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88. We thank R. Moser for the figures and J. S. Weissman, K. Simons, and A. Nicola for valuable input. Support was obtained from the Swiss National Science Foundation, ETH-Zürich, Lavens Kemiske Fabriks Forskningsfond, and the Roche Foundation.

REVIEW

Posttranslational Quality Control: Folding, Refolding, and Degrading Proteins

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Polypeptides emerging from the ribosome must fold into stable three-dimensional structures and maintain that structure throughout their functional lifetimes. Maintaining quality control over protein structure and function depends on molecular chaperones and proteases, both of which can recognize hydrophobic regions exposed on unfolded polypeptides. Molecular chaperones promote proper protein folding and prevent aggregation, and energy-dependent proteases eliminate irreversibly damaged proteins. The kinetics of partitioning between chaperones and proteases determines whether a protein will be destroyed before it folds properly. When both quality control options fail, damaged proteins accumulate as aggregates, a process associated with amyloid diseases.

The appearance and maintenance of functional proteins within cells depends on more than the fidelity of transcription and translation. The initial folding of proteins and assembly of multiprotein complexes can be helped and sometimes requires the participation of mo-

lecular chaperones—proteins that catalyze protein folding. By binding exposed hydrophobic patches on proteins, chaperones prevent proteins from aggregating into insoluble, nonfunctional inclusions and help them reach their stable native state. After initial folding and assembly, proteins may suffer damage in response to various stresses or insults. For such damaged proteins, as for proteins misfolded because of mutations in the gene encoding the protein, lack of fidelity in transcription, or translational errors, a number of fates are possible: rescue by chaperones, de-

struction by energy-dependent cytoplasmic proteases, or aggregation. The efficiency and cost of protein quality control depends on the balance among these processes.

There is significant overlap in the functional and physical features of the prokaryotic and eukaryotic chaperone and proteolytic machinery. Similar principles govern the mechanisms of substrate selection and unfolding by molecular chaperones and the adenosine triphosphatase (ATPase) components of proteases. In fact, the ATPase components of proteases can function as molecular chaperones [reviewed in (1)]. We propose a unified model for partitioning of nonnative proteins between chaperones (for remodeling) and proteases (for degradation), which we refer to as protein triage (2). In discussing quality control, we do not address the numerous biologically important regulatory functions of chaperones and proteases.

When Is Quality Control Necessary?

Most native cellular proteins probably do not interact with chaperones and are resistant to

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the degradative machinery because of the intrinsic stability of their structures, stabilization by ligands and metabolic effectors, or interactions with protein and other macromolecular partners. However, about 10 to 20% of newly made polypeptides are found associated with chaperones, which implies a role for chaperones in the initial folding pathways for some polypeptides (3, 4). Also, about 20% of newly synthesized polypeptides are degraded; this highly unstable fraction may comprise incomplete proteins resulting from errors in transcription or translation (5, 6). Most abnormal and incompletely synthesized proteins are degraded by adenosine triphosphate (ATP)-dependent proteases, such as Lon in *Escherichia coli* or the proteasome in eukaryotic cells. Posttranslationally, chemical damage by oxidative mechanisms involving reactive oxygen species and structural damage caused by heat shock and other stresses results in protein inactivation and protein unfolding or misfolding. In addition, incomplete assembly or incorrect cellular distribution of proteins results in exposure of protein-protein interaction domains that are often amphipathic in nature. The quality control system has evolved to recognize similar characteristics in all these nonnative proteins—namely surface-exposed hydrophobic regions.

Mutational loss of the chaperones or proteases often leads to intracellular accumulation of protein inclusion bodies, particularly after heat stress. In addition, chaperone mutants frequently adversely affect cell growth, again especially at elevated temperatures. Because chaperones and proteases are required for expression of specific essential activities in cells as well as for general protein quality control, it is difficult to know whether growth defects are due to loss of specific functions or to a general accumulation of defective protein aggregates.

Although the activities of the quality control system are generally advantageous to the cell, on occasion the stringent monitoring system can lead to destruction of salvageable proteins. The observation that mutations in protease genes can suppress functional defects resulting from certain missense mutations indicates that abnormal but functional forms of proteins are sometimes targeted by quality control proteases (7). Such phenomena may affect the severity of certain diseases. For example, in a common form of familial cystic fibrosis, misfolding of the transmembrane conductance regulator CFTR causes the misfolded protein to be targeted by the degradative apparatus before it can be translocated to the plasma membrane. Growth conditions that favor proper folding of the mutant CFTR allow increased recovery of function (8).

Similarities of Chaperones and Protease ATPase Components

There are several major families of ATP-dependent chaperones that interact with large numbers of nonnative proteins and assist in protein folding and remodeling, including the Hsp60 or GroEL family (chaperonins), the Hsp70 or DnaK family, and the Clp or Hsp100 family. These chaperones are highly conserved and are present in all cells.

Chaperonins are barrel-shaped protein complexes that consist of two stacked rings of seven to nine subunits each, with large internal chambers that serve as the sites for recognition and sequestration of unfolded proteins. In prokaryotic GroEL, the entrance to the chamber is controlled by reversible association of a small capping protein, GroES. During cycles of ATP binding and hydrolysis, changes in the conformation of the GroEL chamber result in binding and release of folded or partially folded substrate (9). Distantly related homologs in the eukaryotic cytosol also form barrel-like structures and may function similarly. In contrast, Hsp70 (or DnaK) chaperones function as monomers but work in conjunction with a cochaperone Hsp40 (or DnaJ) and are modulated in some cases by a nucleotide exchange factor, GrpE. In response to ATP binding and hydrolysis, Hsp70 (or DnaK) binds and releases small hydrophobic regions of misfolded proteins, allowing the damaged protein another chance to refold (10).

Clp chaperones are structurally similar to GroEL in having an interior chamber formed by one or two stacked rings of six or seven protomers (11). The *Saccharomyces cerevisiae* Clp protein Hsp104 and the closely related ClpB of *E. coli* apparently act exclusively as molecular chaperones, whereas other Clp family members—namely ClpA and ClpX of *E. coli*—function both as chaperones and as components of ATP-dependent proteases [reviewed in (1, 12)]. ClpA catalyzes ATP-dependent protein unfolding (13), which suggests that the function of Clp chaperones is to bind and unfold misfolded proteins that can then be released or transferred to a proteolytic component. Thus, for GroEL, Hsp70, and Clp chaperones, cycles of ATP binding and hydrolysis allow cycles of binding of nonnative proteins and release of folded, partially folded, or unfolded forms. Released folding intermediates may spontaneously fold into a stable conformation, rebound to the same or another chaperone, or be degraded by proteases.

In addition to classic ATP-dependent chaperones, other protein modeling enzymes involved in helping proteins reach their native state are prolyl cis-trans isomerases and protein disulfide isomerases. One such prolyl cis-trans isomerase, a ribosome-associated protein called trigger factor, appears to act

redundantly with DnaK to recognize and presumably aid the folding of nascent and newly synthesized protein (3, 14). Binding of chaperones to ribosome-associated polypeptides or newly synthesized proteins may be particularly important in cases in which folding is delayed—for example, for secreted proteins, or when assembly into larger protein complexes is required.

The alternative fate for misfolded proteins is destruction by the cytosolic ATP-dependent proteases. The relationship between the structural organization and mechanism of action of prokaryotic ATP-dependent proteases is best illustrated by the Clp proteases ClpAP and ClpXP (Fig. 1). The protease component ClpP consists of two stacked heptameric rings of identical subunits that enclose an internal chamber with 14 proteolytic sites (15). Regulatory ATPase component ClpA or ClpX flanks both ends of the proteolytic component, thereby providing gateways to the proteolytic sites (11, 16). ClpA binds and unfolds substrates, facilitating translocation of a significant fraction of unfolded substrate to the associated ClpP in an ATP-dependent reaction (13, 17–19) (Fig. 1). Once the polypeptide enters the proteolytic chamber, it is rapidly degraded to short peptides without further utilization of ATP (20). At least five separate energy-dependent proteases have been identified in *E. coli*; the same proteases are found in chloroplasts and mitochondria of eukaryotic cells [reviewed in (21)]. In addition to ClpAP and ClpXP, a third multiple-component protease, HslUV (ClpYQ), consists of a Clp ATPase associated with a proteolytic component related to the proteasome. The other prokaryotic proteases or their eukaryotic organellar homologs, Lon and FtsH, are homo-oligomers of single polypeptide chains that fold into separate domains, one with ATPase and chaperone activities and the other with proteolytic activity (22, 23). The mechanism of action of these proteases conforms to the general pathway defined for ClpAP (2, 24).

In the eukaryotic cytosol, the protease responsible for most protein degradation is the 26S proteasome, a complex with a domain organization similar to that of ClpAP but containing additional regulatory and modulating components (Fig. 1). The 20S proteasome has the proteolytic active sites situated in a central chamber formed by two rings of catalytic subunits flanked by two rings of noncatalytic subunits of unknown function (25, 26). On either end of the 20S proteasome is a ring of six ATPases that, together with two other proteins, forms the base of the 19S regulatory cap. Proteasomal ATPases and Clp ATPases have two domains that are predicted to have folds similar to

those found in AAA proteins, an extended family of ATPases that mediate assembly or disassembly of macromolecular complexes (27, 28). The apical surface of the 26S proteasome has a final layer of proteins, termed the lid, that is necessary for substrate recognition. The lid includes proteins that recognize ubiquitin, several proteins with homology to signal transduction complexes, and at least one ubiquitin isopeptidase (29). The ATPases of the proteasome share with the Clp ATPases and the molecular chaperones the ability to preferentially bind inactive proteins and to promote reactivation (30). Very likely the mechanism of action of the 26S proteasome is similar to that of ClpAP and ClpXP (Fig. 1).

Substrate Recognition by Chaperones and Proteases

Both chaperones and proteases need to recognize regions that are commonly found on misfolded or unfolded proteins but not on native proteins. The recognition of substrates is general enough that a significant fraction of newly synthesized proteins interact with chaperones. Between 10 and 15% of newly translated polypeptides are found associated with GroEL and this fraction increases to about 30% under heat stress conditions (4). A similar proportion (15 to 20%) of newly synthesized polypeptides associate with DnaK; of these, about 20% are nascent polypeptides (3, 14).

Hsp70 (or DnaK) binds short peptides composed of clusters of hydrophobic residues flanked by basic residues and a distinct absence of acidic residues (10). Such motifs occur with a frequency of about 1 in 36 amino acids in most proteins (31). Thus, they are likely to be found exposed on nascent polypeptides during translation and to become available after a variety of structural perturbations. GroEL binds to short

amphipathic peptides that can adopt α -helical conformations when bound (9, 10). Although extrapolation of peptide binding properties to interactions with intact proteins is problematic, there is a general correlation between surface hydrophobicity and binding to chaperones. Mutational studies indicate that GroEL residues important for substrate binding tend to be hydrophobic, which further supports direct involvement of hydrophobic interactions in substrate binding (9).

Evidence is accumulating that the ATPase components of Clp and Lon proteases also interact with polypeptide regions with a hydrophobic patch adjacent to basic residues (32–35). Location of such regions near the NH₂-terminus and the COOH-terminus of substrates favors interaction with the proteases and may be necessary for their degradation.

Some classes of damaged proteins that should not be subject to chaperone rescue are specifically and efficiently identified by degradation systems and not by chaperones. The presence of certain amino acids (bulky hydrophobic or basic residues) at the NH₂-terminus of proteins results in targeting those proteins to the degradative apparatus, whereas proteins with methionine and small or hydrophilic residues at the NH₂-terminus are not targeted (called the N-end rule) (36). Errors in processing or other events that lead to destabilizing NH₂-terminal amino acids are not subject to attempts to refold but are degraded by the ClpAP protease in bacteria and, after ubiquitin tagging, by the 26S proteasome in eukaryotes (36). In bacteria, interference with completion of translation can lead to rapid degradation of the truncated protein by ClpAP or ClpXP (37, 38). In this case, cotranslational addition of 11 amino acids to the COOH-terminus of nascent polypeptides occurs when ribosomes are stalled in a

position without a termination codon—for example, at the end of a truncated mRNA or within stretches of rare codons (39, 40).

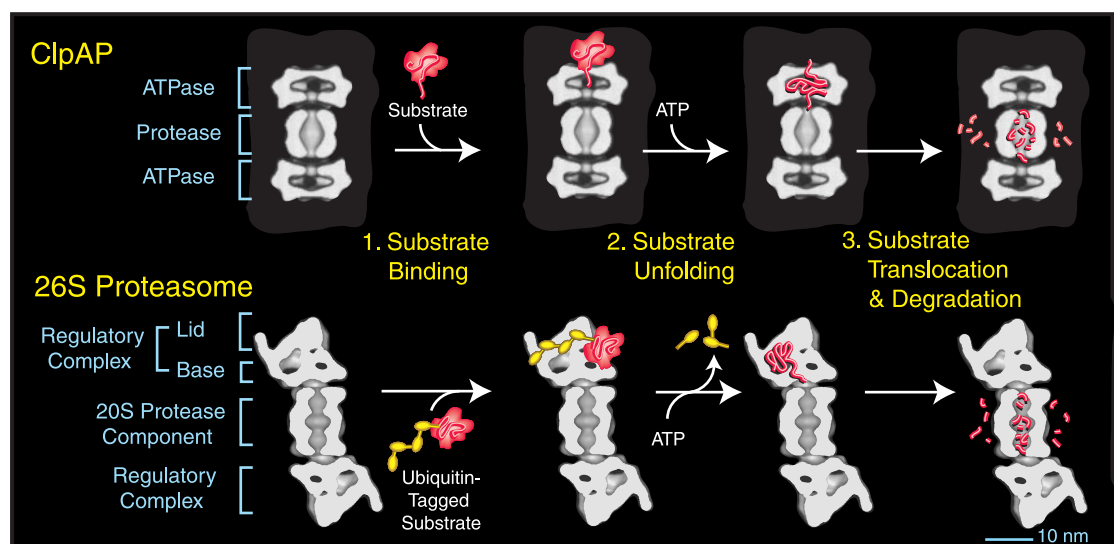
Substrate Recognition in Eukaryotes

Although the ATPase components of the prokaryotic Clp and Lon proteases interact directly with their protein targets, direct interaction between the ATPases of the proteasome and protein substrates has not been well characterized. To be recognized and degraded by the 26S proteasome, most proteins are modified by attachment of a polyubiquitin chain. The ubiquitin conjugating enzymes (E2s) and ubiquitin ligases (E3s) are jointly responsible for substrate recognition and ubiquitination (41, 42); thus these enzymes are central to the selection of proteins for degradation. For example, in yeast, degradation of proteins containing destabilizing NH₂-terminal amino acids requires the E3 protein Ubr1 and the E2 protein Ubc2 (43). Mutations in other specific E2 proteins lead to defects in degradation of proteins with different classes of artificial hydrophobic degradation signals, which suggests that the ubiquitination system also can target exposed hydrophobic regions in proteins (43). More than one E2 protein can cooperate in the degradation of certain proteins (44).

Protein Triage Model for Quality Control

The dictionary defines triage as “sorting and allocation of treatment to patients.” The patients in this case are cellular proteins. The first level of triage must be identification of the proteins that are damaged and require treatment. The quality control system must be able to distinguish between native (properly folded, assembled, and modified) proteins and everything else that might be considered nonnative, including partially unfolded, misfolded, incorrectly modified, unassembled subunits of complexes, or

Fig. 1. ATP-dependent proteolysis. Schematic model of steps in protein degradation, based on our current understanding of the mechanism of action of these proteases. Proteases are shown as cross sections of reconstructed electron microscopic images of *E. coli* ClpAP (60) and the eukaryotic 26S proteasome (61). Ubiquitin is shown in yellow; substrates are red. Details about organization and function of these proteases are found in the text and in reviews (24, 42).



proteins misdirected to cellular compartments. The system must be able to efficiently recognize such nonnative forms of any cellular protein.

Once damaged proteins have been identified, a second level of decision must be made: Can the patient be saved? Chaperones or chaperone components of proteases should have the first opportunity to correct misfolded proteins. Hopeless cases in which structural damage cannot be repaired need to be degraded by the cytoplasmic ATP-dependent proteases. Aggregation is the likely default outcome when both the chaperone and protease systems fail.

How is the decision made to refold or to degrade a protein? The ability of both chaperones and proteases to interact with damaged or misfolded proteins in similar ways allows the pathways for either repair or degradation of a given target to operate in parallel. We propose that, in the prokaryotic systems, these pathways function stochastically and that the fate of the protein depends on the kinetics of interaction (binding and release) of the protein with molecular chaperones or the chaperone components of the proteases.

In the general pathway for a triage system for damaged proteins (2) (Fig. 2), proteins with exposed hydrophobic surfaces or other binding motifs interact with a chaperone or with a regulatory component of a protease. If the nonnative protein is converted to its native conformation by a chaperone, it is removed from the triage system, because the hydrophobic recognition sites are buried. If it is released by a chaperone before it reaches its native form, it remains in the pool of nonnative proteins, perhaps able to rebind to a chaperone for another attempt at remodeling. If the nonnative protein encounters a protease before it reaches its native state, its likely fate is complete degradation, although a fraction of protein unfolded by the chaperone components of proteolytic complexes also may be released without degradation (19).

If correct folding is not possible and degradation is not initiated rapidly, the protein may interact with other unfolded or partially folded proteins, leading to formation of aggregates. Thus, the relative affinity of a nonnative protein for a chaperone versus a protease, coupled with the protein's propensity to fold to a state that does not bind the protease, will determine the probability of remodeling versus degradation. This model does not specify or depend on the source of the protein damage; proteins that are unable to fold because they are mutant or those that have been damaged as a result of high temperature, for instance, will appear the same to the system. However, a mutant protein may never fold correctly and therefore in time is more likely to be degraded or to aggregate, because it cannot leave the pool of nonnative polypeptides. The model also suggests that classic chaperones promote degradation of proteins recalcitrant to refolding by improving their

solubility and minimizing their aggregation, making them more accessible to proteases, instead of by directly presenting substrates to the proteases (2).

Although the triage model was originally developed based on data from prokaryotes (2), a similar model may apply to the more complex eukaryotic systems as well. In this case, the critical partitioning step between refolding and degradation will be a competition between chaperones and the components of the ubiquitination system that mediate substrate recognition by the eukaryotic degradation system (Fig. 3). Ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) recognize and ubiquitinate specific substrates, including a variety of abnormal proteins. We propose that competition between some E3 and E2 proteins and chaperones for binding to nonnative proteins will provide the basis for protein triage decisions in the eukaryotic cytosol.

Setting the Proper Level of Surveillance

The quality control system, consisting of broad specificity chaperones and proteases, is a robust apparatus meant to repair or remove most forms of damaged proteins. The effectiveness of the system depends in large part on the ability of functional proteins to avoid capture because their hydrophobic regions are generally buried. It is important that the cell keep chaperones and proteases at, but not above, the level needed; at higher levels, they

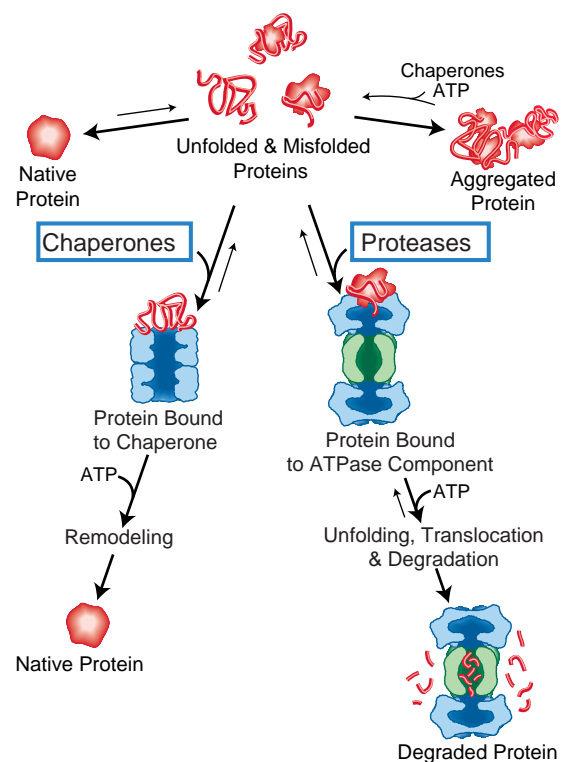
may interact with properly folded proteins as well as misfolded ones. Chaperones such as DnaK and GroEL are among the most abundant proteins in cells, reflecting their role in folding newly synthesized proteins as well as the high demand for maintenance of properly folded proteins. ATP-dependent proteases are somewhat less abundant. Synthesis of both chaperones and proteases are induced as part of the heat shock response, and the inducing signal is likely to be, directly or indirectly, the accumulation of unfolded proteins that bind the chaperones [reviewed in (45, 46)]. Because the amounts of chaperones and proteases increase in parallel, the competitive balance between them, the hallmark of the protein triage model, is not disturbed.

When the balance is perturbed by either mutation or overproduction, cell growth can be affected. Mutations in chaperones or proteases lead to accumulation of insoluble protein aggregates (inclusion bodies) and increase sensitivity to treatments such as heat shock or exposure to amino acid analogs. Thus, both quality control systems are required for normal protein homeostasis.

Aggregation: Failure of Quality Control

In our model, quality control mechanisms are directed at preventing protein aggregation, which ordinarily is not a biologically useful endpoint for the cell. Protein aggregates remove an otherwise recyclable pool of amino

Fig. 2. Protein triage model for quality control. As hydrophobic regions of polypeptides are exposed, either as the newly made proteins emerge from the ribosome or because of subsequent misfolding or failure to assemble properly, they are subject to binding by any of a variety of chaperones or by the ATP-dependent proteases. Chaperone binding and release of folding intermediates may allow proteins to reach their native conformation or may return them to the pool of nonnative proteins that can rebind chaperones or proteases. Protease binding followed by ATP-dependent unfolding and subsequent degradation removes the protein from the pool of nonnative proteins. In eukaryotes, the protease pathway includes initial ubiquitination by components of the ubiquitin tagging system, allowing recognition by the protease (see text and Fig. 3). Some misfolded or partially folded proteins will eventually aggregate. Although the chaperones act most generally to prevent aggregation, they are also able to dissolve aggregates (50–52). Because proteins in aggregates are relatively resistant to proteolysis, chaperones promote proteolysis indirectly by maintaining misfolded proteins in an unaggregated state. Steps in boxes represent major processes at which competition takes place for protein triage.



acids, may form structures that disrupt other cellular functions, can serve as nuclei for aggregation of other unrelated proteins, and can tie up cellular chaperones and proteases. In some cases, such as the ordered aggregates found in prion and other amyloid diseases, there are severe consequences for the organism as a whole [see below and reviewed in (47, 48)]. Various models of aging predict the accumulation over time of aberrant chemical- or structurally damaged proteins (49).

Both in vivo and in vitro, chaperones protect proteins from damage and aggregation by binding to them before irreversible damage is done. However, in some cases, chaperones have been shown to reverse aggregation (Fig. 2, reverse arrow). In *S. cerevisiae*, protein aggregates formed after a heat shock are resolved over time in a reaction dependent on the ClpB chaperone Hsp104, which is known to be important for thermotolerance (12). In vitro, resolution of protein aggregates by Hsp104 requires Hsp70 and Hsp40 (DnaK and DnaJ equivalents) as well (50). Similarly, ClpB from *Thermus thermophilus* and *E. coli* can reactivate heat-inactivated proteins with the participation of the DnaK chaperone system (51, 52).

Although aggregation generally has been thought of as an unregulated default pathway, controlled protein aggregation and membrane inclusion of the aggregates may occur in cells with abnormal proteins in excess of what the

proteolytic system can handle. Inhibition of the proteasome in human cells transfected with a gene for CFTR leads to accumulation of CFTR in a pericentriolar structure called the aggresome, surrounded by the intermediate filament protein vimentin (53). CFTR in the inclusions is highly ubiquitinated, attesting to the cytosolic origin of the aggresomes. Whether aggresomes are formed in response to independently formed protein inclusions or are part of an ordered pathway, which might include regulated promotion of inclusion body formation, remains to be determined.

Pathological Aggregates: Prions and Amyloid Fibrils

Protein aggregation associated with prion and amyloid diseases [reviewed in (47, 48, 54)] can be considered biologically relevant failures of posttranslational quality control. Mutant forms of some proteins and, under conditions not yet understood, even some wild-type proteins, can form ordered aggregates called amyloid fibrils, protease-resistant structures characterized by a high content of β sheets. To date 15 or 20 proteins have been found to form amyloids, which are associated with Creutzfeldt-Jakob, Alzheimer's, Huntington's, and Parkinson's diseases as well as systemic amyloidoses.

How do prionogenic and amyloidogenic proteins escape the proteases and chaperones of the quality control systems? One possibility is

that these alternatively folded precursor proteins have surface structures that are not recognized by the chaperones and proteases. A second possibility is that amyloidogenic forms of the proteins aggregate more quickly than they can be degraded or remodeled. The latter is consistent with current models for conversion of the cellular prion protein (PrP^c) to the scrapie form (PrP^{sc}), which propose that a highly specific physical interaction (either a nucleation-dependent process or a template-mediated conformational transition) occurs between the two protein forms. This interaction results in essentially simultaneous structural conversion and aggregation (47). Finally, because the most abundant chaperones and proteases in cells do not appear to act on aggregates, fibril formation will not be easily reversed once initiated.

Not only does the quality control system allow formation of the amyloid, but evidence suggests that some chaperones may have a role in the generation of amyloid states. In *S. cerevisiae*, Hsp104 is required for maintenance of a prion-like state, [PSI⁺], of the translation termination factor Sup35 (55). [PSI⁺] appears to propagate like a prion, in which the amyloid form of Sup35 promotes the conversion of soluble Sup35 to a form that is incorporated into insoluble fibrils. Conversion of Sup35 to [PSI⁺] leads to its inactivation, resulting in inefficient translation termination and therefore suppression of nonsense mutations. Hsp104 may function by helping to form or stabilize prionogenic folding intermediates of Sup35. However, high-level expression of Hsp104 or yeast Hsp70 cures cells of [PSI⁺], presumably by dissolving aggregated Sup35 (55, 56).

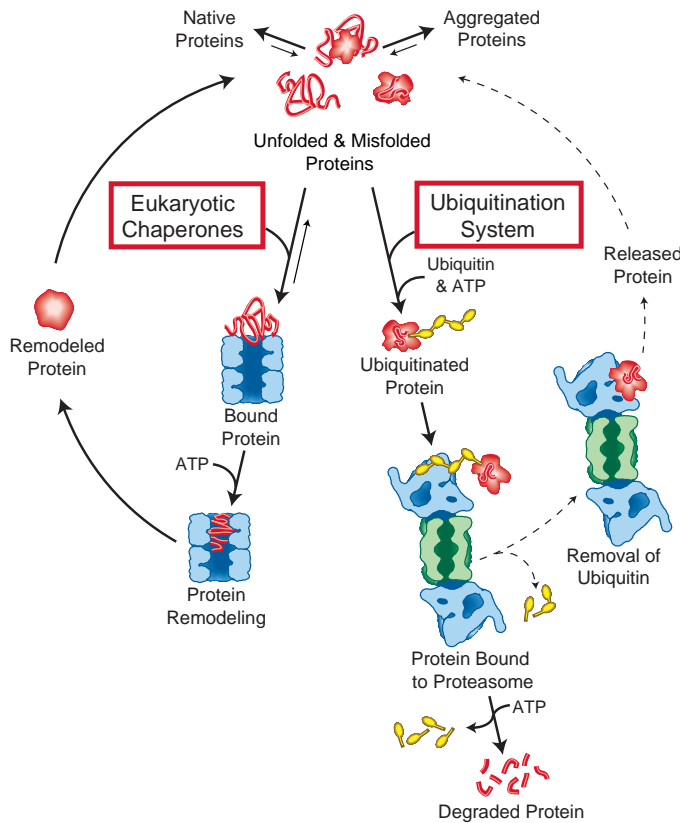
Although it is not known whether chaperones are involved in mammalian prion diseases, GroEL and Hsp104 promote conversion of PrP^c to a protease-resistant form in vitro, demonstrating that, in principle, chaperones have the ability to regulate conformational transitions in PrP (57). Association of chaperone and proteasome components with amyloid deposits have been observed in the nonprion dominant neurological disease spinocerebellar ataxia type I and in a model system for spinal bulbar muscular atrophy. In both cases, amyloid inclusions can be reduced by increasing the intracellular concentration of the Hsp40 chaperones, reinforcing the idea that the chaperones have failed to keep pace with misfolded proteins, leading to aggregation (58, 59).

These and other data suggest that failure of proper functioning of the quality control system to repair or remove misfolded proteins can lead to or allow progression of those diseases associated with protein inclusions.

Conclusions

Cells expend a substantial amount of energy in the form of ATP to enable correct protein folding or, failing that, to rid cells of misfolded forms. The ability of chaperones, domains of

Fig. 3. Substrate recognition and capture in the eukaryotic cytosol. Recognition of abnormal and misfolded proteins in eukaryotes is carried out by the ubiquitin conjugating and ligating enzymes allowing delivery of the substrates to the 26S proteasome for degradation (see Fig. 1). Ubiquitin ligases may compete with chaperones for binding to unfolded proteins. Because ubiquitin chains may need to be a minimum size to promote degradation, proteins targeted to the proteasome with short ubiquitin chains may undergo further ubiquitination before degradation. A further level of quality control is offered by the deubiquitinating enzymes. A protein that has lost its original defect through repair or refolding can be deubiquitinated and returned to the pool of free cytosolic proteins (62).



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proteases, and, in eukaryotes, the ubiquitin conjugating system, to distinguish nonnative from native proteins allows a kinetic partitioning of misfolded proteins between these systems, leading to preferential degradation of those proteins that cannot readily fold into native conformations. Degradation of properly folded proteins is avoided because the motifs recognized by the regulatory components of the degradative machinery have characteristics of regions normally buried within folded proteins and because the proteolytic sites themselves are sequestered within internal chambers that are not directly accessible to proteins in the surrounding medium.

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- We thank C.-C. Li for comments on the manuscript.

REVIEW

Quality Control Mechanisms During Translation

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Translation uses the genetic information in messenger RNA (mRNA) to synthesize proteins. Transfer RNAs (tRNAs) are charged with an amino acid and brought to the ribosome, where they are paired with the corresponding trinucleotide codon in mRNA. The amino acid is attached to the nascent polypeptide and the ribosome moves on to the next codon. The cycle is then repeated to produce a full-length protein. Proofreading and editing processes are used throughout protein synthesis to ensure the faithful translation of genetic information. The maturation of tRNAs and mRNAs is monitored, as is the identity of amino acids attached to tRNAs. Accuracy is further enhanced during the selection of aminoacyl-tRNAs on the ribosome and their base pairing with mRNA. Recent studies have begun to reveal the molecular mechanisms underpinning quality control and go some way to explaining the phenomenal accuracy of translation first observed over three decades ago.

Translation is the process by which the genetic information contained in mRNA is used to determine the sequential order of amino acids in a protein (Fig. 1). Translation is a key facet of the Central Dogma of molecular biology (1) and must be relatively error free in order to allow the accurate flow of genetic

information. Experimental measurements have suggested that, overall, an amino acid is misincorporated at about 1 in every 10,000 codons under normal growth conditions (2). This high level of accuracy is seemingly at odds with the limited ability of enzymes to distinguish structurally similar molecules

such as, for example, the amino acids valine and isoleucine, both of which are substrates for translation (3). This particular problem is solved by the enzyme isoleucyl-tRNA synthetase, which is able to almost completely prevent the misincorporation of valine at isoleucine codons during translation (4). While this represents the first identified, and perhaps best understood, example of quality control during translation, numerous other

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