REVIEW

From hatching to dispatching: the multiple cellular roles of the Hsp70 molecular chaperone machinery

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Abstract

Molecular chaperones are best recognized for their roles in de novo protein folding and the cellular response to stress. However, many molecular chaperones, and in particular the Hsp70 chaperone machinery, have multiple diverse cellular functions. At the molecular level, chaperones are mediators of protein conformational change. To facilitate conformational change of client/substrate proteins, in manifold contexts, chaperone power must be closely regulated and harnessed to specific cellular locales – this is controlled by cochaperones. This review considers specialized functions of the Hsp70 chaperone machinery mediated by its cochaperones. We focus on vesicular trafficking, protein degradation and a potential role in G protein-coupled receptor processing.

Introduction

To become functional and fulfil their cellular roles, the vast majority of proteins must fold into complex three-dimensional shapes. Proteins may not be able to fold fully until their polypeptide chain and/or other subunits are fully synthesized. Partially folded proteins may have undesirable interactions with other essential cellular molecules. Thus, incorrect folding leads to a reduction in levels of functional proteins. Furthermore, additional detrimental effects may be caused by the aggregation of misfolded polypeptides (Dobson 2003). To combat the problems of cellular protein folding helper proteins, which function as ‘molecular chaperones’, have evolved.

The best recognized role for chaperones in the context of molecular endocrinology is their essential involvement in the functional maturation of steroid receptors, where it has become clear that receptor folding, maturation, nuclear trafficking and disassembly of transcriptional regulatory complexes are all chaperone dependent (Freeman & Yamamoto 2002, Prescott & Coetzee 2006, Grad & Picard 2007). The chaperone system that is responsible for the maturation of steroid receptors, to a conformation that is capable of high-affinity hormone binding, is comprised of Hsc70 (the cytoplasmic cognate Hsp70), Hsp90 and a number of cofactors known as cochaperones (Pratt & Toft 2003, Prescott & Coetzee 2006, Grad & Picard 2007, Smith & Toft 2008). The paradigm of Hsc70 and Hsp90 chaperone systems functioning throughout the life cycle of steroid receptors clearly illustrates that chaperones do not function solely in the folding of newly translated proteins, but are required for multiple cellular processes.

In fact, for the Hsc70 chaperone machinery, a very broad range of localized functions have been identified (Young et al. 2003), that go well beyond steroid receptor processing. Here, we give an overview of the Hsp70 molecular chaperone machinery and then describe examples of specialized Hsc70 functions, concentrating on endocytosis, exocytosis and protein degradation. Finally, we also consider whether the Hsp70 machinery may have a role in the processing of G protein-coupled receptors (GPCRs).

Hsp70 proteins and their cochaperones

The majority of genomes code for multiple members of the Hsp70 molecular chaperone family, with higher organisms processing more Hsp70s. In humans, there are 13 Hsp70 proteins (whilst there are only three in Escherichia coli), plus four related Hsp110 proteins (Vos et al. 2008). The first Hsp70 proteins to be identified were strongly induced in response to heat shock e.g. Hsp70/HspA1A. These included some family members with high levels of constitutive expression. Further family members have been found in multiple cellular
compartments and with tissue specific localizations. These include cytoplasmic (Hsc70/Hspa8), endoplasmic reticulum (ER; BiP/Grp78/Hspa5) and mitochondrial (mtHsp70/Grp75/Hspa9) Hsp70s.

When proteins are not natively folded, hydrophobic amino acids, which are normally buried in the core of folded globular proteins, may be exposed. This may lead to undesirable interactions with peptides, nucleic acids and other macromolecules. Chaperones, including Hsc70, solve this problem by binding to nascent peptides soon after they exit the ribosome, shielding hydrophobic residues from unproductive interactions (Young et al. 2004, Bukau et al. 2006).

For Hsp70 chaperones (in common with Hsp90 and chaperonins), cycles of client protein binding and release are coupled to conformational change of the chaperone, driven by ATP hydrolysis and exchange (Fig. 1). Chaperone ATPase activity and binding is regulated by the action of cochaperones, some of which have chaperone activity in their own right. Hsc70 is regulated by cochaperones (Table 1) including DnaJ/Hsp40 proteins (subsequently referred to as DnaJ proteins), Bag-family proteins, Hip, Hop and C-terminus of Hsp70 interacting protein (CHIP; Young et al. 2004, Bukau et al. 2006). DnaJ proteins contain a conserved 70 amino acid J-domain that can stimulate the ATPase activity of Hsp70 proteins. Hsc70 binds short regions of peptides with a certain position and pattern of hydrophobic residues. DnaJ proteins play a role in presenting clients to Hsc70 and when clients are delivered to Hsc70 stabilize binding. This is achieved by switching Hsc70 from an ATP bound state, where client peptide has access to an open substrate-binding pocket, to an ADP bound state where conformational change causes an α-helical lid structure to ‘clamp’ the peptide (Liu & Hendrickson 2007, Saibil 2008). Interestingly, it has been suggested that clamp-like structural features used to grip substrate proteins are a feature of many chaperone systems (Stirling et al. 2006).

Hsc70 has a flexible interdomain linker connecting a substrate-binding domain (SBD) and a nucleotide-biding domain (NBD). Structural analyses of the interaction between Hsc70 and the J-domain of DnaJ/C6/auxilin (the cellular function of this DnaJ protein is discussed later in this review) show that the Hsc70 SBD and NBD interact in the ADP-bound state, and that the auxilin J-domain activates Hsc70 by directing the interdomain linker towards a hydrophobic patch on the NBD surface (Jiang et al. 2007). Thus, the J-domain displaces the SBD from the NBD. It has been suggested that this conformational change may free the SBD to capture client proteins (Jiang et al. 2007). This study indicates some mechanistic differences to the activation of prokaryotic Hsp70, where SBD and NBD do not interact in the ADP-bound state (Swain et al. 2007). Intriguingly, it has been suggested that the equilibrium between conformation states could be set differently in different states.

Figure 1 Hsc70 and its cochaperones function in both protein folding and degradation. (A) Schematic of the Hsc70 ATPase cycle. For Hsc70 mediated folding cycles of client protein binding and release are coupled to conformational change of the chaperone, driven by ATP hydrolysis and exchange. (1) Hsp40 (shown as a dimer in the schematic) captures nascent or misfolded client proteins and presents them to Hsc70. Other DnaJ proteins can substitute for Hsp40. (2) J-domain stimulates the ATPase activity of Hsc70 resulting in conformational change and ‘clamping’ of client protein in the substrate-binding pocket. (3) Hip stabilizes the client protein bound ADP state of Hsc70 further promoting folding. (4) Bag protein binding stimulates nucleotide exchange. In humans HspBP1 and Hsp110 can also act as Hsc70 nucleotide exchange factors. (5) Client proteins may undergo multiple cycles of Hsc70 binding and release before reaching a natively folded state. (6) ATP-bound Hsc70 is in the ‘open’ conformation. (B) Hsc70 cochaperone complexes that promote client protein ubiquitination and proteasomal degradation are illustrated. CHIP interacts with Hsc70 via a TPR domain and acts as an ubiquitin ligase for Hsc70 clients, recruiting ubiquitin conjugating enzyme (Ubc) family members through its U-box domain. HSJ1 binds ubiquitin chains on Hsc70 clients via its ubiquitin interaction motifs, protecting them from chain trimming by ubiquitin hydrolases. HSJ1 also presents client to Hsc70 and is also able to stimulate ATPase activity via its J-domain. Bag-1 binds to Hsc70 via its Bag domain and interacts directly with the proteasome via an ubiquitin-like domain (Arndt et al. 2007).
Table 1 Cochaperones of human Hsc70

<table>
<thead>
<tr>
<th>Cochaperone</th>
<th>Cellular role</th>
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<tr>
<td><strong>DnaJ/Hsp40 proteins</strong></td>
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<tr>
<td><strong>Type I</strong></td>
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<tr>
<td>DnaJA1/DJ-2</td>
<td>Protein folding</td>
<td>Ohtsuka &amp; Hata (2000)</td>
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<td>DnaJA2/Rdj2</td>
<td>Protein folding has been shown to interact with G proteins</td>
<td>Rosales-Hernandez et al. (2008)</td>
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<td>DnaJA3/Tid1</td>
<td>Mitochondrial DnaJ protein, however, has been reported to also interact with cytosolic Hsc70</td>
<td>Lu et al. (2006)</td>
</tr>
<tr>
<td>DnaJA4/Dj4</td>
<td>Protein folding may have different client specificity from DnaJA2</td>
<td>Hafizur et al. (2004)</td>
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<td><strong>Type IIa</strong></td>
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<tr>
<td>DnaJB1/Hsp40</td>
<td>Protein folding. Stress inducible. Role in steroid receptor maturation</td>
<td>Michels et al. (1997)</td>
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<td>DnaJB2/HSJ1</td>
<td>Contains ubiquitin interaction motifs and promotes UPS mediated degradation of Hsc70 clients. Prenylated isoform localizes to cytoplasmic side of ER membrane and can interact with the GPCR rhodopsin. Highly expressed in neuronal tissues</td>
<td>Westhoff et al. (2005)</td>
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<td>DnaJB4/hlj1</td>
<td>Housekeeping Hsp40. Reported to bind the GPCR mu opioid receptor</td>
<td>Ancevska-Taneva et al. (2006)</td>
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<tr>
<td>DnaJB6/Mrj</td>
<td>Binds to keratin and plays a role in placental development</td>
<td>Izawa et al. (2000)</td>
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<td><strong>Type IIIb</strong></td>
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<td>DnaJC5B/Csp-b</td>
<td>Involved in regulated exocytosis</td>
<td>Evans et al. (2003)</td>
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<td>DnaJC6/Auxilin</td>
<td>Uncoating of clathrin-coated vesicles</td>
<td>Jiang et al. (2007)</td>
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<td>DnaJC13/Rme8</td>
<td>Intracellular trafficking</td>
<td>Zhang et al. (2001a)</td>
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<tr>
<td>DnaJC22</td>
<td>Human homologue of the Drosophila protein wurst that functions in endocytosis</td>
<td>Behr et al. (2007)</td>
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<tr>
<td>DnaJC28/GAK</td>
<td>Uncoating of clathrin-coated vesicles</td>
<td>Greener et al. (2000)</td>
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<td><strong>Nucleotide exchange factors</strong></td>
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<td>Bag-1 (Bcl-2 associated athanogene 1)</td>
<td>Initially identified as an interacting partner of the apoptosis inhibitor Bcl-2. Stimulates nucleotide exchange during the Hsc70 ATPase cycle. Binds to the proteasome via a ubiquitin-like domain and stimulates CHIP mediated degradation of Hsc70 clients</td>
<td>Alberti et al. (2004)</td>
</tr>
<tr>
<td>Bag-2 (Bcl-2 associated athanogene 2)</td>
<td>Binds to the ATPase domain of Hsc70 inhibiting chaperone activity. Inhibits CHIP-dependent ubiquitin ligase activity</td>
<td>Arndt et al. (2005)</td>
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<tr>
<td>Bag-3 (Bcl-2 associated athanogene 3)</td>
<td>Inhibits Hsc70 dependent proteasomal degradation. Binds phospholipase C-γ</td>
<td>Doong et al. (2000)</td>
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<tr>
<td>Bag-4 (Bcl-2 associated athanogene 4)</td>
<td>Regulator of cell death dependent on Hsp70</td>
<td>Esser et al. (2004)</td>
</tr>
<tr>
<td>Bag-5 (Bcl-2 associated athanogene 5)</td>
<td>Binds Hsc70. Inhibits the parkin E3 ligase and Hsc70 chaperone activity</td>
<td>Kalia et al. (2004)</td>
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<tr>
<td>Bag-6 (Bcl-2 associated athanogene 6)</td>
<td>Involved in apoptosis and cell proliferation. Interacts with Hsc70 and SGT in prometaphase</td>
<td>Winnefeld et al. (2006)</td>
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<tr>
<td>HspBP1 (Hsp70 binding protein 1)</td>
<td>Nucleotide exchange factor of Hsc70. Binds ATPase domain of Hsc70 competitively with Bag and Hip. Inhibits CHIP E3 ligase activity</td>
<td>Kabani et al. (2002)</td>
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<td>Hsp110</td>
<td>Atypical Hsp70 like proteins that act as a nucleotide exchange factors, significantly enhancing Hsc70 ATPase cycle</td>
<td>Vos et al. (2008)</td>
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<td><strong>Other Hsc70 cochaperones</strong></td>
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<td>Hip (Hsp70 interacting protein)</td>
<td>Stabilizes the ADP client bound state of Hsc70 and stimulates Hsc70 mediated protein folding. Hsc70 binding via a tetratricopeptide repeat (TPR) domain</td>
<td>Hohfeld et al. (1995)</td>
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<td>Hop (Hsp70/Hsp90 organizing protein)</td>
<td>Can bind both Hsc70 and Hsp90 via TPR domains. Acts as an adaptor protein that couples the Hsp70 and Hsp90 chaperone systems</td>
<td>Onuoha et al. (2008)</td>
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Table 1  Continued

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<thead>
<tr>
<th>Cellular role</th>
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<tr>
<td>CHIP (C-terminus of Hsp70 interacting protein)</td>
<td>Ubiquitin E3 ligase that can bind both Hsc70 and Hsp90. Acts to switch the Hsp70 chaperone machinery from a protein folding to protein degradation role</td>
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<td>SGT (small glutamine rich tetratricopeptide repeat domain protein)</td>
<td>Functions in exocytosis. Cysteine string protein binding. TPR protein</td>
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*Type II DnaJ proteins with unknown function are not included.
*There are more than 20 type III DnaJ proteins and only those discussed in this review are listed.

various Hsp70/DnaJ systems, adapting them for specific cellular roles (Jiang et al. 2007).

The diverse functions of Hsc70 rely on cochaperones, and in particular DnaJ proteins, harnessing its ATP-dependent ability to perform ‘conformational work’. DnaJ proteins are the largest class of Hsp70 chaperones (Vos et al. 2008), with more than 40 members in humans, compared with six family members in E. coli. However, it is unclear how many of these DnaJ are partners for Hsc70 as opposed to other Hsp70s. Furthermore, some DnaJ proteins are able to stimulate the ATPase activity of more than one Hsp70 (Hennessy et al. 2005). Outside of the J-domain DnaJ proteins are diverse, with motifs that target them to specific client proteins and cellular locales (Cheetham & Caplan 1998, Kelley 1998, Walsh et al. 2004). Thus, DnaJ proteins deliver the power of the Hsp70 chaperone machinery to multiple cellular functions. DnaJ proteins have been divided into three classes based on possession of domains, in addition to the J-domain, which are conserved with the archetypal E. coli DnaJ. Type I and type II DnaJ proteins have a N-terminal J-domain whilst in type III DnaJ proteins the J-domain may occur anywhere in the protein. Type III DnaJ proteins are highly divergent in size, sequence and structure and tend to serve highly specialized functions (Cheetham & Caplan 1998).

In some cases, there is functional cooperation between chaperone machines with clients being passed between different chaperone systems. For example, in yeast the de novo folding of WD40 β-propeller proteins requires engagement of both an Hsc70 and the yeast cytosolic chaperonin complex (CCT; Siegers et al. 2003). Furthermore, Hsc70 and CCT can form a stable complex that has been suggested to serve to deliver unfolded clients from Hsc70 to the client protein-binding region of CCT (Cuellar et al. 2008).

The interaction between chaperone systems is also regulated by cochaperones. The cochaperone Hop binds Hsc70 and Hsp90 through its tetratricopeptide repeat (TPR) domain, mediating interaction between them (Odumuga et al. 2004). It is also able to modulate the ATPase activity of both chaperones, thus facilitating the transfer of client proteins. It has recently been suggested that for client protein transfer Hop may align the bound client on Hsp70 with the middle domain of Hsp90, which is involved in client protein binding (Onuoha et al. 2008).

Hsc70-mediated endocytosis

Clathrin-coated vesicles transport proteins from the plasma membrane (PM) to endosomes and between the endosomal and trans-golgi compartment (Rappoport et al. 2004, Benmerah & Lamaze 2007). They must be uncoated for vesicle fusion and release of their cargo. It is known that Hsc70, with either auxilin (DnaJ(C6) or cyclin G-associated kinase (GAK/auxilin-2/DnaJ(C28) catalyses disassembly of clathrin cages (Chappell et al. 1986, Ungewickell et al. 1995, Greener et al. 2000, Umeda et al. 2000, Eisenberg & Greene 2007). However, the mechanism by which Hsc70 disrupts the clathrin cage remains to be fully resolved. Recently, it has been reported that Hsc70 binds the motif QLMLT at the C-terminus of the clathrin heavy chain after it is recruited by auxilin (Rapoport et al. 2008). Studies where GAK has been depleted support its role in uncoating and suggest further involvement in endocytosis (Lee et al. 2005, 2008). In particular GAK, together with Hsc70, functions in the recruitment of clathrin adapter proteins and the binding of clathrin to the PM and trans-golgi network. Interestingly, in GAK conditional knockout mouse embryonic fibroblasts, adaptor proteins including AP2, epsin, Eps15 and Eps15R have been reported to be mislocalized in clusters at the PM (Lee et al. 2008).

The Drosophila protein wurst (human orthologue, DnaJ(C22), a six span transmembrane protein with a C-terminal cytoplasmic J-domain, has also been implicated in endocytosis (Behr et al. 2007). Mutations in wurst cause an increase in Drosophila respiratory tube length due to defective extracellular matrix organization in the tracheal tubes. The wurst J-domain has been shown to interact with fly Hsc70 and to contain a functional clathrin-binding motif. Wurst mutation or knockdown resulted in a reduction in endocytosis and an
accumulation of clathrin at the PM. The apical PM of tracheal cells from \textit{wurust} mutant \textit{Drosophila} is disorganized and appears to contain additional membrane (Behr et al. 2007). Behr \textit{et al.} (2007) suggest a model where wurst recruits Hsc70 and clathrin to the apical PM to coordinate the early stages of clathrin-mediated endocytosis.

Additional roles for Hsc70 in intracellular trafficking have recently been identified. The DnaJ protein RME-8/DnaJC13 (receptor mediated endocytosis 8) was first identified in a screen for endocytic defects in \textit{Caenorhabditis elegans} (Zhang et al. 2001). RME-8 has subsequently been co-localized with endosomal markers (Girard et al. 2005). Fujibayashi et al. (2008) showed that human RME8 co-localizes with early endosome markers and was not associated with late endosomes. Knockdown of RME-8 did not affect cellular levels of receptors that primarily recycle to the PM after clathrin-mediated endocytosis, for example transferrin receptor and insulin receptor. However, RME-8 knockdown has been shown to decrease cellular levels of epidermal growth factor receptor (EGFR) by increasing its degradation, possibly by altering levels of EGFR that are targeted from early endosomes to late endosomes/lysosomes (Girard & McPherson 2008). RME-8 knockdown has also been reported to alter trafficking of cation-dependent mannose 6-phosphate receptor and cause improper sorting of cathepsin D (Girard et al. 2005).

**Hsc70 in exocytosis**

There is strong evidence for Hsc70 playing a role in exocytosis, mediated by the J-domain containing, cysteine string proteins (CSP/DnaJC5; Evans et al. 2005). CSP associates with secretory vesicles, including synaptic vesicles, chromaffin granules, pancreatic zymogen granules, insulin-containing granules and mucin granules (Mastrogiacomo et al. 1994, Braun & Scheller 1995, Chamberlain et al. 1996, Brown et al. 1998, Park et al. 2008). CSPz knockout mice develop normally, but then undergo severe neurodegeneration starting 2–4 weeks after birth (Fernandez-Chacon et al. 2004). Interestingly, this neurodegeneration phenotype was observed at photoreceptor synapses where CSPz is expressed in wild-type mice, but not in ribbon synapses of auditory hair cells that contain another CSP isoform (Schmitz et al. 2006). CSP was initially shown to function in the calcium-dependent exocytosis of synaptic vesicles when it was reported that in \textit{CSP Drosophila} mutants Ca\textsuperscript{2+}–dependent neurotransmission is impaired (Umbach et al. 1994). Interestingly, a similar phenotype was observed in flies with Hsc70 mutations (Bronk et al. 2001). It has since been suggested that CSP is an organizer of protein–protein interactions at different stages of the secretory vesicle cycle, stabilizing exocytotic proteins and their complexes (Evans et al. 2003). Indeed, CSP has been shown to bind soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins, which are components of the core membrane fusion machinery. Specifically, CSP has been detected in a complex with syntaxin by immunoprecipitation and has been shown to suppress a syntaxin overexpression phenotype in \textit{Drosophila} (Nie et al. 1999, Wu et al. 1999). CSP has also been immunoprecipitated with vesicle associated membrane protein, although the interaction with this SNARE is not direct (Leveque et al. 1998). Functions for CSP at several different stages of exocytosis have been identified. These include a role for CSP in GABA uptake into synaptic vesicles, based on data demonstrating, that with Hsc70, it is in a complex with the glutamate decarboxylase (GAD, that catalyses the decarboxylation of glutamate to GABA), the vesicular GABA transporter and the Ca\textsuperscript{2+}–calmodulin-dependent kinase (CaMKII; Hsu et al. 2000, Jin et al. 2003). CSP and Hsc70 have also been identified in a complex with the \(\alpha\)-GDP-dissociation inhibitor (\(\alpha\)GDI) where Hsp90 is also present. \(\alpha\)GDI regulates the cycling of Rab3a, one of a number of Rabs involved in the targeting and docking of vesicles. Interestingly, Hsp90 inhibitors have been shown to block Rab3a cycling (Sakisaka et al. 2002). Another cochaperone has been identified in a trimeric complex with CSP and Hsc70. This protein, small glutamine-rich TPR protein (SGT), is able to bind Hsc70 through its TPR domain and CSP through its N-terminus. SGT overexpression in cultured neurons inhibits neurotransmitter release (Tobaben et al. 2001). There is also evidence that CSP may associate with N-type Ca\textsuperscript{2+}–channels via heterotrimeric GTP-binding proteins (G proteins). CSPz has been shown to bind G protein subunits and stimulate GDP/GTP exchange of \(\alpha\)s, dependent on Hsc70 and SGT (Natochin et al. 2005).

**Molecular chaperones as regulators of protein degradation**

Cells utilize a variety of pathways for the degradation of proteins both during normal protein turnover and as part of their response to proteins that become damaged by environmental stress or are mutated. Cytosolic, nuclear and ER proteins are primarily degraded by the ubiquitin-proteasome system (UPS), however, most cells also rely on lysosomal proteolysis pathways, particularly under nutrient-limiting conditions (Majeski & Dice 2004). The Hsc70 chaperone machinery plays a role in the targeting of client proteins to both of these systems.

Hsc70 is linked to the UPS by its cochaperones (Fig. 1) and in particular CHIP. CHIP negatively regulates Hsc70 chaperone activity and acts as an E3 ubiquitin ligase for Hsc70 client proteins. \textit{In vitro} CHIP has been shown to target a number of proteins for degradation including oestrogen receptor-\(\alpha\) (in its non ligand bound state;
Tateishi et al. 2004) and the cystic fibrosis transmembrane conductance regulator (CFTR). Several other Hsc70 cochaperones also interact with the UPS. These include Bag-1 that acts as a nucleotide exchange factor for Hsc70 (causing client protein release) and contains an ubiquitin-like domain through which it interacts with the proteasome, stimulating CHIP mediated degradation of Hsc70 clients (Alberti et al. 2003) including the glucocorticoid hormone receptor (Demand et al. 2001). HsJ1b also promotes the degradation of Hsp70 clients as it contains two ubiquitin interaction motifs, through which it binds ubiquitinated proteins, shielding ubiquitin chains from the action of chain trimming ubiquitin hydrolases and facilitating sorting of ubiquitinated Hsp70 clients to the proteasome (Westhoff et al. 2005). The Hsc70 cochaperones HspBP1, Bag-2 and Bag-5 have an opposing effect to Bag-1 and HsJ1, acting as inhibitors of chaperone-mediated degradation (Esser et al. 2004). For example, HspBP1 and Bag-2 have been shown to inhibit CHIP mediated degradation of CFTR (Alberti et al. 2004, Arndt et al. 2005). Thus, the targeting of client proteins from the Hsc70 chaperone machine to the UPS is closely regulated by cochaperones.

Membrane proteins and luminal proteins retro-translocated from the ER are also targeted for degradation by the UPS by a process called ER-associated degradation (ERAD). Again cytosolic chaperones play a role, for example in yeast a cytosolic Hsp70, Ssa1p, has been shown to be required for ERAD of membrane proteins including CFTR (Zhang et al. 2001b). It has been suggested Ssa1p maintains the solubility of misfolded cytoplasmic membrane protein domains, facilitating targeting to the UPS (Nishikawa et al. 2005).

The lysosomal proteolysis pathway of chaperone-mediated autophagy (CMA) also relies on Hsc70 and its cochaperones (Majeski & Dice 2004). CMA differs from other lysosomal degradation pathways in that it is independent of vesicular transport. In CMA client proteins, which contain a KFERQ like motif, are recognized by an Hsc70/Hsp90 chaperone complex that includes the cochaperones Hsp40/DnaJ B1, Hip, Hop and Bag-1. This complex then binds with the lysosomal membrane receptor Lamp2a via the client protein. The chaperone complex is likely to unfold lysosomal membrane receptor Lamp2a via the client Hop and Bag-1. This complex then binds with the HspA1L, Hsc70/Hsp90 chaperone machine to the UPS (Nishikawa et al. 2005).

As the majority of hormones signal through GPCRs, many of the diseases associated with mutations in GPCRs disrupt endocrine systems. These included nephrogenic diabetes insipidus caused by mutations in the vasopressin type 2 receptor, familial glucocorticoid deficiency caused by mutations in the melanocortin 1 receptor, and obesity caused by mutations in the melanocortin 4 receptor. In human diseases, where GPCRs are mutated it is common for the receptor to fail to traffic to the cell surface (Conn et al. 2007). A failure to transit from the ER to the PM occurs because mutated proteins, including GPCRs, are detected as ‘misfolded’ by the ER quality control system recently been shown to be activated by oxidative stress coupled to an increase in Hsc70 in the lysosomal lumen and Lamp2a in the lysosomal membrane (Kiffin et al. 2004).

### A role for Hsp70 proteins in GPCR trafficking?

The human genome encodes ~450 GPCR proteins with endogenous ligands (plus a further 500 odorant receptors). These receptors are particularly relevant to human health as they represent the target for ~30% of marketed drugs. For GPCRs to transmit extracellular signals into the cell they must be trafficked to the cell surface. The processing of some GPCRs has been shown to be influenced by specific accessory proteins (Tan et al. 2004, Clark et al. 2005). There is evidence that many of these GPCR-interactors have a molecular chaperone function. For example, in Drosophila, a cyclophilin homologue, *Nina A*, with peptidyl-prolyl isomerase activity is essential for rhodopsin Rh1 expression (Stamnes et al. 1991, Baker et al. 1994). In addition to specific accessory proteins/interacting partners facilitating GPCR processing, more ubiquitous components of the cellular molecular chaperone machinery can modulate their folding, trafficking and degradation. Hsp70 proteins have been suggested to promote the processing of both wild-type and mutant GPCRs to the PM. For example, the testis enriched Hsp70 variant, Hsc70t/HspA1L, enhances the expression and trafficking of odorant receptors (Neuhaus et al. 2006); whilst Hsc70 has been demonstrated to interact with cytoplasmic domains of non-glycosylated angiotensin II type 1 receptor (Lanctot et al. 2006). Furthermore, in cultured cells, the Hsp70 cochaperone HsJ1b can modulate the processing of the archetypal GPCR rhodopsin. HsJ1b interacts with rhodopsin and prevents the protein transiting from the ER to the Golgi and on to the PM (Chapple & Cheetham 2003). Another DnaJ protein, hlj1/Dna JB4 has been shown to interact with the human mu opioid receptor via binding its C-terminal domain (Ancenska-Taneva et al. 2006). Interestingly, hlj1 has tumour suppressor activity (Tsai et al. 2006).
(Conn et al. 2007, Anelli & Sitia 2008). ER resident chaperones (e.g. BiP) play a major role in regulating this quality control, recognizing aberrantly folded protein, preventing their routing to the Golgi and subsequently targeting them for ERAD. The apparent role of the cytosolic Hsp70 machinery in GPCR processing could suggest these chaperones are also involved in determining the fate of misfolded transmembrane proteins. Both ER and cytosolic Hsp70 chaperone networks represent potential sites for therapeutic intervention in diseases where GPCRs fail to transit to the PM.

Conclusions

Hsc70 is a chaperone multi-tool with involvement in a still expanding list of cellular pathways. This versatility is achieved through Hsc70’s cochaperones and in particular DnaJ proteins. Although Hsc70 is a cytoplasmic protein it can be recruited to intracellular membranes by membrane associated cochaperones, such as the prenylated DnaJ proteins HsJ1b (Chapple & Cheetham 2003), and the cytoskeleton. For example the DnaJ protein Mrj connects Hsc70 with intermediate filaments (Izawa et al. 2000). Interestingly, the fact that Hsc70 is closely linked to endocytosis, vesicular trafficking and exocytosis suggests it may be involved in the regulation of multiple cellular signalling pathways, in addition to those mediated by nuclear steroid receptors.

For GPCRs endocytosis can control signal termination, propagation and resensitization (Wolfe & Trejo 2007, Hanyaloglu & von Zastrow 2008). Also both Hsp70 proteins and DnaJ proteins have been shown to directly interact with GPCRs modulating their processing, whilst CSP binds G protein subunits. Furthermore, another DnaJ protein, Rdj2 (DnaJA2), has recently been shown to interact with G proteins and can modulate G protein signalling (Rosales-Hernandez et al. 2008).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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