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BiP (GRP78), an essential hsp70 resident protein in the endoplasmic reticulum

Dedicated to Marcel Marceau alias Monsieur Bip

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Abstract. BiP is a constitutively-expressed resident protein of the endoplasmic reticulum (ER) of all eucaryotic cells, and belongs to the highly conserved hsp70 protein family. In the ER, BiP is involved in polypeptide translocation, protein folding and presumably protein degradation as well. These functions are essential to cell viability, as has been shown for yeast. In this review, I will summarize the structural features of hsp70 proteins and focus on those experiments which revealed the biological function of BiP.
Key words. ER-translocation; folding and assembly of polypeptide chains; hsp70 structure; ER-degradation.

Introduction

The immunoglobulin (Ig) heavy (H) chain **Bi**nding **P**rotein (BiP), a soluble ER-component, was the first member of the hsp70 multigene family that was initially identified on the basis of its specific interaction with a known ligand[31,32,64]. This finding provided a long-sought example for a physiological activity of this highly conserved group of ancient proteins, and contributed to the development of the concept of 'molecular chaperones'[20,21]. These proteins, many of which belong to different HSP classes, are involved in the process of in vivo protein folding (for a detailed review, see ref. 36). BiP is encoded by a single copy gene in mouse and rat[31] and is identical with the glucose regulated protein GRP78 (refs 31, 64). Glucose-regulated proteins exhibit elevated expression levels when cells are starved of glucose (for review on GRPs, see ref. 46). As discussed below, underglycosylation of polypeptides, and a variety of other stress conditions, increase the number of potential BiP ligands and therefore decrease the pool of free BiP in the ER. Since BiP has an essential function in yeast[73,78], a low expression level of BiP and of other GRPs may cause severe damage to the ER[53]. To overcome this problem, eukaryotic cells possess a feed-back regulation mechanism that controls the expression level of BiP to the amount actually required by the cells[44].

By a co- or post-translational event, proteins destined for export or for location at a particular site along the secretory pathway are translocated into the ER-lumen. In this particular environment, BiP appears to be functionally involved in multiple processes (schematically summarized in fig. 1). First of all, BiP seems to participate in the process of ER-translocation of nascent polypeptides in yeast[69,80,85] and possibly in mammalian cells as well[70]. Furthermore, BiP is involved in processes of polypeptide chain folding and assembly in the ER[29,30,79] in which it may be part of a quality control system[17,38]. Recent data suggest that BiP plays a role in ER-degradation of polypeptide chains that do not reach a mature conformation (see ref. 43a). All the different functions might be founded on a unique property of BiP, an ability to bond polypeptide chains as long as they exhibit unfolded stretches. The exact mode of BiP action however, is not yet understood.

The structure of hsp70 proteins

BiP of a given species (e.g. rat BiP) has more in common with its homologues from different species (e.g. hamster BiP: 98% sequence identity) than with other members of the hsp70 protein family expressed in the same organism (e.g. rat hsp73: 62% identity). Amino acid sequence comparison of 34 hsp70 proteins from 17 species revealed that BiP genes share a common ancestor, which diverged from other hsp70 genes near the time when eukaryotes first appeared[71]. However, all members of the highly conserved hsp70 protein family (with one exception so far; see below) exhibit similar properties.

Characteristically, hsp70 proteins bind ATP and possess ATPase activity[7,13,64,90]. The ATPase activity of hsp70 proteins is stimulated in vitro by bound peptides which mimic protein binding[24]. Thus, BiP and other hsp70 proteins possess both a substrate recognition site and an ATPase activity. First insights into the structural basis of these properties came from Rothman's group, which identified the clathrin uncoating ATPase as an hsp70 protein cognate (hsc71)[13]. A 44K N-terminal fragment obtained by proteolytic cleavage of the hsc71 protein was devoid of clathrin binding but had retained the ATPase activity, indicating a segregation of the two functions into separate domains[12]. The amino-terminal 44K fragment was cristallized and the three-dimensional structure described solved down to a resolution of 2.2 Å

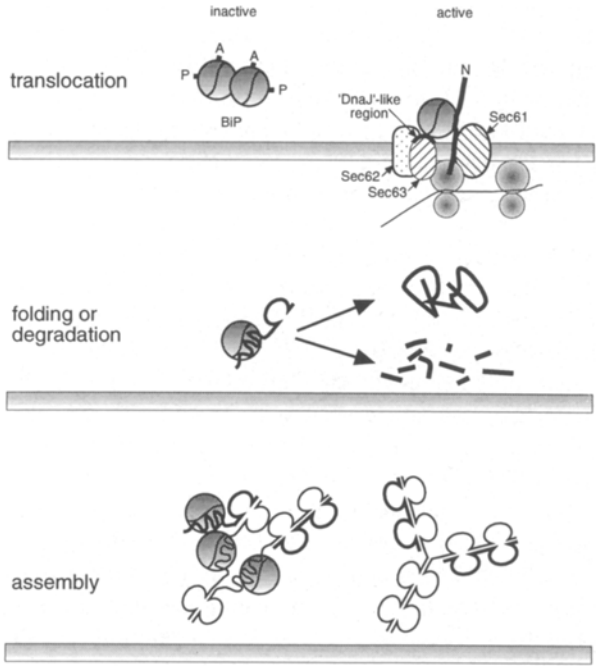

Figure. Model of the multiple functions of BiP in the ER-lumen. Translocation: In its inactive form, BiP is an ADP-ribosylated and phosphorylated dimer. In contrast, the active form is an unmodified monomer. In yeast, translocating polypeptides have been reported to bind to the transmembrane protein Sec61p and to BiP. Other proteins such as Sec62p and Sec63p participate in the translocation process whereby, through its 'J-region', Sec63p may directly interact with BiP. This could specifically position soluble BiP molecules at the ER-translocation sites.
Folding or degradation: BiP interacts in a transient fashion with folding polypeptide chains, whereby the chain can undergo post-translational modifications such as N-linked glycosylation or disulfide bond formation. When folding cannot be achieved, polypeptides remain bound to BiP until they are degraded.
Assembly: In the formation of antibody molecules, BiP binding to Ig H chains serves to ensure the controlled assembly of H and L chains. H chains are only released from BiP when association with L chains is possible. More details on each of the items are given in the text.

in David McKay's group. The ATPase domain consists of two lobes (I and II) of approximately equal size which are separated by a deep cleft with the ATP-binding site at the bottom of the cleft. Each of the two lobes can be subdivided into two topological domains (IA, IB, IIA, IIB)[22]. It was surprising to find a structural similarity between this ATPase domain and two functionally unrelated proteins, hexokinase[22] and actin[39]. From the superposition of the crystal structures and from the alignment of the many known homologous sequences in each of the families, a pattern consisting of five motifs involved in ATP binding and in a putative interdomain hinge was derived. Furthermore, a sequence data base search revealed that this pattern occurred in other proteins as well, such as procaryotic cell cycle proteins and sugar kinases, indicating a common evolutionary origin[4].

A novel microsome-associated member of the hsp70 protein family was recently isolated from a human cDNA library[74]. The single open reading frame encodes a protein of 471 amino acids, designated **st**ress **ch**aperone (STCH) that contains a putative signal peptide and has 33% identity (and 44% homology) with the amino acid sequence of both human BiP and human hsp70 protein. The conserved regions localize primarily to the five ATPase consensus motifs. Surprisingly, STCH lacks the carboxy-terminal portion and seems to consist of a 'core ATPase' only. In comparison with the usual hsp70 structure, the sequence is truncated just downstream of the last ATPase consensus motif. Consistent with the lack of a portion that bears the putative peptide binding domain of hsp70 proteins, the ATPase activity of STCH is not enhanced in the presence of peptides. STCH contains an unusual stretch of 50 amino acids within the ATPase domain, the biological significance of which is unknown. Features resembling BiP include its constitutive expression and intracellular distribution as well as its mRNA induction following cell incubation with the calcium ionophore A23187[74].

The peptide binding activity of all other hsp70 proteins is most probably associated with their carboxy-terminal portions. It is this region which exhibits the greatest sequence divergence amongst the different hsp70 proteins, which are located in different subcellular compartments and are expressed either in a constitutive fashion or under a variety of different stress conditions (for review, see ref. 55). Variations in the peptide binding site might therefore reflect that the different hsp70 proteins interact with distinct subsets of polypeptide chains. This notion correlates with the finding that BiP and a cytosolic hsp70 protein exhibit different peptide binding affinites in vitro[23]. Competition experiments performed by Gaut and Hendershot demonstrated that such peptides can block the binding of BiP to Ig H chains which is consistent with the hypothesis that the peptides interact with the protein binding site[27]. A consensus secondary structure of the putative peptide binding site was deduced from the alignment of the carboxy-terminal sequences of 33 hsp70 proteins[77]. Interestingly, this structure could be superimposed on the secondary structure of the peptide binding domain of a major histocompatibility antigen (MHC) class I molecule[77]. In line with this model are the in vitro data on BiP-peptide interaction, showing that the minimal length of an artificial peptide for it to be efficiently bound by BiP comprises 7–8 amino acids[23]. In contrast to the physiological situation with MHC class I molecules, however, the peptide stretches that bind to hsp70 molecules are embedded inside a long polypeptide chain, so that free N- and C-termini are not available for hydrogen-bonding to residues of the peptide binding cleft. This reasoning led Gething and coworkers to suggest that the peptide binding domain of hsp70 proteins might more closely

resemble MHC class II molecules[2]. A more detailed discussion on the peptide binding site model is given in the introductory chapter of this Multi-author Review.

BiP is a soluble protein and represents a major luminal component of the ER. Similarly to other soluble ER-residents, such as GRP94 or protein disulfide isomerase (PDI), BiP possesses a defined carboxy-terminal tetrapeptide (KDEL in mammalian cells)[65]. Proteins that contain this carboxy-terminal signal are retrieved from post-ER compartments via the interaction with a specific receptor[52,51]. To what extent this retrieval mechanism is important with respect to BiP function remains to be elucidated. STCH lacks such a signal[74].

In vitro studies

BiP's ATPase and autophosphorylation activities

Owing to its ATPase function, it is possible to specifically dissociate a complex composed of BiP and a ligand in the presence of ATP, but not of non-hydrolysable ATP-analogs[64]. The ATPase activity of BiP requires magnesium as the divalent ion, and is inhibited by calcium[40]. On the basis of the three-dimensional structure of hsc71 and its similarity to actin, it was possible to predict which residues would be important for nucleotide interaction[22]. In fact, Gaut and Hendershot identified key residues in the ATP binding cleft that are involved in the in vitro ATP-release of proteins by BiP. The analysis of recombinant BiP molecules that carried specific mutations revealed that the mutant proteins were still able to bind ATP as well as immunoglobulin heavy (Ig H) chains but were not capable of releasing bound H chains in the presence of MgATP[27]. These results strongly suggest that the BiP residues corresponding to Thr-13, Glu-175, and Thr-204 in hsc71 do play an essential role in ATP hydrolysis. Moreover, it is the ATPase activity that drives the release of bound proteins, which suggests that the ATPase domain transfers information to the peptide binding domain of the hsp70 protein molecule. On the other hand, peptide interaction with the carboxy-terminal binding domain also modulates the ATPase activity of BiP. It was already postulated by Pelham[50,75] that hsp70 proteins undergo conformational changes depending on the presence of adenine nucleotides. This was confirmed by protease susceptibility experiments performed by Kassenbrock and Kelly[40]. The demonstration of a protein-mediated ATP transport into isolated rough ER vesicles provided the first evidence for an in vivo relevance of BiP's ATPase activity[14].

BiP has recently been reported to possess autophosphorylating activity as was also shown for three other members of the hsp70 protein family[49,48]. Interestingly, the cation dependence for the autophosphorylation of BiP is exactly the opposite to that reported for BiP's ATPase activity. In vitro phosphorylation is stimulated in the presence of calcium and inhibited by magnesium[49]. The in vitro phosphorylation site of BiP has been mapped to the threonine residue (Thr-229)[26] which corresponds in its relative position to the threonine residue (Thr-199) known to be the autophosphorylation site in the bacterial hsp70, DnaK[59]. However, this site is not used when BiP is phosphorylated in vivo[26].

BiP as peptide binding protein

The nature of peptide binding to hsp70 proteins is not yet understood. Particularly, it remains puzzling how chaperones discriminate unfolded or incompletely folded polypeptides from the native structure with which they no longer interact. In order to prevent aggregation reactions, chaperones might bind to hydrophobic stretches of unfolded or incompletely folded polypeptides that are buried inside when the protein is correctly folded. Peptides bound to hsp70 proteins seem to exhibit a linear conformation, as demonstrated by NMR spectroscopy[45]. No specific amino acid sequence seems to be involved in substrate recognition, as was shown by the analysis of BiP interaction with a set of random peptides. Instead, binding seems to correlate with the content and the accessibility of hydrophobic residues[24]. An elegant approach to investigating the peptide binding specificity of BiP has been chosen by the group of Mary-Jane Gething. They screen a bacteriophage library displaying random octa- or dodecapeptide sequences as the N-terminus of the adsorption protein for high affinity binding to BiP. The peptides scored in this way contain a subset of aromatic and hydrophobic residues in alternating positions. The authors proposed a scoring system to predict BiP binding sequences in naturally occuring polypeptides[2]. The conclusions drawn from these data are based on the assumption that the 'best' peptide is the one which binds BiP with the highest affinity. This might, however, not necessarily apply to the physiological function of the hsp70 protein which exerts its function in a particular micro-environment. It is therefore important to keep in mind that hydrophilic peptides were also bound by BiP, although with low affinity (ref. 23). In this context, in vivo studies on ER-degradation of Ig L chains are of interest[43a]. Two particular L chains that exhibit different half lives were found to be quantitatively associated with BiP when the cellular proteins were analyzed by size chromatography. Surprisingly, only one of these L chains was quantitatively co-immunoprecipitated with BiP, which indicated that the two BiP/L chain complexes exhibited different physical stabilities. Those L chains that exhibited the higher affinity to BiP were degraded more slowly, which suggested that the physical stability of a BiP/ligand complex correlates with the biological fate of ligand. However, the physiological conditions required to dissociate a BiP/ligand complex in vivo remain to be established.

In vivo studies

Factors that influence the experimental detection of BiP-binding to cellular proteins

The question whether a particular protein does or does not interact with BiP is usually investigated by immunoprecipitation experiments performed using lysates of biosynthetically labeled cells. The latter manufacture thousands of different proteins, and cell biologists often encounter the problem of discriminating between specific and non-specific protein/protein interactions. The detection of a specific interaction between BiP and a polypeptide chain that transits the ER is often impeded by additional problems. First, BiP only binds immature folded forms of a polypeptide chain. Thus, in a steady state situation, only small amounts of the protein will be associated with BiP. In addition, BiP is an abundant protein with a very long half life ($t\frac{1}{2} > 48$ h)[16] and will therefore be labeled only to a low level of specific activity. Furthermore, some BiP/ligand complexes are unstable and are therefore not included in the immunoprecipitation procedure (see above). The coincidence of the following items may impede or even completely prevent the detection of a particular polypeptide/BiP interaction: 1) the protein under investigation folds rapidly (so that the steady state levels of immature forms are very low) 2) the particular BiP/ligand complex exhibits a low physical stability, and 3) an antiserum is used that preferentially recognizes mature folded structures. On the other hand, proteins will accumulate in a malfolded or incompletely folded state if they are prevented from reaching a mature conformation (see below) allowing a much easier detection of potential BiP/ligand complexes. These reasons explain why the first molecules reported to bind BiP were abundant proteins such as Ig H chains[32,3] or folding mutants of the influenza virus hemagglutinin[28].

Permanent interaction of BiP with incompletely folded or malfolded proteins

Molecular chaperones interact with immature folded structures but are not part of the final product. BiP, for example, was found to bind Ig H chains as long as those have not assembled together with L chains[32]. The reason why mutant influenza virus hemagglutinin remains associated with BiP in the ER instead of following its normal export pathway is that it cannot reach a mature folding state[28]. BiP also recognizes proteins with incomplete disulfide bonds, as was shown for the vesicular stomatitis virus G protein[58]. Other post-translational modifications such as protein glycosylation also appear to play a role in the process of in vivo protein folding. Polypeptides that are prevented from being correctly glycosylated exhibit a prolonged BiP interaction and are not exported from the ER[18,38,88]. However, the results obtained by mutations of individual N-glycosylation sequences indicate that not all the potential sites have the same impact on protein maturation[88,91,92]. For example, the human transferrin receptor possesses three N-linked oligosaccharides in its mature form. Only the elimination of the most C-terminal glycosylation site had a profound negative effect on cell surface expression of the receptor[91]. These molecules are retained in the ER and exhibit an increased association with BiP. Subunits of some oligomeric proteins that are expressed in the absence of their partner chains also exhibit increased BiP binding, indicating that oligomeric assembly affects the folding state of at least some polypeptide chains. This applies to wild-type Ig H chains, which occur as H chain dimers when expressed in the absence of L chains, and to some Ig L chains in the absence of H chains as well[32,3,57,43]. A more detailed analysis of L chains that require H chain association for ER exit revealed that, in the absence of H chain expression, they remain associated with BiP as partially folded molecules until they are degraded in the ER[43a]. Concerning the time correlation between BiP dissociation and degradation of the non-secreted L chains, we assume that BiP is required for ER-degradation of incompletely folded structures. However, the major function of BiP might not be to retain malfolded proteins in the ER, since this phenomenon is only observed when the normal folding pathway is disturbed. Permanent complexes rather represent an arrested step of a usually transient process. The most recent data support the notion that BiP transiently interacts with folding polypeptide chains, possibly to protect the nascent chains from irreversible aggregation and to stabilize incompletely folded polypeptide stretches in a folding or assembly-competent conformation.

Transient interaction of BiP with folding polypeptides

What are the specific properties of a polypeptide chain that lead to BiP binding and what are the criteria that lead to a release? The earliest stage of polypeptide chain interaction with BiP has not yet been determined. If BiP exhibits properties similar to those of its cytosolic or mitochondrial counterparts[1,61], it should bind to nascent chains as soon as they enter the ER. Immunoprecipitation of BiP from lysates prepared from biosynthetically labeled cells reveals the co-precipitation of a number of different polypeptide chains which disappear during the chase period. These data indicate that BiP transiently interacts with various polypeptide chains; however, only a few have been identified (reviewed in ref. 30). A very early interaction of nascent polypeptides with BiP seems to be essential since kinetic data on BiP interaction with particular polypeptides only reveal the process of the dissociation of complexes[19,32,43]. In contrast, the in vivo formation of short- or long-lived complexes between BiP and its ligands has never been observed. Furthermore, genetic and biochemical data suggest that Kar2p/BiP is involved in the process of

polypeptide chain translocation into the lumen of the yeast ER[8,69,80,85], and possibly into the ER of mammalian cells as well[70]. The exact mechanism by which BiP influences the translocation process is as yet unknown.

BiP interaction with polypeptides appears to accompany the folding process of the prospective proteins. In the beginning of the folding process, more than one molecule of BiP may interact with larger polypeptide chains[42]. An inverse time correlation between the polypeptide chain acquiring its mature structure and its dissociation from BiP was observed[19,41,42,66,67,68]. Cornelia Kaloff in my laboratory has analyzed the folding pathway of antibody molecules with regard to the particular role of the first constant (C_H1) domain in BiP/Ig H chain interaction. As was known before, removal of the C_H1 leads to secretion of H chains even in the absence of L chain assembly, which does not occur with wild type H chains[34]. We observed that such mutant chains only weakly interact with BiP, presumably because the disulfide bonds are rapidly formed and the protein gains an export-competent conformation. The formation of disulfide-bonds on nascent polypeptides in the ER requires the action of PDI, as evidenced by the work of Bulleid and Freedman[10]. The group of Helenius has recently reported that it is possible to (reversibly) manipulate disulfide bond formation and protein folding in the ER by culturing cells in the presence of dithiothreitol (DTT)[5,6]. Due to the effect of DTT, disulfide-bonded proteins are reduced and retained in the ER while proteins that lack disulfide bonds are still secreted[56]. Taking advantage of the possibility of retarding the process of in vivo protein folding, we could show that DTT-reduced wild type and mutant Ig H chains are quantitatively associated with BiP. Upon removal of the reducing agent, the mutant chains that lack the C_H1 domain oxidize, and are simultaneously released from BiP interation. In contrast, wild type chains remain bound to BiP even as covalently linked Ig H chain dimers. This shows that the C_H1 domain interferes with a 'complete folding' of the wild type H chains when these are prevented from pairing with L chains. From these findings, we concluded that the process of Ig chain folding is coupled to that of Ig chain assembly. Analyses performed with Ig chains that carried defined variable domains demonstrated that the C_H1 domain is essential to ensure the formation of a homogeneous population of antibody molecules that are functional with respect to antigen binding. Interestingly, this control function is exclusive to the C_H1 domain since it is not maintained when this domain is replaced by another H chain constant domain (Kaloff and Haas, submitted). Taking into account that correct folding of antibody molecules is only possible once subunit assembly has taken place, it is tempting to speculate that the C_H1 domain has evolved in order to control this process via its specific interaction with BiP.

The expression level of BiP is adapted to the cellular requirements

As already mentioned, both glucose starvation and tunicamycin treatment of cells lead to underglycosylation of proteins, and the association of these proteins with BiP is then prolonged. As a consequence, the BiP pool available for ligand binding is decreased. The same effect is observed when folding mutants of influenza virus hemagglutinin accumulate in the ER. Direct evidence for a feed-back regulation mechanism responsible for transcriptional activation of the BiP gene has been provided by the group of Joe Sambrook[44]. Induction of BiP synthesis due to the accumulation of non-native proteins in the ER correlates with the formation of complexes between BiP and its substrates[68]. However, it is not only the accumulation of malfolded structures that increases the cellular requirement for BiP. Enhanced transcription of the BiP gene was found to accompany the infection of cells with paramyxoviruses[76]. By transfection of cDNAs encoding individual viral proteins, it turned out that the expression of wild type hemagglutinin-neuraminidase-glycoprotein was sufficient to cause BiP activation[87]. Thus an increased flux of normal proteins that require chaperone action for folding can also cause the activation of BiP gene transcription. Analyses of the yeast KAR2 promotor sequence have revealed that the element responsible for the feed-back response lies within a 22bp fragment[63]. Based on this finding, it was possible to identify the first component involved in the mechanism that transfers the signal from the ER to the nucleus. It is a large transmembrane protein which carries a putative protein kinase domain in the carboxy-terminal cytosolic portion[15,62]. This protein is encoded by a gene originally cloned by complementation of a yeast mutant auxotrophic for inositol[72]. An attractive speculation about the link between the unfolded protein response pathway and the inositol metabolism was proposed by Peter Walter's group. They suggested that the synthesis of ER resident proteins and the regulation of phospholipid biogenesis are coupled[15]. This coupling mechanism would allow that under conditions where additional ER functions are required, the relative concentrations of the overexpressed ER proteins remain constant. How misfolding polypeptides transfer the signal to the transmembrane kinase, and how this molecule finally translates and conducts this signal to the nucleus, remain exciting problems to be solved.

Interconversion of different BiP forms

BiP can undergo post-translational modifications such as ADP-ribosylation and phosphorylation[11,89]. Both modifications appear to be modulated as a function of the individual cellular requirement. Under conditions

where BiP synthesis is increased, both ADP-ribosylation and phosphorylation are diminished, suggesting that the active form of BiP is not modified[35,47,84,81]. The group of Linda Hendershot analyzed BiP in cells that produce a high amount of Ig H chains[35]. Indeed, they could show that different forms of BiP correlated to its different states of activity. Whereas H chain-bound BiP molecules which are considered to represent the active state were neither phosphorylated nor ADP-ribosylated, both modifications were associated with the remaining fraction which most probably represents the inactive BiP pool. In addition, the modified molecules appear to be dimers whereas the ligand-bound BiP molecules are in a monomeric form[25]. The latter finding was confirmed by the result of chemical cross-linking analyses: the apparent molecular weight of a specific BiP/L chain cross-link product corresponded to the size expected for a complex containing one molecule of each component[16]. Slow folding proteins such as the extremely large thyreoglobulin molecule, however, might require the concomitant action of several monomeric BiP molecules[42].

Newly synthesized BiP molecules may first enter the inactive modified pool from which the active form is subsequently recruited. This assumption is supported by our results obtained by pulse chase experiments. Newly synthesized Ig L chains preferentially interact with pre-existing unlabeled BiP. Steady state levels of labeled BiP co-isolated with L chains were only obtained during the chase period[43]. If all inactive BiP molecules are phosphorylated and ADP-ribosylated, these modifications have to be reversible to allow BiP to interact with a ligand. On the other hand, the dissociation of BiP from its ligand and its re-entering the inactive pool would be accompanied by dimerization and two covalent modification steps. We have demonstrated that, once dissociated from its ligand, BiP is indeed recycled[43]. The exact mode of interconversion of BiP into the different forms however, remains to be established. In this context it is interesting to note that BiP expression is included by okadaic acid, which suppresses protein glycosylation[37] but is also a potent inhibitor of protein phosphatases.

Perspectives

Despite their high degree of homology, different hsp70 proteins are not interchangeable with respect to their specific functions. This highly specific competency has for example been demonstrated for BiP, yeast cytosolic hsc70 and the bacterial hsp70, DnaK[8]. Although this might be indicative for the previously discussed substrate specificities, it is more likely to reflect the inability of the different hsp70 proteins to interact with specific accessory proteins. It was shown that the bacterial DnaK cooperates with additional proteins such as DnaJ and GrpE to exert its function[36,82]. Polypeptides that are in the process of translocating into the yeast ER can be crosslinked with both the transmembrane proteins Sec61p and KAR2p. Interestingly, in addition to BiP, two other membrane proteins affect the cross-linking efficiency between sec61p and the translocating chains[80]. One of these, Sec63p, contains a region in a luminal domain that closely resembles the 'J-region' of DnaJ[83]. The highly conserved amino-terminal 'J region' of DnaJ is indeed necessary and sufficient for stimulating both DnaK's ATPase activity and λ-DNA replication[86]. It will be of general interest to analyze whether the heterologous bacterial proteins DnaJ and GrpE also have an effect on the ATPase activity of mammalian or yeast BiP, and likewise, to investigate whether it is indeed the 'J-region' of sec63 that binds to BiP[9]. It is tempting to speculate that sec63-binding positions BiP close to the luminal side of the ER translocation site; subsequent binding to the nascent chain would alter BiP conformation in such a way that sec63 can no longer interact but binds to a free BiP molecule; in this way, sequential binding and release reactions could provide the driving force for the hsp70 protein to 'pull' the translocating chain into the lumen of the ER.

Furthermore, additional proteins play a role. We recently described two other proteins that interact with BiP and its ligands, respectively[16]. One of these represents the luminal ER resident GRP94, a constitutively expressed member of the hsp90 protein family[16,60]; the other is the recently characterized GRP 170 protein[54]. Finally, a novel chaperone acting in the ER is calnexin, a transmembrane protein which specifically interacts with incompletely folded forms of transmembrane glycoproteins[33]. Comparative in vitro and in vivo structure/function analyses on hsp70 proteins, as well as a more detailed understanding of the interactions between the different actors involved in the process of ER protein folding, will provide future insights into the specific functions of BiP.

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