

Dean's Lecture

The ER Chaperone BiP Is a Master Regulator of ER Function

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Abstract

The endoplasmic reticulum (ER) is a command center of the cell that is second only to the nucleus in terms of the breadth of its influence on other organelles and activities. It is a major site of protein synthesis, contains the cellular calcium stores that are an essential component of many signaling pathways, and is the proximal site of a signal transduction cascade that responds to cellular stress conditions and serves to maintain homeostasis of the cell. All eucaryotic cells possess an ER, which can comprise nearly 50% of the membranes of a cell. Its functions can be divided into those that occur on the cytosolic side of the membrane (where protein translation and signal transduction cascades occur) and the luminal space (where most other ER functions take place). Our studies during the past several years have revealed that the ER molecular chaperone BiP is a master regulator of ER function. It is responsible for maintaining the permeability barrier of the ER during protein translocation, directing protein folding and assembly, targeting misfolded proteins for retrograde translocation so they can be degraded by the proteasome, contributing to ER calcium stores, and sensing conditions of stress in this organelle, to activate the mammalian unfolded protein response.

Key Words: ER chaperones, BiP, secretory pathway, ER stress, unfolded protein response.

Glossary of Abbreviations

ADP: adenosine diphosphate
ATF6: activating transcription factor 6
ATP: adenosine triphosphate
ATPase: adenosine triphosphatase
BAP: BiP-associated protein
BiP: binding protein
bZIP: basic leucine zipper
C_H1: first domain of the heavy chain constant region
EDEM: ER-degradation enhancing alpha-mannosidase-like protein
ER: endoplasmic reticulum
ERAD: ER-associated degradation
ER_p72: ER resident protein
Ig: immunoglobulin
LC: light chain
PDI: protein disulfide isomerase
PERK: PKR-like kinase
SERCA: sarco-ER CA²⁺ ATPase
SRP: signal recognition particle
UPR: unfolded protein response

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NEARLY ALL CELL SURFACE and secreted proteins, as well as the resident proteins of all organelles connected with the secretory pathway (endoplasmic reticulum, Golgi, lysosomes, and secretory vesicles), are synthesized on membrane-bound polyribosomes, where they are first translocated across the endoplasmic reticulum (ER) membrane and then folded and assembled in the ER lumen (Fig. 1). The environment that the nascent chain enters would appear to be a very hostile setting for protein folding, and yet even dedicated secretory cells like β islet cells of the pancreas, hepatocytes, and antibody secreting plasma cells, which can produce thousands of proteins/minute, do so with very few catastrophes. Unlike the cytosol, the ER is an oxidizing environment, which promotes disulfide bond formation between cysteine residues, contains large calcium stores, and is crowded with numerous partially folded proteins and subunits. All of these conditions have been shown to dramatically and adversely affect protein folding *in vitro*. It is now clear that the ER contains a large and diverse group of molecular chaperones, which recognize various features on unfolded proteins thereby ensuring that disulfide bonds are not formed between the wrong proteins or between the wrong cysteines within a protein. They also guarantee that exposed hydrophobic regions that will ulti-

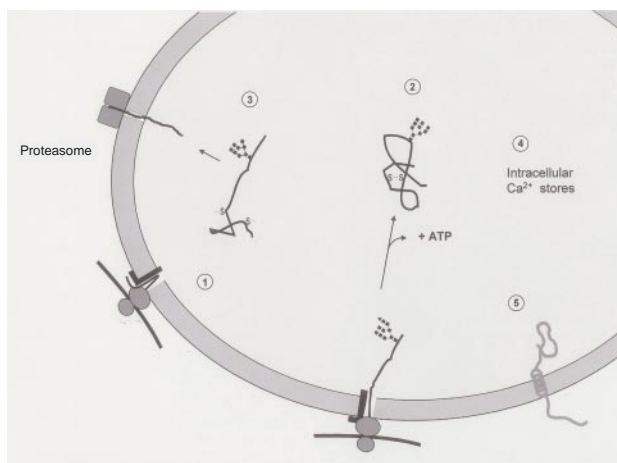


Fig. 1. Functions of the endoplasmic reticulum. (1) The endoplasmic reticulum (ER) is a major site of protein biosynthesis with translation occurring on the cytosolic face of the ER. Nascent proteins are co-translationally translocated across the ER membrane through aqueous channels. (2) Once inside the ER the polypeptide chain is modified by branched N-linked glycans and begins to fold. Folding for many proteins is adenosine triphosphate (ATP)-dependent and the tertiary structure is stabilized by intra- and inter-chain disulfide (S) bonds. (3) If folding fails, the protein must be identified and targeted for retro-translocation back to the cytosol, where it will be degraded by the 26S proteasome. (4) The ER is the storage site for the calcium that will be used in many signal transduction cascades. (5) Finally, if the cell encounters adverse physiological conditions that ultimately affect the environment of the ER, protein folding will be hampered and unfolded proteins will accumulate. A signal transduction response, called the unfolded protein response (UPR), is initiated in the ER, to protect the cell until normal conditions are restored.

mately be buried in the mature, properly folded protein do not lead to aggregate formation in the ER. In addition to their role in aiding and monitoring the maturation of secretory pathway proteins, the chaperones also identify those proteins that fail to mature properly and target them back through the ER membrane for degradation by the cytosolic 26S proteasome in a process that has been referred to as “ER quality control.” When adverse physiological conditions arise that prevent proper folding in the ER and thus lead to the accumulation of unfolded proteins in this organelle, a signal transduction cascade is activated which results in the coordinately up-regulation of all ER chaperones, to prevent their aggregation and promote their re-folding when normal conditions are restored.

Translocation into the ER

The first step in the synthesis of secretory pathway proteins involves targeting it to the ER

membrane, usually via an N-terminal signal peptide. The ER-targeting signal sequence is recognized as it emerges from the ribosome by signal recognition particle (SRP), which halts translation until the ribosome/mRNA, polypeptide chain complex can be attached to the ER membrane via association with SRP and ribosome receptors (1). The nascent chain is then transferred to a proteinaceous channel in the ER membrane called the translocon (2). In a dedicated secretory cell, thousands of ribosomes can be seen attached to the ER membrane, where they begin translocating the N-terminal end of the polypeptide chain, well before translation of the rest of the protein is accomplished. This co-translational translocation would appear to provide many breaches in the permeability barrier that separates the ER lumen from the cytosol and serves to maintain the unique environment of the ER. However, studies reveal that the permeability barrier is carefully preserved during the translocation process. As the nascent chain binds to the ER membranes and begins to be translocated across the ER membrane, the ribosome forms a tight seal with the cytosolic side of the translocon (3). At this time, the luminal side of the translocon is also gated, thus resulting in a translocon that is sealed on both ends. Once the translocating chain reaches a length of ~70 amino acids (~40 inside the ribosome channel and ~30 in the translocon), the luminal end of the translocon is opened (3). To identify the molecule responsible for gating the translocon on the lumen side, ER vesicles were isolated and opened, and luminal contents were removed before the vesicles were resealed. Studies with these vesicles revealed that the translocon was no longer sealed on the luminal side, demonstrating that something released with the luminal contents was responsible (4). By adding back the whole luminal contents or various luminal proteins, it was determined that the ER chaperone binding protein (BiP) was responsible for maintaining the permeability barrier of the translocon during the early stages of protein translocation (4). BiP was released once the nascent chain reached a length of ~70 amino acids, thus opening the channel so that the protein could be translocated into the luminal space. The remaining ribosomal seal on the cytosolic face of the translocon ensures that the environment of the ER is maintained and that nascent proteins move into the ER, as opposed to sliding back to the cytosol.

Protein Folding and Assembly Inside the ER

As the translocating chain enters the ER, well before translation is complete, it is often modified

by the addition of N-linked glycans, which serve to direct or limit the pathways along which the protein can fold (5). Folding can begin as soon as a portion of the protein enters the ER, making it very different from *in vitro* folding assays, which only involve full-length proteins. This co-translational folding introduces special constraints and additional problems, since in many cases the N-terminal region of a protein must interact with more C-terminal regions to yield the correctly folded form of the mature protein. In order to inhibit “inappropriate interactions” between the “wrong” regions of a protein during its biosynthesis, a group of resident ER proteins, which have been termed “molecular chaperones” for this very reason, bind and protect the nascent chain until the “correct” region becomes assessable. To date more than 10 chaperones, as well as numerous regulators and co-factors for these chaperones, have been identified in the mammalian ER (6, 7). The Hsp70 family member BiP was the first eukaryotic ER chaperone to be identified, due to its association with the incompletely assembled subunits of antibody molecules (8, 9), a system that continues to be a model for studying BiP’s interactions with unfolded proteins. Antibodies are composed of a series of immunoglobulin (Ig) domains that fold independently of each other into a compact structure composed of two twisted β sheets stabilized by a single disulfide bond (10). Although the overall structure of the different domains is extremely similar, the folding requirements and dependence on BiP are very different. BiP binds transiently to some Ig domains (e.g., most C_H domains and some V_L and V_H domains) (11, 12), while other domains appear to fold rapidly and stably without ever interacting with BiP (e.g., C_L) (13). Only the C_H1 domain, which pairs with the C_L domain of the light chain and provides the site for its covalent attachment, interacts stably with BiP in the absence of light chains (LCs) (14). The interaction of this domain with BiP is crucial to controlling Ig assembly and transport, because deletion of this domain, and the resulting ablation of BiP binding, allows the secretion of unassembled and partially assembled Ig intermediates (14).

Two theories were proposed to account for this unique feature of the C_H1 domain. First, in addition to BiP’s normal binding to extended, hydrophobic sequences that exist on nascent chains before they fold, BiP might also bind to hydrophobic faces on folded protein subunits before they assembled into multimeric complexes. Alternatively, if BiP only interacted with unfolded linear sequences, then the C_H1 domain might remain unfolded until it paired with LCs. This as-

sembly-dependent folding, first proposed by the Haas lab (15), was somewhat at odds with the belief that ER quality control only allowed properly folded subunits to assemble. By monitoring disulfide bond formation in each heavy chain domain as an indication of folding, we demonstrated that indeed the C_H1 domain is not folded in the absence of light chains, while all the other domains fold and form their intra-domain disulfide bond (16). Interestingly, LCs are not required as a scaffold to induce C_H1 domain folding, but instead act in some undefined way to displace BiP from the heavy chain, thus allowing it to fold (16). Only LCs in which both domains are folded can perform this function. This constraint might be important in controlling the assembly of the pre-B cell antigen receptor, where each LC domain is provided by a separate protein (i.e., V_{preB} and λ_5) (17), as well as in monitoring the variable region of the LC, which can undergo extensive mutation during Ig repertoire development.

BiP (18, 19), like all Hsp70 family members (20), binds and hydrolyzes adenosine triphosphate (ATP), which controls its chaperoning function (20). The ATP-bound state of Hsp70 proteins represents an “open” configuration, which allows them to associate with unfolded substrates. The presence of a DnaJ co-factor in the complex catalyzes the rapid hydrolysis of ATP to adenosine diphosphate (ADP), “locking” the Hsp70 protein onto the unfolded protein. DnaJ proteins exist in all species and organelles: the yeast ER contains three DnaJ proteins (21), while the mammalian ER possesses at least five DnaJ orthologues. The next step in the Hsp70 adenosine triphosphatase (ATPase) cycle is the exchange of ATP back into the nucleotide binding cleft, which “reopens” the Hsp70 protein and releases the unfolded protein. In bacteria and the mitochondria of eukaryotic cells, GrpE proteins regulate the exchange of nucleotide and promote the folding of Hsp70 substrates (22). Although no GrpE homologues have been identified in the yeast or mammalian ER, other nucleotide-releasing factors exist (23, 24). It is believed that the exchange factors and DnaJ proteins continuously turn the Hsp70 ATPase cycle to promote binding and release of Hsp70 proteins, thus allowing the substrate protein to proceed to an increasingly folded state. In support of this idea, we found that BiP ATPase mutants bind to nascent proteins in the ER and protect them from aggregation. However, the mutants could not be released from these proteins, and as a result the substrate protein failed to complete its folding or be secreted (12).

Thus, BiP binds and protects newly synthesized proteins when they are in an unfolded state, but it must release them so that they can fold (Fig. 2).

When studying the folding and assembly of Ig molecules, we found that the C_{H1} domain of unassembled heavy chains remained unfolded in the cell, even though it was capable of folding if BiP was released *in vitro* with ATP (16). This was shown to be due to the fact that BiP did not cycle from the heavy chain *in vivo* and suggested that BiP's ATPase cycle was somehow "stalled" when BiP was associated with unassembled heavy chains (25). This led us to look for regulators of BiP's ATPase cycle. Using chemical cross-linkers to stabilize protein:protein interactions, we found that a luminal DnaJ orthologue, ERdj3, was stably associated with unassembled Ig heavy chains (26). As DnaJ proteins stimulate ATP hydrolysis by Hsp70 proteins and help "lock" them onto unfolded substrates, it is possible that its presence in the BiP:heavy chain complex ensures that BiP is not released prematurely. Using a 2-hybrid screen to identify proteins that interacted with the ATPase domain of BiP, we identified BiP-associated protein (BAP), the first mammalian ER nucleotide-releasing factor (24). BAP binds preferentially to BiP when it is in the ADP-bound form and induces ADP re-

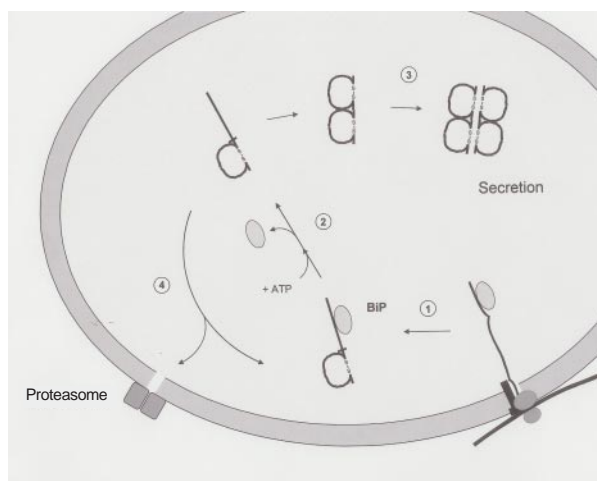


Fig. 2. The molecular chaperone binding protein (BiP) binds to unfolded regions of proteins in the ER. (1) Although proteins can begin to fold co-translationally, some regions of proteins are often slow to fold. BiP binds to these regions and keeps them from aggregating. (2) BiP is released in an ATP-dependent manner allowing the protein to fold completely. (3) If folding occurs properly, the protein will be transported from the ER for eventual cell surface expression or secretion. (4) If it is unable to fold, BiP can either rebind to give the protein another chance or target it for retrotranslocation and degradation.

lease so that ATP can be substituted back. This leads to the release of BiP from heavy chains *in vitro*. It is possible that light chain association with heavy chains is required to either release ERdj3 so that BAP can enter the complex, or that light chains in some way escort BAP to the complex (Fig. 3).

Disposing of Proteins That Fail to Meet ER Quality Control Standards

Although the vast majority of proteins entering the ER fold quickly and accurately into their proper tertiary structure, protein subunits produced in excess, mutant proteins, and even occasionally normal proteins fail to mature properly. A major function of the ER is to detect these "aberrant" proteins and remove them from the secretory pathway, so they can be disposed of in a process called ER-associated degradation (ERAD) (27). Studies of yeast revealed that the *sec61* protein, which is a major component of the translocon, is required for retrotranslocation and degradation of proteins

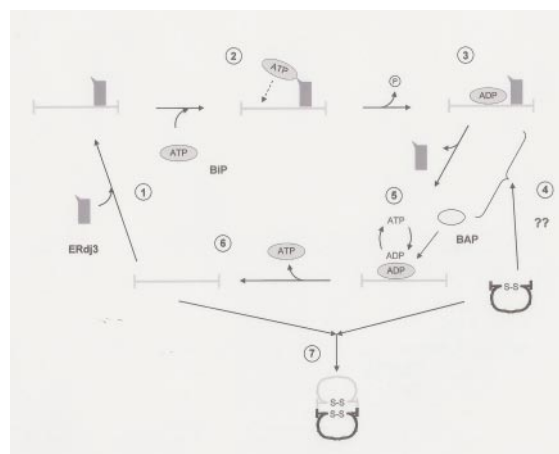


Fig. 3. The ATPase cycle of BiP controls its binding to and release from unfolded proteins and is regulated by co-factors. (1) The co-factor ERdj3 binds to unfolded regions of a nascent Ig heavy chain. (2) It recruits ATP-bound BiP to the substrate. (3) By stimulating BiP's ATPase activity and converting it to the adenosine diphosphate (ADP)-bound form, it "locks" BiP onto the C_{H1} domain of the unassembled heavy chain and keeps it in an unfolded protein. (4) In the case of immunoglobulin (Ig) heavy chains, the cycle is stalled here until light chains associate and restart the cycle by an unknown mechanism. (5) The nucleotide-releasing factor, BiP-associated protein (BAP), catalyzes the release of ADP and rebinding of ATP by BiP, shifting it to the "open" conformation. (6) This leads to the release of BiP from the substrate. (7) If the heavy chain is able to combine properly with the light chain, it will assemble into an antibody molecule and be transported to the Golgi for secretion. If not, it can reassemble with ERdj3 for another round or be targeted for degradation.

that fail ER quality control (28). This suggests that the unfolded proteins move back through the translocon or at least through a structure that contains some of the same components as the translocon. How proteins that have ultimately failed to fold are distinguished from those that are in the process of folding is not completely understood. In the case of the calnexin/calreticulin chaperone family, this involves monitoring the N-linked glycans that decorate most secretory proteins and also serve as binding sites for these chaperones. The N-linked glycan is a complex branched oligosaccharide structure that undergoes modification in the ER and processing in the Golgi as the protein bearing it moves through the secretory pathway. A single glucose sugar is continuously added to and removed from the glycan in the ER as long as a portion of the protein is unfolded (29). Calnexin or calreticulin bind to this monoglucosylated glycan on the unfolded protein (30). If however, the mannose residues are trimmed from n9 residues back to 8 before a glucose residue can be re-added, the protein will be recognized by an ER-degradation enhancing alpha-mannosidase-like protein (EDEM) and pulled from the “folding” pathway. EDEM then targets the protein for retrotranslocation and degradation (31). For proteins that utilize BiP during folding, it is much less clear how terminally unfolded proteins are distinguished from proteins that have not yet folded. Studies of yeast demonstrate that BiP is required for the retrotranslocation of a number of ERAD substrates (28). By making a number of chimeras between domains of unfolded proteins with various half-lives, we found that in mammalian cells the BiP binding domain controls the rate of degradation (32), which strongly implies that BiP plays a role in targeting proteins for retrotranslocation in mammalian cells as well. Since BiP also interacts with nascent proteins as they fold, the question immediately arises as to how these two BiP functions are separated. One possibility is that the different DnaJ co-factors provide the specificity of function (Fig. 2). To date, five DnaJ homologues have been identified in the mammalian ER (ERdj1–5). Three of these (ERdj3–5) are induced by ER stress, a condition that leads to the degradation of an increased number of ER proteins, and one of these (ERdj4) is a membrane-anchored protein that is expressed at very low levels in the absence of ER stress, but is potently up-regulated when folding is adversely affected in the ER

(33). ERdj3 associates directly with a number of unfolded and partially folded proteins in the ER along with BiP (26). It is possible that proteins could be transferred from ERdj3 to ERdj4 when folding has ultimately failed, redirecting them from a soluble state in the ER lumen to the ER membrane, so they can be retrotranslocated for degradation. However, there are no data available at this point to either support or refute this hypothesis.

Controlling and Maintaining ER Calcium Stores

The ER plays a vital role in controlling both the level and the release of the calcium stores that are used in many signal transduction programs. The concentration of calcium in the ER has been calculated to be between 0.3 and 1.5 mmol⁻¹ (34), a concentration that places it several orders of magnitude higher than those found in the cytosol. The high ER levels are controlled by several transmembrane proteins; the ryanodine and the IP₃ receptors release calcium from the ER stores when they are activated by signal transduction cascades, and the sarco-ER CA₂⁺ ATPase (SERCA) pump, which transports calcium back into the ER (35). Drugs like thapsigargin and A23187 lower ER calcium levels by “poisoning” the SERCA pump and releasing calcium stores, respectively. Interestingly, both of these drugs potently activate the unfolded protein response (UPR) (36), demonstrating that secretory pathway proteins require the high ER calcium levels to fold properly. The calcium in the ER exists largely in complexes with both low-affinity calcium-binding proteins (like calnexin, calreticulin and GRP94), which are also molecular chaperones, and with high-affinity calcium-binding proteins (like reticulocalbin and calstosin) (34, 35). Because BiP is a highly abundant ER protein and possesses scattered clusters of acidic amino acids like several of the other ER chaperones that bind calcium, we measured the ability of BiP to contribute to ER calcium stores by overexpressing BiP and other chaperones in HeLa cells and measured the effect on ER calcium levels. Our data reveal that BiP and calreticulin both contribute to the ER calcium stores, whereas overexpression of ERp72 did not significantly increase ER calcium levels (37). The increase observed with BiP was directly due to its ability to bind calcium and not to its chaperoning of another ER protein, since both wild-type and ATPase mutants of BiP were able to perform this function. We calculated that

under normal physiological conditions, BiP contributes to ~25% of the stores, with a stoichiometry of 1B2 moles of calcium/mole of BiP.

Monitoring and Responding to Conditions That Affect Protein Folding in the ER

The ER and the rest of the secretory pathway possess a unique intracellular environment, one that is similar to the extracellular milieu that many of the proteins being synthesized will ultimately inhabit. In addition to being influenced by this oxidizing environment that promotes intra- and inter-chain disulfide bonds in secretory pathway proteins, the vast majority of proteins synthesized in this organelle are also modified by N-linked glycans. These conditions and modifications regulate the stability and folding of the nascent polypeptide. Changes in physiological conditions that affect the storage of calcium, the oxidative state or the pH of the ER lumen, or conditions that alter the availability of N-linked glycans can very dramatically and adversely alter protein maturation and lead to the accumulation of unfolded proteins in this organelle. The cell responds to this accumulation by activating a signal transduction pathway, termed the "unfolded protein response" (UPR). The response consists of coordinately up-regulating the transcription of all ER chaperones (36) to protect unfolded proteins and prevent them from aggregating, and also transiently inhibiting protein synthesis (38) to limit the accumulation of unfolded proteins. The inhibition of protein synthesis also induces cell cycle arrest (39), so that cells encountering stress conditions are not propagated. Finally, apoptotic pathways are activated if the stress conditions are not alleviated (40) in order to protect the organism.

The signal for inducing the UPR was understood long before the proteins that transmit the signal were identified. In 1988, Kozutsumi et al. reported that by transiently overexpressing a single unfolded protein they were able to induce the UPR in mammalian cells (41). Follow-up studies by other groups revealed that only a subset of unfolded proteins activated the response; proteins that bound to BiP did (42), whereas proteins that associated with one of the other chaperones like calnexin did not (43). A role for BiP in signaling ER stress was further supported by data showing that the overexpression of BiP, but not of other ER chaperones like protein disulfide isomerase (PDI) or GRP94, was sufficient to block activation of the UPR during ER stress conditions (44). Together

these data suggested that the cell somehow determined the amount of BiP that was available or free to monitor the presence of conditions in the ER. If sufficient amounts of BiP were available, conditions must have been good, whereas if BiP became limited, conditions must have been taxing the chaperones present, and therefore the UPR would be activated to coordinately up-regulate all ER chaperones. The molecular basis for this level of control awaited the identification of the signaling molecules.

Activation of the mammalian UPR is achieved by three ER-localized transmembrane proteins that "sense" stress through their luminal domains and transduce the stress signal via their cytosolic domains. The Ire1 kinases (α is ubiquitously expressed and β is limited to gut epithelium) (45, 46) are homologues of the single ER stress transducer in yeast, Ire1p (47, 48). Activation of mammalian Ire1 during ER stress stimulates a unique endonuclease activity in its C-terminal region, an activity that is conserved with yeast Ire1p (49). The XBP-1 mRNA, which encodes a basic leucine zipper transcription factor, is the single known target of the mammalian Ire1 endonuclease. Activated Ire1 excises a 26-nucleotide segment from the XBP-1 transcript, which is then relegated to encode a larger remodeled transcription factor, sXBP-1, with an altered transactivation domain tethered to the original DNA binding domain (50, 51). The second signal transducer of the UPR is the double-stranded RNA-activated protein kinase (PKR)-like kinase (PERK), which phosphorylates eIF-2 α in response to ER stress (52, 53). This modification serves to transiently block most protein translation, thereby diminishing the accumulation of unfolded proteins in the ER when conditions are not favorable for folding. The third component of the signaling apparatus, ATF6, is a transmembrane protein with a luminal domain and a cytosolic domain that encodes another basic leucine zipper (bZIP) transcription factor (54). In response to ER stress, ATF6 is transported to the Golgi, where it is cleaved by the S1P and S2P proteases, resulting in the release of its cytosolic domain, which then traffics to the nucleus and up-regulates transcription of ER chaperones and folding enzymes, to prevent the aggregation of unfolded proteins and aid in their subsequent folding if stress conditions are resolved (55).

With the identification of the upstream signaling molecules of the UPR and the production of reagents to isolate them, it was possible to determine the molecular basis for sensing stress in the ER. We found that in unstressed cells PERK

and Ire1 form a stable complex with BiP (Fig. 4). Agents that alter normal protein folding in the ER promote the reversible dissociation of BiP from the luminal domains of these kinases, which leads to their oligomerization and activation (56). In situations where BiP is overexpressed, the higher levels of BiP allow it to remain bound to the kinases even under stress conditions and therefore to inhibit their activation. The abatement of ER stress results in BiP rebinding to the kinases, attenuating the response (56). Other studies reveal that Ire1p is similarly regulated by BiP in yeast (57, 58). When ATF6 was examined, we found that, like the ER kinases, BiP formed complexes with the luminal domain of this membrane-anchored transcription factor under normal physiological conditions. Induction of ER stress led to a rapid release of BiP from the luminal domain of ATF6, which resulted in its transport to the Golgi where it could be acted on by the S1P and S2P proteases that reside there, thus liberating the cytosolic-oriented transcription factor domain (59). This required an ATP-dependent re-

lease of BiP from the luminal domain of ATF6, as BiP ATPase mutants blocked transport and cleavage of ATF6 (59). Together these findings are consistent with BiP serving to inhibit signaling through PERK, Ire1, and ATF6 in the absence of unfolded proteins. Protein misfolding relieves this inhibitory signal and triggers the response either indirectly, by competing for BiP, or directly, by altering the conformation of the signal transducers themselves (Fig. 4). The ATP-dependent release of BiP from the transducers suggests that they may possess unfolded regions in the absence of stress, which serve as conventional BiP binding sites. Thus, stress conditions in the ER appear to be sensed by monitoring the availability of a single molecular chaperone, BiP. When it becomes limiting and the transducers are activated, a signal transduction cascade is put in motion that results in the up-regulation of all ER chaperones and many of their regulators. This ensures that the appropriate chaperone will be available regardless of the requirements of the individual protein, without having to monitor levels of all the chaperones.

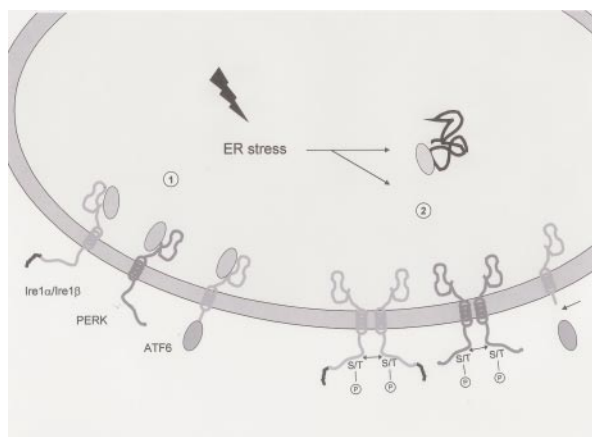


Fig. 4. Sensing stress conditions in the ER. (1) Researchers have identified three resident ER transmembrane proteins that serve as signal transducers for ER stress. They include Ire1 α/β , PKR-like kinase (PERK), and activating transcription factor 6 (ATF6). During normal physiological conditions their luminal domains are bound to BiP, which keeps them in an inactive state. (2) When unfolded proteins accumulate in the ER due to stress conditions, BiP is released from the luminal domains to interact with the unfolded proteins. This results in the dimerization or oligomerization of Ire1 and PERK, which leads to their activation and downstream signaling. The release of BiP from ATF6 allows it to traffic to the Golgi, where the cytosolic transcription factor domain is liberated from the membranes, so that it can drive transcription of UPR target genes.

P=phosphate, S/T=serine or threonine residue.

Summary

The ER houses protein complexes that allow it to carry out its diverse multitude of functions, which include protein translocation, protein folding, the identifying and degrading of proteins that fail to mature properly, the storing of calcium, and finally sensing and responding to abnormal physiological conditions that adversely affect protein biosynthesis in this organelle. A single ER chaperone, BiP, plays a critical role in controlling or contributing to each of these functions (Fig. 5). With the exception of calcium storage, BiP's ATPase activity is required for it to perform all of these different roles, suggesting that BiP may recognize the specific proteins involved as though they were unfolded proteins, and further, that its binding and release will be regulated by DnaJ and nucleotide-releasing factors (as has been demonstrated for its role in protein folding). Five mammalian ER DnaJ orthologues have been identified; it is possible that each is specific for a different function and that they provide an additional layer of specificity to BiP.

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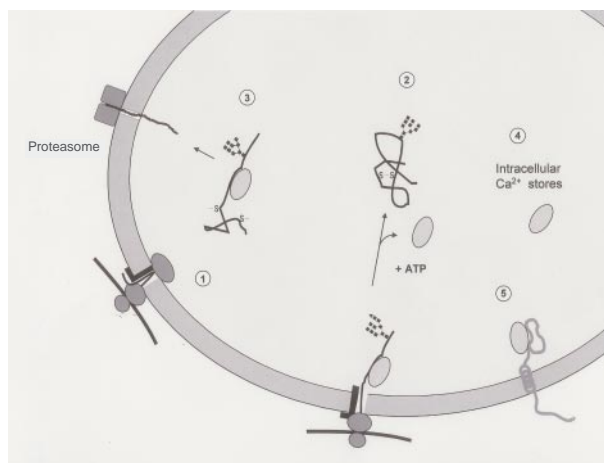


Fig. 5. BiP is integrally involved in all major ER functions. (1) BiP binds to the translocon and seals the luminal end until the ribosome is seated on the cytosolic side and the elongating polypeptide is ~70 amino acids long. (2) BiP binds to unfolded regions of nascent proteins and directs their folding and assembly in an ATP-dependent manner. (3) BiP recognizes improperly folded proteins and targets them for degradation. (4) BiP is a calcium-binding protein and contributes to the ER calcium stores. (5) BiP binds to the luminal domains of the ER stress transducers and keeps them in an inactive state. When levels of free BiP become limiting, BiP is released and the transducers are activated.

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