

Chapter 31

Completing the Protein Life Cycle: Folding, Processing and Degradation

Biochemistry

by

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Essential Question

- How are newly synthesized polypeptide chains transformed into mature, active proteins? How are undesired proteins removed from cells?

Outline

- How Do Newly Synthesized Proteins Fold?
- How Are Proteins Processed Following Translation?
- How Do Proteins Find Their Proper Place in the Cell?
- How Does Protein Degradation Regulate Cellular Levels of Specific Proteins?

31.1 – How Do Newly Synthesized Proteins Fold?

- Chaperones help some proteins fold
- Hsp70 chaperones bind to hydrophobic regions of extended polypeptides
- *E. coli* GroES-GroEL is an Hsp60 chaperonin
- Eukaryotic Hsp90 is a signal transduction protein chaperone

Figure 31.1

Protein folding pathways. **(a)**

Chaperone-independent folding.

The protein folds as it is synthesized on the ribosome (*green*) (or shortly thereafter).

(b) Hsp70-assisted protein folding. Hsp70 (*gray*) binds to nascent polypeptide chains as they are synthesized and assists their folding.

(c) Folding assisted by Hsp70 and chaperonin complexes. The chaperonin complex in *E.coli* is GroES-GroEL. The chaperonin complex in eukaryotic cells is known as TRiC (for TCP-1 ring complex) or CCT (cytosolic chaperonin-containing TCP-1). The majority of proteins fold by pathways (a) or (b).

(Adapted from Figure 2 in Netzer, W.J., and Hartl, F.U., 1998. *Protein folding in the cytosol: Chaperonin-dependent and independent mechanisms*. Trends in Biochemical Sciences **23**: 68-73; and Figure 2 in Hartl, F.U., and Hayer-Hartl, M., 2002 *Molecular chaperones in the cytosol: From nascent chain to folded proteins*, Science **295**:1852-1858.)

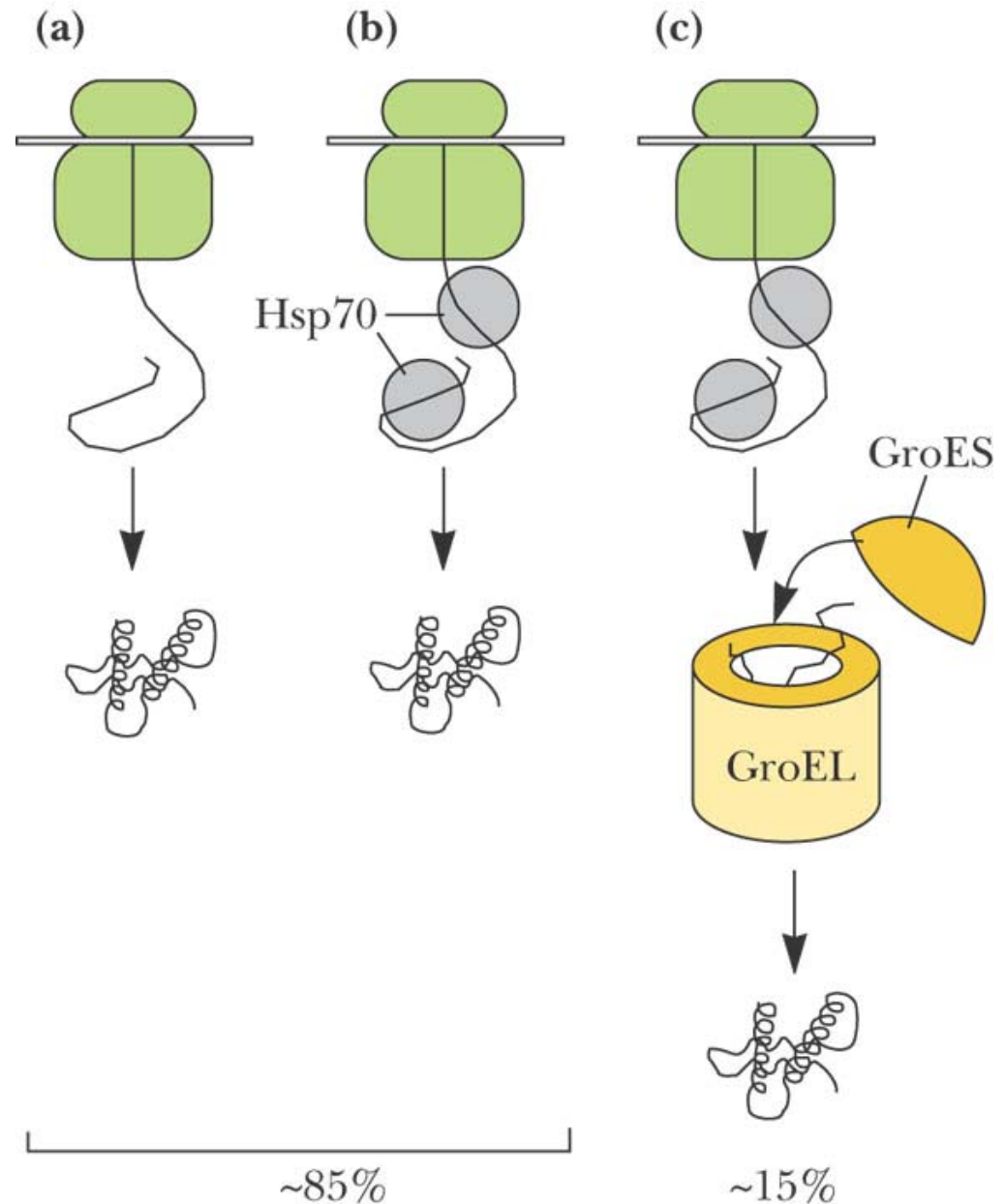
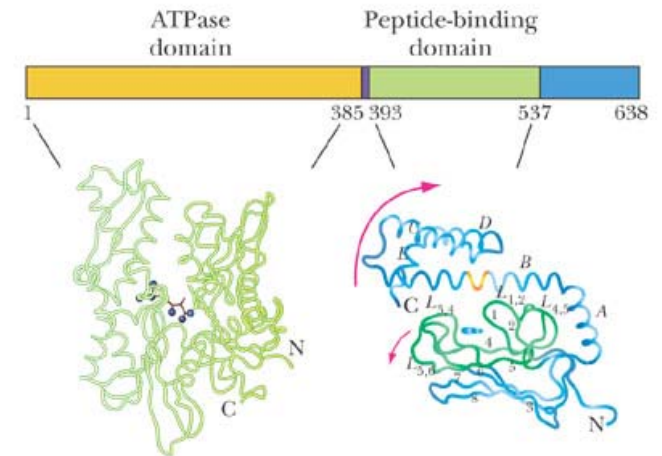
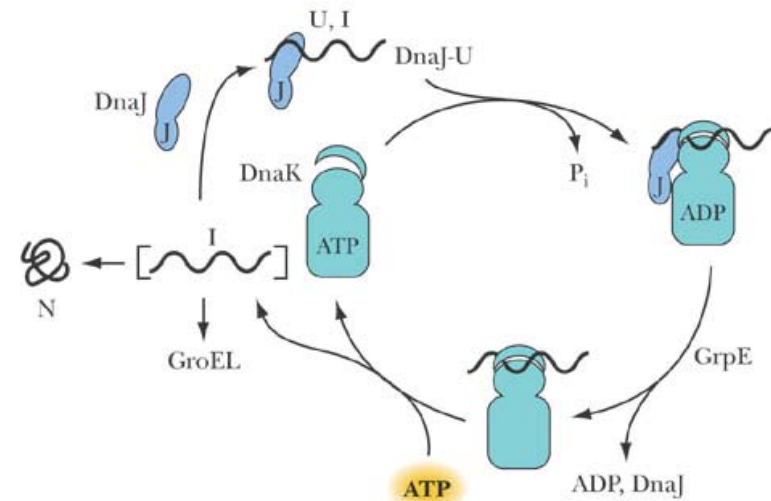


Figure 31.2 Structure and function of DnaK: (a) Domain organization and structure of the Hsp70 family member, DnaK. The N-terminal domain (residues 1-385) of DnaK is the ATP-binding domain; the polypeptide-binding domain encompasses residues 393 to 537 of the 638-residue protein. The ribbon diagram on the lower left is the ATP-binding domain of the DnaK analog, bovine Hsc70; bound ADP is shown as a stick diagram (*purple*). The ribbon diagram on the lower right is the polypeptide-binding domain of DnaK. The small blue ovals highlight the position of the polypeptide substrate; the protein regions that bind the polypeptide substrate are blue-green. **(b)** DnaK mechanism of action: DnaJ binds an unfolded protein (U) or partially folded intermediate (I) and delivers it to the DnaK:ATP complex. DnaJ:DnaK interaction stimulates the ATPase activity of DnaK, converting bound ATP to ADP, which stabilizes the DnaK:unfolded polypeptide association. The nucleotide exchange protein GrpE replaces ADP with ATP on DnaK and the partially folded intermediate ([I]) is released. I has several possible fates: It may fold into the native state, N; it may undergo another cycle of interaction with DnaJ and DnaK; or it may become a substrate for folding by the GroEL chaperonin system. (Adapted from Figures 1a and 2a in Frydman, J., 2001. *Folding of newly translated proteins in vivo: The role of molecular chaperones*. Annual Review of Biochemistry 70:603-647.)

(a) Domain organization and structure of the Hsp70 family member, DnaK



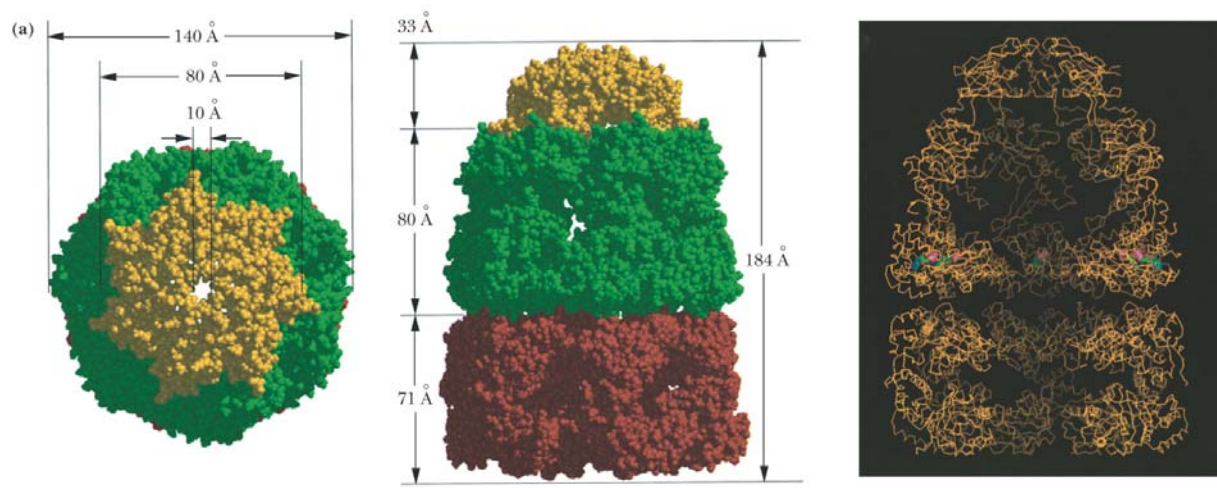
(b) DnaK mechanism of action



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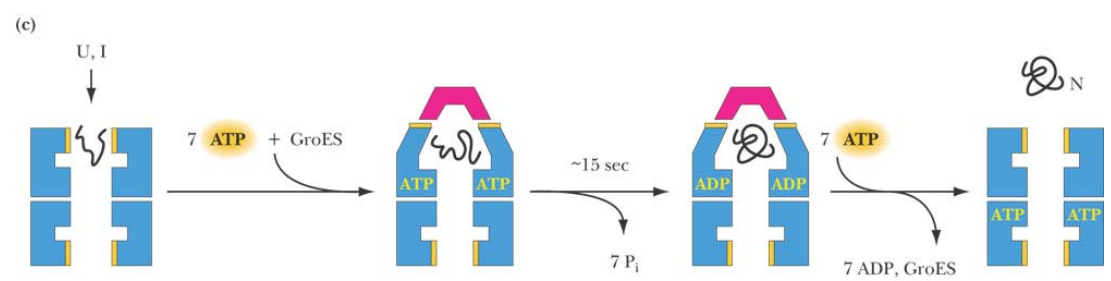
Figure 31.3 Structure and Function of the GroEL-GroES complex. **(a)** Space-filling representation and overall dimensions of GroEL-GroES (top view, left; side view, right). GroES is *gold*; the top, or apical, GroEL ring is *green*, and the bottom GroEL ring is *red*. **(b)** Section through the center of the complex to reveal the central cavity. The GroEL-GroES structure is shown as a C α Carbon trace. ADP molecules bound to GroEL are shown as space-filling models. **(c)** Model of the GroEL cylinder (*blue*) in action. An unfolded (U) or partially folded (I) polypeptide binds to hydrophobic patches on the apical ring of α_7 -subunits, followed by ATP binding and GroES (*red*) association. ATP binding triggers a conformational change that buries the α_7 -subunit hydrophobic patches (*yellow*), releasing the polypeptide into the central activity (“Anfinsen cage”). After about 15 seconds, ATP hydrolysis takes place, followed by binding of ATP to the lower α_7 -subunit ring, which causes dissociation of GroEL:ADP:GroES.

Loss of the GroES cap allows the folded protein to escape from GroEL into the cytosol. If it has not folded completely, successive cycles of protein binding, GroES recruitment and ATP-dependent release of polypeptide into the central cavity, ATP hydrolysis, and complex dissociation will take place until the protein achieves its fully folded form. (Figure parts [a] and [b] adapted from Figure 1 in Xu, Z., Horwich, A.L., and Sigler, P.B., 1997. The crystal structure of the asymmetric GroEL-GroES-(ADP)₇ chaperonin complex. Nature **388**:741-750. Molecular graphics courtesy of Paul B. Sigler, Yale University.)



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31.2 – How Are Proteins Processed Following Translation?

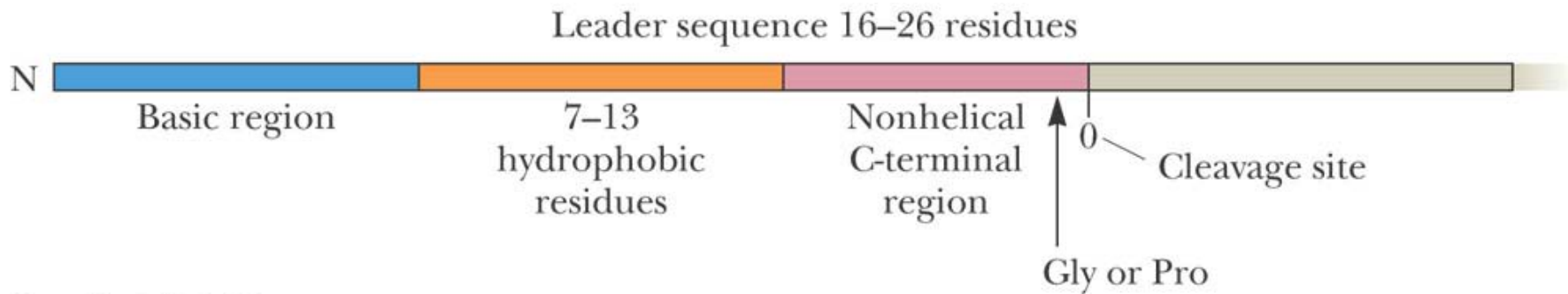
- Hundreds of different post-translational amino acid modifications are known - glycosylation & phosphorylation are 2 prominent examples
- Proteolytic cleavage is the most common form of post-translational processing

31.3 – *How Do Proteins Find Their Proper Place in the Cell?*

- Proteins are delivered to their proper cell compartment by translocation
- Prokaryotic proteins destined for translocation are synthesized as preproteins with amino-terminal leader sequences
- Eukaryotic proteins are routed through protein sorting and translocation
- Synthesis of secretory & membrane proteins is coupled to translocation across the ER membrane
- Signal recognition particles (SRPs), signal receptors (SRs), and translocons participate in secretory protein translocation
- Mitochondria have distinct translocons for their outer & inner membranes

Figure 31.4

General features of the N-terminal signal sequences on *E. coli* proteins destined for translocation: a basic N-terminal region, a central apolar domain, and a nonhelical C-terminal region.



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Figure 31.5

Synthesis of a eukaryotic secretory protein and its translocation into the endoplasmic reticulum. **(1)** The signal recognition particle (SRP, *red*) recognizes the signal sequence emerging from a translating ribosome (ribosome nascent complex [RNC], *green*). **(2)** The RNS-SRP interacts with the signal receptor (SR, *purple*) and is transferred to the translocon (*pink*). **(3)** Release of the SRP and alignment of the peptide exit tunnel of the RNC with the protein-conducting channel of the translocon stimulates the ribosome to resume translation. **(4)** The membrane-associated

signal peptidase (*black*) clips off the N-terminal signal sequence, and BiP (the ER lumen Hsp70 chaperone, *blue*) binds the nascent chain mediating its folding into its native conformation. **(5)** Following dissociation of the ribosome, BiP plugs the translocon channel. Not shown are subsequent secretory protein maturation events, such as glycosylation. (Adapted from Figures 1a and 2a in Frydman, J., 2001. *Folding of newly translated proteins in vivo: The role of molecular chaperones*. Annual Review of Biochemistry **70**:603-647.)

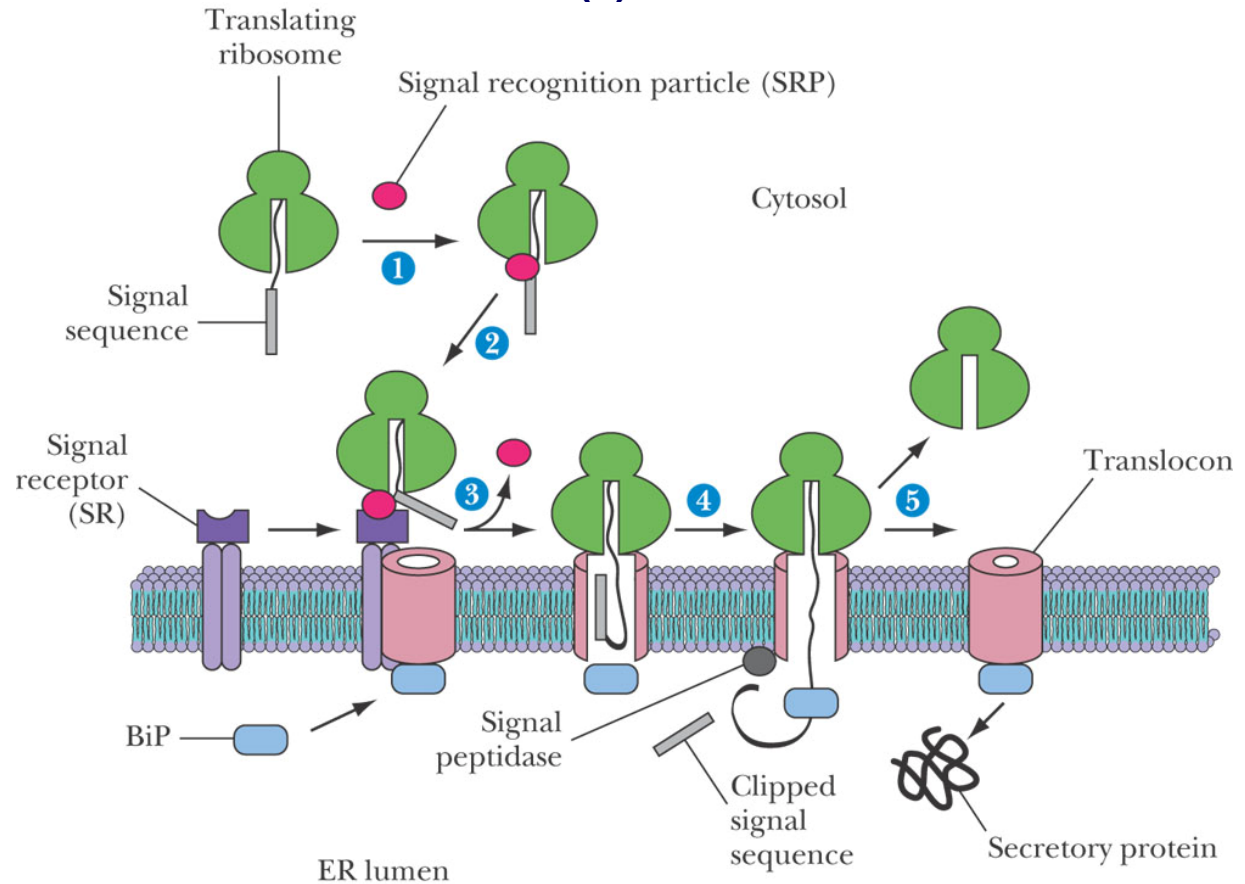


Figure 31.6
Structure of an
amphipathic α -helix
having basic (+)
residues on one side
and uncharged and
hydrophobic (R)
residues on the
other.

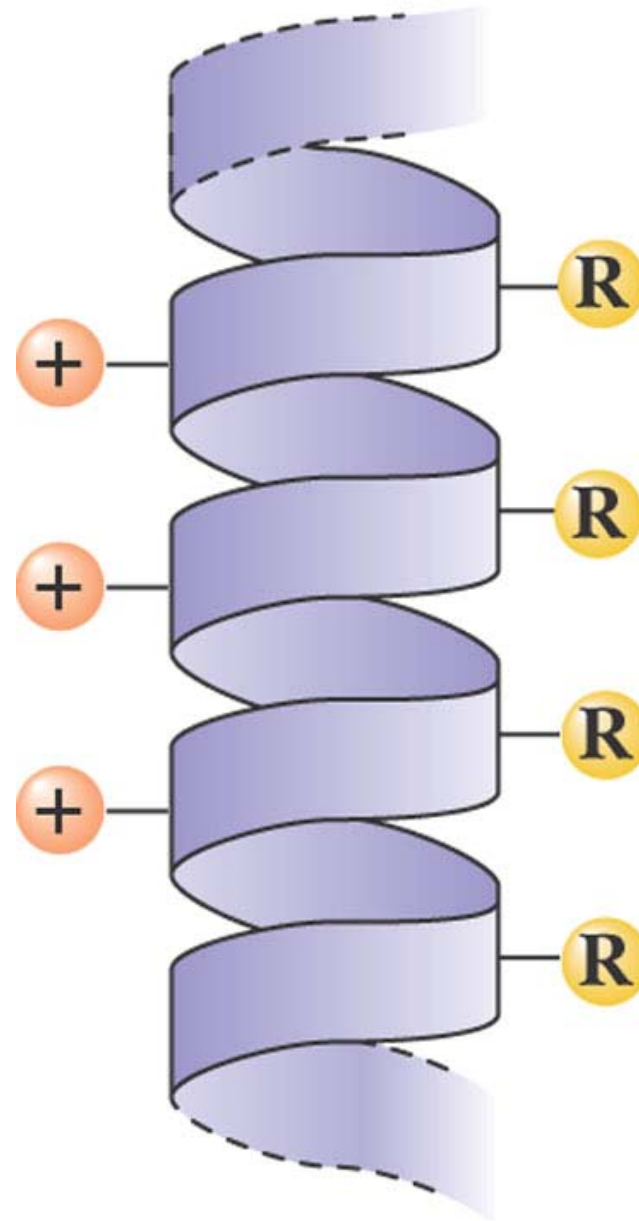
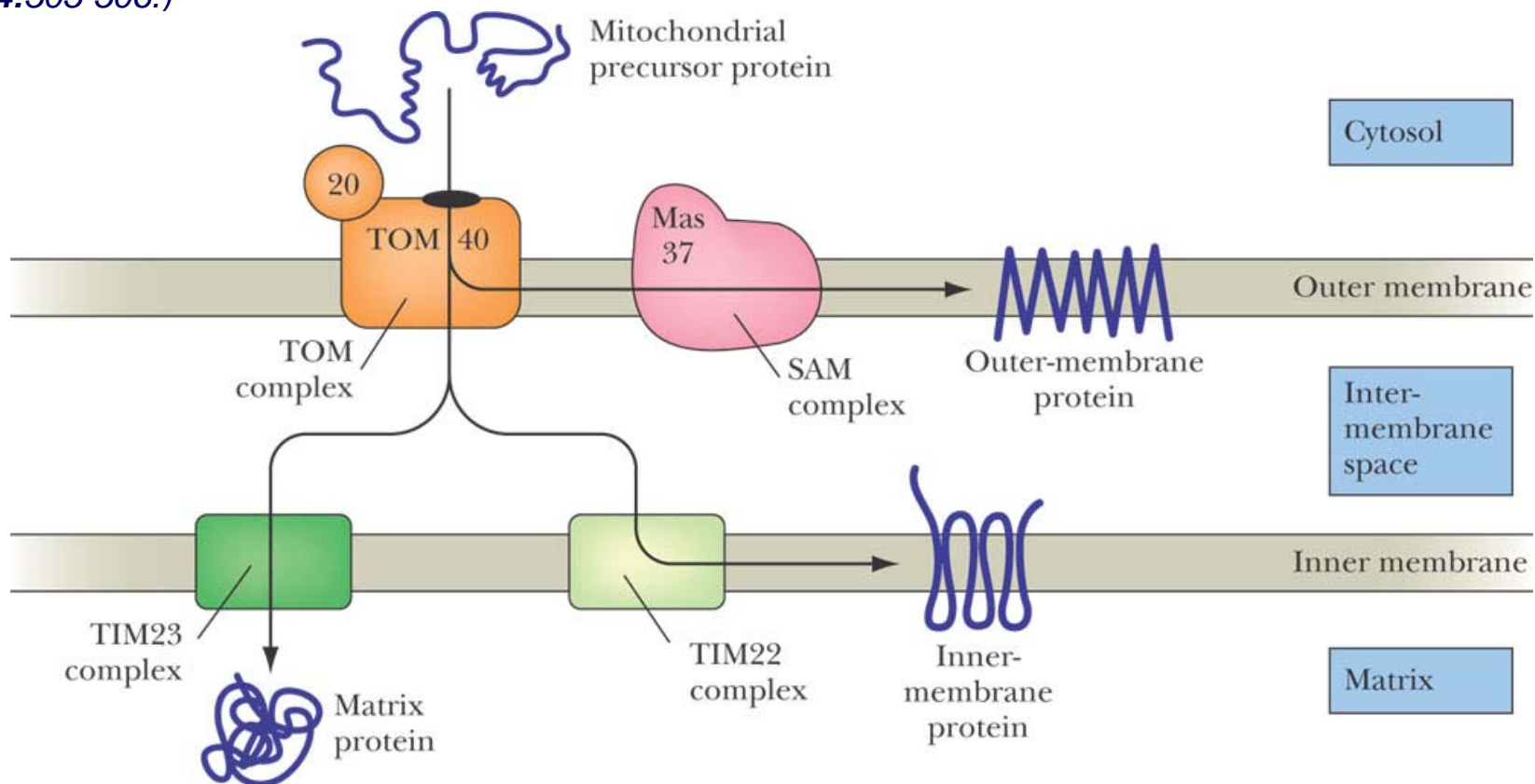


Figure 31.7

Translocation of mitochondrial preproteins involves distinct translocons. All mitochondrial proteins must interact with the outer mitochondrial membrane (TOM). From there, depending on their destiny, they are (1) passed to the SAM complex if they are integral proteins of the outer mitochondrial membrane or (2) traverse the TOM and enter the intermembrane space, where they are taken up by either TIM22 or TIM23, depending on whether they are integral membrane proteins of the inner mitochondrial membrane (TIM22) or mitochondrial matrix proteins (TIM23). (Adapted from Figure 1 in Mihara, K., 2003. Moving inside membranes. Nature 424:505-506.)



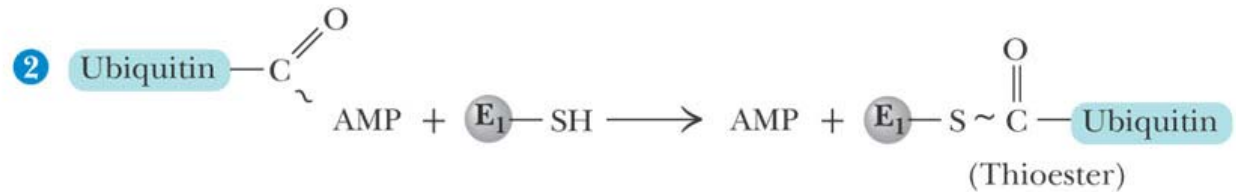
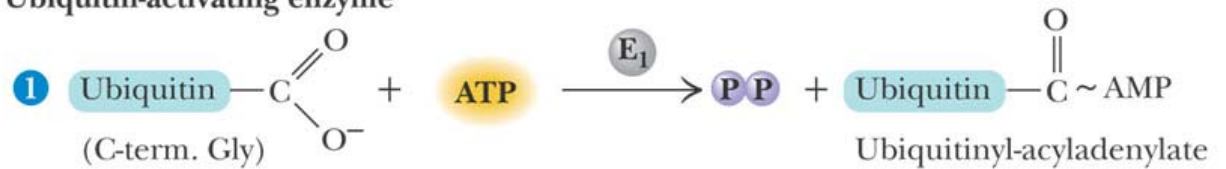
31.4 – How Does Protein Degradation Regulate Cellular Levels of Specific Proteins?

- Eukaryotic proteins are degraded by lysosomes or proteasomes
- Eukaryotic proteins are targeted for proteasome destruction by ubiquitination
- The proteasome core structure is an cylindrical stack of $\alpha_7\beta_7\beta_7\alpha_7$ subunits
- Cap structures on the ends of proteasomes select ubiquitinated proteins for degradation in the core cavity

Figure 31.8

Enzymatic reactions in the ligation of ubiquitin to proteins. Ubiquitin is attached to selected proteins via isopeptide bonds formed between the ubiquitin carboxy-terminus and free amino groups (α -NH₂ terminus, Lys ϵ -NH₂ side chains) on the protein.

E₁ : Ubiquitin-activating enzyme



E₂ : Ubiquitin-carrier protein



E₃ : Ligase

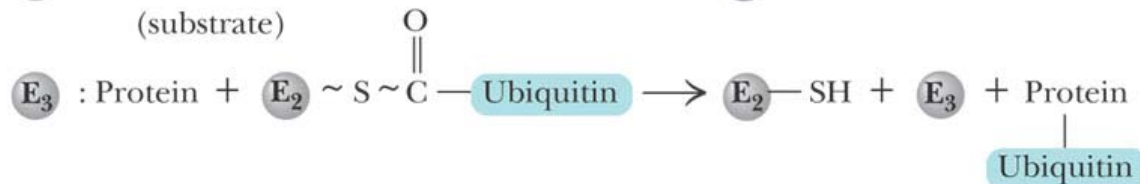
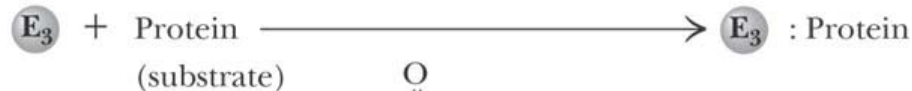


Figure 31.9

Proteins with acidic N-termini show a tRNA requirement for degradation. Arginyl-tRNA^{Arg}: protein transferase catalyzes the transfer of Arg to the free α -NH₂ of proteins with Asp or Glu N-terminal residues. Arg-tRNA^{Arg}: protein transferase serves as part of the protein degradation recognition system.

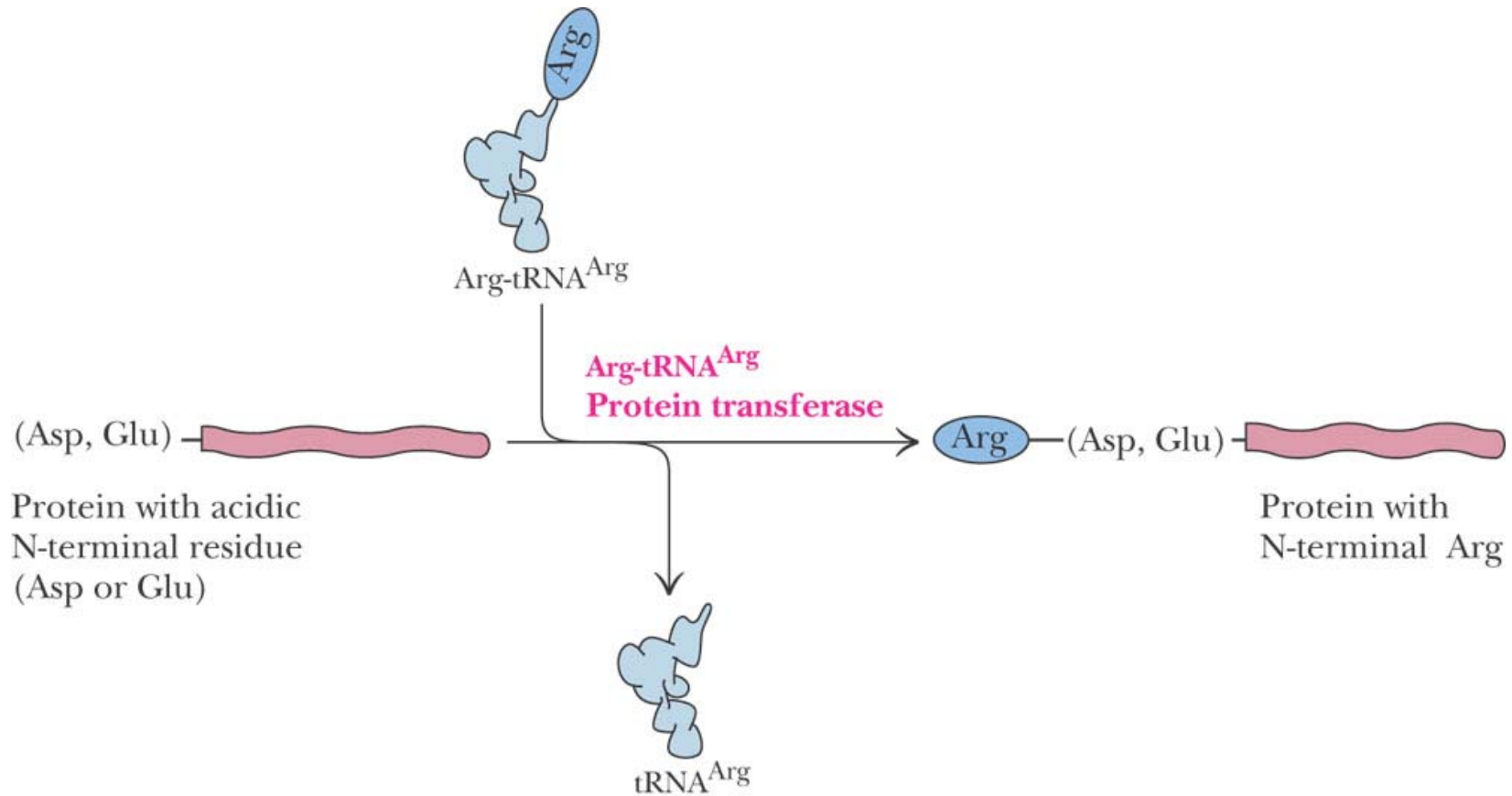


Figure 31.10

Model for the structure of the 26S proteasome. **(a)** The *Thermoplasma acidophilum* 20S proteasome core structure. **(b)** Composite model of the 26S proteasome. The 20S proteasome core is shown in yellow; the 19S regulator (19S cap) structures are in blue.

(Adapted from Figures 3 and 5 in Voges, D., Zwickl, P. and Baumeister, W., 1999. The 26S proteasome: A molecular machine designed for controlled proteolysis. Annual Review of Biochemistry **68**:1015-1068.)

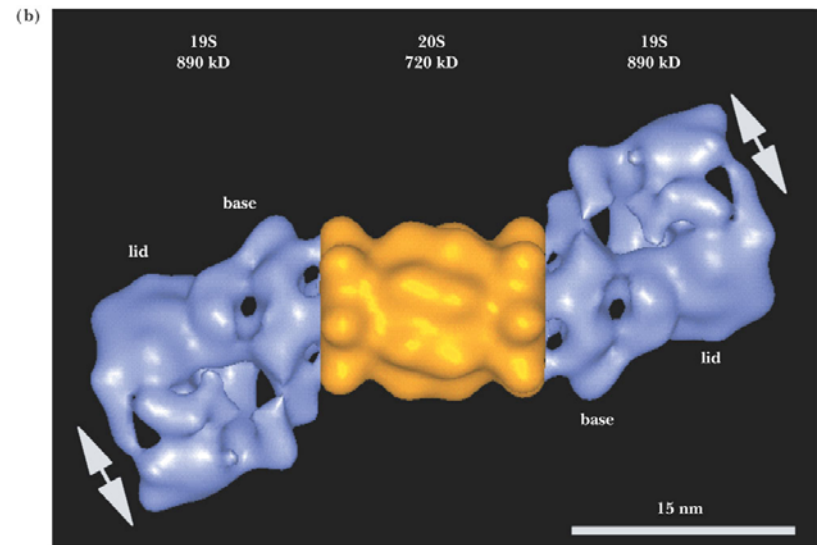
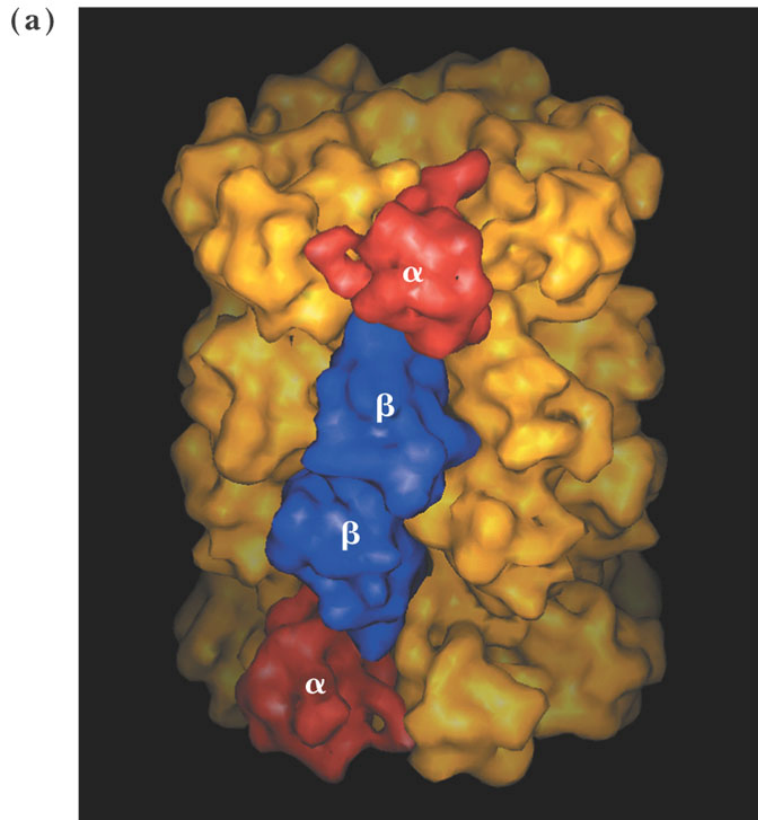


Figure 31.11
Diagram of the ubiquitin-proteasome degradation pathway. Pink “lollipop” structures symbolize ubiquitin molecules. (Adapted from Figure 1 in Hilt, W., and Wolf, D.H., 1996. *Proteasomes: Destruction as a program*. Trends In Biochemical Sciences **21**:96-102.)

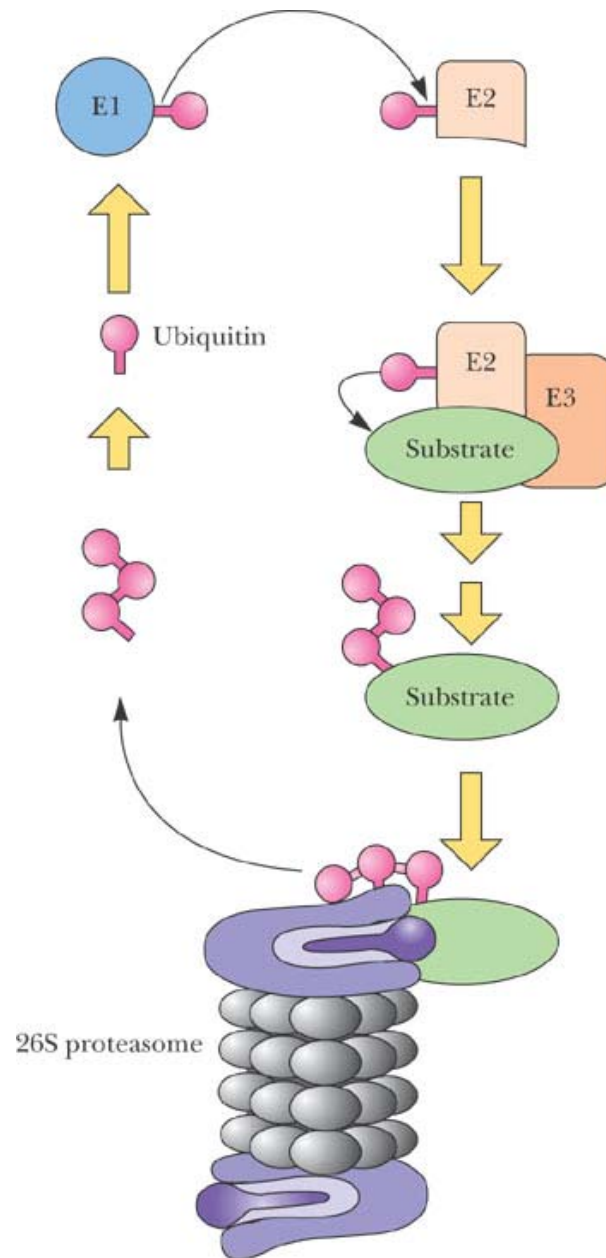


Figure 31.12

The HtrA protease structure. **(a)** A trimer of DegP subunits represents the HtrA functional unit. The different domains are color-coded: The protease domain is green, PDZ domain 1 (PDZ1) is yellow, and PDZ domain 2 (PDZ2) is orange. Protease active sites are highlighted in blue. The trimer has somewhat of a funnel shape, with the protease in the center and the PDZ domains on the rim. **(b)** Two HtrA trimers come together to form a hexameric structure in which the two protease domains form a rigid molecular cage (*blue*) and the six PDZ domains are like tentacles (*red*) that both bind protein substrate targets and control lateral access into the protease cavity. (Adapted from Figure 3 in Clausen, T., Southan, C., and Ehrmann, M., 2002. *The HtrA family of proteases: Implications for protein composition and cell fate*. *Molecular Cell* **10**:443-455.)

