MICROBIOLOGICAL REVIEWS, June 1993, p. 402–414
0146-0749/93/020402-13$02.00/0

Vol. 57, No. 2

Heat Shock Proteins: Molecular Chaperones of Protein Biogenesis

ELIZABETH A. CRAIG,* B. DIANE GAMBILL, AND R. JOHN NELSON

Department of Biomolecular Chemistry, University of Wisconsin-Madison, 1300 University Avenue, Madison, Wisconsin 53706

INTRODUCTION

Over the past several years an understanding of the general roles of the major heat shock proteins (Hsps) in cell physiology has begun to evolve: Hsps are directly involved in the biogenesis of proteins from the time of synthesis as nascent chains until the assembly of multimeric complexes and have therefore been termed molecular chaperones. The budding yeast *Saccharomyces cerevisiae* has proven to be a very productive model system with which to study the roles of Hsps by using both genetic and biochemical tools. This review focuses on the recent advances made in the understanding of the function of the three major classes of Hsp, Hsp90, Hsp70, and Hsp60, thought to be involved in protein biogenesis. In addition, the relationship between Hsps and components of the proteolytic systems is addressed. Here the Hsps of *S. cerevisiae* are emphasized; however, since these proteins are so highly conserved, information obtained from the study of other organisms, the subject of other recent reviews (36, 58, 124), is also discussed.

Hsp FUNCTION IN PROTEIN BIOGENESIS AS MOLECULAR CHAPERONES

Protein folding and the assembly of multimeric structures in vivo is not a completely spontaneous process but is facilitated by proteins called molecular chaperones. Molecular chaperones bind transiently and noncovalently to nascent polypeptides and unfolded or unassembled proteins, aiding in protein biogenesis in two general ways: they block nonproductive protein-protein interactions, and they mediate the folding of proteins to their native state by sequestering folding intermediates, allowing the concerted folding by domains and assembly of oligomers. The 70-kDa family of Hsps (Hsp70s) and the 60-kDa family of Hsps (termed chaperonins or Cpn60s) make up the two major groups of molecular chaperones. The Hsp70s, found in all major cellular compartments of eucaryotes and every bacterium examined to date, bind partially unfolded proteins. They appear to bind nascent chains in the process of protein synthesis and completed polypeptides upon release from ribosomes. In addition, Hsp70s located inside the mitochondria and endoplasmic reticulum (ER) play a critical role in translocation of proteins from the cytosol into those organelles by binding during the initial stages of translocation. Cpn60s, found in both eucaryotes and procaryotes, have been shown both in vivo and in vitro to bind unfolded proteins, preventing their aggregation and facilitating folding.

Although Hsp70s and Cpn60s are the best characterized, evidence implicates other proteins, including Hsp90, proline isomerases, and disulfide isomerases, in protein folding. In addition, Hsp104, an Hsp synthesized only during times of

* Corresponding author.

stress, has been postulated to be involved in similar processes and therefore is also discussed in this section.

Hsp70s

Hsp70s are highly conserved among all species from bacteria to humans (30), with the DnaK from *Escherichia coli* having about 50% identity with all eucaryotic Hsp70s. The eucaryotic proteins are between 50 and 98% identical. A comparison of all the known Hsp70 sequences reveals that the N-terminal two-thirds of the protein is more highly conserved than the C-terminal one-third (9, 27). As suggested by the high degree of conservation of primary structure, Hsp70s from different sources have similar biochemical properties; each has a high-affinity ATP-binding site and a peptide-binding site (reviewed in reference 58). Hsp70s possess a weak ATPase activity, which, in at least some cases, is stimulated by the binding of peptide (50). The ATP-binding and peptide-binding sites appear to be localized in two separate domains of the protein.

Biochemical and structural studies of mammalian cytosolic Hsp70 have shown that an N-terminal 44-kDa proteolytic fragment has ATPase activity, but this activity is not stimulated by peptide binding (16); this provides evidence that the ATP-binding site is localized in the N terminus whereas the more divergent C terminus contains the peptide-binding site. It is thought that this protease-sensitive site, which was used to generate the 44-kDa fragment, is in a flexible hinge region connecting the ATP-binding and peptide-binding domains. The structure of the N-terminal proteolytic fragment has been found by X-ray crystallography (46) to consist of a two-lobed domain with a deep cleft in which ATP binds; its structure is very similar to the ATP-binding domain of G actin (47). Many of the highly conserved residues of the N-terminal domain lie in the ATP-binding cleft.

The C-terminal domain of Hsp70 has not been structurally characterized. However, two groups have proposed that the structure of the Hsp70 C terminus is similar to that of the well-characterized major histocompatibility complex class I antigen-presenting molecule on the basis of the slight similarities in primary sequence and secondary-structure predictions (48, 120). Major histocompatibility complex class I molecules have been shown to bind peptides in an extended conformation (51, 91); interestingly, preliminary nuclear magnetic resonance studies indicate that DnaK binds at least one peptide in a conformation lacking a defined structure (80a). Recent results of Flynn et al. (49) indicate that 7 amino acids is the smallest optimal binding size for a peptide interacting with the ER-localized mammalian Hsp70 (BiP). Analysis of random peptides that bound to BiP showed a preference for amino acids with aliphatic side chains but a toleration of both charged and polar residues. The release of target peptide or protein bound to Hsp70 requires the hydrolysis of ATP, implying an interaction between the C-terminal peptide-binding domain and the N-terminal ATPase domain. This hydrolysis and release of peptide is accompanied by a conformational change of Hsp70 that has been detected by partial digestion with protease (85).

S. cerevisiae has multiple Hsp70 species (Fig. 1), as do most, if not all, eucaryotes (24). Two of these are organelle localized: Ssc1p in the matrix of the mitochondrion and Kar2p in the lumen of the ER. The other six yeast Hsp70 subfamilies are divided between the *SSA* subfamily, which has four members, and the *SSB* subfamily, which has two members. The members of both these subfamilies are localized primarily in the cytosol. The DNA sequence identities among the eight yeast stress proteins are depicted schematically in Fig. 1.

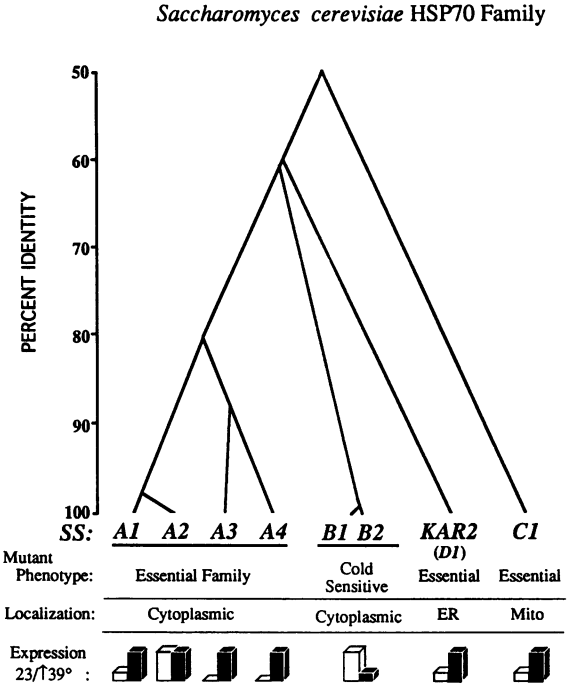

FIG. 1. Hsp70 multigene family of *S. cerevisiae*. Nucleotide identities are based on the complete nucleotide sequence data: *SSA1*, *SSA2* (140); *SSA3* (9); *SSA4* (7); *SSB1* (7), *SSB2* (9), *SSC1* (29); and *KAR2* (107, 121). The placement of genes into functional groups is based on analysis of mutants and is compiled from references 26, 27, and 152. Data for the levels of mRNA are from references 6, 26–28, 37, 107, 121, 139, 151, and 152. Adapted from reference 25 with permission.

The mitochondrial Hsp70, Ssc1p. An Hsp70 has been found in the mitochondrion of *S. cerevisiae* (29), as well as in mammalian cells (82, 96) and trypanosomes (38). Yeast mitochondrial Hsp70 is encoded by the nuclear gene, *SSC1*. The full-length *SSC1* gene product has a 28-amino-acid N-terminal leader that is cleaved upon import of Ssc1p into the mitochondrial matrix (28, 29, 97). Ssc1p is more similar to the *E. coli* Hsp70 DnaK than to any other Hsp70, consistent with the endosymbiont hypothesis, which holds that mitochondria are of procaryotic origin.

Gene disruption experiments have shown that *SSC1* is essential under all growth conditions tested (28); more recently, temperature-sensitive alleles have been used in vivo and in vitro for analysis of Ssc1p function (78). In this study, after shift of the mutant *ssc1-2* cells to the nonpermissive temperature, the precursor form of a number of proteins that are normally imported into mitochondria accumulated. Pulse-labeling experiments indicated that the processing of precursors was inhibited within minutes of the shift. Fractionation of cellular components showed that the accumulated precursor was associated with mitochondria. However, since the precursors remained sensitive to exogenously added protease, it was concluded that they were localized on the cytoplasmic surface of the outer mitochondrial membrane. Together, these results indicated that inactivation of Ssc1p leads to a block in mitochondrial import; experiments with isolated mitochondria confirmed and ex-

tended this idea. Mitochondria isolated from *ssc1-2* cells grown at the permissive temperature were competent for import, but, unlike wild-type mitochondria, they became defective for import when incubated at 37°C for 10 min prior to the addition of labeled precursor. Only a small amount of precursor was completely translocated into the mutant mitochondria; however, binding and cleavage of the targeting sequence were not reduced.

To place these results in the context of what is understood of the translocation of proteins from the cytosol into the matrix of the mitochondria, the process can be experimentally divided into two sequential reactions. First, the precursor protein is partially inserted across the outer and inner membranes such that the N terminus reaches the matrix, where the targeting sequence is proteolytically removed. The remainder of the protein remains on the cytosolic side of the outer membrane (reviewed in reference 114). The completion of mitochondrial import is much less well defined. For most precursors to be transported, they must be in an unfolded or loose conformation (34, 131). The mitochondrial import machinery must then vectorially transport the precursor across both the outer and inner membranes. How this is accomplished is still largely a mystery, but the in vitro analysis of the *ssc1-2* mutant indicates that Ssc1p is required for this second step (78). Ssc1p appears to act in this process through a direct interaction with the precursor, as indicated by immunoprecipitation and cross-linking experiments showing an association of Ssc1p with polypeptides before they have been completely translocated into the matrix (108, 130). In addition, Ssc1p has been shown to associate transiently with authentic precursors prior to becoming folded (89). If the precursor protein was first denatured in urea and then added to mutant mitochondria, the precursor was imported with an efficiency comparable to that of the wild type (78). Thus, if the precursor is in a very loose conformation, there is a reduced dependence on Hsp70 activity in the matrix.

How does Ssc1p function in the translocation process? According to a current model (Fig. 2), Ssc1p binds to the unfolded precursor proteins as the N terminus enters the matrix. The binding of Ssc1p, which is a large, globular protein, may provide directionality or irreversibility to the import process. Once it is bound, the translocating polypeptide cannot move backwards toward the cytosol, because it is sterically blocked by Ssc1p. Continued movement into the matrix might be accomplished simply by Brownian motion, with movement back toward the cytosol being blocked by the binding of Ssc1p to internal sites of the translocating polypeptide as it enters the matrix.

While Ssc1p is required for the translocation of proteins from the cytosol into mitochondria, it could be involved in other processes as well. One such role, that of the noncatalytic subunit of an endonuclease, has been established (97). Endo.*Sce*I causes double-stranded scissions at well-defined sites on DNA, cleaving mitochondrial DNA at several specific sites in in vitro assays, and is thought to be involved in general recombination of mitochondrial DNA (101, 102). In a purified system the catalytic subunit of endonuclease Endo.*Sce*I, a heterodimeric enzyme, has very little activity in the absence of Ssc1p. The role played by Ssc1p in the activity of the endonuclease is not known.

The ER-localized Hsp70, Kar2p. ER-localized Hsp70, termed BiP in mammalian cells, was first identified because of its stable association with unassembled immunoglobulin heavy-chain molecules (65). More recent studies indicate that it associates transiently with a wide range of proteins

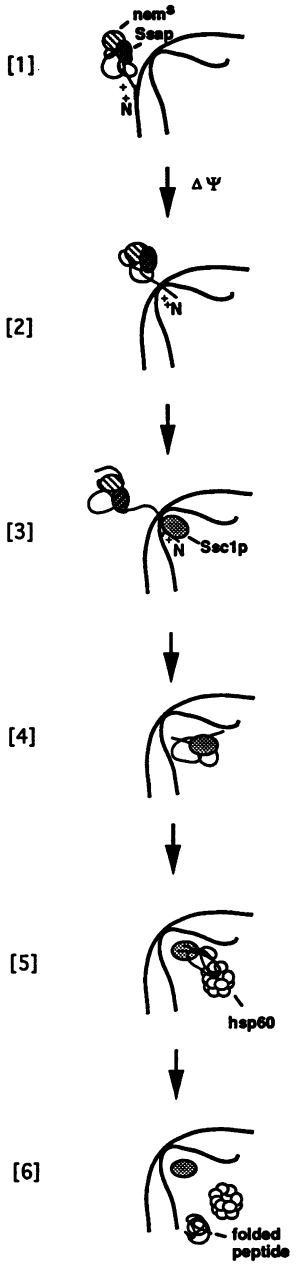

FIG. 2. Model of the role of Ssc1p and Hsp60 in protein translocation into mitochondria. (Panel 1) The precursor, bound to cytosolic Hsp70s and perhaps additional factors which aid in maintaining a relatively unfolded conformation, binds to a receptor on the cytosolic surface of the outer mitochondrial membrane. (Panel 2) The N-terminal presequence is inserted into the outer membrane and, because of the membrane potential across the inner membrane, is translocated across the membranes. (Panels 3 and 4) Ssc1p binds tightly to the precursor protein in the matrix, perhaps at a number of sites as translocation progresses, preventing movement back toward the cytoplasm. (Panel 5) Ssc1p "passes off" the protein to Hsp60, where folding and/or assembly occurs. (Panel 6) The folded protein is released. The proposed model is based on data from references 78, 89, 108, and 109 and adapted from reference 25 with permission.

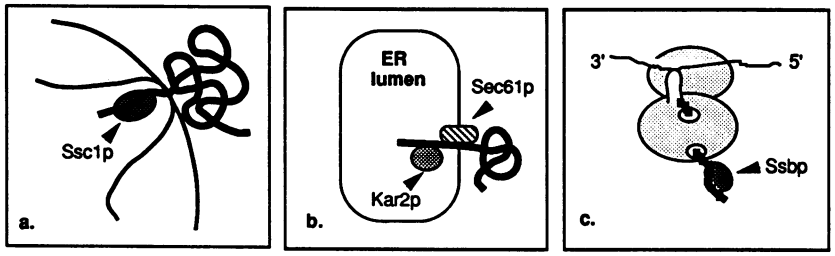

FIG. 3. Similarity in proposed action of Ssc1p, Kar2p, and Ssbps in the translocation of proteins from the cytoplasm into the matrix of the mitochondria or the lumen of the ER or emergence from the 60S subunit of the ribosome. In each case the Hsp70 is binding to an unfolded polypeptide and facilitating the translocation across the membranes (a and b) or perhaps in facilitating movement of the nascent chain out of the tunnel of the 60S subunit (c).

(reviewed in reference 58). This association is prolonged if the substrate protein has an aberrant conformation or cannot be assembled into the appropriate oligomeric complex (5, 57). Association of BiP with proteins is disrupted by the addition of ATP but not by nonhydrolyzable ATP analogs, in keeping with the general model of Hsp70 function (98).

The ER-localized yeast Hsp70 is called Kar2p; a mutation named *kar2-1*, because it resulted in a defect in karogamy, nuclear fusion (22), was later determined to be in the gene encoding ER-localized Hsp70 (122). Kar2p is synthesized with a 42-amino-acid presequence on the amino terminus (145), presumably responsible for targeting to the ER and a C-terminal HDEL sequence, which is the "canonical" yeast ER retention sequence (112). Null alleles which indicated that *KAR2* is essential for viability (107, 122) were constructed. As with *SSC1*, temperature-sensitive alleles have been isolated and exploited to study the function of Kar2p.

When grown at the restrictive temperature, *kar2-159* strains accumulated the precursor forms to several proteins which normally are translocated across the ER membrane (149), indicating a direct involvement of Kar2p in protein translocation. To define more precisely the role of Kar2p in membrane transport, an in vitro ER translocation system was used (129). A translocation intermediate that becomes jammed during transport was used to freeze the translocation machinery, allowing a study of its components. An association between the jammed precursor and the ER membrane protein Sec61p was detected, indicating that precursors interact with Sec61p during their translocation. Three different *KAR2* alleles were studied to determine their effect on translocation and on the formation of the precursor-Sec61p intermediate. Although all of the alleles exhibited defects in translocation, their effects on the formation of the Sec61p-precursor complex were different. Two of the alleles, *kar2-113* and *kar2-159*, resulted in a severe reduction in complex formation. However, the third allele, *kar2-203*, did not significantly reduce the formation of the Sec61p-precursor complex. These results suggest that Kar2p acts during two steps of translocation, in the formation of the Sec61p-precursor complex and in some undefined event after the precursor has become associated with Sec61p. It was also found in this study that Kar2p antiserum precipitated the jammed precursor molecule as well as other fully translocated proteins, indicating a direct interaction between newly translocated proteins and Kar2p. This observation is in keeping with the results of the study of BiP in the mammalian cell.

In sum, the roles that are emerging for mitochondrial and ER-localized Hsp70 are similar (Fig. 3); both Hsp70s are needed for transport of proteins across the membrane and act through a direct interaction with the protein being transported. Although the extent of the parallels between the roles of Hsp70 in these two organelles is not clear, the similarity of their roles in these two different settings suggests that the general model for Hsp70 function may be applicable to the biogenesis of proteins of other organelles and, perhaps, the cytosol as well.

Cytosolic Hsp70s. The yeast cytosol contains two classes of Hsp70, the *SSA* subfamily, with four members, and the *SSB* subfamily, with two members. The *SSA* and *SSB* subfamilies are not functionally equivalent, although there may be some overlap between their function. The regulation of expression of the *SSA* and *SSB* genes is quite different (26, 27, 151). Expression of three of the four *SSA* genes is induced on heat shock, whereas that of the *SSB* genes is turned off.

(i) ***SSA* subfamily.** The yeast *SSA* Hsp70 subfamily is essential (152); at least one of the proteins encoded by this subfamily must be present at high levels for cell viability. These four cytoplasmic proteins are encoded by genes with DNA sequence similarity ranging from 80 to 97% (23). Strains in which both the *SSA1* and *SSA2* genes have been inactivated grow more slowly than the wild-type strain at all temperatures and are unable to form colonies at 37°C (26). These strains are viable at temperatures lower than 37°C as a result of high expression of the *SSA4* gene, which is not normally expressed under optimal growth conditions. If the *SSA4* gene is then inactivated, the triple-mutant cell is inviable. Ssa3p can restore the viability of *ssa1 ssa2 ssa4* mutants only when the *SSA3* gene is expressed at higher than normal levels, for example by being under the control of the strong constitutive *SSA2* promoter (152).

Although the study of the *SSA* subfamily shows that there is functional overlap among the members, there are also suggestions that the different proteins may not be functionally identical. When individual Ssaps were tested for the ability to dissassemble the clathrin cage of mammalian coated vesicles, it was determined that Ssa2p had the highest uncoating activity followed by Ssa4p and then Ssa1p (53). It is likely that each Ssap is optimized for a specific activity or activities in the cell but that the other members have the ability to substitute for each other. This idea is consistent with the observation that Ssa3p and Ssa4p can maintain the viability of a *ssa1 ssa2* strain but are unable to completely suppress the temperature sensitive phenotype even when overepressed (103).

Yeast cells depleted of *SSA* proteins accumulate unimported precursor of the mitochondrial F_1-ATPase β subunit ($F_1\beta$) and the secreted protein prepro-α-factor (ppαF) (32), suggesting an involvement of Ssaps in protein translocation

into both the ER and mitochondria. In vitro, translocation of proteins into mitochondria (99) and the ER (19) is facilitated by the addition of *SSA* Hsp70s, which act in an ATP-dependent manner, along with an undefined *N*-ethylmaleimide (NEM)-sensitive activity, to stimulate translocation. Significantly, denaturation of ppαF in urea prior to addition to the microsomes was able to substitute for Ssap and the NEM-sensitive activity (19), suggesting that the role of Hsp70 and the NEM-sensitive factor is either to unfold the precursor or maintain it in an unfolded, translocation-competent, conformation.

Evidence from other systems has also pointed to a similar role for cytosolic Hsp70 in the transport of proteins across membranes. In an in vitro system, mammalian Hsp70, along with an NEM-sensitive factor, stimulates the translocation of M13 procoat protein into mammalian microsomes (158). Similarly, in a mammalian in vitro mitochondrial protein import system in which rat preornithine carbamyl carboxylase fused to dihydrofolate reductase is used as a precursor, Hsp70 and an NEM-sensitive activity were needed to confer import competence (136). Although the role of Hsp70 in this process is not yet known, it seems likely that Hsp70 interacts directly with the precursor. Consistent with this possibility, Beckmann et al. have shown that mammalian cytosolic Hsp70 associates with newly synthesized proteins (2).

The signal recognition particle (SRP) of mammalian cells recognizes and binds directly to the signal sequence of proteins destined for the ER. When complexed with the ribosome, SRP causes a slowing of translation and acts to target the protein to the ER membrane (reviewed in reference 150). *S. cerevisiae* also seems to have an SRP which is functionally homologous to its mammalian counterpart (67). Strains containing deletions of genes encoding components of SRP are viable, albeit slow growing (42, 67). Hann and Walter (67) have speculated that in the absence of SRP, proteins can be targeted to the ER in an Hsp70-dependent manner; however, as yet there is no evidence to support this idea.

It is very likely that the *SSA* Hsp70s have functions in addition to their role in protein translocation, given the abundance and complexity of the *SSA* subfamily. Suppressors of the temperature sensitive phenotype of the *ssa1 ssa2* mutant were found to affect the regulation of the remaining *SSA* subfamily members (104), rather than the translocation pathway per se, as expected if no single function could be altered through reversion to allow for growth of the mutant at high temperature.

(ii) *SSB* subfamily. The two genes in the *SSB* subfamily encode proteins that are 99% identical (8); there is no evidence indicating a functional difference between the two proteins. Strains with a single *SSB* gene disrupted have no mutant phenotype; however, a mutant with both genes disrupted grows slowly compared with the wild type at all temperatures and is cold sensitive (27). The *SSB* Hsp70s are among the most abundant cellular proteins, approximately as abundant as the *SSA* proteins during growth at 30°C.

Up to 73% of the total cellular content of Ssbp has been found associated with translating ribosomes (105). This association was disrupted by the drug puromycin, an aminoacyl-tRNA analog that causes release of the nascent chain, suggesting that *SSB* Hsp70 is associated with the ribosomes by virtue of its direct binding to the nascent polypeptide. Phenotypic analysis of the *ssb1 ssb2* mutant suggested that Ssbps play a role in translation. The slow-growth phenotype of the *ssb1 ssb2* mutant is suppressed by increased expression of a gene which encodes a translation elongation factor 1 alpha-related protein. In addition, *ssb1 ssb2* cells are hypersensitive to several translation inhibitors such as the aminoglycosides and verucerrin A and have a small number of translating ribosomes.

Nelson et al. (105) propose that Ssbps bind to the nascent chain as it emerges from the "exit site" of the channel in the large ribosomal subunit. An analogy was drawn between the emergence of the polypeptide chain from the ribosome channel, which contains approximately 40 amino acids in an extended conformation (3, 88, 95), and the transport of polypeptide chains across a lipid bilayer. In both cases, the polypeptide is passed through a tunnel or channel in an extended conformation. As discussed above, the Hsp70s residing in the mitochondrial matrix or the lumen of the ER bind transiently to polypeptides as they are being transported into the organelle from the cytosol (78, 129, 130). Yeast strains which bear mutations in their mitochondrial or ER-localized Hsp70s fail to transport proteins across the membrane (78, 149), suggesting that this interaction is required for transport. A very similar role for cytosolic Hsp70 in protein synthesis has been proposed: as the nascent polypeptide chain emerges into the cytosol, it interacts with Hsp70, and this interaction is important for continuous, smooth transport of the polypeptide through the ribosome channel into the cytosol (Fig. 3). According to the model (105), emergence of the peptide from the ribosome may be slower in the *ssb1 ssb2* mutant and thus cause a backing up of the polypeptide in the channel perhaps changing the kinetic parameters of translation and perturbing protein synthesis.

There is ample evidence from a variety of systems implicating Hsp70 in folding and assembly by binding to unfolded proteins. Therefore it appears likely that association of Hsp70 with nascent cytosolic proteins aids in their folding and prevents aberrant protein-protein interactions, as has been a topic of discussion for some years (113, 124). The analysis of *ssb1 ssb2* mutants has led to the idea that there is additional significance to the association of Hsp70 with nascent polypeptides, i.e., prevention of the nascent polypeptide from interfering with translation. However, much remains to be resolved about the exact roles of the cytosolic Hsp70s, particularly the biogenesis of cytosolic proteins. Do Ssbps interact with all nascent chains? Are the Ssbps involved in the subsequent folding of cytosolic proteins, or is this perhaps a function of Ssaps?

Proteins that interact with Hsp70s: DnaJ homologs. The greatest progress on understanding the role of other factors in Hsp70 function has come from study of *E. coli* (reviewed in reference 55). This work shows that two other factors, DnaJ and GrpE, are intimately involved with the function of DnaK (56, 84, 143). DnaJ stimulates the ATP hydrolysis activity of DnaK, and GrpE is required for efficient release of the bound nucleotide. DnaJ-like proteins have been found in a variety of organisms, including humans and *S. cerevisiae*.

S. cerevisiae contains a family of at least four DnaJ-like proteins known as: *YDJ1/MAS5* (1, 14), *SIS1* (87), *SCJ1* (4), and *SEC63/NPL1* (126). Although there are structural differences between these four proteins, all share a conserved DnaJ motif. Inactivation of *YDJ1/MAS5* is not lethal but has pleiotropic effects, resulting in temperature-sensitive growth (1, 14). *YDJ1/MAS5* mutants have a defect in the import of $F_1\beta$ into mitochondria (1, 13) and ppαF into the ER (13). Since the *SSA* Hsp70s have also been implicated in transport of the same proteins (32), *YDJ1/MAS5* may operate in the cell in concert with the *SSA* Hsp70s. In support of this idea,

Ydj1p has been shown to stimulate the ATPase activity of Ssa1p and influence its interaction with a denatured protein (31). Ydj1p is farnesylated, a modification that is at least partially responsible for its association with the cytoplasmic side of the ER and nuclear membrane (15). The farnesylation may play a role in the localization of Ydj1p at higher temperatures, since a mutation in the *YDJ1* gene which prevents farnesylation causes inviability at 37°C.

SEC63/NPL1 encodes an essential protein necessary for transport of proteins across the ER membrane (123). Sec63p, an integral membrane protein (41), is present in a complex with two other integral membrane proteins, Sec61p and Sec62p (33). As described above, Sec61p and Sec62p interact with translocating polypeptides (100, 129). Studies on the topology of Sec63p suggest that the conserved DnaJ motif domain faces the ER lumen (41), raising the possibility that Sec63p plays an important role in utilization of Kar2p in membrane transport. Consistent with this notion, genetic interactions have been observed between *KAR2* and *SEC63* (129).

Studies of the other DnaJ homologs are not as extensive. The *SIS1* gene is essential. Depletion of Sis1p has pleiotropic effects, including a defect in nuclear migration (87). However, a direct role for Sis1p in this process has not been established. The *SCJ1* gene is not essential. Increased expression of Scj1p can alter protein sorting, but the normal cellular function of this protein is obscure (4).

Hsp60

Mitochondrial Hsp60. The matrix of the mitochondria of many different organisms, including *S. cerevisiae*, contains a heat shock protein known as Hsp60 (118), which is structurally and functionally related to the heat-inducible GroEL protein of *E. coli*. Collectively, this highly conserved group of proteins, which also includes the ribulose biphosphate carboxylase subunit-binding protein of the chloroplast stroma (RUBISCO), is known as the chaperonins (Cpn60s). Besides having primary structure similarity, all these chaperonins are found as homo-oligomers of two stacked rings, each ring having sevenfold symmetry (reviewed in reference 36). The most extensive biochemical studies indicating a role for chaperonins in facilitating protein folding have been carried out with the *E. coli* system. The 65-kDa GroEL protein is encoded in an operon with another heat-inducible protein, the 15-kDa GroES protein. GroEL and GroES act together in protein folding and assembly (62, 90). A general model that has emerged holds that GroEL interacts directly with an unfolded polypeptide. GroES then binds to the GroEL-polypeptide complex. After becoming folded, the polypeptide is released. The release requires hydrolysis of ATP. GroES is thought to influence the release by modulating the ATPase activity of GroEL. Although a GroES homolog has been identified in mammalian mitochondria (86), one has yet to be isolated from yeasts.

Yeast Hsp60 is encoded by the essential nuclear *MIF4* gene (17). Hsp60 is synthesized with a characteristic mitochondrial amino-terminal targeting sequence that is cleaved when Hsp60 enters the matrix. Hsp60 is assembled into a typical chaperonin structure of two stacked rings of seven subunits each (94), a process that appears to require functional Hsp60 itself (18). Mutations in the *MIF4* gene were first identified as conditional-lethal mutations that allowed transport and processing of mitochondrial proteins (17); however, once translocated, the proteins failed to fold properly or assemble into their respective oligomeric structures. The similarities between the functions of Hsp60 and GroEL are further borne out by biochemical studies. In a study with *Neurospora* mitochondria (109), newly imported proteins were found complexed with Hsp60 when the mitochondria were depleted of ATP. Complexed proteins were in a highly protease-sensitive and thus loosely folded conformation. Upon addition of ATP, the accumulated unfolded protein was released from Hsp60 in a protease-resistant, fully folded conformation. Therefore, Hsp60 facilitates protein folding and assembly in an ATP-dependent manner by interacting directly with the unfolded protein. Although Hsp60 clearly functions in the folding and assembly of proteins whose final destination is the matrix, its role in the translocation of protein passing from the matrix into the inner membrane space remains to be resolved (Fig. 2) (59, 68, 79).

Cytosolic Hsp60. Although the understanding of protein folding in the eucaryotic cytosol has lagged behind that in mitochondria and bacteria, it seemed likely that a similar machinery would be used in all these different settings. Thus, the existence of a Cpn60-like molecule of the eucaryotic cytosol has been the subject of much conjecture. Now, work from a number of different laboratories points to a protein known as TCP1 as being at least one component of the cytoplasmic protein-folding machinery.

TCP1 (for tailless complex polypeptide) was initially identified in mice as a 57-kDa protein that is especially abundant in the testes but is found in all cell types (137). TCP1 in mice is a cytosolic protein, which, depending on the genetic background, is found in two isoforms. The presence of one of these isoforms is correlated with defects in embryonic development as well as in sperm structure and function. The mouse *TCP1* gene was cloned (155), and, more recently, homologs have been isolated from fruit flies (148), humans (154), and *S. cerevisiae* (147). The first indication that TCP1 might be a cytoplasmic chaperonin came from a comparison of the sequences of Cpn60 genes with data bases of known sequences, which revealed a similarity between Cpn60s and TCP1 (35, 64). Although the sequence similarity between TCP1 and the chaperonins is weak, it suggests that the two proteins may have had a common progenitor; however, it is not known whether the similarity has any meaning with regard to the function of TCP1.

Several lines of evidence indicate that TCP1 is functionally similar to Cpn60. The major heat inducible protein of the thermophilic archaebacterium *Sulfolobus shibatae*, TF55, has a high degree of similarity with TCP1 (146). As expected for a chaperonin, TF55 can bind unfolded proteins and has ATPase activity, although it has not yet been demonstrated to facilitate protein folding. Very recently, two independent studies, one on the folding of actin (54) and the other on the folding of tubulin (157), suggest that TCP1 does function in protein biogenesis. Both of these studies involved assays in which the protein whose folding and assembly being investigated was radiolabeled during synthesis in cytosolic extracts and its association with other proteins was monitored by electrophoresis in nondenaturing polyacrylamide gels. Newly synthesized actin and tubulin were found to be associated with a high-molecular-mass component, which contained as a subunit a protein of approximately 60 kDa that reacted with antibody generated against TCP1. Although complex formation was ATP independent, release to a folded, protease-resistant form was dependent on ATP hydrolysis. TCP1 is a cytosolic protein, which is one of the components of a high-molecular-mass heteromeric complex reminiscent of Cpn60 oligomers (83). It has been purified

from the cytosol of bovine testes as part of a heteromeric 970-kDa complex containing several structurally related subunits of 52 to 65 kDa. This complex binds unfolded polypeptides, preventing aggregation, and mediates the ATP-dependent renaturation of unfolded firefly luciferase and tubulin (52). Together, these experiments strongly suggest that TCP1 acts as a cytoplasmic chaperonin.

Although TCP1 has not been purified from *S. cerevisiae*, genetic experiments have shown that *TCP1* is an essential gene (147). At the nonpermissive temperature, cold-sensitive mutants accumulate multinucleated and nonnucleated cells, and staining with anti-tubulin antibodies revealed aberrant tubulin structures. The relevance of these structures to the function of TCP1 is underscored by the sensitivity of the mutant to antimitotic drugs such as benomyl, which is believed to bind directly to α-tubulin, resulting in an increase in tubulin depolymerization. In light of the recent in vitro experiments, it is likely that TCP1 is directly involved in the folding of tubulin monomers into forms competent for dimer formation and assembly into microtubules.

Relationship between Actions of Hsp70 and Hsp60

Evidence from a number of quarters suggests that Hsp70 and Hsp60 function sequentially in a common pathway (Fig. 2). In mitochondria, mutations in *SSC1* and *MIF4* affect the translocation and folding or assembly of at least some of the same proteins, including $F_1\beta$ and the Fe/S protein, as well as the heterologous protein dihydrofolate reductase (17, 78). In vitro translocation experiments with radiolabeled precursors show an association first with Ssc1p and second with Hsp60, as demonstrated by coimmunoprecipitation experiments (89). In vitro experiments with purified DnaK, DnaJ, GrpE, GroEL and GroES suggest that the mechanism is as follows. First, DnaK and DnaJ interact with an unfolded polypeptide, stabilizing a nonaggregated, unfolded conformation (81; reviewed in reference 55). Then, in a reaction mediated by GrpE, the protein is transferred to GroE, which mediates proper folding. Such pathways probably exist in the cytosol of eucaryotic cells as well. As yet, no Hsp60 homolog has been found in the ER. Perhaps it has simply eluded detection; on the other hand, it is possible that such a function is not required in the ER, where a limited number of proteins are processed for secretion and the action of ER-localized Hsp70 may be sufficient.

Hsp90

Like the Hsp70s, members of the Hsp90 class of Hsps are highly conserved in bacteria, yeasts, and mammals (reviewed in reference 30). Hsp90 is an abundant protein, localized primarily in the cytoplasm. There are two Hsp90 homologs in *S. cerevisiae*, *HSC82*, which is constitutively expressed, and *HSP82*, which has a low basal level of expression and is induced 10- to 15-fold on heat induction (10, 40). If either of these genes is inactivated, the cell is unable to grow at high temperature (>37.5°C). If both genes are inactivated, the cell is inviable.

Hsp90 in vertebrate cells has been studied extensively (reviewed in reference 117). These studies have shown that dimeric Hsp90 is found in association with a wide range of proteins including tyrosine kinases and steroid hormone receptors (reviewed in references 30, 116, and 117). Initially, this work led to the hypothesis that the role of Hsp90 was to serve a regulatory function, blocking activation by steric interference and stabilizing the unfolded conformation of these proteins until they were properly localized. It now appears that Hsp90 is also required for their functional activation, perhaps for folding into a potentially active conformation. In vitro studies of Hsp90 activity on the glucocorticoid receptor of murine L cells have shown that Hsp90 binding is a prerequisite for the binding of hormone, which in turn is required for receptor binding to DNA (11). Subsequent release of Hsp90 from the hormone-receptor complex results in the conversion of the receptor from a non-DNA-binding form to a DNA-binding form. Interestingly, Hsp70 is also present in the Hsp90-receptor complex and may play an active role in receptor biogenesis (74, 140a).

Further support for the role of Hsp90 as a molecular chaperone has come from the study of two heterologous systems constructed in *S. cerevisiae*. The functions of both oncogenic tyrosine kinases (156) and glucocorticoid receptors (115) require the expression of yeast *HSC82*. Biochemical evidence also suggests that Hsp90 can facilitate the folding of proteins such as denatured citrate synthase and the Fab fragment of a monoclonal antibody (153).

Proline and Disulfide Isomerases

In addition to the Hsps, two other classes of highly conserved proteins which play a role in protein folding in vivo have been identified, the protein disulfide isomerases (PDIs) and the peptidyl-prolyl *cis-trans* isomerases (PPI). Since little work has been carried out with *S. cerevisiae*, these enzymes are only briefly discussed here; work with other systems is reviewed in references 71, 106, and 142.

PDIs, which catalyze the formation of disulfide bonds in reduced proteins, are localized in the lumen of the ER of eucaryotic cells. Their association with newly synthesized immunoglobulins and their ability to restore cotranslational disulfide bond formation to microsomes depleted of PDIs suggest that they play an important role in protein folding in vivo. Recently, yeast PDI was shown to be encoded by a single, essential gene, *PDI1* (39, 144). However, the role of PDI in *S. cerevisiae* has yet to be established.

PPIs catalyze the *cis-trans* isomerization at X-Pro peptide bonds and accelerate in vitro a slow phase of folding of several proteins that is dependent on proline isomerization. Recently, two classes of proteins which bind immunosuppressive drugs were shown to be related but distinct PPIs. One class, called cyclophilins, bind cyclosporin A; the second class, called FKBPs, bind the immunosuppressant FK506 (132). Although much attention has been given to the role of these proteins in immunosuppression, little progress has been made toward understanding the role of the PPIs in protein folding in vivo. However, recently the *Drosophila ninaA* locus has provided the first clues concerning the action of cyclophilins in protein folding in vivo. NinA, related to the cyclophilin type of PPI, is localized in the ER of photoreceptor cells and is required for proper trafficking and folding of a particular class (R1 to R6) of rhodopsins (21).

In *S. cerevisiae*, numerous PPI homologs have been identified, none of which appear to be essential. Three cyclophilin-related genes, *CYP1* (66), *CYP2* (80), and *CYP3* (93), have been cloned and sequenced. Cyp1p is cytosolic, whereas the amino-terminal sequences of Cyp2p and Cyp3p suggest that they are localized to subcellular compartments. Another member of this multigene family is *FKB1*, encoding and FKBP homolog (72). *fkb1* single mutants, as well as *fkb1 cyp1* double mutants, are viable. Strains carrying mutations in the cyclophilin and FKBP genes are resistant to cyclospo-

rin A and rapamycin, an FK506-related drug, respectively. Resistance to FK506 itself is modulated by additional proteins unrelated to PPIs, including Fkr1, Fkr2, and Fkr3ps (12) and Tor1 and Tor2 (70). However, the role of these other genes in causing resistance to FK506 is unresolved.

Although many isomerases have been identified in a variety of organisms their in vivo role in protein folding remains unclear. The yeast system will probably prove to be valuable in elucidating the function of this interesting class of proteins.

Hsp104

The Hsps discussed thus far have been shown to be involved in processes, such as protein biogenesis, which are essential under normal growth conditions. Hsp104, however, provides an exception to this general theme. Although Hsp104 is strongly heat inducible, under conditions of optimal growth it is expressed only at very low levels (128). Hsp104 deletion mutants grow at the same rate as wild-type cells at 25, 30, 35, and 37°C, demonstrating that Hsp104 function is dispensable under normal growth conditions.

However, without Hsp104, cells have difficulty surviving under less than optimal conditions such as high temperature. Normal yeast cells are rapidly killed by being shifted to 50°C, but if they are pretreated at a sublethal temperature such as 37°C, they are much better able to survive this treatment. Without Hsp104, yeast cells are defective for this induced thermotolerance. After a pre-heat shock, Hsp104 mutant cells are killed at about 100 times the rate of wild-type cells. In addition, expression of Hsp104 is necessary for the naturally high thermotolerance of stationary phase cells and spores, as well as for tolerance to ethanol (128).

Some clues to possible functions of Hsp104 come from analysis of its primary structure. Hsp104 belongs to a family of proteins (111) which includes the ClpA and ClpB proteins of *E. coli* (63). Many, including ClpB, are heat inducible (111, 141). The greatest region of similarity within this family surrounds two putative nucleotide-binding sites which, in the case of yeast Hsp104, are necessary for function. The Clp proteins of *E. coli* are involved in protein degradation. ClpA, while having no known protease activity itself (63), functions as an ATPase which is important in regulating the activity of the protease ClpP. Parsell et al. (111) do not suggest that Hsp104 is a protease, but, rather, they suggest that it may regulate a protease or, by some other mechanism, be involved in preventing or resolving aggregation of vital cellular structures during times of stress. Thus, like the Hsps discussed above, the picture that is emerging for Hsp104 function seems to be one of mediating protein-protein interactions. Interestingly, the defect in induced thermotolerance in Hsp104 cells can be partially suppressed by overexpression of the Hsp70 Ssa1p, suggesting that the functions of these two proteins are related (127).

PROTEOLYSIS

If, through environmental conditions such as an increase in temperature or misfunction of a normal cellular process, a protein becomes unfolded and nonfunctional, it has two possible fates. It could regain its native structure, perhaps with the aid of molecular chaperones; for example, the *E. coli* protein DnaK is able to salvage denatured RNA polymerase (138). Alternatively, it could be treated as cellular garbage and undergo proteolysis; in lieu of being able to repair the damaged protein, the molecular chaperones may aid in its proteolysis. Thus the activities of the molecular chaperones and the proteolytic machinery are likely to be intimately entwined. This notion is supported by the observation that yeast *ssa1 ssa2* mutants are sensitive to the amino acid analog canavanine, which, upon incorporation into proteins, presumably causes misfolding (103).

The ubiquitin pathway is a major route for protein degradation in eucaryotes. Like Hsps, expression of at least some of the components of the proteolytic machinery of the cells is induced by a variety of stresses, including heat and the presence of amino acid analogs. Ubiquitin mediated protein degradation is a complex multistep process (reviewed in references 20, 44, 73, 75, 76, and 119). Ubiquitin, a highly conserved 76-amino-acid protein, is activated by the formation of a reactive thiol ester with a ubiquitin-activating enzyme (encoded by the *UBA* genes). The activated ubiquitin is transferred to one of several ubiquitin conjugating-enzymes (encoded by the *UBC* genes), from which it is transferred to a protein substrate. A variety of evidence indicates that the protein targeted by ubiquitination is degraded by a large, multiprotein subunit complex known as the proteasome (reviewed in reference 61). This system of protein degradation is highly conserved. Much of the biochemistry was initially investigated in mammalian cells; more recently, genetic analyses, as described below, have proven productive in dissecting the roles of individual genes involved.

Ubiquitin Genes

In *S. cerevisiae*, ubiquitin is encoded by four genes. The *UBI4* gene, the only heat-inducible ubiquitin gene, encodes a fusion protein of five tandem ubiquitin repeats, which are cleaved to monoubiquitin (110). The other yeast ubiquitin genes, *UBI1*, *UBI2*, and *UBI3*, encode ubiquitin–ribosomal-protein fusions (43). A *ubi4* deletion strain grows at rates comparable to wild-type strains under optimal growth conditions between 23 and 36°C (45). However, under adverse conditions, the *UBI4* gene is essential for survival. For example, after 16 h at 38.5°C, a borderline growth temperature, about 60% of wild-type cells maintain colony-forming ability. In contrast, only 1 to 5% of *ubi4* mutant cells survive this treatment. *ubi4* strains are also more sensitive than wild-type strains to amino acid analogs and starvation for nitrogen and carbon; spore viability is also reduced in these strains.

In summary, the *UBI4* gene is specialized to provide the cell with sufficient ubiquitin under conditions of stress. However, the *UBI1*, *UBI2*, and *UBI3* genes play a dual role in *S. cerevisiae*. They supply free ubiquitin on cleavage of the fusion protein, but, probably more importantly, they aid ribosome assembly. Finley et al. (43) propose that ubiquitin may play a chaperone function in assembly of ribosomal proteins into the ribosome, an extremely complex multimeric structure.

Ubiquitin-Activating and Conjugating Enzymes

A yeast gene (*UBA1*) encoding a ubiquitin-activating enzyme has been isolated. In keeping with the involvement of ubiquitin in many essential cellular processes, *UBA1* is essential (92).

S. cerevisiae contains at least 10 different but related ubiquitin-conjugating enzymes encoded by the *UBC* genes (reviewed in reference 75); all share a conserved domain which contains the cysteine residue to which ubiquitin is

covalently attached. The wide array of biological functions of the ubiquitin system is underscored by the diversity of the conjugating enzymes. For example, *UBC2* and *UBC3* are the previously identified *RAD6* (77) and *CDC34* (60) genes, which are involved in DNA repair and the G_1-to-S progression of the cell cycle, respectively.

The proteins encoded by *UBC1*, *UBC4*, and *UBC5* are an essential subgroup of conjugating enzymes (135). Analysis of the *ubc4 ubc5* double mutant indicates that this class of activating enzymes is involved in the degradation of damaged proteins (133). Both genes are expressed constitutively and are significantly induced by heat shock. *ubc4 ubc5* double mutants fail to synthesize high-molecular-mass ubiquitin-protein conjugates; instead, they accumulate free ubiquitin. *ubc4 ubc5* mutants have a slow-growth phenotype, are inviable at 37°C or in the presence of amino acid analogs, and are constitutively thermotolerant. These phenotypes are very reminiscent of the phenotype of the *ssa1 ssa2* double mutant, pointing to the possibility that the roles of the *SSA* Hsp70s and the *UBC4* and *UBC5* conjugating enzymes are related.

Proteasome

The proteasome, best characterized in mammalian cells, is a large, cylindrical 20S particle that is composed of at least 12 different protein subunits arranged in four stacked rings. In vitro this particle exhibits three distinct endopeptidase activities. However, it seems as though additional protein components allow degradation of ubiquitin-protein conjugates (reviewed in reference 61). This form, known as the 26S complex, probably mediates degradation of ubiquitin-protein conjugates in vivo. Although the proteasome has been most extensively studied in mammalian cells, progress has been made on the genetic analysis of the proteasome in *S. cerevisiae*. Several of the genes which encode subunits of the proteasome are essential. A missense mutation in one of these essential genes (*PRE1*) affects the chymotrypsin-like activity of the proteasome, as well as causing inviability at elevated temperatures and hypersensitivity to amino acid analogs (69). In addition, derivatives of β-galactosidase that are known to be degraded very rapidly by a ubiquitin-mediated mechanism are stabilized in the *pre1* mutant. This stabilization indicates that the proteasome mediates the degradation of ubiquitin-conjugated proteins in vivo (125, 134).

CONCLUDING REMARKS

The research carried out over the past few years has resulted in a basic understanding of the roles of the major Hsps in the cell. However, much remains to be understood. Although Hsp70s, Hsp60s, and Hsp90s have been shown to interact with a variety of polypeptides with physiologically important consequences, the chemistry of the interactions is only very superficially understood. The specificity of the chaperone-polypeptide interactions and the triggering of polypeptide release are not known. In a more physiological context, the interrelationships among the Hsps themselves is unclear. For example, there are two classes of Hsps70s in the yeast cytoplasm, Ssaps and Ssbps. Ssbps probably interact with nascent chains on the ribosome, and Ssaps are important for translocation of at least some proteins across the ER and mitochondrial membranes, but whether a single polypeptide reacts sequentially, simultaneously, or uniquely with a particular class of Hsp70s is not resolved. Similarly, interaction between Hsps and the proteolytic system is not established. At present one can only speculate that a pathway may exist, first an interaction of a partially unfolded protein with an Hsp70 and then either an interaction with a Cpn60, resulting in proper folding, or with the ubiquitin system, resulting in degradation mediated by the proteasome. Research carried out over the next few years should yield the answers to these questions.

ACKNOWLEDGMENTS

We thank our colleagues for providing us with unpublished information.

Work from our laboratory was supported by grants from the National Institutes of Health.

REFERENCES

1. **Atencio, D., and M. Yaffe.** 1992. *MAS5*, a yeast homolog of *dnaJ* involved in mitochondrial import. Mol. Cell. Biol. **12:** 283–291.
2. **Beckmann, R. P., L. Mizzen, and W. Welch.** 1990. Interaction of Hsp70 with newly synthesized proteins: implications for protein folding and assembly. Science **248:**850–856.
3. **Blobel, G., and D. D. Sabatini.** 1970. Controlled proteolysis of nascent polypeptides in rat liver cell fractions. J. Cell Biol. **45:**130–145.
4. **Blumberg, H., and P. Silver.** 1991. A homologue of the bacterial heat-shock gene *dnaJ* that alters protein sorting in yeast. Nature (London) **349:**627–629.
5. **Bole, D., G., L. M. Hendershot, and J. F. Kearney.** 1986. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. J. Cell Biol. **102:**1558–1566.
6. **Boorstein, W., and E. A. Craig.** 1990. Transcriptional regulation of *SSA3*, an HSP70 gene from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**3262–3267.
7. **Boorstein, W., and E. A. Craig.** 1990. Structure and regulation of the *SSA4* gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **265:**18912–18921.
8. **Boorstein, W., T. Ziegelhoffer, and E. A. Craig.** Unpublished results.
9. **Boorstein, W., T. Ziegelhoffer, and E. A. Craig.** Molecular evolution of the HSP70 multigene family. J. Mol. Evol., in press.
10. **Borkovich, K., F. Farrelly, D. Finkelstein, J. Taulien, and S. Lindquist.** 1989. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperature. Mol. Cell. Biol. **9:**3919–3930.
11. **Bresnick, E., F. C. Dalman, E. R. Sanchez, and W. B. Pratt.** 1989. Evidence that the 90-kDa heat shock protein is necessary for the steroid binding conformation of the L cell glucocorticoid receptor. J. Biol. Chem. **264:**4992–4997.
12. **Brizuela, L., G. Chrebet, K. A. Bostian, and S. A. Parent.** 1991. Antifungal properties of the immunosuppressant FK-506: identification of an FK-506 responsive yeast gene distinct from *FKB1*. Mol. Cell. Biol. **11:**4616–4626.
13. **Caplan, A., D. Cyr, and M. Douglas.** 1992. $YDJ1_p$ facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. Cell **71:**1143–1155.
14. **Caplan, A., and M. Douglas.** 1991. Characterization of *YDJ1*: a yeast homologue of the bacterial *dnaJ* protein. J. Cell Biol. **114:**609–621.
15. **Caplan, A., J. Tsai, P. Casey, and M. Douglas.** 1992. Farnesylation of YDJ1p is required for function at elevated growth temperatures in *S. cerevisiae*. J. Biol. Chem. **267:**18890–18895.
16. **Chappell, T. G., B. B. Konforti, S. L. Schmid, and J. E. Rothman.** 1987. The ATPase core of a clathrin uncoating protein. J. Biol. Chem. **262:**746–751.
17. **Cheng, M., F. Hartl, J. Martin, R. Pollock, F. Kalousek, W. Neupert, E. Hallberg, R. Hallberg, and A. Horwich.** 1989. Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature

(London) **337**:620–625.
18. **Cheng, M. Y., F.-U. Hartl, and A. Horwich.** 1990. The mitochondrial chaperonin hsp60 is required for its own assembly. Nature (London) **348**:455–458.
19. **Chirico, W., M. G. Waters, and G. Blobel.** 1988. 70K heat shock related proteins stimulate protein translocation into microsomes. Nature (London) **332**:805–810.
20. **Ciechanover, A., and A. L. Schwartz.** 1988. How are substrates recognized by the ubiquitin-mediated proteolytic system? Trends Biochem. Sci. **14**:483–488.
21. **Colley, N. J., E. K. Baker, M. A. Stamnes, and C. S. Zuker.** 1991. The cyclophilin homolog ninaA is required in the secretory pathway. Cell **67**:255–263.
22. **Conde, J., and G. R. Fink.** 1976. A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. USA **73**:3651–3655.
23. **Craig, E. A.** 1985. The heat shock response. Crit. Rev. Biochem. **18**:239–280.
24. **Craig, E. A.** 1989. Essential roles of 70kDa heat inducible genes. Bioessays **11**:48–52.
25. **Craig, E. A.** The heat shock response of *Saccharomyces cerevisiae*. *In* J. R. Broach, J. R. Pringle, and E. W. Jones (ed.), The molecular biology of the yeast *Saccharomyces*, in press.
26. **Craig, E. A., and K. Jacobsen.** 1984. Mutations of the heat-inducible 70 kilodalton genes of yeast confer temperature-sensitive growth. Cell **38**:841–849.
27. **Craig, E. A., and K. Jacobsen.** 1985. Mutations in cognate gene of *Saccharomyces cerevisiae* HSP70 result in reduced growth rates at low temperatures. Mol. Cell. Biol. **5**:3517–3524.
28. **Craig, E. A., J. Kramer, and J. Kosic-Smithers.** 1987. *SSC1*, a member of the 70-kDa heat shock protein multigene family of *Saccharomyces cerevisiae*, is essential for growth. Proc. Natl. Acad. Sci. USA **84**:4156–4160.
29. **Craig, E. A., J. Kramer, J. Shilling, M. Werner-Washburne, S. Holmes, J. Kosic-Smither, and C. M. Nicolet.** 1989. *SSC1*, an essential member of the *S. cerevisiae* HSP70 multigene family, encodes a mitochondrial protein. Mol. Cell. Biol. **9**:3000–3008.
30. **Craig, E. A., and S. Lindquist.** 1988. The heat-shock proteins. Annu. Rev. Genet. **22**:631–677.
31. **Cyr, D., X. Lu, and M. Douglas.** 1992. Regulation of Hsp70 function by a eukaryotic DnaJ homolog. J. Biol. Chem. **267**: 20927–20931.
32. **Deshaies, R., B. Koch, M. Werner-Washburne, E. Craig, and R. Schekman.** 1988. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature (London) **332**:800–805.
33. **Deshaies, R., S. Sanders, D. Feldheim, and R. Schekman.** 1991. Assembly of yeast Sec proteins involved in translocation into the endoplasmic reticulum into a membrane-bound multisubunit complex. Nature (London) **349**:806–808.
34. **Eilers, M., and G. Schatz.** 1986. Binding of a specific ligand inhibits the import of a purified precursor protein into mitochondria. Nature (London) **322**:228–232.
35. **Ellis, R. J.** 1990. Molecular chaperones: the plant connection. Science **250**:954–959.
36. **Ellis, R. J., and S. M. van der Vies.** 1991. Molecular chaperones. Annu. Rev. Biochem. **60**:321–347.
37. **Ellwood, M. S., and E. A. Craig.** 1984. Differential regulation of the 70 K heat shock gene and related genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1454–1459.
38. **Engman, D., L. V. Kirchhoff, and J. E. Donelson.** 1989. Molecular cloning of *mtp70*, a mitochondrial member of the hsp70 family. Mol. Cell. Biol. **9**:5163–5168.
39. **Farquhar, R., N. Honey, S. J. Murant, P. Bossier, L. Schultz, D. Montgomery, R. W. Ellis, R. B. Freedman, and M. F. Tuite.** 1991. Protein disulfide isomerase is essential for viability in Saccharomyces cerevisiae. Gene **108**:81–89.
40. **Farrelly, F., and D. Finkelstein.** 1984. Complete sequence of the heat-inducible HSP90 gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **259**:5745–5751.
41. **Feldheim, D., J. Rothblatt, and Schekman.** 1992. Topology and functional domains of Sec63p, an ER membrane protein required for secretory protein translocation. Mol. Cell. Biol. **12**:3288–3296.
42. **Felici, F., G. Cesareni, and J. Hughes.** 1989. The most abundant small cytoplasmic RNA of *Saccharomyces cerevisiae* has an important function required for normal cell growth. Mol. Cell. Biol. **9**:3260–3268.
43. **Finley, D., B. Bartel, and A. Varshavsky.** 1989. The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. Nature (London) **338**:394–401.
44. **Finley, D., and V. Chau.** 1991. Ubiquitination. Annu. Rev. Cell Biol. **7**:25–69.
45. **Finley, D., E. Ozkaynak, and A. Varshavsky.** 1987. The yeast polyubiquitin gene is essential for resistance to high temperature, starvation and other stresses. Cell **49**:1035–1046.
46. **Flaherty, K. M., C. DeLuca-Flaherty, and D. B. McKay.** 1990. Three dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. Nature (London) **346**:623–628.
47. **Flaherty, K. M., D. B. McKay, W. Kabash, and K. Holmes.** 1991. Similarity of the three-dimensional structure of actin and the ATPase fragment of a 70kDa heat shock cognate protein. Proc. Natl. Acad. Sci. USA **88**:5041–5045.
48. **Flajnik, M., C. Canel, J. Kramer, and M. Kasahara.** 1991. Hypothesis: which came first, MHC class I or class II. Immunogenetics **33**:295–300.
49. **Flynn, G., J. Pohl, M. Flocco, and J. Rothman.** 1991. Peptide-binding specificity of the molecular chaperone BiP. Nature (London) **353**:726–730.
50. **Flynn, G. C., T. G. Chappell, and J. E. Rothman.** 1989. Peptide binding and release by proteins implicated as catalysts of protein assembly. Science **245**:385–390.
51. **Fremont, D., M. Matsumura, E. Strua, P. Petersen, and I. Wilson.** 1992. Crystal structure of two viral peptides in complex with murine MHC class I H-2Kb. Science **257**:919–926.
52. **Frydman, J., E. Nimmesgern, H. Erdjument-Bromage, J. Wall, P. Tempst, and F.-U. Hartl.** 1992. Function in protein folding of TriC, a cytosolic ring complex containing TCP-1 and structurally related subunits. EMBO J. **11**:4767–4778.
53. **Gao, B., J. Biosca, E. A. Craig, L. E. Greene, and E. Eisenberg.** 1991. Uncoating of coated vesicles by yeast hsp70 proteins. J. Biol. Chem. **266**:19565–19571.
54. **Gao, Y., J. O. Thomas, R. L. Chow, G.-H. Lee, and N. J. Cowan.** 1992. A cytoplasmic chaperonin that catalyzes β-actin folding. Cell **69**:1043–1050.
55. **Georgopoulos, C.** 1992. The emergence of the chaperone machines. Trends Biochem. Sci. **17**:294–299.
56. **Georgopoulos, C., D. Ang, K. Liberek, and M. Zylicz.** 1991. Properties of the *Escherichia coli* heat shock proteins and their role in bacteriophage lambda growth, p. 199–221. *In* R. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
57. **Gething, M.-J., K. McCammon, and J. Sambrook.** 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. Cell **46**:939–950.
58. **Gething, M.-J., and J. Sambrook.** 1992. Protein folding in the cell. Nature (London) **355**:33–45.
59. **Glick, B., A. Brandt, K. Cunningham, S. Muller, R. Hallberg, and G. Schatz.** 1992. Cytochromes c_1 and b_2 are sorted to the intermembrane space of yeast mitochondria by a stop-transfer mechanism. Cell **69**:809–822.
60. **Goebl, M., J. Yochem, S. Jentsch, J. McGrath, A. Varshavsky, and B. Byers.** 1988. The yeast cell cycle gene *CDC34* encodes a ubiquitin-conjugating enzyme. Science **241**:1331–1335.
61. **Goldberg, A.** 1992. The mechanism and functions of the ATP-dependent proteases in bacterial and animal cells. Eur. J. Biochem. **203**:9–23.
62. **Goloubinoff, P., A. Gatenby, and G. Lorimer.** 1989. GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate oligomers in *Escherichia coli*. Nature (London) **337**:44–47.
63. **Gottesman, S., C. Squires, E. Pichersky, M. Carrington, M.**

Hobbs, J. S. Mattick, B. Dalrymple, H. Kuramitsu, T. Shiroza, T. Foster, W. P. Clark, C. Ross, C. L. Squires, and M. Maurrizi. 1990. Conservation of the regulatory subunit for the Clp ATP-dependent protease in prokaryotes and eukaryotes. Proc. Natl. Acad. Sci. USA **87**:3513–3517.

64. **Gupta, R.** 1990. Sequence and structural homology between a mouse t-complex protein TCP-1 and the 'chaperonin' family of bacterial (groEL, 60–65 kDa heat shock antigen) and eukaryotic proteins. Biochem. Int. **20**:833–841.
65. **Haas, I. G., and M. Wabl.** 1983. Immunoglobulin heavy chain binding protein. Nature (London) **306**:387–389.
66. **Haendler, B., R. Keller, P. C. Hiestand, H. P. Kocher, G. Wegmann, and N. R. Movva.** 1989. Yeast cyclophilin: isolation and characterization of the protein, cDNA and gene. Gene **83**:39–46.
67. **Hann, B. C., and P. Walter.** 1991. The signal recognition particle in *S. cerevisiae*. Cell **67**:131–144.
68. **Hartl, F.-U., J. Ostermann, B. Guiard, and W. Neupert.** 1987. Successive translocation into and out of the mitochondrial matrix: targeting of proteins to the intermembrane space by a bipartite signal peptide. Cell **51**:1027–1037.
69. **Heinemeyer, W., J. Kleinschmidt, J. Saidowsky, C. Escher, and D. Wolf.** 1991. Proteinase yscE, the yeast proteasome/multicatalytic multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival. EMBO J. **10**:555–562.
70. **Heitman, J., N. R. Movva, and M. N. Hall.** 1991. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science **253**:905–909.
71. **Heitman, J., N. R. Movva, and M. N. Hall.** 1992. Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. New Biol. **4**:448–460.
72. **Heitman, J., N. R. Movva, P. C. Hiestand, and M. N. Hall.** 1991. FK506-binding protein proline rotamase is a target for the immunosuppressive agent FK506 in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **88**:1948–1952.
73. **Hershko, A.** 1991. The ubiquitin pathway for protein degradation. Trends Biochem. Sci. **16**:265–268.
74. **Hutchison, K., M. Czar, L. Scherrer, and W. Pratt.** 1992. Monovalent cation selectivity for the ATP-dependent association of the glucocorticoid receptor with hsp70 and hsp90. J. Biol. Chem. **267**:14047–14053.
75. **Jentsch, S.** 1992. The ubiquitin-conjugation system. Annu. Rev. Genet. **26**:179–207.
76. **Jentsch, S.** 1992. Ubiquitin-dependent protein degradation: a cellular perspective. Trends Cell Biol. **2**:98–103.
77. **Jentsch, S., J. McGrath, and A. Varshavsky.** 1987. The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature (London) **329**:131–134.
78. **Kang, P. J., J. Ostermann, J. Shilling, W. Neupert, E. A. Craig, and N. Pfanner.** 1990. Hsp70 in the mitochondrial matrix is required for translocation and folding of precursor proteins. Nature (London) **348**:137–143.
79. **Koll, H., B. Guirard, J. Rassow, J. Ostermann, A. Horwich, W. Neupert, and F. U. Hartl.** 1992. Antifolding activity of hsp60 couples protein import into the mitochondrial matrix with export to the intermembrane space. Cell **68**:1163–1175.
80. **Koser, P. L., D. J. Bergsma, R. Cafferkey, W.-K. Eng, M. M. McLaughlin, A. Ferrara, C. Silverman, K. Kasyan, M. J. Bossard, R. K. Johnson, T. G. Porter, M. A. Levy, and G. P. Livi.** 1991. The *CYP2* gene of *Saccharomyces cerevisiae* encodes a cyclosporin A-sensitive peptidyl-prolyl cis-trans isomerase with an N-terminal signal sequence. Gene **108**:73–80.

80a. **Landry, S. J., and L. M. Gierasch.** Peptides, in press.

81. **Langer, T., C. Lu, H. Echols, J. Flanagan, M. K. Hayer, and F. U. Hartl.** 1992. Successive action of DnaK, DnaJ, and GroEL along the pathway of chaperone-mediated protein folding. Nature (London) **356**:683–689.
82. **Leustek, T., B. Dalie, D. Amir-Shapira, N. Brot, and H. Weissbach.** 1989. A member of the hsp70 family is localized in mitochondria and resembles *Escherichia coli* DnaK. Proc. Natl. Acad. Sci. USA **86**:7805–7808.
83. **Lewis, V. A., G. M. Hynes, D. Zheng, H. Saibil, and K. Willison.** 1992. T-complex polypeptide-1 is a subunit of a heteromeric particle in the eucaryotic cytosol. Nature (London) **358**:249–252.
84. **Liberek, K., J. Marszalek, D. Ang, and C. Georgopoulos.** 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA **88**:2874–2878.
85. **Liberek, K., D. Skowyra, M. Zylicz, C. Johnson, and C. Georgopoulos.** 1991. The Escherichia coli DnaK chaperone, the 70-kDa heat shock protein eukaryotic equivalent, changes conformation upon ATP hydrolysis, thus triggering its dissociation from a bound target protein. J. Biol. Chem. **266**:14491–14496.
86. **Lubben, T., A. Gatenby, G. Donaldson, G. Lorimer, and P. Viitanen.** 1990. Identification of a groES-like chaperonin in mitochondria that facilitates protein folding. Proc. Natl. Acad. Sci. USA **87**:7683–7687.
87. **Luke, M., A. Suttin, and K. Arndt.** 1991. Characterization of *SIS1*, a *Saccharomyces cerevisiae* homologue of bacterial *dnaJ* proteins. J. Cell Biol. **114**:623–638.
88. **Malkin, L. I., and A. J. Rich.** 1967. Partial resistance of nascent polypeptide chains to proteolytic digestion due to ribosomal shielding. J. Mol. Biol. **26**:329–346.
89. **Manning-Krieg, U. C., P. Scherer, and G. Schatz.** 1991. Sequential action of mitochondrial chaperones in protein import into the matrix. EMBO J. **10**:3273–3280.
90. **Martin, J., T. Langer, R. Boteva, A. Schramel, A. L. Horwich, and F.-U. Hartl.** 1991. Chaperonin-mediated protein folding at the surface of groEL through a 'molten globule'-like intermediate. Nature (London) **352**:36–42.
91. **Matsumura, M., D. Fremont, P. Petersen, and I. Wilson.** 1992. Emerging principles for the recognition of peptide antigens by MHC class I molecules. Science **257**:927–934.
92. **McGrath, J. P., S. Jentsch, and A. Varshavsky.** 1991. *UBA1*: an essential yeast gene encoding ubiquitin-activating enzyme. EMBO J. **10**:227–236.
93. **McLaughlin, M. M., M. J. Bossard, P. L. Koser, R. Cafferkey, R. A. Morris, L. M. Miles, J. Strickler, D. J. Bergsma, M. A. Levy, and G. P. Livi.** 1992. The yeast cyclophilin multigene family: purification, cloning and characterization of a new isoform. Gene **111**:85–92.
94. **McMullin, T., and R. Hallberg.** 1988. A highly conserved mitochondrial protein is structurally related to the protein encoded by the *Escherichia coli groEL* gene. Mol. Cell. Biol. **8**:371–380.
95. **Milligan, R. A., and P. N. T. Unwin.** 1986. Location of the exit channel for nascent protein in 80S ribosome. Nature (London) **319**:693–695.
96. **Mizzen, L. A., C. Chang, J. I. Garrels, and W. Welch.** 1989. Identification, characterization, and purification of two mammalian stress proteins present in mitochondria: one related to hsp70, the other to GroEL. J. Biol. Chem. **264**:20664–20675.
97. **Morishima, N., K. Nakagawa, E. Yamamoto, and T. Shibata.** 1990. A subunit of yeast site-specific endonuclease *SceI* is a mitochondrial version of the 70-kDa heat shock protein. J. Biol. Chem. **265**:15189–15197.
98. **Munro, S., and H. Pelham.** 1986. An hsp70-like protein in the ER: identity with the 78kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell **46**:291–300.
99. **Murakami, H., D. Pain, and G. Blobel.** 1988. 70-kD heat shock-related protein is one of at least two distinct cytosolic factors stimulating protein import into mitochondria. J. Cell Biol. **107**:2051–2057.
100. **Musch, A., M. Wiedmann, and T. Rapoport.** 1992. Yeast Sec proteins interact with polypeptides traversing the endoplasmic reticulum membrane. Cell **69**:343–352.
101. **Nakagawa, K., N. Morishima, and T. Shibata.** 1990. A maturase-like subunit of the sequence-specific endonuclease Endo.*SceI* from yeast mitochondria. J. Biol. Chem. **265**:1977–1984.
102. **Nakagawa, K., N. Morishima, and T. Shibata.** 1992. An endonuclease with multiple cutting sites, Endo.*SceI* initiates ge-

netic recombination at its cutting site in yeast mitochondria. EMBO J. **11**:2707–2715.
103. **Nelson, R. J., and E. A. Craig.** Unpublished results.
104. **Nelson, R. J., M. Heschl, and E. A. Craig.** 1992. Isolation and characterization of extragenic suppressors of mutations in the *SSA* hsp70 genes of *Saccharomyces cerevisiae*. Genetics **131:** 277–285.
105. **Nelson, R. J., T. Ziegelhoffer, C. Nicolet, M. Werner-Washburne, and E. A. Craig.** 1992. The translation machinery and seventy kilodalton heat shock protein cooperate in protein synthesis. Cell **71:**97–105.
106. **Noiva, R., and W. J. Lennarz.** 1992. Protein disulfide isomerase. J. Biol. Chem. **267:**3553–3556.
107. **Normington, K., K. Kohno, Y. Kozutsumi, M. J. Gething, and J. Sambrook.** 1989. *S. cerevisiae* encodes an essential protein homologous in sequence and function to mammalian BiP. Cell **57:**1223–1236.
108. **Ostermann, J., W. Voos, P. J. Kang, E. A. Craig, W. Neupert, and N. Pfanner.** 1990. Precursor proteins in transit through mitochondrial contact sites interact with hsp70 in the matrix. FEBS Lett. **277:**281–284.
109. **Ostermann, J. H., W. Neupert, and F.-U. Hartl.** 1989. Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis. Nature (London) **341:**125–130.
110. **Ozkaynak, E., D. Finley, and A. Varshavsky.** 1984. The yeast ubiquitin gene: head-to-tail repeats encoding a polyubiquitin precursor protein. Nature (London) **312:**663–666.
111. **Parsell, D., Y. Sanchez, J. Stitzel, and S. Lindquist.** 1991. Hsp104 is a highly conserved protein with two essential nucleotide binding sites. Nature (London) **353:**270–273.
112. **Pelham, H., K. Hardwick, and M. Lewis.** 1988. Sorting of soluble ER proteins in yeast. EMBO J. **7:**1757–1762.
113. **Pelham, H. R. B.** 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell **46:**959–961.
114. **Pfanner, N., and W. Neupert.** 1990. The mitochondrial import apparatus. Annu. Rev. Biochem. **59:**331–353.
115. **Picard, D., B. Khursheed, M. Garabedian, M. Fortin, S. Lindquist, and K. Yamamoto.** 1990. Reduced levels of hsp90 compromise steroid receptor action *in vivo*. Nature (London) **348:**166–168.
116. **Pratt, W.** 1987. Transformation of glucocorticoid and progesterone receptors to the DNA-Binding State. J. Cell. Biochem. **35:**51–68.
117. **Pratt, W. B., L. C. Scherrer, K. A. Hutchison, and F. C. Dalman.** 1992. A model of glucocorticoid receptor unfolding and stabilization by a heat shock protein complex. J. Steroid Biochem. **41:**223–229.
118. **Reading, D., R. Hallberg, and A. Meyers.** 1989. Characterization of the yeast HSP60 gene coding for a mitochondrial assembly factor. Nature (London) **337:**655–659.
119. **Rechsteiner, M.** 1991. Natural substrates of the ubiquitin proteolytic pathway. Cell **66:**615–618.
120. **Rippmann, F., W. Taylor, J. Rothbard, and N. M. Green.** 1991. A hypothetical model for the peptide binding domain of hsp70 based on the peptide binding domain of HLA. EMBO J. **10:**1053–1059.
121. **Rose, M., J. Vogel, M. Scidmore, and L. Misra.** 1989. *KAR2*, the yeast homologue of mammalian BiP/GRP78, is required for translocation of proteins into the endoplasmic reticulum. J. Cell. Biochem. Suppl. **13E:**273.
122. **Rose, M. D., L. M. Misra, and J. P. Vogel.** 1989. *KAR2*, a karyogamy gene, is the yeast homologue of the mammalian BiP/GRP78 gene. Cell **57:**1211–1221.
123. **Rothblatt, J., R. Deshaies, S. Sanders, G. Daum, and R. Schekman.** 1989. Multiple genes are required for proper insertion of secretory proteins into the endoplasmic reticulum. J. Cell Biol. **109:**2641–2652.
124. **Rothman, J.** 1989. Polypeptide chain binding proteins: catalysts of protein folding and related processes in cells. Cell **59:**591–601.
125. **Ruoff, B., W. Heinemeyer, and D. Wolf.** 1992. The proteasome/multicatalytic-multifunctional proteinase: *in vivo* function in the ubiquitin-dependent N-end rule pathway of protein degradation in eukaryotes. FEBS Lett. **302:**192–196.
126. **Sadler, I., A. Chiang, T. Kurihara, J. Rothblatt, J. Way, and P. Silver.** 1989. A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to *DnaJ*, an *Escherichia coli* heat shock protein. J. Cell Biol. **109:**2665–2675.
127. **Sanchez, Y., E. A. Craig, and S. Lindquist.** Unpublished results.
128. **Sanchez, Y., and S. Lindquist.** 1990. HSP104 required for induced thermotolerance. Science **248:**1112–1115.
129. **Sanders, S., K. Whitfield, J. Vogel, M. Rose, and R. Schekman.** 1992. Sec61p and BiP directly facilitate polypeptide translocation into the ER. Cell **69:**353–366.
130. **Scherer, P., U. Krieg, S. Hwang, D. Vestweber, and G. Schatz.** 1990. A precursor protein partially translocated into yeast mitochondria is bound to a 70kd mitochondrial stress protein. EMBO J. **9:**4315–4322.
131. **Schleyer, M., and W. Neupert.** 1985. Transport of proteins into mitochondria: translocation intermediates spanning contact sites between inner and outer membranes. Cell **43:**339–350.
132. **Schreiber, S. L.** 1991. Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science **251:**283–287.
133. **Seufert, W., and S. Jentsch.** 1990. Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. EMBO J. **9:**543–550.
134. **Seufert, W., and S. Jentsch.** *In vivo* function of the proteasome in the ubiquitin pathway. EMBO J., in press.
135. **Seufert, W., J. P. McGrath, and S. Jentsch.** 1990. *UBC1* encodes a novel member of an essential subfamily of yeast ubiquitin-conjugating enzymes involved in protein degradation. EMBO J. **9:**4535–4541.
136. **Sheffield, W. P., G. C. Shore, and S. K. Randall.** 1990. Mitochondrial precursor protein: effects of 70-kilodalton heat shock protein on polypeptide folding, aggregation, and import competence. J. Biol. Chem. **265:**11069–11076.
137. **Silver, L. M., K. Artzt, and D. Bennet.** 1979. A major testicular cell protein specified by a mouse T/t complex gene. Cell **17:**275–284.
138. **Skowyra, D., C. Georgopoulos, and M. Zylicz.** 1990. The *E. coli dnak* gene product, the hsp70 homolog, can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner. Cell **62:**939–944.
139. **Slater, M., and E. Craig.** 1987. Transcriptional regulation of an hsp70 heat shock gene in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**1906–1916.
140. **Slater, M. R., and E. A. Craig.** 1989. The *SSA1* and *SSA2* genes of the yeast Saccharomyces cerevisiae. Nucleic Acids Res. **17:**805–806.
140a. **Smith, D. F., B. A. Stensgordt, W. Welch, and D. O. Toft.** 1992. Assembly of progesterone receptor with heat shock proteins and receptor activation are ATP-mediated events. J. Biol. Chem. **267:**1–7.
141. **Squires, C., S. Pedersen, B. Ross, and C. Squires.** 1991. ClpB is the *Escherichia coli* heat shock protein F84.1. J. Bacteriol. **173:**4254–4262.
142. **Stammes, M. A., and C. S. Zuker.** 1992. Peptidyl-prolyl *cis-trans* isomerases, cyclophilin, FK506-binding protein, and ninaA: four of a kind. Curr. Biol. **2:**1104–1107.
143. **Straus, D., W. Walter, and C. Gross.** 1990. DnaK, DnaJ and GrpE negatively regulate heat shock protein gene expression by controlling the synthesis and stability of sigma-32. Genes Dev. **4:**2202–2209.
144. **Tachikawa, H., R. Miura, Y. Katakura, and T. Mizunaga.** 1991. Molecular structure of a yeast gene, *PDI1*, encoding protein disulfide isomerase that is essential for cell growth. J. Biochem. (Tokyo) **110:**306–313.
145. **Tokunda, M., A. Kawamura, and K. Kohno.** Purification and characterization of BiP/Kar2 protein from *Saccharomyces cerevisiae*. J. Biol. Chem., in press.
146. **Trent, J. D., E. Nimmesgern, J. S. Wall, F.-U. Hartl, and A. L. Horwich.** 1991. A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex

polypeptide-1. Nature (London) **354**:490–493.
147. **Ursic, D., and M. R. Culbertson.** 1991. The yeast homolog to mouse Tcp-1 affects microtubule-mediated processes. Mol. Cell. Biol. **11**:2629–2640.
148. **Ursic, D., and B. Ganetzky.** 1988. A *Drosophila melanogaster* gene encodes a protein homologous to the mouse t complex polypeptide 1. Gene **68**:267–274.
149. **Vogel, J. P., L. M. Misra, and M. D. Rose.** 1990. Loss of BiP/Grp78 function blocks translocation of secretory proteins in yeast. J. Cell Biol. **110**:1885–1895.
150. **Walter, P., and V. Lingappa.** 1986. Mechanisms of translocation across the ER membrane. Annu. Rev. Cell Biol. **2**:499–516.
151. **Werner-Washburne, M., J. Becker, J. Kosics-Smithers, and E. A. Craig.** 1989. Yeast Hsp70 RNA levels change in response to the physiological status of the cell. J. Bacteriol. **171**:2680–2688.
152. **Werner-Washburne, M., D. E. Stone, and E. A. Craig.** 1987. Complex interactions among members of an essential subfamily of hsp70 genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:2568–2577.
153. **Wiech, H., J. Buchner, R. Zimmerman, and U. Jakob.** 1992. Hsp90 chaperones protein folding *in vitro*. Nature (London) **358**:169–170.
154. **Willison, K., A. Kelly, K. Dudley, P. Goodfellow, N. Spurr, V. Groves, P. Gorman, D. Sheer, and J. Trowsdale.** 1987. The human homolog of the mouse t-complex gene, *TCP1*, is located on human chromosome 6 but is not near the HLA region. EMBO J. **6**:1967–1974.
155. **Willison, K. R., K. Dudley, and J. Potter.** 1986. Molecular cloning and sequence analysis of a haploid expressed gene encoding *t* complex polypeptide 1. Cell **44**:727–738.
156. **Xu, Y., and S. Lindquist.** Personal communication.
157. **Yaffe, M. B., G. W. Farr, D. Miklos, A. L. Horwich, M. L. Sternlich, and H. Sternlich.** 1992. TCP1 complex is a molecular chaperone in tubulin biogenesis. Nature (London) **358**:245–248.
158. **Zimmerman, R., M. Sagstetter, M. J. Lewis, and H. R. B. Pelham.** 1988. Seventy-kilodalton heat shock proteins and an additional component from reticulocyte lysate stimulate import of M13 procoat protein into microsomes. EMBO J. **7**:2875–2880.