The role of stress proteins in membrane biogenesis

Raymond J. Deshaies, Bruce D. Koch and Randy Schekman

Stress proteins are synthesized by eukaryotic cells in response to diverse environmental insults. The properties of the 70 kDa and 90 kDa members of the heat shock and glucose-regulated protein families have been studied intensively, though their functions have proved difficult to determine. Recent work has revealed that several constitutively expressed homologues of these proteins participate in the assembly of biological membranes.

In cells exposed to noxious chemicals or heat there is a large transient increase in the synthesis of a family of polypeptides referred to as heat shock (HSPs) or stress proteins (reviewed in Refs 1 and 2). Likewise, cells exposed to calcium ionophores, low glucose concentrations, and inhibitors of N-linked glycosylation synthesize a set of polypeptides named glucose-regulated proteins (GRPs). Molecular cloning and DNA sequencing of genes encoding GRPs and HSPs have revealed structural relationships between members of these families. In several cases potential roles for these proteins have been proposed on the basis of genetic and biochemical experiments. Surprisingly, many of these roles are related to the assembly of biological membranes. In this review, we describe the characteristics of members of the 70 kDa and 90 kDa families of GRPs and HSPs, and discuss recent evidence concerning their functions.

70 kDa stress proteins and protein translocation across membranes

For post-translational import into mitochondria, precursor proteins must be in an unfolded conformation. There is also a requirement for NTP hydrolysis outside the mitochondrial inner membrane. (For references concerning the role of protein folding in mitochondrial precursor import, protein translocation into the yeast endoplasmic reticulum, and characterization of the yeast HSP70 gene family see Ref 7.) Rothman and Kornberg suggested that an ATPase may use the energy of the phosphodiester bond to unravel mitochondrial precursors prior to translocation. This proposal is reminiscent of Pelham’s model for HSP70 action, which states that HSP70s couple ATP hydrolysis to the disruption of quaternary structure in multisubunit complexes or denatured aggregates. A simple extension of this model could accommodate a function for HSP70 in the relaxation of tertiary structure within a single polypeptide chain. It was therefore speculated that an HSP70-like protein might fulfill the presumed requirement for unfoldases in mitochondrial protein import, and that HSP70-like proteins might also act in the post-translational translocation of certain secretory proteins into the lumen of the ER.

This idea was easy to test, since all of the HSP70 genes of the yeast Saccharomyces cerevisiae have been cloned, and null alleles of each locus have been constructed in vitro and translocated into the yeast genome. This mutational analysis revealed that a subset of the HSP70 family, the SSA genes, are required for viability. The SSA sub-
group is comprised of four genes, SSA1−SSA4. Genetic interactions between these genes suggest that the SSA gene products perform interchangeable functions in vivo. SSA1 and SSA2 are transcribed constitutively, though the level of SSA1 mRNA increases several fold after a heat shock. SSA3 and SSA4 are only expressed in cells exposed to higher temperatures. Whereas yeast strains bearing single disruptions in any one of the four genes are phenotypically indistinguishable from wild type, ssal ssa2 strains grow slowly at 24°C and do not grow at 37°C, and ssal ssa2 ssa4 cells are inviable. SSA1, present on a single copy plasmid, restored viability to ssal ssa2 ssa4 meiotic segregants. This observation was exploited to construct a conditionally-lethal ssa mutant strain. SSA1-coding sequences were inserted downstream of the GAL1 promoter on a yeast plasmid. Transcription from the GAL1 promoter is repressed by glucose and induced by galactose, thus allowing the level of SSA1 mRNA to be regulated. A strain (MW141) that harbors disrupted copies of ssa1, ssa2 and ssa4, as well as the GALI-SSA1 chimera on a single copy plasmid was constructed. MW141 cells grow normally in galactose-containing medium, but are inviable when glucose is supplied as a carbon source. By growing MW141 in galactose medium and shifting to glucose medium, we could examine the consequences of depleting yeast cells of the essential SSA1 protein (Ssa1p).

Cells depleted of Ssa1p accumulate prepro-a-factor, a protein that is normally secreted after it has undergone glycosylation and proteolytic cleavage. This precursor species is not detected in wild-type cells. Pulse-labeling studies indicate that the defect in prepro-a-factor secretion becomes more pronounced as Ssa1p is progressively depleted. Protease treatment of extracts prepared under conditions that preserve the integrity of the ER membrane confirms that Ssa1p depletion causes prepro-a-factor to accumulate in the cysolic compartinent. Besides prepro-a-factor, an unmodified precursor of the vacuolar protein carboxypeptidase Y accumulates in Ssa1p-depleted cells, though most carboxypeptidase Y is still transported. Ssa1p-deficient cells also accumulate the precursor of the mitochondrial F1-ATPase β-subunit. Whereas previously isolated mutations specifically block translocation and processing of either secretory or mito-

A clue to the function of the Ssa proteins is indicated by the ability to mimic their action by treating the a-factor precursor with urea before adding yeast membranes. Though the effect of urea on the structure of the precursor is not certain, this result was interpreted to mean that Ssa1p and Ssa2p use energy derived from ATP hydrolysis to inhibit folding of nascent chains or to unfold precursor molecules prior to membrane insertion. This result dovetails nicely with the observation that folded precursor proteins cannot be imported into mitochondria. Destabilization of a mitochondrial precursor protein with urea or point mutations, however, dramatically increases its rate of import into mitochondria. Since NTP hydrolysis is required for precursor import into mitochondria and ATP renders mitochondrial precursor proteins more sensitive to protease digestion, it is possible that NTP hydrolysis catalysed by HSP70-like proteins may power the unfolding of precursors during import. Purification of Ssa1p and Ssa2p was on the basis of their ability to augment the rate of translocation into the ER, but it is tempting to speculate that they might also accelerate the rate of precursor import into mitochondria. A model suggesting a role for the Ssa proteins in the import of precursor proteins into both the ER and mitochondria is depicted in Fig. 1. In addition to their documented roles in ER and mitochondrial protein import, it remains possible that Ssa polypeptides also participate in other biochemical reactions associated with the cellular stress response.

Besides the SSA gene products, other yeast HSP70 homologues may be involved in membrane biogenesis pathways. SSCI, a yeast HSP70 gene that is required for viability, encodes a 70 kDa stress protein preceded by a mitochondrial leader sequence (J. Kramer, M. Werner-Washburne and E. Craig, pers. commun.). In vivo import assays suggested that this polypeptide was delivered to mitochondria, though its compartmental location was not determined (J. Kramer, M. W. Washburne and E. Craig, pers. commun.). Perhaps Sss1p is required to redirect matrix-localized precursors across the inner membrane to their final destinations. A constitutively synthesized homologue of HSP70 has been purified from bovine brain based on its ability to remove clathrin coats from purified
coated vesicles. This enzyme, uncoating ATPase, is presumably required for the recycling of membrane between the cell surface and endosomes during receptor-mediated endocytosis. Unfortunately, this proposed role has not been confirmed in vivo. The mechanism of clathrin depolymerization catalysed by uncoating ATPase has been described in detail, and is reviewed elsewhere. Briefly, uncoating ATPase uses energy derived from ATP hydrolysis to displace each leg of a clathrin triskelion from the cage lattice in succession, which results in its release from the vesicle coat.

**Stress protein function in ER–Golgi transport**

Molecular cloning of cDNAs homologous to HSP70 identified a gene that encodes a polypeptide, p72, which is targeted to the secretory pathway. Peptide mapping and DNA sequencing indicate that p72 (which shows ~60% identity with HSP70s) is similar, if not identical to, immunoglobulin heavy chain binding protein (BiP) and the 78 kDa glucose-regulated protein (GRP78). These proteins, which for convenience we will refer to collectively as BiP, contain a typical N-terminal signal sequence which directs their transfer into the lumen of the ER. Owing to the presence of a four amino acid signal KDEL at its C-terminus, BiP is permanently retained in the ER lumen. Though the function of BiP is uncertain, it appears to restrict transport of malfolded secretory proteins from the ER to the Golgi body. In lymphoid cells expressing immunoglobulin heavy chain or in non-lymphoid cells expressing viral envelope polypeptides, BiP is found associated with unassembled components of these multisubunit proteins. BiP appears to retain unfolded proteins in the ER, since immunoglobulin heavy chains lacking the Cys1 domain no longer associate with BiP, and consequently are externalized more rapidly. B cells expressing these mutant heavy chains secrete immunoglobulin molecules at various stages of assembly, whereas normal B cells secrete only intact antibodies.

Treatments that may perturb the folding of secretory proteins, such as thiol reagents and glycosylation inhibitors, induce the expression of BiP mRNA and result in an increased association of secretory proteins with BiP. The synthesis of BiP is also induced by the expression of mutant forms of influenza hemagglutinin in simian cells. Only malfolded hemagglutinin molecules that fail to be transported from the ER result in increased synthesis of BiP. These data suggest that the signal for the induction of BiP is the presence of malfolded proteins in the ER. A biochemical description of BiP’s role in the retention of unfolded secretory proteins in the ER should be aided by the study of ER to Golgi transport in cell-free systems. The interaction between BiP and secretory proteins has recently been reconstituted in a co-translational protein translocation reaction. Preliminary experiments suggest that inhibition of core glycosylation and disulfide bond formation promotes association of translocated polypeptides with BiP.

A second approach to unravelling the role of BiP in intercompartmental transport is to employ reverse genetics to analyze the phenotype of cells expressing mutant forms of BiP. Two groups have identified a gene (KAR2) that encodes a homologue of BiP in S. cerevisiae (M. Rose, pers. commun.: M-J. Getting and J. Sambrook, pers. commun.). Spores bearing a disrupted copy of KAR2 failed to form colonies, suggesting that BiP is essential for cell growth. Unexpectedly, certain mutant alleles of KAR2 blocked nuclear fusion after haploid cells mated to form diploids (M. Rose, pers. comm.). The relation of this observation to BiP’s proposed role in ER–Golgi transport is obscure, perhaps Kar2 protein serves to retain proteins in the nuclear envelope and thus aid in the assembly of the spindle pole body, a nuclear envelope structure that seems crucial for the alignment of the two haploid nuclei prior to their fusion in a zygote.

**90 kDa stress protein homologues and membrane biogenesis**

Besides the 70 kDa stress proteins discussed above, all eukaryotes express soluble stress proteins with a molecular mass of ~90 kDa (HSP90s), which are structurally and biochemically distinct from HSP70s. Though the function of HSP90 is not yet known, it has been detected in complexes with a variety of proteins, including oncogenic tyrosine kinases (v-src, v-fps, v-yes), unoccupied steroid hormone receptors and actin. Our discussion here focuses on the interaction between HSP90 and the plasma membrane protein pp60v-src. Newly synthesized pp60v-src is soluble, inactive, and complexed to both HSP90 and an uncharacterized phosphoprotein pp50 (Ref. 25). Though the exact sequence of events is unclear, kinetic studies suggest that this complex is disassociated and pp60v-src is activated and inserted into the plasma membrane in a concerted manner. One function of HSP90 may be to act as a cytoplasmic carrier of precursor proteins that are destined for delivery to organelles or association with intracellular membranes. Such a model would predict a role for HSP90 in the biogenesis of diverse molecules including nuclear pore proteins, the precursor of the mating pheromone produced.
by yeast a cells, and ras proteins27. Since the yeast homologues of HSP90 (HSP83 and HSC83) have been shown to be required for viability (S. Lindquist, pers. commun.), these possibilities could be evaluated in conditionally defective hsp83/ras83 mutant strains.

Endoplasmic (ERP99), which is perhaps the most abundant protein of mammalian ER, is a glycoprotein with a molecular mass of 90–100 kDa.26,29 Sequence analysis of cDNA clones encoding endoplasmic indicate that it is similar, if not identical to the glucose-regulated 94 kDa protein (GRP94), and ~50% homologous to yeast and Drosophila HSP90s.21,26

The sequence of GRP94 predicts a stretch of 21 hydrophobic amino acids that is proposed to function as a membrane anchor domain.26,29 Proteolysis experiments support the contention that GRP94 spans the ER membrane, though others have reported that GRP94 is readily released by mechanical disruption of ER membranes,28 suggesting that it is freely soluble in the ER lumen. The C terminus of GRP94 concludes with the tetrapeptide KDEL,29 which is known to act as an ER retention signal,13 and is also found at the C termini of the luminal proteins GRP78 and protein disulfide isomerase.14

GRP94 and GRP78 (BiP) are induced simultaneously by various treatments, suggesting that they are co-regulated3. Whereas GRP78 is thought to regulate transport of proteins from the ER to the Golgi body, the function of GRP94 remains elusive. Since known inducers of GRP94/GRP78 are related by their potential to interfere with protein folding in the ER20, it is logical to propose that GRP94 may participate in either post-translational modification of secretory proteins, retention of improperly modified (possibly unfolded) proteins within the ER, denaturation and renaturation of malformed proteins, or disposal of malformed protein aggregates.

Enzymes involved in the modification and recombination of translocated polypeptides may coexist in a complex. Preliminary evidence suggests that GRP76, GRP94 and protein disulfide isomerase are physically associated (M.-J. Gething and J. Sambrook, pers. commun.). Intimate association of ER enzymes involved in post-translational modification of secretory proteins has already been demonstrated for human prolyl-4-hydroxylase. This enzyme is an α2β2 tetramer that catalyses the formation of 4-hydroxyproline in collagen. Mammalian cloning and sequence analysis of the β-subunit indicated that it is identical to protein disulfide isomerase.31

Conclusions
Dissection of an assay that reconstitutes post-translational protein translocation into the yeast ER, and phenotypic characterization of hsp70- mutant strains have established that the yeast 70 kDa stress proteins Ssa1p and Ssa2p facilitate import of precursor polypeptides into the ER and mitochondria. Coupled with previous work on how precursor structure influences mitochondrial import, these results imply that HSP70-like proteins unfold untranslocated precursors, or retain them in an unstructured, translocation-resistant state. This function is reminiscent of the proposed action of HSP70s on coated vesicles and denatured protein aggregates. Molecular cloning of HSP70-like genes has identified BiP as another HSP70 homologue that participates in membrane biogenesis. Members of the HSP90 family of homologous proteins, though less defined, potentially perform functions related to the biosynthesis and transport of membrane and extracytoplasmic proteins.

Genes encoding homologues of the heat shock and glucose-regulated proteins have been identified in the yeast, S. cerevisiae. Genetic and biochemical characterization of these genes and their encoded products has advanced our understanding of their roles in normal cell physiology. A summary of the properties of the yeast HSP70 and HSP90 homologues is presented in Table I. Reconstitution of stress protein function in vitro and characterization of yeast strains expressing mutant stress proteins should unravel the elusive role of these molecules in the assembly of biological membranes.

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References