In eukaryotic cells, accumulation of unfolded proteins in the endoplasmic reticulum (ER) results in a transcriptional induction of a number of ER chaperone proteins. In Saccharomyces cerevisiae, the putative transmembrane receptor kinase, Ire1p (Ern1p), has been implicated as the sensor of unfolded proteins in the ER that initiates transmittance of the unfolded protein signal from the ER to the nucleus. We have shown that the cytoplasmic domain of Ire1p receptor indeed has intrinsic Ser/Thr kinase activity and contains Ser/Thr phosphorylation sites as well. The cytoplasmic domain is also shown to form oligomers in vivo and in vitro. The ability to form oligomers primarily resides within the last 130 amino acids of the cytoplasmic domain, a region that is dispensable for in vitro kinase activity of the receptor. Oligomerization of the cytoplasmic domains is required for receptor trans-phosphorylation and subsequent activation of the kinase function. The activated kinase may transmit the unfolded protein signal from the ER to the nucleus to activate the transcription of the chaperone genes in the nucleus.

SECRETORY AND TRANSMEMBRANE PROTEINS ARE CO-TRANSLATIONALLY TRANSLOCATED INTO THE LUMEN OF THE ENDOPLASMIC RETICULUM (ER). Upon entry into the lumen of the ER, these proteins must be folded properly and frequently assembled into large complexes before they resume their journey to the cell surface (1). The ER provides an oxidizing environment that favors formation of disulfide bonds and contains a number of chaperones that facilitate protein folding and subunit assembly (2), including immunoglobulin binding protein (BiP or GRP78), GRP94, protein disulfide isomerase, and calnexin. Protein folding in the ER can be perturbed by (a) preventing protein glycosylation with drugs such as tunicamycin and 2-deoxyglucose, (b) preventing formation of disulfide bonds with reducing agents such as β-mercaptoethanol, (c) depleting the ER calcium with ionophores, or (d) expression of some wild-type or mutant proteins that transit the secretory compartment. Such perturbations result in accumulation of unfolded proteins in the lumen of the ER (3). When misfolded proteins accumulate in the ER lumen, transcription of many genes encoding ER resident chaperone proteins is induced in the nucleus (4, 5). This unfolded protein response has been observed in a variety of different organisms as divergent as yeast and humans, underscoring its importance for the survival of the organism. In the budding yeast, Saccharomyces cerevisiae, accumulation of unfolded proteins in the lumen of the ER induces transcription of BiP (encoded by KAR2 (6, 7)), PDI (encoded by PDI1 (8, 9)), and peptidyl-prolyl cis-trans-isomerase (encoded by FKB2 (10)).

The genes that are transcriptionally activated in response to unfolded proteins contain a cis-acting promoter element that is necessary and sufficient to mediate the response. In S. cerevisiae, such an element was initially identified by deletion analysis of the KAR2 promoter region. A 22-base pair element, referred to as the unfolded protein responsive element (UPRE), is necessary and sufficient to activate the transcription of KAR2 gene in response to unfolded proteins in the lumen of the ER (11). Subsequently, potential UPRE elements were identified in a number of yeast chaperone genes. However, only the element from FKB2 has been shown experimentally to function as an authentic UPRE (10).

Pathways that sense protein folding status in the ER and transmit a signal to the nucleus are virtually unknown. Therefore, it is unclear how the unfolded protein signal is transmitted from the ER. Using a genetic selection for mutants defective in the UPR pathway, two independent groups isolated the same gene which was named as Ire1p, previously identified as a gene required for inositol autotrophy (9, 12) or ERN1 (13) for ER to nucleus signaling pathway. The identification of Ire1 protein (Ire1p) was established using antibodies that recognize different regions of the molecule. The N-terminal half of the protein is in the lumen of the ER, whereas the C-terminal is in the cytoplasm (13). These two regions are connected by a short transmembrane domain that spans the ER membrane once. The cytoplasmic domain of Ire1p contains a region that is significantly homologous to the catalytic domains of known Ser/Thr kinases. Although Ire1p is the most homologous to CDC28, it is distinctive by being a more distant member of the Ser/Thr protein kinase family (14). On the basis of these findings, it was proposed that Ire1p is the proximal sensor of unfolded proteins in the lumen of the ER and that it directly or
Ire1p is structurally similar to class I growth factor receptors that undergo ligand-mediated oligomerization (15). Ligand binding and subsequent conformational alterations in the extracellular domain of class I receptors induce receptor oligomerization that stabilizes interaction between adjacent cytoplasmic domains (16). These intermolecular interactions lead to receptor trans-phosphorylation and activation of kinase function. Based on these structural homologies, we hypothesized that the Ire1p cytoplasmic domain can form oligomers to elicit trans-phosphorylation and kinase activation as essential requirements in this signal transduction response. To test the above hypothesis, we investigated the mechanism of Ire1p receptor activation utilizing a combination of genetic, molecular biology, and biochemical approaches. Our data show that the cytoplasmic domain of Ire1p receptor forms oligomers in vivo and in vitro and has Ser/Thr kinase activity. Formation of oligomers leads to receptor trans-phosphorylation and subsequent activation of the kinase function.

**EXPERIMENTAL PROCEDURES**

Media, Strains, and Plasmid Constructions—The Escherichia coli strain DH5α was used for the propagation of plasmids. The genotype and the source of S. cerevisiae strains used in this study are as follows. EGY48, Matα his3 (e2a::3-LexAop-LEU2 ura3 trpl lys2) (17). The genetic methods and standard media were previously described (18). The yeast plasmids pJG4–5 (prey plasmid) and pEG202 (bait plasmid) were described by Gyuris et al. (17). To create plasmids for two-hybrid analysis, the cytoplasmic domain (aa 556-1115), the kinase domain (aa 677-985), the N-linker region (aa 556–676), the C-tail (aa 986-1115), the kinase domain plus the N-linker (aa 556–985), and the C-tail plus the N-linker (aa 677-1115) were amplified by polymerase chain reaction using Ire1p DNA from either wild-type (pERN1GL) or K702A mutant (pERN1BC) as the template (generously provided by Mary-Jane Gething). The polymerase chain reaction fragments were subcloned into the EcoRI and XhoI sites of plasmid G4–5 and pEG202. To create GST fusion constructs, cytoplasmic domain, kinase domain, kinase domain plus N-linker region, and kinase domain plus C-tail were amplified by polymerase chain reaction using either pERN1GL or pERN1BC as the template DNA. Amplified fragments were subcloned into the EcoRI site of the bacterial expression vector, pGEX1T (Pharmacia Biotech Inc.).

Preparation of GST Fusion Proteins—All recombinant GST proteins were produced using the expression vector, pGEX1T, that contains the GST gene under control of tac promoter. Plasmids containing different fusions were transformed into E. coli strain BL21. After induction with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 4 h at 37°C, the fusion proteins were purified by GSH-Sepharose chromatography (19) using glutathione-Sepharose beads from Pharmacia.

Protein Kinase Assays—Equal amounts of purified recombinant GST-Ire1p fusion proteins were incubated at 30°C for 30 min in 20 μl of kinase buffer containing 18 mM HEPES (pH 7.5), 10 mM MgOAc, 10 μM ATP, and 5 μCi of [γ-32P]ATP (3000 Ci/mmol, Amersham Corp.). The phosphorylated proteins were analyzed by SDS-PAGE (10% gel) and autoradiography. Phosphorylation was quantified by PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Western Blotting—Glutathione-Sepharose beads (2 μl) saturated with GST-Ire1p fusion proteins were incubated in the kinase buffer with 20 μl of in vitro kinase assay (Sigma) for 10 min at room temperature. Western blotting was performed by standard procedures (24) using horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (Life Technologies, Inc.). Bands were detected using the ECL kit (Amersham Corp.) and quantified using NIH Image.

**RESULTS**

Ire1p Cytoplasmic Domain Forms Oligomers in Vivo and in Vitro—Ire1p is structurally similar to class I growth factor receptors that undergo ligand-mediated oligomerization (16). To test the ability of Ire1p to form oligomers, we utilized the two-hybrid system (17), a modified version of the yeast two-hybrid system first described by Fields and Song (25). The two-hybrid system is based upon the fact that transcription factors have two distinct separable domains that mediate either DNA binding or transcription activation. When the protein of interest (bait) is expressed as a LexA DNA binding domain fusion protein in yeast it cannot activate transcription because the hybrid protein lacks a transcription activation domain. A second protein that is a fusion between the B42 transcription activator and a protein (prey), presumed to interact with the first protein, can activate transcription but cannot bind to DNA. Upon expression of the fusion proteins in yeast, interaction between bait and prey reconstitute a transcription factor that binds to the LexA operator and activates transcription of the LEU2 reporter gene.

To construct the bait hybrid proteins, different regions of the cytoplasmic domain of Ire1p were fused to the DNA binding domain of LexA. The domain structure of the resulting fusion proteins are depicted in Fig. 1A. To create the prey hybrids, the same regions of Ire1p were fused with the acidic transcription activation domain B42. The resulting fusion proteins contained SV40 large T antigen nuclear localization signal (NLS), influenza virus HA epitope tag, and B42 transcriptional activation domain at their amino terminus. Since the molecular masses of as described by Ooi et al. (20). Phosphoamino acids standards were obtained from Sigma.

**Immunoprecipitations—**Yeast strains containing bait and prey plasmids were grown in selective media containing 2% galactose and 1% raffinose for 12 h. Cells were extracted in buffer (50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 7 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin) as described by Williams et al. (21). Immunoprecipitations were performed according to Harlow and Lane (22) using anti-LexA antibody (generously provided by Dr. Erica Golemis). Pellets were boiled for 3 min with 1 × Laemmli buffer (23), separated by SDS-PAGE (10% gel), and analyzed by Western blotting with anti-HA antibody (Boehringer Mannheim).

Western Blots—Yeast cells were broken as described by Williams et al. (21) in extraction buffer (50 mM Tris-HCl, (pH 8.0), 5 mM EDTA, 7 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin), and proteins were extracted by boiling with an equal volume of 2 × Laemmli buffer (23) for 3 min. The supernatants were recovered, and the proteins were quantified by using the protein assay kit from Bio-Rad.

After SDS-PAGE was electroelugraphically transferred to nitrocellulose membranes, and Western blotting was performed by standard procedures (24) using horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (Life Technologies, Inc.). Bands were detected using the ECL kit (Amersham Corp.) and quantified using NIH Image.

In Vitro Pull Down Assays—Extracts were made as above from yeast strains expressing different regions of Ire1p cytoplasmic domain in the prey plasmid pG4–5. Each recombinant protein was quantified by Western blotting with anti-HA antibody (Boehringer Mannheim). Extracts were preceeded with glutathione-Sepharose beads, and equal amounts of each recombinant protein was incubated at 4°C for 10 h with glutathione-Sepharose beads containing equal amounts of either GST or GST-Ire1p cytoplasmic domain fusion protein. Beads were recovered, washed twice with phosphate-buffered saline containing 10% glycerol and 0.05% Triton X-100 and once with phosphate-buffered saline, and boiled for 3 min in 1 × Laemmli (23) buffer. Extracts were run on 10% reducing SDS-polyacrylamide gels and analyzed by Western blotting with anti-HA antibody (Boehringer Mannheim).

The yeast two-hybrid assays were performed as described previously (17). Yeast reporter strain EGY48 was sequentially transformed with derivatives of pG202 and pG4–5 containing different regions of the Ire1p cytoplasmic domain. Interactions were monitored by the ability of the reporter strain to grow on media lacking leucine.

**RESULTS**

Ire1p Cytoplasmic Domain Forms Oligomers in Vivo and in Vitro—In vitro pull down assays with two-hybrid systems were performed as described above from yeast strains expressing different regions of Ire1p cytoplasmic domain in the prey plasmid pG4–5. Each recombinant protein was quantified by Western blotting with anti-HA antibody (Boehringer Mannheim). Extracts were preceeded with glutathione-Sepharose beads, and equal amounts of each recombinant protein was incubated at 4°C for 10 h with glutathione-Sepharose beads containing equal amounts of either GST or GST-Ire1p cytoplasmic domain fusion protein. Beads were recovered, washed twice with phosphate-buffered saline containing 10% glycerol and 0.05% Triton X-100 and once with phosphate-buffered saline, and boiled for 3 min in 1 × Laemmli buffer. Extracts were run on 10% reducing SDS-polyacrylamide gels and analyzed by Western blotting with anti-HA antibody (Boehringer Mannheim). Extracts were run on 10% reducing SDS-polyacrylamide gels and analyzed by Western blotting with anti-HA antibody (Boehringer Mannheim).

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**FIG. 1. In vivo genetic interaction of Ire1p cytoplasmic domain.** Panel A, schematic representation of LexA-Ire1p and B42-HA-Ire1p fusion proteins and their ability to form oligomers as detected by the two-hybrid analysis (Genetic) and co-immunoprecipitation experiments (Physical). WC, wild-type cytoplasmic domain; MC, K702A mutant cytoplasmic domain; NK, N-linker plus kinase domain; NL, N-linker region; WK, wild-type kinase domain; MK, K702A mutant kinase domain; KC, kinase plus C-terminal tail; CT, C-terminal tail; K, lysine; A, alanine; ND, not detected. Panel B, expression of LexA-Ire1p and B42-HA-Ire1p fusion proteins in S. cerevisiae EGY48 strain harboring different regions of Ire1p cytoplasmic domain in vectors, pEG202 and pJG4–5, was grown in His2 Trp2 media containing galactose. Cells were harvested and broken, and extracts were analyzed by Western blotting with either anti-HA or anti-LexA antibody. Migration of the fusion proteins is indicated by kDa. Panel C, two-hybrid analysis. Growth of EGY48 strain harboring different regions of Ire1p cytoplasmic domain as LexA fusions (in pEG202) and as B42-HA fusions (in pJG4–5) on His' Trp' media (Control) and His' Trp' Leu' media containing either galactose plus raffinose (Gal) or glucose (Glu).
LexA DNA binding domain and NLS-HA-B42 are 22.5 and 11.9 kDa, respectively, the bait hybrids are larger than their prey hybrid counterparts. Expression of prey hybrids was under the GAL 1 promoter which is galactose-inducible and glucose-repressible. Plasmids expressing these proteins were introduced into the yeast strain EGY48 that carries a chromosomal copy of LexAop-LEU2. The expression of these proteins in yeast was confirmed by Western blotting with either anti-HA to detect prey or anti-LexA antibody to detect bait (see Fig. 1B). Interaction between the hybrids was assayed by the ability of the transformants to grow on media lacking leucine. Expression of the Ire1p wild-type cytoplasmic domain as bait (LexA-WC) in the presence of NLS-HA-B42 (B42-HA) did not activate the reporter constructs as demonstrated by the absence of growth on media lacking leucine, indicating that the bait was transcriptionally inert (Fig. 1C). In contrast, expression of the cytoplasmic domain as bait (LexA-WC) in the presence of the cytoplasmic domain as prey (B42-HA-WC) yielded a leu+ phenotype on galactose media but not on glucose containing media (see Fig. 1C), indicating interaction between wild-type cytoplasmic domains. Hybrids that contain a catalytically inactive cytoplasmic domain (K702A in MC, a mutation that prevents ATP hydrolysis but not ATP binding) did not affect the interaction, indicating that a functional kinase was not required. To identify the interactive site(s) for oligomerization, the N-terminal and/or C-terminal portions of the cytoplasmic domain that border the kinase domain were deleted. Deletion of either the N-terminal linker region (KC) or the C-terminal tail (NK) did not affect the homotypic interaction, whereas deletion of both the N-terminal linker and the C-terminal tail destroyed the homotypic interaction for the wild-type (WK) as well as the mutant (MK) kinase. Expression of the C-terminal tail (CT) as prey and bait was sufficient to mediate the interaction. Due to the acidic nature of the N-terminal linker region (NL), it could not be used alone as a bait in the two-hybrid analysis (data not shown). These data indicate that the ability to form oligomers resides primarily in the region outside the kinase domain.

To confirm a physical interaction between the wild-type cytoplasmic domain and its subdomains, immunoprecipitations from cell lysates containing the fusion proteins described above were performed with anti-LexA antibody. Immunoprecipitated proteins were analyzed by Western blotting with anti-HA antibody. The HA tagged WC, MC, NK, KC, and CT fusion proteins (prey fusions) were co-immunoprecipitated with anti-LexA antibody that recognized the bait fusions (Fig. 2A). These results demonstrate that in vivo physical interactions between different regions of Ire1p cytoplasmic domain exist and that formation of homotypic oligomers occurs. The N-linker region, however, did not form oligomers and did not interact with the C-tail suggesting that the ability to form oligomers resides primarily in the C-terminal tail.

To further substantiate these observations, we performed affinity adsorption experiments with the wild-type cytoplasmic domain expressed as a glutathione S-transferase (GST) fusion protein in E. coli. The GST-Ire1p cytoplasmic domain fusion protein (GST-WC) was absorbed to glutathione-Sepharose beads and incubated with extracts made from yeast strains expressing different regions of Ire1p cytoplasmic domain in the prey plasmid pJ G4–5. As a control, glutathione-Sepharose beads containing GST (comparable to the amount of GST-WC) were incubated with the same extracts. Beads were washed, washed, and extracted, and the extracts were analyzed by Western blotting with anti-HA antibody. The GST-WC fusion protein brought down WC, WK, MC, MK, and KC (see Fig. 2B) demonstrating a physical interaction between these proteins. Although GST alone did bring down WC and KC, the amounts of WC and KC pulled down with GST were significantly lower than that with GST-WC. The N-linker, NK, and C-tail proteins were present in the supernatant (data not shown), but not in the precipitate indicating that WC could not interact with N-linker, NK, and C-terminal tail in vitro.

Ire1p Is a Ser/Thr Kinase—The cytoplasmic domain of Ire1p contains a sequence that shows a significant similarity to the catalytic domain of Ser/Thr protein kinases (26). To investigate whether Ire1p is indeed a kinase, we constructed a series of GST fusion proteins containing either the cytoplasmic domain of Ire1p or different truncations of it. These fusion proteins were expressed in E. coli and purified by absorbing to glutathione beads. Kinase activity was analyzed by measuring autophosphorylation in an in vitro kinase assay. Western blot analysis with an anti-GST antibody detected the expression of fusion proteins in E. coli (Fig. 3A). The molecular weights of these fusion proteins were in agreement with the predicted size for such proteins. The GST fusion proteins containing the wild-type cytoplasmic domain (GST-WC) and the N-terminal linker plus the kinase domain (GST-NK) incorporated $^{32}$P from $[^{32}$P]ATP indicating autophosphorylation in vitro (Fig. 3B, lanes 2 and 6). In contrast, neither the fusion partner, GST alone, nor the GST-K702A mutant cytoplasmic domain hybrid (GST-MC) exhibited kinase activity. Similarly, the fusions containing the kinase domain alone (GST-WK, GST-MK) or the kinase domain plus the C-terminal tail (GST-KC) did not show
The cytoplasmic domain of Ire1p was labeled by autophosphorylation using \( [\gamma-^{32}P]ATP \) and the labeled cytoplasmic domain was subjected to phosphoamino acid analysis (see “Experimental Procedures”). Position of ninhydrin stained standards are shown. \( pY \), phosphotyrosine; \( pS \), phosphoserine; \( pT \), phosphothreonine.

**Fig. 5.** trans-Phosphorylation of the K702A mutant cytoplasmic domain by the wild-type cytoplasmic domain of Ire1p. Fixed amounts of GST-WC bound to the glutathione beads was mixed with increasing amounts of thrombin-cleaved mutant cytoplasmic domain (MC) and analyzed by in vitro kinase assay (see “Experimental Procedures”). The ratios of GST-WC to MC used in the assays are indicated.

**Fig. 4.** Phosphoamino acid analysis of Ire1p cytoplasmic domain. The cytoplasmic domain of Ire1p was labeled by autophosphorylation using \( [\gamma-^{32}P]ATP \) and the labeled cytoplasmic domain was subjected to phosphoamino acid analysis (see “Experimental Procedures”). Position of ninhydrin stained standards are shown. \( pY \), phosphotyrosine; \( pS \), phosphoserine; \( pT \), phosphothreonine.

**Fig. 3.** In vitro kinase activity of the GST-Ire1p fusion proteins. Panel A, expression of GST-Ire1p fusion proteins in E. coli. GST fusion proteins were purified and detected by Western blotting with anti-GST antibodies as described under “Experimental Procedures.” Panel B, autophosphorylation of GST-Ire1p fusion protein. Equal amounts of the purified GST-Ire1p fusion proteins were assayed for autophosphorylation in an in vitro kinase assay (see “Experimental Procedures”). Phosphoproteins were separated by SDS-PAGE and detected by autoradiography.

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The requirement for oligomerization on the kinase activity of Ire1p. Relative kinase activity of GST-WC and thrombin-cleaved GST-WC was compared. GST-WC was cleaved with thrombin, and equal amounts of intact GST-WC or thrombin-cleaved GST-WC were used in the kinase assay. Phosphorylated proteins were analyzed by SDS-PAGE, Coomassie Blue staining, and autoradiography. Bands were quantified for protein and $^{32}$P incorporation using NIH Image (version 1.55b10). Undiluted GST-WC (lane 1), undiluted thrombin-cleaved GST-WC (lane 2), and thrombin-cleaved GST-WC that was diluted 2-fold (lane 3), 3-fold (lane 4), and 16.7-fold (lane 5). The migration of GST-WC and thrombin-cleaved GST-WC (WC and GST) is indicated.

Activity of the cytoplasmic domain was further reduced by 3-fold. Dilution of the intact GST-WC into the kinase assay did not result in a reduction of the kinase activity (data not shown). Thus, in the absence of the GST dimerizing component, the specific activity of the kinase was concentration-dependent. These results demonstrate a direct correlation between the ability of the receptor to form oligomers and its kinase activity.

DISCUSSION

Ire1p is the only known receptor kinase that mediates the response to unfolded proteins in the ER to the nucleus (9, 13). On the basis of its structural similarity to other receptor kinases whose activation is dependent upon ligand-mediated oligomerization and trans-phosphorylation, we hypothesized a similar mechanism for the activation of Ire1p. In this study, we have presented evidence obtained by three independent methods, namely the yeast two-hybrid analyses, co-immunoprecipitations, and in vitro binding assays, that the cytoplasmic domain of Ire1p has an inherent ability to form oligomers. On the basis of the yeast two-hybrid analyses that detect the in vivo genetic interactions, and co-immunoprecipitation experiments that detect in vivo physical association between the interacting domains, the ability to form oligomers primarily resides within the last 130 residues (C-terminal tail) of the cytoplasmic tail. However, the N-linker region and the kinase domain can also form oligomers when they are physically attached but not when separated. This would argue that the cytoplasmic domain contains multiple sites capable of forming oligomers. The inability to detect a physical interaction between the wild-type cytoplasmic domain and the C-terminal tail in vitro may be due to a conformational change in the wild-type cytoplasmic domain in vitro; perhaps the C-terminal tail of the wild-type cytoplasmic domain folds back on the kinase domain, and consequently, it is not available for interaction with the C-terminal tail.

We have shown that the wild-type cytoplasmic domain of Ire1p has a functional kinase domain as well as autophosphorylation sites. Lys-702 corresponds to the invariant Lys of all protein kinases that contacts the $\alpha$- and $\beta$-phosphates of ATP and is essential for catalysis. The loss of this Lys in K702A mutant corresponds with loss of kinase activity, confirming that the phosphorylation of the GST-Ire1p fusion proteins was due to the intrinsic kinase activity of Ire1p, and ruling out the possibility that the $^{32}$P labeling of the fusion proteins was due to another contaminating kinase. The observation that the N-linker region but not the C-tail was required to generate a $^{32}$P-labeled protein in vitro suggested a number of possibilities.

It may be that the N-linker region either contained some amino acid residues that were critical for kinase function or helped to keep the kinase domain in a proper conformation. Alternatively, the N-linker region may harbor the major phosphorylation sites. We intend to resolve this issue by mapping the phosphorylation sites. If the N-linker region indeed contains such sites, it may serve as a potential docking site for downstream signaling molecules in the unfolded protein response pathway.

There are several indications in the Ire1p sequence that serine and threonine residues are the targets for phosphorylation. These results demonstrate a direct correlation between the ability of the receptor to form oligomers and its kinase activity.

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teins act as ligands for the Ire1p receptor to induce cross-linking. Alternatively, a ligand specific for Ire1p may be generated when conditions in the ER are not favorable for protein folding. It is also possible that chaperones like BiP associate with Ire1p in the absence of malfolded proteins and thereby prevent Ire1p from oligomerizing. When malfolded proteins accumulate, BiP dissociates from the receptor allowing it to oligomerize. There is a precedence for such a model since a number of studies (30, 31, 32) have suggested that the initial signal for unfolded protein response is indeed the decrease of free BiP concentration in the ER. Regardless of what triggers receptor oligomerization, once it is oligomerized, the cytoplasmic domain undergoes trans-phosphorylation at serine and threonine residues. Phosphorylation activates the kinase function which apparently leads to direct or indirect phosphorylation of a transcription factor whose phosphorylation status regulates the transcription of ER chaperone genes.

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