid residues in client proteins (Fig. 1.1c) (Rüdiger et al. 1997; Zhuravleva et al. 2012). Substrate binding increases

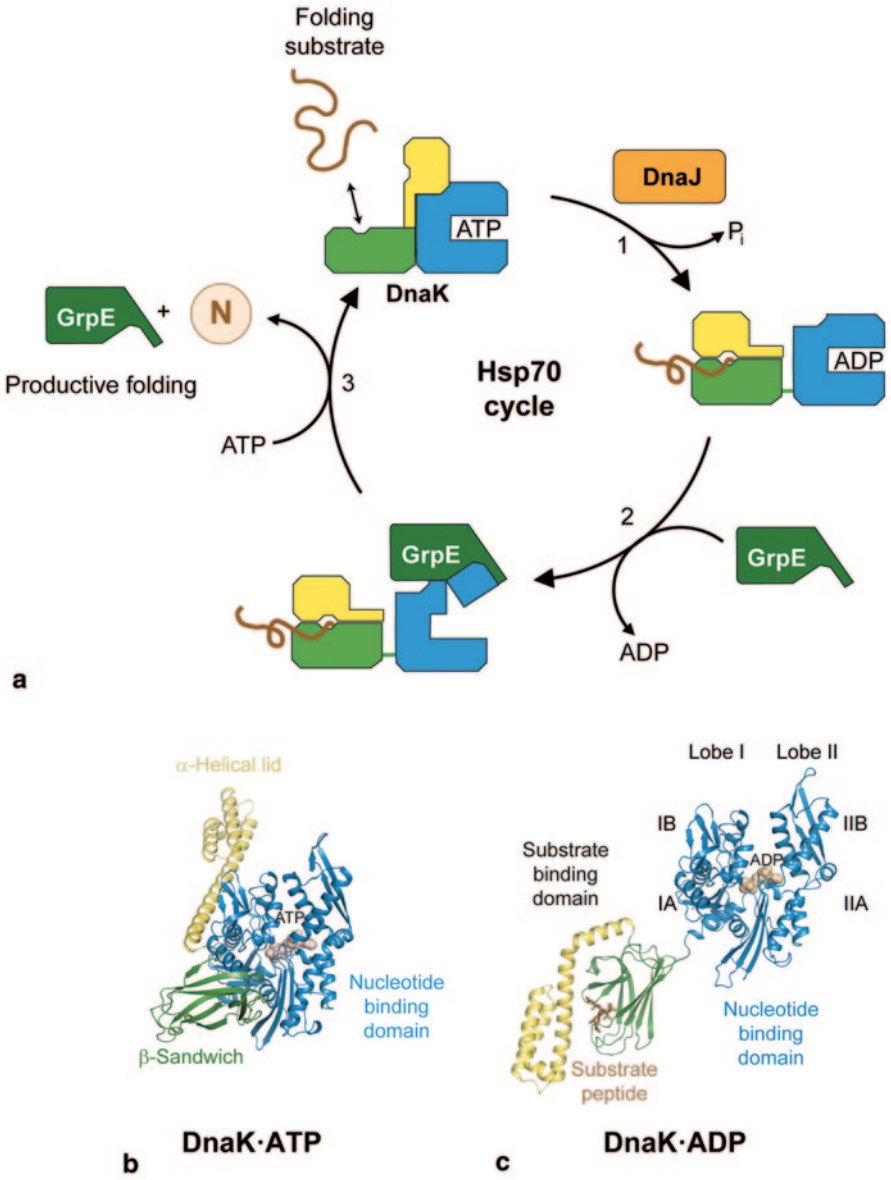


Fig. 1.1 DnaK structure and folding cycle. **a** Model for the Hsp70 folding cycle. The DnaK·ATP complex has weak substrate affinity. ATP binding to the NBD (*blue*) stabilizes a compact domain arrangement, which leaves the SBD (*yellow* and *green*) in an open conformation. This conformation exhibits dynamic interactions with the substrate (indicated in *brown*). ATP hydrolysis stimulated by DnaJ (*1*) causes a conformational change in the NBD that triggers formation of the closed SBD conformation, which has higher affinity for the substrate, resulting in a stable substrate complex. The binding of the NEF GrpE (*2*) promotes a slight opening of the NBD, which results in the release of ADP from DnaK. The cycle is reset (*3*) when a new ATP molecule binds to the NBD, triggering the release of NEF and substrate. **b** Crystal structure of the DnaK·ATP complex. The

the ATP hydrolysis rate of DnaK substantially. The spontaneous transition between the two states is slow as Hsp70 has intrinsically only weak ATPase activity. This prevents substrate-free cycling. The cycle is reset with the release of ADP and replacement with ATP, which releases the client protein for a new folding attempt.

DnaK, DnaJ and GrpE: The Eubacterial Hsp70 System

For its proper functioning in protein folding, DnaK is dependent on the ATPase-stimulating cochaperone DnaJ and the nucleotide exchange function of GrpE (Fig. 1.1a). Although interactions with substrate protein trigger ATP hydrolysis in DnaK, meaningful folding rates with model proteins are only achieved in presence of DnaJ, the prototypical Hsp40 protein (Laufen et al. 1999). Hsp40 and other J-domain proteins are reviewed in Chapter 4. Because of DnaK's slow off-rate for ADP, additional presence of GrpE is essential for *E. coli* cells to reset the Hsp70-folding cycle (Ang and Georgopoulos 1989). The combined action of the two cofactors is thought to drive the folding cycle of the molecular chaperone, resulting in repetitive rounds of substrate binding and release.

GrpE functions as the nucleotide exchange factor for DnaK by stabilizing a NBD conformation with an open nucleotide binding cleft (Harrison et al. 1997) (Fig. 1.1a). The crystal structure revealed that subdomain IIB of DnaK is rotated outwards in the complex, which weakens the contacts to ADP (Fig. 1.2).

The *E. coli* cytosol comprises of two additional isoforms of Hsp70, HscA and HscC, and five more proteins containing a J-domain. These isoforms and their associated J-protein cofactors have more specialized functions than DnaK, such as incorporation of Fe–S clusters into substrates using the IscU scaffold protein. In contrast, DnaK appears to be the more general-purpose protein-folding machine. Interestingly, functioning of HscA does not require the NEF GrpE (Brehmer et al. 2001).

The Evolution of Eukaryotic Hsp70 Systems

In eukaryotes, close sequence homologs to GrpE are only found in mitochondria and chloroplast, i.e. organelles of eubacterial origin, whereas orthologs to DnaK and DnaJ are found in the cytosol/nucleus and the ER lumen. These endosymbiont-derived organelles have thus preserved an eubacterial protein folding machinery (homologs to GroEL, GroES, HtpG and ClpA are further evidence for this), al-

peptide backbone is shown in ribbon representation, and the bound nucleotide as space-filling model (PDB code 4B9Q (Kityk et al. 2012)). The nucleotide binding, β-sandwich and α-helical domains are indicated in *blue*, *green* and *yellow*, respectively. **c** NMR model for the DnaK·ADP complex. In this state, the NBD and SBD are loosely associated (PDB code 2KHO (Bertelsen et al. 2009)). The representation mode is the same as in **b**. The peptide NRLLLTG from the complex structure with the SDB alone (PDB code 1DKZ (Zhu et al. 1996)) is superposed

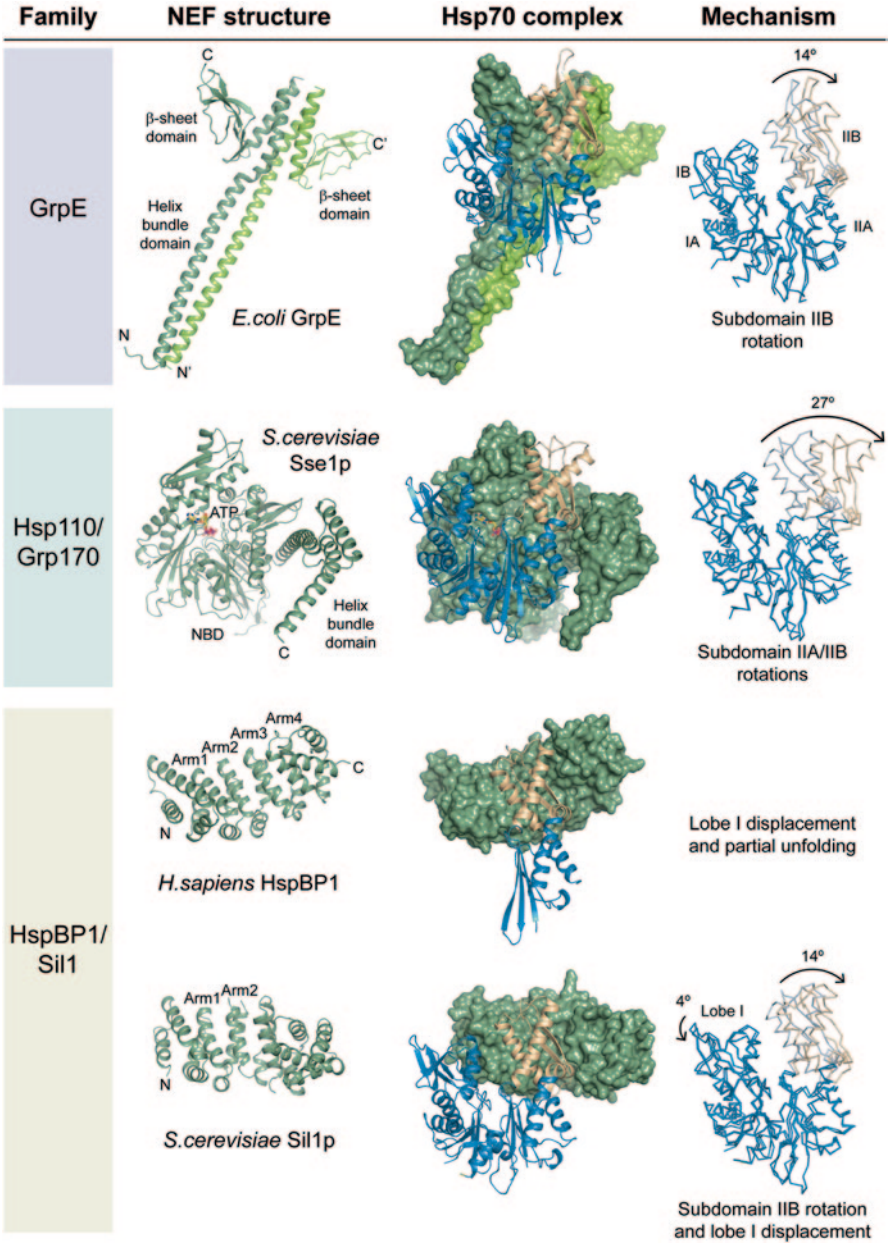
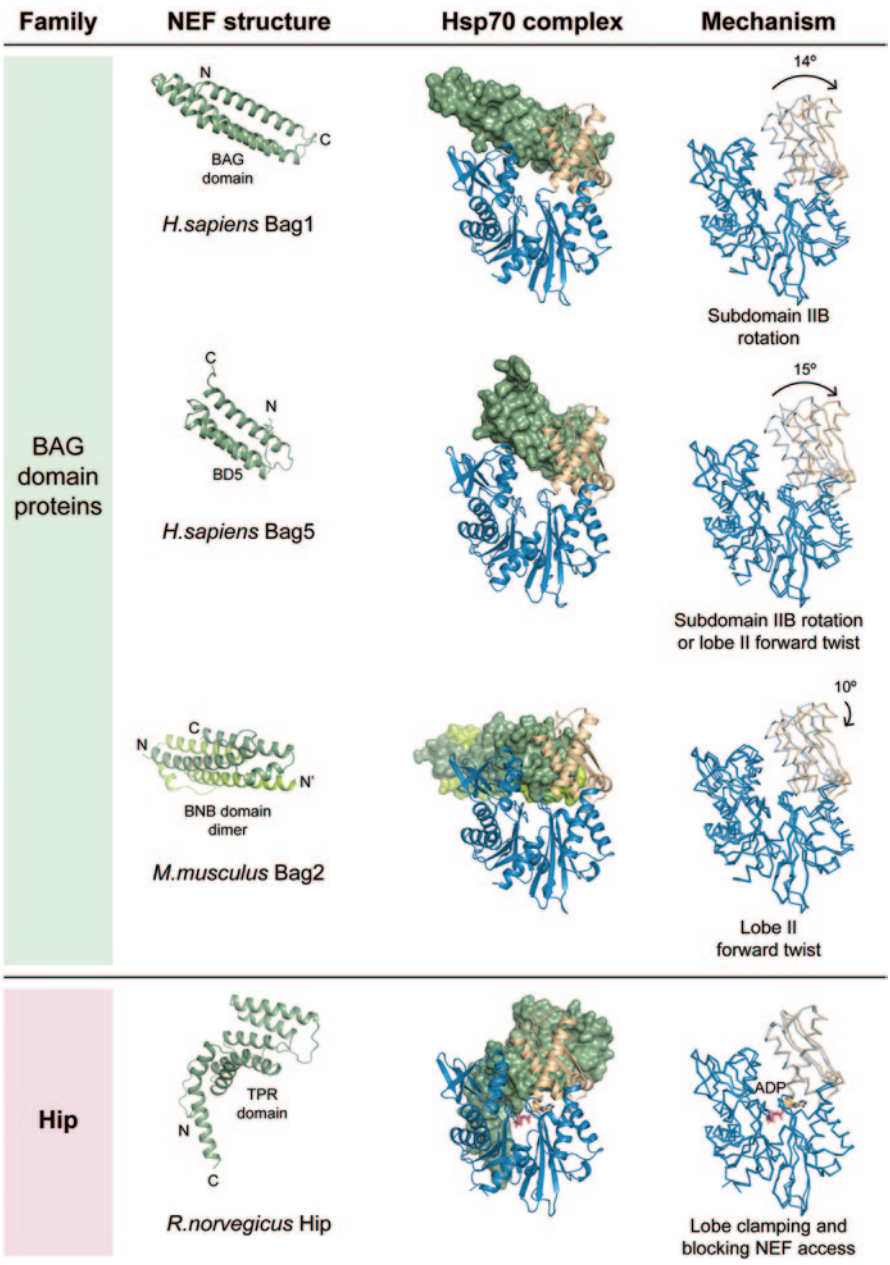


Fig. 1.2 Structure and mechanism of nucleotide exchange factors Structures for the four NEF families are shown together the respective Hsp70 complexes. The NEF is always shown in *green*; the Hsp70 NBD in *blue* with subdomain IIB highlighted in *beige*. On the *right* the structure of the NBD in the complex is superposed with the ADP-bound conformation, and the putative nucleotide exchange mechanism indicated. For comparison, the structure of the NEF-antagonist Hip is shown. The drawings are based on the PDB coordinate sets 1DKG (GrpE·DnaK (Harrison



et al. 1997)), 2V7Y (DnaK·ADP (Chang et al. 2008)), 3D2F (Sse1p·Hsp70 (Polier et al. 2008)), 1HPM (Hsc70·ADP (Wilbanks and McKay 1995)), 1XQS (HspBP1·Hsp70-lobeII (Shomura et al. 2005)), 3QML (Sil1p·Kar2p (Yan et al. 2011)), 1HX1 (Bag1·Hsc70 (Sondermann et al. 2001)), 3A8Y (Bag5·Hsp70 (Arakawa et al. 2010)), 3CQX (Bag2·Hsp70 (Xu et al. 2008)) and 4J8F (Hip·Hsp70 (Li et al. 2013))

though the respective genes were eventually transferred to the host nuclear genome. The "paralogs" of DnaK, Hsp70, Hsc70 and Bip, have somewhat different properties and are only found in eukaryotes. These proteins might thus have derived from an independent genetic transfer to the archaeal progenitor of eukaryotes, perhaps of a more specialized isoform or without the NEF. Note that the genes of DnaJ and DnaK are often part of an operon in bacteria, whereas GrpE is independently transcribed. Consistently, archaea in general do not harbor components of the Hsp70 system, unless presence of other typical bacterial genes suggests a relatively recent fusion event with an eubacterium. These archaeal Hsp70 are clearly more closely related to their eubacterial counterparts than to the Hsp70 proteins of the eukaryotic cytosol and ER lumen.

For a long time the eukaryotic Hsp70 proteins were assumed to require no NEF assistance. The measured ADP off-rates were at least one order of magnitude higher than for *E.coli* DnaK. Hence it came as quite a surprise when the first cytosolic NEF was discovered, Bag1, which belongs to a large family of BAG proteins (Höhfeld and Jentsch 1997; Takayama et al. 1999). Soon after, Sil1p and Fes1p of *Saccharomyces cerevisiae* were recognized as members of a second family of NEF proteins, the HspBP1/Sil1 proteins (Kabani et al. 2000; Kabani et al. 2002b). Finally, the Grp170/Hsp110 family of Hsp70 homologs was identified as potent NEFs to ER-lumenal and cytosolic Hsp70, respectively (Dragovic et al. 2006a; Raviol et al. 2006b; Steel et al. 2004). The fascinating details of this discovery process were reviewed earlier (Brodsky and Bracher 2007).

Now it is clear that under cellular conditions, the function of eukaryotic Hsp70 proteins is strongly dependent on nucleotide exchange factors. The combined deletion of the yeast Hsp110 homologs, Sse1p and Sse2p, is lethal (Raviol et al. 2006b; Shaner et al. 2004); the deletion of Fes1p results in a temperature-sensitive phenotype, suggesting severe problems in protein folding (Shomura et al. 2005; Kabani et al. 2000). The probable reason for the early misconception of NEF expendability is the presence of considerable amounts of inorganic phosphate (P_i) in cellular fluids (17–27 mM in *S. cerevisiae* according to ^{31}P-NMR measurements (Gonzalez et al. 2000)). Additional binding of P_i lowers the spontaneous off-rate of ADP from eukaryotic Hsp70 by approximately one order of magnitude, apparently through reduced nucleotide binding domain (NBD) dynamics (Arakawa et al. 2011; Gässler et al. 2001). Thus the spontaneous off-rate of eukaryotic Hsp70 under physiological conditions is actually close to that of DnaK.

The Grp170/Hsp110 family of Hsp70 NEFs appears to be the most ancient and universal type of eukaryotic Hsp70 NEFs (Table 1.1). Coding sequences for probable homologs were identified in virtually every eukaryotic genome so far. Humans have three genes for cytosolic isoforms (Hsp105/Hsp110, Apg-1 and Apg-2) and one ER-lumenal form (Grp170); *S. cerevisiae* has two cytosolic (Sse1p and Sse2p) and one ER-resident form (Lhs1p). Grp170/Hsp110 family proteins are distantly related to eukaryotic Hsp70. Apparently they have emerged from functional specialization of Hsp70 paralogs. The other NEF families, BAG domain proteins and Sil1/HspBP1 homologs, have rather generic structures frequently found in the eukaryotic proteome, specifically helix bundles and successions of Armadillo repeats,

Table 1.1 Eukaryotic NEFs for Hsp70 proteins

		Species	Name	Proposed involvement in	References
GrpE	Mitochondria	*S. cerevisiae*	Mge1p	Protein import and maturation	
		A. thaliana	*At*Mge1	Possible UV-B stress tolerance	(Hu et al. 2012)
			*At*Mge2	Chronic heat stress tolerance	
		H. sapiens	Mge1	Possible thermosensor function	
	Chloroplast	*Chlamydomonas reinhardtii*	Cge1	Protein import, temperature tolerance, VIPP1 oligomer assembly	(Liu et al. 2010; Willmund et al. 2007)
		Physcomitrella patens	*Pp*Cge1, *Pp*Cge2	Protein import and maturation	(Shi and Theg 2010)
Hsp110/Grp170	Cytosol/nucleus	*Plasmodium falciparum*	*Pf*Hsp110c	Proteome stabilization in malarial fevers	(Muralidharan et al. 2012)
		S. cerevisiae	Sse1p	Substrate binding, constitutive expression	(Mukai et al. 1993)
			Sse2p	Stress-inducible isoform	
		S. pombe	Pss1	Ras1 GTPase signaling	(Chung et al. 1998)
		N. crassa	Hsp88	Direct binding to small Hsp Hsp30	(Plesofsky-Vig and Brambl 1998)
		A. thaliana	Hsp91/Hsp70-14	Possible role in thermotolerance	(Jungkunz et al. 2011; Storozhenko et al. 1996)
		D. melanogaster	HSC70cb	Suppresses aggregation-induced toxicity along with DnaJ-1	(Kuo et al. 2013)
		H. sapiens	Hsp105α,β/Hsp110/HspH1	Dissaggregase activity, substrate binding	
			Apg-1/Osp94/HspA4L	Spermatogenesis	
			Apg-2/HspA4	Spermatogenesis, dissaggregase activity	
	ER	*S. cerevisiae*	Lhs1p	Protein translocation, lumenal folding, UPR, ERAD	
		H. sapiens	Grp170/Hyou1/Orp150	Response to oxygen deprivation	

Table 1.1 (continued)

		Species	Name	Proposed involvement in	References
HspBP1/Sil1	Cytosol/nucleus	*S. cerevisiae*	Fes1p	Co-translational folding, degradation of misfolded proteins	
		A. thaliana	*At*Fes1A	Thermotolerance, response to salt stress	(Zhang et al. 2010)
		H. sapiens	HspBP1	Hsp70 inhibition?	
	ER	*S. cerevisiae*	Sls1p/Sil1p	Protein translocation, lumenal folding, UPR, ERAD	
		H. sapiens	Sil1/BAP	Neuronal morphology, migration and axon growth	
BAG-domain proteins	Cytosol/nucleus	*S. cerevisiae*	Snl1p	Ribosomal editing, co-translational folding	
		S. pombe	Bag101/Bag-1A	Protein QC and degradation	
			Bag102/Bag-1B	Kinetochore integrity	
		A. thaliana	*At*Bag1–*At*Bag4	Plant programmed cell death	(Kabbage and Dickman 2008; Doukhanina et al. 2006)
			*At*Bag5	Calmodulin signaling?	
			*At*Bag6	Calmodulin signaling?	
		C. elegans	Bag-1	Protein QC and degradation?	
			Bag-2/unc-23	Muscle maintenance?	
		D. melanogaster	Starvin	Muscle maintenance, recovery from cold stress	(Coulson et al. 2005)
		H. sapiens	Bag1/Rap46/HAP	Apoptosis, protein QC and degradation	
			Bag2	CHIP Ub-ligase inhibition	
			Bag3/CAIR-1	Autophagy, cell adhesion and migration	
			Bag4/SODD	Apoptosis	
			Bag5	Parkin Ub-ligase inhibition	
			Bag6/Scythe/BAT-3	GET pathway, protein degradation	
	ER	*A. thaliana*[a]	*At*Bag7	UPR	(Williams et al. 2010)

[a] *A. thaliana* has homologs to Sil1 (F4F15.90) and Grp170 (AtHsp70-17). These were however not characterized at the protein level yet

respectively. Such scaffolds can rapidly (on an evolutionary timescale) adapt to a new function after a gene duplication event, and have been employed over and over again in eukaryotic protein evolution. Helix bundles are for example also found in syntaxin SNARE proteins; Armadillo and HEAT repeat proteins in nuclear transport factors and β-catenin (Tewari et al. 2010). It is conceivable that BAG proteins have emerged multiple times, having short and long 3-helix bundle structures (Bag1 and Bag4/Bag5), insertions or 4-helix bundle dimer structures (Bag2). Their few common signature residues are forced by the evolutionary constraints on the binding partner, the NBD of Hsp70, which exhibits high surface conservation (for details see below). It moreover appears that the ER-lumenal NEF Sil1 from yeast and animals have evolved independently: Although yeast Sil1p resembles the mammalian HspBP1 at the secondary and tertiary structure level (Shomura et al. 2005; Yan et al. 2011), it appears to employ a binding mode and mechanism of action distinct from mammalian Sil1, which acts more similar to HspBP1 (Hale et al. 2010; Howes et al. 2012). Consequently, the ancestry and exact functional role of BAG and Sil1/HspBP1 protein homologs in different species is difficult to rationalize on sequence data alone. Humans and *Arabidopsis thaliana* have six and seven known cytosolic BAG isoforms, respectively (Table 1.1); yeast has one ER-membrane-bound homolog, Snl1p, but exact functional homologs to Snl1p have not been identified in humans and *Arabidopsis* either (Sondermann et al. 2002; Takayama et al. 1999).

In addition to the emergence of three Hsp70 NEF families in multiple isoforms in eukaryotes, an even more dramatic expansion in J-domain protein diversity has occurred, resulting in approximately 40 isoforms in humans (see review in (Kampinga and Craig 2010)).

Molecular Structure and Function of Eukaryotic NEFs

Eukaryotic GrpE Homologs

Structural data for eukaryotic GrpE homologs are not yet available. Judging from sequence alignments, their structures are likely fairly similar to bacterial GrpE proteins, which have been solved for the *E. coli* (Harrison et al. 1997), *Thermus thermophilus* (Nakamura et al. 2010) and *Geobacillus kaustophilus* (Wu et al. 2012) homologs. All these proteins have dimeric two-domain structures composed of a coiled-coil helix bundle and a wing-like β-domain (Fig. 1.2). One β-domain engages in contacts with subdomains IB and IIB, assisted by additional contacts from the helix bundle, stabilizing a NBD conformation with an open nucleotide binding cleft. Opening is enabled by an outwards rotation of subdomain IIB.

Simulations suggest a highly dynamic structure for the NBD of Hsp70 proteins, allowing shearing motions between the lobes and an outwards rotation of subdomain IIB around an inbuilt hinge, which likely influence the nucleotide exchange rate (Ung et al. 2013). GrpE and the other Hsp70 NEFs appear to capture and

stabilize open states in which a subset of the interactions between NBD and ADP is disabled, thereby lowering ADP affinity. Substantial parts of the NBD contact area with GrpE become buried near the lobe interface in the ADP-bound conformation of DnaK, suggesting that GrpE captures open conformations, but cannot 'force' the NBD to open. ATP binding induces a conformational change in the NBD of DnaK, displacing the binding sites on lobes I and II by inter-lobe shearing, resulting in strongly decreased affinity to GrpE. So both ADP and ATP compete with GrpE for binding to DnaK.

The Hsp110 Family of Nucleotide Exchange Factors

The Hsp110/Grp170 proteins belong to the Hsp70 protein family (Easton et al. 2000). Crystal structures of the yeast Hsp110 protein Sse1p revealed a shared domain composition comprising a N-terminal actin-type nucleotide binding domain, followed by a β-domain and a α-helix bundle (Liu and Hendrickson 2007; Polier et al. 2008; Schuermann et al. 2008). Hsp110 family protein sequences are however much less conserved than canonical Hsp70, with the greatest divergence found in the C-terminal domains. Backbone extensions compared to canonical Hsp70 proteins are found at the C-terminus and within the β-domain (Fig. 1.3). The Grp170 homologs have even larger extensions than cytosolic homologs and always bear N-terminal import and C-terminal ER-retention signal sequences (Table 1.1).

In the crystal structures of Sse1p, the α-helix bundle is associated with the flank of the NBD, resulting in a compact conformation (Fig. 1.2). The β-domain undergoes extensive interactions with the bottom of the NBD, but not with the α-helix bundle domain, which extends in the opposite direction. Sse1p exhibits a pronounced twist of the NBD lobes, revealing a bound ATP molecule in the center. Structures of an ATPase-inactive DnaK mutant later demonstrated that the binding of ATP induces a very similar conformation in canonical Hsp70 proteins (Kityk et al. 2012; Qi et al. 2013).

In the crystal structures of the complex, the NBDs of Sse1p and mammalian Hsp70 face each other in a pseudo-symmetrical fashion (Polier et al. 2008; Schuermann et al. 2008). The NBD of Hsp70 is captured in an open conformation by additional interactions of subdomain IIB with the α-helix bundle domain of Sse1p. In this conformation, ADP cannot simultaneously engage in direct interactions with all four subdomains and is thus more likely to dissociate, explaining the nucleotide exchange activity of Sse1p. The residues mediating key contacts to Hsp70 are conserved in all Hsp110/Grp170 proteins (Andreasson et al. 2010; Hale et al. 2010). Only the compact, ATP-bound conformation of Hsp110/Grp170 proteins provides the necessary geometry required for simultaneous interactions between NBD·NBD and α-helix bundle·subdomain IIB of Hsp110/Grp170 and Hsp70, respectively (Raviol et al. 2006b; Shaner et al. 2004; Andreasson et al. 2008).

Besides serving as essential NEFs for Hsp70, Hsp110/Grp170 proteins potently stabilize denatured proteins against aggregation (Goeckeler et al. 2002;

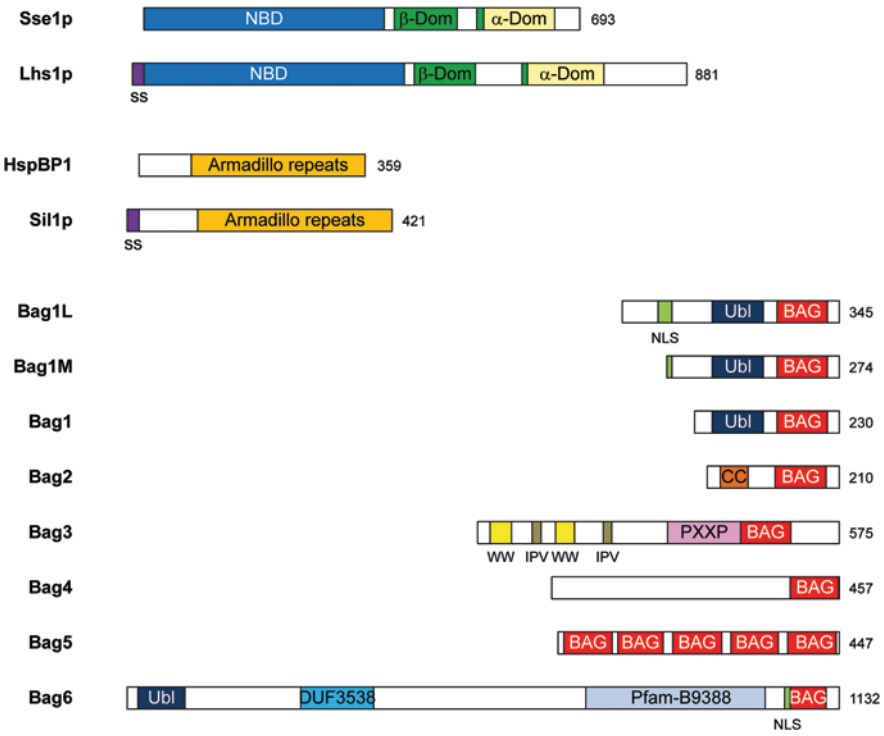


Fig. 1.3 Domain architectures of different NEF families As examples for Hsp110 and Grp170 proteins the yeast homologs Sse1p and Lhs1p are shown, respectively. Both consist of an N-terminal nucleotide binding domain (NBD, *blue*), a β-sandwich (β-Dom, *green*) and a α-helix bundle domain (α-Dom, *pale yellow*). SS indicates a signal sequence for ER import. The HspBP1/Sil family proteins have characteristic Armadillo repeat folds (*orange*). All members of the BAG family in humans, Bag1–6, contain C-terminal Hsp70-binding BAG domains (*red*), but have otherwise divergent domain architecture. Bag5 has four additional BAG domains of unknown function. Bag1 isoforms and the large Bag6 contain Ubiquitin-like domains (Ubl, *dark blue*), which might associate with the regulatory particle of the 26S proteasome. Bag6 has furthermore two probable domains, which have not yet been characterized further. Bag2 contains a coiled-coil dimerization domain (CC, *orange*). Bag3 comprises multiple N-terminal sequence motifs, WW domains (WW, *yellow*), IPV sequence motifs (*brown*) and PXXP repeats (*pink*). Bag1 L and Bag6 have NLS sequences (*light green*) for nuclear targeting

Oh et al. 1997; Oh et al. 1999). The molecular basis for this holdase activity is still controversial. Canonical Hsp70 proteins stably interact with substrate proteins only in the ADP state, enclosing hydrophobic peptide segments between β-domain and α-helix bundle. While Sse1p appears to have no intrinsic ATPase activity—bound ATP survived in the crystallization experiments for weeks—ATPase stimulation by J-domain proteins has been observed (Mattoo et al. 2013; Raviol et al. 2006a). Consistently, binding of Sse1p and human Hsp105 to hydrophobic peptides has been reported, although with a preference towards aromatic residues in contrast to canonical

Hsp70s, which prefer aliphatic sidechains and prolines (Goeckeler et al. 2008; Xu et al. 2012; Rüdiger et al. 1997; Zahn et al. 2013). Because of their low sequence conservation in the β-sheet domain, Hsp110 orthologs may differ considerably in their substrate binding properties. For example, Sse1p potently stabilizes the model protein firefly luciferase (FLuc) at 42 °C for subsequent refolding, while its close homolog Sse2p is inactive (Polier et al. 2010). The reason for this surprising difference seems to be that Sse1p unfolds partially at 37 °C with a concomitant increase in aggregation prevention capacity, while the paralogous Sse2p is stable until 46 °C, similar to human Apg-2, which unfolds at 51 °C (Polier et al. 2010; Raviol et al. 2006a).

While the Hsp110 holdase activity appears to be important, its NEF function is critical (Raviol et al. 2006b; Shaner et al. 2004). Only mutant forms of Sse1p that abolish interactions with Hsp70 and nucleotide exchange were lethal in the *SSE1*/*SSE2* deletion background (Polier et al. 2008). Similar requirements were found for the 'mammalian disaggregase' function of Hsp110, Hsp70 and Hsp40 (see below).

Sil1/HspBP1 homologs

HspBP1 (Hsp70 binding protein 1) is the mammalian homolog of the cytosolic Fes1p protein in *S. cerevisiae* (Kabani et al. 2002a; Kabani et al. 2002b; Raynes and Guerriero 1998). The ER-lumenal paralogs are named Sls1p/Sil1p or Sil1/BAP (Bip associated protein) in yeast and mammals, respectively (Kabani et al. 2000; Chung et al. 2002). Sil1 homologs occur almost ubiquitously in eukaryotes. Homologs to HspBP1 are found in most animal, plant, algal and fungal genomes. Sil1/HspBP1 proteins are composed of a divergent N-terminal part of ~85 residues and a conserved C-terminal core domain, which alone is sufficient to mediate nucleotide exchange (Fig. 1.3). Crystal structures showed that the core domains of human HspBP1 and yeast Sil1p consist of Armadillo repeats flanked by capping helix pairs (Shomura et al. 2005; Yan et al. 2011) (Fig. 1.2). Surprisingly, the complex structures with the respective Hsp70 binding partner revealed distinct binding modes for the paralogs. The curved-shaped HspBP1 associates so extensively with subdomain IIB of the Hsp70 NBD that the bulk of the NEF clashes severely with lobe I, thereby destabilizing its fold as judged from tryptophan fluorescence quenching and increased sensitivity against protease degradation (Shomura et al. 2005). Yeast Sil1p also embraces subdomain IIB, however using different molecular contacts, resulting in a distinct region covered by the NEF (Yan et al. 2011). This binding mode just induces an outward rotation of subdomain IIB and a slight sideways displacement of lobe I, more similar to the complexes with GrpE (Harrison et al. 1997) and the Hsp110 protein Sse1p (Polier et al. 2008; Schuermann et al. 2008). The binding mode of animal and plant Sil1 appears to resemble HspBP1 closer than yeast Sil1, as judged from mutational analysis (Hale et al. 2010; Howes et al. 2012).

BAG domain-containing NEFs

BAG (Bcl-2 associated athanogene) family proteins have a modular domain architecture comprising a conserved region of ~100 amino acids at the C-terminus, called the BAG domain (Takayama et al. 1999). In the N-terminal part diverse domains and sequence motifs were found for BAG domain proteins (Fig. 1.3). The human genome comprises six BAG family protein sequences, which were numbered Bag1–6 (Takayama and Reed 2001) (Table 1.1). As pointed out above, these proteins are structurally and functionally quite heterogeneous, and will be discussed here one after the other. Only Bag1 and Bag3 appear to be conserved in most metazoans. Homologs have been described in the fruit fly *Drosophila melanogaster* (Arndt et al. 2010), the nematode worm *Caenorhabditis elegans* (Nikolaidis and Nei 2004) and the tunicate *Ciona intenstinalis* (Wada et al. 2006).

The first structures to be solved were the BAG domain of Bag1 in isolation and in complex with the NBD of Hsc70, revealing a bundle structure with three long α-helices for the BAG domain (Sondermann et al. 2001; Briknarova et al. 2001) (Fig. 1.2). Interactions with α-helices 2 and 3 of Bag1 stabilize a conformational change in the Hsc70 NBD similar to the GrpE·DnaK complex (Harrison et al. 1997; Sondermann et al. 2001). Three different isoforms of Bag1 exist in cells, which are generated by alternative translation initiation from a single mRNA (Fig. 1.3). All Bag1 isoforms contain an ubiquitin-like (Ubl) domain that serves as a sorting signal to facilitate interaction with the 26S proteasome (Alberti et al. 2003). The Bag1 L isoform contains an additional nuclear localization signal (NLS) at the extreme N-terminus, whereas the other two isoforms are present in the cytosol (Takayama et al. 1998). Interestingly, the BAG domain shares binding sites with Hsc70 and Raf1, a stress-signaling anti-apoptotic kinase, and the two proteins bind Bag1 in a mutually exclusive manner (Song et al. 2001). The structure of the Ubl domain from mouse Bag1 has been solved by NMR, revealing a characteristic ubiquitin-like fold (Huang and Yu 2013). In mice, this domain of Bag1 mediates interaction with the cytoplasmic tail of the heparin-binding EGF-like growth factor (HB-EGF) precursor, thereby altering cell adhesion and secretion of the mitogen HB-EGF (Lin et al. 2001).

Bag3 is expressed prominently in striated muscle tissue, but is also necessary for development and blood cell formation. Bag3 deletion in mice resulted in severe myopathy (Homma et al. 2006) and loss of hematopoietic stem cells (Kwon et al. 2010). Interestingly, Bag3 is the only heat stress-inducible BAG-domain protein (Franceschelli et al. 2008; Jacobs and Marnett 2009). Bag3 contains various sequence motifs and domains, such as WW domains and proline-rich repeats (PXXP), which mediate interactions with numerous partner proteins other than Hsp70. For example, the first WW domain was shown to interact with PXXP motifs at the C-terminus of PDZGEF2, a regulatory protein involved in cell adhesion (Iwasaki et al. 2010); binding to the small heat shock proteins HspB8 and HspB6 is mediated by two IPV motifs (Fuchs et al. 2010). The PXXP repeats of Bag3 likely interact with SH3 domains found in regulatory proteins of cell adhesion and migration (Doong et al. 2000). These interactions link Bag3 to processes such as development, autophagy and cytoskeletal organization (reviewed in (Rosati et al. 2011)). The

complex of Bag3, Hsc70 and HspB8 was strongly implicated in macroautophagy (Arndt et al. 2010; Lamark and Johansen 2012), a process in which portions of the cytosol are engulfed by a membrane and digested. The complex appears to be involved in targeting aggregated proteins to aggresomes for degradation. Aggresomes are microtubule-dependent collection points for such terminally misfolded proteins in the cell (Kopito 2000). Details are unclear, but Bag3 interacts and co-localizes with p62/SQSTM1, a key regulator of the macroautophagy pathway (Gamerdinger et al. 2009). An association of Bag3 with the adaptor protein 14-3-3γ is dependent on phosphorylation at Ser136 and Ser173, and may serve to attach aggregates to the motor protein Dynein that travels along microtubules (Xu et al. 2013). Macroautophagy appears to be vitally important for muscle maintenance. In *D. melanogaster* muscles, the Bag3 ortholog Starvin is required for Z-disk maintenance through a process named 'chaperone-assisted selective autophagy' (CASA) (Arndt et al. 2010). A complex of Bag3, Hsc70 and HspB8 is needed for autophagy of the large muscle protein filamin after mechanical tension-induced unfolding (Ulbricht et al. 2013). Autophagosome formation is dependent on the interaction of the Bag3 WW domain with the filamin-interacting protein synaptopodin-2.

Bag4 is alternatively named "silencer of death domains" (SODD) as it binds to the cytoplasmic regions of receptors that signal cell death, namely TNFR1 and DR3, and prevents ligand-independent receptor signaling and apoptosis (Jiang et al. 1999). Surprisingly, NMR structures showed that the three-helix bundle in Bag4 is about 25 amino acids shorter than in Bag1, although it comprises the signature residues needed for interaction with Hsp70 proteins, suggesting that it might have evolved independently (Brockmann et al. 2004; Briknarova et al. 2002). Bag1, Bag3 and Bag4 have been shown to bind the anti-apoptotic protein Bcl-2 (Antoku et al. 2001). Together with their ability to interact with Hsp70, which also has an anti-apoptotic function, this suggests linked mechanisms for apoptosis inhibition (Antoku et al. 2001). It is not known whether these BAG domain proteins can simultaneously bind Hsp70 and Bcl-2, but it has been hypothesized that these two proteins compete for binding as they both can interact with the BAG domain (Doong et al. 2002).

Among the Bag proteins, Bag5 is unique in containing five consecutive short BAG domains similar in structure to the BAG domains of Bag3 and Bag4 (Arakawa et al. 2010). Of these, only the fifth BAG domain is active in Hsp70 NBD binding and assisting Hsp70-mediated substrate refolding. The crystal structure of this domain with the Hsp70 NBD revealed two distinct conformations of the complex; one where the NBD is in an open state similar to the Bag1 complex and the other with a NBD exhibiting a binding pocket distorted by inter-lobe shearing (Arakawa et al. 2010; Fig. 1.2). Both conformational states likely have reduced affinity for ADP. The functional consequences that could result from the shorter BAG domain structures in Bag3, Bag4 and Bag5 are currently unknown. Interestingly, Bag5 was shown to associate with the E3-ubiquitin ligase Parkin, modulating substrate protein ubiquitylation (Kalia et al. 2004).

Bag2 is the most distantly related member of the BAG family. In the crystal structures, what was supposed to be the BAG domain adopted an unanticipated dimeric

structure formed by pairs of long antiparallel helices intersected by a short additional helix (Xu et al. 2008 Fig. 1.2). Considering these differences from the canonical BAG domain and the low sequence homology, the respective fold was termed 'brand new BAG' (BNB) domain. Binding of Bag2 to Hsp70 also elicits a different conformational change in the NBD—a rotation of the entire lobe II (Fig. 1.2). The BNB domain was also implicated in substrate binding (Xu et al. 2008). Bag2 has clearly lower affinity for Hsp70 proteins than other NEFs (Rauch and Gestwicki 2014), but might compensate by being a dimeric protein with two Hsp70 interaction sites. Thus, Bag2 might be considered the most 'eccentric' BAG family protein. Consistently, Bag2 was found to impair the function of the Hsp70-associated E3-ubiquitin ligase 'carboxyl terminus of Hsp70-interacting protein' (CHIP), in contrast to Bag1, which seems to target substrate proteins for degradation in collaboration with CHIP (Arndt et al. 2005; Dai et al. 2005). Complex formation with CHIP and Hsc70 is dependent on an N-terminal coiled-coil region that forms a dimer structure on its own (Page et al. 2012). Overexpression of Bag2 inhibited CHIP activity and thereby stimulated chaperone-assisted maturation of the model protein cystic fibrosis transmembrane conductance channel regulator (CFTR) (Arndt et al. 2005). Employing a different mechanism, Bag2 has also been suggested to facilitate degradation of Tau, an aggregation-prone protein that accumulates in neurons of Alzheimer's disease patients (Carrettiero et al. 2009). The microtubule-tethered Bag2·Hsp70 complex was proposed to deliver Tau to the proteasome for degradation in an ubiquitin-independent manner.

Multiple functions have been ascribed to the human Bag6/Scythe/BAT3 protein (reviewed in Binici and Koch 2013). Bag6 comprises an Ubl domain similar to Bag1; the predicted domain DUF3538 is found in several proteins associated with the ubiquitin-proteasome (UPS) pathway. Bag6 contains a nuclear localization signal that is masked by its interaction with a cofactor named TRC35 allowing the protein to be mostly cytosolic (Wang et al. 2011). In the cytosol, Bag6 appears to be specifically recruited to the ribosome to shield the transmembrane domains of ER-localized tail-anchored membrane proteins (TA proteins) from the aqueous cytosolic environment. TA proteins are inserted post-translationally into the ER membrane. In this so-called '*g*uided *e*ntry of *T*A proteins' (GET) pathway, Bag6 transfers the TA protein to TRC40 for membrane targeting by association with TRC35 and Ubl4A (Mariappan et al. 2010).

In other organisms only few BAG domain proteins have been studied. Snl1p is the only known BAG domain-containing protein in *S. cerevisiae* (Table 1.1). This protein contains an N-terminal single transmembrane (TM) domain localizing it to the ER and nuclear membranes. The BAG domain faces the cytosol. Biochemical and genetic experiments have shown Snl1p to interact with cytosolic Hsp70s and components of the nuclear pore, respectively, but no phenotype could be associated with the deletion of Snl1p (Sondermann et al. 2002). Interestingly, a short lysine-rich motif at the beginning of the Snl1p BAG domain facilitates its interaction with intact ribosomes (Verghese and Morano 2012). This motif common with a *Candida albicans* homolog, which lacks the TM region, is independent from the Hsp70 interaction region. It was proposed that the Bag homologs in fungi may serve a

previously unknown role in protein biogenesis based on the recruitment of Hsp70 and ribosomes to the ER membrane.

Bag1 has two putative orthologs in the fission yeast *Schizosaccharomyces pombe*, Bag101/Bag1A and Bag102/Bag1B. Both proteins have an Ubl and a C-terminal BAG domain and associate with the 26S proteasome and Hsp70 respectively. Interestingly, Bag102 contains an additional N-terminal single transmembrane helix localizing it to the ER/nuclear membrane, similar to Snl1p. It was recently found that Bag102 but not Bag101 was able to suppress the temperature-sensitive growth phenotype and the DNA segregation defect of a *spc7-23* strain, which contains a point mutation in a conserved kinetochore component Spc7 (Kriegenburg et al. 2014). This suggests that these two BAG proteins in fission yeast have separate and specific cellular functions.

The crystal structure of the BAG domain of the *C. elegans* Bag1 homolog revealed a dimeric structure of two protomers forming mixed three-helix bundles (Symersky et al. 2004). A small β-sheet between helices 2 and 3 interferes with the formation of an intramolecular three-helix bundle in this ortholog. However, the function of this BAG domain protein as well as the putative Bag3 homolog unc-23 remains poorly characterized.

In *D. melanogaster*, the Bag3 homolog starvin was shown to be regulated in a highly developmental-stage specific manner and is expressed in larval somatic muscles. The name starvin was coined as this protein was essential for viability and was required by newly hatched larvae to ingest food and grow (Coulson et al. 2005). Starvin expression correlates with the response to cold exposure in *D. melanogaster*, but the precise role of this protein in the pathway is not known (Colinet and Hoffmann 2010). It was proposed that it plays a role in modulating Hsp70 chaperone activity during recovery, although this will have to be experimentally verified.

Plants contain a large variety of BAG family proteins. Sequences for seven isoforms, named AtBAG1-7, have been identified in the *A. thaliana* genome (Kabbage and Dickman 2008). A comparative structural study on the AtBAG1-4 proteins, which share an architecture consisting of a Ubl and a BAG domain, showed that the respective BAG domains have short three-helix bundle structures similar to human Bag3, Bag4 and Bag5 (Fang et al. 2013). All the proteins lower the binding affinity of ADP with the NBD to a similar degree, suggesting functional redundancy. The structure of the complex of AtBAG1 with the NBD of human Hsp70 revealed for the first time Ubl and BAG domain in context, showing an extended conformation (Fang et al. 2013). The NBD conformation was similar to the Bag1 and Bag5 complexes, with the subdomain IIB rotated 15° away from the nucleotide binding site. AtBAG2 mutant plants are larger than wildtype counterparts, implicating a function of this isoform in plant programmed cell death (PCD). Similarly, AtBAG4 confers tolerance to salt stress, apparently also by inhibiting PCD (Doukhanina et al. 2006). AtBAG7 is the only known ER-lumenal BAG domain protein (Williams et al. 2010).

Thus, in cells the diversity of BAG domain-containing proteins appears to serve in recruiting Hsp70 to specific locations and for specific functions. How the combinatorial assembly with multiple co-chaperones governs the biochemical properties of Hsp70 will be a fertile field for further studies.

Antagonism Between Hip and NEFs

It was recognized early on that the protein Hip (Hsc70-interacting protein, alternatively named ST13, suppressor of tumorigenicity) antagonizes Bag1, then the only known eukaryotic NEF (Kanelakis et al. 2000). Hip is present in protozoa, plants and animals, and is composed of a dimerization domain, a tetratricopeptide repeat (TPR) domain for interactions with the NBD of Hsp70 and a C-terminal DP domain (DP stands for Asp-Pro motifs) connected by flexible peptide linkers (Velten et al. 2002). The crystal structure of the core complex consisting of the Hip middle domain and the NDB of Hsp70, showed that Hip slows ADP dissociation by forming a bracket over the nucleotide binding cleft (Li et al. 2013), functionally opposing NEFs (Fig. 1.3). Moreover the binding area on the NBD overlaps substantially with the known contact regions for NEFs, indicating that interactions are mutually exclusive. The binding affinity between Hip and Hsp70 is however approximately two orders of magnitude lower than with NEFs, which exhibit dissociation constants of around 0.1 μM (Raviol et al. 2006b; Shomura et al. 2005; Sondermann et al. 2001). Therefore, Hip would only slow the Hsp70 cycle substantially, when the mutual affinity is increased, for example by additional interactions with Hsp70-bound substrates (via the DP domains of Hip) or simultaneous interaction with two Hsp70 molecules attached to the same client protein or aggregate. Such hallmarks might indicate substrates, which require downstream chaperones like Hsp90 for folding, or are hopeless clients that need to be degraded (Wang et al. 2013).

Cellular Functions of NEF Proteins in *S. cerevisiae*

A comprehensive picture of NEF function is only available for one eukaryotic organism, *S. cerevisiae*. This budding yeast comprises seven NEFs associated with the isoforms of Hsp70 found in the mitochondrial matrix (Ssc1p and Ssq1p), the ER lumen (Kar2p) and the cytosol/nucleus (Ssa1-4, Ssb1/2), namely the GrpE homolog Mge1p, Lhs1p (Grp170 homolog) and Sil1p in the ER lumen, and Sse1p and Sse2p (Hsp110 homologs), Fes1p (HspBP1 homolog) and Snl1p, the only known BAG domain protein, in yeast cytosol.

Mge1p plays an important role in both import and maturation of mitochondrial matrix proteins encoded in the nucleus (Laloraya et al. 1995; Laloraya et al. 1994), and is therefore essential for yeast viability (Ikeda et al. 1994). During the final step of import through the inner mitochondrial membrane, Ssc1p and Mge1p form a complex with the import channel-associated protein Tim44p (Horst et al. 1997). Protein import is furthermore dependent on the activity of the membrane-anchored J-protein complex Tim16p/Tim14p (also known as Pam18p/Pam16p) (Mokranjac et al. 2006). The mechanism of protein import—whether ATP-hydrolysis driven power strokes, entropic pulling or a Brownian ratchet, which would only prevent back-sliding—is still under discussion. Overexpression of Mge1p leads to reduced pre-protein translocation into the mitochondria, likely due to excessive acceleration

of nucleotide exchange and premature conversion of Ssc1p to the low affinity state, thus causing rapid release of the protein in transit (Schneider et al. 1996). In vitro studies have shown that ATP but not ADP effectively releases Mge1p interaction with mtHsp70 (Miao et al. 1997). In the mitochondrial matrix, Mge1p furthermore helps to fold newly imported proteins. A strain harboring the temperature sensitive allele *mge1-100* showed reduced rates of maturation of the Yfh1p protein, similar to the defect observed in a deletion strain for the mitochondrial Hsp70 isoform Ssq1p, suggesting a close relationship between the two proteins in substrate folding (Schmidt et al. 2001). Ssc1p and the much less abundant Ssq1p compete for binding to Mge1p (Schmidt et al. 2001). Overexpression of Mge1p increases the activity of Ssq1p, indicating that Mge1p availability is limiting for Ssq1p function. A role of Mge1p as a possible sensor of stress is attributed to the reversible cessation of the interaction between Mge1p and mitochondrial Hsp70s at heat shock temperatures and under conditions of oxidative stress (Marada et al. 2013; Moro and Muga 2006). A similar role has been discussed for bacterial GrpE (Nakamura et al. 2010). The loss of interaction with Hsp70 has been attributed to a transition from active dimer to inactive monomer.

The ER is a major folding compartment of the cell, handling the folding, maturation and post-translational modification of secretory and membrane proteins, and is therefore rich in molecular chaperones. Through the 'unfolded protein response' (UPR) pathway, the folding capacity is adapted to the protein load. Aberrant proteins are efficiently cleared by ER-associated decay (ERAD), a process which retrotranslocates substrates into the cytosol for proteasomal degradation. Therefore it is not surprising that the combined function of the ER-NEFs Lhs1p and Sil1p is essential and the double-deletion lethal (Tyson and Stirling 2000). Both proteins contribute to co-translational import of proteins into the ER lumen together with the luminal Kar2p and the translocon-associated J-domain protein Sec63p, and to subsequent folding. Both *LHS1* and *SIL1* are up-regulated by the ER stress response, the 'unfolded protein response' (UPR), thus increasing the folding capacity of the ER. Deletion of either factor triggers the UPR. This may explain why the single deletions have comparatively mild phenotypes such as altered protein maturation in the ER lumen and increased ERAD. Lhs1p and Sil1p are however only partially redundant (de Keyzer et al. 2009; Tyson and Stirling 2000). Although Sil1p appears to be about one order of magnitude more abundant than Lhs1p (but constitutes less than 0.1 % of the Kar2p content) under normal growth conditions (Ghaemmaghami et al. 2003), *lhs1*Δ cells exhibit a slight import defect, as indicated by the accumulation of pre-proteins.

The cytosolic Hsp110 family proteins, Sse1p and Sse2p, are closely related, having 76 % sequence identity (Mukai et al. 1993). Sse1p and Sse2p probably originated from a recent genome duplication event in *S. cerevisiae*. Sse2p is 10 times less abundant that Sse1p, although both proteins are expressed under normal conditions. Sse1p is the most abundant NEF in the yeast cytosol, but the concentration of cytosolic Hsp70 proteins is one order of magnitude higher. Under stress, *SSE2* gene expression is strongly induced by the heat shock response (HSR) pathway, while that of *SSE1* is only modestly increased (Mukai et al. 1993). The loss of Sse1p, but not

Sse2p, renders cells slow-growing, which is exacerbated by temperature stress (Liu et al. 1999). Overexpression of Sse1p also results in a slow-growth phenotype. The simultaneous deletion of both genes is lethal (Raviol et al. 2006b). Over-expression of the HspBP1 homolog Fes1p can partially compensate *sse1,2*Δ (Raviol et al. 2006b). Sse1p collaborates with both forms of cytosolic Hsp70, Ssa1-4 and the ribosome-associated Ssb1/2 to fold newly synthesized proteins (Yam et al. 2005; Shaner et al. 2005). This is dependent on the ribosome-associated complex (RAC) (Koplin et al. 2010; Willmund et al. 2013), containing the J-protein Zuo1p, and the type-I J-domain protein Ydj1p, respectively (Shaner et al. 2006). Interactions of Sse1p with 1940 potential substrate proteins were listed in a proteomics survey (Gong et al. 2009), a substantial part of the yeast proteome (~6600 proteins). Sse1p function also appears to have an impact on Hsp90 client proteins such as kinases and nuclear receptors (Goeckeler et al. 2002; Liu et al. 1999), probably by upstream client processing through the Hsp70 system. Specific examples are the growth control kinase Sch9p (Trott et al. 2005) and the MAP kinase Slt2p (Shaner et al. 2008), enabling Slt2p interaction with downstream effectors required for yeast cell wall integrity and morphogenesis. The NEF-function of Sse1p for Ssa1/2 is required for proper distribution of the kinesin-5 motor during bipolar spindle assembly, thus preventing premature spindle elongation during mitosis (Makhnevych et al. 2012). Besides de novo protein folding, Sse1p is also deeply involved in cellular protein quality control, as shown by the impact of its absence on the proteasomal clearance of the von-Hippel-Lindau (VHL) tumor suppressor protein (McClellan et al. 2005), a model substrate for the chaperonin TRiC, which cannot stably fold in absence of its complex partners Elongin-BC, and a folding-defective mutant version of the Hsp90 client protein Ste11p (Mandal et al. 2010).

The cellular concentration of Fes1p, the yeast homolog of HspBP1, is ~5-fold lower than Sse1p, and its expression is up-regulated upon stress. Fes1p catalyzes nucleotide exchange both on Ssa and Ssb-type Hsp70 proteins (Dragovic et al. 2006b; Kabani et al. 2002a) and associates with translating ribosomes (Kabani et al. 2002a). Fes1p and RAC appear to compete for binding to Ssb Hsp70 proteins, perhaps indicating the necessity for sequential interactions—RAC and Ssb·ATP upon emergence of the nascent chain at the ribosomal exit channel and Fes1p and Ssb·ADP towards completion of translation (Dragovic et al. 2006b). Deletion of *FES1* causes a growth defect under heat stress and a folding defect in the reporter protein firefly luciferase (FLuc) (Ahner et al. 2005; Shomura et al. 2005). Binding to Hsp70 and nucleotide exchange activity are critical for Fes1p function, since the inactive, but structurally intact mutant Fes1p(A79R/R195A) cannot complement the phenotype (Shomura et al. 2005). Deletion of *FES1* induces a massive heat shock response with strong up-regulation of molecular chaperones under standard growth conditions, while *sse1*Δ triggers only a mild induction, suggesting a critical function of Fes1p in the heat shock factor (Hsf1) activation pathway (Abrams et al. 2014; Gowda et al. 2013). This might explain why the growth defect of *fes1*Δ is relatively mild compared to the latter strain. Interestingly, Fes1p was implicated in the proteasome-mediated clearance of the constitutively misfolded proteins DHFRmutC, DHFRmutD and the protein fragment Rpo41(T920-L1217), but not in the

clearance of folded proteins such as FLuc, Stp1p and fructose-1,6-bisphosphatase 1 (Abrams et al. 2014; Ahner et al. 2005; Gowda et al. 2013). Inconsistently however, the misfolded test substrate CpY*-GFP, a mutant form of Carboxypeptidase Y fused to GFP, is efficiently degraded in *fes1*Δ cells (Abrams et al. 2014). Thus Sse1p and Fes1p both contribute to protein quality control, but with different specificities. This could be based on the distinct nucleotide exchange mechanisms or the additional holdase activity of the Hsp110 homolog.

The BAG domain protein Snl1p is expressed at low levels, similar to Sse2p. The ER-membrane protein interacts directly with the ribosome (Verghese and Morano 2012). Many of its surprisingly numerous interactors are integral membrane proteins, suggesting perhaps a role in faithful targeting of secretory proteins. Normally, these clients should not get in contact with cytosolic Hsp70 proteins.

The cytosolic NEFs of Hsp70 have a strong impact on the maintenance and propagation of prions in yeast. These fibrous polymeric forms of protein have a rather generic amyloid core structure, which is inherited in a non-Mendelian fashion to daughter cells. Interestingly, the fibers morphologically resemble pathologic protein deposits from amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's disease in humans. The fibers grow at their ends by incorporation of unfolded monomers and multiply by fracturing into seeds. The AAA protein disaggregase Hsp104, in collaboration with the Hsp70 system, contributes in a complex manner both to the fracturing and the disassembly of the filaments (Masison et al. 2009). The Hsp70 system might also erroneously deliver unfolded protein to the growing ends. Faithful maintenance of the [*PSI*$^+$] prion is dependent on the presence of *Sse1* (Fan et al. 2007; Sadlish et al. 2008). Complementation of the *sse1*Δ effect by overexpression of nucleotide-exchange active Sse1p mutants, Fes1p and Snl1p(ΔN) suggests that the NEF function is the main requirement for propagation (Sadlish et al. 2008).

Aspects of NEF Function in Mammalian Protein Folding and Quality Control

Compared to yeast, much less is known about the integrated function of mammalian NEFs because of the increased complexity of higher organisms. Mutations or deletions might affect specific cell types differentially, or might prevent development to an adult organism. Simultaneous deletion of the Hsp110 isoforms Apg-1 and Apg-2 in mice resulted in neonatal death (Mohamed et al. 2014); deletion of Apg-1 alone causes faulty spermatogenesis (Held et al. 2011). Deletion of the third isoform Hsp110/Hsp105 causes no obvious defects (Nakamura et al. 2008), but truncation of the human Hsp105 gene by micro-satellite instability in intestinal cancer cell lines sensitizes these cells towards chemotherapy (Dorard et al. 2011). The inactivating mutations found in the Sil1 gene of patients with Marinesco-Sjögren syndrome affect visibly only the Purkinje cells of the brain (Anttonen et al. 2005; Senderek et al. 2005). Deletion of Grp170/Hyou1 in mice is lethal (Kitao et al. 2004).

At high concentrations, NEFs inhibit Hsp70 by competing with nucleotide binding (Dragovic et al. 2006a; Rampelt et al. 2012; Gässler et al. 2001; Polier et al. 2008; Raviol et al. 2006b). It is unclear whether competition with J-domain protein binding will also occur. The concentration of Hsp70 components determines if accelerated Hsp70 cycling will occur or if cycling is inhibited. Experiments employing overexpression of specific NEFs should be treated with caution for this reason. There are numerous reports about inhibitory effects of Bag1 and HspBP1 (Bimston et al. 1998; Raynes and Guerriero 1998), although these do accelerate ATP hydrolysis of Hsp70 in conjunction with Hsp40 at appropriate concentrations *in vitro* (Höhfeld and Jentsch 1997; Shomura et al. 2005). The expression levels vary between cell types, and cancer cells lines typically have severely abnormal chaperone levels.

Comparative studies with model proteins shed light on the differential effects of members of the different NEF families on the folding and degradation of specific proteins. Young and coworkers showed that the BAG domain of Bag1 (cBag1), HspBP1 and Hsp105 all trigger ATP hydrolysis by Hsc70/HSPA8 in presence of the constitutive cytosolic type-I J-domain proteins DNAJA1/Hdj2 and DNAJA2/Hdj3. However, only the combinations Hsc70/DNAJA2 with cBag1 or Hsp105 improved refolding of chemically denatured FLuc compared to the control without NEF (Tzankov et al. 2008). Protein folding however improved only in a narrow NEF/Hsc70 concentration range. Later, combinations of the NEF proteins Bag1, Bag2, Bag3 and Hsp105 with the J-domain proteins DNAJA1, DNAJA2, DNAJB1/Hdj1 and DNAJB4 were investigated, using the stress-inducible Hsp70 form HSPA1A/Hsp72 (Rauch and Gestwicki 2014). All NEFs but Hsp105 accelerated ATP hydrolysis in presence of the J-domain proteins. DNAJA1 was inactive in FLuc refolding in any combination, as noted before by the Young group. In absence of phosphate, low concentrations of Bag1 and Bag3 together with the type-II J-domain proteins DNAJB1 and DNAJB4 were most efficient in FLuc refolding. Type-II J-domain proteins have a slightly different domain composition than type-I paralogs. In the presence of phosphate, the dependency on NEFs increased dramatically, and all combinations with Bag1-3 worked. Hsp105 did not increase FLuc refolding in any combination of the four J-domain proteins with HSPA1A. Taken together, this indicates that a combinatorial library of mammalian Hsp70 components might enable adaptation to a spectrum of substrates with different folding needs such as assistance by holdase activity and suitable cycling rates (Brehmer et al. 2001). In a comparative study, evidence for selective interaction of steroid receptors, which are prototypical Hsp90 client proteins that progress through the Hsp70 system beforehand, with the Hsp70 complexes of Bag1-M was found (Knapp et al. 2014). The respective HspBP1 complex with Hsp70 failed both to interact with the downstream factor, Hsp70–Hsp90-organzing protein (HOP) and Hsp90 as well as the (upstream) Hsp40 protein DNAJB1/Hdj1 (the latter might however be an indirect effect).

The folding of the ABC transporter Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) sheds light on the roles of NEFs in protein quality control and degradation. In addition to two transmembrane helix bundles, CFTR has large cytoplasmic domains, which on average require 10 min for folding and employ the cytoplasmic Hsp70 and Hsp90 machinery (Wang et al. 2006). Mutations in CFTR are the molecular basis for cystic fibrosis in humans, causing a lack of functional protein

in mucous membranes. Hsp105 appears to have a prominent role in early CFTR folding events and later at the epithelial membrane, employing its holdase activity (Saxena et al. 2012). Ineffective folding results in proteasomal degradation of most CFTR molecules before reaching the plasma membrane. CFTR is targeted for degradation by the dimeric E3-ubiquitin (Ub) ligase CHIP (Meacham et al. 2001), which attaches to the C-termini of Hsp70 and Hsp90 and ubiquitylates client proteins (and Hsp70). In cells, most of the CHIP protein appears to be associated with Bag2 and Hsp70, which form a large complex that is Ub-ligase-inactive (Dai et al. 2005). Binding of Bag2 prevents association of CHIP with the E2 enzyme (Ub donor) Ub-cH5b (Arndt et al. 2010; Dai et al. 2005). In a ternary complex with Hsc70, HspBP1 also inhibits CHIP Ub-ligase activity, however by a different mechanism (Alberti et al. 2004), whereas Bag1 collaborates with CHIP in targeting substrate proteins for proteasomal degradation (Demand et al. 2001). Therefore Bag2 and HspBP1 might help to keep CHIP in check while productive protein folding is ongoing.

Because of its considerable holdase capability, Hsp110 appears to play a special role among the NEFs. All Hsp110 isoforms were found attached to large aggregates of mutant superoxide dismutase (SOD1) that are a hallmark of Lou Gehrig's Disease (also named Amyotrophic lateral sclerosis (ALS)), a protein deposition disease in which the motor neurons degenerate (Wang et al. 2009). *In vitro*, Hsp105 can suppress mutant SOD aggregation to some degree (Yamashita et al. 2007). A transgenic mouse lacking Hsp110/Hsp105 was shown to accumulate hyper-phosphorylated tau protein in an age-dependent manner, which in turn forms neurofibrillary tangles and causes neurodegeneration similar to Alzheimer's disease. This phenotype was comparable to mice deficient in Hsp70, confirming the role for Hsp70·Hsp110 complexes in maintaining tau in an unphosphorylated form during aging (Eroglu et al. 2010). Hsp110 was also enriched in aggregates of an artificial model protein for protein deposition diseases, β23 (Olzscha et al. 2011). In a similar functional role but with a distinct interaction partner, Hsp110 was required to suppress polyglutamine-induced cell death in a *Drosophila* model of polyglutamine (polyQ) diseases. This class of neurodegenerative diseases, which includes Huntington's disease, is characterized by cellular deposition of aggregated mutant proteins containing expanded polyQ regions. Together with the Hsp40 family member DNAJ-1, Hsp110 protected cells from neural degeneration while either protein expressed alone had little effect, suggesting an Hsp110·Hsp40 complex is required to maintain protein homeostasis (Kuo et al. 2013). Hsp105α was also found associated with deposits of polyQ-androgen receptor in spinal and bulbar muscular atrophy (Kennedy's disease) (Ishihara et al. 2003). Interestingly, Hsp110 along with Hsp70 and Hsp40 was found to form a disaggregase system capable of dissolving amorphous aggregates in mammalian cells that are resistant to Hsp70 and Hsp40 alone (Rampelt et al. 2012; Shorter 2011). This system seems to partially replace the function of ClpB/Hsp104 proteins found in bacteria, plants and fungi, which together with the Hsp70 system remodel large aggregates and amyloids in an ATP-dependent process. Animals lack cytosolic ClpB/Hsp104 homologs. The most effective system for solubilization of aggregates of FLuc and GFP consisted of the isoforms Apg-2, Hsc70 and Hdj1/DNAJB1. Since the functional interfaces between Hsp110, Hsp70 and Hsp40

proteins are conserved, this suggests that specific interactions with the substrate proteins are important for activity. Perhaps the substrate specificities of the chaperones have to be complementary, enabling simultaneous interactions with different regions in the substrate protein. Additionally, substrate interactions with small heat shock proteins increase the refolding yields. Co-aggregation with small heat shock proteins seems to make the aggregates more accessible to the disaggregase system. The disaggregase activity is dependent on Hsp70-binding and the NEF capability of the Hsp110 component. Other NEFs can partially substitute for Hsp110 in *in vitro* disaggregation reactions (Rampelt et al. 2012). The studies disagreed on the requirement for ATP hydrolysis by the Hsp110 component (Rampelt et al. 2012; Shorter 2011). Taken together the association of Hsp110 and Hsp70 with cellular aggregate deposits might be a sign for on-going remodeling activity.

Both Bag3 and Hsp110 are strongly up-regulated upon stress by the heat shock response. It is unclear to what extent autophagy and disaggregation/UPS-mediated degradation contribute to recovery of the cell and the clearance of aggregated protein after heat shock. Another important aspect might be the cellular localization of respective proteostasis machineries. Hsp110 proteins are found both in the cytoplasm and nucleus, whereas Bag3 is found exclusively in the cytosol. Therefore the two compartments might follow different strategies for recovery.

Conclusions

The Hsp70 system represents the central hub in the proteostasis network by interacting with polypeptides at various stages of their existence from birth to ultimate demise. The Hsp70 folding cycle is fine-tuned by cochaperones adapting it to the divergent folding requirements of individual substrates. Recent structural and biochemical evidence has shown that especially the different nucleotide exchange factors serve additional roles by linking Hsp70 to other branches of the proteostasis network. We have now first insights how functional diversity might be encoded in their distinct binding modes to Hsp70. What is still missing is the integration of all these processes. For this we would need good estimates for the local concentrations of the players, in healthy cells and under conditions of stress or disease. It will be exciting to dissect this complex interplay between NEFs, Hsp70 and client proteins.

References

Abrams JL, Verghese J, Gibney PA, Morano KA (2014) Hierarchical functional specificity of cytosolic heat shock protein 70 (Hsp70) nucleotide exchange factors in yeast J Biol Chem 289:13155–13167

Ahner A, Whyte FM, Brodsky JL (2005) Distinct but overlapping functions of Hsp70, Hsp90, and an Hsp70 nucleotide exchange factor during protein biogenesis in yeast. Arch Biochem Biophys 435:32–41

Alberti S, Esser C, Höhfeld J (2003) BAG-1- a nucleotide exchange factor of Hsc70 with multiple cellular functions. Cell Stress Chaperones 8:225–231

Alberti S, Bohse K, Arndt V, Schmitz A, Höhfeld J (2004) The co-chaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. Mol Biol Cell 15:4003–4010

Andreasson C, Fiaux J, Rampelt H, Mayer MP, Bukau B (2008) Hsp110 is a nucleotide-activated exchange factor for Hsp70. J Biol Chem 283:8877–8884

Andreasson C, Rampelt H, Fiaux J, Druffel-Augustin S, Bukau B (2010) The endoplasmic reticulum Grp170 acts as a nucleotide exchange factor of Hsp70 via a mechanism similar to that of the cytosolic Hsp110. J Biol Chem 285:12445–12453

Ang D, Georgopoulos C (1989) The heat-shock-regulated grpE gene of Escherichia coli is required for bacterial growth at all temperatures but is dispensable in certain mutant backgrounds. J Bacteriol 171:2748–2755

Antoku K, Maser RS, Scully WJ Jr, Delach SM, Johnson DE (2001) Isolation of Bcl-2 binding proteins that exhibit homology with BAG-1 and suppressor of death domains protein. Biochem Biophys Res Commun 286:1003–1010

Anttonen AK, Mahjneh I, Hamalainen RH, Lagier-Tourenne C, Kopra O, Waris L, Anttonen M, Joensuu T, Kalimo H, Paetau A, Tranebjaerg L, Chaigne D, Koenig M, Eeg-Olofsson O, Udd B, Somer M, Somer H, Lehesjoki AE (2005) The gene disrupted in Marinesco-Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet 37:1309–1311

Arakawa A, Handa N, Ohsawa N, Shida M, Kigawa T, Hayashi F, Shirouzu M, Yokoyama S (2010) The C-terminal BAG domain of BAG5 induces conformational changes of the Hsp70 nucleotide-binding domain for ADP-ATP exchange. Structure 18:309–319

Arakawa A, Handa N, Shirouzu M, Yokoyama S (2011) Biochemical and structural studies on the high affinity of Hsp70 for ADP. Protein Sci 20:1367–1379

Arndt V, Daniel C, Nastainczyk W, Alberti S, Höhfeld J (2005) BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. Mol Biol Cell 16:5891–5900

Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, Fürst DO, Saftig P, Saint R, Fleischmann BK, Hoch M, Höhfeld J (2010) Chaperone-assisted selective autophagy is essential for muscle maintenance. Curr Biol 20:143–148

Balch WE, Morimoto RI, Dillin A, Kelly JW (2008) Adapting proteostasis for disease intervention. Science 319:916–919

Bertelsen EB, Chang L, Gestwicki JE, Zuiderweg ER (2009) Solution conformation of wild-type E. coli Hsp70 (DnaK) chaperone complexed with ADP and substrate. Proc Natl Acad Sci U S A 106:8471–8476

Bimston D, Song J, Winchester D, Takayama S, Reed JC, Morimoto RI (1998) BAG-1, a negative regulator of Hsp70 chaperone activity, uncouples nucleotide hydrolysis from substrate release. EMBO J 17:6871–6878

Binici J, Koch J (2013) BAG-6, a jack of all trades in health and disease. Cell Mol Life Sci. doi:10.1007/s00018–013-1522-y

Brehmer D, Rüdiger S, Gässler CS, Klostermeier D, Packschies L, Reinstein J, Mayer MP, Bukau B (2001) Tuning of chaperone activity of Hsp70 proteins by modulation of nucleotide exchange. Nat Struct Biol 8:427–432

Briknarova K, Takayama S, Brive L, Havert ML, Knee DA, Velasco J, Homma S, Cabezas E, Stuart J, Hoyt DW, Satterthwait AC, Llinas M, Reed JC, Ely KR (2001) Structural analysis of BAG1 cochaperone and its interactions with Hsc70 heat shock protein. Nat Struct Biol 8:349–352

Briknarova K, Takayama S, Homma S, Baker K, Cabezas E, Hoyt DW, Li Z, Satterthwait AC, Ely KR (2002) BAG4/SODD protein contains a short BAG domain. J Biol Chem 277:31172–31178

Brockmann C, Leitner D, Labudde D, Diehl A, Sievert V, Büssow K, Kühne R, Oschkinat H (2004) The solution structure of the SODD BAG domain reveals additional electrostatic interactions in the HSP70 complexes of SODD subfamily BAG domains. FEBS Lett 558:101–106

Brodsky JL, Bracher A (2007) Nucleotide Exchange Factors for Hsp70 Molecular Chaperones. In: Balch GL (ed) Networking of chaperones by co-chaperones. Molecular Biology Intelligence Unit. Landes Biosciences, Austin, pp 1–12

Bukau B, Weissman J, Horwich A (2006) Molecular chaperones and protein quality control. Cell 125:443–451

Carrettiero DC, Hernandez I, Neveu P, Papagiannakopoulos T, Kosik KS (2009) The cochaperone BAG2 sweeps paired helical filament- insoluble tau from the microtubule. J Neurosci 29:2151–2161

Chang YW, Sun YJ, Wang C, Hsiao CD (2008) Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation. J Biol Chem 283:15502–15511

Chung KS, Hoe KL, Kim KW, Yoo HS (1998) Isolation of a novel heat shock protein 70-like gene, pss1 + of Schizosaccharomyces pombe homologous to hsp110/SSE subfamily. Gene 210:143–150

Chung KT, Shen Y, Hendershot LM (2002) BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. J Biol Chem 277:47557–47563

Colinet H, Hoffmann A (2010) Gene and protein expression of Drosophila Starvin during cold stress and recovery from chill coma. Insect Biochem Mol Biol 40:425–428

Coulson M, Robert S, Saint R (2005) Drosophila starvin encodes a tissue-specific BAG-domain protein required for larval food uptake. Genetics 171:1799–1812

Dai Q, Qian SB, Li HH, McDonough H, Borchers C, Huang D, Takayama S, Younger JM, Ren HY, Cyr DM, Patterson C (2005) Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. J Biol Chem 280:38673–38681

de Keyzer J, Steel GJ, Hale SJ, Humphries D, Stirling CJ (2009) Nucleotide binding by Lhs1p is essential for its nucleotide exchange activity and for function in vivo. J Biol Chem 284:31564–31571

Demand J, Alberti S, Patterson C, Höhfeld J (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr Biol 11:1569–1577

Doong H, Price J, Kim YS, Gasbarre C, Probst J, Liotta LA, Blanchette J, Rizzo K, Kohn E (2000) CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. Oncogene 19:4385–4395

Doong H, Vrailas A, Kohn EC (2002) What's in the 'BAG'?–A functional domain analysis of the BAG-family proteins. Cancer Lett 188:25–32

Dorard C, de Thonel A, Collura A, Marisa L, Svrcek M, Lagrange A, Jego G, Wanherdrick K, Joly AL, Buhard O, Gobbo J, Penard-Lacronique V, Zouali H, Tubacher E, Kirzin S, Selves J, Milano G, Etienne-Grimaldi MC, Bengrine-Lefevre L, Louvet C, Tournigand C, Lefevre JH, Parc Y, Tiret E, Flejou JF, Gaub MP, Garrido C, Duval A (2011) Expression of a mutant HSP110 sensitizes colorectal cancer cells to chemotherapy and improves disease prognosis. Nat Med 17:1283–1289

Doukhanina EV, Chen S, van der Zalm E, Godzik A, Reed J, Dickman MB (2006) Identification and functional characterization of the BAG protein family in Arabidopsis thaliana. J Biol Chem 281:18793–18801

Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU (2006a) Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. EMBO J 25:2519–2528

Dragovic Z, Shomura Y, Tzvetkov N, Hartl FU, Bracher A (2006b) Fes1p acts as a nucleotide exchange factor for the ribosome-associated molecular chaperone Ssb1p. Biol Chem 387:1593–1600

Easton DP, Kaneko Y, Subjeck JR (2000) The hsp110 and Grp1 70 stress proteins: newly recognized relatives of the Hsp70s. Cell Stress Chaperones 5:276–290

Eroglu B, Moskophidis D, Mivechi NF (2010) Loss of Hsp110 leads to age-dependent tau hyperphosphorylation and early accumulation of insoluble amyloid beta. Mol Cell Biol 30:4626–4643

Fan Q, Park KW, Du Z, Morano KA, Li L (2007) The role of Sse1 in the de novo formation and variant determination of the [PSI+] prion. Genetics 177:1583–1593

Fang S, Li L, Cui B, Men S, Shen Y, Yang X (2013) Structural insight into plant programmed cell death mediated by BAG proteins in Arabidopsis thaliana. Acta Crystallogr D Biol Crystallogr 69:934–945

Flaherty KM, DeLuca-Flaherty C, McKay DB (1990) Three-dimensional structure of the ATPase fragment of a 70 K heat-shock cognate protein. Nature 346:623–628

Franceschelli S, Rosati A, Lerose R, De Nicola S, Turco MC, Pascale M (2008) Bag3 gene expression is regulated by heat shock factor 1. J Cell Physiol 215:575–577

Fuchs M, Poirier DJ, Seguin SJ, Lambert H, Carra S, Charette SJ, Landry J (2010) Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. Biochem J 425:245–255

Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C (2009) Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. EMBO J 28:889–901

Gässler CS, Wiederkehr T, Brehmer D, Bukau B, Mayer MP (2001) Bag-1M accelerates nucleotide release for human Hsc70 and Hsp70 and can act concentration-dependent as positive and negative cofactor. J Biol Chem 276:32538–32544

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS (2003) Global analysis of protein expression in yeast. Nature 425:737–741

Goeckeler JL, Stephens A, Lee P, Caplan AJ, Brodsky JL (2002) Overexpression of yeast Hsp110 homolog Sse1p suppresses ydj1–151 thermosensitivity and restores Hsp90-dependent activity. Mol Biol Cell 13:2760–2770

Goeckeler JL, Petruso AP, Aguirre J, Clement CC, Chiosis G, Brodsky JL (2008) The yeast Hsp110, Sse1p, exhibits high-affinity peptide binding. FEBS Lett 582:2393–2396

Gong Y, Kakihara Y, Krogan N, Greenblatt J, Emili A, Zhang Z, Houry WA (2009) An atlas of chaperone–protein interactions in Saccharomyces cerevisiae: implications to protein folding pathways in the cell. Mol Syst Biol 5:275

Gonzalez B, de Graaf A, Renaud M, Sahm H (2000) Dynamic in vivo (31)P nuclear magnetic resonance study of *Saccharomyces cerevisiae* in glucose-limited chemostat culture during the aerobic-anaerobic shift. Yeast 16:483–497

Gowda NK, Kandasamy G, Froehlich MS, Dohmen RJ, Andreasson C (2013) Hsp70 nucleotide exchange factor Fes1 is essential for ubiquitin-dependent degradation of misfolded cytosolic proteins. Proc Natl Acad Sci U S A 110:5975–5980

Gupta RS (1998) Protein phylogenies and signature sequences: A reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol Mol Biol Rev 62:1435–1491

Hale SJ, Lovell SC, de Keyzer J, Stirling CJ (2010) Interactions between Kar2p and its nucleotide exchange factors Sil1p and Lhs1p are mechanistically distinct. J Biol Chem 285:21600–21606

Harrison CJ, Hayer-Hartl M, Di Liberto M, Hartl F, Kuriyan J (1997) Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. Science 276:431–435

Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475:324–332

Held T, Barakat AZ, Mohamed BA, Paprotta I, Meinhardt A, Engel W, Adham IM (2011) Heat-shock protein HSPA4 is required for progression of spermatogenesis. Reproduction 142:133–144

Höhfeld J, Jentsch S (1997) GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. EMBO J 16:6209–6216

Homma S, Iwasaki M, Shelton GD, Engvall E, Reed JC, Takayama S (2006) BAG3 deficiency results in fulminant myopathy and early lethality. Am J Pathol 169:761–773

Horst M, Oppliger W, Rospert S, Schönfeld HJ, Schatz G, Azem A (1997) Sequential action of two hsp70 complexes during protein import into mitochondria. EMBO J 16:1842–1849

Howes J, Shimizu Y, Feige MJ, Hendershot LM (2012) C-terminal mutations destabilize SIL1/BAP and can cause Marinesco-Sjogren syndrome. J Biol Chem 287:8552–8560

Hu C, Lin SY, Chi WT, Charng YY (2012) Recent gene duplication and subfunctionalization produced a mitochondrial GrpE, the nucleotide exchange factor of the Hsp70 complex, specialized in thermotolerance to chronic heat stress in Arabidopsis. Plant Physiol 158:747–758

Huang HW, Yu C (2013) The NMR solution structure of the ubiquitin homology domain of Bcl-2-associated athanogene 1 (BAG-1-UBH) from Mus musculus. Biochem Biophys Res Commun 431:86–91

Ikeda E, Yoshida S, Mitsuzawa H, Uno I, Toh-e A (1994) YGE1 is a yeast homologue of Escherichia coli grpE and is required for maintenance of mitochondrial functions. FEBS Lett 339:265–268

Ishihara K, Yamagishi N, Saito Y, Adachi H, Kobayashi Y, Sobue G, Ohtsuka K, Hatayama T (2003) Hsp105alpha suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. J Biol Chem 278:25143–25150

Iwasaki M, Tanaka R, Hishiya A, Homma S, Reed JC, Takayama S (2010) BAG3 directly associates with guanine nucleotide exchange factor of Rap1, PDZGEF2, and regulates cell adhesion. Biochem Biophys Res Commun 400:413–418

Jacobs AT, Marnett LJ (2009) HSF1-mediated BAG3 expression attenuates apoptosis in 4-hydroxynonenal-treated colon cancer cells via stabilization of anti-apoptotic Bcl-2 proteins. J Biol Chem 284:9176–9183

Jiang Y, Woronicz JD, Liu W, Goeddel DV (1999) Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 283:543–546

Jungkunz I, Link K, Vogel F, Voll LM, Sonnewald S, Sonnewald U (2011) AtHsp70–15-deficient Arabidopsis plants are characterized by reduced growth, a constitutive cytosolic protein response and enhanced resistance to TuMV. Plant J 66:983–995

Kabani M, Beckerich JM, Gaillardin C (2000) Sls1p stimulates Sec63p-mediated activation of Kar2p in a conformation-dependent manner in the yeast endoplasmic reticulum. Mol Cell Biol 20:6923–6934

Kabani M, Beckerich JM, Brodsky JL (2002a) Nucleotide exchange factor for the yeast Hsp70 molecular chaperone Ssa1p. Mol Cell Biol 22:4677–4689

Kabani M, McLellan C, Raynes DA, Guerriero V, Brodsky JL (2002b) HspBP1, a homologue of the yeast Fes1 and Sls1 proteins, is an Hsc70 nucleotide exchange factor. FEBS Lett 531:339–342

Kabbage M, Dickman MB (2008) The BAG proteins: a ubiquitous family of chaperone regulators. Cell Mol Life Sci 65:1390–1402

Kalia SK, Lee S, Smith PD, Liu L, Crocker SJ, Thorarinsdottir TE, Glover JR, Fon EA, Park DS, Lozano AM (2004) BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. Neuron 44:931–945

Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579–592

Kanelakis KC, Murphy PJ, Galigniana MD, Morishima Y, Takayama S, Reed JC, Toft DO, Pratt WB (2000) hsp70 interacting protein Hip does not affect glucocorticoid receptor folding by the hsp90-based chaperone machinery except to oppose the effect of BAG-1. BioChemistry 39:14314–14321

Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355

Kitao Y, Hashimoto K, Matsuyama T, Iso H, Tamatani T, Hori O, Stern DM, Kano M, Ozawa K, Ogawa S (2004) ORP150/HSP12A regulates Purkinje cell survival: a role for endoplasmic reticulum stress in cerebellar development. J Neurosci 24:1486–1496

Kityk R, Kopp J, Sinning I, Mayer MP (2012) Structure and dynamics of the ATP-bound open conformation of Hsp70 chaperones. Mol Cell 48:863–874

Knapp RT, Wong MJ, Kollmannsberger LK, Gassen NC, Kretzschmar A, Zschocke J, Hafner K, Young JC, Rein T (2014) Hsp70 cochaperones HspBP1 and BAG-1M differentially regulate steroid hormone receptor function. PLoS One 9:e85415

Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10:524–530

Koplin A, Preissler S, Ilina Y, Koch M, Scior A, Erhardt M, Deuerling E (2010) A dual function for chaperones SSB-RAC and the NAC nascent polypeptide-associated complex on ribosomes. J Cell Biol 189:57–68

Kriegenburg F, Jakopec V, Poulsen EG, Nielsen SV, Roguev A, Krogan N, Gordon C, Fleig U, Hartmann-Petersen R (2014) A chaperone-assisted degradation pathway targets kinetochore proteins to ensure genome stability. PLoS Genet 10:e1004140

Kuo Y, Ren S, Lao U, Edgar BA, Wang T (2013) Suppression of polyglutamine protein toxicity by co-expression of a heat-shock protein 40 and a heat-shock protein 110. Cell Death Dis 4:e833

Kwon KR, Ahn JY, Kim MS, Jung JY, Lee JH, Oh IH (2010) Disruption of bis leads to the deterioration of the vascular niche for hematopoietic stem cells. Stem Cells 28:268–278

Laloraya S, Gambill BD, Craig EA (1994) A role for a eukaryotic GrpE-related protein, Mge1p, in protein translocation. Proc Natl Acad Sci U S A 91:6481–6485

Laloraya S, Dekker PJ, Voos W, Craig EA, Pfanner N (1995) Mitochondrial GrpE modulates the function of matrix Hsp70 in translocation and maturation of preproteins. Mol Cell Biol 15:7098–7105

Lamark T, Johansen T (2012) Aggrephagy: selective disposal of protein aggregates by macroautophagy. Int J Cell Biol. doi:10.1155/2012/736905

Laufen T, Mayer MP, Beisel C, Klostermeier D, Mogk A, Reinstein J, Bukau B (1999) Mechanism of regulation of hsp70 chaperones by DnaJ cochaperones. Proc Natl Acad Sci U S A 96:5452–5457

Li Z, Hartl FU, Bracher A (2013) Structure and function of Hip, an attenuator of the Hsp70 chaperone cycle. Nat Struct Mol Biol 20:929–935

Lin J, Hutchinson L, Gaston SM, Raab G, Freeman MR (2001) BAG-1 is a novel cytoplasmic binding partner of the membrane form of heparin-binding EGF-like growth factor: a unique role for proHB-EGF in cell survival regulation. J Biol Chem 276:30127–30132

Liu Q, Hendrickson WA (2007) Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. Cell 131:106–120

Liu XD, Morano KA, Thiele DJ (1999) The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. J Biol Chem 274:26654–26660

Liu Y, Gierasch LM, Bahar I (2010) Role of Hsp70 ATPase domain intrinsic dynamics and sequence evolution in enabling its functional interactions with NEFs. PLoS Comput Biol 6. doi:10.1371/journal.pcbi.1000931

Makhnevych T, Wong P, Pogoutse O, Vizeacoumar FJ, Greenblatt JF, Emili A, Houry WA (2012) Hsp110 is required for spindle length control. J Cell Biol 198:623–636

Mandal AK, Gibney PA, Nillegoda NB, Theodoraki MA, Caplan AJ, Morano KA (2010) Hsp110 chaperones control client fate determination in the hsp70-Hsp90 chaperone system. Mol Biol Cell 21:1439–1448

Marada A, Allu PK, Murari A, PullaReddy B, Tammineni P, Thiriveedi VR, Danduprolu J, Sepuri NB (2013) Mge1, a nucleotide exchange factor of Hsp70, acts as an oxidative sensor to regulate mitochondrial Hsp70 function. Mol Biol Cell 24:692–703

Mariappan M, Li X, Stefanovic S, Sharma A, Mateja A, Keenan RJ, Hegde RS (2010) A ribosome-associating factor chaperones tail-anchored membrane proteins. Nature 466:1120–1124

Masison DC, Kirkland PA, Sharma D (2009) Influence of Hsp70s and their regulators on yeast prion propagation. Prion 3:65–73

Mattoo RU, Sharma SK, Priya S, Finka A, Goloubinoff P (2013) Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates. J Biol Chem 288:21399–21411

Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684

McClellan AJ, Scott MD, Frydman J (2005) Folding and quality control of the VHL tumor suppressor proceed through distinct chaperone pathways. Cell 121:739–748

Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nat Cell Biol 3:100–105

Miao B, Davis JE, Craig EA (1997) Mge1 functions as a nucleotide release factor for Ssc1, a mitochondrial Hsp70 of Saccharomyces cerevisiae. J Mol Biol 265:541–552

Mohamed BA, Barakat AZ, Held T, Elkenani M, Muhlfeld C, Manner J, Adham IM (2014) Respiratory distress and early neonatal lethality in hspa4 l/hspa4 double-mutant mice. Am J Respir Cell Mol Biol 50:817–824

Mokranjac D, Bourenkov G, Hell K, Neupert W, Groll M (2006) Structure and function of Tim14 and Tim16, the J and J-like components of the mitochondrial protein import motor. EMBO J 25:4675–4685

Moro F, Muga A (2006) Thermal adaptation of the yeast mitochondrial Hsp70 system is regulated by the reversible unfolding of its nucleotide exchange factor. J Mol Biol 358:1367–1377
Mukai H, Kuno T, Tanaka H, Hirata D, Miyakawa T, Tanaka C (1993) Isolation and characterization of SSE1 and SSE2, new members of the yeast HSP70 multigene family. Gene 132:57–66
Muralidharan V, Oksman A, Pal P, Lindquist S, Goldberg DE (2012) Plasmodium falciparum heat shock protein 110 stabilizes the asparagine repeat-rich parasite proteome during malarial fevers. Nat Commun 3:1310
Nakamura J, Fujimoto M, Yasuda K, Takeda K, Akira S, Hatayama T, Takagi Y, Nozaki K, Hosokawa N, Nagata K (2008) Targeted disruption of Hsp110/105 gene protects against ischemic stress. Stroke 39:2853–2859
Nakamura A, Takumi K, Miki K (2010) Crystal structure of a thermophilic GrpE protein: insight into thermosensing function for the DnaK chaperone system. J Mol Biol 396:1000–1011
Nikolaidis N, Nei M (2004) Concerted and nonconcerted evolution of the Hsp70 gene superfamily in two sibling species of nematodes. Mol Biol Evol 21:498–505
Oh HJ, Chen X, Subjeck JR (1997) Hsp110 protects heat-denatured proteins and confers cellular thermoresistance. J Biol Chem 272:31636–31640
Oh HJ, Easton D, Murawski M, Kaneko Y, Subjeck JR (1999) The chaperoning activity of hsp110. Identification of functional domains by use of targeted deletions. J Biol Chem 274:15712–15718
Olzscha H, Schermann SM, Woerner AC, Pinkert S, Hecht MH, Tartaglia GG, Vendruscolo M, Hayer-Hartl M, Hartl FU, Vabulas RM (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144:67–78
Page RC, Xu Z, Amick J, Nix JC, Misra S (2012) Crystallization and preliminary X-ray crystallographic analysis of the Bag2 amino-terminal domain from Mus musculus. Acta Crystallogr Sect F Struct Biol Cryst Commun 68:647–651
Plesofsky-Vig N, Brambl R (1998) Characterization of an 88-kDa heat shock protein of Neurospora crassa that interacts with Hsp30. J Biol Chem 273:11335–11341
Polier S, Dragovic Z, Hartl FU, Bracher A (2008) Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. Cell 133:1068–1079
Polier S, Hartl FU, Bracher A (2010) Interaction of the Hsp110 molecular chaperones from S. cerevisiae with substrate protein. J Mol Biol 401:696–707
Qi R, Sarbeng EB, Liu Q, Le KQ, Xu X, Xu H, Yang J, Wong JL, Vorvis C, Hendrickson WA, Zhou L, Liu Q (2013) Allosteric opening of the polypeptide-binding site when an Hsp70 binds ATP. Nat Struct Mol Biol 20:900–907
Rampelt H, Kirstein-Miles J, Nillegoda NB, Chi K, Scholz SR, Morimoto RI, Bukau B (2012) Metazoan Hsp70 machines use Hsp110 to power protein disaggregation. EMBO J 31:4221–4235
Rauch JN, Gestwicki JE (2014) Binding of human nucleotide exchange factors to heat shock protein 70 (Hsp70) generates functionally distinct complexes in vitro. J Biol Chem 289:1402–1414
Raviol H, Bukau B, Mayer MP (2006a) Human and yeast Hsp110 chaperones exhibit functional differences. FEBS Lett 580:168–174
Raviol H, Sadlish H, Rodriguez F, Mayer MP, Bukau B (2006b) Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. EMBO J 25:2510–2518
Raynes DA, Guerriero V (1998) Inhibition of Hsp70 ATPase activity and protein renaturation by a novel Hsp70-binding protein. J Biol Chem 273:32883–32888
Rosati A, Graziano V, De Laurenzi V, Pascale M, Turco MC (2011) BAG3: a multifaceted protein that regulates major cell pathways. Cell Death Dis 2:e141
Rüdiger S, Germeroth L, Schneider-Mergener J, Bukau B (1997) Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. EMBO J 16:1501–1507
Sadlish H, Rampelt H, Shorter J, Wegrzyn RD, Andreasson C, Lindquist S, Bukau B (2008) Hsp110 chaperones regulate prion formation and propagation in S. cerevisiae by two discrete activities. PLoS One 3:e1763
Saxena A, Banasavadi-Siddegowda YK, Fan Y, Bhattacharya S, Roy G, Giovannucci DR, Frizzell RA, Wang X (2012) Human heat shock protein 105/110 kDa (Hsp105/110) regulates

biogenesis and quality control of misfolded cystic fibrosis transmembrane conductance regulator at multiple levels. J Biol Chem 287:19158–19170
Schmidt S, Strub A, Rottgers K, Zufall N, Voos W (2001) The two mitochondrial heat shock proteins 70, Ssc1 and Ssq1, compete for the cochaperone Mge1. J Mol Biol 313:13–26
Schneider HC, Westermann B, Neupert W, Brunner M (1996) The nucleotide exchange factor MGE exerts a key function in the ATP-dependent cycle of mt-Hsp70-Tim44 interaction driving mitochondrial protein import. EMBO J 15:5796–5803
Schuermann JP, Jiang J, Cuellar J, Llorca O, Wang L, Gimenez LE, Jin S, Taylor AB, Demeler B, Morano KA, Hart PJ, Valpuesta JM, Lafer EM, Sousa R (2008) Structure of the Hsp110:Hsc70 nucleotide exchange machine. Mol Cell 31:232–243
Senderek J, Krieger M, Stendel C, Bergmann C, Moser M, Breitbach-Faller N, Rudnik-Schoneborn S, Blaschek A, Wolf NI, Harting I, North K, Smith J, Muntoni F, Brockington M, Quijano-Roy S, Renault F, Herrmann R, Hendershot LM, Schroder JM, Lochmuller H, Topaloglu H, Voit T, Weis J, Ebinger F, Zerres K (2005) Mutations in SIL1 cause Marinesco-Sjogren syndrome, a cerebellar ataxia with cataract and myopathy. Nat Genet 37:1312–1314
Shaner L, Trott A, Goeckeler JL, Brodsky JL, Morano KA (2004) The function of the yeast molecular chaperone Sse1 is mechanistically distinct from the closely related hsp70 family. J Biol Chem 279:21992–22001
Shaner L, Wegele H, Buchner J, Morano KA (2005) The yeast HSP110 SSE1 functionally interacts with the HSP70 chaperones SSA and SSB. J Biol Chem 279:21992–22001
Shaner L, Sousa R, Morano KA (2006) Characterization of Hsp70 binding and nucleotide exchange by the yeast Hsp110 chaperone Sse1. BioChemistry 45:15075–15084
Shaner L, Gibney PA, Morano KA (2008) The Hsp110 protein chaperone Sse1 is required for yeast cell wall integrity and morphogenesis. Curr Genet 54:1–11
Shi LX, Theg SM (2010) A stromal heat shock protein 70 system functions in protein import into chloroplasts in the moss Physcomitrella patens. Plant Cell 22:205–220
Shomura Y, Dragovic Z, Chang HC, Tzvetkov N, Young JC, Brodsky JL, Guerriero V, Hartl FU, Bracher A (2005) Regulation of Hsp70 function by HspBP1: Structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. Mol Cell 17:367–379
Shorter J (2011) The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. PLoS One 6:e26319
Sondermann H, Scheufler C, Schneider C, Höhfeld J, Hartl FU, Moarefi I (2001) Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science 291:1553–1557
Sondermann H, Ho AK, Listenberger LL, Siegers K, Moarefi I, Wente SR, Hartl FU, Young JC (2002) Prediction of novel Bag-1 homologs based on structure/function analysis identifies Snl1p as an Hsp70 co-chaperone in Saccharomyces cerevisiae. J Biol Chem 277:33220–33227
Song J, Takeda M, Morimoto RI (2001) Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. Nat Cell Biol 3:276–282
Steel GJ, Fullerton DM, Tyson JR, Stirling CJ (2004) Coordinated activation of Hsp70 chaperones. Science 303:98–101
Storozhenko S, De Pauw P, Kushnir S, Van Montagu M, Inze D (1996) Identification of an Arabidopsis thaliana cDNA encoding a HSP70-related protein belonging to the HSP110/SSE1 subfamily. FEBS Lett 390:113–118
Swain JF, Dinler G, Sivendran R, Montgomery DL, Stotz M, Gierasch LM (2007) Hsp70 chaperone ligands control domain association via an allosteric mechanism mediated by the interdomain linker. Mol Cell 26:27–39
Symersky J, Zhang Y, Schormann N, Li S, Bunzel R, Pruett P, Luan CH, Luo M (2004) Structural genomics of Caenorhabditis elegans: structure of the BAG domain. Acta Crystallogr D Biol Crystallogr 60:1606–1610
Takayama S, Reed JC (2001) Molecular chaperone targeting and regulation by BAG family proteins. Nat Cell Biol 3:E237–E241

Takayama S, Krajewski S, Krajewska M, Kitada S, Zapata JM, Kochel K, Knee D, Scudiero D, Tudor G, Miller GJ, Miyashita T, Yamada M, Reed JC (1998) Expression and location of Hsp70/Hsc-binding anti-apoptotic protein BAG-1 and its variants in normal tissues and tumor cell lines. Cancer Res 58:3116–3131

Takayama S, Xie Z, Reed JC (1999) An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. J Biol Chem 274:781–786

Tewari R, Bailes E, Bunting KA, Coates JC (2010) Armadillo-repeat protein functions: questions for little creatures. Trends Cell Biol 20:470–481

Trott A, Shaner L, Morano KA (2005) The molecular chaperone Sse1 and the growth control protein kinase Sch9 collaborate to regulate protein kinase A activity in Saccharomyces cerevisiae. Genetics 170:1009–1021

Tyson JR, Stirling CJ (2000) LHS1 and SIL1 provide a lumenal function that is essential for protein translocation into the endoplasmic reticulum. EMBO J 19:6440–6452

Tzankov S, Wong MJ, Shi K, Nassif C, Young JC (2008) Functional divergence between co-chaperones of Hsc70. J Biol Chem 283:27100–27109

Ulbricht A, Eppler FJ, Tapia VE, van der Ven PF, Hampe N, Hersch N, Vakeel P, Stadel D, Haas A, Saftig P, Behrends C, Fürst DO, Volkmer R, Hoffmann B, Kolanus W, Höhfeld J (2013) Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy. Curr Biol 23:430–435

Ung PM, Thompson AD, Chang L, Gestwicki JE, Carlson HA (2013) Identification of key hinge residues important for nucleotide-dependent allostery in E. coli Hsp70/DnaK. PLoS Comput Biol 9:e1003279

Velten M, Gomez-Vrielynck N, Chaffotte A, Ladjimi MM (2002) Domain structure of the HSC70 cochaperone, HIP. J Biol Chem 277:259–266

Verghese J, Morano KA (2012) A lysine-rich region within fungal BAG domain-containing proteins mediates a novel association with ribosomes. Eukaryot Cell 11:1003–1011

Vogel M, Mayer MP, Bukau B (2006) Allosteric regulation of Hsp70 chaperones involves a conserved interdomain linker. J Biol Chem 281:38705–38711

Wada S, Hamada M, Satoh N (2006) A genomewide analysis of genes for the heat shock protein 70 chaperone system in the ascidian Ciona intestinalis. Cell Stress Chaperones 11:23–33

Wang X, Venable J, LaPointe P, Hutt DM, Koulov AV, Coppinger J, Gurkan C, Kellner W, Matteson J, Plutner H, Riordan JR, Kelly JW, Yates JR 3rd, Balch WE (2006) Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell 127:803–815

Wang J, Farr GW, Zeiss CJ, Rodriguez-Gil DJ, Wilson JH, Furtak K, Rutkowski DT, Kaufman RJ, Ruse CI, Yates JR 3rd, Perrin S, Feany MB, Horwich AL (2009) Progressive aggregation despite chaperone associations of a mutant SOD1-YFP in transgenic mice that develop ALS. Proc Natl Acad Sci U S A 106:1392–1397

Wang Q, Liu Y, Soetandyo N, Baek K, Hegde R, Ye Y (2011) A ubiquitin ligase-associated chaperone holdase maintains polypeptides in soluble states for proteasome degradation. Mol Cell 42:758–770

Wang AM, Miyata Y, Klinedinst S, Peng HM, Chua JP, Komiyama T, Li X, Morishima Y, Merry DE, Pratt WB, Osawa Y, Collins CA, Gestwicki JE, Lieberman AP (2013) Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. Nat Chem Biol 9:112–118

Wilbanks SM, McKay DB (1995) How potassium affects the activity of the molecular chaperone Hsc70. II. Potassium binds specifically in the ATPase active site. J Biol Chem 270:2251–2257

Williams B, Kabbage M, Britt R, Dickman MB (2010) AtBAG7, an Arabidopsis Bcl-2-associated athanogene, resides in the endoplasmic reticulum and is involved in the unfolded protein response. Proc Natl Acad Sci U S A 107:6088–6093

Willmund F, Muhlhaus T, Wojciechowska M, Schroda M (2007) The NH2-terminal domain of the chloroplast GrpE homolog CGE1 is required for dimerization and cochaperone function in vivo. J Biol Chem 282:11317–11328

Willmund F, del Alamo M, Pechmann S, Chen T, Albanese V, Dammer EB, Peng J, Frydman J (2013) The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. Cell 152:196–209

Wu CC, Naveen V, Chien CH, Chang YW, Hsiao CD (2012) Crystal structure of DnaK protein complexed with nucleotide exchange factor GrpE in DnaK chaperone system: insight into intermolecular communication. J Biol Chem 287:21461–21470

Xu Z, Page RC, Gomes MM, Kohli E, Nix JC, Herr AB, Patterson C, Misra S (2008) Structural basis of nucleotide exchange and client binding by the Hsp70 cochaperone Bag2. Nat Struct Mol Biol 15:1309–1317

Xu X, Sarbeng EB, Vorvis C, Kumar DP, Zhou L, Liu Q (2012) Unique peptide substrate binding properties of 110-kDa heat-shock protein (Hsp110) determine its distinct chaperone activity. J Biol Chem 287:5661–5672

Xu Z, Graham K, Foote M, Liang F, Rizkallah R, Hurt M, Wang Y, Wu Y, Zhou Y (2013) 14-3-3 protein targets misfolded chaperone-associated proteins to aggresomes. J Cell Sci 126:4173–4186

Yam AY, Albanese V, Lin HT, Frydman J (2005) HSP110 cooperates with different cytosolic HSP70 systems in a pathway for de novo folding. J Biol Chem 280:41252–41261

Yamashita H, Kawamata J, Okawa K, Kanki R, Nakamizo T, Hatayama T, Yamanaka K, Takahashi R, Shimohama S (2007) Heat-shock protein 105 interacts with and suppresses aggregation of mutant Cu/Zn superoxide dismutase: clues to a possible strategy for treating ALS. J Neurochem 102:1497–1505

Yan M, Li J, Sha B (2011) Structural analysis of the Sil1-Bip complex reveals the mechanism for Sil1 to function as a nucleotide-exchange factor. Biochem J 438:447–455

Zahn M, Berthold N, Kieslich B, Knappe D, Hoffmann R, Sträter N (2013) Structural studies on the forward and reverse binding modes of peptides to the chaperone DnaK. J Mol Biol 425:2463–2479

Zhang JX, Wang C, Yang CY, Wang JY, Chen L, Bao XM, Zhao YX, Zhang H, Liu J (2010) The role of Arabidopsis AtFes1A in cytosolic Hsp70 stability and abiotic stress tolerance. Plant J 62:539–548

Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrickson WA (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. Science 272:1606–1614

Zhuravleva A, Clerico EM, Gierasch LM (2012) An interdomain energetic tug-of-war creates the allosterically active state in Hsp70 molecular chaperones. Cell 151:1296–1307

Chapter 2
Functions of the Hsp90-Binding FKBP Immunophilins

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Abstract Hsp90 functionally interacts with a broad array of client proteins, but in every case examined Hsp90 is accompanied by one or more co-chaperones. One class of co-chaperone contains a tetratricopeptide repeat domain that targets the co-chaperone to the C-terminal region of Hsp90. Within this class are Hsp90-binding peptidylprolyl isomerases, most of which belong to the FK506-binding protein (FKBP) family. Despite the common association of FKBP co-chaperones with Hsp90, it is now clear that the client protein influences, and is influenced by, the particular FKBP bound to Hsp90. Examples include Xap2 in aryl hydrocarbon receptor complexes and FKBP52 in steroid receptor complexes. In this chapter, we discuss the known functional roles played by FKBP co-chaperones and, where possible, relate distinctive functions to structural differences between FKBP members.

Keywords Immunophilin · FKBP · Hsp90 · Steroid hormone receptor

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,* Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_2

Introduction

Immunophilins are a large, functionally diverse group of proteins that are defined by their ability to bind immunosuppressive ligands. The immunophilins minimally contain a peptidyl-prolyl cis-trans isomerase (PPIase; also termed rotamase) domain to which the immunosuppressive drugs bind. Early investigations into the PPIase enzymatic activity led to the belief that the immunosuppressive drugs elicited their effects by inhibiting the PPIase activity. However, some compounds binding the PPIase active site efficiently inhibit PPIase activity without inducing immunosuppression, so PPIase activity is not critical for immune responses. It is now known that effector domains on the immunosuppressive drugs project from the PPIase pocket. This allows the immunophilin-drug complex to bind tightly to and inhibit calcineurin or target of rapamycin, signal transduction proteins required for immune responses (see Hamilton and Steiner 1998 for a detailed review on the mechanisms by which immunophilins and their ligands suppress immune responses).

Since the initial identification of the immunophilin proteins, multiple family members have been identified in all major branches of life. Some immunophilins are small proteins containing only a single PPIase domain while others are large multidomain proteins that contain one or more PPIase domains, as well as additional functional domains. The immunophilins are divided into two groups based on their ability to bind different immunosuppressive ligands: the FK506 binding proteins (FKBP), which also bind rapamycin, and the cyclosporin-A binding proteins or cyclophilins (CyP). The PPIase domains of FKBP and cyclophilins are structurally distinct and likely evolved independently. On the other hand, some members of either the FKBP or cyclophilin families contain a structurally similar tetratricopeptide repeat (TPR) domain that targets binding to heat shock protein 90 (Hsp90).

Hsp90 is an abundant molecular chaperone that interacts with a broad array of protein clients that regulate numerous important cellular pathways. Among the known Hsp90 clients are transcription factors (e.g., steroid hormone receptors, heat shock transcription factor 1, aryl hydrocarbon receptor), both serine/threonine and tyrosine kinases (e.g., Raf and Src-related kinases), and key regulatory enzymes (e.g., nitric oxide synthase and telomerase). A compilation of known Hsp90 clients maintained by Didier Picard at Univ. of Geneva can be accessed at: http://www.picard.ch/downloads/Hsp90interactors.pdf.

In concert with other chaperone proteins, Hsp90 facilitates client folding and proteolytic stability but can also promote client degradation. In the case of steroid receptors, Hsp90 and its associated co-chaperones also regulate receptor activity. Hsp90 binding to steroid receptors must be preceded by transient receptor interactions with Hsp40, Hsp70, and associated co-chaperones. Hsp90, which is recruited as a dimer in the latter stages of complex assembly, binds directly to the receptor ligand binding domain and stabilizes a receptor conformation that is competent for hormone binding. Proteins that are associated with Hsp90 in the functionally mature

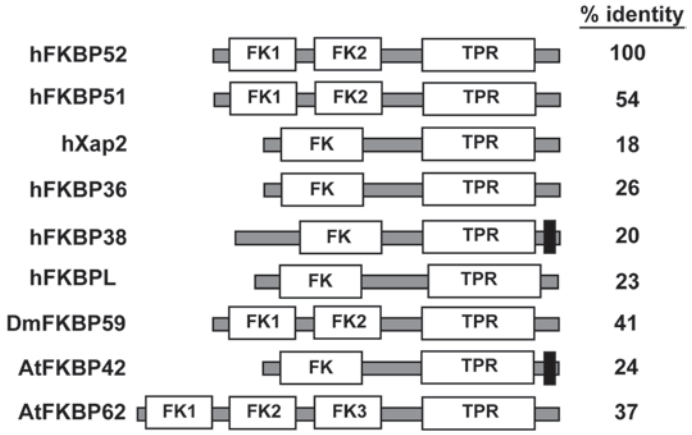

Fig. 2.1 Domain organization of representative Hsp90-binding TPR-containing FKBPs from vertebrate, insect, and plant sources were selected for comparison of domain organizations. The proteins are human FKBP52 (acc. # NP_002005), human FKBP51 (acc. # Q13451), human FKBPL (acc. # NP_071393.2), human Xap2 (acc. # O00170), human FKBP36 (acc. # NP_003593), human FKBP38 (acc. # NP_036313.3), *Drosophila melanogaster* FKBP59 (acc. # AAF18387), *Arabadopsis thaliana* FKBP42 (acc. # CAC00654), and *Arabadopsis thaliana* FKBP62 (acc. # AAB82062). The percent amino acid identity of each compared to human FKBP52 was determined from ClustalW2 alignments (http://www.ebi.ac.uk/clustalw). Each protein shown has at least one FKBP12-like domain (FK), which in some cases has peptidylprolyl isomerase activity and is the binding site for the immunosuppressant drug FK506, and one tetratricopeptide repeat domain (TPR), which is typically an Hsp90 binding site. The black box in the C-terminus of AtFKBP42 is a transmembrane domain used for anchoring the protein to the plasma and vacuolar membranes

receptor complex are p23, a co-chaperone that stabilizes Hsp90 binding to receptor, and any one of several TPR co-chaperones, including the immunophilin/PPIases FKBP52 (also termed p59, Hsp56, p50, HBI, FKBP59, and FKBP4), FKBP51 (also termed p54, FKBP54, and FKBP5), and CyP40, or the protein phosphatase PP5. As discussed below, receptor activity can vary depending on the particular TPR co-chaperone in mature receptor heterocomplexes.

The domain organization for several TPR co-chaperones is compared in Fig. 2.1. These co-chaperones compete for a common binding site in the C-terminal region of Hsp90 that includes the highly conserved -MEEVD sequence that terminates Hsp90. Co-crystallographic structures have shown how an MEEVD pentapeptide associates with the TPR binding pocket (Scheufler et al. 2000; Wu et al. 2004). Although the TPR domains for each of these co-chaperones are structurally similar and interact in a similar manner with Hsp90, the client protein bound by Hsp90 can influence the rank order of co-chaperone recruitment to Hsp90-client complexes (reviewed in Riggs et al. 2004). For instance, PP5 and FKBP51 are preferred components in glucocorticoid receptor (GR) complexes, FKBP51 is preferred in progesterone receptor (PR) complexes, and CyP40 is relatively enhanced in estrogen receptor (ER) complexes (Silverstein et al. 1997; Barent et al. 1998). On the other

hand, another TPR-containing FKBP, the hepatitis B virus protein X associated protein 2 (Xap2; also termed AIP, ARA9, and FKBP37) shows little interaction with steroid receptors but is strongly associated with the aryl hydrocarbon receptor-Hsp90 complex (Ma and Whitlock 1997; Meyer et al. 1998). The distinctive patterns of preference for co-chaperone association in client complexes is one line of evidence that the co-chaperones bound to Hsp90 can also interact with the Hsp90-bound client.

In addition to FKBP52, FKBP51, and XAP2, several other FKBP family members contain TPR domains that are known or likely to bind Hsp90. FKBP36 is structurally similar to XAP2 but is required for male fertility and homologous chromosome pairing in meiosis (Crackower et al. 2003). FKBP38 is a unique family member that is anchored to the mitochondrial and endoplasmic reticulum membranes, and is involved in a variety of processes including protein folding and trafficking, apoptosis, neural tube formation, cystic fibrosis transmembrane conductance regulator (CFTR) trafficking, and viral replication (reviewed in Edlich and Lucke 2011). FK506-binding protein like (FKBPL) protein is a divergent member of the FKBP family that can associate and functionally regulate steroid hormone receptors, has antiangiogenic properties, has a role in the DNA damage response, and controls tumor growth (reviewed in Robson and James 2012). *Drosophila melanogaster* express a TPR-containing immunophilin (DmFKBP59) that has high similarity to FKBP52/51 in vertebrates (Goel et al. 2001; Zaffran 2000). Plants have several FKBP genes that encode TPR domains; for example, in *Arabidopsis thaliana* there are 4 such genes: AtFKBP42, AtFKBP62, AtFKBP65 and AtFKBP72 (Romano et al. 2005; He et al. 2004). Although prokaryotic and Archaeal genomes also contain FKBP family members (Maruyama et al. 2004), none of these genes encode a TPR domain.

Structure/Function Relationships of Steroid Receptor-Associated FKBPs

X-ray crystallographic structures have been resolved for full-length FKBP51 and for overlapping fragments of FKBP52 (Fig. 2.2). FKBP51 and FKBP52 share greater than 60% amino acid sequence similarity, and individual domains do not differ markedly between FKBP51 and FKBP52. Both share a similar TPR domain composed of three tandem repeats of the degenerate 34-amino acid motif, which is a typical characteristic of TPR proteins (Blatch and Lassle 1999). Each repeat adopts a helix-turn-helix conformation and adjacent units stack in parallel to form a saddle-shaped domain with a concave binding pocket for Hsp90. In addition to the TPR domain, both FKBP51 and FKBP52 have two N-terminal domains, each of which is structurally similar to FKBP12. FK506-binding and PPIase activities reside in the most N-terminal domain (FK1), which has a pocket and active site residues similar to FKBP12. Due to several amino acid differences, the second domain (FK2) lacks drug binding and PPIase activity (Sinars et al. 2003).

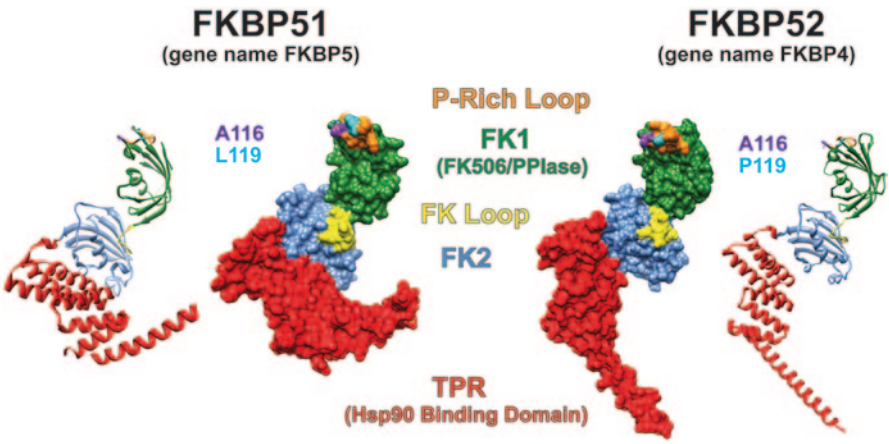

Fig. 2.2 Structural and functional characteristics of FKBP51 and FKBP52. Both ribbon and molecular surface depictions of the X-ray crystallographic structures for human FKBP51 (A; protein data bank number 1KT0) and a composite of two partial structures for human FKBP52 (B; protein data bank numbers 1Q1C and 1P5Q) are shown. In either protein the two FKBP12-like domains (FK1 and FK2, *green* and *blue* respectively) are indicated, the first of which has FK506 binding and PPIase activities. PPIase activity is not required for receptor regulation. The proline-rich loop (*orange*) that overhangs the PPIase catalytic pocket is critical for FKBP52 function and is responsible for the functional difference between FKBP51 and FKBP52. Two functionally critical residues (A116 and L119 in FKBP51 and A116 and P119 in FKBP52) within this loop are highlighted. The FK1 domain, the proline-rich loop in particular, is hypothesized to serve as an interaction surface within the Hsp90-receptor heterocomplex. A loop structure containing a CKII phosphorylation site in the hinge region between FK1 and FK2 is pointed out (*yellow*). The C-terminal TPR domain (*red*) consists of three helix-loop-helix motifs that form the Hsp90 binding pocket. Structures of the individual domains are highly similar between the two proteins, but the angle between FK2 and TPR domains of FKBP51 is more acute and probably more constrained than in FKBP52. The FKBP51 and FKBP52 structure models shown were constructed using UCSF Chimera version 1.5

The most striking difference in crystal structures relates to apparent domain:domain orientations. The FKBP52 structure shown in Fig. 2.2 is a composite model derived from merging the separate FK1-FK2 and FK2-TPR structures. The composite model suggests that the FKBP52 TPR domain is aligned in a more linear fashion with the FK domains rather than in the kinked conformation seen with FKBP51 (Fig. 2.2). In fact, the static orientations shown in crystal structures are likely more dynamic in solution, but the different crystal orientations are perhaps telling. Amino acid side chains unique to FKBP51 form a salt bridge between FK2 and TPR that would stabilize the domain:domain interaction in FKBP51 relative to FKBP52, which lacks this salt bridge. The apparently more malleable structure of FKBP52 might allow interactions within the receptor heterocomplex that are strained in FKBP51.

Significant progress has been made in understanding functionally important domains and residues on FKBP52 that contribute to the distinct ability to regulate steroid hormone receptor activity. Random mutagenesis studies in *S. cerevisiae*

demonstrated that two point mutations (A116V and L119P) in the FKBP51 FK1 domain, which does not potentiate steroid hormone receptor activity under normal conditions, confer full receptor potentiating ability to FKBP51, similar to that of FKBP52 (Riggs et al. 2007). This suggests that FKBP51 and FKBP52 functionally diverged at some point in evolution by only a few residues. A recent study suggests that there are differences in conformational dynamics between FKBP51 and FKBP52 within the proline-rich loop (Mustafi et al. 2014). 15N NMR relaxation measurements demonstrated that only the proline-rich loop in FKBP51 displays significantly larger line broadening, which is completely suppressed in the presence of the L11P mutation. These data suggest not only that differences in the proline-rich loop confer distinct functions to FKBP51 and FKBP52, but also that the proline-rich loop is functionally important for FKBP52 regulation of receptor activity. The current hypothesis holds that the FKBP52 proline-rich loop serves as an interaction surface, and the interaction partner is likely the receptor hormone binding domain (Sivils et al. 2011; De Leon et al. 2011).

Recent evidence by Bracher et al. demonstrate that the FK1-FK2 domains portray a flexible hinge that may account for regulatory differences between FKBP51 and FKBP52 (Bracher et al. 2013). It is hypothesized that the FK2 domain of FKBP52 contains an activation mechanism based on the calmodulin-binding motif at the C-terminus, yet this region is unable to bind FK506 and rapamycin, and lacks PPIase activity (Chambraud et al. 1993; Pirkl and Buchner 2001; Rouviere et al. 1997).

FKBP51 and FKBP52 also differ in the hinge region connecting FK1 and FK2 domains (FK loop). The FK loop of FKBP52 contains a -TEEED- sequence that has been identified as an *in vitro* substrate for casein kinase II; the corresponding sequence in FKBP51, -FED-, lacks the threonine phosphorylation site. Phosphorylation of FKBP52 is potentially important since the phospho-protein is reported to lose Hsp90 binding (Miyata et al. 1997). This difference was further tested using comparative analysis of FKBP51 and FKBP52 FK linker sequences (Cox et al. 2007). While the phosphomimetic mutation T143E had no effect on FKBP52 binding to Hsp90 in this study, the mutation did abrogate FKBP52 regulation of receptor activity. It is predicted that phosphorylation of residue T143 in the FKBP52 FK linker reorients the entire FK1 conformation, thereby eliminating FK1 interactions with the receptor hormone binding domain.

Cellular and Physiological Functions of Hsp90-Associated FKBPs

FKBP52

FKBP52 is expressed in most vertebrate tissues and cell lines, although its expression can be up-regulated by heat stress (Sanchez 1990), by estrogen in MCF-7 breast

cancer cells (Kumar et al. 2001), and by the homeobox transcription factor HoxA-10 in the peri-implantation mouse uterus (Daikoku et al. 2005). FKBP52 associates with steroid receptor complexes in an Hsp90-dependent manner, but FKBP52 is not required in a defined cell-free assembly system for receptor to reach the mature conformation that is competent for hormone binding (Dittmar et al. 1996; Kosano et al. 1998). Nonetheless, FKBP52 in cells potentiates hormone-dependent reporter gene activation by GR (Riggs et al. 2003), AR (Cheung-Flynn et al. 2005), and PR (Tranguch et al. 2005). Potentiation of hormone signaling can be related to an increase in receptor affinity for hormone (Riggs et al. 2003; Davies et al. 2005), but there may be additional mechanisms by which FKBP52 enhances receptor activity.

In concordance with hormone binding affinity changes, domain-swapping experiments between GR and ER, which is not potentiated by FKBP52, demonstrated that FKBP52 potentiation is localized to the ligand binding domain of GR (Riggs et al. 2003). FKBP52-dependent potentiation of receptor activity is abrogated in point mutants that are defective for Hsp90 binding, and potentiation is blocked by the PPIase inhibitor FK506 (Riggs et al. 2003; Cheung-Flynn et al. 2005). One model to explain these findings is that Hsp90 recruits FKBP52 to the receptor heterocomplex such that the FK1 PPIase can effectively catalyze isomerization of one or more proline substrates in the receptor ligand binding domain. However, studies have shown that point mutations within the FKBP52 PPIase pocket that eliminate PPIase activity have no effect on FKBP52 potentiation of receptor activity (Riggs et al. 2007). Thus, FK506-mediated inhibition of FKBP52 function likely occurs through the inhibition of FK1 interactions as opposed to inhibition of PPIase enzymatic activity. As discussed above, the FKBP52 FK1 domain as a whole is functionally important and the proline-rich loop that overhangs the PPIase pocket could serve as a functionally important interaction surface that contacts the receptor hormone binding domain within the receptor-chaperone heterocomplex. A structure-based screen for small molecules targeting an alternative surface of the androgen receptor hormone binding domain identified a series of fenamic acid molecules that allosterically affect coactivator binding at the activation function 2 (AF2) site through interaction with a surface cleft termed binding function 3 (BF3) (Estebanez-Perpina et al. 2007). Steroid hormone receptor structural comparisons identified this region to be a highly conserved regulatory surface that could serve as a therapeutic target for hormone-dependent diseases (Buzon et al. 2012). Interestingly, mutations within the AR BF3 surface (F673P, P723S, and C806Y) result in increased dependence on FKBP52 for function. In addition, a drug termed MJC13 that specifically inhibits FKBP52-regulated AR activity is predicted to target the BF3 surface (De Leon et al. 2011). Thus, the BF3 surface is a putative FKBP52 interaction and/or regulatory surface, and FKBP52 interaction with the receptor BF3 surface could allosterically affect receptor interactions at the AF2 site. In addition to the AR BF3 surface, recent studies suggest that the Helix 1–3 (H1-H3) loop in the GR LBD is an important site of FKBP regulation. Glucocorticoid insensitivity in guinea pig has been linked to sequence differences in the H1-H3 loop and substitution of the guinea pig H1-H3 loop into rat GR resulted in increased FKBP51-mediated repression of receptor activity. It is hypothesized that changes in the H1-H3 loop result in

changes within the GR-Hsp90 heterocomplex that favor FKBP51 repression over FKBP52 potentiation (Cluning et al. 2013).

FKBP52 has been shown by *in vitro* studies to have a chaperone activity that is independent of Hsp90 binding or PPIase (Bose et al. 1996; Pirkl and Buchner 2001). Like Hsp90 and numerous other chaperone components, FKBP52 can hold misfolded proteins in a non-aggregated state that is amenable to refolding. The possibility that chaperone holding activity displayed by FKBP52 plays some role in altering receptor activity cannot be dismissed, but this appears unlikely since holding activity is highly redundant among chaperone components. Furthermore, holding activity, unlike FKBP52-dependent potentiation of receptor activity, is neither PPIase- nor Hsp90-dependent. Unfortunately, no one has identified an FKBP52 mutation that disrupts holding activity in a discrete manner.

In an effort to extend biochemical and cellular data to the physiological level FKBP52 gene knockout (52KO) mice were generated, independently, by two groups (Cheung-Flynn et al. 2005; Yong et al. 2007). The mutant mice have striking reproductive phenotypes that can be attributed, at least in part, to loss of steroid receptor activity. Male 52KO mice are infertile and display abnormal virilization with persistent nipples, ambiguous external genitalia, and dysgenic seminal vesicles and prostate (Cheung-Flynn et al. 2005; Yong et al. 2007). These developmental defects are consistent with androgen insensitivity in these tissues. Testicular morphology, descent, histology, and spermatogenesis are normal and androgen production and release from testes is unimpaired; these developmental features are not highly androgen-dependent. On the other hand, sperm isolated from the epididymis have abnormal tail morphology and reduced motility suggestive of a defect in sperm maturation within the epididymis, a process that is androgen-dependent. Cellular studies confirm that FKBP52 is required for full AR function, which provides a rational explanation for androgen insensitivity in tissues of 52KO males.

52KO females have no gross morphological abnormalities, yet are completely infertile (Tranguch et al. 2005). Oocyte formation and release are not markedly impaired, and oocytes are competent for *in vitro* and *in vivo* fertilization. Infertility is due, at least in part, to a maternal failure of embryonic implantation and uterine decidualization. During the early stages of pregnancy, the 52KO uterus does not display the usual molecular or physiological markers for implantation. These events are largely dependent on progesterone actions, and both molecular and cellular studies confirm that FKBP52 is required for full PR activity. Additionally, FKBP52 is related to the etiology of endometriosis given that 52KO mice display increased endometrial lesions, inflammation, cell proliferation, and angiogenesis, and FKBP52 protein levels are reduced in human endometrial tissues (Hirota et al. 2008).

FKBP52 is critical for reproductive development and success in both male and female mice and its role can be traced to support of AR and PR function. Although GR-related phenotypes are not readily apparent, cellular and biochemical studies suggest that 52KO animals should display phenotypes related to reduced GR activity. Given that abnormal Mendelian ratios are not observed for heterozygous crosses,

the 52KO phenotype does include partial embryonic lethality. This combined with the reproductive defects leads to difficulty in obtaining sufficient numbers of 52KO animals for experiments. Thus, heterozygous fkbp52-deficient mice (52+/–) were generated to determine the *in vivo* roles for FKBP52 in GR-mediated physiology. 52+/– mice displayed phenotypes associated with reduced GR signaling including increased susceptibility to high-fat diet-induced hepatic steatosis, hyperglycemia, hyperinsulinemia, and behavioral alterations under basal and chronic stress conditions (Wadekar et al. 2004; Warrier et al. 2010).

Although FKBP52 does not alter ER function in cellular studies and 52KO mice show no signs of estrogen insensitivity, FKBP52 expression is upregulated by estrogens and FKBP52 is over-expressed in breast tumors (Ward et al. 1999). In addition, the FKBP52 gene is methylated in ER-negative, but not in ER-positive breast cancer cells (Ostrow et al. 2009). Thus, a few studies have identified FKBP52 as a potential regulator of at least ER expression in breast cancer.

Despite the fact that FKBP52 was initially discovered in the immune system, it is ubiquitously expressed and particularly abundant in the central nervous system. Thus, it is not surprising that FKBP52 is involved in neurodegenerative tauopathies including Alzheimer's (AD) and Pick's disease, fronto-temporal dementia and Parkinsonism linked to chromosome 17 (FTDP), and progressive supranuclear palsy (Haelens et al. 2007; Hernandez and Avila 2007). The defining neuropathological characteristic of tauopathies is the aberrant aggregation of insoluble hyperphosphorylated microtubule-associated protein (MAP) tau within the neurons, which is termed neurofibrillary tangles (NFTs) and is also referred to as paired helical filaments (PHF) (Cao and Konsolaki 2011). Recent studies have shown FKBP52's direct interaction with tau, particularly with its hyperphosphorylated form, has antagonistic effects on tubulin polymerization and microtubule assembly (Chambraud et al. 2007; Chambraud et al. 2010). In addition, FKBP52 was recently shown to induce Tau-P301L oligimerization and assembly into filaments (Giustiniani et al. 2014). More importantly, knockdown of FKBP52 was shown to restore axonal outgrowth and branching caused by Tau-P301L expression, thereby validating FKBP52 as an attractive therapeutic target in tauopathies. FKBP52 is known to be involved in subcellular rearrangement. Studies by Quintá et al. demonstrated that the overexpression of FKBP52 can induce neuronal differentiation and neurite outgrowth (Quintá et al. 2010).

Recent reports have shown that copper (Cu) contributes to the neuropathology of AD by interacting with copper binding domains of amyloid precursor proteins (APPs) and beta-amyloid (Aβ) peptides causing the formation of amyloid plaques and disrupting metal ion homeostasis (Barnham and Bush 2008; Drago et al. 2008; Kong et al. 2007). FKBP52 is involved in the regulation of cellular Cu homeostasis by interacting directly with the copper transport protein Atox1 (Sanokawa-Akakura et al. 2004), which is part of the Cu efflux machinery in neurons. In addition, both genetic and cellular data in Drosophila suggest a novel role for FKBP52 in the regulation of intracellular Cu homeostasis via binding to APP, thus, modulating the toxicity level of Aβ peptides (Sanokawa-Akakura et al. 2010).

S100A proteins belong to the EF-hand type calcium (Ca^{2+}) sensing protein family that are linked to regulation of various intracellular processes and are often expressed in a cell- and tissue-specific fashion (Santamaria-Kisiel et al. 2006; Wright et al. 2009). Based on biochemical evidence, it has been demonstrated that S100A1 and S100A6 interact with FKBP52 by competing with Hsp90 for the TPR domain in a Ca^{2+}-dependent manner (Shimamoto et al. 2010). Cellular data has linked S100A1s involvement in the neuronal cell dysfunction/death that occurs in AD by reducing APP expression and stabilizing the intracellular Ca^{2+} homeostasis (Zimmer et al. 2005). It seems that the function of FKBP52 can be regulated by Ca^{2+} homeostasis within the cell leading to effects on the phosphorylation of tau and pathology in AD. Interestingly, a Drosophila orthologue of FKBP52 termed FKBP59 was found to interact with the Ca^{2+} channel protein TRPL in photoreceptor cells and to influence Ca^{2+} influx (Goel et al. 2001). Subsequent studies revealed that FKBP52 similarly interacts with a subset of rat transient receptor potential channel (TRPC) proteins that form Ca^{2+} channels in the mammalian brain (Sinkins et al. 2004). The C-terminus of FKBP52 contains a predicted calmodulin binding domain, which enables the protein to bind to calmodulin-Sepharose in a Ca^{2+}-dependent manner, the biological function of which is still unknown (Silverstein et al. 1999).

Apart from the well-established roles of FKBP52 in steroid hormone receptor function, FKBP52, as with other Hsp90 co-chaperones, has been identified in a variety of client-Hsp90 heterocomplexes such as those containing kinases, aryl hydrocarbon receptor, and heat shock transcription factor; however, many of these interactions might reflect passive, transient association of the protein with Hsp90 and have no functional impact on client activity. FKBP52 is also linked to various Hsp90-independent interactions. Aside from the aforementioned Hsp90-independent interactors, FKBP52 has been found to interact directly with the interferon regulatory factor 4 (Mamane et al. 2000), which regulates gene expression in B and T lymphocytes, forms a complex with tyrosine kinase receptor RET51, which is involved in the development and maintenance of the nervous system (Fusco et al. 2010) and FKBP associated protein 48 (Chambraud et al. 1996), which influences proliferation of Jurkat T cells (Krummrei et al. 2003). Each of these interactions was found to be disrupted by FK506 and to target the FKBP52 PPIase domain to specific proline sites in each partner protein. Phenotypes potentially related to these interactions have not yet been assessed in 52KO mice. Not only does FKBP52 interact with proteins, but also FKBP52 is capable of directly binding adeno-associated virus DNA and regulating replication of the viral genome (Qing et al. 2001; Zhong et al. 2004). The relevant DNA binding site in FKBP52 has not been identified.

FKBP51

FKBP51/p54/FKBP54 was originally identified as a component of chicken PR complexes (Smith et al. 1990; Smith et al. 1993a; Smith et al. 1993b) and is now

known to assemble as an Hsp90 co-chaperone with all steroid receptors and other Hsp90-client complexes. FKBP51 is functionally similar in some ways to FKBP52; both have similar PPIase activity in the presence of model peptide substrates, both hold misfolded proteins in a folding competent state, and they compete for binding a common site on Hsp90 (Nair et al. 1997; Pirkl et al. 2001). As noted above, the overall structural similarity of these FKBPs is consistent with these shared functional properties, yet their distinct effects on steroid receptor activity belie these similarities. In addition to the aforementioned structural differences between FKBP51 and FKBP52, another distinction is that the FKBP51 gene is highly inducible by glucocorticoids, androgens, and progesterone (Baughman et al. 1995; Kester et al. 1997; Zhu et al. 2001; Yoshida et al. 2002; Vermeer et al. 2003; Hubler et al. 2003; Febbo et al. 2005).

FKBP51 acts as an inhibitor of GR, PR, and MR function excluding AR. The first indication of its inhibitory role came from studies by Scammell and colleagues of glucocorticoid resistance in New World primates (Reynolds et al. 1999; Denny et al. 2000). In squirrel monkeys, GR has a relatively low affinity for hormone yet the cloned monkey GR has an affinity similar to human GR *in vitro*. This observation led to a search for cellular factors in monkey cells that reduced GR binding affinity. A key factor identified was FKBP51, which is constitutively overexpressed in squirrel monkey cells as well as cells of other New World primates, all of which display some degree of glucocorticoid resistance. Human FKBP51 was also found to inhibit GR function but not to the degree of squirrel monkey FKBP51, which differs in amino acid sequence from its human counterpart at 15 of 457 amino acids. These differences are scattered fair evenly along the sequence, and mapping studies have shown that amino acid changes in several domains contribute to the more potent inhibitory actions of squirrel monkey FKBP51 (Denny et al. 2005). Crystal structures for both human and squirrel monkey FKBP51 have been solved (Sinars et al. 2003); although functionally relevant structural changes are not yet apparent, comparison of these structures should ultimately help to understand why inhibitory potencies differ. The function of FKBP51 is dichotomous with respect to regulation of the steroid hormone receptors. *In vitro* experiments have shown that overexpression of human FKBP51 reduces glucocorticoid binding affinity and nuclear translocation of GR which forms an ultra-short negative feedback loop for receptor activity (Wochnik et al. 2005). This model is in agreement with the aforementioned data from squirrel monkeys that have a general resistance to glucocorticoids even though they express GR that has the full potential to bind cortisol with high affinity. Another interesting possibility by which FKBP51 decreases overall GR signaling is by promoting nuclear translocation of the transcriptionally inactive β isoform of GR (Zhang et al. 2008). Interestingly, FKBP51 has an opposing effect on AR; it increases the receptor signaling in prostate cancer cells. Using both recombinant protein- and cell-based assays, Ni *et al*. demonstrated that FKBP51 stimulates chaperone complex association with AR, which further enhances AR ligand binding and androgen-dependent transcription and cell growth, resulting in an ultra-short positive feedback loop (Ni et al. 2010).

In a yeast model for studying functional interactions between steroid receptors and human FKBPs, FKBP51 does not inhibit the activity of GR; however, FKBP51 can effectively reverse the potentiation of GR activity conferred by FKBP52 (Riggs et al. 2003). Therefore, FKBP51 acts as an antagonist of FKBP52. FKBP51 has also been shown to inhibit PR function (Hubler et al. 2003), presumably through a similar inhibition of FKBP52-mediated potentiation. The mechanism by which FKBP51 antagonizes FKBP52's ability to enhance steroid receptor function is not understood. Other Hsp90-binding TPR proteins do not block FKBP52 actions, so it does not appear that competitive displacement of FKBP52 from receptor complexes by FKBP51 can fully account for antagonism. On the other hand, FKBP51 is known to preferentially associate with PR and GR complexes (Nair et al. 1997; Barent et al. 1998). Domain swapping studies indicate that the FK1 PPIase domain partially contributes to antagonism but sequences in the FK2 and TPR domain also play a role (Riggs et al. 2003; Denny et al. 2005).

Given that FKBP51 gene expression is inducible by some steroid hormones and FKBP51 can both activate and inhibit receptor function, one can reasonably speculate that FKBP51 serves as a cellular modulator of hormone responsiveness. In cells unexposed to hormone, FKBP52 actions would predominate and promote a robust response to hormone. As a consequence, FKBP51 levels would rise and partially desensitize cells to a secondary hormone exposure in most systems excluding AR-mediated prostate cancer cells. These effects can be demonstrated in cellular models, but the physiological importance of this mechanism must be established with animal models. Toward this goal, FKBP51 gene knockout (51KO) mice were generated. Homozygous mutant animals are grossly normal and reproductively viable, so FKBP51 does not appear to be critical in the same physiological processes as FKBP52. Nonetheless, modulatory actions of FKBP51 are relevant but subject to compensatory physiological mechanisms. Interestingly, double knockout of both FKBP51 and FKBP52 genes is embryonic lethal in mice, suggesting either that FKBP51 and FKBP52 have a critical, mutually redundant function or that FKBP51 and FKBP52 function in a common developmental pathway that requires the distinct actions of both immunophilins.

The hypothalamic-pituitary-adrenal (HPA) axis controls stress response and is associated with susceptibility to depression as well as antidepressant efficacy (Touma et al. 2011; O'Leary et al. 2011). The HPA axis is regulated via negative feedback of GR activity and FKBP51. GR resistance is conferred by the overexpression of FKBP51, which is associated with an impaired negative feedback mechanism (Denny et al. 2005). Polymorphisms in the FKBP5 gene are associated with an increased susceptibility for depression, an increased response to antidepressants, and an increased risk of posttraumatic stress disorder in response to adverse early life events (Binder et al. 2008; Binder et al. 2004). In addition, genotype-directed environment-induced gene programming through FKBP5 gene methylation was recently shown to mediate gene-childhood trauma interactions (Klengel et al. 2013). Recent studies have shown that FKBP51 is a modulator of the cortisol-HPA axis response to chronic stress and related psychiatric disorders (Hartmann et al. 2012; O'Leary et al. 2011; Tatro et al. 2009; Touma et al. 2011). Indeed, 51KO mice

displayed diminished physiological and neuroendocrine response to the adverse effects of chronic stress with fast recovery from acute stress episodes. The null mice also showed reduced adrenal gland weight and lower levels of basal corticosterone suggesting an enhanced sensitivity of GR due to the loss of FKBP51.

As aforementioned, aggregation of MAP tau into neurofibrillary tangles in neurons is the hallmark of tauopathies. *In vitro* studies demonstrated that PPIase activity of FKBP51 regulates and balances the phosphorylation state of tau for microtubule stabilization (Jinwal et al. 2010; Koren et al. 2011). Interestingly, knockdown of FKBP51 dramatically reduced tau levels while inhibiting its PPIase activity led to increased stability and accumulation of phosphorylated tau (Jinwal et al. 2010). In addition, overexpression of FKBP51 prevented tau clearance and produced oligomeric tau in the brain, facilitating its neurotoxicity (Blair et al. 2013; Jinwal et al. 2010). Studies by Blair et al. demonstrated that upregulation of FKBP51 expression is attributed to a decrease in FKBP5 methylation in which the process appears to be inversely proportional over time (Blair et al. 2013). This provides an explanation for the detection of increased FKBP51 protein levels in aged murine brains, and the manifestation of depression and cognitive deficits in AD patients.

Aside from its role in steroid receptor function, FKBP51 has been identified in a wide array of Hsp90-independent complexes. Biochemical and cellular studies have demonstrated that FKBP51 inhibits apoptosis in irradiated melanoma cells (Romano et al. 2010), promotes dephosphorylation of Akt and downregulation of the Akt pathway (Pei et al. 2009), and is associated with polymorphisms in *fkbp5* as seen in affective and anxiety disorders (Binder 2009). Furthermore, FKBP51 has been shown to regulate NFκB pathways. FKBP51 was identified (Bouwmeester et al. 2004) by a proteomic approach in complex with IKKα, one of the serine/threonine kinases that stimulates phosphorylation and degradation of the NFκB inhibitor IκB. Knockdown of FKBP51 expression was shown to inhibit IKKα activation and thereby block TNFα-induced activation of NFκB, which confirmed the functional significance of FKBP51 in IKKα complexes. Perhaps related to FKBP51-dependent regulation of NFκB pathways, overexpression of FKBP51 has been correlated (Giraudier et al. 2002) with idiopathic myelofibrosis, a rare clonal stem cell disorder. Experimental overexpression of FKBP51 was subsequently shown to stimulate NFκB activity and, as a consequence, to increase secretion of pro-fibrotic TGF-β1 (Komura et al. 2005). IKKα had previously been shown to be an Hsp90 client (Broemer et al. 2004), so it is possible that, analogous to steroid receptor complexes, FKBP51 assembles with IKKα as a heterocomplex with Hsp90. Whether FKBP51 Hsp90 binding or PPIase is required for regulation of IKKα has not been determined.

Cytoplasmic Transport

There is strong evidence that Hsp90-binding immunophilins play a key role in the subcellular relocalization of some transcription factors, the pioneer studies having

been performed with steroid receptors. In the absence of ligand, some members of the steroid-receptor family such as GR or MR reside primarily in the cytoplasm, whereas others such as ER or PR are mostly nuclear in a constitutive manner even in the absence of hormone. Regardless of their primary localization, receptors are constantly shuttling in a highly dynamic manner between the nucleus and the cytoplasm (Elbi et al. 2004; Galigniana et al. 2010a; Madan and DeFranco 1993). Therefore, the final localization of a given receptor under a certain biological condition is the resultant of the proper displacement of that dynamic equilibrium between both cellular compartments. Accordingly, the presence of hormone favors the import driven mechanism that results in the nuclear concentration of GR. Although some molecules can escape to the cytoplasm, they are transported back to the nucleus and vice versa, the opposite situation is also true when receptors are primarily cytoplasmic. In summary, the degree of cytoplasmic or nuclear localization reflects both the rate of nuclear import and the rate of nuclear export in a given moment (Galigniana et al. 2010a).

It has always been assumed that simple diffusion is the driving force for steroid hormone receptor movement. The classic model for receptor trafficking was posited several years ago (Dahmer et al. 1984) and supported the heuristic notion that the receptor-chaperone heterocomplex is dissociated immediately after steroid binding (a process usually referred to as 'transformation'). Therefore, transformation was originally thought to be a key cytoplasmic requirement to favor the release of the receptor from the cytoplasmic anchoring sites and to permit its consequent nuclear translocation. Today, the experimental evidence shows that rather than an early event in the molecular mechanism of activation of steroid receptors, transformation is a nuclear process (Galigniana et al. 2010a; Grossmann et al. 2012; Presman et al. 2014).

The original finding that some TPR-domain proteins such as FKBP52, CyP40 and the PPIase-like protein phosphatase PP5 are able to interact with the motor protein dynein (Galigniana et al. 2002), led to the idea that they may be involved in the retrotransport of the receptors. It was demonstrated that dynein is also present in the native GR/Hsp90/FKBP52 heterocomplex (Galigniana et al. 2001), and that such association is FKBP52-dependent via the peptidyl-prolyl isomerase domain of the immunophilin (Galigniana et al. 2001; Galigniana et al. 2010b). The enzymatic activity, however, is related neither to the protein-protein interaction nor to the molecular mechanism of transport. The disruption of such complex or the lack of expression of FKBP52 impairs (but not totally abolishes) the cytoplasmic transport of GR to the nucleus (Galigniana et al. 2001; Galigniana et al. 2010b; Tatro et al. 2009), such that the half-life for nuclear translocation is increased one order of magnitude under this abnormal situation (from 5 min to 40–50 min for GR). This means that the cytoplasmic retention of the receptor when the transportosome is inactivated is indeed a temporal event. Thus, it can be predicted that incubation times with steroid longer than 40–60 min will show the receptor in the nucleus anyway. A retrotransport delay may have physiological consequences when the biological response should be fast, for example, in stressing situations, and can be envisaged when travelling distances are long, such as in axons. In this case, GR

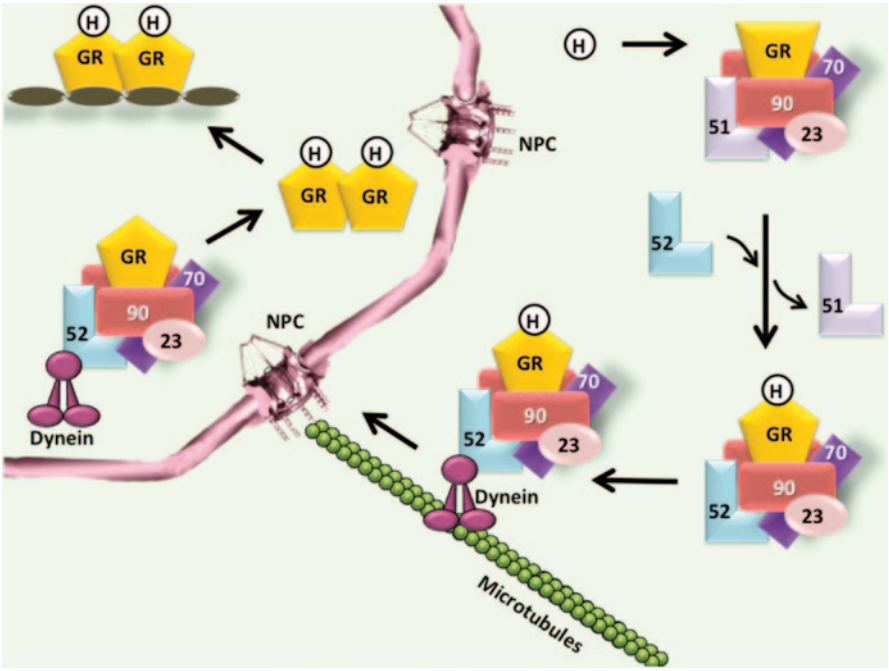

Fig. 2.3 Model of glucocorticoid receptor activation. In the absence of hormone (H), the GR exists in the cytoplasm associated with the Hsp90-based heterocomplex formed by a dimer of Hsp90, and one molecule of Hsp70, p23 and FKBP51. Upon steroid binding, FKBP51 is replaced by FKBP52, an immunophilin able to recruit the dynein/dynactin motor complex. The whole GR heterocomplex is retrotransported on microtubules tracks and translocates through the nuclear pore complex (NPC) to the nucleoplasm still associated to the heterocomplex. Transformation (i.e., Hsp90-complex dissociation) occurs in the nuclear compartment followed by receptor dimerization. The receptor is targeted to the promoter binding-sites to trigger the proper biological response and the heterocomplex is recycled

does not reach the nucleus because it is targeted to proteosomal degradation along its pathway (Galigniana et al. 2004a).

On the other hand, FKBP51, the highly homologous partner that shows low affinity for dynein motors (Wochnik et al. 2005; Galigniana et al. 2010b), acts as a competitive inhibitor of FKBP52. Therefore, it is not surprising that upon ligand binding FKBP51 is released from steroid receptor complexes and replaced by FKBP52 (Davies et al. 2002), which in turn recruits the dynein/dynactin motor complex (Fig. 2.3). In line with this fact, it has been proposed that the FKBP52/FKBP51 expression ratio may be one of the key regulatory factors for the nuclear retention of steroid receptors (Galigniana et al. 2010b; Tatro et al. 2009; Gallo et al. 2007).

It is possible that other TPR-domain immunophilins that are also able to interact with dynein, such as CyP40 and PP5, may replace FKBP52 in the transport machinery, although this has not been demonstrated. Nonetheless, recent evidence showed

that the Hsp90-binding immunophilin FKBPL/WISp39 also favors GR retrotransport in a similar fashion as FKBP52 (McKeen et al. 2008).

Importantly, the active Hsp90-, FKBP52-dependent mechanism for cytoplasmic transport first described for GR has also been found for other factors such as MR (Galigniana et al. 2010b), AR (Thomas et al. 2006), ecdysone receptor (Vafopoulou and Steel 2012), p53 (Galigniana et al. 2004b), RAC3 (Colo et al. 2008), and adeno-associated virus-2 (AAV) (Zhao et al. 2006). This immunophilin-dependent model for soluble protein trafficking implies that the proteins of the heterocomplex should remain associated to the client cargo during the passage through the nuclear pore complex. In line with this speculation, it was demonstrated that the whole Hsp90-FKBP52 heterocomplex cross-linked to corticosteroid receptors (Galigniana et al. 2010b; Echeverria et al. 2009) is able to translocate intact in a hormone-dependent manner through the nuclear pore of digitonin-permeabilized cells, suggesting that steroid-receptor transformation and its subsequent dimerization must be a nuclear event. This was recently confirmed by using different methodologies (Galigniana et al. 2010b; Grossmann et al. 2012; Presman et al. 2014; Presman et al. 2010).

Studies of reconstitution of the Hsp90-FKBP52 heterocomplex with purified proteins or reticulocyte lysate as a source of chaperones (Echeverria et al. 2009), demonstrated that the interaction of GR with structures of the nuclear pore such as nucleoporins (NUPs) is strengthened when both factors, GR and NUPs, are chaperoned. On the other hand, the discovery that NUPs are Hsp90- and FKBP52-interacting proteins also suggests a potential regulatory role of these chaperones for the nuclear import process. In this regard, it has always been very difficult to explain how single factors such as importins could shield the multitude of different protein-, RNA- and DNA-binding domains in transport cargoes that are import substrates. It could be speculated that these chaperones associated to importins, NUPs, and the cargo itself may act as a cooperative system to prevent aggregation of cargoes when a hydrophobic domain is exposed during the translocation step. This may justify why there is a more efficient interaction between NUPs and GR when both proteins are associated to the Hsp90-FKBP52 complex compared to both ‘naked’ proteins (Echeverria et al. 2009).

The association of FKBP52 and PP5 with Nup62 seems to be Hsp90-dependent, as was suggested by the almost-complete dissociation of these immunophilins from Nup62 in the presence of the Hsp90-disrupting agent radicicol (Echeverria et al. 2009). However, indirect immunofluorescence assays performed with intact cells treated with radicicol still show the presence of the immunophilins in the perinuclear ring, suggesting that they may also bind in an Hsp90-independent manner to other perinuclear structures. Nonetheless, competition experiments with the TPR domain overexpressed in intact cells showed that the perinuclear signal of FKBP52 was totally abolished, indicating that most, if not all, types of association of the immunophilin with any structure of the nuclear envelope require the TPR domain.

Xap2

Apart from the highly characterized steroid hormone receptor-associated FKBPs, several other TPR-containing FKBPs are present in higher vertebrates. As mentioned in earlier sections of this chapter, Xap2 is a TPR-containing immunophilin that is found almost extensively in AhR complexes. As the name implies, Xap2 also functionally interacts with the hepatitis B virus protein X (Kuzhandaivelu et al. 1996). Recently, Xap2 was shown to exert an inhibitory effect on both GR and ERα, but not ERβ activity, and may inhibit AR and PR as well (Cai et al. 2011; Laenger et al. 2009; Schulke et al. 2010). In addition, Xap2 is known to have functional interactions with peroxisome proliferator activated receptor α (PPARα) (Sumanasekera et al. 2003) and thyroid hormone receptor β, however, these interactions have not been extensively characterized. AhR is a ligand-dependent transcription factor that mediates the physiological response to specific environmental contaminants termed polycyclic aromatic hydrocarbons, the most notorious of which is 2,3,7,8-tetrachlorodibenzo-p-dioxin. Similar to steroid receptors, AhR requires assembly with Hsp90 and p23 to achieve a mature ligand-binding conformation (reviewed in Petrulis and Perdew 2002), although the AhR ligand binding domain is unrelated to steroid receptor ligand binding domains. AhR complexes also contain an FKBP component, but in this case it is Xap2 rather FKBP52 or FKBP51.

As with FKBP51 and FKBP52, Xap2 has a C-terminal TPR domain that is known to facilitate binding to the MEEVD motif on Hsp90 (Carver et al. 1998) (Fig. 2.1). In addition Xap2 contains one N-terminal FK domain that lacks drug binding and also likely lacks PPIase activity. Although the FK domain is not required for Hsp90 binding, it is required for an interaction with the AhR-Hsp90 complex that functionally influences receptor activity (Carver et al. 1998; Kazlauskas et al. 2002). In a cell-free assembly system that lacks Xap2, AhR is capable of assembling with Hsp90 and binding ligand, and upon ligand binding AhR is capable of binding AhR response elements on DNA (Meyer et al. 1998). Again, similar to FKBP52 or FKBP51 in steroid receptor complexes, Xap2 is not required for basal maturation of AhR activity, but in both yeast and mammalian systems, Xap2 can modulate AhR-mediated reporter gene expression (Miller 2002; Ma and Whitlock 1997; Meyer et al. 1998; Carver et al. 1998). By titrating the relative level of Xap2 protein in cells, AhR activity can be enhanced or decreased. For example, when Xap2 is expressed at a level 2- to 3-fold higher than normal, binding of p23 in the AhR-Hsp90 complex is reduced (Hollingshead et al. 2004). Displacement of p23 by high levels of Xap2 would destabilize binding of Hsp90 to AhR and reduce the proportion of AhR in functionally mature complexes. Conversely, there is also evidence that at elevated Xap2 levels, AhR is protected from ubiquitination and proteosomal degradation which would increase total AhR levels (Lees et al. 2003; LaPres et al. 2000; Meyer et al. 2000; Meyer and Perdew 1999; Kazlauskas et al. 2000). Finally, several studies suggest that Xap2 facilitates nucleocytoplasmic shuttling of AhR following ligand binding (Berg and Pongratz 2002; Petrulis et al. 2000; Kazlauskas et al. 2000; Kazlauskas et al. 2001; Petrulis et al. 2003).

The physiological relevance of Xap2 interactions with AhR complexes has not been examined in a whole animal model, but Xap2 could potentially influence any of several physiological and pathological pathways mediated by AhR. Mice that are homozygous for a disrupted AhR gene have many physiological and developmental defects; among these are immune system impairment, hepatic fibrosis, cardiac hypertrophy, impaired insulin regulation, and defects in ovarian and vascular development (Fernandez-Salguero et al. 1995; Lahvis et al. 2005; Thackaberry et al. 2003; Benedict et al. 2000). In addition, many of the toxic and teratogenic effects produced by AhR ligands require an intact AhR signaling pathway (Mimura and Fujii-Kuriyama 2003; Fernandez-Salguero et al. 1996). For example, dioxin induced defects in prostate development are absent in AhR knockout mice (Lin et al. 2002). In a conditional Xap2 hepatic knockout mouse model, AhR and Cyp1b1 levels were significantly reduced, however Cyp1a1 and Cyp1a2 were induced to levels seen in wild type mice in response to dioxin challenge (Nukaya et al. 2010). Development of a mouse strain lacking Xap2 would aid in determining the role Xap2 plays in these processes and might validate Xap2 as a potential target for therapeutic intervention. In addition to the above functional interactions, Xap2 has several other interacting partners including, but not limited to, PDE4A5 and 2A3, HSC70, TIF-2, TRβ1, RET, and TOMM20; thereby modulating a host of physiological functions (Reviewed in Trivellin and Korbonits 2011).

FKBP36

FKBP36 (gene name *FKBP6* in humans) is another TPR-containing FKBP that is structurally similar to Xap2, yet functionally distinct. FKBP36 has a single N-terminal FK domain and a C-terminal TPR domain. *In vitro* studies show that FKBP36 binds Hsp90 and can assemble with steroid receptor complexes (unpublished observation), but there is currently no evidence that FKBP36 alters receptor activity. FKBP36 mRNA is broadly expressed in vertebrate tissues with an exceptionally high level observed in the testis; male *FKBP6* knockout mice lack sperm and FKBP36 was shown to be a critical component in meiotic synaptonemal complexes (Crackower et al. 2003). FKBP36 interacts with and inhibits GAPDH activity and expression (Jarczowski et al. 2009). FKBP36 forms a complex with Hsp90 and GAPDH and this complex may regulate GAPDH activity in a manner akin to FKBP/Hsp90/ steroid receptor complexes (Jarczowski et al. 2009). FKBP36 can exert an effect on GAPDH in an Hsp90 independent manner by either directly inhibiting NAD^+ binding to GAPDH or by decreasing GAPDH expression (Jarczowski et al. 2009). Patients with Williams syndrome, which is characterized by congenital cardiovascular defects, dysmorphic facial features, mental retardation, growth defects, azoospermia, and hypercalcemia, are typically haploinsufficient for *FKBP6* (Meng et al. 1998); however, the contribution of

FKBP6 deletion in this syndrome is not clear since several contiguous genes on chromosome 11, including genes for elastin and LIM-Kinase 1, are also deleted in these patients and clearly contribute to some phenotypic aspects.

FKBP38

FKBP38 (gene name *FKBP8*) contains a glutamate-rich domain, FK domain, three TPR domains, and a calmodulin-binding motif. FKBP38 is ubiquitously expressed in all tissues, with high expression in neuronal tissues. Among the FKBP family, FKBP38 is novel in several respects, including a unique C-terminal transmembrane anchor domain, used to localize FKBP38 to both the mitochondrial and ER membranes. Although FKBP38 contains a PPIase domain, PPIase activity is regulated. The structure of the PPIase domain is similar to the prototypical family member, FKBP12; however, there are important differences in the three-dimensional structure of the loop and the binding pocket of the active site (Maestre-Martinez et al. 2006; Kay 1996). The loss of several aromatic residues in the active site leads to lower PPIase activity, even upon activation, and low affinity for FK506 (Maestre-Martinez et al. 2006; Edlich et al. 2006). FKBP38 PPIase activation is dependent on the calmodulin-binding domain and calmodulin/Ca^{2+} binding stimulates PPIase activity (Edlich et al. 2005; Edlich et al. 2007b; Maestre-Martinez et al. 2010).

FKBP38 participates in a number of cellular processes involving protein folding and trafficking, apoptosis, neural tube formation, CFTR trafficking, and viral replication (Edlich and Lucke 2011; Banasavadi-Siddegowda et al. 2011). FKBP38 interacts with the anti-apoptotic proteins Bcl-2 in regulating apoptosis and appears to have both pro- and anti-apoptotic activity that is likely tissue specific (Shirane and Nakayama 2004). In general, FKBP38 anti-apoptotic activity appears to regulate apoptosis by transporting Bcl-2 to the mitochondrial membrane stabilizing Bcl-2 and inhibiting apoptosis (Shirane and Nakayama 2004). Two mechanisms on how FKBP38 protects Bcl-2 from degradation have been explored. One involves the interaction between FKBP38 and a caspase cleavage site located within Bcl-2 (Choi et al. 2010). When FKBP38 is associated with Bcl-2 access to the caspase cleavage site may be blocked, preventing caspase-mediated Bcl-2 degradation (Choi et al. 2010). The second mechanism is through an interaction between the S4 subunit of the 19S proteasome complex, thereby regulating proteasome activity. However, in neuroblastoma cells the active FKBP38/calmodulin/Ca^{2+} complex has a pro-apoptotic affect by interfering with the ability of Bcl-2 to interact with and block pro-apoptotic proteins (Edlich et al. 2005). In this case, an interaction between Hsp90 and the FKB38/calmodulin/Ca^{2+} complex interferes with FKP38 pro-apoptotic activity, which could impede apoptosis (Edlich et al. 2007a).

FKBP38 is also implicated in the regulation of mTOR signaling through an interaction with Rheb (Rosner et al. 2003). mTOR regulates a wide range of cellular processes, including cell cycle and cell growth, in response to various conditions,

including fluctuations in nutrient and energy levels, and growth factors (Yang and Guan 2007). The FKBP12/rapamycin complex interacts with and inhibits mTOR activity (Brown et al. 1994). However, FKBP38 interacts with and antagonizes mTOR in a rapamycin-independent manner (Bai et al. 2007). Overexpression of FKBP38 decreases the induction of mTOR-regulated genes, and siRNA-induced reduction of FKBP38 increased mTOR activity (Bai et al. 2007). Rheb disrupts the mTOR/FKBP38 complex by binding to FKBP38 in a nutrient-dependent manner leading to an induction of mTOR-responsive genes (Bai et al. 2007).

FKBP38 is also involved in neural tube formation as the loss of FKBP38 leads to gross abnormalities during embryonic formation of the nervous system (Wong et al. 2008). It has been speculated that this is due to deregulation of the Sonic hedgehog (SHH) pathway during neural tube formation, where FKBP38 is a SHH antagonist, and the loss of FKBP38 function leads to over activity of SHH during development resulting in neuronal malformation (Cho et al. 2008).

In addition to the regulatory role in response to nutritional conditions, FKBP38 is also involved in the cellular response to hypoxia. Hypoxia-inducible transcription factors (HIFs) are involved in the cellular response to low oxygen levels, and, under normal conditions, are quickly degraded by prolyl-4-hydroxylase (PDH) enzymes (Wenger et al. 2005). FKBP38 interacts with PHD2 at the endoplasmic reticulum and mitochondrial membranes, and regulates PDH2 activity through proteasomal degradation, thereby regulating HIF stability and downstream gene expression in response to hypoxic conditions (Barth et al. 2009).

FKBP38 is involved in CFTR synthesis and folding by negatively regulating CFTR synthesis and positively regulating folding (Banasavadi-Siddegowda et al. 2011). Knockdown of FKBP38 increased CFTR production, but reduced post-translational modification, resulting in a lower expression of functional CFTR (Banasavadi-Siddegowda et al. 2011). Interestingly, FKBP38 PPIase activity is required for the regulation of CFTR folding.

Finally, FKBP38 is required for replication of the hepatitis C virus (HCV). In HCV infection the viral nonstructural protein 5A (NS5A) has been shown to form a complex with FKBP38 and Hsp90 at the mitochondrial and endoplasmic reticulum membranes (Wang et al. 2006). Either knockdown of FKBP38 with siRNA or inhibition of Hsp90 activity with geldanamycin results in decreased HCV RNA replication (Okada et al. 2004).

FKBPL

FKBPL shares the same general structure as other members of the FKBP family, including a TPR domain that facilitates Hsp90 binding and a PPIase domain, which lacks catalytic activity (Robson et al. 1999; Sunnotel et al. 2010). FKBPL was initially discovered while screening for genes that were protective against ionizing radiation (Robson et al. 1997; Robson et al. 1999). FKBPL is most closely related

to the larger FKBP52 (26% identity) (Robson and James 2012). However, the PPIase domain only shares 17% identity with the FKBP52 PPIase region (Robson and James 2012). The FKBPL TPR domain shares 33% amino acid identity with FKBP52 and has the ability to interact with Hsp90 stabilizing steroid hormone receptor conformations as well as stabilizing newly synthesized p21 preventing its degradation (Robson and James 2012; Jascur et al. 2005). There is conflicting data on FKBPL and its role in conferring radiation resistance. Jascur *et al.* originally showed that, in response to high-dose radiation, the FKBPL/Hsp90/p21 complex stabilized p21 leading to G2 cell cycle arrest, which conferred a pro-survival effect. However, more recent data has demonstrated that there is a down-regulation of p21 in response to radiation exposure and decreased p21 was involved in pro-survival after radiation exposer (Chu et al. 2004; Robson et al. 1999; Robson et al. 2000). In addition to radiation resistance, FKBPL plays a significant role in tumor progression (Robson et al. 1997; Robson et al. 1999; Robson et al. 2000; Jascur et al. 2005). In tumor cells, FKBPL appears to participate in not only growth of the tumor, but also in the sensitivity of the tumor to various chemotherapeutic agents (Bublik et al. 2010). For example, high levels of GSTE-1 interact with the FKBPL/Hsp90/p21 complex, which leads to p21 stabilization leading to resistance to the chemotherapeutic agent Taxane (Bublik et al. 2010). Although the exact radio- and chemo-protective role of FKBPL needs to be elucidated, the data clearly show that FKBPL is an important factor in cell-cycle progression, cell survival, and tumor progression.

Like other Hsp90-associated FKBP proteins, FKBPL also forms complexes with various steroid hormone receptors (reviewed in Erlejman et al. 2014). FKBPL and Hsp90 appear to stabilize AR, ER, and GR/Hsp90 complexes (Sunnotel et al. 2010; McKeen et al. 2008; McKeen et al. 2010). Similar to FKBP52, FKBPL affects the AR-dependent expression of prostate-specific antigen (Sunnotel et al. 2010). Sunnotel *et al.* demonstrated that two populations of azoospermic males had alterations in their FKBPL gene, which may alter FKBPL interaction with AR and contribute to infertility in the two populations. FKBPL was also shown to colocalize with the GR/Hsp90 complex (McKeen et al. 2008). Dexamethasone treatment resulted in the colocalization of FKBPL and GR in the nucleus and the up-regulation of GR-response genes in a prostate cancer cell line (McKeen et al. 2008). Translocation of the FKBPL/GR complex appears to be mediated by an interaction with dynamitin motor proteins, similar to the mechanism described for FKBP52 (McKeen et al. 2008).

FKBPL expression is regulated by estrogen and FKBPL functionally interacts with the ER/Hsp90 complex (McKeen et al. 2010). In addition, FKBPL expression correlates with breast cancer tumor growth as FKBPL and ER expression are inversely related; increased FKBPL levels lead to decreased ER expression (McKeen et al. 2010; Abukhdeir et al. 2008). Overexpression of FKBPL is associated with increased survival of untreated breast cancer patients and sensitizes cancer cells to the anti-proliferative effect of both tamoxifen and fulvestrant, which promotes increased recurrence-free survival (McKeen et al. 2011;

Han et al. 2006). Interestingly, overexpression of related FKBP proteins in tumors is associated with a poor treatment outcome and prognosis (Romano et al. 2010; Solassol et al. 2011). Conversely, increased levels of FKBPL correlate to a more positive response to treatment and a more favorable prognosis (McKeen et al. 2010; McKeen et al. 2011; Han et al. 2006). FKBPL stability is regulated by RBCK1, and as with FKBPL, RBCK1 is up-regulated by estrogen and can interact with the FKBPL/ER/Hsp90 complex (Donley et al. 2013). Increased expression of both FKBPL and RBCK1 appear to correlate with increased survival; however, elevated RBCK1 levels reduce the efficacy of tamoxifen (Donley et al. 2013). The interactions leading to tumor survival and progression still need to be explored further.

Finally, FKBPL possesses anti-angiogenic properties (Yakkundi et al. 2013). In a mouse xenograft tumor model overexpression of FKBPL resulted in decreased tumor growth and tumor necrosis (Crabb et al. 2009). The anti-angiogenic effects of FKBPL are mediated through the N-terminal portion of the protein comprised of amino acids 34–58, termed peptide AD-01, which is currently being explored as a novel anti-angiogenic drug (Valentine et al. 2011; Yakkundi et al. 2013).

Plant FKBPs

Hsp90-binding TPR immunophilins have been identified in all eukaryotes examined. A few examples of plant TPR-containing FKBPs are shown in Fig. 2.1. The TPR domain of each FKBP is very similar in amino acid sequence to that of vertebrate proteins; these are presumed to bind Hsp90, but that has not been determined in all cases. The plant and insect FKBPs contain one or more PPIase-related domain and can contain other functional domains. For example, AtFKBP42 contains a C-terminal transmembrane domain that localizes the protein to the inner plasma membrane and the vacuolar membrane (Kamphausen et al. 2002; Geisler et al. 2003; Geisler et al. 2004).

There is ample evidence to suggest that the plant and insect FKBPs are physiologically important. Mutations in AtFKBP42 cause the severe developmental phenotypes termed twisted dwarf 1 (TWD) (Geisler et al. 2003) and ultracurvata (UCU2) (Perez-Perez et al. 2004). The mechanism by which these phenotypes occur likely involves impairment of membrane transport of the growth hormone auxin, as AtFKBP42 is known to interact with several ATP-binding cassette transporters on the plasma and vacuolar membranes (Geisler et al. 2004; Geisler et al. 2003; Liu et al. 2001). Mutations in AtFKBP72 result in a class of mutants termed pasticcino or pas mutants, which are characterized by a wide variety of developmental defects (Vittorioso et al. 1998). Two Hsp90-binding TPR FKBPs in wheat, wFKBP72 and the heat shock-inducible wFKBP77, have been shown in transgenic plants to distinctively influence developmental patterns (Kurek et al. 2002).

Summary

In addressing the physiological importance of PPIases, Heitman and colleagues (Dolinski et al. 1997) generated an *S. cerevisiae* strain that lacked all 12 PPIase genes in the FKBP and cyclophilin families; the pluri-mutant strain displayed some growth abnormalities but was viable, thus demonstrating that these genes collectively are non-essential in yeast. Nonetheless, it has become increasingly clear that the Hsp90-binding FKBP immunophilins, through interactions with steroid receptors, kinases, and other cellular factors, play important physiological and pathological roles in mammals. Significant progress has been made on the elucidation of these roles and the definition of underlying molecular mechanisms. The identification of specific inhibitors will likely quicken in the coming few years and lead to therapeutic targeting of individual Hsp90-associated FKBP immunophilins for the treatment of a variety of human diseases.

Acknowledgements Studies in the authors' laboratory were supported by grants to the Border Biomedical Research Center from the National Center for Research Resources (5 G12 RR008124) and from the National Institute on Minority Health and Health Disparities (8 G12 MD007592) from the National Institutes of Health. The authors were also supported in part by the Cancer Prevention and Research Institute of Texas by grant number RP110444-P2 (M.B.C). M.D.G. was supported by grants PICT 2011-1715, UBACYT 2011-14-GC, and the Fundación Roemmers. The text in this chapter contains sections reproduced with kind permission from Springer Science + Business Media: Networking of Chaperones by Co-chaperones; Chapter 2: Functions of the Hsp90-Binding FKBP Immunophilins; 2006; page 13–21; Marc B. Cox and David F. Smith

References

Abukhdeir AM, Vitolo MI, Argani P, De Marzo AM, Karakas B, Konishi H, Gustin JP, Lauring J, Garay JP, Pendleton C, Konishi Y, Blair BG, Brenner K, Garrett-Mayer E, Carraway H, Bachman KE, Park BH (2008) Tamoxifen-stimulated growth of breast cancer due to p21 loss. Proc Natl Acad Sci U S A 105(1):288–293

Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y, Jiang Y (2007) Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. Science 318(5852):977–980

Banasavadi-Siddegowda YK, Mai J, Fan Y, Bhattacharya S, Giovannucci DR, Sanchez ER, Fischer G, Wang X (2011) FKBP38 peptidylprolyl isomerase promotes the folding of cystic fibrosis transmembrane conductance regulator in the endoplasmic reticulum. J Biol Chem 286(50):43071–43080

Barent RL, Nair SC, Carr DC, Ruan Y, Rimerman RA, Fulton J, Zhang Y, Smith DF (1998) Analysis of FKBP51/FKBP52 chimeras and mutants for Hsp90 binding and association with progesterone receptor complexes. Mol Endocrinol 12(3):342–354

Barnham KJ, Bush AI (2008) Metals in Alzheimer's and Parkinson's diseases. Curr Opin Chem Biol 12(2):222–228. doi:10.1016/j.cbpa.2008.02.019

Barth S, Edlich F, Berchner-Pfannschmidt U, Gneuss S, Jahreis G, Hasgall PA, Fandrey J, Wenger RH, Camenisch G (2009) Hypoxia-inducible factor prolyl-4-hydroxylase PHD2 protein abundance depends on integral membrane anchoring of FKBP38. J Biol Chem 284(34):23046–23058

Baughman G, Wiederrecht GJ, Campbell NF, Martin MM, Bourgeois S (1995) FKBP51, a novel T-cell-specific immunophilin capable of calcineurin inhibition. Mol Cell Biol 15(8):4395–4402

Benedict JC, Lin TM, Loeffler IK, Peterson RE, Flaws JA (2000) Physiological role of the aryl hydrocarbon receptor in mouse ovary development. Toxicol Sci 56(2):382–388

Berg P, Pongratz I (2002) Two parallel pathways mediate cytoplasmic localization of the dioxin (aryl hydrocarbon) receptor. J Biol Chem 277(35):32310–32319

Binder EB (2009) The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology 34(Suppl 1):S186–195. doi:10.1016/j.psyneuen.2009.05.021

Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Kunzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Kohnlein O, Dabitz H, Bruckl T, Muller N, Pfister H, Lieb R, Mueller JC, Lohmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet 36(12):1319–1325

Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 299(11):1291–1305

Blair LJ, Nordhues BA, Hill SE, Scaglione KM, O'Leary JC 3rd, Fontaine SN, Breydo L, Zhang B, Li P, Wang L, Cotman C, Paulson HL, Muschol M, Uversky VN, Klengel T, Binder EB, Kayed R, Golde TE, Berchtold N, Dickey CA (2013) Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. J Clin Invest 123(10):4158–4169. doi:10.1172/JCI69003

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21(11):932–939

Bose S, Weikl T, Bugl H, Buchner J (1996) Chaperone function of Hsp90-associated proteins. Science 274(5293):1715–1717

Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S, Hopf C, Huhse B, Mangano R, Michon AM, Schirle M, Schlegl J, Schwab M, Stein MA, Bauer A, Casari G, Drewes G, Gavin AC, Jackson DB, Joberty G, Neubauer G, Rick J, Kuster B, Superti-Furga G (2004) A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. Nat Cell Biol 6(2):97–105

Bracher A, Kozany C, Hahle A, Wild P, Zacharias M, Hausch F (2013) Crystal structures of the free and ligand-bound FK1-FK2 domain segment of FKBP52 reveal a flexible inter-domain hinge. J Mol Biol 425(22):4134–4144

Broemer M, Krappmann D, Scheidereit C (2004) Requirement of Hsp90 activity for IkappaB kinase (IKK) biosynthesis and for constitutive and inducible IKK and NF-kappaB activation. Oncogene 23(31):5378–5386

Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 369(6483):756–758

Bublik DR, Scolz M, Triolo G, Monte M, Schneider C (2010) Human GTSE-1 regulates p21(CIP1/WAF1) stability conferring resistance to paclitaxel treatment. J Biol Chem 285(8):5274–5281

Buzon V, Carbo LR, Estruch SB, Fletterick RJ, Estebanez-Perpina E (2012) A conserved surface on the ligand binding domain of nuclear receptors for allosteric control. Mol Cell Endocrinol 348(2):394–402

Cai W, Kramarova TV, Berg P, Korbonits M, Pongratz I (2011) The immunophilin-like protein XAP2 is a negative regulator of estrogen signaling through interaction with estrogen receptor alpha. PLoS One 6(10):e25201

Cao W, Konsolaki M (2011) FKBP immunophilins and Alzheimer's disease: a chaperoned affair. J Biosci 36(3):493–498

Carver LA, LaPres JJ, Jain S, Dunham EE, Bradfield CA (1998) Characterization of the Ah receptor-associated protein, ARA9. J Biol Chem 273(50):33580–33587

Chambraud B, Rouviere-Fourmy N, Radanyi C, Hsiao K, Peattie DA, Livingston DJ, Baulieu EE (1993) Overexpression of p59-HBI (FKBP59), full length and domains, and characterization of PPlase activity. Biochem Biophys Res Commun 196(1):160–166

Chambraud B, Radanyi C, Camonis JH, Shazand K, Rajkowski K, Baulieu EE (1996) FAP48, a new protein that forms specific complexes with both immunophilins FKBP59 and FKBP12. Prevention by the immunosuppressant drugs FK506 and rapamycin. J Biol Chem 271(51):32923–32929

Chambraud B, Belabes H, Fontaine-Lenoir V, Fellous A, Baulieu EE (2007) The immunophilin FKBP52 specifically binds to tubulin and prevents microtubule formation. FASEB J 21(11):2787–2797. doi:10.1096/fj.06-7667com

Chambraud B, Sardin E, Giustiniani J, Dounane O, Schumacher M, Goedert M, Baulieu EE (2010) A role for FKBP52 in Tau protein function. Proc Natl Acad Sci U S A 107(6):2658–2663

Cheung-Flynn J, Prapapanich V, Cox MB, Riggs DL, Suarez-Quian C, Smith DF (2005) Physiological role for the cochaperone FKBP52 in androgen receptor signaling. Mol Endocrinol 19(6):1654–1666

Cho A, Ko HW, Eggenschwiler JT (2008) FKBP8 cell-autonomously controls neural tube patterning through a Gli2- and Kif3a-dependent mechanism. Dev Biol 321(1):27–39

Choi BH, Feng L, Yoon HS (2010) FKBP38 protects Bcl-2 from caspase-dependent degradation. J Biol Chem 285(13):9770–9779

Chu K, Teele N, Dewey MW, Albright N, Dewey WC (2004) Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. Radiat Res 162(3):270–286

Cluning C, Ward BK, Rea SL, Arulpragasam A, Fuller PJ, Ratajczak T (2013) The helix 1–3 loop in the glucocorticoid receptor LBD is a regulatory element for FKBP cochaperones. Mol Endocrinol 27(7):1020–1035

Colo GP, Rubio MF, Nojek IM, Werbajh SE, Echeverria PC, Alvarado CV, Nahmod VE, Galigniana MD, Costas MA (2008) The p160 nuclear receptor co-activator RAC3 exerts an anti-apoptotic role through a cytoplasmatic action. Oncogene 27(17):2430–2444

Cox MB, Riggs DL, Hessling M, Schumacher F, Buchner J, Smith DF (2007) FK506-binding protein 52 phosphorylation: a potential mechanism for regulating steroid hormone receptor activity. Mol Endocrinol 21(12):2956–2967

Crabb SJ, Patsios D, Sauerbrei E, Ellis PM, Arnold A, Goss G, Leighl NB, Shepherd FA, Powers J, Seymour L, Laurie SA (2009) Tumor cavitation: impact on objective response evaluation in trials of angiogenesis inhibitors in non-small-cell lung cancer. J Clin Oncol 27(3):404–410

Crackower MA, Kolas NK, Noguchi J, Sarao R, Kikuchi K, Kaneko H, Kobayashi E, Kawai Y, Kozieradzki I, Landers R, Mo R, Hui CC, Nieves E, Cohen PE, Osborne LR, Wada T, Kunieda T, Moens PB, Penninger JM (2003) Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis. Science 300(5623):1291–1295

Dahmer MK, Housley PR, Pratt WB (1984) Effects of molybdate and endogenous inhibitors on steroid-receptor inactivation, transformation, and translocation. Annu Rev Physiol 46:67–81.

Daikoku T, Tranguch S, Friedman DB, Das SK, Smith DF, Dey SK (2005) Proteomic analysis identifies immunophilin FK506 binding protein 4 (FKBP52) as a downstream target of Hoxa10 in the periimplantation mouse uterus. Mol Endocrinol 19(3):683–697

Davies TH, Ning YM, Sanchez ER (2002) A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem 277(7):4597–4600

Davies TH, Ning YM, Sanchez ER (2005) Differential control of glucocorticoid receptor hormone-binding function by tetratricopeptide repeat (TPR) proteins and the immunosuppressive ligand FK506. Biochemistry 44(6):2030–2038

De Leon JT, Iwai A, Feau C, Garcia Y, Balsiger HA, Storer CL, Suro RM, Garza KM, Lee S, Kim YS, Chen Y, Ning YM, Riggs DL, Fletterick RJ, Guy RK, Trepel JB, Neckers LM, Cox MB (2011) Targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. Proc Natl Acad Sci U S A 108(29):11878–11883. doi:10.1073/pnas.1105160108

Denny WB, Valentine DL, Reynolds PD, Smith DF, Scammell JG (2000) Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding. Endocrinology 141(11):4107–4113

Denny WB, Prapapanich V, Smith DF, Scammell JG (2005) Structure-function analysis of squirrel monkey FK506-binding protein 51, a potent inhibitor of glucocorticoid receptor activity. Endocrinology 146(7):3194–3201

Dittmar KD, Hutchison KA, Owens-Grillo JK, Pratt WB (1996) Reconstitution of the steroid receptor hsp90 heterocomplex assembly system of rabbit reticulocyte lysate. J Biol Chem 271(22):12833–12839

Dolinski K, Muir S, Cardenas M, Heitman J (1997) All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 94(24):13093–13098

Donley C, McClelland K, McKeen HD, Nelson L, Yakkundi A, Jithesh PV, Burrows J, McClements L, Valentine A, Prise KM, McCarthy HO, Robson T (2013) Identification of RBCK1 as a novel regulator of FKBPL: implications for tumor growth and response to tamoxifen. Oncogene 5(10):306

Drago D, Bolognin S, Zatta P (2008) Role of metal ions in the abeta oligomerization in Alzheimer's disease and in other neurological disorders. Curr Alzheimer Res 5(6):500–507

Echeverria PC, Mazaira G, Erlejman A, Gomez-Sanchez C, Piwien Pilipuk G, Galigniana MD (2009) Nuclear import of the glucocorticoid receptor-hsp90 complex through the nuclear pore complex is mediated by its interaction with Nup62 and importin beta. Mol Cell Biol 29(17):4788–4797

Edlich F, Lucke C (2011) From cell death to viral replication: the diverse functions of the membrane-associated FKBP38. Curr Opin Pharmacol 11(4):348–353

Edlich F, Weiwad M, Erdmann F, Fanghanel J, Jarczowski F, Rahfeld JU, Fischer G (2005) Bcl-2 regulator FKBP38 is activated by Ca2+/calmodulin. EMBO J 24(14):2688–2699

Edlich F, Weiwad M, Wildemann D, Jarczowski F, Kilka S, Moutty MC, Jahreis G, Lucke C, Schmidt W, Striggow F, Fischer G (2006) The specific FKBP38 inhibitor N-(N′,N′-dimethylcarboxamidomethyl)cycloheximide has potent neuroprotective and neurotrophic properties in brain ischemia. J Biol Chem 281(21):14961–14970

Edlich F, Erdmann F, Jarczowski F, Moutty MC, Weiwad M, Fischer G (2007a) The Bcl-2 regulator FKBP38-calmodulin-Ca2+ is inhibited by Hsp90. J Biol Chem 282(21):15341–15348

Edlich F, Maestre-Martinez M, Jarczowski F, Weiwad M, Moutty MC, Malesevic M, Jahreis G, Fischer G, Lucke C (2007b) A novel calmodulin-Ca2+ target recognition activates the Bcl-2 regulator FKBP38. J Biol Chem 282(50):36496–36504

Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL, DeFranco DB (2004) Molecular chaperones function as steroid receptor nuclear mobility factors. Proc Natl Acad Sci U S A 101(9):2876–2881

Erlejman AG, Lagadari M, Toneatto J, Piwien-Pilipuk G, Galigniana MD (2014) Regulatory role of the 90-kDa-heat-shock protein (Hsp90) and associated factors on gene expression. Biochim Biophys Acta 1839(2):71–87

Estebanez-Perpina E, Arnold LA, Nguyen P, Rodrigues ED, Mar E, Bateman R, Pallai P, Shokat KM, Baxter JD, Guy RK, Webb P, Fletterick RJ (2007) A surface on the androgen receptor that allosterically regulates coactivator binding. Proc Natl Acad Sci U S A 104(41):16074–16079

Febbo PG, Lowenberg M, Thorner AR, Brown M, Loda M, Golub TR (2005) Androgen mediated regulation and functional implications of fkbp51 expression in prostate cancer. J Urol 173(5):1772–1777

Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science 268(5211):722–726

Fernandez-Salguero PM, Hilbert DM, Rudikoff S, Ward JM, Gonzalez FJ (1996) Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. Toxicol Appl Pharmacol 140(1):173–179

Fusco D, Vargiolu M, Vidone M, Mariani E, Pennisi LF, Bonora E, Capellari S, Dirnberger D, Baumeister R, Martinelli P, Romeo G (2010) The RET51/FKBP52 complex and its involvement in Parkinson disease. Hum Mol Genet 19(14):2804–2816. doi:10.1093/hmg/ddq181

Galigniana MD, Radanyi C, Renoir JM, Housley PR, Pratt WB (2001) Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus. J Biol Chem 276(18):14884–14889

Galigniana MD, Harrell JM, Murphy PJ, Chinkers M, Radanyi C, Renoir JM, Zhang M, Pratt WB (2002) Binding of hsp90-associated immunophilins to cytoplasmic dynein: direct binding and *in vivo* evidence that the peptidylprolyl isomerase domain is a dynein interaction domain. Biochemistry 41(46):13602–13610

Galigniana MD, Harrell JM, Housley PR, Patterson C, Fisher SK, Pratt WB (2004a) Retrograde transport of the glucocorticoid receptor in neurites requires dynamic assembly of complexes with the protein chaperone hsp90 and is linked to the CHIP component of the machinery for proteasomal degradation. Brain Res Mol Brain Res 123(1–2):27–36

Galigniana MD, Harrell JM, O'Hagen HM, Ljungman M, Pratt WB (2004b) Hsp90-binding immunophilins link p53 to dynein during p53 transport to the nucleus. J Biol Chem 279(21):22483–22489

Galigniana MD, Echeverria PC, Erlejman AG, Piwien-Pilipuk G (2010a) Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore. Nucleus 1(4):299–308

Galigniana MD, Erlejman AG, Monte M, Gomez-Sanchez C, Piwien-Pilipuk G (2010b) The hsp90-FKBP52 complex links the mineralocorticoid receptor to motor proteins and persists bound to the receptor in early nuclear events. Mol Cell Biol 30(5):1285–1298

Gallo LI, Ghini AA, Pilipuk GP, Galigniana MD (2007) Differential recruitment of tetratricorpeptide repeat domain immunophilins to the mineralocorticoid receptor influences both heat-shock protein 90-dependent retrotransport and hormone-dependent transcriptional activity. Biochemistry 46(49):14044–14057

Geisler M, Kolukisaoglu HU, Bouchard R, Billion K, Berger J, Saal B, Frangne N, Koncz-Kalman Z, Koncz C, Dudler R, Blakeslee JJ, Murphy AS, Martinoia E, Schulz B (2003) TWISTED DWARF1, a unique plasma membrane-anchored immunophilin-like protein, interacts with Arabidopsis multidrug resistance-like transporters AtPGP1 and AtPGP19. Mol Biol Cell 14(10):4238–4249

Geisler M, Girin M, Brandt S, Vincenzetti V, Plaza S, Paris N, Kobae Y, Maeshima M, Billion K, Kolukisaoglu UH, Schulz B, Martinoia E (2004) Arabidopsis immunophilin-like TWD1 functionally interacts with vacuolar ABC transporters. Mol Biol Cell 15(7):3393–3405

Giraudier S, Chagraoui H, Komura E, Barnache S, Blanchet B, LeCouedic JP, Smith DF, Larbret F, Taksin AL, Moreau-Gachelin F, Casadevall N, Tulliez M, Hulin A, Debili N, Vainchenker W (2002) Overexpression of FKBP51 in idiopathic myelofibrosis regulates the growth factor independence of megakaryocyte progenitors. Blood 100(8):2932–2940

Giustiniani J, Chambraud B, Sardin E, Dounane O, Guillemeau K, Nakatani H, Paquet D, Kamah A, Landrieu I, Lippens G, Baulieu EE, Tawk M (2014) Immunophilin FKBP52 induces Tau-P301L filamentous assembly *in vitro* and modulates its activity in a model of tauopathy. Proc Natl Acad Sci U S A 111(12):4584–4589

Goel M, Garcia R, Estacion M, Schilling WP (2001) Regulation of drosophila trpl channels by immunophilin fkbp59. J Biol Chem 276(42):38762–38773

Grossmann C, Ruhs S, Langenbruch L, Mildenberger S, Stratz N, Schumann K, Gekle M (2012) Nuclear shuttling precedes dimerization in mineralocorticoid receptor signaling. Chem Biol 19(6):742–751

Haelens A, Tanner T, Denayer S, Callewaert L, Claessens F (2007) The hinge region regulates DNA binding, nuclear translocation, and transactivation of the androgen receptor. Cancer Res 67(9):4514–4523. doi:10.1158/0008-5472.CAN-06-1701

Hamilton GS, Steiner JP (1998) Immunophilins: beyond immunosuppression. J Med Chem 41(26):5119–5143

Han W, Han MR, Kang JJ, Bae JY, Lee JH, Bae YJ, Lee JE, Shin HJ, Hwang KT, Hwang SE, Kim SW, Noh DY (2006) Genomic alterations identified by array comparative genomic hybridization as prognostic markers in tamoxifen-treated estrogen receptor-positive breast cancer. BMC Cancer 6(92):92

Hartmann J, Wagner KV, Liebl C, Scharf SH, Wang XD, Wolf M, Hausch F, Rein T, Schmidt U, Touma C, Cheung-Flynn J, Cox MB, Smith DF, Holsboer F, Muller MB, Schmidt MV (2012) The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress. Neuropharmacology 62(1):332–339. doi:10.1016/j.neuropharm.2011.07.041

He Z, Li L, Luan S (2004) Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in Arabidopsis. Plant Physiol 134(4):1248–1267

Hernandez F, Avila J (2007) Tauopathies. Cell Mol Life Sci 64(17):2219–2233. doi:10.1007/s00018-007-7220-x

Hirota Y, Tranguch S, Daikoku T, Hasegawa A, Osuga Y, Taketani Y, Dey SK (2008) Deficiency of immunophilin FKBP52 promotes endometriosis. Am J Pathol 173(6):1747–1757. doi:10.2353/ajpath.2008.080527

Hollingshead BD, Petrulis JR, Perdew GH (2004) The aryl hydrocarbon (Ah) receptor transcriptional regulator hepatitis B virus X-associated protein 2 antagonizes p23 binding to Ah receptor-Hsp90 complexes and is dispensable for receptor function. J Biol Chem 279(44):45652–45661

Hubler TR, Denny WB, Valentine DL, Cheung-Flynn J, Smith DF, Scammell JG (2003) The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progestin and attenuates progestin responsiveness. Endocrinology 144(6):2380–2387

Jarczowski F, Jahreis G, Erdmann F, Schierhorn A, Fischer G, Edlich F (2009) FKBP36 is an inherent multifunctional glyceraldehyde-3-phosphate dehydrogenase inhibitor. J Biol Chem 284(2):766–773

Jascur T, Brickner H, Salles-Passador I, Barbier V, El Khissiin A, Smith B, Fotedar R, Fotedar A (2005) Regulation of p21(WAF1/CIP1) stability by WISp39, a Hsp90 binding TPR protein. Mol Cell 17(2):237–249

Jinwal UK, Koren J III, Borysov SI, Schmid AB, Abisambra JF, Blair LJ, Johnson AG, Jones JR, Shults CL, O'Leary JC III, Jin Y, Buchner J, Cox MB, Dickey CA (2010) The Hsp90 cochaperone, FKBP51, increases Tau stability and polymerizes microtubules. J Neurosci 30(2):591–599

Kamphausen T, Fanghanel J, Neumann D, Schulz B, Rahfeld JU (2002) Characterization of Arabidopsis thaliana AtFKBP42 that is membrane-bound and interacts with Hsp90. Plant J 32(3):263–276

Kay JE (1996) Structure-function relationships in the FK506-binding protein (FKBP) family of peptidylprolyl cis-trans isomerases. Biochem J 314:361–385

Kazlauskas A, Poellinger L, Pongratz I (2000) The immunophilin-like protein XAP2 regulates ubiquitination and subcellular localization of the dioxin receptor. J Biol Chem 275(52):41317–41324

Kazlauskas A, Sundstrom S, Poellinger L, Pongratz I (2001) The hsp90 chaperone complex regulates intracellular localization of the dioxin receptor. Mol Cell Biol 21(7):2594–2607

Kazlauskas A, Poellinger L, Pongratz I (2002) Two distinct regions of the immunophilin-like protein XAP2 regulate dioxin receptor function and interaction with hsp90. J Biol Chem 277(14):11795–11801

Kester HA, van der Leede BM, van der Saag PT, van der Burg B (1997) Novel progesterone target genes identified by an improved differential display technique suggest that progestin-induced growth inhibition of breast cancer cells coincides with enhancement of differentiation. J Biol Chem 272(26):16637–16643

Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci 16(1):33–41

Komura E, Tonetti C, Penard-Lacronique V, Chagraoui H, Lacout C, Lecouedic JP, Rameau P, Debili N, Vainchenker W, Giraudier S (2005) Role for the nuclear factor kappaB pathway in

transforming growth factor-beta1 production in idiopathic myelofibrosis: possible relationship with FK506 binding protein 51 overexpression. Cancer Res 65(8):3281–3289
Kong GK, Adams JJ, Cappai R, Parker MW (2007) Structure of Alzheimer's disease amyloid precursor protein copper-binding domain at atomic resolution. Acta Crystallogr Sect F Struct Biol Cryst Commun 63(Pt 10):819–824. doi:10.1107/S1744309107041139
Koren J 3rd, Jinwal UK, Davey Z, Kiray J, Arulselvam K, Dickey CA (2011) Bending tau into shape: the emerging role of peptidyl-prolyl isomerases in tauopathies. Mol Neurobiol 44(1):65–70. doi:10.1007/s12035-011-8182-4
Kosano H, Stensgard B, Charlesworth MC, McMahon N, Toft D (1998) The assembly of progesterone receptor-hsp90 complexes using purified proteins. J Biol Chem 273(49):32973–32979
Krummrei U, Baulieu EE, Chambraud B (2003) The FKBP-associated protein FAP48 is an antiproliferative molecule and a player in T cell activation that increases IL2 synthesis. Proc Natl Acad Sci U S A 100(5):2444–2449
Kumar P, Mark PJ, Ward BK, Minchin RF, Ratajczak T (2001) Estradiol-regulated expression of the immunophilins cyclophilin 40 and FKBP52 in MCF-7 breast cancer cells. Biochem Biophys Res Commun 284(1):219–225
Kurek I, Stoger E, Dulberger R, Christou P, Breiman A (2002) Overexpression of the wheat FK506-binding protein 73 (FKBP73) and the heat-induced wheat FKBP77 in transgenic wheat reveals different functions of the two isoforms. Transgenic Res 11(4):373–379
Kuzhandaivelu N, Cong YS, Inouye C, Yang WM, Seto E (1996) XAP2, a novel hepatitis B virus X-associated protein that inhibits X transactivation. Nucleic Acids Res 24(23):4741–4750
Laenger A, Lang-Rollin I, Kozany C, Zschocke J, Zimmermann N, Ruegg J, Holsboer F, Hausch F, Rein T (2009) XAP2 inhibits glucocorticoid receptor activity in mammalian cells. FEBS Lett 583(9):1493–1498
Lahvis GP, Pyzalski RW, Glover E, Pitot HC, McElwee MK, Bradfield CA (2005) The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. Mol Pharmacol 67(3):714–720
LaPres JJ, Glover E, Dunham EE, Bunger MK, Bradfield CA (2000) ARA9 modifies agonist signaling through an increase in cytosolic aryl hydrocarbon receptor. J Biol Chem 275(9):6153–6159
Lees MJ, Peet DJ, Whitelaw ML (2003) Defining the role for XAP2 in stabilization of the dioxin receptor. J Biol Chem 278(38):35878–35888
Lin TM, Ko K, Moore RW, Simanainen U, Oberley TD, Peterson RE (2002) Effects of aryl hydrocarbon receptor null mutation and in utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on prostate and seminal vesicle development in C57BL/6 mice. Toxicol Sci 68(2):479–487
Liu G, Sanchez-Fernandez R, Li ZS, Rea PA (2001) Enhanced multispecificity of arabidopsis vacuolar multidrug resistance-associated protein-type ATP-binding cassette transporter, AtMRP2. J Biol Chem 276(12):8648–8656
Ma Q, Whitlock JP Jr (1997) A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Biol Chem 272(14):8878–8884
Madan AP, DeFranco DB (1993) Bidirectional transport of glucocorticoid receptors across the nuclear envelope. Proc Natl Acad Sci U S A 90(8):3588–3592
Maestre-Martinez M, Edlich F, Jarczowski F, Weiwad M, Fischer G, Lucke C (2006) Solution structure of the FK506-binding domain of human FKBP38. J Biomol NMR 34(3):197–202
Maestre-Martinez M, Haupt K, Edlich F, Jahreis G, Jarczowski F, Erdmann F, Fischer G, Lucke C (2010) New structural aspects of FKBP38 activation. Biol Chem 391(10):1157–1167
Mamane Y, Sharma S, Petropoulos L, Lin R, Hiscott J (2000) Posttranslational regulation of IRF-4 activity by the immunophilin FKBP52. Immunity 12(2):129–140
Maruyama T, Suzuki R, Furutani M (2004) Archaeal peptidyl prolyl cis-trans isomerases (PPIases) update 2004. Front Biosci 9:1680–1720
McKeen HD, McAlpine K, Valentine A, Quinn DJ, McClelland K, Byrne C, O'Rourke M, Young S, Scott CJ, McCarthy HO, Hirst DG, Robson T (2008) A novel FK506-like binding protein

interacts with the glucocorticoid receptor and regulates steroid receptor signaling. Endocrinology 149(11):5724–5734
McKeen HD, Byrne C, Jithesh PV, Donley C, Valentine A, Yakkundi A, O'Rourke M, Swanton C, McCarthy HO, Hirst DG, Robson T (2010) FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. Cancer Res 70(3):1090–1100
McKeen HD, Brennan DJ, Hegarty S, Lanigan F, Jirstrom K, Byrne C, Yakkundi A, McCarthy HO, Gallagher WM, Robson T (2011) The emerging role of FK506-binding proteins as cancer biomarkers: a focus on FKBPL. Biochem Soc Trans 39(2):663–668
Meng X, Lu X, Morris CA, Keating MT (1998) A novel human gene FKBP6 is deleted in Williams syndrome. Genomics 52(2):130–137
Meyer BK, Perdew GH (1999) Characterization of the AhR-hsp90-XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization. Biochemistry 38(28):8907–8917
Meyer BK, Pray-Grant MG, Vanden Heuvel JP, Perdew GH (1998) Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity. Mol Cell Biol 18(2):978–988
Meyer BK, Petrulis JR, Perdew GH (2000) Aryl hydrocarbon (Ah) receptor levels are selectively modulated by hsp90-associated immunophilin homolog XAP2. Cell Stress Chaperones 5(3):243–254
Miller CA (2002) Two tetratricopeptide repeat proteins facilitate human aryl hydrocarbon receptor signalling in yeast. Cell Signal 14(7):615–623
Mimura J, Fujii-Kuriyama Y (2003) Functional role of AhR in the expression of toxic effects by TCDD. Biochim Biophys Acta 1619(3):263–268
Miyata Y, Chambraud B, Radanyi C, Leclerc J, Lebeau MC, Renoir JM, Shirai R, Catelli MG, Yahara I, Baulieu EE (1997) Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52. Proc Natl Acad Sci U S A 94(26):14500–14505
Mustafi SM, LeMaster DM, Hernandez G (2014) Differential conformational dynamics in the closely homologous FK506-binding domains of FKBP51 and FKBP52. Biochem J 461(1):115–123
Nair SC, Rimerman RA, Toran EJ, Chen S, Prapapanich V, Butts RN, Smith DF (1997) Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. Mol Cell Biol 17(2):594–603
Ni L, Yang CS, Gioeli D, Frierson H, Toft DO, Paschal BM (2010) FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells. Mol Cell Biol 30(5):1243–1253
Nukaya M, Lin BC, Glover E, Moran SM, Kennedy GD, Bradfield CA (2010) The aryl hydrocarbon receptor-interacting protein (AIP) is required for dioxin-induced hepatotoxicity but not for the induction of the Cyp1a1 and Cyp1a2 genes. J Biol Chem 285(46):35599–35605
Okada M, Hatakeyama T, Itoh H, Tokuta N, Tokumitsu H, Kobayashi R (2004) S100A1 is a novel molecular chaperone and a member of the Hsp70/Hsp90 multichaperone complex. J Biol Chem 279(6):4221–4233. doi:10.1074/jbc.M309014200
O'Leary JC 3rd, Dharia S, Blair LJ, Brady S, Johnson AG, Peters M, Cheung-Flynn J, Cox MB, de Erausquin G, Weeber EJ, Jinwal UK, Dickey CA (2011) A new anti-depressive strategy for the elderly: ablation of FKBP5/FKBP51. PLoS One 6(9):e24840. doi:10.1371/journal.pone.0024840
Ostrow KL, Park HL, Hoque MO, Kim MS, Liu J, Argani P, Westra W, Van Criekinge W, Sidransky D (2009) Pharmacologic unmasking of epigenetically silenced genes in breast cancer. Clin Cancer Res 15(4):1184–1191
Pei H, Li L, Fridley BL, Jenkins GD, Kalari KR, Lingle W, Petersen G, Lou Z, Wang L (2009) FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. Cancer Cell 16(3):259–266

Perez-Perez JM, Ponce MR, Micol JL (2004) The ULTRACURVATA2 gene of Arabidopsis encodes an FK506-binding protein involved in auxin and brassinosteroid signaling. Plant Physiol 134(1):101–117

Petrulis JR, Perdew GH (2002) The role of chaperone proteins in the aryl hydrocarbon receptor core complex. Chem Biol Interact 141(1–2):25–40

Petrulis JR, Hord NG, Perdew GH (2000) Subcellular localization of the aryl hydrocarbon receptor is modulated by the immunophilin homolog hepatitis B virus X-associated protein 2. J Biol Chem 275(48):37448–37453

Petrulis JR, Kusnadi A, Ramadoss P, Hollingshead B, Perdew GH (2003) The hsp90 Co-chaperone XAP2 alters importin beta recognition of the bipartite nuclear localization signal of the Ah receptor and represses transcriptional activity. J Biol Chem 278(4):2677–2685

Pirkl F, Buchner J (2001) Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. J Mol Biol 308(4):795–806

Pirkl F, Fischer E, Modrow S, Buchner J (2001) Localization of the chaperone domain of FKBP52. J Biol Chem 276(40):37034–37041

Presman DM, Alvarez LD, Levi V, Eduardo S, Digman MA, Marti MA, Veleiro AS, Burton G, Pecci A (2010) Insights on glucocorticoid receptor activity modulation through the binding of rigid steroids. PLoS One 5(10):e13279

Presman DM, Ogara MF, Stortz M, Alvarez LD, Pooley JR, Schiltz RL, Grontved L, Johnson TA, Mittelstadt PR, Ashwell JD, Ganesan S, Burton G, Levi V, Hager GL, Pecci A (2014) Live cell imaging unveils multiple domain requirements for *in vivo* dimerization of the glucocorticoid receptor. PLoS Biol 12(3):e1001813

Qing K, Hansen J, Weigel-Kelley KA, Tan M, Zhou S, Srivastava A (2001) Adeno-associated virus type 2-mediated gene transfer: role of cellular FKBP52 protein in transgene expression. J Virol 75(19):8968–8976

Quintá HR, Maschi D, Gomez-Sanchez C, Piwien Pilipuk G, Galigniana MD (2010) Subcellular rearrangement of hsp90-binding immunophilins accompanies neuronal differentiation and neurite outgrowth. J Neurochem 115:716–734

Reynolds PD, Ruan Y, Smith DF, Scammell JG (1999) Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. J Clin Endocrinol Metab 84(2):663–669

Riggs DL, Roberts PJ, Chirillo SC, Cheung-Flynn J, Prapapanich V, Ratajczak T, Gaber R, Picard D, Smith DF (2003) The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling *in vivo*. EMBO J 22(5):1158–1167

Riggs DL, Cox MB, Cheung-Flynn J, Prapapanich V, Carrigan PE, Smith DF (2004) Functional specificity of co-chaperone interactions with Hsp90 client proteins. Crit Rev Biochem Mol Biol 39:279–295

Riggs DL, Cox MB, Tardif HL, Hessling M, Buchner J, Smith DF (2007) Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling. Mol Cell Biol 27(24):8658–8669

Robson T, James IF (2012) The therapeutic and diagnostic potential of FKBPL; a novel anticancer protein. Drug Discov Today 17(11–12):544–548

Robson TA, Lohrer H, Bailie JR, Hirst DG, Joiner MC, Arrand JE (1997) Gene regulation by low-dose ionizing radiation in a normal human lung epithelial cell line. Biochem Soc Trans 25(1):335–342

Robson T, Joiner MC, Wilson GD, McCullough W, Price ME, Logan I, Jones H, McKeown SR, Hirst DG (1999) A novel human stress response-related gene with a potential role in induced radioresistance. Radiat Res 152(5):451–461

Robson T, Price ME, Moore ML, Joiner MC, McKelvey-Martin VJ, McKeown SR, Hirst DG (2000) Increased repair and cell survival in cells treated with DIR1 antisense oligonucleotides: implications for induced radioresistance. Int J Radiat Biol 76(5):617–623

Romano P, Gray J, Horton P, Luan S (2005) Plant immunophilins: functional versatility beyond protein maturation. New Phytol 166(3):753–769

Romano S, D'Angelillo A, Pacelli R, Staibano S, De LE, Bisogni R, Eskelinen EL, Mascolo M, Cali G, Arra C, Romano MF (2010) Role of FK506-binding protein 51 in the control of apoptosis of irradiated melanoma cells. Cell Death Differ 17(1):145–157

Rosner M, Hofer K, Kubista M, Hengstschlager M (2003) Cell size regulation by the human TSC tumor suppressor proteins depends on PI3K and FKBP38. Oncogene 22(31):4786–4798

Rouviere N, Vincent M, Craescu CT, Gallay J (1997) Immunosuppressor binding to the immunophilin FKBP59 affects the local structural dynamics of a surface beta-strand: time-resolved fluorescence study. Biochemistry 36(24):7339–7352

Sanchez ER (1990) Hsp56: a novel heat shock protein associated with untransformed steroid receptor complexes. J Biol Chem 265(36):22067–22070

Sanokawa-Akakura R, Dai H, Akakura S, Weinstein D, Fajardo JE, Lang SE, Wadsworth S, Siekierka J, Birge RB (2004) A novel role for the immunophilin FKBP52 in copper transport. J Biol Chem 279(27):27845–27848

Sanokawa-Akakura R, Cao W, Allan K, Patel K, Ganesh A, Heiman G, Burke R, Kemp FW, Bogden JD, Camakaris J, Birge RB, Konsolaki M (2010) Control of Alzheimer's amyloid beta toxicity by the high molecular weight immunophilin FKBP52 and copper homeostasis in Drosophila. PLoS One 5(1):e8626. doi:10.1371/journal.pone.0008626

Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS (2006) Calcium-dependent and -independent interactions of the S100 protein family. Biochem J 396(2):201–214. doi:10.1042/BJ20060195

Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. Cell 101(2):199–210

Schulke JP, Wochnik GM, Lang-Rollin I, Gassen NC, Knapp RT, Berning B, Yassouridis A, Rein T (2010) Differential impact of tetratricopeptide repeat proteins on the steroid hormone receptors. PLoS One 5(7):e11717. doi:10.1371/journal.pone.0011717

Shimamoto S, Kubota Y, Tokumitsu H, Kobayashi R (2010) S100 proteins regulate the interaction of Hsp90 with Cyclophilin 40 and FKBP52 through their tetratricopeptide repeats. FEBS Lett 584(6):1119–1125

Shirane M, Nakayama KI (2004) Immunophilin FKBP38, an inherent inhibitor of calcineurin, targets Bcl-2 to mitochondria and inhibits apoptosis. Nippon Rinsho 62(2):405–412

Silverstein AM, Galigniana MD, Chen MS, Owens-Grillo JK, Chinkers M, Pratt WB (1997) Protein phosphatase 5 is a major component of glucocorticoid receptor hsp90 complexes with properties of an FK506-binding immunophilin. J Biol Chem 272(26):16224–16230

Silverstein AM, Galigniana MD, Kanelakis KC, Radanyi C, Renoir JM, Pratt WB (1999) Different regions of the immunophilin FKBP52 determine its association with the glucocorticoid receptor, hsp90, and cytoplasmic dynein. J Biol Chem 274(52):36980–36986 [In Process Citation]

Sinars CR, Cheung-Flynn J, Rimerman RA, Scammell JG, Smith DF, Clardy J (2003) Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes. Proc Natl Acad Sci U S A 100(3):868–873

Sinkins WG, Goel M, Estacion M, Schilling WP (2004) Association of immunophilins with mammalian TRPC channels. J Biol Chem 279(33):34521–34529

Sivils JC, Storer CL, Galigniana MD, Cox MB (2011) Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52). Curr Opin Pharmacol 19:19

Smith DF, Faber LE, Toft DO (1990) Purification of unactivated progesterone receptor and identification of novel receptor-associated proteins. J Biol Chem 265(7):3996–4003

Smith DF, Albers MW, Schreiber SL, Leach KL, Deibel MR Jr (1993a) FKBP54, a novel FK506-binding protein in avian progesterone receptor complexes and HeLa extracts. J Biol Chem 268(32):24270–24273

Smith DF, Baggenstoss BA, Marion TN, Rimerman RA (1993b) Two FKBP-related proteins are associated with progesterone receptor complexes. J Biol Chem 268(24):18365–18371

Solassol J, Mange A, Maudelonde T (2011) FKBP family proteins as promising new biomarkers for cancer. Curr Opin Pharmacol 11(4):320–325

Sumanasekera WK, Tien ES, Turpey R, Vanden Heuvel JP, Perdew GH (2003) Evidence that peroxisome proliferator-activated receptor alpha is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2. J Biol Chem 278(7):4467–4473

Sunnotel O, Hiripi L, Lagan K, McDaid JR, De Leon JM, Miyagawa Y, Crowe H, Kaluskar S, Ward M, Scullion C, Campbell A, Downes CS, Hirst D, Barton D, Mocanu E, Tsujimura A, Cox MB, Robson T, Walsh CP (2010) Alterations in the steroid hormone receptor co-chaperone FKBPL are associated with male infertility: a case-control study. Reprod Biol Endocrinol 8(22):22

Tatro ET, Everall IP, Kaul M, Achim CL (2009) Modulation of glucocorticoid receptor nuclear translocation in neurons by immunophilins FKBP51 and FKBP52: implications for major depressive disorder. Brain Res 1286:1–12

Thackaberry EA, Bedrick EJ, Goens MB, Danielson L, Lund AK, Gabaldon D, Smith SM, Walker MK (2003) Insulin regulation in AhR-null mice: embryonic cardiac enlargement, neonatal macrosomia, and altered insulin regulation and response in pregnant and aging AhR-null females. Toxicol Sci 76(2):407–417

Thomas M, Harrell JM, Morishima Y, Peng HM, Pratt WB, Lieberman AP (2006) Pharmacologic and genetic inhibition of hsp90-dependent trafficking reduces aggregation and promotes degradation of the expanded glutamine androgen receptor without stress protein induction. Hum Mol Genet 15(11):1876–1883

Touma C, Gassen NC, Herrmann L, Cheung-Flynn J, Bull DR, Ionescu IA, Heinzmann JM, Knapman A, Siebertz A, Depping AM, Hartmann J, Hausch F, Schmidt MV, Holsboer F, Ising M, Cox MB, Schmidt U, Rein T (2011) FK506 binding protein 5 shapes stress responsiveness: modulation of neuroendocrine reactivity and coping behavior. Biol Psychiatry 70(10):928–936. doi:10.1016/j.biopsych.2011.07.023

Tranguch S, Cheung-Flynn J, Daikoku T, Prapapanich V, Cox MB, Xie H, Wang H, Das SK, Smith DF, Dey SK (2005) Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. Proc Natl Acad Sci U S A 102(40):14326–14331

Trivellin G, Korbonits M (2011) AIP and its interacting partners. J Endocrinol 210(2):137–155

Vafopoulou X, Steel CG (2012) Cytoplasmic travels of the ecdysteroid receptor in target cells: pathways for both genomic and non-genomic actions. Front Endocrinol (Lausanne) 3(43):43

Valentine A, O'Rourke M, Yakkundi A, Worthington J, Hookham M, Bicknell R, McCarthy HO, McClelland K, McCallum L, Dyer H, McKeen H, Waugh DJ, Roberts J, McGregor J, Cotton G, James I, Harrison T, Hirst DG, Robson T (2011) FKBPL and peptide derivatives: novel biological agents that inhibit angiogenesis by a CD44-dependent mechanism. Clin Cancer Res 17(5):1044–1056

Vermeer H, Hendriks-Stegeman BI, van der Burg B, van Buul-Offers SC, Jansen M (2003) Glucocorticoid-induced increase in lymphocytic FKBP51 messenger ribonucleic acid expression: a potential marker for glucocorticoid sensitivity, potency, and bioavailability. J Clin Endocrinol Metab 88(1):277–284

Vittorioso P, Cowling R, Faure JD, Caboche M, Bellini C (1998) Mutation in the Arabidopsis PASTICCINO1 gene, which encodes a new FK506- binding protein-like protein, has a dramatic effect on plant development. Mol Cell Biol 18(5):3034–3043

Wadekar SA, Li D, Sanchez ER (2004) Agonist-activated glucocorticoid receptor inhibits binding of heat shock factor 1 to the heat shock protein 70 promoter *in vivo*. Mol Endocrinol 18(3):500–508

Wang J, Tong W, Zhang X, Chen L, Yi Z, Pan T, Hu Y, Xiang L, Yuan Z (2006) Hepatitis C virus non-structural protein NS5A interacts with FKBP38 and inhibits apoptosis in Huh7 hepatoma cells. FEBS Lett 580(18):4392–4400

Ward BK, Mark PJ, Ingram DM, Minchin RF, Ratajczak T (1999) Expression of the estrogen receptor-associated immunophilins, cyclophilin 40 and FKBP52, in breast cancer. Breast Cancer Res Treat 58(3):267–280

Warrier M, Hinds TD Jr, Ledford KJ, Cash HA, Patel PR, Bowman TA, Stechschulte LA, Yong W, Shou W, Najjar SM, Sanchez ER (2010) Susceptibility to diet-induced hepatic steatosis and glucocorticoid resistance in FK506-binding protein 52-deficient mice. Endocrinology 151(7):3225–3236

Wenger RH, Stiehl DP, Camenisch G (2005) Integration of oxygen signaling at the consensus HRE. Sci STKE 2005(306):re12

Wochnik GM, Ruegg J, Abel GA, Schmidt U, Holsboer F, Rein T (2005) FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J Biol Chem 280(6):4609–4616

Wong RL, Wlodarczyk BJ, Min KS, Scott ML, Kartiko S, Yu W, Merriweather MY, Vogel P, Zambrowicz BP, Finnell RH (2008) Mouse Fkbp8 activity is required to inhibit cell death and establish dorso-ventral patterning in the posterior neural tube. Hum Mol Genet 17(4):587–601

Wright NT, Cannon BR, Zimmer DB, Weber DJ (2009) S100A1: structure, function, and therapeutic potential. Curr Chem Biol 3(2):138–145

Wu B, Li P, Liu Y, Lou Z, Ding Y, Shu C, Ye S, Bartlam M, Shen B, Rao Z (2004) 3D structure of human FK506-binding protein 52: implications for the assembly of the glucocorticoid receptor/Hsp90/immunophilin heterocomplex. Proc Natl Acad Sci U S A 101(22):8348–8353

Yakkundi A, McCallum L, O'Kane A, Dyer H, Worthington J, McKeen HD, McClements L, Elliott C, McCarthy HO, Hirst DG, Robson T (2013) The anti-migratory effects of FKBPL and its peptide derivative, AD-01: regulation of CD44 and the cytoskeletal pathway. PLoS One 8(2):e55075

Yang Q, Guan KL (2007) Expanding mTOR signaling. Cell Res 17(8):666–681

Yong W, Yang Z, Periyasamy S, Chen H, Yucel S, Li W, Lin LY, Wolf IM, Cohn MJ, Baskin LS, Sanchez ER, Shou W (2007) Essential role for co-chaperone Fkbp52 but not Fkbp51 in androgen receptor-mediated signaling and physiology. J Biol Chem 282(7):5026–5036

Yoshida NL, Miyashita T, U M, Yamada M, Reed JC, Sugita Y, Oshida T (2002) Analysis of gene expression patterns during glucocorticoid-induced apoptosis using oligonucleotide arrays. Biochem Biophys Res Commun 293(4):1254–1261

Zaffran S (2000) Molecular cloning and embryonic expression of dFKBP59, a novel Drosophila FK506-binding protein. Gene 246(1–2):103–109

Zhang X, Clark AF, Yorio T (2008) FK506-binding protein 51 regulates nuclear transport of the glucocorticoid receptor beta and glucocorticoid responsiveness. Invest Ophthalmol Vis Sci 49(3):1037–1047. doi:10.1167/iovs.07-1279

Zhao W, Zhong L, Wu J, Chen L, Qing K, Weigel-Kelley KA, Larsen SH, Shou W, Warrington KH Jr, Srivastava A (2006) Role of cellular FKBP52 protein in intracellular trafficking of recombinant adeno-associated virus 2 vectors. Virology 353(2):283–293

Zhong L, Qing K, Si Y, Chen L, Tan M, Srivastava A (2004) Heat-shock treatment-mediated increase in transduction by recombinant adeno-associated virus 2 vectors is independent of the cellular heat-shock protein 90. J Biol Chem 279(13):12714–12723

Zhu W, Zhang JS, Young CY (2001) Silymarin inhibits function of the androgen receptor by reducing nuclear localization of the receptor in the human prostate cancer cell line LNCaP. Carcinogenesis 22(9):1399–1403

Zimmer DB, Chaplin J, Baldwin A, Rast M (2005) S100-mediated signal transduction in the nervous system and neurological diseases. Cell Mol Biol (Noisy-le-grand) 51(2):201–214

Chapter 3
Hsp70/Hsp90 Organising Protein (Hop): Beyond Interactions with Chaperones and Prion Proteins

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Abstract The Hsp70/Hsp90 organising protein (Hop), also known as stress-inducible protein 1 (STI1), has received considerable attention for diverse cellular functions in both healthy and diseased states. There is extensive evidence that intracellular Hop is a co-chaperone of the major chaperones Hsp70 and Hsp90, playing an important role in the productive folding of Hsp90 client proteins. Consequently, Hop is implicated in a number of key signalling pathways, including aberrant pathways leading to cancer. However, Hop is also secreted and it is now well established that Hop also serves as a receptor for the prion protein, PrP^C. The intracellular and extracellular forms of Hop most likely represent two different isoforms, although the molecular determinants of these divergent functions are yet to be identified. There is also a growing body of research that reports the involvement of Hop in cellular activities that appear independent of either chaperones or PrP^C. While Hop has been shown to have various cellular functions, its biological function remains elusive. However, recent knockout studies in mammals suggest that Hop has an important role in embryonic development. This review provides a critical overview

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_3

of the latest molecular, cellular and biological research on Hop, critically evaluating its function in healthy systems and how this function is adapted in diseases states.

Keywords Hop · STIP1 · STI1 · Tetratricopeptide repeat

Assisted-Protein Folding by the Hsp70/Hsp90 Chaperone Complex

Living cells synthesize large amounts of protein in a very short time. If the hydrophobic residues of proteins are exposed, they can aggregate with each other which could lead to precipitation (Martin 2004; Kampinga 2006). Specialised proteins, known as molecular chaperones, have evolved to prevent this from happening. They assist nascent or stress-denatured proteins in folding, conformational assembly, translocation and degradation (Ellis 1988; Welch 1991; Hendrick and Hartl 1995; Clarke 1996; Hartl 1996; Picard 2002; Wandinger et al. 2008; Taipale et al. 2010; Hartl et al. 2011). The heat shock proteins, Hsp70 and Hsp90, form an important molecular chaperone network required for folding and maturation of key regulatory proteins, many of which are signalling intermediates or transcription factors (Kimmins and MacRae 2000; Wegele et al. 2004; Carrigan et al. 2005). Whereas Hsp90 is primarily involved in conformational regulation and stabilisation of proteins that are almost completely folded, Hsp70 is required for earlier stages of assisted folding of nascent or denatured proteins (Whitelaw et al. 1991; Stepanova et al. 2000; Park et al. 2003; Pratt and Toft 2003; Citri et al. 2006).

Both Hsp70 and Hsp90 are dependent on ATP hydrolysis and association with a range of accessory proteins, known as co-chaperones, for chaperone activity (Nadeau et al. 1993; Jakob et al. 1996; Scheibel et al. 1997; Obermann et al. 1998; Panaretou et al. 1998; Prodromou et al. 2000; McLaughlin et al. 2004; Onuoha et al. 2008; Prodromou 2012). The Hsp70/Hsp90 protein folding cycle has been described for hormone receptors (GR) (Smith et al. 1993; Dittmar et al. 1996; Johnson et al. 1998; Wegele et al. 2004; Li et al. 2012a) and is currently widely accepted as the mechanism followed for most client proteins. The early stages of the chaperone assisted folding cycle occur when Hsp70, together with one of the Hsp40 co-chaperone isoforms, capture nascent or denatured proteins. The next stage involves the formation of the intermediate complex, in which the client protein is transferred from the Hsp70 complex to the open Hsp90 complex. Hsp90 is constitutively dimerised at the C terminus, while the N terminal nucleotide binding domains (NBD) of the dimers are disassociated (resembling a “V” shape). This is followed by ATP binding to the nucleotide binding domain (NBD) of Hsp90. Subsequent conformational changes result in N terminal dimerization, docking of the middle domain and binding of the client protein. Hsp90 in this complex is in the closed conformation. Hydrolysis of ATP occurs and the protein reverts to the open conformation and the client protein is released (Wegele et al. 2004; Wegele et al. 2006; Richter et al. 2008; Graf et al. 2009; Hessling et al. 2009). Progression through the

different stages of this cycle is regulated by a variety of co-chaperones, including Hsp70 interacting protein (HIP), C-terminus of Hsp70 interacting protein (CHIP), Hsp70-Hsp90 organizing protein (Hop), activator of Hsp90 ATPase 1 (AHA1), CDC37 and p23 (Chen et al. 1996; Chang et al. 1997; Chen and Smith 1998; Johnson et al. 1998; van der Spuy et al. 2000; Angeletti et al. 2002; Richter et al. 2003; Lee et al. 2004; Hildenbrand et al. 2010). Hop and CDC37 are intermediate stage co-chaperones controlling entry of clients into the pathway, while p23 and AHA1 are involved in the later stages of the cycle involving client protein maturation (Li et al. 2012a). In this way, co-chaperones indirectly modulate the function of the Hsp70/Hsp90 complex by controlling the progression of client proteins through the chaperone cycle.

Hop (Hsp70-Hsp90 Organising Protein)

The Hsp70-Hsp90 organising protein (henceforth referred to as Hop; but also known as stress-inducible protein 1 [STI1], stress-inducible phosphoprotein 1 [STIP1] or p60) is a ubiquitous protein and one of the most widely dispersed co-chaperones of Hsp90 (Johnson and Brown 2009). First identified in yeast (Nicolet and Craig 1989), Hop has been demonstrated or predicted to be encoded in the genome of many organisms. This includes model organisms used for genetic studies of human disease [nematode (Song et al. 2009), fruit fly (Grigus et al. 1998), zebrafish (Woods et al. 2005; Tastan Bishop et al. 2014) and mouse (Blatch et al. 1997)], as well as rats (Demand et al. 1998), frogs (Klein et al. 2002), fish (Andreassen et al. 2009), parasites (Webb et al. 1997; Hombach et al. 2013), and plants (Zhang et al. 2003; Chen et al. 2010). The gene and nucleotide sequence for Hop was also recently identified in the genome and transcriptome of the Coelacanth (*Latimeria* spp), an organism largely unchanged for many years (Amemiya et al. 2013; Tastan Bishop et al. 2013). The human homologue of Hop was isolated in 1992 (Honore et al. 1992). Despite the conservation of Hop in these species, there is some evidence that Hop is structurally and functionally different in different organisms. For example, Hop is an essential gene in the mouse (Beraldo et al. 2013), but not in yeast (Chang et al. 1997).

Hop is predominantly a cytoplasmic protein, but can also be found in the nucleus (Longshaw et al. 2004), Golgi (Honore et al. 1992), in the extracellular environment and associated with cell membranes (Hajj et al. 2013). Current dogma suggests that the nuclear and extracellular Hop species derive from changes in the subcellular localisation of cytoplasmic Hop. Indeed, mammalian Hop contains a bipartite nuclear localisation signal (NLS) which has been proposed to facilitate translocation from the cytoplasm to the nucleus in response to stress. Hop also contains potential export signals, and inhibition of nuclear export enhances the nuclear localisation of Hop (Longshaw et al. 2004). Hop translocates to the nucleus during G1/S transition through phosphorylation by casein kinase II whereas phosphorylation by cell division cycle 2 kinase retains Hop in the cytoplasm (Longshaw et al. 2004; Daniel

et al. 2008). Recently, studies using astrocyte cell lines identified PIAS1 (protein inhibitor of activated STAT1) as a nuclear retention factor for Hop (Soares et al. 2013). The mechanism by which Hop is transported to the plasma membrane and extracellular environment is currently undefined, although there is evidence for export of Hop from mouse astrocytes in exosomes derived from multivesicular bodies (Hajj et al. 2013).

Structure of Hop

Structurally, Hop is composed of repeating units of two different types of domain, namely the tetratricopeptide repeat (TPR) motif and the aspartate-proline (DP) motif domains. Hop contains three TPR domains (designated TPR1, TPR2A and TPR2B) each of which is formed from three TPR motifs. There are two DP domains, the DP1 and DP2 domains, which are positioned between TPR1 and TPR2A and C terminal to TPR2B of Hop, respectively. The TPR domains of Hop are amongst the best characterised (Scheufler et al. 2000; Brinker et al. 2002; Odunuga et al. 2003; Odunuga et al. 2004; Onuoha et al. 2008). The TPR motif is a protein-protein interaction module that is found in a range of proteins, which are involved in diverse cellular processes, from transcription to protein degradation (Allan and Ratajczak 2011). The structure of the TPR domain consists of modules of anti-parallel α-helices arranged in tandem creating an amphipathic groove which is the main site of protein-protein interactions (Allan and Ratajczak 2011). In co-chaperones, TPR domains mediate the interaction with Hsp70 or Hsp90 by binding to the conserved C terminal EEVD motif of the cytosolic isoforms of the chaperones. Among co-chaperones of Hsp70 and Hsp90, the TPR motif is not unique to Hop, and is also found in CHIP and HIP.

Mutational studies in both yeast and murine systems have demonstrated that the TPR domains of Hop display different affinity for the Hsp70 and Hsp90 chaperones (Odunuga et al. 2003; Song and Masison 2005). Mutations in TPR1 but not TPR2AB impair Hsp70 binding, while the converse is true for Hsp90 binding. The ability of Hop to discriminate between Hsp70 and Hsp90 EEVD motifs is mediated by specific TPR residues which interact with residues immediately upstream of the EEVD (GPTIEEVD in the case of Hsp70 and MEEVD in the case of Hsp90) (Odunuga et al. 2003; Carrigan et al. 2004). Hop is therefore differentiated from other TPR-containing co-chaperones in that its TPR domains can discriminate between Hsp70 and Hsp90 (Odunuga et al. 2003; Carrigan et al. 2004). Conserved residues in the TPR domains form a carboxylate clamp with the C-terminal EEVD motif in the chaperones. Adjacent residues in TPR1 and TPR2A promote high affinity binding to either the GPTIEEVD peptide of Hsp70 or the MEEVD peptide of Hsp90, respectively (Scheufler et al. 2000; Brinker et al. 2002; Odunuga et al. 2003).

More recent evidence proposes a model in which Hop binding to Hsp90 is not restricted only to the C-terminal EEVD motif. Hop also appears to interact with N

terminal regions of Hsp90, with residues in TPR2A (VISK, residues 334–337) and TPR2B (EIDQLYYKASQQR, residues 505–517) coming within 13 Å of residue 57 in the NBD during binding (Lee et al. 2012). This observation at first appears unlikely given that TPR2A is simultaneously involved in binding of the C-terminal EEVD motif of Hsp90. However, it is explained by the fact that the rate of Hop-Hsp90 binding is dependent on the length of the linker region between the C-terminal dimerization domain of Hsp90 and the MEEVD (Lee et al. 2012; Schmid et al. 2012). This suggests a model in which the C terminus of Hsp90 has conformational flexibility and can therefore support simultaneous interactions of Hop TPR2 with both the C-terminal and N-terminal domains. In addition, Hop inhibits the ATPase activity of Hsp90 by preventing N-terminal dimerization, by a mechanism that depends on the presence of TPR2A and TPR2B but does not require the MEEVD of Hsp90 (Lee et al. 2012).

In mammals, discrimination between TPR-containing co-chaperones by Hsp70 or Hsp90 depends on relative affinities, and is regulated by phosphorylation (Muller et al. 2013). Phosphorylation of serine and threonine residues located close to the C-terminal EEVD motifs of Hsp70 and Hsp90 promotes association with Hop over CHIP. Therefore, the C-terminal phosphorylation of Hsp70 or Hsp90 controls the balance between protein folding (Hop-based) and protein degradation (CHIP-based) pathways.

The DP domains (also known as STI domains) are rich in aspartic acid and proline residues and also adopt alpha helical structures (Fig. 3.1a). The role of these two motifs is less clear (Song and Masison 2005; Allan and Ratajczak 2011; Willmer et al. 2013), although DP2 mutants showed reduced ability to bind HSP70 (Carrigan et al. 2004) and the DP2 segment is required for client activation in *vivo* (Carrigan et al. 2005; Flom et al. 2006; Schmid et al. 2012). There is sequence similarity between the DP2 domain of Hop and a C-terminal DP domain in HIP, although the two domains are not functionally equivalent (Nelson et al. 2003). More recent studies suggest that the TPR1-DP1 module of Hop is directly involved in translocation of the client protein within the complex (Schmid et al. 2012).

The overall structure of Hop as described above is conserved in the human, mouse and yeast proteins (Fig. 3.1b). Interestingly, not all Hop orthologues share this structure. For example, Hop in *Drosophila* lacks the DP1 domain, while Hop in *C. elegans* lacks the TPR1 domain and the short linker region containing the DP1 domain that precedes the TPR2A domain. Nevertheless, Hop in *C. elegans* is able to bind both Hsp70 and Hsp90 via the TPR2AB domain, although unlike most organisms, the TPR domains of Hop in *C. elegans* do not discriminate between Hsp70 and Hsp90 (Gaiser et al. 2009). This suggests that the transfer of client proteins between Hsp70 and Hsp90 chaperone systems in these organisms may be different. As a consequence of these differences, the study of Hop, especially using genetic approaches has been limited to metazoans that are amenable to genetic manipulation.

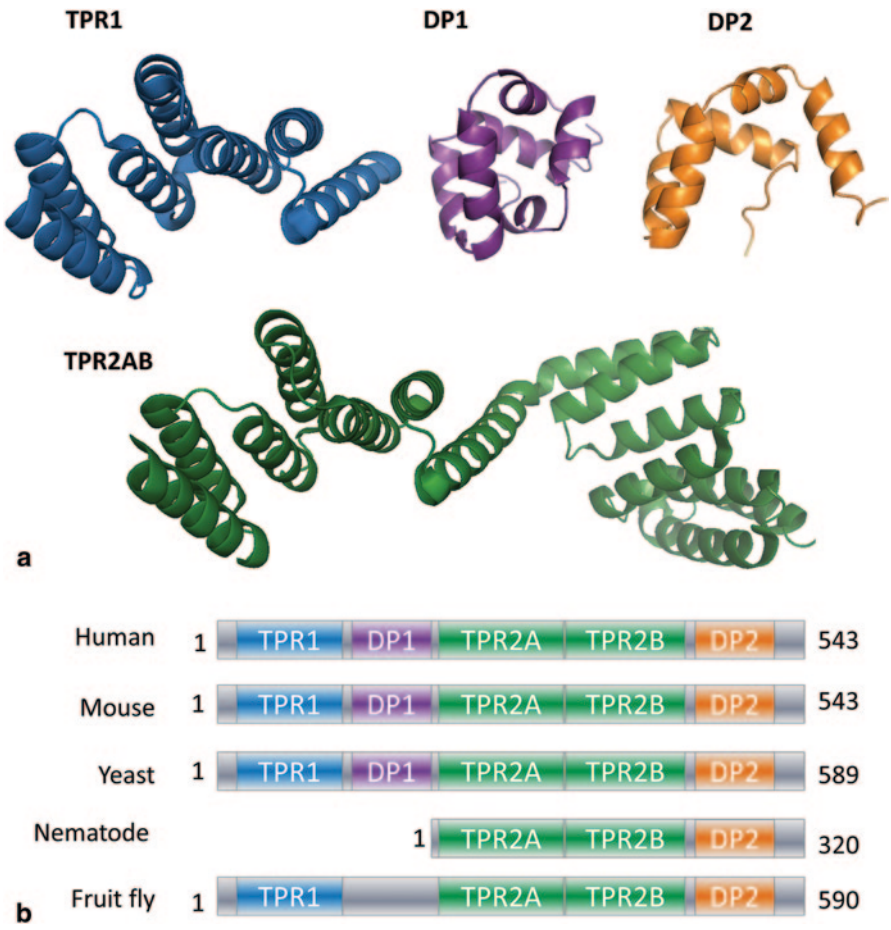

Fig. 3.1 Structural domains and architecture of Hop proteins. **a** Three dimensional structure of Hop domains. Images generated using Pymol (Delano Scientific). The PDB codes for the structures are: 3ESK for TPR1; 3UQ3 for TPR2AB; 2LLV for DP1; and 2LLW for DP2. **b** Comparison of Hop domain structure across model organisms. TPR1: tetratricopeptide repeat domain 1; DP1: aspartate-proline motif domain 1; TPR2AB: tetratricopeptide repeat domains 2A and B; DP2: aspartate-proline motif domain 2. The N terminus is indicated by the number 1, while the numbers at the C terminus gives the total number of amino acids in the proteins

Functions of Hop

The roles of Hop as a co-chaperone for Hsp70/Hsp90 complex and as a receptor for the prion protein, PrP^C, are the best described. However, there is a growing body of literature that reports the involvement of Hop in cellular activities that appear independent of either chaperones or PrP^C (Table 3.1). However, it should be noted that many of these studies do not demonstrate that Hsp70 or Hsp90 are not involved, but rather fail to provide any evidence that they are involved. Therefore, it is possible

Table 3.1 Summary of the roles for Hop in human cellular function and disease

Condition	Hop status[a]	Model system	Cellular function[b]	Proposed biological mechanism[b]	Reference
Aging	KO	*C. elegans* model	Stress response	Impairment of stress tolerance and fertility	Song et al. (2009)
Angiogenesis	KD	HUVEC endothelial cell lines	Cell migration	Cytoskeletal dynamics (tubulin binding)	Li et al. (2012b)
Cancer	KD	Hs578T breast cancer lines	Cell migration	Cytoskeletal dynamics (actin binding)	Willmer et al. (2013)
	I	Isolated proteins and in *vitro* breast cancer cell lines	Cell growth	Disruption of interaction between Hop and Hsp90	Pimienta et al. (2011)
	CO	Clinical colon cancer versus non-tumour samples; in *vitro* cell lines	ND	Increased complex formation with Hsp90 and Hsp70	Kubota et al. (2010)
	UP S	Glioma cell lines	Cell proliferation	Activation of cell signalling pathways (MAPK)	Erlich et al. (2007)
	UP (protein)	Hepatocellular carcinoma clinical tumour versus non-tumour samples	ND	ND	Sun et al. (2007)
	UP	Bohemine resistant versus sensitive lymphoblastic leukaemia cell lines	Drug resistance	ND	Skalnikova et al. (2011)
	UP (serum); S	Clinical ovarian cancer versus non-tumour samples	Cell proliferation	Activation of cell signalling pathways (ERK, ALK)	Wang et al. (2010); Tsai et al. (2012); Chao et al. (2013)
	UP (protein) KD	Clinical pancreatic cancer samples versus normal controls: invasive versus non-invasive clones of pancreatic cell line MiaPaCa-2	Cell invasion	MMP activation	Walsh et al. (2009); Walsh et al. (2011)
Cystic fibrosis	KD	CFBE41o–cells expressing ΔF508 CFTR cell line model	Mutant protein dynamics	Prevention of mutant CFTR variant (Δ F508) maturation	Marozkina et al. (2010)

Table 3.1 (continued)

Condition	Hop status[a]	Model system	Cellular function[b]	Proposed biological mechanism[b]	Reference
Development	S?	Neural stem cell culture	Self-renewal and proliferation	Inhibition reduced neurosphere formation; self-renewal and proliferation	Lopes et al. (2012)
	KD	Murine ESC culture	Pluripotency	Blocked embryoid body formation	Longshaw et al. (2009)
	KO	Mouse model	Embryonic development	Embryonic lethal (E10.5)	Beraldo et al. (2013)
	UP, S, I	Developing versus mature retinal tissues (rat)	Cell proliferation and death	Retinal development independent of PrP^C	Arruda-Carvalho et al. (2007)
Memory	I (antibodies); UP	Intra-hippocampal infusion	Reduced performance	Short term and long term memory inhibited	Coitinho et al. (2007)
	OE (Peptide 230–245)	Peptide 230–245 from Hop (including PrP^C binding site)	Enhanced performance	ND (but involves PrP^C binding site)	
Neuronal function	S?	Mouse model and in *vitro* cell lines	Neuroprotection, neurite formation	PrP^C dependent activation of signalling pathways, endocytosis	Lopes et al. (2005); Lima et al. (2007); Roffe et al. (2010); Caetano et al. (2008)

[a] *KD* knockdown by RNA interference, *S* secreted or extracellular Hop, *KO* gene knockout, *OE* Hop overexpressed (recombinant form), *UP* Hop upregulated (endogenous form), *CO* Hop in complex with Hsp90 and Hsp70, *I* pharmacological inhibition, *MUT* mutated

[b] *ND* not determined

that Hsp70/Hsp90, or indeed PrP^C, may fulfil as yet undefined roles in these seemingly alternative functions of Hop.

Most recently, evidence has emerged to suggest that Hop has independent ATPase activity (Yamamoto et al. 2014). Hop bound ATP with a similar affinity to Hsp90 and Hsp70 but hydrolysis of ATP took place at a slower rate than in the two chaperones. The ATPase activity of Hop was associated with the N terminal regions of the protein, encompassing the TPR1, DP1 and TPR2A domains. While the DP1 domain was essential for ATPase activity, the mutation of a putative Walker B motif in this domain did not abolish the ATPase activity of Hop (Yamamoto et al. 2014). The consequences of this ATPase activity for the function of Hop remain to be determined. However, ATP binding by Hop induced a conformational change in the protein. The domains which display ATPase activity are those involved in binding both Hsp70 (TPR1) and Hsp90 (TPR2A) and therefore it is plausible that the ATP induced conformational changes may be involved in the transfer of client protein between Hsp70 and Hsp90.

Hop as a Co-chaperone for Hsp70 and Hsp90

Hsp90 substrates include a diverse set of proteins, many of which have been implicated in regulation of apoptosis (Samali and Cotter 1996; Mosser and Morimoto 2004; Lanneau et al. 2008), proliferation (Caplan et al. 2007; DeZwaan and Freeman 2008; Lanneau et al. 2008), autophagy (Agarraberes and Dice 2001; Qing et al. 2006; Joo et al. 2011; Xu et al. 2011) and cell cycle progression (Francis et al. 2006; Reikvam et al. 2009) as well as in tumorigenesis (Kamal et al. 2004; Müller et al. 2004; Whitesell and Lindquist 2005; Chiosis 2006; Neckers 2007; Mahalingam et al. 2009; Trepel et al. 2010; Miyata et al. 2013). In early studies it was found that Hsp90 interacted with the yeast and vertebrate homologues of Hop in lysates of these cells (Chang et al. 1997). Deletion of the gene encoding Hop reduced the in *vivo* activity of the Hsp90 target proteins, glucocorticoid receptor (GR) and the oncogenic tyrosine kinase, v-Src (Chang et al. 1997). Hop was also shown to stimulate the refolding of luciferase by Hsp70 and a much more dramatic effect was seen when Hsp90 was also included (Johnson et al. 1998). This led to the conclusion that Hop is a general factor in the maturation of Hsp90 target proteins. Since then it has been clearly demonstrated that Hop regulates the molecular chaperone activities of Hsp70 and Hsp90 and thus plays a crucial role in the productive folding of client proteins (Johnson et al. 1998; Kimmins and MacRae 2000; Wegele et al. 2004; Song and Masison 2005; Wegele et al. 2006; Kubota et al. 2010; Lee et al. 2012). These client proteins include a variety of kinases, transcription factors and steroid hormone receptors, many of which are deregulated in cancer (Pratt and Toft 2003; Lee et al. 2004; Song and Masison 2005; Tan et al. 2011; Walsh et al. 2011; Ruckova et al. 2012; Willmer et al. 2013). The central role of Hop in these processes is demonstrated by mutations in Hop that impair the client folding pathway (Song and Masison 2005). Hop connects Hsp90 and Hsp70 in a ternary multichaperone

complex, where it facilitates the transfer of client proteins from the early complex (Hsp70-Hsp40) to the intermediate complex (Hsp70-Hsp90) (Chen and Smith 1998; Johnson et al. 1998; Song and Masison 2005; Wegele et al. 2006) Depletion of Hop levels using RNA interference leads to a dramatic reduction in the levels of obligate Hsp90 client proteins, HER2, Bcr-Abl, c-MET and v-Src (Walsh et al. 2011).

Extracellular Hop has Cytokine-like Activity

Chaperones have been found in the extracellular environment and play physiological roles such as modulation of the stress response and cell survival (Arruda-Carvalho et al. 2007; Lima et al. 2007; Beraldo et al. 2013; Hajj et al. 2013). Hop is secreted by various cells types, including neuronal stem cells (Santos et al. 2011), microglia (da Fonseca et al. 2012), astrocytes (Lima et al. 2007; Arantes et al. 2009) and cancerous cells such as gliomas (Erlich et al. 2007) and ovarian cancer cells (Wang et al. 2010; Tsai et al. 2012). Despite evidence of an extracellular Hsp90 complex, in the extracellular environment Hop appears to act more like a cytokine than a co-chaperone. Secreted Hop activates numerous different signalling pathways (Caetano et al. 2008; Arantes et al. 2009; Beraldo et al. 2010; Wang et al. 2010; Tsai et al. 2012).

Many, but not all, of the activities of extracellular Hop involve an interaction with normal cellular prion protein PrP^C. Extracellular Hop and PrP^C interact directly with each other via an interaction site that maps to residues 230–245 in Hop (encompassing the start of TPR2A domain) and 113–128 in PrP^C (Zanata et al. 2002). The Hop- PrP^C complex has been found to play a role in a number of different processes such as cell growth, survival and differentiation. In particular, the interaction between Hop and PrP^C is linked to processes that involve neuronal development and cognitive function. Interestingly, these roles of Hop appear to be independent of the Hsp70/Hsp90 chaperones.

Hop induced signalling was able to protect a range of neuronal cell types from apoptosis using mechanisms that were dependent on the presence of wild type PrP^C (Zanata et al. 2002; Lopes et al. 2005; Arantes et al. 2009). Studies using cells from PrP^C null mice have demonstrated that the effects of Hop on neural stem cell renewal and differentiation (Santos et al. 2011; Lopes and Santos 2012), proliferation and survival (Lima et al. 2007), neuritogenesis (Lopes et al. 2005; Lima et al. 2007; Santos et al. 2013) and response to ischemic stress (Beraldo et al. 2013) are all dependent on an interaction with PrP^C. These interactions appear to have an important impact on cognitive functions, as disruption of the Hop–PrP^C interaction led to defects in memory and learning in rats (Coitinho et al. 2007). Extracellular Hop also acts in a PrP^C independent manner in certain cases. The control of retinal proliferation by extracellular Hop for example was found to be independent of PrP^C (Arruda-Carvalho et al. 2007), as are some of the functions of extracellular Hop in cancer (da Fonseca et al. 2012; Tsai et al. 2012).

The effects of extracellular Hop appear to be mediated primarily by activation of downstream signalling pathways. Hop interacting with PrP^C or other receptors has been shown to induce activation of a range of signalling pathways, including SMAD (Tsai et al. 2012), ERK (Americo et al. 2007; Caetano et al. 2008), PKA (Chiarini et al. 2002; Zanata et al. 2002) and PI3K/Akt (Erlich et al. 2007; Roffé et al. 2010) pathways. In this way, Hop appears to function like a classical cytokine, binding to a transmembrane receptor to induce cellular signalling cascades. A similar effect has been noted with extracellular chaperones like Hsp90, which are able to induce signalling from cellular receptors like LRP-1 (Tsen et al. 2013). The studies on extracellular Hop are particularly interesting since nothing is known about the mechanism of export or the isoform specificity of extracellular Hop. If indeed extracellular Hop is derived from intracellular Hop, then it begs the question of the mechanism and conditions under which Hop is exported from the cell? It is tempting to speculate that there may be alternative isoforms of Hop; one isoform that functions as the intracellular co-chaperone of Hsp70/Hsp90, the other, as an extracellular cytokine for which PrP^C is the receptor.

Hop in Human Cellular Function and Disease

Cancer Cell Biology

Transformed cells rely on molecular chaperones together with co-chaperones to stabilise their mutant, unstable proteins (Soti and Csermely 1998; Tytell and Hooper 2001; Daugaard et al. 2005; Chiosis 2006; Boschelli et al. 2010). Recent studies have demonstrated that Hop may regulate multiple biological processes in a range of cancer cell types (Table 3.1). In most cases, Hop levels are increased in cancer cells compared to normal cell equivalents, as well as being upregulated in metastatic, drug resistant or aggressive tumours (Sims et al. 2011). This was true of breast (Sims et al. 2011), colon (Kubota et al. 2010), pancreatic (Walsh et al. 2009; Walsh et al. 2011), ovarian (Wang et al. 2010; Tsai et al. 2012) and hepatocellular carcinomas (Sun et al. 2007). Concomitant with the increased expression levels, Hop appeared to function to promote or support malignancy in tumours, while depletion of Hop levels in cancer cell lines was sufficient to ameliorate some of these pro-cancer activities.

There is growing evidence to support a major role for intracellular Hop in cellular functions relating to metastatic processes, such as cell migration and invasion. Depletion of intracellular Hop levels in endothelial (Li et al. 2012b) and breast cancer cells (Willmer et al. 2013) reduced pseudopodia formation and inhibited cell migration and polarisation. These effects were predicted to be via regulation of different cell processes, including a direct interaction with cytoskeletal proteins like actin and tubulin. Hop also regulates the activity of specific proteins, such as matrix metalloproteinase 2 (MMP2), which are involved in the degradation of the extracellular matrix during cancer cell invasion (Walsh et al. 2011). Interestingly, the cur-

rent literature suggests that intracellular Hop does not seem to have a major role in cell proliferation, leading to the suggestion that intracellular Hop may be a selective target for inhibition of processes associated with metastasis (e.g. migration, invasion). These data are in contrast with the functions proposed for extracellular Hop.

Extracellular Hop in cancer does not appear to induce a major migratory phenotype, but instead leads to an increase in cancer cell proliferation. Hop is secreted into the extracellular environment by a range of cell types, including ovarian carcinomas (Wang et al. 2010; Tsai et al. 2012) and glioblastomas (Erlich et al. 2007). The ability of extracellular Hop to induce cell proliferation appears to be mediated by the ability of the co-chaperone to activate intracellular signalling pathways. In both glioma and ovarian cancer cells, Hop activated mitogenic pathways, including MAPK (Erlich et al. 2007), a major signal transduction pathway required for cell growth. The difference in biological response to intracellular versus extracellular Hop may, in part, be due to the involvement of PrP^C as a receptor, for which extracellular Hop is a major ligand. The proliferative effect of Hop in glioma occurs, at least in part via a PrP^C dependent mechanism (Erlich et al. 2007), although PrP^C -independent growth has been observed in different cell lines (da Fonseca et al. 2012).

Many of the studies of the role of Hop in cancer do not include a direct analysis of the contributions to the phenotype of the chaperones Hsp90 and Hsp70. However, Hop has been shown to be constitutively incorporated into an Hsp90 complex in some cancer cells and many of the proteins affected by Hop inhibition or depletion are in fact client proteins of the Hsp90 complex (Kubota et al. 2010). Therefore, it is likely that many of the activities of Hop in cancer are linked to perturbations in the function of the Hsp70/Hsp90 complex. This conclusion is supported by the observations that compounds that disrupt interactions between Hop and the Hsp90 or Hsp70 chaperone are toxic to cancer cells (Horibe et al. 2011; Horibe et al. 2012).

The link between Hop and oncogenic activity has led to the proposal that Hop itself may be a viable drug target for cancer. Indeed, studies in which Hop levels were reduced using RNA interference in cancer cells demonstrated that depletion of Hop could reverse oncogenic properties (Walsh et al. 2011; Willmer et al. 2013). Despite this, there are currently no small molecule inhibitors that directly inhibit Hop. This may be partly due to the fact that until recently, Hop did not have any known enzymatic activity that could be targeted by inhibitors. The recent discovery that Hop is an ATPase (Yamamoto et al. 2014) means that it may now be possible to design ATPase inhibitors that specifically target Hop. The domains required for Hop ATPase function have been determined (TPR1-DP1-TPR2A) and structures for these domains (albeit as separate units) are available. Therefore it should be theoretically possible to begin to design inhibitors of these domains. The exact residues involved in Hop ATPase remain to be determined, although a predicted Walker B motif in the DP1 domain has been shown *not* to be involved (Yamamoto et al. 2014).

Currently, the most common strategy used for anti-cancer compounds is to inhibit the interaction of Hsp90 and Hop, as an alternative to inhibiting Hsp90. Hsp90 is considered a promising drug target for cancer treatment because Hsp90 is the main

chaperone required for the stabilization of multiple oncogenic kinases (Reikvam et al. 2009). Over-expression of Hsp90 in cancer cells stabilizes mutant oncoproteins, promoting cancer cell survival. Given that Hop is required for entry of these client proteins into the Hsp90 complex, targeting the interaction of Hop and Hsp90 is likely to inactivate client proteins. However, inhibition of Hsp90 (particularly by blocking the N terminal ATP binding site) has been associated with unwanted compensatory upregulation of Hsp70, which can lead to drug resistance (Pimienta et al. 2011). Therefore, the targeting of protein-protein interactions with co-chaperones rather than ATPase activity has been considered as an alternative strategy for the treatment of cancer (Reikvam et al. 2009; Maciejewski et al. 2013).

Compounds specifically inhibiting the interaction of Hop with the Hsp70/Hsp90 complex have been identified. A hybrid peptide comprising a sequence based on the TPR2A region of Hop was designed to competitively inhibit the interaction between Hsp90 and Hop (Horibe et al. 2011). This peptide induced cell death in a range of cancer cell lines *in vitro,* as well as displaying anti-tumour activity in a pancreatic cancer xenograft model (Horibe et al. 2012). The compound also showed differential toxicity in that it did not affect the viability of normal cells, which might be attributed to the constitutive formation of the Hsp90 complex in cancer cells as opposed to normal cells (Barrott and Haystead 2013; Jego et al. 2013). Unlike other inhibitors of the Hsp90 complex, this compound did not alter Hsp70 expression. It has also been possible to inhibit Hop interaction with Hsp90 via small molecules, like Sansalvamide A analogues (Ardi et al. 2011) and a compound termed C9 (1,6-dimethyl-3-propylpyrimido [5,4-e] [1,2,4] triazine-5,7-dione) (Pimienta et al. 2011). The Sansalvamide A analogue bound Hsp90 at a region between the N terminal and middle domains, inducing allosteric changes that blocked the binding of Hop (and two other TPR containing proteins) to the Hsp90 MEEVD. The compound C9 also blocked the interaction of Hsp90 with Hop *in vitro*. Six compounds containing a 7-azapteridine ring were similarly able to inhibit the interaction between Hsp90 and Hop (Yi and Regan 2008). All of these compounds were shown to have anti-cancer activity in cell lines, demonstrating that prevention of the interaction between Hsp90 and Hop may be a viable target for anti-cancer therapies (Pimienta et al. 2011; Ardi et al. 2011; Yi and Regan 2008).

Developmental and Protein Folding Disorders

Hop has an established role in cellular development. Knockout of Hop in the mouse is embryonic lethal and Hop null mice fail to develop beyond E10.5 (Beraldo et al. 2013). Hop has also been linked with a role in embryonic stem cell biology *in vitro*. Transient silencing of Hop in embryonic stem cells led to a reduction in the ability to form embryoid bodies, suggesting a more differentiated phenotype (Longshaw et al. 2009; Prinsloo et al. 2009). This was attributed to a decrease in the phosphorylation and concomitant extranuclear accumulation of signal transducer and activator of transcription 3 (STAT3), a protein shown to interact directly with Hsp90

in vitro and in embryonic cells during leukaemia inhibitory factor (LIF)-induced pluripotency signalling (Setati et al. 2010; Prinsloo et al. 2011). The role of Hop in stem cell biology suggests that Hop may play a fundamental role in embryonic development. Hop is also required for neurosphere self-renewal and differentiation in neuronal cells which is linked to neuronal development and conceptual processes such as memory (Coitinho et al. 2007). These findings are consistent with recent evidence that Hop interacts with Rnd1 GTPase to enhance neurite outgrowth in neuronal cell lines, leading to the proposal that Hop may be involved in neuronal development (de Souza et al. 2014).

Interestingly, linked to its role in foetal development through neuritogenesis, a decrease in Hop could be involved in autism-spectrum disorders (ASD) (Braunschweig et al. 2013). The production of maternal IgG antibodies against a number of foetal brain antigens, including Hop, has been linked to ASD in the children born to these mothers. Children from mothers with specific reactivity to these had increased ASD-type stereotypical behaviours. It was suggested these antigens could serve as a panel of markers for risk of maternal-autoantibody-related autism (Braunschweig et al. 2013).

The role of Hop as a co-chaperone has linked it to disorders in which Hsp90 client protein stability or misfolding are a hallmark. The leading cause of cystic fibrosis is the presence of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. A variant of CFTR harbouring a phenylalanine deletion (CFTR ΔF508) has been shown to interact directly with Hop (Marozkina et al. 2010). Hop captures CFTR ΔF508 and prevents its maturation, thereby blocking its function. The maturation of CFTR ΔF508 could be rescued by treatment with S-nitrosoglutathione (GSNO), which reduced Hop levels, without affecting Hsp70 or Hsp90; a phenotype recapitulated by siRNA mediated knockdown of Hop (Marozkina et al. 2010).

In Alzheimer's disease, soluble β-amyloid oligomers (AβOs) bind to PrP^C and trigger neurotoxicity. Hop was found to prevent the binding of AβOs to PrP^C, both *in vitro* and to mouse hippocampal neuronal PrP^C in *vivo* (Ostapchenko et al. 2013). Hop was able to prevent AβO-induced synaptic loss and neuronal death, and neurons that were haploinsufficient in Hop were more sensitive to AβO-induced death which could be rescued by treatment with recombinant Hop. The toxicity induced by AβOs could also be prevented by TPR2A which is the domain in Hop that interacts with PrP^C (Ostapchenko et al. 2013).

Hop has also been implicated in other protein conformational diseases, in which various proteins are converted into a common toxic conformational state similar to β-amyloid (Wolfe et al. 2013). Molecular chaperones have been found to suppress the toxicity of β-amyloid-like proteins by packaging the toxic proteins into protein-handling depots. Hop was found to be a component of the Hsp70/Hsp90 system in the control of spatial organisation of amyloid-like protein assemblies, leading to a suppression of toxicity by proteins such as the glutamine-rich yeast prion [RNQ+] and polyglutamine-expanded Huntingtin (Htt103Q) (Wolfe et al. 2013).

Parasitic Diseases

Hsp70 and Hsp90 are considered drug targets for the treatment of infectious diseases like malaria and trypanosomiasis. Hop is conserved across species, including a number of parasitic organisms that cause disease in humans, such as *Plasmodium* and *Leishmania* species. Hop from *Leishmania donovani* is expressed during the amastigote stage (Joshi et al. 1993) which is important for adaption of the parasite to the human host (Morales et al. 2010). *Plasmodium falciparum* Hop (PfHop) shares a similar domain architecture with human Hop and the residues that are known to be important in the interaction with Hsp70 or Hsp90 (Odunuga et al. 2003) are conserved. However, despite the fact that chaperone and co-chaperone systems are highly conserved, there is evidence that the proteins are sufficiently biochemically different to be considered as putative drug targets. For example, the sequence of plasmodial Hop proteins was different to those of yeast and mammals, despite the structural conservation (Gitau et al. 2012). If these differences result in functional changes, antimalarial compounds could be designed to selectively target distinct regions of PfHop (Gitau et al. 2012). Similarly, deletion of specific residues in *Leishmania donovani* Hop blocked phosphorylation and led to parasite death (Morales et al. 2010). If these residues are unique to the parasitic Hop, they may indeed be targets for therapy. Furthermore, it may be relevant that the Hop interaction motif of Hsp90 which is crucial for survival of the parasite is MEQVD in *Leishmania* spp. instead of the MEEVD seen in the human host (Hombach et al. 2013).

Conclusion

While the exact biological function of Hop remains elusive, recent evidence from knockout studies in mammals suggests that it is important in embryonic development in this system at least. A role in development would be consistent with the reported link between Hop and cancer characteristics. The biological function of Hop will be system dependent, and while there are conserved features across species, the sequence and domain variations suggest that it could have been recruited by evolution for a number of different biological roles. The diverse functions of Hop in mammalian cells, suggests that at least two major isoforms may exist, one intracellular and the other extracellular, although direct evidence for this has yet to be presented. Identification and elucidation of the molecular basis for these isoforms and their seemingly divergent cellular functions is an exciting area for future research. How has this dynamic scaffold protein been functionally adapted to such different roles and processes? A deeper structural and functional understanding of these Hop isoforms will assist research on the role of Hop in cancer. The intracellular isoform appears to be involved in processes important for successful metastasis while the extracellular isoform appear to enhance proliferation of cancer cells. The identification of small molecules that can specifically disrupt Hop and its partner protein interactions are starting to emerge. These Hop modulators represent novel

molecular tools for functional analyses as well as novel hit compounds for use in anti-cancer drug discovery research. Elucidation and targeting of the recently identified Hop ATP-binding site will be a rich area for future drug discovery research. Finally, there is growing evidence that Hop has functions that are independent of its major partner proteins (Hsp70, Hsp90 and PrP^C). Many of the recently defined activities of Hop, including ATPase activity, direct interaction and stabilisation of substrate proteins, are those that are more associated with chaperone function than co-chaperone function. As we learn more about this protein, it may be appropriate to evaluate whether it is time to reclassify Hop as chaperone, rather than a co-chaperone. This beckons a fresh approach to understanding the biological function of Hop, especially if its global function is in the area of early development.

Acknowledgments SB-H was supported to conduct this research under the Australian Commonwealth Collaborative Research Network (CRN) funding to Victoria University. ALE and GLB were supported by grants from the National Research Foundation (NRF) South Africa and the Cancer Research Initiative of South Africa (CARISA). ALE was also supported by grants from the Medical Research Council (MRC) South Africa and Cancer Association of South Africa (CANSA).

References

Agarraberes FA, Dice JF (2001) A molecular chaperone complex at the lysosomal membrane is required for protein translocation. J Cell Sci 114:2491–2499

Allan RK, Ratajczak T (2011) Versatile TPR domains accommodate different modes of target protein recognition and function. Cell Stress Chaperones 16:353–367

Amemiya CT, Alfoldi J, Lee AP et al (2013) The African coelacanth genome provides insights into tetrapod evolution. Nature 496:311–316

Americo TA, Chiarini LB, Linden R (2007) Signaling induced by hop/STI-1 depends on endocytosis. Biochem Biophys Res Commun 358:620–625

Andreassen R, Lunner S, Hoyheim B (2009) Characterization of full-length sequenced cDNA inserts (FLIcs) from Atlantic salmon (*Salmo salar*). BMC Genomics 10:502

Angeletti PC, Walker D, Panganiban AT (2002) Small glutamine-rich protein/viral protein U-binding protein is a novel cochaperone that affects heat shock protein 70 activity. Cell Stress Chaperones 7:258–268

Arantes C, Nomizo R, Lopes MH et al (2009) Prion protein and its ligand stress inducible protein 1 regulate astrocyte development. Glia 57:1439–1449

Ardi VC, Alexander LD, Johnson VA et al (2011) Macrocycles that inhibit the binding between heat shock protein 90 and TPR-containing proteins. ACS Chem Biol 6:1357–1366

Arruda-Carvalho M, Njaine B, Silveira MS et al (2007) Hop/STI1 modulates retinal proliferation and cell death independent of PrPC. Biochem Biophys Res Commun 361:474–480

Barrott JJ, Haystead TAJ (2013) Hsp90, an unlikely ally in the war on cancer. FEBS J 280:1381–1396

Beraldo FH, Arantes CP, Santos TG et al (2010) Role of α7 nicotinic acetylcholine receptor in calcium signaling induced by prion protein interaction with stress-inducible protein. J Biol Chem 285:36542–36550

Beraldo FH, Soares IN, Goncalves DF et al (2013) Stress-inducible phosphoprotein 1 has unique cochaperone activity during development and regulates cellular response to ischemia via the prion protein. FASEB J 27:3594–3607

Blatch GL, Lässle M, Zetter BR et al (1997) Isolation of a mouse cDNA encoding mSTI1, a stress-inducible protein containing the TPR motif. Gene 194:277–282

Boschelli F, Golas JM, Petersen R et al (2010) A cell-based screen for inhibitors of protein folding and degradation. Cell Stress Chaperones 15:913–927
Braunschweig D, Krakowiak P, Duncanson P et al (2013) Autism-specific maternal autoantibodies recognize critical proteins in developing brain. Transl Psychiatry 3:e277
Brinker A, Scheufler C, Von Der Mülbe F et al (2002) Ligand discrimination by TPR domains. Relevance and selectivity of EEVD-recognition in Hsp70·Hop·Hsp90 complexes. J Biol Chem 277:19265–19275
Caetano FA, Lopes MH, Hajj GNM et al (2008) Endocytosis of prion protein is required for ERK1/2 signaling induced by stress-inducible protein 1. J Neurosci 28:6691–6702
Caplan AJ, Mandal AK, Theodoraki MA (2007) Molecular chaperones and protein kinase quality control. Trends Cell Biol 17:87–92
Carrigan PE, Nelson GM, Roberts PJ et al (2004) Multiple domains of the Co-chaperone Hop are important for Hsp70 binding. J Biol Chem 279:16185–16193
Carrigan PE, Riggs DL, Chinkers M et al (2005) Functional comparison of human and Drosophila Hop reveals novel role in steroid receptor maturation. J Biol Chem 280:8906–8911
Chang HCJ, Nathan DF, Lindquist S (1997) In vivo analysis of the Hsp90 cochaperone Sti1 (p60). Mol Cell Biol 17:318–325
Chao A, Lai CH, Tsai CL et al (2013) Tumor stress-induced phosphoprotein1 (STIP1) as a prognostic biomarker in ovarian cancer. PLoS One 8:e57084
Chen S, Smith DF (1998) Hop as an adaptor in the heat shock protein 70 (Hsp70) and Hsp90 chaperone machinery. J Biol Chem 273:35194–35200
Chen S, Prapapanich V, Rimerman RA et al (1996) Interactions of p60, a mediator of progesterone receptor assembly, with heat shock proteins Hsp90 and Hsp70. Mol Endocrinol 10:682–693
Chen L, Hamada S, Fujiwara M et al (2010) The Hop/Sti1-Hsp90 chaperone complex facilitates the maturation and transport of a PAMP receptor in rice innate immunity. Cell Host Microbe 7:185–196
Chiarini LB, Freitas ARO, Zanata SM et al (2002) Cellular prion protein transduces neuroprotective signals. EMBO J 21:3317–3326
Chiosis G (2006) Targeting chaperones in transformed systems—a focus on Hsp90 and cancer. Expert Opin Ther Targets 10:37–50
Citri A, Harari D, Shohat G et al (2006) Hsp90 recognizes a common surface on client kinases. J Biol Chem 281:14361–14369
Clarke AR (1996) Molecular chaperones in protein folding and translocation. Curr Opin Struct Biol 6:43–50
Coitinho AS, Lopes MH, Hajj GNM et al (2007) Short-term memory formation and long-term memory consolidation are enhanced by cellular prion association to stress-inducible protein 1. Neurobiol Dis 26:282–290
da Fonseca ACC, Romão L, Amaral RF et al (2012) Microglial stress inducible protein 1 promotes proliferation and migration in human glioblastoma cells. Neuroscience 200:130–141
Daniel S, Bradley G, Longshaw VM et al (2008) Nuclear translocation of the phosphoprotein Hop (Hsp70/Hsp90 organizing protein) occurs under heat shock, and its proposed nuclear localization signal is involved in Hsp90 binding. Biochim Biophys Acta 1783:1003–1014
Daugaard M, Jäättelä M, Rohde M (2005) Hsp70-2 is required for tumor cell growth and survival. Cell Cycle 4:877–880
de Souza LER, Moura Costa MD, Bilek ES et al (2014) STI1 antagonizes cytoskeleton collapse mediated by small GTPase Rnd1 and regulates neurite growth. Exp Cell Res 324:84–91
Demand J, Lüders J, Höhfeld J (1998) The carboxy-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors. Mol Cell Biol 18:2023–2028
DeZwaan DC, Freeman BC (2008) HSP90: the Rosetta stone for cellular protein dynamics? Cell Cycle 7:1006–1012
Dittmar KD, Hutchison KA, Owens-Grillo JK et al (1996) Reconstitution of the steroid receptor hsp90 heterocomplex assembly system of rabbit reticulocyte lysate. J Biol Chem 271:12833–12839
Ellis J (1988) Proteins as molecular chaperones. Nature 328:378–379

Erlich RB, Kahn SA, Lima FRS et al (2007) STI1 promotes glioma proliferation through MAPK and PI3K pathways. Glia 55:1690–1698

Flom G, Weekes J, Williams JJ et al (2006) Effect of mutation of the tetratricopeptide repeat and asparatate-proline 2 domains of Sti1 on Hsp90 signaling and interaction in *Saccharomyces cerevisiae*. Genetics 172:41–51

Francis LK, Alsayed Y, Leleu X et al (2006) Combination mammalian target of rapamycin inhibitor rapamycin and HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin has synergistic activity in multiple myeloma. Clin Cancer Res 12:6826–6835

Gaiser AM, Brandt F, Richter K (2009) The non-canonical Hop protein from *Caenorhabditis elegans* exerts essential functions and forms binary complexes with either Hsc70 or Hsp90. J Mol Biol 391:621–634

Gitau GW, Mandal P, Blatch GL et al (2012) Characterisation of the *Plasmodium falciparum* Hsp70-Hsp90 organising protein (PfHop). Cell Stress Chaperones 17:191–202

Graf C, Stankiewicz M, Kramer G et al (2009) Spatially and kinetically resolved changes in the conformational dynamics of the Hsp90 chaperone machine. EMBO J 28:602–613

Grigus S, Burnett B, Margot N et al (1998) Drosophila homolog of Hsp70/Hsp90 Organizing Protein. GenBank accession number AF056198.1. NCBI. http://www.ncbi.nlm.nih.gov/nuccore/AF056198.1. Cited 17 Jan 2014

Hajj GNM, Arantes CP, Dias MVS et al (2013) The unconventional secretion of stress-inducible protein 1 by a heterogeneous population of extracellular vesicles. Cell Mol Life Sci 70:3211–3227

Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–580

Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475:324–332

Hendrick JP, Hartl FU (1995) The role of molecular chaperones in protein folding. FASEB J 9:1559–1569

Hessling M, Richter K, Buchner J (2009) Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. Nat Struct Mol Biol 16:287–293

Hildenbrand ZL, Molugu SK, Paul A et al (2010) High-yield expression and purification of the Hsp90-associated p23, FKBP52, HOP and SGTα proteins. J Chromatogr B Analyt Technol Biomed Life Sci 878:2760–2764

Hombach A, Ommen G, Chrobak M et al (2013) The Hsp90-Sti1 interaction is critical for *Leishmania donovani* proliferation in both life cycle stages. Cell Microbiol 15:585–600

Honore B, Leffers H, Madsen P et al (1992) Molecular cloning and expression of a transformation-sensitive human protein containing the TPR motif and sharing identity to the stress-inducible yeast protein STI1. J Biol Chem 267:8485–8491

Horibe T, Kohno M, Haramoto M et al (2011) Designed hybrid TPR peptide targeting Hsp90 as a novel anticancer agent. J Transl Med 9:8

Horibe T, Torisawa A, Kohno M et al (2012) Molecular mechanism of cytotoxicity induced by Hsp90-targeted Antp-TPR hybrid peptide in glioblastoma cells. Mol Cancer 11:59

Jakob U, Scheibel T, Bose S et al (1996) Assessment of the ATP binding properties of Hsp90. J Biol Chem 271:10035–10041

Jego G, Hazoumé A, Seigneuric R et al (2013) Targeting heat shock proteins in cancer. Cancer Lett 332:275–285

Johnson JL, Brown C (2009) Plasticity of the Hsp90 chaperone machine in divergent eukaryotic organisms. Cell Stress Chaperones 14:83–94

Johnson BD, Schumacher RJ, Ross ED et al (1998) Hop modulates Hsp70/Hsp90 interactions in protein folding. J Biol Chem 273:3679–3686

Joo J, Dorsey F, Joshi A et al (2011) Hsp90-Cdc37 chaperone complex regulates Ulk1- and Atg13-mediated mitophagy. Mol Cell 43:572–585

Joshi M, Dwyer DM, Nakhasi HL (1993) Cloning and characterization of differentially expressed genes from in vitro-grown 'amastigotes' of *Leishmania donovani*. Mol Biochem Parasitol 58:345–354

Kamal A, Boehm MF, Burrows FJ (2004) Therapeutic and diagnostic implications of Hsp90 activation. Trends Mol Med 10:283–290
Kampinga HH (2006) Chaperones in preventing protein denaturation in living cells and protecting against cellular stress. Handb Exp Pharmacol 172:1–42
Kimmins S, MacRae TH (2000) Maturation of steroid receptors: an example of functional cooperation among molecular chaperones and their associated proteins. Cell Stress Chaperones 5:76–86
Klein SL, Strausberg RL, Wagner L et al (2002) Genetic and genomic tools for Xenopus research: the NIH xenopus initiative. Dev Dyn 225:384–391
Kubota H, Yamamoto S, Itoh E et al (2010) Increased expression of co-chaperone HOP with HSP90 and HSC70 and complex formation in human colonic carcinoma. Cell Stress Chaperones 15:1003–1011
Lanneau D, Brunet M, Frisan E et al (2008) Heat shock proteins: essential proteins for apoptosis regulation: apoptosis review series. J Cell Mol Med 12:743–761
Lee P, Shabbir A, Cardozo C et al (2004) Sti1 and Cdc37 can Stabilize Hsp90 in chaperone complexes with a protein kinase. Mol Biol Cell 15:1785–1792
Lee CT, Graf C, Mayer FJ et al (2012) Dynamics of the regulation of Hsp90 by the co-chaperone Sti1. EMBO J 31:1518–1528
Li J, Soroka J, Buchner J (2012a) The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta 1823:624–635
Li J, Sun X, Wang Z et al (2012b) Regulation of vascular endothelial cell polarization and migration by Hsp70/Hsp90-organizing protein. PLoS One 7:e36389
Lima FRS, Arantes CP, Muras AG et al (2007) Cellular prion protein expression in astrocytes modulates neuronal survival and differentiation. J Neurochem 103:2164–2176
Longshaw VM, Chapple JP, Balda MS et al (2004) Nuclear translocation of the Hsp70/Hsp90 organizing protein mSTI1 is regulated by cell cycle kinases. J Cell Sci 117:701–710
Longshaw VM, Baxter M, Prewitz M et al (2009) Knockdown of the co-chaperone Hop promotes extranuclear accumulation of Stat3 in mouse embryonic stem cells. Eur J Cell Biol 88:153–166
Lopes MH, Santos TG (2012) Prion potency in stem cells biology. Prion 6:142–146
Lopes MH, Hajj GNM, Muras AG et al (2005) Interaction of cellular prion and stress-inducible protein 1 promotes neuritogenesis and neuroprotection by distinct signaling pathways. J Neurosci 25:11330–11339
Maciejewski A, Prado MA, Choy WY (2013) ^{1}H, ^{15}N and ^{13}C backbone resonance assignments of the TPR1 and TPR2A domains of mouse STI1. Biomol NMR Assign 7:305–310
Mahalingam D, Swords R, Carew JS et al (2009) Targeting HSP90 for cancer therapy. Br J Cancer 100:1523–1529
Marozkina NV, Yemen S, Borowitz M et al (2010) Hsp 70/Hsp 90 organizing protein as a nitrosylation target in cystic fibrosis therapy. Proc Natl Acad Sci U S A 107:11393–11398
Martin J (2004) Chaperonin function—effects of crowding and confinement. J Mol Recognit 17:465–472
McLaughlin SH, Ventouras LA, Lobbezoo B et al (2004) Independent ATPase activity of Hsp90 subunits creates a flexible assembly platform. J Mol Biol 344:813–826
Miyata Y, Nakamoto H, Neckers L (2013) The therapeutic target Hsp90 and cancer hallmarks. Curr Pharm Des 19:347–365
Morales MA, Watanabe R, Dacher M et al (2010) Phosphoproteome dynamics reveal heat-shock protein complexes specific to the *Leishmania donovani* infectious stage. Proc Natl Acad Sci U S A 107:8381–8386
Mosser DD, Morimoto RI (2004) Molecular chaperones and the stress of oncogenesis. Oncogene 23:2907–2918
Müller L, Schaupp A, Walerych D et al (2004) Hsp90 regulates the activity of wild type p53 under physiological and elevated temperatures. J Biol Chem 279:48846–48854
Muller P, Ruckova E, Halada P et al (2013) C-terminal phosphorylation of Hsp70 and Hsp90 regulates alternate binding to co-chaperones CHIP and HOP to determine cellular protein folding/degradation balances. Oncogene 32:3101–3110

Nadeau K, Das A, Walsh CT (1993) Hsp90 chaperonins possess ATPase activity and bind heat shock transcription factors and peptidyl prolyl isomerases. J Biol Chem 268:1479–1487
Neckers L (2007) Heat shock protein 90: the cancer chaperone. J Biosci 32:517–530
Nelson GM, Huffman H, Smith DF (2003) Comparison of the carboxy-terminal DP-repeat region in the co-chaperones Hop and Hip. Cell Stress Chaperones 8:125–133
Nicolet CM, Craig EA (1989) Isolation and characterization of STI1, a stress-inducible gene from *Saccharomyces cerevisiae*. Mol Cell Biol 9:3638–3646
Obermann WMJ, Sondermann H, Russo AA et al (1998) In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. J Cell Biol 143:901–910
Odunuga OO, Hornby JA, Bies C et al (2003) Tetratricopeptide repeat motif-mediated Hsc70-mSTI1 interaction. Molecular characterization of the critical contacts for successful binding and specificity. J Biol Chem 278:6896–6904
Odunuga OO, Longshaw VM, Blatch GL (2004) Hop: more than an Hsp70/Hsp90 adaptor protein. Bioessays 26:1058–1068
Onuoha SC, Coulstock ET, Grossmann JG et al (2008) Structural studies on the co-chaperone Hop and its complexes with Hsp90. J Mol Biol 379:732–744
Ostapchenko VG, Beraldo FH, Mohammad AH et al (2013) The prion protein ligand, stress-inducible phosphoprotein 1, regulates amyloid-β oligomer toxicity. J Neurosci 33:16552–16564
Panaretou B, Prodromou C, Roe SM et al (1998) ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. EMBO J 17:4829–4836
Park JW, Yeh MW, Wong MG et al (2003) The heat shock protein 90-binding geldanamycin inhibits cancer cell proliferation, down-regulates oncoproteins, and inhibits epidermal growth factor-induced invasion in thyroid cancer cell lines. J Clin Endocrinol Metab 88:3346–3353
Picard D (2002) Heat-shock protein 90, a chaperone for folding and regulation. Cell Mol Life Sci 59:1640–1648
Pimienta G, Herbert KM, Regan L (2011) A compound that inhibits the HOP-Hsp90 complex formation and has unique killing effects in breast cancer cell lines. Mol Pharm 8:2252–2261
Pratt WB, Toft DO (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med 228:111–133
Prinsloo E, Setati MM, Longshaw VM et al (2009) Chaperoning stem cells: a role for heat shock proteins in the modulation of stem cell self-renewal and differentiation? Bioessays 31:370–377
Prinsloo E, Kramer AH, Edkins AL et al (2011) STAT3 interacts directly with Hsp90. IUBMB Life 64:266–273
Prodromou C (2012) The 'active life' of Hsp90 complexes. Biochim Biophys Acta—Mol Cell Res 1823:614–623
Prodromou C, Panaretou B, Chohan S et al (2000) The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. EMBO J 19:4383–4392
Qing G, Yan P, Xiao G (2006) Hsp90 inhibition results in autophagy-mediated proteasome-independent degradation of IκB kinase (IKK). Cell Res 16:895–901
Reikvam H, Ersvær E, Bruserud Ø (2009) Heat shock protein 90–a potential target in the treatment of human acute myelogenous leukemia. Curr Cancer Drug Targets 9:761–776
Richter K, Muschler P, Hainzl O et al (2003) Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. Binding prevents the N-terminal dimerization reaction during the ATPase cycle. J Biol Chem 278:10328–10333
Richter K, Soroka J, Skalniak L et al (2008) Conserved conformational changes in the ATPase cycle of human Hsp90. J Biol Chem 283:17757–17765
Roffé M, Beraldo FH, Bester R et al (2010) Prion protein interaction with stress-inducible protein 1 enhances neuronal protein synthesis via mTOR. Proc Natl Acad Sci U S A 107:13147–13152
Ruckova E, Muller P, Nenutil R et al (2012) Alterations of the Hsp70/Hsp90 chaperone and the HOP/CHIP co-chaperone system in cancer. Cell Mol Biol Lett 17:446–458
Samali A, Cotter TG (1996) Heat shock proteins increase resistance to apoptosis. Exp Cell Res 223:163–170
Santos TG, Silva IR, Costa-Silva B et al (2011) Enhanced neural progenitor/stem cells self-renewal via the interaction of stress-inducible protein 1 with the prion protein. Stem Cells 29:1126–1136

Santos TG, Beraldo FH, Hajj GNM et al (2013) Laminin-γ1 chain and stress inducible protein 1 synergistically mediate PrPC-dependent axonal growth via Ca2+ mobilization in dorsal root ganglia neurons. J Neurochem 124:210–223

Scheibel T, Neuhofen S, Weikl T et al (1997) ATP-binding properties of human Hsp90. J Biol Chem 272:18608–18613

Scheufler C, Brinker A, Bourenkov G et al (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. Cell 101:199–210

Schmid AB, Lagleder S, Gräwert MA et al (2012) The architecture of functional modules in the Hsp90 co-chaperone Sti1/Hop. EMBO J 31:1506–1517

Setati MM, Prinsloo E, Longshaw VM et al (2010) Leukemia inhibitory factor promotes Hsp90 association with STAT3 in mouse embryonic stem cells. IUBMB Life 62:61–66

Sims JD, McCready J, Jay DG (2011) Extracellular heat shock protein (Hsp)70 and Hsp90α assist in matrix metalloproteinase-2 activation and breast cancer cell migration and invasion. PLoS One 6:e18848

Skalnikova H, Martinkova J, Hrabakova R et al (2011) Cancer drug-resistance and a look at specific proteins: Rho GDP-dissociation inhibitor 2, Y-box binding protein 1, and HSP70/90 organizing protein in proteomics clinical application. J Proteome Res 10:404–415

Smith DF, Sullivan WP, Marion TN et al (1993) Identification of a 60-kilodalton stress-related protein, p60, which interacts with hsp90 and hsp70. Mol Cell Biol 13:869–76.

Soares IN, Caetano FA, Pinder J et al (2013) Regulation of stress-inducible phosphoprotein 1 nuclear retention by protein inhibitor of activated STAT PIAS1. Mol Cell Proteomics 12:3253–3270

Song Y, Masison DC (2005) Independent regulation of Hsp70 and Hsp90 chaperones by Hsp70/Hsp90-organizing protein Sti1 (Hop1). J Biol Chem 280:34178–34185

Song HO, Lee W, An K et al (2009) C. elegans STI-1, the Homolog of Sti1/Hop, is involved in aging and stress response. J Mol Biol 390:604–617

Soti C, Csermely P (1998) Molecular chaperones in the etiology and therapy of cancer. Pathol Oncol Res 4:316–321

Stepanova L, Finegold M, DeMayo F et al (2000) The oncoprotein kinase chaperone CDC37 functions as an oncogene in mice and collaborates with both c-myc and cyclin D1 in transformation of multiple tissues. Mol Cell Biol 20:4462–4473

Sun W, Xing B, Sun Y et al (2007) Proteome analysis of hepatocellular carcinoma by two-dimensional difference gel electrophoresis. Mol Cell Proteomics 6:1798–1808

Taipale M, Jarosz DF, Lindquist S (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11:515–528

Tan SS, Ahmad I, Bennett HL et al (2011) GRP78 up-regulation is associated with androgen receptor status, Hsp70-Hsp90 client proteins and castrate-resistant prostate cancer. J Pathol 223:81–87

Tastan Bishop O, Edkins AL, Blatch GL (2014) Sequence and domain conservation of the coelacanth Hsp40 and Hsp90 chaperones suggests conservation of function. J Exp Zool B Mol Dev Evol. 322:359–378

Trepel J, Mollapour M, Giaccone G et al (2010) Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer 10:537–549

Tsai CL, Tsai CN, Lin CY et al (2012) Secreted Stress-Induced Phosphoprotein 1 Activates the ALK2-SMAD signaling pathways and promotes cell proliferation of ovarian cancer cells. Cell Rep 2:283–293

Tsen F, Bhatia A, O'Brien K et al (2013) Extracellular heat shock protein 90 signals through subdomain II and the NPVY motif of LRP-1 receptor to Akt1 and Akt2: a circuit essential for promoting skin cell migration in vitro and wound healing in vivo. Mol Cell Biol 33:4947–4959

Tytell M, Hooper PL (2001) Heat shock proteins: new keys to the development of cytoprotective therapies. Expert Opin Ther Targets 5:267–287

van der Spuy J, Kana BD, Dirr HW et al (2000) Heat shock cognate protein 70 chaperone-binding site in the co-chaperone murine stress-inducible protein 1 maps to within three consecutive tetratricopeptide repeat motifs. Biochem J 345:645–651

Walsh N, O'Donovan N, Kennedy S et al (2009) Identification of pancreatic cancer invasion-related proteins by proteomic analysis. Proteome Sci 7:3

Walsh N, Larkin A, Swan N et al (2011) RNAi knockdown of Hop (Hsp70/Hsp90 organising protein) decreases invasion via MMP-2 down regulation. Cancer Lett 306:180–189

Wandinger SK, Richter K, Buchner J (2008) The Hsp90 chaperone machinery. J Biol Chem 283:18473–18477

Wang TH, Chao A, Tsai CL et al (2010) Stress-induced phosphoprotein 1 as a secreted biomarker for human ovarian cancer promotes cancer cell proliferation. Mol Cell Proteomics 9:1873–1884

Webb JR, Campos-Neto A, Skeiky YAW et al (1997) Molecular characterization of the heat-inducible LmSTI1 protein of Leishmania major. Mol Biochem Parasitol 89:179–193

Wegele H, Müller L, Buchner J (2004) Hsp70 and Hsp90-a relay team for protein folding. Rev Physiol Biochem Pharmacol 151:1–44

Wegele H, Wandinger SK, Schmid AB et al (2006) Substrate transfer from the chaperone Hsp70 to Hsp90. J Mol Biol 356:802–811

Welch WJ (1991) The role of heat-shock proteins as molecular chaperones. Curr Opin Cell Biol 3:1033–1038

Whitelaw ML, Hutchison K, Perdew GH (1991) A 50-kDa cytosolic protein complexed with the 90-kDa heat shock protein (hsp90) is the same protein complexed with pp60v-src hsp90 in cells transformed by the rous sarcoma virus. J Biol Chem 266:16436–16440

Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5:761–772

Willmer T, Contu L, Blatch GL et al (2013) Knockdown of Hop downregulates RhoC expression, and decreases pseudopodia formation and migration in cancer cell lines. Cancer Lett 328:252–260

Wolfe KJ, Rena HY, Trepte P et al (2013) The Hsp70/90 cochaperone, Sti1, suppresses proteotoxicity by regulating spatial quality control of amyloid-like proteins. Mol Biol Cell 24:3588–3602

Woods IG, Wilson C, Friedlander B et al (2005) The zebrafish gene map defines ancestral vertebrate chromosomes. Genome Res 15:1307–1314

Xu C, Liu J, Hsu LC et al (2011) Functional interaction of Heat Shock protein 90 and beclin 1 modulates toll-like receptor-mediated autophagy. FASEB J 25:2700–2710

Yamamoto S, Subedi GP, Hanashima S et al (2014) ATPase Activity and ATP-dependent conformational change in the co-chaperone HSP70/HSP90-organizing protein (HOP). J Biol Chem 289:9880–9886

Yi F, Regan L (2008) A novel class of small molecule inhibitors of Hsp90. ACS Chem Biol 3:645–654

Zanata SM, Lopes MH, Mercadante AF et al (2002) Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. EMBO J 21:3307–3316

Zhang Z, Quick MK, Kanelakis KC et al (2003) Characterization of a plant homolog of hop, a cochaperone of hsp90. Plant Physiol 131:525–535

Chapter 4
Specification of Hsp70 Function by Type I and Type II Hsp40

Douglas M. Cyr and Carlos H. Ramos

Abstract Cellular homeostasis and stress survival requires maintenance of the proteome and suppression of proteotoxicity. Molecular chaperones promote cell survival through repair of misfolded proteins and cooperation with protein degradation machines to discard terminally damaged proteins. Hsp70 family members play an essential role in cellular protein metabolism by binding and releasing nonnative proteins to facilitate protein folding, refolding and degradation. Hsp40 family members are Hsp70 co-chaperones that determine the fate of Hsp70 clients by facilitating protein folding, assembly, and degradation. Hsp40s select substrates for Hsp70 via use of an intrinsic chaperone activity to bind non-native regions of proteins. During delivery of bound cargo Hsp40s employ a conserved J-domain to stimulate Hsp70 ATPase activity and thereby stabilize complexes between Hsp70 and nonnative proteins. Type I and Type II Hsp40s direct Hsp70 to preform multiple functions in protein homeostasis. This review describes the mechanisms by which Type I and Type II sub-types of Hsp40 bind and deliver substrates to Hsp70.

Keywords Hsp70 · Hsp40 · Protein folding · Molecular chaperone

Introduction

The Hsp40 family of co-chaperone proteins plays a role in cell stress protection, folding of nascent polypeptides, refolding of denatured or aggregated proteins, modulation of amyloid formation, protein degradation, and protein translocation. There are 44 *Hsp40* genes present in the human genome and 20 Hsp40s identified in the yeast genome (Kampinga and Craig 2010; Buchberger et al. 2010;

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_4

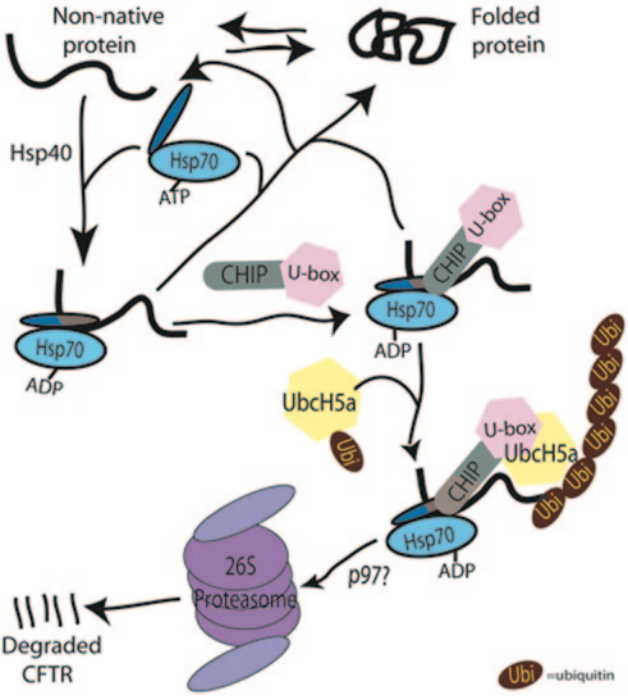

Fig. 4.1 Model for regulation of the Hsp70 polypeptide binding and release cycle by Hsp40. Hsp70 has low substrate affinity in the ATP bound state but upon hydrolysis of ATP stable Hsp-70substrate complexes are formed. Hsp70-substrate complexes then disassociate upon regeneration of Hsp70-ATP. In this model, Hsp40 acts to (1) deliver substrates to Hsp70 and (2) stimulate the ATPase activity of Hsp70. This cycle is repeated numerous times until the substrate protein is able to reach a native state. Co-chaperones such as the E3 ligase CHIP act downstream of Hsp40 to target Hsp70 clients to the proteasome for degradation

Kim et al. 2013; Cyr et al. 1994). These proteins were identified by the presence of a conserved J-domain that stimulates the ATPase activity of the Hsp70 (Fig. 4.1; Cyr et al. 1992; Liberek et al. 1991). Type I and Type II Hsp40s also have the conserved ability to bind and deliver non-native proteins to Hsp70, which is essential for life (Johnson and Craig 2001).

Type I Hsp40s are descendants of bacterial DnaJ and contain the J domain, followed by a glycine/phenylalanine rich region (G/F), a zinc finger like region (ZFLR), and a conserved C-terminal domain. The Type II Hsp40's are similar to the type I Hsp40s, but instead of the zinc finger like region they contain a glycine/methionine rich region. Type III Hsp40s contain the J-domain, but none of the other conserved domains found in Type I or II Hsp40s. Instead, they often have specialized domains that localize them to certain areas of the cell and provide specificity in substrate binding (Grove et al. 2011; Houck et al. 2014; Summers et al. 2013; Douglas et al. 2009). Type I and Type II Hsp40s contain a C-terminal dimerization

domain, but this does not mean that all Hsp40s function as dimers. However, the J-domains of Type III Hsp40s form dimers (Mokranjac et al. 2003), and the transmembrane Hsp40s DnaJB12 and DnaJB14, which lack a canonical dimerization domain, form heterodimers (Goodwin et al. 2014; Sopha et al. 2012). Thus, in many instances dimeric Hsp40s interact with Hsp70, but a general requirement for dimerization in Hsp40 function has not been demonstrated.

Hsp40s are conserved across species and are found in organisms from bacteria to humans, and a variety of Type I, Type II, and Type III Hsp40s are found in the same subcellular organelles where they can play specialized roles (Kampinga and Craig 2010). In order to better understand the cellular processes that these chaperones facilitate, we must first understand the mechanism by which Hsp40s bind substrates and regulate Hsp70 function. In the following sections, we will review the genetic, biochemical, cell biological, and structural data that have helped elucidate the unique mechanisms that different Hsp40s use to maintain protein homeostasis.

Hsp70 Co-Chaperone Activity of Hsp40s

The affinity of Hsp70 for polypeptides is regulated by its nucleotide bound state. In the ATP bound form, Hsp70 has a low affinity for substrate proteins. However, upon hydrolysis of the ATP to ADP, Hsp70 undergoes a conformational change that increases its affinity for substrate proteins (Fig. 4.1). Hsp70 goes through repeated cycles of ATP hydrolysis and nucleotide exchange, which permits cycles of substrate binding and release.

The Hsp70 proteins are assisted and regulated by several different co-chaperones. These co-chaperones have been shown to not only regulate different steps of the ATPase cycle of Hsp70 (Fig. 4.1), but they also have an individual specificity such that one co-chaperone may promote folding of a substrate while another may promote degradation. For example, the Hsp40 DnaJB12 and ubiquitin ligase CHIP both promote the degradation of Hsp70 bound substrates (Meacham et al. 2001; Cyr et al. 2002; Grove et al. 2011). On the other hand, the Hsp40 co-chaperones Hdj2 and Ydj1 promote protein folding (Meacham et al. 1999; Cyr and Douglas 1994; Fan et al. 2005a). The yeast Hsp40 Sis1 functions in spatial protein quality control (Douglas et al. 2008, 2009) and promotes protective aggregation of amyloid-like proteins (Wolfe et al. 2013, 2014). The Hsp40 proteins are classified as co-chaperones for Hsp70 due to the fact that they can use their various domain structures to (1) bind Hsp70 (2) help load the substrates on Hsp70 and (3) stimulate the ATPase activity of Hsp70 (Summers et al. 2009a; Cyr 2008). The general ability of Hsp40s to load substrates onto Hsp70 explains why Hsp40s are essential. The mechanism by which Hsp40s bind and interact with Hsp70s has been reviewed in detail, but many questions remain unanswered (Ramos et al. 2008; Sha et al. 2000; Fan et al. 2003, 2004, 2005a; Lee et al. 2002)..Therefore, in the remainder of this chapter we will focus on the question of how Hsp40s bind unfolded proteins.

Do Hsp40s Act as Chaperones?

It is established that Hsp40s specify Hsp70 function, but the manner by which Hsp40s bind and deliver substrates to Hsp40 is not completely understood (Fan et al. 2003; Summers et al. 2009a). Type I and Type II Hsp40s act independently as chaperones, so we will discuss the data that describes how they bind and transfer substrates to Hsp70.

The first observations of intrinsic chaperone activity of an Hsp40 came from studying the bacterial type I Hsp40, DnaJ (Langer et al. 1992) when purified DnaJ protein was shown to suppress the aggregation of denatured rhodanese. Subsequently, the yeast Hsp40 Ydj1 was shown to have the conserved ability to suppress protein aggregation (Cyr 1995; Lu and Cyr 1998a, b) and assist Hsp70 in refolding denatured proteins. These studies were the first to show that DnaJ and its eukaryotic relatives could not only bind denatured substrates, but it could also prevent the aggregation of those denatured substrates, thereby categorizing Type I Hsp40s as chaperones.

Studies with the yeast Sis1 protein, have shown that Type II Hsp40s can also bind chemically denatured luciferase and reduced α-lactalbumin and that this binding is dependent on specific residues within the C-terminal peptide-binding domain (Sha et al. 2000; Lee et al. 2002). This ability of Sis1 to recognize and bind non-native polypeptides classifies Sis1 as a chaperone. However, Sis1 alone is not as effective of a chaperone as the Type I Hsp40s because Sis1 cannot prevent the aggregation of thermally denatured luciferase nor does it hold the thermally denatured luciferase in a folding competent state. However, Sis1 is able to hold chemically denatured luciferase in a folding competent state (Lee et al. 2002) and also binds specific residues in yeast prions to promote prion propagation (Douglas et al. 2008). The human Hsp40, Hdj-1, also has the ability to bind non-native proteins and it's ability to recognize proline-rich regions of proteins (Lee et al. 2002) appears to make it susceptible to inactivation by huntingtin protein (Park et al. 2013). The inactivation of Sis1 by huntingtin is associated with inhibition of the proteasome and may contribute to huntingtin toxicity in the brain.

Determination of Functional Specificity

Type I and Type II Hsp40s bind exhibit different substrate specificity and direct Hsp70 to preform different functions *in vivo*. (Theodoraki and Caplan 2012; Fan et al. 2004; Rudiger et al. 2001; Caplan et al. 1992a, b, 1993; Luke et al. 1991). Sequence analysis reveals two possible regions that may be responsible for specifying this difference in function between the Type I and Type II Hsp40s. First the G/F rich region of Ydj1 and Sis1 are different, with that of Sis1 containing a 10 residue long insert containing the amino acids, GHAFSNEDAF (Yan and Craig 1999). Second, as mentioned previously, the protein modules located in the middle of Ydj1 and Sis1 are different such that Ydj1 contains the ZFLR and Sis1 contains the G/M region

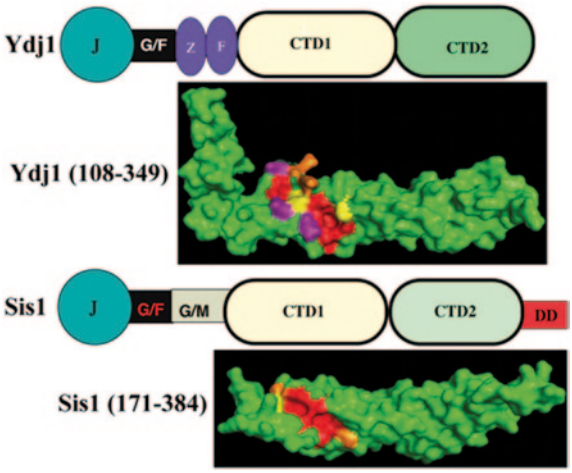

Fig. 4.2 Domain structures of Type I and Type II Hsp40 proteins. Ydj1 and Sis1 are yeast Hsp40s that are representative of Type I and Type II Hsp40 sub-types. The panels below the cartoons are models of Hsp40 substrate-binding domains built from X-ray structures of indicated Sis1 and Ydj1 fragments. *Red* denotes solvent exposed hydrophobic residues on the surface of the models. *J* J-domain, *G/F* glycine/phenylalanine rich region, *ZFLR* zinc finger-like region, *G/M* glycine/methionine rich region, *CTD1* carboxyl-terminal domain I, *CTD11* carboxyl terminal domain II, *DD* dimerization domain

(Fig. 4.2). Thus, it is plausible that either the G/F domain or the central domain (ZFLR vs G/M CTD1) of Ydj1 and Sis1 serve to specify their *in vivo* functions (Ramos et al. 2008; Fan et al. 2004). Below we will discuss the studies that were carried out in order to determine whether either of these differences has a role in specifying the functions of the Type I proteins versus the Type II proteins.

The G/F Region

To determine whether the G/F regions of Type I and Type II Hsp40s help specify Hsp70 functions the Craig group has carried out a number of complementation studies with Hsp40 fragments (Johnson and Craig 2001; Yan and Craig 1999). In these studies, which were conducted with a sis1Δ strain, the G/F region of Sis1, but not that of Ydj1, was shown to be important for suppression of lethality caused by the loss of Sis1 function. Deletion of the G/F region also prevents Sis1 from maintaining the prion state of RNQ1, while truncated versions of Sis1 containing just the J domain and G/F region (Sis1 1-121) can functionally substitute for wild type Sis1 (Aron et al. 2007). Deletion of one of the unique insertions of the Sis1 G/F region (Sis1 1-121 Δ101-113) causes a defect in cell growth in the absence of wild type Sis1, thereby suggesting that the unique insertion of the G/F region is at least partially responsible for specifying the *in vivo* functions of the Sis1 protein.

Sis1 ΔG/F still binds denatured luciferase and the RNQ1 protein, and Sis1 ΔG/F still stimulates the Hsp70 ATPase activity. The function that was lacking in the Sis1 ΔG/F protein was the ability to cooperate with Hsp70 to refold denatured substrates (Aron et al. 2005, 2007; Sondheimer et al. 2001; Johnson and Craig 2001). Sis1 ΔG/F can still bind substrates and stimulate ATPase activity, so the defect likely comes from an inability to efficiently transfer substrates from Sis1 to Hsp70. In support of this conclusion mutation of the conserved ASP-ILE-PHE (DIF) motif in the G/F region interferes with functions of Hsp40s that occur after J-domain dependent hydrolysis of ATP by Hsp70 (Cajo et al. 2006). Molecular details of G/F region action in Hsp40 function require further study (Wall et al. 1995), and this is an important topic because this domain clearly plays a critical role in regulation of Hsp70 function.

Central Domains

In addition to the differences found in the G/F regions, the central domains of the Type I and Type II Hsp40s also have dramatic structural differences(Borges et al. 2012; Silva et al. 2011; Ramos et al. 2008). The central domain of the Type II Hsp40s contains the G/M region and a polypeptide-binding site found in CTD1, while the Type I Hsp40s contain a ZFLR that is adjacent to CTDI. The differences in the substrate binding domains will be discussed in the next section, so for now we will concentrate on how the G/M region versus the ZFLR may help specify function. Studies with the full length Sis1 protein indicate that the G/M region has some overlapping function with the G/F region. As discussed above, deletion of unique residues within the G/F region has deleterious effects on cell growth in cells that only have a truncated version of Sis1 containing the J domain and G/F region (Aron et al. 2005; Johnson and Craig 2001). However, in cells expressing the full length Sis1, deletion of the same unique residues, Sis1 Δ101-113, no longer effects cell growth at normal temperatures. These cells also maintain the prion state of RNQ1. Likewise, deletion of the G/M region from the full-length protein (Sis 1 ΔG/M) has no effect on cell growth at normal temperatures and has a very mild effect on the maintenance of the RNQ1 prion. However, deletion of both the G/M and the unique residues within the G/F region from the full-length protein (Sis 1 ΔG/M Δ101-113) prevents the maintenance of the RNQ1 prion. These studies indicate that the essential function of Sis1 is actually specified by both the G/M region and the unique residues within the G/F region (Aron et al. 2005; Johnson and Craig 2001).

Studies of the ZFLR of Type I Hsp40s has also provided clues as to why the function of the Type I proteins is unique from the Type II proteins. While the central domain of the Type I Hsp40s, the ZFLR, has been implicated as a component of the polypeptide binding site in combination with CTDI. The exact role of the ZFLR is not completely clear. A NMR structure of the ZFLR reveals a V-shaped groove with an extended B-hairpin topology, which could potentially be involved in protein:protein interactions (Martinez-Yamout et al. 2000). A proteolytic fragment of Ydj1, Ydj1 (179–384), which is missing the J-domain and the first zinc binding

module of the ZFLR is capable of suppressing protein aggregation and therefore must retain the ability to bind substrates (Lu and Cyr 1998a, b). Therefore, while these studies do not rule out the possibility that the ZFLR is involved with polypeptide binding, it is definitely not required for polypeptide binding.

Mutation of the ZFLR does reveal that this domain is necessary to cooperate with Hsp70 in folding reactions (Fan et al. 2005b; Linke et al. 2003). In order to determine why the ZFLR is necessary to cooperate with Hsp70, yeast cells expressing a zinc-binding domain 2 (ZBD2) mutant of Ydj1 were examined. These cells show a decrease in the activity of the androgen receptor (AR), which is a known Hsp70 substrate. Isolation of androgen receptor complexes revealed that mutation of the ZFLR of Ydj1 leads to the accumulation of Hsp40-AR complexes with the concomitant decrease in Hsp70-AR complexes. Therefore, it seems that one important role of the ZFLR is to stimulate the transfer of substrates from Hsp40 to Hsp70 (Summers et al. 2009a).

In order to directly decipher the involvement of the ZFLR versus the G/M CTD1 central domains in specifying Hsp40 function, chimeric forms of Ydj1 and Sis1 were constructed in which the central domains were swapped to form YSY and SYS (Fan et al. 2004). Purified SYS and YSY were found to exhibit protein-folding activity and substrate specificity that mimicked that of Ydj1 and Sis1, respectively. *In vivo* studies also showed that YSY exhibited a gain of function, and unlike Ydj1, could complement the lethal phenotype of sis1Δ and promote the propagation of the yeast prion [RNQ1 +]. SYS exhibited a loss of function and was unable to maintain [RNQ1 +]. These *in vitro* and *in vivo* data suggest that the central domain of Ydj1 and Sis1 are exchangeable and that they help specify Hsp40's cellular functions.

Substrate Binding Domains

The studies discussed above suggest that the unique residues in the G/F region and the different central domains may help specify the function of the Type I vs Type II proteins by affecting the manner in which the individual chaperones interact with or transfer substrates to Hsp70. Another important determinant of specificity could obviously come from the substrate binding domains themselves. Since the Type I proteins do prefer to bind peptides that are distinct from those that the Type II proteins bind (Fan et al. 2004), one would hypothesize that there are differences in the substrate binding domains of these two types of proteins. Studies have shown that the substrate binding domains of both Type I and Type II Hsp40s are found in CTDI (Fig. 4.2; Sha et al. 2000; Qian et al. 2002). For example, the carboxyl terminus of the Ydj1 protein (residues 206-380) was shown to be at least partially responsible for polypeptide binding, and a single point mutation in this C-terminal domain (Ydj1 G315D) exhibits severe defects in cellular function and polypeptide binding (Lu and Cyr 1998a; Kimura et al. 1995). A fragment of Ydj1 consisting of residues 179-384 was also shown to be able to suppress rhodanese aggregation to the same level as the full length protein (Lu and Cyr 1998a). This fragment lacks the J domain, the G/F region and the first zinc-binding domain, but contains the

C-terminal domain. Studies of the yeast Type II Hsp40, Sis1, have also localized the polypeptide-binding site to CTDI (Sha et al. 2000; Lee et al. 2002). Therefore, similar regions within Ydj1 and Sis1 are implicated in polypeptide binding.

Crystal structures of the C-terminal domains of both Ydj1 and Sis1 have been solved (Fig. 4.2). These structures confirm that the C-terminal domain is a site for peptide binding for both types of Hsp40s and they suggest similar yet unique mechanisms for substrate binding. The Ydj1 crystal structure is of the monomeric form of a truncated C-terminal domain (Ydj1 102-350) in complex with a short peptide substrate, GWLYEIS (Li et al. 2003; Li and Sha 2003). There are two hydrophobic depressions, one in domain 1 and one in domain 3. The crystal structure shows that the peptide substrate binds to Ydj1 by forming an extra β-strand in the domain 1 depression. There is also an interaction in which the L from the peptide is buried in a small hydrophobic pocket found in this surface depression. The pocket that the L is buried in is formed by a variety of highly conserved hydrophobic residues (I116, L135, L137, L216, and P249), thereby suggesting that the pocket may be a common feature found in Type I Hsp40s, and may play a role in determining the substrate specificity.

The X-ray crystal structure of Sis1 171-352 was also solved and it depicts a homodimer that has a crystallographic two-fold axis (Sha et al. 2000; Qian et al. 2002). Sis1 171-352 monomers are elongated and constructed from two barrel-like domains that have similar folds and mostly β-structure. Sis1 dimerizes through a short C-terminal α-helical domain, and the dimer has a wishbone shape with a cleft that separates the arms of the two elongated monomers. CTDI on each monomer also contains two shallow depressions that are lined by highly conserved solvent exposed hydrophobic residues (Fig. 4.2). Mutational analysis of the residues that line the hydrophobic depression in Sis1 has identified K199, F201 and F251 as amino acids that are essential for cell viability and required for Sis1 to both bind denatured substrates and cooperate with Hsp70 to refold those substrates (Lee et al. 2002; Fan et al. 2004). Interestingly, peptides from the C-terminal lid domain of Hsp70 are also bound by in the hydrophobic depression on CTDI (Qian et al. 2002). It is therefore possible that Hsp70 and substrates interact with Sis1 at similar sites. If true, then Hsp70 might displace substrate from Hsp40s to drive substrate transfer from Hsp40 to Hsp70 polypeptide binding domain (Kota et al. 2009; Summers et al. 2009a).

Hsp40 Quaternary Structure

A common feature of Type I and Type II Hsp40s is that dimerization is important for them to function *in vivo* (Summers et al. 2009a, b). There are no crystal structures of full length Type I or Type II Hsp40s, but small angle X-ray scattering (SAXS) and protein modeling have been used to build models of the quaternary structure of Type I and Type II Hsp40s (Borges et al. 2005; Ramos et al. 2008). These models suggest that there are substantial differences in the quaternary structure of the Type

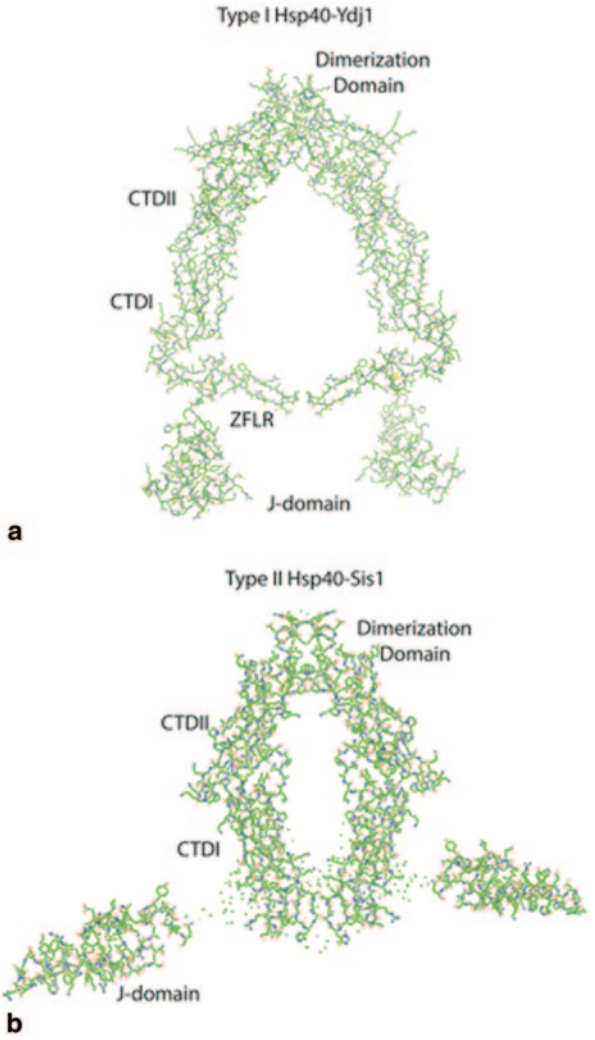

Fig. 4.3 Models of Type I and Type II quaternary structures. *Ab initio* models of Type I and Type II Hsp40s were generated by SAXS and molecular modeling of Ydj1 and Sis1. Models depicted were of Ydj1 and Sis1 dimers generated by pymol from data presented in Ramos et al. 2008

I and Type II Hsp40s that may help account for their ability to direct Hsp70 to preform different cellular functions (Fig. 4.3). In Type I Hsp40 the interface between CTDI and CTD2 and the ZFLR space the polypeptide binding pockets in CTDI and appear to impact the orientation for the J-domain relative to the long-axis of the chaperone (Silva et al. 2011). In Type II Hsp40s CDTI on the arms of the different dimers are closer together, and the J-domains are splayed to the side of the chaperone. It appears that J-domains can exist in a dimeric state, while the models depicted show the J-domains of Ydj1 and Sis1 as monomers. It is therefore possible that these models depict an inactive state of Ydj1 and Sis1 and that forms of these Hsp40s that regulate Hsp70 ATPase activity under go a conformational change to permit J-domain dimerization (Mokranjac et al. 2003; Goodwin et al. 2014).

A driving force for this putative conformational change might be the binding of polypeptides to the hydrophobic pocket in CTDI and downstream conformational changes. This model is hypothetical, and requires that an Hsp40 dimerize, and it is not clear that all Hsp40s are dimers. Never the less, this hypothesis suggests a substrate dependent mechanism for regulating some of the interactions that occur between Hsp40 and Hsp70.

While it appears that the unique structures of Type I and Type II Hsp40s almost certainly specify function, the exact mechanism by which these structures specify function is not clear. A combination of all the unique characteristics of the Type I and Type II chaperones discussed above likely explains the difference levels of chaperone and co-chaperone activity observed for these different types of Hsp40s.

Acknowledgements Work in the laboratory of DMC is supported by the National Institutes of Health 5R01GM056981. CHR is supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Ministério da Ciência e Tecnologia/Conselho Nacional de Pesquisa e Desenvolvimento (MCT/CNPq), and NIH-R03TW007437 through the Fogarty International Center.

References

Aron R, Lopez N, Walter W, Craig EA, Johnson J (2005) In vivo Bipartite Interaction between the Hsp40 Sis1 and Hsp70 in *Saccharomyces cerevisiae*. Genetics 169:1873–1882

Aron R, Higurashi T, Sahi C, Craig EA (2007) J-protein co-chaperone Sis1 required for generation of [RNQ(+)] seeds necessary for prion propagation. EMBO J 26(16):3794–3803

Borges JC, Fischer H, Craievich AF, Ramos CH (2005) Low-resolution structural study of two human Hsp40 chaperones in solution. DjA1 from subfamily A and DjB4 from subfamily B have different quaternary structures. J Biol Chem 280:13671–13681

Borges JC, Seraphim TV, Mokry DZ, Almeida FC, Cyr DM, Ramos CH (2012) Identification of regions involved in substrate binding and dimer stabilization within the central domains of yeast Hsp40 Sis1. PLoS One 7:e50927

Buchberger A, Bukau B, Sommer T (2010) Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell 40:238–252

Cajo GC, Horne BE, Kelley WL, Schwager F, Georgopoulos C, Genevaux P (2006) The role of the DIF motif of the DnaJ (Hsp40) co-chaperone in the regulation of the DnaK (Hsp70) chaperone cycle. J Biol Chem 281:12436–12444

Caplan AJ, Cyr DM, Douglas MG (1992a) YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. Cell 71:1143–1155

Caplan AJ, Tsai J, Casey PJ, Douglas MG (1992b) Farnesylation of YDJ1p is required for function at elevated growth temperatures in *Saccharomyces cerevisiae*. J Biol Chem 267:18890–18895

Caplan AJ, Cyr DM, Douglas MG (1993) Eukaryotic homologues of *Escherichia coli* dnaJ: a diverse protein family that functions with hsp70 stress proteins. Mol Biol Cell 4:555–563

Cyr DM (1995) Cooperation of the molecular chaperone Ydj1 with specific Hsp70 homologs to suppress protein aggregation. FEBS Lett 359:129–132

Cyr DM (2008) Swapping nucleotides, tuning Hsp70. Cell 133(6):945–947

Cyr DM, Douglas MG (1994) Differential regulation of Hsp70 subfamilies by the eukaryotic DnaJ homologue YDJ1. J Biol Chem 269:9798–9804

Cyr DM, Lu X, Douglas MG (1992) Regulation of Hsp70 function by a eukaryotic DnaJ homolog. J Biol Chem 267:20927–20931

Cyr DM, Langer T, Douglas MG (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. Trends Biochem Sci 19:176–181

Cyr DM, Hohfeld J, Patterson C (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. Trends Biochem Sci 27:368–375

Douglas PM, Treusch S, Ren HY, Halfmann R, Duennwald ML, Lindquist S, Cyr DM (2008) Chaperone-dependent amyloid assembly protects cells from prion toxicity. Proc Natl Acad Sci U S A 105:7206–7211

Douglas PM, Summers DW, Ren HY, Cyr DM (2009) Reciprocal efficiency of RNQ1 and polyglutamine detoxification in the cytosol and nucleus. Mol Biol Cell 20:4162–4173

Fan CY, Lee S, Cyr DM (2003) Mechanisms for regulation of Hsp70 function by Hsp40. Cell Stress Chaperones 8:309–316

Fan CY, Lee S, Ren HY, Cyr DM (2004) Exchangeable chaperone modules contribute to specification of type I and type II Hsp40 cellular function. Mol Biol Cell 15:761–773

Fan CY, Ren HY, Lee P, Caplan AJ, Cyr DM (2005a) The type I Hsp40 zinc finger-like region is required for Hsp70 to capture non-native polypeptides from Ydj1. J Biol Chem 280:695–702

Fan CY, Ren HY, Lee P, Caplan AJ, Cyr DM (2005b) The type I Hsp40 zinc finger-like region is required for Hsp70 to capture non-native polypeptides from Ydj1. J Biol Chem 280:695–702

Goodwin EC, Motamedi N, Lipovsky A, Fernandez-Busnadiego R, DiMaio D (2014) Expression of DNAJB12 or DNAJB14 causes coordinate invasion of the nucleus by membranes associated with a novel nuclear pore structure. PLoS One 9:e94322

Grove DE, Fan CY, Ren HY, Cyr DM (2011) The endoplasmic reticulum-associated Hsp40 DNAJB12 and Hsc70 cooperate to facilitate RMA1 E3-dependent degradation of nascent CFTRDeltaF508. Mol Biol Cell 22:301–314

Houck SA, Ren HY, Madden VJ, Bonner JN, Conlin MP, Janovick JA, Conn PM, Cyr DM (2014) Quality control autophagy degrades soluble ERAD-resistant conformers of the misfolded membrane protein GnRHR. Mol Cell 54:166–179

Johnson JL, Craig EA (2001) An essential role for the substrate-binding region of Hsp40s in *Saccharomyces cerevisiae*. J Cell Biol 152:851–856

Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579–592

Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355

Kimura Y, Yahara I, Lindquist S (1995) Role of the protein chaperone YDJ1 in establishing Hsp90-mediated signal transduction pathways. Science 268:1362–1365

Kota P, Summers DW, Ren HY, Cyr DM, Dokholyan NV (2009) Identification of a consensus motif in substrates bound by a Type I Hsp40. Proc Natl Acad Sci U S A 106:11073–11078

Langer T, Lu C, Echols H, Flanagan J, Hayer MK, Hartl FU (1992) Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding. Nature 356:683–689

Lee S, Fan CY, Younger JM, Ren H, Cyr DM (2002) Identification of essential residues in the type II Hsp40 Sis1 that function in polypeptide binding. J Biol Chem 277:21675–21682

Li J, Sha B (2003) Preliminary X-ray crystallographic studies of yeast Hsp40 Ydj1 complexed with its peptide substrate. Acta Crystallogr D Biol Crystallogr 59:1317–1319

Li J, Qian X, Sha B (2003) The crystal structure of the yeast hsp40 ydj1 complexed with its Peptide substrate. Structure 11:1475–1483

Liberek K, Marszalek J, Ang D, Georgopoulos C, Zylicz M (1991) *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc Natl Acad Sci U S A 88:2874–2878

Linke K, Wolfram T, Bussemer J, Jakob U (2003) The roles of the two zinc binding sites in DnaJ. J Biol Chem 278:44457–44466

Lu Z, Cyr DM (1998a) The conserved carboxyl terminus and zinc finger-like domain of the co-chaperone Ydj1 assist Hsp70 in protein folding. J Biol Chem 273:5970–5978

Lu Z, Cyr DM (1998b) Protein folding activity of Hsp70 is modified differentially by the hsp40 co-chaperones Sis1 and Ydj1. J Biol Chem 273:27824–27830

Luke MM, Sutton A, Arndt KT (1991) Characterization of SIS1, a *Saccharomyces cerevisiae* homologue of bacterial dnaJ proteins. J Cell Biol 114:623–638

Martinez-Yamout M, Legge GB, Zhang O, Wright PE, Dyson HJ (2000) Solution structure of the cysteine-rich domain of the *Escherichia coli* chaperone protein DnaJ. J Mol Biol 300:805–818

Meacham GC, Lu Z, King S, Sorscher E, Tousson A, Cyr DM (1999) The Hdj-2/Hsc70 chaperone pair facilitates early steps in CFTR biogenesis. EMBO J 18:1492–1505

Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nature Cell Biol 3:100–105

Mokranjac D, Sichting M, Neupert W, Hell K (2003) Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria. EMBO J 22:4945–4956

Park SH, Kukushkin Y, Gupta R, Chen T, Konagai A, Hipp MS, Hayer-Hartl M, Hartl FU (2013) PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. Cell 154:134–145

Qian X, Hou W, Zhengang L, Sha B (2002) Direct interactions between molecular chaperones heat-shock protein (Hsp) 70 and Hsp40: yeast Hsp70 Ssa1 binds the extreme C-terminal region of yeast Hsp40 Sis1. Biochem J 361:27–34

Ramos CH, Oliveira CL, Fan CY, Torriani IL, Cyr DM (2008) Conserved central domains control the quaternary structure of type I and type II Hsp40 molecular chaperones. J Mol Biol 383:155–166

Rudiger S, Schneider-Mergener J, Bukau B (2001) Its substrate specificity characterizes the DnaJ co-chaperone as a scanning factor for the DnaK chaperone. EMBO J 20:1042–1050

Sha B, Lee S, Cyr DM (2000) The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1. Structure 8:799–807

Silva JC, Borges JC, Cyr DM, Ramos CH, Torriani IL (2011) Central domain deletions affect the SAXS solution structure and function of yeast Hsp40 proteins Sis1 and Ydj1. BMC Struct Biol 11:40. doi:10.1186/1472-6807-11-40

Sondheimer N, Lopez N, Craig EA, Lindquist S (2001) The role of Sis1 in the maintenance of the [RNQ+] prion. EMBO J 20:2435–2442

Sopha P, Kadokura H, Yamamoto YH, Takeuchi M, Saito M, Tsuru A, Kohno K (2012) A novel mammalian ER-located J-protein, DNAJB14, can accelerate ERAD of misfolded membrane proteins. Cell Struct Funct 37:177–187

Summers DW, Douglas PM, Ramos CH, Cyr DM (2009a) Polypeptide transfer from Hsp40 to Hsp70 molecular chaperones. Trends Biochem Sci 34:230–233

Summers DW, Douglas PM, Ren HY, Cyr DM (2009b) The type I Hsp40 Ydj1 utilizes a farnesyl moiety and zinc finger-like region to suppress prion toxicity. J Biol Chem 284:3628–3639

Summers DW, Wolfe KJ, Ren HY, Cyr DM (2013) The Type II Hsp40 Sis1 cooperates with Hsp70 and the E3 ligase Ubr1 to promote degradation of terminally misfolded cytosolic protein. PLoS One 8:e52099

Theodoraki MA, Caplan AJ (2012) Quality control and fate determination of Hsp90 client proteins. Biochim Biophys Acta 1823:683–688

Wall D, Zylicz M, Georgopoulos C (1995) The conserved G/F motif of the DnaJ chaperone is necessary for the activation of the substrate binding properties of the DnaK chaperone. J Biol Chem 270:2139–2144

Wolfe KJ, Ren HY, Trepte P, Cyr DM (2013) The Hsp70/90 cochaperone, Sti1, suppresses proteotoxicity by regulating spatial quality control of amyloid-like proteins. Mol Biol Cell 24:3588–3602

Wolfe KJ, Ren HY, Trepte P, Cyr DM (2014) Polyglutamine-rich suppressors of huntingtin toxicity act upstream of hsp70 and sti1 in spatial quality control of amyloid-like proteins. PLoS One 9:e95914

Yan W, Craig EA (1999) The glycine-phenylalanine-rich region determines the specificity of the yeast Hsp40 Sis1. Mol Cell Biol 19:7751–7758

Chapter 5
Cdc37 as a Co-chaperone to Hsp90

Stuart K. Calderwood

Abstract The co-chaperone p50/Cdc37 is an important partner for Hsp90, assisting in molecular chaperone activities, particularly with regard to the regulation of protein kinases. The Hsp90/Cdc37complex controls the folding of a large proportion of protein kinases and thus stands at the hub of a multitude of intracellular signaling networks. Its effects thus reach beyond the housekeeping pathways of protein folding into regulation of a wide range of cellular processes. Due to its influence in cell growth pathways Cdc37 has attracted much attention as a potential intermediate in carcinogenesis. Cdc37 is an attractive potential target in cancer due to: (1) it may be expressed to high level in some types of cancer and (2) Cdc37 controls multiple signaling pathways. This indicates a potential for: (1) selectivity due to its elevated expression and (2) robustness as the co-chaperone may control multiple growth signaling pathways and thus be less prone to evolution of resistance than other oncoproteins. Cdc37 may also be involved in other aspects of pathophysiology. Protein aggregation disorders have been linked to molecular chaperones and to age related declines in molecular chaperones and co-chaperones. Cdc37 appears to be a potential agent in longevity due to its links to protein folding and autophagy and it will be informative to study the role of Cdc37 maintenance/decline in aging organisms.

Keywords Cdc37 · Hsp90 · Protein kinase · Cancer

This work was supported by NIH research grants RO-1CA047407, R01CA119045 and RO-1CA094397

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_5

Introduction

Folding of many proteins in the cell to a fully functional conformation requires the influence of molecular chaperones (Ellis 2007). Such molecules appear to be required to inhibit the formation of alternatively folded conformations that lack canonical gene function, and permit the majority of the translated protein to assume its functional shape. It is now accepted by many that molecular chaperones play essential roles in cellular pathology as well as in normal function and roles for chaperone overexpression in tumorigenesis and tumor progression have been described while failure in chaperone function may underlie processes in aging (Calderwood et al. 2009; Ciocca et al. 2013; Ciocca and Calderwood 2005; Jinwal et al. 2012). The underlying causes of increased chaperone expression in cancer and loss of chaperone activity in aging have not currently been deduced. It has been assumed that elevated amounts of proteins with or without oncogenic mutations accumulate in cancer increasing the "folding burden placed on cancer cells (Calderwood and Gong 2012). However direct proof for such a hypothesis is required. It is known that chaperones such as Hsp27, Hsp70 and Hsp90 often undergo enhanced expression during cancer development and play roles in many of the key steps in cancer development such as acquisition of independent growth, escape from oncogene mediated programmed cell death and senescence, *de novo* angiogenesis invasion and metastasis (Calderwood et al. 2006; Ciocca and Calderwood 2005). This may point to key regulatory roles for the chaperones in cancer. In addition Hsp27, Hsp70 and Hsp90 are all regulated at the transcriptional level primarily by heat shock factor 1 (HSF1) a protein that responds to both stress and cancer signals, leading to potent HSP synthesis and enhanced tumorigenesis (Ciocca et al. 2013; Santagata et al. 2011). Interactions between HSF1 and Hsp90 are particularly intriguing, as transcriptional activation of HSF1 leads to Hsp90 increases, while Hsp90 is a potent HSF1 repressor, a tautology with some significance in cellular responses to Hsp90 targeting drugs (Boellmann et al. 2004; Zou et al. 1998). Many members of the molecular chaperone family require accessory proteins known as co-chaperones in order to function at significant rates in cells (Calderwood 2013). Co-chaperones may be decisive in the selection of Hsp90 clients within the cell and may determine the rate of polypeptide folding and the ability of chaperones to stably interact with unstable proteins (Cox and Johnson 2011). Optimal Hsp90 activity involves a wide range of co-chaperones including Sgt1, p23, Aha1, Cdc37, Hop, Cyp40, FKBP1, FKBP2, PP5 phosphatase, TTC4, TTC5 and XAP2 which regulate the chaperoning cycle as well as function and localization in the cells (Calderwood 2013; Cox and Johnson 2011). The subject of the current review is the Hsp90 co-chaperone role of Cdc37.

As would be surmised from its name, the *CDC37* gene was discovered in a screen for cell division cycle genes in S. *cereviseae* (Reed 1980). It has since been shown to be conserved to man, although Cdc37 homologs in plants have not been reported. Despite its discovery in a cell cycle screen, the Cdc37 protein apparently does not perform a traditional cell cycle checkpoint role, such as has been attributed to the cyclins and cell division kinases (cdk), and instead appears to function largely

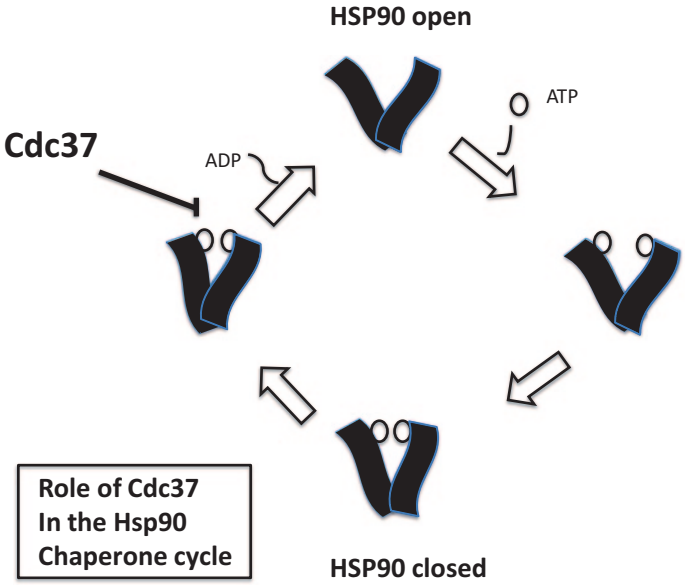

Fig. 5.1 Role of Cdc37 in the Hsp90 chaperone cycle. The figure depicts the HSP90 dimer going through a cycle of ATP binding and hydrolysis. ATP bound HSP90 is capable of client binding while ATP hydrolysis and ADP dissociation leads to client release. The bound client undergoes folding during this cycle. Cdc37 inhibits the ATP hydrolysis step and permits prolonged association of Hsp90 dimers with client proteins and more effective chaperone activity. In addition to association with Hsp90, Cdc37 also binds the client in a ternary complex (not shown). Client binding to Hsp90 and to Cdc37 both assist in molecular chaperone function

by enhancing the stability and activities of protein kinases, including cdks (Caplan et al. 2007a, b; Pearl 2005). Cdc37 has emerged as a co-chaperone that is required for the stable folding of a wide spectrum of protein kinases when complexed with Hsp90 and has thus emerged as an important signal transduction molecule (Caplan et al. 2007a; Gray et al. 2008). Indeed recent proteomic studies confirmed that Hsp90 interacted with a wide range of kinases and that the majority of these interactions were shared with Cdc37 (Taipale et al. 2012). This was in contrast with the findings regarding transcription factors, including steroid hormone receptors, only a few of which proteins seemed to interact significantly with Hsp90 or Cdc37 (Taipale et al. 2012). Hsp90 has been shown to bind to client proteins and lead to their optimal folding in a series of reactions that can be regarded as involving a cycle of ATP-dependent conformational changes within the Hsp90 molecule (Fig. 5.1; Calderwood 2013; Cox and Johnson 2011; Taipale et al. 2010). Hsp90 carries out its molecular chaperone functions as a dimer. Such Hsp90 dimers bind to the client while in an open conformation and then are converted to a more closed conformation on binding of ATP, a conformation in which substrates are tightly bound. The exact nature of the Hsp90-client binding interaction is however still somewhat obscure. Release of bound, folded client proteins from the Hsp90 involves ATP

hydrolysis, a reaction that leads to loss of affinity for the client protein (Fig. 5.1). Cdc37 binding to Hsp90 appears to inhibit this latter ATPase activity of Hsp90 and to permit prolonged interactions between chaperone and client (Fig. 5.1)(Cox and Johnson 2011). During the interaction with Hsp90, Cdc37 binds both to the client kinase as well as to hsp90 itself and both interactions are required for chaperone function (Gray et al. 2008). Cdc37 binds to the highly conserved N-loop of protein kinases and is thought to stabilize the aC-b4 loop, while Hsp90 binds both the N and C lobes (Discussed in more detail in Gray et al 2008). , Two distinct client interaction domains have been described in mammalian Cdc37, including a conserved N-terminal domain as well as a C terminal domain that is not conserved in yeast Cdc37 (Calderwood 2013). Thus Cdc37 is a molecular chaperone itself that, at least in Yeast appears to have independent protein interaction functions but that in mammalian cells more commonly operates in cooperation with Hsp90 to optimally fold the structures of protein kinases (MacLean and Picard 2003; Turnbull et al. 2005). Cdc37 has been shown to bind to the catalytic domains of a large number of client kinases, structures that appear to be conserved in all protein kinases suggesting a common mode of interaction with a range of such enzymes (Caplan et al. 2007a; Taipale et al. 2012; Vaughan et al. 2006). However, such Cdc37-kinase interactions are by no means uniform in nature. For instance Hsp90-Cdc37 complexes are required to maintain Cdk4 in folded conformation only until such kinases encounter the regulatory Cyclin D1 subunits when they then become chaperone-independent rev. (Caplan et al. 2007a). The oncogenic receptor tyrosine kinase ERBB2 by contrast requires persistent association with Hsp90-Cdc37 complexes for stability and activity (Caplan et al. 2007a). Indeed quantitative studies of Hsp90/Cdc37 complex/kinase client interactions show that even closely related kinase family members interact with quite different affinities. Another principle involved in Cdc37/client interactions seems to be that the chaperone complex binds most avidly to the more unstable protein kinases and stabilization of clients led to reduced association (Taipale et al. 2012). In addition, it has been shown that the exchange of ATP in activated kinase clients during enzymatic activity leads to their increased instability and enhanced chaperone binding (Gray et al. 2008). Interestingly, but in accordance with the prior statements, it would appear that protein kinase catalytic activities may be reduced under Cdc37/Hsp90 complex-bound conditions, as determined for the LKB1 kinase (Taipale et al. 2012). Dissociation of LKB1 from the Cdc37/Hsp90 complex led to transient activation of kinase activity prior to degradation via a pathway involving association with Hsp70 family proteins, recruitment of ubiquitin ligase CHIP and breakdown in the proteasome (Xu and Neckers 2012).

Posttranslational Modifications of Cdc37 and Hsp90

The Hsp90/Cdc37 interaction is also regulated by posttranscriptional modifications (PTM) that affect profoundly the chaperoning cycle. Both proteins appear to be substrates for casein kinase 2 (CK2), an enzyme that is in fact also a client (Miyata and

Nishida 2005). CK2 phosphorylates Cdc37 on serine 13, a modification with profound impact on function, leading to formation of stable complexes with the clients (Miyata and Nishida 2005). In addition CK2 phosphorylates Hsp90 on threonine (T) 22 in yeast (human T36), an interaction that stabilizes binding to co-chaperones Cdc37 and Aha1 (Mollapour et al. 2011). CK2 is thus a key enzyme in Hsp90/Cdc37 client folding. Recently it has been shown that tyrosine (Y) phosphorylation also has profound effects on Hsp90/Cdc37 activities. Cdc37 phosphorylation on Y4 and Y298 disrupts client association while Hsp90 phosphorylation on Y197 leads to dissociation of Cdc37. (Xu et al. 2012; Xu and Neckers 2012) The enzyme implicated in these modifications is the non-receptor tyrosine kinase Yes (Summy et al. 2003; Xu et al. 2012). The findings thus suggest profound regulation of each step of the Cdc37/Hsp90 chaperoning cycle by PTMs.

Cdc37 in Cell Proliferation and Cancer

As a cell cycle division protein, required to drive cell proliferation, it is probably not surprising that *CDC37* appears to play a positive role in tumorigenesis (Gray et al. 2008; Stepanova et al. 2000a). An early hint suggesting such a role was provided by the finding of a requirement for Cdc37, along with Hsp90 in the transforming functions of the viral oncogene p60v-src (Dey et al. 1996; Perdew et al. 1997). More conclusive evidence for a transforming role for Cdc37 was next provided by the finding that overexpression of the *cdc37* gene in transgenic mice could lead to elevated rates of prostate tumorigenesis, a process that was amplified by co-expression of the proto-oncogene c-Myc (Stepanova et al. 2000b). Subsequently other cancer types such as anaplastic large cell lymphoma, acute myeloblastic leukemia, multiple myeloma and hepatocellular carcinoma have been shown to express high levels of cdc37 (rev.) (Gray et al. 2008). The exact upstream mediator of Cdc37 tumorigenesis might be currently difficult to tie down due to the large numbers of potential Hsp90/CDc37 targets with potential roles in carcinogenesis. Probable candidates could include: (1) Activity of the androgen receptor (AR) (Heinlein and Chang 2004). While most steroid hormone receptors require Hsp90 for optimal folding and activity, only AR has been shown to be dependent on Cdc37 (Fliss et al. 1997; Rao et al. 2001). Indeed Cdc37 knockdown in AR + LnCaP cells was shown to lead to the loss of androgen-dependent AR-mediated transcriptional activity and to a reduction in target PSA expression (Gray et al. 2007). It may be significant that Cdc37 has been found to be associated with the AR co-activating protein Vav3 (Wu et al. 2013). Disruption of AR-Vav3 interactions inhibited the co-activating effects of Vav3 (Wu et al. 2013). AR has been shown to be essential for the early stages in prostate tumorigenesis and to even play unpredicted roles in castration-resistant forms of PCa. Thus a role for Cdc37 in fostering AR activity and prostate carcinogenesis might be postulated. However other promising candidates for CDC37 targets exist. Protein kinases are the preferred clients of Cdc37 and upward of 50 %

of kinases may require Cdc37 to a greater or lesser degree (Gray et al. 2008). It has been shown that the phosphatidyl inositol—3 kinase (PI-3K) pathway plays a key driving role in prostate carcinogenesis and that the PI-3K inhibitory pathway, mediated through the lipid phosphatase PTEN inhibits this process (Bitting and Armstrong 2013). Indeed, inactivation of PTEN leads to spontaneous prostate carcinogenesis. It was also shown that knockdown of Cdc37 led to inhibition of Akt, the kinase directly downstream of PI-3K as well as to inhibition of the S6 ribosomal protein, a substrate of the mTORC1 kinase complex, another enzyme regulated downstream of PI-3K (Gray et al. 2007). The mTORC1 pathway has been shown to play key roles in cancer progression by boosting the rate of translation and permitting elevated protein synthesis in cancer cells. Other potential Cdc37 dependent targets could include receptor tyrosine kinases such as EGFR and HER2/*neu* that are CDC37 clients and could also play roles in prostate cancer (Calderwood S. K. et al. in preparation), (Lavictoire et al. 2003). However, as there is currently no definitive proof for any of these pathways and other candidates such as no-receptor tyrosine kinases of the Src family as well as mutant or over-expressed KIT, MET, ALK and RAF could play roles.

Cdc37 and Cancer Treatment

The dependence of cancer, particularly prostate cancer cells, on Cdc37 suggests this molecule as a potential target. This approach would have the decided advantage of leading to multi-targeting and the potential for evasion of resistance in contrast to targeting individual oncoproteins, in which evolution of resistance is problematic. Cdc37 knockdown was shown to reduce proliferation to minimal levels in a range of malignant cell types (Gray et al. 2008; Gray et al. 2007; Smith and Workman 2009). A natural product-based drug has recently been isolated that can disrupt Hsp90/Cdc37 interactions. This compound Celastrol could thus be envisaged as a potential drug for targeting Cdc37 activity in cancer (Salminen et al. 2010). However, this compound is lacking in specificity, was shown to directly inhibit both IκκB kinase activity and the function of the proteasome and to induce HSF1 activity (Calderwood 2013). No doubt future endeavors will lead to further Cdc37-targeted drugs with higher specificity.

Roles for Cdc37 in Autophagy and Protein Aggregation Disorders

Unsurprisingly, with its versatile role in kinase activation, Cdc37 appears to play roles in cell pathology outside of cancer. The Hsp90-Cdc37 complex appears to participate in the upstream activation of autophagy, one of the primary pathways

in protein quality control and longevity (Calderwood et al. 2009). Autophagy is significant in protein homeostasis in that bulky protein aggregates or damaged organelles that cannot enter the lumen of the proteasome for proteolytic digestion can be enveloped by autophagosomes and broken down (Calderwood et al. 2009). The Cdc37-Hsp90 complex was shown to stabilizes and activate ULK1, a protein kinase that phosphorylates Atg1 one of the first steps in initiating the autophagy pathway and in this way regulated mitophagy, a specialize autophagy-like process involved in breaking down damaged mitochondria (Joo et al. 2011). Cdc37-fostered autophagy may be important in neurodegenerative diseases such as Amyotrophic Lateral Sclerosis and Alzheimer disease, that are components of the aging process and chaperone complexes may be involved in clearance of misfolded proteins through the autophagy pathway (Jinwal et al. 2012).

Conclusions

Thus Cdc37, as a major component of the protein complex that controls the folding of protein kinases in the cell stands at the hub of a multitude of intracellular signaling networks (Caplan et al. 2007a; Gray et al. 2008; Karnitz and Felts 2007). Its effects thus reach beyond the housekeeping pathways of protein folding into a wide range of cellular processes. Further developments may await more information as to the exact role of Cdc37 in molecular chaperone function.

Due to its influence in cell growth pathways Cdc37 has attracted much attention as a potential intermediate in carcinogenesis and indeed proof of concept studies in cell lines indicate that Cdc37 is required for cancer cell signaling and that quenching the influence of the co-chaperone prevents malignant cell growth (Gray et al. 2008). Cdc37 might be an attractive potential target in cancer due to (1) the fact that it may be expressed to high level in some types of cancer and (2) controls multiple signaling pathways. This indicates a potential for: (1) selectivity due to its elevated expression and (2) robustness as the co-chaperone may control multiple growth signaling pathways and may thus be less prone to evolution of resistance than for other oncoproteins. Currently specific agents to target Cdc37 are not available.

Protein aggregation disorders have been linked to molecular chaperones and to age related declines in molecular chaperones and co-chaperones (Calderwood et al. 2009). Cdc37 appears to be a potential agent in longevity due to its links to protein folding and autophagy and it will be informative to study the role of Cdc37 maintenance/decline in aging organisms. The development of agents that might increase Cdc37 levels may thus be called for to remedy aging relate shortfalls (Fig. 5.2).

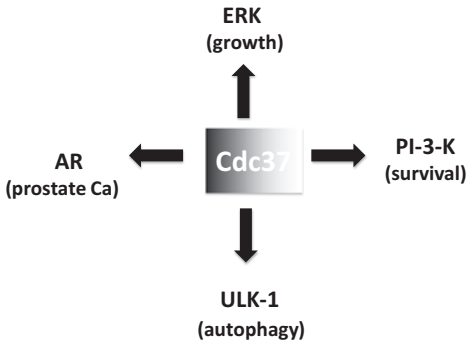

Fig. 5.2 Cdc37 controls a network of intracellular protein kinases. Cdc37 is able to bind to a wide spectrum of protein kinases through their highly conserved catalytic domain. Depicted here is Cdc37 regulation of the ERK-MAP kinase pathway, the phosphatidylinositol-3-kinase (PI-3-K) pathway, Unc-51-like kinase (ULK-1) activity and the activity of AR. The interactions with ERK and PI-3-K are not direct but involve other members of these cascade reactions. In this way, Cdc37 is able to control a wide spectrum of intracellular metabolic pathways involved in cell growth, survival, autophagy and carcinogenesis. AR seems to be exceptional in being a non-kinase client of Cdc37

References

Bitting RL, Armstrong AJ (2013) Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer. Endocr Relat Cancer 20:83–99

Boellmann F, Guettouche T, Guo Y, Fenna M, Mnayer L, Voellmy R (2004) DAXX interacts with heat shock factor 1 during stress activation and enhances its transcriptional activity. Proc Natl Acad Sci U S A 101:4100–4105

Calderwood SK (2013) Molecular cochaperones: tumor growth and cancer treatment. Scientifica 2013:217513

Calderwood SK, Gong J (2012) Molecular chaperones in mammary cancer growth and breast tumor therapy. J Cell Biochem 113:1096–1103

Calderwood SK, Khaleque MA, Sawyer DB, Ciocca DR (2006) Heat shock proteins in cancer: chaperones of tumorigenesis. Trends Biochem Sci 31:164–172

Calderwood SK, Murshid A, Prince T (2009) The shock of aging: molecular chaperones and the heat shock response in longevity and aging-a mini-review. Gerontology 55:550–558

Caplan AJ, Ma'ayan A, Willis IM (2007a) Multiple kinases and system robustness: a link between Cdc37 and genome integrity. Cell Cycle 6:3145–3147

Caplan AJ, Mandal AK, Theodoraki MA (2007b) Molecular chaperones and protein kinase quality control. Trends Cell Biol 17:87–92

Ciocca DR, Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10:86–103

Ciocca DR, Arrigo AP, Calderwood SK (2013) Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. Arch Toxicol 87:19–48

Cox MB, Johnson JL (2011) The role of p23, Hop, immunophilins, and other co-chaperones in regulating Hsp90 function. Methods Mol Biol 787:45–66

Dey B, Lightbody JJ, Boschelli F (1996) CDC37 is required for p60v-src activity in yeast. Mol Biol Cell 7:1405–1417

Ellis RJ (2007) Protein misassembly: macromolecular crowding and molecular chaperones. Adv Exp Med Biol 594:1–13

Fliss AE, Fang Y, Boschelli F, Caplan AJ (1997) Differential in vivo regulation of steroid hormone receptor activation by Cdc37p. Mol Biol Cell 8:2501–2509

Gray PJ Jr, Stevenson MA, Calderwood SK (2007) Targeting Cdc37 inhibits multiple signaling pathways and induces growth arrest in prostate cancer cells. Cancer Res 67:11942–11950

Gray PJ Jr, Prince T, Cheng J, Stevenson MA, Calderwood SK (2008) Targeting the oncogene and kinome chaperone CDC37. Nat Rev Cancer 8:491–495

Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25:276–308

Jinwal UK, Abisambra JF, Zhang J, Dharia S, O'Leary JC, Patel T, Braswell K, Jani T, Gestwicki JE, Dickey CA (2012) Cdc37/Hsp90 protein complex disruption triggers an autophagic clearance cascade for TDP-43 protein. J Biol Chem 287:24814–24820

Joo JH, Dorsey FC, Joshi A, Hennessy-Walters KM, Rose KL, McCastlain K, Zhang J, Iyengar R, Jung CH, Suen DF et al (2011) Hsp90-Cdc37 chaperone complex regulates Ulk1- and Atg13-mediated mitophagy. Mol Cell 43:572–585

Karnitz LM, Felts SJ (2007) Cdc37 regulation of the kinome: when to hold 'em and when to fold 'em. Sci STKE 2007:pe22

Lavictoire SJ, Parolin DA, Klimowicz AC, Kelly JF, Lorimer IA (2003) Interaction of Hsp90 with the nascent form of the mutant epidermal growth factor receptor EGFRvIII. J Biol Chem 278:5292–5299

MacLean M, Picard D (2003) Cdc37 goes beyond Hsp90 and kinases. Cell Stress Chaperones 8:114–119

Miyata Y, Nishida E (2005) CK2 binds, phosphorylates, and regulates its pivotal substrate Cdc37, an Hsp90-cochaperone. Mol Cell Biochem 274:171–179

Mollapour M, Tsutsumi S, Truman AW, Xu W, Vaughan CK, Beebe K, Konstantinova A, Vourganti S, Panaretou B, Piper PW et al (2011) Threonine 22 phosphorylation attenuates Hsp90 interaction with cochaperones and affects its chaperone activity. Mol Cell 41:672–681

Pearl LH (2005) Hsp90 and Cdc37- a chaperone cancer conspiracy. Curr Opin Gen Dev 15:55–61

Perdew GH, Wiegand H, Vanden Heuvel JP, Mitchell C, Singh SS (1997) A 50 kilodalton protein associated with raf and pp60(v-src) protein kinases is a mammalian homolog of the cell cycle control protein cdc37. Biochemistry 36:3600–3607

Rao J, Lee P, Benzeno S, Cardozo C, Albertus J, Robins DM, Caplan AJ (2001) Functional interaction of human Cdc37 with the androgen receptor but not with the glucocorticoid receptor. J Biol Chem 276:5814–5820

Reed SI (1980) The selection of amber mutations in genes required for completion of start, the controlling event of the cell division cycle of S. cerevisiae. Genetics 95:579–588

Salminen A, Lehtonen M, Paimela T, Kaarniranta K (2010) Celastrol: molecular targets of Thunder God Vine. Biochem Biophys Res Commun 394:439–442

Santagata S, Hu R, Lin NU, Mendillo ML, Collins LC, Hankinson SE, Schnitt SJ, Whitesell L, Tamimi RM, Lindquist S et al (2011) High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer. Proc Natl Acad Sci U S A 108:18378–18383

Smith JR, Workman P (2009) Targeting CDC37: an alternative, kinase-directed strategy for disruption of oncogenic chaperoning. Cell Cycle 8:362–372

Stepanova L, Finegold M, DeMayo F, Schmidt EV, Harper JW (2000a) The oncoprotein kinase chaperone CDC37 functions as an oncogene in mice and collaborates with both c-myc and cyclin D1 in transformation of multiple tissues. Mol Cell Biol 20:4462–4473

Stepanova L, Yang G, DeMayo F, Wheeler TM, Finegold M, Thompson TC, Harper JW (2000b) Induction of human Cdc37 in prostate cancer correlates with the ability of targeted Cdc37 expression to promote prostatic hyperplasia. Oncogene 19:2186–2193

Summy JM, Sudol M, Eck MJ, Monteiro AN, Gatesman A, Flynn DC (2003) Specificity in signaling by c-Yes. Front Biosci 8:185–205

Taipale M, Jarosz DF, Lindquist S (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11:515–528

Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, Lindquist S (2012) Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell 150:987–1001

Turnbull EL, Martin IV, Fantes PA (2005) Cdc37 maintains cellular viability in Schizosaccharomyces pombe independently of interactions with heat-shock protein 90. FEBS J 272:4129–4140

Vaughan CK, Gohlke U, Sobott F, Good VM, Ali MM, Prodromou C, Robinson CV, Saibil HR, Pearl LH (2006) Structure of an Hsp90-Cdc37-Cdk4 complex. Mol Cell 23:697–707

Wu F, Peacock SO, Rao S, Lemmon SK, Burnstein KL (2013) Novel interaction between the co-chaperone Cdc37 and Rho GTPase exchange factor Vav3 promotes androgen receptor activity and prostate cancer growth. J Biol Cell 288:5463–5474

Xu W, Neckers L (2012) The double edge of the HSP90-CDC37 chaperone machinery: opposing determinants of kinase stability and activity. Future Oncol 8:939–942

Xu W, Mollapour M, Prodromou C, Wang S, Scroggins BT, Palchick Z, Beebe K, Siderius M, Lee MJ, Couvillon A et al (2012). Dynamic tyrosine phosphorylation modulates cycling of the HSP90-P50(CDC37)-AHA1 chaperone machine. Mol Cell 47:434–443

Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 94:471–480

Chapter 6
p23 and Aha1

Alexandra Beatrice Rehn and Johannes Buchner

Abstract Hsp90 is a conserved molecular chaperone and is responsible for the folding and activation of several hundred client proteins, involved in various cellular processes. The large number and the diversity of these client proteins demand a high adaptiveness of Hsp90 towards the need of the individual client. This adaptiveness is amongst others mediated by more than 20 so-called cochaperones that differ in their actions towards Hsp90. Some of these cochaperones are able to modulate the ATPase activity of Hsp90 and/or its client protein binding, folding and activation. p23 and Aha1 are two prominent examples with opposing effects on the ATPase activity of Hsp90. p23 is able to inhibit the ATP turnover while Aha1 is the strongest known activator of the ATPase activity of Hsp90. Even though both cochaperones are conserved from yeast to man and have been studied for years, some Hsp90-related as well as Hsp90-independent functions are still enigmatic and under current investigation. In this chapter, we first introduce the ATPase cycle of Hsp90 and then focus on the two cochaperones integrating them in the Hsp90 cycle.

Keywords Hsp90 · p23 · Aha1 · Hch1

The Hsp90 ATPase Cycle

The molecular chaperone Hsp90 is required for the folding, activation and maturation of several hundred client proteins with diverse cellular functions (McClellan et al. 2007; Taipale et al. 2012). It is a flexible dimer in which each protomer consists of an N-terminal- (ND), middle- (MD) and C-terminal domain (CD). The N-terminal domain, responsible for ATP binding and hydrolysis (Prodromou et al.

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_6

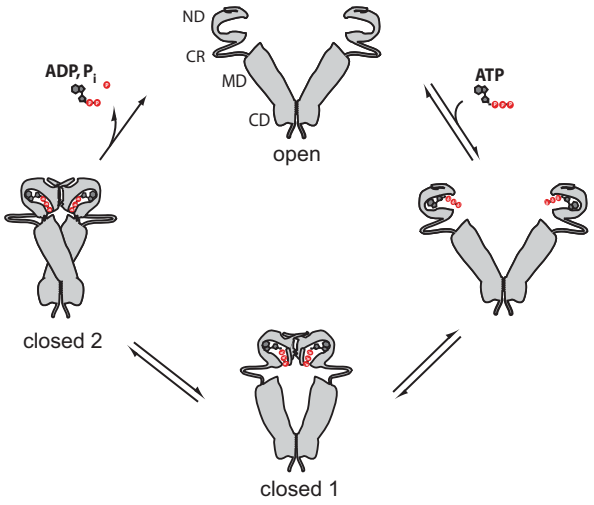

Fig. 6.1 The ATPase cycle of Hsp90. During one ATPase cycle, Hsp90 changes from an open, nucleotide-free state to a closed, ATP-bound conformation. After ATP binding, the N-terminal domains (ND) dimerize, leading to the closed 1 state. Rearrangement of the middle domains (MD) leads to the hydrolysis-competent closed 2 conformation. ADP and Pi are released after hydrolysis, leaving Hsp90 again in an open conformation, ready for a further cycle

1997), is connected via a charged region (CR) to the middle domain (Hainzl et al. 2009; Wayne and Bolon 2010; Tsutsumi et al. 2012). The middle domain is thought to be important for client protein binding (Meyer et al. 2003; Hawle et al. 2006; Lorenz et al. 2014). The C-terminal domains are responsible for the dimerization of the protein (Nemoto et al. 1995). They are essential for viability and client activation (Wayne and Bolon 2007).

During one cycle of ATP hydrolysis, as shown in Fig. 6.1, Hsp90 changes from an open, nucleotide-free conformation to a closed, ATP-bound state (Sullivan et al. 1997; Ali et al. 2006; Hessling et al. 2009; Mickler et al. 2009). It has been recently shown that binding of ATP to both subunits, but not the hydrolysis of both nucleotides is essential (Mishra and Bolon 2014). Binding of the nucleotides induces the closing of the ATP lid and the subsequent dimerization of the N-terminal domains, leading to a conformation called “closed 1” state (Li et al. 2013). This is only partially closed and ATP exchange is still possible. Next, a reorientation of the middle domains occurs, leading to contacts between the N- and M-domains. This “closed 2” position represents the state in which ATP is hydrolyzed (Li et al. 2013). After hydrolysis, Hsp90 returns to the open state thereby releasing the hydrolysis products ADP and Pi.

Even though Hsp90’s chaperone function relies on the ATPase activity (Obermann et al. 1998; Panaretou et al. 1998), the ATP turnover is very slow. For yeast Hsp90 1 ATP per minute is hydrolyzed (Panaretou et al. 1998). For human Hsp90 one turnover takes 10 min (McLaughlin et al. 2002). This slow ATPase activity is not a consequence of a slow binding or hydrolysis of ATP, but rather due to the large conformational changes within the protein (Weikl et al. 2000; Hessling et al. 2009). These conformational changes and hence the ATPase activity of Hsp90 can be modulated by the cohort of Hsp90 cochaperones.

Hsp90 cochaperones are divided into two subgroups according to the presence of a TPR-domain (tetratricorepeat): TPR-cochaperones and non-TPR-cochaperones

(Wandinger et al. 2008). Most Hsp90 cochaperones are TPR-proteins and therefore interact with the C-terminal MEEVD-motif of Hsp90. The only exception here is the cochaperone Sgt1, which interacts despite the presence of a TPR-domain with the N-terminal domain of Hsp90. In contrast to the TPR-cochaperones, the number of known non-TPR cochaperones is much smaller. p23 and Aha1 are two prominent examples of non-TPR proteins.

The Cochaperone p23

Discovery and Occurrence

p23 is a highly acidic protein which was first identified in a complex with Hsp90 and the avian progesterone receptor (Johnson et al. 1994). It was named, like several other chaperones, according to its molecular weight and is hence the smallest cochaperone of the Hsp90 machinery. p23's existence is however not confined to Hsp90 complexes, as it fulfills several Hsp90-independent functions in the cell (Echtenkamp et al. 2011). The identification of p23 from yeast to man suggests that p23 is a conserved protein (Johnson et al. 1994). Subsequent studies and the increasing existence of genome sequences allowed the identification of p23-like proteins in plants (Krishna and Gloor 2001; Cha et al. 2009; Zhang et al. 2010), which may differ in their effects on Hsp90. p23 is expressed in all mammalian tissues with the exception of striated muscles, where its homologue tsp23 is expressed instead (Freeman et al. 2000; Grad et al. 2006). Within the eukaryotic cell, the localization of this small protein is still not completely clear. The fact that the p23 sequence does not contain any known localization signals, defines the protein as cytoplasmatic. Nevertheless, it was also found in the nucleus (Picard 2006b; Ge et al. 2011), which is in agreement with newly identified functions of p23 in this compartment (Echtenkamp et al. 2011; Zelin et al. 2012). Questions like how p23 reaches these destinations and what portions of cellular p23 are localized in these cellular compartments are still elusive. Moreover, p23-Hsp90-complexes were also found outside the cell, implying an extracellular, not yet completely understood function of p23 (Eustace and Jay 2004; Sims et al. 2011).

Structure

First structural investigations of p23 based on protease sensitivity and CD-spectroscopy suggested a β-sheet fold with an unstructured C-terminal region (Weikl et al. 1999). This view was confirmed by crystal structures of human and yeast p23 (Weaver et al. 2000; Ali et al. 2006) as well as NMR studies (Martinez-Yamout et al. 2006). According to atomic structures, p23 can be divided in a stably folded N-terminal domain, organized as a seven stranded β-sandwich and a

flexible, unstructured C-terminal tail. The flexible nature of the C-terminal region required the 35 C-terminal amino acids to be deleted to obtain crystals of human p23 (Weaver et al. 2000), while the C-terminal tail could simply not be resolved in the structure of the yeast protein (Ali et al. 2006).

The folded β-sandwich structure is not only conserved within the p23 family (see Fig. 6.2a) but is also highly homologous to the core domain of small heat shock proteins like MjHsp16.5, from *Methanococcus jannaschii* (Kim et al. 1998), and Hsp16.9B, from wheat (van Montfort et al. 2001), even though sequence alignments between p23 and the sHsps show little similarities. This conserved fold was suggested to be part of an ancient and large family of proteins with diverse size and functions (Garcia-Ranea et al. 2002). Subsequently, the "p23-domain" has been found in other protein families, including proteins like Sgt1, Rar1 and NudC (Garcia-Ranea et al. 2002). All identified proteins use the p23 domain for interactions with their corresponding partners (Matsuzawa and Reed 2001; Garcia-Ranea et al. 2002; Zhu et al. 2010). For p23 and Hsp90 it had been first shown biochemically that the absence of the C-terminal tail does not interfere with Hsp90 binding (Weikl et al. 1999). It was later confirmed in the crystal structure of yeast p23 and Hsp90 that the interaction sites are located in the folded N-terminal domain of p23 (Ali et al. 2006).

The C-terminal domain of p23 however, which is essential for the Hsp90-independent chaperone activity (Weikl et al. 1999; Forafonov et al. 2008), is quite diverse (see Fig. 6.2b). The reconstruction of a phylogenetic tree of p23 sequences showed that p23 can be further divided into 3 subgroups as depicted in Fig. 6.2b (Zhu and Tytgat 2004):

1. Human p23-like homologues
2. Yeast p23-like homologues
3. B-ind1-like-homologues

The characteristic of the first group is an acidic region at the very C-terminus (Weaver et al. 2000). In the yeast p23-like proteins, this region is interjected with a GM/A rich region (Fang et al. 1998). This additional domain might be the result of an exon shuffling (Zhu and Tytgat 2004). B-ind1-like (butyrate-induced 1) homologues are composed of two domains, where the N-terminal domain is homologous to p23 and the C-terminal domain possesses homology with putative tyrosine phosphatase-like domain, member A (PTPLA) (Courilleau et al. 2000; Taguwa et al. 2008). The reason for the diversity of the C-terminal tail in length and sequence composition is still unclear, even though it is tempting to speculate that this diversity correlates with its chaperone activity.

Functions of p23

Interaction with Hsp90

Even though p23 was first identified in complex with Hsp90 and the progesterone receptor (Johnson et al. 1994) and later also shown to be important for

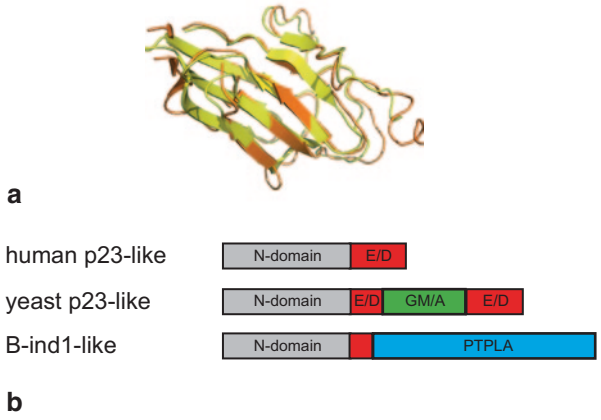

Fig. 6.2 Structural insights into p23. **a** Overlay of the N-terminal domains of yeast and human p23 demonstrate the conserved β-sheet fold. **b** Schematic presentation of p23 indicating the diverse C-terminal domains of p23, modified from Zhu and Tytgat (2004). Human p23 carries a characteristic acidic region at the very C-terminus, which is interjected by a GM/A rich region in yeast p23-like proteins. B-ind1-like variants have a PTPLA-domain (putative tyrosine phosphatase-like, member A) fused to an N-terminal domain with significant homology to p23

the folding of further steroid hormone receptors (Hutchison et al. 1995; Knoblauch and Garabedian 1999), it was also shown to bind to Hsp90 in the absence of client proteins (Johnson et al. 1994; Sullivan et al. 1997). This interaction is nucleotide-dependent *in vivo* as well as *in vitro* and ATP-competing inhibitors of Hsp90 like geldanamycin or macbecin prevent complex formation (Sullivan et al. 1997; Johnson and Toft 1994; Fang et al. 1998). These observations suggested for the first time a two-state model for Hsp90, where either ATP/p23 or ADP are bound (Sullivan et al. 1997; Prodromou et al. 2000; Sullivan et al. 2002). Since then, numerous studies were performed to analyze the Hsp90 ATPase cycle and to dissect the conformational states cochaperones are able to bind to (Hessling et al. 2009; Li et al. 2011; Li et al. 2013). p23 recognizes and binds to the closed, N-terminally dimerized conformation of Hsp90, more specifically the closed 2 state, thus being a conformation-specific cochaperone (Li et al. 2013). The crystal structure of yeast p23 and Hsp90 in the presence of the non-hydrolysable ATP-analogue AMP-PNP shows that the folded domain of p23 binds to the junction of the two N-domains of Hsp90, thereby interacting with both monomers (Ali et al. 2006). Two out of three identified interactions sites between p23 and Hsp90 are located in the N-domain of Hsp90, while the third one shows interaction with residues of the middle domain. NMR studies with human p23 and Hsp90 suggested additional interactions with the middle domain (Martinez-Yamout et al. 2006; Karagöz et al. 2011) and some of them could even be formed in the absence of nucleotides (Martinez-Yamout et al. 2006). Whether the unstructured C-terminal tail

is attached to Hsp90 is not completely certain, even though no further peak shifts were observed in NMR studies with full-length p23 compared to a C-terminal deletion mutant (Martinez-Yamout et al. 2006).

A further enigma is the stoichiometry of the p23-Hsp90 complex. A 1:1 as well as a 2:1 [p23:Hsp90-Dimer] stoichiometry had been suggested (Sullivan et al. 2002; Richter et al. 2004; Siligardi et al. 2004; Karagöz et al. 2011). Also, the binding mode is still unclear, as a negative as well as a positive allosteric effect had been described (Richter et al. 2004; Karagöz et al. 2011). It is also imaginable that the stoichiometry of the complex changes according to cellular conditions like stress, client protein folding or protein availability (Lorenz et al. 2014).

The interaction between Hsp90 and p23 leads to inhibition of the ATPase of Hsp90 (Richter et al. 2004; Siligardi et al. 2004; McLaughlin et al. 2006). p23 differs greatly in its inhibition mechanism as compared to other inhibitory cochaperones like Hop/Sti1. While Hop/Sti1 keeps Hsp90 in an open conformation by inhibiting association of the N-terminal domains and hence ATP hydrolysis (Richter et al. 2003), p23 solely binds to the ATP-bound, hydrolysis-competent state of Hsp90. Consequently, only two possible inhibition mechanisms come into question: Either p23 inhibits the hydrolysis process itself or impedes the subsequent release of the products ADP and Pi and hence the re-opening of Hsp90. There is experimental evidence for both mechanisms. The crystal structure shows the catalytic loop in a hydrolysis-active position in the presence of p23 (Ali et al. 2006), thereby favoring a decelerated release. NMR data on the other hand show a change in the nucleotide environment upon p23 binding (Karagöz et al. 2011). Plant p23-like proteins are conserved in their binding to plant Hsp90, but unlike their animal counterparts, they do not inhibit the plant Hsp90 activity, implying that different modes of actions have been evolved for the function of p23 proteins (Zhang et al. 2010).

How p23 Assists Hsp90 in Client Protein Folding

The class of steroid hormone receptors (SHR) is one of the most stringent and best studied classes of Hsp90 clients (Pratt et al. 1996; Pratt and Toft 1997; Smith and Toft 2008). p23 is next to Hsp70, Hsp40, Hsp90, Hop/Sti1 part of a minimal set of proteins needed to chaperone the SHRs to their active state in cell-free extracts (Dittmar et al. 1996; Pratt and Dittmar 1998). In current models, the unfolded SHR is first associated with Hsp70 and Hsp40 forming the so called early complex. With the aid of the co-chaperone Hop, the client protein is brought into contact with Hsp90, resulting in the intermediate complex. After transfer to Hsp90 and exchange of Hop by p23 the late-stage complex is formed (Picard 2006a). p23 stabilizes this complex, keeping the ligand binding cleft open and thus prolonging the time for steroid binding (Hutchison et al. 1995; Dittmar et al. 1997; Grad and Picard 2007). p23 dissociation and SHR release are coupled with the hydrolysis of ATP (Young and Hartl 2002). The absence of p23 resulted in either SHR-Hsp90 disassembly or SHR aggregation (Johnson and Toft 1994; Dittmar and Pratt 1997; Morishima et al. 2003). In cellular studies however, the role of p23 is not that clear. Activating

(Freeman et al. 2000) as well as inhibiting (Wochnik et al. 2004) effects of p23 were shown for glucocorticoid receptor (GR) activity. The fact that p23-null mice die shortly after birth due to an incompletely formed skin barrier and a deficient lung development, a phenotype comparable to GR deficient mice, shows that GR is an important target (Grad et al. 2006). p23 acted stimulatory on estrogen receptor (ER) activity (Oxelmark et al. 2003; Oxelmark et al. 2006), while it inhibited androgen receptor- (AR) and thyroid hormone receptor (TR) activities (Freeman et al. 2000).

The C-terminal tail of p23 might also play a role in this process, as SHR-complexes showed a reduced hormone binding activity in the presence of C-terminal deletion mutants (Weaver et al. 2000; Oxelmark et al. 2003).

The minimal set of components was also used to chaperone the reverse transcriptase (RT) of the hepadnavirus (Hu et al. 2002). Here p23 was not needed for RT activity and only enhanced the kinetics of the reconstitution, demonstrating that not every Hsp90 client is stringently dependent on p23 as a cochaperone.

Hsp90 Independent Functions

Using genetic and proteomic screening methods together with gene ontology analysis, the cellular interactome of p23 was analyzed (Echtenkamp et al. 2011). Surprisingly, p23 did not only show an overlapping interactome with Hsp90 but an even larger number of interactions independent of Hsp90 were identified. Thus p23 possesses its own set of interacting proteins, implying a biological role broader than that of an Hsp90 cochaperone, as described below (Freeman et al. 1996; Freeman et al. 2000; Echtenkamp et al. 2011).

The Function of p23 as a Chaperone

The function of p23 to suppress protein aggregation and thus exhibiting chaperoning activity on its own was already reported in 1996 (Bose et al. 1996; Freeman et al. 1996). This function is conserved from yeast to man and was also shown to apply for the plant homologues (Cha et al. 2009). As p23 acts independently of ATP, it is not able to refold proteins, but it is able to hold the protein in a refolding-competent state, as demonstrated for the model substrate β-galactosidase (Freeman et al. 1996). In this context, the C-terminal tail of p23 plays an essential role, as deletions in this region resulted in loss of this function (Weikl et al. 1999; Weaver et al. 2000). Nevertheless, the C-terminal tail is not able to suppress aggregation on its own, suggesting an interplay of the C-terminal tail with the N-terminal core domain (Weikl et al. 1999). The detailed mechanism of this chaperoning activity remains however elusive. It is striking that caspases, which are a hallmark of apoptosis, are able to cleave within the C-terminus of p23, thereby attacking its chaperoning function (Gausdal et al. 2004; Mollerup and Berchtold 2005). Truncated p23 is then further degraded by the proteasome and this might therefore be a way to attack the Hsp90 machinery (Mollerup and Berchtold 2005), which is known

to play a protective role during apoptosis (Pandey et al. 2000; Lanneau et al. 2007). The fact that p23's chaperone function has evolved as a point of attack demonstrates the importance of this protein.

Nuclear Functions of p23

As mentioned before, p23 is not only localized in the nucleus but also shows a bias for nuclear processes (Freeman et al. 2000; Echtenkamp et al. 2011). p23 was found to play a role in telomere maintenance (Toogun et al. 2007), DNA repair (Echtenkamp et al. 2011) and transcription processes (Zelin et al. 2012). Even though Hsp90 and p23 have long been known to be involved in altering telomerase activity (Holt et al. 1999), the actions of both chaperones are independent and opposed. While Hsp90 promotes the assembly of the DNA-telomerase complex, p23 fosters its disassembly (Toogun et al. 2008). Furthermore, this function relies on its chaperoning activity (Toogun et al. 2007).

p23 also initiates the disassembly of various other protein-DNA complexes and teams up with the acetyltransferase GCN5 to regulate transcriptional activities in response to cellular signals (Zelin et al. 2012). In this recently discovered function of p23, GCN5 acetylates the released protein after the p23-induced disassembly of the protein-DNA complex. To control GCN5 action and re-assembly, p23 interacts with GCN5. Only the continuing existence of the transcription activating signal removes both the GCN5 and the inhibiting acetyl moiety to allow re-assembly of the protein to the DNA (Zelin et al. 2012).

By destabilizing various protein-DNA complexes in the nucleus, p23 provides the possibility to stop signaling and re-assemble protein-DNA complexes in response to new internal and external stimuli. Hence, p23 contributes to a dynamic signaling-response environment in the nucleus. Since the nuclear functions of p23 have only been recently addressed, future investigations may unravel further functions of p23 in this context.

The Cochaperone Aha1

Discovery and Occurrence

Compared to the discovery date of p23, Aha1 is a relative young cochaperone of Hsp90, as it was first described in this context in 2002 (Panaretou et al. 2002). One year earlier it was identified as a 38-kDa protein (p38) interacting with the vesicular stomatitis virus glycoprotein (VSV-G) and modulating its transit from the ER to the Golgi apparatus (Sevier and Machamer 2001). Due to its prominent function as an *a*ctivator of the *H*sp90 *A*TPase, the name Aha1 prevailed. Its identification as an Hsp90 cochaperone was based on a homology search of Hch1. This protein has been previously described as a "*h*igh *c*opy suppressor of *H*sp90

temperature-sensitive mutants" and was thus suggested to be an unidentified co-chaperone (Nathan et al. 1999). The N-terminal part of Aha1 (aa 1–153) shares 36 % sequence identity and 50 % similarity with Hch1, but possesses an additional C-terminal part (aa 154–350) (Panaretou et al. 2002).

Aha1 homologues are, like p23, conserved from yeast to man, whereas Hch1 seems to be restricted to lower eukaryotes like *S. cerevisiae* and *Candida albicans* (Panaretou et al. 2002; Lotz et al. 2003). In higher eukaryotes (mainly mammels) an additional hitherto uncharacterized Aha1-related protein, namely Aha2, is furthermore present. Aha1 is expressed in numerous tissues including kidney, brain, heart- and skeletal muscles (Sevier and Machamer 2001). Within the eukaryotic cell, Hch1 as well as Aha1 are mainly located in the cytoplasm, even though a small fraction of Aha1 was also found to associate with intracellular membranes (Sevier and Machamer 2001) and GFP-tagged Hch1 was also observed in the nucleus (Huh et al. 2003). Like for p23, it is still not clear how Aha1 and Hch1 migrate to different cellular compartments.

Structure

To date no structure of full-length Aha1 is available. Nevertheless, the isolated N- and C-domain were investigated and it was shown that Aha1 is a mixed α+β protein. Its N-terminal domain (1–153) was crystallized and revealed an N-terminal α-helix, followed by a four stranded, antiparallel β-sheet leading into a C-terminal α-helix (PDB code 1usu and 1usv) (Meyer et al. 2004). NMR studies of the C-terminal domain also revealed the presence of several α-helices and β-sheets [(PDB code 1×53 and (Retzlaff et al. 2010)]. Both domains are linked by a coil-structure. Interestingly, phylogenetic analysis of Aha1 revealed that the two domains have evolved separately (Singh et al. 2014). It was hypothesized that the Aha1 N-domain originates from the protozoan *Giardia* and the Aha1 C-domain from the parasitic protozoan *Entamoeba*, as both organisms possess only the N-domain or C-domain, respectively (see Fig. 6.3a). Early eukaryotes have both domains, but expressed as separate proteins. Apicomplexan protozoan like *P. falciparum* or *T. gondii* even have Aha1 homologues containing two Aha1 N-domains. Higher eukaryotes express the fusion of both proteins, known as Aha1 (Singh et al. 2014). As mentioned above, organisms like *S. cerevisiae* separately express Hch1 and even higher eukaryotes express an additional Aha2 protein. The significance of these additional proteins is still not fully understood.

Functions of Aha1

Interaction with Hsp90

Aha1 as well as Hch1 have been identified as Hsp90-interactors by yeast-two-hybrid and co-immunoprecipitation assays (Panaretou et al. 2002). In complex with

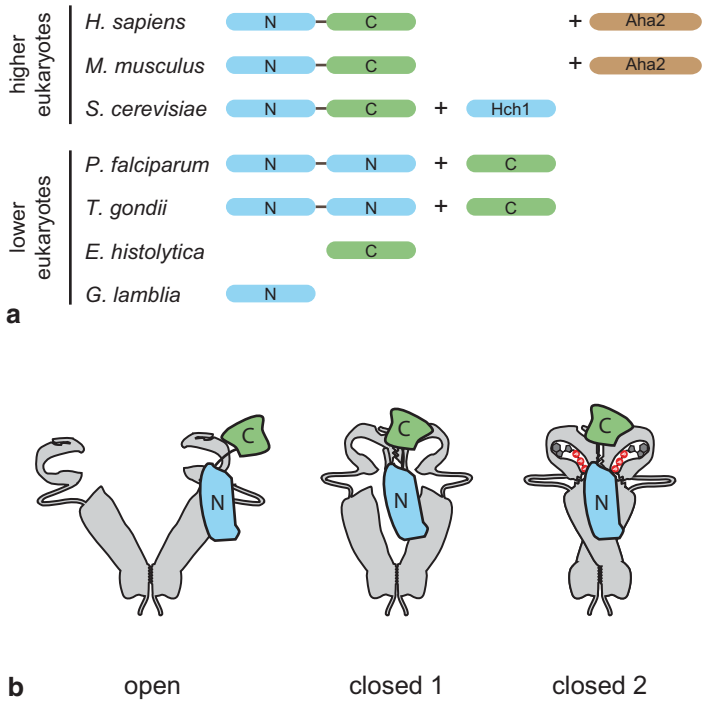

Fig. 6.3 Evolutionary development of Aha1 and its binding to Hsp90. **a** Aha1 homologues from higher and lower eukaryotes are schematically displayed to visualize the origin and evolution of the Aha1 domains. The figure is modified from Singh et al. (2014). **b** Aha1 is able to bind in the presence or absence of ATP. The N-domain of Aha1 interacts with the M-domain of Hsp90. Upon dimerization of Hsp90, the C-domain of Aha1 is able to bind to the N-domains of Hsp90

Hsp90, the N-terminal domain of Aha1 interacts with the middle domain of Hsp90, while the C-terminal domain of Aha1 contacts the N-domains of Hsp90 (Meyer et al. 2004; Retzlaff et al. 2010; Koulov et al. 2010). In our current model, the Aha1 N-domain serves as the primary binding site which is then followed by a binding of the C-terminal domain of Aha1 to the N-domains of Hsp90 once they dimerize (Retzlaff et al. 2010). Consequently, Aha1 is able to interact with Hsp90 independent of its nucleotide state (see Fig. 6.3b), even though higher binding affinities were observed for the nucleotide-bound state (Li et al. 2013). It had been recently reported that N-domain SUMOylation of Hsp90 is able to initiate the recruitment of Aha1 to Hsp90 (Mollapour et al. 2014). Many of the observed interactions between Aha1 and Hsp90 are of polar nature, thus explaining the salt-dependence of this complex (Panaretou et al. 2002; Meyer et al. 2004).

Via binding to Hsp90, Aha1 and Hch1 activate the ATPase activity of Hsp90 even though Hch1 and the isolated N-domain of Aha1 do so to a significant lower

extent than full-length Aha1 (Panaretou et al. 2002; Retzlaff et al. 2010). The isolated C-terminal domain of higher eukaryotes is not able to stimulate the ATPase activity of Hsp90 on its own (Panaretou et al. 2002; Retzlaff et al. 2010), but potentiates the activity of a preformed Hsp90/Aha1-N-domain complex, implying an interplay between the two domains (Retzlaff et al. 2010). In the case of the lower eukaryote *Entamoeba histolytica* however, which only expresses the C-terminal domain of Aha1, a weak stimulation of Hsp90 by the C-domain of Aha1 was observed (Singh et al. 2014). The required interplay between the N- and C-domain of Aha1 to fully stimulate the ATPase of Hsp90 is further supported by two independent observations. First, mutations in the C-domain were identified that impaired the stimulation of the ATPase without altering the binding to Hsp90 (Koulov et al. 2010). Secondly, a chimeric fusion of Hch1 and the C-terminal domain of Aha1 was not able to reach Aha1-WT levels (Horvat et al. 2014). This result also shows that differences between Aha1 and Hch1 concerning their stimulation activities must derive in part from the interaction with the middle domain of Hsp90. For Aha1 we have structural information on the interaction of the Aha1 N-domain with the middle domain of Hsp90 (Meyer et al. 2004). In the presence of Aha1, the catalytic loop of Hsp90 harboring residue R380, which contacts the γ-phosphate of ATP, is switched to a conformation in which R380 is able to orient and polarize the γ-phosphate of ATP, necessary for ATP hydrolysis (Meyer et al. 2004). Even though the exact role of R380 is controversially discussed in the literature (Cunningham et al. 2012), the role of Aha1 in stabilizing the catalytic loop is indisputable. Responsible for that action is the RKxK-motif within Aha1, which is furthermore conserved within the Aha1 family (Meyer et al. 2004). Mutation of this motif in Aha1 as well as in Hch1 lead to impaired stimulation of the Hsp90 ATPase, indicating that both proteins activate the ATPase by stabilization of the catalytic loop (Meyer et al. 2004; Horvat et al. 2014). Additionally, binding of Aha1 facilitates N-terminal dimerization of Hsp90 thus accelerating the conformational rearrangements in Hsp90 and hence the ATP turnover (Hessling et al. 2009; Retzlaff et al. 2010). It is important to know, that only one molecule of Aha1 is sufficient to induce the full stimulatory effect as Aha1 is able to activate the ATP hydrolysis in both N-domains (*cis* and *trans*) (Retzlaff et al. 2010). Since full-length Aha1 is required for full stimulation, Hch1 may have further functions. Recent studies revealed that deletion of Hch1 but not Aha1 conferred resistance to Hsp90 inhibitors (Armstrong et al. 2012), demonstrating that Aha1 and Hch1 have common as well as individual functions.

Client Protein Activation

Hitherto, Aha1 has been implicated to play a role in the folding and activation of several Hsp90 client proteins. A well-studied Hsp90 client is the viral tyrosine kinase v-src. Experiments with Aha1-depleted yeast cells revealed that phosphorylation levels in the cell, as a read-out for v-src activity, are decreased, while ΔHch1-cells showed no effect compared to WT (Panaretou et al. 2002). This indicates that Aha1, in contrast to Hch1, is an important factor for v-src activation. Notably, v-src activity was not enhanced by overexpression of Aha1 (Sun et al. 2012). For the

Hsp90 client GR, deletions of Aha1 and Hch1 in yeast as well as overexpression and silencing of Aha1 in human cells demonstrated that Aha1 contributes to the activation of this steroid hormone receptor (Harst et al. 2005). These findings led to the conclusion that Aha1 generally enhances Hsp90 function due to its ability to activate the ATPase activity. Nevertheless and in contrast to p23, no client protein complexes with Hsp90 and Aha1 were found for v-src or SHRs, even under complex stabilizing conditions like the presence of molybdate (Sun et al. 2012). A current study even shows that simultaneous binding of GR and Aha1 is not possible due to an overlapping binding site on Hsp90 (Lorenz et al. 2014). The absence of evidence for a Aha1/Hsp90/client-complex in combination with the observed *in vivo* effects raises the possibility that the interaction is either indirect, happens after client maturation or is simply too transient to detect (Sun et al. 2012).

Even though no direct interaction between Aha1, Hsp90 and the classic clients (v-src and SHRs) are detectable, Aha1 was found in Hsp90-complexes with the cystic fibrosis regulator CFTR (Wang et al. 2006; Koulov et al. 2010), the adenine nucleotide transporter ANT1 (Bhangoo et al. 2007) and Akt kinase (Sun et al. 2012). In the case of CFTR and especially its disease-related mutant ΔF508, the knockdown of Aha1 promoted the translocation to the plasma membrane (Wang et al. 2006). This result is consistent with the observation that Aha1 overexpression led to a decrease in Akt activation, implying that stimulation of the ATPase is not generally correlated to Hsp90's ability to facilitate protein folding or activation (Sun et al. 2012).

Pull-down of Aha1 and subsequent MS analysis revealed several more Aha1 interacting proteins that play a role in several cellular processes, among them DNA-PKcs and Topo IIa. Most of the identified proteins are part of oligomeric complexes that contain components known to interact with Hsp90 (Sun et al. 2012). Therefore, Aha1 is thought to be involved in several further Hsp90-related processes. Until today no Hsp90-independent function of Aha1 has been described.

The Interplay Between p23 and Aha1 in the Hsp90 Cycle

p23 and Aha1 are Hsp90 cochaperones with opposing effects on Hsp90. While p23 inhibits the ATPase to about 50% (Richter et al. 2004; Siligardi et al. 2004), Aha1 stimulates the ATP turnover up to 30-fold (Panaretou et al. 2002; Retzlaff et al. 2010). It therefore seems obvious that both cochaperones should not influence Hsp90 simultaneously. As Aha1 is able to bind to Hsp90 in the nucleotide-free as well as in the ATP-bound state, the Aha1-Hsp90 complex seems to be present during different steps of the cycle while the p23-Hsp90 complex can only be formed in the nucleotide-bound state. The ability of Aha1 to bind to different conformations of Hsp90 entails at the same time the challenge of releasing this cochaperone to enable further cofactor or client protein binding. In this regard, p23 was shown to replace Aha1 from Hsp90 in a nucleotide-dependent manner. This is possible because both cochaperones share a binding site in the middle domain of Hsp90 (Meyer et al. 2004; Ali et al. 2006; Röhl et al. 2013) and therefore compete for Hsp90 binding.

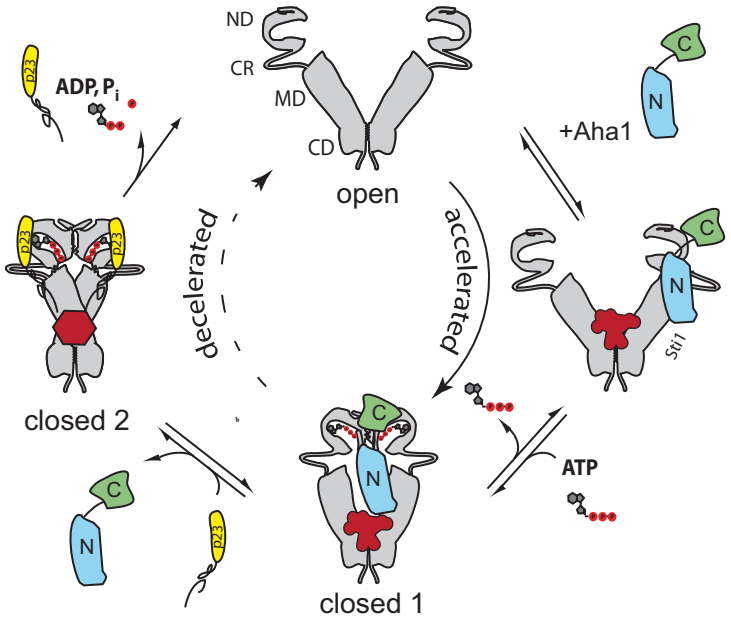

Fig. 6.4 Integration of Aha1 and p23 into the Hsp90 cycle. Aha1 binds to the open conformation of Hsp90 and accelerates dimerization of the N-terminal domains of Hsp90, leading to the closed 1 state. p23 is able to displace Aha1 from Hsp90 during the rearrangements leading to the closed 2 state. p23 inhibits the ATP turnover thereby decelerating the cycle. After ATP hydrolysis, ADP, Pi and p23 dissociate from Hsp90, thereby enabling another cycle of ATP turnover. This model shall not imply that both cochaperones *have* to be present in one ATPase cycle, but if they do, some conformational changes will be accelerated while others will be decelerated

Consistent with p23's selective binding to the nucleotide-bound conformation of Hsp90, p23 was able to fully replace Aha1 from Hsp90 in the presence of the ATP analogues ATPγS and AMP-PNP (Harst et al. 2005; Li et al. 2013), while only a partial release of Aha1 was observed in the presence of ATP (Li et al. 2013). Whether the formation of a mixed ternary complex of Hsp90, Aha1 and p23 is possible is controversial as the previous *in vitro* and *in vivo* results have come to different conclusions (Harst et al. 2005; Sun et al. 2012; Li et al. 2013).

The current knowledge allows the integration of p23 and Aha1 in the Hsp90 cycle (see Fig. 6.4). First, the N-domain of Aha1 binds to the open conformation of Hsp90 thereby promoting N-terminal dimerization of Hsp90 leading to the closed 1 conformation. The dimerized N-domains of Hsp90 provide the binding site for the C-terminal domain of Aha1. By conformational rearrangements of the middle domains, closed 1 progresses to the closed 2 state, which is the p23-competent binding state of Hsp90. During this progression, p23 competes with Aha1 for the Hsp90 binding site, resulting in a p23-bound state. p23 dissociates upon ATP hydrolysis and Hsp90 can undergo another round of cycling (Li et al. 2013).

Conclusions

Hsp90 is a tightly regulated molecular chaperone. Especially a unique set of co-chaperones regulates the ATPase cycle of Hsp90. p23 and Aha1 are two prominent cochaperones with opposing effects on the ATP turnover. As described above, both cochaperones act on different conformations of Hsp90. Additionally, the set of client proteins they influence is not the same, indicating that different Hsp90 clients may need cochaperones to a different extent for folding, activation or maturation. This observation opens the opportunity to inhibit client protein folding in the context of diseases by cochaperone-specific, instead of Hsp90-specific, inhibitors. The natural product gedunin was recently found to be an inhibitor of p23 (Patwardhan et al. 2013), while no Aha1-specific inhibitor is known to date. In contrast to Hsp90 inhibitors, this strategy might be more powerful as only a specific subset of clients would be addressed.

References

Ali MM, Roe SM, Vaughan CK, Meyer P, Panaretou B, Piper PW, Prodromou C, Pearl LH (2006) Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. Nature 440(7087):1013–1017. doi:10.1038/nature04716

Armstrong H, Wolmarans A, Mercier R, Mai B, LaPointe P (2012) The co-chaperone Hch1 regulates Hsp90 function differently than its homologue Aha1 and confers sensitivity to yeast to the Hsp90 inhibitor NVP-AUY922. PloS One 7(11):e49322. doi:10.1371/journal.pone.0049322

Bhangoo MK, Tzankov S, Fan AC, Dejgaard K, Thomas DY, Young JC (2007) Multiple 40-kDa heat-shock protein chaperones function in Tom70-dependent mitochondrial import. Mol Biol Cell 18(9):3414–3428. doi:10.1091/mbc.E07-01-0088

Bose S, Weikl T, Bugl H, Buchner J (1996) Chaperone function of Hsp90-associated proteins. Science 274(5293):1715–1717

Cha JY, Ermawati N, Jung MH, Su'udi M, Kim KY, Kim JY, Han CD, Lee KH, Son D (2009) Characterization of orchardgrass p23, a flowering plant Hsp90 cohort protein. Cell Stress Chaperones 14(3):233–243. doi:10.1007/s12192-008-0077-6

Courilleau D, Chastre E, Sabbah M, Redeuilh G, Atfi A, Mester J (2000) B-ind1, a novel mediator of Rac1 signaling cloned from sodium butyrate-treated fibroblasts. J Biol Chem 275(23):17344–17348. doi:10.1074/jbc.M000887200

Cunningham CN, Southworth DR, Krukenberg KA, Agard DA (2012) The conserved arginine 380 of Hsp90 is not a catalytic residue, but stabilizes the closed conformation required for ATP hydrolysis. Protein Sci 21(8):1162–1171. doi:10.1002/pro.2103

Dittmar KD, Pratt WB (1997) Folding of the glucocorticoid receptor by the reconstituted Hsp90-based chaperone machinery. The initial hsp90.p60.hsp70-dependent step is sufficient for creating the steroid binding conformation. J Biol Chem 272(20):13047–13054

Dittmar KD, Hutchison KA, Owens-Grillo JK, Pratt WB (1996) Reconstitution of the steroid receptor.hsp90 heterocomplex assembly system of rabbit reticulocyte lysate. J Biol Chem 271(22):12833–12839

Dittmar KD, Demady DR, Stancato LF, Krishna P, Pratt WB (1997) Folding of the glucocorticoid receptor by the heat shock protein (hsp) 90-based chaperone machinery. The role of p23 is to stabilize receptor.hsp90 heterocomplexes formed by hsp90.p60.hsp70. J Biol Chem 272(34):21213–21220

Echtenkamp FJ, Zelin E, Oxelmark E, Woo JI, Andrews BJ, Garabedian M, Freeman BC (2011) Global functional map of the p23 molecular chaperone reveals an extensive cellular network. Mol Cell 43(2):229–241. doi:10.1016/j.molcel.2011.05.029

Eustace BK, Jay DG (2004) Extracellular roles for the molecular chaperone, hsp90. Cell Cycle 3(9):1098–1100

Fang Y, Fliss AE, Rao J, Caplan AJ (1998) SBA1 encodes a yeast hsp90 cochaperone that is homologous to vertebrate p23 proteins. Mol Cell Biol 18(7):3727–3734

Forafonov F, Toogun OA, Grad I, Suslova E, Freeman BC, Picard D (2008) p23/Sba1p protects against Hsp90 inhibitors independently of its intrinsic chaperone activity. Mol Cell Biol 28(10):3446–3456. doi:10.1128/MCB.02246-07

Freeman BC, Toft DO, Morimoto RI (1996) Molecular chaperone machines: chaperone activities of the cyclophilin Cyp-40 and the steroid aporeceptor-associated protein p23. Science 274(5293):1718–1720

Freeman BC, Felts SJ, Toft DO, Yamamoto KR (2000) The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies. Genes Dev 14(4):422–434

Garcia-Ranea JA, Mirey G, Camonis J, Valencia A (2002) p23 and HSP20/alpha-crystallin proteins define a conserved sequence domain present in other eukaryotic protein families. FEBS Lett 529(2–3):162–167

Gausdal G, Gjertsen BT, Fladmark KE, Demol H, Vandekerckhove J, Doskeland SO (2004) Caspase-dependent, geldanamycin-enhanced cleavage of co-chaperone p23 in leukemic apoptosis. Leukemia 18(12):1989–1996. doi:10.1038/sj.leu.2403508

Ge X, Rameix-Welti MA, Gault E, Chase G, dos Santos AE, Picard D, Schwemmle M, Naffakh N (2011) Influenza virus infection induces the nuclear relocalization of the Hsp90 co-chaperone p23 and inhibits the glucocorticoid receptor response. PloS One 6(8):e23368. doi:10.1371/journal.pone.0023368

Grad I, McKee TA, Ludwig SM, Hoyle GW, Ruiz P, Wurst W, Floss T, Miller CA 3rd, Picard D (2006) The Hsp90 cochaperone p23 is essential for perinatal survival. Mol Cell Biol 26(23):8976–8983. doi:10.1128/MCB.00734-06

Grad I, Picard D (2007) The glucocorticoid responses are shaped by molecular chaperones. Mol Cell Endocrinol 275(1–2):2–12. doi:10.1016/j.mce.2007.05.018

Hainzl O, Lapina MC, Buchner J, Richter K (2009) The charged linker region is an important regulator of Hsp90 function. J Biol Chem 284(34):22559–22567. doi:10.1074/jbc.M109.031658

Harst A, Lin H, Obermann WM (2005) Aha1 competes with Hop, p50 and p23 for binding to the molecular chaperone Hsp90 and contributes to kinase and hormone receptor activation. Biochem J 387(Pt 3):789–796. doi:10.1042/BJ20041283

Hawle P, Siepmann M, Harst A, Siderius M, Reusch HP, Obermann WM (2006) The middle domain of Hsp90 acts as a discriminator between different types of client proteins. Mol Cell Biol 26(22):8385–8395. doi:10.1128/MCB.02188-05

Hessling M, Richter K, Buchner J (2009) Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. Nat Struct Mol Biol 16(3):287–293. doi:10.1038/nsmb.1565

Holt SE, Aisner DL, Baur J, Tesmer VM, Dy M, Ouellette M, Trager JB, Morin GB, Toft DO, Shay JW, Wright WE, White MA (1999) Functional requirement of p23 and Hsp90 in telomerase complexes. Genes Dev 13(7):817–826

Horvat NK, Armstrong H, Lee BL, Mercier R, Wolmarans A, Knowles J, Spyracopoulos L, LaPointe P (2014) A mutation in the catalytic loop of hsp90 specifically impairs ATPase stimulation by aha1p, but not hch1p. J Mol Biol 426(12):2379–2392. doi:10.1016/j.jmb.2014.04.002

Hu J, Toft D, Anselmo D, Wang X (2002) In vitro reconstitution of functional hepadnavirus reverse transcriptase with cellular chaperone proteins. J Virol 76(1):269–279

Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK (2003) Global analysis of protein localization in budding yeast. Nature 425(6959):686–691. doi:10.1038/nature02026

Hutchison KA, Stancato LF, Owens-Grillo JK, Johnson JL, Krishna P, Toft DO, Pratt WB (1995) The 23-kDa acidic protein in reticulocyte lysate is the weakly bound component of the hsp

foldosome that is required for assembly of the glucocorticoid receptor into a functional heterocomplex with hsp90. J Biol Chem 270(32):18841–18847
Johnson JL, Toft DO (1994) A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23. J Biol Chem 269(40):24989–24993
Johnson JL, Beito TG, Krco CJ, Toft DO (1994) Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes. Mol Cell Biol 14(3):1956–1963
Karagöz GE, Duarte AM, Ippel H, Uetrecht C, Sinnige T, van Rosmalen M, Hausmann J, Heck AJ, Boelens R, Rudiger SG (2011) N-terminal domain of human Hsp90 triggers binding to the cochaperone p23. Proc Natl Acad Sci U S A 108(2):580–585. doi:10.1073/pnas.1011867108
Kim R, Kim KK, Yokota H, Kim SH (1998) Small heat shock protein of Methanococcus jannaschii, a hyperthermophile. Proc Natl Acad Sci U S A 95(16):9129–9133
Knoblauch R, Garabedian MJ (1999) Role for Hsp90-associated cochaperone p23 in estrogen receptor signal transduction. Mol Cell Biol 19(5):3748–3759
Koulov AV, LaPointe P, Lu B, Razvi A, Coppinger J, Dong MQ, Matteson J, Laister R, Arrowsmith C, Yates JR 3rd, Balch WE (2010) Biological and structural basis for Aha1 regulation of Hsp90 ATPase activity in maintaining proteostasis in the human disease cystic fibrosis. Mol Biol Cell 21(6):871–884. doi:10.1091/mbc.E09-12-1017
Krishna P, Gloor G (2001) The Hsp90 family of proteins in Arabidopsis thaliana. Cell Stress Chaperones 6(3):238–246
Lanneau D, de Thonel A, Maurel S, Didelot C, Garrido C (2007) Apoptosis versus cell differentiation: role of heat shock proteins HSP90, HSP70 and HSP27. Prion 1(1):53–60
Li J, Richter K, Buchner J (2011) Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle. Nat Struct Mol Biol 18(1):61–66. doi:10.1038/nsmb.1965
Li J, Richter K, Reinstein J, Buchner J (2013) Integration of the accelerator Aha1 in the Hsp90 co-chaperone cycle. Nat Struct Mol Biol 20(3):326–331. doi:10.1038/nsmb.2502
Lorenz OR, Freiburger L, Rutz DA, Krause M, Zierer BK, Alvira S, Cuellar J, Valpuesta JM, Madl T, Sattler M, Buchner J (2014) Modulation of the hsp90 chaperone cycle by a stringent client protein. Mol Cell 53(6):941–953. doi:10.1016/j.molcel.2014.02.003
Lotz GP, Lin H, Harst A, Obermann WM (2003) Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. J Biol Chem 278(19):17228–17235. doi:10.1074/jbc.M212761200
Martinez-Yamout MA, Venkitakrishnan RP, Preece NE, Kroon G, Wright PE, Dyson HJ (2006) Localization of sites of interaction between p23 and Hsp90 in solution. J Biol Chem 281(20):14457–14464. doi:10.1074/jbc.M601759200
Matsuzawa SI, Reed JC (2001) Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. Mol Cell 7(5):915–926
McClellan AJ, Xia Y, Deutschbauer AM, Davis RW, Gerstein M, Frydman J (2007) Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. Cell 131(1):121–135. doi:10.1016/j.cell.2007.07.036
McLaughlin SH, Smith HW, Jackson SE (2002) Stimulation of the weak ATPase activity of human hsp90 by a client protein. J Mol Biol 315(4):787–798. doi:10.1006/jmbi.2001.5245
McLaughlin SH, Sobott F, Yao ZP, Zhang W, Nielsen PR, Grossmann JG, Laue ED, Robinson CV, Jackson SE (2006) The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins. J Mol Biol 356(3):746–758. doi:10.1016/j.jmb.2005.11.085
Meyer P, Prodromou C, Hu B, Vaughan C, Roe SM, Panaretou B, Piper PW, Pearl LH (2003) Structural and functional analysis of the middle segment of hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. Mol Cell 11(3):647–658
Meyer P, Prodromou C, Liao C, Hu B, Roe SM, Vaughan CK, Vlasic I, Panaretou B, Piper PW, Pearl LH (2004) Structural basis for recruitment of the ATPase activator Aha1 to the Hsp90 chaperone machinery. EMBO J 23(6):1402–1410. doi:10.1038/sj.emboj.7600141
Mickler M, Hessling M, Ratzke C, Buchner J, Hugel T (2009) The large conformational changes of Hsp90 are only weakly coupled to ATP hydrolysis. Nat Struct Mol Biol 16(3):281–286. doi:10.1038/nsmb.1557

Mishra P, Bolon DN (2014) Designed Hsp90 heterodimers reveal an asymmetric ATPase-driven mechanism in vivo. Mol Cell 53(2):344–350. doi:10.1016/j.molcel.2013.12.024

Mollapour M, Bourboulia D, Beebe K, Woodford MR, Polier S, Hoang A, Chelluri R, Li Y, Guo A, Lee MJ, Fotooh-Abadi E, Khan S, Prince T, Miyajima N, Yoshida S, Tsutsumi S, Xu W, Panaretou B, Stetler-Stevenson WG, Bratslavsky G, Trepel JB, Prodromou C, Neckers L (2014) Asymmetric Hsp90 N domain SUMOylation recruits Aha1 and ATP-competitive inhibitors. Mol Cell 53(2):317–329. doi:10.1016/j.molcel.2013.12.007

Mollerup J, Berchtold MW (2005) The co-chaperone p23 is degraded by caspases and the proteasome during apoptosis. FEBS Lett 579(19):4187–4192. doi:10.1016/j.febslet.2005.06.045

Morishima Y, Kanelakis KC, Murphy PJ, Lowe ER, Jenkins GJ, Osawa Y, Sunahara RK, Pratt WB (2003) The hsp90 cochaperone p23 is the limiting component of the multiprotein hsp90/hsp70-based chaperone system in vivo where it acts to stabilize the client protein: hsp90 complex. J Biol Chem 278(49):48754–48763. doi:10.1074/jbc.M309814200

Nathan DF, Vos MH, Lindquist S (1999) Identification of SSF1, CNS1, and HCH1 as multicopy suppressors of a Saccharomyces cerevisiae Hsp90 loss-of-function mutation. Proc Natl Acad Sci U S A 96(4):1409–1414

Nemoto T, Ohara-Nemoto Y, Ota M, Takagi T, Yokoyama K (1995) Mechanism of dimer formation of the 90-kDa heat-shock protein. Eur J Biochem 233(1):1–8

Obermann WM, Sondermann H, Russo AA, Pavletich NP, Hartl FU (1998) In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. J Cell Biol 143(4):901–910

Oxelmark E, Knoblauch R, Arnal S, Su LF, Schapira M, Garabedian MJ (2003) Genetic dissection of p23, an Hsp90 cochaperone, reveals a distinct surface involved in estrogen receptor signaling. J Biol Chem 278(38):36547–36555. doi:10.1074/jbc.M305960200

Oxelmark E, Roth JM, Brooks PC, Braunstein SE, Schneider RJ, Garabedian MJ (2006) The cochaperone p23 differentially regulates estrogen receptor target genes and promotes tumor cell adhesion and invasion. Mol Cell Biol 26(14):5205–5213. doi:10.1128/MCB.00009-06

Panaretou B, Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1998) ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. EMBO J 17(16):4829–4836. doi:10.1093/emboj/17.16.4829

Panaretou B, Siligardi G, Meyer P, Maloney A, Sullivan JK, Singh S, Millson SH, Clarke PA, Naaby-Hansen S, Stein R, Cramer R, Mollapour M, Workman P, Piper PW, Pearl LH, Prodromou C (2002) Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1. Mol Cell 10(6):1307–1318

Pandey P, Saleh A, Nakazawa A, Kumar S, Srinivasula SM, Kumar V, Weichselbaum R, Nalin C, Alnemri ES, Kufe D, Kharbanda S (2000) Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. EMBO J 19(16):4310–4322. doi:10.1093/emboj/19.16.4310

Patwardhan CA, Fauq A, Peterson LB, Miller C, Blagg BS, Chadli A (2013) Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. J Biol Chem 288(10):7313–7325. doi:10.1074/jbc.M112.427328

Picard D (2006a) Chaperoning steroid hormone action. Trends Endocrinol Metab 17(6):229–235. doi:10.1016/j.tem.2006.06.003

Picard D (2006b) Intracellular dynamics of the Hsp90 co-chaperone p23 is dictated by Hsp90. Exp Cell Res 312(2):198–204. doi:10.1016/j.yexcr.2005.10.009

Pratt WB, Dittmar KD (1998) Studies with purified chaperones advance the understanding of the mechanism of glucocorticoid receptor-hsp90 heterocomplex assembly. Trends Endocrinol Metab 9(6):244–252

Pratt WB, Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 18(3):306–360. doi:10.1210/edrv.18.3.0303

Pratt WB, Gehring U, Toft DO (1996) Molecular chaperoning of steroid hormone receptors. Exs 77:79–95

Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 90(1):65–75

Prodromou C, Panaretou B, Chohan S, Siligardi G, O'Brien R, Ladbury JE, Roe SM, Piper PW, Pearl LH (2000) The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. EMBO J 19(16):4383–4392. doi:10.1093/emboj/19.16.4383

Retzlaff M, Hagn F, Mitschke L, Hessling M, Gugel F, Kessler H, Richter K, Buchner J (2010) Asymmetric activation of the hsp90 dimer by its cochaperone aha1. Mol Cell 37(3):344–354. doi:10.1016/j.molcel.2010.01.006

Richter K, Muschler P, Hainzl O, Reinstein J, Buchner J (2003) Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. Binding prevents the N-terminal dimerization reaction during the atpase cycle. J Biol Chem 278(12):10328–10333. doi:10.1074/jbc.M213094200

Richter K, Walter S, Buchner J (2004) The Co-chaperone Sba1 connects the ATPase reaction of Hsp90 to the progression of the chaperone cycle. J Mol Biol 342(5):1403–1413. doi:10.1016/j.jmb.2004.07.064

Röhl A, Rohrberg J, Buchner J (2013) The chaperone Hsp90: changing partners for demanding clients. Trends Biochem Sci 38(5):253–262. doi:10.1016/j.tibs.2013.02.003

Sevier CS, Machamer CE (2001) p38: A novel protein that associates with the vesicular stomatitis virus glycoprotein. Biochem Biophys Res Commun 287(2):574–582. doi:10.1006/bbrc.2001.5621

Siligardi G, Hu B, Panaretou B, Piper PW, Pearl LH, Prodromou C (2004) Co-chaperone regulation of conformational switching in the Hsp90 ATPase cycle. J Biol Chem 279(50):51989–51998. doi:10.1074/jbc.M410562200

Sims JD, McCready J, Jay DG (2011) Extracellular heat shock protein (Hsp)70 and Hsp90alpha assist in matrix metalloproteinase-2 activation and breast cancer cell migration and invasion. PloS One 6(4):e18848. doi:10.1371/journal.pone.0018848

Singh M, Shah V, Tatu U (2014) A novel C-terminal homologue of Aha1 co-chaperone binds to heat shock protein 90 and stimulates its ATPase activity in Entamoeba histolytica. J Mol Biol 426(8):1786–1798. doi:10.1016/j.jmb.2014.01.008

Smith DF, Toft DO (2008) Minireview: the intersection of steroid receptors with molecular chaperones: observations and questions. Mol Endocrinol 22(10):2229–2240. doi:10.1210/me.2008-0089

Sullivan W, Stensgard B, Caucutt G, Bartha B, McMahon N, Alnemri ES, Litwack G, Toft D (1997) Nucleotides and two functional states of hsp90. J Biol Chem 272(12):8007–8012

Sullivan WP, Owen BA, Toft DO (2002) The influence of ATP and p23 on the conformation of hsp90. J Biol Chem 277(48):45942–45948. doi:10.1074/jbc.M207754200

Sun L, Prince T, Manjarrez JR, Scroggins BT, Matts RL (2012) Characterization of the interaction of Aha1 with components of the Hsp90 chaperone machine and client proteins. Biochim Biophys Acta 1823(6):1092–1101. doi:10.1016/j.bbamcr.2012.03.014

Taguwa S, Okamoto T, Abe T, Mori Y, Suzuki T, Moriishi K, Matsuura Y (2008) Human butyrate-induced transcript 1 interacts with hepatitis C virus NS5A and regulates viral replication. J Virol 82(6):2631–2641. doi:10.1128/JVI.02153-07

Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, Lindquist S (2012) Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell 150(5):987–1001. doi:10.1016/j.cell.2012.06.047

Toogun OA, Zeiger W, Freeman BC (2007) The p23 molecular chaperone promotes functional telomerase complexes through DNA dissociation. Proc Natl Acad Sci U S A 104(14):5765–5770. doi:10.1073/pnas.0701442104

Toogun OA, Dezwaan DC, Freeman BC (2008) The hsp90 molecular chaperone modulates multiple telomerase activities. Mol Cell Biol 28(1):457–467. doi:10.1128/MCB.01417-07

Tsutsumi S, Mollapour M, Prodromou C, Lee CT, Panaretou B, Yoshida S, Mayer MP, Neckers LM (2012) Charged linker sequence modulates eukaryotic heat shock protein 90 (Hsp90) chaperone activity. Proc Natl Acad Sci U S A 109(8):2937–2942. doi:10.1073/pnas.1114414109

van Montfort RL, Basha E, Friedrich KL, Slingsby C, Vierling E (2001) Crystal structure and assembly of a eukaryotic small heat shock protein. Nat Struct Biol 8(12):1025–1030. doi:10.1038/nsb722

Wandinger SK, Richter K, Buchner J (2008) The Hsp90 chaperone machinery. J Biol Chem 283(27):18473–18477. doi:10.1074/jbc.R800007200

Wang X, Venable J, LaPointe P, Hutt DM, Koulov AV, Coppinger J, Gurkan C, Kellner W, Matteson J, Plutner H, Riordan JR, Kelly JW, Yates JR 3rd, Balch WE (2006) Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell 127(4):803–815. doi:10.1016/j.cell.2006.09.043

Wayne N, Bolon DN (2007) Dimerization of Hsp90 is required for in vivo function. Design and analysis of monomers and dimers. J Biol Chem 282(48):35386–35395. doi:10.1074/jbc.M703844200

Wayne N, Bolon DN (2010) Charge-rich regions modulate the anti-aggregation activity of Hsp90. J Mol Biol 401(5):931–939. doi:10.1016/j.jmb.2010.06.066

Weaver AJ, Sullivan WP, Felts SJ, Owen BA, Toft DO (2000) Crystal structure and activity of human p23, a heat shock protein 90 co-chaperone. J Biol Chem 275(30):23045–23052. doi:10.1074/jbc.M003410200

Weikl T, Abelmann K, Buchner J (1999) An unstructured C-terminal region of the Hsp90 co-chaperone p23 is important for its chaperone function. J Mol Biol 293(3):685–691. doi:10.1006/jmbi.1999.3172

Weikl T, Muschler P, Richter K, Veit T, Reinstein J, Buchner J (2000) C-terminal regions of Hsp90 are important for trapping the nucleotide during the ATPase cycle. J Mol Biol 303(4):583–592. doi:10.1006/jmbi.2000.4157

Wochnik GM, Young JC, Schmidt U, Holsboer F, Hartl FU, Rein T (2004) Inhibition of GR-mediated transcription by p23 requires interaction with Hsp90. FEBS Lett 560(1–3):35–38. doi:10.1016/S0014-5793(04)00066-3

Young JC, Hartl FU (2002) Chaperones and transcriptional regulation by nuclear receptors. Nat Struct Biol 9(9):640–642. doi:10.1038/nsb0902-640

Zelin E, Zhang Y, Toogun OA, Zhong S, Freeman BC (2012) The p23 molecular chaperone and GCN5 acetylase jointly modulate protein-DNA dynamics and open chromatin status. Mol Cell 48(3):459–470. doi:10.1016/j.molcel.2012.08.026

Zhang Z, Sullivan W, Felts SJ, Prasad BD, Toft DO, Krishna P (2010) Characterization of plant p23-like proteins for their co-chaperone activities. Cell Stress Chaperones 15(5):703–715. doi:10.1007/s12192-010-0182-1

Zhu S, Tytgat J (2004) Evolutionary epitopes of Hsp90 and p23: implications for their interaction. FASEB J 18(9):940–947. doi:10.1096/fj.04-1570hyp

Zhu XJ, Liu X, Jin Q, Cai Y, Yang Y, Zhou T (2010) The L279P mutation of nuclear distribution gene C (NudC) influences its chaperone activity and lissencephaly protein 1 (LIS1) stability. J Biol Chem 285(39):29903–29910. doi:10.1074/jbc.M110.105494

Chapter 7
UCS Proteins: Chaperones for Myosin and Co-Chaperones for Hsp90

Weiming Ni and Odutayo O. Odunuga

Abstract The UCS (***U***NC-45/***C***RO1/***S***he4p) family of proteins has emerged as chaperones that are specific for the folding, assembly and function of myosin. These proteins participate in various important myosin-dependent cellular processes that include myofibril organization and muscle functions, cell differentiation, cardiac and skeletal muscle development, cytokinesis and endocytosis. Mutations in the genes that code for UCS proteins cause serious defects in these actomyosin-based processes. Homologs of UCS proteins can be broadly divided into (1) animal UCS proteins, generally known as UNC-45 proteins, which contain an N-terminal tetratricopeptide repeat (TPR) domain in addition to the canonical UCS domain, and (2) fungal UCS proteins, which lack the TPR domain. Structurally, except for TPR domain, both sub-classes of UCS proteins comprise of several irregular armadillo (ARM) repeats that are divided into two-domain architecture: a combined central-neck domain and a C-terminal UCS domain. Structural analyses suggest that UNC-45 proteins form elongated oligomers that serve as scaffolds to recruit Hsp90 and/or Hsp70 to form a multi-protein chaperoning complex that assists myosin heads to fold and simultaneously organize them into myofibrils. Similarly, fungal UCS proteins may dimerize to promote folding of non-muscle myosins as well as determine their step size along actin filaments. These findings confirm UCS proteins as a new class of myosin-specific chaperones and co-chaperones for Hsp90. This chapter reviews the implications of the outcome of studies on these proteins in cellular processes such as muscle formation, and disease states such as myopathies and cancer.

Keywords UCS · Myosin · TPR · Chaperones · Co-chaperones

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_7

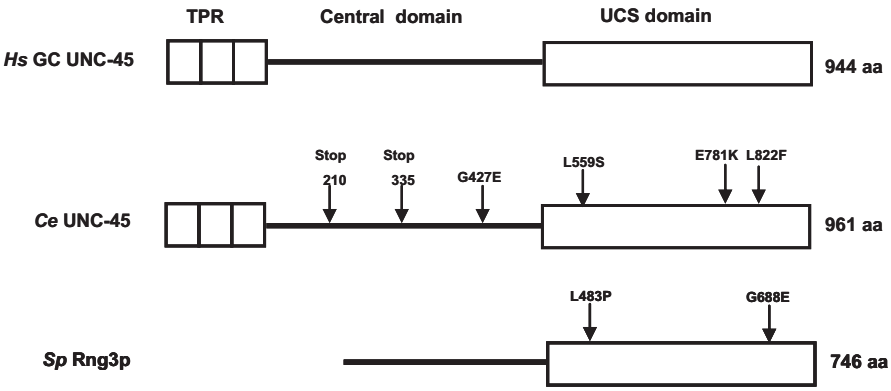

Fig. 7.1 Domain organization of homologs of UCS proteins *Hs* GC UNC-45, *Ce* UNC-45 and *Sp* Rng3p represent the human general cell UNC-45A (NP_061141) (Price et al. 2002), *C. elegans* UNC-45 (NP_497205) (Epstein and Thomson 1974; Barral et al. 2002), and *S. pombe* ring assembly protein 3 (O74994) (Balasubramanian et al. 1998), respectively. The TPR and UCS domains are represented by *small* and *large* boxes, respectively. The *horizontal line* represents the central region. The positions of amino acid substitutions are indicated by *vertical arrows*. (Note: Fig. 7.1 and its legends were used with kind permission from Springer Science + Business Media: Networking of Chaperones by Co-Chaperones, UNC-45: a chaperone for myosin and a co-chaperone for Hsp90, 2007, pp. 62–74, Odutayo O. Odunuga & Henry F. Epstein, Fig. 3)

Introduction

UNC-45 is a prototype of a class of proteins known as the UCS-(UNC-45 in *Caenorhabditis elegans*, CRO1 in *Podospora anserina* and She4p in *Saccharomyces cerevisiae*) domain containing proteins (Hutagalung et al. 2002; Yu and Bernstein 2003). The UCS-domain-containing proteins are emerging as essential for a wide spectrum of myosin- and actin-related cellular processes in many eukaryotes, ranging from fungi to humans (Table 7.1). They are necessary for important cellular processes such as myofibril and sarcomere organization, cell differentiation, embryonic development, cytokinesis, endocytosis, and syncytial-cellular stage transition (Hutagalung et al. 2002; Yu and Bernstein 2003; Odunuga and Epstein 2007). The UCS proteins can be divided into two broad sub-classes; animal and fungal UCS-containing proteins (Table 7.1). The similarity between these two sub-classes of proteins is the presence of the canonical C-terminal UCS domain that is absolutely required for their interaction with myosins. The animal UCS proteins, generally referred to as UNC-45 proteins, contain an N-terminal tetratricopeptide repeat (TPR) (Das et al. 1998) domain which is absent in the fungal proteins (Fig. 7.1). While, only one copy of the gene is found in invertebrates, vertebrates have two copies encoding differentially expressed isoforms (Price et al. 2002). Mutations in the UCS proteins result in various defective actomyosin-based processes such as cytokinesis in *Schizosaccharomyces pombe* (Balasubramanian et al. 1998), endocytosis and trafficking in *S. cerevisiae* (Jansen et al. 1996; Wendland et al. 1996), syncytial-cellular stage transition in *P.* anserina (Berteaux-Lecellier et al. 1998), and

Table 7.1 UCS proteins and their characteristics

UCS proteins	Organisms	Myosin substrates	Loss of function phenotypes
Animal TPR-containing UCS proteins (*UNC-45 proteins*)			
General cell (GC) UNC-45 (UNC-45A/a)	Vertebrates (mouse, human, zebrafish)	Cytoskeletal myosin II	Inhibition of cell proliferation and fusion
Striated muscle (SM) UNC-45 (UNC-45B/b, Steif)	Vertebrates (mouse, human, zebrafish, frog)	Sarcomeric and cytoskeletal myosin II	Loss of sarcomere organization
DUNC-45	*D. melanogaster* (fruitfly)	Sarcomeric and cytoskeletal myosin II	Embryonic and late larval stage lethality, reduced body size and defects in motility
UNC-45	*C. elegans* (Nematode)	Sarcomeric and cytoskeletal myosin II	Fewer thick filaments, myofibril disorganization, paralysis and failure of embryonic cytokinesis
Fungal TPR-lacking UCS proteins			
She4p	*S. cerevisiae* (budding yeast)	Myosin types 1 & V	Loss of actin polarization, defective internalization of membrane, defects in endocytosis
Rng3p	*S. pombe* (fission yeast)	Cytoskeletal myosin II	Defective actomyosin ring, failure of cytokinesis
CRO1	*P. anserina* (filamentous fungus)	?	Inability to form septum, defective syncytial-cellular transition

GC UNC-45: Price et al. 2002; Bazzaro et al. 2007; Comyn and Pilgrim 2012, ***SM UNC-45***: Wohlgemuth et al. 2007; Geach and Zimmerman 2010; Chen et al. 2012; Comyn and Pilgrim 2012, ***DUNC-45***: Yu et al. 2003; Lee et al. 2011b; Melkani et al. 2011, ***UNC-45***: Epstein and Thomson 1974; Venolia and Waterston 1990; Guo and Kemphues 1996; Barral et al. 1998; Venolia et al. 1999; Ao and Pilgrim 2000; Kachur et al. 2004, 2008, ***She4p***: Jansen et al. 1996; Wendland et al. 1996; Toi et al. 2003; Wesche et al. 2003; Lord et al. 2008, ***Rng3p:*** Balasubramanian et al. 1998; Wong et al. 2000, 2002; Lord and Pollard 2004; Mishra et al. 2005; Lord et al. 2008; Stark et al. 2013, ***CRO1***: Berteaux-Lecellier et al. 1998

myofibril organization and cytokinesis in *C. elegans* (Epstein and Thomson 1974; Kachur et al. 2004). *C. elegans* UNC-45 has been established as a chaperone for the motor domain of myosin (Barral et al. 2002). Other UCS proteins such as Rng3p in *S. pombe* (Mishra et al. 2005), She4p in *S. cerevisiae* (Wesche et al. 2003), and DUNC-45 (Yu et al. 2003) in *Drosophila melanogaster* have also been shown to interact with myosin and modulate its function. The interaction of UCS proteins is not limited to sarcomeric myosins alone; cytoskeletal myosins including both

conventional and (filament assembling) myosin II and unconventional (non-assembling) myosins I and V are known to require UCS proteins for their functions (Barral et al. 2002; Wesche et al. 2003). Myosins require the UCS-containing chaperones due to their large size, complexity of their structures and the need to form highly organized oligomeric assemblies which are sometimes composed of different isoforms and other associating proteins (Landsverk and Epstein 2005). Furthermore, UNC-45 recruits Hsp90 to form a ternary complex with myosin (Barral et al. 2002; Mishra et al. 2005). Put together, genetic, biochemical and recent structural studies have established UCS proteins as chaperones that are required in myosin folding, organization and function, and UNC-45 as a co-chaperone (recruiter) for Hsp90 to optimize these processes where the thick filaments are involved.

Myosin Folding and Assembly are Chaperone-Dependent Processes

The myosin family is a large group of motor proteins that interact with actin, hydrolyze ATP and produce movement along the actin filament. Myosins are involved in a broad spectrum of cellular processes that include cellular trafficking, phagocytosis, muscle contraction, cytokinesis and cytoskeletal assembly. The full protein complement of a myosin is composed of two parts: the myosin heavy chains (MHC) and the myosin light chains (MLC). Typically, a myosin heavy chain is comprised of three functional regions: (1) a conserved (catalytic) motor or head that contains actin and ATP binding sites, (2) the neck domain which binds myosin light chains, and (3) the tail domain, which forms an α-helical coiled-coil rod in some myosin types, and serves to anchor and position the motor domain to interact with actin. Myosin II includes the classical conventional myosin first isolated from muscle, but subsequently found in nonmuscle cells and protists (Kuczmarski and Spudich 1980). The sarcomeric muscle myosin II is the only member of the myosin family that is assembled into thick filaments of skeletal and cardiac muscles. Myosin is a multidomain protein; therefore its folding pathway may be expected to be complex (Srikakulam and Winkelmann 1999). The myosin head itself contains multiple domains connected by flexible loops (Rayment et al. 1993a, b) while the light chains and rod are simpler in structures (Atkinson and Stewart 1991; Saraswat and Lowey 1991). Myosin light chains and rod when expressed in bacteria, fold into functionally active structures (Atkinson and Stewart 1991; Saraswat and Lowey 1991). Regardless of their origin, expression of myosin motors has proved difficult in bacteria; this may be due to the complex nature of their structure (McNally et al. 1988; Mitchell et al. 1989). Using the baculovirus expression system in insect cells, considerable success was achieved in expressing heavy meromyosin (HMM) from cytoskeletal types II, V and VI, and to a limited extent, cardiac myosins (Trybus 1994; Sweeney et al. 1998; Wang et al. 2000). These HMMs have properly folded motor domains that are capable of binding actin (Trybus 1994; Sweeney et al. 1998; Wang et al. 2000). However, the fast skeletal muscle myosin head has been expressed in

active form in C2C12 mouse cell line suggesting that this myosin requires additional cofactor(s) which are present in myogenic cells for folding (Kinose 1996; Chow et al. 2002). *In vitro*, chimeric fast skeletal muscle myosin head fused to green fluorescent protein (GFP) folds very slowly and transits through multiple intermediates in a temperature-dependent manner that strongly suggests a high susceptibility to off-pathway interactions and aggregations and hence the need for chaperone-assisted folding (Chow et al. 2002). Expression of the protein *in vivo* is cell-dependent: C2C12 myogenic cell lines yield a folded and active protein that exhibits Mg^{2+} ATP-sensitive actin-binding and myosin motor activity, while epithelia cell lines yield inactive protein aggregates (Chow et al. 2002). This observation suggests that the myosin motor requires cytosolic molecular chaperones to fold correctly under physiological conditions and that the required factor(s) are optimized in muscle cells (Chow et al. 2002). In addition, during *de novo* folding and assembly of striated muscle myosin heavy chain, Hsp70 and Hsp90 colocalize with the myosin intermediates but not the mature myofibrils (Srikakulam and Winkelmann 2004). Using biochemical analyses, Liu et al. (2008) and Srikakulam and Winkelmann (2008) showed that UNC-45 forms a stable complex with Hsp90 that specifically binds unfolded myosin motor and promotes its folding.

UCS Proteins-Hsp90 Interaction in Myosin Folding, Assembly and Function

Genetic, biochemical and structural studies have confirmed that UCS proteins, especially UNC-45 interact with Hsp90 chaperone (Barral et al. 2002; Mishra et al. 2005; Srikakulam and Winkelmann 2004, 2008; Liu et al. 2008; Gazda et al. 2013). Full-length UNC-45 from *C. elegans* binds both endogenous Hsp70 and Hsp90 from Sf9 insect cell lysates (Barral et al. 2002). Mutant UNC-45 protein lacking the TPR domain interacts with Hsp70 but not Hsp90 also from Sf9 insect cell lysates, indicating that the interaction of UNC-45 with Hsp90 is specifically mediated by the TPR domain (Barral et al. 2002). In surface plasmon resonance spectroscopy experiments, the binding of Hsp90 to the TPR domain of UNC-45 is preferentially competed by Hsp90 C-terminal peptides in comparison to the analogous Hsp70 peptides (Barral et al. 2002). C-terminal peptides of both Hsp70 and Hsp90 have been co-crystallized with the TPR domain of UNC-45 (Gazda et al. 2013). Purified Hsp90, myosin and UNC-45 can form the three possible binary complexes in pull-down assays (Barral et al. 2002). When expressed in striated muscle cells, UNC-45 was isolated as a stable complex with Hsp90 (Liu et al. 2008; Srikakulam and Winkelmann 2008). Both the myosin binding and chaperoning activities of UCS proteins have been mapped to the UCS domain of the protein (Barral et al. 2002; Mishra et al. 2005; Srikakulam and Winkelmann 2004, 2008; Liu et al. 2008; Shi and Blobel 2010; Ni et al. 2011). Therefore both UNC-45 and Hsp90 (Du et al. 2008; Gaiser et al. 2011) are capable of interacting directly with and exerting chaperoning activity on myosin motor domain and thick filament assembly. However,

experimental evidence showed that UNC-45s chaperoning activity on myosin heads is also necessary for myosin contractile function and turnover *in vivo* (Du et al. 2008; Kachur et al. 2008; Shi and Blobel 2010; Gaiser et al. 2011; Melkani et al. 2011; Ni et al. 2011; Gazda et al. 2013). The interaction of UNC-45 with Hsp70 appears to be a classical chaperone-client interaction, in which the promiscuous Hsp70 binds nonspecifically to various unfolded myosin polypeptides to assist in folding them. The interaction of UNC-45 with Hsp90 is a specific TPR-mediated co-chaperone-chaperone association (Barral et al. 2002; Gazda et al. 2013). Interestingly, studies on *S. pombe* and *S. cerevisiae* showed that even the TPR-less Rng3p and She4p UCS proteins are found in complexes involving Hsp90 and myosin (Lord and Pollard 2004; Millson et al. 2005; Melkani et al. 2011). Hsp90 is capable of acting as a chaperone on its own without assistance from other proteins. However, there seems to be a distinction between TPR-less and TPR-containing UCS proteins on their dependence on Hsp90 for chaperoning activities. It appears that UNC-45 recruits and retains Hsp90 via its TPR domain to not only assist in folding myosin heads but to maintain the integrity of myosin assemblies such as the thick filaments. On the other hand, fungal TPR-less UCS proteins may not necessarily need to retain Hsp90 in close proximity to isolated myosin heads (Gazda et al. 2013).

Structural Organization and Versatility of UCS Proteins as Myosin Chaperones

Structurally, UNC-45 protein contains three domains: an N-terminal domain characterized by the presence of three TPR repeats (Figs. 7.1 and 7.2a), a central domain comprising of central and neck regions, and a C-terminal UCS domain (Shi and Blobel 2010; Lee et al. 2011a; Gazda et al. 2013). The TPR repeat is a degenerate motif that occurs in multiple copies in proteins and forms scaffolds that mediate protein-protein interactions (Sikorski et al. 1990; Das et al. 1998; Blatch and Lassle 1999; Scheufler et al. 2000). The TPR domain of UNC-45 preferentially binds Hsp90 (Barral et al. 2002; Gazda et al. 2013). Both the central and the UCS domains of *C. elegans* UNC-45 share strong sequence conversation only with other animal UCS-containing proteins. Prior to determination of their three-dimensional structures, the UCS domain was identified based on the positions of temperature-sensitive (*ts*) mutations in *C. elegans* UNC-45 and the presence of blocks of sequence identity among UNC-45, CRO1 and She4p (Fig. 7.1; Barral et al. 1998; Berteaux-Lecellier et al. 1998; Wong et al. 2000). It was later confirmed by the presence of analogous *ts* mutation in *S. pombe* Rng3p (Wong et al. 2000). The two UNC-45 null mutations that result in stop codons are both located in the central region while three of the four UNC-45 *ts* mutations are found in the UCS domain. UNC-45 proteins share sequence conservation with fungal UCS-proteins at key sites within the UCS domains. Recently, the three-dimensional structures of three UCS proteins, She4p (Shi and Blobel 2010), DUNC-45 (Lee et al. 2011a), and UNC-45 from *C. elegans* (CeUNC-45) (Gazda et al. 2013) were determined

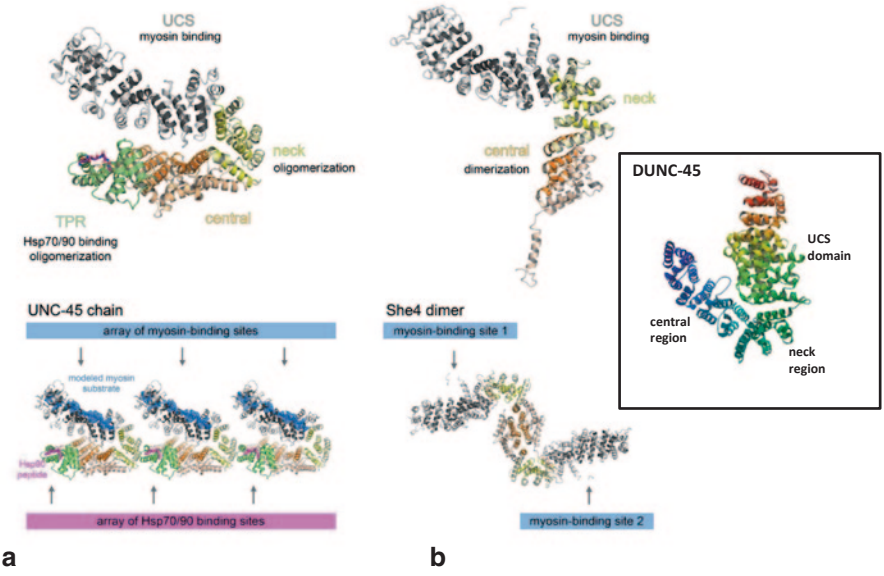

Fig. 7.2 Structures of representative UCS proteins (**a**) *Upper panel*: cartoon representation of a protomer of *C. elegans* UNC-45 (PDB code: 4i2z) with co-crystallized Hsp90 peptide (*magenta*). *Lower panel*: UNC-45 chain formed by three protomers linked via a TPR domain-neck region interaction as seen in crystal lattice (Gazda et al. 2013). Co-crystallized Hsp90 peptide is shown in magenta while a modeled myosin substrate based on beta-catenin/E-cadherin co-crystal structure (PDB code 1i7×) is shown in *blue*. **b** *Upper panel*: cartoon representation of a She4 monomer (PDB code: 3opb). *Lower panel*: Dimer of She4 formed by central domain interaction as seen in the crystal packing (Shi and Blobel 2010). Insert: structure of *D. melanogaster* UNC-45 lacking the TPR domain (Lee et al. 2011a). (Note: this figure, without the insert, was adapted from Hellerschmied and Clausen 2014)

by X-ray crystallography (Fig. 7.2a, b). In these structures, apart from the easily recognizable N-terminal TPR domain in CeUNC-45 (not resolved in DUNC-45), the rest of the protein is composed of repeating helices that organize themselves into irregular three-helix armadillo (ARM) repeats. An ARM repeat is a 40-amino acid long sequence comprising of three helices that mediates a variety of protein-protein interactions, for example in β-catenin (Peifer et al. 1994). The central domain of CeUNC-45, which can be further divided into central and neck (or bend in She4p) regions, forms a rigid and somewhat flat backbone structure to which is attached the canonical UCS domain, a more cylindrical and flexible superhelix, on the C terminus. In the CeUNC-45 structure, the rigid central-neck backbone thus provide binding grooves oriented in two directions; the TPR domain on the N-terminus to bind Hsp90 and the UCS domain on the C-terminus to bind myosin. In addition to providing rigidity to maintain the orientation of TPR and UCS domains, the central-neck domain serves as dimerization region in She4p (Shi and Blobel 2010) and possibly oligomerization in UNC-45 proteins (Fig. 7.2a, b; Gazda et al. 2013). In She4p, the UCS domain groove can accommodate a 27-residue epitope, located

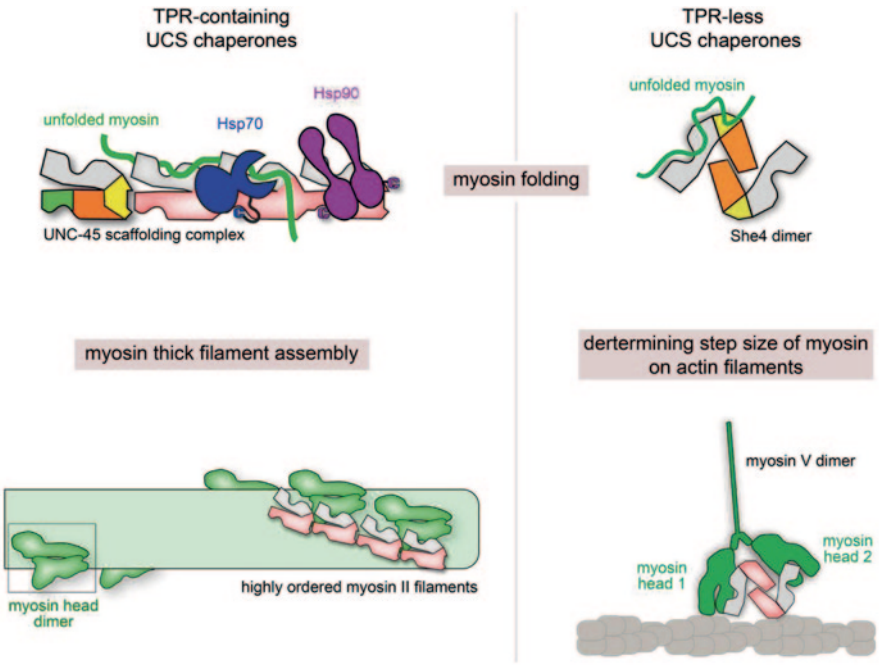

Fig. 7.3 Proposed models for myosin-specific chaperoning activities of UCS proteins *Left panel*: TPR-containing UCS proteins, UNC-45, oligomerize to form scaffolds that serve as sites to recruit multiprotein chaperone complexes, comprising of Hsp90, Hsp70 and UNC-45 itself, that simultaneously assist in myosin folding and myofilament formation. *Right panel*: TPR-less UCS proteins, e.g. She4, act as adaptors that physically link two myosin heads to assist in folding them as well as regulate their step size along actin filaments. (Note: this figure was adapted from Hellerschmied and Clausen 2014)

in the ATP- and actin-binding region, of a myosin V from yeast with a binding affinity of approximately 1 μM (Shi and Blobel 2010). Previous biochemical study established that the TPR domain in UNC-45 binds Hsp90 peptide with a 10-fold higher affinity than Hsp70 peptide (Barral et al. 2002), suggesting a more specific interaction with the Hsp90 molecular chaperone. These biochemical and structural studies have provided critical insights into the versatility of UCS proteins as myosin-specific chaperones in general and perhaps molecular explanation of the evolutionary requirement for TPR domain in UNC-45. In TPR-less homologs of UCS proteins, typified by She4p, the neck region participates in dimerization such that two L-shaped She4p molecules form a Z-shaped zigzag molecule. In this dimeric form, She4p can act as adaptor that physically links two myosin heads to assist in folding them as well as regulate their step size along actin filaments (Fig. 7.3; Shi and Blobel 2010; Hellerschmied and Clausen 2014). In this model, Hsp90 can bind in a TPR-independent manner to She4p-myosin complex to perform its chaperone function. On the other hand, the chaperoning activity of UNC-45 appears to be more sophisticated in higher eukaryotes in which highly organized myosin filaments

have evolved (Gazda et al. 2013; Hellerschmied and Clausen 2014). Structural and biochemical analyses suggested that CeUNC-45 (Gazda et al. 2013) forms transient linear protein structures (essentially short filaments) by oligomerization that is mediated by a repeating TPR domain-neck region interaction (Gazda et al. 2013). The tilting of both the TPR and UCS domains at specific angles relative to the rigid central-neck domain enables two helices (7H2 and 8H2) in the neck region of one molecule of CeUNC-45 to bind to two helices (TPR3B and kinked helix) in the TPR domain of adjacent molecule in a defined array, and simultaneously allowing for interaction with Hsp90/or Hsp70 and myosin motors (Fig. 7.3). The resulting multimeric scaffolds can thus serve as sites to recruit multiprotein chaperone complexes that simultaneously assist in myosin folding and myofilament formation (Gazda et al. 2013; Hellerschmied and Clausen 2014). Interestingly, it was observed that the spacing (periodicity) of UNC-45 molecules in the so-called 'UNC-45 filaments' may closely match the spacing of neighboring myosin heads in the thick filaments under certain conditions (Gazda et al. 2013). Put together, these experimental observations provide strong evidence for the requirement for UCS proteins not only in the folding of myosin heads but also in myofibrillar assembly (Myhre et al. 2014) and regulation of myosin-dependent processes. UCS proteins thus appear to be active participants in sarcomere assembly (Myhre et al. 2014). The regulation of the level of UNC-45 protein in muscle cells is important (Hoppe et al. 2004; Janiesch et al. 2007; Landsverk et al. 2007). Overexpression of UNC-45 in muscle cells resulted in increased myosin degradation and thus reduced or disorganized myofibrils (Janiesch et al. 2007; Landsverk et al. 2007). The chaperoning activity of UNC-45 on myosin seems to be dependent on regulation by the ubiquitin/proteasome system. A novel E3/E4-multiubiquitylation complex comprising of CDC-48, UFD-2 and CHN-1 proteins has been shown to link the turnover of both UNC-45 and myosin to functional muscle formation (Hoppe et al. 2004; Janiesch et al. 2007). Similar effects were observed in yeasts (Lord et al. 2008). From these studies, it is clear that, apart from its involvement in myosin folding, myofibrillogenesis and functional muscle formation, UNC-45 is important in muscle repair and perhaps aging (Hoppe et al. 2004; Janiesch et al. 2007; Landsverk et al. 2007; Lord et al. 2008).

UNC-45 Proteins in Invertebrates

The *unc-45* gene was originally identified in *C. elegans* through the recessive, *ts* mutant allele, *e286* (Epstein and Thomson 1974). *C. elegans* possesses only one copy of the *unc-45* gene. The *e286* mutant worms are paralyzed, with disorganized thick filaments in their muscles when grown at 25 °C, but at the permissive temperature of 15 °C, the worms display phenotypes essentially similar to the wild-type (Epstein and Thomson 1974). These phenotypes can be reversed by temperature shifts in developing embryos and larvae but not in adult worms, implying that UNC-45 possesses a function essential for proper myofilament arrays to form (Epstein and Thomson 1974). Detailed genetic analysis revealed three additional

recessive *ts* mutations and two lethal mutations in the *unc-45* gene (Venolia and Waterston 1990; Barral et al. 1998). All of the *ts* alleles, which show similar effects on myofibril formation, contain missense substitutions in the UCS domain of the UNC-45 protein (Barral et al. 1998). The lethal alleles each contains a stop codon located within the central region of the protein (Fig. 7.1) preventing further translation of the unc-*45* gene product (Barral et al. 1998). Genetic analysis confirmed functional relationships between the protein products of *unc-45* and of the *unc-54* and *myo-3* genes, which code for myosin heavy chain A and B respectively that form homodimeric myosins in the body wall muscle of *C. elegans* (Waterston 1988). The *unc-45 ts* mutants directly affect myosin B, the major isoform, by generating an incorrectly folded myosin B which drastically reduces the number of intact thick filaments and therefore incapable of forming proper myofilament assemblies (Waterston et al. 1980; Waterston 1988). Null mutations in the *unc-54* gene generate defects in muscle structure and functions similar to that of the *unc-45 ts* alleles, implying that the two genes may be epistatic (Waterston 1988). In the normal *C. elegans* thick filament, the two myosin isoforms are differentially assembled such that myosin B flanks a central myosin A zone (Schachat et al. 1977; Miller et al. 1983). However, in worms harboring the *unc-45 ts* mutant genes, this differential assembly is lost and instead, there is a scrambling of the myosins (Barral et al. 1998), which might be due to improper folding of the myosins and consequent decreases in their concentrations (Hoppe et al. 2004; Janiesch et al. 2007; Landsverk et al. 2007). The lethal *unc-45* alleles cause arrest of development at the two-fold embryonic stage resulting in inability to produce functional body wall muscle (Venolia and Waterston 1990). Interestingly, mutant worms lacking the essential *myo-3* encoded myosin heavy chain A, the minor isoform of myosins found in *C. elegans* body wall muscle, also manifest severely impaired thick filament assembly with little or no body movement (Waterston 1989). This observation suggests the necessity for myosin A in the formation of the nematode thick filament; moreover, myosin B cannot substitute for myosin A to reverse the phenotype (Waterston 1989). CFP tagged UNC-45 could be detected in all bands of the nematode muscle ultrastructure and this fusion UNC-45 protein stably binds to myosin B containing bands but nematode Hsp90 seems to function in the maintenance of muscle structures as a transiently accompanying diffusible factor with unstable association with the I-band and the M-line (Gaiser et al. 2011). In addition, decreased pharyngeal pumping in worms containing the *unc-45 ts* mutant genes suggests that myosin C and D, which are exclusively found in the pharyngeal muscles of *C. elegans* (Ardizzi and Epstein 1987), may be affected (Venolia and Waterston 1990). Localization by both antibodies and GFP tagging showed that UNC-45 protein is expressed in all *C. elegans* muscle cells at the adult stage (Venolia et al. 1999; Ao and Pilgrim 2000). In the developing body wall muscle of *C. elegans* larvae, UNC-45 is found in the cytosol, whereas in the mature adult muscle, it is localized to the A bands of the sarcomere, apparently chiefly with myosin B (Ao and Pilgrim 2000). *C. elegans* UNC-45 also colocalizes in the cleavage furrow with the conventional cytoskeletal myosin II (NMY-2), a protein that plays an essential role during embryonic cytokinesis (Guo and Kemphues 1996). *C. elegans* germline and embryo have abundant

UNC-45 protein and RNA interference studies reveal that the UNC-45 protein (1) is maternally contributed, hence rescue can occur to some extent and, (2) that it plays a role in cytokinesis in addition to muscle development (Kachur et al. 2004). The interaction between NMY-2 and maternally supplied UNC-45 has been further proven to be necessary for embryonic polarity establishment and germline cellularization (Kachur et al. 2008).

In *D. melanogaster* embryos, high levels of *dunc-45* (*D. melanogaster* homolog of *unc-45* gene) RNA were detected in mesodermal precursors to muscle; with accumulation in other tissues as well (Yu et al. 2003). This suggests that *dunc-45* gene product (DUNC-45) may be important in multiple cell types. Similarly to *C. elegans*, *D. melanogaster* possesses only one copy of the *dunc-45* gene whose protein product, DUNC-45, has been shown to possess classical chaperone activity (Melkani et al. 2010). DUNC-45 is constitutively expressed during development in *D. melanogaster* and peaks at pupation, when adult tissues are being formed. DUNC-45 associates with nonmuscle myosin in embryonic blastoderm of 2-h-old embryos and then DUNC-45 enriches mostly in striated muscles of 14-h-old embryos, which is similar to muscle myosin (Lee et al. 2011b). Mutations in the *dunc-45* gene leading to DUNC-45 deficiency cause embryonic as well as late larval stage lethality, and reduced body size and defects in motility appear to be the results of embryonic body wall muscle dysfunction and defective myosin accumulation (Yu et al. 2003; Lee et al. 2011b). The *unc-45* knockdown experiment via RNA specifically targeted to *Drosophila* heart revealed that adult heart had been severely affected owing to reduced muscle contractility, decreased cardiac myosin accumulation, disassembled myofibrils, and myofibrillar disarray while there were just mild cardiac abnormalities in third instar larvae and young pupal heart (Melkani et al. 2013). Severe cardiac problems develop during metamorphosis as *unc-45* knockdown after metamorphosis led to less serious phenotypes, which suggests that DUNC-45 is indispensible for myosin accumulation and folding during remodeling of the forming adult *Drosophila* heart (Melkani et al. 2011). It is also reported that co-expression of UNC-45 with expanded polyQ-72 (Huntington's disease-causing poly-glutamine repeats which lead to protein unfolding in cardiac cells) in *Drosophila* heart could reverse polyQ-72 induced cardiac dysfunction such as protein aggregation and myofibril disassembly (Melkani et al. 2013). Thus, DUNC-45 is important for myosin to be fully functional through all stages of *Drosophila*'s lifespan.

UNC-45 Proteins in Vertebrates

Vertebrates have two copies of *unc-45*-like genes encoding distinct isoforms of UNC-45 (Price et al. 2002). The genes are designated as *UNC45A* and *UNC45B* in human, and *UNC45a* and *UNC45b* in mouse respectively. In mouse, *UNC45a* encodes an isoform that is expressed in multiple adult organs including uterus, kidney, lung and liver, hence the designation general cell (GC) UNC-45 (Price et al. 2002). The second isoform encoded by *UNC45b* is found almost exclusively in

heart and skeletal muscles, and was therefore designated as striated muscle (SM) UNC-45 (Price et al. 2002). The two isoforms share 50–55 % sequence identity in both human and mouse. There is > 90 % sequence identity among similar isoforms between these species. When compared with *C. elegans* UNC-45, both isoforms show 30–40 % identity with the worm protein. In eight-day old mouse embryo, UNC-45b is predominantly expressed in the contractile heart and is hardly found in other organs; whereas UNC-45a is diffusely expressed and later concentrates in regions of intense development such as the branchial arches and forelimb bud (Price et al. 2002).

Studies on *Danio rerio* (zebrafish) confirmed that UNC-45a has important function in pharyngeal arch and aortic arch development and UNC-45a is involved in the generation of arteriovenous malformation (AVM) (Anderson et al. 2008). Knockdown of UNC-45b in zebrafish by morpholino-oligonucleotide caused myofibril disassembly in the sarcomeres of the trunk muscle and the ventral displacement of jaw cartilages, thus demonstrating that the protein is required for skeletal, cranial and cardiac muscle contraction (Wohlgemuth et al. 2007). Therefore, UNC-45b is necessary for zebrafish motility and it is also essential for morphogenesis and function of the developing heart and jaw. Overexpression of *D. rerio* UNC-45b mimicked results of similar experiments in *C. elegans* as transgenic zebrafish embryos had defective myofilament assembly in skeletal muscles (Bernick et al. 2010). The defect by UNC-45b overexpression was shown to depend on the UCS domain but not the TPR domain since deletion of the UCS domain revoked myofibril disorganization by UNC-45b overexpression and deletion of the TPR domain had no effect (Bernick et al. 2010). Further studies on zebrafish revealed that the central region of UNC-45b mediated Z line association and interacted with Apo2a (the cytidine deaminase Apobec 2a) (Etard et al. 2008, 2010). The interaction between UNC-45b and Apo2a is necessary for integrity of the myosepta and myofiber attachment in zebrafish, which is Hsp90-independent (Etard et al. 2010). The *apo2* mutant embryos share similar dystrophic muscle phenotypes with *unc45b* mutants but not with *hsp90α* mutants as *hsp90α* mutant had normal myosepta structure and a beating heart (Etard et al. 2010). Missense mutation of *Xenopus tropicalis* UNC-45b caused skeletal muscle myofibril disruption, paralysis and heart beat problem, suggesting that UNC-45b participates in the Z-body maturation (Geach and Zimmerman 2010). A major implication of these studies is that loss of function of UNC-45 may lead to defective muscle formation and thus myopathies (Etard et al. 2008, 2010; Geach and Zimmerman 2010).

In C2C12 myogenic cells, only UNC-45a mRNA is detected in proliferating myoblasts, with the level decreasing as the cells progress to form myotubes (Price et al. 2002). In contrast, UNC-45b mRNA is detected only after the cells start fusing, peaking in young myotubes and dropping off as the myotubes age (Price et al. 2002). These observations in both mouse embryo and C2C12 myoblasts implicate stage-specific expression and functions of the UNC-45 isoforms in embryogenesis and muscle differentiation. The UNC-45a isoform may be involved in cell division and related cytoskeletal formation while the UNC-45b isoform may be related to striated muscle differentiation and myofibril formation. In fact, in C2C12

knock-down experiments using anti-sense oligonucleotides, UNC-45a antisense severely reduced myoblast proliferation and fusion while UNC-45b antisense results in significant loss of sarcomere organization (Price et al. 2002). Reduction of UNC-45b mRNA did not affect the level of skeletal myosin heavy chain, whereas lowering of UNC-45a levels by antisense did (Price et al. 2002). Low levels of UNC-45a result in reduction of cell proliferation and differentiation which decreases the expression of sarcomeric myosin. The expression of UNC-45b, however, starts at the fusion stage; therefore it may not affect myosin synthesis but rather its organization into thick filaments. Thus the differential expression of the two UNC-45 isoforms separates myosin synthesis from its organization into myofilaments. Interestingly, UNC-45a has been reported to have higher apparent affinity and greater folding capability of smooth muscle myosin motor domain, compared to UNC-45b (Liu et al. 2008). In addition, UNC-45A prefers to bind with Hsp90β *in vitro* and it was shown that UNC-45A is essential for Hsp90β but not Hsp90α to assume normal cellular distribution in HeLa cell via siRNA knockdown experiments (Chadli et al. 2008).

Two isoforms of UNC-45A were detected in several breast carcinomas: a 929-amino-acid protein isoform and a 944-amino-acid protein isoform, which differ by an N-terminal proline-rich 15-amino-acid sequence (Guo et al. 2011). The protein level of the 929-amino-acid protein was found to be 3-fold higher due the 944-amino acid protein being degraded at a 5-fold higher rate that the former by the ubiquitin-proteosome system (Guo et al. 2011). Immunoprecipitation experiments showed that both UNC-45A isoforms could interact with non-muscle myosin IIA, non-muscle myosin IIB, and Hsp90β, suggesting that both UNC-45A isoforms are capable to play functional roles in cell motility (Guo et al. 2011). Further experimental evidence that proved that the two isoforms of UNC-45 are functionally divergent was obtained from studies using zebrafish (Comyn and Pilgrim 2012). The authors found out that the singly homologous zygotic mutant *unc45b*$^{-/-}$ and the doubly homologous mutant u*nc45b*$^{-/-;}$ u*nc45a*$^{-/-}$ displayed identical defects in cardiac and skeletal muscle and jaw formation Comyn and Pilgrim 2012). They therefore concluded that unc-45a did not appear to play an important role in muscle development and that the two gene paralogs are functionally divergent.

To elucidate the importance of muscle-specific UNC-45b's function *in vivo*, studies were carried out on three UNC-45b recessive loss-of-function lines in C3H and C57BL/6 inbred mouse strains (Chen et al. 2012). These mutations caused arrest of cardiac morphogenesis at the formation of right heart structures and failure of contractile function (Chen et al. 2012). A novel outcome of these experiments was the finding that UNC-45b was essential for sufficient accumulation and function of the cysteine-zinc finger protein GATA4 (an important transcription factor during development) in mouse embryonic cardiac morphogenesis (Chen et al. 2012). Pull-down experiments confirmed a direct physical interaction between UNC-45b and GATA4 (Chen et al. 2012). Therefore, the heart-specific UNC-45b isoform functions as a molecular chaperone mediating contractile function of the sarcomere and gene expression in cardiac development. Recently, UNC-45B has been reported to be involved in lens development and pathogenesis of autosomal dominant juvenile cataract in humans (Hansen et al. 2014). It is hypothesized that developmental cataract

might be induced by defective non-muscle myosin organization during maturation of the lens fiber cells, which is caused by UNC-45B mutation (Hansen et al. 2014). At the molecular level, these phenotypes could be due to defective direct or indirect myosin-UNC-45 interactions (Kaiser et al. 2012; Fratev et al. 2013).

UCS Proteins in Yeasts and Fungi

UCS domain proteins have been identified in *S. cerevisiae* (She4p) (Jansen et al. 1996; Wendland et al. 1996), *P. anserina* (CRO1) (Berteaux-Lecellier et al. 1998), and *S. pombe* (Rng3p) (Balasubramanian et al. 1998). Sequence similarity among the three fungal proteins and UNC-45 is restricted to the C-terminal UCS domain. Despite the lack of TPR motifs, the N-terminal sequence of fungal UCS proteins may contain sequences capable of recruiting chaperones (Young et al. 2003). However, all three TPR-less UCS proteins are linked by their common association with cellular processes involving myosins. Although sequence similarity among the fungal UCS proteins is low (Lord and Pollard 2004), the *ts* mutations in *S. pombe*, like *C. elegans* are in conserved residues (Fig. 7.1).

The *she4p* gene was identified and named differently in two independent screens in *S. cerevisiae* (Jansen et al. 1996; Wendland et al. 1996). The first screen was for the expression of the HO endonuclease in mother cells yielding the She4p-encoding gene (SHE: Swi5p-dependent HO expression) (Jansen et al. 1996), and the other for defects in endocytosis identifying the *dim1* gene (*dim*: defective internalization of membrane) (Wendland et al. 1996). Both null and *ts* mutants of the *she4p* gene caused defects in endocytosis and loss of actin polarization in the cell. Two-hybrid and biochemical experiments showed that She4p interacts, via its UCS domain, with the motor domains of conventional type II myosin (Myo1p) as well as unconventional types 1 (Myo3p/Myo5p) and V (Myo2p/Myo4p) myosins in an actin-dependent manner for proper endocytosis and cytokinesis to occur (Toi et al. 2003; Wesche et al. 2003). She4p was also reported to be important for myosin stability and interactions with actin (Lord et al. 2008). In addition, She4p interacts with Hsp90 in yeast two-hybrid assays (Millson et al. 2005). The She4p protein is composed of 789 amino acids and shares about 33 % similarities with other fungal UCS proteins (Lord and Pollard 2004).

The *rng3* gene was identified in a large-scale screen for genes whose products function in cytokinesis (Balasubramanian et al. 1998). The gene encodes a protein of 746 amino acid residues. Actin ring formation was found defective in *S. pombe* cells harboring mutants of both *rng3* (*rng3–65*) and *rng5* (*rng5-E1*) which encodes for Myo2 (a type II myosin heavy chain), suggesting a functional interaction between the protein productions of the two genes. Null mutants in *rng3* resemble deletion mutants in *myo2* while *ts rng3* mutants show strong adverse interactions with Myo2-E1 mutant myosin (Wong et al. 2000). Rng3p colocalizes with *myo2-E1* mutant myosin at the cell division site in an F-actin-dependent manner. More importantly, Rng3p has been shown to be necessary for the formation of progenitor

'spots' that form the actomyosin ring assembly in interphase *S. pombe* cells (Wong et al. 2002). Maintenance of the myosin-containing spots however, is independent of F-actin. While the actomyosin ring has a rapid turnover, the interphase spot does not, showing that this progenitor structure in the interphase is necessary to ensure proper assembly of the actomyosin ring and successful cell division. Recombinant full-length Rng3p or its UCS domain alone are necessary and sufficient to activate the actin-based motility of myosin *in vitro* and double its actin-activated Mg^{2+}-ATP activity (Lord and Pollard 2004; Lord et al. 2008). Although Rng3p is specifically necessary to maintain the activity of intrinsically unstable Myo2, it may possess the capability of responding to changes in the stability of other myosins (Stark et al. 2013). Rng3p was also found to associate with polysomes and bind to mRNAs encoding all types of myosin heavy chains, which suggest that Rng3p may be involved in myosin folding cotranslationally (Amorim and Mata 2009).Whether Rng3p and other fungal UCS proteins require Hsp90 for their myosin-dependent functions is uncertain. However, *in vivo*, Swo1p (Hsp90 homolog in *S. pombe*) and Rng3p have been shown to be both required for Myo2 assembly in the contractile ring (Mishra et al. 2005). These observations suggest that some functional relationship exists between the *S. pombe* UCS protein and Hsp90.

The CRO1 protein of the filamentous fungus, *P. anserina*, is a 702-residue protein that is required for sexual sporulation (Berteaux-Lecellier et al. 1998). GFP-tagging of the CRO1 protein reveals that it is a cytosolic protein expressed mainly at the beginning of the dikaryotic stage and at the time of ascospore maturation. The primary defect of null mutant allele of the gene, *cro1-1* is the inability to form septa between the daughter nuclei after mitotic division. The mutant also results in abortive meiosis of resultant polyploidy nuclei and lack of progression from the syncytial (vegetative) state to the cellular (sexual) state (Berteaux-Lecellier et al. 1998). Unlike the wild type fungal filaments, disorganization of the actin prevents microtubule disassembly.

UNC-45 and Cancers

Recently, more and more results have been emerging to link UNC-45 to different cancers. The possible non-myosin function of UNC-45 was first described in progesterone receptor (PR) chaperoning pathway (Chadli et al. 2006). UNC-45A was identified as a new factor to regulate Hsp90-dependent PR chaperoning in a yeast two-hybrid screen for additional PR binding factors (Chadli et al. 2006). UNC-45A can interact with PRs *in vivo* and *in vitro*. It was shown that UNC-45A inhibits the activation of Hsp90 by the cochaperone Aha1 and blocks progression of PR chaperoning to its hormone binding state in the simplified cell-free system, which limit Hsp90-dependent PR chaperoning (Chadli et al. 2006). Since PR has been implicated in breast cancer for a long time, and not only tumor cell proliferation but also tumor metastasis depend on myosin's function, researchers began to explore the relationship between cancer and UNC-45. Ovarian cancer was discovered to have

correlation with UNC-45A levels (Bazzaro et al. 2007). Severe carcinoma exhibited increased expression level of UNC-45A compared to normal ovarian surface epithelium and benign cystadenoma (Bazzaro et al. 2007). High-stage carcinoma had UNC-45A expressed in greater amount compared with low-stage serous carcinoma (Bazzaro et al. 2007). There were also enhanced UNC-45A transcripts and protein levels in ovarian cell lines in comparison with those in immortalized ovarian surface epithelia cells (Bazzaro et al. 2007). Ovarian cancer cell proliferation was elevated by the ectopic expression of UNC-45A but was decreased by siRNA knockdown of UNC-45A without changing myosin II levels (Bazzaro et al. 2007). Similarly, knockdown of UNC-45A attenuated the spreading of ovarian cancer cells and overexpression of UNC-45A increased the spreading ability (Bazzaro et al. 2007). UNC-45A was next discovered to be able to confer transferred cells resistance to histone deacetylase inhibitors (HDACIs) treatment in a large-scale gain-of-function genetic screen in order to identify potential biomarkers of therapy response (Epping et al. 2009). UNC-45A also inhibits the signaling transduction pathway of the retinoic acid receptor α, which hints that HDACIs employ retinoic acid pathway at least partly for antitumor effect (Epping et al. 2009). It was shown that retinoic acid-induced proliferation arrest could be relieved by UNC-45A expression and UNC-45A expression could also inhibit retinoic acid-induced differentiation of human neuroblastoma cells (Epping et al. 2009). In addition, the expression of UNC-45A further suppressed the induced expression of endogenous retinoic acid receptor target genes (Epping et al. 2009). Human breast carcinoma and cell lines derived from breast carcinoma metastases also had enhanced UNC-45A mRNA and protein expression levels (Guo et al. 2011). RNAi knockdown of endogenously overexpressed UNC-45A caused significantly reduced cell proliferation and invasion in the most metastatic cell line (Guo et al. 2011).

Conclusions and Future Work

The discovery of *C. elegans* UNC-45 and other UCS-domain proteins has led to the new research area of myosin-targeted chaperones. The complexity of myosin motor domain in terms of its structure and the multiplicity of its conformational states suggest that it is a target for molecular chaperones. Evidences presented above strongly confirm that the UCS proteins function as chaperones for myosins. In addition to assisting myosins to fold properly, UCS proteins participate in myosin assembly, and modulate its contractile function and turnover. While research needs to continue to unravel the importance of UCS-myosin interactions in muscle formation and function, the involvement of these interactions in muscle repair and aging and disease states such as myopathies and cancer need to be pursued.

Acknowledgements This work is dedicated to the memory of the late Dr. Henry F. Epstein who was a pioneer of work on UNC-45 and myosins. The authors are grateful to Drs. Hellerschmied and Clausen for providing the original copies of Figs. 7.2 (without the insert) and 7.3. Texts and

figures from the first edition of this book were used with kind permission from Springer Science + Business Media: Networking of Chaperones by Co-Chaperones, UNC-45: a chaperone for myosin and a co-chaperone for Hsp90, 2007, pp. 62–74, Odutayo O. Odunuga & Henry F. Epstein.

References

Amorim MJ, Mata J (2009) Rng3, a member of the UCS family of myosin co-chaperones, associates with myosin heavy chains cotranslationally. EMBO Rep 10(2):186–191

Anderson MJ, Pham VN, Vogel AM et al (2008) Loss of unc45a precipitates arteriovenous shunting in the aortic arches. Dev Biol 318(2):258–267

Ao W, Pilgrim D (2000) *Caenorhabditis elegans* UNC-45 is a component of muscle thick filaments and colocalizes with myosin heavy chain B, but not myosin heavy chain A. J Cell Biol 148:375–384

Ardizzi JP, Epstein HF (1987) Immunochemical localization of myosin heavy chain isoforms and paramyosin in developmentally and structurally diverse muscle cell types of the nematode *Caenorhabditis elegans*. J Cell Biol 105:2763–2770

Atkinson SJ, Stewart M (1991) Expression in *Escherichia coli* of fragments of the coiled-coil rod domain of rabbit myosin: influence of different regions of the molecule on aggregation and paracrystal formation. J Cell Sci 99:823–836

Balasubramanian MK, McCollum D, Chang L et al (1998) Isolation and characterization of new fission yeast cytokinesis mutants. Genetics 149:1265–1275

Barral JM, Bauer CC, Ortiz I et al (1998) Unc-45 mutations in *Caenorhabditis elegans* implicate a CRO1/She4p-like domain in myosin assembly. J Cell Biol 143(5):1215–1225

Barral JM, Hutagalung AH, Brinker A et al (2002) Role of myosin assembly protein UNC-45 as a molecular chaperone for myosin. Science 295:669–671

Bazzaro M, Santillan A, Lin Z et al (2007) Myosin II co-chaperone general cell UNC-45 overexpression is associated with ovarian cancer, rapid proliferation, and motility. Am J Pathol 171(15):1640–1649

Bernick EP, Zhang PJ, Du S (2010) Knockdown and overexpression of Unc-45b result in defective myofibril organization in skeletal muscles of zebrafish embyos. BMC Cell Biol 11:70

Berteaux-Lecellier V, Zickler D, Debuchy R et al (1998) A homologue of the yeast SHE4 gene is essential for the transition between the syncytial and cellular stages during sexual reproduction of the fungus *Podospora anserina*. EMBO J 17:1248–1258

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. BioEssays 21:932–939

Chadli A, Graham JD, Abel MG et al (2006) GCUNC-45 is a novel regulator for the progesterone receptor/hsp90 chaperoning pathway. Mol Cell Biol 26(5):1772–1730

Chadli A, Felts SJ, Toft DO (2008) GCUNC45 is the first Hsp90 co-chaperone to show alpha/beta isoform specificity. J Biol Chem 283(15):9509–9512

Chen D, Li S, Singh R et al (2012) Dual function of the UNC-45b chaperone with myosin and GATA4 in cardiac development. J Cell Sci 125(Pt 16):3893–3903

Chow D, Srikakulam R, Chen Y et al (2002) Folding of the striated muscle myosin motor domain. J Biol Chem 277:36799–36807

Comyn SA, Pilgrim D (2012) Lack of developmental redundancy between Unc45 proteins in zebrafish muscle development. PLoS One 7(11):e48861

Das AK, Cohen PTW, Barford D (1998) The structure of the tetratricopeptide repeats of protein phosphatase 5, implications for TPR-mediated protein-protein interactions. EMBO J 17:1192–1199

Du SJ, Li H, Bian Y et al (2008) Heat-shock protein 90alpha1 is required for organized myofibril assembly in skeletal muscles of zebrafish embryos. Proc Natl Acad Sci U S A 105(2):554–559

Epping MT, Mejer LA, Bos JL et al (2009) UNC45A confers resistance to histone deacetylase inhibitors and retinoic acid. Mol Cancer Res 7(11):1861–1870
Epstein HF, Thomson JN (1974) Temperature-sensitive mutation affecting myofilament assembly in *Caenorhabditis elegans*. Nature 250:579–580
Etard C, Roostalu U, Strahle U (2008) Shuttling of the chaperone Unc45b and Hsp90a between the A band and the Z line of the myofibril. J Cell Biol 180(6):1163–1175
Etard C, Roostalu U, Strahle U (2010) Lack of Apobec2-related proteins causes a dystrophic muscle phenotype in zebrafish embryos. J Cell Biol 189(3):527–539
Fratev F, Osk Jonsdottir S, Pajeva I (2013) Structural insight into the UNC-45-myosin complex. Proteins 81(7):1212–1221
Gaiser AM, Kaiser CJ, Haslbeck V et al (2011) Downregulation of the Hsp90 system causes defects in muscle cells of *Caenorhabditis elegans*. PLoS One 6(9):e25485
Gazda L, Pokrzywa W, Hellerschmied D et al (2013) The myosin chaperone UNC-45 is organized in tandem modules to support myofilament formation in *C. elegans*. Cell 152(1–2):183–195
Geach TJ, Zimmerman LB (2010) Paralysis and delayed Z-disc formation in the *Xenopus tropicalis* unc45b mutant dicky ticker. BMC Dev Biol 10:75
Guo S, Kemphues KJ (1996) A nonmuscle myosin required for embryonic polarity in *Caenorhabditis elegans*. Nature 382:455–458
Guo W, Chen D, Fan Z et al (2011) Differential turnover of myosin chaperone UNC-45A isoforms increases in metastatic human breast cancer. J Mol Biol 412(3):365–378
Hansen L, Comyn S, Mang Y et al (2014) The myosin chaperone UNC45B is involved in lens development and autosomal dominant juvenile cataract. Eur J Hum Genet doi:10.1038/ejhg.2014.21
Hellerschmied D, Clausen T (2014) Myosin chaperones. Curr Opin Struct Biol 25:9–15
Hoppe T, Cassata G, Barral JM et al (2004) Regulation of the myosin-directed chaperone UNC-45 by a novel E3/E4-multiubiquitylation complex in *C. elegans*. Cell 118(3):337–349
Hutagalung AH, Landsverk ML, Price MG et al (2002) The UCS family of myosin chaperones. J Cell Sci 115:3983–3990
Janiesch PC, Kim J, Mouysset J et al (2007) The ubiquitin-selective chaperone CDC-48/p97 links myosin assembly to human myopathy. Nat Cell Biol 9(4):379–390
Jansen RP, Dowzer C, Michaelis C et al (1996) Mother cell-specific HO expression in budding yeast depends on the unconventional myosin myo4p and other cytoplasmic proteins. Cell 84:651–654
Kachur T, Ao W, Berger J et al (2004) Maternal UNC-45 is involved in cytokinesis and colocalizes with nonmuscle myosin in the early *Caenorhabditis elegans* embryo. J Cell Sci 117:5313–5321
Kachur TM, Audhya A, Pilgrim DB (2008) UNC-45 is required for NMY-2 contractile function in early embryonic polarity establishment and germline cellularization in *C. elegans*. Dev Biol 314(2):287–299
Kaiser CM, Bujalowski PJ, Ma L et al (2012) Tracking UNC-45 chaperone-myosin interaction with a titin mechanical reporter. Biophys J 102(9):2212–2219
Kinose F, Wang SX, Kidambi US (1996) Glycine 699 is pivotal for the motor activity of skeletal muscle myosin. J Cell Biol 134:895–909
Kuczmarski ER, Spudich JA (1980) Regulation of myosin self-assembly: phosphorylation of Dictyostelium heavy chain inhibits formation of thick filaments. Proc Natl Acad Sci U S A 77:7292–7296
Landsverk ML, Epstein HF (2005) Genetic analysis of myosin II assembly and organization in model organisms. Cell Mol Life Sci 62:2270–2282
Landsverk ML, Li S, Hutagalung AH et al (2007) The UNC-45 chaperone mediates sarcomere assembly through myosin degradation in *Caenorhabditis elegans*. J Cell Biol 177(2):205–210
Lee CF, Hauenstein AV, Fleming JK et al (2011a) X-ray crystal structure of the UCS domain-containing UNC-45 myosin chaperone from *Drosophila melanogaster*. Structure 19(3):397–408
Lee CF, Melkani GC, Yu Q et al (2011b) Drosophila UNC-45 accumulates in embryonic blastoderm and in muscles, and is essential for muscle myosin stability. J Cell Sci 124(Pt 5):699–705

Liu L, Srikakulam R., Winklemann DA (2008) UNC45 activates Hsp90-dependent folding of the myosin motor domain. J Biol Chem 283(19):13185–13193
Lord M, Pollard TD (2004) UCS proteins Rng3p activates actin filament gliding by fission yeast myosin-II. J Cell Biol 167(2):315–325
Lord M, Sladewski TE, Pollard TD (2008) Yeast UCS proteins promote actomyosin interactions and limit myosin turnover in cells. Proc Natl Acad Sci U S A 105(23):8014–8019
McNally EM, Goodwin EB, Spudich JA et al (1988) Coexpression and assembly of myosin heavy chain and myosin light chain in *Escheichia coli*. Proc Natl Acad Sci U S A 85:7270–7273
Melkani GC, Lee CF, Cammarato A et al (2010) Drosophila UNC-45 prevents heat-induced aggregation of skeletal muscle myosin and facilitates refolding of citrate synthase. Biochem Biophys Res Commun 396(2):317–322
Melkani GC, Bodmer R, Ocorr K et al (2011) The UNC-45 chaperone is critical for establishing myosin-based myofibrillar organization and cardiac contractility in the Drosophila heart model. PLoS One 6(7):e22579
Melkani GC, Trujillo AS, Ramos R et al (2013) Huntington's disease induced cardiac amyloidosis is reversed by modulating protein folding and oxidative stress pathways in the Drosophila heart. PLoS Genet 9(12):e1004024
Miller DM, Ortiz I, Berliner GC et al (1983) Differential localization of two myosins within nematode thick filaments. Cell 34:477–490
Millson SH, Truman AW, King V et al (2005) A two-hybrid screen of the yeast proteome for Hsp90 interactors uncovers a novel Hsp90 chaperone requirement in the activity of a stress-activated mitogen-activated protein kinase, Slt2p (Mpk1p). Eukaryot Cell 4:849–860
Mishra M, D'souza VM, Chang KC et al (2005) Hsp90 protein in fission yeast Swo1p and UCS protein Rng3p facilitate myosin II assembly and function. Eukaryot Cell 4:567–576
Mitchell EJ, Karn J, Brown DM et al (1989) Regulatory and essential light-chain-binding sites in myosin heavy chain subfragment-1 mapped by site-directed mutagenesis. J Mol Biol 208:199–25
Myhre JL, Hills JA, Jean F et al (2014) Unc45b is essential for early myofibrillogenesis and costamere formation in zebrafish. Dev Biol 390(1):26–40
Ni W, Hutagalung AH, Li S et al (2011) The myosin-binding UCS domain but not the Hsp90-binding TPR domain of the UNC-45 chaperone is essential for function in *Caenorhabditis elegans*. J Cell Sci 124(Pt 18):3164–3173
Odunuga OO, Epstein HF (2007) UNC-45: a chaperone for myosin and a co-chaperone for Hsp90. In: Blatch GL (ed) Networking of chaperones by co-chaperones. Springer, New York
Peifer M, Berg S, Ryenolds AB (1994) A repeating amino acid motif shared by proteins with diverse cellular roles. Cell 76(5):789–791
Price MG, Landsverk ML, Barral JM et al (2002) Two mammalian UNC-45 isoforms are related to distinct cytoskeletal and muscle-specific functions. J Cell Sci 115:4013–4023
Rayment I, Rypniewski WR, Schmidt-Base K et al (1993a) Three-dimensional structure of myosin subfragment-1: a molecular motor. Science 261:50–58
Rayment I, Holden HM, Whittaker M et al (1993b) Structure of the actin-myosin complex and its implication for muscle contraction. Science 261:58–65
Saraswat LD, Lowey S (1991) Engineered cysteine mutants of myosin light chain 2: fluorescent analogues for structural studies. J Biol Chem 266:19777–19785
Schachat F, Harris HE, Epstein HF (1977) Two homogeneous myosins in body-wall muscle of *Caenorhabditis elegans*. Cell 10:721–728
Scheufler C, Brinker A, Bourenkov G et al (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. Cell 101:199–210
Shi H, Blobel G (2010) UNC-45/CRO1/She4p (UCS) protein forms elongated dimer and joins two myosin heads near their actin binding region. Proc Natl Acad Sci U S A 107(50):21382–21387
Sikorski RS, Boguski MS, Goebl M (1990) A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell 60:307–317

Srikakulam R, Winkelmann DA (1999) Myosin II folding is mediated by a molecular chaperone. J Biol Chem 274:27265–27273
Srikakulam R, Winklemann DA (2004) Chaperone-mediated folding and assembly of myosin in striated muscle. J Cell Sci 117:641–652
Srikakulam R, Liu L, Winkelmann DA (2008) UNC45b forms a cytosolic complex with Hsp90 and targets the unfolded myosin motor domain. PLoS One 3(5):e2137
Stark BC, James ML, Pollard LW (2013) UCS protein Rng3p is essential for myosin-II motor activity during cytokinesis in fission yeast. PLoS One 8(11):e79593
Sweeney HL, Rosenfeld SS, Brown F et al (1998) Kinetic tuning of myosin via a flexible loop adjacent to the nucleotide binding pocket. J Biol Chem 273:6262–6270
Toi H, Fujimura-Kamada K, Irie K et al (2003) She4p/Dim1p interacts with the motor domain of unconventional myosins in the budding yeast, *Saccharomyces cerevisiae*. Mol Biol Cell 14:2237–2249
Trybus KM (1994) Regulation of expressed truncated smooth muscle myosins. Role of the essential light chain and tail length. J Biol Chem 269:20819–20822
Venolia L, Waterston RH (1990) The unc-45 gene of *Caenorhabditis elegans* is an essential muscle-affecting gene with maternal expression. Genetics 126:345–353
Venolia L, Ao W, Kim S et al (1999) unc-45 gene of *Caenorhabditis elegans* encodes a muscle-specific tetratricopeptide repeat-containing protein. Cell Mot Cytoskeleton 42:163–177
Wang F, Chen L, Arcucci O et al (2000) Effect of ADP and ionic strength on the kinetic and motile properties of recombinant mouse myosin V. J Biol Chem 275:4329–4335
Waterston RH (1988) Muscle. In: Wood WB (ed) The nematode *Caenorhabditis elegans*. Cold Spring Harbor, New York
Waterston RH (1989) The minor myosin heavy chain, mhcA, of *Caenorhabditis elegans* is necessary for the initiation of thick filament assembly. EMBO J 8:3429–3436
Waterston RH, Thomson JN, Brenner S (1980) Mutants with altered muscle structure in *Caenorhabditis elegans*. Dev Biol 77:271–302
Wendland B, McCaffrey JM, Xiao Q et al (1996) A novel fluroscence-activated cell sorter-based screen for yeast endocytosis mutants identifies a yeast homologue of mammalian eps15. J Cell Biol 135:1485–1500
Wesche S, Arnold M, Jansen RP (2003) The UCS domain protein She4p binds to myosin motor domains and is essential for class I and class V myosin function. Curr Biol 13:715–724
Wohlgemuth SL, Crawford BD, Pilgrim DB (2007) The myosin co-chaperone UNC-45 is required for skeletal and cardiac muscle function in zebrafish. Dev Biol 303(2):483–492
Wong KCY, Naqvi NI, Lino Y et al (2000) Fission yeast Rng3p: an UCS-domain protein that mediates myosin II assembly during cytokinesis. J Cell Sci 113:2421–2432
Wong KCY, D'souza VM, Naqvi NI et al (2002) Importance of a myosin II-containing progenitor for actomyosin ring assembly in fission yeast. Curr Biol 12:724–729
Young JC, Barral JM, Hartl FU (2003) More than unfolding: localized functions of cytosolic chaperones. Trends Biochem Sci 28:541–547
Yu QI, Bernstein SI (2003) UCS proteins: managing the myosin. Curr Biol 13:525–527
Yu Q, Hipolito LC, Kronert WA et al (2003) Characterization and functional analysis of the *Drosophila melanogaster* unc-45 (dunc-45) gene. Mol Biol Cell 14:45

Chapter 8
Chaperonin—Co-chaperonin Interactions

Aileen Boshoff

Abstract Co-chaperonins function together with chaperonins to mediate ATP-dependant protein folding in a variety of cellular compartments. GroEL and its co-chaperonin GroES are the only essential chaperones in *Escherichia coli* and are the archetypal members of this family of protein folding machines. The unique mechanism used by GroEL and GroES to drive protein folding is embedded in the complex architecture of double-ringed complexes, forming two central chambers that undergo structural rearrangements as part of the folding mechanism. GroES forms a lid over the chamber, and in doing so dislodges bound substrate into the chamber, thereby allowing non-native proteins to fold in isolation. GroES also modulates allosteric transitions of GroEL. A significant number of bacteria and eukaryotes house multiple chaperonin and co-chaperonin proteins, many of which have acquired additional intracellular and extracellular biological functions. In some instances co-chaperonins display contrasting functions to those of chaperonins. Human Hsp60 continues to play a key role in the pathogenesis of many human diseases, in particular autoimmune diseases and cancer. A greater understanding of the fascinating roles of both intracellular and extracellular Hsp10, in addition to its role as a co-chaperonin, on cellular processes will accelerate the development of techniques to treat diseases associated with the chaperonin family.

Keywords Chaperonins · Co-chaperonins · GroEL · GroES · Hsp60 · Hsp10

Introduction

Chaperonins are ubiquitous protein folding machines characterised by a large multi-subunit ring structure. They prevent aggregation by binding non-native proteins, and facilitate folding and unfolding of proteins. They form part of the Hsp60 family of heat shock proteins and are related by homology to the GroEL proteins of *E. coli* (Hemmingsen et al. 1988; Hartl and Hayer-Hartl 2002). The *E. coli* chaperonin

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,* Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_8

GroEL and its co-chaperonin GroES are the quintessential members of this family of protein folding machines (Hartl and Hayer-Hartl 2002; Hartl 1996; Horwich et al. 2007). Hemmingsen first used the term 'chaperonin' (Cpn) in 1988 to represent this family of molecular chaperones (Hemmingsen et al. 1988). The Hsp60 family of chaperones is one of the most abundant classes of molecular chaperone present in the plastids, mitochondria, and cytoplasm of all eukaryotes and eubacteria. GroEL, the only essential molecular chaperone in *E. coli*, is indispensable for viability at all temperatures (Fayet et al. 1989; Ang and Georgopoulos 1989) and *S. cerevisiae* is non-viable without CCT subunits (Stoldt et al. 1996). Mitochondrial Hsp60 inactivation results in embryonic lethality in mice (Christensen et al. 2010).

The terms GroEL and GroES were initially applied strictly to the two proteins found in *E. coli* and have been extended to include homologues from other bacterial species. The GroEL protein functions as a typical molecular chaperone as it binds and folds proteins, whilst GroES exhibits no autonomous role as a chaperone but modulates the activity of GroEL and is referred to as a co-chaperone. The term chaperonin is applied to bacterial proteins that are homologous to the *E. coli* GroEL and are also referred to as Cpn60, whilst co-chaperonins refer to homologues of *E. coli* GroES, also known as Cpn10. Whilst the mitochondrial homologues are called Hsp60 and Hsp10, the archeal chaperonins are referred to as thermosomes (Trent et al. 1991). In the eukaryotic group, chaperonins found in the cytosol were first called TCP-1 and are now also called CCT (chaperonin containing TCP-1) (Kubota et al. 1994), TRiC (TCP containing ring complex) (Frydman et al. 1992) and c-cpn (Gao et al. 1992). The human HSP60/HSP10 proteins have been renamed HSPD/E (Kampinga et al. 2009). The chloroplast chaperonin is referred to as Cpn60 protein, whilst two types of co-chaperonins Cpn10 and Cpn20 are present (Koumoto et al. 2001). Prior to its recognition as chloroplast Cpn60, it was known as Rubisco binding protein (Barraclough and Ellis 1980).

The chaperonins share a common subunit organisation and structure. They are a family of ATPases consisting of twin heptameric rings stacked back-to-back to create a characteristic cylindrical structure and function by assisting in the folding of nascent and misfolded proteins (Hartl and Martin 1995; Houry et al. 1999). Each ring creates a large cavity for unfolded proteins to bind and undergo productive folding to the native state in a highly cooperative and ATP-dependent manner (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002). Co-chaperonins form a single heptameric ring of 10 kDa subunits and are present in all bacterial and eukaryotic organisms (Hartl 1996). The *E. coli* GroEL/GroES complex consists of two stacked heptameric rings of GroEL capped by a single heptameric ring of GroES that forms the lid over the folding cage (Fig. 8.1). The functional cycle requires the binding of chaperonin 10 to one or both chaperonin rings which forms a lid-like structure on top of the cyclinder when ATP is bound that causes the chamber to enlarge to allow for protein folding (Chandrasekhar et al. 1986; Saibil 1996). A vital part of the structure of each subunit is a flexible mobile loop that mediates binding to the chaperonin (Landry et al. 1996). The flexibility and the structure of the complex is conserved amongst co-chaperonins and sequence variations impose differences in binding affinity (Richardson et al. 2001). Protein substrates first bind to the apical

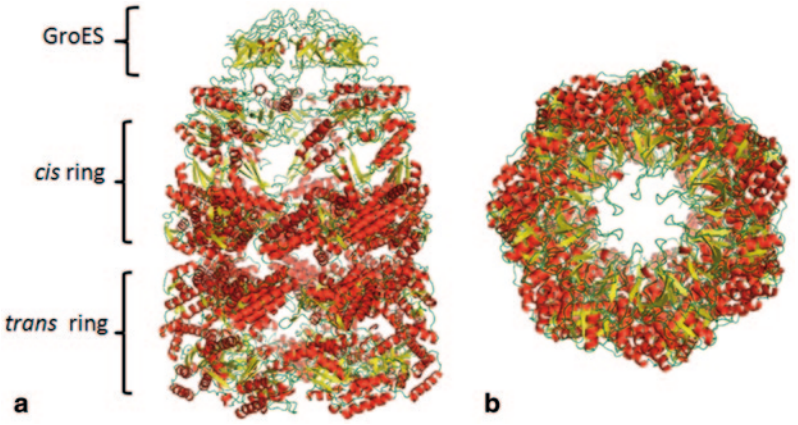

Fig. 8.1 Structure of the GroEL/GroES complex. The GroEL/GroES complex comprises of two heptameric rings of GroEL stacked back-to-back with the GroES 'lid' bound to the *cis* ring to form a *barrel-shaped* complex, showing *the side* (**a**) and *the top* (**b**) views of the complex. Alpha helices are shown in *red* and β-sheets in *yellow*. The images were generated using PyMol (DeLano Scientific) from coordinates in PDB: 1AON

domain and are then dislodged and driven into the cavity by the binding of the co-chaperonin to the same area (Hartl and Hayer-Hartl 2002). The folding process is driven by the binding and hydrolysis of ATP which triggers a complex set of allosteric signals both within and between the stacked rings (Gray and Fersht 1991; Todd et al. 1993). GroEL is critical for the correct folding of many proteins in the cell, under both normal and stress conditions. The folding of nascent polypeptides often requires the cooperation of both the Hsp70 and Hsp60 families and these families are also responsible for most of the general folding events in the cell (Hartl et al. 1992; Fink 1999). While CCT is not upregulated during heat shock (Horwich et al. 2007), GroEL and mitochondrial Hsp60 are heat inducible. In addition to ensuring the correct folding of proteins, chaperonins play a role in the assembly of protein complexes (Seo et al. 2010) and trafficking of proteins (Xu et al. 2011).

The Gp31 protein from bacteriophage T4, a functional co-chaperonin that promotes the assembly of the T4 major capsid protein, can functionally substitute for GroES resulting in an increase in size and hydrophilicity of the enclosed chamber (van der Vies et al. 1994; Hunt et al. 1997). Another co-chaperonin from bacteriophage RB49 called CocO is distantly related to GroES (Ang et al. 2001). Both of these bacteriophage co-chaperonins utilize host encoded GroEL to assemble capsid proteins and both proteins could functionally replace GroES in *E. coli* (Keppel et al. 2002). Interestingly, the first viral-encoded chaperonin was identified in the genome of *Pseudomonas aeruginosa* bacteriophage EL (Hertveldt et al. 2005), and later demonstrated to have functional properties similar to GroEL except that it does not require a co-chaperonin for activity (Kurochkina et al. 2012). A wide range of newly identified functions have been attributed to eukaryotic Hsp60, including roles in carcinogenesis, immunity and cell signalling (reviewed by (Calderwood et al. 2007;

Chandra et al. 2007). The roles played by both intracellular and extracellular forms of human HSP10 (HSPE) in pregnancy, cancer and autoimmune diseases continue to receive attention (Jia et al. 2011; Corrao et al. 2010).

Whilst the *E. coli* chaperonins are encoded by only two genes, *groEL* and *groES*, both Cpn60 and Cpn10 found in green algae and plants are encoded by numerous genes (Boston et al. 1996; Hill and Hemmingsen 2001; Schroda 2004). The complexity of chloroplast chaperonins has been viewed by (Vitlin Gruber et al. 2013a). It also appears that approximately 30% of bacteria encode more than one *groEL* gene (Hill and Hemmingsen 2001). The biological significance of several chaperonin genes has yet to be revealed (Lund 2009), however the literature has expanded in recent years in this area of research. In general, it appears as though major subunits play housekeeping roles and minor subunits have more specialised functions and fold specific proteins (Peng et al. 2011).

The chaperonins can be further sub-divided into two distantly related groups. Group I chaperonins are found in eubacteria, mitochondria and chloroplasts, of which GroEL from *E. coli* is the best studied and understood (Leroux 2001). They form homooligomeric complexes consisting of two stacked heptameric rings together with the heptameric Hsp10 co-chaperonin that forms the lid for the folding cage (Braig et al. 1994). Group II chaperonins are present in archaebacteria and in the eukaryotic cytosol (Horwich et al. 1993; Frydman 2001). Although both subgroups form ring-like structures with cavities for sequestered protein folding, Group II chaperonins form heterooligomeric complexes (Spiess et al. 2004; Archibald et al. 1999). The Group II chaperonins consist of two eight or nine-membered rings consisting of one to three subunit types in the archeal thermosome rings (Phipps et al. 1991), while TRiC/CCT rings consist of eight subunit types (Frydman et al. 1992; Spiess et al. 2004). An important difference between the two groups is the lack of a GroES homologue in the Group II chaperonins (Horwich and Saibil 1998). Group I chaperonins utilize an independently expressed co-chaperonin that functions as a lid to aid the encapsulation of unfolded protein, whilst Group II chaperonins have a built-in lid in the form of a particular α-helical protrusion and do not require additional protein subunits to function (Vabulas et al. 2010; Meyer et al. 2003). However, the activity of CCT is regulated by a number of co-chaperones, including prefoldin, phosducin-like proteins and BAG3 (Vainberg et al. 1998; Martin-Benito et al. 2002; Stirling et al. 2006; Fontanella et al. 2010). In 2010, a third group was proposed in bacteria and are conserved in the genomes of 11 bacteria (Techtmann and Robb 2010). These novel chaperonins are capable of refolding denatured proteins in a GroES-independant manner. Group III chaperoinins are highly divergent and distantly related to Group I and Group II and they might represent an ancient horizontal gene transfer event from archaea to bacteria, and this may revise the current paradigm for chaperonin classification (Techtmann and Robb 2010).

Over the past 25 years many researchers have demonstrated the abilities of the *E. coli* GroEL and GroES machine to bind and refold a wide range of aggregation prone proteins both *in vivo* and *in vitro*. Early *in vitro* experiments demonstrating

the abilities of *E. coli* GroEL and GroES to refold denatured proteins were carried out using heat denatured Rubisco enzyme (Goloubinoff et al. 1989a); and following this seminal paper the GroEL-GroES cycle has been scrutinised *in vitro*. Chaperonins continue to also play an important role in recombinant protein production and this has been well documented in the literature. *E. coli* is a frequently used host and the folding of proteins in the cytoplasm is assisted primarily by Hsp70 and Hsp60 (Vabulas et al. 2010). They aid in functional expression and retain solubility by assisting the refolding of aggregated target proteins. The chaperonin GroEL and its co-chaperonin GroES have been used extensively for this purpose and are often co-expressed with the protein of interest. Some of these proteins include malate dehydrogenase (Hartman et al. 1993; Ranson et al. 1997), citrate synthase (Buchner et al. 1991), rhodanese (Martin et al. 1991), carbamoylase (Sareen et al. 2001) and aconitase (Chaudhuri et al. 2001). The presence of *E. coli* GroEL and GroES significantly improved the yields of soluble protein in most instances; however large amounts of the chaperonins are often required, exceeding endogenous concentrations. Extensive optimisation of the reaction conditions is also vital and the requirements of each chaperonin are variable. A greater understanding of the effects of over-expressing chaperonins on cell growth, and conditions for optimum recombinant protein production, need to be investigated (Gupta et al. 2006). Despite these drawbacks, the *E. coli* chaperonins have been used successfully for the production of a wide range of recombinant proteins. More recently the co-expression of GroEL/ES appreciably enhanced the expression of human tumor necrosis factor, CD 137 ligand (Wang et al. 2012). The solubility of *Plasmodium falciparum* 1-deoxy-D-xylulose-5-phosphate reductoisomerase was significantly increased by the co-production of GroEL/ES (Goble et al. 2013).

To date, the structure and mechanism of chaperonin and co-chaperonin functions has centred on the GroEL and GroES system of *E. coli* (Hartl and Hayer-Hartl 2002; Hartl 1996; Horwich et al. 2007). This system has received the most attention and serves as a model for chaperonin and co-chaperonin interactions. The GroEL and GroES folding machine will be discussed in the following section with emphasis on the role of GroES. Group I chaperonins will be the focus of this chapter as the functional activity of Group II chaperonins is not assisted by co-chaperonins. The biological impact of chaperonins extends beyond protein folding as they are the dominant immunogens present during human bacterial infections, and there is considerable interest in their role in cancer and autoimmune diseases (Kaufmann 1992). Data on the extensive roles of both extracellular and intracellular Hsp10 has left no doubt that the functions of this protein extends beyond its role as a co-chaperonin, and these roles have been reviewed by (Corrao et al. 2010). Research on bacterial chaperonins has expanded in recent years as more bacterial genomes have been sequenced. Our understanding of co-chaperonins in other organisms and organelles is gaining momentum and recent findings on bacterial and eukaryotic co-chaperonins will be addressed.

Activities of the *E. coli* GroEL/GroES Folding Machine

One of the most efficient chaperone systems is the well characterised *E. coli* chaperonin machine composed of GroEL and its co-chaperonin GroES. Three different functions have been assigned to this folding machine, binding to non-native proteins preventing aggregation (Buchner et al. 1991), facilitating protein folding by encapsulating the protein in a sequestered environment (Weissman et al. 1995), and finally unfolding of kinetically trapped intermediates so that they can refold (Todd et al. 1993; Sparrer and Buchner 1997; Sparrer et al. 1997; Shtilerman et al. 1999). The *groE* genes of *E. coli* were the first chaperonin genes to be discovered. These genes were first identified when temperature sensitive mutant strains of *E. coli* could not support the growth of bacteriophage λ (Georgopoulos et al. 1972), afterwards it was determined that the two genes are encoded on the same operon *groE*. The importance of these GroEL and GroES proteins is emphasised by the fact that they are the only chaperones that are essential for the viability of *E. coli* at all temperatures (Fayet et al. 1989). Additionally, host GroEL and GroES play a role in both phage infection and defence strategies of the host (Ang et al. 2000), as well as protecting viral proteins at high temperatures (Chen et al. 2013). *E. coli* GroEL/ES was previously known to play a role in the regulation of sigma-32 by enhancing proteolysis (Guisbert et al. 2004). Recently an additional proteolytic role was demonstrated whereby interaction with the cold shock RNA chaperone (CspC) lead to proteolysis (Lenz and Ron 2014).

It is estimated that under normal cellular growth conditions 10–15 % of all cytoplasmic proteins rely on GroEL in order to fold correctly, and this increases to 30 % under conditions of stress (Ewalt et al. 1997). Many of the cytoplasmic proteins that interact with GroEL have been identified (Houry et al. 1999) and GroEL acts downstream of the *E. coli* molecular chaperones, DnaK (prokaryotic Hsp70) and trigger factor, in the folding of 10 % of cytosolic proteins (Houry et al. 1999; Ewalt et al. 1997). The mechanism of action is different to that of Hsp70 as the protein is sequestered from its environment. In a proteomic study of *E. coli* proteins, ~250 different proteins interact with GroEL, of these ~85 proteins are dependent on GroEL for folding and 13 of these are essential proteins (Kerner et al. 2005). These 85 proteins were scrutinised further and ~60 % were found to be absolutely dependent on GroEL and GroES for folding and an additional 8 proteins were classified as obligate substrates (Fujiwara et al. 2010). Most of the substrates are characterised by a size range of 20–50 kDa and complex α/β or α+β topologies, and tend to populate kinetically trapped folding intermediates (Kerner et al. 2005).

Structure of GroEL and GroES

Several crystal structures of GroEL are available (Braig et al. 1994), including GroEL complexed with ATP (Boisvert et al. 1996), GroEL bound to GroES and ADP (Xu et al. 1997) and a GroEL-peptide complex (Chen and Sigler 1999), as well as NMR (nuclear magnetic resonance) spectroscopy (Fiaux et al. 2002; Nishida

et al. 2006) and cryo-electron microscopy structures (Ranson et al. 2006; Chen et al. 2006). Co-chaperonin structures alone have been reported for GroES (Boudker et al. 1997; Hunt et al. 1996; Seale et al. 1996).

The ability of GroEL and GroES to enhance protein folding is embedded in the unique quaternary structures of these proteins (Fig. 8.1a). The arrangement of the GroEL subunits results in an oligomeric structure consisting of fourteen subunits arranged in two inverted rings, while the GroES subunits are arranged into a single ring of seven subunits, and both structures display seven-fold rotationally symmetric ring-shaped oligomers (Fig. 8.1b). The GroEL subunits are composed mainly of α-helices and the arrangement of the subunits into two stacked GroEL rings create a central channel that is split into two functionally separate cavities at the ring interface (Braig et al. 1994; Braig et al. 1993). Each subunit is divided into three distinct domains (Fig. 8.2): an ATP-binding equatorial domain that mediates interactions between subunits of each ring, a substrate-binding apical domain including co-chaperone binding sites, and an intermediate domain that connects both domains and transmits conformational changes generated by nucleotide binding between the equatorial and apical domains (Braig et al. 1994; Fenton et al. 1994). The apical domains are positioned on the outside of each ring, the intermediate domains are in the middle and the equatorial domains are positioned at the interface of both rings.

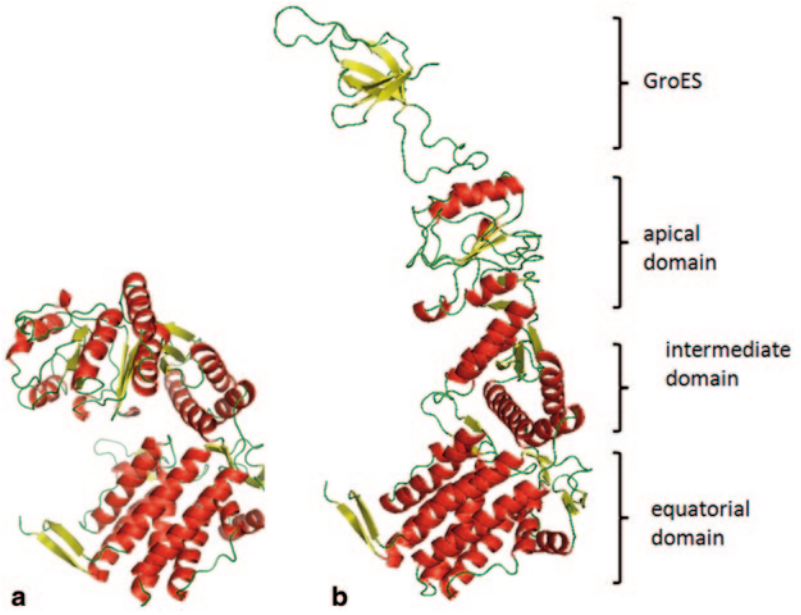

Fig. 8.2 Binding of GroES induces a large conformational change in GroEL. Each subunit of GroEL is divided into three distinct domains: an apical domain, an equatorial domain and an intermediate domain that connects both domains. Unbound GroEL (**a**) undergoes large rigid body movements of the apical domain upon binding of GroES (**b**). Apical domain is twisted 90° relative to the open ring not bound to GroES. Alpha helices are shown in *red* and β-sheets in *yellow*. The images were generated using PyMol (DeLano Scientific) from coordinates in PDB: 1AON

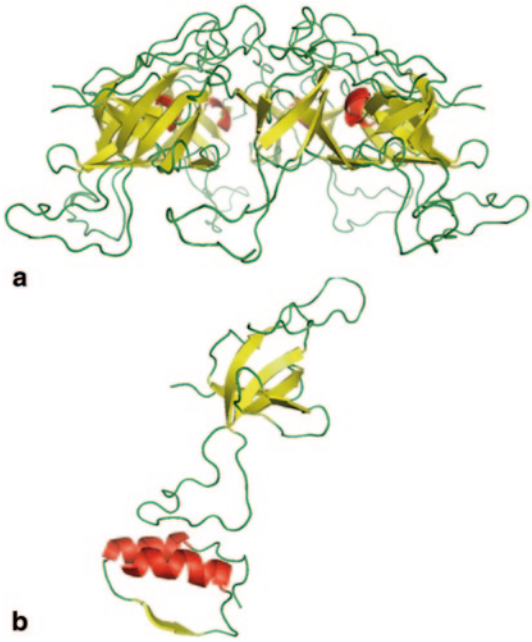

Fig. 8.3 Structure of GroES. Side view of GroES heptameric structure, as it occurs bound to GroEL and ATP, showing the flexible loops that interact with GroEL pointing *downwards* (**a**). The backbone structure of the GroES monomer interacting with the top of the apical domain of GroEL (**b**). Alpha helices are shown in *red* and β-sheets in *yellow*. The images were generated using PyMol (DeLano Scientific) from coordinates in PDB: 1AON

Coalescence of the disordered and flexible C-terminal segments of the subunits in each ring blocks the central channel at the equatorial domain causing discontinuity between the cavities turning them into two separate chambers for folding (Chen et al. 1994). Specific hydrophilic amino acid residues in the C-terminal region were identified as being vital in maintaining an appropriate environment for protein folding within the central cavity of GroEL (Machida et al. 2009).

GroES is composed of seven identical 10 kDa subunits that form a lid-like structure (Hunt et al. 1996; Mande et al. 1996). These subunits form an irregular β-barrel structure formed by five β-strands with anti-parallel pairing of the last β-strand of one subunit with the first β-strand of the following subunit (Landry et al. 1993). Each subunit includes two loop regions, one facing upwards that forms the roof of the lid and one extending downwards from the bottom of the lid that constitutes a highly flexible mobile loop 16 amino acids in length (Fig. 8.3; Landry et al. 1993). Binding of GroES to GroEL is mediated by the seven flexible loops which are induced to form a β-hairpin structure upon formation of the GroEL/GroES/ATP complex (Fig. 8.3; Richardson et al. 2001). Mutations in the mobile loop disrupted GroES binding to GroEL (Zeilstra-Ryalls et al. 1994). The contribution of the mobile loop was studied using a synthetic peptide resembling the loop, which lacked structure until induced to form the β-hairpin structure when bound to GroEL

(Landry et al. 1996). The functional contribution of the flexibility of the mobile loop to chaperonin function was investigated by restricting the flexibility by the formation of disulphide bonds within the loop, and the results revealed that they play an important role in inducing substrate release into the cavity (Nojima et al. 2012).

The GroEL ring that is bound to GroES is termed the *cis* ring and opposite ring free of GroES is termed the *trans* ring (Fig. 8.1a). The GroEL rings are subject to intra-ring cooperativity and inter-ring allostery (Rye et al. 1997). The two GroEL rings are staggered such that each subunit contacts two subunits on the other ring that facilitates negative cooperativity between rings (Braig et al. 1994; Roseman et al. 2001). A review of the unfolding and refolding of GroEL and GroES in the presence of ligands and different solvents has highlighted differences in behaviour between these two proteins (Ryabova et al. 2013). The crystal structure of the bullet-shaped GroEL-GroES-ADP complex revealed that the apical domains are twisted 90° relative to the open ring not bound to GroES (Fig. 8.2; Roseman et al. 1996; Xu et al. 1997). The transmission of conformational changes between the apical and equatorial domains of GroEL via the intermediate domain are essential as mutations in this domain compromised the folding capacities of GroEL/GroES (Kawata et al. 1999). Movement of the apical domains shifts the hydrophobic substrate binding site from a position facing the cavity to an elevated and rotated position to facilitate the binding of the mobile loop of GroES to cap the folding camber (Fig. 8.2). Mutational mapping revealed that there is an overlap between substrate and GroES binding to the hydrophobic binding site (Fenton et al. 1994). Another study suggested that rotation of the hydrophobic binding site weakens substrate binding (Ranson et al. 2001). However, mapping the trajectories of domain movements of the GroEL-ATP complex showed that the apical domains are linked by salt bridges that allows the binding sites to separate from each other in an extended conformation, at the same time maintaining the binding surface facing the cavity, providing a potential binding site for GroES which triggers a final rotation that provides the "power stroke" to eject substrate in the chamber (Clare et al. 2012). The effects of interactions between the cavity wall of GroEL and rhodanese was investigated with the result that these interactions slowed down the folding rate of rhodanese (Sirur and Best 2013).

The Role of GroES in the Reaction Cycle

GroES functions as a co-chaperonin of GroEL to mediate the folding of unfolded or partially unfolded proteins. GroEL captures substrates at a site in the apical domain that exposes hydrophobic amino acid residues to facilitate substrate binding towards the ring centre (Fenton et al. 1994). GroES binds at the apical domain of ATP-bound GroEL, at a site that overlaps largely with the substrate binding site, and in doing so displaces the substrate into the binding cavity (Fenton et al. 1994). The distortion of the GroEL ring caused by the binding of GroES causes the hydrophobic residues that bind non-native protein to become inaccessible creating a hydrophilic lined cavity (Xu et al. 1997). The result is the eviction of the protein into the cage for folding, also referred to as the Anfinsen cage (Ellis and Hartl 1996). GroES

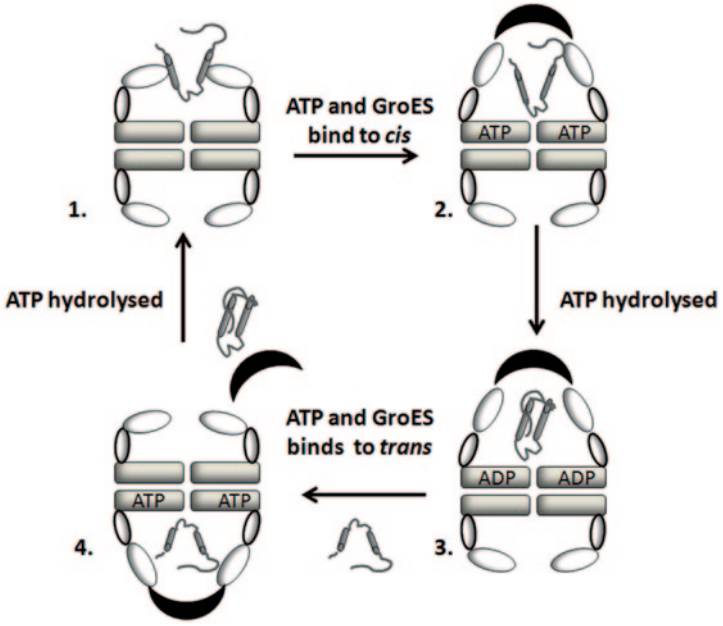

Fig. 8.4 The GroEL/GroES folding cycle. The binding of substrate to one GroEL ring is followed by the binding of ATP and GroES to the *cis* ring. The substrate is released into the cavity closed by GroES and allowed to fold. ATP is hydrolysed and the complex is ready to dissociate. The binding of ATP to the *trans* ring triggers release of substrate and dissociation of GroES from the *cis* ring and allows GroES to bind releasing substrate into the cavity

then forms a lid over the central cavity entrapping the protein. GroES binding is faster than ATP-induced release of the substrate and this provides a mechanism for the entrapment of proteins in the *cis* cavity (Burston et al. 1995). The complete GroEL/GroES folding cycle is shown in Fig. 8.4.

The transition between the open conformation, that is receptive to protein binding, and the closed state, in which the protein is isolated, is induced by ATP binding and hydrolysis (Horovitz and Willison 2005). ATP binds with positive cooperativity within rings but with negative cooperativity between rings (Yifrach and Horovitz 1995). Allosteric transitions support the ATP-dependant control of the affinity of GroEL for its substrate and the subsequent folding (Saibil et al. 1993; Roseman et al. 1996; Yifrach and Horovitz 1995). ATP binding initiates bending and twisting of subunit domains that distorts the ring structure. ATP binds to a ring with positive cooperativity, and movements of the interlinked subunit domains are concerted. In contrast, there is negative cooperativity between the rings, so that they act in alternation (Rye et al. 1997; Horovitz et al. 2001). Understanding the pathways of allosteric communication in GroEL has been the subject of intense research, and this was viewed more recently by (Saibil et al. 2013).

Once the substrate is encapsulated in the chamber, the slow rate of ATP hydrolysis dictates the length of time for folding to take place, however ATP hydrolysis is not required for protein folding but is required to complete the reaction cycle

(Fenton and Horwich 2003; Frydman 2001; Hartl and Hayer-Hartl 2002, 2009). GroEL exhibits weak ATPase activity that is lowered in the presence of GroES (Chandrasekhar et al. 1986; Goloubinoff et al. 1989b). GroEL provides energy in the form of ATP as the energy released during hydrolysis assists in the folding of non-native proteins (Xu et al. 1997). Transformational changes in the *trans* ring caused by binding of ATP, substrate and GroES to the *cis* ring results in the *trans* ring not being able to bind substrate (Tyagi et al. 2009). ATP hydrolysis of the GroES-bound ring is required for the binding of ATP to the *trans* ring, negative cooperativity is displayed between the two GroEL rings which favours dissociation of GroES, ADP and substrate from the *cis* ring (Rye et al. 1997). If the substrate is not folded correctly it can rebind to another or the same GroEL for successive cycles of folding (Rye et al. 1997). GroES can now bind to the *trans* ring and this ring then becomes the new *cis* ring in the subsequent round of substrate folding events. Thus both rings alternate to become the *cis* ring during folding cycles and this has led to the term "two-stroke engine" for the GroEL/GroES folding machine (Lorimer 1996; Xu and Sigler 1998).

In addition to its role as a lid for the folding chamber in the chaperonin complex, GroES controls the cooperativity by directing conformational changes in GroEL that are orchestrated by the seven mobile loops binding to each of the seven GroEL subunits, followed by release of substrate into the cage (Gray and Fersht 1991; Todd et al. 1994; Yifrach and Horovitz 1995). GroES also plays a key role in controlling the competence and specificity of protein folding by GroEL (Richardson et al. 2001).

Based on the GroEL-GroES-ADP complex, the binding of GroES causes large rigid body movements of the apical domains of GroES that results in doubling of the volume of the *cis* ring cavity compared to the *trans* ring (Fig. 8.2; Xu et al. 1997). This increased volume is capable of binding a native protein of 70 kDa (Houry et al. 1999). Most of the *E. coli* proteins that require GroEL-GroES for folding are ~60 kDa and larger proteins that cannot be accommodated within the folding cavity can be folded by binding to the uncapped *trans* ring of GroEL (Sigler et al. 1998). As a result of the limited number of GroEL-GroES dependent substrates, it has been suggested that the complex may actively rescue proteins from kinetic folding traps thereby facilitating their refolding (Hartl and Hayer-Hartl 2009; Jewett and Shea 2010; Chakraborty et al. 2010). Without GroES and ATP, the most dependent or stringent GroEL substrates do not fold and remain tightly associated with the GroES as they need to transit through *cis* in order to fold (Rye et al. 1997). Binding of GroES also causes a dramatic change in the walls of the cavity as the hydrophobic binding sites are rotated towards the interfaces of adjacent subunits and GroES resulting in a hydrophilic wall; and the intermediate domain twists downwards capping the nucleotide binding site (Xu et al. 1997).

Roles of Bacterial Chaperonins

Due to their importance in protein homeostasis, chaperonins are essential and universally distributed in all bacteria. Bacterial chaperonins are required for the correct assembly of the cell division apparatus (Ogino et al. 2004). In contrast to *E. coli*

which possesses a single operon-encoded *groEL* gene with a *groES* gene, nearly 30% of all bacterial genomes contain multiple chaperonin genes (Lund 2009). The mycobacteria were the first bacteria revealed to have multiple chaperonins (Lund 2001; Kong et al. 1993). *M. tuberculosis* encodes two chaperonin genes, *cpn60.1* in an operon with the co-chaperonin gene *cpn10* and *cpn60.2* in a different position on the chromosome (Kong et al. 1993), while *M. smegmatis* has 3 copies of *cpn60* (Fan et al. 2012). In bacteria with multiple *groEL* genes, such as mycobacteria, the essential copy is unexpectedly often not the operon-encoded gene and this has resulted in much interest and speculation about the functions of these additional chaperonins (Hu et al. 2008; Ojha et al. 2005). It is possible that one copy preserves the essential chaperone function, while the others diverge to take on altered roles (Lund 2001). Biophysical studies of the chaperonins from *M. tuberculosis* and *M. smegmatis* provide support of novel functions for Cpn60.1 as Cpn60.2 proteins assemble into oligomers and are able to replace GroEL in *E. coli* when co-expressed with GroES or the cognate Cpn10; while neither Cpn60.1 nor Cpn60.3 found in *M. smegmatis* could functionally replace GroEL (Fan et al. 2012). Based on the fact that Cpn60.1 appears to chaperone a discrete set of key enzymes involved in the synthesis of the complex cell wall and differences in protein sequence, this novel mycobacterial chaperonin may provide a unique target for drug development [reviewed by (Colaco and MacDougall 2014)].

One of the five GroEL paralogs in *Sinorhizobium meliloti* is required for NodD protein folding (Ogawa and Long 1995), whilst *Bradyrhizobium japonicum* possess at least five *groESL* operons that can partially compensate for the lack of one or other genes (Fischer et al. 1993). These duplicated proteins have evolved specific roles in different bacteria but the mechanism involved in functional divergence has not been elucidated (Wang et al. 2013). *Myxococcus xanthus* DK1622 displayed functional divergence with respect to substrate specificity and this was as a result of differences in the apical and C-terminal regions of the two GroEL proteins (Wang et al. 2013). Interestingly, monomeric Cpn60 from *Thermus thermophilus* was able to support protein folding independently of both ATP and a co-chaperonin (Taguchi et al. 1994). The crystal structures of the *T. thermophilus* Cpn60/Cpn10 complex alone (Shimamura et al. 2003) and with bound proteins has been reported (Shimamura et al. 2004). Despite a destabilised structure, Cpn60 proteins from *M. tuberculosis* also displayed activity in the absence of ATP or co-chaperonin (Qamra and Mande 2004).

Cpn60s are dominant immunogens present during human bacterial infections. Moreover, Cpn60s of *M. tuberculosis* are potent inducers of host inflammatory responses and behave as antigens and cytokines (Qamra et al. 2005). The host immune response to exogenous chaperonins may be both protective and damaging (Ranford and Henderson 2002). It has been hypothesised that due to sequence conservation, the host immune response mounted against bacterial co-chaperonins may result in cross-reactivity to human Cpn60 causing an autoimmune reaction (van Eden et al. 1998). There is convincing evidence for the case in the development of atherosclerosis (Wick 2006). The roles of chaperonins in disease, including models and potential treatments are addressed in a review by (Ranford and Henderson 2002).

Specific Functions of Bacterial Co-chaperonins

In addition to co-chaperonin activity, a number of diverse roles played by bacterial co-chaperonins are emerging, in particular during host-pathogen interactions. The possible reasons for numerous chaperonins in bacteria were reviewed by Lund in 2009 and the evolution of so many different functions were highlighted by Henderson and Martin (2011; Henderson and Martin 2011; Lund 2009). Most bacterial Cpn10 proteins are stimulators of the immune system and the response varies between different species, with human and *E. coli* Cpn10 proteins being poor immunogens and *M. tuberculosis* and *M. leprae* being strong immunogens (Cavanagh and Morton 1994). These proteins also play a role in apoptosis, cytokine secretion and cellular growth and development (Cavanagh 1996). Cpn10 of *M. tuberculosis*, a secreted protein with cell signalling functions, is an important virulence factor during infection and it plays a key role in the pathology of spinal tuberculosis by inhibiting the growth of osteoblasts (Meghji et al. 1997; Roberts et al. 2003). Structures have been reported for *M. leprae*, and *M. tuberculosis* cpn10 proteins and immunodominant epitopes have been mapped to the mobile loop (Mande et al. 1996; Roberts et al. 1999). Further structural analysis of *M. tuberculosis* Cpn10, in the presence of divalent cations, showed the existence of a heptamer (Taneja and Mande 2001, 2002). The crystal structure of *T. thermophilus* HB8 Cpn10 showed disordered loops in five subunits (Numoto et al. 2005). Comparison of *M. tuberculosis* Cpn10 to that of *T. thermophilus* HB8 Cpn10 revealed a similar overall structure, however the dome loops and mobile loops are different (Fig. 8.5). The Cpn10 from *Aquifex aeolicus* has a 25-residue C-terminal extension present in each monomer that is absent from

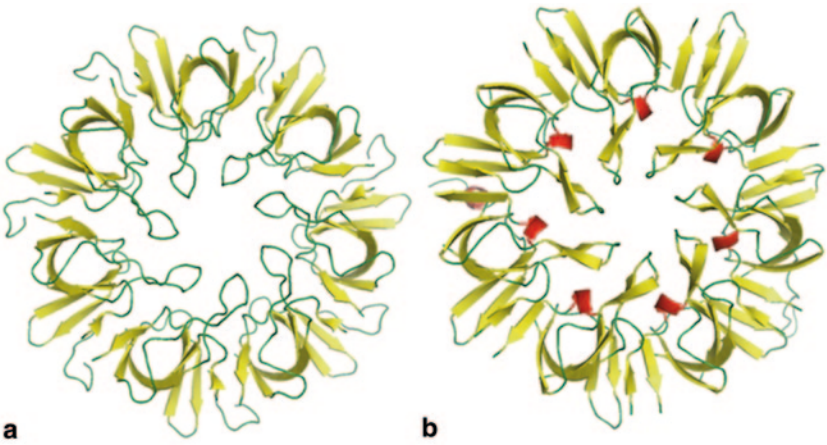

Fig. 8.5 The overall structures of *M. tuberculosis* Cpn10 (**a**) and *T. thermophilus* Cpn10 (**b**) conform to the GroES-fold. Differences are evident in the mobile loops and a partially helical structure is present in the *T. thermophilus* Cpn10 monomer. Breaks are evident in the structures due to a lack of electron density in the highly flexible mobile loops. Alpha helices are shown in *red* and β-sheets in *yellow*. The images were generated using PyMol (DeLano Scientific) from coordinates in PDB: 1HX5 and WNR

any other known Cpn10 protein that is not essential for function, but plays a role in preventing aggregation at high temperatures (Luke et al. 2005; Chen et al. 2008).

Roles of Eukaryotic Group I Chaperonins

In eukaryotes, Hsp60 is found in the cytosol and the mitochondria and also in chloroplasts of plants. It interacts with its co-chaperonin Hsp10 or Cpn10 to promote protein folding in the cell. Most mitochondria and chloroplasts in higher plants appear to possess multiple chaperonin subunits (Hill and Hemmingsen 2001). Chaperonins in the eukaryotic cytosol are frequently situated near the ribosome and are found in complexes with other chaperones such as Hsc70 (Thulasiraman et al. 1999). In recent years a number of novel functions and interacting molecules have been assigned to Hsp60 (Czarnecka et al. 2006). Some of these are associated with carcinogenesis as its role in the survival and proliferation of tumor cells has increased (Czarnecka et al. 2006; Cappello et al. 2008).

The mitochondrial Hsp60 protein is essential for the folding of proteins imported into the mitochondria and prevention of denaturation during stress (Cheng et al. 1989; Levy-Rimler et al. 2001). They are also characterised by a host of additional functions, including extracellular functions. Mutations of human Hsp60 are linked to severe genetic diseases (Bross et al. 2007; Hansen et al. 2007; Magen et al. 2008). It also plays a role in the production of pro-inflammatory cytokines (Chun et al. 2010). In addition, it plays both pro-apoptotic and anti-apoptotic roles, depending on localisation (Xanthoudakis et al. 1999; Knowlton and Gupta 2003). The mitochondrial Hsp60 in eukaryotic cells is composed of a double-ring structure capped by one ring of Hsp10 (Azem et al. 1995; Coluzza et al. 2006). In contrast, human Hsp60 exists basically in a single-ring to double-ring equilibrium and Hsp10 can bind to both single and double-ring Hsp60 in the presence of ATP (Azem et al. 1995). The mitochondrial chaperonin complex that is composed of a single ring of seven subunits and a ring of Hsp10 subunits cannot exploit binding of ATP to the *trans* ring as a mechanism for releasing *cis* GroES (Nielsen and Cowan 1998). This complex may have evolved an intrinsically lower affinity for the co-chaperonin but the presence of a high affinity mobile loop on Hsp10 may offset the low affinity (Nielsen and Cowan 1998). Despite the fact that mitochondrial Hsp60 can functionally replace GroEL, it is incapable of interacting with GroES (Nielsen et al. 1999). The elements that dictate the specificity of mitochondrial Hsp60 for Hsp10 appear to lie in the apical domain (Parnas et al. 2012). Analysis of *in vivo* substrates of yeast mitochondrial chaperonins revealed divergent chaperonin requirements, indicating that Hsp60 and Hsp10 do not always operate as a functional unit (Dubaquie et al. 1998). Yeast mitochondrial Hsp60 can bind to single-stranded DNA *in vitro* and play a role in the structure and transmission of nucleoids (Kaufman et al. 2003). A number of parasites affecting human health have demonstrated an up-regulation of Hsp60, which is possibly linked to diverse environmental conditions encountered

during its transition from a mammalian to an insect vector (Maresca and Carratu 1992). Induction of Hsp60 was found to occur during the entire course of infection of *Trypanosoma brucei*, a protozoan parasite responsible for causing sleeping sickness in humans (Radwanska et al. 2000).

The chloroplast type I chaperonin complex (Cpn60) is structurally similar to GroEL and also forms two stacked heptameric rings (Tsuprun et al. 1991), however these are composed of two different subunit types, Cpn60α and Cpn60β (Martel et al. 1990) which are ~50% identical to each other (Hill and Hemmingsen 2001). *Arabidopsis thaliana* encodes several Cpn60α and Cpn60β families and both are required for plastid division (Suzuki et al. 2009). A unique chaperonin subunit present in *A. thaliana* confers substrate specificity, while the dominant subunits retain housekeeping functions (Peng et al. 2011).

Specific Functions of Eukaryotic Group I Co-chaperonins

A single copy of the Cpn10 co-chaperonin is present in the mitochondria of yeast and mammals, in contrast to chloroplasts that have two different co-chaperonin homologs (Rospert et al. 1993; Hansen et al. 2003). The chloroplast co-chaperonins are varied with *cpn10* encoding the conventional 10 kDa protein that is similar in structure and function to GroES, as well as *cpn20* encoding tandem fusions of Cpn10 domains that form tetrameric ring structures that function with GroEL and Cpn60 (Bertsch et al. 1992; Koumoto et al. 2001; Sharkia et al. 2003). Interestingly, the green alga *Chlamydomonas reinhardtii* has three co-chaperonins, Cpn10, Cpn20 and Cpn23 that are individually non-functional (Tsai et al. 2012). In studies using recombinant co-chaperonins of *A. thaliana and C. reinhardtii* hetero-oligomeric ring complexes formed by combinations of Cpn10, Cpn20 and Cpn23 were able to serve as co-chaperonins in order to perhaps modify the chaperonin folding cage for specific client proteins (Tsai et al. 2012).

Whilst our understanding of the roles of human Hsp10 in disease continues to receive research attention, little is known about the roles of its homologues in virulence and pathogenicity of protozoan parasites affecting human health; and they may interact with the human chaperone system. The first protozoan CPN10 protein characterised was from *Leishmania donovani* and was shown to interact with CPN60.2 with increased concentrations detected during the amastigote stage of the lifecycle (Zamora-Veyl et al. 2005). Cpn20 proteins were known to exist only in chloroplasts, however sequencing of the malarial genome revealed a single Cpn20 protein which correlates with the algal origin of the parasite (Sato and Wilson 2005; Janouskovec et al. 2010). Since the *Plasmodium falciparum* genome encodes only one *cpn20* gene, it functions as a homo-oligomeric co-chaperonin that can functionally replace GroES (Vitlin Gruber et al. 2013b). Characterisation of HSP10 from *Strongyloides ratti,* an intestinal nematode infecting humans, revealed a strong immunogenic response and the inability to bind to *S. ratti* HSP 60 provided evidence of a role in host-parasite interactions (Tazir et al. 2009).

The structure of human mitochondrial Hsp10 has been solved and mutations in the first and last β-strands altered both the oligomeric and folded states (Guidry et al. 2003). In contrast to human mitochondrial Hsp60, Hsp10 stimulates the production of anti-inflammatory cytokines and exerts immunosuppressive activity (Johnson et al. 2005). One of the first extracellular heat shock proteins to be isolated was a circulating immunosuppressive protein, termed early pregnancy factor (EPF), which was later identified as HSP10 (HSPE) after the isolation and demonstration of its role as a co-chaperonin for Hsp60 (Cavanagh and Morton 1994; Morton et al. 1977). The isolation of EPF was also the first evidence that heat shock proteins could function as cell signalling agonists (Morton et al. 1977). EFP appeared in the maternal serum within 24 h after fertilisation in some mammalians and has been found to exhibit growth factor qualities and anti-inflammatory properties essential for protecting the embryo from the mother's own immune system (Athanasas-Platsis et al. 2004; Morton et al. 1977; Quinn et al. 1990). The relationship between Hsp10 and EPF is discussed in a review by (Corrao et al. 2010). Recombinant human Hsp10 has been used for the treatment of rheumatoid arthritis (Vanags et al. 2006) and multiple sclerosis (Broadley et al. 2009). Extracellular Hsp10 influences endothelial cell differentiation (Dobocan et al. 2009). There is growing evidence to suggest that extracellular Hsp10 plays an active role in cell signalling (David et al. 2013). A number of reviews have been written on Hsp60 chaperonopathies, diseases that arise from abnormal chaperonins (Cappello et al. 2008; Cappello et al. 2013; Cappello et al. 2011; Cappello et al. 2014; Macario and Conway de Macario 2005, 2007).

Conclusion

Continued research on the influence of the cellular environment on the GroEL/ES folding machine and factors affecting the rate of protein folding will enhance our understanding of this system. The evolution of moonlighting functions of bacterial chaperonins and co-chaperonins needs to be addressed. The structural states of Hsp10, including mixed oligomeric or fragmented, appears to influence the function as well as location. Hsp10 often functions as an antagonist to Hsp60 and possibly other molecular chaperones. Further knowledge of the extracellular functions of Hsp10, including secretion pathways and cell signalling, will definitely be of benefit in the development of treatments for cancer and auto-immune diseases related to this protein.

Acknowledgments Financial support from the National Research Foundation (NRF), Rhodes University and Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged. The views reflected in this document are those of the author and should in no way be attributed to the NRF, Rhodes University or DFG.

References

Ang D, Georgopoulos C (1989) The heat-shock-regulated grpE gene of *Escherichia coli* is required for bacterial growth at all temperatures but is dispensable in certain mutant backgrounds. J Bacteriol 171:2748–2755

Ang D, Keppel F, Klein G, Richardson A, Georgopoulos C (2000) Genetic analysis of bacteriophage-encoded cochaperonins. Annu Revi Genet 34:439–456

Ang D, Richardson A, Mayer MP, Keppel F, Krisch H, Georgopoulos C (2001) Pseudo-T-even bacteriophage RB49 encodes CocO, a cochaperonin for GroEL, which can substitute for *Escherichia coli's* GroES and bacteriophage T4's Gp31. J Biol Chem 276:8720–8726

Archibald JM, Logsdon JM, Doolittle WF (1999) Recurrent paralogy in the evolution of archaeal chaperonins. Curr Biol 9:1053–1056

Athanasas-Platsis S, Somodevilla-Torres MJ, Morton H, Cavanagh AC (2004) Investigation of the immunocompetent cells that bind early pregnancy factor and preliminary studies of the early pregnancy factor target molecule. Immunol Cell Biol 82:361–369

Azem A, Diamant S, Kessel M, Weiss C, Goloubinoff P (1995) The protein-folding activity of chaperonins correlates with the symmetric GroEL14(GroES7)2 heterooligomer. Proc Natl Acad Sci U S A 92:12021–12025

Barraclough R, Ellis RJ (1980) Protein synthesis in chloroplasts. IX. Assembly of newly-synthesized large subunits into ribulose bisphosphate carboxylase in isolated intact pea chloroplasts. Biochim Biophys Acta 608:19–31

Bertsch U, Soll J, Seetharam R, Viitanen PV (1992) Identification, characterization, and DNA sequence of a functional "double" groES-like chaperonin from chloroplasts of higher plants. Proc Natl Acad Sci U S A 89:8696–8700

Boisvert DC, Wang J, Otwinowski Z, Horwich AL, Sigler PB (1996) The 2.4 A crystal structure of the bacterial chaperonin GroEL complexed with ATP gamma S. Nat Struct Biol 3:170–177

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32:191–222

Boudker O, Todd MJ, Freire E (1997) The structural stability of the co-chaperonin GroES. J Mol Biol 272:770–779.

Braig K, Simon M, Furuya F, Hainfeld JF, Horwich AL (1993) A polypeptide bound by the chaperonin groEL is localized within a central cavity. Proc Natl Acad Sci U S A 90:3978–3982

Braig K, Otwinowski Z, Hegde R, Boisvert DC, Joachimiak A, Horwich AL, Sigler PB (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 A. Nature 371:578–586

Broadley SA, Vanags D, Williams B, Johnson B, Feeney D, Griffiths L, Shakib S, Brown G, Coulthard A, Mullins P, Kneebone C (2009) Results of a phase IIa clinical trial of an anti-inflammatory molecule, chaperonin 10, in multiple sclerosis. Mult Scler 15:329–336

Bross P, Li Z, Hansen J, Hansen JJ, Nielsen MN, Corydon TJ, Georgopoulos C, Ang D, Lundemose JB, Niezen-Koning K, Eiberg H, Yang H, Kolvraa S, Bolund L, Gregersen N (2007) Single-nucleotide variations in the genes encoding the mitochondrial Hsp60/Hsp10 chaperone system and their disease-causing potential. J Hum Genet 52:56–65

Buchner J, Schmidt M, Fuchs M, Jaenicke R, Rudolph R, Schmid FX, Kiefhaber T (1991) GroE facilitates refolding of citrate synthase by suppressing aggregation. Biochemistry 30:1586–1591

Bukau B, Horwich AL (1998) The Hsp70 and Hsp60 chaperone machines. Cell 92:351–366

Burston SG, Ranson NA, Clarke AR (1995) The origins and consequences of asymmetry in the chaperonin reaction cycle. J Mol Biol 249:138–152

Calderwood SK, Mambula SS, Gray PJ Jr (2007) Extracellular heat shock proteins in cell signaling and immunity. Ann N Y Acad Sci 1113:28–39

Cappello F, Conway de Macario E, Marasa L, Zummo G, Macario AJ (2008) Hsp60 expression, new locations, functions and perspectives for cancer diagnosis and therapy. Cancer Biol Ther 7:801–809

Cappello F, Caramori G, Campanella C, Vicari C, Gnemmi I, Zanini A, Spanevello A, Capelli A, La Rocca G, Anzalone R, Bucchieri F, D'Anna SE, Ricciardolo FL, Brun P, Balbi B, Carone

M, Zummo G, Conway de Macario E, Macario AJ, Di Stefano A (2011) Convergent sets of data from in vivo and in vitro methods point to an active role of Hsp60 in chronic obstructive pulmonary disease pathogenesis. PloS one 6:e28200

Cappello F, Angileri F, de Macario EC, Macario AJ (2013) Chaperonopathies and chaperonotherapy. Hsp60 as therapeutic target in cancer: potential benefits and risks. Curr Pharm Des 19:452–457

Cappello F, Marino Gammazza A, Palumbo Piccionello A, Campanella C, Pace A, Conway de Macario E, Macario AJ (2014) Hsp60 chaperonopathies and chaperonotherapy: targets and agents. Expert Opin Ther Targets 18:185–208

Cavanagh AC (1996) Identification of early pregnancy factor as chaperonin 10: implications for understanding its role. Rev Reprod 1:28–32

Cavanagh AC, Morton H (1994) The purification of early-pregnancy factor to homogeneity from human platelets and identification as chaperonin 10. Eur J Biochem 222:551–560

Chakraborty K, Chatila M, Sinha J, Shi Q, Poschner BC, Sikor M, Jiang G, Lamb DC, Hartl FU, Hayer-Hartl M (2010) Chaperonin-catalyzed rescue of kinetically trapped states in protein folding. Cell 142:112–122

Chandra D, Choy G, Tang DG (2007) Cytosolic accumulation of HSP60 during apoptosis with or without apparent mitochondrial release: evidence that its pro-apoptotic or pro-survival functions involve differential interactions with caspase-3. J Biol Chem 282:31289–31301

Chandrasekhar GN, Tilly K, Woolford C, Hendrix R, Georgopoulos C (1986) Purification and properties of the groES morphogenetic protein of *Escherichia coli*. J Biol Chem 261:12414–12419

Chaudhuri TK, Farr GW, Fenton WA, Rospert S, Horwich AL (2001) GroEL/GroES-mediated folding of a protein too large to be encapsulated. Cell 107:235–246

Chen L, Sigler PB (1999) The crystal structure of a GroEL/peptide complex: plasticity as a basis for substrate diversity. Cell 99:757–768

Chen S, Roseman AM, Hunter AS, Wood SP, Burston SG, Ranson NA, Clarke AR, Saibil HR (1994) Location of a folding protein and shape changes in GroEL-GroES complexes imaged by cryo-electron microscopy. Nature 371:261–264

Chen DH, Song JL, Chuang DT, Chiu W, Ludtke SJ (2006) An expanded conformation of single-ring GroEL-GroES complex encapsulates an 86 kDa substrate. Structure 14:1711–1722

Chen DH, Luke K, Zhang J, Chiu W, Wittung-Stafshede P (2008) Location and flexibility of the unique C-terminal tail of Aquifex aeolicus co-chaperonin protein 10 as derived by cryo-electron microscopy and biophysical techniques. J Mol Biol 381:707–717

Chen DH, Madan D, Weaver J, Lin Z, Schroder GF, Chiu W, Rye HS (2013) Visualizing GroEL/ES in the act of encapsulating a folding protein. Cell 153:1354–1365

Cheng MY, Hartl FU, Martin J, Pollock RA, Kalousek F, Neupert W, Hallberg EM, Hallberg RL, Horwich AL (1989) Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature 337:620–625

Christensen JH, Nielsen MN, Hansen J, Fuchtbauer A, Fuchtbauer EM, West M, Corydon TJ, Gregersen N, Bross P (2010) Inactivation of the hereditary spastic paraplegia-associated Hspd1 gene encoding the Hsp60 chaperone results in early embryonic lethality in mice. Cell Stress Chaperones 15:851–863

Clare DK, Vasishtan D, Stagg S, Quispe J, Farr GW, Topf M, Horwich AL, Saibil HR (2012) ATP-triggered conformational changes delineate substrate-binding and -folding mechanics of the GroEL chaperonin. Cell 149:113–123

Colaco CA, MacDougall A (2014) Mycobacterial chaperonins: the tail wags the dog. FEMS Microbiol Lett 350:20–24

Coluzza I, van der Vies SM, Frenkel D (2006) Translocation boost protein-folding efficiency of double-barreled chaperonins. Biophys J 90:3375–3381

Corrao S, Campanella C, Anzalone R, Farina F, Zummo G, Conway de Macario E, Macario AJ, Cappello F, La Rocca G (2010) Human Hsp10 and Early Pregnancy Factor (EPF) and their relationship and involvement in cancer and immunity: current knowledge and perspectives. Life Sci 86:145–152

Czarnecka AM, Campanella C, Zummo G, Cappello F (2006) Heat shock protein 10 and signal transduction: a "capsula eburnea" of carcinogenesis? Cell Stress Chaperones 11:287–294

David S, Bucchieri F, Corrao S, Czarnecka AM, Campanella C, Farina F, Peri G, Tomasello G, Sciume C, Modica G, La Rocca G, Anzalone R, Giuffre M, Conway De Macario E, Macario AJ, Cappello F, Zummo G (2013) Hsp10: anatomic distribution, functions, and involvement in human disease. Front Biosci (Elite Ed) 5:768–778

Dobocan MC, Sadvakassova G, Congote LF (2009) Chaperonin 10 as an endothelial-derived differentiation factor: role of glycogen synthase kinase-3. J Cellular Physiol 219:470–476

Dubaquie Y, Looser R, Funfschilling U, Jeno P, Rospert S (1998) Identification of in vivo substrates of the yeast mitochondrial chaperonins reveals overlapping but non-identical requirement for hsp60 and hsp10. EMBO J 17:5868–5876

Ellis RJ, Hartl FU (1996) Protein folding in the cell: competing models of chaperonin function. FASEB J 10:20–26

Ewalt KL, Hendrick JP, Houry WA, Hartl FU (1997) In vivo observation of polypeptide flux through the bacterial chaperonin system. Cell 90:491–500

Fan M, Rao T, Zacco E, Ahmed MT, Shukla A, Ojha A, Freeke J, Robinson CV, Benesch JL, Lund PA (2012) The unusual mycobacterial chaperonins: evidence for in vivo oligomerization and specialization of function. Mol Microbiol 85:934–944

Fayet O, Ziegelhoffer T, Georgopoulos C (1989) The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. J Bacteriol 171:1379–1385

Fenton WA, Horwich AL (2003) Chaperonin-mediated protein folding: fate of substrate polypeptide. Q Rev Biophys 36:229–256

Fenton WA, Kashi Y, Furtak K, Horwich AL (1994) Residues in chaperonin GroEL required for polypeptide binding and release. Nature 371:614–619

Fiaux J, Bertelsen EB, Horwich AL, Wuthrich K (2002) NMR analysis of a 900K GroEL GroES complex. Nature 418:207–211

Fink AL (1999) Chaperone-mediated protein folding. Physiol Rev 79:425–449

Fischer HM, Babst M, Kaspar T, Acuna G, Arigoni F, Hennecke H (1993) One member of a gro-ESL-like chaperonin multigene family in Bradyrhizobium japonicum is co-regulated with symbiotic nitrogen fixation genes. EMBO J 12:2901–2912

Fontanella B, Birolo L, Infusini G, Cirulli C, Marzullo L, Pucci P, Turco MC, Tosco A (2010) The co-chaperone BAG3 interacts with the cytosolic chaperonin CCT: new hints for actin folding. Int J Biochem Cell Biol 42:641–650

Frydman J (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. Annu Rev Biochem 70:603–647

Frydman J, Nimmesgern E, Erdjument-Bromage H, Wall JS, Tempst P, Hartl FU (1992) Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. EMBO J 11:4767–4778

Fujiwara K, Ishihama Y, Nakahigashi K, Soga T, Taguchi H (2010) A systematic survey of in vivo obligate chaperonin-dependent substrates. EMBO J 29:1552–1564

Gao Y, Thomas JO, Chow RL, Lee GH, Cowan NJ (1992) A cytoplasmic chaperonin that catalyzes beta-actin folding. Cell 69:1043–1050

Georgopoulos CP, Hendrix RW, Kaiser AD, Wood WB (1972) Role of the host cell in bacteriophage morphogenesis: effects of a bacterial mutation on T4 head assembly. Nat New Biol 239:38–41

Goble JL, Johnson H, de Ridder J, Stephens LL, Louw A, Blatch GL, Boshoff A (2013) The druggable antimalarial target PfDXR: overproduction strategies and kinetic characterization. Protein Pept Lett 20:115–124

Goloubinoff P, Christeller JT, Gatenby AA, Lorimer GH (1989a) Reconstitution of active dimeric ribulose bisphosphate carboxylase from an unfoleded state depends on two chaperonin proteins and Mg-ATP. Nature 342:884–889

Goloubinoff P, Gatenby AA, Lorimer GH (1989b) GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in *Escherichia coli*. Nature 337:44–47

Gray TE, Fersht AR (1991) Cooperativity in ATP hydrolysis by GroEL is increased by GroES. FEBS Lett 292:254–258

Guidry JJ, Shewmaker F, Maskos K, Landry S, Wittung-Stafshede P (2003) Probing the interface in a human co-chaperonin heptamer: residues disrupting oligomeric unfolded state identified. BMC Biochem 4:14

Guisbert E, Herman C, Lu CZ, Gross CA (2004) A chaperone network controls the heat shock response in *E. coli*. Genes Dev 18:2812–2821

Gupta P, Aggarwal N, Batra P, Mishra S, Chaudhuri TK (2006) Co-expression of chaperonin GroEL/GroES enhances in vivo folding of yeast mitochondrial aconitase and alters the growth characteristics of *Escherichia coli*. Int J Biochem Cell Biol 38:1975–1985

Hansen JJ, Bross P, Westergaard M, Nielsen MN, Eiberg H, Borglum AD, Mogensen J, Kristiansen K, Bolund L, Gregersen N (2003) Genomic structure of the human mitochondrial chaperonin genes: HSP60 and HSP10 are localised head to head on chromosome 2 separated by a bidirectional promoter. Human Genet 112:71–77

Hansen J, Svenstrup K, Ang D, Nielsen MN, Christensen JH, Gregersen N, Nielsen JE, Georgopoulos C, Bross P (2007) A novel mutation in the HSPD1 gene in a patient with hereditary spastic paraplegia. J Neurol 254:897–900

Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–579

Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:1852–1858

Hartl FU, Hayer-Hartl M (2009) Converging concepts of protein folding in vitro and in vivo. Nat Struct Mol Biol 16:574–581

Hartl FU, Martin J (1995) Molecular chaperones in cellular protein folding. Curr Opin Struct Biol 5:92–102

Hartl FU, Martin J, Neupert W (1992) Protein folding in the cell: the role of molecular chaperones Hsp70 and Hsp60. Annu Rev Biophys Biomol Struct 21:293–322

Hartman DJ, Surin BP, Dixon NE, Hoogenraad NJ, Hoj PB (1993) Substoichiometric amounts of the molecular chaperones GroEL and GroES prevent thermal denaturation and aggregation of mammalian mitochondrial malate dehydrogenase in vitro. Proc Natl Acad Sci U S A 90:2276–2280

Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333:330–334

Henderson B, Martin A (2011) Bacterial virulence in the moonlight: multitasking bacterial moonlighting proteins are virulence determinants in infectious disease. Infect Immun 79:3476–3491

Hertveldt K, Lavigne R, Pleteneva E, Sernova N, Kurochkina L, Korchevskii R, Robben J, Mesyanzhinov V, Krylov VN, Volckaert G (2005) Genome comparison of *Pseudomonas aeruginosa* large phages. J Mol Biol 354:536–545

Hill JE, Hemmingsen SM (2001) Arabidopsis thaliana type I and II chaperonins. Cell Stress Chaperones 6:190–200

Horovitz A, Willison KR (2005) Allosteric regulation of chaperonins. Curr Opin Struct Biol 15:646–651

Horovitz A, Fridmann Y, Kafri G, Yifrach O (2001) Review: allostery in chaperonins. J Struct Biol 135:104–114

Horwich AL, Saibil HR (1998) The thermosome: chaperonin with a built-in lid. Nat Struct Biol 5:333–336

Horwich AL, Low KB, Fenton WA, Hirshfield IN, Furtak K (1993) Folding in vivo of bacterial cytoplasmic proteins: role of GroEL. Cell 74:909–917

Horwich AL, Fenton WA, Chapman E, Farr GW (2007) Two families of chaperonin: physiology and mechanism. Annu Rev Cell Dev Biol 23:115–145

Houry WA, Frishman D, Eckerskorn C, Lottspeich F, Hartl FU (1999) Identification of in vivo substrates of the chaperonin GroEL. Nature 402:147–154

Hu Y, Henderson B, Lund PA, Tormay P, Ahmed MT, Gurcha SS, Besra GS, Coates AR (2008) A Mycobacterium tuberculosis mutant lacking the groEL homologue cpn60.1 is viable but fails to induce an inflammatory response in animal models of infection. Infect Immun 76:1535–1546

Hunt JF, Weaver AJ, Landry SJ, Gierasch L, Deisenhofer J (1996) The crystal structure of the GroES co-chaperonin at 2.8 A resolution. Nature 379:37–45

Hunt JF, Earnest TN, Bousche O, Kalghatgi K, Reilly K, Horvath C, Rothschild KJ, Engelman DM (1997) A biophysical study of integral membrane protein folding. Biochemistry 36:15156–15176

Janouskovec J, Horak A, Obornik M, Lukes J, Keeling PJ (2010) A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. Proc Natl Acad Sci U S A 107:10949–10954

Jewett AI, Shea JE (2010) Reconciling theories of chaperonin accelerated folding with experimental evidence. Cell Mol Life Sci 67:255–276

Jia H, Halilou AI, Hu L, Cai W, Liu J, Huang B (2011) Heat shock protein 10 (Hsp10) in immune-related diseases: one coin, two sides. Int J Biochem Mol Biol 2:47–57

Johnson BJ, Le TT, Dobbin CA, Banovic T, Howard CB, Flores Fde M, Vanags D, Naylor DJ, Hill GR, Suhrbier A (2005) Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. J Biol Chem 280:4037–4047

Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14:105–111

Kaufman BA, Kolesar JE, Perlman PS, Butow RA (2003) A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in Saccharomyces cerevisiae. J Cell Biol 163:457–461

Kaufmann SH (1992) The cellular immune response to heat shock proteins. Experientia 48:640–643

Kawata Y, Kawagoe M, Hongo K, Miyazaki T, Higurashi T, Mizobata T, Nagai J (1999) Functional communications between the apical and equatorial domains of GroEL through the intermediate domain. Biochemistry 38:15731–15740

Keppel F, Rychner M, Georgopoulos C (2002) Bacteriophage-encoded cochaperonins can substitute for *Escherichia coli's* essential GroES protein. EMBO Rep 3:893–898

Kerner MJ, Naylor DJ, Ishihama Y, Maier T, Chang HC, Stines AP, Georgopoulos C, Frishman D, Hayer-Hartl M, Mann M, Hartl FU (2005) Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. Cell 122:209–220

Knowlton AA, Gupta S (2003) HSP60, Bax, and cardiac apoptosis. Cardiovasc Toxicol 3:263–268

Kong TH, Coates AR, Butcher PD, Hickman CJ, Shinnick TM (1993) Mycobacterium tuberculosis expresses two chaperonin-60 homologs. Proc Natl Acad Sci U S A 90:2608–2612

Koumoto Y, Shimada T, Kondo M, Hara-Nishimura I, Nishimura M (2001) Chloroplasts have a novel Cpn10 in addition to Cpn20 as co-chaperonins in Arabidopsis thaliana. J Biol Chem 276:29688–29694

Kubota H, Hynes G, Carne A, Ashworth A, Willison K (1994) Identification of six Tcp-1-related genes encoding divergent subunits of the TCP-1-containing chaperonin. Curr Biol 4:89–99

Kurochkina LP, Semenyuk PI, Orlov VN, Robben J, Sykilinda NN, Mesyanzhinov VV (2012) Expression and functional characterization of the first bacteriophage-encoded chaperonin. J Virol 86:10103–10111

Landry SJ, Zeilstra-Ryalls J, Fayet O, Georgopoulos C, Gierasch LM (1993) Characterization of a functionally important mobile domain of GroES. Nature 364:255–258

Landry SJ, Taher A, Georgopoulos C, van der Vies SM (1996) Interplay of structure and disorder in cochaperonin mobile loops. Proc Natl Acad Sci U S A 93:11622–11627

Lenz G, Ron EZ (2014) Novel interaction between the major bacterial heat shock chaperone (GroESL) and an RNA chaperone (CspC). J Mol Biol 426:460–466

Leroux MR (2001) Protein folding and molecular chaperones in archaea. Adv Appl Microbiol 50:219–277

Levy-Rimler G, Viitanen P, Weiss C, Sharkia R, Greenberg A, Niv A, Lustig A, Delarea Y, Azem A (2001) The effect of nucleotides and mitochondrial chaperonin 10 on the structure and chaperone activity of mitochondrial chaperonin 60. EurJ Biochem 268:3465–3472

Lorimer GH (1996) A quantitative assessment of the role of the chaperonin proteins in protein folding in vivo. FASEB J 10:5–9

Luke K, Apiyo D, Wittung-Stafshede P (2005) Role of the unique peptide tail in hyperthermostable Aquifex aeolicus cochaperonin protein 10. Biochemistry 44:14385–14395

Lund PA (2001) Microbial molecular chaperones. Adv Microb Physiol 44:93–140

Lund PA (2009) Multiple chaperonins in bacteria–why so many? FEMS Microbiol Rev 33:785–800

Macario AJ, Conway de Macario E (2005) Sick chaperones, cellular stress, and disease. N Engl J Med 353:1489–1501

Macario AJ, Conway de Macario E (2007) Molecular chaperones: multiple functions, pathologies, and potential applications. Front Biosci 12:2588–2600

Machida K, Fujiwara R, Tanaka T, Sakane I, Hongo K, Mizobata T, Kawata Y (2009) Gly192 at hinge 2 site in the chaperonin GroEL plays a pivotal role in the dynamic apical domain movement that leads to GroES binding and efficient encapsulation of substrate proteins. Biochim Biophys Acta 1794:1344–1354

Magen D, Georgopoulos C, Bross P, Ang D, Segev Y, Goldsher D, Nemirovski A, Shahar E, Ravid S, Luder A, Heno B, Gershoni-Baruch R, Skorecki K, Mandel H (2008) Mitochondrial hsp60 chaperonopathy causes an autosomal-recessive neurodegenerative disorder linked to brain hypomyelination and leukodystrophy. Am J Hum Genet 83:30–42

Mande SC, Mehra V, Bloom BR, Hol WG (1996) Structure of the heat shock protein chaperonin-10 of Mycobacterium leprae. Science 271:203–207

Maresca B, Carratu L (1992) The biology of the heat shock response in parasites. Parasitol Today 8:260–266

Martel R, Cloney LP, Pelcher LE, Hemmingsen SM (1990) Unique composition of plastid chaperonin-60: alpha and beta polypeptide-encoding genes are highly divergent. Gene 94:181–187

Martin J, Langer T, Boteva R, Schramel A, Horwich AL, Hartl FU (1991) Chaperonin-mediated protein folding at the surface of groEL through a 'molten globule'-like intermediate. Nature 352:36–42

Martin-Benito J, Boskovic J, Gomez-Puertas P, Carrascosa JL, Simons CT, Lewis SA, Bartolini F, Cowan NJ, Valpuesta JM (2002) Structure of eukaryotic prefoldin and of its complexes with unfolded actin and the cytosolic chaperonin CCT. EMBO J 21:6377–6386

Meghji S, White PA, Nair SP, Reddi K, Heron K, Henderson B, Zaliani A, Fossati G, Mascagni P, Hunt JF, Roberts MM, Coates AR (1997) Mycobacterium tuberculosis chaperonin 10 stimulates bone resorption: a potential contributory factor in Pott's disease. J Exp Med 186:1241–1246

Meyer AS, Gillespie JR, Walther D, Millet IS, Doniach S, Frydman J (2003) Closing the folding chamber of the eukaryotic chaperonin requires the transition state of ATP hydrolysis. Cell 113:369–381

Morton H, Rolfe B, Clunie GJ (1977) An early pregnancy factor detected in human serum by the rosette inhibition test. Lancet 1:394–397

Nielsen KL, Cowan NJ (1998) A single ring is sufficient for productive chaperonin-mediated folding in vivo. Mol Cell 2:93–99

Nielsen KL, McLennan N, Masters M, Cowan NJ (1999) A single-ring mitochondrial chaperonin (Hsp60-Hsp10) can substitute for GroEL-GroES in vivo. J Bacteriol 181:5871–5875

Nishida N, Motojima F, Idota M, Fujikawa H, Yoshida M, Shimada I, Kato K (2006) Probing dynamics and conformational change of the GroEL-GroES complex by 13C NMR spectroscopy. J Biochem 140:591–598

Nojima T, Ikegami T, Taguchi H, Yoshida M (2012) Flexibility of GroES mobile loop is required for efficient chaperonin function. J Mol Biol 422:291–299

Numoto N, Kita A, Miki K (2005) Crystal structure of the Co-chaperonin Cpn10 from Thermus thermophilus HB8. Proteins 58:498–500

Ogawa J, Long SR (1995) The Rhizobium meliloti groELc locus is required for regulation of early nod genes by the transcription activator NodD. Genes Dev 9:714–729

Ogino H, Wachi M, Ishii A, Iwai N, Nishida T, Yamada S, Nagai K, Sugai M (2004) FtsZ-dependent localization of GroEL protein at possible division sites. Genes Cells 9:765–771

Ojha A, Anand M, Bhatt A, Kremer L, Jacobs WR Jr, Hatfull GF (2005) GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. Cell 123:861–873

Parnas A, Nisemblat S, Weiss C, Levy-Rimler G, Pri-Or A, Zor T, Lund PA, Bross P, Azem A (2012) Identification of elements that dictate the specificity of mitochondrial Hsp60 for its co-chaperonin. PloS one 7:e50318

Peng L, Fukao Y, Myouga F, Motohashi R, Shinozaki K, Shikanai T (2011) A chaperonin subunit with unique structures is essential for folding of a specific substrate. PLoS Biol 9:e1001040.

Phipps BM, Hoffmann A, Stetter KO, Baumeister W (1991) A novel ATPase complex selectively accumulated upon heat shock is a major cellular component of thermophilic archaebacteria. EMBO J 10:1711–1722

Qamra R, Mande SC (2004) Crystal structure of the 65-kilodalton heat shock protein, chaperonin 60.2, of Mycobacterium tuberculosis. J Bacteriol 186:8105–8113

Qamra R, Mande SC, Coates AR, Henderson B (2005) The unusual chaperonins of Mycobacterium tuberculosis. Tuberculosis 85:385–394

Quinn KA, Athanasas-Platsis S, Wong TY, Rolfe BE, Cavanagh AC, Morton H (1990) Monoclonal antibodies to early pregnancy factor perturb tumour cell growth. Clin Exp Immunol 80:100–108

Radwanska M, Magez S, Michel A, Stijlemans B, Geuskens M, Pays E (2000) Comparative analysis of antibody responses against HSP60, invariant surface glycoprotein 70, and variant surface glycoprotein reveals a complex antigen-specific pattern of immunoglobulin isotype switching during infection by Trypanosoma brucei. Infect Immun 68:848–860

Ranford JC, Henderson B (2002) Chaperonins in disease: mechanisms, models, and treatments. Mol Pathol 55:209–213

Ranson NA, Burston SG, Clarke AR (1997) Binding, encapsulation and ejection: substrate dynamics during a chaperonin-assisted folding reaction. J Mol Biol 266:656–664

Ranson NA, Farr GW, Roseman AM, Gowen B, Fenton WA, Horwich AL, Saibil HR (2001) ATP-bound states of GroEL captured by cryo-electron microscopy. Cell 107:869–879

Ranson NA, Clare DK, Farr GW, Houldershaw D, Horwich AL, Saibil HR (2006) Allosteric signaling of ATP hydrolysis in GroEL-GroES complexes. Nat Struct Mol Biol 13:147–152

Richardson A, Schwager F, Landry SJ, Georgopoulos C (2001) The importance of a mobile loop in regulating chaperonin/ co-chaperonin interaction: humans versus *Escherichia coli*. J Biol Chem 276:4981–4987

Roberts MM, Coker AR, Fossati G, Mascagni P, Coates AR, Wood SP (1999) Crystallization, x-ray diffraction and preliminary structure analysis of Mycobacterium tuberculosis chaperonin 10. Acta Crystallogra D Biol Crystallogr 55:910–914

Roberts MM, Coker AR, Fossati G, Mascagni P, Coates AR, Wood SP (2003) Mycobacterium tuberculosis chaperonin 10 heptamers self-associate through their biologically active loops. J Bacteriol 185:4172–4185

Roseman AM, Chen S, White H, Braig K, Saibil HR (1996) The chaperonin ATPase cycle: mechanism of allosteric switching and movements of substrate-binding domains in GroEL. Cell 87:241–251

Roseman AM, Ranson NA, Gowen B, Fuller SD, Saibil HR (2001) Structures of unliganded and ATP-bound states of the *Escherichia coli* chaperonin GroEL by cryoelectron microscopy. J Struct Biol 135:115–125

Rospert S, Glick BS, Jeno P, Schatz G, Todd MJ, Lorimer GH, Viitanen PV (1993) Identification and functional analysis of chaperonin 10, the groES homolog from yeast mitochondria. Proc Natl Acad Sci U S A 90:10967–10971

Ryabova NA, Marchenkov VV, Marchenkova SY, Kotova NV, Semisotnov GV (2013) Molecular chaperone GroEL/ES: unfolding and refolding processes. Biochemistry Biokhimiia 78:1405–1414

Rye HS, Burston SG, Fenton WA, Beechem JM, Xu Z, Sigler PB, Horwich AL (1997) Distinct actions of cis and trans ATP within the double ring of the chaperonin GroEL. Nature 388:792–798
Saibil H (1996) The lid that shapes the pot: structure and function of the chaperonin GroES. Structure 4:1–4
Saibil HR, Zheng D, Roseman AM, Hunter AS, Watson GM, Chen S, Auf Der Mauer A, O'Hara BP, Wood SP, Mann NH, Barnett LK, Ellis RJ (1993) ATP induces large quaternary rearrangements in a cage-like chaperonin structure. Curr Biol 3:265–273
Saibil HR, Fenton WA, Clare DK, Horwich AL (2013) Structure and allostery of the chaperonin GroEL. J Mol Biol 425:1476–1487
Sareen D, Sharma R, Vohra RM (2001) Chaperone-assisted overexpression of an active D-carbamoylase from Agrobacterium tumefaciens AM 10. Protein Expr Purif 23:374–379
Sato S, Wilson RJ (2005) Organelle-specific cochaperonins in apicomplexan parasites. Mol Biochem Parasitol 141:133–143
Schroda M (2004) The Chlamydomonas genome reveals its secrets: chaperone genes and the potential roles of their gene products in the chloroplast. Photosynth Res 82:221–240
Seale JW, Gorovits BM, Ybarra J, Horowitz PM (1996) Reversible oligomerization and denaturation of the chaperonin GroES. Biochemistry 35:4079–4083
Seo S, Baye LM, Schulz NP, Beck JS, Zhang Q, Slusarski DC, Sheffield VC (2010) BBS6, BBS10, and BBS12 form a complex with CCT/TRiC family chaperonins and mediate BBSome assembly. Proc Natl Acad Sci U S A 107:1488–1493
Sharkia R, Bonshtien AL, Mizrahi I, Weiss C, Niv A, Lustig A, Viitanen PV, Azem A (2003) On the oligomeric state of chloroplast chaperonin 10 and chaperonin 20. Biochim Biophys Acta 1651:76–84
Shimamura T, Koike-Takeshita A, Yokoyama K, Yoshida M, Taguchi H, Iwata S (2003) Crystallization of the chaperonin GroEL-GroES complex from Thermus thermophilus HB8. Acta Crystallogr D Biol Crystallogr 59:1632–1634
Shimamura T, Koike-Takeshita A, Yokoyama K, Masui R, Murai N, Yoshida M, Taguchi H, Iwata S (2004) Crystal structure of the native chaperonin complex from Thermus thermophilus revealed unexpected asymmetry at the cis-cavity. Structure 12:1471–1480
Shtilerman M, Lorimer GH, Englander SW (1999) Chaperonin function: folding by forced unfolding. Science 284:822–825
Sigler PB, Xu Z, Rye HS, Burston SG, Fenton WA, Horwich AL (1998) Structure and function in GroEL-mediated protein folding. Annu Rev Biochem 67:581–608
Sirur A, Best RB (2013) Effects of interactions with the GroEL cavity on protein folding rates. Biophys J 104:1098–1106
Sparrer H, Buchner J (1997) How GroES regulates binding of nonnative protein to GroEL. J Biol Chem 272:14080–14086
Sparrer H, Rutkat K, Buchner J (1997) Catalysis of protein folding by symmetric chaperone complexes. Proc Natl Acad Sci U S A 94:1096–1100
Spiess C, Meyer AS, Reissmann S, Frydman J (2004) Mechanism of the eukaryotic chaperonin: protein folding in the chamber of secrets. Trends Cell Biol 14:598–604
Stirling PC, Bakhoum SF, Feigl AB, Leroux MR (2006) Convergent evolution of clamp-like binding sites in diverse chaperones. Nature Struct Mol Biol 13:865–870
Stoldt V, Rademacher F, Kehren V, Ernst JF, Pearce DA, Sherman F (1996) Review: the Cct eukaryotic chaperonin subunits of Saccharomyces cerevisiae and other yeasts. Yeast 12:523–529
Suzuki K, Nakanishi H, Bower J, Yoder DW, Osteryoung KW, Miyagishima SY (2009) Plastid chaperonin proteins Cpn60 alpha and Cpn60 beta are required for plastid division in Arabidopsis thaliana. BMC Plant Biol 9:38
Taguchi H, Makino Y, Yoshida M (1994) Monomeric chaperonin-60 and its 50-kDa fragment possess the ability to interact with non-native proteins, to suppress aggregation, and to promote protein folding. J Biol Chem 269:8529–8534
Taneja B, Mande SC (2001) Metal ions modulate the plastic nature of Mycobacterium tuberculosis chaperonin-10. Protein Eng 14:391–395

Taneja B, Mande SC (2002) Structure of Mycobacterium tuberculosis chaperonin-10 at 3.5 A resolution. Acta Crystallogr Section D, Biol Crystallogr 58:260–266
Tazir Y, Steisslinger V, Soblik H, Younis AE, Beckmann S, Grevelding CG, Steen H, Brattig NW, Erttmann KD (2009) Molecular and functional characterisation of the heat shock protein 10 of Strongyloides ratti. Mol Biochem Parasitol 168:149–157
Techtmann SM, Robb FT (2010) Archaeal-like chaperonins in bacteria. Proc Natl Acad Sci U S A 107:20269–20274
Thulasiraman V, Yang CF, Frydman J (1999) In vivo newly translated polypeptides are sequestered in a protected folding environment. EMBO J 18:85–95
Todd MJ, Viitanen PV, Lorimer GH (1993) Hydrolysis of adenosine 5'-triphosphate by *Escherichia coli* GroEL: effects of GroES and potassium ion. Biochemistry 32:8560–8567
Todd MJ, Viitanen PV, Lorimer GH (1994) Dynamics of the chaperonin ATPase cycle: implications for facilitated protein folding. Science 265:659–666
Trent JD, Nimmesgern E, Wall JS, Hartl FU, Horwich AL (1991) A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex polypeptide-1. Nature 354:490–493
Tsai YC, Mueller-Cajar O, Saschenbrecker S, Hartl FU, Hayer-Hartl M (2012) Chaperonin cofactors, Cpn10 and Cpn20, of green algae and plants function as hetero-oligomeric ring complexes. J Biol Chem 287:20471–20481
Tsuprun VL, Boekema EJ, Samsonidze TG, Pushkin AV (1991) Electron microscopy of the complexes of ribulose-1,5-bisphosphate carboxylase (Rubisco) and Rubisco subunit-binding protein from pea leaves. FEBS Lett 289:205–209
Tyagi NK, Fenton WA, Horwich AL (2009) GroEL/GroES cycling: ATP binds to an open ring before substrate protein favoring protein binding and production of the native state. Proc Natl Acad Sci U S A 106:20264–20269
Vabulas RM, Raychaudhuri S, Hayer-Hartl M, Hartl FU (2010) Protein folding in the cytoplasm and the heat shock response. Cold Spring Harb Perspect Biol 2:a004390
Vainberg IE, Lewis SA, Rommelaere H, Ampe C, Vandekerckhove J, Klein HL, Cowan NJ (1998) Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. Cell 93:863–873
van der Vies SM, Gatenby AA, Georgopoulos C (1994) Bacteriophage T4 encodes a co-chaperonin that can substitute for *Escherichia coli* GroES in protein folding. Nature 368:654–656
van Eden W, van der Zee R, Taams LS, Prakken AB, van Roon J, Wauben MH (1998) Heat-shock protein T-cell epitopes trigger a spreading regulatory control in a diversified arthritogenic T-cell response. Immunol Rev 164:169–174
Vanags D, Williams B, Johnson B, Hall S, Nash P, Taylor A, Weiss J, Feeney D (2006) Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. Lancet 368:855–863
Vitlin Gruber A, Nisemblat S, Azem A, Weiss C (2013a) The complexity of chloroplast chaperonins. Trends Plant Sci 18:688–694
Vitlin Gruber A, Nisemblat S, Zizelski G, Parnas A, Dzikowski R, Azem A, Weiss C (2013b) P. falciparum cpn20 is a bona fide co-chaperonin that can replace GroES in *E. coli*. PloS one 8:e53909
Wang S, Tan A, Lv J, Wang P, Yin X, Chen Y (2012) Soluble expression of recombinant human CD137 ligand in *Escherichia coli* by co-expression of chaperones. J Ind Microbiol Biotechnol 39:471–476
Wang Y, Zhang WY, Zhang Z, Li J, Li ZF, Tan ZG, Zhang TT, Wu ZH, Liu H, Li YZ (2013) Mechanisms involved in the functional divergence of duplicated GroEL chaperonins in Myxococcus xanthus DK1622. PLoS Genet 9:e1003306
Weissman JS, Hohl CM, Kovalenko O, Kashi Y, Chen S, Braig K, Saibil HR, Fenton WA, Horwich AL (1995) Mechanism of GroEL action: productive release of polypeptide from a sequestered position under GroES. Cell 83:577–587
Wick G (2006) The heat is on: heat-shock proteins and atherosclerosis. Circulation 114:870–872

Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, Cassady R, Tawa P, Ruel R, Rosen A, Nicholson DW (1999) Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. The EMBO J 18:2049–2056

Xu Z, Horwich AL, Sigler PB (1997) The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex. Nature 388:741–750

Xu Z, Sigler PB (1998) GroEL/GroES: structure and function of a two-stroke folding machine. J Struct Biol 124:129–141

Xu XM, Wang J, Xuan Z, Goldshmidt A, Borrill PG, Hariharan N, Kim JY, Jackson D (2011) Chaperonins facilitate KNOTTED1 cell-to-cell trafficking and stem cell function. Science 333:1141–1144

Yifrach O, Horovitz A (1995) Nested cooperativity in the ATPase activity of the oligomeric chaperonin GroEL. Biochemistry 34:5303–5308

Zamora-Veyl FB, Kroemer M, Zander D, Clos J (2005) Stage-specific expression of the mitochondrial co-chaperonin of *Leishmania donovani*, CPN10. Kinetoplastid Biol Dis 4:3

Zeilstra-Ryalls J, Fayet O, Georgopoulos C (1994) Two classes of extragenic suppressor mutations identify functionally distinct regions of the GroEL chaperone of *Escherichia coli*. J Bacteriol 176:6558–6565

Chapter 9
Co-chaperones of the Mammalian Endoplasmic Reticulum

Armin Melnyk, Heiko Rieger and Richard Zimmermann

Abstract In mammalian cells, the rough endoplasmic reticulum or ER plays a central role in the biogenesis of most extracellular plus many organellar proteins and in cellular calcium homeostasis. Therefore, this organelle comprises molecular chaperones that are involved in import, folding/assembly, export, and degradation of polypeptides in millimolar concentrations. In addition, there are calcium channels/pumps and signal transduction components present in the ER membrane that affect and are affected by these processes. The ER lumenal Hsp70, termed immunoglobulin-heavy chain binding protein or BiP, is the central player in all these activities and involves up to seven different co-chaperones, i.e. ER-membrane integrated as well as ER-lumenal Hsp40s, which are termed ERj or ERdj, and two nucleotide exchange factors.

Keywords Human endoplasmic reticulum · Cellular calcium hoemostasis · Protein transport · Protein folding · Protein degradation

Introduction

In all nucleated human cells the endoplasmic reticulum or ER forms a vast and dynamic membrane network (Palade 1975; English and Voeltz 2013). The rough ER is studded with 80S ribosomes. These ribosomes are engaged in the biosynthesis of most secretory and many organellar proteins by cotranslationally inserting nascent polypeptides into the membrane and lumen of the ER, thus defining one major function of the rough ER. The peripheral ER contacts the plasma membrane, the tubular ER contacts mitochondria (Kornmann et al. 2009; Hayashi et al. 2009; Bakowski

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_9

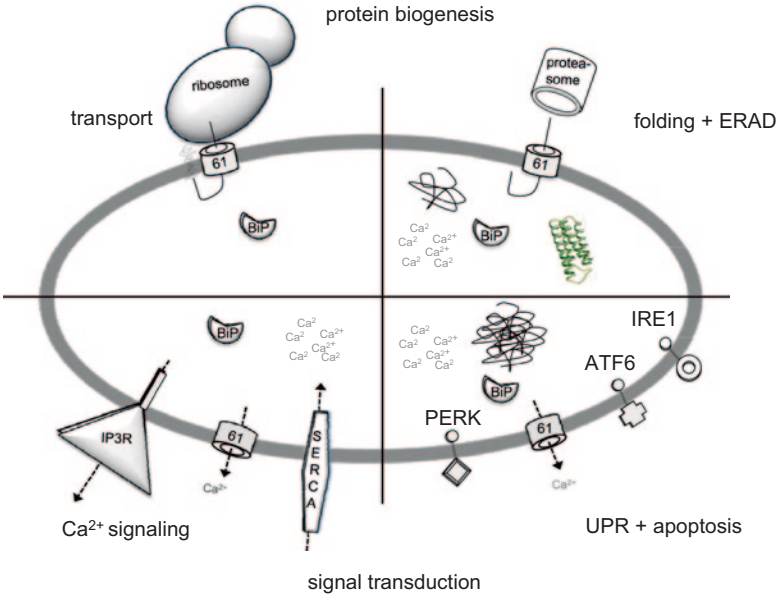

Fig. 9.1 Cross section through the ER, highlighting the central role of Sec61 complex and BiP in protein biogenesis and calcium homeostasis in human cells. *ERAD* ER-associated protein degradation, *SERCA* sarcoplasmic endoplasmic reticulum calcium ATPases, *UPR* unfolded protein response

et al. 2012). These contacts play important roles in cellular calcium homeostasis, thus defining another major function of the mammalian ER. In addition, the ER membrane forms a continuum with the outer nuclear envelope membrane.

Protein translocation into the ER is the first step in the biogenesis of many proteins of eukaryotic cells (such as proteins of the ER, ERGIC, Golgi apparatus, endosome, lysosome, nucleus, peroxisome, plasma membrane) as well as of most extracellular proteins (Fig. 9.1, "transport") (Blobel and Dobberstein 1975a, b). Typically, protein translocation into the ER involves amino-terminal signal peptides in the precursor polypeptides and a complex machinery of transport components, most notably the heterotrimeric Sec61 complex in the ER-membrane and the ER-lumenal Hsp70-type molecular chaperone BiP and its co-chaperones plus nucleotide exchange factors or NEFs.

Protein transport into the ER is followed by folding and assembly of the newly imported polypeptides (Fig. 9.1, "folding"). Typically, this folding and assembly of proteins involve some of the above-mentioned components, such as the calcium-dependent chaperone BiP and its co-chaperones plus NEFs (Haas and Wabl 1983; Bole et al. 1986; Weitzmann et al. 2007; Zahedi et al. 2009; Bulleid 2012). Except for resident proteins of the ER, the native proteins are delivered to their functional location by vesicular transport (Schekman 2004, 2005; Sambrook 1990; Pelham 1990).

In cases of mis-folding or mis-assembly of polypeptides in the ER membrane or lumen, the polypeptides are exported to the cytosol and degraded by the proteasome

(Fig. 9.1, "ERAD") (Smith et al. 2011; Bagola et al. 2011; Thibault and Ng 2012; Olzmann et al. 2012). Export of mis-folded polypeptides from the ER lumen to the cytosol can also involve some of the above-mentioned components, such as the Sec61 complex and BiP and its co-chaperones (Pilon et al. 1997; Plemper et al. 1997; Schäfer and Wolf 2009).

When protein mis-folding or mis-assembly prevail, a complex signal transduction pathway is activated and leads to an increase of the folding and degradation capacity of the ER and to a decrease of global protein synthesis (Fig. 9.1, "UPR") (Gardner et al. 2013; Ron and Harding 2012; Ma and Hendershot 2001; Schröder and Kaufman 2005). In mammals, UPR involves the three ER membrane proteins PERK, ATF6 and IRE1, respectively. These proteins comprise lumenal domains, which are not structurally related to J-domains, that interact with BiP and cytosolic domains that attenuate global translation (PERK) or induce selective transcription (ATF6, IRE1) in the absence of BiP.

When the protein mis-folding problem persists, however, the programmed cell death pathway or apoptosis is activated in the respective cell to protect the organism (Fig. 9.1, "apoptosis) (Madeo and Kroemer 2009; Tabas and Ron 2011). This switch involves efflux of calcium ions (Ca^{2+}) from the ER. Indirect evidence from various laboratories has first suggested that the Sec61 complex may transiently contribute to the ER Ca^{2+}leak after completion of protein translocation (Lomax et al. 2002; van Coppenolle et al. 2004; Flourakis et al. 2006; Giunti et al. 2007; Ong et al. 2007; Lang et al. 2011). Recently, this concept was confirmed by the observations that the open Sec61 complex is indeed Ca^{2+}permeable and that silencing the *SEC61A1* gene in HeLa cells prevents the Ca^{2+}leakage linked to completion of protein translocation (Lang et al. 2011; Erdmann et al. 2011; Schäuble et al. 2012). Under physiological conditions, BiP and its co-chaperones are involved in limiting Sec61 complex-mediated Ca^{2+}leakage or passive Ca^{2+}efflux. Therefore, it is tempting to speculate that the intrinsic Ca^{2+}permeability of the Sec61 complex and its regulation by BiP play an important role at the interface between protein biogenesis and Ca^{2+}homeostasis in mammalian cells (summarized in Fig. 9.1). Since the more than thousand-fold Ca^{2+}gradient between ER lumen and cytosol allows $Ca^{2+}$to play its central role as a second messenger in cellular signaling (Berridge 2002; Rizzuto and Pozzan 2006), it is the function of the sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) to counteract both the receptor-mediated Ca^{2+}release and the Ca^{2+}leakage from the ER in order to maintain the Ca^{2+}gradient of the resting cell (Wuytack et al. 2002).

The Chaperone Network of the ER

Both the yeast and the mammalian ER contain molecular chaperones and folding catalysts in millimolar concentrations (Van et al. 1989; Bies et al. 1999; Weitzmann et al. 2007). Many of these molecular chaperones belong to the classical Hsp40, Hsp70, and Hsp90 protein families (Table 9.1, Fig. 9.2). However, the ER also

Table 9.1 BiP and its interaction partners in the mammalian ER

Function	Protein (synonym)	Related human disease	OMIM	Animal model	First reference
Hsp70-type chaperone	BiP (Grp78, HspA5)	Haemolytic uraemic syndrome	235400	Embryonic lethality or surfactant deficiency	Haas and Wabl (1983)
Hsp40-type co-chaperones	ERj1 (Htj1, DNAJC1)				Brightman et al. (1995)
	ERj2 (Sec63, ERdj2)	Polycystic liver disease colorectal cancer	174050	Embryonic lethality	Skowronek et al. (1999)
	ERj3 (ERdj3, DnaJB11, HEDJ, Dj9)				Bies et al. (1999)
	ERj4 (ERdj4, DnaJB9, MDG1)			Postnatal lethality (surfactant deficiency)	Shen et al. (2002)
	ERj5 (ERdj5, DnaJC10, JPDI)			No phenotype	Hosoda et al. (2003); Cunnea et al. (2003)
	ERj6 ($p58^{IPK}$, DnaJC3, ERdj6)			Diabetic mouse	Rutkowski et al. (2007)
	ERj7 (Gng10, DnaJC25, ERdj7)				Zahedi et al. (2009)
Nucleotide exchange factors	Grp170 (ORP150, HYOU1)			Embryonic lethality	Lin et al. (1993)
	Sil1 (BAP)	Marinesco-Sjögren syndrome	248800	Woozy mouse	Chung et al. (2002)
Additional co-chaperones	Sig-1R (sigma-1 receptor)				Hayashi and Su 2007
	HspA5BP1 (GBP)				Oh-hashi et al. (2003)
Additional chaperones	Grp94 (CaBP4, ERp99, gp96, endoplasmin)			Embryonic lethality	Shiu et al. (1977)
	Calnexin (IP90, p88)			Postnatal lethality	Degen and Williams (1991)
	Calreticulin (CaBP3, ERp60)			Embryonic lethality	Burns et al. (1992)

Table 9.1 (continued)

Function	Protein (synonym)	Related human disease	OMIM	Animal model	First reference
UPR signal transducers	IRE1α/β (ERN1/2)				Tirasophon et al. (1998)
	IRE2				Wang et al. (1998)
	ATF6α/β				Yoshida et al. (1998)
	PERK (EIF2AK3, PEK)	Wolcott-Rallison syndrome breast cancer	226980	Diabetic mouse	Shi et al. (1998); Harding et al. (1999)
Sec proteins	Sec61α1			Diabetic mouse	Görlich et al. (1992)
	Sec61β				Hartmann et al. (1994)
	Sec61γ	Glioblastoma			
	Sec62 (TLOC1)	Prostate/lung/thyroid cancer			Mayer et al. (2000); Tyedmers et al. (2000)

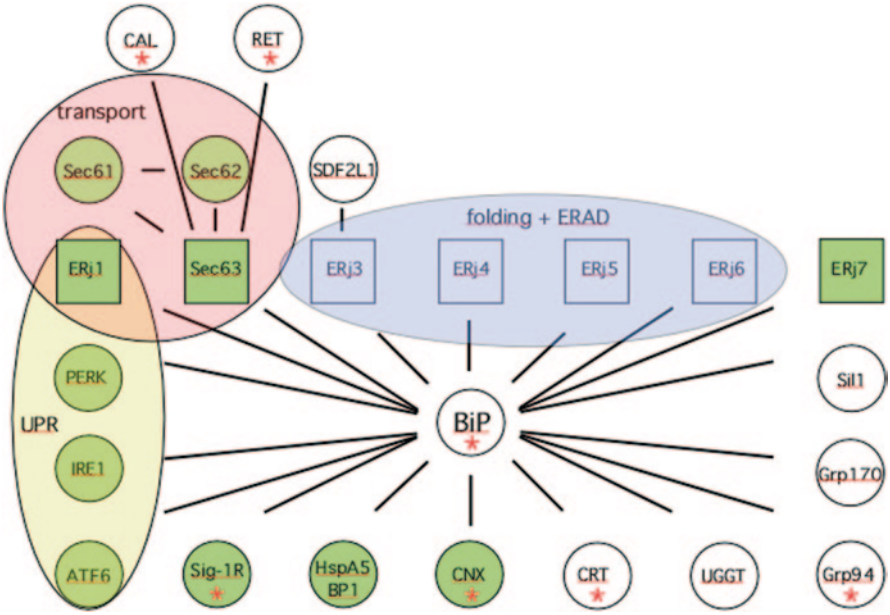

Fig. 9.2 Interaction partners of BiP that are involved in protein biogenesis and calcium homeostasis. The proteins that are involved in protein transport, folding, *ERAD*, and *UPR* are indicated, all other proteins are involved in protein folding or calcium homeostasis (*red asterisk*). Membrane proteins are depicted in green; ER-lumenal Hsp40s are represented as *squares*, all other proteins as *circles*

comprises a special class of molecular chaperones or lectins that are dedicated to the folding of glycoproteins. The mammalian ER, contains a soluble (calreticulin or CRT) as well as a membrane integrated (calnexin or CNX) lectin (Degen and Williams 1991; Burns et al. 1992). The folding catalysts of the ER deal with either the formation of disulfide bonds (protein disulfide isomerases or PDI) or the isomerization of proline-containing peptide bonds (peptidyprolyl-*cis/trans*-isomerases or PPIase). The PPIases belong to either the cyclosporin A- or the FK506-sensitive protein family (cylophilin or FK506-binding protein). All these chaperones and folding catalysts have been observed to be present in larger complexes in various combinations (Tatu and Helenius 1997; Meunier et al. 2002).

The Hsp70/Hsp40 Network of the ER

Just like the bacterial cytosol or the mitochondrial matrix, the ER contains the typical Hsp70 triad, comprising the Hsp70 itself (BiP in mammals) as well as a Hsp40-type co-chaperone, which stimulates the ATPase activity of BiP, and a NEF, which catalyzes the exchange of ADP for ATP (Tables 9.1 and 9.2, Fig. 9.3). These proteins have also been shown to be able to perform the classical Hsp70 reaction cycle, thereby mediating the folding and assembly of newly-synthesized and –imported polypeptides. Similarly to the two above-mentioned cellular compartments, there are two Hsp70-type chaperones in both the yeast as well as the mammalian ER (Haas and Wabl 1983; Bole et al. 1986; Munro and Pelham 1986; Weitzmann et al. 2007; Mimura et al. 2007; Luo et al. 2006). One of these, however, may also be referred to as a Hsp110 protein family member (Grp170 in mammals) and serves as a NEF for BiP (Lin et al. 1993; Kitao et al. 2004; Weitzmann et al. 2006). There also seems to be a *bona fide* functional homolog to bacterial GrpE in the ER lumen (BAP or Sil1 in mammals) (Chung et al. 2002; Zhao et al. 2005, 2010), i.e. there is redundancy at the level of the NEFs, which may explain the non-lethal phenotype of loss of Sil1 function that is associated with the neurodegenerative disease, Marinesco-Sjögren syndrome (Table 9.1, see below). The structures of the two cytosolic paralogs of the two NEFs were recently solved and revealed distinct interacting surfaces with the top of the nucleotide-binding domain (NBD) of BiP (Shomura et al. 2005; Polier et al. 2008); thus, the NEF binding sites on Hsp70 are different from the J-domain binding site, which resides at the NBD bottom. Based on these structural data, the two NEFs may even be able to bind simultaneously to BiP.

There may be up to nine different Hsp40 type molecular chaperones present in the human ER, although not necessarily simultaneously in the same cell (Tables 9.1 and 9.2, Fig. 9.3). To date, seven of these have been characterized in some detail and were termed ERj1 through ERj7 (or ERdj). The two additional candidates for ERj proteins are DnaJC14 or HDJ3 and DnaJC16, the latter also containing two thioredoxin domains. The Hsp40-type co-chaperones in the ER can be divided into membrane proteins with a lumenal J-domain and into lumenal proteins (Fig. 9.3). Furthermore, they can be classified according to the domains they have in common

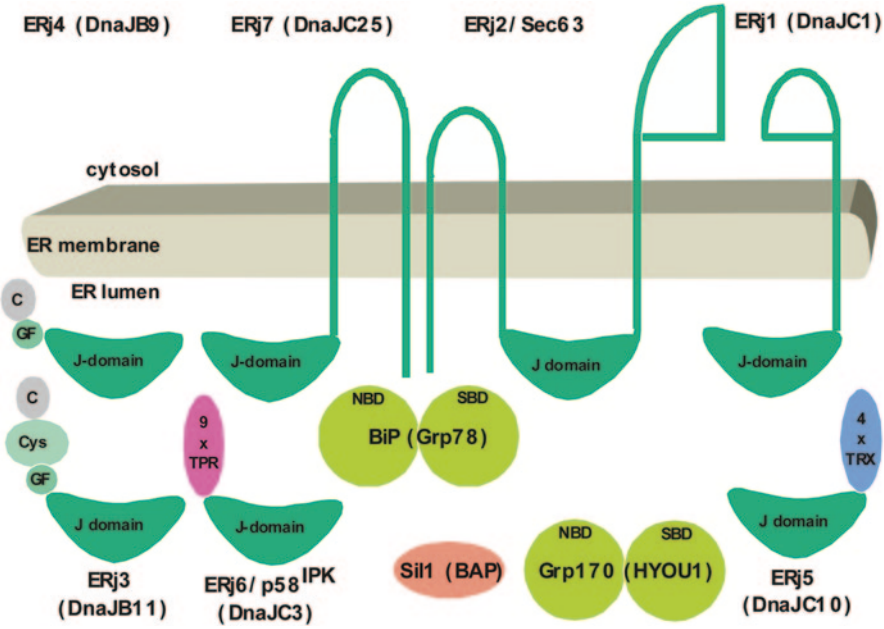

Fig. 9.3 Topology and domain organisation of BiP and its co-chaperones and nucleotide exchange factors. *C*, carboxy-terminal substrate binding domain, *Cys* cysteine-repeat domain, *GF* glycine-phenylalanine rich domain, *NBD* nucleotide binding domain, *SBD* substrate binding domain, *TPR* tetratricopeptide repeat, *TRX* thioredoxin domain. We note that ERj1 and Sec63 both comprise large cytosolic domains that are structurally un-related. In the case of ERj1, this domain is involved in ribosome binding; (Blau et al. 2005; Dudek et al. 2005) (Fig. 9.6), the cytoslic domain of Sec63 is structurally related to certain helicases (Pena et al. 2009) and is involved in interaction with Sec62 (Müller et al. 2010) (Fig. 9.5)

with the bacterial DnaJ protein (i.e. besides the actual J-domain) (Hennessy et al. 2005). Type I Hsp40s contain four domains: an amino-terminal J-domain, a glycine-phenylalanine (G/F) rich domain, a Zn-finger- or cysteine repeat-domain, and a carboxy-terminal substrate binding domain. Type II Hsp40s contain three domains: an amino-terminal J-domain, a glycine-phenylalanine (G/F) rich domain, and a carboxy-terminal substrate binding domain. Type III Hsp40s contain only the J-domain and, in general, have more specialized functions compared to type I and II Hsp40s. Thus, only the type I and II ER-lumenal Hsp40s, ERj3 (Bies et al. 1999, 2004; Yu et al. 2000; Shen and Hendershot 2005; Jin et al. 2008, 2009) and ERj4 (Shen et al. 2002 Kurisu et al. 2003; Dong et al. 2008; Lai et al. 2012; Fritz et al. 2014), have the ability to bind substrate polypeptides and deliver them to BiP, that is, to facilitate polypeptide folding, analogous to the paradigm of Hsp40, the DnaJ in *E. coli*. However, the four thioredoxin domains within ERj5 (Cunnea et al. 2003; Hosoda et al. 2003; Dong et al. 2008; Ushioda et al. 2008; Ladiges et al. 2005; Hagiwara et al. 2011; Oka et al. 2013) and the tetratricopeptide repeat (TPR) domains in ERj6 ($p58^{IPK}$) (Kang et al. 2006; Rutkowski et al. 2007; Petrova et al. 2008;

Table 9.2 Properties of BiP and its co-chaperones and NEFs. We note that the given concentrations refer to a suspension of rough microsomes, which was isolated from canine pancreas and adjusted to a concentration of 1 equivalent/µl. In the ER lumen, the concentrations are approximately thousand-fold higher. The data were taken from Weitzmann et al. 2007; Zahedi et al. 2009). *GST* glutathione-S-transferase

Protein	UPR controlled	Cellular function(s)	Concentration in suspension of RM (µM)	Recombinant protein (amino acid residues)	Rate constants for interaction with BiP in the presence of ATP	
					k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})
BiP	+	ERAD, folding, Sec61-gating, transport, UPR	5.00	BiP-Hexahis (20-655)	–	–
ERj1	–	Unknown	0.36	GST-J-domain (44-140)	6.00×10^3	2.60×10^{-3}
ERj2	–	Transport	1.98	GST-J-domain (91-189)	0.81×10^3	2.60×10^{-3}
ERj3	+	ERAD, folding	0.29	GST-ERj3 (18-336)	1.25×10^3	3.60×10^{-3}
ERj4	+++	ERAD, folding	Not detectable	GST-ERj4 (23-222)		
ERj5	+	ERAD, folding	2.00	GST-ERj5 (26-793)	6.20×10^3	2.80×10^{-3}
ERj6	+	ERAD, folding	Not determined	GST-ERj6 (32-504)	64.4	3.97×10^{-3}
ERj7	+	Unknown	2.30	GST-J-domain (39-149)	5.07×10^3	5.70×10^{-3}
Grp170	+	Folding, NEF	0.60	–	Not determined	
Sil1	–	NEF	0.005	GST-39-461	Not detectable	

Dong et al. 2008; Svard et al. 2011) may also play a role in substrate binding. Thus, ERj3 through ERj6 are involved in protein folding under physiological as well as stress conditions and in ERAD (Table 9.2, Fig. 9.2). This is consistent with the fact that these four BiP co-chaperones are over-produced together with BiP under stress conditions, i.e. when there is an increased demand for chaperone and degradation activity towards mis-folded polypeptides (Table 9.2). Therfore, it is not surprising that these members of the resident ER Hsp70-cycle have been found in large complexes with each other, with other chaperones and folding catalysts, and with other resident ER proteins that are involved in N- or O-glycosylation (UDP-glucose-glycoprotein-glycosyltransferase or UGGT, SDF2L1) and calcium homeostasis (calumenin, reticulocalbin), respectively (Fig. 9.2).

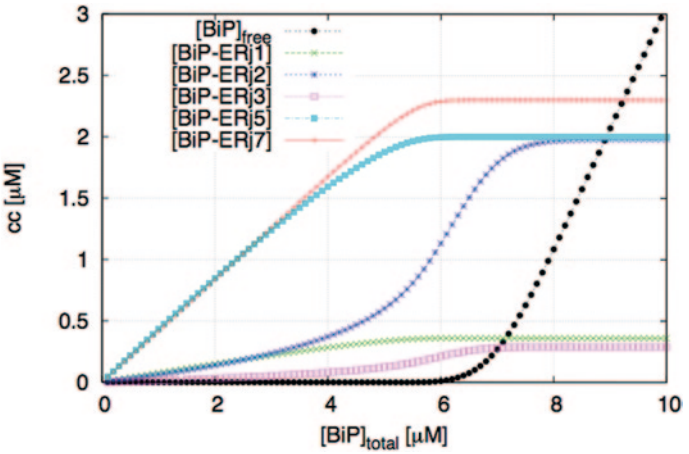

Fig. 9.4 Equilibrium concentrations for (free) BiP and reaction products BiP-ERjX (X = 1,2,3,5,7) as a function of the initial concentration of BiP as calculated numerically with the reaction equations, shown below, and using the experimentally determined rate constants k_a and k_d and initial concentrations [ERjX] in rough microsomes from canine pancreas (Table 9.2). The time evolution of the concentrations is then given by a coupled set of ordinary differential equations:

$$\frac{d}{dt}[BiP] = \sum_{X=1}^{7}\left\{k_d^{(X)}\,[BiP-ERjX] - k_a^{(X)}[BiP]\cdot[ERjX]\right\},$$

and

$$\frac{d}{dt}[ERjX] = k_d^{(X)}[BiP-ERjX] - k_a^{(X)}[BiP]\cdot[ERjX],$$

$$\frac{d}{dt}[BiP-ERjX] = -k_d^{(X)}[BiP-ERjX] + k_a^{(X)}[BiP]\cdot[ERjX],$$

where [BiP], [ERjX], and [BiP–ERjX] denote the concentrations of BiP, ERjX (X = 1,2,…,7), and [BiP–ERjX], respectively. Due to the lack of data we set [ERj6] and [BiP-ERj6] constant to zero. Using the measured values for the initial concentrations [ERjX](t = 0) and the rate constants k_a and k_d from Table 9.1 we solved the above differential equations numerically for various initial concentrations [BiP](t = 0) and zero initial concentrations of the reaction products [BiP-ERjX](t = 0). In Fig. 9.1 we show the results of the stationary (equilibrium) concentrations of BiP and the reaction products, $[BiP]_{eq}$ and $[BiP\text{-}ERjX]_{eq}$, respectively, as a function of the initial BiP concentration [BiP](t = 0)—which is equal to the total BiP concentration $[BiP]_{total}$, since [BiP–ERjX](t = 0) is zero for X = 1,…7

In Fig. 9.4, we have modelled the equlibrium concentrations of free BiP and complexes of BiP with its co-chaperones for canine pancreatic microsomes, based on the determined concentrations of the various proteins and the rate constants for their interacttion with BiP (Table 9.2). The complexes are formed transiently in order to stimulate the ATPase activity of BiP, thus creating the form of BiP with high substrate affinity. Typically, the ER lumenal concentrations of BiP are in the millimolar range and similar to the total concentration of ERjs (Weitzmann et al. 2007). The model illustrates that under normal conditions there is enough BiP available for interaction with all ERjs and that under conditions of UPR induction, where BiP and ERj3 through ERj6 are over-produced, BiP becomes limiting for ERj2, thus, selectively preventing import of additional precursor polypeptides. This can be deduced

from the observation that complex formation between BiP and ERj2 requires much higher concentrations of BiP as compared to complex formation between BiP and e.g. ERj5 or ERj7.

The Putative Role of BiP and its Co-chaperones in Protein Transport into the ER as an Example of Chaperone/Co-chaperone Action in the Mammalian ER

The structure of the Sec61 complex suggests a potential mechanism for BiP-mediated gating, i.e. opening and closing, of the Sec61 channel (Figs. 9.1 and 9.5) (Pfeffer et al. 2012, 2014; Zimmermann et al. 2011). We suggest that the ribosome

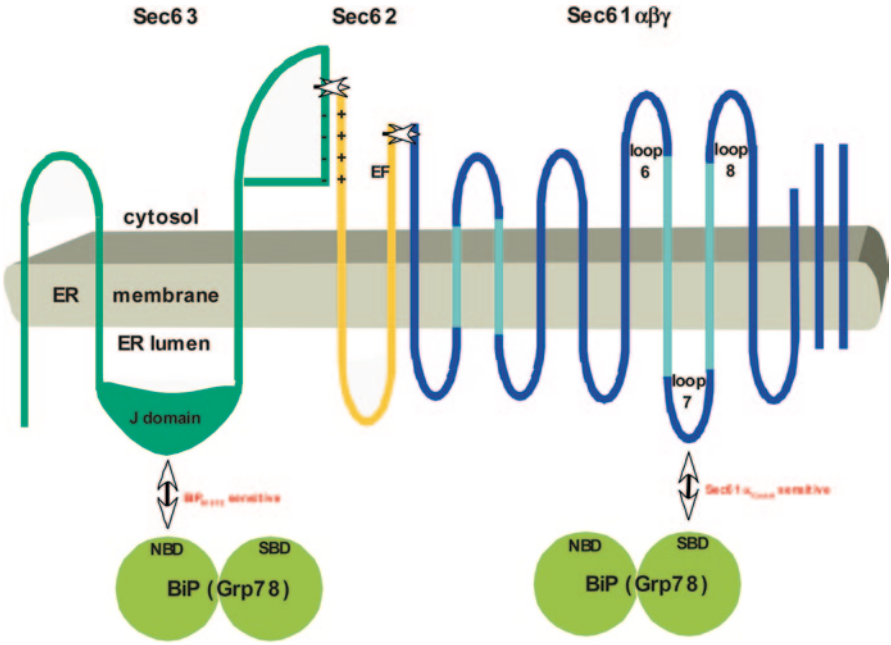

Fig. 9.5 Protein-protein interactions that are involved in gating of the Sec61-complex in the human ER membrane. The shown interactions of BiP with Sec61α (Schäuble et al. 2012), Sec62 with Sec61α (Linxweiler et al. 2013) and Sec62 with Sec63 (Müller et al. 2010) as well as their sensitivities to mutations were previously described. The BiP-Sec63 interaction was described by Tyedmers et al. (2000) and the effect of the R197E mutation by Awad et al. (2008). So far, the latter interaction as well as the Sec62-Sec63 interaction were found to be relevant only for protein transport into the ER, i.e. gating of the Sec61 complex from the closed to the open conformation; in contrast, the BiP co-chaperone for gating to the closed state is still elusive. Interactions are indicated by *arrows*, the transmembrane helices that form the lateral gate are shown in *light blue*, the cytosolic and ER luminal loops, which form the binding sites for ribosomes and BiP, respectively, are indicated. *NBD* nucleotide binding domain, *SBD* substrate binding domain

in cotranslational transport and the Sec62/Sec63 complex in posttranslational transport can prime the closed Sec61 complex for opening (Lang et al. 2012). The current view on opening of the Sec61 complex in protein translocation, i.e. channel gating from the closed to the open conformation, is that signal peptides of nascent presecretory polypeptides intercalate between the Sec61α transmembrane (tm) helices 2 and 7, opening the lateral gate of the Sec61 complex that these two tm helices form (van den Berg et al. 2004; Gumbart and Schulten 2007). It has been proposed that the minihelix within loop 7 plays a role in gating of the Sec61 complex from closed to open and that BiP binding to this minihelix may be required for gating from the closed state to the open state in the case of some precursor polypeptides, while others may be able to trigger gating on their own (Schäuble et al. 2012). Here, BiP binds the native Sec61α as a substrate and facilitates its conformational change. At this point of translocation, the nascent precursor polypeptide chain can be fully inserted into the Sec61 complex and initiate translocation. Next, BiP binds to the precursor polypeptide in transit and acts as a molecular ratchet, thus mediating completion of translocation (Nicchitta and Blobel 1993; Tyedmers et al. 2003; Shaffer et al. 2005). Here, BiP binds the non-native precursor polypeptide as a substrate and prevents it from sliding back into the cytosol. Subsequently, i.e. in the absence of a precursor polypeptide in transit, binding of BiP to loop 7 can facilitate closing of the Sec61 channel to limit ion efflux from the ER (Schäuble et al. 2012). We find this view attractive, because loop 7 connects tm helices 7 and 8, and is thus close enough to the lateral gate to influence gate movements. Interestingly, mutation of tyrosine 344 to histidine within the minihelix of loop 7 leads to diabetes in mice (Lloyd et al. 2010).

There is no doubt that the physical and mechanistic link between the Sec61- and the BiP-reaction cycles is most efficiently provided by a membrane integrated Hsp40 with a lumenal J-domain. Indeed in yeast, Sec63p has been shown to provide the lumenal J-domain that allows Kar2p (BiP in yeast) to play its roles in insertion of precursors into the Sec61 complex as well as in completion of translocation (Lyman and Schekman 1995, 1997). Since in pancreatic microsomes Sec63 or ERj2 was found in association with the Sec61 complex and to be present in approximately stoichiometric amounts as compared to heterotrimeric Sec61 complexes, we expect mammalian Sec63 to play a similar role, i.e. recruit BiP to the Sec61 complex and stimulate ATPase activity of BiP for conversion to the high substrate affinity (Mayer et al. 2000; Tyedmers et al. 2000; Pena et al. 2009; Lang et al. 2012). However, it remains open, whether or not a single BiP molecule can first bind loop 7 of Sec61α and, subsequently, the incoming precursor polypeptide within one functional cycle (Schlecht et al. 2011). Interestingly, it has been shown that human ERj1 can complement the otherwise lethal deletion of Sec63p in yeast (Kroczynska et al. 2004). Therefore, ERj1 may play a similar role as Sec63 in the mammalian ER, thereby providing at least partial redundancy for this essential function that may explain the non-lethal phenotype of loss of Sec63 function, associated with polycystic liver disease (Table 9.1, see below). ERj1 was observed in association with translating ribosomes (Fig. 9.6; Dudek et al. 2002, 2005; Blau et al. 2005; Benedix et al. 2010). Therefore, we propose that in the mammalian ER two different membrane

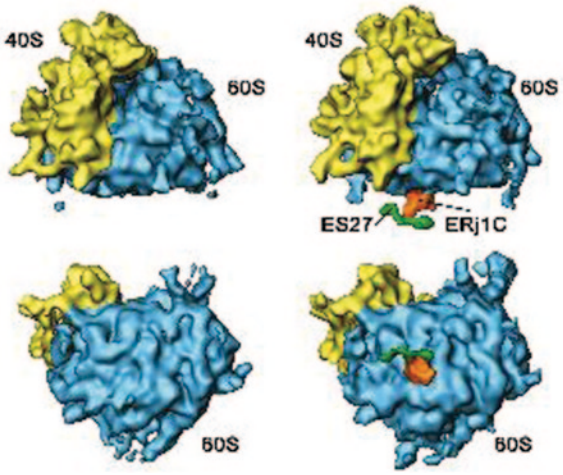

Fig. 9.6 ERj1's ribosomal contacts, overall position and conformational changes. Cryo-EM map of the dog pancreas 80S ribosome at a resolution of 23 Å. Left side: Yellow indicates the small (40S) ribosomal subunit, blue indicates the large (60S) subunit (Blau et al. 2005; Dudek et al. 2005). Top, side view; bottom, rotated 90° backwards, exposing the membrane attachment side of the ribosome. Right side: Cryo-EM map of the 80S ribosome- ERj1C complex at a resolution of 20 Å. ERj1C refers to the cytoslic domain of ERj1. Orange and green indicate the densities for ERj1C and the expansion segment 27 or ES27, respectively

proteins provide J-domains in the neighborhood of translating ribosomes and Sec61 complexes and allow BiP to play its roles in protein import. In addition, ERj1 appears to have regulatory roles that are related to transcription as well as to translation. The cytosolic domain of ERj1 has the ability to allosterically inhibit translation at the stage of initiation when it is not bound to BiP (Fig. 9.6). Thus, ERj1 would be ideally suited to allow initiation of synthesis of precursor polypeptides on ER bound ribosomes only when BiP is available on the other side of the membrane. Furthermore, ERj1 has all the features of a membrane-tethered transcription factor that can be activated by regulated intra-membrane proteolysis (Zupicich et al. 2001). The cytosolic domain has actually been shown to be able to enter the nucleus (Zupicich et al. 2001, Dudek et al. 2005). Last but not least, it was observed that a resident ER protein with a lumenal J-domain is also involved in sealing of the Sec61 complex in the mammalian system (Schäuble et al. 2012). At present, we only can exclude ERj1 as the co-chaperone for this BiP activity (Lang et al. 2011).

Regulatory Mechanisms

It has been known for some time that the genes of many of the protein transport components of the mammalian ER are under control of the unfolded protein response (see Table 9.2 for examples). In addition, various miRNAs apparently target

some of these same genes and there may be splice variants for some of these genes according to the respective data bases. But there apparently also is regulation on the protein level. In the case of mammalian BiP, ADP ribosylation was shown to be a mechanism for reversible inactivation of BiP when the concentration of unfolded polypeptides is low (Chambers et al. 2012). Various modifications have been observed for mammalian as well as yeast protein transport components, most notably phosphorylation. Phosphorylation of mammalian proteins ERj1 and Sec63 by CK2 was reported, but the functional consequences of these phosphorylations was not addressed (Götz et al. 2009; Ampofo et al. 2013). A first hint for the importance of CK2-dependent phosphorylation of components of the transport machinery may come from studies in yeast (Wang and Johnsson 2005). The essential Sec63p is phosphorylated by the protein kinase CK2 and non-phosphorylatable Sec63p causes a protein translocation defect. Taken together, these findings suggest a general role of phosphorylation for a network of transport factors in regulation of protein translocation across the ER-membrane.

Medical Aspects

Shiga toxigenic *Escherichia coli* (STEC) strains cause morbidity and mortality in developing countries (Paton et al. 2006). Some of these pathogens produce AB_5 toxin or subtilase AB and are responsible for gastrointestinal diseases, including the life-threatening haemolytic uraemic syndrome (HUS) (OMIM 235400). During an infection, the bacterial cytotoxin enters human cells by endocytosis and retrograde transport to the ER. In the ER, BiP is the major target of the catalytic subunit, which inactivates BiP by limited proteolysis. Eventually, all BiP functions are lost, and the affected cells die.

Autosomal dominant polycystic liver disease (PLD) (OMIM 174050) is a rare human inherited disease that is characterized by the progressive development of multiple biliary epithelial liver cysts (Davila et al. 2004). It usually remains asymptomatic at young ages and manifests between the ages of 40 and 60 years. Liver function is usually preserved. A loss of Sec63 function has been postulated in several genetic mutations. Although no mechanism has been firmly established for PLD, the disease can be explained by a two-hit mechanism: patients with one inherited mutant allele and one wild-type allele may lose the wild-type allele in some liver cells through somatic mutation. A plausible scenario is that Sec63 is essential for the ER import of a subset of non-essential secretory or plasma membrane proteins that are involved in the control of biliary cell growth or cell polarity. Thus, without functional Sec63, these proteins do not reach the correct location at the cell surface. This view was confirmed by recent results and it was concluded that the secondary lack of polycystins 1 and 2 results in disrupted cell adhesion and, therefore, cyst formation (Fedeles et al. 2011; Lang et al. 2012).

Marinesco-Sjögren syndrome (MSS) (OMIM 248800) is a rare autosomal recessively inherited neurodegenerative disease (Anttonen et al. 2005; Senderek et al.

2005). The hallmarks of MSS are cerebellar ataxia, cataracts, developmental and mental retardation, and progressive myopathy (Roos et al. 2014). The cause of the disease in the majority of MSS patients has been characterized as a mutation in the *SIL1* gene that results in mutated or truncated Sil1. Sil1 is a nucleotide exchange factor for BiP, and its role is to provide BiP with ATP (Weitzmann et al. 2006). Thus, the loss of Sil1 function results in a reduction of functional BiP. Several possible consequences are: (i) some precursor proteins may not be transported into the ER, causing precursor polypeptides to accumulate in the cytosol; (ii) some proteins that are successfully transported into the ER may not be folded correctly, leading to accumulation of mis-folded polypeptides in the ER; (iii) some essential secretory or plasma membrane proteins may not reach their functional location, leading to secondary loss of functions; or (iv) Sec61 channel gating to the closed state may be compromised, thus, leading to apoptosis.

Wolcott-Rallison syndrome (WRS) (OMIM 226980) is a rare autosomal recessive disorder characterized by permanent neonatal and early infant insulin dependent diabetes associated with various multisystemic clinical manifestations (Brickwood et al. 2003). The cause of the disease has been characterized as a mutation in the *PERK* gene that results in a mutated or truncated PERK protein. Based on the analysis of some of the mutant proteins, a loss of PERK function is expected in all of these cases. PERK seems to be essential in postnatal pancreatic β cells and may play a role in pancreatic development *in utero*. Because PERK is only one of four kinases that are known to phosphorylate eIF2A, it was argued that PERK may also have an important metabolic function and that the latter may be the essential function in β cells.

Due to poor vascularization and the resulting hypoxia and glucose starvation, tumor cells are prone to ER stress and UPR (Macario and Conway de Macario 2007; Aridor 2007). In cultured cells, BiP is one of the proteins involved in protecting cancer cells against ER stress-induced apoptosis (Fu et al. 2007). In addition to this general link between BiP and cancer, some of the above-mentioned BiP interacting proteins have been connected to certain tumors. Sec63 is an ER-membrane resident Hsp40 that, together with BiP, plays a role in gating of the Sec61 complex (Lang et al. 2012; Schäuble et al. 2012). The *SEC63* gene was found among the most frequently mutated genes in cancers that had deficient DNA mismatch repair, such as hereditary nonpolyposis colorectal cancer (HNPCC)-associated malignancies and sporadic cancers with frequent microsatellite instability (Mori et al. 2002; Schulmann et al. 2005). These genetic alterations may be associated with a more or less pronounced loss of Sec63 function. This alone may contribute to tumorigenesis or it may result in a non-physiological Sec62-Sec63-ratio. This hypothesis is supported by a study on the gene expression signatures of sporadic colorectal cancers; they recognized the over-expression of *SEC62* as part of a 43-gene cDNA panel that was used for predicting the long-term outcome of colorectal cancer patients (Eschrich et al. 2005). Sec62 forms a complex with Sec63 and Sec61 and is also involved in Sec61 channel gating (Linxweiler et al. 2013). Gene amplification at chromosome 3q25-q26 commonly occurs in prostate- as well as several other cancers. Mapping the 3q25-q26 amplification and identifying candidate genes with

quantitative real-time PCR revealed that the *SEC62* gene had the highest known amplification frequency (50 %) in prostate cancer and was found to be up-regulated at the mRNA and protein level in all tumors analyzed (Jung et al. 2006). Recently, this was also observed for cancers of the lung and thyroid (Greiner et al. 2011a, 2011b; Linxweiler et al. 2012, 2013) and *SEC62* (*TLOC1*) was characterized as a cancer driver gene (Hagenstrand et al. 2013). Thus, *SEC62* over-expression appears to be associated with a proliferative advantage for various cancer cells, which appears to be due to the role of Sec62 in cellular calcium homeostasis. In summary, a Sec62-Sec63 imbalance is likely to contribute to the development of various human malignancies.

A common theme seems to emerge from some of the described patho-physiological situations in mice and men (summarized in Table 9.1): Mammalian cells, which are highly active in protein secretion, may be particularly sensitive towards problems in Sec61 channel closure and, therefore, constantly on the verge to apoptosis, e.g. seen in the β-cells of the mouse with the Sec61αY344H mutation. On the other hand, the secretory active cells may be particularly sensitive to imbalances in the Sec62 to Sec63 ratio, which result in over-efficient Sec61 channel closure and, thus, a proliferative advantage that can lead to cancer, e.g. seen after over-epression of *SEC62* in prostate or lung cancer. However, it remains to be seen to what extent the other diseases that are listed in Table 9.1 fit into this scheme.

Acknowledgements We are grateful to Drs Roland Beckmann (Munich), Gregory L. Blatch (Melbourne, Australia), Adolfo Cavalié (Homburg), Johanna Dudek (Homburg), Friedrich Förster (Martinsried), Markus Greiner (Homburg), Volkhard Helms (Saarbrücken), Stephen High (Manchester, UK), Martin Jung (Homburg), James C. Paton (Adelaide, Australia) Stefan Pfeffer (Martinsried), Albert Sickmann (Dortmund), Jörg Tatzelt (Bochum), Richard Wagner (Osnabrück), and René P. Zahedi (Dortmund) for many years of fruitful collaborations. This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

References

Ampofo E, Welker S, Jung M, Müller L, Greiner M, Zimmermann R, Montenarh M (2013) CK2 phosphorylation of human Sec63 regulates its interaction with Sec62. Biochim Biophys Acta 1830:2938–2945

Anttonen A-K, Mahjneh I, Hämäläinen RH, Lagier-Tourenne C, Kopra O, Waris L, Anttonen M, Joensuu T, Kalimo H, Paetau A, Tranebjaerg L, Chaigne D, Koenig M, Eeg-Olofsson O, Udd B, Somer M, Somer H, Lehesjoki A-E (2005) The gene disrupted in Marinesco-Sjögren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet 37:1309–1311

Aridor M (2007) Visiting the ER: the endoplasmic reticulum as a target for therapeutics in traffic related diseases. Adv Drug Deliv Rev 59:759–781

Awad W, Estrada I, Shen Y, Hendershot LM (2008) BiP mutants that are unable to interact with endoplasmic reticulum DnaJ proteins provide insights into interdomain interactions in BiP. Proc Natl Acad Sci U S A 105:1164–1169

Bagola K, Mehnert M, Jarosch E, Sommer T (2011) Protein dislocation from the ER. Biochim Biophys Acta 1808:925–936

Bakowski D, Nelson C, Parekh AB (2012) Endoplasmic reticulum-mitochondria coupling: local Ca^{2+} signalling with functional consequences. Eur J Physiol 464:27–32

Benedix J, Lajoie P, Jaiswal H, Burgard C, Greiner M, Zimmermann R, Rospert S, Snapp EL, Dudek J (2010) BiP modulates the affinity of its co-chaperone ERj1 to ribosomes. J Biol Chem 285:36427–36433

Berridge MJ (2002) The endoplasmic reticulum: a multifunctional signalling organelle. Cell Calcium 32:235–249

Bies C, Guth S, Janoschek K, Nastainczyk W, Volkmer J, Zimmermann, R (1999) AScj1p homolog and folding catalysts present in dog pancreas microsomes. Biol Chem 380:1175–1182

Bies C, Blum R, Dudek J, Nastainczyk W, Oberhauser S, Jung M, Zimmermann R (2004) Characterization of pancreatic ERj3p, a homolog of yeast DnaJ-like protein Scj1p. Biol Chem 385:389–395

Blau M, Mullapudi S, Becker T, Dudek J, Zimmermann R, Penczek PA, Beckmann R (2005) ERj1p uses a universal ribosomal adaptor site to coordinate the 80S ribosome at the membrane. Nat Struct Mol Biol 12:1015–1016

Blobel G, Dobberstein B (1975a) Transfer of proteins across membranes I Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J Cell Biol 67:835–851

Blobel G, Dobberstein B (1975b) Transfer of proteins across membranes II. Reconstitution of functional rough microsomes from heterologous components. J Cell Biol 67:852–862

Bole DG, Hendershot LM, Kearney JF (1986) Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. J Cell Biol 102:1558–1566

Brickwood S, Bonthron DT, Al-Gazali LI, Piper K, Hearn T, Wilson DI, Hanley NA (2003) Wolcott-Rallison syndrome: pathogenic insights into neonatal diabetes from new mutation and expression studies of EIF2AK3. J Med Genet 40:685–689

Brightman SE, Blatch GL, Zetter BR (1995) Isolation of a mouse cDNA encoding MTJ1, a new murine member of the DnaJ family of proteins. Gene 153:249–254

Bulleid NJ (2012) Disulfide bond formation in the mammalian endoplasmic reticulum. Cold Spring Harb Perspect Biol 4:a013219

Burns K, Helgason CD, Bleakley RC, Michalak M (1992) Calreticulin in T-lymphocytes. Identification of calreticulin in T-lymphocytes and demonstration that activation of T-cells correlates with increased levels of calreticulin mRNA and protein. J Biol Chem 267:19039–19042

Chambers JE, Petrova K, Tomba G, Vendruscolo M, Ron D (2012) ADP ribosylation adapts an ER chaperone response to short-term fluctuations in unfolded protein load. J Cell Biol 198:371–385

Chung KT, Shen Y, Hendershot H (2002) BAP, a mammalian BiP associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. J Biol Chem 277:47557–47563

Cunnea PM, Miranda-Vizuete A, Bertoli G, Simmen T, Damdimopoulos AE, Hermann S, Leinonen S, Huikko MP, Gustafsson J-A, Sitia R, Spyrou G (2003) ERdj5, an endoplasmic reticulum (ER)-resident protein containing DnaJ and thioredoxin domains, is expressed in secretory cells or following ER stress. J Biol Chem 278:1059–1066

Davila S, Furu L, Gharavi AG, Tian X, Onoe T, Qian Q, Li A, Cai Y, Kamath PS, King BF, Azurmendi PJ, Tahvanainen P, Kääriäinen H, Höckerstedt K, Devuyst O, Pirson Y, Martin RS, Lifton RP, Tahvanainen E, Tores VE, Somlo S (2004) Mutations in SEC63 cause autosomal dominant polycystic liver disease. Nat Genet 36:575–577

Degen E, Williams DB (1991) Participation of a novel 88-kD protein in the biogenesis of murine class I histocompatibility proteins. J Cell Biol 112:1099–1115

Dong M, Bridges JP, Apsley K, Xu Y, Weaveret TE (2008) ERdj4 and ERdj5 are required for endoplasmic reticulum-associated protein degradation of misfolded surfactant protein C. Mol Biol Cell 19:2620–2630

Dudek J, Volkmer J, Bies C, Guth S, Müller A, Lerner M, Feick P, Schäfer KH, Morgenstern E, Hennessy F, Blatch GL, Janoscheck K, Heim N, Scholtes P, Frien M, Nastainczyk W, Zimmermann R (2002) A novel type of cochaperone mediates transmembrane recruitment of DnaK-like chaperones to ribosomes. EMBO J 21:2958–2967

Dudek J, Greiner M, Müller, A, Hendershot LM, Kopsch K, Nastainczyk W, Zimmermann R (2005) ERj1p plays a basic role in protein biogenesis at the endoplasmic reticulum. Nat Struct Mol Biol 12:1008–1014

English AR, Voeltz GK (2013) Endoplasmic reticulum structure and interconnections with other organelles. Cold Spring Harb Perspect Biol 5:a013227

Erdmann F, Schäuble N, Lang S, Jung M, Honigmann A, Ahmad M, Dudek J, Benedix J, Harsmann A, Kopp A, Helms V, Cavalié A, Wagner R, Zimmermann R (2011) Interaction of calmodulin with Sec61a limits Ca^{2}+ leakage from the endoplasmic reticulum. EMBO J 30:17–31

Eschrich S, Yang I, Bloom G, Kwong KY, Boulware D, Cantor A, Coppola D, Kruhoffer M, Aaltonen L, Orntoft TF, Quackenbush J, Yeatman TJ (2005) Molecular staging or surival prediction of colorectal cancer patients. J Clin Oncol 23:3526–3535

Fedeles SV, Tian X, Gallagher A-R, Mitobe M, Nishio S, Lee SH, Cai Y, Geng L, Crews CM, Somlo S (2011) A genetic interaction network of five genes for human polycystic kidney and liver disease defines polycystin-1 as the central determinant of cyst formation. Nat Genet 43:639–647

Flourakis M, Van Coppenolle F, Lehen'kyi V, Beck B, Skryma R (2006) Passive calcium leak via translocon is a first step for iPLA2-pathway regulated store operated channels activation. FASEB J 20:1215–1217

Fritz JM, Dong M, Apsley KS, Martin EP, Na CL, Sitaraman S, Weaver TE (2014) Deficiency of the BiP cochaperone ERdj4 causes constitutive endoplasmic reticulum stress and metabolic defects. Mol Biol Cell 25:431–440

Fu Y, Li J, Lee AS (2007) GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen starvation-induced apoptosis. Cancer Res 67:3734–3740

Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P (2013) Endoplasmic reticulum stress sensing in the unfolded protein response. Cold Spring Harb Perspect Biol 5:a013169

Giunti R, Gamberucci A, Fulceri R, Banhegyi G (2007) Both translocon and a cation channel are involved in the passive Ca^{2+} leak from the endoplasmic reticulum: a mechanistic study on rat liver microsomes. Arch Biochem Biophys 462:115–121

Görlich D, Prehn S, Hartmann E, Kalies K-U, Rapoport TA (1992) A mammalian homolog of SEC61p and SECYp is associated with ribosomes and nascent polypeptides during translocation. Cell 71:489–503

Götz G, Müller A, Montenarh M, Zimmermann R, Dudek J (2009) ERj1 is a substrate of phosphorylation by CK2. Biochem Biophys Res Commun 388:637–642

Greiner M, Kreutzer B, Jung V, Grobholz R, Hasenfus A, Stöhr R, Franz R, Tornillo L, Dudek J, Stöckle M, Unteregger G, Kamradt J, Wullich B, Zimmermann R (2011a) Silencing of the *SEC62* gene inhibits migratory and invasive potential of various tumor cells. Int J Cancer 128:2284–2295

Greiner M, Kreutzer B, Lang S, Jung V, Cavalié A, Unteregger G, Zimmermann R, Wullich B (2011b) Sec62 protein level is crucial for ER-stress tolerance of prostate cancer. The Prostate 71:1074–1083

Gumbart J, Schulten K (2007) Structural determinants of lateral gate opening in the protein translocon. Biochemistry 46:11147–11157

Haas IG, Wabl M (1983) Immunoglobulin heavy chain binding protein. Nature 306:387–389

Hagerstrand D, Tong A, Schumacher SE, Ilic N, Shen RR, Cheung HW, Vazquez F, Shrestha Y, Kim SY, Giacomelli AO, Rosenbluh J, Schinel AC, Spardy NA, Barbie DA, Mermel CH, Weir BA, Garraway LA, Tamayo P, Mesirov JP, Beroukhim R, Hahn WC (2013) Systematic interrogation of 3q26 identifies TLOC1 and SKL as cancer drivers. Cancer Discov 3:1044–1057

Hagiwara M, Maegawa K-i, Suzuki M, Ushioda R, Araki K, Matsumoto JH, Nagata K, Inaba K (2011) Structural basis of an ERAD pathway mediated by the ER-resident protein disulfide reductase ERdj5. Mol Cell 41:432–444

Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397:271–274

Hartmann E, Sommer T, Prehn S, Görlich D, Jentsch S, Rapoport TA (1994) Evolutionary conservation of components of the protein translocation complex. Nature 367:654–657

Hayashi T, Su T-P (2007) Sigma-1 receptor chaperones at the ER- mitochondrion interface regulate Ca(2+) signaling and cell survival. Cell 131:596–610

Hayashi T, Rizzuto R, Hajnoczky G, Su T-P (2009) MAM: more than just a housekeeper. Trends Cell Biol 19:81–88

Hennessy F, Nicoll WS, Zimmermann R, Cheetham ME, Blatch GL (2005) Not all J domains are created equal: implications for the specificity of Hsp40-Hsp70 interactions. Protein Sci 14:1697–1709

Hosoda A, Kimata Y, Tsuru A, Kohno K (2003) JPDI, a novel endoplasmic reticulum-resident protein containing both a BiP-interacting J-domain and thioredoxin-like motifs. J Biol Chem 278:2669–2676

Jin Y, Awad W, Petrova K, Hendershot LM (2008) Regulated release of ERdj3 from unfolded proteins by BiP. EMBO J 27:2873–2882

Jin Y, Zhuang M, Hendershot LM (2009) Erdj3, a luminal ER DnaJ homologue, binds directly to unfolded proteins in the mammalian ER: identification of critical residues. Biochemistry 48:41–49

Jung V, Kamradt J, Kindich R, Jung M, Mueller M, Schulz WA, Engers R, Stoeckle M, Zimmermann R, Wullich B (2006) Genomic and expression analysis of the 3q25-q26 amplicon reveals TLOC1/SEC62 as a probable target gene in prostate cancer Mol Cancer Res 4:169–176

Kang SW, Ran NS, Kim SJ, Garrison JL, Taunton J,.Hegde RS (2006) Substrate-specific translocational attenuation during ER stress defines a pre-emptive quality control pathway. Cell 127:999–1013

Kitao Y, Hashimoto K, Matsuyama T, Iso H, Tamatani T, Hori O, Stern DM, Kano M, Ozawa K, Ogawa S (2004) ORP150/HSP12A regulates purkinje cell survival: a role for endoplasmic reticulum stress in cerebellar development. J Neurosci 24:1486–1496

Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS, Walter P (2009) An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325:477–481

Kroczynska B, Evangelista CM, Samant SS, Elguindi EC, Blond SY (2004) The SANT2 domain of murine tumor cell DnaJ-like protein 1 human homologue interacts with α1-antichymotrypsin and kinetically interferes with its serpin inhibitory activity. J Biol Chem 279:11432–11443

Kurisu J, Honma A, Miyajima H, Kondo S Okumura M, Imaizumi K (2003) MDG1/ERdj4, an ER-resident DnaJ family member, suppresses cell death induced by ER stress. Genes Cells 8:189–202

Ladiges WC, Knoblaugh SE, Morton JF, Korth MJ, Sopher BL, Baskin CR, MacAuley A, Goodman AG, LeBoeuf RC, Katze MG (2005) Pancreatic beta-cell failure and diabetes in mice with a deletion mutation of the endoplasmic reticulum molecular chaperone gene P58IPK. Diabetes 54:1074–1081

Lai CW, Otero JH, Hendershot LM, Snapp E (2012) Erdj4 protein is a soluble endoplasmic reticulum (ER) DnaJ family protein that interacts with ER-associated degradation machinery. J Biol Chem 287:7969–7978

Lang S, Erdmann F, Jung M, Wagner R, Cavalié A, Zimmermann R (2011) Sec61 complexes form ubiquitous ER Ca^{2+} leak channels. Channels 5:228–235

Lang S, Benedix J, Fedeles SV, Schorr S, Schirra C, Schäuble N, Jalal C, Greiner M, Haßdenteufel S, Tatzelt J, Kreutzer B, Edelmann L, Krause E, Rettig J, Somlo S, Zimmermann R, Dudek J (2012) Different effects of Sec61a-, Sec62 and Sec63-depletion on transport of polypeptides into the endoplasmic reticulum of mammalian cells. J Cell Sci 125:1958–1969

Lin H-Y, Masso-Welch P, Di Y-P, Cai J-W, Shen J-W, Subjeck JR (1993) The 170-kDa glucose-regulated stress protein is an endoplasmic reticulum protein that binds immunoglobulin. Mol Biol Cell 4:1109–1119

Linxweiler M, Linxweiler J, Barth M, Benedix J, Jung V, Kim Y-J, Bohle R, Zimmermann R, Greiner M (2012) Sec62 bridges the gap from 3q amplification to molecular cell biology in non-small cell lung cancer. Am J Pathol 180:473–483

Linxweiler M, Schorr S, Jung M, Schäuble N, Linxweiler J, Langer F, Schäfers H-J, Cavalié A, Zimmermann R, Greiner M (2013) Targeting cell migration and the ER stress response with

calmodulin antagonists: A clinically tested small molecule phenocopy of SEC62 gene silencing in human tumor cells. BMC–Cancer 13:574
Lloyd DJ, Wheeler MC, Gekakis N (2010) A point mutation in Sec61 α leads to Diabetes and Hepatosteatisis in Mice. Diabetes 59:460–470
Lomax RB, Camello C, Van Coppenolle F, Petersen OH, Tepikin AV (2002) Basal and physiological Ca^{2+} leak from the endoplasmic reticulum of pancreatic acinar cells. Second messenger-activated channels and translocons. J Biol Chem 277:26479–26485
Luo S, Mao C, Lee B, Lee AS (2006) GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. Mol Cell Biol 15:5688–5697
Lyman SK, Schekman R (1995) Interaction between BiP and Sec63p is required for the completion of protein translocation into the ER of Saccharomyces cerevisiae. J Cell Biol 131:1163–1171
Lyman SK, Schekman R (1997) Binding of secretory precursor polypeptides to a translocon subcomplex is regulated by BiP. Cell 88:85–96
Ma Y, Hendershot LM (2001) The unfolding tale of the unfolded protein response. Cell 107:827–830
Macario AJ, Conway de Macario E (2007) Molecular chaperones: multiple functions, pathologies, and potential applications. Front Biosci 12:2588–2600
Madeo F, Kroemer G (2009) Intricate links between ER stress and apoptosis. Mol Cell 33:669–670
Mayer H-A, Grau H, Kraft R, Prehn S, Kalies K-U, Hartmann E (2000) Mammalian Sec61 is associated with Sec62 and Sec63. J Biol Chem 275:14550–14557
Meunier L, Usherwood Y-K, Chung KT, Hendershot LM (2002) A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. Mol Biol Cell 13:4456–4469
Mimura N, Hamada H, Kashio M, Jin H, Toyama Y, Kimura K, Iida M, Goto S, Saisho H, Toshimori K, Koseki H, Aoe T (2007) Aberrant quality control in the endoplasmic reticulum impairs the biosynthesis of pulmonary surfactant in mice expressing mutant BiP. Cell Deat Differ 14:1475–1485
Mori Y, Sato F, Selaru FM, Olaru A, Perry K, Kimos MC, Tamura G, Matsubara N, Wang S, Xu Y, Yin J, Zou T-T, Leggett B, Young J, Nukiwa T, Stine OC, Abraham JM, Shibata D, Meltzer SJ (2002) Instabilotyping reveals unique mutational spectra in microsatellite-unstable gastric cancers. Cancer Res 62:3641–3645
Müller L, Diaz de Escauriaza M, Lajoie P, Theis M, Jung M, Müller A, Burgard C, Greiner M, Snapp EL, Dudek J, Zimmermann R (2010) Evolutionary gain of function of the ER membrane protein Sec62 from yeast to humans. Mol Biol Cell 21:691–703
Munro S, Pelham HRB (1986) An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell 46:291–300
Nicchitta CV, Blobel G (1993) Lumenal proteins of the mammalian endoplasmic reticulum are required to complete protein translocation. Cell 73:989–998
Oh-hashi K, Naruse Y, Amaya F, Shimosato G, Tanaka M (2003) Cloning and Characterization of a novel GRP78-binding protein in the rat brain. J Biol Chem 278:10531–10537
Oka OBV, Pringle MA, Schopp IM, Braakman I, Bulleid NJ (2013) ERdj5 is the ER reductase that catalyzes the removal of non-native disulfides and correct folding of the LDL receptor. Mol Cell 50:793–804
Olzmann JA, Kopito RR, Christianson JA (2012) The mammalian endoplasmic reticulum-associated degradation system. Cold Spring Harb Perspect Biol. doi:10.1101/cshperspect.a013185
Ong HL, Liu X, Sharma A, Hegde RS, Ambudkar IS (2007) Intracellular Ca^{2+} release via the ER translocon activates store-operated calcium entry. Pflugers Arch 453:797–808
Palade G (1975) Intracellular aspects of protein synthesis. Science 189:347–358
Paton AW, Beddoe T, Thorpe CM, Whisstock JC, Wilche MC, Rossjohn J, Talbot UM, Paton JC (2006) AB5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. Nature 443:548–552
Pelham HRB (1990) The retention signal for soluble proteins of the endoplasmic reticulum. Trends Biochem Sci 15:483–486

Pena V, Jovin SM, Fabrizio P, Orlowski J, Bujnicki JM, Lührmann R, Wahl MC (2009) Common design principles in the spliceosomal RNA helicase Brr2 and in the Hel308 DNA helicase. Mol Cell 35:454–466

Petrova K, Oyadomari S, Hendershot LM, Ron D (2008) Regulated association of misfolded endoplasmic reticulum lumenal proteins with P58/DNAJc3. EMBO J 27:2862–2872

Pfeffer S, Brandt F, Hrabe T, Lang S, Eibauer M, Zimmermann R, Förster F (2012) Structure and 3D arrangement of ER-membrane associated ribosomes. Structure 20:1508–1518

Pfeffer S, Dudek J, Gogala M, Schorr S, Linxweiler J, Lang S, Becker T, Beckmann R, Zimmermann R, Förster F (2014) Structure of the mammalian oligosaccharyl-transferase in the native ER protein translocon. Nature Comm. doi:10.1038/ncomms4072

Pilon M, Schekman R, Römisch K (1997) Sec61p mediates export of a misfolded secretory protein from the endoplasmic reticulum to the cytosol for degradation. EMBO J 16:4540–4548

Plemper RK, Böhmler S, Bordallo J, Sommer T, Wolf DH (1997) Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. Nature 388:891–895

Polier S, Dragovic Z, Hartl FU, Bracher, A (2008) Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. Cell 133:1068–1079

Rizzuto R, Pozzan T (2006) Microdomains of intracellular Ca2+: molecular determinants and functional consequences. Physiol Rev 86:369–408

Ron D, Harding HP (2012) Protein-folding homeostasis in the endoplasmic reticulum and nutritional regulation. Cold Spring Harb Perspect Biol 4:a013177

Roos A, Buchlremer S, Labisch T, Gatz C, Brauers E, Nolte K, Goebel HH, Zimmermann R, Senderek J, Weis J (2014) Severe degenerative myopathy in woozy mice: chaperonopathy and specific nuclear envelope pathology due to Sil1 dysfuction in a model for Marinesco-Sjögren syndrome. Acta Neuropathologica 127:761–777

Rutkowski DT, Kang SW, Goodman AG, Garrison JL, Taunton J, Katze MG, Kaufman RJ, Hedge RS (2007) The role of p58IPK in protecting the stressed endoplasmic reticulum. Mol Biol Cell 18:3681–3691

Sambrook JF (1990) The involvement of calcium in transport of secretory proteins from the endoplasmic reticulum. Cell 61:197–199

Schäfer A, Wolf DH (2009) Sec61p is part of the endoplasmic reticulum-associated degradation machinery. EMBO J 28:2874–2884

Schäuble N, Lang S, Jung M, Cappel S, Schorr S, Ulucan Ö, Linxweiler J, Dudek J, Blum R, Helms V, Paton A W, Paton J C, Cavalié A, Zimmermann R (2012) BiP-mediated closing of the Sec61 channel limits Ca^{2+} leakage from the ER. EMBO J 31:3282–3296

Schekman R (2004) Merging cultures in the study of membrane traffic. Nature Cell Biol 6:483–486

Schekman R (2005) Peroxisomes: Another branch of the secretory pathway? Cell 122:1–7

Schlecht R, Erbse AH, Bukau B, Mayer MP (2011) Mechanics of Hsp70 chaperones enables differential interaction with client proteins. Nat Struct Mol Biol 18:345–351

Schulmann K, Brasch FE, Kunstmann E, Engel C, Pagenstecher C, Vogelsang H, Krüger S, Vogel T, Knaebel H-P, Rüschoff J, Hahn SA, von Knebel-Doeberitz M, Moeslein G, Meltzer SJ, Schackert HK, Tympner C, Mangold E, Schmiegel W for the German HNPCC consortium (2005) HNPCC-associated small bowel cancer: Clinical and molecular characteristics. Gastroenterol 128:590–599

Senderek J, Krieger M, Stendel C, Bergmann C, Moser M, Breitbach-Faller N, Rudinik-Schoneborn S, Blaschek A, Wolf N, Harting I, North K, Smith J, Muntoni F, Brockington M, Quijano-Roy S, Renault F, Herrmann R, Hendeshot LM, Schröder JM, Lochmüller H, Topaloglu H, Voit T, Weis J, Ebinger F, Zerres K (2005) Mutations in Sil1 cause Marinesco-Sjögren syndrome, a cerebellar ataxia with cataract and myopathy. Nat Gen 37:1312–1314

Shaffer KL, Sharma A, Snapp EL, Hegde RS (2005) Regulation of protein compartmentalization expands the diversity of protein function. Dev Cell 9:545–554

Shen Y, Hendershot LM (2005) ERdj3p, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP's interactions with unfolded substrates. Mol Biol Cell 16:40–50

Shen Y, Meunier L, Hendershot LM (2002) Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress. J Biol Chem 277:15947–15956

Shi Y, Vattem K M, Sood R, An J, Liang J, Stramm L, Wek RC (1998) Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. Mol Cell Biol 18:7499–7509

Shiu RP, Pouyssegur J, Pasta I (1977) Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibrobalsts. Proc Natl Acad Sci U S A 74:3840–3844

Shomura Y, Dragovic Z, Chang HC, Tzvetkov N, Young JC, Brodsky JL, Guerriero V, Hartl FU, Bracher A (2005) Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. Mol Cell 17:367–379

Skowronek MH, Rotter M, Haas IG (1999) Molecular characterization of a novel mammalian DnaJ-like Sec63p homolog. Biol Chem 380:1133–1138

Smith MH, Ploegh HL, Weissman JS (2011) Road to ruin: targeting proteins for degradation in the endoplasmic reticulum. Science 334:1086–1090

Svärd, M, Biterova EI, Bourhis J-M, Guy JE (2011) The crystal structure of the human co-chaperone P58^{IPK}. PloS One 6:e22337

Tabas I, Ron D (2011) Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nature Cell Biol 13:184–190

Tatu U, Helenius A (1997) Interactions between newly synthesized glycoproteins, calnexin and a network of resident chaperones in the endoplasmic reticulum. J Cell Biol 136:555–565

Thibault G, Ng DTW (2012) The ERAD pathways of budding yeast. Cold Spring Harb Perspect Biol. doi:10.1101/cshperspect.a013193

Tirasophon W, Welihinda AA, Kaufman RJ (1998) A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinas/endoribonuclease (Ire1p) in mammalian cells. Genes Develop 12:1812–1824

Tyedmers J, Lerner M, Bies C, Dudek J, Skowronek MH, Haas IG, Heim N, Nastainczyk W, Volkmer J, Zimmermann R (2000) Homologs of the yeast Sec complex subunits Sec62p and Sec63p are abundant proteins in dog pancreas microsomes. Proc Natl Acad Sci U S A 97:7214–7219

Tyedmers J, Lerner M, Wiedmann M, Volkmer J, Zimmermann R (2003) Polypeptide chain binding proteins mediate completion of cotranslational protein translocation into the mammalian endoplasmic reticulum. EMBO Rep 4:505–510

Ushioda R, Hoseki J, Araki K, Jansen G, Thomas DY, Nagata K (2008) ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. Science 321:569–572

Van PN, Peter F, Söling H-D (1989) Four intracisternal calcium-binding glycoproteins from rat liver microsomes with high affinity for calcium. J Biol Chem 264:17494–17501

Van Coppenolle F, Vanden Abeele F, Slomianny C, Flourakis M, Hesketh J, Dewailly E, Prevarskaya N (2004) Ribosome-translocon complex mediates calcium leakage from endoplasmic reticulum stores. J Cell Sci 117:4135–4142

van den Berg B, Clemons WM, Collinson I, Modis Y, Hartmann E, Harrison SC, Rapoport TA (2004) X-ray structure of a protein-conducting channel. Nature 427:36–44

Wang X, Johnsson N (2005) Protein kinase CK2 phosphorylates Sec63p to stimulate the assembly of the endoplasmic reticulum protein translocation apparatus. J Cell Sci 118:723–732

Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D (1998) Cloning of mammalian Ire1 reveals diversity in the ER stress response. EMBO J 17:5708–5717

Weitzmann A, Volkmer J, Zimmermann R (2006) The nucleotide exchange factor activity of Grp170 may explain the non-lethal phenotype of loss of Sil1 function in man and mouse. FEBS Lett 580:5237–5240

Weitzmann A, Baldes C, Dudek J, Zimmermann R (2007) The heat shock protein 70 molecular chaperone network in the pancreatic endoplasmic reticulum—a quantitative approach. FEBS J 274:5175–5187

Wuytack F, Raeymaekers L, Missiaen L (2002) Molecular physiology of the SERCA and SPCA pumps. Cell Calcium 32:279–305

Yoshida H, Haze K, Yanagi H, Yura T, Mori K (1998) XBP1 Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional iduction of mammalian glucose-regulated proteins: involvement of basic-leucine zipper transcription factors. J Biol Chem 273:33741–33749

Yu M, Haslam RHA, Haslam DB (2000) HEDJ, an Hsp40 Co-chaperone localized to the endoplasmic reticulum of human cells. J Biol Chem 275:24984–24992

Zahedi RP, Völzing C, Schmitt A, Frien M, Jung M, Dudek J, Wortelkamp S, Sickmann A, Zimmermann R (2009) Analysis of the membrane proteome of canine pancreatic rough microsomes identifies a novel Hsp40, termed ERj7. Proteomics 9:3463–3473

Zhao L, Longo-Guess C, Harris BS, Lee JW, Ackerman SL (2005) Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. Nat Genet 37:974–979

Zhao L, Rosales C, Seburn K, Ron D, Ackerman S L (2010) Alteration of the unfolded protein response modifies neurodegeneration in a mouse model of Marinesco-Sjögren syndrome. Human Mol Gen 19:25–35

Zimmermann R, Eyrisch S, Ahmad M, Helms V (2011) Protein translocation across the ER membrane. Biochim Biophys Acta 1808:912–924

Zupicich J, Brenner SE, Skarnes WC (2001) Computational prediction of membrane-tethered transcription factors. Genome Biol 2:501–506

Chapter 10
The Evolution and Function of Co-Chaperones in Mitochondria

Neta Regev-Rudzki, Kip Gabriel and Dejan Bursać

Abstract Mitochondrial chaperones mediate and affect critical organellar processes, essential for cellular function. These chaperone systems have both prokaryotic and eukaryotic features. While some of the mitochondrial co-chaperones have clear homologues in prokaryotes, some are unique to eukaryotes and have no homologues in the chaperone machinery of other cellular compartments. The mitochondrial co-chaperones are required for protein import into the organelle and in enforcing the structure of the main chaperones. In addition to novel types of interaction with their senior partners, unexpected and essential interactions between the co-chaperones themselves have recently been described.

Keywords Co-chaperones · Mitochondria · Protein translocation · Evolution

Introduction

The mitochondrion is an endosymbiotically derived double membrane organelle of prokaryotic origin, characteristic of eukaryotic organisms. The organelle still retains many prokaryotic features, such as 70S ribosomes, the machinery to synthesize FeS clusters and its own circular chromosomal DNA. The mitochondrion is enveloped by two membranes, the outer and the inner membrane. All mitochondrial co-chaperones identified to date are localized in the lumen of the inner membrane, the mitochondrial matrix. While comparative analyses of mitochondrial chaperones

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_10

with their bacterial homologues have yielded rich understanding of the processes of protein folding and FeS cluster assembly, the co-chaperones regulating protein import are unique to mitochondria and must have evolved some time after the original endosymbiont began to assume the form of an organelle. Much of the understanding of mitochondrial function comes from studies in yeast *Saccharomyces cerevisiae*, and their co-chaperone complement will be examined in this chapter.

The Mitochondrial Homologue of DnaK and its Co-Chaperones

The major mitochondrial Hsp70 chaperone (called Ssc1 in yeast) is regulated by 5 co-chaperones of the J protein family. These co-chaperones define the spectrum of processes Ssc1 mediates, such as protein folding, import and degradation. Protein folding is a conserved process, mediated in the mitochondrial matrix in an analogous way to the process in the bacterial cytoplasm, and two co-chaperones regulating the process in mitochondria (Mdj1 and Mge1/Yge1) are mitochondrial equivalents of the bacterial co-chaperones DnaJ and GrpE. Mdj1, a DnaJ homologue, is the only type I J protein in mitochondria (Rowley et al. 1994). Mge1, a GrpE homologue is the only bacterial-type nucleotide exchange factor in eukaryotes (Bolliger et al. 1994).

The mitochondrial Ssc1/Mdj1/Mge1 chaperone system shares biochemical, as well as functional properties with the bacterial DnaK/DnaJ/GrpE (Fig. 10.1). Like DnaJ, Mdj1 binds to and prevents the aggregation of unfolded firefly luciferase in *in vitro* assays (Prip-Buus et al. 1996). The unfolded substrate is delivered to the major chaperone and a typical J/Hsp70 interaction follows: Mdj1, through its J domain stimulates the ATPase activity of Ssc1, coupling it with substrate delivery (Kubo et al. 1999). Firefly luciferase folding is facilitated by Ssc1, but the substrate is efficiently released only in the presence of Mge1, which affects ADP release from the chaperone. Mge1 dimer is sensitive to reactive oxygen species, and may act as an oxidative state sensor (Marada et al. 2013). Nucleotide release factors in other eukaryotic compartments are not of bacterial origin, and the presence of this bacterial-type co-chaperone further underlines the prokaryotic nature of the mitochondrial chaperone system.

Mdj1 can both deliver substrate to DnaK, and stimulate the ATPase activity of DnaK *in vitro*, resulting in a productive folding interaction (Deloche et al. 1997b). It rapidly binds to ATP bound form of Ssc1, and disassociates more slowly. The rate of Mdj1 release from Ssc1 is significantly increased in the presence of a protein substrate (Mapa et al. 2010). If expressed in bacterial cells, Mge1 can replace GrpE. Also functional in bacterial cells is a hybrid J protein composed of the glycine-rich and zinc finger domains of DnaJ fused to the J domain of Mdj1 (Deloche et al. 1997a). Despite this apparent similarity between the co-chaperones of mitochondria and bacteria, the two chaperone machines are not completely equivalent. Ssc1 can not, even in the presence of Mdj1, complement the loss of DnaK *in vivo*, and

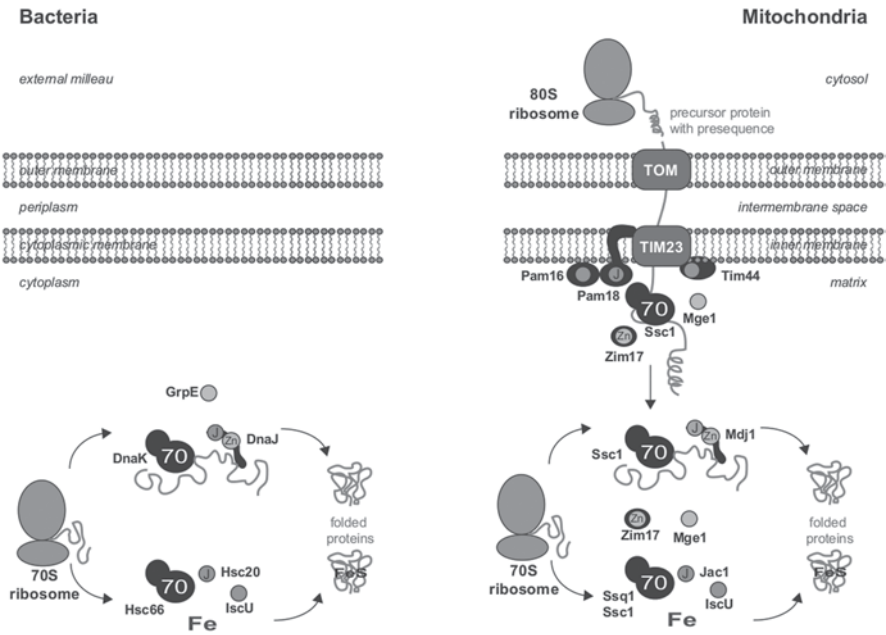

Fig. 10.1 Co-chaperones in *Escherichia coli* and mitochondria**.** The two bacterial Hsp70 chaperone systems, mediating protein folding and FeS cluster assembly, are shown in the panel on the *left*. The J domain (J) and zinc finger domain (Zn) of DnaJ are shown. Hsc20, a type III J protein has only the J domain. DnaK also requires the action of GrpE to efficiently complete its ATP hydrolysis cycles. In mitochondria (*right* panel), two Hsp70 isoforms derived from a DnaK-like ancestor, mediate protein folding and FeS cluster assembly. Both require the assistance of the Mge1 co-chaperone. Ssq1 acts exclusively in FeS cluster assembly, while Ssc1 also mediated the translocation of proteins into the matrix and folding of protein substrates (derived either from the mitochondrial 70S ribosomes or from the protein import machinery). In all three processes, multiple rounds of ATP hydrolysis are required to handle a single protein substrate molecule. Ssc1 can function as the motor for protein import only with the assistance of the co-chaperones of the PAM complex (Pam18, Pam16), the TIM23 complex (Tim44) and Mge1. The mitochondrial co-chaperone Zim17 is required to enforce the structure of Ssc1 and Ssq1. Note: Fig. 10.1 and its legend have been adapted with kind permission from Springer Science + Business Media: Networking of Chaperones by Co-chaperones; Chap. 9: The evolution and function of co-chaperones in mitochondria; 2007; pp. 99–108; Dejan Bursać, and Trevor Lithgow; Fig. 1

full-length Mdj1 can not complement the loss of DnaJ (Deloche et al. 1997a). The glycine rich region of Mdj1 can not fully complement the function of the same region of DnaJ (Cajo et al. 2006).

Folding of proteins within the mitochondrial matrix is a major function of Ssc1(Liu et al. 2001) and is regulated by Mdj1. Analysis of mutant forms of Mdj1 has revealed that folding of mitochondrial proteins following their import into the organelle largely depends on the co-chaperone, but that it only interacts with fully translocated substrates and does not play a role in protein import itself (Rowley et al. 1994; Horst et al. 1997). Increased levels of misfolded proteins and protein aggregates were observed *in organello* upon heat shock of mitochondria in yeast cells

genetically depleted of Mdj1, confirming its importance in this process (Prip-Buus et al. 1996). Protein folding in mitochondria is not solely dependent on Ssc1; some substrates are handed over to the conserved chaperonin system, Hsp60/Hsp10 in yeast (homologues of bacterial GroES and GroEL, respectively), which completes the folding reactions (Heyrovska et al. 1998; Manning-Krieg et al. 1991).

Ssc1 also assists the folding of proteins translated from the mitochondrial 70S ribosomes. Mdj1 and Ssc1 were found associated with the nascent chain of Var1 emerging from the mitochondrial ribosomes and are proposed to protect the emerging protein from aggregation in the protein-dense mitochondrial matrix. In the absence of functional Mdj1, Var1 readily aggregates and does not assume its biologically active form, suggesting that the co-chaperone is also essential for its folding (Westermann et al. 1996).

Yeast cells genetically depleted of Mdj1 either completely (*rho*^{o}) or partially (*rho*$^{-}$) lose mitochondrial DNA, though the details of how this occurs remain to be understood. Partial loss of mtDNA seems likely due, at least in part, to inefficient assembly of fidelity regulating components with the DNA polymerase, and the polymerase itself, in the absence of the co-chaperone (Duchniewicz et al. 1999). Binding of Mdj1 to DNA, and localization to the nucleoid are critical for the maintenance of mtDNA. Disruption of the zinc finger domain, or the peptide binding cleft of Mdj1 do not affect this process, but the loss of the C-terminal domain or J domain function result in loss of mtDNA (Ciesielski et al. 2013). In bacteria, DnaJ mediates disassembly of the DNA replication machinery during λ phage DNA replication, but Mdj1 has not been shown to exert such direct effects on the mtDNA replication components.

Mitochondrial Protein Import Motor with a Highly Advanced Hsp70 Machine at its Core

Protein translocation across membranes is an essential cellular process, in some compartments mediated by Hsp70/J protein partnerships (Walsh et al. 2004). Nearly all mitochondrial proteins are encoded by the nuclear genome and synthesized on cytosolic ribosomes, making protein import crucial for the biogenesis and function of the organelle.

Depending on their final localization, mitochondrial precursor proteins must cross either the outer or both mitochondrial membranes via dedicated protein complexes, TOM and SAM (Pfanner et al. 2004) in the outer membrane, and TIM22 and TIM23 in the inner membrane (Truscott et al. 2003a). Most matrix-targeted precursor proteins carry an N-terminal targeting signal known as the presequence (Pfanner and Geissler 2001; Pfanner 2000). These precursor proteins are synthesized on 80S ribosomes in the cytosol (Lithgow 2000), recognized by the receptors of the TOM complex (Tom20, Tom22 and Tom70) and translocated across the outer membrane via the import pore (Tom40) (Hill et al. 1998). The presequence then interacts with the components of the TIM23 complex (Tim23 and Tim50) and traverses the inner

membrane import pore (Tim23) (Koehler 2004; Jensen and Dunn 2002). Further translocation of the precursor is driven by the action of the presequence-associated import motor, the PAM complex. In yeast, Ssc1 is a central component of the PAM complex, which is a highly specialized and extensively studied Hsp70/J protein machine.

Two mechanisms that explain PAM driven protein import have been proposed: the "Brownian ratchet" and the "power stroke" models (Mayer 2004). Both accept precursor binding by Ssc1 to be a central feature of the process, but differ in accounts of the mechanism that pulls the precursor into the matrix. The Brownian ratchet model proposes that a precursor slides back and forth through the Tim23 channel, with binding of Ssc1 preventing the retrograde movement resulting in the net movement toward the matrix (Liu et al. 2003). The power stroke model stipulates a more active role for Ssc1: the chaperone exerts the force to pull the precursor through the pore (Voos et al. 1996). Recently, a new model of action of Ssc1 in protein import was proposed, the entropic pulling, which proposes that the increase in entropy favours dissociation of Ssc1-precursor complex from the site of import and prevents its back-sliding toward the pore (De Los Rios et al. 2006). This model seems to explain contradictory observations that lead to the formulation of two previous incompatible models.

The protein import function of the DnaK homologue Ssc1 is a novel, eukaryotic adaptation, mediated by unique proteins of the mitochondrial inner membrane. The essential integral membrane protein Pam18/Tim14, is a type III J protein which stimulates the ATPase activity of Ssc1, enabling tight interaction between the chaperone and the precursor, necessary for the successful import reaction (D'Silva et al. 2003; Truscott et al. 2003b; Mokranjac et al. 2003). It consists of an intermembrane space domain, followed by a transmembrane domain (embedded in the mitochondrial inner membrane), a conserved "arm" region, and the C-terminal J domain located in the mitochondrial matrix (Pais et al. 2011). In addition to stimulating the ATPase activity of Ssc1, J domain of Pam18 interacts with the J-like domain of a peripheral membrane co-chaperone protein Pam16/Tim16 (D'Silva et al. 2008, 2005; Mokranjac et al. 2003, 2006). This domain of Pam16 has both sequence and structural homology to helices one through three of the canonical fold, but does not contain the catalytic HPD motif (D'Silva et al. 2005; Walsh et al. 2004; Mokranjac et al. 2006). It does not stimulate the ATPase activity of Ssc1, and the addition of the HPD to the J-like domain at the end of helix II does not result in a functional J domain (Li et al. 2004).

Pam18 and Pam16 are structurally unstable as monomers ("marginally stable"), and the formation of the heterodimer is thermodynamically favored and increases their stability (Iosefson et al. 2007; Mokranjac et al. 2003, 2006). The three dimensional structure of the Pam18/Pam16 complex and mutagenesis approaches have defined crucial regions of these proteins involved in the heterodimer formation (Mokranjac et al. 2006). The J and J-like domains of Pam18 and Pam16 have a nearly identical fold, comprising three alpha helices and two connecting loops. When in complex, they are tightly packed in a pseudo-symmetrical arrangement. Their interaction is mediated by highly conserved residues in helices I, II and III which form the surface of the interface (Mokranjac et al. 2006).

Disruption of heterodimer formation was observed when residues in the conserved "arm" region, and the J-domain of Pam18, and C-terminal region containing the J-like domain of Pam16 were mutated or deleted. In all cases, these disruptions were either lethal or severely affected the rates of protein import and cell growth (D'Silva et al. 2005, 2008; Hayashi et al. 2011; Pais et al. 2011). While expression of the C-terminal domain of Pam16 alone can complement the depletion of the wild-type protein, this region can be substituted by the J domain of Pam18, but under those circumstances the N-terminal domain of Pam16 becomes indispensable (D'Silva et al. 2008; Mokranjac et al. 2006, 2007). These findings demonstrate that the interaction of the J and J-like domains of Pam18 and Pam16 is functionally indispensable for the protein import process. Interestingly, this type of interaction between co-chaperones has not been observed in any other Hsp70 system.

In *in vitro* assays, the Pam18/Pam16 heterodimer stimulates the ATPase activity of Ssc1 to a significantly lesser degree than Pam18 alone (Li et al. 2004; Mokranjac et al. 2006; Chacinska et al. 2005). This finding led to the proposal that Pam16 acts as regulator of Pam18, preventing it from interacting with Ssc1 in the absence of the substrate, i.e. preventing the unnecessary activity of the protein import motor. Regulation of the Pam18 activity, under this model, is mediated by the changes in the conformation of the heterodimer, specifically the J/J-like domain interface, which alternates between the Ssc1 stimulating form and the inactive form (Endo et al. 2011; Mokranjac and Neupert 2010; van der Laan et al. 2010). However, it remains unclear whether the reduction in the ATPase stimulating activity of the heterodimer plays an important role *in vivo*. Mutations in the Pam18 J domain that reduce its ATPase stimulating activity were deleterious when the "arm" region (critical for binding to Pam16) was also disrupted. ATPase activity enhancing mutations in the J domain alleviated the effects of mutations that destabilize the heterodimer formation (Pais et al. 2011). It is possible that *in vivo*, the heterodimer can stimulate Ssc1 sufficiently for the ATP hydrolysis to occur, especially considering the relatively high effective concentration of these proteins at the import site.

In yeast, a duplicate gene (*MDJ2*) encodes a paralogue of Pam18 (Westermann and Neupert 1997; Mokranjac et al. 2005). It is not clear if there is any functional advantage gained from this isoform of Pam18, arisen in the common ancestor of *Saccharomycotina* (Hayashi et al. 2011). There are informative differences in the way the two isoforms of Pam18 perform act. Although the Mdj2 isoform interacts with Pam16, the latter does not antagonize, but rather enhances the Mdj2 mediated stimulation of ATP hydrolysis by Ssc1 (Mokranjac et al. 2005). Furthermore, while Mdj2 can act in the protein import motor in place of Pam18, it cannot fully substitute its function under anaerobic conditions *in vivo* (Hayashi et al. 2011).

Pam18 is associated with the TIM23 complex during the protein import reaction. The key role of Pam16 is to mediate this interaction (Kozany et al. 2004; Li et al. 2004). When the Pam18/Pam16 interaction is disrupted by site directed mutagenesis targeting either protein, Pam18's binding to the TIM23 complex is reduced (D'Silva et al. 2008). The same effect is observed when Pam16's N-terminal domain was deleted, suggesting that Pam16 binds to the TIM23 complex via this domain (Mokranjac et al. 2007). The two N-terminal domains of Pam18 are also

involved in interaction of the heterodimer with this complex. Disruption of either the intermembrane space or the transmembrane domains of Pam18 weaken this association (Hayashi et al. 2011; Mokranjac et al. 2007; Chacinska et al. 2005; D'Silva et al. 2008). The intermembrane space domain likely binds directly to the C-terminal region of Tim17, a component of the TIM23 complex (Chacinska et al. 2005; Schilke et al. 2012). Simultaneous disruption of the Pam16 and Pam18 mediated interactions of the heterodimer with the TIM23 complex is lethal to yeast cells (Schilke et al. 2012).

Two further mitochondrial matrix proteins regulate the interaction of the Pam18/Pam16 heterodimer with the TIM23 complex, Tim44 and Pam17. The peripheral membrane located protein Tim44 was the first co-chaperone component of the protein import machinery to be identified (Maarse et al. 1992; Scherer et al. 1992; Weiss et al. 1999). It has a matrix-exposed segment with limited similarity to a J domain, mostly in the second helix of the canonical fold. This segment of the protein does not contain the catalytic HPD motif, but is essential for Tim44 function (Merlin et al. 1999; Rassow et al. 1999, 1994). The three-dimensional structure of the C-terminal domain of Tim44 revealed a large hydrophobic pocket that was proposed to mediate binding to the mitochondrial inner membrane. Subsequent *in vitro* studies have demonstrated that the N-terminal helices of this domain, rather that the hydrophobic pocket, act as a critical lipid-binding site of Tim44 (Josyula et al. 2006; Marom et al. 2009; Cui et al. 2011).

Tim44 plays a dual role in the initial stages of import mediated by the PAM complex: sensing and binding the precursor in the Tim23 channel, and recruiting Ssc1 and Pam18/16 to the TIM23 complex (D'Silva et al. 2004; Marom et al. 2011). Tim44 can bind Ssc1 at sites both in the ATPase and peptide-binding domains, but does not stimulate its ATPase activity, nor interact with Ssc1 in a substrate-like manner (Krimmer et al. 2000; Moro et al. 2002; Strub et al. 2002). In its ADP bound form, Ssc1 binds to Tim44 with greater affinity than in ATP bound form (Slutsky-Leiderman et al. 2007). During the protein import reaction, the complex formed between the ADP-bound form of Ssc1 and the imported precursor dissociates from Tim44 and is released into the matrix (D'Silva et al. 2004). The nucleotide exchange factor Mge1 then mediates subsequent dissociation of the precursor from Ssc1, (Laloraya et al. 1995, 1994; Westermann et al. 1995; Voos et al. 1994; Schneider et al. 1996) with this entire cycle repeated until the precursor molecule is imported fully into the matrix. Disruption of the N-terminal domain of Tim44 reduces its binding affinity for Ssc1, and affects the release of Ssc1 from Tim44 upon substrate binding (Schiller et al. 2008).

Several lines of evidence point to the direct interaction of Tim44 with Pam16. Pam16 and Tim44 can be cross-linked *in vivo* (Mokranjac et al. 2007; Kozany et al. 2004). Association of Pam18/Pam16 heterodimers with TIM23 complexes is significantly reduced when Tim44, or its N-terminal region is disrupted (Schilke et al. 2012; D'Silva et al. 2008; Kozany et al. 2004; Hutu et al. 2008). Point mutations in this region of Tim44 can suppress deleterious effects of mutations in the N-terminal region of Pam16 (D'Silva et al. 2008). By interacting with the precursor proteins, Ssc1 and the Pam18/Pam16 complex, the Tim44 co-chaperone plays a critical scaffolding and regulatory role in the mitochondrial protein import machinery.

Pam17 is an integral membrane protein with no homology to known Hsp70 co-chaperones (van der Laan et al. 2005). Disruption of the Pam17 function by genetic depletion or mutagenesis results in the decrease in the formation of the Pam18/Pam16 heterodimer and its association with the TIM23 machinery (Hutu et al. 2008; Popov-Celeketic et al. 2008; Schilke et al. 2012). Pam17 likely plays a role in regulating the dynamics of this machinery during protein import, and does not interact with the mitochondrial chaperones (Chacinska et al. 2010; Schiller 2009; Lytovchenko et al. 2013).

The PAM components Ssc1, Mge1, Tim44, Pam16 and Pam18 appear to be found in all eukaryotic organisms, and are highly conserved (Chen et al. 2013; Clements et al. 2009; Dolezal et al. 2005; Rada et al. 2011; Sinha et al. 2010). Two proteins found in extant alphaproteobacteria, TimA and TimB, have significant structural similarities to Tim44 and Pam18, respectively. The J-domain of the bacterial TimB can fully replace the J domain of yeast Pam18 upon single amino acid mutation enabling the interaction with Pam16's J- like domain (Clements et al. 2009). These proteins, with LivH (amino acid translocon) and DnaK are proposed to be the prokaryotic progenitors of the mitochondrial protein import machinery (Clements et al. 2009). The J and J-like domains of Pam18 and Pam16 are interchangeable between human and yeast proteins in their ability to form a functional heterodimer and stimulate Ssc1 (Elsner et al. 2009). Similarly, highly conserved proteins are present in plants, and in parasitic organisms with highly divergent mitochondria (Rada et al. 2011; Dolezal et al. 2005; Chen et al. 2013). These findings strongly indicate the importance of the function of mitochondrial co-chaperones in essential cellular processes and the conservation of interactions between these components.

The number of components involved, and the unique nature of their interactions, makes the PAM complex the most complicated Hsp70 machine known to date. Its further study is likely to lead to greater understanding of the molecular and biochemical properties of Hsp70 systems in general.

Molecular Chaperones and FeS Cluster Assembly

Iron-sulfur (FeS) cluster proteins are essential cellular components found in virtually all organisms studied so far (Balk and Lill 2004). In mitochondria, they are involved in redox chemistry as components of respiratory chain (NADH dehydrogenase, succinate dehydrogenase, Rieske protein) and metabolic conversions (aconitase, a key enzyme in the citric acid cycle). Machinery devoted to the assembly of FeS clusters is highly conserved from prokaryotes to humans (Muhlenhoff and Lill 2000); in bacteria and mitochondria alike, a dedicated Hsp70/J protein system mediates this essential process (Craig and Marszalek 2002).

In *Escherichia coli*, the *Isc* operon encodes proteins essential for FeS cluster assembly, such as IscU (Agar et al. 2000) as well as the Hsp70 protein Hsc66 (Seaton and Vickery 1994) and the type III J protein Hsc20 (Kawula and Lelivelt 1994). These two proteins interact with IscU and are important in FeS assembly in bacteria

(Fig. 10.1), but their disruption leads to only moderate growth defects, possibly due to the existence of an alternative assembly pathway (Takahashi and Tokumoto 2002; Frazzon and Dean 2003; Tokumoto and Takahashi 2001). Hsc66 and Hsc20 have mitochondrial orthologs: in yeast these are the Hsp70 Ssq1 (Knight et al. 1998) and the type III J protein Jac1 (Voisine et al. 2001).

Loss of function of either Ssq1 or Jac1 results in similarly severe phenotypic consequences. Biological activity and the steady state levels of FeS proteins such as aconitase drop dramatically, accompanied by iron uptake and accumulation in mitochondria. Additionally, maturation of yeast frataxin and ferredoxin are impaired and the function of the respiratory chain function is heavily compromised (Voisine et al. 2001, 2000; Lutz et al. 2001; Schilke et al. 1999;Kim et al. 2001). Some of these effects are secondary consequences, rather than direct effects: with iron uptake regulated at the transcriptional level, it may be dependent on FeS assembly, rather than cellular levels of iron.

The precise role of Ssq1 and Jac1 in the assembly of FeS clusters is not yet clear. Based on iron exchange and labeling studies, these chaperones were proposed to act after the assembly of FeS clusters was complete, (Muhlenhoff et al. 2003) to assist only their incorporation into holo-proteins, but their involvement in the assembly of the clusters themselves cannot be ruled out. Part of the confusion stems from the fact that the only well-studied substrate of the chaperone system is the IscU homolog, the scaffold protein that accepts the nascent FeS cluster before its transfer into other apo-proteins (Frazzon and Dean 2003). Whether IscU homologues should be considered a substrate or an assembly factor is not entirely clear. Jac1 binds to the mitochondrial IscU protein and targets it to Ssq1 *in vitro*, coupling its delivery with ATPase activity stimulation (Andrew et al. 2006). Interaction of the IscU with both Ssc1 and Jac1 is dependent on conserved residues in IscU, (Dutkiewicz et al. 2004) and the IscU is fully folded when delivered to Ssq1. The IscU binding pocket of Jac1 has been identified by structural and mutagenesis studies. It is located in the C-terminal domain of Jac1 and contains eight residues. Three residues are hydrophobic, and are essential for the Jac1/IscU interaction, which is critical for the function of Jac1 *in vivo* (Andrew et al. 2006; Majewska et al. 2013; Ciesielski et al. 2012). This binding site and the corresponding binding site on IscU are highly conserved in eukaryotic and prokaryotic proteins (Fuzery et al.2008, 2011). Ssq1 seems to mediate disassembly of the IscU/FeS cluster complex, enabling loading of the FeS cluster into the newly imported apo-enzymes (Dutkiewicz et al. 2006). This might be just one of the many functions Ssq1 and Jac1 perform in this complex process. They have also been proposed to enforce the structure of other components of the assembly machinery, as well as preventing the aggregation of the apo-enzymes themselves.

Given the conserved nature of the process of FeS cluster assembly between bacteria and mitochondria, the evolutionary origin of Ssq1 is rather surprising. Phylogenetic analysis reveals that it is more closely related to Ssc1 than it is to Hsc66, and that it arose from a gene duplication event in a subset of fungal lineages (Huynen et al. 2001; Schilke et al. 2006). Consistent with this evolutionary scenario, biochemical differences between Hsc66 and Ssq1 are significant: Hsc66 only weakly

interacts with nucleotide and does not need a nucleotide exchange factor, but Ssq1, like Ssc1, interacts strongly with nucleotides and requires Mge1 as a nucleotide exchange factor (Dutkiewicz et al. 2003). This sharing of a nucleotide exchange factor between two Hsp70s in the same cellular compartment is unique to mitochondria (Schmidt et al. 2001). Disruption of Ssq1 is less detrimental to cells than depletion of Jac1, and it can be partially complemented by over-expression of Ssc1 (Schilke et al. 1996). Ssq1 is absent from most eukaryotic organisms, and is likely a specialized isoform of Ssc1 devoted to FeS cluster assembly. The J domain of Jac1 in yeast is shorter than that in organisms that do not have Ssq1 (Pukszta et al. 2010). When expressed in yeast, these Jac1 proteins can complement the loss of Ssq1 function to a greater degree than yeast Jac1, suggesting that they recruit Ssc1 to perform the FeS cluster assembly role. Consistent with these findings is the observation that Ssc1 can interact with Jac1 *in vitro*, but to a lesser extent than Ssq1 (Schilke et al. 2006). These observations strongly suggest that Hsp70 function is essential for FeS cluster assembly.

Zim17, a Unique Regulator of Hsp70

Hsp70 chaperones require various co-chaperone regulators to carry out their cellular role. Since the discovery of bacterial Hsp70 system, until recently, the only known regulators of mitochondrial Hsp70 were members of the J protein family or the nucleotide exchange factors. The first Hsp70 co-chaperone identified that does not belong to either group was the Hsp70/Hsp90 organizing protein Hop. Recently, a novel protein essential for function of Hsp70s has been described in mitochondria (Burri et al. 2004). The 17 kD peripheral membrane protein, Zim17/Tim15/Hep1, has an essential zinc finger domain homologous to that of bacterial DnaJ. Loss of Zim17 function leads to aggregation of Ssc1 and Ssq1, disruption of mitochondrial protein import, loss of function of FeS cluster proteins and aberrant mitochondrial morphology (Sanjuan Szklarz et al. 2005; Lewrenz et al. 2013). Decrease in FeS cluster biosynthesis due to Zim17 disruption also results in the increased rate of nuclear genome recombination and alterations in ribosome biogenesis (Diaz de la Loza Mdel et al. 2011).

Since Pam18 is a type III J protein, Zim17 was hypothesized to be the substrate-binding domain of a “fractured” co-chaperone, with the coordinated action of the two proteins resembling that of a type I J protein (Burri et al. 2004). Initially, it was suggest Zim17 might play a role in protein import by binding precursors emerging from the TIM23 pore and mediating their interaction with Ssc1 (Burri et al. 2004; Yamamoto et al. 2005). Subsequent studies have established that aggregation of Hsp70s (and to some extent Pam16) is the first effect of Zim17 depletion, and that other disruptions of mitochondrial processes result from the loss of Hsp70 function (Sichting et al. 2005; Sanjuan Szklarz et al. 2005). Most recent work, utilizing temperature sensitive mutants of Zim17, revealed that Zim17 might play a more

direct role in mitochondrial protein import in addition to stabilizing Ssc1, but the mechanisms of this process are yet to be elucidated (Lewrenz et al. 2013).

Function of Zim17 is dependent on the binding of Zn^{2+} to the core domain following import into the mitochondrial matrix (where Zn^{2+} concentration is relatively high) (Fraga et al. 2013). The core domain of Zim17 contains conserved basic residues and an aspartic acid residue in proximity of a flexible loop and the zinc finger domains. Mutations in these residues lead to the loss of Zim17 function, cell viability and aggregation of Ssc1 (Momose et al. 2007).

During its ATP hydrolysis cycle, Hsp70 can either be nucleotide-free or have either ATP or ADP bound to the ATPase domain. Nucleotide-bound forms of Hsp70 are not prone to aggregation, while nucleotide-free Hsp70 appears to aggregate. Zim17 forms a complex with nucleotide-free Ssc1, and is released upon nucleotide binding, and prevents formation of non-functional Ssc1 oligomers (Sichting et al. 2005; Blamowska et al. 2010). The peptide binding domain of Ssc1 can fold rapidly upon import into mitochondria in the absence of Zim17, but the ATPase and linker domains show a strong dependence on Zim17 for folding. Zim17 binds to these regions of the major chaperone and disruption of this interaction leads to an increase in Ssc1 aggregation (Blamowska et al. 2010, 2012). To some extent, Mdj1 might be able to minimize aggregation of Ssc1 (but not Ssq1) and compensate for the loss of Zim17, since over-expression of Mdj1 minimizes the otherwise lethal consequences of down-regulating the gene encoding Zim17 (Burri et al. 2004). Similarly, co-expression of Mge1 and Ssc1 in *Escherichia coli cells* prevents aggregation of Ssc1 (Momose et al. 2007). These observations suggest that the interaction of Hsp70 with its co-chaperones might promote folding and prevent aggregation of the major chaperone.

Homologues of Zim17 are present in all eukaryotic organisms, and are found in chloroplasts as well as mitochondria (Willmund et al. 2008). No homologues have yet been identified in bacteria, and are absent from other eukaryotic compartments. Ssc1 and Ssq1 are unusual amongst Hsp70s in their tendency to aggregate at elevated temperatures and requirement of the Zim17 co-chaperone for optimal function. These observations strongly suggest that stabilization of nucleotide-free form of Hsp70, like nucleotide exchange, might not be essential for all Hsp70 chaperones.

Concluding Remarks

Mitochondria belie their bacterial ancestry in their Hsp70 (DnaK/DnaJ/GrpE) and Hsp60 (GroEL/GroES) complement of chaperone systems. These chaperones continue to mediate the protein folding pathways that were already established in bacteria at a time before the α proteobacterial endosymbiont ancestor of mitochondria was taken up by the first eukaryote.

The need to drive protein import, for substrate polypeptides now made externally in the cytosol, placed a demand on mitochondria that has been met with a series of novel co-chaperones. The uniquely eukaryotic proteins Pam16, Pam18 and

Tim44 enabled the existing Hsp70 (Ssc1) to be recruited as a protein import motor. That this motor is truly ubiquitous in eukaryotes is made certain from the finding of Pam18 in the anaerobic protists *Giardia intestinalis* and *Trichomonas vaginalis* (Dolezal et al. 2005).

In the course of evolution, the progenitor DnaK-type chaperone has been modified to perform novel functions: protein import and FeS cluster assembly. In some organisms such as *Saccharomyces cerevisiae,* two isoforms of the DnaK ancestor have specialized to perform one of these novel tasks, Ssc1 mediating protein import and Ssq1 mediating FeS cluster assembly. It is intriguing that a novel co-chaperone, Zim17, has arisen to stabilize mitochondrial (and chloroplast) Hsp70 chaperones. It is not clear why the Hsp70s in organelles of endosybiotic origin require their structure to be enforced in a manner so distinct from their counterparts prokaryotes, and in other eukaryotic organelles.

Acknowledgements The text and figure in this chapter contain sections reproduced with kind permission from Springer Science + Business Media: Networking of Chaperones by Co-chaperones; Chap. 9: The evolution and function of co-chaperones in mitochondria; 2007; pp. 99–108; Dejan Bursać, and Trevor Lithgow.

References

Agar JN, Krebs C, Frazzon J et al (2000) IscU as a scaffold for iron-sulfur cluster biosynthesis: sequential assembly of [2Fe-2S] and [4Fe-4S] clusters in IscU. Biochemistry 39:7856–7862

Andrew AJ, Dutkiewicz R, Knieszner H et al (2006) Characterization of the interaction between the J-protein Jac1p and the scaffold for Fe-S cluster biogenesis, Isu1p. J Biol Chem 281:14580–14587

Balk J, Lill R (2004) The cell's cookbook for iron–sulfur clusters: recipes for fool's gold? Chembiochem 5:1044–1049

Blamowska M, Sichting M, Mapa K et al (2010) ATPase domain and interdomain linker play a key role in aggregation of mitochondrial Hsp70 chaperone Ssc1. J Biol Chem 285:4423–4431

Blamowska M, Neupert W, Hell K (2012) Biogenesis of the mitochondrial Hsp70 chaperone. J Cell Biol 199:125–135

Bolliger L, Deloche O, Glick BS et al (1994) A mitochondrial homolog of bacterial GrpE interacts with mitochondrial hsp70 and is essential for viability. Embo J 13:1998–2006

Burri L, Vascotto K, Fredersdorf S et al (2004) Zim17, a novel zinc finger protein essential for protein import into mitochondria. J Biol Chem 279:50243–50249

Cajo GC, Horne BE, Kelley WL et al (2006) The role of the DIF motif of the DnaJ (Hsp40) co-chaperone in the regulation of the DnaK (Hsp70) chaperone cycle. J Biol Chem 281:12436–12444

Chacinska A, Lind M, Frazier AE et al (2005) Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17. Cell 120:817–829

Chacinska A, van der Laan M, Mehnert CS et al (2010) Distinct forms of mitochondrial TOM-TIM supercomplexes define signal-dependent states of preprotein sorting. Mol Cell Biol 30:307–318

Chen X, Ghazanfar B, Khan AR et al (2013) Comparative analysis of putative orthologues of mitochondrial import motor subunit: Pam18 and Pam16 in plants. PloS One 8:e78400

Ciesielski SJ, Schilke BA, Osipiuk J et al (2012) Interaction of J-protein co-chaperone Jac1 with Fe-S scaffold Isu is indispensable in vivo and conserved in evolution. J Mol Biol 417:1–12

Ciesielski GL, Plotka M, Manicki M et al (2013) Nucleoid localization of Hsp40 Mdj1 is important for its function in maintenance of mitochondrial DNA. Biochim Biophys Acta 1833:2233–2243

Clements A, Bursac D, Gatsos X et al (2009) The reducible complexity of a mitochondrial molecular machine. Proc Natl Acad Sci U S A 106:15791–15795

Craig EA, Marszalek J (2002) A specialized mitochondrial molecular chaperone system: a role in formation of Fe/S centers. Cell Mol Life Sci 59:1658–1665

Cui W, Josyula R, Li J et al (2011) Membrane binding mechanism of yeast mitochondrial peripheral membrane protein TIM44. Protein Pept Lett 18:718–725

De Los Rios P, Ben-Zvi A, Slutsky O et al (2006) Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. Proc Natl Acad Sci U S A 103:6166–6171

Deloche O, Kelley WL, Georgopoulos C (1997a) Structure-function analyses of the Ssc1p, Mdj1p, and Mge1p Saccharomyces cerevisiae mitochondrial proteins in *Escherichia coli*. J Bacteriol 179:6066–6075

Deloche O, Liberek K, Zylicz M et al (1997b) Purification and biochemical properties of Saccharomyces cerevisiae Mdj1p, the mitochondrial DnaJ homologue. J Biol Chem 272:28539–28544

Diaz de la Loza Mdel C, Gallardo M, Garcia-Rubio ML et al (2011) Zim17/Tim15 links mitochondrial iron-sulfur cluster biosynthesis to nuclear genome stability. Nucleic Acids Res 39:6002–6015

Dolezal P, Smid O, Rada P et al (2005) Giardia mitosomes and trichomonad hydrogenosomes share a common mode of protein targeting. Proc Natl Acad Sci U S A 102:10924–10929

D'Silva PD, Schilke B, Walter W et al (2003) J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix. Proc Natl Acad Sci U S A 100:13839–13844

D'Silva P, Liu Q, Walter W et al (2004) Regulated interactions of mtHsp70 with Tim44 at the translocon in the mitochondrial inner membrane. Nat Struct Mol Biol 11:1084–1091

D'Silva PR, Schilke B, Walter W et al (2005) Role of Pam16's degenerate J domain in protein import across the mitochondrial inner membrane. Proc Natl Acad Sci U S A 102:12419–12424

D'Silva PR, Schilke B, Hayashi M et al (2008) Interaction of the J-protein heterodimer Pam18/Pam16 of the mitochondrial import motor with the translocon of the inner membrane. Mol Biol Cell 19:424–432

Duchniewicz M, Germaniuk A, Westermann B et al (1999) Dual role of the mitochondrial chaperone Mdj1p in inheritance of mitochondrial DNA in yeast. Mol Cell Biol 19:8201–8210

Dutkiewicz R, Schilke B, Cheng S et al (2004) Sequence-specific interaction between mitochondrial Fe-S scaffold protein Isu and Hsp70 Ssq1 is essential for their in vivo function. J Biol Chem 279:29167–29174

Dutkiewicz R, Schilke B, Knieszner H et al (2003) Ssq1, a mitochondrial Hsp70 involved in iron-sulfur (Fe/S) center biogenesis. Similarities to and differences from its bacterial counterpart. J Biol Chem 278:29719–29727

Dutkiewicz R, Marszalek J, Schilke B et al (2006) The Hsp70 chaperone Ssq1p is dispensable for iron-sulfur cluster formation on the scaffold protein Isu1p. J Biol Chem 281:7801–7808

Elsner S, Simian D, Iosefson O et al (2009) The mitochondrial protein translocation motor: structural conservation between the human and yeast Tim14/Pam18-Tim16/Pam16 co-chaperones. Int J Mol Sci 10:2041–2053

Endo T, Yamano K, Kawano S (2011) Structural insight into the mitochondrial protein import system. Biochim Biophys Acta 1808:955–970

Fraga H, Papaleo E, Vega S et al (2013) Zinc induced folding is essential for TIM15 activity as an mtHsp70 chaperone. Biochim Biophys Acta 1830:2139–2149

Frazzon J, Dean DR (2003) Formation of iron-sulfur clusters in bacteria: an emerging field in bioinorganic chemistry. Curr Opin Chem Biol 7:166–173

Fuzery AK, Tonelli M, Ta DT et al (2008) Solution structure of the iron-sulfur cluster cochaperone HscB and its binding surface for the iron-sulfur assembly scaffold protein IscU. Biochemistry 47:9394–9404

Fuzery AK, Oh JJ, Ta DT et al (2011) Three hydrophobic amino acids in *Escherichia coli* HscB make the greatest contribution to the stability of the HscB-IscU complex. BMC Biochem 12:3
Hayashi M, Schilke B, Marszalek J et al (2011) Ancient gene duplication provided a key molecular step for anaerobic growth of Baker's yeast. Mol Biol Evol 28:2005–2017
Heyrovska N, Frydman J, Hohfeld J et al (1998) Directionality of polypeptide transfer in the mitochondrial pathway of chaperone-mediated protein folding. Biol Chem 379:301–309
Hill K, Model K, Ryan MT et al (1998) Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins [see comment]. Nature 395:516–521
Horst M, Oppliger W, Rospert S et al (1997) Sequential action of two hsp70 complexes during protein import into mitochondria. Embo J 16:1842–1849
Hutu DP, Guiard B, Chacinska A et al (2008) Mitochondrial protein import motor: differential role of Tim44 in the recruitment of Pam17 and J-complex to the presequence translocase. Mol Biol Cell 19:2642–2649
Huynen MA, Snel B, Bork P et al (2001) The phylogenetic distribution of frataxin indicates a role in iron-sulfur cluster protein assembly. Hum Mol Genet 10:2463–2468
Iosefson O, Levy R, Marom M et al (2007) The Pam18/Tim14-Pam16/Tim16 complex of the mitochondrial translocation motor: the formation of a stable complex from marginally stable proteins. Protein Sci 16:316–322
Jensen RE, Dunn CD (2002) Protein import into and across the mitochondrial inner membrane: role of the TIM23 and TIM22 translocons. Biochim Biophys Acta 1592:25–34
Josyula R, Jin Z, Fu Z et al (2006) Crystal structure of yeast mitochondrial peripheral membrane protein Tim44p C-terminal domain. J Mol Biol 359:798–804
Kawula TH, Lelivelt MJ (1994) Mutations in a gene encoding a new Hsp70 suppress rapid DNA inversion and bgl activation, but not proU derepression, in hns-1 mutant *Escherichia coli*. J Bacteriol 176:610–619
Kim R, Saxena S, Gordon DM et al (2001) J-domain protein, Jac1p, of yeast mitochondria required for iron homeostasis and activity of Fe-S cluster proteins. J Biol Chem 276:17524–17532
Knight SA, Sepuri NB, Pain D et al (1998) Mt-Hsp70 homolog, Ssc2p, required for maturation of yeast frataxin and mitochondrial iron homeostasis. J Biol Chem 273:18389–18393
Koehler CM (2004) New developments in mitochondrial assembly. Annu Rev Cell Dev Biol 20:309–335
Kozany C, Mokranjac D, Sichting M et al (2004) The J domain-related cochaperone Tim16 is a constituent of the mitochondrial TIM23 preprotein translocase. Nat Struct Mol Biol 11:234–241
Krimmer T, Rassow J, Kunau WH et al (2000) Mitochondrial protein import motor: the ATPase domain of matrix Hsp70 is crucial for binding to Tim44, while the peptide binding domain and the carboxy-terminal segment play a stimulatory role. Mol Cell Biol 20:5879–5887
Kubo Y, Tsunehiro T, Nishikawa S et al (1999) Two distinct mechanisms operate in the reactivation of heat-denatured proteins by the mitochondrial Hsp70/Mdj1p/Yge1p chaperone system. J Mol Biol 286:447–464
Laloraya S, Gambill BD, Craig EA (1994) A role for a eukaryotic GrpE-related protein, Mge1p, in protein translocation. Proc Natl Acad Sci U S A 91:6481–6485
Laloraya S, Dekker PJ, Voos W et al (1995) Mitochondrial GrpE modulates the function of matrix Hsp70 in translocation and maturation of preproteins. Mol Cell Biol 15:7098–7105
Lewrenz I, Rietzschel N, Guiard B et al (2013) The functional interaction of mitochondrial Hsp70s with the escort protein Zim17 is critical for Fe/S biogenesis and substrate interaction at the inner membrane preprotein translocase. J Biol Chem 288:30931–30943
Li Y, Dudek J, Guiard B et al (2004) The presequence translocase-associated protein import motor of mitochondria. Pam16 functions in an antagonistic manner to Pam18. J Biol Chem 279:38047–38054
Lithgow T (2000) Targeting of proteins to mitochondria. FEBS Lett 476:22–26
Liu Q, Krzewska J, Liberek K et al (2001) Mitochondrial Hsp70 Ssc1: role in protein folding. J Biol Chem 276:6112–6118

Liu Q, D'Silva P, Walter W et al (2003) Regulated cycling of mitochondrial Hsp70 at the protein import channel. Science 300:139–141
Lutz T, Westermann B, Neupert W et al (2001) The mitochondrial proteins Ssq1 and Jac1 are required for the assembly of iron sulfur clusters in mitochondria. J Mol Biol 307:815–825
Lytovchenko O, Melin J, Schulz C et al (2013) Signal recognition initiates reorganization of the presequence translocase during protein import. EMBO J 32:886–898
Maarse AC, Blom J, Grivell LA et al (1992) MPI1, an essential gene encoding a mitochondrial membrane protein, is possibly involved in protein import into yeast mitochondria. EMBO J 11:3619–3628
Majewska J, Ciesielski SJ, Schilke B et al (2013) Binding of the chaperone Jac1 protein and cysteine desulfurase Nfs1 to the iron-sulfur cluster scaffold Isu protein is mutually exclusive. J Biol Chem 288:29134–29142
Manning-Krieg UC, Scherer PE, Schatz G (1991) Sequential action of mitochondrial chaperones in protein import into the matrix. Embo J 10:3273–3280
Mapa K, Sikor M, Kudryavtsev V et al (2010) The conformational dynamics of the mitochondrial Hsp70 chaperone. Mol Cell 38:89–100
Marada A, Allu PK, Murari A et al (2013) Mge1, a nucleotide exchange factor of Hsp70, acts as an oxidative sensor to regulate mitochondrial Hsp70 function. Mol Biol Cell 24:692–703
Marom M, Safonov R, Amram S et al (2009) Interaction of the Tim44 C-terminal domain with negatively charged phospholipids. Biochemistry 48:11185–11195
Marom M, Dayan D, Demishtein-Zohary K et al (2011) Direct interaction of mitochondrial targeting presequences with purified components of the TIM23 protein complex. J Biol Chem 286:43809–43815
Mayer MP (2004) Timing the catch. Nat Struct Mol Biol 11:6–8
Merlin A, Voos W, Maarse AC et al (1999) The J-related segment of tim44 is essential for cell viability: a mutant Tim44 remains in the mitochondrial import site, but inefficiently recruits mtHsp70 and impairs protein translocation. J Cell Biol 145:961–972
Mokranjac D, Sichting M, Neupert W et al (2003) Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria. Embo J 22:4945–4956
Mokranjac D, Sichting M, Popov-Celeketic D et al (2005) The import motor of the yeast mitochondrial TIM23 preprotein translocase contains two different J proteins, Tim14 and Mdj2. J Biol Chem 280:31608–31614
Mokranjac D, Bourenkov G, Hell K et al (2006) Structure and function of Tim14 and Tim16, the J and J-like components of the mitochondrial protein import motor. EMBO J 25:4675–4685
Mokranjac D, Berg A, Adam A et al (2007) Association of the Tim14.Tim16 subcomplex with the TIM23 translocase is crucial for function of the mitochondrial protein import motor. J Biol Chem 282:18037–18045
Mokranjac D, Neupert W (2010) The many faces of the mitochondrial TIM23 complex. Biochim Biophys Acta 1797:1045–1054
Momose T, Ohshima C, Maeda M et al (2007) Structural basis of functional cooperation of Tim15/Zim17 with yeast mitochondrial Hsp70. EMBO Rep 8:664–670
Moro F, Okamoto K, Donzeau M et al (2002) Mitochondrial protein import: molecular basis of the ATP-dependent interaction of MtHsp70 with Tim44. J Biol Chem 277:6874–6880
Muhlenhoff U, Lill R (2000) Biogenesis of iron-sulfur proteins in eukaryotes: a novel task of mitochondria that is inherited from bacteria. Biochim Biophys Acta 1459:370–382
Muhlenhoff U, Gerber J, Richhardt N et al (2003) Components involved in assembly and dislocation of iron-sulfur clusters on the scaffold protein Isu1p. Embo J 22:4815–4825
Pais JE, Schilke B, Craig EA (2011) Reevaluation of the role of the Pam18:Pam16 interaction in translocation of proteins by the mitochondrial Hsp70-based import motor. Mol Biol Cell 22:4740–4749
Pfanner N (2000) Protein sorting: recognizing mitochondrial presequences. Curr Biol 10:R412–415
Pfanner N, Geissler A (2001) Versatility of the mitochondrial protein import machinery. Nat Rev Mol Cell Biol 2:339–349

Pfanner N, Wiedemann N, Meisinger C et al (2004) Assembling the mitochondrial outer membrane. Nat Struct Mol Biol 11:1044–1048
Popov-Celeketic D, Mapa K, Neupert W et al (2008) Active remodelling of the TIM23 complex during translocation of preproteins into mitochondria. EMBO J 27:1469–1480
Prip-Buus C, Westerman B, Schmitt M et al (1996) Role of the mitochondrial DnaJ homologue, Mdj1p, in the prevention of heat-induced protein aggregation. FEBS Lett 380:142–146
Pukszta S, Schilke B, Dutkiewicz R et al (2010) Co-evolution-driven switch of J-protein specificity towards an Hsp70 partner. EMBO Rep 11:360–365
Rada P, Dolezal P, Jedelsky PL et al (2011) The core components of organelle biogenesis and membrane transport in the hydrogenosomes of Trichomonas vaginalis. PloS One 6:e24428
Rassow J, Maarse AC, Krainer E et al (1994) Mitochondrial protein import: biochemical and genetic evidence for interaction of matrix hsp70 and the inner membrane protein MIM44. J Cell Biol 127:1547–1556
Rassow J, Dekker PJ, van Wilpe S et al (1999) The preprotein translocase of the mitochondrial inner membrane: function and evolution. J Mol Biol 286:105–120
Rowley N, Prip-Buus C, Westermann B et al (1994) Mdj1p, a novel chaperone of the DnaJ family, is involved in mitochondrial biogenesis and protein folding. Cell 77:249–259
Sanjuan Szklarz LK, Guiard B, Rissler M et al (2005) Inactivation of the mitochondrial heat shock protein zim17 leads to aggregation of matrix hsp70s followed by pleiotropic effects on morphology and protein biogenesis. J Mol Biol 351:206–218
Scherer PE, Manning-Krieg UC, Jeno P et al (1992) Identification of a 45-kDa protein at the protein import site of the yeast mitochondrial inner membrane. Proc Natl Acad Sci U S A 89:11930–11934
Schilke B, Forster J, Davis J et al (1996) The cold sensitivity of a mutant of Saccharomyces cerevisiae lacking a mitochondrial heat shock protein 70 is suppressed by loss of mitochondrial DNA. J Cell Biol 134:603–613
Schilke B, Voisine C, Beinert H et al (1999) Evidence for a conserved system for iron metabolism in the mitochondria of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 96:10206–10211
Schilke B, Williams B, Knieszner H et al (2006) Evolution of mitochondrial chaperones utilized in Fe-S cluster biogenesis. Curr Biol 16:1660–1665
Schilke BA, Hayashi M, Craig EA (2012) Genetic analysis of complex interactions among components of the mitochondrial import motor and translocon in Saccharomyces cerevisiae. Genetics 190:1341–1353
Schiller D (2009) Pam17 and Tim44 act sequentially in protein import into the mitochondrial matrix. Int J Biochem Cell Biol 41:2343–2349
Schiller D, Cheng YC, Liu Q et al (2008) Residues of Tim44 involved in both association with the translocon of the inner mitochondrial membrane and regulation of mitochondrial Hsp70 tethering. Mol Cell Biol 28:4424–4433
Schmidt S, Strub A, Rottgers K et al (2001) The two mitochondrial heat shock proteins 70, Ssc1 and Ssq1, compete for the cochaperone Mge1. J Mol Biol 313:13–26
Schneider HC, Westermann B, Neupert W et al (1996) The nucleotide exchange factor MGE exerts a key function in the ATP-dependent cycle of mt-Hsp70-Tim44 interaction driving mitochondrial protein import. EMBO J 15:5796–5803
Seaton BL, Vickery LE (1994) A gene encoding a DnaK/hsp70 homolog in *Escherichia coli*. Proc Natl Acad Sci U S A 91:2066–2070
Sichting M, Mokranjac D, Azem A et al (2005) Maintenance of structure and function of mitochondrial Hsp70 chaperones requires the chaperone Hep1. EMBO J 24:1046–1056
Sinha D, Joshi N, Chittoor B et al (2010) Role of Magmas in protein transport and human mitochondria biogenesis. Hum Mol Genet 19:1248–1262
Slutsky-Leiderman O, Marom M, Iosefson O et al (2007) The interplay between components of the mitochondrial protein translocation motor studied using purified components. J Biol Chem 282:33935–33942
Strub A, Rottgers K, Voos W (2002) The Hsp70 peptide-binding domain determines the interaction of the ATPase domain with Tim44 in mitochondria. Embo J 21:2626–2635

Takahashi Y, Tokumoto U (2002) A third bacterial system for the assembly of iron-sulfur clusters with homologs in archaea and plastids. J Biol Chem 277:28380–28383
Tokumoto U, Takahashi Y (2001) Genetic analysis of the isc operon in *Escherichia coli* involved in the biogenesis of cellular iron-sulfur proteins. J Biochem (Tokyo) 130:63–71
Truscott KN, Brandner K, Pfanner N (2003a) Mechanisms of protein import into mitochondria. Curr Biol 13:R326–337
Truscott KN, Voos W, Frazier AE et al (2003b) A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria. J Cell Biol 163:707–713
van der Laan M, Chacinska A, Lind M et al (2005) Pam17 is required for architecture and translocation activity of the mitochondrial protein import motor. Mol Cell Biol 25:7449–7458
van der Laan M, Hutu DP, Rehling P (2010) On the mechanism of preprotein import by the mitochondrial presequence translocase. Biochim Biophys Acta 1803:732–739
Voisine C, Schilke B, Ohlson M et al (2000) Role of the mitochondrial Hsp70s, Ssc1 and Ssq1, in the maturation of Yfh1. Mol Cell Biol 20:3677–3684
Voisine C, Cheng YC, Ohlson M et al (2001) Jac1, a mitochondrial J-type chaperone, is involved in the biogenesis of Fe/S clusters in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 98:1483–1488
Voos W, Gambill BD, Laloraya S et al (1994) Mitochondrial GrpE is present in a complex with hsp70 and preproteins in transit across membranes. Mol Cell Biol 14:6627–6634
Voos W, von Ahsen O, Muller H et al (1996) Differential requirement for the mitochondrial Hsp70-Tim44 complex in unfolding and translocation of preproteins. EMBO J 15:2668–2677
Walsh P, Bursac D, Law YC et al (2004) The J-protein family: modulating protein assembly, disassembly and translocation. EMBO Rep 5:567–571
Weiss C, Oppliger W, Vergeres G et al (1999) Domain structure and lipid interaction of recombinant yeast Tim44. Proc Natl Acad Sci U S A 96:8890–8894
Westermann B, Neupert W (1997) Mdj2p, a novel DnaJ homolog in the mitochondrial inner membrane of the yeast Saccharomyces cerevisiae. J Mol Biol 272:477–483
Westermann B, Prip-Buus C, Neupert W et al (1995) The role of the GrpE homologue, Mge1p, in mediating protein import and protein folding in mitochondria. EMBO J 14:3452–3460
Westermann B, Gaume B, Herrmann JM et al (1996) Role of the mitochondrial DnaJ homolog Mdj1p as a chaperone for mitochondrially synthesized and imported proteins. Mol Cell Biol 16:7063–7071
Willmund F, Hinnenberger M, Nick S et al (2008) Assistance for a chaperone: Chlamydomonas HEP2 activates plastidic HSP70B for cochaperone binding. J Biol Chem 283:16363–16373
Yamamoto H, Momose T, Yatsukawa Y et al (2005) Identification of a novel member of yeast mitochondrial Hsp70-associated motor and chaperone proteins that facilitates protein translocation across the inner membrane. FEBS Lett 579:507–511

Chapter 11
CHIP: A Co-chaperone for Degradation by the Proteasome

Adrienne L. Edkins

Abstract Protein homeostasis relies on a balance between protein folding and protein degradation. Molecular chaperones like Hsp70 and Hsp90 fulfil well-defined roles in protein folding and conformational stability via ATP dependent reaction cycles. These folding cycles are controlled by associations with a cohort of non-client protein co-chaperones, such as Hop, p23 and Aha1. Pro-folding co-chaperones facilitate the transit of the client protein through the chaperone mediated folding process. However, chaperones are also involved in ubiquitin-mediated proteasomal degradation of client proteins. Similar to folding complexes, the ability of chaperones to mediate protein degradation is regulated by co-chaperones, such as the C terminal Hsp70 binding protein (CHIP). CHIP binds to Hsp70 and Hsp90 chaperones through its tetratricopeptide repeat (TPR) domain and functions as an E3 ubiquitin ligase using a modified RING finger domain (U-box). This unique combination of domains effectively allows CHIP to network chaperone complexes to the ubiquitin-proteasome system. This chapter reviews the current understanding of CHIP as a co-chaperone that switches Hsp70/Hsp90 chaperone complexes from protein folding to protein degradation.

Keywords CHIP · STUB1 · Ubiquitin · Proteasome

Introduction to Ubiquitin Modification and Proteasomal Degradation of Proteins

The ubiquitin proteasome system is a highly conserved mechanism through which eukaryotic cells facilitate the controlled enzymatic degradation of unwanted proteins (Amm et al. 2014; Ciechanover 1998). The ubiquitin and proteasome systems work in concert to regulate protein levels in eukaryotic cells (Roos-Mattjus and Sistonen 2004; Wolf et al. 2004; Lecker et al. 2006). The proteasome not

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,* Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_11

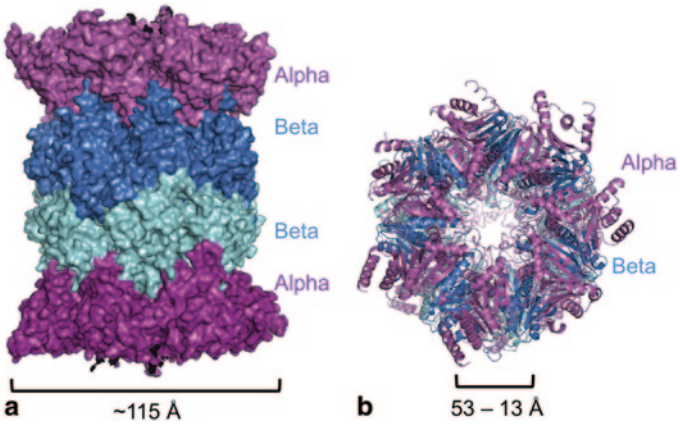

Fig. 11.1 Structure of the 20S proteasome. The proteasome forms a barrel shaped structure in which the proteolytic residues are deep within the central channel through which substrates to be degraded must pass. The 20S subunit is formed of two heptameric ring structures composed of beta subunits, which is bounded by single rings of a similar heptameric ring structure composed of alpha subunits. **a** Surface rendering of a side view and **b** cartoon representation of the top view of the 20S core catalytic particle of the proteasome. The alpha and beta subunits are shown in shades of purple and blue respectively. The image was generated using Pymol (DeLano Scientific). PDB code: 1FNT

only regulates the degradation of incorrectly folded proteins, but is also important for the degradation of proteins whose expression needs to be temporally regulated (such as cell cycle kinases) (Wagner et al. 2011). The proteasome is a large (>2 MDa), multiprotein complex that comprises the major non-lysosomal degradation machinery for cytosolic and nuclear proteins (Fig. 11.1). The structure resembles a barrel-like assembly with a central proteolytic cavity through which substrate proteins are degraded (Bedford et al. 2010; Tanaka 2009). The proteasome can be divided into two components, namely the 20S core proteasome and the 19S regulatory components. A single 20S core proteasome associates with two 19S regulatory particles to form the active 26S proteasome (Murata et al. 2009; Walz et al. 1998). The location of the proteolytic sites within the central cavity of the 20S proteasome allows protein degradation to be compartmentalised (da Fonseca and Morris 2008; Heinemeyer et al. 2004). The core proteasome particle contains stacked ring structures built from 7 copies of either an alpha or beta subunit (Fig. 11.1). The inner two heptameric beta rings form the central catalytic component of the proteasome. This central unit is bounded on either side by an equivalent outer ring structure made up of 7 copies of the structural alpha subunit (Tanaka 2009; Unverdorben et al. 2014; da Fonseca and Morris 2008). Whereas the beta subunit complex contains the catalytic protease sites, the outer ring of alpha subunits serves as a 'gate' to restrict unregulated entry of proteins into the catalytic cavity (da Fonseca and Morris 2008). The alpha subunit rings also act as the docking sites for interaction with the regulatory 19S particle of the

proteasome. The 19S regulatory subunit is a protein complex that functions to activate the 20S particle for protein degradation (Unverdorben et al. 2014). The size of the central pore of the proteasome ranges between 50–13 Å and entry is restricted by the alpha subunit gate. The 19S proteasome is required to open the gate in the 20S proteasome, thereby permitting entry of substrate proteins into the catalytic sites within the proteasome core (da Fonseca and Morris 2008; Sledz et al. 2013; Unverdorben et al. 2014). Polyubiquitinated proteins targeted for degradation need to be deubiquitinated and delivered to the proteolytic active site of the proteasome that is buried within the 20S core particle. The substrate is thought to be partially unfolded during translocation into the cavity. Within the proteasome core, substrate peptide bonds are hydrolysed by nucleophilic attack dependent on catalytic threonine residues that extend into the cavity from the beta subunits. The resultant peptides released from the proteasome range between 4 and 25 residues (Babbitt et al. 2005; Ortega et al. 2005; Goldberg et al. 1997).

Degradation of proteins by the proteasome is preceded by the conjugation of ubiquitin to the substrate via a series of sequential enzyme catalysed reactions (Fig. 11.2) (Hershko and Ciechanover 1998). Ubiquitin is a small, abundant protein (~8 kDa) found in all eukaryotic cells which, when added to proteins in a polyubiquitin chain, functions as the degradation signal (Smith 1988; Johnson et al. 1992; Johnson et al. 1995). Ubiquitin is initially activated by conjugation to an ubiquitin activating enzyme (E1) in an ATP dependent manner via a thioester linkage (Lee and Schindelin 2008). Ubiquitin is subsequently transferred via an ubiquitin conjugating enzyme (E2) intermediate to the substrate protein targeted for degradation (Olsen and Lima 2013). This reaction is catalysed by an ubiquitin ligase enzyme (E3) and results in the formation of a peptide bond between a glycine residue in the C-terminus of ubiquitin and lysine residues within the substrate protein (Scheffner et al. 1995; Wilkinson 2000). There are a range of different E2 and E3 isoforms that may combine for different substrate proteins, suggesting a diverse and discriminatory recognition system for ubiquitin conjugation (Spratt et al. 2012). This process may be repeated a number of times, often involving the conjugation of subsequent ubiquitin molecules to lysines within ubiquitin itself, leading to the formation of covalently linked polyubiquitin chains. A fourth enzyme may also be involved in this cascade. Known as E4, this protein acts as an ubiquitin chain elongation enzyme to catalyse the assembly of polyubiquitin chains on protein substrates (Koegl et al. 1999). While monoubiquitination may induce changes in activity or subcellular localisation of proteins, the conjugation of a polyubiquitin chain to a substrate protein is required for degradation by the proteasome (Johnson et al. 1992). Ubiquitin contains 7 lysines residues (K6, K11, K27, K29, K33, K48, and K63), in addition to its N-terminus, which act as potential sites of conjugation. The lysine residue involved in the bond can also impact on the outcome of ubiquitination (Hershko and Ciechanover 1998). K48 linked ubiquitin chains, where the covalent linkage of the ubiquitin chain is via the K48 residue of ubiquitin, is the canonical signal for proteasomal degradation (Jacobson et al. 2009). K48 linkage is regulated

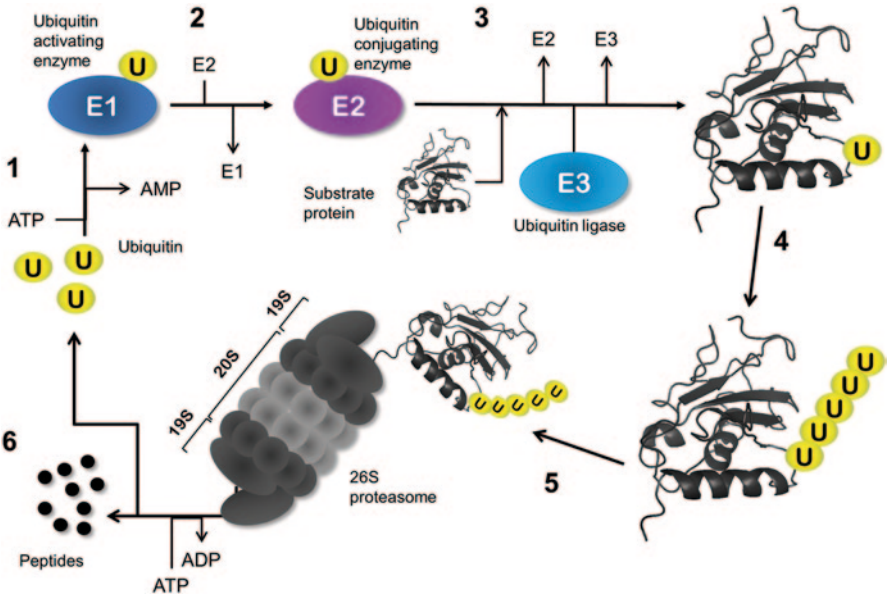

Fig. 11.2 Degradation of proteins via the ubiquitin-proteasome system. Conjugation of polyubiquitin chains to substrate proteins is catalysed as part of an ATP dependent enzyme catalysed cascade that precedes protein recognition and degradation by the proteasome. (*1*) The process is initiated by the activation of ubiquitin via conjugation to an ubiquitin activating enzyme (E1) via a thioester bond. (*2*) The ubiquitin moiety is subsequently transferred to ubiquitin conjugating enzyme (E2), which (*3*) subsequently forms a complex with a specific ubiquitin ligase (E3) and a substrate protein. (*4*) The E3 ligase transfers the ubiquitin to lysine residues within the substrate protein. This cycle is repeated multiple times to generate substrate proteins linked to polyubiquitin chains. (*5*) Polyubiquitination, particularly via K48 linkages, is the signal for transfer of substrate proteins to the proteasome. (*6*) At the proteasome, ubiquitinated substrates interact with the 19S regulatory particle which deubiquitinates them and passes them into the central cavity of the core 20S proteasome. Here in the active site, proteins are degraded and peptides released. Ubiquitin molecules can subsequently be recycled. 19S: regulatory particle, 20S: core particle

by members of the Ubc4, Ubc5 and Ubc7 E2 ubiquitin conjugating enzymes. In contrast, K63 ubiquitination may have a regulatory function (Jacobson et al. 2009) and is catalysed by the Ubc13 E2 in complex with other Ubc proteins (e.g. Uev1a) (Hofmann and Pickart 1999; Sun and Chen 2004). Most often, the selectivity of protein degradation is controlled by the E3 ligase. These E3 ligases integrate with the cellular molecular chaperone system, which is used as the recognition system of the misfolded substrate during this process. In this way, the numerous different E3 isoforms, each of which may be specific for certain protein substrates, are able to utilise the innate ability of chaperones to capture a range of misfolded substrates, to target specific proteins for degradation (Kriegenburg et al. 2012; Esser et al. 2004).

Molecular Chaperones and the Ubiquitin-Proteasome System

A molecular chaperone is a protein that participates in the conformational regulation and folding of a range of substrate proteins, known as client proteins. Chaperones function under physiological conditions to maintain protein homeostasis, and are also vital during or after stressful conditions to prevent or reverse the potentially disastrous consequences of protein aggregation for the cell (Agashe and Hartl 2000; Landry and Gierasch 1994; Hartl 1996; Welch and Brown 1996; Fedorov and Baldwin 1997; Ellis 1997). Many molecular chaperones are members of the heat shock protein family (HSP). In particular, the Hsp70 and Hsp90 chaperones, as part of multiprotein complexes, regulate both *de novo* and stress-related protein folding and stability. Hsp70 and Hsp90 are ATP-dependent chaperones who participate in protein folding cycles that involve multiple phases of client binding and release (Wegele et al. 2004). The activities of both chaperone complexes is dependent on interactions with a number of protein cofactors, known as co-chaperones. A co-chaperone is defined as a non-client accessory protein that lacks intrinsic chaperone activity, but functions to modulate the activity of a known chaperone (Caplan 2003). Co-chaperones act at every stage of the chaperone folding cycle and control progression of the client protein through these cycles by a range of mechanisms, including regulation of ATPase activity, direct protein-protein interactions and posttranslational modifications. Co-chaperones may be selective for one particular family of chaperones, or may interact with multiple chaperone families (Li et al. 2012).

The Hsp70-Hsp40 chaperone complex is one of the main foldase complexes in the cell, participating in both *de novo* and stress-related protein folding (Landry and Gierasch 1994; Cheetham et al. 1994; Strickland et al. 1997; Hiromura et al. 1998). Hsp40 co-chaperones deliver client proteins to Hsp70 and regulate the affinity of Hsp70 for these client proteins by stimulating the ATPase activity of Hsp70 (Cheetham et al. 1994). The stimulation of Hsp70 ATPase converts Hsp70 into the high affinity substrate binding form and leads to binding of the client protein by Hsp70 and prevention of misfolding or aggregation. This form of Hsp70 is ADP bound and is stabilised by another co-chaperone, known as Hsc70/Hsp70 interacting protein (Hip). Client proteins are subsequently released from Hsp70 via nucleotide exchange, which returns the Hsp70 to the ATP bound form, which has low affinity for the substrate. This stage is catalysed by the GrpE (in prokaryotes) or BAG1/HspBP1 (in eukaryotes) co-chaperones (Hohfeld 1998; Chang et al. 2010; Mao et al. 2006; Kabani et al. 2002).

A subset of client proteins will be passed from the Hsp70 chaperone complex to the Hsp90 chaperone complex. Hsp90 is also an ATP-dependent chaperone, but its function primarily relates to the maintenance of protein stability of labile client proteins prior to their activation (Prodromou et al. 1997; Panaretou et al. 1998) The Hsp90 complex is also able to stabilise a number of mutated client proteins, thereby preventing their degradation (Whitesell et al. 1998). Hsp90 is constitutively

dimerised at the C-terminus and exists in an open conformation (resembling a V shape) when inactive (Ali et al. 2006). Once bound by Hsp90, the client protein then transitions through the Hsp90 cycle, a process which involves the sequential interaction with a range of co-chaperones (McLaughlin et al. 2002; Li et al. 2012). The early stages of Hsp90 mediated folding involve the transfer of client proteins between Hsp70 and Hsp90, catalysed by the co-chaperone Hop (Frydman and Hohfeld 1997; Brinker et al. 2002; Siligardi et al. 2004). Hop is a member of the TPR (tetratricopeptide repeat) domain containing co-chaperones that is able to simultaneously bind to both Hsp70 and Hsp90 (Prodromou et al. 1999). The TPR domain is a protein-protein interaction module that is found in a wide range of proteins (Allan and Ratajczak 2011). TPR domains are comprised of multiple copies of a TPR motif, which gives rise to a particular alpha helical structure. The TPR motif is loosely defined by a 34 residue degenerate consensus sequence (Tpr-Leu-Gly-Tyr-Ala-Phe-Ala-Pro). Therefore, while the primary sequence varies substantially between different TPR domains, the overall structure is conserved. Most TPR domains are comprised of three TPR motifs, each of which contributes 6 alpha helices, which pack together to form an alpha helical amphipathic groove. This groove is the site of interaction with the target peptide (Brinker et al. 2002). TPR containing chaperones bind to the C terminal EEVD motifs contained in both Hsp70 and Hsp90 (Odunuga et al. 2003; Blatch and Lassle 1999; Van Der Spuy et al. 2000). Hop binds to the complex as a monomer via one EEVD motif in the Hsp90 dimer, while the other EEVD motif may be bound by a peptidyl prolyl isomerase (PPIase) leading to the formation of the asymmetric intermediate complex. The binding of ATP and the late co-chaperones p23 stimulate conformational change in Hsp90 to the closed conformation, with a concomitant dissociation of Hop from the complex (Prodromou et al. 2000). ATP hydrolysis by Hsp90 is subsequently stimulated by Aha1, which returns Hsp90 to the open conformation and results in release of the client protein (Lotz et al. 2003). Co-chaperones like Hop, p23 and Aha1 are considered general co-chaperones, as they constitute the core co-chaperones required for the transition of general client proteins through the cycle. In addition, there exist a range of other co-chaperones that may associate with Hsp90 complex for specific functions, such as regulation of specific classes of client proteins (like Cdc37) or post-translational modification (e.g. PP5) (Li et al. 2012).

The term chaperone is associated with protein folding. However, chaperones also participate in the degradation of proteins via the proteasome (Kriegenburg et al. 2012; Esser et al. 2004; Kettern et al. 2010; Kastle and Grune 2012). This role for chaperones is opposing, yet complementary, to their role in promoting protein folding and is consistent with a role as regulators of global protein homeostasis (Hohfeld et al. 2001; Imai et al. 2003). Molecular chaperones are thought to be able to identify and capture misfolded protein substrates in order for them to be directed to the proteasome (Bercovich et al. 1997; Kriegenburg et al. 2012; Meimaridou et al. 2009). The involvement of chaperones, particularly Hsp70 and Hsp90, in proteasomal mediated protein degradation is also regulated by co-chaperones, including the Hsp70/Hsp90 co-chaperone, carboxyl terminus of Hsp70-interacting protein (CHIP) (Murata et al. 2001; McDonough and Patterson 2003).

The Carboxyl Terminus of Hsp70-Interacting Protein (CHIP)

The carboxyl terminus of Hsp70-interacting protein (CHIP; also known as STIP1 homology and U-box containing protein 1 or STUB1) has dual functions, one as a co-chaperone of Hsp70 and Hsp90, and the other as an E3 ubiquitin ligase to regulate proteasomal degradation of chaperone client proteins (McDonough and Patterson 2003; Ballinger et al. 1999). In this way, CHIP is a major link between chaperone mediated folding and protein degradation. CHIP is distinguished from the other Hsp90 co-chaperones in that it is primarily involved in tuning the chaperone complexes towards protein degradation, rather than supporting protein folding (Demand et al. 2001). The CHIP gene is conserved in a range of eukaryotes, being demonstrated or predicted to exist in the genomes of the human, monkey, mouse, zebrafish, fruit fly, frog, and even the genome and transcriptome of the recently sequenced Coelacanth (*Latimeria sp.*) (Tastan Bishop et al. 2014). CHIP knockout mice were viable and displayed normal development, suggesting that CHIP is not an essential gene (Morishima et al. 2008; Dai et al. 2003). However, there was increased peripartum mortality of CHIP null mice compared to wild type mice. This was attributed to wasting of the thymus, which is an indicator of reduced ability to cope with stress. The link between peripartum death of CHIP null mice and stress was subsequently supported by the fact that CHIP-/- mice were temperature sensitive and that induction of stress in these animals induced apoptosis in multiple organs after challenge (Dai et al. 2003). CHIP overexpression activated the stress response by specifically inducing trimerization and nuclear translocation of HSF-1 and activation of HSE containing stress responsive promoters, like Hsp70 (Dai et al. 2003). This suggests that the role of CHIP is not exclusively linked to protein degradation, but also involves regulation of the stress response.

Notwithstanding its role in activation of the stress response, CHIP appears to be a master regulator of protein degradation via chaperones, although it is by no means the only co-chaperone associated with protein degradation. The Hsp40 isoform, Hsj1 (DNAJB2) (Chapple et al. 2004; Westhoff et al. 2005; Gao et al. 2011) and the nucleotide exchange factor, BAG-1, both have defined roles in proteasome-mediated protein degradation (Luders et al. 2000; Alberti et al. 2002; Alberti et al. 2003; Elliott et al. 2007). In addition, there are other E3 ubiquitin ligase proteins (e.g. Ubr1, Cul5, Parkin, Mdm2) that may associate with Hsp90 and/or Hsp70 chaperone complexes to target client proteins for ubiquitination and degradation (Nillegoda et al. 2010; Eisele and Wolf 2008; Ehrlich et al. 2009). These proteins are able to induce ubiquitination in the absence of CHIP, although there is also evidence that inhibition of these E3 ligases can affect protein folding and degradation even in the presence of CHIP. It is clear that functional redundancy exists between the E3 ligases and is possible that multiple members will collaborate as a complex to control degradation of specific proteins (Morishima et al. 2008).

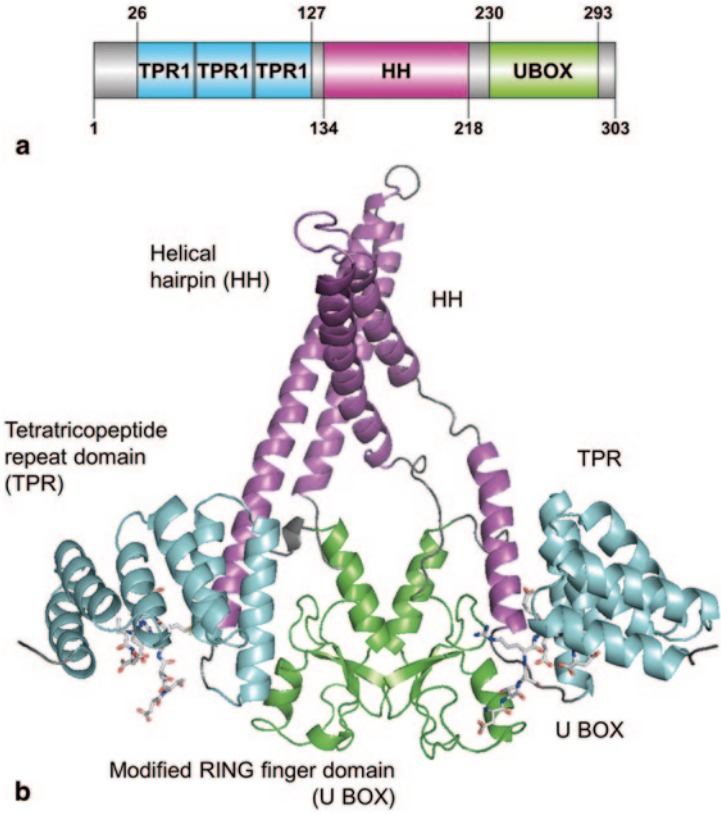

Fig. 11.3 Domain architecture and structure of CHIP. **a** Domain structure of CHIP showing the N-terminal TPR domain (composed of 3 TPR motifs) and the C terminal U-box separated by the helical hairpin region. **b** The asymmetric dimer structure observed in the crystal structure of murine CHIP. The U-box domain (green) is the point of dimerization. The structure of the TPR (cyan; helices 1–6) and U-box domains are largely conserved between the two protomers. The helical hairpin (magenta, helices 7–8) region differs substantially. The C-terminal MEEVD peptide from Hsp90 in shown in stick format and coloured grey. The image was generated using Pymol (DeLano Scientific). PDB code: 2C2L

Structure of CHIP

CHIP is a 35 kDa protein, expressed as a single isoform containing an N terminal TPR domain together with a U-box domain linked via a long helical region (Ballinger et al. 1999). The crystal structure of murine CHIP was determined in 2005 by Pearl and colleagues (Zhang et al. 2005b). This structure demonstrates that CHIP is dimerised at the C-terminus via the U-box domains (Fig. 11.3). The murine CHIP homodimer from this study was shown to be asymmetrical, with the two monomers of the dimer adopting different structures. The structural difference

in the two monomers is predominantly in the structure of a long helical region that links the TPR and U-box domains. This region, termed the helical hairpin, is formed from two continuous antiparallel alpha helices in an extended conformation in one protomer. In contrast, in the other monomer, the helical hairpin adopts a bent conformation and the seventh helix is split into two helices (as opposed to being a single continuous helix) (Fig. 11.3). C-terminal to the helical hairpin domain is the U-box domain, which is composed of beta hairpins separated by alpha helices. Interestingly, the structure of the helical hairpin and U-box region of *D. rerio* CHIP present a symmetrical dimer that differs from the asymmetric dimer observed in the crystal structure of the full length mouse CHIP (Xu et al. 2006). This discrepancy may be a consequence of the absence of the TPR domains in this structure, or may reflect the fact that crystal structures are static and may capture only one form of a dynamic structure. Consistent with this, recent studies demonstrate that the full length human CHIP homodimer appears highly flexible in solution (Graf et al. 2010). Specific changes in the CHIP conformation were noted upon interaction with either chaperones or E2 enzymes. Binding of Hsp70/Hsp90 chaperones or chaperone-derived peptides to CHIP promoted stabilisation of the TPR domains, while distinct changes were observed upon interaction with E2 conjugating enzymes (UbcH5a and Ubc13) (Graf et al. 2010).

Interaction of CHIP with Chaperones and E2 Ligases

The crystal structure of the CHIP dimer in complex with peptides from both Hsp90 and enzymes of the ubiquitin pathway demonstrates how the structure of CHIP has evolved to allow simultaneous interaction with chaperones and proteasomal substrates (Zhang et al. 2005a). The dimeric E3 ubiquitin ligase CHIP bound with its TPR domain the C-terminus of molecular chaperones Hsp70 and Hsp90 and with its U-box region E2 ubiquitin-conjugating enzymes (Zhang et al. 2005b; Ballinger et al. 1999; Xu et al. 2008). This unique combination of domains allows CHIP to bind to both chaperones Hsp70 and Hsp90, via its TPR domain, and to interact with the proteasome by acting as an E3 ligase using its U-box domain, effectively crosslinking the chaperones to the proteasome via ubiquitination of substrates for degradation.

CHIP was originally identified as co-chaperone for Hsp70 and Hsp90 in a screen for novel TPR-containing proteins (Ballinger et al. 1999). CHIP interacts with the C terminal EEVD motifs in both Hsp90 and Hsp70 via its TPR domains (Ballinger et al. 1999), a feature in common with other TPR containing co-chaperones, like Hop, PP5 and Hip (Allan and Ratajczak 2011; Brinker et al. 2002; Cortajarena and Regan 2006; Odunuga et al. 2003). The CHIP monomer contains only a single TPR domain which can bind indiscriminately to both Hsp90 and Hsp70. CHIP can bind two molecules of Hsp70 in a dynamic and flexible complex in which both CHIP and Hsp70 move independently of each other and is predicted to provide space

to accommodate additional client proteins (Smith et al. 2013). This independent movement is demonstrated by the fact that only the extreme C terminal residues (IEEVD) of Hsp70 appear to be involved in the interaction with CHIP. The IEEVD motif of Hsp70 was also required for ubiquitination of Hsp70, although a reduction in the length of the C terminal tail preceding the IEEVD led to reduced capacity to ubiquitinate Hsp70 (Smith et al. 2013). Although the TPR motif is the chaperone binding site of CHIP, there is evidence to suggest that allosteric interactions with the U-box domain are required for CHIP association with Hsp70 (Matsumura et al. 2013). Despite this, there was little difference in the affinity of binding to CHIP between full length Hsc70 and the C terminal IEEVD peptide (Smith et al. 2013). There are conflicting reports regarding the affinity of the interaction between CHIP and different chaperones, with reports that both Hsp70 binding (Kundrat and Regan 2010a) and Hsp90 binding to CHIP (Stankiewicz et al. 2010) is the greater affinity of the two.

As an E3 ubiquitin ligase, CHIP also interacts with members of the E2 family of ubiquitin conjugating enzymes during ubiquitination of substrates. These interactions between E2 and E3 proteins are highly specific and will ultimately determine the nature of ubiquitination that occurs, as well as the identity of the substrate protein. CHIP has been shown to interact with specific E2 from the UBCH5 family (which are involved in K48 mediated ubiquitination which promotes proteasomal degradation) (Cyr et al. 2002; Wiederkehr et al. 2002; Xu et al. 2008) and Ubc13 (which regulates K63 ubiquitination and has a regulatory role) (Alberti et al. 2002; Jiang et al. 2001; Murata et al. 2001; Zhang et al. 2005b). CHIP also displays E4 ligase activity, in that it can catalyse the extension of polyubiquitin chains on substrate proteins (Murata et al. 2001; Murata et al. 2003; Jiang et al. 2001; Koegl et al. 1999). CHIP interacts with E2 enzymes via its U-box domain. The U-box is composed of 70 amino acids and is structurally similar to the RING finger domains found in other ubiquitin ligases (Ohi et al. 2003). U-box containing ligases are distinct from HECT and RING finger E3 ligases and appear to associate almost exclusively with chaperones during ubiquitination of client proteins (Hatakeyama et al. 2004b; Hatakeyama et al. 2001; Kriegenburg et al. 2012).

CHIP binding can inhibit both Hsp90 and Hsp70- thereby preventing both protein folding by Hsp70 and conformational regulation of client proteins by Hsp90. CHIP blocked the Hsp40 mediated stimulation of Hsp70 ATPase activity and attenuated the function of Hip (Ballinger et al. 1999). The consequence of this is that CHIP promotes accumulation of the ATP-bound form of Hsp70. This prevented substrate binding and refolding of denatured luciferase *in vitro* (Ballinger et al. 1999). CHIP therefore regulates ubiquitination of Hsp70 client proteins through regulation of client protein affinity. CHIP did not affect ATP or ADP association, but blocked the Hsp40 mediated stimulation of Hsp70 ATPase activity (Stankiewicz et al. 2010). The consequence of this is that, indirectly, CHIP promotes an ATP bound form of Hsp70, one which has low affinity for client proteins. CHIP also works in concert with certain members of the Hsp70 nucleotide exchange family of proteins, including the Bcl2-associated athanogene (BAG). BAG-1

could bind simultaneously to both Hsp70 and the 26S proteasome (Luders et al. 2000). In doing so, BAG-1 induces the release of ubiquitinated client proteins from Hsp70 to the proteasome.

Addition of CHIP is also sufficient to modify the co-chaperone complement of the Hsp90 complex and induce degradation of canonical Hsp90 client proteins (Connell et al. 2001). CHIP did not affect the ATPase activity of Hsp90 (Stankiewicz et al. 2010), but CHIP binding did reduce Hop binding and prevented binding of p23 completely (Ballinger et al. 1999). Displacement of p23 relies on the presence of the CHIP TPR domains, while the ubiquitination of glucocorticoid receptor is U-box dependent; demonstrating defined functions for the two different domains, but that cooperation between them is required for protein degradation. This is interesting, as p23 and CHIP bind to different termini of the chaperone. CHIP may antagonise the action of p23, thereby stabilising the substrate protein within the complex for ubiquitination. CHIP activity does not require the N terminal domain of Hsp90 and an interaction with the C terminal region of Hsp90 is sufficient to allow CHIP-mediated ubiquitination of the client protein. This may suggest that client proteins that associate with different Hsp90 regions may be differentially susceptible to ubiquitination by CHIP.

The ability of CHIP to induce degradation requires the presence of the substrate protein in a denatured form, in addition to the chaperone, as demonstrated by the fact that denatured, but not native, luciferase could be ubiquitinated *in vitro* in the presence of Ubc4/5 (E2 enzyme) and the Hsp90 or Hsp70 complex (Murata et al. 2001). In this way, Hsp70/Hsp90 are involved in the recognition and delivery of substrates for ubiquitination. Indeed, binding of CHIP to the Hsp70 EEVD motif was favoured when Hsp70 was in the ADP form, which has higher affinity for client proteins (Matsumura et al. 2013). In addition, Hsp70-bound peptides are preferentially targeted for degradation by CHIP compared to Hsp90 bound substrates (Qian et al. 2006; Stankiewicz et al. 2010). Therefore, the chaperones would act as sensors for denatured protein substrates which could subsequently be targeted for degradation by CHIP. Interestingly, CHIP also mediates the degradation of Hsp70 itself, once the misfolded client proteins have been degraded.

CHIP Substrates and Human Disease

The role of CHIP as a major regulator of proteasome-mediated degradation has been cemented by the recent identification of numerous protein substrates that are dependent on CHIP for proteasomal degradation (Table 11.1). One of the best described client proteins for CHIP is CFTR, upon which many of the early fundamental studies on CHIP function were performed. An updated list of CHIP substrate proteins includes a number of transcription factors, signalling intermediates and cytoskeletal or structural proteins (Table 11.1). The substrates, many of which are known client proteins of either Hsp90 or Hsp70, fulfil important

Table 11.1 Selection of the proteins targeted for proteasomal degradation by CHIP

Substrate/Client	Classification	Disease association	Reference
Androgen receptor (AR)	Receptor	Cancer	Sarkar et al. (2014)
Cystic fibrosis transmembrane conductance regulator (CFTR)	Receptor	Cystic fibrosis	Younger et al. (2004); Meacham et al. (2001)
Katanin-p60	Cytoskeleton		Yang et al. (2013)
Profilin	Cytoskeleton		Choi et al. (2014)
Tau	Cytoskeleton	Neurodegeneration	Elliott et al. (2007); Dickey et al. (2007); Hatakeyama et al. (2004a); Petrucelli et al. (2004); Shimura et al. (2004)
Alpha-synuclein	Cytoskeleton	Neurodegeneration	Kalia et al. (2011); Shin et al. (2005)
Keratin	Cytoskeleton		Loffek et al. (2010)
Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	Enzyme		Wang et al. (2012)
Histone deacetylase 6 (HDAC6)	Enzyme		Cook et al. (2012)
Nitric-oxide synthase (NOS)	Enzyme	Neurodegeneration	Chen et al. (2009); Peng et al. (2004)
NAD(P)H:quinone oxidoreductase 1 (NQO1)	Enzyme		Tsvetkov et al. (2011)
V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 2 (Her2)	Receptor	Cancer	Zhou et al. (2003); Xu et al. (2002)
Phosphatidylinositol 3-kinase P85 (P13K)	Signalling intermediate	Cancer	Ko et al. (2014)
Interferon regulatory factor 1 (IRF-1)	Signalling intermediate		Gao et al. (2013)
Liver kinase B1 (LKB1)	Signalling intermediate	Cancer	Gaude et al. (2012)
Phosphatase and tensin homolog (PTEN)	Signalling intermediate	Cancer	Ahmed et al. (2012)
Protein kinase B (PKB/Akt)	Signalling intermediate	Cancer	Su et al. (2011)
TNF receptor-associated factor 2 (TRAF2)	Signalling intermediate		Jang et al. (2011b)
Met receptor	Signalling intermediate	Cancer	Jang et al. (2011a)
Immature BCR-ABL	Signalling intermediate	Cancer	Tsukahara and Maru (2010)

Table 11.1 (continued)

Substrate/Client	Classification	Disease association	Reference
MAPK/ERK kinase kinase 2 (MEKK2)	Signalling intermediate		Maruyama et al. (2010)
Apoptosis signal-regulating kinase 1 (ASK1)	Signalling intermediate		Gao et al. (2010); Hwang et al. (2005)
Leucine-rich repeat kinase 2 (LRRK2)	Signalling intermediate	Neurodegeneration	Ding and Goldberg (2009); Ko et al. (2009)
Eukaryotic translation initiation factor 5A (eIF5A)	Transcription factor		Shang et al. (2014)
Estrogen Receptor (ER)	Transcription factor	Cancer	Fan et al. (2005)
Glucocorticoid receptor (GR)	Transcription factor		Wang and DeFranco (2005); Galigniana et al. (2004); Connell et al. (2001)
Tumour protein 53 (p53)	Transcription factor	Cancer	Wang et al. (2011); Sisoula et al. (2011); Muller et al. (2008); Esser et al. (2005)
V-Myc Avian Myelocytomatosis Viral Oncogene Homolog (c-Myc)	Transcription factor	Cancer	Paul et al. (2013)
Forkhead transcription factor p (FOXp)	Transcription factor		Chen et al. (2013)
Hypoxia-inducible factor 1 alpha (HIF-1alpha)	Transcription factor	Cancer	Luo et al. (2010)
Forkhead transcription factor 1 (FoxO1)	Transcription factor		Li et al. (2009)

roles in fundamental cellular processes. What is also striking is that many of these proteins are linked with diseases including cancer and neurodegenerative diseases like Alzheimer's disease. CHIP may therefore be of therapeutic value given the potential ability to regulate the degradation of disease relevant proteins like tau or p53 (Hatakeyama et al. 2004a; Petrucelli et al. 2004; Shimura et al. 2004; Esser et al. 2005).

In particular, the role of CHIP in neurodegenerative diseases, where protein aggregates are a hallmark, has been a main focus of research. CHIP can bind directly to and ubiquitinate the protein tau, actions that mediate the dissolution of tau aggregates (Hatakeyama et al. 2004a; Petrucelli et al. 2004; Shimura et al. 2004). These tau aggregates are associated with the pathology of Alzheimer's disease (Kosik and Shimura 2005; Medeiros et al. 2011; Martin et al. 2011; Salminen et al. 2011). CHIP may also regulate the aggregation of tau via ubiquitination of other client

proteins, like HDAC6 and Akt (Cook et al. 2012; Dickey et al. 2008). Therefore overexpression of CHIP may represent a therapeutic strategy to prevent neuronal cell death and ameliorate the symptoms and onset of the disease (Dickey et al. 2007; Sahara et al. 2005). The role of CHIP in cancer has been relatively less well studied than neurodegeneration. However, CHIP also controls the proteasomal degradation of a number of important oncogenic transcription factors or signalling intermediates, including p53, PTEN, Akt and c-Myc (Paul et al. 2013; Kajiro et al. 2009; Ahmed et al. 2012). These classes of proteins often act as nodes for the activation of a host of downstream proteins in the cellular reactions that lead to oncogenesis. Therefore, CHIP may in fact indirectly regulate a larger cohort of cellular proteins via degradation of central transcription factors or signalling intermediates. Indeed, analysis of the function of CHIP in breast cancer has demonstrated that the protein can regulate cellular responses, many of which are considered cancer hallmarks. Overexpression of CHIP blocked oncogenic signalling pathways, inhibited cancer associated processes like cell migration and anchorage independent growth, and induced cell death. Conversely, depletion of CHIP protein levels increased tumour formation and metastasis in mouse models (Kajiro et al. 2009; Choi et al. 2014; Sarkar et al. 2014).

In addition to classical substrate proteins, CHIP also ubiquitinates the chaperones Hsp70 and Hsp90 on multiple solvent exposed, but clustered lysine residues (6 in Hsp70 and 13 in Hsp90) (Kundrat and Regan 2010b). The polyubiquitination of these chaperones by CHIP occurs via K6, K11, K48, and K63 linkages. The canonical signal for protein degradation is ubiquitination via K48 linkages, and it is known that CHIP can mediate degradation of Hsp70 via this mechanism (Jiang et al. 2001). This reduction in Hsp70 plays a central regulatory role to return Hsp70 levels to basal after the induction of the stress response. However, non-canonical ubiquitin linkages (like K6, K11 and K63) have not been demonstrated to induce protein degradation, but may mediate other functions. In some experiments, ubiquitination via K63 resulted in recruitment of Hsp70, Hsp90 and BAG-1 to the proteasome but did not lead to their degradation (Alberti et al. 2002; Jiang et al. 2001). This suggested that K63 linkage may be a proteasome targeting sequence and represent a mechanism by which CHIP uses the chaperone to deliver its clients to the proteasome (Saeki et al. 2009; Chen and Sun 2009).

Ubiquitination of substrates by CHIP does not always lead to proteasomal degradation via the canonical K48 ubiquitiation. There are some examples in the literature than demonstrate a role for CHIP in non-canonical ubiquitination of substrates. One example is the protein, sirtuin, which underwent non-canonical CHIP-mediated ubiquitination that culminated in its stabilisation and promotion of DNA repair (Ronnebaum et al. 2013). CHIP also mediated T cell activation by ubiquitination of CARMA1 (Caspase recruitment domain (CARD) containing membrane-associated guanylate kinase protein 1), a receptor important in antigen receptor linked NF-kappaB signalling. The CHIP mediated ubiquitination of CARMA1 via K27 was determined to be important for activation of this pathway (Wang et al. 2013).

Hsp70 and Hsp90: To Degrade or to Refold?

The chaperone folding and ubiquitin-proteasome degradation pathways work competitively during protein homeostasis (Marques et al. 2006). The major question that remains unanswered is how is it determined whether proteins enter refolding or degradation pathways? Pro-folding chaperone complexes are largely associated with the co-chaperone Hop, which facilitates entry of client proteins from the Hsp70 complex into the Hsp90 complex (Siligardi et al. 2004). In contrast, chaperone complexes containing CHIP are considered to be pro-degradation complexes. Hop and CHIP cannot bind simultaneously to the Hsp90 complex, which indicates that the complexes controlling either protein folding or protein degradation are mutually exclusive and possibly competitive (Kundrat and Regan 2010a). Therefore, the simplest mechanism to control the choice of folding or degradation may be via regulation of the levels of the specific co-chaperone (Marques et al. 2006). Indeed, simply increasing the CHIP concentration by overexpression increased proteasomal degradation of client proteins, including hormone receptors (Connell et al. 2001; Adachi et al. 2007).

Hop and CHIP compete with each other for binding to Hsp70 and Hsp90, which could determine whether pro-folding or pro-degradation complexes form. There is evidence that these associations are regulated by post-translational modification of the C terminal region of these chaperones. Phosphorylation of sites in the C-terminus of either Hsp70 or Hsp90 blocked CHIP binding and promoted association of Hop with the chaperones. This observation is particularly important in the context of cancer, where increased levels of phosphorylated Hsp70 and Hsp90 chaperones have been linked with high cell proliferation rates (Muller et al. 2013). Indeed, many of the kinases that phosphorylated Hsp90, including CK1, CK2 and GSK3β, are linked to the cell cycle or mitogenic signalling pathways. Hop has also been shown to be upregulated in numerous cancers, suggesting that increased levels of this co-chaperone may out-compete CHIP for chaperone binding in these cells (Willmer et al. 2013; Ruckova et al. 2012). The net consequence of this would be to create a cellular environment that promoted protein folding over degradation. The fact that this is observed in cancer cells may explain the dependency of proteins on the Hsp70-Hsp90 folding complex and support the high cellular growth rates observed in most malignancies.

However, protein folding also predominates under physiological conditions. This is as a result of a greater concentration of chaperone complexes containing Hop or Hip, than CHIP or BAG-1 (Kundrat and Regan 2010a). Despite this, ubiquitination of substrates by CHIP proceeds at a basal level under physiological conditions. Recent studies by the Regan group suggest that the switch to degradation of protein clients was largely determined by partitioning of the client protein between Hsp70 and Hsp90 chaperone complexes (Kundrat and Regan 2010a). Hsp70 and Hsp90 can both bind CHIP, but the purported greater affinity of the CHIP-Hsp70 interaction predicts that this is the dominant complex that mediates the degradation pathway (Kundrat and Regan 2010a). Therefore, the interaction

between Hsp90 and CHIP is predicted to play a minor role in direct protein triage decisions. This is supported by the fact that CHIP has a preference for ubiquitination of Hsp70-bound client proteins compared to Hsp90-bound substrates (Stankiewicz et al. 2010). Rather, degradation of Hsp90 clients is proposed to be induced indirectly by inhibition of the Hsp90 complex, meaning that client proteins associate with the Hsp70 complex for an extended duration, leading to increased potential for degradation via CHIP (Kundrat and Regan 2010a). This is consistent with the observation that the time spent by the client protein in the Hsp70 complex determines its stability (Matsumura et al. 2013). Interestingly, the TPR containing co-chaperone, DNAJC7, which has been proposed to catalyse retrograde transfer of client proteins from Hsp90 back to Hsp70 (Brychzy et al. 2003), can interact directly with CHIP (Hatakeyama et al. 2004b). Hsp90 inhibition using compounds that induce the stress response (such as 17-AAG) lead to increased Hsp70 levels, which could subsequently associate with clients released from the non-functional Hsp90 complex and trigger their degradation via CHIP. The fact that CHIP is also known to induce both the expression and turnover of Hsp70 as part of the stress response, supports the major role for Hsp70 over Hsp90 in this process (Qian et al. 2006).

The increase in substrate degradation in response to higher levels of CHIP could also be explained by the fact that increased levels of CHIP result in increased levels of Hsp70-CHIP degradation complexes to a concentration that exceeds Hsp70-Hop-Hsp90 folding complexes (Kundrat and Regan 2010a). The addition of CHIP to the Hsp90 complex induces a similar response to treatment with GA, promoting dissociation of stabilising co-chaperones, like p23, and promoting proteasomal degradation of the client protein (Connell et al. 2001; Whitesell and Cook 1996). This suggests that, in addition to an increase in Hsp70-CHIP complexes, increased CHIP levels may also block Hsp90 complexes and push client proteins towards association with Hsp70.

The balance between folding and degradation can also be controlled by naturally occurring regulators of CHIP. The activity of CHIP is regulated by BAG-1 and HspBP1, which are both nucleotide exchange factors for Hsp70 (Kabani et al. 2002; Alberti et al. 2003). CHIP can bind directly to the proteasome (Connell et al. 2001; Meacham et al. 2001), or it may interact with the proteasome via BAG-1. BAG-1 binds simultaneously to CHIP and the 26S proteasome, thereby recruiting a complex that delivers CHIP bound complexes to the proteasome (Luders et al. 2000). Interestingly, the association of BAG-1 with the proteasome is mediated in part by CHIP-mediated ubiquitination of BAG-1 (Alberti et al. 2002). HspBP1 is a negative regulator of CHIP activity (Alberti et al. 2004). HsBP1 interacts with the Hsp70 ATPase domain (Raynes and Guerriero 1998) and induces conformational changes in the chaperone (McLellan et al. 2003), leading to binding of CHIP to the C terminal site of Hsp70. This complex abolished the CHIP mediated ubiquitination and degradation of the substrate protein, CFTR (Alberti et al. 2004). Hsp40 and client protein (steroid receptors) preferentially associate with BAG-1 over HspBP1. HspBP1, not BAG-1, also reduced binding of Hsp70 to client proteins and inhibited the activity of steroid hormone receptors at both high and low concentrations. In

contrast, the effect of BAG-1 on steroid receptor function was concentration dependant, being stimulatory at low levels of BAG-1 and inhibitory at higher concentrations of BAG-1 (Knapp et al. 2014).

Conclusions

While the molecular mechanisms that are involved in defining the balance between protein folding and protein degradation are not fully understood, the existence of CHIP suggests that chaperones actively participate in protein degradation via the proteasome. This suggests that an as yet undefined mechanism exists to determine which pathway, folding or degradation, should be followed under certain conditions. Recent studies have demonstrated that CHIP mediated the degradation of a wide range of cellular proteins, which signifies a central role for this co-chaperone in protein degradation. Many of these client proteins are important factors in a range of human diseases; an association that suggests CHIP may be a putative drug target. The potential applications of CHIP to human disease are likely to be largely restricted to those that involve either the overexpression or activation of CHIP. While some experiments demonstrate that CHIP depletion results in degradation of CHIP substrates, other reports demonstrate that CHIP clients remain unaffected in a CHIP depleted background. This hints at functional redundancy whereby other E3 ligase factors may compensate for the loss of CHIP. These data may also suggest that some substrates are more reliant on CHIP for their degradation, whereas others may be promiscuous with respect to the E3 ligase required for their degradation. The application of CHIP as a drug target will be limited until we are able to define the mechanisms which regulate whether chaperones function in protein folding or protein degradation.

Acknowledgments Financial support for research activities in the laboratory of the author from the South African National Research Foundation (NRF), Medical Research Council (MRC) South Africa, Rhodes University and Cancer Association of South Africa (CANSA) is gratefully acknowledged. The views reflected in this document are those of the author and should in no way be attributed to the NRF, MRC, Rhodes University or CANSA.

References

Adachi H, Waza M, Tokui K et al (2007) CHIP overexpression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. J Neurosci 27:5115–5126

Agashe VR, Hartl FU (2000) Roles of molecular chaperones in cytoplasmic protein folding. Semin Cell Dev Biol 11:15–25

Ahmed SF, Deb S, Paul I et al (2012) The chaperone-assisted E3 ligase C terminus of Hsc70-interacting protein (CHIP) targets PTEN for proteasomal degradation. J Biol Chem 287:15996–16006

Alberti S, Demand J, Esser C et al (2002) Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. J Biol Chem 277:45920–45927

Alberti S, Esser C, Hohfeld J (2003) BAG-1–a nucleotide exchange factor of Hsc70 with multiple cellular functions. Cell Stress Chaperones 8:225–231

Alberti S, Bohse K, Arndt V et al (2004) The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. Mol Biol Cell 15:4003–4010

Ali MM, Roe SM, Vaughan CK et al (2006) Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. Nature 440:1013–1017

Allan RK, Ratajczak T (2011) Versatile TPR domains accommodate different modes of target protein recognition and function. Cell Stress Chaperones 16:353–367

Amm I, Sommer T, Wolf DH (2014) Protein quality control and elimination of protein waste: the role of the ubiquitin-proteasome system. Biochim Biophys Acta 1843:182–196

Babbitt SE, Kiss A, Deffenbaugh AE et al (2005) ATP hydrolysis-dependent disassembly of the 26S proteasome is part of the catalytic cycle. Cell 121:553–565

Ballinger CA, Connell P, Wu Y et al (1999) Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol Cell Biol 19:4535–4545

Bedford L, Paine S, Sheppard PW et al (2010) Assembly, structure, and function of the 26S proteasome. Trends Cell Biol 20:391–401

Bercovich B, Stancovski I, Mayer A et al (1997) Ubiquitin-dependent degradation of certain protein substrates *in vitro* requires the molecular chaperone Hsc70. J Biol Chem 272:9002–9010

Blatch GL, Lassle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21:932–939

Brinker A, Scheufler C, Von Der Mulbe F et al (2002) Ligand discrimination by TPR domains. Relevance and selectivity of EEVD-recognition in Hsp70 x Hop x Hsp90 complexes. J Biol Chem 277:19265–19275

Brychzy A, Rein T, Winklhofer KF et al (2003) Cofactor Tpr2 combines two TPR domains and a J domain to regulate the Hsp70/Hsp90 chaperone system. EMBO J 22:3613–3623

Caplan AJ (2003) What is a co-chaperone? Cell Stress Chaperones 8:105–107

Chang L, Thompson AD, Ung P et al (2010) Mutagenesis reveals the complex relationships between ATPase rate and the chaperone activities of Escherichia coli heat shock protein 70 (Hsp70/DnaK). J Biol Chem 285:21282–21291

Chapple JP, van der Spuy J, Poopalasundaram S et al (2004) Neuronal DnaJ proteins HSJ1a and HSJ1b: a role in linking the Hsp70 chaperone machine to the ubiquitin-proteasome system? Biochem Soc Trans 32:640–642

Cheetham ME, Jackson AP, Anderton BH (1994) Regulation of 70-kDa heat-shock-protein ATPase activity and substrate binding by human DnaJ-like proteins, HSJ1a and HSJ1b. Eur J Biochem 226:99–107

Chen ZJ, Sun LJ (2009) Nonproteolytic functions of ubiquitin in cell signaling. Mol Cell 33:275–286

Chen L, Kong X, Fu J et al (2009) CHIP facilitates ubiquitination of inducible nitric oxide synthase and promotes its proteasomal degradation. Cell Immunol 258:38–43

Chen Z, Barbi J, Bu S et al (2013) The ubiquitin ligase Stub1 negatively modulates regulatory T cell suppressive activity by promoting degradation of the transcription factor Foxp3. Immunity 39:272–285

Choi YN, Lee SK, Seo TW et al (2014) C-terminus of Hsc70-interacting protein regulates profilin1 and breast cancer cell migration. Biochem Biophys Res Commun 446:1060–1066

Ciechanover A (1998) The ubiquitin-proteasome pathway: on protein death and cell life. EMBO J 17:7151–7160

Connell P, Ballinger CA, Jiang J et al (2001) The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat Cell Biol 3:93–96

Cook C, Gendron TF, Scheffel K et al (2012) Loss of HDAC6, a novel CHIP substrate, alleviates abnormal tau accumulation. Hum Mol Genet 21:2936–2945
Cortajarena AL, Regan L (2006) Ligand binding by TPR domains. Protein Sci 15:1193–1198
Cyr DM, Hohfeld J, Patterson C (2002) Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. Trends Biochem Sci 27:368–375
da Fonseca PC, Morris EP (2008) Structure of the human 26S proteasome: subunit radial displacements open the gate into the proteolytic core. J Biol Chem 283:23305–23314
Dai Q, Zhang C, Wu Y et al (2003) CHIP activates HSF1 and confers protection against apoptosis and cellular stress. EMBO J 22:5446–5458
Demand J, Alberti S, Patterson C et al (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr Biol 11:1569–1577
Dickey CA, Kamal A, Lundgren K et al (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Invest 117:648–658
Dickey CA, Koren J, Zhang YJ et al (2008) Akt and CHIP coregulate tau degradation through coordinated interactions. Proc Natl Acad Sci U S A 105:3622–3627
Ding X, Goldberg MS (2009) Regulation of LRRK2 stability by the E3 ubiquitin ligase CHIP. PLoS One 4:e5949
Ehrlich ES, Wang T, Luo K et al (2009) Regulation of Hsp90 client proteins by a Cullin5-RING E3 ubiquitin ligase. Proc Natl Acad Sci U S A 106:20330–20335
Eisele F, Wolf DH (2008) Degradation of misfolded protein in the cytoplasm is mediated by the ubiquitin ligase Ubr1. FEBS Lett 582:4143–4146
Elliott E, Tsvetkov P, Ginzburg I (2007) BAG-1 associates with Hsc70.Tau complex and regulates the proteasomal degradation of Tau protein. J Biol Chem 282:37276–37284
Ellis RJ (1997) Molecular chaperones: avoiding the crowd. Curr Biol 7:R531–533
Esser C, Alberti S, Hohfeld J (2004) Cooperation of molecular chaperones with the ubiquitin/proteasome system. Biochim Biophys Acta 1695:171–188
Esser C, Scheffner M, Hohfeld J (2005) The chaperone-associated ubiquitin ligase CHIP is able to target p53 for proteasomal degradation. J Biol Chem 280:27443–27448
Fan M, Park A, Nephew KP (2005) CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor-alpha. Mol Endocrinol 19:2901–2914
Fedorov AN, Baldwin TO (1997) Cotranslational protein folding. J Biol Chem 272:32715–32718
Frydman J, Hohfeld J (1997) Chaperones get in touch: the Hip-Hop connection. Trends Biochem Sci 22:87–92
Galigniana MD, Harrell JM, Housley PR et al (2004) Retrograde transport of the glucocorticoid receptor in neurites requires dynamic assembly of complexes with the protein chaperone hsp90 and is linked to the CHIP component of the machinery for proteasomal degradation. Brain Res Mol Brain Res 123:27–36
Gao Y, Han C, Huang H et al (2010) Heat shock protein 70 together with its co-chaperone CHIP inhibits TNF-alpha induced apoptosis by promoting proteasomal degradation of apoptosis signal-regulating kinase1. Apoptosis 15:822–833
Gao XC, Zhou CJ, Zhou ZR et al (2011) Co-chaperone HSJ1a dually regulates the proteasomal degradation of ataxin-3. PLoS One 6:e19763
Gao B, Wang Y, Xu W et al (2013) Inhibition of histone deacetylase activity suppresses IFN-gamma induction of tripartite motif 22 via CHIP-mediated proteasomal degradation of IRF-1. J Immunol 191:464–471
Gaude H, Aznar N, Delay A et al (2012) Molecular chaperone complexes with antagonizing activities regulate stability and activity of the tumor suppressor LKB1. Oncogene 31:1582–1591
Goldberg AL, Akopian TN, Kisselev AF et al (1997) New insights into the mechanisms and importance of the proteasome in intracellular protein degradation. Biol Chem 378:131–140
Graf C, Stankiewicz M, Nikolay R et al (2010) Insights into the conformational dynamics of the E3 ubiquitin ligase CHIP in complex with chaperones and E2 enzymes. Biochemistry 49:2121–2129
Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–579

Hatakeyama S, Yada M, Matsumoto M et al (2001) U box proteins as a new family of ubiquitin-protein ligases. J Biol Chem 276:33111–33120
Hatakeyama S, Matsumoto M, Kamura T et al (2004a) U-box protein carboxyl terminus of Hsc70-interacting protein (CHIP) mediates poly-ubiquitylation preferentially on four-repeat Tau and is involved in neurodegeneration of tauopathy. J Neurochem 91:299–307
Hatakeyama S, Matsumoto M, Yada M et al (2004b) Interaction of U-box-type ubiquitin-protein ligases (E3s) with molecular chaperones. Genes Cells 9:533–548
Heinemeyer W, Ramos PC, Dohmen RJ (2004) The ultimate nanoscale mincer: assembly, structure and active sites of the 20S proteasome core. Cell Mol Life Sci 61:1562–1578
Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479
Hiromura M, Yano M, Mori H et al (1998) Intrinsic ADP-ATP exchange activity is a novel function of the molecular chaperone, Hsp70. J Biol Chem 273:5435–5438
Hofmann RM, Pickart CM (1999) Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. Cell 96:645–653
Hohfeld J (1998) Regulation of the heat shock conjugate Hsc70 in the mammalian cell: the characterization of the anti-apoptotic protein BAG-1 provides novel insights. Biol Chem 379:269–274
Hohfeld J, Cyr DM, Patterson C (2001) From the cradle to the grave: molecular chaperones that may choose between folding and degradation. EMBO Rep 2:885–890
Hwang JR, Zhang C, Patterson C (2005) C-terminus of heat shock protein 70-interacting protein facilitates degradation of apoptosis signal-regulating kinase 1 and inhibits apoptosis signal-regulating kinase 1-dependent apoptosis. Cell Stress Chaperones 10:147–156
Imai J, Yashiroda H, Maruya M et al (2003) Proteasomes and molecular chaperones: cellular machinery responsible for folding and destruction of unfolded proteins. Cell Cycle 2:585–590
Jacobson AD, Zhang NY, Xu P et al (2009) The lysine 48 and lysine 63 ubiquitin conjugates are processed differently by the 26 s proteasome. J Biol Chem 284:35485–35494
Jang KW, Lee JE, Kim SY et al (2011a) The C-terminus of Hsp70-interacting protein promotes Met receptor degradation. J Thorac Oncol 6:679–687
Jang KW, Lee KH, Kim SH et al (2011b) Ubiquitin ligase CHIP induces TRAF2 proteasomal degradation and NF-kappaB inactivation to regulate breast cancer cell invasion. J Cell Biochem 112:3612–3620
Jiang J, Ballinger CA, Wu Y et al (2001) CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. J Biol Chem 276:42938–42944
Johnson ES, Bartel B, Seufert W et al (1992) Ubiquitin as a degradation signal. EMBO J 11:497–505
Johnson ES, Ma PC, Ota IM et al (1995) A proteolytic pathway that recognizes ubiquitin as a degradation signal. J Biol Chem 270:17442–17456
Kabani M, McLellan C, Raynes DA et al (2002) HspBP1, a homologue of the yeast Fes1 and Sls1 proteins, is an Hsc70 nucleotide exchange factor. FEBS Lett 531:339–342
Kajiro M, Hirota R, Nakajima Y et al (2009) The ubiquitin ligase CHIP acts as an upstream regulator of oncogenic pathways. Nat Cell Biol 11:312–319
Kalia LV, Kalia SK, Chau H et al (2011) Ubiquitinylation of alpha-synuclein by carboxyl terminus Hsp70-interacting protein (CHIP) is regulated by Bcl-2-associated athanogene 5 (BAG5). PLoS One 6:e14695
Kastle M, Grune T (2012) Interactions of the proteasomal system with chaperones: protein triage and protein quality control. Prog Mol Biol Transl Sci 109:113–160
Kettern N, Dreiseidler M, Tawo R et al (2010) Chaperone-assisted degradation: multiple paths to destruction. Biol Chem 391:481–489
Knapp RT, Wong MJ, Kollmannsberger LK et al (2014) Hsp70 cochaperones HspBP1 and BAG-1M differentially regulate steroid hormone receptor function. PLoS One 9:e85415
Ko HS, Bailey R, Smith WW et al (2009) CHIP regulates leucine-rich repeat kinase-2 ubiquitination, degradation, and toxicity. Proc Natl Acad Sci U S A 106:2897–2902
Ko HR, Kim CK, Lee SB et al (2014) P42 Ebp1 regulates the proteasomal degradation of the p85 regulatory subunit of PI3K by recruiting a chaperone-E3 ligase complex HSP70/CHIP. Cell Death Dis 5:e1131

Koegl M, Hoppe T, Schlenker S et al (1999) A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96:635–644
Kosik KS, Shimura H (2005) Phosphorylated tau and the neurodegenerative foldopathies. Biochim Biophys Acta 1739:298–310
Kriegenburg F, Ellgaard L, Hartmann-Petersen R (2012) Molecular chaperones in targeting misfolded proteins for ubiquitin-dependent degradation. FEBS J 279:532–542
Kundrat L, Regan L (2010a) Balance between folding and degradation for Hsp90-dependent client proteins: a key role for CHIP. Biochemistry 49:7428–7438
Kundrat L, Regan L (2010b) Identification of residues on Hsp70 and Hsp90 ubiquitinated by the cochaperone CHIP. J Mol Biol 395:587–594
Landry SJ, Gierasch LM (1994) Polypeptide interactions with molecular chaperones and their relationship to *in vivo* protein folding. Annu Rev Biophys Biomol Struct 23:645–669
Lecker SH, Goldberg AL, Mitch WE (2006) Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol 17:1807–1819
Lee I, Schindelin H (2008) Structural insights into E1-catalyzed ubiquitin activation and transfer to conjugating enzymes. Cell 134:268–278
Li F, Xie P, Fan Y et al (2009) C terminus of Hsc70-interacting protein promotes smooth muscle cell proliferation and survival through ubiquitin-mediated degradation of FoxO1. J Biol Chem 284:20090–20098
Li J, Soroka J, Buchner J (2012) The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta 1823:624–635
Loffek S, Woll S, Hohfeld J et al (2010) The ubiquitin ligase CHIP/STUB1 targets mutant keratins for degradation. Hum Mutat 31:466–476
Lotz GP, Lin H, Harst A et al (2003) Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. J Biol Chem 278:17228–17235
Luders J, Demand J, Hohfeld J (2000) The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J Biol Chem 275:4613–4617
Luo W, Zhong J, Chang R et al (2010) Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1alpha but Not HIF-2alpha. J Biol Chem 285:3651–3663
Mao Y, Deng A, Qu N et al (2006) ATPase domain of Hsp70 exhibits intrinsic ATP-ADP exchange activity. Biochemistry (Mosc) 71:1222–1229
Marques C, Guo W, Pereira P et al (2006) The triage of damaged proteins: degradation by the ubiquitin-proteasome pathway or repair by molecular chaperones. FASEB J 20:741–743
Martin L, Latypova X, Terro F (2011) Post-translational modifications of tau protein: implications for Alzheimer's disease. Neurochem Int 58:458–471
Maruyama T, Kadowaki H, Okamoto N et al (2010) CHIP-dependent termination of MEKK2 regulates temporal ERK activation required for proper hyperosmotic response. EMBO J 29:2501–2514
Matsumura Y, Sakai J, Skach WR (2013) Endoplasmic reticulum protein quality control is determined by cooperative interactions between Hsp/c70 protein and the CHIP E3 ligase. J Biol Chem 288:31069–31079
McDonough H, Patterson C (2003) CHIP: a link between the chaperone and proteasome systems. Cell Stress Chaperones 8:303–308
McLaughlin SH, Smith HW, Jackson SE (2002) Stimulation of the weak ATPase activity of human hsp90 by a client protein. J Mol Biol 315:787–798
McLellan CA, Raynes DA, Guerriero V (2003) HspBP1, an Hsp70 cochaperone, has two structural domains and is capable of altering the conformation of the Hsp70 ATPase domain. J Biol Chem 278:19017–19022
Meacham GC, Patterson C, Zhang W et al (2001) The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. Nat Cell Biol 3:100–105
Medeiros R, Baglietto-Vargas D, LaFerla FM (2011) The role of tau in Alzheimer's disease and related disorders. CNS Neurosci Ther 17:514–524

Meimaridou E, Gooljar SB, Chapple JP (2009) From hatching to dispatching: the multiple cellular roles of the Hsp70 molecular chaperone machinery. J Mol Endocrinol 42:1–9
Morishima Y, Wang AM, Yu Z et al (2008) CHIP deletion reveals functional redundancy of E3 ligases in promoting degradation of both signaling proteins and expanded glutamine proteins. Hum Mol Genet 17:3942–3952
Muller P, Hrstka R, Coomber D et al (2008) Chaperone-dependent stabilization and degradation of p53 mutants. Oncogene 27:3371–3383
Muller P, Ruckova E, Halada P et al (2013) C-terminal phosphorylation of Hsp70 and Hsp90 regulates alternate binding to co-chaperones CHIP and HOP to determine cellular protein folding/degradation balances. Oncogene 32:3101–3110
Murata S, Minami Y, Minami M et al (2001) CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. EMBO Rep 2:1133–1138
Murata S, Chiba T, Tanaka K (2003) CHIP: a quality-control E3 ligase collaborating with molecular chaperones. Int J Biochem Cell Biol 35:572–578
Murata S, Yashiroda H, Tanaka K (2009) Molecular mechanisms of proteasome assembly. Nat Rev Mol Cell Biol 10:104–115
Nillegoda NB, Theodoraki MA, Mandal AK et al (2010) Ubr1 and Ubr2 function in a quality control pathway for degradation of unfolded cytosolic proteins. Mol Biol Cell 21:2102–2116
Odunuga OO, Hornby JA, Bies C et al (2003) Tetratricopeptide repeat motif-mediated Hsc70-mSTI1 interaction. Molecular characterization of the critical contacts for successful binding and specificity. J Biol Chem 278:6896–6904
Ohi MD, Vander Kooi CW, Rosenberg JA et al (2003) Structural insights into the U-box, a domain associated with multi-ubiquitination. Nat Struct Biol 10:250–255
Olsen SK, Lima CD (2013) Structure of a ubiquitin E1-E2 complex: insights to E1-E2 thioester transfer. Mol Cell 49:884–896
Ortega J, Heymann JB, Kajava AV et al (2005) The axial channel of the 20S proteasome opens upon binding of the PA200 activator. J Mol Biol 346:1221–1227
Panaretou B, Prodromou C, Roe SM et al (1998) ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone *in vivo*. EMBO J 17:4829–4836
Paul I, Ahmed SF, Bhowmik A et al (2013) The ubiquitin ligase CHIP regulates c-Myc stability and transcriptional activity. Oncogene 32:1284–1295
Peng HM, Morishima Y, Jenkins GJ et al (2004) Ubiquitylation of neuronal Nitric-oxide synthase by CHIP, a chaperone-dependent E3 ligase. J Biol Chem 279:52970–52977
Petrucelli L, Dickson D, Kehoe K et al (2004) CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum Mol Genet 13:703–714
Prodromou C, Roe SM, O'Brien R et al (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 90:65–75
Prodromou C, Siligardi G, O'Brien R et al (1999) Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. EMBO J 18:754–762
Prodromou C, Panaretou B, Chohan S et al (2000) The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. EMBO J 19:4383–4392
Qian SB, McDonough H, Boellmann F et al (2006) CHIP-mediated stress recovery by sequential ubiquitination of substrates and Hsp70. Nature 440:551–555
Raynes DA, Guerriero V Jr (1998) Inhibition of Hsp70 ATPase activity and protein renaturation by a novel Hsp70-binding protein. J Biol Chem 273:32883–32888
Ronnebaum SM, Wu Y, McDonough H et al (2013) The ubiquitin ligase CHIP prevents SirT6 degradation through noncanonical ubiquitination. Mol Cell Biol 33:4461–4472
Roos-Mattjus P, Sistonen L (2004) The ubiquitin-proteasome pathway. Ann Med 36:285–295
Ruckova E, Muller P, Nenutil R et al (2012) Alterations of the Hsp70/Hsp90 chaperone and the HOP/CHIP co-chaperone system in cancer. Cell Mol Biol Lett 17:446–458
Saeki Y, Kudo T, Sone T et al (2009) Lysine 63-linked polyubiquitin chain may serve as a targeting signal for the 26S proteasome. EMBO J 28:359–371
Sahara N, Murayama M, Mizoroki T et al (2005) *In vivo* evidence of CHIP up-regulation attenuating tau aggregation. J Neurochem 94:1254–1263

Salminen A, Ojala J, Kaarniranta K et al (2011) Hsp90 regulates tau pathology through co-chaperone complexes in Alzheimer's disease. Prog Neurobiol 93:99–110
Sarkar S, Brautigan DL, Parsons SJ et al (2014) Androgen receptor degradation by the E3 ligase CHIP modulates mitotic arrest in prostate cancer cells. Oncogene 33:26–33
Scheffner M, Nuber U, Huibregtse JM (1995) Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature 373:81–83
Shang Y, Zhao X, Tian B et al (2014) CHIP/Stub1 interacts with eIF5A and mediates its degradation. Cell Signal 26:1098–1104
Shimura H, Schwartz D, Gygi SP et al (2004) CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. J Biol Chem 279:4869–4876
Shin Y, Klucken J, Patterson C et al (2005) The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. J Biol Chem 280:23727–23734
Siligardi G, Hu B, Panaretou B et al (2004) Co-chaperone regulation of conformational switching in the Hsp90 ATPase cycle. J Biol Chem 279:51989–51998
Sisoula C, Trachana V, Patterson C et al (2011) CHIP-dependent p53 regulation occurs specifically during cellular senescence. Free Radic Biol Med 50:157–165
Sledz P, Unverdorben P, Beck F et al (2013) Structure of the 26S proteasome with ATP-gammaS bound provides insights into the mechanism of nucleotide-dependent substrate translocation. Proc Natl Acad Sci U S A 110:7264–7269
Smith HT (1988) A "new" protein: ubiquitin. Science 242:787–788
Smith MC, Scaglione KM, Assimon VA et al (2013) The E3 ubiquitin ligase CHIP and the molecular chaperone Hsc70 form a dynamic, tethered complex. Biochemistry 52:5354–5364
Spratt DE, Wu K, Kovacev J et al (2012) Selective recruitment of an E2~ubiquitin complex by an E3 ubiquitin ligase. J Biol Chem 287:17374–17385
Stankiewicz M, Nikolay R, Rybin V et al (2010) CHIP participates in protein triage decisions by preferentially ubiquitinating Hsp70-bound subtrates. FEBS J 277:3353–3367
Strickland E, Qu BH, Millen L et al (1997) The molecular chaperone Hsc70 assists the *in vitro* folding of the N-terminal nucleotide-binding domain of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 272:25421–25424
Su CH, Wang CY, Lan KH et al (2011) Akt phosphorylation at Thr308 and Ser473 is required for CHIP-mediated ubiquitination of the kinase. Cell Signal 23:1824–1830
Sun L, Chen ZJ (2004) The novel functions of ubiquitination in signaling. Curr Opin Cell Biol 16:119–126
Tanaka K (2009) The proteasome: overview of structure and functions. Proc Jpn Acad Ser B Phys Biol Sci 85:12–36
Tastan Bishop O, Edkins AL, Blatch GL (2014) Sequence and domain conservation of the coelacanth Hsp40 and Hsp90 chaperones suggests conservation of function. J Exp Zool B Mol Dev Evol 322(6):359–378 doi:10.1002/jez.b.22541
Tsukahara F, Maru Y (2010) Bag1 directly routes immature BCR-ABL for proteasomal degradation. Blood 116:3582–3592
Tsvetkov P, Adamovich Y, Elliott E et al (2011) E3 ligase STUB1/CHIP regulates NAD(P)H:quinone oxidoreductase 1 (NQO1) accumulation in aged brain, a process impaired in certain Alzheimer disease patients. J Biol Chem 286:8839–8845
Unverdorben P, Beck F, Sledz P et al (2014) Deep classification of a large cryo-EM dataset defines the conformational landscape of the 26S proteasome. Proc Natl Acad Sci U S A 111:5544–5549
Van Der Spuy J, Kana BD, Dirr HW et al (2000) Heat shock cognate protein 70 chaperone-binding site in the co-chaperone murine stress-inducible protein 1 maps to within three consecutive tetratricopeptide repeat motifs. Biochem J 345(Pt 3):645–651
Wagner SA, Beli P, Weinert BT et al (2011) A proteome-wide, quantitative survey of *in vivo* ubiquitylation sites reveals widespread regulatory roles. Mol Cell Proteomics 10:M 111 013284
Walz J, Erdmann A, Kania M et al (1998) 26S proteasome structure revealed by three-dimensional electron microscopy. J Struct Biol 121:19–29

Wang X, DeFranco DB (2005) Alternative effects of the ubiquitin-proteasome pathway on glucocorticoid receptor down-regulation and transactivation are mediated by CHIP, an E3 ligase. Mol Endocrinol 19:1474–1482

Wang J, Zhao Q, Qi Q et al (2011) Gambogic acid-induced degradation of mutant p53 is mediated by proteasome and related to CHIP. J Cell Biochem 112:509–519

Wang Y, Guan S, Acharya P et al (2012) Multisite phosphorylation of human liver cytochrome P450 3A4 enhances its gp78- and CHIP-mediated ubiquitination: a pivotal role of its Ser-478 residue in the gp78-catalyzed reaction. Mol Cell Proteomics 11:M 111 010132

Wang S, Li Y, Hu YH et al (2013) STUB1 is essential for T-cell activation by ubiquitinating CARMA1. Eur J Immunol 43:1034–1041

Wegele H, Muller L, Buchner J (2004) Hsp70 and Hsp90–a relay team for protein folding. Rev Physiol Biochem Pharmacol 151:1–44

Welch WJ, Brown CR (1996) Influence of molecular and chemical chaperones on protein folding. Cell Stress Chaperones 1:109–115

Westhoff B, Chapple JP, van der Spuy J et al (2005) HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. Curr Biol 15:1058–1064

Whitesell L, Cook P (1996) Stable and specific binding of heat shock protein 90 by geldanamycin disrupts glucocorticoid receptor function in intact cells. Mol Endocrinol 10:705–712

Whitesell L, Sutphin PD, Pulcini EJ et al (1998) The physical association of multiple molecular chaperone proteins with mutant p53 is altered by geldanamycin, an hsp90-binding agent. Mol Cell Biol 18:1517–1524

Wiederkehr T, Bukau B, Buchberger A (2002) Protein turnover: a CHIP programmed for proteolysis. Curr Biol 12:R26–28

Wilkinson KD (2000) Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. Semin Cell Dev Biol 11:141–148

Willmer T, Contu L, Blatch GL et al (2013) Knockdown of Hop downregulates RhoC expression, and decreases pseudopodia formation and migration in cancer cell lines. Cancer Lett 328:252–260

Wolf DH, Sommer T, Hilt W (2004) Death gives birth to life: the essential role of the ubiquitin-proteasome system in biology. Biochim Biophys Acta 1695:1–2

Xu W, Marcu M, Yuan X et al (2002) Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB2/Neu. Proc Natl Acad Sci U S A 99:12847–12852

Xu Z, Devlin KI, Ford MG et al (2006) Structure and interactions of the helical and U-box domains of CHIP, the C terminus of HSP70 interacting protein. Biochemistry 45:4749–4759

Xu Z, Kohli E, Devlin KI et al (2008) Interactions between the quality control ubiquitin ligase CHIP and ubiquitin conjugating enzymes. BMC Struct Biol 8:26

Yang SW, Oh KH, Park E et al (2013) USP47 and C terminus of Hsp70-interacting protein (CHIP) antagonistically regulate katanin-p60-mediated axonal growth. J Neurosci 33:12728–12738

Younger JM, Ren HY, Chen L et al (2004) A foldable CFTRΔF508 biogenic intermediate accumulates upon inhibition of the Hsc70-CHIP E3 ubiquitin ligase. J Cell Biol 167:1075–1085

Zhang C, Xu Z, He XR et al (2005a) CHIP, a cochaperone/ubiquitin ligase that regulates protein quality control, is required for maximal cardioprotection after myocardial infarction in mice. Am J Physiol Heart Circ Physiol 288:H2836–2842

Zhang M, Windheim M, Roe SM et al (2005b) Chaperoned ubiquitylation–crystal structures of the CHIP U box E3 ubiquitin ligase and a CHIP-Ubc13-Uev1a complex. Mol Cell 20:525–538

Zhou P, Fernandes N, Dodge IL et al (2003) ErbB2 degradation mediated by the co-chaperone protein CHIP. J Biol Chem 278:13829–13837

Chapter 12
The Role of HSP70 and Its Co-chaperones in Protein Misfolding, Aggregation and Disease

Emma J. Duncan, Michael E. Cheetham, J. Paul Chapple and Jacqueline van der Spuy

Abstract Molecular chaperones and their associated co-chaperones are essential in health and disease as they are key facilitators of protein folding, quality control and function. In particular, the HSP70 molecular chaperone networks have been associated with neurodegenerative diseases caused by aberrant protein folding. The pathogenesis of these disorders usually includes the formation of deposits of misfolded, aggregated protein. HSP70 and its co-chaperones have been recognised as potent modulators of inclusion formation and cell survival in cellular and animal models of neurodegenerative disease. In has become evident that the HSP70 chaperone machine functions not only in folding, but also in proteasome mediated degradation of neurodegenerative disease proteins. Thus, there has been a great deal of interest in the potential manipulation of molecular chaperones as a therapeutic approach for many neurodegenerations. Furthermore, mutations in several HSP70 co-chaperones and putative co-chaperones have been identified as causing inherited neurodegenerative and cardiac disorders, directly linking the HSP70 chaperone system to human disease.

Keywords HSP70 · Co-chaperone · Protein misfolding and aggregation · Neurodegeneration

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,* Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7_12

Introduction

Molecular chaperone networks have been shown to be fundamentally important to many aspects of human health and disease. In a large number of disease studies, changes in chaperone expression profiles have been observed, such that almost no other class of proteins have been linked to such a large array of human disorders. The HSP70 family of chaperone proteins, and their co-chaperone regulators, have received particular interest in the field of cancer biology, heart disease and neurodegeneration. HSP70 biology has not only contributed to our understanding of the molecular mechanism of these conditions, but has also led to the identification of biomarkers for disease states and potential targets for therapeutic intervention.

Given their importance in protein folding and quality control it is perhaps unsurprising that molecular chaperones have been identified as key modulators of human misfolding disease and in particular neurodegenerations (Bonini 2002; Barral et al. 2004; Muchowski and Wacker 2005). The majority of neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), amytrophic lateral sclerosis (ALS) and polyglutamine (polyQ) expansion diseases, are characterised by conformational changes in proteins that result in misfolding and aggregation (Taylor et al. 2002; Barral et al. 2004; Muchowski and Wacker 2005). Some of these aggregates share a propensity to assemble into amyloid fibrils, which are characterised by detergent insolubility, protease resistance, and high β sheet content and cross β sheet structure (Dobson 2003; Stefani and Dobson 2003). It has been suggested that during the formation of amyloid fibrils 'off-pathway' assembly may occur resulting in misfolded protein monomers or higher-order aggregates that are not required intermediates in amyloid fibril production (Muchowski and Wacker 2005). It is unclear why neurons are particularly vulnerable to the accumulation of these off-pathway species, although it has been suggested it may partly be because as post-mitotic cells they cannot dilute the toxic proteins during cell division (Muchowski and Wacker 2005). In neurons and other cells molecular chaperones represent the first line of defence against aberrant protein accumulation (Fig. 12.1). They are central to the three main cellular defences against protein aggregation (Ross and Poirier 2005); protein folding and refolding (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002); proteasome dependent degradation (Connell et al. 2001; Alberti et al. 2002, 2004; Chapple et al. 2004); inclusion formation and lysosome-mediated autophagy (Cuervo et al. 2004; Cuervo 2004; Fig. 12.1). Furthermore, folding and proteasomal degradation of proteins are linked through co-chaperones, such as C-terminus of HSP70-interacting protein (CHIP) and HSJ1 (DNAJB2) (Westhoff et al. 2005), which regulate triage decisions determining whether misfolded proteins are refolded or degraded.

In this chapter we focus on links between the HSP70 molecular chaperone network and neurodegenerative diseases. Firstly, we consider evidence for the ability of HSP70 and its co-chaperones to act as suppressors of neurodegeneration, with an

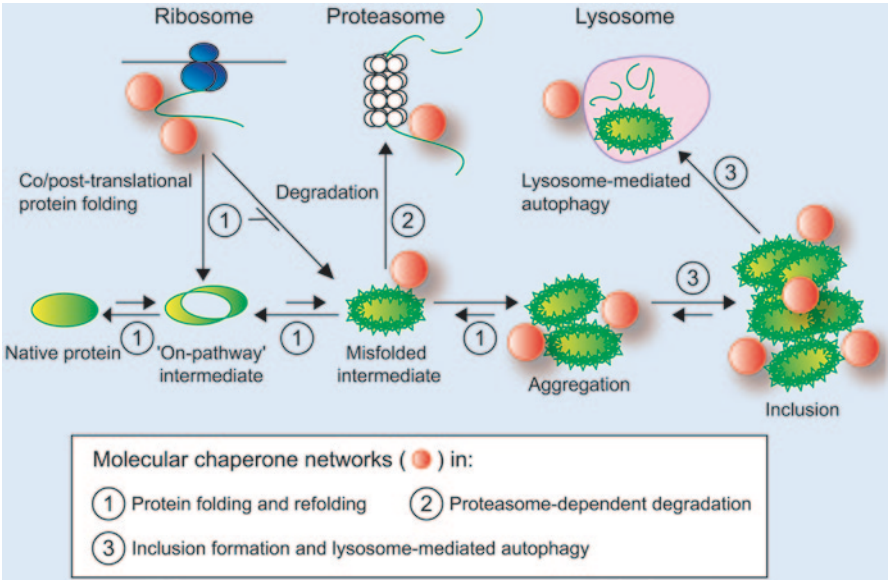

Fig. 12.1 Molecular chaperones in protein misfolding and aggregation. Molecular chaperones and their associated co-chaperones are essential in the cellular defences against protein aggregation. Molecular chaperone networks participate in protein folding and refolding, proteasome-dependent degradation, and inclusion formation and lysosome-mediated autophagy (reproduced with kind permission from Springer Science + Business Media: networking of chaperones by co-chaperones; Chap. 11: the role of Hsp70 and its co-chaperones in protein misfolding, aggregation and disease; 2007; pp. 122–127; Jacqueline van der Spuy, Michael E, Cheetham and J. Paul Chapple; Fig. 11.1)

emphasis on polyQ misfolding diseases. Secondly, we look at a direct link between the HSP70 chaperone machine and disease by considering co-chaperones and putative co-chaperones that are mutated in human genetic disorders.

In this chapter, the chaperone families will be referred to generally as HSP70 (HSPA), HSP110 (HSPH), HSP40 (DNAJ), HSP90 (HSPC) and small HSP (HSPB). Where appropriate, specific members of the chaperone family and co-chaperones will be designated in parentheses according to the HUGO Gene Nomenclature Committee (HGNC) (Kampinga et al. 2009).

HSP70 and Its Co-chaperones in Neurodegenerative Disease

In misfolding disease the ubiquitin proteasome system (UPS) can become compromised and/or overloaded, thereby exacerbating the sequestration of ubiquitylated proteins in inclusions (Fig. 12.1). The impairment of the proteasome itself as well as proteasome impairment as a consequence or cause of inclusion formation

in polyQ misfolding diseases has been controversial. More recent experimental evidence supports a model in which the proteasomal activity is not directly affected by the mutant expanded protein, but rather maintaining the solubility of high levels of the aggregation-prone protein places a burden on the total protein homeostasis machinery, indirectly leading to the collapse of the proteolysis network (Hipp et al. 2012; Schipper-Krom et al. 2012). It is moreover now more certain that such inclusion bodies are a beneficial cellular coping response. The observation that the formation of inclusion bodies in a model of Huntington's disease (HD) reduced the level of mutant polyQ-expanded huntingtin and prolonged cellular survival coincident with less proteasomal impairment points toward inclusions being part of the cellular defence mechanism (Arrasate et al. 2004; Ross and Poirier 2005; Mitra et al. 2009). However, it is clear that the presence of inclusions reveals problems of protein processing and could be viewed as surrogate markers of protein misfolding. There is increasing evidence that the cytotoxic agent in polyQ expansion diseases, including HD are the soluble oligomeric precursors of the aggregated proteins, rather than the insoluble fibrillar species that are sequestered into inclusions (Schaffar et al. 2004; Takahashi et al. 2008; Lajoie and Snapp 2010). Recently, Leitman et al. (2013) revealed that soluble oligomeric polQ-expanded pathogenic huntingtin inhibited endoplasmic reticulum (ER)-associated degradation and consequently induced ER stress prior to the formation of visible aggregates. This supports emerging consensus that the overall, overwhelming disturbance of cellular proteostasis is the trigger for cytotoxicity and pathogenesis prior to cell death.

Chaperones have been shown to co-localise with protein inclusions in cellular and animal models, as well as the lesions observed in human brain tissue. The specific cohort of chaperones associated with inclusions appears to be disease dependent, presumably because although the inclusions have similar biochemical characteristics, the disease protein and cellular context varies. The small heat shock protein (HSPB), the HSP70 and HSP40 families of molecular chaperones have been most frequently associated with misfolding disease. It has been proposed that the interaction of molecular chaperones and other components of the cellular protein quality control machinery with misfolded proteins may deplete them sufficiently that their normal cellular functions are impaired (Sakahira et al. 2002). Other essential cellular proteins, such as transcription factors, are also recruited to inclusions and this may be detrimental to cell survival (McCampbell et al. 2000; Nucifora et al. 2001; Schaffar et al. 2004). Of the proteins which are known to be recruited to inclusions molecular chaperones appear to be unique, as they also have the ability to modulate the formation of the inclusions and cell survival. Interestingly, HSP70 has been demonstrated to be transiently associated with polyQ protein aggregates, exhibiting rapid kinetics of association and dissociation, raising the possibility it may be involved in a pathway rescuing sequestered transcription factors and/or other essential cellular proteins (Kim et al. 2002).

The potential mechanisms by which the HSP70 chaperone machine is neuroprotective are manifold and complex. It seems likely that the HSP70 chaperone machine prevents the conversion of native protein species into toxic intermediates and either facilitates their degradation or, instead, pushes them towards a folding pathway where non-toxic disordered aggregates form. The prominent role played by HSP70 in the removal of toxic protein species by the UPS means that it helps prevent unwanted interactions between misfolded proteins and important cellular proteins such as transcription factors (Schaffar et al. 2004).

The HSP70 Chaperone Machine

The HSP70 chaperone machine is a key component of the cellular protein production and quality control machinery. The frequent association of HSP70 proteins with inclusions of misfolded disease protein suggests this chaperone machine is particularly important in dealing with toxic misfolding disease proteins. HSP70 proteins bind short regions of peptides with a certain position and pattern of hydrophobic residues in a substrate-binding pocket, assisting in their stabilisation and folding (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002). Substrate binding is cyclic with HSP70 switching from a low substrate affinity, fast substrate exchange state when bound to ATP to a high substrate affinity, slow substrate exchange state upon the hydrolysis of ATP to ADP. HSP70 undergoes a conformational change resulting in closure of its substrate binding pocket upon ATP hydrolysis (Bukau and Horwich 1998; Hartl and Hayer-Hartl 2002), dependent on interdomain communication via an allosteric mechanism (Jiang et al. 2005). This cycle is regulated by HSP70 co-chaperones and in particular HSP40 proteins, which are characterised by a highly conserved 70-amino acid domain called the J-domain (Cheetham and Caplan 1998). The J-domain interacts with HSP70 protein, stimulating ATP hydrolysis and altering substrate binding.

Currently, up to 13 HSP70 and 4 HSP70-related HSP110 (HSPH) genes have been identified in humans coding for different members of the HSP70 family, including the cytosolic constitutive heat shock 70 cognate, HSC70 (HSPA8), several stress inducible forms of HSP70 and the endoplasmic reticulum resident glucose-regulated protein 78, GRP78 or BiP (HSPA5). Many more HSP40 proteins have been identified. As well as stimulating HSP70 ATPase activity, HSP40 proteins can bind client proteins independently, directly facilitating targeting to HSP70 (Cheetham and Caplan 1998). Thus HSP40 proteins may provide a mechanism for recruiting the HSP70 machine to its many cellular roles. Interestingly, some type II HSP40 proteins, such as HSJ1 (DNAJB2) and MRJ (DNAJB6), are expressed at higher levels in the brain than other tissues suggesting a specificity and/or particular requirements for HSP70 function in neurons (Chuang et al. 2002; Chapple and Cheetham 2003).

HSP70 and HSP40 Proteins as Modulators of polyQ Protein Aggregation and Toxicity

In 1998 Cummings et al demonstrated that molecular chaperones could be potent modulators of polyQ disease (Cummings et al. 1998). This report showed that in a cellular model of spinocerebellar ataxia type 1 (SCA1) overexpression of the HSP40 protein, hdj-2 (DNAJA1), caused a significant decrease in the incidence of ataxin-1 inclusions. Subsequently, co-expression of hdj-2 (DNAJA1) was demonstrated to reduce inclusion incidence in a model of spinal bulbar muscular atrophy (SBMA) (Stenoien et al. 1999).The overexpression of either hdj-1 (DNAJB1) or hdj-2 (DNAJA1) in a cell model of spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD) suppressed the aggregation and toxicity of polyQ-expanded full-length or truncated mutant ataxin-3 (Chai et al. 1999). Interestingly, not all the studies of HSP40 co-chaperone overexpression have shown beneficial effects on protein aggregation and inclusion formation in cells. For example, it has been reported that overexpression of hdj-2 (DNAJA1) caused increased inclusion formation in a cell model of HD, and had little effect on inclusion formation in cell models of SBMA (Kobayashi et al. 2000; Wyttenbach et al. 2000; Bailey et al. 2002). However, hdj-1 (DNAJB1) in combination with HSP70 reduced inclusion incidence and provided cellular protection, suggesting that members of the HSP70 and HSP40 families might function together in chaperoning aggregation-prone proteins. There are multiple reports of the cooperation of HSP70 and HSP40 proteins in reducing inclusion incidence and toxicity in cellular models of polyQ diseases, including HD (Jana et al. 2000; Rujano et al. 2000).

It seems likely that HSP40 proteins which are enriched in neuronal tissues, or have a neuronal specific expression profile, may be particularly relevant in neurodegenerative diseases. In particular, HSJ1a (DNAJB2a) has been shown to increase ubiquitylation and effectively reduce the incidence of polyQ protein aggregation in both cell models and *in vivo* models of polyQ and protein misfolding diseases dependent on its ubiquitin interaction motifs (UIMs) and a functional J domain (Westhoff et al. 2005; Howarth et al. 2007; Gao et al. 2011; Labbadia et al. 2012; Novoselov et al. 2013). The transgenic overexpression of HSJ1a (DNAJB2a) in the R6/2 mouse model of HD led to improved neurological performance, significantly reduced mutant huntingtin aggregation and enhanced solubility dependent on HSJ1a (DNAJB2a) client binding, ubiquitin interaction and functional co-operation with HSP70 (Labbadia et al. 2012). Interestingly, the overexpression of HSJ1a (DNAJB2a) in a mouse model of ALS was also recently shown to improve motor performance and the survival of motor neurons at the late stages of disease progression (Novoselov et al. 2013). HSJ1a (DNAJB2a) was shown to interact with mutant superoxide dismutase (SOD1) in spinal cord lysates from the transgenic animals and to suppress SOD1 aggregation. In a neuroblastoma cell model, HSJ1a (DNAJB2a) suppressed SOD1 aggregation and enhanced SOD1 ubiquitylation dependent on a functional J-domain and UIMs.

Interestingly, the closely related MRJ (DNAJB6), which lacks UIMs but is similarly expressed at higher levels in the brain, has also been shown to suppress polyQ dependent protein aggregation, and cellular toxicity (Chuang et al. 2002; Hageman et al. 2010). A report by Hageman et al. (2010) compared the efficiency of members of the HSP70, HSP110 and HSP40 chaperone families in suppressing the aggregation of polyQ-expanded huntingtin, and found that members of the HSP40 DNAJB subfamily were comparably potent suppressors of protein aggregation and polyQ-associated cytotoxicity. In particular, DNAJB6b and DNAJB8 suppressed the aggregation of polyQ-expanded huntingtin, ataxin-3 and androgen receptor (AR), as well as unexpanded AR, which also aggregated to some extent. In assessing the dependence of the anti-aggregation activity on the cooperation with the HSP70 machinery, it was found that the anti-aggregation activity did not depend on a J-domain mediated direct interaction with HSP70, although collaboration with the HSP70 machinery for proteasomal degradation of the polyQ substrate was required for the full anti-aggregation activity. These data suggest that the role of HSP40 proteins in suppressing protein misfolding and polyQ toxicity may rely to varying extents on the regulation of the HSP70 machine, depending on the specific identity of the HSP40 member.

In the first *in vivo* investigation of HSP70's effect on polyQ disease, the amount of neurodegeneration was reduced but inclusion formation was not affected. This study of a *Drosophila* model of SCA3 was partially rescued by co-expression of HSP70 (Warrick et al. 1999). Furthermore, an HSP70 mutant without ATPase activity had a dominant negative effect making neurodegeneration worse. In the same *Drosophila* model hdj-1 (DNAJB1) but not hdj-2 (DNAJA1) was able to suppress degeneration and was also observed to have a synergistic effect with HSP70, again without altering inclusion formation (Chan et al. 2000). When another *Drosophila* model was used to screen for genetic factors modifying degeneration caused by expression of polyQ in the fly eye, two HSP40 proteins were identified, dHDJ-1 and dTPR2, which are potentially homologous to human hdj-1 (DNAJB1) and TPR2 (DNAJC7) (Kazemi-Esfarjani and Benzer 2000).

The ability of HSP70 to reduce the severity of polyQ-mediated degeneration has also been demonstrated in mouse models. For example, when a SCA1 transgenic model was crossed with a hemizygous model overexpressing HSP70 at approximately 10-fold normal levels, behavioural and neuropathological symptoms improved (Cummings et al. 2001). When animals homozygous and hemizygous for HSP70 overexpression were compared, results suggested HSP70 ameliorated polyQ pathologies in a dose dependent manner. Not all mouse models of polyQ disease, however, appear to be equally affected by increasing HSP70 levels. In a mouse model of HD, overexpression of HSP70 by 5- to 15-fold only had modest effects on disease progression (Hansson et al. 2003). Conversely, the deletion of HSP70 in the same mouse model of HD exacerbated the behavioural and neuropathological defects, but did not correlate with the levels of fibrillar aggregates although the size of inclusions in the neocortex increased (Wacker et al. 2009).

The Role of the HSP70 Co-chaperones in Modulating polyQ Protein Aggregation and Toxicity

Other regulatory components of the HSP70 chaperone machine have also been recognised as potentially playing important roles in the chaperone response to misfolded disease protein, including the co-chaperones HIP and CHIP, and the HSP70 nucleotide exchange factors (NEFs) Bag-1, HspBP1 and HSP110 (HSPH1). HIP interacts with the nucleotide binding domain of substrate-bound HSP70 to slow the dissociation of ADP and therefore delay the release of the substrate from HSP70 (Li et al. 2013). The interaction of HIP and the HSP70 NEFs with the HSP70-substrate complex occurs in a mutually exclusive manner such that HIP attenuates the active cycling of the HSP70 complex thereby facilitating further downstream processing of the substrate by chaperones or the proteasome (Li et al. 2013). It has been shown that HIP, in collaboration with HSP70, can modulate the ubiquitylation and proteasomal degradation of polyQ-expanded AR thereby reducing the formation of cytotoxic aggregates (Howarth et al. 2009; Wang et al. 2013). Similarly CHIP, a co-chaperone that negatively regulates HSP70 chaperone activity and acts as an ubiquitin ligase for HSP70 client proteins, was reported to suppress the aggregation of polyQ-expanded huntingtin or ataxin-3 by increasing the ubiquitination and subsequent degradation of these mutant proteins (Jana et al. 2005). Interestingly, the context of the polyQ expansion in the full-length protein may be an important factor in CHIP modulation of protein solubility and stability in conferring protection against aggregation-induced neurotoxicity (Al-Ramahi et al. 2006; Choi et al. 2007). CHIP over-expression was reported to promote the ubiquitylation and degradation of both expanded and unexpanded ataxin-1 in cell models,dependent on chaperone interaction (Choi et al. 2007). However, the importance of CHIP in the modulation of polyQ disease *in vivo* is supported by numerous studies. HD transgenic mice haploinsufficient for CHIP display a markedly accelerated disease phenotype (Miller et al. 2005). Moreover, CHIP haploinsufficiency in a hemizygous mouse model of SCA3 exacerbated neuropathological markers and accelerated the progression of the neurobehavioural phenotype through increased levels of pre-fibrillar microaggregates that correlated with disease severity (Williams et al. 2009). Conversely, the hemizygous or homozygous overexpression of full-length human CHIP in transgenic SBMA mice ameliorated motor symptoms by enhancing the degradation and consequently inhibiting the neuronal nuclear accumulation of mutant expanded AR (Adachi et al. 2007).

The HSP70 NEF Bag-1 interacts with the ATPase domain of HSP70 via a C-terminal BAG domain to stimulate HSP70 ATPase activity. Bag-1 has also been shown to interact with CHIP and contains an integral ubiquitin-like domain, which enable it to promote the ubiquitination of HSP70-bound substrates and recruit HSP70 chaperone complexes to the proteasome for degradation of the substrate by the UPS (Luders et al. 2000; Demand et al. 2001). Bag-1 has been shown to accelerate the degradation and reduce the aggregation of polyQ-expanded huntingtin

in cells, and to protect cells against polyQ-induced cytotoxicity (Jana et al. 2005; Jana and Nukina 2005; Sroka et al. 2009). In a *Drosophila* model of HD, Bag-1 was reported to prevent cell loss induced by mutant huntingtin and therefore ameliorate toxicity *in vivo* (Sroka et al. 2009). The HSP70 NEF HSP110 (HSPH1) is structurally related to HSP70, but similar to Bag-1, stabilizes the HSP70 NBD cleft in an open conformation. HSP110 (HSPH1) has been reported to cooperate with HSP70 and HSP40 in protein disaggregation (see Links between HSP70 and other chaperone machines). Recently, it has been reported that the interaction of the *Drosophila* HSP110 protein HSC70cb with the *Drosophila* HSP40 protein DNAJ-1 is both necessary for and enhances the DNAJ-1 mediated suppression of polyQ-induced neurodegeneration in a *Drosophila* model of polyQ disease (Kuo et al. 2013). Moreover, the expression of human HSP110 or human HSP40 DNAJB1 alone had little effect on suppressing polyQ-induced neurodegeneration in the fly model, but together exerted a neuroprotective effect (Kuo et al. 2013). Finally, mutations in Sil1, which functions as the NEF for ER resident GRP78/BiP (HSPA5) and is homologous to the cytosolic NEF HspBP1, cause the neurodegenerative disease Marinesco-Sjögren syndrome, highlighting the critical importance of the nucleotide exchange function. It is hence evident that the co-chaperone regulators and interacting partners of the HSP70 chaperone machine likely represent modulators of misfolding disease.

Links Between HSP70 and Other Chaperone Machines

In many of its cellular roles the HSP70 chaperone machine functions in conjunction with other molecular chaperones systems. For example, the modulation of neurodegeneration by HSP70 chaperones could be performed in concert with the HSPB family, a diverse group of proteins under 40 kDa in size, that include the α-crystallins (CRYAA) and HSP27 (HSBP1). The HSPB family share a C-terminal domain of approximately 100 amino acids, which mediates assembly into large oligomeric structures. Upon cellular stress it is believed that these oligomers reorganise into smaller, active complexes which interact with misfolded proteins preventing them from aggregating and maintaining them in a state from which they can potentially be refolded or degraded, by the HSP70 chaperone machine (Ehrnsperger et al. 1997). There is evidence that HSPB can modulate models of misfolding disease. For example, Hsp27 (HSPB1) has been shown to prevent cellular polyQ toxicity associated with the formation of reactive oxygen species caused by huntingtin, suggesting that Hsp27 (HSPB1) protects against oxidative stress (Wyttenbach et al. 2002). Moreover, a cell model of MJD stably expressing full-length polyQ-expanded ataxin-3 reported defects in Hsp27 (HSPB1) protein synthesis and impairment in the cell stress response, as Hsp27 (HSPB1) over-expression protected against apoptosis (Chang et al. 2009). A comparison of the efficacy of members of the HSPB family to prevent aggregation of expanded huntingtin or ataxin-3 revealed potent suppression of polyQ aggregation and cytotoxicity by cvHSP (HSPB7) in cells

and a *Drosophila* SCA3 model (Vos et al. 2010). However, in a mouse model of HD, the constitutive and ubiquitous overexpression of transgenic Hsp27 (HSPB1) did not rescue the HD phenotype, suggesting the differential modulation of Hsp27 (HSPB1) activation *in vivo* in acute models of disease versus chronic models of disease (Zourlidou et al. 2007).

In yeast it has been demonstrated that Hsp26 alters the nature of polyQ aggregation to facilitate reactivation by the chaperones Hsp104 with the assistance of Hsp70 and Hsp40 proteins (Cashikar et al. 2005). Although no mammalian orthologue of Hsp104 has yet been identified, it has recently been reported that a disaggregase machinery comprised of HSP40, HSP70 and HSP110 (HSPH1), a NEF for HSP70 that also exhibits independent chaperone activity, couples protein disaggregation to protein renaturation in metazoan (Shorter 2011). Together, HSP110, HSP70 and HSP40 synergise to dissolve disordered amorphous aggregates and exploit the slow monomer exchange dynamics of amyloid to slowly depolymerize amyloid fibrils from their ends (Shorter 2011). Both the amyloid depolymerase activity and the disaggregation of disordered aggregates are stimulated by HSPB (Cashikar et al. 2005; Haslbeck et al. 2005; Duennwald et al. 2012). Notably, the mechanistic details of the disaggregase machinery have primarily been elucidated *in vitro* or *in vivo* in yeast using urea- or heat-denatured substrates including luciferase, GFP or citrate synthase, although activity against the infectious amyloid form of the yeast prion protein Sup 35 and amyloid conformers of PD-associated α-synuclein and polyQ-expanded huntingtin have also been tested. The overexpression of Hsp104 together with Hsp26 in yeast strongly reduced the aggregation of polyQ-expanded huntingtin and the associated cytotoxicity (Cashikar et al. 2005). More direct evidence of a disaggregase activity was recently reported by Duennwald et al. (2012). The authors also reported the potentiation of Hsp104 activity by the yeast sHSPs (Hsp26 and Hsp46), but against pre-formed disease-associated amyloid fibrils of polyQ-expanded huntingtin as measured by a decline in thioflavin T fluorescence *in vitro*. In metazoa, it is hence possible that the simultaneous activation or induction of the components of the disaggregase system is necessary to suppress toxicity after the onset of aggregation and degeneration, and could conceivably be beneficial in the treatment of several neurodegenerative disorders. Interestingly, exogenous Hsp104 can interface with the metazoan disaggregase machinery to rapidly eliminate disease-associated amyloid. Hsp104 prevented the aggregation and toxicity of polyglutamine in *C. elegans* (Satyal et al. 2000), and the introduction of Hsp104 into *Drosophila* models of SCA3 suppressed toxicity of a C-terminal ataxin-3 fragment when expressed even after the onset of polyglutamine-induced degeneration (Cushman-Nick et al. 2013).

Pharmacological Manipulation of HSP70 and Other Chaperones

The neuroprotective potential of molecular chaperones may be exploited for the treatment of neurodegenerative diseases. Several drugs have been identified that

induce the expression of HSP70 and other chaperones (Soti et al. 2005). These include the hydroxylamine derivative bimoclomol and its analogue, arimoclomol, and the benzoquinone ansamycin antibiotic, geldanymycin and derivatives thereof. These compounds potentiate chaperone expression by activating heat shock transcription factor Hsf-1 (Soti et al. 2005). Hydroxylamine derivatives bind Hsf-1 to stabilize the active phosphorylated trimer and prolong its binding to the heat shock response element found in the heat shock gene promoters. In contrast, the benzoquinone ansamycins bind to the ATP site on HSP90 and block its interaction with Hsf-1 and other clients, thereby leading to Hsf-1 trimerization and stimulating the transcription of heat shock proteins.

These pharmacological compounds have been tested in several models of polyQ disease. The geldanamycin derivative 17-AAG (17-allylamino-17-demethoxygeldanamycin) (Tanespimycin) suppressed neurodegeneration and rescued lethality in a *Drosophila* model of SCA3 and HD dependent on Hsf-1 activation and HSP induction (Fujikake et al. 2008). 17-AAG and the derivative 17-DMAG (17-(dimethylaminoethylamino)-17-demethoxygeldanamycin) (Alvespimycin), were reported to significantly ameliorate polyQ-mediated motor neuron degeneration in the SBMA transgenic mouse model without detectable toxicity through HSP90 chaperone complex formation and preferential proteasome-dependent degradation of polyQ-expanded mutant AR (Waza et al. 2005; Tokui et al. 2009). Arimoclomol has also shown very encouraging results in the transgenic mouse model of SBMA, significantly improving motor neuron survival and rescuing the neuromuscular phenotype (Malik et al. 2013). Moreover, arimoclomol has been reported to delay disease progression in ALS mice, resulting in a 22 % increase in lifespan (Kieran et al. 2004). This correlated with a slight increase in Hsp27 (HSPB1) levels and a significant increase in both HSP70 and HSP90 levels in the spinal cord of the treated ALS mice. Arimocolomal has reached clinical testing in ALS (Cudkowicz et al. 2008; Lanka et al. 2009). However, the complete mechanism of action of arimoclomol (and other heat shock response activating drugs) is uncertain at present, and it is not clear that all the beneficial effects of arimoclomol are related to the potentiation of the heat shock response; for example, recent evidence showed that arimoclomol can also potentiate the unfolded protein response when there is ER stress (Parfitt et al. 2014).

The clinical development of geldanamycin has been limited by its poor pharmacokinetic profile, including poor solubility and blood-brain-barrier permeability. Geldanamycin derivatives, including 17-AAG and 17- DMAG, have better pharmacokinetic profiles but their use is limited by poor blood-brain-barrier permeability and toxicity, respectively (Kim et al. 2009; Porter et al. 2010). Moreover, there is evidence that these drugs may directly affect the interaction of HSP90 with client proteins leading to undesirable cellular effects. For example, Aquilá et al. (2014) recently reported that inhibition of HSP90 by 17-AAG and the HSP90 inhibitor 2-amino-7, 8-dihydro-6H-pyrido[4,3-D]pyrimidin-5-one NVPHSP990 (HSP990) post-transcriptionally down-regulated HSP90 client proteins essential for vision, including the G-protein-coupled receptor kinase (GRK1) and the β subunit of retinal phosphodiesterase (PDE6β). Interestingly, the stability of retinal PDE6 requires

the cooperation of HSP90 with the retina-specific HSP90 co-chaperone AIPL1, mutations in which cause the devastating disease Leber congenital amaurosis (LCA) characterised by the loss or severe impairment of vision at birth (Sohocki et al. 2000; Hidalgo-de-Quintana et al. 2008; Kolandaivelu et al. 2009). Therefore, despite encouraging results in disease models, the clinical utility of these compounds for the treatment of neurodegenerative diseases may be limited. Arimoclomol has a superior pharmacokinetic profile and biodistribution in humans to bimoclomal (Visy et al. 2002). Another important therapeutic consideration is that unlike other HSR inducers, which induce the HSR in both unstressed and stressed cells, compounds such as arimocolomal co-induce the HSR only under cellular stress conditions (Hargitai et al. 2003).

As chaperones are fundamentally important in many essential cellular processes it would not be surprising if pharmacological interference in their expression had deleterious effects, although as yet none have been reported with arimoclomol. Given the importance of Hsf-1 as the master regulator of chaperone gene transcription and the limitations of global HSP90 inhibition, small molecules that directly modulate Hsf-1 may be advantageous. A high-throughput screen for small molecule activators of Hsf-1 recently identified the compound HSF1A, a small benzyl pyrazole-based molecule, which was shown to promote Hsf-1 activation in the absence of HSP90 inhibition and did not induce undesirable proteotoxic activity (Neef et al. 2010). HSF1A-mediated HSP induction reduced protein aggregates and ameliorated polyQ-induced cytotoxicity in both a neuronal precursor cell and *Drosophila* model of HD (Neef et al. 2010). Another therapeutic approach may be to target specific co-chaperones, such as HSJ1 (DNAJB2) proteins, which have been demonstrated to modulate protein aggregation, but are not ubiquitously expressed.

Mutations in Putative HSP70 Co-chaperones Which Cause Inherited Disease

Multiple human disorders have been identified that are associated with mutations in genes encoding chaperones or putative chaperones (Table 12.1). As yet, no mutations associated with disease have been identified in HSP70 proteins, possibly because these molecular chaperones are so fundamentally important to cellular survival that mutations would be lethal. However, mutations in several HSP70 co-chaperones have been identified as causing disease. The following is a brief description of some of these proteins.

The BiP Nucleotide Exchange Factor SIL1

In the lumen of the ER the HSP70 family member GRP78/BiP (HSPA5) plays a crucial role in protein folding, protein translocation and quality control (Kleizen

Table 12.1 Genetic disorders associated with mutations in putative chaperones or co-chaperones

Protein	Disease	Main phenotypic features	Chaperone system	Function/putative function	References
Hsp60/HSPD1	Hereditary spastic paraplegia-13	Progressive spasticity	Type I chaperonin	Mitochondrial protein folding	Hansen et al. (2002)
		Weakness of lower limbs			
BBS6/MKKS	McKusick-Kaufman syndrome and Bardet-Biedl syndrome-6	Retinal degeneration	Type II chaperonin homology	Localized to the centrosome and cilia basal body	Katsanis et al. (2000)
		Obesity		Important for normal cilia function and cytokinesis	Stone et al. (2000)
		Post-axial poldactyly			Kim et al. (2005)
					Ross et al. (2005)
Tubulin specific chaperone E (TBCE)	Sanjad-Sakati and Kenny-Caffrey	Hypoparathyroidism	Post-CCT tubulin specific chaperone pathway	Folding of α-tubulin and its heterodimerization with β-tubulin Regulation of microtubules	Martin et al. (2002)
		Mental retardation			Parvari et al. (2002)
		Facial dysmorphism			
		Growth failure			
RP2	X-linked retinitis pigmentosa-2	Retinal degeneration	Homology to tubulin specific chaperone C (TBCC)	GTPase activating protein (GAP) for the ADP ribosylation factor like 3 protein (Arl3)	Schwahn et al. (1998)
				Regulates the assembly and traffic of membrane associated protein complexes	Schwarz et al. (2012)

Table 12.1 (continued)

Protein	Disease	Main phenotypic features	Chaperone system	Function/putative function	References
SIL1/BAP	Marinesco-Sjögren syndrome	Cerebellar ataxia	Hsp70 co-chaperone	Regulation of BiP mediated protein folding in the ER	Senderek et al. (2005)
		Cataracts			Anttonen et al. (2005, 2008)
		Developmental delay			Karim et al. (2006)
		Myopathy			Eriguchi et al. (2008)
					Takahata et al. (2010)
Tim14/DNAJC19	Dilated Cardiomyopathy with Ataxia (DCMA)	Cardiomyopathy	Hsp70 co-chaperone	Translocation of preproteins across the inner membrane of mitochondria	Davey et al. (2006)
		Cerebellar ataxia			Ojala et al. (2012)
		Growth failure			
HSJ1a/DNAJB2	Distal hereditary motor neuropathy (dHMN)	Motor neuron degeneration in anterior horn spinal cord	Hsp70 co-chaperone	Dual regulation of protein folding mediated by J-domain dependent Hsp70 interaction and ubiquitylation via UIM motifs	Blumen et al. (2012)
		Muscle weakness and atrophy			
MRJ/DNAJB6	Limb-girdle muscular dystrophy	Proximal dominant muscle weakness	Hsp70 co-chaperone	Neural stem cell renewal, reduces the formation and toxicity of misfolded protein aggregates	Harms et al. (2012)

Table 12.1 (continued)

Protein	Disease	Main phenotypic features	Chaperone system	Function/putative function	References
		Muscle atrophy		Role in cell cycle regulation and malignancies such as breast cancer and melanoma	Sarparanta et al. (2012)
					Sato et al. (2013)
					Couthouis et al. (2014)
Sacsin/SACS	Spastic Ataxia of Charlevoix-Saguenay (ARSACS/SACS)	Motor neuropathy	Putative DnaJ domain and Hsp90 homology	Possible Hsp70 co-chaperone	Engert et al. (2000)
		Sensory neuropathy		Role in mitochondrial dynamics	Vermeer et al. (2008)
		Retinal hypermyelination			Bouhlal et al. (2011)
					Pyle et al. (2012)
					Girard et al. (2012)
Aryl hydrocarbon receptor interacting protein-like 1 (AIPL1)	Leber congenital amaurosis	Early-onset, severe retinal degeneration	Hsp90 TPR co-chaperone, also able to interact with Hsp70	Chaperone for the post-translational stability of the catalytic subunit and assembly of photoreceptor phosphodiesterase (PDE)	Sohocki et al. (2000)
					Hidalgo-de-Quintana et al. (2008)
					Kolandaivelu et al. (2009)

Table 12.1 (continued)

Protein	Disease	Main phenotypic features	Chaperone system	Function/putative function	References
αA-Crystallin/	Congenital cataracts	Cataracts	Small heat shock protein	Lens protein biogenesis and protection from damage	Litt et al. (1998)
HSBP4					Mackay et al. (2003)
αB-Crystallin/	Desmin Related myopathy and Congenital cataracts	Skeletal myopathy	Small heat shock protein	Chaperone for the assembly of desmin filaments	Vicart et al. (1998)
HSPB5		Cardiomyopathy		Lens protein biogenesis and protection from damage	
		Cataracts			
Hsp27/HSPB1	Charcot-Marie-Tooth disease	Weakness	Small heat shock protein	Oligomeric complex formation with other sHSP, thus mutations lead to the formation of cytotoxic amyloid protein oligomers and precipitates	Evgrafov et al. (2004)
	Distal hereditary motor neuropathy (dHMNIIb)	Limb muscle atrophy		Abnormally organized intermediate filaments and disrupted microtubule dynamics	Tang et al. (2005a)
				Disruption of cytoskeletal networks	Kijima et al. (2005)
				Increased downstream enzymatic activities	Houlden et al. (2008)

Table 12.1 (continued)

Protein	Disease	Main phenotypic features	Chaperone system	Function/putative function	References
Hsp22/HSPB8	Distal hereditary motor neuropath (dHMNIIa)	Weakness	Small heat shock protein	Oligomeric complex formation with other sHSP, thus mutations lead to the formation of cytotoxic amyloid protein oligomers and precipitates	Tang et al. (2005b)
		Limb muscle atrophy		Disruption of cytoskeletal networks	Irobi et al. (2010)
				Increased downstream enzymatic activities	
HspL27/HSPB3	Distal hereditary motor neuropath (dHMNIIc)	Weakness	Small heat shock protein	Oligomeric complex formation with other sHSP, thus mutations lead to the formation of cytotoxic amyloid protein oligomers and precipitates	Kolb et al. (2010)
		Limb muscle atrophy		Disruption of cytoskeletal networks	
				Increased downstream enzymatic activities	

and Braakman 2004). Mutations in the GRP78/BiP (HSPA5) co-chaperone SIL1 (or BAP, for BiP associated protein) have been identified as causing the multi-system autosomal recessive disorder Marinesco-Sjögren syndrome (MSS; OMIM 248800) (Anttonen et al. 2005, 2008; Senderek et al. 2005; Karim et al. 2006; Eriguchi et al. 2008; Takahata et al. 2010). This disease is a multi-system disorder affecting multiple tissues with key features including: cerebellar ataxia, due to Purkinje and granule cell loss; progressive myopathy and psychomotor delay; hypotonia; early-onset cataracts; mental retardation and short stature. One report of a novel SIL1 homozygous premature stop mutation reported the absence cerebellar ataxia in two affected maternal cousins, although cellular atrophy could not be assessed (Karim et al. 2006). Moreover, additional and novel atypical findings in MSS patients with SIL1 mutations suggest a broader clinical spectrum caused by SIL1 mutations than previously thought (Ezgu et al. 2014). These include facial dysmorphism and dental abnormalities; coloboma; seizures and diffuse eczemea. The 461 amino acid N-glycosylated SIL1 protein contains an N-terminal ER targeting sequence and a divergent C-terminal ER retention signal (LLKELR) (Chung et al. 2002). In the ER, SIL1 interacts with the ATPase domain of GRP78/BiP (HSPA5) and induces ADP release and subsequent exchange for ATP, thus acting as a nucleotide exchange factor for GRP78/BiP (HSPA5) and regulating the chaperones substrate binding cycle (Chung et al. 2002). SIL1 mutations in MSS patients include premature stops, frame shifts, splice site mutations and large intragenic deletions (Anttonen et al. 2005, 2008; Senderek et al. 2005; Karim et al. 2006; Eriguchi et al. 2008; Takahata et al. 2010).The majority of SIL1 mutations are truncating mutations that lead to the loss of SIL1 function and the consequent disruption of ER protein quality control. Using homology mapping and replacement based on the co-crystal structure of the cytosolic BiP/SIL1 homologs HSP70 and HspBP1, SIL1 mutant proteins clustering in the major interaction site (exons 6 and 9) or minor interaction site (exon 10) were predicted to disrupt binding to GRP78/BiP (HSPA5) and induce protein folding defects in the ER (Anttonen et al. 2005; Senderek et al. 2005). Mutations affecting the C-terminal ER retention signal were initially predicted to interfere with the ER localisation through disruption of the ER retention signal, leading to inappropriate secretion of SIL1 and depletion from the ER. However in contrast, it was recently reported that mutations that disrupt the ER retention signal result in unstable mutant proteins that either form large aggregates in the ER or are rapidly degraded by the proteasome, suggesting that the C-terminal residues of SIL1 are important for its structural integrity and that the clinical defect is unlikely to arise from depletion of SIL1 from the ER (Howes et al. 2012). Interestingly, prior to the identification of mutations in SIL1 in MSS, a spontaneous recessive mouse mutation, woozy (*wz*), was identified as being caused by disruption of the mouse SIL1 gene. The *wz* mouse has an overlapping phenotype with MSS, including adult onset ataxia with loss of cerebellar Purkinje cells (Zhao et al. 2005). Affected cells have intracellular protein inclusions in the ER and nucleus and upregulation of the unfolded protein response (Zhao et al. 2005). These data suggest that BiP mediated protein folding is compromised in the ER of affected cells. SIL1 appears to be ubiquitously expressed, so it is unclear

why only certain cell types are affected in MSS. Possible mechanisms that could explain this differential cell sensitivity could include: lack of a compensatory factor for mutated SIL1; enhanced sensitivity to an impaired ER chaperone machinery or UPR; or specialised client protein requirements (Anttonen et al. 2005). The precise basis remains to be defined, yet the identification of mutations in SIL1 highlight the importance of correct chaperone networking in the ER as well as the cytosol.

The Mitochondrial HSP40 Protein DNAJC19

Splice site and frameshift mutations in the *DNAJC19* gene have been shown to be associated with a rare autosomal recessive disorder, Dilated Cardiomyopathy with Ataxia (DCMA; OMIM 608977) (Davey et al. 2006; Ojala et al. 2012). Features of this condition have been characterised as early onset dilated cardiomyopathy with conductance defects, non-progressive cerebellar ataxia, testicular dysgenesis, growth failure and 3-methylglutaconic aciduria (Davey et al. 2006). DNAJC19 has previously been identified as a component of the mitochondrial proteome (Taylor et al. 2003) and is a human orthologue of the yeast HSP40 protein Tim14/Pam18. Tim14/Pam18 is essential for cell viability in yeast, as it functions as a component of the Tim23 complex, a membrane-bound translocase machinery that mediates the import of presequence-containing preproteins across the inner membrane of the mitochondria (D'Silva et al. 2003; Mokranjac et al. 2003; Truscott et al. 2003). In yeast, the essential core channel, composed of Tim23 and Tim17, interacts cooperatively with the import motor. The central motor component, Tim44, recruits the mitochondrial HSP70 (mtHSP70), which together with the accessory J proteins plays a central critical role in protein import. The J protein Tim14/Pam18 stimulates the ATPase activity of mtHSP70 thereby facilitating efficient binding of mtHSP70 to the incoming preproteins. Tim16/Pam16 is a J-like protein that forms a stable J-mediated heterodimeric subcomplex with Tim14/Pam18 to recruit it to the translocase (Mokranjac et al. 2006; Iosefson et al. 2007). Recently, an investigation of Tim23 organisation in mammalian cells reported that the dynamic recruitment of Tim17 paralogues in association with particular J proteins governs the function of three distinct translocase complexes (A, B1 and B2) (Sinha et al. 2014). The Tim17b paralogue together with DNAJC19 (Tim14/Pam18) are recruited to translocase B1 and B2 to drive robust constitutive mitochondrial functions, whereas the Tim17a paralogue together with DNAJC15 (MCJ) are recruited to translocase A, a nonessential translocase with a specific role in the translocation of oncoproteins lacking presequence (Schusdziarra et al. 2013; Sinha et al. 2014). Consistent with this function, the loss of DNAJC15 expression is observed in several tumors, including ovarian tumors, Wilm's tumors, malignant paediatric brain tumors and in melanomas (Schusdziarra et al. 2013).

Interestingly, there are significant similarities between the DCMA phenotype and another disease in which abnormal mitochondria and respiratory chain defects are observed, X-linked Barth syndrome (BTHS: OMIM 302060). In BTHS the *TAZ1* gene for the evolutionary conserved Tafazzin protein is mutated (Bione et al. 1996; Mazurová et al. 2013). Tafazzin is localized to both the mitochondrial inner and outer membranes, but always facing the intermembrane space, where it functions as an phospholipid acyltransferase involved in the remodeling of cardiolipin (CL) (Brandner et al. 2005; Claypool et al. 2006; Xu et al. 2006). CL is similarly a constituent of the inner and outer mitochondrial membranes where it has a pleiotropic function in the maintenance of membrane complexes and cristae morphology, and is critical for the biogenesis of respiratory chain supercomplexes (Xu et al. 2006; Aechan et al. 2007; Gebert et al. 2009; Dudek et al. 2013). Thus, the inactivation of Tafazzin affects both the assembly and stability of mitochondrial respiratory chain complexes.The knockdown of Tafazzin in mice results in a CL deficiency, reduced mitochondrial respiratory chain activity and ATP production, decreased cardiac contractility, hypertrophy and cell death (He 2010).While the DCMA phenotype reflects a defect in mitochondrial protein import, whether there is a direct link between the pathways involved in this disease and BTHS is unresolved.

Another mitochondrial HSP40 protein Tid1 (DNAJA3) has been identified as an interacting partner with the α-subunit of the mitochondrial DNA polymerase γ (Polga) (Hayashi et al. 2006). Furthermore, polga has been identified as a client of the yeast homolog of Tid1 (Duchniewicz et al. 1999). Tid1 (DNAJA3) functions to maintain the integrity of mitochondrial DNA (mtDNA) and regulate the steady-state homogeneity of the mitochondrial membrane potential in a J-domain dependent manner (Ng et al. 2014). The knockdown of Tid1 (DNAJA3) in mice leads to embryonic lethality, whereas mice with a conditional loss of Tid1 (DNAJA3) in the heart have a decreased copy number of mitochondrial DNA and succumb at 10 weeks to dilated cardiomyopathy, further illustrating the importance of HSP40 proteins in protein folding in the mitochondria (Lo et al. 2004; Hayashi et al. 2006). Finally, genetic defects in the HSP70 co-chaperone BAG3 have recently been shown to cause adult onset dilated cardiomyopathy (Norton et al. 2011), highlighting the importance of specific chaperone—co-chaperone networks in mitochondrial function and physiology in human cardiac health.

The Spastic Ataxia Protein SACS

Mutations in the *SACS* gene have been identified as causing the inherited ataxia, autosomal recessive spastic ataxia of Charlevoix-Saguenay (SACS/ARSACS: OMIM 270550) (Engert et al. 2000). ARSACS is characterised by early onset neurodegeneration with absent sensory-nerve conduction; reduced motor-nerve velocity and hypermyelination of retinal-nerve fibres. Pathological features include atrophy of the upper cerebellar vermis (Martin et al. 2007; Bouhlal et al. 2011), and absence of Purkinje cells post mortem (Bouchard et al. 2000). Although mutations in this

protein were identified as causing ARSACS in a Canadian population, ARSACS patients have now been identified worldwide with cases in Europe, North Africa, Turkey, Japan and Brazil (Bouhlal et al. 2011; Pyle et al. 2012). Patients outside of Canada display considerable phenotypic heterogeneity and a later disease onset is more common (Pyle et al. 2012; Synofzik et al. 2013). The *SACS* gene was initially thought to be encoded by a single gigantic exon (Engert et al. 2000), but a further eight coding exons and a tenth non-coding exon have been identified upstream of this, forming a 13,737 bp open reading frame (Ouyang et al. 2006; Vermeer et al. 2008). *SACS* encodes the protein sacsin (SACS), a multimodular protein of 4579 amino acids (520 kDa), one of the largest known proteins in the human genome. SACS is expressed in many tissues, with high expression in large neurons, particularly cerebellar Purkinje cells (Parfitt et al. 2009). Subcellular localisation shows a predominantly cytoplasmic localisation with a significant mitochondrial component (Vermeer et al. 2008; Girard et al. 2012).

SACS is a putative co-chaperone of the HSP40 family based upon the presence of a J domain at the C-terminus of its predicted amino acid sequence (~60% identity over 30 residues compared to hdj-1 (DNAJB1)). Although the SACS J domain is divergent from that of HSP40 it does contain the highly conserved His-Pro-Asp (HPD) motif essential for the stimulation of HSP70 ATPase activity. The SACS J-domain was found to function with bacterial HSP70 (DnaK) by an *in vivo* complementation assay (Parfitt et al. 2009), and a recombinant version of the J-domain from mouse SACS increased the ATPase activity of HSP70 (Anderson et al. 2010). This confirmed that SACS is a type III HSP40 protein. Type III HSP40 proteins recruit HSP70s to specialised roles. Interestingly, mutations in the HSP40 family *DNAJB2* gene have recently been shown to cause distal hereditary motor neuropathy (dHMN), characterised by motor neuron degeneration in the anterior horn spinal cord, muscle weakness and atrophy (Blumen et al. 2012).

SACS consists of three repeated regions, known as the SACS-repeating region (SRR), which cover ~84% of the protein sequence (Romano et al. 2013). Each repeated region contains discrete sub-repeats, the first of which is homologous to the HATPase (Histidine kinase-like ATPases; SMART acc. no. SM00387) domain of HSP90 (Anderson et al. 2010; Romano et al. 2013). Biochemical characterisation identified the repeating regions to be ATPase active. Furthermore, a disease causing mutation (D168Y) within the first sub-repeat region, homologous to HSP90, abolished the ability to hydrolyse ATP. From this, it was suggested that ATPase activity is a requirement for SACS function, as this mutation leads to essentially the same clinical phenotype as nonsense mutations close to the N-terminus of the protein that result in truncations of the protein (Anderson et al. 2010). In HSP90, the middle domain contains an arginine residue that accepts phosphate after ATP hydrolysis (Pearl and Prodromou 2006). This arginine is conserved in each of the three SRR domains and a mutation occurring in one of these conserved arginines, namely c.1420C>T (p.R474C), leads to a severe clinical phenotype (Romano et al. 2013). Previously, HSP90 and the hdj-2 (DNAJA1) protein have been implicated to function together in folding pathways, for example in the maturation of the glucocorticoid receptor.

At the C-terminus of SACS is a higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domain (Grynberg et al. 2003), which binds to various nucleotides, such as ATP, ADP, and GTP, but does not exhibit any GTPase or ATPase activity (Kozlov et al. 2011). Crystal structure of the human SACS-HEPN domain revealed that it exists as a dimer. An ARSACS patient mutation, N4549D, at the dimer interface hinders protein folding and dimerization of the domain, which is likely responsible for its loss of function (Kozlov et al. 2011). HEPN domains are widely distributed in eubacteria and archaea but are restricted to animals in eukaryotes (Grynberg et al. 2003). In humans, the HEPN domain occurs only in the protein SACS. It has been suggested that the close proximity of the SACS J-domain and HEPN domain could be important for the hypothesised function of SACS as a co-chaperone. The HEPN domain may increase the local concentration of GTP or ATP to promote nucleotide exchange onto HSP70 (Kozlov et al. 2011).

Additionally, the N-terminus of SACS contains an ubiquitin-like (UbL) domain, which shares 43 % homology to the Rad23A UbL domain over 65 residues, and has been shown by co-immunoprecipitation to interact with the 20S proteosomal alpha subunit C8 (Parfitt et al. 2009). Towards the C-terminus is the XPCB domain, which shares 35 % homology with the hHR23 XPCB domain (Kamionka and Feigon 2004). Interestingly, the hHR23 protein also contains an UbL domain, which similar to SACS, interacts with the 19S regulatory subunit of the 26S proteasome (Mueller and Feigon 2002). The SACS-XPCB domain was recently identified as a potential binding domain for the E3 ubiquitin ligase Ube3A, which is non-functional in Angelman's syndrome (Greer et al. 2010). In Ube3A KO mice levels of ubiquitinated SACS were severely reduced, suggesting Ube3A is responsible for ubiquitinating SACS (Jana 2012). Bioinformatic analyses also suggests two UIMs in SACS, located either side of the XPCB domain. The presence of the UbL domain, XPCB domain and UIM motifs all point towards a function for SACS in the ubiquitin-proteosome pathway of protein degradation. This along with the presence of the J-domain and HSP90 like chaperone domains suggest that SACS plays a role in proteostasis. Interestingly, other proteins that function in the ubiquitin-proteosome pathway are linked to ataxias, for example, ataxin-3 contains a UIM and functions as a deubiquitination enzyme (Burnett et al. 2003).

SACS's localisation indicates it may function at mitochondria. This is supported by recent findings which show that siRNA-mediated SACS knockdown in SH-SY5Y cells leads to a more interconnected mitochondrial network (Girard et al. 2012). Similarly, fibroblasts from ARSACS patients display a hyperfused mitochondrial phenotype indicated by the presence of balloon-like or bulbed mitochondria (Girard et al. 2012). This mitochondrial phenotype parallels that seen when the mitochondrial fission protein Drp1 is silenced or when mutant forms of Drp1 are overexpressed (Frank et al. 2001; Smirnova et al. 2001; Lee et al. 2004; Estaquier and Arnoult 2007). This suggests that loss of SACS function leads to a disruption in normal mitochondrial dynamics. Furthermore, a decrease in mitochondrial fission seems more likely than enhanced fusion due to the identification

of an interaction between SACS and Drp1 by coimmunoprecipitation (Girard et al. 2012). Mitochondrial fission and fusion are essential processes for quality control of mitochondria and disruption of this leads to detrimental cellular consequences.

Transgenic SACS knockout (KO) mice display an age-dependent loss of Purkinje cells in the cerebellum compared to wild-type littermates (Girard et al. 2012), consistent with the progressive ataxia seen in patients. Loss of SACS in neurons disrupts mitochondrial transport. It has been hypothesised that larger, unusually shaped mitochondria cannot distribute into the narrow dendrites to reach synapses and instead accumulate in the soma and proximal dendrites. This leads to striking alterations in the organization of dendritic fields in the cerebellum of SACS knockout mice, which precedes Purkinje cell death (Girard et al. 2012). Neurons are very sensitive to changes that disturb mitochondria because of their high metabolic activity and significant energy demand at locations distant from the cell body. In addition, SACS knockdown cells and neurons from SACS knockout mice have a loss in mitochondrial membrane potential, which is generated by oxidative phosphorylation, thereby indicating a loss in mitochondrial function (Girard et al. 2012). Although the role of SACS in the brain is unknown, it is intriguing to speculate that it may be a chaperone for proteins involved in related ataxias and/or components of the machinery that regulate mitochondrial dynamics. Moreover, there is increasing evidence that perturbation of the equilibrium between mitochondrial fission and fusion underlie mitochondrial defects observed in age-related neurodegenerative diseases, including Alzheimer's, Parkinson's and Huntington's (Lu 2009).

Conclusions

The molecular chaperones and their associated co-chaperones are of central importance to protein function from facilitating folding, transport and translocation, through functional maturation to the clearance of misfolded species. Failure of chaperones to fulfil these vital roles may ultimately contribute to a number of devastating human diseases. A number of inherited human disorders have also been associated with mutations in molecular co-chaperones, the modulatory function of which is essential for the normal regulation of the molecular chaperone networks. Therefore, the central importance of the molecular chaperones and their associated co-chaperones in protein misfolding, aggregation and disease makes them a prime target for pharmacological intervention for the treatment of these diseases.

Acknowledgements The text in this chapter contains sections reproduced with kind permission from Springer Science + Business Media: Networking of Chaperones by Co-chaperones; Chap. 11: The role of Hsp70 and its co-chaperones in protein misfolding, aggregation and disease; 2007; page 122–127; Jacqueline van der Spuy, Michael E, Cheetham and J. Paul Chapple.

References

Adachi H, Waza M, Tokui K et al (2007) CHIP overexpression reduces mutant androgen receptor protein and ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. J Neurosci 27:5115–5126

Aechan D, Xu Y, Stokes DL et al (2007) Comparison of lymphoblast mitochondria from normal subjects and patients with Barth syndrome using electron microscopic tomography. Lab Invest 87:40–48

Alberti S, Demand J, Esser C et al (2002) Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. J Biol Chem 277:45920–45927

Alberti S, Bohse K, Arndt V et al (2004) The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. Mol Biol Cell 15:4003–4010

Al-Ramahi I, Lam YC, Chen HK et al (2006) CHIP protects from the neurotoxicity of expanded and wild-type ataxin-1 and promotes their ubiquitination and degradation. J Biol Chem 281:26714–224

Anderson JF, Siller E, Barral JM (2010) The SACS repeating region (SRR): a novel HSP90-related supra-domain associated with neurodegeneration. J Mol Biol 400:665–674

Anttonen AK, Mahjneh I, Hamalainen RH et al (2005) The gene disrupted in Marinesco-Sjogren syndrome encodes SIL1, an HSPA5 cochaperone. Nat Genet 37:1309–1311

Anttonen AK, Siintola E, Tranebjaerg L et al (2008) Novel SIL1 mutations and exclusion of functional candidate genes in Marinesco-Sjögren syndrome. Eur J Hum Genet 16:961–969

Aquilá M, Bevilacqua D, McCulley et al (2014) Hsp90 inhibition protects against inherited retinal degeneration. Hum Mol Gen 23:2164–2175

Arrasate M, Mitra S, Schweitzer ES et al (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431:805–810

Bailey CK, Andriola IF, Kampinga HH et al (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. Hum Mol Genet 11:515–523

Barral JM, Broadley SA, Schaffar G et al (2004) Roles of molecular chaperones in protein misfolding diseases. Semin Cell Dev Biol 15:17–29

Bione S, D'Adamo P, Maestrini E et al (1996) A novel X-linked gene, G4.5. is responsible for Barth syndrome. Nat Genet 12:385–389

Blumen SC, Astord S, Robin V et al (2012) A rare recessive distal hereditary motor neuropathy with HSJ1 chaperone mutation. Ann Neurol 71:509–519

Bonini NM (2002) Chaperoning brain degeneration. Proc Natl Acad Sci U S A 99(Suppl 4):16407–16411

Bouchard JP, Richter A, Melancon SB et al (2000) Autosomal recessive spastic ataxia (Charlevoix-Saguenay). In: Klockether T (ed) Handbook of Ataxia disorders. Marcel Dekker, New York, pp 312–324

Bouhlal Y, Amouri R, El Euch-Fayeche G et al (2011) Autosomal recessive spastic ataxia of Charlevoix-Saguenay: an overview. Parkinsonism Relat Disord 17:418–422

Brandner K, Mick DU, Frazier AE et al (2005) Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth Syndrome. Mol Biol Cell 16:5202–5214

Bukau B, Horwich AL (1998) The Hsp70 and Hsp60 chaperone machines. Cell 92:351–366

Burnett B, Li F, Pittman RN (2003) The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Hum Mol Gen 12:3195–3205

Cashikar AG, Duennwald M, Lindquist SL (2005) A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. J Biol Chem 280:23869–23875

Chai Y, Koppenhafer SL, Bonini NM et al (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. J Neurosci 19:10338–10347
Chan HY, Warrick JM, Gray-Board et al (2000) Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in Drosophila. Hum Mol Genet 9:2811–2820
Chang W-H, Tien C-L, Chen T-J et al (2009) Decreased protein synthesis of Hsp27 associated with cellular toxicity in a cell model of Machado-Joseph disease. Neurosci Lett 454:152–156
Chapple JP, Cheetham ME (2003) The chaperone environment at the cytoplasmic face of the endoplasmic reticulum can modulate rhodopsin processing and inclusion formation. J Biol Chem 278:19087–19094
Chapple JP, van der Spuy J, Poopalasundaram S et al (2004) Neuronal DnaJ proteins HSJ1a and HSJ1b: a role in linking the HSP70 chaperone machine to the ubiquitin-proteasome system? Biochem Soc Trans 32:640–642
Cheetham ME, Caplan AJ (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress Chaperones 3:28–36
Choi JY, Ryu JH, Kim HS et al (2007) Co-chaperone CHIP promotes aggregation of ataxin-1. Mol Cell Neurosci 34:69–79
Chuang JZ, Zhou H, Zhu M et al (2002) Characterization of a brain-enriched chaperone, MRJ, that inhibits Huntingtin aggregation and toxicity independently. J Biol Chem 277:19831–19838
Chung KT, Shen Y, Hendershot LM (2002) BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. J Biol Chem 277:47557–47563
Claypool SM, McCaffery JM, Koehler CM (2006) Mitochondrial mislocalization and altered assembly of a cluster of Barth syndrome mutant tafazzins. J Cell Biol 174:379–390
Connell P, Ballinger CA, Jiang J et al (2001) The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat Cell Biol 3:93–96
Couthouis J, Raphael AR, Siskind C et al (2014) Exome sequencing identifies a DNAJB6 mutation in a family with dominantly-inherited limb-girdle muscular dystrophy. Neuromuscul Disord 24:431–435
Cudkowicz ME, Shefner JM, Simpson E et al (2008) Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. Muscle Nerve 38:837–844
Cuervo AM (2004) Autophagy: in sickness and in health. Trends Cell Biol 14:70–77
Cuervo AM, Stefanis L, Fredenburg R et al (2004) Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 305:1292–1295
Cummings CJ, Mancini MA, Antalffy B et al (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19:148–154
Cummings CJ, Sun Y, Opal P et al (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. Hum Mol Genet 10:1511–1518
Cushman-Nick M, Bonini NM, Shorter J (2013) Hsp104 suppresses polyglutamine-induced degeneration post onset in a drosophila MJD/SCA3 model. PLoS Genet 9:e1003781
Davey KM, Parboosingh JS, McLeod DR et al (2006) Mutation of DNAJC19, a human homolog of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. J Med Genet 43:385–393
Demand J, Alberti S, Patterson C, Hohfeld J (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr Biol 11:1569–1577
Dobson CM (2003) Protein folding and misfolding. Nature 426:884–890
D'Silva PD, Schilke B, Walter W et al (2003) J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix. Proc Natl Acad Sci U S A 100:13839–13844
Duchniewicz M, Germaniuk A, Westermann B et al (1999) Dual role of the mitochondrial chaperone Mdj1p in inheritance of mitochondrial DNA in yeast. Mol Cell Biol 19:8201–8210
Dudek J, Cheng IF, Balleininger M et al (2013) Cardiolipin deficiency affects respiratory chain function and organization in an induced pluripotent stem cell model of Barth syndrome. Stem Cell Res 11:806–819

Duennwald ML, Echeverria A, Shorter J (2012) Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans. PLoS Biol 10:e1001346
Ehrnsperger M, Graber S, Gaestel M et al (1997) Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. EMBO J 16:221–229
Engert JC, Berube P, Mercier J et al (2000) ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. Nat Gen 24:120–125
Eriguchi M, Mizuta H, Kurohara K et al (2008) Identification of a new homozygous frameshift insertion mutation in the SIL1 gene in 3 Japanese patients with Marinesco-Sjögren syndrome. J Neurol Sci 270:197–200
Estaquier J, Arnoult D (2007) Inhibiting Drp1-mediated mitochondrial fission selectively prevents the release of cytochrome c during apoptosis. Cell Death Diff 14:1086–1094
Evgrafov OV, Mersiyanova I, Irobi J et al (2004) Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat Genet 36:602–606
Ezgu F, Krejci P, Li S et al (2014) Phenotype-genotype correlations in patients with Marinesco-Sjögren syndrome. Clin Genet 86:74–84
Frank S, Gaume B, Bergmann-Leitner ES et al (2001) The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev Cell 1:515–525
Fujikake N, Nagai Y, Popiel HA et al (2008) Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. J Biol Chem 283:26188–26197
Gao XC, Zhou CJ, Zhou ZR et al (2011) Co-chaperone HSJ1a dually regulates the proteasomal degradation of ataxin-3. PLoS One 6:e19763
Gebert N, Joshi AS, Kutik S et al (2009) Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: implications for Barth syndrome. Curr Biol 19:2133–2139
Girard M, Lariviere R, Parfitt DA et al (2012) Mitochondrial dysfunction and Purkinje cell loss in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). Proc Nat Acad Sci U S A 109:1661–1666
Greer PL, Hanayama R, Bloodgood BL et al (2010) The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating arc. Cell 140:704–716
Grynberg M, Erlandsen H, Godzik A (2003) HEPN: a common domain in bacterial drug resistance and human neurodegenerative proteins. Trends Bioch Sci 28:224–226
Hageman J, Rujano MA, van Waarde MAWH et al (2010) A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. Mol Cell 37:355–369
Hansen JJ, Durr A, Cournu-Rebeix I et al (2002) Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. Am J Hum Genet 70:1328–1332
Hansson O, Nylandsted J, Castilho RF et al (2003) Overexpression of heat shock protein 70 in R6/2 Huntington's disease mice has only modest effects on disease progression. Brain Res 970:47–57
Hargitai J, Lewis H, Boros I et al (2003) Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. Biochem Biophys Res Commun 307:689–695
Harms MB, Sommerville RB, Allred P et al (2012) Exome sequencing reveals DNAJB6 mutations in dominantly-inherited myopathy. Ann Neurol 71:407–416
Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:1852–1858
Haslbeck M, Miess A, Stromer T et al (2005) Disassembling protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and Hsp104. J Biol Chem 280:23861–23868
Hayashi M, Imanaka-Yoshida K, Yoshida T et al (2006) A crucial role of mitochondrial Hsp40 in preventing dilated cardiomyopathy. Nat Med 12:128–132
He Q (2010) Tafazzin knockdown causes hypertrophy of neonatal ventricular myocytes. Am J Physiol Heart Circ Physiol 299:H210–229
Hidalgo-de-Quintana J, Evans RJ, Cheetham ME et al (2008) The Leber congenital amaurosis protein AIPL1 functions as part of a chaperone heterocomplex. Invest Ophthalmol Vis Sci 49:2878–2887

Hipp MS, Patel CN, Bersuker K et al (2012) Indirect inhibition of 26S proteasome activity in a cellular model of Huntington's disease. J Cell Biol 96:573–587
Houlden H, Laura M, Wavrant-De Vrieze F et al (2008) Mutations in the HSP27 (HSPB1) gene cause dominant, recessive, and sporadic distal HMN/CMT type 2. Neurology 71:1660–1668
Howarth JL, Kelly S, Keasey MP et al (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. Mol Ther 15:1100–1105
Howarth JL, Glover CP, Uney JB (2009) HSP70 interacting protein prevents the accumulation of inclusions in polyglutamine disease. J Neurochem 108:945–951
Howes J, Shimizu Y, Feige MJ, Hendershot LM (2012) C-terminal mutations destabilize SIL1/BAP and can cause Marinesco-Sjögren syndrome. J Biol Chem 287:8552–8560
HUGO Gene Nomenclature Committee (HGNC) (2014) http://www.genenames.org/genefamilies/HSP. Accessed 23 April 2014.
Iosefson O, Levy R, Marom M et al (2007) The Pam18/Tim14–Pam16/Tim16 complex of the mitochondrial translocation motor: the formation of a stable complex from marginally stable proteins. Protein Sci 16:316–322
Irobi J, Almeida-Souza L, Asselbergh B et al (2010) Mutant HSPB8 causes motor neuron-specific neurite degeneration. Hum Mol Genet 19:3254–3265
Jana NR (2012) Understanding the pathogenesis of Angelman syndrome through animal models. Neur Plas 2012:710943
Jana NR, Nukina N (2005) BAG-1 associates with the polyglutamine-expanded huntingtin aggregates. Neurosci Lett 378:171–175
Jana NR, Tanaka M, Wang G et al (2000) Polyglutamine length-dependent interaction of Hsp40 and HSP70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. Hum Mol Genet 9:2009–2018
Jana NR, Dikshit P, Goswami A et al (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J Biol Chem 280:11635–11640
Jiang J, Prasad K, Lafer EM et al (2005) Structural basis of interdomain communication in the Hsc70 chaperone. Mol Cell 20:513–524
Kamionka M, Feigon J (2004) Structure of the XPC binding domain of hHR23A reveals hydrophobic patches for protein interaction. Protein Sci 13:2370–2377
Kampinga HH, Hageman J, Vos MJ et al (2009) Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14:105–111
Karim MA, Parsian AJ, Cleves MA et al (2006) A novel mutation in BAP/SIL1 gene causes Marinesco-Sjögren syndrome in an extended pedigree. Clin Genet 70:420–423
Katsanis N, Beales PL, Woods MO et al (2000) Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. Nat Genet 26:67–70
Kazemi-Esfarjani P, Benzer S (2000) Genetic suppression of polyglutamine toxicity in Drosophila. Science 287:1837–1840
Kieran D, Kalmar B, Dick JR et al (2004) Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med 10:402–405
Kijima K, Numakura C, Goto T et al (2005) Small heat shock protein 27 mutation in a Japanese patient with distal hereditary motor neuropathy. J Hum Genet 50:473–476
Kim S, Nollen EA, Kitagawa K et al (2002) Polyglutamine protein aggregates are dynamic. Nat Cell Biol 4:826–831
Kim JC, Ou YY, Badano JL et al (2005) MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis. J Cell Sci 118:1007–1020
Kim YS, Alarcon SV, Lee S (2009) Update on HSP90 inhibitors in clinical trial. Curr Top Med Chem 9:1479–1492
Kleizen B, Braakman I (2004) Protein folding and quality control in the endoplasmic reticulum. Curr Opin Cell Biol 16:343–349

Kobayashi Y, Kume A, Li M et al (2000) Chaperones HSP70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. J Biol Chem 275:8772–8778
Kolandaivelu S, Huang J, Hurley JB et al (2009) AIPL1, a protein associated with childhood blindness, interacts with alpha-subunit of rod phosphodiesterase (PDE6) and is essential for its proper assembly. J Biol Chem 284:30853–30861
Kolb SJ, Snyder PJ, Poi EJ et al (2010) Mutant small heat shock protein B3 causes in motor neuropathy. Neurology 74:502–506
Kozlov G, Denisov AY, Girard M et al (2011) Structural basis of defects in the SACS HEPN domain responsible for autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). J Biol Chem 286:20407–20412
Kuo Y, Ren S, Lao U et al (2013) Suppression of polyglutamine protein toxicity by co-expression of a heat-shock protein 40 and a heat-shock protein 110. Cell Death Dis 4:e833
Labbadia J1, Novoselov SS, Bett JS et al (2012) Suppression of protein aggregation by chaperone modification of high molecular weight complexes. Brain 135:1180–1196
Lajoie P, Snapp EL (2010) Formation and toxicity of soluble polyglutamine oligomers in living cells. PLoS One 5:e15245
Lanka V, Wieland S, Barber J et al (2009) Arimoclomol: a potential therapy under development for ALS. Expert Opin Investig Drugs 18:1907–1918
Lee YJ, Jeong SY, Karbowski M et al (2004) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol Biol Cell 15:5001–5011
Leitman J, Hartl FU, Lederkremer GZ (2013) Soluble forms of polyQ-expanded huntingtin rather than large aggregates cause endoplasmic reticulum stress. Nat Comm 4:2753
Li Z, Hartl FU, Bracher A (2013) Structure and Function of Hip, an attenuator of the Hsp70 chaperone cycle. Nat Struc Mol Biol 20:929–935
Litt M, Kramer P, LaMorticella DM et al (1998) Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 7:471–474
Lo JF, Hayashi M, Woo-Kim S et al (2004) Tid1, a cochaperone of the heat shock 70 protein and the mammalian counterpart of the Drosophila tumor suppressor l(2) tid, is critical for early embryonic development and cell survival. Mol Cell Biol 24:2226–2236
Lu B (2009) Mitochondrial dynamics and neurodegeneration. Curr Neurol Neurosci Rep 9:212–219
Luders J, Demand J, Hohfeld J (2000) The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J Biol Chem 275:4613–4617
Mackay DS, Andley UP, Shiels A (2003) Cell death triggered by a novel mutation in the alphaA-crystallin gene underlies autosomal dominant cataract linked to chromosome 21q. Eur J Hum Genet 11:784–793
Malik B, Nirmalananthan N, Gray AL et al (2013) Co-induction of the heat shock response ameliorates disease progression in a mouse model of human spinal and bulbar muscular atrophy: implications for therapy. Brain 136:926–943
Martin N, Jaubert J, Gounon P et al (2002) A missense mutation in Tbce causes progressive motor neuronopathy in mice. Nat Genet 32:443–447
Martin MH, Bouchard JP, Sylvain M et al (2007) Autosomal recessive spastic ataxia of Charlevoix-Saguenay: a report of MR imaging in 5 patients. AJNR Am J Neurorad 28:1606–1608
Mazurová S, Tesařová M, Magner M et al (2013) Novel mutations in the TAZ gene in patients with Barth syndrome. Prague Med Rep 114:139–153
McCampbell A, Taylor JP, Taye AA et al (2000) CREB-binding protein sequestration by expanded polyglutamine. Hum Mol Genet 9:2197–2202
Miller VM, Nelson RF, Gouvion CM et al (2005) CHIP suppresses polyglutamine aggregation and toxicity *in vitro* and *in vivo*. J Neurosci 25:9152–9161
Mitra S, Tsvetkov AS, Finkbeiner S (2009) Single neuron ubiquitin-proteasome dynamics accompanying inclusion body formation in Huntington disease. J Biol Chem 284:4398–4403

Mokranjac D, Sichting M, Neupert W et al (2003) Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria. EMBO J 22:4945–4956
Mokranjac D, Bourenkov G, Hell K et al (2006) Structure and function of Tim14 and Tim16, the J and J-like components of the mitochondrial protein import motor. EMBO J 25:4675–4685
Muchowski PJ, Wacker JL (2005) Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci 6:11–22
Mueller TD, Feigon J (2002) Solution structures of UBA domains reveal a conserved hydrophobic surface for protein-protein interactions. J Mol Biol 319:1243–1255
Neef DW, Turski ML, Thiele DJ (2010) Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in Neurodegenerative disease. PLoS Biol 8:e1000291
Ng AC, Baird SD, Screaton RA (2014) Essential role of TID1 in maintaining mitochondrial membrane potential homogeneity and mitochondrial DNA integrity. Mol Cell Biol 34:1427–1437
Norton N, Li D, Rieder MJ et al (2011) Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. Am J Hum Genet 88:273–288
Novoselov SS, Mustill WJ, Gray AL et al (2013) Molecular chaperone mediated late-stage neuroprotection in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis PLoS One 8:e73944
Nucifora FC Jr, Sasaki M, Peters MF et al (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science 291:2423–2428
Ojala T, Polinati P, Manninen T et al (2012) New mutation of mitochondrial DNAJC19 causing dilated and noncompaction cardiomyopathy, anemia, ataxia, and male genital anomalies. Pediatr Res 72:432–437
Ouyang Y, Takiyama Y, Sakoe K et al (2006) SACS-related ataxia (ARSACS): expanding the genotype upstream from the gigantic exon. Neurol 66:1103–1104
Parfitt DA, Michael GJ, Vermeulen EG et al (2009) The ataxia protein SACS is a functional co-chaperone that protects against polyglutamine-expanded ataxin-1. Hum Mol Gen 18:1556–1565
Parfitt DA, Aguila M, McCulley CH et al (2014) The heat-shock response co-inducer arimoclomol protects against retinal degeneration in rhodopsin retinitis pigmentosa. Cell Death Dis 5:e1236
Parvari R, Hershkovitz E, Grossman N et al (2002) Mutation of TBCE causes hypoparathyroidism-retardation-dysmorphism and autosomal recessive Kenny-Caffey syndrome. Nat Genet 32:448–452
Pearl LH, Prodromou C (2006) Structure and mechanism of the HSP90 molecular chaperone machinery. Ann Rev Bioch 75:271–294
Porter JR, Fritz CC, Depew KM (2010) Discovery and development of HSP90 inhibitors: a promising pathway for cancer therapy. Curr Opin Chem Biol 14:412–420
Pyle A, Griffin H, Yu-Wai-Man P et al (2012) Prominent sensorimotor neuropathy due to SACS mutations revealed by whole-exome sequencing. Arch Neurol 69:1351–1354
Romano A, Tessa A, Barca A et al (2013) Comparative analysis and functional mapping of SACS mutations reveal novel insights into SACS repeated architecture. Hum Mut 34:525–537
Ross CA, Poirier MA (2005) Opinion: what is the role of protein aggregation in neurodegeneration? Nat Rev Mol Cell Biol 6:891–898
Ross AJ, May-Simera H, Eichers ER et al (2005) Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. Nat Genet 37:1135–1140
Rujano MA, Kaminga HH, Salomons FA (2000) Modulation of polyglutamine inclusion formation by the HSP70 chaperone machine. Exp Cell Res 313:3568–3578
Sakahira H, Breuer P, Hayer-Hartl MK et al (2002) Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. Proc Natl Acad Sci U S A 99(Suppl 4):16412–16418
Sarparanta J, Jonson PH, Golzio C et al (2012) Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy. Nat Genet 44:450–455, S1–S2

Sato T, Hayashi YK, Oya Y et al (2013) DNAJB6 myopathy in an Asian cohort and cytoplasmic/nuclear inclusions. Neuromuscul Disord 23:269–276
Satyal SH, Schmidt E, Kitagawa K et al (2000) Polyglutamine aggregates alter protein folding homeostasis in Caenorhabditis elegans. Proc Natl Acad Sci U S A 97:5750–5755
Schaffar G, Breuer P, Boteva R et al (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. Mol Cell 15:95–105
Schipper-Krom S, Juenemann K, Reits EA (2012) The ubiquitin-proteasome system in Huntington's disease: are are proteasomes impaired, initiators of disease, or coming to the Rescuerescue? Biochem Res Int 2012:837015
Schusdziarra C, Blamowska M, Azem A et al (2013) Methylation-controlled J-protein MCJ acts in the import of proteins into human mitochondria. Hum Mol Genet 22:1348–1357
Schwahn U, Lenzner S, Dong J et al (1998) Positional cloning of the gene for X-linked retinitis pigmentosa 2. Nat Genet 19:327–332
Schwarz N, Hardcastle AJ, Cheetham ME (2012) Arl3 and RP2 mediated assembly and traffic of membrane associated cilia proteins. Vision Res 75:2–4
Senderek J, Krieger M, Stendel C et al (2005) Mutations in SIL1 cause Marinesco-Sjogren syndrome, a cerebellar ataxia with cataract and myopathy. Nat Genet 37:1312–1314
Shorter J (2011) The mammalian disaggregase machinery: Hsp110 synergizes with HSP70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. PLoS One 6:e26319
Sinha D, Srivastava S, Krishna L et al (2014) Unraveling the intricate organization of mammalian mitochondrial presequence translocases: existence of multiple translocases for maintenance of mitochondrial function. Mol Cell Biol 34:1757–1775
Smirnova E, Griparic L, Shurland DL et al (2001) Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol Biol Cell 12:2245–2256
Sohocki MM, Bowne SJ, Sullivan LS et al (2000) Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. Nat Genet 24:79–83
Soti C, Nagy E, Giricz Z et al (2005) Heat shock proteins as emerging therapeutic targets. Br J Pharmacol 146:769–780
Sroka K, Voigt A, Deeg S et al (2009) BAG1 modulated huntingtin toxicity, aggregation, degradation, and subcellular distribution. J Neurochem 111:801–807
Stefani M, Dobson CM (2003) Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med 81:678–699
Stenoien DL, Cummings CJ, Adams HP et al (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. Hum Mol Genet 8:731–741
Stone DL, Slavotinek A, Bouffard GG et al (2000) Mutation of a gene encoding a putative chaperonin causes McKusick-Kaufman syndrome. Nat Genet 25:79–82
Synofzik M, Soehn AS, Gburek-Augustat J et al (2013) Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. Orphanet J Rare Dis 8:41
Takahashi T et al (2008) Soluble polyglutamine oligomers formed prior to inclusion body formation are cytotoxic. Hum Mol Genet 17:345–356
Takahata T, Yamada K, Yamada Y et al (2010) Novel mutations in the SIL1 gene in a Japanese pedigree with the Marinesco-Sjögren syndrome. J Hum Genet 55:142–146
Tang B, Liu X, Zhao G et al (2005a) Mutation analysis of the small heat shock protein 27 gene in Chinese patients with Charcot-Marie-Tooth disease. Arch Neurol 62:1201–1207
Tang B, Zhao G, Luo W et al (2005b) Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L. Hum Genet 116:222–224
Taylor JP, Hardy J, Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. Science 296:1991–1995
Taylor SW, Fahy E, Zhang B et al (2003) Characterization of the human heart mitochondrial proteome. Nat Biotechnol 21:281–286

Tokui K, Adachi H, Waza M et al (2009) 17-DMAG ameliorates polyglutamine-mediated motor neuron degeneration through well-preserved proteasome function in an SBMA model mouse. Hum Mol Genet 18:898–910

Truscott KN, Voos W, Frazier AE et al (2003) A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria. J Cell Biol 163:707–713

Vermeer S, Meijer RP, Pijl BJ et al (2008) ARSACS in the Dutch population: a frequent cause of early-onset cerebellar ataxia. Neurogen 9:207–214

Vicart P, Caron A, Guicheney P et al (1998) A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 20:92–95

Visy J, Fitos I, Mády G et al (2002) Enantioselective plasma protein binding of bimoclomol. Chirality 14:638–642

Vos MJ, Zijlstra MP, Kanon B et al (2010) HSPB7 is the most potent polyQ aggregation suppressor within the HSPB family of molecular chaperones. Hum Mol Genet 19:4677–4693

Wacker JL, Huang SY, Steele AD et al (2009) Loss of HSP70 exacerbates pathogenesis but not levels of fibrillar aggregates in a mouse model of Huntington's disease. J Neurosci 29:9104–9114

Wang AM, Miyata Y, Klinedinst S et al (2013) Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. Nat Chem Biol 9:112–118

Warrick JM, Chan HY, Gray-Board et al (1999) Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. Nat Genet 23:425–428

Waza M, Adachi H, Katsuno M et al (2005) 17-AAG, an HSP90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nat Med 11:1088–1095

Westhoff B, Chapple JP, van der Spuy J et al (2005) HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. Curr Biol 15:1058–1064

Williams AJ, Knutson TM, Colomer Gould VF et al (2009) *In vivo* suppression of polyglutamine neurotoxicity by C-terminus of HSP70-interacting protein (CHIP) supports an aggregation model of pathogenesis. Neurobiol Dis 33:342–353

Wyttenbach A, Carmichael J, Swartz J et al (2000) Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. Proc Natl Acad Sci U S A 97:2898–2903

Wyttenbach A, Sauvageot O, Carmichael J et al (2002) Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. Hum Mol Genet 11:1137–1151

Xu A, Malhotra M, Ren M (2006) The enzymatic function of tafazzin J Biol Chem 281:39217–3922

Zhao L, Longo-Guess C, Harris BS et al (2005) Protein accumulation and neurodegeneration in the woozy mutant mouse is caused by disruption of SIL1, a cochaperone of BiP. Nat Genet 37:974–979

Zourlidou A, Gidalevitz T, Kristiansen M et al (2007) Hsp27 overexpression in the R6/2 mouse model of Huntington's disease: chronic neurodegeneration does not induce Hsp27 activation. Hum Mol Genet 16:1078–1090

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G. L. Blatch, A. L. Edkins (eds.), *The Networking of Chaperones by Co-chaperones,*
Subcellular Biochemistry 78, DOI 10.1007/978-3-319-11731-7