Protein folding: **Chaperones get Hip**
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The discovery of a new co-chaperone, Hip, that interacts with Hsp70 underscores the complexity of the Hsp70 'chaperone machine' that mediates early steps of protein folding in cells.

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In the cell, nascent and completed polypeptides may misfold and aggregate. A class of proteins called molecular chaperones has evolved to facilitate the production of native, functional forms of proteins. These chaperones also aid in the translocation of proteins across biological membranes. The ubiquitous heat-shock protein (Hsp) 70 class of chaperones has been the subject of intensive study over the last decade. Hsp70s participate in the processes of protein folding and translocation through their ability to recognize non-native conformations of proteins. Hsp70s bind preferentially to short, extended, peptide segments containing hydrophobic amino acids that may become exposed during translation, translocation or unfolding.

To understand the diverse roles of Hsp70, we must also understand the interaction of Hsp70 with the co-chaperones that are essential for its function. Results from diverse experimental systems support the notion that Hsp70 is in fact the 'core' of a multisubunit chaperone complex. Recent papers from the laboratories of Franz-Ulrich Hartl [1] and David Smith [2] add a new component to the eukaryotic Hsp70 protein-folding machine. The observations reported by these two groups have important implications for our understanding of Hsp70 function in the eukaryotic cytosol.

The DnaK–DnaJ–GrpE model of Hsp70 function

Studies of the Hsp70 of *Escherichia coli*, DnaK, and its cohort proteins, DnaJ and GrpE, have provided a paradigm for the function of the Hsp70 chaperone (reviewed in [3]). Like all other Hsp70 proteins that have been studied, DnaK consists of two major domains: a 44 kDa amino-terminal ATPase domain followed by a smaller peptide-binding domain. Although the peptide-binding domain is defined as such for empirical reasons, its most likely ligand *in vivo* is an unfolded polypeptide chain. The ligand-binding properties of the peptide-binding domain reflect the occupancy of the ATPase domain by either ADP, which promotes stable peptide binding, or ATP, which results in unstable binding [4]. Although the crystal

structure of intact Hsp70 remains elusive, indirect evidence supports the existence of a nucleotide-dependent conformational switch. DnaJ serves to accelerate the hydrolysis of DnaK-bound ATP, thereby driving the equilibrium towards the ADP-bound state, whereas GrpE interacts with the ATPase domain of DnaK to accelerate the rate of nucleotide exchange and facilitate the rebinding of ATP [5] (Fig. 1a). These two co-chaperones serve, therefore, to efficiently interconvert DnaK between two conformational states.

How do these cycles of peptide binding and release relate to the ability of these proteins to modulate protein folding *in vitro*? In studies of the refolding of rhodenase and luciferase by DnaK and its cohorts, DnaJ bound to the unfolded protein and prevented its aggregation but was unable to restore the native conformation [6,7]. For refolding to occur, interaction with DnaK was required, a process facilitated by DnaJ. The DnaK–unfolded-protein complex

Figure 1
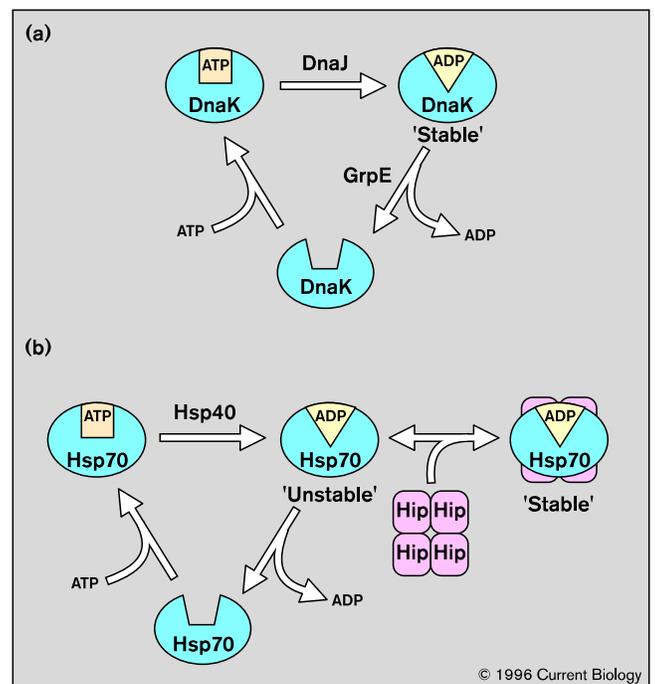
Models for the ATPase reaction cycle of DnaK **(a)** and cytosolic Hsp70 ATPase **(b)**. In (a), DnaJ stimulates the hydrolysis of bound ATP to ADP, and GrpE stimulates the dissociation of the relatively stable DnaK–ADP complex. In (b), Hsp40 (DnaJ homolog) stimulates ATP hydrolysis, and Hip binds to the resulting Hsp70–ADP complex, forming a relatively stable Hip–Hsp70–ADP complex.

must, in turn, dissociate to allow the completion of folding, either through the assistance of the GroE–Hsp60 complex, as in the case of rhodenase, or in the absence of additional proteins, as is observed with luciferase. GrpE acts at this dissociation step, facilitating the release of bound ADP and, consequently, the unfolded polypeptide from DnaK.

Is there a distinct set of co-chaperones for cytosolic Hsp70?
The apparent simplicity of the DnaK–DnaJ–GrpE system is very appealing and, given the high degree of conservation among Hsp70 proteins across phylogenetic groups, might be expected to extend to the Hsp70 chaperone complexes of eukaryotes as well. In keeping with the endosymbiont hypothesis, a very similar set of proteins does indeed exist in mitochondria [8,9]. The eukaryotic cytosol appears to be another matter, however. Although cytosolic DnaJ homologs have been identified in species as diverse as yeast and human (reviewed in [10]), no cytosolic GrpE homologs have been identified, despite the efforts of several laboratories over recent years.

The biochemical properties of cytosolic Hsp70s may provide an explanation for the apparent absence of cytosolic GrpE homologs. For example, although the intrinsic ATPase activities of many Hsp70s are very similar, the ability of the yeast DnaJ homolog, Ydj1p, to stimulate the ATPase activity of its corresponding Hsp70 partner is significantly greater than that reported for the DnaK–DnaJ system [11], in which GrpE is also required for maximal stimulation of the steady-state ATPase activity. Furthermore, polypeptide substrates decrease the stability of nucleotide binding to eukaryotic Hsp70 [12,13], an effect not observed with DnaK. These results suggest that there are significant differences in the nucleotide-binding properties of DnaK and eukaryotic Hsp70. More compellingly perhaps, studies of the Hsp70-dependent refolding of luciferase have shown that eukaryotic Hsp70 requires only an appropriate DnaJ homolog for folding activity [14,15].

Our understanding of the differences between prokaryotic and eukaryotic Hsp70 chaperones moves a step forward with the recent results of the Hartl and Smith laboratories [1,2]. In a search for Hsp70-interacting proteins using the yeast two-hybrid system, Hartl and colleagues identified a previously unknown gene that encodes a protein, dubbed Hip, able to interact with the amino-terminus of rat Hsc70 [1]. Independently, Smith and colleagues [2] cloned the gene for human p48, a Hsp70-binding protein that interacts transiently with steroid-hormone receptor complexes. The human and rat proteins are 90 % identical, strongly suggesting that they are functional homologs.

As reported by Hartl and colleagues [1], the tetrameric Hip protein can suppress the aggregation of unfolded rhodenase *in vitro*. Thus, much like DnaJ, Hip by itself functions as a molecular chaperone. In addition to the expected interaction with Hsp70, Hip also bound to bovine liver Hsp40 (a DnaJ homolog) and Hsp90. The identification of Hsp40 in the Hip–Hsp70 complex prompted examination of the effects of Hip on luciferase refolding. Under conditions where relatively little reactivation of luciferase was observed in the presence of purified bovine Hsc70 and recombinant Hsp40, the addition of Hip increased the yield of active luciferase about five-fold, resulting in the recovery of more than half of the initial activity.

The effect of Hip on the efficiency of luciferase reactivation is intriguing, especially in light of the biochemical differences observed between DnaK and cytosolic Hsp70. From studies examining the nucleotide dependence of the Hip–Hsp70 interaction, it appears that Hip binds preferentially to the ADP-bound form of Hsp70. In the presence of 1 mM ADP, the formation of the Hip–Hsp70 complex required no additional factors. In the presence of 1 mM ATP, however, the Hip–Hsp70 complex was recovered efficiently only in the presence of Hsp40. The effect of Hip on the stability of Hsp70–nucleotide complexes was assessed by separating bound from free nucleotide and determining the ADP:ATP ratio in the complex. Although the presence of Hip appeared not to affect the rate of ATP hydrolysis by Hsp70, it did result in a higher proportion of ADP in the Hsp70–nucleotide complex. Given the demonstrated ability of Hsp40 to stimulate the ATPase activity of Hsp70, interaction with Hsp40 would serve to convert Hsp70 to its ADP-bound state, which would, in turn, be stabilized by the binding of Hip (Fig. 1b).

The Hsp70–Hip–hormone connection
How can these studies of purified chaperone components *in vitro* be compared to what is known about the complex interactions of chaperones *in vivo*? The identification of Hip as a component of a hormone receptor complex provides a context for understanding the Hip–Hsp70 interaction in an *in vivo* system. In the absence of hormone, steroid hormone receptors form large multi-protein complexes that are inactive, but able to bind and respond to hormone. Among the components of the complex are the heat shock proteins Hsp70 and Hsp90 (reviewed in [16]). Upon hormone binding, the complex disassembles, leading to the activation of the steroid hormone receptors as transcription factors. The interaction of Hsp90 and other associated proteins is essential for hormone binding and receptor activation, suggesting they function to maintain the receptor in the proper hormone-binding conformation [17].

The inactive progesterone receptor complex can be reconstituted *in vitro* by incubation of progesterone receptor in rabbit reticulocyte lysate in the presence of ATP [18]. In the paper by Smith and colleagues [2], p48 (Hip) is identified as a component of progesterone receptors assembled in rabbit reticulocyte lysate. Co-immunoprecipitation studies support the direct interaction between Hip and

Figure 2
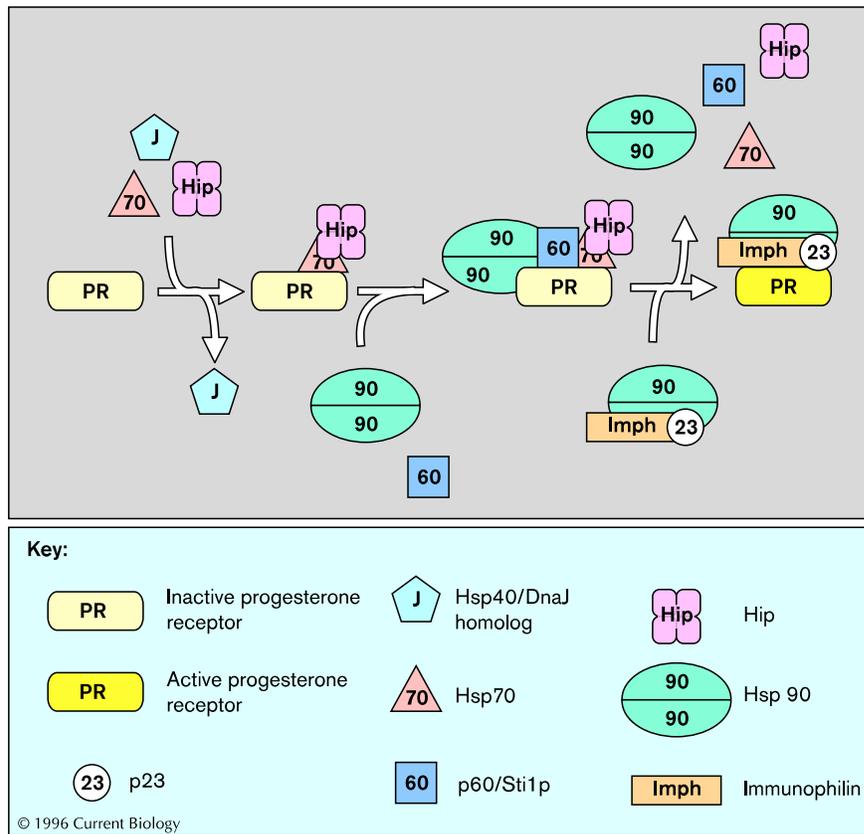
Model for the sequential interaction of the progesterone receptor (PR) with chaperones, leading to the formation of active receptor that can bind hormone. See text for further details.

Hsp70. In agreement with Hartl and colleagues [1], the co-precipitation of Hsp70 with Hip was enhanced by the presence of ADP.

Hip and Hsp70 bind early in the assembly of progesterone receptor complexes, along with Hsp90 and p60, the homolog of yeast Sti1 (which has been shown to interact with Hsp70 and Hsp90 in yeast [19]). This interaction of the progesterone receptor with Hsp70, Hip, p60 and Hsp90 is transient, however, and does not convey the ability to bind progesterone. The form of the progesterone receptor complex that binds hormone lacks Hip and p60 and contains the additional factors p23 and immunophilins (Fig. 2). The formation of this mature form of the progesterone receptor is dependent upon ATP hydrolysis. Notably, when ATP levels in rabbit reticulocyte lysate are limiting, the predominant form of the progesterone receptor is bound to Hsp70, Hip, p60 and Hsp90. These results are consistent with the increased interaction of Hip with Hsp70 in the presence of ADP, and could represent the stabilization of the ADP-bound form of Hsp70 in a complex with the progesterone receptor.

What do these recent observations tell us about the mechanism of Hsp70 action in the eukaryotic cytosol? First,

they support the view that cytosolic Hsp70 forms a dynamic complex with numerous co-chaperones. Clearly, Hip/p48 and DnaJ homologs [17,20] are co-chaperones that interact directly with cytosolic Hsp70 to modulate its activity. Other components of this dynamic complex include Hsp90 and p60/Sti1. The fact that Hip has some limited sequence similarity to p60/Sti1 raises interesting questions about the evolutionary origins of these uniquely cytosolic co-chaperones. Second, these observations further demonstrate that cytosolic Hsp70 has a different requirement for co-chaperones than does its bacterial equivalent, DnaK. Although the comparative analysis of the biochemistry of these Hsp70s is far from complete, the apparent stability of the ADP form of DnaK compared to the ADP form of cytosolic Hsp70 provides a plausible explanation for the requirement for a nucleotide release factor — GrpE — in the DnaK reaction cycle. Hip may be required, as Hartl and colleagues [1] suggest, to compensate for the relative instability of the ADP form of cytosolic Hsp70. In both systems, co-chaperones function to maintain a delicate balance between the different conformational states of Hsp70. The continued study of these diverse Hsp70 systems will undoubtedly lead to a better understanding of the rules governing chaperone function in the cell.

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