CHAPTER 3

Hop:
An Hsp70/Hsp90 Co-Chaperone That Functions Within and Beyond Hsp70/Hsp90 Protein Folding Pathways

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Abstract

Molecular chaperones and their co-chaperones are crucial for the facilitation of efficient protein folding, and prevention of denaturation and aggregation of nascent polypeptides. Hsp70/Hsp90 organizing protein (Hop), a co-chaperone of the two major molecular chaperones, heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90), facilitates their interaction by acting as an adaptor between the two chaperones, so that substrate is efficiently transferred from Hsp70 to Hsp90. Although initial studies reported its scaffolding properties to be its primary function, recent findings suggest an additional modulatory effect of Hop on the activities of Hsp70 and Hsp90. In addition, a more diverse role of Hop, involving structurally and functionally unrelated biomolecules and complexes, is currently being revealed. This review focuses on the integratory and modulatory effects of Hop on the Hsp70 and Hsp90 protein folding pathways, and puts forward evidence and theories regarding its multifaceted roles within various biological systems.

Introduction

The efficient folding of polypeptides is extremely challenging within the complex cellular environment due to various reasons, including proteotoxic conditions such as heat stress, anoxia, exposure to heavy metals or other chemical agents. The assistance of molecular chaperones, a group of proteins that are adapted to facilitate protein folding, has thus proven to be critical in this regard. Molecular chaperones are known to interact reversibly with nascent polypeptide chains in an attempt to reduce inappropriate interactions that can otherwise lead to poorly reversible conformations and aggregations.[1] Heat shock proteins (Hsps) are a group of cytoprotective proteins synthesized in response to various kinds of cell stress, and they form the central components of the molecular chaperone machinery.[2] They protect functional proteins from irreversible denaturation as well as assist them in renaturation. Two of the most studied heat shock protein families are Hsp70, a structurally conserved protein with a role in the survival of the organism and Hsp90, one of the most abundant cytosolic proteins in eukaryotes, essential for its viability.[3]

Hsp70, found in eukaryotes, eubacteria and many archaea, is primarily involved in protecting proteins against misfolding and aggregation within the cell's overcrowded environment.[4] It

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is well known for its role in recognizing, binding and stabilizing unfolded proteins, translocation of newly synthesized proteins, protein degradation and protection of the cell against the effects of cellular stress.[5-7] The Hsp70 family is composed of four major members: cytosolic constitutive heat shock cognate 70 (Hsc70), cytosolic inducible heat shock protein 70 (Hsp70), endoplasmic reticulum Hsp70 which is also known as the immunoglobulin heavy-chain binding protein (BiP), and mitochondrial Hsp70 (mtHsp70).[8]

The chaperone activity of Hsp70 is regulated by co-chaperones, which usually act by modifying the ATPase cycle of Hsp70. Hsp70 in the ATP bound state has a lower affinity for substrates than the ADP bound state. ATP hydrolysis converts Hsp70 to its higher affinity substrate-binding state and subsequent nucleotide exchange allows for substrate release and return of Hsp70 to its lower affinity state.[9] Hsp40 proteins are well-known co-chaperones of Hsp70, regulating the activity of Hsp70 by stimulating its ATP hydrolysis step (see Chapter by Rosser and Cyr). GrpE is an additional co-chaperone of prokaryotic Hsp70 that acts by stimulating nucleotide exchange thereby enhancing the basal ATPase activity of Hsp70 up to 50 times.[10] A functional equivalent of GrpE in eukaryotes, Bag-1 (Bcl2-associated anthanogene), also regulates Hsp70 nucleotide exchange in a similar manner to GrpE (see Chapter by Brodsky and Bracher).[11] In eukaroyotes, Hsp70-interacting protein (Hip) stimulates the assembly of the Hsp70-Hsp40-substrate complex and stabilizes the ADP-bound form of Hsp70 so that the unfolded polypeptide has more time to attain its proper conformation before being released from the chaperone complex. Once released, the polypeptide either folds to its native state, or is passed on to other molecular chaperones, which include the Hsp90 chaperone machinery.[12]

Hsp90 is a ubiquitous and abundant cytosolic molecular chaperone that is conserved from bacteria to mammals.[13,14] It plays a variety of roles in processes such as protein restoration, protein degradation, signaling, cytoplasmic organization, nuclear transport, DNA rearrangements, DNA-protein interactions, the cell cycle and apoptosis. Hsp90 interacts with a diverse range of proteins (referred to as client and/or substrate proteins) and ensures the folding and maturation of these molecules, which includes steroid receptors, phosphatases, protein kinases and other signaling intermediates of the mitogenic signal transduction pathway.[15,16] In vitro experiments have shown binding and anti-aggregation properties of purified Hsp90 to denatured protein, however a cohort of co-chaperones, which form several subcomplexes with Hsp90, are necessary for it to carry out its functions in vivo. These co-chaperones enable Hsp90 to attend to such a versatile range of client proteins.[17,18] Some of these co-chaperones also interact directly with Hsp90 substrates, as well as display chaperone activity on their own.[19]

Hsp90 contains two ATP binding sites within the N and C- terminal domains and ATP hydrolysis is of crucial importance for Hsp90 functioning in vivo.[17,20-23] The ADP-bound form of Hsp90 is described as "relaxed" and therefore ideal for client protein loading, whereas the ATP-bound form of Hsp90 is described as a "closed" conformation, which is capable of tightly retaining the substrate.[22] Conversion of the ATP state of Hsp90 to its ADP form allows for the efficient release of substrate,[21,24] and this is in stark contrast to the ATP regulated substrate-binding cycle of Hsp70. The benzoquinone ansamycin antibiotic geldanamycin blocks this cycle by maintaining Hsp90 in an ADP-bound state, thereby acting as a specific inhibitor of Hsp90.[17] The Hch1/Aha1 proteins have been identified as Hsp90 co-chaperones, accelerating the ATPase activity of yeast Hsp90 to 12 times its basal level.[25] Cdc37 (p50) is an inhibitor of the Hsp90 ATPase activity, and this suppression is restored to normal levels when Cdc37/p50 is displaced by Cpr6.[24]

Although both Hsp70 and Hsp90 protein folding systems act independently of each other and on different substrates, some protein substrates are processed by Hsp70 and then transferred to Hsp90. The collaboration between the two major chaperone machineries, Hsp70 and Hsp90, is coordinated by a number of co-chaperones. This review will focus on Hop, the Hsp70/Hsp90 organizing protein, which is a unique co-chaperone that interacts with both Hsp70 and Hsp90, bringing them together in a molecular chaperone complex.

Hop (Hsp70/Hsp90 Organizing Protein)

The 60-kDa protein Hop, was first identified by Nicolet and Craig[26] during a genetic screen for proteins that were involved in the heat shock response in yeast. Hop has been found to associate with Hsp70 and Hsp90 within intermediate steroid receptor complexes and appears to be essential for the in vitro assembly of steroid receptors with Hsp90.[27,28] Homologues of Hop have also been identified in humans,[29] mice,[30] rats,[31] insects,[32] plants,[33] and parasites[34] and are classified as belonging to the stress-inducible protein 1 (STI1) family.[26] In this review, Hop will refer to the protein of mammalian origin, and that of a specific species such as yeast or mouse, will be designated with the first letter of the species next to Hop, eg. yHop (yeast Hop) and mHop (mouse Hop).

The presence of nine tetratricopeptide repeat (TPR) motifs structurally defines homologues of Hop, in which the TPR motifs are grouped into three domains, each comprising three TPRs (Fig. 1A). TPRs are protein-protein interaction modules, characterized by a loose, 34-amino acid consensus motif that is found in varying numbers of tandem repeats.[35] The N terminal TPR domain of Hop (TPR1) is required for Hsp70 binding[36] and a central TPR motif-containing region (TPR2A, Fig. 1A,B) is essential for Hsp90 binding.[37,38] The TPR-acceptor site on both Hsp70 and Hsp90 is comprised of an EEVD motif on the C-terminus.[38,39] It is also possible that there are networks of interactions between Hop and the chaperones Hsp70 and Hsp90, apart from those mediated by the TPR domains, which allow for its functionality as a scaffolding protein.[38]

Hop possesses insignificant chaperoning capabilities[40] and despite an increase in mRNA levels of mHop in mouse cells there is no change in the steady state levels of this protein following heat shock.[41] A similar occurrence has been described for human Hop (hHop) upon viral transformation.[29] Hop appears to be regulated between a monomeric and dimeric state, interacting with the dimeric Hsp90 as a dimer while associating with Hsp70 as a monomer.[42]

Initial studies of Hop focused on its role as an adaptor between Hsp70 and Hsp90 (Fig. 2) and the functioning of this multi-chaperone complex in steroid receptor (SR) regulation. SRs comprise of soluble intracellular proteins, which shuttle between the cytosol and the nucleus. They exist in an inactive or nontransformed state in the absence of their particular steroid hormone. Diffusion of the appropriate hormone into the cell transforms the receptor into an active transcription factor, which is capable of activating or repressing the expression of the steroid response genes.[43] The assembly of the progesterone receptor (PR) and the glucocorticoid receptor (GR) requires the participation of Hsp70, which brings the substrate protein into contact with Hsp90 via the scaffolding function of Hop. In the initial stage of the models proposed by both the Smith and Toft groups[42,44-46] Hsp40 binds to free SR, and facilitates the binding of the SR to Hsp70 through modulation of the ATPase cycle of Hsp70. Hip stabilizes this complex formation. Hsp70 then interacts with Hop, which is already in complex with Hsp90, and in this way, allows for the SR to come into contact with Hsp90. Hop is thus able to act as a "bridge" between the two major Hsps. This complex is generally referred to as the "intermediate complex".[46] Recent reports demonstrate the need for both TPR1 and TPR2 domains of yHop to be present on the same polypeptide, in order to maintain regulation of steroid receptor activation by Hsp70 and Hsp90.[47]

The intermediate step is followed by the release of Hop, Hsp70 and its co-chaperones Hsp40 and Hip and the formation of a mature complex that is stabilized by the presence of p23 and one of its TPR-containing immunophilins (immunophilins are a group of proteins which bind to immunosuppressive ligands; see Chapter by Cox and Smith). The result is a high affinity hormone binding conformation of the receptor. Hormone binding to the receptors releases them from Hsp90, and in the absence of bound hormone, dissociated receptor subunits reassociate with Hsp70 and proceed through the cycle again.[1,48]

Hop Modulates the Activities of Hsp70 and Hsp90

Some studies have suggested that Hop may change its conformation during the assembly of the Hsp70-Hop-Hsp90 chaperone heterocomplex, due to the fact that the affinity and

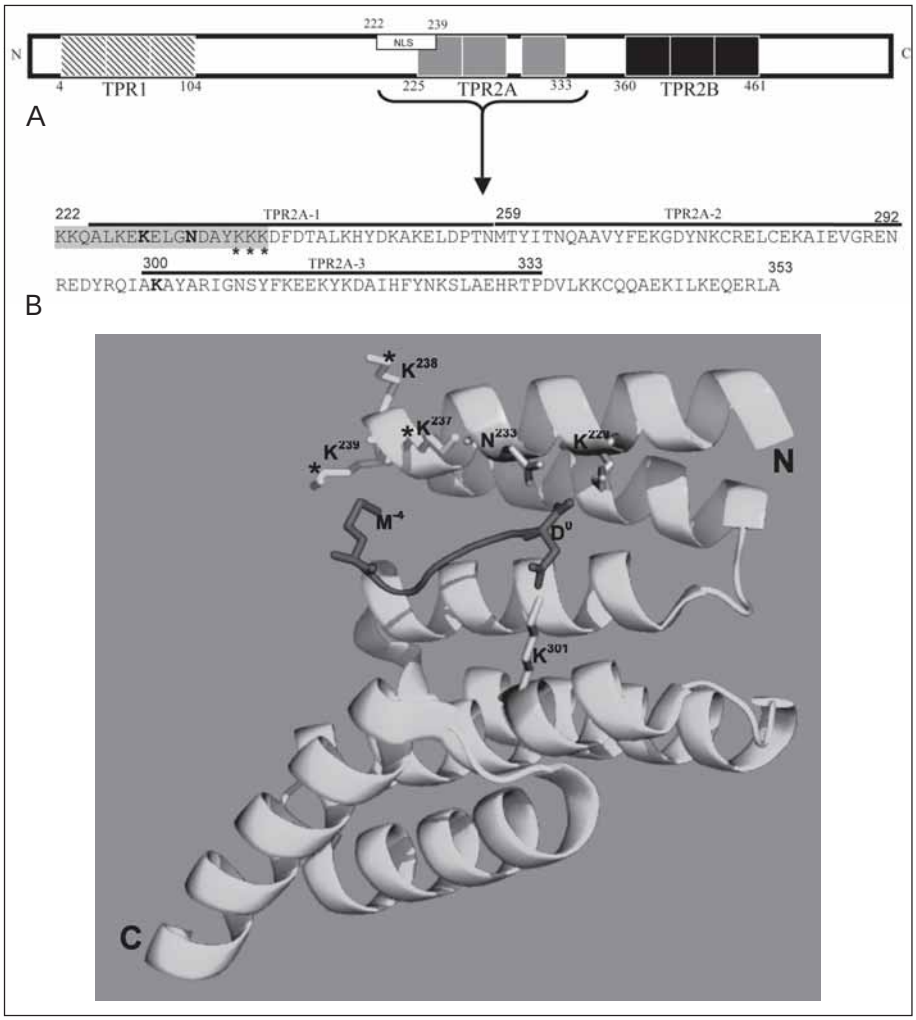

Figure 1. The Hop TPR2A domain overlaps with a putative NLS. A) Schematic diagram showing the TPR domain organization of Hop. The three TPR motifs which form part of the TPR1 domain are denoted by boxes with diagonal patterns, while those that form TPR2A and TPR2B domains are depicted as grey and black boxes, respectively. A solid white rectangle denotes the proposed NLS domain, which overlaps with TPR2A. TPR2A has been further enlarged diagrammatically, to show the amino acid sequence of this domain and a flanking C-terminal helix. TPR2A comprises of three TPR motifs (residues are shown by a thick black overline) denoted as TPR2A-1, TPR2A-2 and TPR2A-3.[80] The residues against a gray background are those of the proposed bipartite NLS[70,71] and which overlap with TPR2A-1 motif. The major arm of the bipartite NLS is denoted by an asterisk under each residue. The residues shown in bold, K229, N233 and K301 have been shown to be important for Hsp90 binding.[38] B) Ribbon representation of the structure of the TPR2A domain of hHop. The TPR2A domain and a flanking C-terminal helix (green) are shown interacting with the C-terminal MEEVD peptide (red) of Hsp90 (Protein Database code: 1ELR).[80] Residues shown in gold are those that are important for interaction with Hsp90 (refer to A). Residues denoted with an asterisk correspond to those that form the major arm of the bipartite NLS, which overlaps with the TPR2A domain. The figure was generated using Pymol Molecular Graphics Software (http://pymol.sourceforge.net).[81] A color version of this figure is available online at www.eurekah.com.

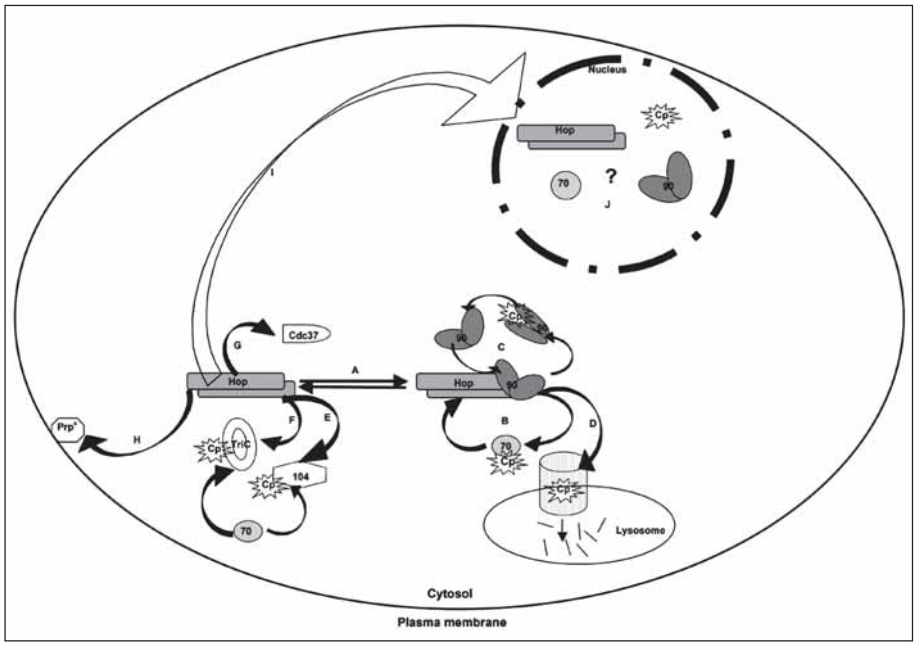

Figure 2. A summary of known cellular functions of Hop. Hop appears to exist either in the bound form to Hsp90, or as free Hop (A). B) reflects a simplistic view of its function as a scaffolding protein in bringing Hsp70 with the client protein into contact with Hsp90. Once the client protein is transferred onto Hsp90, Hop and Hsp70 dissociate, and the refolded client protein is then released, freeing Hsp90 (C). The Hsp70-Hop-Hsp90 chaperone complex is also involved in chaperone-mediated autophagy (D). Free Hop in yeast, interacts directly with Hsp104 (E), which is a stress tolerance factor that acts in concert with Hsp40 and Hsp70 to reactivate denatured proteins. Hop also interacts directly with TRiC (F), which is otherwise involved together with Hsp70, in the refolding of certain specific client proteins. Yeast Hop interacting with Cdc37 (G), a molecular chaperone and co-chaperone of Hsp90, appears to be important for Cdc37 to enter the chaperone dependent-folding pathway. Mouse Hop binds to Prp^c both in vitro and in vivo, and this interaction was found to transduce neuroprotective signals (H). Hop has been speculated to shuttle bi-directionally between the nucleus and cytoplasm (I). It is now known that both Hsp70 and Hsp90 also move into the nucleus during specific conditions. The nuclear role of Hop within its co-chaperoning context remains to be elucidated (J). Although Hop is generally depicted as a dimer, it must be noted that the stoichiometry of Hop in some of these interactions has not been determined. Heat shock proteins are identified by their molecular mass stated as numbers. CP stands for client protein. A color version of this figure is available online at www.eurekah.com.

stoichiometry of Hsp70-Hop binding is dramatically affected by binding of Hsp90. Although Hsp70 binds to Hop with a relatively lower affinity than Hsp90, this affinity is increased five fold in the presence of Hsp90.[42] Hsp90 may thus be altering the conformation of Hop to one that better accommodates interactions of Hop with Hsp70. Another possibility is that Hop binding to Hsp90 may open up a new conformation of Hsp90 that provides contact sites for Hsp70 binding.[42,49]

Despite findings that show that hHop and yHop are capable of functionally complementing for each other,[45,50] the involvement of Hop within the chaperone machineries of mammalian and yeast cells have shown some differences. Practically all of the yHop protein exists in a complex with Hsp90.[42,51] The basic elements of the Hsp90 chaperone complex in yeast are

similar to that of vertebrates;[51] however it has been suggested that yHop is not essential for mediating associations between Hsp70, Hsp90 and target proteins in yeast.[52] This is in contrast to the mammalian system in which it has been shown that Hop is necessary for efficient assembly of steroid receptor-Hsp90 complexes in vitro[27,28,38] and that Hop is essential in integrating Hsp70-Hsp90 interactions.[45,47]

Morishima et al[53] reported that Hop, rather than being essential for GR folding by the Hsp90 based chaperone system, enhances the rate of this phenomenon. Furthermore, the same authors have shown that the protein levels of Hop were increased in geldanamycin-blocked GR complexes of Hsp90 compared to GR-Hsp90 complexes in the absence of geldanamycin. This may be due to the fact that geldanamycin-inhibited Hsp90 is in an ADP-bound conformation, which has higher affinity for Hop than the ATP-conformation.[53,54] In a Hop-depleted system, GR that was incubated with Hsp90 inhibitor geldanamycin, displayed little or no association with Hsp90, whereas the same system showed a more stable GR-Hsp90 association in the presence of Hop.[53] The same authors reported that the effect of Hop on GR-Hsp90 interactions in the presence of geldanamycin could either be due to the stable retention of Hsp90 by Hop, or due to some kind of influence that Hop may be exerting on the geldanamycin-Hsp90 conformation such that its affinity for GR is increased. Exclusion of Hop results in the reduced activity, but not accumulation, of two structurally and functionally unrelated Hsp90 client proteins, the steroid receptor GR and the oncogenic tyrosine kinase v-Src. The exclusion of Hop did not, however, have an effect on the activity of c-Src which is a protein closely related to v-Src but less dependent on Hsp90. This suggests that Hop is an important factor in promoting the maturation of Hsp90 client proteins.[52]

In the human system, hHop has no effect on Hsp70's ATPase activity, alone or in combination with Hsp90.[37] hHop does not affect Hsp90's ATPase cycle in the human system either, although it is capable of inhibiting client protein-stimulated ATPase activity of Hsp90.[55] yHop however, in direct contrast, stimulates ATP hydrolysis of Hsp70, enhancing its ATPase activity by a factor of 200 and is a noncompetitive inhibitor of Hsp90's ATPase activity.[56,57] Studies conducted by Wegele et al [56] showed that yHop is capable of accelerating ATP hydrolysis of Hsp70 to a greater extent than any other stimulation factor including yeast Hsp40. Even if yHop was added to a preformed Hsp70-Hsp40 complex, it was still able to activate the ATPase activity of Hsp70 and moreover, yeast Hsp40 was unable to replace yHop in a preformed Hsp70-Hop complex. Binding of yHop to Hsp90 and Hsp70 allowed activation of Hsp70 ATPase and inhibition of Hsp90 ATPase at the same time.[56]

The ATPase inhibition of Hsp90 by yHop is achieved by restricting N-terminal dimerization, which is a necessary conformational change in Hsp90 for ATP hydrolysis. This was confirmed by studies demonstrating a Hop binding site in the N-terminal region of Hsp90, in addition to the already characterized C-terminal peptide region that interacts with the TPR2A domain of Hop.[57] This Hop-mediated suppression of ATP turnover by Hsp90, is the motivation underpinning the postulation that Hop is involved in preparing Hsp90 for fresh "loading" of substrate protein.[24,58]

The Hsp70-Hop-Hsp90 multi-chaperone machinery is also involved in a process called "chaperone-mediated autophagy", one that targets cytosolic proteins to the lysosomes for degradation in response to stress conditions such as prolonged starvation or serum withdrawal (Fig. 2).[59] Protein substrates have to become unfolded in order to be transported into the lysosomal lumen. Hop is speculated to be part of the strategy employed to stabilize the lysosomal Hsp70-substrate complex on the lysosomal surface in such a way that it allows for the complete unfolding of the substrate protein before import into the lysosome.[59]

A recent report by Song and Masison [47] clearly demonstrates impairment of an Hsp70-dependent chaperone pathway upon deletion of the TPR1 domain in yHop, as well as an impaired Hsp90-dependent chaperone pathway upon deletion of the TPR2 domain of yHop. Deletion of TPR1 did not affect Hsp90-dependent client protein activity, and deletion of TPR2 also had no adverse effect on Hsp70-dependent client protein activity. These

deletions, however, impaired client protein folding pathways that involved both Hsp70 and Hsp90.[47] This strongly indicates that Hop regulates Hsp70 and Hsp90 chaperone pathways independently, as well as concurrently. These findings strengthen the potential role of Hop as an active modulator of the functions of both Hsp70 and Hsp90, in addition to its passive role of serving as an "adaptor" between these two chaperone machineries.

Hop Interactions Go Beyond Hsp70 and Hsp90

Hsp70-Hop-Hsp90 interactions are the most well characterized TPR-mediated interactions of Hop, however protein-protein contacts through this domain are not exclusive to the Hsp70-Hsp90 multi-chaperone complex. Studies done with *Saccharomyces cerevisiae*, show that in the presence of nonfermentable carbon sources like ethanol and glycerol, yHop (in addition to other Hsp90 co-chaperones, Cpr7 and Cns1) interacts with Hsp104 through its N-terminal TPR1 domain (Fig. 2) and this interaction has been shown to be independent of Hsp90.[19] Hsp104 is a stress tolerance factor, which acts in concert with Hsp40 and Hsp70 to reactivate denatured proteins.[60]

yHop directly interacts with Cdc37 (Fig. 2), a co-chaperone of Hsp90, and this interaction is speculated to occur via both TPR1 and TPR2 domains of Hop but possibly not on the same binding sites as those involved in binding of Hop to both Hsp70 and Hsp90.[61] Recent work by Harst et al[62] confirmed this interaction with the mammalian homologue of Cdc37, p50, and suggested the presence of a complex that comprises of Hsp90, yHop and p50, in which one of the Hsp90 cofactors acted as the central component. yHop is not essential for growth of yeast cells at 30°C but growth impairment occurs at higher and lower temperatures or in the presence of minimal media.[26,27] Interestingly however, the combination of Cdc37 and yHop mutations is synthetically lethal to yeast under normal conditions, implying that their interaction may contribute to the vital functioning of yeast.[61] On the basis of Cdc37 being a molecular chaperone (see Chapter by Caplan), as well as the findings that prevention of aggregation of polypeptides and folding of protein kinases require the presence of Cdc37 as a co-chaperone to Hsp90, Lee et al[63] have speculated that yHop interaction with Cdc37 may be crucial for Cdc37 to enter the chaperone dependent-folding pathway.

Hop has also shown a direct interaction with the eukaryotic chaperonin-containing TCP1 (CCT), also known as the TCP-1 ring complex (TriC), which seems to be involved in the proper folding of actins and tubulins.[64] This is diagrammatically represented in Figure 2. While Hop showed no effect on the ATPase activity of CCT, it significantly stimulated nucleotide exchange, thereby interfering with substrate-associative capabilities of CCT. CCT cooperates with Hsp70 in refolding of luciferase in vitro, and this phenomenon is proposed to occur in vivo for certain substrates after translation or after stress-induced damage. The interaction of Hop with CCT was mediated through its C-terminal domain, in contrast to its interaction with Hsp70, which is mainly through its N-terminal domain, and this is consistent with the observation that the presence of Hsp70 did not affect Hop-CCT interactions.[64]

Hop contains, in addition to its TPR domains, two smaller domains with characteristic DP repeat motifs comprising four amino acid residues, reflected in an arrangement that corresponds to TPR1-DP1-TPR2A-TPR2B-DP2.[65,66] Recent comparison studies on hHop, yHop and *Drosophila melanogaster* Hop which lacks DP1 (dHop), showed that dHop cannot support GR function in yeast, although it can still bind to both Hsp70 and Hsp90, and can complement for, and thus rescue, growth defects in yeast which lack yHop.[66] Disruption of DP2 abrogates Hsp70 binding (implying an interaction between DP2 and TPR1). The substitution of DP2 of hHop by DP2 from dHop does not affect Hsp70 binding although it fails to support GR activity. A substitution of DP2 from dHop with DP2 from hHop regains the ability to enhance GR activity. It is possible therefore, that the DP2 domains may be responsible for an additional function of Hop to enhance GR activity, besides its Hsp70 binding capabilities. Carrigan et al[66] have thus proposed a novel role for Hop in GR maturation in vivo, which is independent of Hsp70/Hsp90 binding and showed using chimeric studies, that DP2 is critical for this "new" role of Hop.

Another novel role of Hop, in neuroprotection, was proposed by Martins' group.[67] Recombinant mHop was found to bind to Prp^c (a protein whose expression is crucial to the propagation of neurological disease, in particular, Prion disease) both in vitro and in vivo. The interaction of Prp^c with mHop was found to transduce neuroprotective signals (Fig. 2). A number of other molecules have shown in vitro association with Prp^c such as Hsp60, BiP, Bcl-2 and a 37/67 kDa laminin receptor,[68] but physiological relevance in the form of neuroprotection has only been attributed to the Prp^c – laminin complex. The laminin receptor-binding site on the Prp^c molecule maps to a region of amino acids that is significantly distinct from that of the mHop-binding domain. mHop may therefore participate within a Prp^c – laminin complex wherein association of Prp^c with both molecules may supply an additive effect.[67]

Subcellular Localization of Hop Affects Its Activities

The subcellular localization of Hop has definite implications on its various functions, both within, as well as outside the context of the Hsp70/Hsp90 chaperone heterocomplex. hHop has been found in the Golgi apparatus and small vesicles in normal cells, and nucleolar localization of hHop has been described in SV40-transformed cells.[29] In contrast, mHop has been described as being predominantly cytoplasmic with a small percentage of it being identified in the nucleus.[41] Nuclear Hop has been shown to be a crucial component of the OCA-S complex, involved in the regulation of S-phase dependent Histone H2B transcription.[69] An investigation into mHop's subcellular localization by Blatch's group described a predominantly nuclear accumulation of mHop under conditions of G1/S arrest or leptomycin B treatment in mouse fibroblast cells, leading to the proposal that there is a constant shuttling of the protein between nucleus and cytosol, with the export of mHop from the nucleus occurring at a faster rate than its import.[70] A proposed nuclear localization signal (NLS) in mHop, when fused to EGFP (enhanced green fluorescent protein), resulted in the localization of EGFP within the nucleus, suggesting that this NLS was functional in mHop.[70]

Interestingly, the proposed NLS overlaps with the TPR2A domain, which modulates interactions between Hop and Hsp90. Figure 1A shows a partial amino acid sequence of Hop, demonstrating the overlap of the proposed NLS with the TPR2A domain. The NLS, by virtue of its proximity relative to the TPR2A domain, may therefore contain residues involved in Hsp90 binding. A three dimensional representation of the crystal structure of TPR2A interacting with the pentapeptide MEEVD peptide of Hsp90 is presented in Figure 1B, displaying the proximity of the NLS relative to the sites on TPR2A involved in binding to Hsp90. Interactions of Hop with Hsp90, may therefore be involved in the mechanism of the nuclear localization of Hop. A possibility is that Hop binds a nuclear import factor like importin α and Hsp90 alternately and each interaction mediates nuclear import or cytosolic retention, respectively. Binding of Hsp90 to the TPR2A domain may mask the NLS preventing interactions with importin α and thus retaining Hop within the cytosol.

Acidic isoforms of Hop were elevated after viral transformation[29] and heat shock,[41] suggesting that this protein is phosphorylated during stress. There is evidence for the in vitro phosphorylation of mHop by casein kinase II (CKII; S189) and cdc2 kinase (T198) at sites located upstream of a putative NLS.[71] Furthermore, there is evidence that phosphorylation of Hop at these sites regulates its localization.[70] The postulation is that phosphorylation of mHop by CKII or cdc2 kinase promotes the nuclear import or cytosolic retention of mHop, respectively. It is therefore possible that phosphorylation of mHop at either or both of these sites may affect its interactions with Hsp90, thereby regulating the assembly of the Hsp70-Hsp90 chaperone heterocomplex.[72]

In light of Hop's subcellular localization and its interaction with Prp^c, it is interesting to note that wild-type Prp^c is not detected in the cytoplasm and is localized predominantly on cell surfaces or synaptosomal fractions.[67,73] Martins et al[74] reported the presence of a small fraction of the Prp^c ligand, which was unknown at the time, at the cell surface. Using membrane preparations from mouse brain, the same authors have shown through immunoprecipitation that at

least part of mHop, which is postulated to be that "unknown" Prp^c-ligand, is localized at the cell surface, where it interacts with Prp^c.[67] It has been proposed that mHop is taken into the cell membrane as part of a protein complex, or secreted by a pathway that is distinct from the classical route through the ER and Golgi apparatus[75] due to the absence of a transmembrane domain or signal peptide for membrane transport within the amino acid sequence of mHop.[41,67]

Conclusion

Although the function of Hop has conventionally been restricted to that of Hsp70/Hsp90 organizing protein, it is apparent now that even within this system Hop has an active role to play as a modulator of their chaperone activities and protein folding pathways (Fig. 2). The presence of its various isoforms, the strong evidence for post-translational phosphorylation, a varied subcellular localization pattern and the possibility that its localization may be linked to post-translational modifications, strongly suggests complex roles for Hop in different systems and under different cellular conditions.

Members of the Hsp70 family migrate to the nucleus particularly during heat shock, where they are involved in stress-related cytoprotection. Hsp70 is capable of translocating nuclear proteins into the nucleolus during stress, possibly to prevent the random aggregation of thermolabile proteins within the nucleus and thereby preventing damage to other nuclear components.[76] Hsp70 is also known to regulate the activity of certain nuclear DNA-binding transcription factors.[77] Although Hsp90 is predominantly cytosolic, it is also known to translocate into the nucleus and associate with nuclear membranes, under conditions of stress,[15,78,79] thereby maintaining the integrity of the nuclear envelope and possibly other nuclear structures during heat shock.[79] The presence of Hop in the nucleus under prescribed conditions, and the possibility that it may be translocating to the nucleus via a functional NLS, is particularly intriguing and poses a number of questions as to what its functions are within the nucleus. Reports have already been published regarding the involvement of nuclear Hop in complex with Hsp70, in cell cycle-regulated transcription of histone H2B.[69] Considering the potential variety of roles and complexes of Hop in the cytosol, it is likely that Hop may also possess a varied role within the nucleus and may be interacting within a number of other nuclear complexes. However, these proposed new roles of Hop remain to be fully elucidated.

Interactions of Hop with structurally and functionally unrelated proteins makes it increasingly difficult to define Hop as merely a Hsp70/Hsp90 adaptor or co-chaperone, and presents the multifaceted nature of its biological functions (Fig. 2). Questions regarding the actual mechanism(s) by which Hop is able to distinguish between its different interactions as well as its subcellular localization, need to be answered in order to gain further insight into its global function within the biological system.

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