Hsp70 Chaperones

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Via their interaction with client proteins, Hsp70 molecular chaperone machines function in a variety of cellular processes, including protein folding, translocation of proteins across membranes and assembly/disassembly of protein complexes. Such machines are composed of a core Hsp70, as well as a J-protein and a nucleotide exchange factor as co-chaperones. These co-factors regulate the cycle of adenosine triphosphate (ATP) hydrolysis and nucleotide exchange, which is critical for Hsp70's interaction with client proteins. Cellular compartments often contain multiple Hsp70s, J-proteins and nucleotide exchange factors. The capabilities of Hsp70s to carry out diverse cellular functions can result from either specialisation of an Hsp70 or by interaction of a multifunctional Hsp70 with a suite of J-protein co-chaperones. The well-studied Hsp70 systems of mitochondria provide an example of such modes of diversification and specialisation of Hsp70 machinery, which are applicable to other cellular compartments.

Introduction

Hsp70 chaperone machines, a ubiquitous class of molecular chaperones present in all major cellular compartments, assist in critical cellular processes. They act through cycles of binding to exposed hydrophobic stretches of amino acids in polypeptides, called client proteins. Via this common biochemical mechanism, Hsp70 chaperones participate in numerous essential functions, including folding of nascent proteins as they emerge from ribosomes, modulating protein–protein interactions by controlling conformational changes and driving protein transport across membranes. In addition, Hsp70 chaperones can facilitate protein refolding, particularly under conditions of cellular stress. In some cases they also facilitate transfer of client proteins to proteolytic systems, particularly when refolding into the native state is unachievable. **See also**: Chaperones, Chaperonin and Heat-Shock Proteins

The ability of Hsp70 chaperones to be involved in such diverse cellular functions, whereas relying on a single biochemical activity, an adenosine triphosphate (ATP)-dependent client binding and release cycle, is remarkable. Here, to illustrate the molecular mechanisms and evolutionary history behind the specialisation of Hsp70 systems we focus on mitochondrial Hsp70 systems, as they exemplify two major strategies of Hsp70 specialisation: (i) amplification and diversification of HSP70 genes and (ii) multiplication and specialisation of genes encoding their J-proteins co-chaperones (**Figure 1**). Primarily we emphasise fungal mitochondrial Hsp70 systems, as both extensive genomic information for a diverse group of fungi and the results of detailed functional analyses utilising the yeast *Saccharomyces cerevisiae* as model systems are available. However, first we review the basics of the Hsp70:client protein binding cycle and provide a general discussion of the proliferation of Hsp70 and J-protein genes. **See also**: Mitochondria: Structure and Role in Respiration; *Saccharomyces cerevisiae*: Applications

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Hsp70:Client Protein Interaction Cycle

The overall structure of Hsp70s is highly conserved. They have two domains: an *N*-terminal ATPase domain of ~44 kDa, with two lobes forming a deep cleft in which adenine nucleotide binds; and a *C*-terminal peptide-binding domain (PBD) of ~26 kDa (**Figure 2**). The peptide-binding site, which contacts five contiguous residues of a client polypeptide, is within a β-sandwich structure; an α helix folds back upon the sandwich forming a 'lid' over the binding pocket (Mayer *et al*., 2001). The two domains are joined by a flexible linker, which plays an important role in modulating the interaction between them (Bhattacharya *et al*., 2009; Swain *et al*., 2007; Vogel *et al*., 2006). Interdomain communication (Jiang *et al*., 2005) is critical as the adenosine diphosphate (ADP)- and ATP-bound states have profoundly different effects on client protein binding.

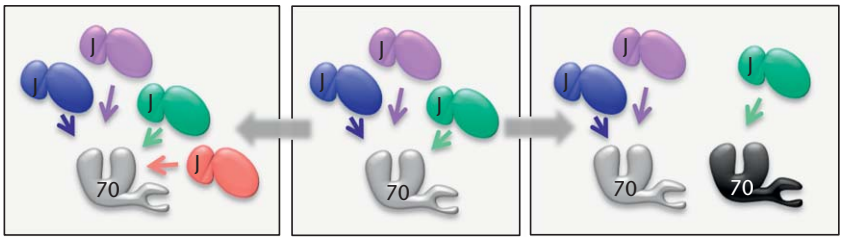

Figure 1 Evolving specificity Hsp70: J-protein machineries. Often a single Hsp70 functions with multiple J-protein partners (centre panel). Additional specificity may evolve either by duplication of an Hsp70 gene to generate a specialised Hsp70 (right panel) or duplication of a J-protein gene to generate an additional J-protein partner for an existing Hsp70.

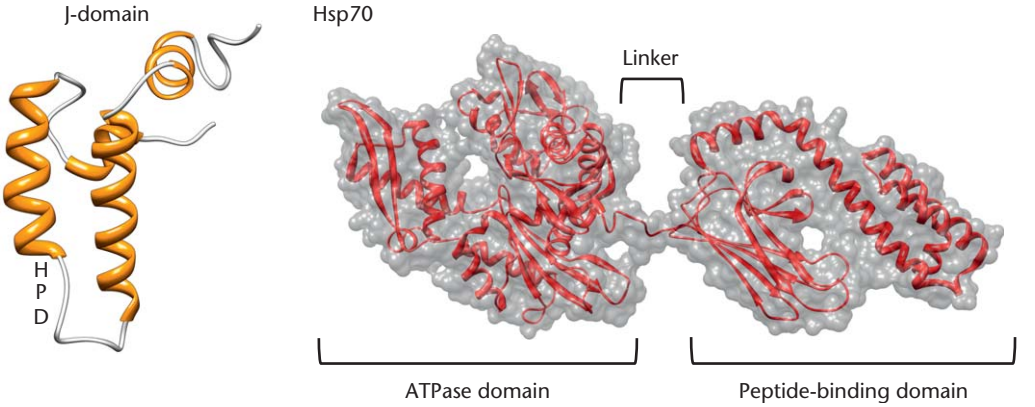

Figure 2 Structure of J-domain and Hsp70. The J-domain is the defining feature of J-proteins. Each functional J-domain has an invariant HPD tripeptide, critical for ATPase stimulatory activity (picture based on PBD id:1XBL). The structure of Hsp70s is conserved: adenine nucleotide binds between the lobes of the ATPase domain. The peptide-binding cleft is covered by an α helical lid. The linker allows interdomain communication (picture based on PBD id :2KHO).

In the ADP-state, Hsp70s exhibit relatively stable binding; in the ATP-state, binding of peptide is unstable. However, *in vivo*, the ATP-bound Hsp70 initiates productive interactions with a client polypeptide, because the on-rate (but also the off-rate) is very rapid, on the order of milliseconds. ATP hydrolysis converts Hsp70 to the ADP-state, with a client protein off-rate on the order of minutes. Exchange of ADP for ATP results in dissociation of the bound peptide/polypeptide (Mayer *et al.*, 2001). Thus, although the molecular basis of interdomain communication remains elusive, it is abundantly clear that this interaction is fundamental to the chaperone activity of Hsp70, as the nucleotide bound to the ATPase domain profoundly affects the character of the PBD's interaction with client proteins.

Hsp70s do not function alone. Rather, they act with obligatory co-chaperones: J-proteins and nucleotide exchange factors (NEFs). The defining feature of a J-protein, and indeed the only domain required to be classified as such, is the 'J domain', a compact antiparallel helical coiled-coil, having an invariant HPD tripeptide (**Figure 2**). The J-domain interacts directly with Hsp70's ATPase domain (Hennessy *et al.*, 2000; Jiang *et al.*, 2007; Landry, 2003). Some J-proteins also contain a domain that binds client proteins, although such domains may be structurally divergent (Craig *et al.*, 2006). In certain instances the binding of a J-protein to a client polypeptide serves to 'target/deliver' it to the partner Hsp70 (**Figure 3**). On the contrary, some J-proteins lack a domain that interacts with client proteins. NEFs serve the essential role of stimulating the release of ADP and client protein from Hsp70, allowing the start of a new cycle (Cyr, 2008). Clearly, the points of action of J-proteins and NEFs in the Hsp70 reaction cycle can serve as points of regulation, for example, by facilitating client protein engagement through stimulation of ATPase activity and fostering release by enhancing nucleotide exchange. **See also**: Chaperones, Chaperonin and Heat-Shock Proteins; Heat Shock Response; Protein Folding and Chaperones

Proliferation of Hsp70 and J-protein Genes

Hsp70/J-proteins are unique amongst molecular chaperones in having evolved into large and varied families in virtually all organisms (Boorstein *et al.*, 1994; Brocchieri *et al.*, 2008; Craig *et al.*, 2006; Genevaux *et al.*, 2007). For example, the yeast *Saccharomyces cerevisiae* encodes 14 Hsp70s and 23 J-proteins, whereas in human cells 17 Hsp70s cooperate with 41 J-proteins (Hageman and

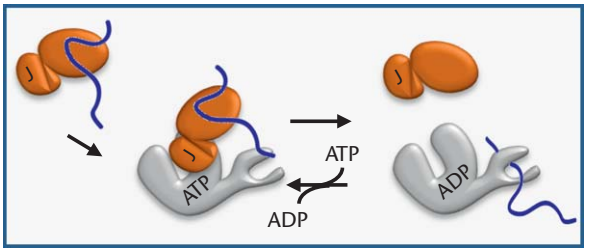

Figure 3 The Hsp70 cycle of interaction with a client protein. The universal function of the conserved J-domain (J) of J-proteins is stimulation of Hsp70's ATPase activity. In addition, many, but not all, J-proteins can also bind directly to client proteins. In this way, they 'target' the client to Hsp70. The stimulation of Hsp70's ATPase activity is critical, because it allows Hsp70 to capture the client protein. This stimulation is important because, although the on-rate of the client when Hsp70 is bound to ATP is rapid, the off-rate is also rapid. However, in the ADP-state the off rate is slow. Exchange of bound ADP for ATP completes the cycle, as Hsp70 dissociates rapidly from the client when ATP is bound. Nucleotide exchange factors (not shown) facilitate this exchange.

Kampinga, 2009). This proliferation of Hsp70 and J-protein genes is in contrast to genes encoding other conserved molecular chaperones. Why? We think the answer lies in the fact that the fundamental biochemical properties of Hsp70 are flexible, and thus very adaptable. First, the specificity of binding can differ, depending on both the character of the peptide-binding cleft and on the specificity of interaction of the J-protein with a client protein, as J-proteins can function as adaptors that deliver specific client proteins to Hsp70. Second, the kinetics of the Hsp70:client protein interaction can be strictly regulated, by tight modulation of the off rates of a client protein via action of nucleotide release factors. And last, but not least, unlike the multimeric GroEL-like folding chambers that encapsulate client proteins (Horwich *et al.*, 2007), Hsp70s function as monomers, thus providing a much higher degree of spatial freedom for action. These features are likely what promoted Hsp70's ability to play roles beyond protein folding per se (e.g. remodelling of protein conformation and protein translocation). **See also**: Chaperonins; Gene Families; Gene Families: Multigene Families and Superfamilies

Function and Evolution of Mitochondrial Hsp70 Systems

The core mtHsp70 system: Mitochondria arose from an endosymbiotic event, the engulfment of an α-proteobacteria (Gray *et al.*, 1999). Therefore, it is not surprising that the components of the core mitochondrial Hsp70 system are closely related to the major bacterial Hsp70 system: the Hsp70 DnaK, the J-protein DnaJ and the NEF GrpE (Genevaux *et al.*, 2007). In yeast these mitochondrial proteins are known as the Hsp70 Ssc1, the J-protein Mdj1 and the NEF Mge1. These proteins have a high level of sequence and structure identity with their respective bacterial counterpart. In some cases individual components can even be exchanged between systems, with little affect on function (Deloche *et al.*, 1997a, b; Lisse and Schwarz, 2000). This core mtHsp70 system resides in the matrix of the mitochondria, where it performs functions similar to its bacterial homologue. For example, *in vitro* and *in vivo* analyses, have revealed that the core mtHsp70 system is involved both in *de novo* protein folding and in protection of mitochondrial proteins against heat stress initiated unfolding (**Figure 4**). Evidence indicates that this role of Ssc1/Mdj1/Mge1 system in protein folding/refolding is performed according to the canonical model of Hsp70 function described earlier. The cycle begins with Mdj1 binding a client protein, and then delivers it to Ssc1, stimulating its ATPase activity, thus facilitating client capture (**Figure 3**). Mge1 then completes the cycle, initiating the release of nucleotide, leading to dissociation of the client from Ssc1 (Kubo *et al.*, 1999; Liu *et al.*, 2001). Although much of this work was done with model heterologous proteins, several mitochondrial proteins have been identified as clients of the Ssc1/Mdj1/Mge1 system. These include the mitochondrial deoxyribonucleic acid (DNA) polymerase Mip1 and a subunit of mitochondrial ribosome, Var1 (Duchniewicz *et al.*, 1999; Germaniuk *et al.*, 2002; Westermann *et al.*, 1996). **See also**: Mitochondria: Origin

The discussion above indicates that the roles played by the core mtHsp70 system of mitochondria are closely related to those played by their bacterial counterpart. There is, however, one function of the mitochondrial system that sets it apart from its bacterial counterpart – the maintenance and propagation of the mitochondrial genome (Rowley *et al.*, 1994). This involvement in DNA maintenance indicates that this core system has evolved in response to specific requirements of organellar biology. Mitochondria are considered semi autonomous organelles, as they contain their own DNA (mtDNA), which encodes a limited number of proteins. Typically mtDNA encodes a subset of those involved in oxidative phosphorylation and ATP synthesis, as well as components of the translational apparatus needed to synthesise these proteins (Gray *et al.*, 1999). The faithful maintenance and replication of mtDNA depends on the function of a nucleo-protein complex termed the mitochondrial nucleoid (Chen and Butow, 2005). The components of the core mtHsp70 system are among the many proteins associated with the nucleoid complex. **See also**: Mitochondrial DNA and Diseases; Mitochondrial Genome

Deletion of *MDJ1* results in the loss of functional mtDNA, even in cells cultivated at optimal temperature (Duchniewicz *et al.*, 1999). Thus involvement of Mdj1 in maintenance of mtDNA is not related to its role in protection of mitochondrial DNA polymerase under heat stress conditions. In fact, mitochondrial extracts isolated from cells lacking Mdj1 that were cultivated at optimal temperatures display a normal level of mtDNA polymerase activity (Duchniewicz *et al.*, 1999). However, localisation of Mdj1 to the nucleoid appears to be critical, as analysis of

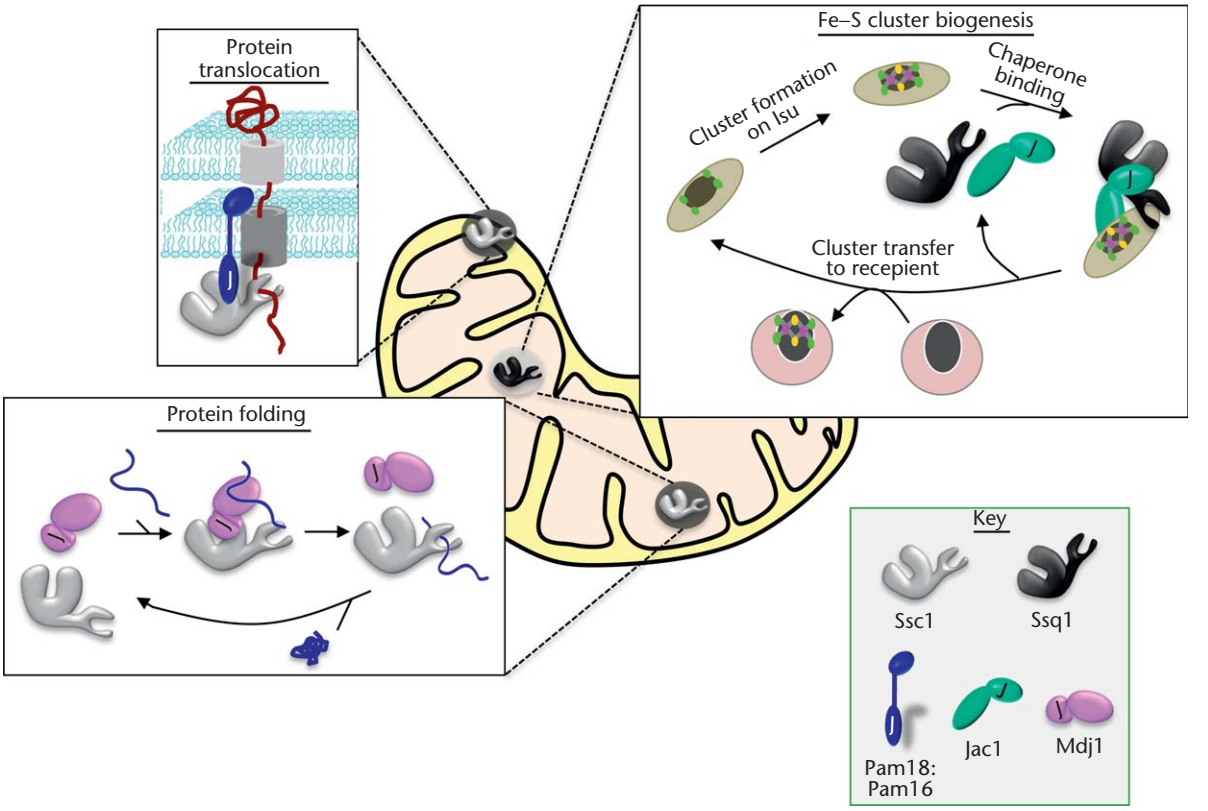

Figure 4 Hsp70s and J-proteins of the mitochondrial matrix. The generalist Hsp70 of the mitochondrial matrix (called Ssc1 in yeast) functions in general protein folding with the J-protein Mdj1. Ssc1 also functions in protein translocation with a transmembrane J-protein partner, Pam18. In both cases, Ssc1 interacts with numerous different client proteins. In addition, the specialised Hsp70 Ssq1 functions with the specialised J-protein Jac1 in Fe-S cluster biogenesis. Jac1 and Ssq1 are thought to have a single client, Isu, the scaffold on which the clusters are built. The chaperones are involved in the transfer of the cluster from Isu to a recipient protein.

MDJ1 mutants shows a positive correlation between Mdj1 nucleoid localisation and maintenance of mtDNA (Marszalek J and Ciesielski G, University of Gdansk, Poland, unpublished results). How Mdj1 is localised to the nucleoid remains unclear. Localisation via direct client protein binding and interaction with a tethering protein are possibilities. The later would be consistent with cases of other J-proteins that, via their localisation to specific suborganellar locations, direct the function of their Hsp70 partner. The molecular details of the role of the core mtHsp70 system in mtDNA transactions remain to be elucidated. But it would be consistent with known functions of other Hsp70s, if it were involved in facilitating assembly or disassembly of a multimeric protein complex.

Specialised mtHsp70 system involved in protein import: Although the core Mdj1:Ssc1:Mge1 system primarily carries out the typical roles in protein folding and refolding, the Hsp70 Ssc1 (and NEF Mge1) also works with other J-proteins to carry out a more specific role – translocation of proteins across the mitochondrial inner membrane (Chacinska *et al.*, 2009; Neupert and Herrmann, 2007). During the conversion of ancestral α proteobacteria to mitochondria many of the genes of the bacterial endosymbiont were transferred to the host cell's nuclear genome (Gray *et al.*, 1999). Once relocated to the nucleus the gene products that still functioned within the mitochondria, but were translated in the cytosol, had to be translocated into the mitochondria. Thus, mitochondria evolved sophisticated molecular machinery for efficient protein import (Dolezal *et al.*, 2006). In the mitochondrial matrix, mtHsp70 was recruited to play a critical role in the translocation process; it became the core of the 'import motor', which is associated with the translocon (a multiprotein complex through which the polypeptide passes), on the matrix side of the inner membrane (**Figure 4**) and is directly responsible for the inward movement of polypeptides through the import channel. The function of the import motor follows the basic biochemical rules for the Hsp70 systems described earlier. In the process of driving polypeptide import, mtHsp70 Ssc1 binds to exposed hydrophobic sequences in unfolded translocating polypeptides and a J-protein partner stimulates Hsp70's ATPase activity to enhance its interaction with the polypeptide. The NEF Mge1 effects nucleotide release, and thus dissociation of the translocating polypeptide (Liu *et al.*, 2003). **See also**: Mitochondria Protein Import: Methods

The import motor consists of two components of the core mtHsp70 system (mtHsp70 Ssc1 and NEF Mge1).

However, Mdj1 does not participate in protein import (Rowley *et al*., 1994). Rather, a different J-protein, Pam18 (also called Tim14), is involved (D'Silva *et al*., 2003; Mokranjac *et al*., 2003; Truscott *et al*., 2003). Although hypothesised to be related to a bacterial J-protein of unknown function (Clements *et al*., 2009), the evolutionary origin of Pam18 remains unclear. Regardless of its origin, Pam18 does have a unique domain structure, which is well conserved in eukaryotes and sets it apart from Mdj1 and bacterial DnaJ. The only sequence they share in common is the J-domain itself. In contrast to Mdj1, Pam18 is not able to bind client proteins. Thus, its sole function during protein import is stimulation of the ATPase activity of mtHsp70 Ssc1.

Pam18 has a topology that maximises its function in protein import. In addition to the matrix-localised J-domain, Pam18 consists of a transmembrane domain, which localises it to the mitochondrial inner membrane, and a short intermembrane space domain, which in the case of the *S. cerevisiae* protein, has been shown to directly interact with the translocon (Chacinska *et al*., 2005). Although the intermembrane space domain stabilises the interaction of Pam18 with the translocon, the positioning at the translocon is most dependent on its interaction with another protein, Pam16 (also called Tim16) (Frazier *et al*., 2004; Kozany *et al*., 2004). Interestingly, Pam16, a peripheral membrane protein, has a 'J-like' domain, that is related in sequence and structure to a typical J-domain, but lacks some distinctive features, such as the conserved HPD motif. Not surprisingly, Pam16 is unable to stimulate the ATPase activity of Hsp70 (Li *et al*., 2004). However, the 'J-like domain' of Pam16 and the J-domain of Pam18 interact (Mokranjac *et al*., 2006). This interaction forms the basis of the stable association of Pam18 with the translocon, as the *N*-terminal region of Pam16 binds to the translocon, and thus indirectly tethers Pam18 to it as well. The evolutionary origin of Pam16 can only be a matter of conjecture. However, because it is present in all eukaryotes, it is likely that duplication of the ancestral Pam gene allowed distribution of functions, stimulation of Hsp70's ATPase activity and tethering to the translocon, between two descendant paralogous genes. Over time, one of them, Pam18 specialised in effective stimulation of the partner mtHsp70 ATPase activity, whereas the other, Pam16, lost its ability to stimulate the ATPase activity of the Hsp70 partner, but instead gained the ability to efficiently interact with the translocon (D'Silva *et al*., 2005). **See also**: Gene Duplication: Evolution

This sophisticated mechanism of Pam18 localisation might lead one to think that an important function of Pam18 might be recruitment of Ssc1 to the import channel. However, Ssc1 is positioned close to the translocon by a mechanism independent of its interaction with Pam18. It directly interacts with a component of the translocon called Tim44 (Chacinska *et al*., 2009; Neupert and Herrmann, 2007). As the Ssc1–Tim44 interaction is not dependent on Ssc1's ability to bind client proteins, it seems reasonable to consider it as a derived feature that evolved in response to selective pressure for efficient protein import into mitochondrial matrix, and was not present in bacterial ancestor of mtHsp70. Thus two strategies were involved in the evolution of mtHsp70 based import motor, recruitment of a new, highly specific J-protein and evolution of new protein–protein interaction between mtHsp70 and a component of the translocon.

mtHsp70 system involved in biogenesis of iron–sulphur (Fe–S) containing proteins: Having inherited several biochemical pathways from their bacterial ancestors, it is not surprising that mitochondria are major metabolic centres of the eukaryotic cell. Among their critical roles is the synthesis of iron–sulphur clusters (Fe–S), prosthetic groups, which are noncovalently linked to proteins via cysteine residues. Fe–S proteins perform critical roles in cells, acting as regulatory factors detecting oxygen or iron levels, and catalysing a variety of enzymatic reactions, in addition to their more familiar role of electron transfer in oxidative phosphorylation (Lill and Muhlenhoff, 2008). Among the three alternative pathways that allow bacteria to produce Fe–S containing proteins, only one, ISC (iron–sulphur-cluster), was inherited by mitochondria. In this pathway, a cluster is first assembled on a scaffold protein, IscU, before transfer to a final recipient protein. Interestingly, this particular pathway of Fe–S biogenesis requires a specialised Hsp70 system for the transfer of the cluster from the scaffold (Craig and Marszalek, 2002; Vickery and Cupp-Vickery, 2007). Although many bacteria and all eukaryotes utilise an Hsp70 system in the biogenesis of Fe–S clusters using the ISC-type scaffold, the changes that this Hsp70 system has undergone in evolution is illustrative of both the plasticity and specialisation of its components. **See also**: Electron Carriers: Proteins and Cofactors in Oxidative Phosphorylation

In *Escherichia coli* the Hsp70 and J-protein involved in Fe–S cluster biogenesis are called HscA and HscB, respectively. This Hsp70 and J-protein are quite distinct from the related components of the core system, DnaK and DnaJ, described earlier. In contrast to DnaK, HscA does not bind an array of different client proteins, instead it appears to interact exclusively with the IscU scaffold, recognising a sequence of five residues found on its surface, LPPVK (Vickery and Cupp-Vickery, 2007). Moreover, HscA does not appear to require a NEF for exchange of ADP for ATP during the reaction cycle, as its interaction with nucleotide is intrinsically transient. HscB is also highly specialised. It consists of not only a J-domain that stimulates the ATPase activity of HscA but also a *C*-terminal client protein binding domain that specifically interacts with IscU (Kim *et al*., 2009). As the site of interaction of HscB with IscU is distinct from that of HscA, it is easy to envision the 'transfer' of IscU client protein from the J-protein to Hsp70 in this system. In addition, the specificity of the interaction of both HscB and HscA with IscU underscores the highly specialised nature of this chaperone system, which functions only in Fe–S biogenesis (Vickery and Cupp-Vickery, 2007).

Mitochondria inherited from their endosymbiont ancestor most, but not all, of the components of ISC pathway. The scaffold (termed Isu in *S. cerevisiae*) and the specialised J-protein (termed Jac1 in *S. cerevisiae*) are present in all eukaryotes. However, the gene encoding HscA was either not inherited or lost early during evolution, as an *hscA* orthologue has not been found in any eukaryotic genome examined thus far (Huynen *et al*., 2001; Schilke *et al*., 2006). However, Fe–S cluster biogenesis is critical in all organisms. Many species, including humans, have a single Hsp70 in the mitochondria. All evidence indicates that the multifunctional mtHsp70, the component of the multifunctional core Hsp70 system, has taken over the role of HscA in Fe–S biogenesis, cooperating in this process with the specialised J-protein Jac1 (Schilke *et al*., 2006).

This evolutionary story does not end here, as a small subset of fungi, including *S. cerevisiae*, contain a highly specialised mtHsp70, Ssq1, which is involved exclusively in the process of Fe–S cluster biogenesis. Evolutionary analysis indicates that Ssq1 is encoded by a gene that arose fairly late in fungal evolution by duplication of the gene that encoded the only mitochondrially localised Hsp70 in a common ancestor of *Candida albicans* and *S. cerevisiae* (Schilke *et al*., 2006). The product of one of the gene duplicates, *SSC1*, remained abundant and multifunctional, involved in protein folding and refolding in the mitochondrial matrix, as well as in the maintenance of mtDNA and as a component the protein translocation motor. In contrast, Ssq1 is much less abundant, it specifically binds the LPPVK motif of Isu, but has lost its ability to interact with other peptides that are typical substrates of Ssc1 (Schilke *et al*., 2006). Ssq1 also lost the ability to efficiently interact with the mitochondrial J-proteins Mdj1 and Pam18 (D'Silva *et al*., 2003), but interact very efficiently with Jac1 (Dutkiewicz *et al*., 2003).

Intriguingly, the presence of Ssq1 in fungal species correlates with changes within the J-domain of Jac1, most notably its 'shortening'. All species encoding this alternative version of the J-domain share a common ancestry, suggesting that all short Jac1 proteins arose from a single deletion event. Data suggests that a dramatic change within the J-domain of Jac1 was a trigger that initiated co-evolution of Jac1 and Ssq1, leading to formation of the specialised Hsp70 system (Pukszta *et al*., 2010).

Conclusions: Versatility of Hsp70 System Allows for Adaptation to New Functions

As illustrated earlier, that adaptability of Hsp70 systems is an outgrowth of two major strategies: (i) evolution of highly specific client interactions either via specialisation of an Hsp70 to interact directly with a specific sequence of a client protein (as illustrated by Ssq1) and/or a specialised J-protein that binds and delivers a specific client to its Hsp70 partner (as illustrated by Jac1/HscB) (ii) tethering of Hsp70 system to its place of action, either by evolving new binding abilities by Hsp70 (as illustrated by mtHsp70:Tim44 interaction) or by recruiting Hsp70 to the place of its action by a properly localised J-protein partner (as illustrated by Mdj1 and the nucleoid). These strategies are not limited to mitochondria, but rather appear general, resulting in highly robust Hsp70 systems throughout the cell.

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