HSP70-HSP90 chaperone networking in protein misfolding disease

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Abstract

Molecular chaperones and their associated co-chaperones are essential in health and disease as they are key facilitators of protein folding, quality control and function. In particular, the heat shock protein (HSP) 70 and HSP90 molecular chaperone networks have been associated with neurodegenerative diseases caused by aberrant protein folding. The pathogenesis of these disorders usually includes the formation of deposits of misfolded, aggregated protein. HSP70 and HSP90, plus their co-chaperones, have been recognised as potent modulators of misfolded protein toxicity, inclusion formation and cell survival in cellular and animal models of neurodegenerative disease. Moreover, these chaperone machines function not only in folding, but also in proteasome mediated degradation of neurodegenerative disease proteins. This chapter gives an overview of the HSP70 and HSP90 chaperones, and their respective regulatory co-chaperones, and explores how the HSP70 and HSP90 chaperone systems form a larger functional network and its relevance to counteracting neurodegenerative disease associated with misfolded proteins and disruption of proteostasis.

Introduction

HSP70 and HSP90 are core components of an extensive molecular chaperone network that is essential for cell survival. They can perform multiple cellular roles as their ability to modulate protein folding and conformational change is recruited to specific functions, including through their co-chaperones. This is exemplified by the HSP70 machinery where in humans approximately 50 co-chaperone J-domain proteins, with cell and tissue specific expression patterns, regulate the function and client protein interactions of 13 different HSP70s to facilitate diverse processes ranging from *de novo* protein folding to clathrin mediated vesicular trafficking (Kampinga & Craig, 2010)

HSP70 and HSP90 have their own exclusive complements of co-chaperones that direct their function but are also directly linked by specific co-chaperones that can interact with both. The systems are also linked to protein degradation mechanisms through co-chaperones. This means that HSP70 and HSP90 can pass client proteins between them and triage them for degradation dependent on levels and activity of network components. This is most likely in combination with the folding state of the client. This flexibility in chaperone networks is particularly relevant in response to disruption of the proteome. This includes perturbations associated with protein misfolding disease and in neurodegenerations.

In this chapter we focus on links between the HSP70 and HSP90 molecular chaperone networks and their roles in neurodegenerative diseases. Firstly, we describe the HSP70 and HSP90 chaperone machines, including cataloguing key co-chaperones. We then consider the role of these chaperones in protein quality control systems in the context of the maintenance of a functional proteome in health and disease. Finally, we consider the evidence for how HSP70 and HSP90 networks are linked in protein misfolding neurodegenerations.

The HSP70 Chaperone Machine

The ability of HSP70 proteins to associate transiently with short hydrophobic peptide segments within their client proteins makes them versatile and provides them with a generalised ‘housekeeping’ chaperone function. However, through association with other chaperone machines such as HSP90 and HSP100, they are also involved in specific tasks (Mayer & Bukau, 2005). Collectively, these functions include protein folding and assembly of newly synthesized proteins, disassembly and refolding of misfolded or aggregated proteins, translocation across membranes and control of regulatory proteins (Bauer, Hofmann, & Neupert, 2002; Bukau, Deuerling, Pfund, & Craig, 2000; Hartl & Hayer-Hartl, 2002; Mayer & Bukau, 2005; Pratt & Toft, 2003; Ryan & Pfanner, 2001; Toft, 1999; Young, Barral, & Ulrich Hartl, 2003).

Homologs of HSP70 consist of two domains, a 45 kDa N-terminal ATPase domain (NTD) and a 25 kDa C-terminal substrate binding domain (SBD) (Mayer & Bukau, 2005). Nucleotide binds at the centre of the NTD, which consists of a two-lobed structure, divided into four subdomains, IA, IB, IIA, and IIB. The SBD itself consists of two subdomains, the larger of which consists of a 15 kDa β-sandwich fold and the other a C-terminal α-helical subdomain (Figure 1A). The α-helical subdomain is particularly important in switching conformation of the chaperone between the high and low substrate binding state during the ATPase cycle of HSP70. The ATP state of HSP70 displays a low affinity and fast exchange rates for substrates, while the ADP state shows high affinity and low exchange rates. The cycle of binding and release of client substrate is itself regulated by J-domain containing proteins, such as HSP40, which also target clients to HSP70, and by nucleotide exchange factors (NEFs), such as bag1, which are essential for displacing the tightly bound ADP from HSP70 (Kampinga & Craig, 2010; Mayer & Bukau, 2005; Radons, 2016). Although, two specialized HSP70 isoforms, HscA and HscC, expressed in *Escherichia coli,* do not depend on GrpE (Brehmer et al., 2001). However, during the ATPase cycle, the α-helical subdomain locks client substrate bound in the β-sandwich fold in the ADP state, whereas in the ATP state the α-helical subdomain docks onto the ATPase domain so opening up the substrate-binding site for release of the client protein. The interplay of HSP70s with co-chaperones determines the life time of the chaperone cycle (Zhu et al., 1996).

It appears that the SBD of HSP70 proteins has high sequence conservation and although differences exist between different HSP70 proteins, it is not clear what the significance of these are. Much of what is known about HSP70 client binding was gained form the X-ray structure of *E. coli* DnaK bound to a heptameric peptide (NRLLLTG) substrate in an extended conformation. The side-chain of the central leucine was found bound to a hydrophobic cleft within the SBD of DnaK, while a further 5 residues are bound between the SBD cleft and the SBD helical lid and are stabilized by a network of both hydrogen bonding and hydrophobic interactions (Zhu et al., 1996). The configuration that peptides adopt when bound to HSP70s appears to be conserved (Cupp-Vickery, Peterson, Ta, & Vickery, 2004; Jiang, Prasad, Lafer, & Sousa, 2005; Morshauser et al., 1999; Pellecchia et al., 2000; Stevens, Cai, Pellecchia, & Zuiderweg, 2003), but variability in both the exact register and orientation of binding has been observed (Clerico, Tilitsky, Meng, & Gierasch, 2015; Tapley, Cupp-Vickery, & Vickery, 2005; Zahn et al., 2013).

Despite the fact that the binding configuration is conserved, substrate preferences are noticeable between HSP70s form different organisms and even compartments (Fourie, Sambrook, & Gething, 1994). For example, while cytosolic HSP70s preferably bind peptides

rich in aliphatic side chains (such as leucine), endoplasmic reticulum (ER) homologs, such as HSPA5/BIP prefers motifs containing aromatic residues (Gragerov & Gottesman, 1994). In contrast, peptides containing proline are preferred by the *E. coli* HscA and *Saccharomyces cerevisiae* mitochondrial Ssq1 homologues (Dutkiewicz et al., 2004; Hoff, Ta, Tapley, Silberg, & Vickery, 2002), and yet other preferences have also been reported (Mok et al., 2018). It appears that this plasticity in binding specific substrate residues is mainly due to alterations in the two amino acid positions, represented by Met 404 and Ala 429 in *E.coli* DnaK and Ala 406 and Tyr 431 in human HSPA1/HSP70-1 and HSPA8/HSC70 (Rosenzweig, Nillegoda, Mayer, & Bukau, 2019; Rudiger, Mayer, Schneider-Mergener, & Bukau, 2000), but also from differences in the length of loops that enclose the substrate (Kluck et al., 2002). There is also evidence that the NTD of HSP70s may play a role in substrate specificity, but further work is required to understand the molecular details of this (Sharma & Masison, 2011). Reiterative cycles of binding and release of the chaperone, regulated by an exchange factor leads to folding of the client protein. This cycle is, however, strictly regulated by J-domain containing proteins and NEFs that together regulate the rate of the folding cycle.

J-domain proteins: J-domain containing proteins form a heterogeneous class of multidomain proteins that interact with HSP70. These proteins share a common conserved sequence of approximately 70 residues that is often located at the N-terminus of the protein and has been named the J-domain. The best described mechanism for J-domain proteins is exemplified by the *E. coli* DnaJ protein. The canonical mechanism of HSP70 relies on an initial interaction with a J-domain protein, that stimulates the rate limiting step of the HSP70 ATPase cycle in most cases (Karzai & McMacken, 1996; Laufen et al., 1999; Liberek, Marszalek, Ang, Georgopoulos, & Zylicz, 1991; Mayer & Bukau, 2005). Binding of a J-domain containing protein serves to deliver client proteins to the HSP70 chaperone, but also prevents client protein aggregation prior to chaperone loading (Kampinga & Craig, 2010). The stimulation of ATPase activity by a J-domain results in hydrolysis of ATP and the formation of a stable ADP-HSP70-client protein complex, in which the α-helical subdomain is locked over the bound client protein (Mayer & Bukau, 2005). The activation of the ATPase activity of DnaK by DnaJ involves a complicated series of conformational couplings between the various domains of the chaperone and the co-chaperone, such that a synergistic effect leads to the hydrolysis of ATP by DnaK. Binding of a substrate to the hydrophobic SBD of DnaK involves coupling to the ATPase domain, this transmits binding to the catalytic centre (Laufen et al., 1999). Furthermore, the coupling activity of DnaJ is dependent on both an interaction with substrate and with the ATPase domain of DnaK (Karzai & McMacken, 1996; Wall & Koger, 1994). DnaJ

itself interacts with substrates in a rapid and transient manner, which facilitates transfer to the DnaK SBD.

NEFs: For most HSP70s the dissociation of ADP from its tightly bound state within the ATPase domain involves a NEF. However, there are differences in the detailed mechanisms by which ADP is displaced form HSP70s, this typically appears to involve opening of the nucleotide binding pocket and has been observed for both DnaK and bovine HSP70, whose respective NEFs are GrpE and bag1 (Gassler, Wiederkehr, Brehmer, Bukau, & Mayer, 2001; Karzai & McMacken, 1996). In common, it appears that all eukaryotic NEFs capture an open NBD conformation (Bracher & Verghese, 2015; Rosenzweig et al., 2019).

GrpE, which represents the NEF in prokaryotes, mitochondria and chloroplasts binds across the NBD of DnaK and literally drives a β-domain into the nucleotide binding cleft (Harrison, Hayer-Hartl, Di Liberto, Hartl, & Kuriyan, 1997). This induces a tilt of subdomain IIB, opening up the nucleotide binding pocket and reducing the affinity for ADP. In the cytoplasm of eukaryotic cells, the situation is more complex. Three structurally distinct families of exchange factor (bag, HSP110 and armadillo) have been described that act on HSP70s to open the nucleotide binding pocket (Sondermann et al., 2001; Takayama, Xie, & Reed, 1999). The bag family of proteins use a conserved 3-helix bundle to bind to both the IB and IIB subdomains of HSP70s, which induces a tilt in these domains in a similar conformation to that induced by GrpE. HSP100-type of NEF actually belong to a HSP70 superfamily, in which the ATP binding domain of both HSP100s and the ER homologue Grp170, resemble the ATP (open) state of the nucleotide binding domain of HSP70s. The nucleotide exchange activities of HSP100s are initiated by a head-to-head interaction between the NBD of both HSP100 and HSP70 of their NBD resulting in an outward tilt of the HSP70s subdomain IIB in a similar manner to that seen for GrpE (Polier, Dragovic, Hartl, & Bracher, 2008; Schuermann et al., 2008). Finally, the core domain of armadillo-type NEFs, which are composed of four α-helical armadillo repeats, bind HSP70s by wrapping around subdomain IIB of the HSP70 NBD. This causes rotation, rather than tilting, of the subdomain around one of its helices and thus weakens the binding for ADP (Yan, Li, & Sha, 2011). Another interesting feature of NEF, is that some of them prevent rebinding of substrate to the SBD of HSP70 (Gowda et al., 2018; Rosam et al., 2018). This is achieved through structural elements able to mimic motifs that are recognized by the SBD of HSP70s and prevent unproductive rebinding. Examples include the N-terminal release domain found in the armadillo-type Fes1p protein of yeast and in human HSPBP1 (Gowda et al., 2018; Rosam et al., 2018) and perhaps with a helical extension at the N-terminal end of GrpE (Harrison et al., 1997; Wu, Naveen, Chien, Chang, & Hsiao, 2012).

Hedgehog-interacting protein (HIP): HIP is a multidomain protein consisting of an N-terminal dimerization domain, a predicted tetratricopeptide (TPR) middle domain, a highly charged segment, and a C-terminal substrate binding domain consisting of a GGMP repeat segment and a DP domain (Irmer & Hohfeld, 1997; Z. Li, Hartl, & Bracher, 2013; Prapapanich, Chen, Toran, Rimerman, & Smith, 1996). The DP domain is also known as the sti1 homology domain and is a region following the TPR domain rich in DP motifs. The TPR domain of HIP interacts with the NBD of HSP70s found in animals, plants and protozoa and delays the release of ADP form HSP70s (Hohfeld, Minami, & Hartl, 1995; Velten, Villoutreix, & Ladjimi, 2000). It appears that HIP in collaboration with HSP70 and its DP domain has been implicated in glucocorticoid receptor (GR) binding (Z. Li et al., 2013; Nelson, Huffman, & Smith, 2003; Schmid et al., 2012).

The crystal structure of the TPR domain of HIP in complex with HSP70 has been determined (Z. Li et al., 2013). HIP was shown to have a strong preference for the ADP conformation of HSP70, preventing protein aggregation and perhaps promoting substrate degradation. The structure showed that the TPR domain of HIP binds across HSP70 and contacts the IA, IB and IIB subdomains of HSP70 and thus locks it in an ADP bound conformation. The binding of HIP was also shown to be mutually exclusive to the NEFs bag1, (Z. Xu et al., 2008) HSP100 and HSPBP1, an armadillo-type NEF (Arakawa et al., 2010; Z. Li et al., 2013; Polier et al., 2008; Schuermann et al., 2008; Shomura et al., 2005; Sondermann et al., 2001).

The HSP90 Chaperone Machine

HSP90 is a multidomain ATP dependant dimeric chaperone (Ali et al., 2006). While dimerization is inherent through the C-terminal domains, the N-terminal domains undergo cycles of dimerization and disassembly in response to ATP binding and hydrolysis. The structural changes that take place within HSP90, in order to establish a closed catalytic state, are thought to occur simultaneously in a co-ordinated fashion and collectively represent the rate limiting step of ATP hydrolysis (Schulze et al., 2016). This conformational cycle is responsible for the activation of a vast array of client proteins, including proteins such as erbB-2, cyclin-dependent kinase 4 (CDK4), braf, structural proteins such as actin and tubulin and steroid hormone receptors. A full list of clients can be found at http://www.picard.ch/Downloads/HSP90interactors.pdf. The N-terminal domain has been shown in interactions with co-chaperones such as p23 (Sba1 in yeast), Cdc37 and aha1, all of which are able to regulate HSP90 ATPase activity and therefore modulate the catalytic cycle of HSP90.

The middle and largest domain of HSP90 contains the catalytic-loop arginine residue that interacts with the γ-phosphate of bound ATP and completes the catalytic unit (Figure 1B). The structure and catalytic cycle of HSP90 has been reviewed in detail in a number of recent reviews (Genest, Wickner, & Doyle, 2019; Hoter, El-Sabban, & Naim, 2018; J. Li & Buchner, 2013; Prodromou, 2012, 2016). Co-chaperones such as Cdc37, p23 and aha1 have all been shown to interact with the middle domain and influence its ATPase activity through these interactions (Ali et al., 2006; Meyer et al., 2004; Roe et al., 2004). This is, at least in part, most likely achieved through interactions with residues on HSP90 preceding the catalytic loop of the middle domain. Structural work has revealed that kinases interact with the middle domain of HSP90, however, other clients may interact more extensively with the chaperone (Karagoz et al., 2014).

The C-terminal domain is inherently dimerized and contains a conserved -MEEVD peptide motif that is responsible for the binding of TPR domain containing co-chaperones. These include HOP (Sti1p in yeast), immunophilins (such as FK-binding protein (FKBP) 51 and FKBP52), protein phosphatase 5 (PP5), Carboxyl terminus of Hsc70-interacting protein (CHIP), Aryl hydrocarbon receptor interacting protein (AIP), Aryl hydrocarbon receptor interacting protein-like 1 (AIPL1), and RNA polymerase II-associated protein 3 (RPAP3) to name but a few. These co-chaperones may be specific for particular client protein complexes and may impart additional enzymatic activities that help regulate the HSP90 complex and activation or degradation of client proteins (Prodromou, 2012).

Cdc37: The Cdc37 co-chaperone is involved in delivering kinase client proteins to the HSP90 machinery. Remarkably, around 60% of the human kinome interacts with HSP90 through participation with Cdc37 (Taipale et al. 2012). Initial structural studies have shown that Cdc37 interacts with the N-terminal domain of HSP90 and inhibits the ATPase driven cycle of the chaperone (Roe et al., 2004). In complex with CDK4, it was shown that the kinase was engaged with the N- and middle-domain of HSP90, while Cdc37 engages with the N-domains and consequently the chaperone remained in an open state (Vaughan et al., 2006). In contrast, recent Cryo-EM studies of the HSP90-Cdc37-CDK4 complex have shown that Cdc37 can engage with HSP90 by binding at the interface of the N- and middle-domains of the chaperone (Verba et al., 2016). In this state HSP90 is in a closed conformation and the kinase is trapped in a partially unfolded state, bound across the HSP90 dimer. The two lobes of the kinase are separated between HSP90 and Cdc37, the latter of which mimics part of the CDK4 N-lobe to stabilize an open conformation of the kinase (Verba et al. 2016). Cdc37 phosphorylation at Ser 13 plays an important role in stabilising the closed conformation of the HSP90-Cdc37-CDK4 complex and is involved in contacts with residues of HSP90 within the

helix preceding the catalytic loop (Verba et al., 2016). The interaction helps stabilise the N-terminal fragment of Cdc37, which also interacts with the catalytic loop directly (Verba et al., 2016). This not only acts as an interaction hub stabilising the complex, but may also influence the rate of ATP hydrolysis in the complex. Dephosphorylation of Ser 13 by PP5 has been shown to cause release of the protein kinase and disassembly of the complex (Vaughan et al., 2008). In contrast, Tyr 4 and Tyr 298 phosphorylation has been shown to lead to client dissociation (Xu et al. 2012).

p23: Unlike Cdc37, the co-chaperone p23 (Sba1p in yeast) is not involved in delivering clients to HSP90, but it appears to stabilize the chaperone-client complex in a closed state. p23 presents a characteristic cysteine and histidine-rich domain known as the CS domain (CHORD and sgt1), a ~100 residue compact antiparallel β-sandwich formed by seven β-strands that shows structural homology to the core domain of several small HSPs and HSP90 interactors such as NudC, sgt1 and rar1 (Garcia-Ranea et al. 2002; Van Montfort et al. 2001; Weaver et al. 2000). p23 is involved in the maturation of protein clients, interacting with and stabilising the ATP-bound, closed-state, of the HSP90 dimer through its CS domain (Ali et al. 2006). Its unstructured C-terminal tail is responsible for inhibiting or down regulating the HSP90 ATP hydrolysis by most likely inducing structural changes in the catalytic loop at the NTD-MD interface of the closed dimer, and consequently stabilizing the interaction with the client (Ali et al., 2006; Biebl et al., 2021).

aha1: The co-chaperone aha1 consists of an NTD and C-terminal domain (CTD) connected by a 60-amino acid residue flexible linker. It remains the most potent ATPase activator of HSP90. Early structural studies showed that the NTD of aha1 binds to the middle domain of Hp90 and influences the conformation of the catalytic loop, bringing about the acceleration of ATPase hydrolysis and thus the conformational cycle of HSP90 (Panaretou et al. 2002). This interaction represents the recruitment step for aha1 into the HSP90 complex. It has been seen that aha1 can accelerate the rate of HSP90 dimer closure and the CTD of HSP90 may play a major role in this, as it is known to interact with the N-terminal domain of the chaperone (Li et al. 2013). More recent cryo-EM studies have now shown that the C-terminal domain of aha1 interacts with the middle domain of both protomers of the HSP90 dimer (Yanxin Liu, 2020). The aha1 CTD induces a semi-closed HSP90 conformation together with the N-terminal domain of aha1, but steric clashes within this conformation cause the NTDs of HSP90 to become undocked form their respective middle domains. The NTDs can now rotate and are now primed for ATP binding and redocking in a conformation that allows N-terminal

dimerization of HSP90. This also causes the NTDs of aha1 to tilt by around 30° to establish new interactions with the dimerized NTDs of HSP90. This not only stabilizes the dimerized state of the NTDs of HSP90, but also facilitates ATP hydrolysis. However, it appears that the NTD of aha1 only interacts with the lid region from only one of the NTDs of the HSP90 dimer. Thus, ATP hydrolysis is stimulated in that protomer alone, which establishes an asymmetric semi-hydrolysed state within HSP90. The open state of HSP90 and disassembly of the complex, probably results following the hydrolysis of the second bound ATP molecule by HSP90.

Within the HSP90- aha1 closed state there are a number of interactions that influence the stability and activity of the complex. In the tilted position, the conserved N-terminal motif, NxNNWHW, interacts across the dimerized N-terminal domains. This motif contacts the 'ATP lid' of one protomer, helps to stabilise the catalytic loop of the middle domain of HSP90 within the same protomer and probably provides additional stability to the β-strand exchange between the NTDs of HSP90, by binding to and locking the helix preceding the β-strand element. The deletion of the conserved NxNNWHW motif of HSP90 has been shown to reduce its ability to stimulate the ATPase activity of HSP90 and its ability to rescue the temperature sensitive S25P mutation of yeast HSP90p (Mercier et al., 2019). A small α-helix (residues 163-170) at the extreme C-terminal end of the NTD of aha1 also interacts with the 'ATP-lid' of the same HSP90 protomer. Furthermore, the CTD of aha1 helps to stabilise the N- and middle-domain interface of HSP90. Previous work has also shown that the F349A mutation, thought to destabilise the N-middle domain interface of HSP90, might be suppressed by aha1 (Siligardi et al., 2004), and this suggestion has now been shown to be fully consistent with the structural model proposed for the HSP90- aha1 complex.

RPAP3 and PIH1 domain-containing protein 1 (PIH1D1): RPAP3 (Tah1p in yeast) and PIH1D1 (Pih1p in yeast) form a complex and act as a co-chaperone of HSP90 (Eckert et al., 2010; Millson et al., 2008; Morgan, Pal, Roe, Pearl, & Prodromou, 2015; Pal et al., 2014). Tah1 has also been shown to activate the HSP90 ATPase activity, but as a complex with Pih1p, it appears to inhibit activity, perhaps allowing client protein loading and complex formation (Eckert et al., 2010; Millson et al., 2008). The RPAP3 and PIH1D1 complex also forms part of the larger chromosome remodelling complexes, ino80 and swr-c, by interacting with Rvb1p and Rvb2p (Martino et al., 2018; Rivera-Calzada et al., 2017). In this way they may act as an adaptor between HSP90 and Rvb1/2p (RUVBL1/2 in humans) client proteins (Morgan et al., 2015; Zhao et al., 2008). In contrast to the inhibition of HSP90, the Tah1p-

Pih1p complex activates the ATPase activity of Rvb1/2p complex (Rivera-Calzada et al., 2017).

Tah1 consists of five α-helices, which constitute a TPR domain, and an unstructured C-terminal domain (Back et al., 2013; Jimenez et al., 2012; Millson et al., 2008; Pal et al., 2014). The TPR domain appears to be specific for HSP90 binding (Millson et al., 2008). Unlike other TPR domains, Tah1p requires a helix swap, which involves the fifth α-helix between two Tah1p molecules, to create the normal binding environment for the methionine from the bound HSP90 -MEEVD motif (Millson et al., 2008). This reconstitutes a TPR binding site similar to that seen in other typical seven-helix-containing TPR domain proteins. It has been suggested that dimerization of Tah1p prevents other monomeric TPR-domain proteins form simultaneously binding to HSP90 (Morgan et al., 2015). The TPR cleft of Tah1p also appears to be accessible and able to accept the -MEEVD motif of HSP90 when bound within the R2TP (Rvb1p-Rvb2p-Tah1p-Pih1p) complex (Rivera-Calzada et al., 2017).

RPAP3 is considerably larger than Tah1p, and appears to be recruited to the R2TP complex through a C-terminal rruvbl2 interacting domain that interacts with the ATPase face of the complex. It also contains two central TPR domains, that are equivalent to the dimerized TPR domains of Tah1p, whose binding maps to the opposite face of the R2 ring where it can interact with PIH1D1, as observed with the yeast R2TP complex (Martino et al., 2018). Similarly, as with Tah1p there is an unstructured segment downstream of the TPR domains of RPAP3, represented by residues 400 to 420, that is sufficient to form a complex with PIH1D1.

PIH1D1 and Pih1p consist of two domains, an N-terminal PIH domain, that recruits Tel2, through a phosphoserine binding site and a CS domain that interacts with the unstructured region of Tah1p and RPAP3 described above (Martino et al., 2018; Pal et al., 2014). The CS domain comprises of residues 264–344 forming a seven-stranded β-sandwich with the topology found in other HSP90 co-chaperones, such as p23/Sba1p (Ali et al., 2006) and sgt1 (M. Zhang et al., 2008; M. Zhang, Kadota, Prodromou, Shirasu, & Pearl, 2010). In contrast, the PIH domain consists of a twisted five-stranded β-sheet where strands 4 and 5 are traversed by a helix-turn-helix segment, and the other face of the β-sheet is traversed by a coil segment that extends from the end of β-strand 5 (Pal et al., 2014). An additional α-helix that connects β-strands 2 and 3 projects from the end of the sheet, packing against the larger of the other two helices. The interaction of Pih1 with Tel2 (and therefore the TTT complex) is through a casein kinase 2 phosphorylated motif in Tel2 (SELDpSDDEF) and the PIH domain (Horejsi et al., 2010; Pal et al., 2014).

PP5: The PP5 Ser/Thr phosphatase plays an important role in the regulation of the HSP90-client kinase loading and unloading cycle. The HSP90 co-chaperone Cdc37 is phosphorylated on residue Ser 13 by casein kinase 2 and is then able to recruit kinases to HSP90 complexes (Miyata & Nishida, 2004; Vaughan et al., 2006). Binding of PP5 to such Cdc37-kinase-HSP90 complexes results in dephosphorylation of Cdc37 at Ser 13 and leads to subsequent release of the kinase (Oberoi et al., 2016; Vaughan et al., 2008). PP5 is also found in HSP90 complexes with the co-chaperones FKBP51, FKBP52 and p23 in the chaperoning of steroid hormone receptors (Banerjee et al., 2008; Kaziales, Barkovits, Marcus, & Richter, 2020; Silverstein et al., 1997) and has been shown to directly dephosphorylate GR on several residues, modulating its activity (Dushukyan et al., 2017; Z. Wang, Chen, Kono, Dang, & Garabedian, 2007).

Immunophilins: Immunophilins are peptidyl-prolyl cis-trans isomerases (PPIases) that catalyse and stabilise the cis-trans isomerization of peptide bonds. PPIase proteins are classified into cyclophilins that bind cyclosporin, and FK506-binding proteins. FKBP52, FKBP51 and Cyp40 are thought to catalyse and stabilise the cis-trans isomerization of peptide bonds in the HSP90 steroid hormone receptor clients GR, estrogen receptor and progesterone receptor as well as tau (Jinwal, Koren, & Dickey, 2013; K. Lee et al., 2021; Nair et al., 1997; Ratajczak et al., 1993). Immunophilins are found together with p23 within HSP90-steroid hormone complex (Ebong, Beilsten-Edmands, Patel, Morgner, & Robinson, 2016; J. L. Johnson & Toft, 1994; Nair et al., 1997). In the closed HSP90 complex, the TPR domain of FKBP51 is bound to the C-terminal domain of HSP90 (J. L. Johnson & Toft, 1994; Noddings, Wang, & Agard, 2020). The TPR cleft is directed away from the HSP90 molecule, but is bound by the conserved -DTSRMEEVD peptide motif of HSP90. Furthermore, the terminal helix of the TPR domain interacts between the terminal helices of the HSP90 protomers and sequence conservation suggests that other immunophilin classes of TPR co-chaperones may collectively represent a conserved mechanism for HSP90-specific recognition. Binding within this C-terminal cleft of HSP90 appears to be favoured in its closed state. Further contacts between the C-terminal domain of FKBP and HSP90 occur in the connecting strand between helix 5 and 6 of FKBP51, including a direct backbone contact between N365 and N655 in HSP90. The FK1 domain is positioned adjacent to HSP90 client interaction sites (Genest et al., 2013; Verba et al., 2016) and may provide a means by which the PPIase activity of the FK1 domain may act on specific client proline residues. In contrast the FK2 domain is inactive (Sinars et al., 2003), but helps transverse the middle domain of HSP90 so that the FK1 domain can dock correctly with the chaperone.

In yeast, Cpr6p, Cpr7p, and Cns1p interact with the intact ribosome (Tenge, Zuehlke, Shrestha, & Johnson, 2015). It has also been reported that an HSP90p-Cpr6p complex can interact with Ura2p, a protein involved in pyrimidine biosynthesis (Zuehlke, Wren, Tenge, & Johnson, 2013). The structure of intact Cpr7p has been reported (Qiu et al., 2017). Cpr7p was also seen to interact with Ure2p and is required for the stability of its Ure3p prion derivative (Kumar, Gaur, Gupta, Puri, & Sharma, 2015). Together with the recruiter Hgh1p, Cns1p links HSP90 to translation elongation by chaperoning Elongation factor 2 (Schopf et al., 2019).

AIP: AIP consists of two domains, an N-terminal immunophilin-like domain and a C-terminal TPR domain (Linnert et al., 2012; Morgan et al., 2012). AIP interacts with a number of different chaperone proteins (HSP90, HSP70, TOMM20) that share a common conserved -EEVD motif (-DDVE in TOMM20) at the C-terminal end of these chaperones (Bolger et al., 2003; Scheufler et al., 2000; M. Zhang et al., 2005). Client proteins, amongst others, include nuclear receptors (aryl hydrocarbon (Ahr), estrogen receptor α), phosphodiesterase 4A5 (rat isoform of human *PDE4A4*) and PDE2A3, survivin, G proteins, ret and Epstein-Barr virus nuclear antigen 3 (Trivellin & Korbonits, 2011). Mutations in AIP have been linked to familial isolated pituitary adenomas (Chahal, Chapple, Frohman, Grossman, & Korbonits, 2010; Daly et al., 2007; Leontiou et al., 2008; Vierimaa et al., 2006), which leads to acromegaly and gigantism. The precise role in predisposition to pituitary adenoma is not well understood, but AhR may act as a tumour suppressor that becomes silenced (Heliovaara et al., 2009; G. Huang & Elferink, 2005; Kolluri, Weiss, Koff, & Gottlicher, 1999; Leontiou et al., 2008; Marlowe, Knudsen, Schwemberger, & Puga, 2004; Pang et al., 2008; Puga et al., 2000; Vierimaa et al., 2006).

AIPL1: AIPL1 shares 49% identity with AIP and similar to AIP is comprised of an N-terminal FKBP-like domain that lacks PPIase activity and a C-terminal TPR domain. AIPL1 has been shown to interact with both HSP70 and HSP90, and similar to other TPR-domain co-chaperones, the terminal -MEEVD and -IEEVD motifs of HSP90 and HSP70, respectively, contribute to the interaction of AIPL1 with the chaperones (Hidalgo-de-Quintana, Evans, Cheetham, & van der Spuy, 2008). The interaction of AIPL1 with HSP90 is nucleotide dependent (Sacristan-Reviriego et al., 2017), though the exact structural features mediating the AIPL1-HSP90 interaction have yet to be characterised. Interestingly, AIPL1 is expressed exclusively in the photoreceptor cells of the neurosensory retina and in the pineal gland, and AIPL1 mutations thus cause a severe and early onset inherited retinal degeneration, Leber congenital amaurosis (Sohocki et al., 2000; van der Spuy et al., 2002; van der Spuy et al.,

2003). To date, the only identified client protein for the AIPL1-HSP90 chaperone complex is retina-specific cGMP phosphodiesterase (PDE6), a critical component of the phototransduction cascade that catalyses the hydrolysis of cGMP to GMP upon light exposure, thus leading to the closure of cGMP-gated cation channels and membrane hyperpolarisation. In Aipl1 knockout or hypomorphic mice, all three subunits of the rod photoreceptor PDE6, including the catalytic α and β subunits and the inhibitory γ subunit, are translated, however the PDE6 holoenzyme is misassembled and the subunits rapidly degraded (Kolandaivelu, Huang, Hurley, & Ramamurthy, 2009; Liu et al., 2004; Ramamurthy, Niemi, Reh, & Hurley, 2004). Similarly, AIPL1 is required for the proper assembly and stability of the cone photoreceptor PDE6 heterocomplex (Kirschman et al., 2010). Inhibition of HSP90 in the rat retina leads to the post-transcriptional loss of PDE6 (Aguila et al., 2014). Within the PDE6-AIPL1-HSP90 complex, the FKBP-like ligand-binding domain of AIPL1 is thought to interact with isoprenyl groups of the PDE6 catalytic subunits (Kolandaivelu et al., 2009; Majumder, Gopalakrishna, Cheguru, Gakhar, & Artemyev, 2013; Yadav, Gakhar, Yu, & Artemyev, 2017), whereas the TPR domain of AIPL1 mediates the interaction with HSP90 (Hidalgo-de-Quintana et al., 2008; Sacristan-Reviriego et al., 2017), although an interaction between the PDE6 inhibitory γ subunit and the TPR domain has also been observed (Yadav, Boyd, Yu, & Artemyev, 2019). It is thought that through these interactions, AIPL1 mediates the specificity of HSP90 for the PDE6 client protein in retinal photoreceptors, and the folding, orientation and organisation of the AIPL1 domains are important for mediating this function (Gopalakrishna, Boyd, Yadav, & Artemyev, 2016; Sacristan-Reviriego et al., 2017).

sgt1: sgt1 is an essential chaperone that can recruit HSP90 in to a range of cellular activities including Skp, Cullin, F-box containing complex E3 ubiquitin ligases and the kinetochore. It appears that in these pathways Skp1, a small protein that heterodimerizes with proteins containing the F-box motif, is associated with sgt1 (A. E. Davies & Kaplan, 2010; Willhoft et al., 2017). Sgt1 has also been implicated in the regulation of innate immunity systems in plants and animals. Sgt1 has been found to associate, together with HSP90, with plant R proteins and related animal Nod-like receptors. The crystal structure and stoichiometry of the core HSP90-sgt1-rar1 CHORD II domain complex in association with the HSP90 N-terminal domain has been determined (Siligardi, Zhang, & Prodromou, 2017; M. Zhang et al., 2008; M. Zhang et al., 2010).

Major co-chaperones involved in both HSP90 and HSP70 complexes

Although many other co-chaperones are involved in the maturation and regulation of client proteins that are both HSP70 and HSP90 dependant, two major co-chaperones that work closely with the HSP70 and HSP90 chaperone systems are worthy of a mention here. The first is the adaptor HOP (Sti1p in yeast), which links the HSP70 and HSP90 systems together. The second is CHIP, which is utilised in client protein degradation by both chaperone systems (Figure 2).

HOP: HOP (Sti1p in yeast) is a TPR repeat containing co-chaperone, which recognizes the C-terminal -EEVD motif in HSP90 and HSP70 (Brinker et al., 2002; Odunuga et al., 2003; Scheufler et al., 2000). HOP/Sti1p contains two Asp-Pro (DP)-rich domains, which are arranged as follows with the three TPR domains: TPR1–DP1–TPR2A–TPR2B–DP2. The TPR1 and TPR2A domains of HOP bind specifically to the C-terminal tails of HSP70 and HSP90, respectively (Brinker et al., 2002). HOP/Sti1p is able to bind simultaneously to HSP90 and HSP70 (Alvira et al., 2014; C. T. Lee, Graf, Mayer, Richter, & Mayer, 2012; Rohl et al., 2015; Scheufler et al., 2000; Schmid et al., 2012) to facilitate client protein transfer. Clients include nuclear receptors (steroid hormone receptors), kinases such as eukaryotic initiation factor 2α-kinase, cyclin-dependent kinases and the p53, HSF-1, pRb transcription factors (Mayer & Bukau, 2005).

HOP is a major TPR domain containing co-chaperone that regulates HSP90's molecular chaperone function. HOP plays an important role in delivering steroid hormone receptors and other clients to HSP90 by working together with HSP70, and by stabilising the open conformation of HSP90 through inhibition of its ATPase activity (Jinwal et al., 2013; Kirschke, Goswami, Southworth, Griffin, & Agard, 2014; Prodromou et al., 1999; Rohl et al., 2015). Sti1p has been reported to be a dimer and a potent inhibitor of the ATPase activity of HSP90 (Prodromou et al., 1999). It has been proposed that HOP interactions with the middle domain of HSP90 may inhibit the conformational changes leading to the N-terminally closed state, but further evidence is required to substantiate these claims. In contrast, the effect of HOP or Sti1p on HSP70s has been reported as variable (Gross & Guerrieri, 1996; B. D. Johnson, Schumacher, Ross, & Toft, 1998).

CHIP: CHIP is a E3 ubiquitin ligase, which contains a TPR repeat domain able to interact with HSP70, but also HSP90 (Stankiewicz, Nikolay, Rybin, & Mayer, 2010). CHIP is a dimeric protein of ~35 kDa consisting of a N-terminal TPR repeat domain and a C-terminal U box domain (Ballinger et al., 1999; Hatakeyama, Yada, Matsumoto, Ishida, & Nakayama, 2001). The structure of CHIP has been determined (M. Zhang et al., 2005): CHIP displays an E3 and

E4 ubiquitin ligase activity (Jiang et al., 2001; Murata, Minami, Minami, Chiba, & Tanaka, 2001) and it is thought to be involved in client protein quality control that facilitates switching between chaperone-mediated folding and maturation to a proteasome-mediated degradation involving lysine 48-linked polyubiquitylation (Cyr, Hohfeld, & Patterson, 2002; Wiederkehr, Bukau, & Buchberger, 2002). Although CHIP has been reported to autoubiquitylate and to facilitate non lysine 48 polyubiquitylation (Alberti et al., 2002; Jiang et al., 2001; Murata et al., 2001). The ubiquitylation of HSP70 and HSP90 substrates targets them for proteolytic degradation by the proteosome (Connell et al., 2001b; Edkins, 2015; Hohfeld, Cyr, & Patterson, 2001; Meacham, Patterson, Zhang, Younger, & Cyr, 2001). Thus, although both HSP70 and HSP90 are involved in protein folding and regulation of clients, both send clients for degradation under appropriate conditions. Details about the mechanism are enigmatic, but it is believed that CHIP might respond to substrates or client proteins that fail to fold or progress within the HSP70 and HSP90 chaperone systems (Connell et al., 2001b; Meacham et al., 2001; Stankiewicz et al., 2010; VanPelt & Page, 2017). Clients responding to CHIP directed degradation include cystic fibrosis transmembrane conductance regulator, GR, the E2A transcription factor, tau, huntingtin (Htt) and ataxin, telomerase, apoptosis signal-regulating kinase 1, phosphatase and tensin homologue and p53 (Ahmed et al., 2012; Connell et al., 2001b; Gao et al., 2010; Z. Huang, Nie, Xu, & Sun, 2004; Jana et al., 2005; J. H. Lee, Khadka, Baek, & Chung, 2010; Meacham et al., 2001; Petrucelli et al., 2004). Cryo-EM structures of HSP70-client and CHIP complexes show that the substrate is located between the chaperone and CHIP. This suggests that client protein is presented to CHIP and flexibility within such complexes is important for the ubiquitylation process (Quintana-Gallardo et al., 2019). Inhibition of HSP90 by ATPase competitive inhibitors, such as geldanamycin (GA), result in its client proteins being directed for proteolytic degradation of proteins such as erbB-2 (Xu et al., 2002). Similarly, addition of CHIP to a HSP90 - client complex promotes proteasomal degradation of the client protein. However, no physical link has been found between the HSP90 - client protein complex and the proteasome (Connell et al., 2001b; Whitesell & Cook, 1996).

Disruption of proteostasis and disease

Given their importance in protein folding and quality control it is unsurprising that molecular chaperones, including those of HSP70 and HSP90 networks, have been identified as key modulators of human misfolding disease and in particular neurodegenerations (Barral, Broadley, Schaffar, & Hartl, 2004; Bonini, 2002; Muchowski & Wacker, 2005). Many neurodegenerative disorders, including Alzheimer's disease (AD), PD, amyotrophic lateral sclerosis (ALS) and polyQ expansion diseases, are characterised by conformational changes

in disease associated proteins that result in their misfolding and aggregation (Barral et al., 2004; Muchowski & Wacker, 2005; Taylor, Hardy, & Fischbeck, 2002). More specifically, although the majority of proteins fold via intermediate states, that can be kinetically stable and form amorphous aggregates, a subset of aggregation prone proteins linked to neurodegenerative disease shares a propensity to assemble into ordered fibrillar aggregates, referred to as amyloid fibrils. These can be more stable than the native state of the protein and protease resistant. Structurally amyloid fibrils are characterised by a cross β-sheet conformation, with β-strands that run perpendicular to the fibril axis forming extended β-sheets (Dobson, 2003; Klaips, Jayaraj, & Hartl, 2018; Stefani & Dobson, 2003). Although fibrillar aggregates are a pathological feature in the brains of patients with neurodegenerative diseases characterised by protein misfolding, evidence suggests they are not the major culprit for neuronal toxicity. Instead, it appears that smaller soluble precursors of the fibrillar aggregates are key drivers of pathogenesis. These precursors are heterogeneous and thought to consist of both on and off pathway intermediates of amyloid fibrils, with variable toxicity (Soto & Pritzkow, 2018). There are multiple examples of chaperone networks counteracting toxic misfolded protein species. These include HIP and HSP70 together, that can prevent cytotoxic aggregates of α-synuclein and poly-Q expanded androgen receptor (AR) that lead to neurological disease (Howarth, Glover, & Uney, 2009; Roodveldt et al., 2009). Furthermore, even where there is no direct evidence for reduction in cytotoxic protein species, chaperone levels are frequently increased in neurodegenerative conditions. For example, upregulation of HSP90, gt1 and CHP-1 has been seen in the majority of cases of PD and dementia with Lewy bodies (Bohush, Niewiadomska, Weis, & Filipek, 2019).

Another feature of amyloid fibrils is that they have the potential to nucleate subsequent fibril formation of their soluble constituent protein. Recently it has been identified that this seeding activity can occur not just at the molecular level but between cells facilitating cell-to-cell spread and pathological transmission in misfolding diseases. There is significant evidence for HSP70 and HSP90 binding to amyloid fibril proteins. This includes the AD protein tau, where HSP70 inhibits nucleation and elongation of fibrils and sequesters oligomers and fibrils into protective complexes (Kundel et al., 2018), while HSP90 promotes formation of small tau oligomers yet inhibits formation of tau fibrils (Weickert, Wawrzyniuk, John, Rudiger, & Drescher, 2020). Chaperones play multiple roles in neurons and other cells in the cellular defences against accumulation of misfolded proteins and aggregation (Ross & Poirier, 2005) (Figure 3). As well as being essential for protein folding and refolding (Bukau & Horwich, 1998; Hartl & Hayer-Hartl, 2002) they also function in disaggregation and the triage of terminally misfolded proteins for degradation by the ubiquitin proteasome or autophagy-lysosome systems (Alberti, Bohse, Arndt, Schmitz, & Hohfeld, 2004; Alberti et al., 2002; Chapple, van der Spuy,

Poopalasundaram, & Cheetham, 2004; Connell et al., 2001a) (Figure 3). This includes that chaperone may promote the sequestration of misfolded aggregated proteins into larger protein inclusions, with relatively decreased surface-to-volume ratios. This accumulation of the soluble oligomers into larger inclusions is thought to be neuroprotective, as it separates the toxic soluble oligomers away from the cellular milieu and into an insoluble compartment that may also be more amenable to clearance by autophagic mechanisms (Arrasate, Mitra, Schweitzer, Segal, & Finkbeiner, 2004; Cohen, Bieschke, Perciavalle, Kelly, & Dillin, 2006; Cuervo, 2004; Cuervo, Stefanis, Fredenburg, Lansbury, & Sulzer, 2004; Kaganovich, Kopito, & Frydman, 2008; Kopito, 2000; Mannini & Chiti, 2017; Sontag, Samant, & Frydman, 2017; Taylor et al., 2003).

Together protein folding and degradation pathways are integral to maintenance of cellular proteostasis (protein homeostasis) with a highly interconnected network of chaperones and degradation factors combating proteotoxicity. HSP70 (Fernandez-Fernandez, Gragera, Ochoa-Ibarrola, Quintana-Gallardo, & Valpuesta, 2017) and HSP90 (Taipale, Jarosz, & Lindquist, 2010) can both be considered hubs in the proteostasis network. The HSP70 chaperone machinery can channel its clients along folding, refolding, maturation, disaggregation, and proteolytic pathways. This is in cooperation with its network of co-chaperones and other chaperone systems, including the HSP90 machinery (Sekhar, Rosenzweig, Bouvignies, & Kay, 2016). Indeed, dependent on chaperone co-chaperone interactions and wider chaperone networking HSP70 may act as a holdase, foldase or disaggregase as well as directing its clients to degradation by both the UPS (Kapustian et al., 2013) and autophagy-lysosome pathways (Fernandez-Fernandez et al., 2017) (Figure 3). In chaperone assisted UPS degradation HSP70 and HSP90 bound clients are ubiquitinated for sorting to the proteasome and subsequent degradation. This is mediated by CHIP, with a number of other co-chaperones, such as the J-domain protein DNAJB2/HSJ1 that acts to sort HSP70 clients to the proteasome (Westhoff, Chapple, van der Spuy, Hohfeld, & Cheetham, 2005). For chaperone mediated autophagy (CMA) (Karzai & McMacken, 1996), which is a specific lysosome-dependent degradation pathway, cytosolic proteins destined for degradation are delivered directly into the lumen of lysosomes through a mechanism which involves interaction with HSPA8 (the constitutively expressed HSP70) and lysosome associated-membrane protein type 2 (LAMP-2A). HSPA8 binds clients with a KFERQ-like domain facilitating their targeting to the transmembrane domain LAMP-2A which oligomerises to form a translocation complex taking the HSPA8 client into the lumen of the lysosome for degradation. There is evidence that other chaperones and co-chaperones play a role in regulating CMA as DNAJB1/HSP40, HOP, HIP and bag1 have been reported to complex with HSPA8 on the lysosomal membrane (Fernandez-Fernandez et al., 2017). HSP70 and its co-

chaperones also play roles in other forms of autophagy, including macroautophagy. For example, bag3 and HSP70 in conjunction with HSPB8 and the ubiquitin receptor p62/SQSTM1 target aggregation prone proteins for macroautophagic degradation through a mechanism that requires the sequestration of misfolded proteins to inclusion bodies (Sturner & Behl, 2017).

Impaired function of proteostasis networks is a common feature of neurodegenerative diseases (Klaips et al., 2018). This can occur through misfolded protein species interfering with the normal function of components of proteostasis networks. A key example is that specific oligomers of the proteins Aβ, α-synuclein and polyQ huntingtin (53Q), that aggregate in AD, PD and Huntington's disease respectively, have a common conformation that has been shown to inhibit the proteasome (Thibaudeau, Anderson, & Smith, 2018). This is through an allosteric mechanism where misfolded oligomers bind the proteasome and stop normal functioning of the substrate gate that regulates entry to the degradation chamber. Thus, in misfolding disease UPS can become compromised and/or overloaded, thereby promoting the special sequestration of ubiquitylated proteins to inclusion bodies. CMA may also be inhibited by misfolded proteins including acetylated tau, which is elevated in tau-mediated neurodegenerations (Min et al., 2010) and instead is cleared by other autophagic pathways (Caballero et al., 2021). The action of HSP70 in the clearance of potentially toxic protein species by targeting to UPS and autophagy-lysosome pathway is likely to reduce unwanted interactions between misfolded proteins and key cellular components such as transcription factors or other essential cellular proteins (Schaffar et al., 2004).

Despite redundancy and plasticity in proteostasis networks, maintaining the solubility and/or clearance of high levels of some aggregation-prone proteins places a burden on the total protein homeostasis machinery, leading to further disruption of proteostasis (Hipp et al., 2012; Schipper-Krom et al., 2012). Moreover, it is known that chaperone activity for HSP70, and other ATP-dependant chaperones, decrease during human brain ageing, which is consistent with prominent neurodegenerative disease being associated with old age (Brehme et al., 2014; Hipp, Kasturi, & Hartl, 2019). This suggests that levels of HSP70 chaperone activity may become limiting in age associated neurodegenerative diseases. It is also consistent with data supporting HSP70 is neuroprotective. Indeed, overexpression of HSP70 and its co chaperones is neuroprotective in multiple model systems supporting that chaperone activity may become limiting. This includes a reduction in Aβ plaque deposition in a mouse model of AD that was crossed with a mouse over expressing HSP70 (Hoshino et al., 2011). Modulation of the HSP70 machinery by altered co-chaperones expression has also shown to be neuroprotective. This include that the transgenic overexpression of DNAJB2a in the R6/2 mouse model of HD led to improved neurological performance, significantly reduced mutant huntingtin (mHtt) aggregation and enhanced solubility dependent on DNAJB2a client binding,

ubiquitin interaction and functional co-operation with HSP70 (Labbadia et al., 2012). Moreover, the overexpression of DNAJB2a in a mouse model of ALS was also shown to improve motor performance and the survival of motor neurons at the late stages of disease progression (Novoselov et al., 2013).

Cooperation of HSP70 and HSP90 chaperone machines and maintenance of proteostasis in neurodegenerative disease

HSP90, HSP70 and their respective co-chaperones, whilst acting as central hubs for protein folding and turnover, also function together in a multiprotein complex to drive the dynamic assembly and coordinate the stabilization of HSP90-client protein heterocomplexes (Moran Luengo, Mayer, & Rudiger, 2019). Substrate recognition is mediated by HSP70 and HSP40, with the substrate passed from HSP70 to HSP90 via HOP (Figure 2). Interestingly, a number of HSP90 client proteins are implicated in adult-onset neurodegenerative diseases, including AD, PD, and the polyQ expansion diseases (HD, spinal and bulbar muscular atrophy (SBMB), dentatorubral-pallidoluysian atrophy and the spinocerebellar ataxias). These HSP90 client proteins include tau (in AD), α-synuclein (in PD), Htt (in HD) and the AR (in SBMB) amongst others, all critical proteins known to unfold and aggregate in intracellular inclusions that are the characteristic hallmarks of these neurodegenerative diseases (Kuiper, de Mattos, Jardim, Kampinga, & Bergink, 2017; Mogk, Bukau, & Kampinga, 2018). HSP90 interacts with these metastable native or near-native clients to stabilize them against ubiquitin-mediated proteasomal degradation, thus facilitating their attainment of a functionally active conformation. However, in neurodegenerative diseases, the intrinsically unstable pro-aggregation segments of these client proteins can unfold to form soluble toxic oligomers before they aggregate into inclusions (M. Li, Chevalier-Larsen, Merry, & Diamond, 2007; Outeiro et al., 2008). Evidence points to the accumulation of these soluble oligomers into larger inclusions being neuroprotective, with the sequestration of the neurotoxic species away from the cellular milieu and into an insoluble compartment to limit the cellular damage (Arrasate et al., 2004; Cohen et al., 2006; Kaganovich et al., 2008; Kopito, 2000; Sontag et al., 2017; Taylor et al., 2003).

The quality control function of the HSP90/HSP70 chaperone machine regulates the turnover of aberrant HSP90 client proteins, with failure in this quality control function contributing to the aetiology of neurodegeneration (Balchin, Hayer-Hartl, & Hartl, 2016; Moran Luengo et al., 2019). When conformational instability of the native or near-native HSP90 client protein is extensive enough such that the client protein can no longer interact and cycle productively with HSP90, protein unfolding proceeds and the client protein is degraded by the proteasome, which is the major route of client protein degradation. Chaperones recognise the unfolding client protein as a substrate for proteasomal degradation, and direct the unfolded client protein to chaperone-dependent E3 ligases, such as CHIP (Connell et al., 2001a; Schipper-Krom et al., 2012). The E3 ligases in turn target a ubiquitin-charged E2 enzyme to the substrate. The N-terminal TPR domain of CHIP interacts with both HSP90 and HSPA8/HSP70 whilst the C-terminal U box interacts with the UBCH5 family of E2 ubiquitin conjugating enzymes (Cyr et al., 2002). Overexpression of CHIP has been shown to increase the ubiquitylation and proteasomal degradation of many HSP90 client proteins (Adachi et al., 2007; Al-Ramahi et al., 2006; Jana et al., 2005; Petrucelli et al., 2004; Sahara et al., 2005; Saidi et al., 2015; Shimura, Schwartz, Gygi, & Kosik, 2004; Shin, Klucken, Patterson, Hyman, & McLean, 2005; S. Zhang, Hu, Mao, Shi, & Xu, 2020). However, there is evidence that there is functional redundancy of CHIP with other E3 ligases, as some HSP90 client proteins, including polyQ expanded AR, are degraded at the same rate in CHIP-/- and CHIP+/+ cells (Morishima et al., 2008). Moreover, it has been shown that CHIP is functionally redundant with parkin, another HSP70-dependent ligase, against polyQ expanded ataxin-3 (Morishima et al., 2008; Tsai, Fishman, Thakor, & Oyler, 2003). However, CHIP is thought to play a key role in the chaperone-dependent ubiquitylation and proteasomal degradation of unfolded client proteins (Al-Ramahi et al., 2006; Dickey et al., 2007; Dickey et al., 2008; Jana et al., 2005; Kalia et al., 2011; Shimura et al., 2004; Shin et al., 2005; S. Zhang et al., 2020). Through this quality control function, the HSP90/HSP70 chaperone machinery plays an important role in triage decisions directing unfolded client proteins for proteasomal degradation. It has been proposed that the triage decision lies with HSP90, as once the unfolding of the client protein progresses to a state where it can no longer interact and cycle with HSP90, HSP70 mediated CHIP-dependent ubiquitylation and proteasomal degradation of the client protein is triggered and proceeds unopposed (Pratt, Gestwicki, Osawa, & Lieberman, 2015).

The model for HSP90/HSP70 coordination in protein quality control is supported by evidence *in vitro* and *in vivo* that show that inhibition of the HSP90 ATPase activity results in the rapid proteasome-dependent degradation of HSP90 client proteins. The ansamycin class of antibiotics (e.g. GA and herbimycin A) bind to the HSP90 nucleotide binding pocket and act as a nucleotide mimic, thus inhibiting the intrinsic ATPase activity, which is essential for

HSP90-client protein heterocomplex assembly (Roe et al., 1999) and preventing the cycling of the client protein with HSP90. HSP90 inhibition leads to the degradation of the soluble client protein thus preventing the formation of oligomers and aggregates, and the treatment of cell or animal models with HSP90 inhibitors has thus been shown to ameliorate neurotoxicity (Auluck, Meulener, & Bonini, 2005; Thomas et al., 2006; Tokui et al., 2009; Waza et al., 2005). For example, HSP90 inhibitors were shown to promote the proteasomal degradation of polyQ AR in cellular models of SBMA, and ameliorate polyglutamine-mediated motor neuron impairment in mouse models of SBMA (Thomas et al., 2006; Tokui et al., 2009; Waza et al., 2005). Moreover, GA was reported to reduce the formation of α-synuclein aggregates and α-synuclein-induced toxicity in a cell model of PD (McLean, Klucken, Shin, & Hyman, 2004). Interestingly, the treatment of cells with pre-existing inclusions did not reduce inclusion formation, confirming that HSP90 inhibition leads to the targeted degradation of the soluble client protein but not aggregated client.

One mechanism whereby HSP90 inhibition is thought to prevent the formation of protein aggregates and alleviate neurotoxicity in neurodegenerative disease models is via induction of the heat shock response (HSR) (Auluck & Bonini, 2002; Hay et al., 2004; Sittler et al., 2001). Inhibition of HSP90 ATPase activity prevents the cycling of HSP90 with heat shock factor 1 (HSF1), which consequently trimerizes and translocates to the nucleus where it upregulates the expression of chaperones including HSP70 and HSP40 (Kijima et al., 2018). The overexpression of HSP70 or its co-chaperone HSP40 in turn decreases the levels of aberrant proteins and is neuroprotective in models of neurodegenerative disorders, including PD, HD and SBMA (Adachi et al., 2003; Auluck, Chan, Trojanowski, Lee, & Bonini, 2002; Bailey, Andriola, Kampinga, & Merry, 2002; Jana, Tanaka, Wang, & Nukina, 2000; Klucken, Shin, Masliah, Hyman, & McLean, 2004; Kobayashi et al., 2000; Muchowski et al., 2000). Hence, it has been proposed that the activation or promotion of HSP70 and CHIP-dependent ubiquitylation could be beneficial in neurodegenerative disorders.

The HSP70 co-chaperone HIP stabilizes HSP70 in its ADP-bound conformation which recognizes unfolded substrates with high affinity, thus facilitating their HSP70-dependent ubiquitylation and degradation. It has been reported that HIP overexpression significantly reduced polyQ expanded AR inclusion formation in a primary neuronal model of SBMA, and promoted the CHIP-mediated ubiquitylation and proteasomal degradation of polyQ expanded AR thus reducing the formation of intranuclear inclusions (Howarth et al., 2009; Wang et al., 2013). HIP was moreover reported to mediate HSP70-dependent suppression of α-synuclein aggregation *in vitro* whilst its knockdown exacerbated α-synuclein aggregation in a *C Caenorhabditis elegans* model of inclusion formation (Roodveldt et al., 2009). YM-1 is a small molecule drug that selectively binds to the nucleotide binding domain of ADP-bound but not

ATP-bound HSP70, thus increasing the affinity of HSP70 for substrate proteins similar to HIP (Rousaki et al., 2011). YM-1 reduced oligomeric and aggregated polyQ AR but soluble polyQ AR was unaffected indicating the preferential targeting of unfolded client protein (Wang et al., 2013). Hence, the rational design of small molecules to stabilise the ADP-bound conformation of HSP70 is a focus of ongoing research to manipulate HSP90/HSP70 quality control in neurodegeneration.

There is also evidence that the protein quality control function of the HSP90/HSP70 chaperone machine *per se* is involved in the clearance of abnormal client proteins in the absence of induction of the HSR. For example, the inhibition of HSP90 by GA inhibited polyQ AR aggregation and promoted the proteasomal degradation of polyQ AR in HSF1-/- cells (Thomas et al., 2006). In this model, polyQ AR aggregates were cleared in the absence of an HSR highlighting the coordination between HSP90 and HSP70-mediated degradation in the quality control of this substrate. However, the role of the HSR versus HSP90/HSP70 quality control *per se* in target protein turnover has been more difficult to dissect for other HSP90 client proteins. Full-length Htt as well as full-length polyQ expanded mHtt are known HSP90 client proteins (Baldo et al., 2012). HSP90 inhibition induced the ubiquitylation and proteasomal degradation of mHtt in a manner that does not require upregulation of HSP70 through HSF1 activation (Baldo et al., 2012). However, Htt undergoes extensive N-terminal proteolytic cleavage and it is not clear whether full-length polyQ expanded mHtt or expanded proteolytic fragments thereof are the main culprits driving the toxic gain-of-function disease pathology. Many investigations of mHtt have been conducted with polyQ expanded exon 1 of Htt, and the R6/2 mouse model, which expresses polyQ expanded Htt exon 1, recapitulates many disease features of HD (Davies et al., 1997; Schilling et al., 1999). However, mHtt exon 1 fragments are not HSP90 client proteins and their proteasomal degradation is entirely due to the HSF1-mediated stress response. Therefore, both the HSR and direct HSP90/HSP70 quality control could play a role in abnormal client protein clearance in neurodegenerative diseases.

Conclusions

The molecular chaperones and their associated co-chaperones are of central importance to protein function from facilitating folding, transport and translocation, through functional maturation to the clearance of misfolded and aggregated species via the UPS, autophagy or lysosomal pathways. Failure of chaperones to fulfil these vital roles may ultimately contribute to a number of devastating human diseases, including neurodegenerative diseases. The coordinated activities of the HSP90 and HSP70 chaperone machineries and their respective co-chaperone networks are particularly important in the quality control of HSP90 client proteins

implicated in the aetiology of neurodegeneration and other diseases. Therefore, the central importance of these molecular chaperones and their associated co-chaperones makes them a prime target for pharmacological intervention for the treatment of these diseases. In particular, targeting the quality control function of the HSP90/HSP70 chaperone machine with rationally designed small molecules might be a therapeutically valid approach to promote the degradation of critical unfolded proteins and ameliorate toxicity in neurodegenerative diseases.

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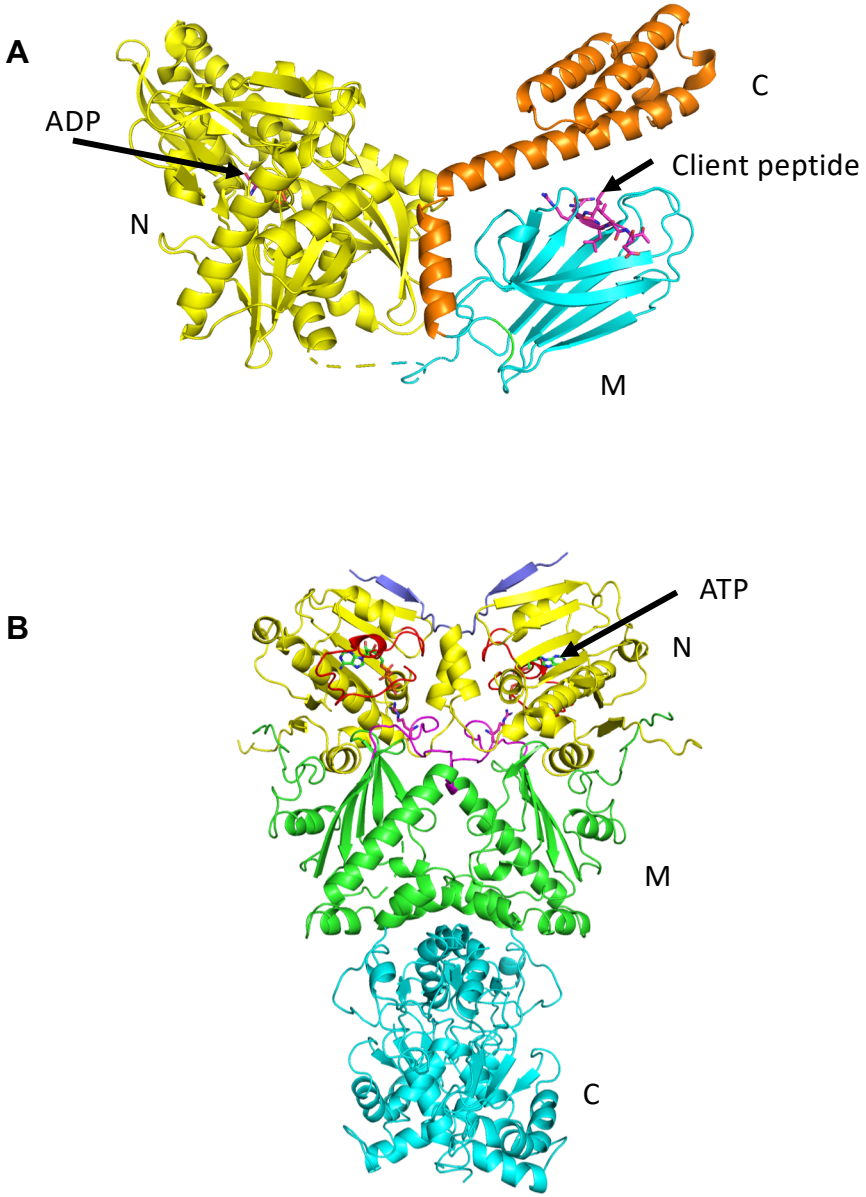
Figure Legends

Figure 1. Structural domains of Hsp70 and Hsp90. A) Hsp70 composite model from pdb structures 3FZF and 1DKX, showing the substrate bound conformation of Hsp70. Yellow, N-terminal ATP binding domain; cyan, substrate binding domain and gold, substrate binding domain helical lid. **B)** PDB 2CG9 structure of Hsp90. Yellow, N-terminal ATP binding domain with ATP bound and shown as green coloured sticks; green, middle domain; cyan, C-terminal domain; blue, fragment of structure involved in the b-strand exchange during N-terminal dimerization, magenta, loop carrying the catalytic arginine residue (shown as magenta sticks) and red, the ATP-lid. N, N-terminal domain, M, middle domain and C, C-terminal domain.

Figure 2. Proteostasis by the Hsp70 and Hsp90 chaperone systems. Unfolded or unmature client proteins are either folded or activated by Hsp70 or Hsp90 chaperones as required. If required by the client protein, HOP acts as a bridge allowing the transfer of the client to Hsp90. Co-chaperones for the Hsp70 and Hsp90 systems are also shown, which aid the chaperone cycle. CHIP can access both chaperone systems to initiate protein degradation by ubiquitylation of client proteins that have stalled in the folding cycle.

Figure 3. Molecular chaperones in protein misfolding and aggregation. Molecular chaperones and their associated co-chaperones are essential in the cellular defences against protein aggregation. Molecular chaperone networks participate in protein folding and refolding, proteasome-dependent degradation, and inclusion formation and lysosome-mediated autophagy.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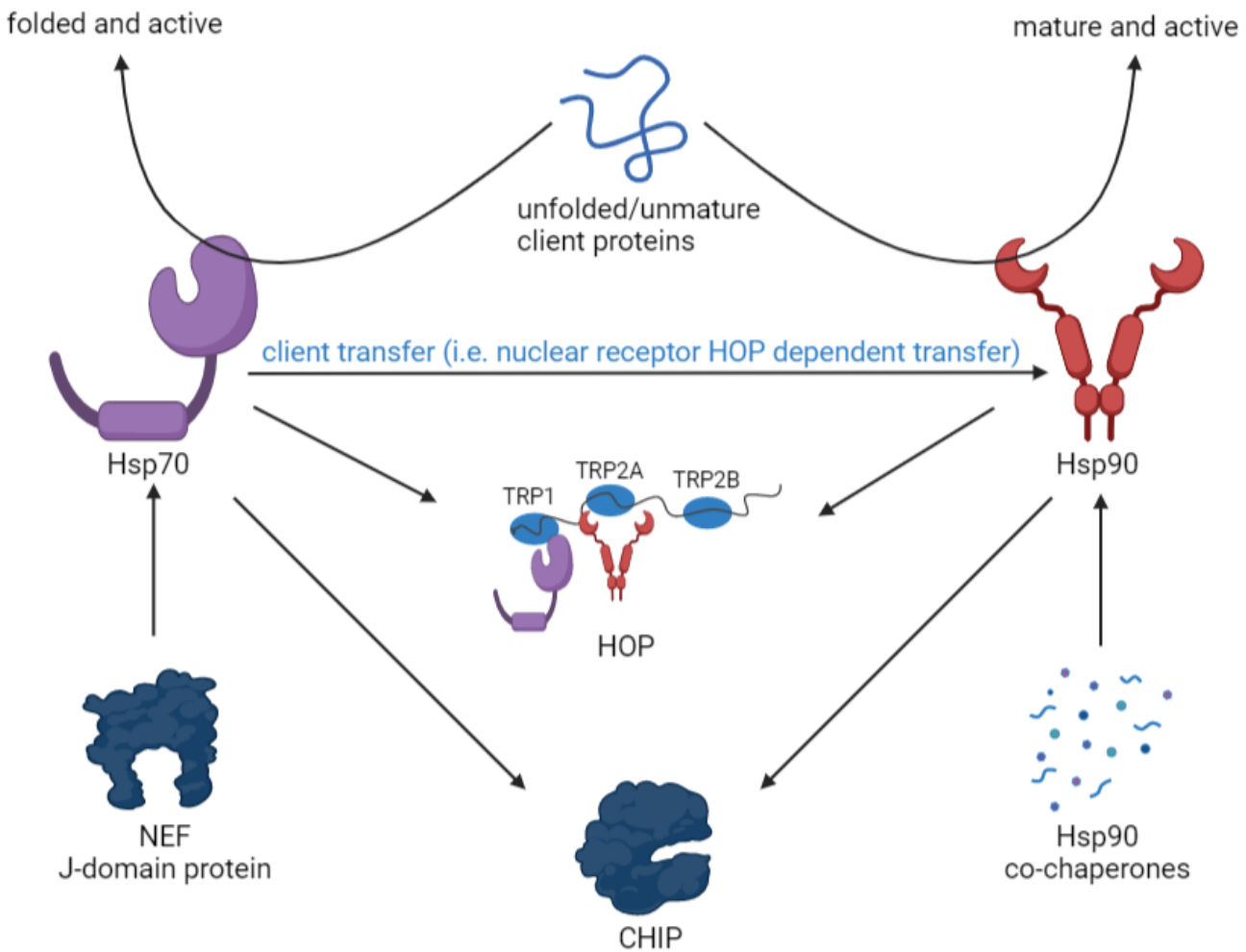
folded and active
mature and active
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client proteins
client transfer (i.e. nuclear receptor HOP dependent transfer)
Hsp70
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TRP1
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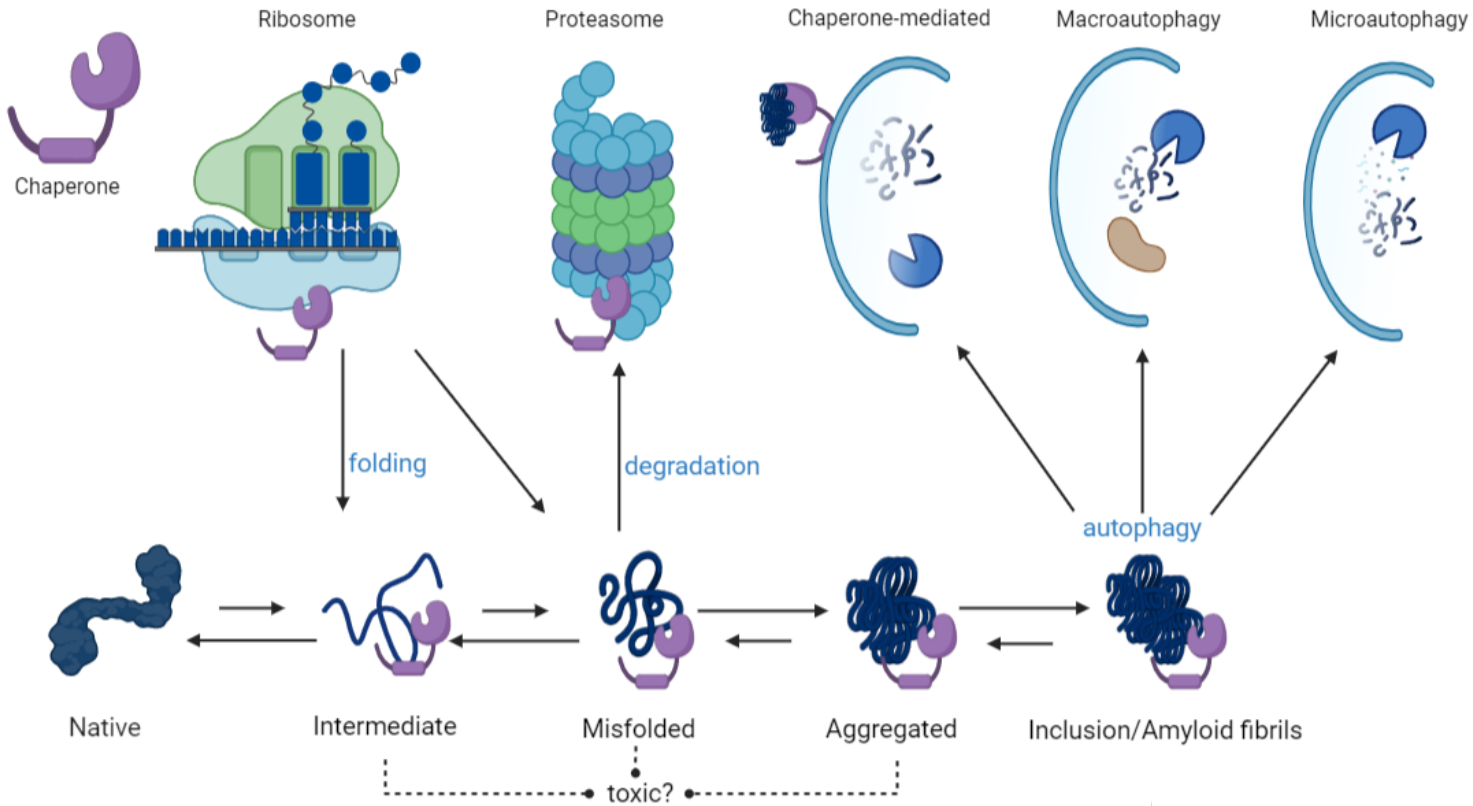
HOP
NEF
J-domain protein
CHIP
Hsp90
co-chaperones


Chaperone
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