Interaction of BiP with the J-domain of the Sec63p Component of the Endoplasmic Reticulum Protein Translocation Complex*

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Hsp70s exist in most cellular compartments. They participate in many different processes, including protein folding, assembly and disassembly of protein complexes, uncoating of clathrin-coated vesicles, and transport of proteins across membranes (for review, see Refs. 1–4). Hsp70s are able to perform these apparently different tasks because of their ability to reversibly bind polypeptides. Hsp70s bind peptides through a peptide binding pocket which is regulated by the NH2-terminal ATPase domain (for review, see Ref. 4). With ATP bound to the ATPase domain, the peptide binding pocket is open and peptides can rapidly bind and dissociate. In the presence of ADP, the pocket is closed and covered by a lid domain (5), and association and dissociation of peptides are much slower (6, 7).

To bind substrates under physiological conditions Hsp70s cooperate with their co-factors, the J-proteins (for review, see Ref. 8). J-proteins are a ubiquitous family defined by a conserved domain of about 70 amino acids, the J-domain. In addition, they contain other, nonconserved domains which are responsible for association of the J-proteins with different polypeptide substrates. It is believed that the J-domain interacts with Hsp70 molecules and stimulates their ATPase activity (9). The ensuing rapid closure of the peptide binding pocket would trap the substrate within the closed pocket of the ADP form of the Hsp70 molecule. The J-domain would thus stimulate peptide binding by Hsp70s (10, 11). Release of the bound Hsp70 molecule from the peptide substrate would occur when exchange of ADP for ATP re-opens the peptide binding pocket (11). Under equilibrium conditions, an isolated Hsp70 has a preference for peptide sequences of at least seven mostly hydrophobic amino acids (12–14). Activation of the J-domain causes a steady-state situation which allows an Hsp70 to bind even polar peptides (11).

Two models have been proposed for how the J-domain interacts with an Hsp70. In one model, the ATP form of the Hsp70 would bind to the J-domain, the nucleotide would be hydrolyzed, and the Hsp70 would be stably bound in its ADP form to the J-domain. This would represent an activated form of the Hsp70 which could subsequently be transferred to a peptide substrate (15–17). This mechanism is supported by experiments in which eukaryotic Hsp70s were found to be bound in their ADP form to fusion proteins of a J-domain to glutathione S-transferase (GST-J)† (16, 17). However, it was not demonstrated that the GST-J-Hsp70-ADP complex represented a precursor for the Hsp70-peptide substrate interaction. In an alternative model, the interaction of the Hsp70 with the J-domain would be extremely transient, even in the absence of a peptide substrate (see Ref. 4, and references therein). In the presence of a substrate, the Hsp70 would immediately be transferred from the J-domain to the peptide, resulting in a stable peptide-Hsp70-ADP complex. This view is supported by experiments involving DnaK, the bacterial Hsp70, and its J-partner DnaJ (10). It is possible that the different results reflect fundamental differences between the eukaryotic and prokaryotic Hsp70 systems.

A well studied system in which an Hsp70 family member plays an essential role is the post-translational transport of proteins across the membrane of the yeast endoplasmic reticulum (for review, see Refs. 18 and 19). The lumenal Hsp70 family member BiP (Kar2p) is required to transport proteins through a channel in the endoplasmic reticulum membrane formed by the Sec complex (20–23). The J-partner for BiP is a lumenal domain of the Sec63p component of the Sec complex (15, 17, 24, 25). Upon interaction with the J-domain of Sec63p, BiP binds to the translocation substrate and prevents its backwards movement through the channel, thus acting as a molecular ratchet.‡ Whether BiP also actively “pulls” on the substrate is unclear (26).

In the present study, we have investigated the binding of yeast BiP to GST-J and to the Sec complex. We demonstrate

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‡ The abbreviations used are: GST, glutathione S-transferase; GST-J, fusion protein containing the J-domain and GST; GSH, glutathione; SPR, surface plasmon resonance; RU, response units; ppF, pre-pro-factor; EDC, N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide hydrochloride.

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that, in the absence of added peptides, BiP binds to GST-J as if it was a peptide substrate. BiP appears to interact with the J-domain only very transiently, but this brief interaction is sufficient to induce nucleotide hydrolysis and activate BiP for peptide binding. Thus, BiP does not “wait” for the substrate while bound to the J-domain. Our data indicate that the transient nature of the Hsp70-J interaction is conserved between eukaryotes and prokaryotes.

**EXPERIMENTAL PROCEDURES**

**Cloning and Purification of GST-J**—DNA encoding the J-domain of SEC63 was amplified by polymerase chain reaction using 5'-primer, CCGCGGGATCCCCACAAAATTATTTGATCCTTATG, and 3'-primer, CGCGGAATTCGCCGATGTTGATGTTGATGTTGATGAGCCGTTATTCAAAAT. The product was cloned into pGEX-3x (Pharmacia). The GST-J fusion protein was purified essentially as described in Ref. 17, using its affinity to a GST column (Pharmacia) and a Ni-NTA column (Qiagen), extensively dialyzed against buffer A (150 mM KCl, 50 mM HEPES, pH 7.0, 5 mM MgCl2), and frozen in aliquots.

**Purification of BiP and BiP Mutants**—The plasmid pMR2660 encoding wild type BiP was a kind gift from Mark Rose. The plasmids encoding BiP mutants T249G, G247D, and T59G have been described (27, 28). Plasmids encoding the BiP peptide binding mutants G488D, V519R, and T448R were made with the P-Alter system (Promega). The proteins were purified as described (22) and gel filtered with a PD10 column (Pharmacia) equilibrated in buffer A. All protein concentrations were determined, using the method described in Ref. 29.

**SPR Experiments**—Experiments were done on a BIAcore upgrade machine, using CM5 research grade chips (BIAcore) at 25 °C. Buffer A was used as the running buffer at a flow rate of 5–7 μl/min. GST-J was immobilized in a sodium acetate buffer, pH 4–5, via NH2-specific cross-linking following the instructions of the manufacturer (BIAcore). The chip was first activated using EDC and N-hydroxysuccinimide, followed by the immobilization of GST-J and quenching of excess reagent with ethanolamine. If not otherwise indicated, the chip was first equilibrated with 1 mM MgCl2, 0.5 mg/ml leupeptin, 0.25 μg/ml elastin, and 0.1 mg/ml pepstatin A as protease inhibitors and 2 mM nucleotide, followed by the injection of BiP in an otherwise identical buffer. The chip was regenerated by a brief injection of 1% Triton and 1 mM ATP in buffer A. The chip did not lose more than 10% of its BiP binding activity after 12 rounds of BiP injections.

For the dissociation experiments, BiP in buffer A including 2 mM ATP and protease inhibitors was injected first, followed by injection of 2 mM nucleotide, with or without 2 mM peptide P5 in an otherwise identical buffer lacking BiP. For the peptide inhibition studies, the chip was equilibrated as described, in the presence of peptide followed by the injection of BiP in an otherwise identical buffer. Peptide binding studies were done as described previously (11). The chip was first equilibrated in buffer with nucleotide and protease inhibitors as described above with or without GST-J, followed by injection of BiP in an otherwise identical buffer.

**Binding of BiP to the Sec Complex**—The Sec complex was purified as described previously (22) from a wild type or a sec63-1 strain. For experiments with the Sec complex, streptavidin chips (BIAcore) were used with buffer B (150 mM KCl, 50 mM HEPES, pH 7.0, 5 mM MgCl2, 1 mM MnCl2, 5 mM CaCl2, 7 mM β-mercaptoethanol, 0.5% digitonin (Merck)). The Sec complex was immobilized by first injecting saturating amounts of biotinylated protein A, followed by a polyclonal antibody raised against a peptide epitope at the cytosolic part of Sec62p, and finally the Sec complex. By this procedure, approximately 1200 RU Sec complex could be immobilized. For the injection of BiP, the chip was first equilibrated in a buffer identical to buffer B, except instead of digitonin the detergent Deoxy-Big-Chap (Calbiochem) was used in the presence of 1 mM ATP or ADP, followed by an injection of BiP in an otherwise identical buffer. BiP also bound specifically to the Sec complex in the presence of the detergent digitonin (data not shown), although somewhat more weakly, most likely because the larger micelles of the detergent digitonin are able to shield parts of the J-domain of Sec63p.

**ATPase Experiments**—BiP (32 μM) was incubated either with or without peptide P5 at 1 mM and the indicated concentration of J-GST in buffer A at room temperature in a final volume of 20 μl. The reactions were started by adding a mixture of 4 mM ATP and [γ-32P]ATP and incubated for 120 min. At different time points, samples were taken and applied to polyethyleneimide-cellulose. The cellulosate sheets were developed by thin layer chromatography in a 0.5 M LiCl, 0.5 M formic acid buffer, and analyzed using a Fujix Phosphorimager. If not otherwise indicated, chemicals were purchased from Sigma.

**Release of pppF from the Sec Complex**—Experiments were done exactly as described previously (28).

**RESULTS**

**BiP Binds to GST-J**—In previous experiments stable interactions between Hsp70 family members and fusion proteins of their J-protein partners with GST were observed (16, 17), but the nature of these complexes remained unclear. To further investigate this interaction, we used recombinant BiP and a fusion of the J-domain of Sec63p with GST (GST-J) in a surface plasmon resonance (SPR) assay (30). In an SPR assay, one component is immobilized on a surface, and the interaction partner is in a solution which is passed over it. Binding between the two increases the refractive index at the surface. The signal (given in response units, RU) is directly proportional to the mass of protein bound to the surface. This assay makes it possible to follow the association and dissociation of proteins in real time.

GST-J was immobilized via NH2-specific cross-linking and binding of BiP was tested in the presence of ATP or ADP. In ATP, BiP was bound with fast kinetics, while in ADP the response was as low as in the absence of GST-J (Fig. 1A, see also Ref. 17). In agreement with previous results (17), the GST-J-BiP complex could also be isolated with GSH beads (data not shown). Previous experiments have demonstrated that BiP binds neither to GST alone nor to a GST-J molecule containing the sec63-1 point mutation in its J-domain (17).

To determine the apparent affinity of BiP for GST-J, we measured the time course of binding for different BiP concentrations in the presence of ATP. The dependence of the plateau values on the BiP concentration could not be fitted with a single binding constant; the half-maximum concentration was approximately 5 μM (Fig. 1B). In the

![Fig. 1. Binding of BiP to GST-J. A, 1000 RU of GST-J were immobilized. At time 0, a solution containing 10 μM BiP and either 2 mM ATP or ADP was introduced (arrow, "BiP"). "Buffer" indicates the point at which the BiP solution was replaced with buffer alone (arrow). The dashed curve gives the background binding of BiP with ATP but in the absence of GST-J (background). B, 1200 RU of GST-J were immobilized. Different concentrations of BiP were introduced in the presence of either 2 mM ADP (circles) or ATP (triangles). The response was followed with time and the maximum values were plotted versus the BiP concentration.](image-url)
Binding of BiP to GST-J Requires ATP Hydrolysis—Since the binding of BiP to GST-J is strictly dependent on ATP, the ATP-bound form of BiP is likely to make the initial interaction with the J-domain. To determine whether ATP hydrolysis is required for the binding of BiP to GST-J, we employed BiP mutants with single amino acid changes in their ATPase domain (27, 28). The mutants are trapped at different stages of the ATPase cycle. BiP G247D is unable to bind ATP, BiP T59G binds ATP, but is unable to undergo a conformational change following ATP binding, and BiP T249G binds ATP and undergoes the conformational change, but fails to hydrolyze the bound nucleotide (Ref. 27, and data not shown). As shown in Fig. 2, none of these mutants interacted with GST-J, demonstrating that ATP hydrolysis is required. Previous experiments with nonhydrolyzable analogs of ATP led to the same conclusion (17), but protease protection and peptide binding experiments indicate that, in contrast to the mutation T249G, the analogs do not induce the genuine ATP conformation of BiP (data not shown). ATP hydrolysis during binding of GST-J is also supported by pull-down experiments with GSH beads which demonstrated that the nucleotide bound to GST-J-BiP is ATP (data not shown, see also Refs. 16 and 17).

Effects of a Hydrophobic Peptide—We next tested whether the GST-J-BiP complex was an activated precursor for BiP’s interaction with peptides as has been suggested (16, 17) or whether it represented a subsequent state with GST-J bound to the peptide binding pocket of BiP. To distinguish between these two models we investigated the effects of the hydrophobic peptide P5 (ALLLSAPRR; Ref. 31). This peptide binds BiP well in ADP (11), but much more weakly in ATP (11). We first tested whether pre-binding of P5 would inhibit BiP’s interaction with GST-J. As shown in Fig. 3a, peptide P5 in solution competed with immobilized GST-J for BiP binding. At the highest peptide concentration used, binding of BiP was reduced to approximately 15% of the original value. We estimate an inhibition constant of approximately 500 μM for the peptide.

To investigate the effect of peptide on the dissociation of BiP from GST-J, BiP was prebound to GST-J with or without ATP, and its dissociation was followed in ATP or ADP in the absence or presence of 2 mM peptide P5 (Fig. 3b). The dissociation in ADP was relatively slow (estimated half-life approximately 100 s, see also Ref. 16) and biphasic, probably due to the different dissociation rates of the multiple BiP molecules bound to GST-J. The peptide had no effect on the dissociation rate. In ATP the release of BiP was significantly faster (half-life approximately 12 s), and the peptide had a small effect, most likely by preventing the re-binding of dissociated BiP to GST-J. The inhibitory effect of the peptide on the formation of the GST-J-BiP complex (Fig. 3a) is therefore not due to an increased off-rate of BiP. In addition, the dissociation kinetics of BiP from GST-J in ADP and ATP are very similar to those for the dissociation of BiP from a synthetic peptide (11). These data were consistent with the view that GST-J is bound to BiP as a peptide substrate, arguing that the complex represents the final BiP-substrate interaction, rather than a precursor to it.

GST-J and Peptide P5 Stimulate BiP's ATPase Activity—To exclude the possibility that the peptide prevents BiP from interacting with the J-domain, we tested whether GST-J could stimulate the ATPase activity of BiP in the presence of peptide. BiP’s ATPase activity was followed with or without peptide P5 and GST-J, allowing each BiP molecule to hydrolyze many molecules of ATP (Fig. 3c). BiP alone hydrolyzed ATP at a rate of approximately 0.1 mol/mol/min. GST-J stimulated this basal rate approximately 5-fold (see also Ref. 17) with maximal stimulation reached at stoichiometric amounts of GST-J relative to BiP. In the absence of GST-J, peptide P5 did not stimulate the ATPase activity of BiP (Fig. 3c). These data suggest that GST-J is bound to BiP as a peptide substrate, and that the complex represents the final BiP-substrate interaction, rather than a precursor to it.

Interaction of BiP with J-proteins

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We next tested whether BiP’s ATPase activity is required for the formation of the GST-J-BiP complex. Point mutations were introduced into BiP’s peptide binding pocket to render it nonfunctional. The mutations G445D and G488D were chosen because analogous mutations were found in a genetic screen for mutations in the peptide-binding domain of DnaK in Escherichia coli (32). The mutations VS19R and T448R should fill the binding pocket with a bulky residue (5), and therefore might inhibit peptide binding. Indeed, none of these BiP mutants was able to bind the hydrophobic peptide P5 (Fig. 4a). They were, however, able to hydrolyze ATP efficiently (data not shown) and were thus clearly different from the three ATPase mutants described above, which can bind peptides (data not shown), but fail to hydrolyze ATP. None of the peptide-binding mutants was able to bind GST-J (Fig. 4b). A similar mutant in DnaK was also unable to bind to DnaJ (33). Furthermore, a truncation mutant of BiP containing only the ATPase domain was unable to bind GST-J (data not shown). On the other hand, a mutant lacking the COOH-terminal lid domain but maintaining the ATPase and peptide-binding domains was still able to bind GST-J (data not shown, see also Refs. 11, 34, and 35). Taken together, BiP’s peptide binding activity appears to be required for stable J-activated BiP’s ATPase activity. These data are consistent with the view that the peptide binding pocket of BiP is already occupied. We conclude that even though the peptide binding pocket is already occupied by the peptide, BiP’s ATPase activity is still required for the formation of the GST-J-BiP complex.

Binding of BiP to the Sec Complex—Based on the experiments with GST-J, we reasoned that, in the absence of a translocation substrate, BiP might bind to the Sec complex itself. In fact, in previous experiments with GST-J, we found that the translocation substrate was required for BiP’s peptide binding activity. In testing a large number of mutants, we have found a perfect correlation between the decrease in BiP binding to GST-J and defects in BiP’s activity in a translocation assay. In addition, our data indicate that the functions of both the ATPase and peptide-binding domain are required for J-activated BiP binding.

We immobilized the soluble, purified Sec complex via an antibody against the Sec63p component of the Sec63p complex. BiP was passed over it in a detergent-containing buffer with either ATP or ADP. As with GST-J, the binding of BiP to the Sec complex was significantly stronger in ATP than in ADP (Fig. 5, solid curves). The lower level of binding in ADP is probably due to the presence of hydrophobic domains of the Sec complex. Binding was specific for the Sec complex since in its absence the residual response due to changes caused by the presence of BiP in solution was
observed (Fig. 5, lower dashed curves).

To further test the specificity of the interaction, we used the Sec complex purified from the sec63-1 mutant, containing a point mutation in the J-domain of Sec63p (36) which makes it defective in translocation (37). This protein was inactive since binding of BiP in the presence of ATP was identical to that in the presence of ADP (Fig. 5, upper dashed curves, see also Ref. 15). ADP-dependent binding was also observed with a subcomplex of the Sec complex (38), containing Sec62p, Sec63p, Sec71p, and Sec72p (data not shown). Taken together, these data indicate that the J-domain of Sec63p can activate BiP to bind to the Sec complex in a manner that is analogous to the interaction observed with the GST-J fusion protein.

DISCUSSION

Our experiments discriminate between two models proposed for the interaction of an Hsp70 molecule with its J-protein partner. Our data are inconsistent with a model in which, in the absence of a substrate, Hsp70 forms an activated, stable J-Hsp70-ADP complex, from which the bound Hsp70 is subsequently transferred to a peptide (15–17). Rather, they suggest that Hsp70 interacts only very transiently with the J-domain and is immediately transferred to a peptide substrate, be it the natural substrate or the J-protein itself (4, 10). Although we confirm the generation of a stable J-protein-Hsp70-ADP complex in an ATP-dependent reaction, we demonstrate that the Hsp70 is bound to the J-protein as if it were a peptide substrate. The complex therefore represents the final, rather than an intermediate, state of the peptide binding reaction.

The following observations support our conclusion. 1) A hydrophobic peptide in solution competes with GST-J for the formation of the GST-J-BiP-ADP complex. The peptide does not accelerate the dissociation of BiP from GST-J, nor does it prevent the interaction of GST-J with BiP that is required for ATPase stimulation. We therefore conclude that the peptide exerts its inhibitory effect by occupying the same peptide-binding domain to which GST-J is ultimately bound. In addition, in agreement with other results (4, 10, 33, 35, 39), these data show that peptide and J-domain interact with BiP at different sites. 2) Since the GST-J-BiP-ADP complex binds a hydrophobic peptide much more weakly than BiP-ADP, GST-J must block BiP's peptide binding pocket. 3) The fact that two BiP molecules are bound per GST-J molecule and the biphasic dissociation kinetics argue against the existence of a stable, defined J-domain-BiP interaction. Rather, these data indicate that BiP molecules are bound to at least two distinct regions of GST-J. 4) The kinetics of dissociation of BiP from GST-J resembles that of BiP from peptide substrates. In both cases dissociation is fast in ATP and slow in ADP, as expected from open and closed peptide binding pockets, respectively (7, 11). 5) Binding of BiP to peptide when co-immobilized with the J-domain (11) is strikingly similar to BiP's interaction with immobilized GST-J. The kinetics of association and dissociation are very similar, ATP hydrolysis is required for the binding reaction, and the final complex contains ADP. We therefore conclude that formation of the GST-J-BiP-ADP complex involves the activation of BiP by the J-domain. 6) BiP mutants whose peptide binding activity is altered by point mutations or deletions fail to bind GST-J. 7) Similarly to the results with GST-J, BiP can be transferred in a J-domain- and ATP-dependent manner to the Sec complex itself. Limited availability of the purified Sec complex and its dissociation from the surface during subsequent SPR experiments have made it impossible to demonstrate directly that the peptide binding pocket of BiP is involved in this interaction. However, the analogy between GST-J and the Sec complex strongly suggests that BiP binds both in an identical manner.

Our results suggest that a J-domain-BiP-ATP complex, which is too short-lived to be detected in conventional binding experiments, is transiently formed. The short half-life is caused by the J-protein inducing rapid ATP hydrolysis by BiP. Upon ATP hydrolysis, BiP binds to peptides in close proximity to the J-domain (11). The final complex contains BiP, ADP, and peptide, but lacks the J-domain. BiP is rather promiscuous following J-activation and can therefore bind to essentially any sub-
strate (11), including polypeptide parts of the J-protein itself. The peptide motifs in the J-proteins to which BiP is bound are unknown, but they are probably outside the J-domain. Our data argue against a previously proposed model in which BiP in its ADP form would wait for the translocation substrate while bound to the J-domain at the lumenal end of the channel (15). Although we have confirmed that BiP associates with the Sec complex or the Sec62/63p subcomplex in a J- and ATP-dependent manner, this complex is likely not a precursor for peptide interaction. Rather, in the absence of a translocation substrate, BiP appears to bind to polypeptide segments of the Sec complex, as observed with the GST-J protein.

During translocation, the translocating polypeptide would emerge from the channel close to the J-domain of Sec63p and J-activated BiP would be transferred to the substrate. Once bound, BiP would prevent backwards movements of the polypeptide chain, thus acting as a molecular ratchet.2 BiP was also proposed to pull on the translocation substrate (26). However, this would require a conformational change of BiP while bound simultaneously to the J-domain and the polypeptide substrate, which seems unlikely given the transient nature of the BiP-J interaction. Simultaneous binding is also rendered unlikely by our finding that more than one BiP molecule could bind to GST-J. Moreover, since the addition of peptide does not stimulate the dissociation of BiP from its complex with GST-J, a complex containing BiP, J-domain, and translocation substrate would be stable, preventing any movement of the polypeptide chain.

J-activated binding of BiP to polypeptide segments of the Sec complex in the absence of a translocation substrate may have physiological significance. Mammalian BiP was proposed to seal the lumenal end of the channel to small ions (40). In an analogous manner, BiP may serve to seal the channel in the post-translational pathway. J-activated BiP would continuously associate with and dissociate from the Sec complex, providing a dynamic lid for the channel, and would switch to binding of a translocation substrate as soon as it emerges from the lumenal end of the channel. “Futile” ATP hydrolysis would not be excessively high because the steady state ATPase activity is only stimulated 5-fold by the J-protein. On the other hand, it is possible that the J-domain is only exposed for BiP interaction once a translocation substrate is bound to the channel.

Our results may also have relevance for studies on the import of proteins into mitochondria (26, 41–44). There, a stable complex of mitochondrial Hsp70 with its partner protein of the inner membrane, Tim44p, is formed following ATP hydrolysis (45). In light of our results, it appears questionable whether it represents a precursor for substrate binding.

A dimeric complex between Hsp70 and peptide appears to be

![Fig. 4. Mutants defective in peptide binding.](image-url)

A, 1000 RU of peptide P5 were immobilized using a 6-amino acid spacer. A buffer containing 2 mM ADP was introduced and, at time 0, 20 μM wild type BiP (wt) or BiP mutants carrying point mutations in their peptide-binding domain were added. B, as in A, except that 1300 RU of GST-J were immobilized and binding was tested in the presence of 2 mM ATP. C, a soluble complex between radiolabeled ppαF and the Sec complex was generated and incubated with increasing concentrations of either wild type (wt, squares) BiP or of BiP mutants containing a defective peptide binding pocket: BiP G445D (diamonds), BiP V519R (triangles), BiP T448R (circles), or BiP G448D (crosses), respectively. The Sec complex was immunoprecipitated with antibodies to Sec62p and the amount of co-precipitated ppαF determined. The data are normalized to the amount of ppαF bound to the Sec complex in the absence of added BiP.

![Fig. 5. BiP binds to the Sec complex.](image-url)

The Sec complex was immobilized via its affinity to an anti-Sec62p antibody. At time 0 30 μM BiP was added to the buffer containing either 1 mM ADP or ATP, which was passing continuously over the surface. At the time point where the curves begin to fall, the BiP solution was replaced by buffer. Controls were performed in the absence of Sec complex (lower dashed curves) or with the Sec complex from the sec63-1 mutant, carrying a point mutation in the J-domain (upper dashed curves).
the general end product in many Hsp70 systems, such as the auxilin-Hsc70 system responsible for the uncoating of clathrin-coated vesicles (16, 46). Our data indicate that such complexes only form efficiently in the presence of a J-domain, despite the presence of high concentrations of Hsp70s in the cell. Previously it has been observed that in the presence of ATP the yeast J-protein Ydj1p releases an unfolded protein from the cytosolic Hsp70 homolog Ssa1p (47). In light of our data it seems possible that, under these conditions, Ydj1p competes with the unfolded protein for binding of activated Ssa1p, thus apparently stimulating peptide dissociation.

In the bacterial system a transient interaction between DnaK and the J-domain of DnaJ has been demonstrated (4, 10, 48). The J-domain alone did not stimulate the ATPase activity of DnaK, in contrast to either the J-domain plus a following domain (G/F-domain) (49), the full-length DnaJ, or the J-domain plus a separately added hydrophobic peptide (10). It is therefore possible that with the longer J-protein constructs DnaK is targeted to segments COOH-terminal of the J-domain (10). The ATP-dependent formation of a complex between DnaK and DnaJ (33, 35, 50) is thus likely to be similar to that between GST-J and BiP, with a peptide segment occupying the peptide-binding domain of the Hsp70. Recently, the binding site for the J-domain has been mapped to a region in the ATPase domain of DnaK (33, 35). In addition, using NMR a nucleotide-independent interaction between the J-domain of DnaJ and the ATPase domain of DnaK was found (39), presumably reflecting the transient interaction that escapes detection in other experiments. Taken together, it appears that the interaction between a J-domain and Hsp70 is highly conserved in evolution.

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