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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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.

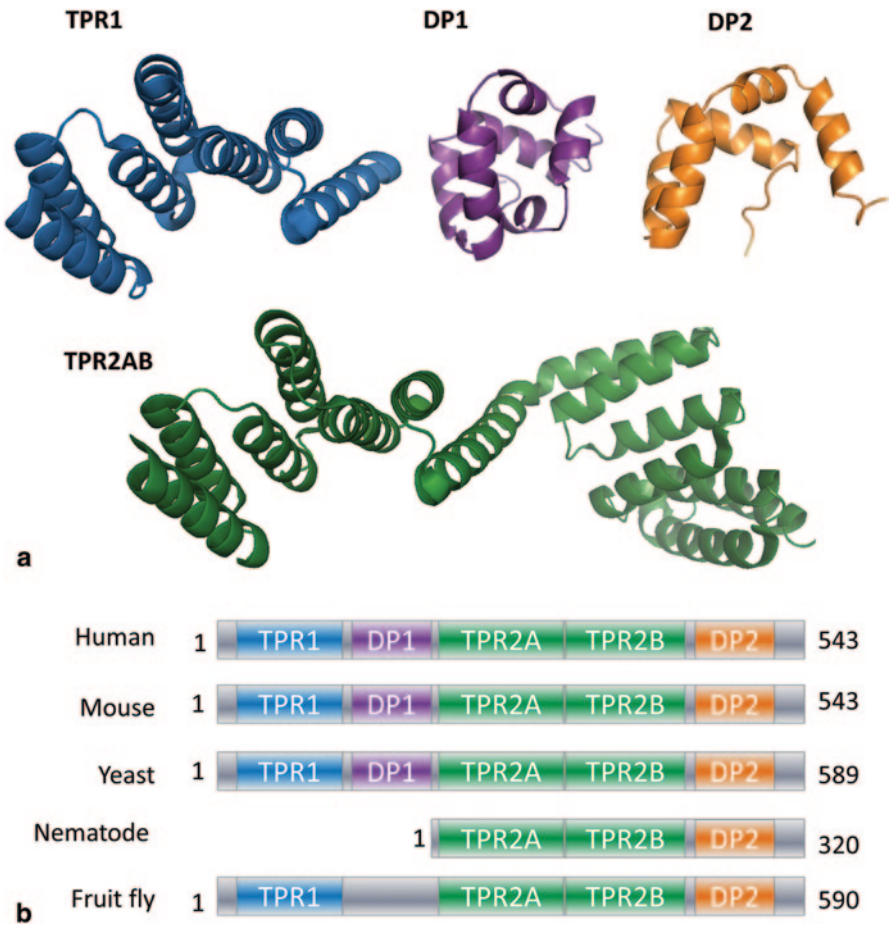

Fig. 3.1 Structural domains and architecture of Hop proteins. **a** Three dimensional structure of Hop domains. Images generated using Pymol (Delano Scientific). The PDB codes for the structures are: 3ESK for TPR1; 3UQ3 for TPR2AB; 2LLV for DP1; and 2LLW for DP2. **b** Comparison of Hop domain structure 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.

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