

# Chapter 9

## Emergence and Characterization of the p23 Molecular Chaperone

Frank J. Echtenkamp and Brian C. Freeman

**Abstract** The p23 molecular chaperone is an abundant eukaryotic protein that shares genetic and physical connections to a variety of proteins working in diverse biological processes including protein transport, ribosome biogenesis, transcription activation, and chromatin remodeling. Here, we describe the initial founding of the p23 chaperone along with the early discoveries related to p23's function, its relationship to small heat shock proteins and other CHORD and Sgt1 (CS)-domain-containing factors, p23's biological relevancies in different cellular pathways, and how this chaperone has been exploited by both parasites and viruses. Finally, we discuss the correlation between p23 levels and cancerous cell growth.

### 1 Introduction

The promiscuous members of the heterogeneous molecular chaperone family manage the conformational status of cellular proteins [1]. Typically, chaperone function is considered through the study of the classic heat shock proteins (HSPs) in preventing the aggregation of nascent or metastable (inherent and stress-induced) polypeptides, thereby allowing low-energy states to be achieved [2]. Yet, seemingly native proteins also require assistance under normal physiological conditions. Most proteins are not single-use factors. Rather, they perform multiple activities by interacting with various partners and/or ligands (i.e., coenzymes). By adopting distinct physical forms, cofactor binding and release is coordinated [3, 4]. While discrete polypeptide shapes can dictate partner selection, the mechanisms triggering the transitions between physical states are not understood. We believe that molecular chaperones facilitate the interchange between structural forms thereby enhancing coenzyme exchange. To illustrate our point, we will describe the p23 molecular chaperone including its apparent rise from the classic small heat shock proteins (sHSPs) and its functional interaction network adapted for manipulating

---

B. C. Freeman (✉) · F. J. Echtenkamp  
Department of Cell and Developmental Biology, University of Illinois,  
Urbana-Champaign, Urbana, IL, USA  
e-mail: bfreeman@life.illinois.edu

the conformations of native or near native client proteins to promote an effective and dynamic protein environment.

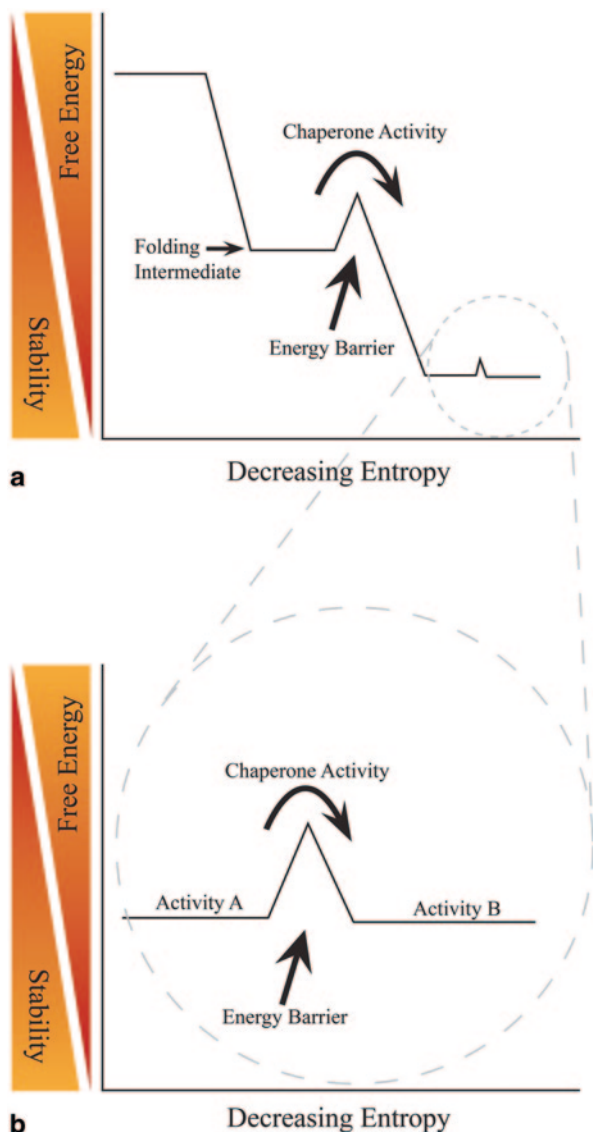
Historically, molecular chaperone activities have been aligned with the process of nascent polypeptide folding where the complexity of a linear chain necessitates assistance to avoid aggregating within the context of a cytosol [2]. For example, a single polypeptide consisting of 149 amino acids can have  $4^{149}$  to  $9^{149}$  different conformations in solution assuming two rotational bonds per residue [5]. However, the likelihood that an elongated polypeptide is exposed to the cytosol is rather limited. When a nascent chain emerges from a ribosome, it is met by a legion of molecular chaperones including Hsp70, TRiC, Hsp90, along with their respective cochaperones. Although the absolute contributions made by each type of chaperone to protein folding is still being resolved, chaperones minimally shield neighboring chains to avoid nonspecific aggregation through the exposed hydrophobic side groups. Significantly, collapse of the chaperone system managing the early nascent chains leads to cell death [6].

The folding process initiates as a naïve polypeptide exits the ribosome with the chain reaching a biologically active, low-energy state by proceeding down a folding landscape pervaded with nuances, which creates a variety of potential folding routes [7]. Regardless of the precise pathway traversed, each polypeptide will encounter unproductive intermediates trapping the molecule in an ineffective state. Fortunately, molecular chaperones circumvent these wasteful intermediaries by overcoming the energy barriers thwarting the capacity of a polypeptide to reach its native state (Fig. 9.1a). The effectiveness of the chaperone system is enhanced by a differential use of the molecular chaperones along the folding process. Early off-pathway species are managed by the Hsp70s, which bind to ribosome-associated nascent chains [8–10]. Presumably, binding by Hsp70 restricts the conformation of the chain and biases the folding towards on-pathway steps. Late maturation events are mediated by the chaperonin (e.g., GroEL, TRiC) and Hsp90 paralogs [2]. For example, chaperonins coordinate the assembly of multi-subunit structures whereas Hsp90 maintains metastable factors in a soluble state until activated (e.g., ligand binding by a steroid hormone receptor, SHR).

In the event that the chaperones fail to guide a naïve polypeptide to a productive structure, the network is designed to deliver the castoffs to the proteolysis machine for disposal [11]. The relationship between the chaperone and proteolysis systems is further exploited to remove damaged proteins occurring under both normal and stressful (e.g., heat shock) circumstances. To effectively handle the aggregation prone polypeptides an additional class of molecular chaperones, the sHSPs, is employed [12]. In general, the expression of sHSPs is highly stress-inducible. Yet, several sHSP derivatives, including p23, have emerged for prominent use during normal growth (discussed below). Overall, molecular chaperones collectively monitor the cellular proteome and maintain homeostasis by minimizing unproductive conformational protein species under all physiological conditions.

In addition to the classic folding activities, molecular chaperones contribute to more subtle protein conformational changes. Many, if not all, cellular proteins utilize distinct structural forms to accomplish their cellular tasks. To partition the

**Fig. 9.1** Molecular chaperones mediate structural changes throughout the lifetime of a polypeptide. Molecular chaperones facilitate protein conformational changes necessary for the folding of nascent polypeptides (a) and for coenzyme interactions separating functional activities of a client protein (b)



different configurations relatively small energy barriers can be used to bias residence in one low-energy state or another (Fig. 9.1b). Hence, the ability of a protein to perform different activities is distinguished. For example, Clathrin heavy and light chains form a triskelion-shaped structure that oligomerizes into a polyhedral lattice around a membrane vesicle [13]. While cooperative interactions foster assembly of the Clathrin triskelion lattice, an incumbent energy barrier prevents the cage from spontaneously dissociating [14]. The clathrin-coated vesicles are an effective means to transport molecules between intracellular compartments or even

to other cells [14]. Upon connection to an acceptor membrane, Hsp70 triggers a conformational change in clathrin promoting disassembly of the coat and allowing the release of the vesicle's contents [15, 16].

While contributions to the protein quality control process are well established, how molecular chaperones manage the more subtle structural fluctuations that drive coenzyme interactions is less well understood. A classic example of client dependence for coenzyme binding involves SHRs, Hsp70, Hsp90, and a plethora of co-chaperones. As p23 was originally discovered while investigating Hsp90-mediated SHR-biogenesis, we will start our discussion of p23 by describing its contributions to SHR biology.

## 2 The Founding of the p23 Molecular Chaperone

SHRs are the protein-binding partners for glucocorticoid, progestin, androgen, and estradiol hormones [17]. SHRs are a subfamily within the intracellular hormone receptor (IR) super family, which also contains binders for retinoids and thyroids along with numerous orphan receptors [18]. In brief, IRs convert hormonal signals into gene regulatory programs important for diverse physiological functions including embryonic development, cell differentiation, inflammation, and memory [19–22]. In addition, IRs are one of the most therapeutically targeted protein families being relevant to numerous diseases including cancer and diabetes [23–27]. Central to SHR function are the abilities to bind hormone, execute ligand-induced allostery, and undergo further structural rearrangements to release the hormone [28, 29]. It is well established that molecular chaperones are required for ligand binding, yet the influence of chaperones in the later hormone steps is not as well understood [30]. Nevertheless, it is now evident that certain molecular chaperones, including p23, have additional roles with the holoreceptors (i.e., receptor-ligand complexes) downstream of the hormone-binding event [31].

Prior to hormone association, aporeceptors (i.e., ligand free) are in heterotypic assemblies with numerous molecular chaperones [32]. The p23 chaperone was discovered as a component of the apo form of the progesterone receptor (PR) [33, 34]. Follow-up studies demonstrated that p23 directly binds to Hsp90 through an adenosine triphosphate (ATP)-dependent mechanism [35]. Importantly, both PR and the glucocorticoid receptor (GR) rely on the weakly associated p23 chaperone for proper long-term maintenance of hormone-binding activity [34, 36]. However, the initial formation of a SHR hormone-binding state only requires Hsp70, Hsp90/Hsp70 organizing protein (HOP), and Hsp90 [37]. Extensive additional research identified Hsp70, Hsp40, HOP, Hsp90, and p23 as the minimum chaperones sufficient for both establishing and maintaining the hormone-binding competence of a SHR *in vitro* [38, 39]. In a biochemical context, Hsp70 acts at an early stage of folding and then the SHR is transferred to Hsp90 in a HOP-dependent manner.

In the typical model, the maturation process continues with p23 interacting with the complex by binding to the (Hsp90-ATP)<sub>2</sub>:SHR assembly where it stabilizes the

closed conformation of the Hsp90 dimer [38, 40, 41]. Initial work suggested that the chaperones could associate, albeit ineffectually, with an aporeceptor independent of ATP but later studies showed the structure to be ATP-dependent [34, 42, 43]. Suggestively, p23 joins the complex to effectively prolong the Hsp90-client association and increase the time period an SHR is maintained in a hormone-binding competent state [40, 44]. Besides SHRs, the tyrosine kinase feline sarcoma (FES), heat shock factor 1 (HSF1), and aryl hydrocarbon receptor (AhR) form similar complexes with the chaperones suggesting that numerous proteins rely on this system [45].

The p23 molecular chaperone is a highly conserved protein being present from yeast to human cells with a broad tissue distribution in mammals [34]. At least in plants, p23 supports thermal stress tolerance [46]. Although p23 is nonessential in yeast, it is essential in vertebrates [47–51]. p23 is perhaps best known as an Hsp90 cochaperone where it binds to the amino-terminus of Hsp90 in a structure dependent manner [52, 53]. Hsp90 works as a highly flexible dimer anchored through carboxyl-termini oligomerization motifs [54]. ATP binding by Hsp90 induces a transition from an open conformation to a closed state by promoting further cross subunit interactions at the amino-termini [53]. Once in proximity the amino-ends form a p23-binding site [56]. Correspondingly, p23 association stabilizes the Hsp90 amino-terminal interaction, which functionally results in a ~2-fold reduction in Hsp90's ATPase rate [57]. Whether p23's relevance in the SHR maturation pathway is its ability to prolong the closed conformation of Hsp90, to impair Hsp90's ATPase, or to bind directly to the SHR will require more work to resolve. Likely, it is a combination of these benefits.

Besides being an Hsp90 cochaperone, p23 has an intrinsic chaperone function. Notably, p23 prevents the *in vitro* aggregation of denatured model substrates at a level comparable to Hsp90 or Hsp70 [58, 59]. Intriguingly, however, the mechanisms used by p23 and the classic HSPs to prevent protein aggregation are different. Hsp90 and Hsp70 both mediate a collapse of non-native polypeptides while keeping the species in a soluble form but p23 maintains non-native proteins in a soluble, extended state [59]. If p23 has a distinct structural consequence on a client, how did this chaperone evolve?

### 3 The Relationship Between p23, Small Heat Shock Proteins, and Other CS-domain Factors

The p23 molecular chaperone family is expressed in most eukaryotes being represented from yeast to humans (29% identity with 54% conservation); however, a prokaryotic counterpart is yet to be described. In general, p23 proteins are acidic (pI~4) and typically range in size from 18 to 25 kDa. The signature motif of the family is a central WPRLTKE sequence, which has been preserved in human, mouse, chicken, budding yeast, fission yeast with elements of the motif remaining in round worms and other species. In fact, the WPRLTKE motif was key for identifying the budding yeast homolog Sba1 (sensitive to benzoquinoid ansamycins 1) since it shares only 24% amino acid identity with the human homolog [47, 48].

However, the potential origin of the p23 homologs did not become apparent until a structure of p23 was solved. The atomic structure of human p23 missing the 35 carboxyl-terminal residues, which are essential for chaperone activity, revealed a compact antiparallel  $\beta$ -sandwich fold containing eight  $\beta$ -strands [60, 61]. A comparison of the p23 structure yielded the *Methanococcus jannaschii* sHSPs Hsp16.5 in which 73  $\alpha$ -carbon atoms of the amino acid backbone displayed a root mean square deviation of 1.27 Å despite only ~10% sequence identity [61]. This fold had been previously coined HSP20 or the  $\alpha$ -crystallin domain (ACD) [62].

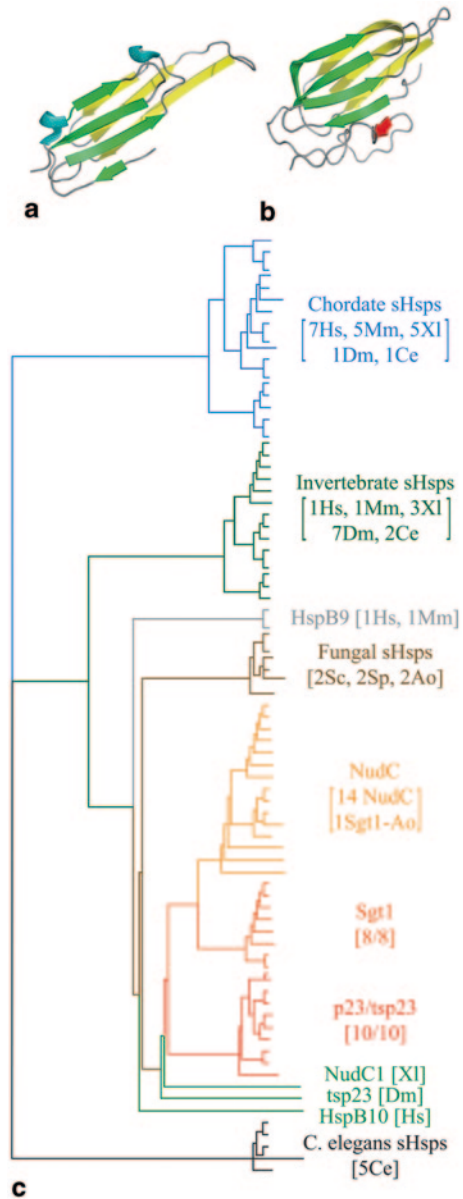
sHSPs are ubiquitously expressed across all three domains of life [63]. The sHSPs are of variable sequence and size (12–42 kDa range) except for a carboxyl-terminal ~90 residue ACD. The sHSPs are ATP-independent molecular chaperones that form polydispersed assemblies ranging from 2–32 subunits that minimally support thermotolerance [64]. The chaperone active oligomeric state of sHSPs is of debate. One proposed mechanism involves the larger structures (i.e., 12–32 subunit complexes) breaking down into active dimers in response to unfolded protein substrates [65]. For example, during heat shock conditions, the yeast Hsp26 is expressed and transitions from a large inactive oligomer (24–26 subunits) to a dimer displaying chaperone activity as unfolded proteins appear [65]. Yet, another yeast sHSP Hsp42 is constitutively expressed assembling into large (24–26 subunits) chaperone-active structures under all growth conditions [66, 67]. Together, Hsp26 and Hsp42 can suppress the aggregation of approximately one third of all yeast proteins [67]. Given their capacity, sHSPs have been referred to as molecular sponges capable of broadly reducing or preventing irreversible protein aggregation [12]. Basically, sHSPs have evolved as a cellular mechanism for functionally interacting with a wide-range of non-native proteins.

The unifying physical characteristic of all sHSPs is the presence of an ACD flanked by variable amino- and carboxyl-terminal extensions [63, 68]. The end regions contribute to functions specific to each sHSP and also are used for self-association [69]. Besides the highly oligomeric sHSPs, the  $\alpha$ -crystallin-like domains are found in a number of monomeric proteins including p23, suppressor of the  $G_2$  allele of *SKP1* (Sgt1), nuclear distribution gene C (NudC), melusin, and b5+b5R flavo-heme cytochrome NAD(P)H oxidoreductase type B [70]. To distinguish between these classes of proteins, the domain in sHSPs is typically referred to as an ACD while the structure is marked as a CHORD and Sgt1 (CS)-like domain in the other proteins. No matter the nomenclature, the structure is typically a compact antiparallel  $\beta$ -sandwich fold consisting of seven  $\beta$ -strands [70] (Fig. 9.2a). Interestingly, p23 contains an eighth  $\beta$ -strand in its CS domain that is critical for modulating the ATPase activity of Hsp90 [53] (Fig. 9.2b). Yet, the eighth  $\beta$ -strand is not essential for a CS protein to interact with Hsp90 since Sgt1, which has a seven  $\beta$ -strand CS domain, associates with Hsp90 [71–73]. However, the mode of assembly is distinct as Sgt1 binds to Hsp90 independent of ATP and Sgt1 has no apparent effect on Hsp90's ATPase activity [74].

Nevertheless, the presence of a common fold opens the possibility that CS and ACD proteins are distantly related [70]. The melusin and cytochrome reductase type B proteins have diversified the use of a CS domain, as these proteins



**Fig. 9.2** Phylogenetic map and structural comparison of CS/ACD-containing proteins. Representative structures of human p23 (accession code 1EJF) (**a**) and the human small heat shock protein (sHSP) alpha B crystallin (accession code 2WJ7) (**b**) were obtained from the protein data bank (<http://www.rcsb.org>) and modified with Pymol Version 1.5.0.4 (Molecular Graphics System, Schrödinger). Homologous secondary structures are highlighted in green ( $\beta$ -stand Face A) and yellow ( $\beta$ -stand Face B) with the p23-specific eighth  $\beta$ -strand depicted in red. Protein sequences of sHSP, NudC, Sgt1, and p23 family members from *Homo sapiens* (Hs), *Mus musculus* (Mm), *Xenopus laevis* (Xl), *Drosophila melanogaster* (Dm), *Caenorhabditis elegans* (Ce), *Saccharomyces cerevisiae* (Sc), *Schizosaccharomyces pombe* (Sp), and *Aspergillus oryzae* (Ao) were aligned and mapped using the ClustalW multiple alignment program (<http://www.bioinformatics.nl/tools/clustalw.html>). The protein clusters, based on relative homology levels, were color coded and the species along with number of homologs within each group are indicated (**c**)



are important for mammalian cell focal adhesions and protection against oxidative stress, respectively [75–78]. Intriguingly, three of the CS domain protein groups have retained chaperone activity—p23, NudC, and Sgt1. A phylogenetic analysis of the full-length sequences of these CS proteins along with numerous sHSPs illustrates the apparent relationships (Fig. 9.2c). While the CS/ACD domains drive

the arrangement of the homology tree, the protein regions outside of this domain demark the various subgroups.

A comparison of the structural organization of the p23s to the sHSPs highlights the absence of an amino-terminal extension and a significant expansion of p23's carboxyl-terminus [61]. As mentioned previously, the CS domain of p23 has been lengthened by an additional  $\beta$ -strand in order to gain a regulatory capacity with Hsp90 [70]. Importantly, a further extension of the carboxyl-terminus is critical for the chaperone activity of p23 used both to prevent the aggregation of model substrates in vitro and to affect clients in vivo [61, 79–81]. On the other hand, the NudC and Sgt1 families can have extensions on either side of the CS domain.

NudC was first identified as a regulator of nuclear movement in the asexual reproductive cycle of the filamentous fungus *Emericella nidulans* [82]. Metazoans express three NudC paralogues (NudC, NudCL, and NudCL2) with a fourth distantly related isoform, NudCD1 (CML66), being a tumor antigen [83]. The NudC proteins are involved in numerous events including nuclear migration, cell cycle progression, cell proliferation, dynein- and kinesin1-mediated transport, and have been implicated in a variety of malignancies [84–86]. All four NudC isoforms have a carboxyl-terminal CS domain, yet only NudC and NudCL display in vitro chaperone activity [83]. Both NudC and NudCL have amino-terminal extensions with a coiled-coil site along with an unstructured region—truncations comprising just the amino-termini and CS domains are sufficient for in vitro chaperone activity [83]. Comparable to p23, NudC can dampen the ATPase activity of Hsp90 [87]. Given the different extensions on p23 and NudC, it seems that CS domains provide an adaptable module to generate a protein capable of managing labile polypeptides and also interacting with Hsp90.

Sgt1 is conserved among eukaryotes being identified as a suppressor of the G<sub>2</sub> allele of SKP1 where it fosters assembly of yeast kinetochores and is a subunit of the SCF (Skp1-Cul1-F-box) ubiquitin ligase complex [88]. Further work demonstrated that Sgt1 supports innate immunity pathways by stabilizing resistance (R) and nucleotide-binding domain and leucine-rich repeat containing (NLR) proteins [89–91]. Sgt1s are extended on both sides of the CS domain with an amino-terminal tetratricopeptide repeat domain (TPR) and a carboxyl-terminal SGT1-specific (SGS) domain [89]. The CS fold is used to associate with Hsp90 while Hsp70 binds to the SGS motif [71, 92]. The ability of Sgt1 to interact with both Hsp90 and Hsp70 suggests that it may serve a role similar to the well-established cochaperone HOP but for specializing the chaperone system for R and NLR proteins [93, 94]. Unlike other cochaperones, Sgt1 does not affect the ATPase activity of Hsp90 [95]. However, similar to p23 and NudC, Sgt1 suppresses the aggregation of a model non-native protein in vitro by working as a molecular chaperone [96].

Among the CS protein family is a poorly understood p23 paralog tsp23 (transcript similar to p23), also referred to as “putative protein PTGES3 Lisoform 5,” with 44% sequence identity to p23 [97]. The tsp23 protein has the WPRLTKE signature motif and a carboxyl-terminal extension with a length intermediate between human and yeast p23. Tsp23 is a poorly studied protein though it is known to stimulate certain SHRs in a manner similar to p23 yet it does not inhibit other



SHRs that are p23-repressive [97]. Interestingly, tsp23 is only expressed in cardiac and skeletal muscle whereas p23 is broadly expressed in tissues except for cardiac and skeletal muscles [34, 97]. In addition, tsp23 can be found in skeletal muscle as a splice variant fused to the alanyl-transfer ribonucleic acid (tRNA) synthetase AlaXp to form p23<sup>H</sup>AlaXp [98]. However, the fusion protein is inactive and has no known function although it was suggested that p23<sup>H</sup>AlaXp might link the Hsp90 machine to the prevention of alanine-tRNAs misactivation [98].

In sum, the CS/ACD domain provides a versatile module to cultivate a protein family with diverse cellular functions using a few basic properties—chaperone activity, partner interaction site (e.g., Hsp90), and self-association capacity. While no single member seems to use all three assets (e.g., p23 is a monomeric chaperone that can bind to Hsp90 yet Hsp26 is an autonomous, oligomeric chaperone), the flexibility of this relatively simple domain appears to condone a fitness advantage. The benefit of the CS/ACD domain is obvious for eukaryotes where the variety of sHSPs significantly increases and the domain is commandeered to develop numerous non-HSPs including p23. Perhaps the broad binding capacity of the ancient sHSPs, which is used to prevent general protein aggregation, provided a readily exploitable module for widely interacting with cellular proteins under normal physiological conditions. At least for p23, we suspect this is the case since the p23 molecular chaperone has a role in a broad array of cellular processes important for mediating cofactor binding and release.

## 4 Cellular Processes Governed by p23 Molecular Chaperones

The exploration of molecular chaperone biology is particularly demanding since these proteins tend to be highly abundant and promiscuous [2]. Further complicating matters is the capacity of chaperones to associate with both coregulators (i.e., cochaperones) and clients (i.e., protein substrates) and therefore discrimination between these two classes is a matter of concern. Often the cellular roles of a protein, including the elucidation of previously unknown functions, are gained by identifying other proteins capable of interacting with the target. Unfortunately, the fundamental properties of molecular chaperones challenge this proposition at least when using classic assays. For example, a 2-hybrid screen incorporating both yeast Hsp90 homologs as baits did not identify any strong interactors and found only four hits (two known cochaperones and two open reading frames (ORFs) with unknown functions) under mild selection conditions [99]. Similar 2-hybrid results were obtained using four different cochaperones, including yeast p23, as bait [99]. However, if the Hsp90 mutant E33A was the bait, then 177 interactors were identified [100]. Presumably, the propensity of the E33A mutant to form the closed conformation of Hsp90, which might trap clients and certain cochaperones, accounts for the increase in hits. Together, these studies support the notion that the transient nature of chaperone-binding limits the ability to detect interacting proteins.

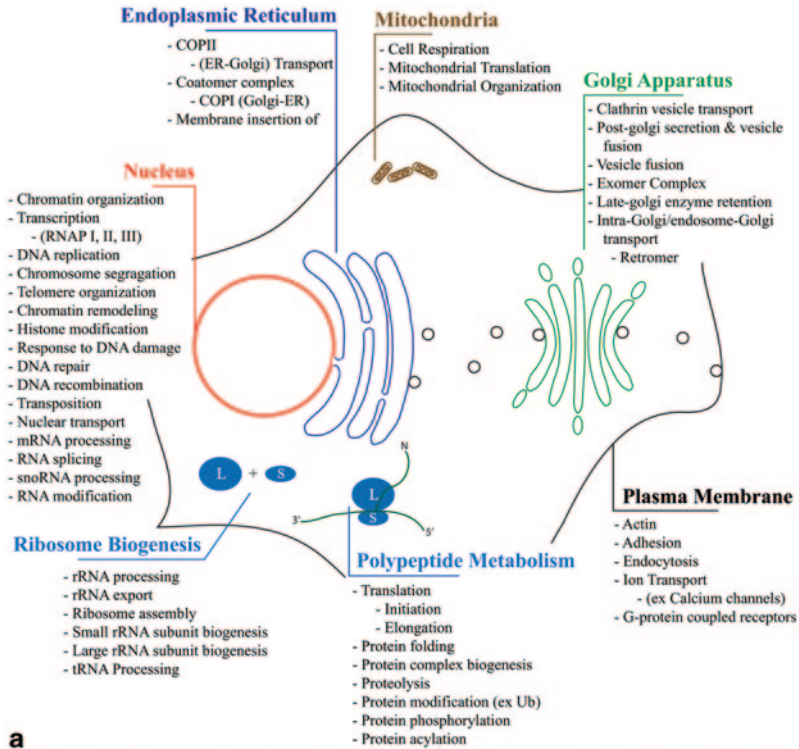
As a comprehensive and reliable interaction network is highly beneficial for comprehending the cellular roles of a given protein, interest in constructing system-wide maps of the molecular chaperones persisted especially in light of their increased medicinal targeting [101–104]. With the recent rise in the types of available high-throughput approaches, it is now feasible to generate a variety of networks for a target protein that are based upon physical, genetic, or functional relationships [105, 106]. The selection of readily accessible large-scale methods along with their declining costs makes it possible to chart the global connections for most, if not all, cellular proteins. Application of tactics such as synthetic genetic array (SGA) analysis, small-molecule chemical screens, protein microarrays, affinity purification/mass spectrometry methods have led to the development of extensive networks for numerous proteins including molecular chaperones [107–112]. For instance, application of such techniques raised the number of known yeast Hsp90 interactors to ~1000 [113, 114]. Given that budding yeast only has ~6000 ORFs, the two studies showed that Hsp90 has a significant relationship with the yeast proteome.

With the application of both high-throughput genetic (SGA) and physical (protein microarrays) tactics to the yeast p23 chaperone, the first functional interaction network for an Hsp90 cochaperone was established [115]. Prior to this work 34 proteins had a known association with p23. After the study, 348 unique p23 interactors had been revealed including 234 new genetic hits and 80 direct binders. A bioinformatic analysis of the compiled p23-interactors using the gene ontology (GO) Slim program freely available at the *Saccharomyces* genome database (SGD) suggests p23 functions within numerous biological processes and is associated with all compartments of the cell (Fig. 9.3a). Such a broad distribution is supported by indirect immunofluorescent images using mouse embryonic fibroblasts and antibodies directed against vertebrate p23 (Fig. 9.3b) [115]. Of note, if the localization pattern of p23 is examined using fluorescent protein fusion technology, the relative level of p23 nuclear occupancy is reduced [116, 117]. As fusion tags on either terminus of p23 abrogate its chaperone activity [118], the altered pattern likely reflects this functional change. The broad cellular localization along with the apparent wide distribution of interactors indicates that p23 might be a general molecular chaperone with a bias for nuclear pathways.

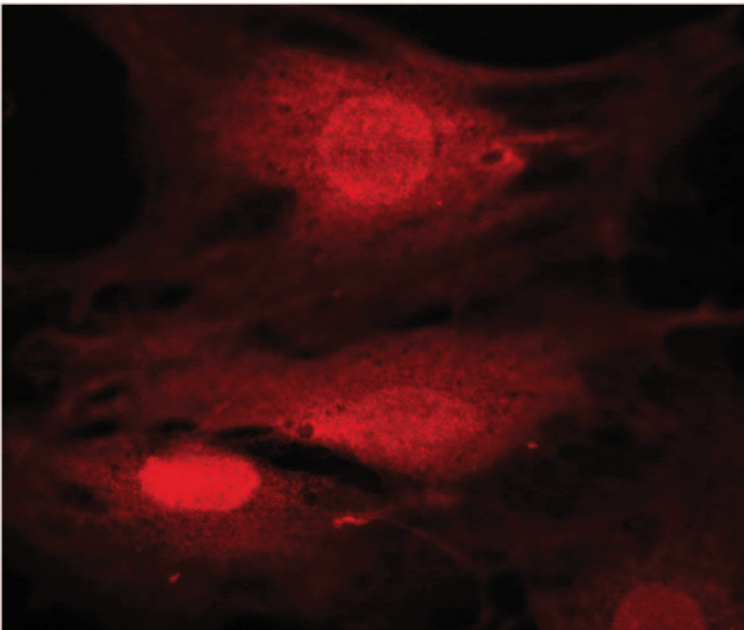
As p23 is typically considered an Hsp90 cochaperone, where it is used to modulate Hsp90's ATPase and possibly direct client selection for Hsp90, it is reasonable to expect a significant overlap between the interactors for p23 and Hsp90. Yet, only ~25% of the p23 hits were found within the Hsp90 network suggesting p23 also functions autonomously of Hsp90 [115]. As the potential for independent activities of several Hsp90 cochaperones has been recently described [119], we will refrain from an elaborate discussion of this topic. Rather, we will delve into less explored areas of the p23 network.

---

with their predicted cellular locations were determined using the Gene Ontology (GO) Slim Mapper program (a). The cellular distribution of mouse p23 was determined by indirect immunofluorescence using wild type mouse embryonic fibroblasts and a monoclonal antibody (JJ3) that recognizes vertebrate p23 [34] (b)



**a**



**b**

**Fig. 9.3** The p23 chaperone is a ubiquitous protein with an interaction network encompassing a variety of biological processes. The expanse of the p23 network is illustrated using select, established p23-interactors [115]. The biological processes associated with various p23 targets along

The most evident biological process noted in the p23 GO Slim analysis was a link to intracellular protein transport underscored by a prevalence of Golgi apparatus associated factors [115]. Yet a rudimentary check of p23's localization pattern does not highlight the Golgi organelle, as p23 seems to be uniformly distributed within the cytosol (Fig. 9.3b). However, comparisons with Golgi and endoplasmic reticulum (ER) markers suggest that p23 preferentially associates with the Golgi. Briefly, p23 displayed a ~98% coincidence with a Golgi marker but only a ~50% overlap with an ER indicator [115]. While the seemingly substantial phase with the ER might normally tempt the conclusion that p23 has an ER interaction, the high abundance and broad distribution of many molecular chaperones, including p23, warrants a more significant overlap to conclude a working relationship.

Significantly, functional studies identified one of the p23 affects in the protein transport process. In both yeast and mammalian backgrounds the efficiency of transport-associated activities (e.g., secretion and processing) were increased in p23 null cells indicating that p23 serves as a negative regulator [115]. Through an epistasis approach one p23 contribution to the transport pathway was discovered—modulation of cargo mannosylation within the Golgi. However, the mannosylation step only accounts for 2 of the 31 transportation-related hits within the p23 network.

Interestingly, normal yeast are fairly unresponsive to the Golgi inhibitor Brefeldin A, yet *p23Δ* cells are sensitive and p23 overexpression is protective [115, 120]. Brefeldin A is a heterocyclic lactone that inhibits coat protein I (COPI)-mediated retrograde transport from the Golgi to the ER [121]. The intracellular transport of vesicles is a complicated process with numerous steps reliant on dynamic changes in protein conformations and in the composition of complexes [122]. To briefly illustrate we will focus on the final step of the pathway when a vesicle merges with its target membrane [123]. Fusion occurs through the pairing of soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) proteins when a vesicle-SNARE (v-SNARE) docks with a target-SNARE (t-SNARE) protein to form a labile trans-SNARE complex [122]. Following membrane fusion, the resultant protein structure (the cis-SNARE complex) must be “primed” and then disassembled minimally by the actions  $\alpha$ -SNAP and NSF homologs [124].

While vesicular trafficking requires numerous conformational changes to proceed, the direct involvement of p23 has not been established. The Brefeldin A phenotypes implicate a p23 involvement in COPI retrograde transport delivering components from the Golgi back to the ER. Intriguingly, p23 shares a genetic connection with *SEC28* [115]. Sec28 (epsilon-COP) stabilizes the cytosolic coatomer complexes that are required for the formation of COPI-coated vesicles [125]. An additional p23-link to vesicular transport is made with Vam7, which stabilizes a t-SNARE complex required for the docking and/or fusion of multiple transport intermediates destined for the vacuole [115, 126, 127]. Besides Sec28 and Vam7, p23 has 27 other interactors linked to the protein transport pathway whose functional connection to p23 is yet to be defined. Moreover, there are over 300 hits within the p23 map yet to be explored. Hence, significant additional work is required to fully appreciate the utility of p23.

An unexpected outcome found within the p23 network was a preponderance of nuclear hits—almost one third of the 348 interactors are considered nuclear proteins [115]. At the time, the predominant model indicated that the Hsp90 molecular chaperone system was a strict cytoplasmic machine [128]. This erroneous theory was proposed to rationalize the cytosolic localization of certain unactivated SHRs [129]. Now, p23 and Hsp90 are known to have a variety of nucleoplasmic roles in diverse activities including transcription activation and telomere deoxyribonucleic acid (DNA) maintenance [119, 130]. A survey of the p23 network reveals a wide-range of nuclear processes including ribosome biogenesis, chromatin structure, transcription events, RNA processing, DNA replication, telomere maintenance, and DNA repair [115]. The simplest explanation for the numerous connections is that p23 serves as a general chaperone overseeing nuclear pathways. But why would there be a need for chaperone activity in the nucleus?

## 5 The Functional Requisite of p23 in the Nucleoplasm

A dynamic protein environment along the genome is essential to appropriately respond to the numerous and diverse conditions that challenge cells [131]. For example, fluctuations in carbon sources or exposure to stress conditions prompt rapid changes in chromatin status and gene expression patterns. Coordinating the activities of these pathways are various transcription factors along with other DNA-binding proteins—for simplicity we will refer to this group as TFs. The TFs use cooperative interactions to bind select DNA sites and recruit various protein machines (e.g., chromatin remodelers, histone acetylases, RNA polymerases) to perform the requisite work (i.e., remodel chromatin or transcribe DNA into RNA). While the cooperativity drives precise and rapid assembly, the integral stability of these structures interferes with the timing of biological systems [132]. Therefore, TF-nucleated DNA complexes must be actively and persistently disassembled to function on a useful time scale. Fortunately, the abundant and promiscuous nature of the p23 molecular chaperone places it in a position to foster a dynamic genomic environment that is sufficiently pliable to meet the requirements of homeostasis.

With the application of modern cell biology techniques it was realized that nuclear factors are highly motile [133]. In a seminal study, Phair and Misteli [134] used green fluorescent protein (GFP) fusions to show that diverse nuclear proteins, regardless of functional purpose, move with near diffusion constants in live cells. Of note, the rapid motilities of the fusions were not restricted by chemical or thermal energy. Yet, the candidates moved slower than GFP alone suggesting that each momentarily interacts with partners, thereby marginally reducing their movements. If a factor is recognizing a partner, why isn't there a more pronounced change in mobility especially when bound to chromatin? While it is clear that dynamic action is a fundamental property of nuclear proteins [135–141], the mechanisms driving the rapid actions are not understood.

To limit the timing of protein interactions and to accelerate pathways, we believe that molecular chaperones, especially p23, create a highly dynamic nuclear environment [142]. The chaperones contribute by circumventing otherwise long-lived, unproductive structures. Significantly, molecular chaperones do not interfere with the formation of transient, functional complexes since chaperone-client-binding events are typically fleeting. Of note, chaperones work over a broad temperature range and do not require chemical energy to functionally impact a client [143, 144]. Hence, chaperones can account for the observed dynamic protein behavior that persists following chemical and thermal energy fluctuations in vivo [134].

Perhaps fittingly, an early activity revealed for any molecular chaperone was the ability to dissociate a protein-DNA complex. While identifying the host proteins supporting the life cycle of the bacterial phage  $\lambda$ , both DnaK and DnaJ were discovered [145]. DnaK and DnaJ are the *Escherichia coli* homologs of Hsp70 and Hsp40. Further work demonstrated that DnaK and DnaJ dissociate the  $\lambda$ P protein from preprimosomal structures leading to firing of origins of  $\lambda$  DNA replication [146]. Subsequent studies led to the suggestion that DnaK, in conjunction with DnaJ, creates a feedback reaction by cyclically building and destroying the  $\lambda$  preprimosomal DNA replication complexes [147]. Hence, one of the first discovered functions for a molecular chaperone was the assembly and disassembly of protein-DNA complexes.

The original report suggesting a role for the Hsp90 chaperone system in DNA-associated events was the finding that Hsp90 fosters the DNA-binding activity of the myogenic transcription factor MyoD in vitro [148]. Further biochemical work showed that Hsp90 might serve as a general chaperone for basic helix-loop-helix (bHLH) transcription factors by manipulating the conformation of preformed dimeric bHLH transcription factors into a DNA-binding competent state [149]. Numerous other in vitro studies indicate that Hsp90 facilitates the DNA-binding activity of diverse transcription factors including p53, hypoxia-inducible factor 1 (HIF-1), and AhR [150–153]. Significantly, in vivo data support the idea that Hsp90 promotes the DNA-binding activity of a transcription factor as it engages a gene promoter [154].

Early p23 studies showed a nucleoplasmic presence of this chaperone as it was found in nuclear extracts prepared from *Mus musculus*, *Xenopus laevis*, and *Schizosaccharomyces pombe* cells [155–157]. In murine erythroleukemia (MEL) cells p23 directly interacted with DNA cytosine-5 methyltransferase independent of Hsp90 [155]. In fission yeast the ubiquitous cellular distribution of p23 minimally was required for suppression of the Wee1 kinase [157]. The frog work provided the first indication that p23 might affect the DNA-binding activity of a transcription factor, as injection of anti-p23 antibodies into oocytes prevented attenuation of stress-induced HSF1 DNA-binding activity [156]. Interestingly, additional research demonstrated that overexpression or deletion of p23 in budding yeast results in chromosome instability [158]. While a mechanism is yet to be discovered, the phenomena suggest p23 has a broad role along the genome.

Subsequent work to these studies showed that p23 overexpression altered ligand efficacy of several intracellular receptors [97]. Typically, molecular chaperones



modulate ligand potency of SHRs [41]. In brief, ligand efficacy gauges the transcription activation potential whereas potency is an indirect measure of hormone-binding activity. Follow-up work using purified thyroid hormone receptor (TR) and p23 showed that the change in efficacy correlated with an ability of p23 to dissociate TR from its cognate DNA element [97]. Additional work indicated that p23 could alter the transcriptional output of non-IR factors including activation protein 1 (AP1), nuclear factor  $\kappa$ B (NF- $\kappa$ B), and vitamin D receptor (VDR) [159, 160].

The breadth of pathways regulated by p23 at the level of DNA binding has been expanded to include proteins involved in telomere biogenesis (Telomerase), DNA repair (RAD51), and DNA replication (CDC6) [79, 81, 161, 162]. The most notable addition to our understanding of p23's range with DNA-binding proteins was recently shown using a high-throughput tactic (DNase-Seq) for comprehensively mapping protein-DNA interactions along a genome. Basically, the preferential cleavage of naked DNA by the DNase I nuclease is exploited along with massive parallel DNA sequencing (Seq). In brief, nuclei are lightly treated with DNase I and the fragmentation pattern of the mildly digested chromatin reveals both areas of open chromatin (i.e., DNase I hypersensitive sites (DHSs)) and TF interactions (bound TFs protect DNA from digestion) [163]. The identities of the bound TFs are made by analogy with their known consensus binding elements. Application of DNase-Seq to both parental and *p23* $\Delta$  yeast revealed changes in open chromatin with the number of DHSs declining upon loss of p23 yet the average size of the prevailing DHSs doubling [81]. Significantly, a dramatic increase in the extent of DNA-bound transcription factors occurs within the affected DHSs since p23 is required to disassemble the otherwise stable protein-DNA structures. Of the 178 TFs followed in yeast using this approach, 110 are associated with DHSs and of those proteins 71 are impacted in *p23* $\Delta$  yeast. Hence, p23 modulates  $\sim 70\%$  of the yeast TFs with known DNA-binding motifs.

Even the seemingly simple feat of a transcription factor finding its appropriate DNA-binding element requires dynamic action. A classic conundrum within the transcription field has been the observation that many DNA motifs are not utilized even when they are near consensus sequences with close proximity to transcription start sites [164–168]. Although many reasons have been postulated to account for this phenomenon including incomplete activation of a TF or a need to dimerize with a partner, these explanations do not account for the broad absence of binding. For example, the human genome contains thousands of potential glucocorticoid-binding elements, yet only a few hundred are apparently utilized in any given cell type [169]. While many regulatory features contribute to these patterns, including the state of the chromatin encompassing each site [170], the ability of the p23 molecular chaperone to broadly impede DNA-binding activities indicates that numerous transcription factors are actively removed from their motifs. Supporting this conclusion is the finding that many transcription factors inappropriately occupy their cognate sites in the absence of the p23 global regulator [81]. Hence, a prominent means to prevent spurious transcription factor-DNA interactions is the active removal of the DNA-binding proteins by p23.

Intriguingly, however, p23 is not necessarily a negative regulator of DNA-binding events. Studies focusing on either estrogen receptor beta (ER $\beta$ ) or androgen receptor (AR) demonstrate that the gene promoter occupancies of these SHR's could increase following an elevation in p23 levels [171, 172]. In addition, p23 overexpression enhances both basal and ligand-induced peroxisome proliferator-activated receptor (PPAR) transcription [173]. Whether these effects are through a direct change in the DNA-binding function of a receptor or through an indirect mechanism (e.g., changes to open chromatin exposing additional receptor response elements) is yet to be revealed.

We believe a broad use of the p23's nuclear activities is to foster the dynamics of protein DNA complexes. Prior studies have shown that the Hsp90 and p23 chaperones facilitate DNA binding and release for a variety of proteins including transcription factors and telomerase [130]. Certainly there is a need for rapid action by DNA-binding proteins as most genome-related pathways utilize multiple steps to function. For instance, both transcription initiation and DNA repair pathways employ a series of histone acetyltransferase- and chromatin remodeling-complexes to clear the chromatin and expose the DNA for RNA polymerase or DNA repair enzymes, respectively [174]. Other events at or near DNA including DNA replication and telomere maintenance also utilize multi-step pathways to function [175, 176]. In our model, DNA-associated cellular processes move forward using high affinity interactions between low abundant proteins, which are specific for a particular path, and transitions between the diverse structures are mediated by transient, low affinity interactions with the highly abundant p23 molecular chaperone.

## 6 Hijacking of p23 by Viruses and Parasites

One indicator of a protein's central importance is the usurping of that factor by a virus or parasite to maintain a pest's life cycle. An early observation on p23 demonstrated that the Hepatitis B virus, a hepadnavirus that can cause liver cancer, packages the host's p23 and Hsp90 chaperones into the viral nucleocapsids along with its RNA genome [177]. Both p23 and Hsp90 bind independently to the polymerase, which is essential to convert the RNA strand into DNA upon entry into a host cell [177].

Nearly one third of the human population is chronically infected with *Toxoplasma gondii* [178]. During infection *T. gondii* undergoes a transition from a rapidly replicating, infectious tachyzoite stage to a slower dividing encysted bradyzoite phase. The stage conversion is critical to the pathogenesis and longevity of infection. Interestingly, p23 transitions from a solely cytosolic protein in tachyzoite to being evenly distributed in the nucleoplasm and cytosol in bradyzoite period [179]. Hsp90's pattern mirrors that of p23's. Of note, the altered localization of p23 and Hsp90, as the cells transition to a more quiescent growth phase, parallels observations in budding yeast [180]. Unfortunately, the functional purpose of the redistribution in either *T. gondii* or *Saccharomyces cerevisiae* is yet to be resolved.

In *Plasmodium falciparum*, which causes a severe form of malaria, the p23 homolog also undergoes changes during the parasites life cycle. In the early ring and

trophozoite stages (actively dividing within blood cells), p23 is expressed but its levels decline at the beginning of schizogony (vector production for entering the mosquito) [181]. While the relevance of the change is not known, the results parallel fission yeast data showing a decline in p23 levels as yeast enter stationary phase [157]. Comparable to the initial studies on mammalian p23, *P. falciparum* p23 does not form a stable interaction with *P. falciparum* Hsp90 in cell extracts [36, 182]. Yet, the purified proteins can assemble and p23 does suppress Hsp90's ATPase in vitro [182]. As *P. falciparum* p23 has independent chaperone activity [182], it too may function autonomously of Hsp90 in live parasites.

## 7 Cancer Relevance of p23

At the clinical level, cancer includes a diverse set of illnesses that share a common feature of inappropriate cell growth and at the molecular level an accumulation of unacceptable genetic alterations [183, 184]. Almost certainly cancerous growth results from the buildup of DNA mutations that eventually initiate and drive the transformation process. In addition to genomic alterations, significant changes in gene expression and epigenome programs often are observed along with increased activities in many enzymes—telomerase being a notable example [185–188].

Although the p23 molecular chaperone has not been shown to be a causative agent for any cancer, increased expression of p23 correlates with cellular transformation, increased cancer cell invasiveness, and reduced prognosis for cancer patients [162, 189–192]. Significantly, p23 influences genome stability and regulates numerous key factors associated with cancer including nuclear hormone receptors and telomerase [79, 81, 97, 158, 159, 161, 162]. In addition, p23 has been linked to endoplasmic reticulum-stress induced apoptosis [193–196]. While a causative association has not been made, mitochondrial RNA (miRNA) mediated upregulation of p23 can act as an anti-apoptotic factor in childhood acute lymphoblastic leukemia [197].

A promising new report identifies Gedunin as a potential small molecule inhibitor of p23 [198]. Although Gedunin had been suggested to inactivate Hsp90 [199, 200], the Patwardhan study reveals p23 as the primary target. Significantly, Gedunin is a more potent growth inhibitor of cancerous lines relative to non-transformed cells. Besides the benefits of directly inhibiting p23, targeting p23 might enhance the effectiveness of Hsp90 inhibitors since p23 has a protective effect against Hsp90 drugs [80]. Hence, strategies targeting p23 have significant therapeutic promise.

## 8 Summary

Homeostasis requires cellular processes to function efficiently and selectively within the crowded milieu of the cell [142]. While cooperative interactions drive rapid and precise assembly, the inherent stability of such organized structures interferes

with the timing of biological systems. Further complicating performance is the nature of the cell interior, it is densely packed and often contains multiple binding partners for each protein—both features increase non-productive or off-pathway interactions. These variables present great challenges for achieving homeostasis especially in the midst of fluctuating internal and external stimuli that must be monitored constantly to appropriately initiate, continue or halt cellular pathways [31]. Hence, biological complexes must be actively and persistently disassembled in order to work on a useful time scale. We suggest that the apparent broad binding specificity and energy-independent chaperone activity provided by the CS/ACD domain has been exploited to allow p23 to govern the kinetic-behavior of the heterologous proteins within a cell [139]. In our view, p23 resolves inherently stable cooperative-complexes into dynamic machinery capable of rapid action that enables efficient and timely biological pathways [142].

## References

1. Ellis JR (2006) Molecular chaperones: assisting assembly in addition to folding. *Trends Biochem Sci* 31:395–401
2. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. *Nature* 475:324–332
3. Henzler-Wildman K, Kern D (2007) Dynamic personalities of proteins. *Nature* 450:964–972
4. Mitternacht S, Berezovsky IN (2011) Coherent conformational degrees of freedom as a structural basis for allosteric communication. *PLoS Comput Biol* 7:1–12
5. Anfinsen CB (1973) Principles that govern the folding of protein chains. *Science* 181:223–230
6. Deuerling E, Schulze-Specking A, Tomoyasu T et al (1999) Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature* 400:693–696
7. Chan HS, Dill KA (1998) Protein Folding in the landscape perspective: chevron plots and non-arrhenius kinetics. *Proteins* 30:2–33
8. Beckmann RP, Mizzen LA, Welch WJ (1990) Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. *Science* 248:850–854
9. Pfund C, Lopez-Hoyo N, Ziegelhoffer T (1998) The molecular chaperone Ssb from *Saccharomyces cerevisiae* is a component of the ribosome-nascent chain complex. *EMBO J* 17:3981–3989
10. Gautschi M, Lilie H, Fünfschilling U (2001) RAC, a stable ribosome-associated complex in yeast formed by the DnaK-DnaJ homologs Ssz1p and zutin. *Proc Natl Acad Sci U S A* 98:3762–2767
11. McClellan AJ, Tam S, Kaganovich D (2005) Protein quality control: chaperones culling corrupt conformations. *Nat Cell Biol* 7:736–741
12. Eyles SJ, Gierasch LM (2010) Nature's molecular sponges: Small heat shock proteins grow into their chaperones roles. *Proc Natl Acad Sci U S A* 107:2727–2728
13. Pearse BMF (1975) Coated vesicles from pig brain: purification and biochemical characterization. *J Mol Biol* 97:93–98
14. Kirchhausen T (2000) Clathrin. *Annu Rev Biochem* 69:699–727
15. Braell WA, Schlossman DM, Schmid SL et al (1984) Dissociation of clathrin coats coupled to the hydrolysis of ATP: role of an uncoating ATPase. *J Cell Biol* 99:734–741
16. Ungewickell E, Ungewickell H, Holstein SEH et al (1995) Role of auxilin in uncoating clathrin-coated vesicles. *Nature* 378:632–635
17. Tsai MJ, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptors superfamily members. *Annu Rev Biochem* 63:451–486

18. Mangelsdorf DJ, Thummel C, Beato et al (1995) The nuclear receptor superfamily: the second decade. *Cell* 83:835–839
19. Jeong Y, Mangelsdorf DJ (2009) Nuclear receptor regulation of stemness and stem cell differentiation. *Exp Mol Med* 41:525–537
20. McEwan IJ (2009) Nuclear receptors: one big family. In: McEwan IJ (ed) *Methods in molecular biology: the nuclear receptor superfamily*, vol 505. Humana, Totowa, pp 3–18
21. Stanišić V, Lonard DM, O'Malley BW (2010) Modulation of steroid hormone receptor activity. In: Martini L (ed) *Progress in brain research*, vol 181. Elsevier, Amsterdam, pp 153–176
22. Kadmiel M, Cidlowski JA (2013) Glucocorticoid receptor signaling in health and disease. *Trends Pharmacol Sci* 34:518–530
23. Stahn C, Löwenberg M, Hommes DW, et al (2007) Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. *Mol Cell Endocrinol* 275:71–78
24. Stingl J (2011) Estrogen and progesterone in normal mammary gland development and in cancer hormones. *Cancer* 2:85–90
25. Ahmadian M, Suh JM, Hah N et al (2013) PPAR $\gamma$  signaling and metabolism: the good, the bad and the future. *Nat Med* 19:557–566
26. Saylor PJ (2013) The androgen receptor remains front and centre. *Nat Rev Clin Oncol* 10:126–128
27. Zennaro MC, Rickard AJ, Boulkroun S (2013) Genetics of mineralocorticoid excess: an update for clinicians. *Eur J Endocrinol* 169:R15–R25
28. Renaud JP, Moras D (2000) Structural studies on nuclear receptors. *Cell Mol Life Sci* 57:1748–1769
29. Bain DL, Heneghan AF, Connaghan-Jones KD (2007) Nuclear receptor structure implications for function. *Annu Rev Physiol* 69:201–220
30. Grad I, Picard D (2007) The glucocorticoid responses are shaped by molecular chaperones. *Mol Cell Endocrinol* 275:2–12
31. Freeman BC, Yamamoto KR (2001) Continuous recycling: a mechanism for modulatory signal transduction. *Trends Biochem Sci* 26:285–290
32. Pratt WB, Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev* 18:306–360
33. Smith DF, Faber LE, Toft DO (1990) Purification of unactivated progesterone receptor and identification of novel receptor-associated proteins. *J Biol Chem* 265:3996–4003
34. Johnson JL, Beito TG, Kreo CJ et al (1994) Characterization of a novel 23-kilodalton protein of unactive progesterone receptor complexes. *Mol Cell Biol* 14:1956–1963
35. Johnson JL, Toft DO (1994) A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23. *J Biol Chem* 269:24989–24993
36. Hutchison KA, Stancat LF, Owens-Grillo JK (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* 270:18841–18847
37. 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* 272:13047–13054
38. 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
39. Kosano H, Stensgard B, Charlesworth MC et al (1998) The assembly of progesterone receptor-Hsp90 complexes using purified proteins. *J Biol Chem* 273:32973–32979
40. McLaughlin SH, Sobott F, Yao Z et al (2006) The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins. *J Mol Biol* 356:746–758
41. Smith DF, Toft DO (2008) The intersection of steroid receptors with molecular chaperones: observations and questions. *Mol Endocrinol* 22:2229–2240
42. Smith DF, Schowalter DB, Kost SL et al (1990) Reconstitution of progesterone receptor with heat shock proteins. *Mol Endocrinol* 4:1704–1711

43. Smith DF, Toft DO (1992) Composition, assembly and activation of the avian progesterone receptor. *J Steroid Biochem* 41:201–207
44. Morishima Y, Kanelakis KC, Murphy PJM et al (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* 278:48754–48763
45. Nair SC, Toran EJ, Rimerman RA et al (1996) A pathway of multi-chaperone interactions common to diverse regulatory proteins: estrogen receptor, Fes tyrosine kinase, heat shock transcription factor Hsf1, and the aryl hydrocarbon receptor. *Cell Stress Chaperones* 1:237–250
46. Cha JY, Ermawati N, Jung MH et al (2009) Characterization of orchardgrass p23, a flowering plant Hsp90 cohort protein. *Cell Stress Chaperones* 14:233–243
47. Bohlen SP (1998) Genetic and biochemical analysis of p23 and ansamycin antibiotics in the function of Hsp90-dependent signaling proteins. *Mol Cell Biol* 18:3330–3339
48. Fang Y, Fliss AE, Rao J et al (1998) SBA1 encodes a yeast Hsp90 cochaperone that is homologous to vertebrate p23 proteins. *Mol Cell Biol* 18:3727–3734
49. Grad I, McKee TA, Ludwig SM et al (2006) The Hsp90 cochaperone p23 is essential for perinatal survival. *Mol Cell Biol* 26:8976–8983
50. Lovgren AK, Kovarova M, Koller BH (2007) cPGES/p23 is required for glucocorticoid receptor function and embryonic growth but not prostaglandin E<sub>2</sub> synthesis. *Mol Cell Biol* 27:4416–4430
51. Nakatani Y, Hokonohara Y, Kakuta S et al (2007) Knockout mice lacking the cPGES/p23, a constitutively expressed PGE<sub>2</sub> synthetic enzyme are peri-natally lethal. *Biochem Biophys Res Commun* 362:387–392
52. 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
53. Ali MMU, Roe SM, Vaughan CK et al (2006) Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. *Nature* 440:1013–1017
54. Minami Y, Kimura Y, Kawasaki H et al (1994) The carboxy-terminal region of mammalian Hsp90 is required for its dimerization and function *in vivo*. *Mol Cell Biol* 14:1459–1464
55. Sullivan WP, Owen BAL, Toft DO (2002) The Influence of ATP and p23 on the conformation of hsp90. *J Biol Chem* 277:45942–45948
56. Zhu S, Tytgat J (2004) Evolutionary epitopes of Hsp90 and p23: implications for their interaction. *FASEB J* 18:940–947
57. 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
58. Böse S, Weikl T, Bügl H, et al (1996) Chaperone function of Hsp90-associated proteins. *Science* 274:1715–1717
59. 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* 274:1718–1720
60. 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* 293:685691
61. Weaver AJ, Sullivan WP, Felts SJ et al (2000) Crystal structure and activity of human p23, a heat shock protein 90 co-chaperone. *J Biol Chem* 275:23045–23052
62. Kim KK, Kim R, Kim SH (1998) Crystal structure of a small heat-shock protein. *Nature* 394:595–599
63. Poulain P, Gelly JC, Flatters D (2010) Detection and architecture of small heat shock protein monomers. *PLoS ONE* 5:1–10
64. Haslbeck M, Franzmann T, Weinfurter D et al (2005) Some like it hot: the structure and function of small heat-shock proteins. *Nat Struct Mol Biol* 12:842–846
65. Haslbeck M, Walke S, Stromer T et al (1999) Hsp26: a temperature-regulated chaperone. *EMBO J* 18:6744–6751
66. Wotton D, Freeman K, Shore D (1996) Multimerization of Hsp42, a novel heat shock protein of *Saccharomyces cerevisiae*, is dependent on a conserved carboxyl-terminal sequence. *J Biol Chem* 271:2717–2723



67. Haslbeck M, Braun N, Stromer et al (2004) Hsp42 is the general small heat shock protein in the cytosol of *Saccharomyces cerevisiae*. *EMBO J* 23:638–649
68. Kriehuber T, Rattei T, Weinmaier T et al (2010) Independent evolution of the core domain and its flanking sequences in small heat shock proteins. *FASEB J* 24:3633–3642
69. Chen J, Feige MJ, Franzmann TM et al (2010) Regions outside the  $\alpha$ -crystallin domain of the small heat shock protein Hsp26 are required for its dimerization. *J Mol Biol* 398:122–131
70. Garcia-Ranea JA, Mirey G, Camonis J et al (2002) p23 and HSP20/ $\alpha$ -crystallin proteins define a conserved sequence domain present in other eukaryotic protein families. *FEBS Lett* 529:162–167
71. Takahashi A, Casais C, Ichimura K et al (2003) Hsp90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in *Arabidopsis*. *Proc Natl Acad Sci U S A* 100:11777–11782
72. Liu Y, Burch-Smith T, Schiff M et al (2004) Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. *J Biol Chem* 279:2101–2108
73. Hubert DA, Tornero P, Belkhadir Y et al (2003) Cytosolic Hsp90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. *EMBO J* 22:5679–5689
74. Lee YT, Jacob J, Michowski W et al (2004) Human Sgt1 binds Hsp90 through the CHORD-Sgt1 domain and not the tetratricopeptide repeat domain. *J Biol Chem* 279:16511–16517
75. Sbroggiò M, Ferretti R, Percivalle E et al (2008) The mammalian CHORD-containing protein melusin is a stress response protein interacting with Hsp90 and Sgt1. *FEBS Lett* 582:1788–1794
76. Deng B, Parthasarathy S, Wang W et al (2010) Study of the individual cytochrome  $b_5$  and cytochrome  $b_5$  reductase domains of Ncb50r reveals a unique heme pocket and a possible role of the CS domain. *J Biol Chem* 285:30181–30191
77. Sbroggiò M, Bertero A, Velasco S et al (2011) ERK1/2 activation in heart is controlled by melusin focal adhesion kinase and the scaffold protein IQGAP1. *J Cell Sci* 124:3515–3524
78. Kálmán FS, Lizák B, Nagy SK, et al (2013) Natural mutations lead to enhanced proteasomal degradation of human Ncb50r, a novel flavoheme reductase. *Biochimie* 95:1403–1410
79. Toogun OA, Zeiger W, Freeman BC (2007) The p23 molecular chaperone promotes functional telomerase complexes through DNA dissociation. *Proc Natl Acad Sci USA* 104:5765–5770
80. Forafonov F, Toogun OA, Grad I et al (2008) p23/Sba1p Protects against Hsp90 inhibitors independently of its intrinsic chaperone activity. *Mol Cell Biol* 28:3446–3456
81. Zelin E, Zhang Y, Toogun OA et al (2012) The p23 molecular chaperone and GCN5 acetylase jointly modulate protein-DNA dynamics and open chromatin status. *Mol Cell* 48:1–12
82. Osmani AH, Osmani SA, Morris NR (1990) The molecular cloning and identification of a gene product specifically required for nuclear movement in *Aspergillus nidulans*. *J Cell Biol* 111:543–551
83. Zheng M, Cierpicki T, Burdette AJ et al (2011) Structural features and chaperone activity of the NudC protein family. *J Mol Biol* 409:722–741
84. Gocke CD, Reaman GH, Stine C et al (2000) The nuclear migration gene NudC and human hematopoiesis. *Leuk Lymphoma* 39:447–454
85. Riera J, Lazo PS (2009) The mammalian *NudC*-like genes: a family with functions other than regulating nuclear distribution. *Cell Mol Life Sci* 66:2383–2390
86. Toba S, Hirotsune S (2012) A unique role of dynein and nud family proteins in corticogenesis. *Neuropathology* 32:432–439
87. Zhu XJ, Liu X, Jin Q et al (2010) The L279P mutation of nuclear distribution gene C (NudC) influences its chaperone activity and lissencephaly protein 1 (Lis1) stability. *J Biol Chem* 285:29903–29910
88. Kitagawa K, Skowrya D, Elledge SJ et al (1999) *SGT1* encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. *Mol Cell* 4:21–33
89. Azevedo C, Sadanandom A, Kitagawa K et al (2002) The RAR1 interactor SGT1, an essential component of *R* gene-triggered disease resistance. *Science* 295:2073–2076

90. da Silva Correia JM, Leonard N et al (2007) SGT1 is essential for Nod1 activation. *Proc Natl Acad Sci U S A* 104:6764–6769
91. Mayor A, Martinon F, De Smedt T et al (2007) A crucial function of SGT1 and Hsp90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol* 8:497–503
92. Spiechowicz M, Zylicz A, Bieganski P et al (2007) Hsp70 is a new target of Sgt1—an interaction modulated by S100A6. *Biochem Biophys Res Commun* 357:1148–1153
93. Botër M, Amigues B, Peart J et al (2007) Structural and functional analysis of SGT1 reveals that its interaction with Hsp90 is required for the accumulation of Rx, an R protein involved in plant immunity. *Plant Cell* 19:3791–3804
94. Stuttmann J, Parker JE, Noël LD (2008) Staying in the fold. *Plant Signal Behav* 3:283–285
95. Catlett MG, Kaplan KB (2006) Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p. *J Biol Chem* 281:33739–33748
96. Żabka M, Leśniak W, Prus W et al (2008) Sgt1 has co-chaperone properties and is up-regulated by heat shock. *Biochem Biophys Res Commun* 370:179–183
97. Freeman BC, Felts SJ, Toft DA et al (2000) The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies. *Gene Dev* 14:422–434
98. Nawaz MH, Merriman E, Yang XL et al (2011) p23<sup>H</sup> implicated as *cis/trans* regulator of AlaXp-directed editing for mammalian cell homeostasis. *Proc Natl Acad Sci U S A* 108:2723–2328
99. Millson SH, Truman AW, Wolfram F et al (2004) Investigating the protein-protein interactions of the yeast Hsp90 chaperone system by two-hybrid analysis: potential uses and limitations of this approach. *Cell Stress Chaperones* 9:359–368
100. 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, Sl2p (Mpk1p). *Eukaryot Cell* 4:849–860
101. Kalia SK, Kalia LV, McLean PJ (2010) Molecular chaperones as rational drug targets for parkinson's disease therapeutics. *CNS Neurol Disord Drug Targets* 9:741–753
102. Hartson SD, Matts RL (2012) Approaches for defining the Hsp90-dependent proteome. *BBA-Mol Cell Res* 1823:656–667
103. Neckers L, Workman P (2012) Hsp90 molecular chaperone inhibitors: are we there yet? *Clin Cancer Res* 18:64–76
104. Patki JM, Pawar SS (2013) Hsp90: chaperone-me-not. *Pathol Oncol Res* 19:631–640
105. Joyce AR, Palsson B (2006) The model organism as a system integrating 'omics' data sets. *Nat Rev Mol Cell Biol* 7:198–210
106. Fraser JS, Gross JD, Krogan NJ (2013) From systems to structure: bridging networks and mechanism. *Mol Cell* 49:222–231
107. Tong AHY, Evangelista M, Parsons AB et al (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* 294:2364–2368
108. Ho Y, Gruhler A, Hellbut A et al (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 415:180–183
109. Kreuzberger J (2006) Protein microarrays: a chance to study microorganisms? *Appl Microbiol Biotechnol* 70:383–390
110. Kawasumi M, Nghiem P (2007) Chemical genetics: elucidating biological systems with small-molecule compounds. *J Invest Dermatol* 127:1577–1584
111. Gong Y, Zhang Z, Houry WA (2011) Bioinformatic approach to identify chaperone pathway relationship from large-scale interaction networks. In: Calderwood SK, Prince TL (eds) *Molecular chaperones: methods and protocols, methods in molecular biology*. Springer, New York, pp 189–203
112. Enserink JM (2012) Chemical genetics: budding yeast as a platform for drug discovery and mapping of genetic pathways. *Molecules* 17:9258–9273

113. Zhao R, Davey M, Hsu YC et al (2005) Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the Hsp90 chaperone. *Cell* 120:715–727
114. McClellan AJ, Xia Y, Deutschbauer AM, Davis RW et al (2007) Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. *Cell* 131:121–135
115. Echtenkamp FJ, Zelin E, Oxelmark E et al (2011) Global functional map of the p23 molecular chaperone reveals an extensive cellular network. *Mol Cell* 43:229–241
116. Knoblauch R, Garabedian MJ (1999) Role for Hsp90-associated cochaperone p23 in estrogen receptor signal transduction. *Mol Cell Biol* 19:3748–3759
117. Picard D, Suslova E, Briand PA (2006) 2-color photobleaching experiments reveal distinct intracellular dynamics of two components of the Hsp90 complex. *Exp Cell Res* 312:3949–3958
118. Freeman BC, Unpublished Data
119. Echtenkamp FJ, Freeman BC (2012) Expanding the cellular molecular chaperone network through the ubiquitous cochaperones. *BBA-Mol Cell Res* 1823:668–673
120. Shah N, Klausner RD (1993) Brefeldin A reversibly inhibits secretion in *Saccharomyces cerevisiae*. *J Biol Chem* 268:5345–5348
121. Jackson CL, Casanova JE (2000) Turning on ARF: the Sec7 family of guanine-nucleotide exchange factors. *Trends Cell Biol* 10:60–67
122. Jahn R, Scheller RH (2006) SNAREs—engines for membrane fusion. *Nat Rev Mol Cell Biol* 7:631–643
123. Cai H, Reinisch K, Ferro-Novick S (2007) Coats, tethers, rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. *Dev Cell* 12:671–682
124. Chen YA, Scheller RH (2001) SNARE-mediated membrane fusion. *Nat Rev Mol Cell Biol* 2:98–106
125. Duden R, Kajikawa L, Wuestehube L et al (1998)  $\epsilon$ -COP is a structural component of coatomer that functions to stabilize  $\alpha$ -COP. *EMBO J* 17:985–995
126. Sato TK, Darsow T, Emr S (1998) Vam7p a SNAP-25-like molecule, and Vam3p, a syntaxin homolog, function together in yeast vacuolar protein trafficking. *Mol Cell Biol* 18:5308–5319
127. Ungermann C, Wickner W (1998) Vam7p, a vacuolar SNAP-25 homolog, is required for SNARE complex integrity and vacuole docking and fusion. *EMBO J* 17:3269–3276
128. Pratt WB (1993) The role of heat shock proteins in regulating the function, folding, and trafficking of the glucocorticoid receptor. *J Biol Chem* 268:21455–21458
129. Sanchez ER, Toft DO, Schlesinger MJ et al (1985) Evidence that the 90-kDa phosphoprotein associated with the untransformed L-cell glucocorticoid receptor is a muring heat shock protein. *J Biol Chem* 260:12398–12401
130. DeZwaan DC, Freeman BC (2010) Hsp90 manages the ends. *Trends Biochem Sci* 35:384–391
131. Misteli T (2001) Protein dynamics: implications for nuclear architecture and gene expression. *Science* 291:843–847
132. Stasevich TJ, McNally JG (2011) Assembly of the transcription machinery: ordered and stable, random and dynamic, or both? *Chromosoma* 120:533–545
133. Dundr M, Misteli T (2001) Functional architecture in the cell nucleus. *Biochem J* 356:297–310
134. Phair RD, Misteli T (2000) High mobility of proteins in the mammalian cell nucleus. *Nature* 404:604–609
135. Houtsmuller AB, Rademakers S, Nigg AL (1999) Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science* 284:958–961
136. McNally JG, Müller WG, Walker D (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* 287:1262–1265
137. Phair RD, Scaffidi P, Elbi C et al (2004) Global nature of dynamic protein-chromatin interactions in vivo: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins. *Mol Cell Biol* 24:6393–6402

138. Gorski SA, Snyder SK, John S et al (2008) Modulation of RNA polymerase assembly dynamics in transcriptional regulation. *Mol Cell* 30:486–497
139. DeZwaan DC, Freeman BC (2008) Hsp90 the rosetta stone for cellular protein dynamics? *Cell Cycle* 7:1006–1012
140. Hager GL, McNally JG, Misteli T (2009) Transcription dynamics. *Mol Cell* 35:741–753
141. Hübner MR, Spector DL (2010) Chromatin dynamics. *Ann Rev Biophys* 39:471–489
142. Echtenkamp FJ, Freeman BC (2013) Molecular chaperone-mediated nuclear protein dynamics. *Curr. Protein Pept. Sci.*, 15, 216–224
143. Freeman BC, Morimoto RI (1996) The human cytosolic molecular chaperones Hsp90, Hsp70 (hsc70) and hsp-1 have distinct roles in recognition of a non-native protein and protein refolding. *EMBO J* 15:2969–2979
144. Hayer-Harl MK, Weber F, Hartl FU (1996) Mechanism of chaperonin action: GroES binding and release can drive GroEL-mediated protein folding in the absence of ATP hydrolysis. *EMBO J* 15:6111–6121
145. Yochem J, Uchida H, Sunshine M et al (1978) Genetic analysis of two genes, *dnaJ* and *dnaK*, necessary for *Escherichia coli* and bacteriophage lambda DNA replication. *Mol Genet Genomics* 164:9–14
146. LeBowitz JH, Zylicz M, Georgopoulos C et al (1985) Initiation of DNA replication on single-stranded DNA templates catalyzed by purified replication proteins of bacteriophage  $\lambda$  and *Escherichia coli*. *Proc Natl Acad Sci U S A* 82:3988–3992
147. Liberek K, Georgopoulos C, Zylicz M (1988) Role of the *Escherichia coli* DnaK and DnaJ heat shock proteins in the initiation of bacteriophage  $\lambda$  DNA replication. *Proc Natl Acad Sci U S A* 85:6632–6636
148. Shaknovich R, Shue G, Kohtz S (1992) Conformational activation of a basic helix-loop-helix protein (MyoD1) by the C-terminal region of murine Hsp90 (HSP84). *Mol Cell Biol* 12:5059–5068
149. Shue G, Kohtz DS (1994) Structural and functional aspects of basic helix-loop-helix protein folding by heat-shock protein 90. *J Biol Chem* 269:2707–2711
150. Antonsson C, Whitelaw ML, McGuire J et al (1995) Distinct roles of the molecular chaperone hsp90 in modulating dioxin receptor function via the basic helix-loop-helix and PAS domains. *Mol Cell Biol* 15:756–765
151. Hur E, Kim HH, Choi SM et al (2002) Reduction of hypoxia-induced transcriptino through the repression of hypoxia-inducible factor-1 $\alpha$ /Aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. *Mol Pharmacol* 62:975–982
152. 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
153. Walerych D, Kudla G, Gutkowska M et al (2004) Hsp90 chaperones wild-type p53 tumor suppressor protein. *J Biol Chem* 279:48836–48845
154. Stavreva DA, Müller WG, Hager GL, et al (2004) Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. *Mol Cell Biol* 24:2682–2697
155. Zhang X, Verdine GL (1996) Mammalian DNA cytosine-5 methyltransferase interacts with p23 protein. *FEBS Lett* 392:178–183
156. Bharadwaj S, Ali A, Ovsenek N (1999) Multiple component of the HSP90 chaperone complex function in regulation of heat shock factor 1 in vivo. *Mol Cell Biol* 19:8033–8041
157. Muñoz MJ, Bejarano ER, Daga RR et al (1999) The identification of Wos2, a p23 homologue that interacts with Wee1 and Cdc2 in the mitotic control of fission yeast. *Genetics* 153:1561–1572
158. Ouspenski II, Elledge SJ, Brinkley BR (1999) New yeast genes important for chromosome integrity and segregation identified by dosage effects on genome stability. *Nucleic Acids Res* 27:3001–3008
159. Freeman BC, Yamamoto KR (2002) Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science* 296:2232–2235

160. Bikle D, Teichert A, Hawker N et al (2007) Sequential regulation of keratinocyte differentiation by  $1,25(\text{OH})_2\text{D}_3$ , VDR, and its coregulators. *J Steroid Biochem* 103:396–404
161. Holt SE, Aisner DL, Baur J et al (1999) Functional requirement of p23 and Hsp90 in telomerase complexes. *Gene Dev* 13:817–826
162. Forsythe HL, Jarvis JL, Turner JW et al (2001) Stable association of hsp90 and p23, but Not hsp70, with active human telomerase. *J Biol Chem* 276:15571–15574
163. Hesselberth JR, Chen X, Zhang Z et al (2009) Global mapping of protein-DNA interactions *in vivo* by digital genomic footprinting. *Nat Methods* 6:283–289
164. Carr A, Biggin MD (1999) A comparison of *in vivo* and *in vitro* DNA-binding specificities suggest a new model for homeoprotein DNA binding in *Drosophila* embryos. *EMBO J* 18:1598–1608
165. Iyer VR, Horak CE, Scafe CS et al (2001) Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. *Nature* 409:533–538
166. Yang A, Zhu Z, Kapranov P et al (2006) Relationships between p63 binding DNA sequence, transcription activity, and biological function in human cells. *Mol Cell* 24:593–602
167. Joseph R, Orlov YL, Huss M et al (2010) Integrative model of genomic factors for determining binding site selection by estrogen receptor- $\alpha$ . *Mol Syst Biol* 456:1–13
168. Kaplan T, Li XY, Sabo PJ et al (2011) Quantitative models of the mechanisms that control genome-wide patterns of transcription factor binding during early *Drosophila* development. *PLoS Genet* 7:1–15
169. Wang JC, Derynck MK, Nonaka DF et al (2004) Chromatin immunoprecipitation (ChIP) scanning identifies primary glucocorticoid receptor target genes. *Proc Natl Acad Sci U S A* 101:15603–15608
170. John S, Sabo PJ, Thurman RE et al (2011) Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. *Nat Genet* 43:264–270
171. Reebye V, Cano LQ, Lavery DN et al (2012) Role of the HSP90-associated cochaperone p23 in enhancing activity of the androgen receptor and significance for prostate cancer. *Mol Endocrinol* 26:1694–1706
172. Simpson NE, Gertz J, Imberg K et al (2012) Research resource: enhanced genome-wide occupancy of estrogen receptor  $\alpha$  by the cochaperone p23 in breast cancer cells. *Mol Endocrinol* 26:194–202
173. Sumanasekera WK, Tien ES, Davis JW et al (2003) Heat shock protein-90 (Hsp90) acts as a repressor of peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) and PPAR $\beta$  activity. *Biochemistry* 42:10726–10735
174. Lee KK, Workman JL (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol* 8:284–295
175. Bell SP, Dutta A (2002) DNA replication in eukaryotic cells. *Annu Rev Biochem* 71:333–374
176. Gilson E, Géli V (2007) How telomeres are replicated. *Nat Rev Mol Cell Biol* 8:825–838
177. Hu J, Toft DO, Seeger C (1997) Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. *EMBO J* 16:59–68
178. Halonen SK, Weiss LM (2013) Toxoplasmosis. In: Garcia HH, Tanowitz HB, Del BOH (eds) *Handbook of clinical neurology*, 3rd series. Elsevier, Amsterdam, pp 125–145
179. Echeverria PC, Figueras MJ, Vogler M et al (2010) The Hsp90 co-chaperone p23 of *Toxoplasma gondii*: identification, functional analysis and dynamic interactome determination. *Mol Biochem Parasit* 172:129–140
180. Tapia H, Morano K (2010) Hsp90 nuclear accumulation in quiescence is linked to chaperone function and spore development in yeast. *Mol Biol Cell* 21:63–72
181. Wisner MF (2003) A *Plasmodium* homologue of cochaperone p23 and its differential expression during the replicative cycle of the malaria parasite. *Parasitol Res* 90:166–170
182. Chua CS, Low H, Goo KS et al (2010) Characterization of *Plasmodium falciparum* co-chaperone p23: its intrinsic chaperone activity and interaction with Hsp90. *Cell Mol Life Sci* 67:1675–1686

183. Luo J, Isaacs WB, Trent JM et al (2003) Looking beyond morphology: cancer gene expression profiling using DNA microarrays. *Cancer Invest* 21:937–949
184. Storchova Z, Pellman D (2004) From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 5:45–54
185. Hermeking H (2003) Serial analysis of gene expression and cancer. *Curr Opin Oncol* 15:44–49
186. Meeker AK, De Marzo A (2004) Recent advances in telomere biology: implications for human cancer. *Curr Opin Oncol* 16:32–38
187. van't Veer LJ, Bernards R (2008) Enabling personalized cancer medicine through analysis of gene-expression patterns. *Nature* 452:564–570
188. Chi P, Allis CD, Wang GG (2010) Covalent histone modification-miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer* 10:457–469
189. Boltze C, Schneider-Stock R, Roessner A et al (2003) Function of HSP90 and p23 in the telomerase complex of thyroid tumors. *Pathol Res Pract* 199:573–579
190. Mollerup J, Krogh TN, Nielsen PF et al (2003) Properties of the co-chaperone protein p23 erroneously attributed to ALG-2 (apoptosis-linked gene 2). *FEBS Lett* 555:478–482
191. Elmore LW, Forsythe R, Forsythe H et al (2008) Overexpression of telomerase-associated chaperone proteins in prostatic intraepithelial neoplasia and carcinomas. *Oncol Rep* 20:613–617
192. Simpson NE, Lambert WM, Watkins R et al (2010) High levels of Hsp90 cochaperone p23 promote tumor progression and poor prognosis in breast cancer by increasing lymph node metastases and drug resistance. *Cancer Res* 70:8446–8456
193. Rao RV, Niazi K, Mollahan P et al (2006) Coupling endoplasmic reticulum stress to the cell-death program: a novel HSP90-independent role for the small chaperone protein p23. *Cell Death Differ* 13:415–425
194. Chinta SJ, Rane A, Poksay KS et al (2008) Coupling endoplasmic reticulum stress to the cell death program in dopaminergic cells: effect of paraquat. *Neuromol Med* 10:333–342
195. Radanyi C, Le Bras G, Bouclier C et al (2009) Tosylcyclohexanone dihydrochloride promotes cleavage of the hsp90-associated cochaperone p23. *Biochem Biophys Res Commun* 379:514–518
196. Poksay KS, Banwait S, Crippen D et al (2012) The small chaperone protein p23 and its cleaved product p19 in cellular stress. *J Mol Neurosci* 46:303–314
197. Liu X, Zou L, Zhu L et al (2012) miRNA mediated up-regulation of cochaperone p23 acts as an anti-apoptotic factor in childhood acute lymphoblastic leukemia. *Leuk Res* 36:1098–1104
198. Patwardhan CA, Fauq A, Peterson LB et al (2013) Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. *J Biol Chem* 288:7313–7325
199. Hieronymus H, Lamb J, Ross KN et al (2006) Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. *Cancer Cell* 10:321–330
200. Brandt GEL, Schmidt MD, Prisinzano TE et al (2008) Gedunin, a novel Hsp90 inhibitor: semisynthesis of derivatives and preliminary structure-activity relationships. *J Med Chem* 51:6495–6502