

## Roles of Molecular Chaperones in Protein Degradation

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**H**HEAT and other forms of stress that cause proteins to denature induce the synthesis of several classes of proteins known as heat shock proteins (hsps)<sup>1</sup> many of which act as molecular chaperones (see Table I). A major role of these molecular chaperones after stress is to catalyze the refolding of denatured proteins (3, 4, 16). However, certain molecular chaperones are produced constitutively indicating that they have important functions even under normal conditions. Such functions are being intensively investigated and include: (a) promotion of proper protein folding after synthesis; (b) stimulation of assembly and disassembly of multimeric proteins; and (c) facilitation of protein translocation across a variety of intracellular membranes including those of the endoplasmic reticulum, mitochondrion, nucleus, and peroxisome (3, 4, 16).

Molecular chaperones also stimulate the breakdown of proteins, and the idea has emerged that when molecular chaperones fail in their functions of protein folding, assembly, or translocation, they then facilitate the degradation of the mishandled protein (4, 17). Although this "refold or degrade" model remains to be critically tested, it now appears that molecular chaperones facilitate protein degradation in many different ways. We will cite examples where molecular chaperones increase the susceptibility of certain substrate proteins to proteolytic attack. In some cases the molecular chaperones simply prevent substrate proteins from forming massive aggregates that are partially shielded from proteolytic attack, but in other cases they function in more specific targeting of substrate proteins to particular proteolytic pathways. In addition to these effects on protein substrates, molecular chaperones can increase the overall activities of certain proteolytic pathways. In some cases the molecular chaperones are physically associated with proteases while in other cases they appear to be required for the interactions between different macromolecules needed for the proteolytic pathway to operate efficiently.

### *Molecular Chaperones and Protein Degradation in Prokaryotes*

**Proteolysis Is Induced by Stress.** Under stress conditions, such as elevated temperature or exposure to amino acid

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1. *Abbreviations used in this paper:* hsp, heat shock protein; mt-hsp, mitochondrial heat shock protein; TF, trigger factor.

analogs, rates of protein degradation increase in bacteria (16). The increased proteolysis is not simply due to effects of stress on the structure of substrate proteins; instead, molecular chaperones and proteases produced during stress are required (1, 7, 13).

Bacterial proteases known to be induced by stress include two ATP-dependent proteases, La and Clp (also called protease Ti; Table I). Protease La, the product of the *lon* gene, acts as a tetramer, and Clp protease acts as a tetradecamer of the protease subunit, Clp P (14) assembled with a hexamer of regulatory ATPase subunits, ClpA or ClpX (8, 14).

**Molecular Chaperones and Proteolysis by Proteases La and Clp.** Degradation of bulk abnormal proteins such as puromycin peptides and proteins containing amino acid analogs is carried out largely by proteases La and Clp and requires DnaK, DnaJ, GrpE, GroEL, and GroES, all of which are heat shock proteins and molecular chaperones (13, 16, 18; Table I). ClpA, ClpB, and ClpX have substantial sequence homology to heat shock proteins of the 100-kD family, and ClpB and ClpX are heat inducible (8).

Interestingly, both ClpA and ClpX can act alone as molecular chaperones. Wickner et al. (23) have shown that ClpA acts as a molecular chaperone in the *in vitro* activation of the plasmid P1 RepA initiator protein. ClpA functions, as do DnaK and DnaJ, by monomerizing RepA thereby allowing it to bind the DNA replication origin of plasmid P1. Additionally, ClpA functions to target RepA for degradation by ClpP. This stimulation of degradation does not occur when RepA is monomerized by DnaJ and DnaK, so ClpA seems to play a direct role in targeting RepA for degradation by ClpP (Fig. 1). ClpX has similarly been shown to target the bacteriophage lambda O replication protein for degradation by ClpP and to act as a molecular chaperone (22). ClpX can also catalyze the disassembly of the Mu virus transposase tetramer and target it for degradation by ClpP (Baker, T. A., personal communication).

The role of ClpA and ClpX in targeting proteins for degradation by ClpP is due, in part, to physical association between the molecular chaperone subunit and the protease subunit. Additional roles for ClpA and ClpX in modifying the protein substrate structure so that it is more susceptible to attack by ClpP are possible but have not been proven.

Other molecular chaperones appear to be involved in the degradation of specific abnormal proteins in *Escherichia coli*, but in these cases the roles of the molecular chaperones are not well understood. PhoA61, a short-

Table I. Molecular Chaperones and Proteases

Protein	Source	Description
<b>Molecular Chaperones</b>		
DnaK	bacteria	hsp70
DnaJ		stimulates DnaK
GrpE		works with DnaJ
GroEL		hsp60
GroES		hsp10; works with GroEL
Trigger Factor		stimulates protein insertion into plasma membrane
Ssa1,2	yeast	cytosolic hsp70s
Ssc1		mitochondrial hsp70
Mdj1		mitochondrial DnaJ homolog
Sis		cytosolic DnaJ homolog
Ydj		cytosolic DnaJ homolog
Hsc73	mammals	major constitutively expressed hsp70
<b>Proteases</b>		
La	bacteria	ATP-dependent protease
ClpP		proteolytic subunit of Clp
ClpA		ATPase subunit of Clp; molecular chaperone
ClpB		molecular chaperone
ClpX		ATPase subunit of Clp; molecular chaperone
Pim1	yeast	mitochondrial protease
proteasome	yeast, mammals	La homolog
		multisubunit ATP-dependent protease

lived, nonsecreted form of alkaline phosphatase, is degraded more rapidly in response to heat shock, and deletion of the *dnaK* gene or the *lon* gene stabilizes *phoA61* (17). This result alone does not prove that DnaK and protease La are involved with degradation of *phoA61*. In the absence of the molecular chaperone or protease many abnormal proteins can accumulate and potentially saturate a proteolytic pathway responsible for the degradation of *phoA61*. However, *phoA61* can also be found associated with DnaK, GrpE (Table I), and protease La, and under a variety of conditions the rate of degradation of *phoA61*

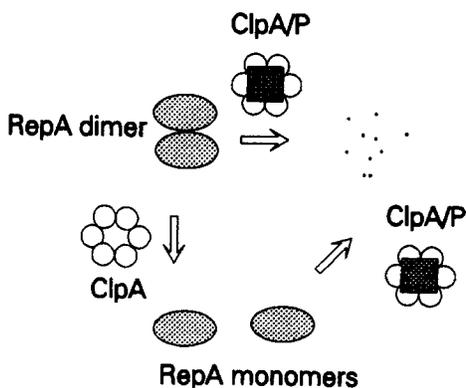


Figure 1. ClpA functions in the monomerization and degradation of RepA. ClpA alone (hexamer) acts as a molecular chaperone in the monomerization and activation of RepA (shaded ovals). ClpA also works together with ClpP (shaded square) in the degradation of RepA. Whether ClpA/P degrades RepA monomers, dimers, or both is not clear. All of the steps shown require ATP.

correlates with the amount of DnaK associated with the protein. For example, mutations in the *dnaJ* gene stabilize *phoA61* and reduce the amount of DnaK associated with the protein.

Other molecular chaperones appear to be involved in the degradation of other specific abnormal proteins in *E. coli*. CRAG is a chimeric protein consisting of 12 amino acids from the *cro* repressor, a truncated form of protein A, and 14 amino acids from  $\beta$ -galactosidase (11). It is efficiently degraded by the Clp protease, but only if GroEL and GroES are also present. Kandror et al. (12) also show that another protein previously not recognized to be a general molecular chaperone, trigger factor (TF; Table I), also associates with CRAG and may be involved in the degradation of this abnormal protein.

From the examples of *phoA61* and CRAG, it might seem that DnaK and GrpE stimulate proteolysis by protease La while GroEL, GroES, and TF stimulate proteolysis by protease Clp. However, there is no evidence for the specific interaction between these molecular chaperones and proteases. Another possibility is that the molecular chaperones in each case may be preventing the massive aggregation of substrate proteins, and, for unknown reasons, *phoA61* may be a good substrate for La and CRAG may be a good substrate for Clp.

The mechanism by which ClpA, or any other chaperone, refolds some protein substrates but stimulates degradation of others is unknown, but will be an important issue to resolve. If the "refold or degrade" idea mentioned earlier proves to be correct, perhaps the stimulation of degradation is based simply on the duration of binding of the chaperone to the substrate protein. If the protein can be readily refolded and the chaperone released, there may be insufficient time for the protease to encounter the protein substrate.

### Molecular Chaperones and Protein Degradation in Eukaryotes

**Proteolytic Pathways Induced by Stress.** Multiple pathways of protein degradation exist in yeast and mammalian cells. Components of the ATP- and ubiquitin-dependent proteolytic pathway (2) are induced by heat shock (16). Such components include ubiquitin, two ubiquitin carrier proteins, and certain subunits of the 26S proteasome. Additionally, in mammalian cells a lysosomal pathway of proteolysis, macroautophagy (15), is induced by heat shock (9). Finally, a selective pathway of lysosomal proteolysis requires the constitutive form of an hsp70, hsc73 (6).

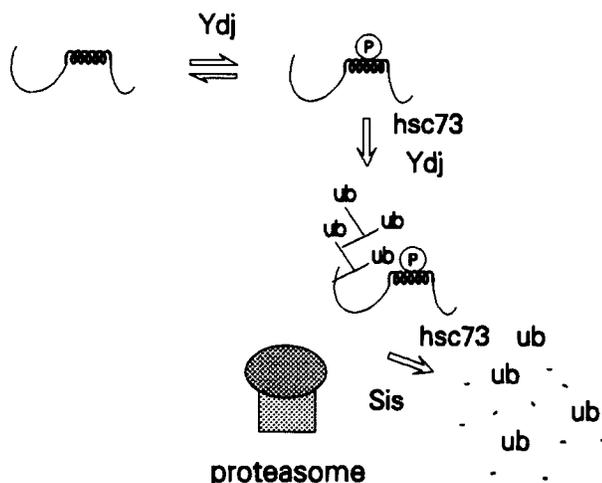
**Molecular Chaperones and Mitochondrial Proteolysis.** Mitochondria can be taken up and degraded by lysosomes, but substantial protein degradation also takes place within the organelle (10). The intramitochondrial proteases are homologous to the prokaryotic proteases, La and Clp, and also require intramitochondrial chaperones for optimal proteolysis (8, 19). The mitochondrial homolog of protease La in yeast, the Pim1 protease, is required for the degradation of the  $\beta$  subunits of ATP synthase and the general matrix peptidase within mitochondria (20). The mitochondrial hsp70 (mt-hsp70; the *ssc1* gene product) and the mitochondrial DnaJ homolog, Mdj1 (Table I), are both required for efficient degradation of two abnormal, mistargeted

proteins by Pim1. In the absence of mt-hsp70 and Mdj1 the abnormal proteins form large aggregates (21). A question that remains is whether or not molecular chaperones are also required for the degradation of normal mitochondrial proteins.

**Molecular Chaperones and the Ubiquitin-Proteasome Proteolytic Pathway.** Chaperones are required for the degradation of certain abnormal proteins by the ubiquitin-proteasome pathway of yeast and mammalian cells. Mutations in hsp70 genes (*ssa1* and *ssa2*) and temperature-sensitive mutants of the DnaJ homologs, Ydj and Sis (Table I), reduce the degradation of proteins containing amino acid analogs and most short-lived, normal proteins (Lee, D. H., M. Y. Sherman, and A. L. Goldberg, 1995. *Cold Spring Harbor Symp. Quant. Biol.* VX:111a.). Degradation of the majority of long-lived proteins is not affected by such mutations. Interestingly, Ydj appears to facilitate ubiquitination of proteins while Sis appears to promote the breakdown of ubiquitinated proteins by the proteasome (Fig. 2).

The ubiquitin-dependent degradation of a yeast cyclin, the *cln3* gene product, is stimulated by the DnaJ homolog, Ydj. In this case, the cyclin is phosphorylated before it can be efficiently ubiquitinated (24), and this phosphorylation step is stimulated by Ydj (Sherman, M., personal communication; Fig. 2). The requirement for Ydj1 appears to be for modifying the structure of the cyclin substrate making it more susceptible to phosphorylation since Ydj binds to cyclin near its phosphorylation site (Sherman, M., personal communication). Most DnaJ homologs work in conjunction with hsp70s, but a role for hsp70s in the degradation of this cyclin remains to be established.

Other studies indicate that hsc73 promotes ubiquitin conjugation to certain denatured proteins in reticulocyte extracts. This conclusion was reached by immunodepleting



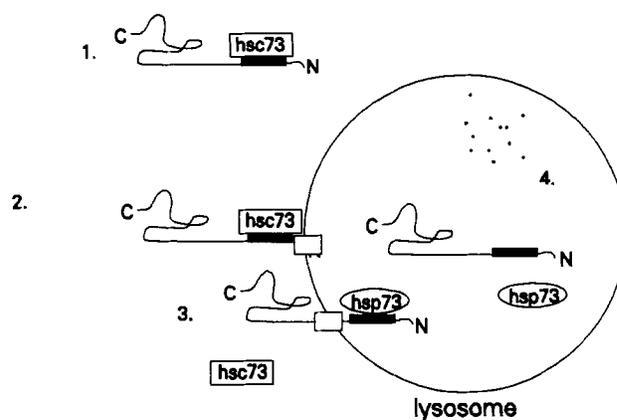
**Figure 2.** Involvement of molecular chaperones in the ubiquitin-proteasome pathway of proteolysis. Ydj is required for efficient phosphorylation of a cyclin (the *cln3* gene product) and is also required for the efficient ubiquitination of certain abnormal proteins. Hsc73 is also required for the efficient ubiquitination of some abnormal proteins. Sis is required for degradation of certain ubiquitinated proteins by the proteasome. Many of the steps shown require ATP. Ubiquitin (ub), and the multiple Ub structure represents a covalent chain of ubiquitins known to be the signal for degradation of certain short-lived proteins.

hsc73 and showing that levels of ubiquitination of certain substrate proteins were greatly diminished. In addition, hsc73 is also required for efficient degradation of certain ubiquitinated proteins in reticulocyte extracts (Ciechanover, A., personal communication; Fig. 2).

**Molecular Chaperones and Lysosomal Proteolysis.** Our own work has focused on the participation of hsc73 in the selective targeting of certain cytosolic proteins to the lysosome where they are degraded (Fig. 3). The role of hsc73 in this degradation pathway appears to be very similar to roles of hsc73 in the import of proteins for residence or transit through other organelles (6). An intralysosomal hsp73 is also required for the import of substrate proteins (6; Fig. 3). Kinetic intermediates in the import of substrate proteins have been identified, and such intermediates are characteristic of protein import through polypeptide channels (5). As for the import of proteins into other organelles, there is likely a complex of receptors and a polypeptide transporter in the lysosomal membrane that recognizes the substrate protein.

### Conclusions

Molecular chaperones are required for the degradation of abnormal and certain normal, short-lived proteins in both prokaryotes and eukaryotes. Distinct molecular chaperones appear to be required for the degradation of different proteins. In some cases the role of the molecular chaperones seems to be simply to prevent the formation of large aggregates of protein substrates which is thought to be a primary function in their roles in protein folding and translocation. However, the evidence presented here suggests that molecular chaperones can also play a more specific role in the breakdown of proteins either by being physically associated with proteases, like Clp, by facilitating a



**Figure 3.** Role of hsc73 in a selective pathway of lysosomal proteolysis. The substrate protein shown is RNase A, and the dark box represents the KFERQ sequence near the amino terminus. (Step 1) Hsc73 interacts with the KFERQ region and surrounding amino acids of RNase A. (Step 2) RNase A (perhaps with hsc73 still bound) interacts with receptors and/or polypeptide transporters on the lysosomal surface (*open rectangle*). (Step 3) Cytosolic hsc73 dissociates from the RNase A, and an hsp73 within the lysosome pulls RNase A from its amino terminus into the lysosomal lumen. (Step 4) RNase A is degraded by lysosomal proteases.

rate-limiting step in degradation, such as phosphorylation of Cln3, or by facilitating selective transport of proteins into lysosomes.

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