Published in final edited form as:

Traffic. 2006 December ; 7(12): 1596–1603. doi:10.1111/j.1600-0854.2006.00497.x.

Keep the Traffic Moving: Mechanism of the Hsp70 Motor

Rui Sousa and Eileen M. Lafer

Department of Biochemistry, University of Texas Health, Science Center at San Antonio, 7703
Floyd Curl Drive, San Antonio, TX 78229, USA

Abstract

Hsp70s are ubiquitous chaperones that use ATP hydrolysis to drive a variety of protein processing reactions, including a number of steps in protein trafficking. Recent studies have shed light on how ATP might generate conformational changes in an Hsp70 molecule and how such changes might be harnessed to drive processes as diverse as protein import into subcellular organelles and uncoating of clathrin-coated vesicles.

Keywords

chaperone; clathrin; coated vesicles; Hsc70; Hsp70; motor protein; protein import

The simplest of the processes in which an Hsp70 plays a role may be the prevention of protein aggregation through the binding and release of unfolded proteins [reviewed by Young et al. (1)]. This activity is mediated by the two domains common to all Hsp70 family members: a nucleotide-binding domain (NBD) and a protein substrate-binding domain (SBD). Binding of ATP to the NBD transmits a conformational change to the SBD that causes the latter to release substrate and primes it to bind a new substrate molecule. Hydrolysis of ATP to ADP (or more probably, release of Pi) reverses this change and locks the SBD onto the substrate [(2-6) and reviewed by Bukau and Horwich (7)].

Hsp70 alone is, however, both poorly active and nonspecific: its rate of ATP hydrolysis or nucleotide exchange is slow, and, on average, sequences that can be bound by Hsp70 occur every ~40 residues in any given protein (8). Specificity of action for the many Hsp70s found in a cell [in yeast, there are 14 representatives (9)] is largely determined by their subcellular location and by their interactions with the even more numerous J co-chaperones (21 in yeast) that contain both a J domain that interacts with and stimulates ATP hydrolysis by Hsp70 and a distinct domain that interacts with different substrates (10). Coupling of the adenosine triphosphatase (ATPase) stimulating and substrate presentation activities in the J co-chaperones is likely important for co-ordinating ATP hydrolysis and substrate binding by the Hsp70, so as to ensure that energy is not wasted in futile ATPase cycles and that the Hsp70 does not spend time in non-productive substrate-free/ ADP-bound states (11). Nucleotide exchange is catalyzed by a distinct group of co-chaperones or nucleotide exchange factors [NEFs: GrpE in *Escherichia coli*, and Bag, HspBP1/Fes1/Slf1 and Sse/

Hsp110 in eukaryotes (12–19)]. Although the description of Hsp70 activity as specified through subcellular location and the substrate presentation domain of a J protein is correct to a first approximation, it is an oversimplification. All Hsp70s are not the same, and variations in their intrinsic kinetics and (weak) substrate specificity or ability to interact with distinct J protein partners or NEFs (10) must tune different Hsp70s for different functions. Similarly, the NEFs and the J domains of different J proteins exhibit structural divergence and are not uniformly interchangeable (10–17,20,21), suggesting that differences in their affinities or modes of binding with distinct Hsp70s and variations in their effects on nucleotide hydrolysis or exchange kinetics modulate Hsp70 function as required for different cellular processes.

Among these processes are a number of protein trafficking events, including the uncoating of clathrin-coated vesicles (CCVs) (22) and the import of proteins into the endoplasmic reticulum (ER) lumen (23,24) and the mitochondrial matrix (25). The latter reactions have, in particular, been studied in an effort to understand how Hsp70s function as motors to pull proteins through transport pores (24–26). Models for this motor function have emphasized either biased diffusion in which the Hsp70 captures spontaneous fluctuations that move the substrate protein in the right direction or directed pulling mechanisms in which the Hsp70 binds a substrate and then undergoes a conformational change that exerts a pull. More recently, a mechanism termed ‘entropic pulling’ has been proposed (27), in which movement of an Hsp70-bound polypeptide segment away from a membrane or protein wall is accompanied by a favorable increase in freedom of movement (and therefore of entropy) that generates a pulling force on the polypeptide.

Recent structural and structure-function studies have markedly advanced our understanding of the mechanism of communication of conformational change between the Hsp70 SBD and NBD and the mechanisms that control ATP hydrolysis and nucleotide exchange kinetics. Characterization of the role of Hsp70s in refolding of aggregated proteins and in uncoating of CCVs suggests that not only protein translocation but also dissociation of protein aggregates or complexes may utilize the force-generating capacity of the Hsp70 motor. Finally, the development of a novel model for how such force is generated offers the possibility of providing a unified description of Hsp70 mechanism in many different processes.

Hsp70 Structure and Mechanism of Interdomain Communication

X-ray and NMR structures of isolated Hsp70 (or Hsc70) SBDs and NBDs have been known for several years (28–30), but the structure of a functionally intact chaperone (that of bovine Hsc70) was only recently revealed (Figure 1) (31). The Hsc70 NBD [amino acid (aa) 1–382] exhibits the characteristic hexokinase fold, with nucleotide binding at the bottom of a deep cleft in its center (Figure 2). ATP binding induces closing of the NBD, which may be the first step in propagating a conformational change to the SBD. Substrate binds in a pocket formed by the SBD β -sheet sandwich subdomain (aa 397–512) and is covered by the helical lid subdomain (aa 513–650) (28). Opening of the lid appears to be required for substrate release (32). The NBD is divided into four subdomains and the groove between subdomains IA and IB forms most of the interface with helix A of the SBD lid subdomain (Figure 1A),

although interactions between aa 191–193 and 415–417 of, respectively, the NBD and SBD are also functionally important. Residues forming the interdomain interface are well conserved, and mutation of almost any of these residues affects communication of conformational changes between the two domains (Figure 1C) (31,33,34). The well-conserved, hydrophobic interdomain linker (aa 387–396: VQDLLLLDV) is also essential for communication between the NBD and the SBD. ATP binding induces the linker to move from a solvent-exposed conformation to a more buried state, concomitant with a conformational change in the SBD that leads to substrate release (31,35). Substrate binding to the SBD then stimulates ATP hydrolysis and reverses this conformational change, causing the linker to become solvent exposed and the SBD to clamp onto substrate. Mutations that abolish this ATP-driven change in linker solvent exposure abolish chaperone function (5,31). Interdomain interactions at the C-terminal end of SBD helix A are primarily ionic, while the interface at the N-terminal end of this helix is hydrophobic and is close to the hydrophobic linker in nucleotide-free Hsc70 (Figure 1B, C). It is possible that the linker can compete for the hydrophobic interdomain interactions and, upon ATP binding, invades and displaces some of these interactions so as to communicate the nucleotide state of the NBD to the SBD. Support for this idea emerges from the properties of an Hsp70 fragment that includes the NBD and interdomain linker but not the SBD. ATP hydrolysis by the NBD alone is slow but the NBD + linker construct exhibits a constitutively fast ATPase rate (35,36) (Maes, Sousa, and Lafer, University of Texas Health Science Center at San Antonio, unpublished observations), suggesting that removal of the SBD allows the linker to make persistent interactions with the hydrophobic interdomain interface of the NBD that stimulate its ATPase rate.

Control of Substrate and Nucleotide Release Kinetics

Spontaneous release of substrate from Hsp70 is slow until ATP binds. There are a handful of instances in which conformational changes in proteins have been shown to be restricted through intrinsically slow proline *cis-trans* isomerization events, and Hsp70 appears to be an example of this. Gly or Ala substitutions of an invariant NBD proline that lies close to the ATP-binding site (P143 or P147 in *E. coli* Hsp70 or bovine Hsc70, respectively) cause Hsp70 to release substrate rapidly, even in the absence of ATP (34). *Cis-trans* isomerization of this proline may relay the ATP-binding signal directly through the polypeptide chain via R155 and D152 (bovine Hsc70 numbering), which interact with Q520, E523 and K524 of the SBD (Figure 2).

Both ATP and ADP bind tightly to Hsp70 and spontaneous dissociation of nucleotide from the chaperone is slow. At physiological ATP concentrations, Hsp70 is expected to exist only transiently in a nucleotide-free state, following the stimulation of nucleotide release by any one of several structurally distinct NEFs. The mechanisms of some of these are fairly well understood thanks to crystal structures of NBD:NEF complexes. The major prokaryotic NEF, GrpE and the eukaryotic Bag protein are structurally unrelated but work similarly, binding to the rim of the IB and IIB subdomains and opening up the NBD (12,13) in a reversal of the closing that accompanies nucleotide binding (Figure 2). Another NEF, HspBP1, works differently, inducing a lateral skewing of NBD subdomains IB and IIB (14). Diversity in NEF mechanism appears to be common. The Sse/Hsp110 eukaryotic co-

chaperones are homologous to Hsp70 and structurally unrelated to Bag, GrpE or HsBP1, but have NEF activity (15,16), and almost certainly operate through a mechanism distinct from other NEFs. The gross conformational changes induced by NEFs appear to be coupled to movements of Hsp70 residues that control nucleotide release. In a nucleotide-free NBD, residues Y15 and R272 at the mouth of the nucleotide-binding site are positioned so as to allow access to this site (Figure 2A) (31), while in the nucleotide-bound state, they are positioned so as to block nucleotide exit (Figure 2B) (29). Substitution of these residues with alanines accelerates nucleotide release (Jiang, Lafer, and Sousa, University of Texas Health Science Center at San Antonio, unpublished observations).

Hsp70 Can Disassociate Protein Aggregates

Hsp70s and other chaperones inhibit protein aggregation by binding to partially unfolded proteins. For some time, it was unclear whether chaperones could also rescue proteins that were already aggregated. However, work in the mid-1990s revealed that not only could Hsp70s (together with their J co-chaperone and NEF partners) inhibit protein aggregation they could also renature unfolded proteins both *in vivo* and *in vitro* (37–39). More recent work has shown directly that Hsp70, Hsp40 (the major *E. coli* J co-chaperone) and GrpE together can break up large aggregates into smaller oligomers (40) and can extract proteins from such aggregates and feed them as unfolded polypeptides into other chaperones or protease complexes (41).

Hsp70 Pulls Proteins into the ER Lumen and Mitochondrial Matrix

Proteins are imported into the ER lumen both co- and post-translationally through the heteromeric Sec61 import channel (42,43). For post-translational import, Sec61 forms a complex with the Sec62/63p tetramer (42) and recruits a luminal Hsp70 [BiP; also known as Kar2p in yeast (23)]. Protein unfolding is required for import, as proteins with tightly folded domains are inefficiently imported, and it is ATP hydrolysis by BiP that drives both post-translational protein unfolding and import (24).

Most mitochondrial proteins are synthesized by cytosolic ribosomes as preproteins with positively charged N-terminal (or multiple internal) targeting sequences and subsequently imported into mitochondria (42). The translocation machinery of the mitochondrial outer membrane (TOM) includes the Tom70, Tom22 and Tom20 preprotein receptors. Preproteins with multiple internal targeting signals are delivered to Tom70 complexed with Hsp70 alone or Hsp70 and Hsp90 and then, in an ATP-requiring process, passed through the Tom20/Tom22/Tom40 pore [reviewed by Rehling et al. and Matouschek et al. (44,45)]. The preprotein is then engaged by the translocation machinery of the inner membrane (TIM). Passage through both the TOM and the TIM pores requires unfolding of the preprotein, and ATP hydrolysis by Hsp70 is believed to drive preprotein unfolding.

Translocation of the preprotein through the outer and inner mitochondrial pores relies on two energy sources. One of these is provided by the electric potential across the inner membrane, which is positive at the outer (intermembrane space) surface and negative at the inner (matrix) surface, thus providing a force for translocation of the positively charged targeting sequence (46). The other force is provided by the mitochondrial Hsp70 (mtHsp70).

MtHsp70 is recruited to the matrix side of the TIM pore through an interaction with Tim44 (25). This positions mtHsp70 to receive the preprotein as it emerges from the pore but on binding the preprotein, mtHsp70 releases Tim44 (47). Also present on the matrix side of the pore is the Pam16/Pam18 J protein heterodimer, which stimulates ATP hydrolysis by mtHsp70, thereby locking it onto the preprotein (48,49).

Models for Force Generation by Hsp70

Debate on how Hsp70 generates the force to disrupt protein aggregates, unfold proteins or move proteins through pores has, until recently, focused on two models (45). These are illustrated in Figure 3 in the context of translocation through a pore, although their extension to protein aggregate disassociation is straightforward.

The biased diffusion model considers that an unfolded protein in a pore is free to diffuse in either direction (Figure 3A). However, binding of an Hsp70 to the emerging protein on the downstream side of the membrane will prevent it moving back up the pore. Diffusion can, however, continue to move the protein in the downstream direction, and whenever another protein segment with an Hsp70-binding site emerges, another Hsp70 molecule can bind and further block upstream movement. Because Hsp70-binding sites occur with high frequency, this process can eventually 'pull' the entire protein through the pore, and as complete domains emerge and Hsp70s are dislodged by nucleotide exchange, the folding of the protein itself will block upstream movement. In support of this mechanism, it has been found that elimination of BiP, depletion of ATP, or use of a BiP mutant that binds substrate weakly allows a translocating protein to slide back and forth within the ER translocation pore, validating the idea that the translocating protein can diffuse in both directions in the absence of a motive force provided by BiP (24). Moreover, vectorial translocation could be reconstituted when an antibody to the translocating protein was added to the luminal side of reconstituted vesicles, indicating that binding and trapping of the protein were sufficient to drive its import, even in the absence of the directed pulling that might be provided by ATP hydrolysis and conformational changes in BiP (24). In a protein aggregate, this mechanism could operate whenever fluctuations cause protein loops or termini to become free of the main body of the aggregate. Hsp70 would bind these transiently accessible segments, preventing their reassociation with the aggregate. Further fluctuations would release more loops and segments that would be bound by more Hsp70 molecules until an entire polypeptide is released.

Studies of protein import into mitochondria have, however, favored the directed pulling model (Figure 3B), at least for the import of tightly folded proteins (50). MtHsp70 mutants that interfere with the mtHsp70:Tim44 interaction block import of tightly folded proteins, although less stably folded proteins could still be imported by the mutant mtHsp70 (25). This is consistent with the idea that mtHsp70 uses Tim44 as a fulcrum when it undergoes the conformational change that pulls proteins through the pore (Figure 3B; in the case of aggregate dissociation, the fulcrum might be either the J co-chaperone or the body of the aggregate itself). Further, these mtHsp70 mutants displayed increased association with substrate proteins but less efficient translocation. Similarly, low levels of ATP led to increased mtHsp70:substrate protein association but inhibited translocation. Thus, in this

instance, there was no correlation between the ability of mtHsp70 to hold proteins and its ability to translocate them, but the latter *was* correlated with mtHsp70 binding to Tim44. The idea that Tim44 acts as a fulcrum was, however, undermined by a subsequent report that, on binding substrate, mtHsp70 releases Tim44 (47).

An ingenious new model (Figure 3C) may be able to reconcile the biased diffusion and directed pulling mechanisms (27). Consider an extended polypeptide segment emerging from a translocation pore. It can wiggle around in a large space limited only by the wall presented by the membrane and its associated proteins. When a bulky Hsp70 binds to this emerging polypeptide, its wiggle room is decreased because the Hsp70 cannot penetrate the space occupied by the membrane wall. Thus, Hsp70 binding decreases the entropy of the polypeptide chain (as well as of the Hsp70). Emergence of more polypeptide from the pore allows the polypeptide and the associated Hsp70 to wiggle around again, thereby increasing the entropy of the system and lowering its energy. Subsequent binding of another Hsp70 to the newly emerged polypeptide segment adjacent to the membrane wall would pull yet more protein through the pore, and repeated cycles would translocate the entire protein (in the case of an aggregate, the 'wall' can be the body of the aggregate itself). Mathematical modeling of entropic pulling reveals that it can generate a larger force than passive diffusion but requires neither a fulcrum nor a force generating conformational change in the Hsp70.

Models for Force Generation in the Context of CCV Uncoating

Definitively establishing a motor protein mechanism is difficult. The observation that antibodies can drive import of a protein into a luminal space, so long as the energetic barrier of protein unfolding is not too large (24), shows that import *can* be achieved by simple trapping, but this does not mean that this is how Hsp70 does it. Similarly, the observation that trapping is not sufficient for import of tightly folded proteins into the mitochondrial matrix, but that interaction with Tim44 is required (25), is not necessarily evidence that Tim44 is used as a fulcrum. The entropic pulling mechanism, for example requires not only that Hsp70 be loaded onto a polypeptide but also that this be done at the right place, i.e. directly adjacent to the membrane pore in the case of a protein import mechanism because the entropic pulling force diminishes as the Hsp70:polypeptide segment moves away from the membrane and gains freedom of motion. The action of Tim44, which binds mtHsp70 and holds it in a form receptive for substrate binding at the mouth of the TIM pore and then releases mtHsp70 as soon as substrate binds, would be at least as consistent with it acting in an entropic pulling mechanism as with it acting as a fulcrum in a directed pulling model.

The dissociation of defined protein complexes may present a set of processes in which these questions may be effectively addressed. In particular, the mechanism of uncoating of CCVs seems ripe for analysis. The structures of all the players in this reaction – Hsc70, auxilin (the J co-chaperone that recruits Hsc70 to the vesicle) and the clathrin basket itself – have been determined over the past 3 years (21,31,51–53). Inspection of the structures of clathrin baskets complexed with auxilin suggested a model in which basket dissociation involves the unstructured C-terminal tails of the clathrin heavy chains (aa 1631–75) that emerge from the helical tripods whose interactions hold together the monomers that form the clathrin triskelia (Figure 4) (53). These tails contain a likely Hsc70-binding sequence. In the cryo-electron

microscopy images from which the basket structures were determined, the tails could not be traced, reflecting either static or dynamic disorder or a limitation in the resolution of these images.

It is not clear if biased diffusion could explain uncoating of CCVs. Apart from the C-terminal tails, other unstructured loops or segments (as might be seen on the surface of an aggregate of unfolded proteins) that could be trapped by Hsc70 are not in evidence, and the extended C-terminal tails themselves are connected to the tripod helices and unable to diffuse as an unfolded protein in an import pore might.

A mechanism in which Hsc70 pulls directly on the C-terminal tails, however, seems plausible. This would not disrupt the tripods since the triskelia remain intact during uncoating, but it could direct a force on the tripod, inducing a distortion of the hub from which the tripod projects. In baskets of different sizes, the crossing angles for the heavy chain legs vary, but hub geometry is invariant (52), suggesting that this geometry is required for basket stability. Distortion of the hub by pulling on the tripod might propagate movements along the legs, leading to basket disassembly. Such a mechanism, however, may run afoul of the requirement for a fulcrum. The fulcrum could be auxilin, but if auxilin operates like a classic J co-chaperone, it would interact with Hsc70 only transiently as it stimulated the latter's ATPase activity and locked it onto the clathrin tail. Even if the auxilin:Hsc70 interaction is more persistent, the utility of auxilin as a fulcrum seems compromised because auxilin binds clathrin through a flexible and extended domain that contains multiple clathrin binding sites (51,54) and may therefore not provide a stable platform against which to push.

In this light, the entropic pulling mechanism becomes attractive. In fact, if Hsc70 mutations that restore Hsc70 interaction with a mutant auxilin (55) are used to model Hsc70 into the basket, we find that Hsc70 is placed so that its access to the C-termini of the tripod helices is blocked by segments of the heavy chain legs (Figure 4). The only way Hsc70 can bind the C-terminal tails is if the latter emerge as extended chains through the portals formed by the leg segments. The analogy to transport through a pore is direct: Hsc70 bound to the tails could abut the portal formed by the heavy chain legs. The mobility of both the Hsc70 and the tails would be restricted, and this would generate a pulling force due to the favorable entropy change that would accompany movement of Hsc70 away from the portal.

Although it is tempting to believe that a common mechanism of force generation underlies the many processes in which Hsp70 plays a role, caution is warranted. Whether this is the case will only be settled by further experimentation, but recent advances have set the stage for continued, rapid progress in our understanding of Hsp70 mechanism.

Acknowledgments

Work in the authors' laboratories is supported by the grant GM52522 and AQ-1486 from the National Institutes of Health and Welch Foundation (to R. S.) and by the grant NS29051 and MDA3473 from the National Institutes of Health and the Muscular Dystrophy Association (to E. M. L.).

References

1. Young JC, Agashe VR, Siegers K, Hartl FU. Pathways of chaperone-mediated protein folding in the cytosol. *Nat Rev Mol Cell Biol.* 2004; 5:781–791. [PubMed: 15459659]
2. Schmid D, Baici A, Gehring H, Christen P. Kinetics of molecular chaperone action. *Science.* 1994; 263:971–973. [PubMed: 8310296]
3. Greene LE, Zinner R, Naficy S, Eisenberg E. Effect of nucleotide on the binding of peptides to 70-kDa heat shock protein. *J Biol Chem.* 1995; 270:2967–2973. [PubMed: 7852376]
4. Revington M, Zhang Y, Yip GN, Kurochkin AV, Zuiderweg ER. NMR investigations of allosteric processes in a two-domain *Thermus thermophilus* Hsp70 molecular chaperone. *J Mol Biol.* 2005; 349:163–183. [PubMed: 15876376]
5. Wei J, Gaut JR, Hendershot LM. In vitro dissociation of BiP-peptide complexes requires a conformational change in BiP after ATP binding but does not require ATP hydrolysis. *J Biol Chem.* 1995; 270:26677–26682. [PubMed: 7592894]
6. Wilbanks SM, Chen L, Tsuruta H, Hodgson KO, McKay DB. Solution small-angle X-ray scattering study of the molecular chaperone Hsc70 and its subfragments. *Biochemistry.* 1995; 34:12095–12106. [PubMed: 7547949]
7. Bukau B, Horwich AL. The Hsp70 and Hsp60 chaperone machines. *Cell.* 1998; 92:351–366. [PubMed: 9476895]
8. Rudiger S, Germeroth L, Schneider-Mergener J, Bukau B. Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. *EMBO J.* 1997; 16:1501–1507. [PubMed: 9130695]
9. James P, Pfund C, Craig EA. Functional specificity among Hsp70 molecular chaperones. *Science.* 1997; 275:387–389. [PubMed: 8994035]
10. Walsh P, Bursac D, Law YC, Cyr D, Lithgow T. The J-protein family: modulating protein assembly, disassembly and translocation. *EMBO Rep.* 2004; 5:567–571. [PubMed: 15170475]
11. Misselwitz B, Staack O, Rapoport TA. J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences. *Mol Cell.* 1998; 2:593–603. [PubMed: 9844632]
12. Harrison CJ, Hayer-Hartl M, Di Liberto M, Hartl F, Kuriyan J. Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. *Science.* 1997; 276:431–435. [PubMed: 9103205]
13. Sondermann H, Scheufler C, Schneider C, Hohfeld J, Hartl FU, Moarefi I. Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. *Science.* 2001; 291:1553–1557. [PubMed: 11222862]
14. Shomura Y, Dragovic Z, Chang HC, Tzvetkov N, Young JC, Brodsky JL, Guerriero V, Hartl FU, Bracher A. Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. *Mol Cell.* 2005; 17:367–379. [PubMed: 15694338]
15. Raviol H, Sadlish H, Rodriguez F, Mayer MP, Bukau B. Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J.* 2006; 25:2510–2518. [PubMed: 16688211]
16. Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU. Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. *EMBO J.* 2006; 25:2519–2528. [PubMed: 16688212]
17. Miao B, Davis JE, Craig EA. Mge1 functions as a nucleotide release factor for Ssc1, a mitochondrial Hsp70 of *Saccharomyces cerevisiae*. *J Mol Biol.* 1997; 265:541–552. [PubMed: 9048947]
18. Kabani M, Beckerich JM, Brodsky JL. Nucleotide exchange factor for the yeast Hsp70 molecular chaperone Ssa1p. *Mol Cell Biol.* 2002; 22:4677–4689. [PubMed: 12052876]
19. Kabani M, Beckerich JM, Gaillardin C. Sls1p stimulates Sec63p-mediated activation of Kar2p in a conformation-dependent manner in the yeast endoplasmic reticulum. *Mol Cell Biol.* 2000; 20:6923–6934. [PubMed: 10958688]
20. Hennessy F, Nicoll WS, Zimmermann R, Cheetham ME, Blatch GL. Not all J domains are created equal: implications for the specificity of Hsp40-Hsp70 interactions. *Protein Sci.* 2005; 14:1697–1709. [PubMed: 15987899]

21. Jiang J, Taylor AB, Prasad K, Ishikawa-Brush Y, Hart PJ, Lafer EM, Sousa R. Structure-function analysis of the auxilin J-domain reveals an extended Hsc70 interaction interface. *Biochemistry*. 2003; 42:5748–5753. [PubMed: 12741832]
22. Rothman JE, Schmid SL. Enzymatic recycling of clathrin from coated vesicles. *Cell*. 1986; 46:5–9. [PubMed: 2872968]
23. Lyman SK, Schekman R. Binding of secretory precursor polypeptides to a translocon subcomplex is regulated by BiP. *Cell*. 1997; 88:85–96. [PubMed: 9019409]
24. Matlack KE, Misselwitz B, Plath K, Rapoport TA. BiP acts as a molecular ratchet during posttranslational transport of prepro-alpha factor across the ER membrane. *Cell*. 1999; 97:553–564. [PubMed: 10367885]
25. Voisine C, Craig EA, Zufall N, von Ahsen O, Pfanner N, Voos W. The protein import motor of mitochondria: unfolding and trapping of preproteins are distinct and separable functions of matrix Hsp70. *Cell*. 1999; 97:565–574. [PubMed: 10367886]
26. Pilon M, Schekman R. Protein translocation: how Hsp70 pulls it off. *Cell*. 1999; 97:679–682. [PubMed: 10380919]
27. De Los Rios P, Ben-Zvi A, Slutsky O, Azem A, Goloubinoff P. Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. *Proc Natl Acad Sci U S A*. 2006; 103:6166–6171. [PubMed: 16606842]
28. Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrickson WA. Structural analysis of substrate binding by the molecular chaperone DnaK. *Science*. 1996; 272:1606–1614. [PubMed: 8658133]
29. Flaherty KM, Wilbanks SM, DeLuca-Flaherty C, McKay DB. Structural basis of the 70-kilodalton heat shock cognate protein ATP hydrolytic activity. II. Structure of the active site with ADP or ATP bound to wild type and mutant ATPase fragment. *J Biol Chem*. 1994; 269:12899–12907. [PubMed: 8175707]
30. Morshauser RC, Hu W, Wang H, Pang Y, Flynn GC, Zuiderweg ER. High-resolution solution structure of the 18 kDa substrate-binding domain of the mammalian chaperone protein Hsc70. *J Mol Biol*. 1999; 289:1387–1403. [PubMed: 10373374]
31. Jiang J, Prasad K, Lafer EM, Sousa R. Structural basis of interdomain communication in the Hsc70 chaperone. *Mol Cell*. 2005; 20:513–524. [PubMed: 16307916]
32. Slepnev SV, Witt SN. Kinetic analysis of interdomain coupling in a lidless variant of the molecular chaperone DnaK: DnaK's lid inhibits transition to the low affinity state. *Biochemistry*. 2002; 41:12224–12235. [PubMed: 12356325]
33. Montgomery DL, Morimoto RI, Gierasch LM. Mutations in the substrate binding domain of the Escherichia coli 70 kDa molecular chaperone, DnaK, which alter substrate affinity or interdomain coupling. *J Mol Biol*. 1999; 286:915–932. [PubMed: 10024459]
34. Vogel M, Bukau B, Mayer MP. Allosteric regulation of Hsp70 chaperones by a proline switch. *Mol Cell*. 2006; 21:359–367. [PubMed: 16455491]
35. Rist W, Graf C, Bukau B, Mayer MP. Amide hydrogen exchange reveals conformational changes in hsp70 chaperones important for allosteric regulation. *J Biol Chem*. 2006; 281:16493–16501. [PubMed: 16613854]
36. Gierasch LM, Swain JF, Smock RG, Dinler G. A multipronged approach to the mechanism of allostery in Hsp70 chaperones. *The FASEB Journal*. 2006; 20:A964.
37. Schroder H, Langer T, Hartl FU, Bukau B. DnaK, DnaJ and GrpE form a cellular chaperone machinery capable of repairing heat-induced protein damage. *EMBO J*. 1993; 12:4137–4144. [PubMed: 7900997]
38. Levy EJ, McCarty J, Bukau B, Chirico WJ. Conserved ATPase and luciferase refolding activities between bacteria and yeast Hsp70 chaperones and modulators. *FEBS Lett*. 1995; 368:435–440. [PubMed: 7635193]
39. Freeman BC, Myers MP, Schumacher R, Morimoto RI. Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. *EMBO J*. 1995; 14:2281–2292. [PubMed: 7774586]

40. Zietkiewicz S, Lewandowska A, Stocki P, Liberek K. Hsp70 chaperone machine remodels protein aggregates at the initial step of Hsp70-Hsp100-dependent disaggregation. *J Biol Chem*. 2006; 281:7022–7029. [PubMed: 16415353]
41. Weibezahn J, Tessarz P, Schlieker C, Zahn R, Maglica Z, Lee S, Zentgraf H, Weber-Ban EU, Dougan DA, Tsai FT, Mogk A, Bukau B. Thermo-tolerance requires refolding of aggregated proteins by substrate translocation through the central pore of ClpB. *Cell*. 2004; 119:653–665. [PubMed: 15550247]
42. Schatz G, Dobberstein B. Common principles of protein translocation across membranes. *Science*. 1996; 271:1519–1526. [PubMed: 8599107]
43. Hanein D, Matlack KE, Jungnickel B, Plath K, Kalies KU, Miller KR, Rapoport TA, Akey CW. Oligomeric rings of the Sec61p complex induced by ligands required for protein translocation. *Cell*. 1996; 87:721–732. [PubMed: 8929540]
44. Rehling P, Brandner K, Pfanner N. Mitochondrial import and the twin-pore translocase. *Nat Rev Mol Cell Biol*. 2004; 5:519–530. [PubMed: 15232570]
45. Matouschek A, Pfanner N, Voos W. Protein unfolding by mitochondria. The Hsp70 import motor. *EMBO Rep*. 2000; 1:404–410.
46. Martin J, Mahlke K, Pfanner N. Role of an energized inner membrane in mitochondrial protein import. Delta psi drives the movement of presequences. *J Biol Chem*. 1991; 266:18051–18057.
47. Liu Q, D'Silva P, Walter W, Marszalek J, Craig EA. Regulated cycling of mitochondrial Hsp70 at the protein import channel. *Science*. 2003; 300:139–141. [PubMed: 12677068]
48. D'Silva PR, Schilke B, Walter W, Craig EA. Role of Pam16's degenerate J domain in protein import across the mitochondrial inner membrane. *Proc Natl Acad Sci U S A*. 2005; 102:12419–12424. [PubMed: 16105940]
49. D'Silva PD, Schilke B, Walter W, Andrew A, Craig EA. J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix. *Proc Natl Acad Sci U S A*. 2003; 100:13839–13844. [PubMed: 14605210]
50. Eilers M, Schatz G. Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria. *Nature*. 1986; 322:228–232. [PubMed: 3016548]
51. Gruschus JM, Han CJ, Greener T, Ferretti JA, Greene LE, Eisenberg E. Structure of the functional fragment of auxilin required for catalytic uncoating of clathrin-coated vesicles. *Biochemistry*. 2004; 43:3111–3119. [PubMed: 15023062]
52. Fotin A, Cheng Y, Sliz P, Grigorieff N, Harrison SC, Kirchhausen T, Walz T. Molecular model for a complete clathrin lattice from electron cryomicroscopy. *Nature*. 2004; 432:573–579. [PubMed: 15502812]
53. Fotin A, Cheng Y, Grigorieff N, Walz T, Harrison SC, Kirchhausen T. Structure of an auxilin-bound clathrin coat and its implications for the mechanism of uncoating. *Nature*. 2004; 432:649–653. [PubMed: 15502813]
54. Scheele U, Kalthoff C, Ungewickell E. Multiple interactions of auxilin 1 with clathrin and the AP-2 adaptor complex. *J Biol Chem*. 2001; 276:36131–36138. [PubMed: 11470803]
55. Suh WC, Burkholder WF, Lu CZ, Zhao X, Gottesman ME, Gross CA. Interaction of the Hsp70 molecular chaperone, DnaK, with its cochaperone DnaJ. *Proc Natl Acad Sci U S A*. 1998; 95:15223–15228. [PubMed: 9860950]
56. Sousa MC, McKay DB. The hydroxyl of threonine 13 of the bovine 70-kDa heat shock cognate protein is essential for transducing the ATP-induced conformational change. *Biochemistry*. 1998; 37:15392–15399. [PubMed: 9799500]

Figure 1. Structure and conformational mechanisms of Hsc70

A) Ribbon model of bovine Hsc70 (aa 1–554) (PDB 1YUW) with NBD (aa 1–382) in cyan, SBD (aa 397–554) in orange and interdomain linker (aa 383–396) in magenta. Helix A of the SBD rests in the groove between NBD subdomains IA and IIA. The construct used for structure determination had 10 kD deleted from its C-terminus and, as a consequence, the C-terminal region is unwound and residues 539–544 (in green) bind as an extended polypeptide in the substrate-binding site. B) Ribbon model of bovine Hsc70 colored as in A but rotated by 90 degrees as indicated. C) Interdomain interface and linker. The main chains of the NBD, SBD and linker are in cyan, orange, and magenta, respectively. Side chains are red for negative, blue for positive, yellow for polar and gray for hydrophobic. Mutations of residues highlighted in yellow affect interdomain communication.

Figure 2. Relaying of the ATP/substrate-binding signals and control of substrate and nucleotide release kinetics

A) Ribbon model of the nucleotide-free NBD and helix A of the SBD (coloring as in Figure 1). Side chains putatively involved in gating nucleotide release or relaying ligand-binding signals between the SBD and the NBD are in ball-and-stick representation. The NBD is in 'open', and residues Y15 and R272 allow access to the nucleotide-binding pocket. B) Ribbon model of the NBD with ATP (in green) bound (PDB 1KAX): the NBD is closed and Y15 and R272 block nucleotide release. Relaying of the ATP-binding signal to the SBD may involve T13, which moves in response to ATP binding and is essential for communicating the nucleotide state of the NBD to the SBD (56). Movement of T13 may affect β -sheet 10 through the latter's interactions with β -sheet 1 (both highlighted in magenta), and conformational information may then be communicated through the polypeptide backbone to R155/D152, which make ionic interactions with Q520/E523/K524 of the SBD. The kinetics of information transmission may be restricted by slow *cis-trans* isomerization of P147 (34).

Figure 3. Models for Hsp70 protein translocation mechanism

A) Trapping or biased diffusion: 1) an unfolded protein moving through a pore can diffuse in either direction, 2) binding of Hsp70 to the protein on the downstream side of the pore blocks its upstream, but not downstream, diffusion and 3) whenever diffusion exposes another protein segment with an Hsp70-binding site on the downstream side of the pore another Hsp70 can bind. Eventually, the entire protein will be transported to the downstream side of the pore. B) Directed pulling: 2) the protein is bound by Hsp70, and the latter physically abuts or binds to the transport pore and 3) using the pore as a fulcrum, Hsp70 undergoes a conformational changes that pulls the protein in the downstream direction. C) Entropic pulling: 1) the unfolded protein segment that has emerged on the downstream side of the pore can wiggle around in a volume limited only by the wall presented by the pore and membrane, 2) binding of Hsp70 near the pore restricts the freedom of movement of the unfolded protein segment and the Hsp70, decreasing the entropy ($\Delta S < 0$) and 3) emergence of more protein on the downstream side of the pore increases the freedom of movement of the unfolded protein and the associated Hsp70, increasing the entropy.

Figure 4. Uncoating of CCVs by Hsc70

A) Structure of clathrin heavy chain/auxilin basket (PDB 1XI5). The smallest unit from which a basket can be generated by symmetry operations is shown, centered on a vertex with the clathrin terminal domain (TD; aa 1–331) in green, the linker and ankle (aa 331–800) in cyan, the distal leg (800–1200) in purple, the proximal leg (aa 1200–1597) in blue and the tripod (aa 1598–1630) in rose. The auxilin J domain is in yellow. Suppression of mutants of auxilin D876 (in red) by mutations of Hsc70 R172 indicates that these two residues approach each other in the complex, allowing Hsc70 (shown with the SBD in red and the NBD in magenta) to be modeled onto the auxilin. B) The structure from A, rotated as indicated so that the view is from the interior of the basket and centered on a vertex. The TD, auxilin and tripod are labeled. One Hsc70 molecule is modeled ('1'), and two others would occupy the threefold related positions in the ellipses labeled '2' and '3'. Elements of the heavy chain legs lie interposed between the tripod and the Hsc70s. C) Expanded view of the boxed region from A, with the Hsc70 removed, but its position indicated by an ellipse. Also suggested is how the extended C-terminal tail (in pink) could emerge from the portal formed by the ankle and distal legs to be bound by the Hsc70 molecule. D) Schematic of the organization of the uncoating machinery: Hsc70 may bind the C-terminal tail in a manner analogous to an unfolded polypeptide emerging from an import pore.