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More than folding: localized functions of cytosolic chaperones

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Compared with other chaperone systems, heat shock proteins Hsp70 and Hsp90 interact with a larger variety of co-chaperone proteins that regulate their activity or aid in the folding of specific substrate proteins. Although many co-chaperones are soluble cytosolic proteins, co-chaperone domains are also found in modular adaptor proteins, which are often localized to intracellular membranes or elements of the cytoskeleton. These specialized co-chaperones include auxilin, cysteine string protein, Tom70, UNC-45 and homologs of Bag-1. The localized co-chaperones can harness the ATP-dependent mechanisms of Hsp70 and Hsp90 to do conformational work in diverse functional contexts, including vesicle secretion and recycling, protein transport and the regulated assembly and/or disassembly of protein complexes. Such flexibility is unique to the cytosolic Hsp70 and Hsp90 chaperone system.

It is generally accepted that molecular chaperones interact with folding intermediates of polypeptides, preventing non-productive interactions that would result in aggregation, and aiding them in reaching their native state. The chaperones belonging to the heat shock protein Hsp70, Hsp90 and chaperonin families act through cycles of substrate binding and release governed by ATP binding and hydrolysis [1–3]. In the eukaryotic cytosol, Hsp70 and Hsc70 (70-kDa heat shock protein and cognate protein) handle a broad range of substrate polypeptides, and contribute generally to the folding of newly synthesized proteins and refolding of proteins after stress denaturation [2]. Hsp90 (90-kDa heat shock protein) and the chaperonin CCT/TRiC are thought to mediate folding of a more limited set of substrates, including various signal-transducing proteins in the case of Hsp90 [2,3]. Because Hsp90 often functions together with Hsc70, the two chaperones might be considered as parts of a larger multi-chaperone system.

Cytosolic Hsc70 and Hsp90 are further distinguished by the large number of regulatory or accessory co-chaperone proteins that they interact with. Many of these co-chaperones have a modular architecture in which a chaperone-interacting domain is fused to other sequences supplying different activities. Interestingly, several of these co-chaperone modules are targeted within the cytosolic compartment to different membrane systems or to cytoskeletal elements. Such localized co-chaperones can then recruit cytosolic Hsc70 or Hsp90 for tasks involving the conformational modulation of specific target proteins at the respective intracellular sites. Recent work has revealed some of the mechanisms and cellular processes supported by the targeted co-chaperones and their cytosolic chaperone partners. This review will focus on the best characterized of these specific co-chaperones, acting in endocytosis and exocytosis, protein targeting, cytoskeletal function and signal transduction.

Three classes of co-chaperone domains appear as protein modules: DnaJ homology or J domains, Bag-1 homology or Bag domains, and so-called tetratricopeptide repeat (TPR) clamp domains. Representative structures of these domains are depicted in Figure 1 [4–6]. DnaJ homologs were the first co-chaperone proteins to be recognized as a family, and contain a conserved His-Pro-Asp tripeptide motif that is essential for function [7]. The J domains of these homologs stimulate ATP hydrolysis via the partner Hsc70 proteins, converting them to the ADP-bound forms and leading to stable binding of substrates by the Hsc70 peptide-binding domains [1,2]. More recently, Bag-1 homologs were also identified as a family of Hsc70 cofactors [8]. Bag domains interact with the ATPase

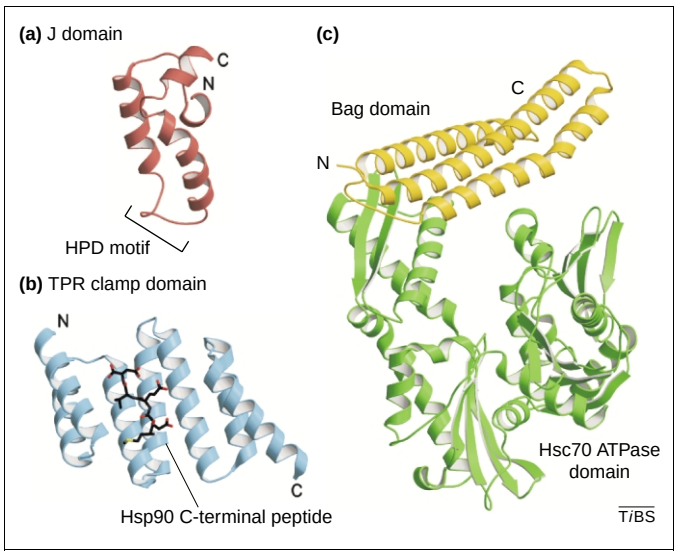

Figure 1. Molecular structures of modular co-chaperone domains. **(a)** Nuclear magnetic resonance structure of the human Hsp40 J domain (red), the position of the conserved His-Pro-Asp (HPD) tripeptide motif necessary for function is marked. **(b)** Crystallographic structure of the central tetratricopeptide (TPR) clamp domain of human Hop (light blue) bound to the C-terminal peptide of Hsp90 (black ball and stick); the N-terminal TPR clamp domain of Hop binds the Hsc70 C-terminal peptide similarly. **(c)** Crystallographic structure of the Bag domain of human Bag-1 (yellow) bound to the Hsc70 ATPase domain (light green). Ribbon diagrams were generated with MOLSCRIPT and RASTER-3D.

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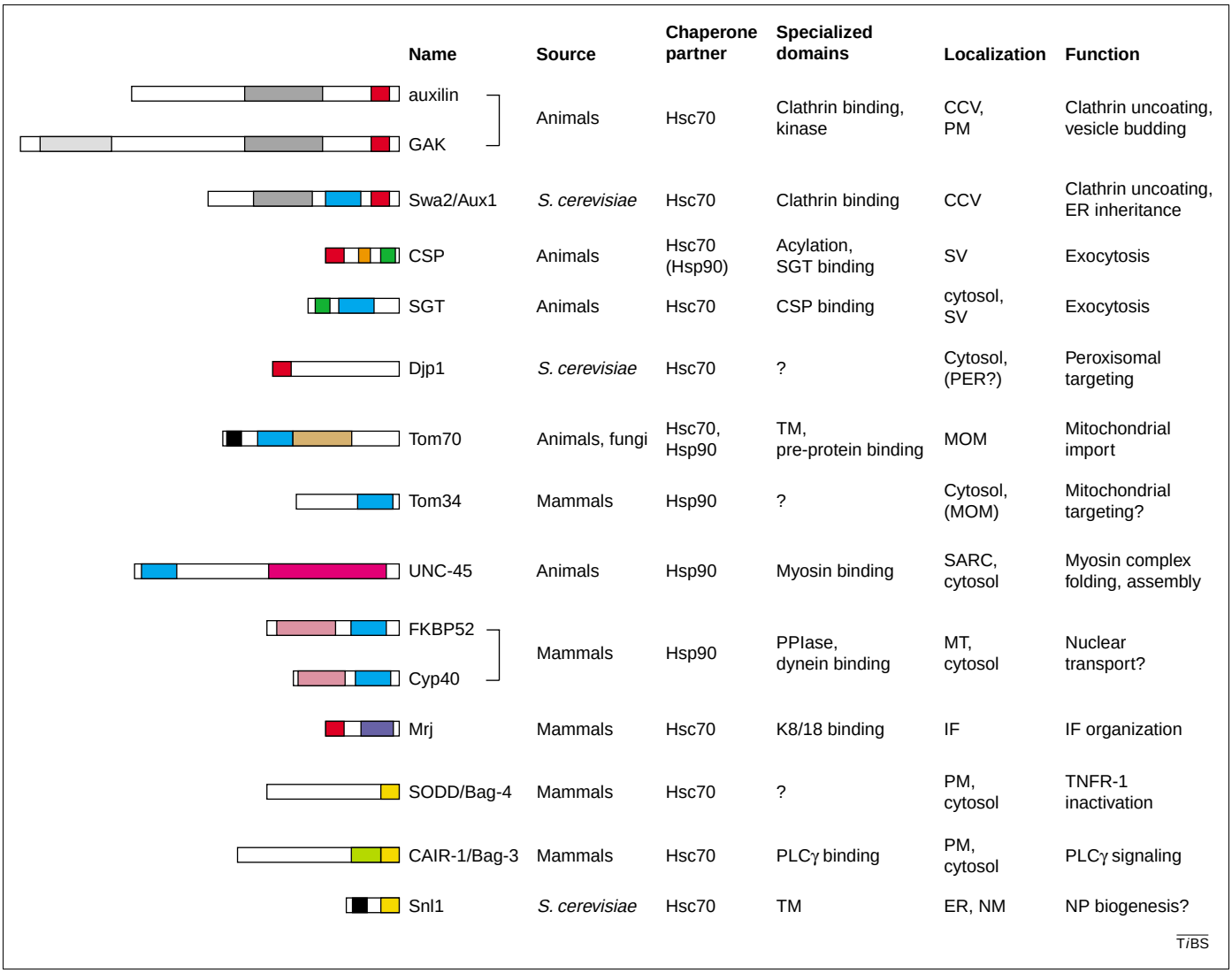

Figure 2. Schematic diagram of localized co-chaperone proteins. N termini are on the left, J domains are shown in red, tetratricopeptide clamp domains in light blue, and Bag domains in yellow. Specialized interaction domains are depicted as follows: clathrin-binding domains of auxilin, GAK and Swa2/Aux1, dark gray; kinase domain of GAK, light gray; acylated cysteines of CSP, light orange; interaction sites between CSP and SGT, green; transmembrane domains of Tom70 and Snl1, black; preprotein-binding domain of Tom70, light brown; myosin-binding domain of UNC-45, magenta; PPIase- and dynein-binding domains of FKBP52 and Cyp40, pink; K8/18-binding domain of Mrj, dark blue; PLCγ-binding site of CAIR-1/Bag-3, light green. Abbreviations: CCV, clathrin-coated vesicles; ER, endoplasmic reticulum; GAK, cyclin G-associated kinase; IF, intermediate filaments; K8/18, keratin 8 and 18 filaments; MOM, mitochondrial outer membrane; MT, microtubules; NM, nuclear membrane; NP, nuclear pore; PER, peroxisomes; PLCγ, phospholipase C-γ; PM, plasma membrane; PPIase, peptidylprolyl isomerase; SARC, sarcomere; SODD, suppressor of death domains; SV, synaptic vesicles; TM, transmembrane domain; TNFR-1, tumor necrosis factor receptor type 1; TPR, tetratricopeptide repeat.

domains of eukaryotic cytosolic Hsc70 and trigger the exchange of ADP for ATP, favoring release of Hsc70-bound peptides [5,9]. TPR clamp domains were first identified as homologous sequences in a series of cytosolic Hsp90 co-chaperones [10], and are now known to recognize the C-terminal peptides of Hsp90 or Hsc70, or both [3,6]. At least one TPR clamp protein, Hop/Sti1, inhibits the ATPase cycle of Hsp90, whereas other such co-chaperones do not [11]. Thus, the localized co-chaperones of Hsc70 and Hsp90 can control the biochemical regulation as well as the intracellular location of the recruited chaperones.

An important comparison can be made to the action of organellar Hsp70s in polypeptide transport across membranes. The unique mitochondrial-inner-membrane co-chaperone Tim44 localizes mitochondrial matrix Hsp70 (mtHsp70/Ssc1) to drive import into mitochondria [12], and Bip/Kar2 (ER lumenal Hsp70) similarly requires the membrane-anchored J domain of Sec63 to act in ER translocation [13]. Analogously, cytosolic Hsc70 and/or Hsp90 can be recruited by their localized co-chaperones to accomplish specific tasks.

Clathrin uncoating

Perhaps the best-known specialized co-chaperone is the J-domain protein auxilin, which functions in the Hsc70-mediated uncoating of clathrin-coated vesicles (CCVs) budded from the plasma membrane [14]. Neuronal auxilin contains a central clathrin-binding domain and a J domain at the extreme C terminus (Figure 2). *In vitro* studies have established that the clathrin-binding domain first assembles onto clathrin cages, and the J domain then stimulates free Hsc70 to hydrolyze ATP. Hsc70 in the ADP state binds tightly to clathrin, presumably in a manner similar to the binding of an unfolded polypeptide, and this distorts the conformation of clathrin leading to disassembly of the cage [14,15] (Figure 3). In the cytosol, Hsc70 continuously

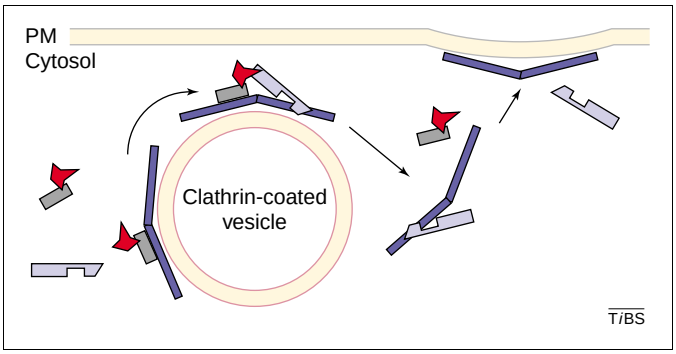

Figure 3. Clathrin uncoating by auxilin and Hsc70. Auxilin targets via its clathrin-binding domain (gray) to clathrin (dark purple) on the surface of vesicles. The J domain of auxilin (red) triggers ATP hydrolysis by Hsc70 (light purple), which then binds clathrin tightly. Hsc70 binding causes dissociation of clathrin from the vesicle surface, and enables it to recycle onto membranes.

releases and re-binds clathrin, and together with clathrin assembly proteins such as AP-180, stabilizes clathrin for subsequent re-assembly on the plasma membrane [16]. The non-neuronal form of auxilin, called GAK (cyclin G-associated kinase) or auxilin 2, contains an additional N-terminal kinase domain (Figure 2) that is not required for clathrin uncoating but might regulate clathrin assembly [17,18].

Different experimental approaches have confirmed the functions of Hsc70 and auxilin in clathrin uncoating *in vivo*: (i) by overexpressing dominant inhibitory mutants of Hsc70 in cultured HeLa cells; (ii) by RNA interference knockdown of auxilin expression in *Caenorhabditis elegans*; (iii) by injection of mutant auxilin lacking a functional J domain into squid presynaptic nerve terminals; and (iii) by a genetic screen in *Drosophila* for endocytosis mutants revealing a point mutation in Hsc70 [19–22]. In all cases, clathrin-dependent endocytosis is markedly inhibited and is accompanied by abnormal vesicular accumulation of clathrin. Disruption of Hsc70 function in HeLa cells causes defects not only in endocytosis, but also in vesicle transport between the *trans*-Golgi, endosomes and plasma membrane, suggesting that Hsc70 together with auxilin or GAK might be generally required for all clathrin-dependent vesicle trafficking [19]. Consistent with this, RNA interference of auxilin in *C. elegans* leads to broad developmental defects and an overall intracellular immobilization of clathrin [20]. However, a new report suggests that Hsc70 and auxilin also form complexes with dynamin, a GTPase that works in the budding and fission of CCVs, and some of the effects of disrupting auxilin *in vivo* might arise from defects in vesicle formation as well as in clathrin uncoating [23].

The *Saccharomyces cerevisiae* auxilin called Swa2/Aux1 differs from the animal forms in several interesting aspects. Between the clathrin-binding and J domains, Swa2 also contains a predicted TPR clamp domain, which is expected to recognize Hsc70 and/or Hsp90 [24,25] (Figure 2). The *swa2–1* mutation bears a single amino-acid exchange in a conserved residue of the TPR domain. Interestingly, the *swa2–1* mutation shows synthetic lethality with a deletion of the gene encoding the ADP-ribosylation factor Arf1 (hence the name Swa2, synthetic lethal with *arf1*), which functions in coated-vesicle transport between the endoplasmic reticulum (ER) and Golgi. Although deletion of the gene encoding Swa2 causes endocytosis defects, the *swa2–1* point mutation does not interfere with clathrin binding or stimulation of the Hsc70 ATPase by the Swa2 J domain [24]. Thus, chaperone binding to the TPR domain might serve a function that is unique to yeast auxilin. One possibility is the involvement of Swa2 in the formation of the ER during cell division in yeast, which is a function not known for auxilin or GAK in animal cells [26].

Synaptic vesicle fusion

Cysteine string protein (CSP) is a specialized J-domain co-chaperone and was first shown to function in calcium-activated exocytosis of synaptic vesicles [27,28]. CSP has been identified in *Drosophila*, *Torpedo* and mammals (with some species having different isoforms), but is not found in *S. cerevisiae*. CSP contains a J domain at its N terminus, and a central cysteine-rich sequence anchored to vesicle membranes by multiple acylations [29] (Figure 2). The involvement of Hsc70 in CSP-mediated exocytosis *in vivo* is now supported by a mutagenesis study in *Drosophila*, which produced several alleles of Hsc70 that caused impaired synaptic transmission at the neuromuscular junction. Importantly, these particular Hsc70 alleles did not cause endocytosis or vesicle recycling defects, suggesting that the phenotype is unrelated to the clathrin-uncoating activity of Hsc70 [30].

The exact mechanism of CSP activity is still unresolved. CSP interacts with some neuronal SNARE proteins [31], which are responsible for vesicle targeting and fusion, and Hsc70 that is recruited by CSP has been proposed to stabilize unstructured monomeric SNAREs before formation of the fusion-active hetero-oligomeric SNARE complex [29]. However, there is also some evidence that CSP might regulate the activity of the calcium channels that trigger neurotransmitter release. These channels are controlled by the heterotrimeric GTP-binding proteins (G proteins) such that, after dissociation from the GTP-bound G_{α} subunit, the free $G_{\beta\gamma}$ subunits bind the channels and down-regulate their activity. CSP forms complexes with both $G_{\beta\gamma}$ and G_{α}, and calcium-channel modulation by CSP overexpression in cultured neuronal cells shows characteristics of $G_{\beta\gamma}$ inhibition [32] (Figure 4).

In some cases, CSP acts with another co-chaperone called SGT (small glutamine-rich TPR protein). SGT has an Hsc70-binding TPR clamp domain in its central region and a glutamine-rich sequence of unknown function near the C terminus (Figure 2). The N-terminal region of SGT binds the C terminus of CSP (Figure 2), localizing a fraction of the cytosolic SGT and Hsc70 to vesicle membranes in a trimeric complex. Together, CSP and SGT regulate the ATPase activity of Hsc70, and SGT overexpression in neurons inhibits neuronal exocytosis [33]. Although it is not yet clear at which step of membrane fusion the SGT-containing complex works, the TPR domain of SGT might transiently anchor Hsc70 to the membrane to handle the relevant polypeptides.

Among these polypeptides could be the small GTPases of the Rab protein family, particularly the neuronal

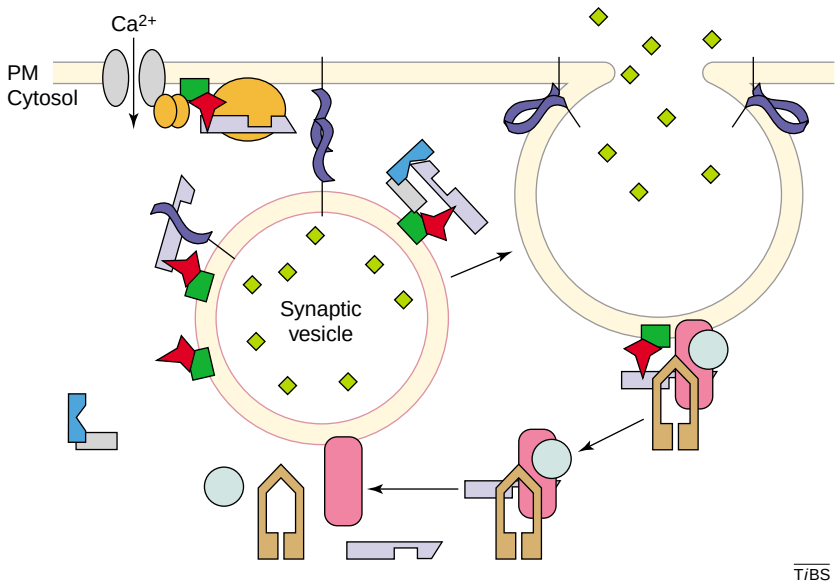

Figure 4. Functions of cysteine string protein (CSP) in synaptic vesicle exocytosis. CSP is attached to membranes by its acylated cysteine residues (dark green). CSP interacts through its J domain (red) with Hsc70 (light purple), and with interacts with the Gα (large orange oval) and Gβγ subunits (small orange ovals) of heterotrimeric G proteins to modulate calcium channel activation of exocytosis. CSP might also recruit Hsc70 to stabilize monomeric SNARE proteins (dark purple) before SNARE-complex formation, vesicle fusion and neurotransmitter release (lime green). After vesicle fusion, CSP works with Hsp90 (brown) and Hsc70 to release the regulatory complex of Rab3a (pink) and αGDI (pale green) from the membrane and recycle Rab3a onto vesicles. Some functions of CSP involve the co-chaperone SGT, which binds to Hsc70 through its TPR clamp domain (blue) and to CSP through its N-terminal domain (light gray rectangle). Abbreviations: αGDI, GDP-dissociation inhibitor; CSP, cysteine string protein; PM, plasma membrane; TPR, tetratricopeptide repeat.

isoform Rab3A, which limits the extent of calcium-stimulated exocytosis. GTP hydrolysis by Rab3A on vesicle membranes is triggered upon vesicle fusion, and the brain-specific GDP-dissociation inhibitor α (αGDI) then transfers Rab3A from the membrane to the cytosol. It seems that the αGDI binds Rab3A on the membrane in a complex containing CSP, Hsc70 and Hsp90. Interestingly, both the removal of Rab3A from membranes and the subsequent dissociation of αGDI are blocked by the anti-tumor drugs geldanamycin and radicicol, which specifically inhibit the Hsp90 ATPase cycle. The binding of αGDI by Hsp90, and probably also by Hsc70, is therefore proposed to facilitate both the membrane extraction of Rab3A and its reassembly on the membrane in the GTP-bound state [34] (Figure 4). It will be interesting to see if a similar mechanism also applies to other Rab proteins, or even to other members of the GTPase superfamily that includes the heterotrimeric G proteins and Arf1.

Protein targeting

Cytosolic Hsc70 is known to be important for the post-translational translocation of proteins across the ER, mitochondrial and peroxisomal membranes [35–37]. This Hsc70 function has been thought to be non-specific for the target organelles, most probably involving the prevention of precursor protein aggregation. However, genetic analysis has shown that the *S. cerevisiae* co-chaperone Djp1 is specifically involved in targeting to peroxisomes, but not to the ER or mitochondria. Djp1 has a J domain at its N terminus, and C-terminal sequences that might interact with the peroxisomal surface (Figure 2), although only a fraction of the protein co-localizes with membranes [38]. The molecular mechanism of Djp1 function with Hsc70 could thus involve cycling with precursor proteins from the cytosol to the peroxisomal membrane.

Recently, the mitochondrial import receptor Tom70 (70-kDa translocase of the outer membrane) has also been shown to act as a localized co-chaperone [39]. From yeast to mammals, Tom70 functions in the post-translational import of mitochondrial proteins having non-classical, internal targeting sequences [40,41]. An N-terminal domain anchors Tom70 to the cytoplasmic face of the mitochondrial outer membrane (MOM), followed by a chaperone-binding TPR clamp domain and another domain that binds directly to preproteins (Figure 2). Mammalian Tom70 recognizes both Hsp90 and Hsc70, but the yeast Tom70 is specific for Hsc70 (the Ssa proteins). When chaperone binding to the Tom70 TPR domain is disrupted by competition with an Hsp90 fragment *in vitro*, or mutation of Tom70 in yeast, mitochondrial targeting is inhibited and the oligomeric preprotein-Tom70 complex that precedes transport across the MOM cannot assemble. Furthermore, mitochondrial import of Tom70-dependent preproteins in mammals is blocked by the Hsp90-specific inhibitor geldanamycin both *in vitro* and in Cos7 cultured cells [39], suggesting that the chaperones might also help transfer preproteins from the Tom70 complex to the intermembrane space (Figure 5).

The mammalian Tom34 protein, which has no counterpart in yeast, might also function in the targeting of mitochondrial proteins with classical N-terminal presequences [42]. Tom34 contains a TPR clamp domain that recognizes Hsp90 [43] (Figure 2). However, Tom34 is almost entirely cytosolic with only a small fraction associated with the MOM [42], and possibly acts with Hsp90 in handling preproteins before they reach the membrane.

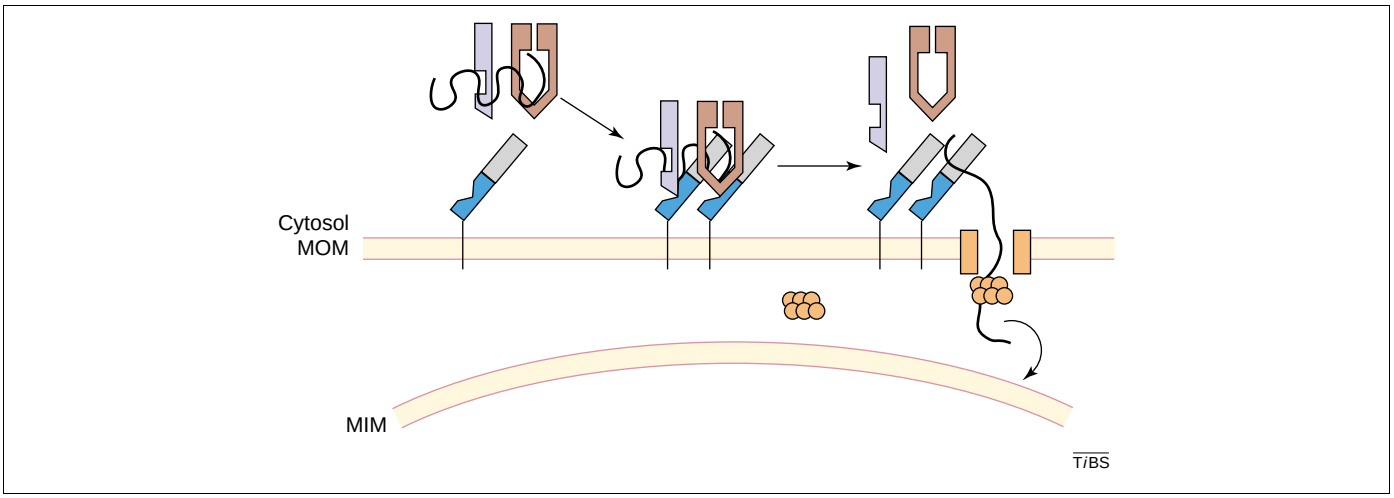

Figure 5. Protein targeting via chaperones and Tom70. Hsc70 (light purple) and also Hsp90 (brown) in mammals, bind some mitochondrial preproteins (black) in the cytosol. The chaperones dock onto the TPR clamp domain (blue) of Tom70 on the mitochondrial outer membrane. The preprotein-binding domain of Tom70 (light gray) then recognizes internal targeting sequences in the preprotein. After ATP cycling by the chaperones, the preprotein is transported by the import machinery (orange) through the outer membrane. Abbreviations: MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; TPR, tetratricopeptide repeat.

Cytoskeleton function

Hsc70 and Hsp90 are recruited not only to intracellular membranes, but also to the cytoskeletal networks, including motor proteins that move along actin filaments and microtubules. Again, the recruited chaperones are thought to fulfill different functions at these macromolecular assemblies.

In *C. elegans*, the *unc-45* gene product (named for the uncoordinated phenotype) is required for the correct assembly of conventional myosin into muscle thick filaments, which then contact the actin-based thin filaments of the sarcomere [44,45]. UNC-45 contains an N-terminal TPR clamp domain and a C-terminal UCS (UNC-45/Cro1/She4) domain. The TPR clamp binds specifically to Hsp90, whereas the C-terminal regions bind and exert chaperone activity on the myosin head (Figure 2). Hsp90 recruited by UNC-45 probably aids in the folding and assembly of myosin complexes, and might also act in their disassembly [46]. Mice and humans each have two UNC-45 isoforms with the same domain configuration. The striated muscle (SM) type is expressed only in cardiac and skeletal muscles and its function might be restricted to the sarcomere, whereas a general cell (GC) type of UNC-45 is expressed in multiple tissues and might have a role in myosin-based cytoskeletal functions [47]. Although fungal UCS domain proteins are also important for the assembly and function of non-muscle myosins [48], they do not contain TPR clamp domains. Instead, they contain N-terminal sequences that might interact with additional proteins, which in turn might recruit molecular chaperones.

Intriguingly, TPR-containing co-chaperones also interact with cytoplasmic dynein, a motor protein that moves along microtubules. These include the immunophilin FKBP52 (52-kDa FK506-binding protein) and cyclophilin Cyp40, both of which bind Hsp90 through their TPR clamp domains. The interaction with dynein is mediated by their central peptidylprolyl isomerase (PPIase) domains (Figure 2), but is independent of this enzymatic activity. These co-chaperones are thought to link Hsp90-bound steroid hormone receptors to the microtubule cytoskeleton for nuclear import [49,50]. Recent work with *S. cerevisiae*, using heterologously expressed glucocorticoid receptor, suggests that this interaction might not be necessary for nuclear transport [51], but dynein binding might still increase the transport efficiency in mammalian cells.

The J domain co-chaperone Mrj (mammalian relative of DnaJ) has recently been found to connect Hsc70 with the intermediate filament cytoskeleton. Mrj contains an N-terminal Hsc70-regulatory J domain, and its C-terminal domain specifically recognizes the keratin 18 component of keratin 8 and 18 (K8/18) intermediate filaments (Figure 2). Notably, microinjection of antibodies against Mrj into HeLa cells disrupts the K8/18 filament network without affecting the actin or microtubule cytoskeletons. Thus, it has been proposed that Hsc70 that has been recruited by Mrj acts in the regulated assembly of the keratin filament cytoskeleton [52].

Bag domain co-chaperones

The Bag-1-related family of proteins form another family of modular co-chaperones [4]. As nucleotide exchange factors for Hsc70, they probably act by stimulating ATP cycling by Hsc70 rather than stabilizing its binding to polypeptides. At least two Bag domain proteins are associated with plasma membrane proteins (Figure 2): SODD (silencer of death domains) or Bag-4 with the cytosolic region of TNFR1 (tumor necrosis factor receptor type 1), and CAIR-1 (calcium-influx inhibitor response regulated) or Bag-3 with PLCγ (phospholipase C-γ). In both cases, the Bag co-chaperones bind the inactive forms of these signal transducers, and are released after activation upon binding of cytokine ligand to TNFR1, or phosphorylation of PLCγ by EGF-R (epidermal growth factor receptor) [53,54]. The function of Hsc70 in these pathways is not yet clear, but could be related to conformational changes required for the switching mechanisms. In *S. cerevisiae*, the Bag domain co-chaperone Snl1 (suppressor of Nup116C lethality) is localized to the cytosolic face of nuclear and ER membranes, and can

suppress growth defects caused by some mutant nuclear pore proteins, perhaps by stimulating Hsc70 to stabilize them [55]. The physiological function of Snl1 under normal growth conditions, however, remains to be established.

Concluding remarks

The list of localized co-chaperones of Hsc70 and Hsp90 continues to grow, and knowledge of how they act will broaden our understanding of the functions of these chaperones. Important questions concern how the recruited Hsc70 or Hsp90 proteins work mechanistically, and how the chaperone-mediated steps are integrated with the functions of other components.

A few principles can be derived from what is known about the chaperone-dependent mechanisms discussed above. First, the action of the chaperones must be regulated as well as localized within the cytosol. For example, there is no evidence that auxilin and Hsc70 remove clathrin from clathrin-coated pits in the plasma membrane, but only from vesicles after budding [14,15]. Such regulation probably requires interactions in addition to those between the chaperones and co-chaperones. In the case of auxilin, it could be speculated that the interaction with dynamin during the vesicle budding process, but not afterwards [23], might be such a regulatory interaction. Another example might be the chaperone-dependent removal of Rab3a from synaptic vesicle membranes [34] because the complex with CSP, Hsp90 and Hsp70 recognizes GDP-bound Rab3a together with αGDI, but probably not GTP-bound Rab3a without αGDI. For other functions of CSP and Hsp70, including the interaction with the heterotrimeric G proteins [32], the regulatory steps remain to be discovered.

Second, additional interactions centered on the co-chaperones will also provide further specificity to the chaperone functions. A clear example is the function of Tom70: although Hsp90 and Hsc70 help to bring mitochondrial preproteins to the import receptor, sorting of preproteins from other chaperone substrates must be performed by Tom70 itself or other components of the import machinery [39]. The PPIases FKBP52 and Cyp40, which connect to dynein and to Hsp90, might similarly contribute to the specificity of microtubule-based transport because the steroid hormone receptors, but not other Hsp90-bound substrates, are targeted to the nucleus [49,50]. It is not known how the dynein-bound complex distinguishes between liganded receptors, which must be targeted, and unliganded receptors, which must remain cytosolic, and the co-chaperones might also act in this regulatory step. A crucial challenge will be to discover how the specificity and regulation of chaperone function are determined by the components of these co-chaperone complexes.

Third, the chaperones must fulfill their localized functions through the same biochemical mechanisms that they use for protein folding. Hsc70 is known to recognize short hydrophobic polypeptide stretches in an extended conformation [1,2], and cellular processes that require Hsc70, such as clathrin uncoating, probably require the presentation of such chaperone-binding sites. However, as cytosolic clathrin remains largely folded [16], binding by Hsc70 does not necessarily imply a gross loss of protein structure. The features recognized by Hsp90 have not yet been clearly defined, but this chaperone appears to bind some of its substrates when they are in a compact state close to their native conformation [3]. This characteristic suggests, for instance, that Hsp90 that has been recruited to myosin by UNC-45 [45] might act in the final stages of myosin assembly into thick filaments. The key to understanding these processes will be to determine which polypeptides are handled by the chaperones and what conformational changes are involved.

Fourth, localization of the chaperone partners by their specialized co-chaperones is most probably necessary to accomplish the specified task. The localized co-chaperones must provide a particular orientation, local concentration or regulatory step of the chaperones that is needed for their relevant functions. In the absence of a localized co-chaperone, binding of chaperones to a crucial site might be too infrequent, or mechanically ineffective without a coordinating anchor. The modular domain structure of the co-chaperones imparts a unique flexibility to the Hsc70 and Hsp90 chaperone system that enables it to act not only in conventional protein folding, but also in adjusting protein conformational switches in a multitude of cellular processes.

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