

Chapter 31

Completing the Protein Life Cycle: Folding, Processing, and Degradation

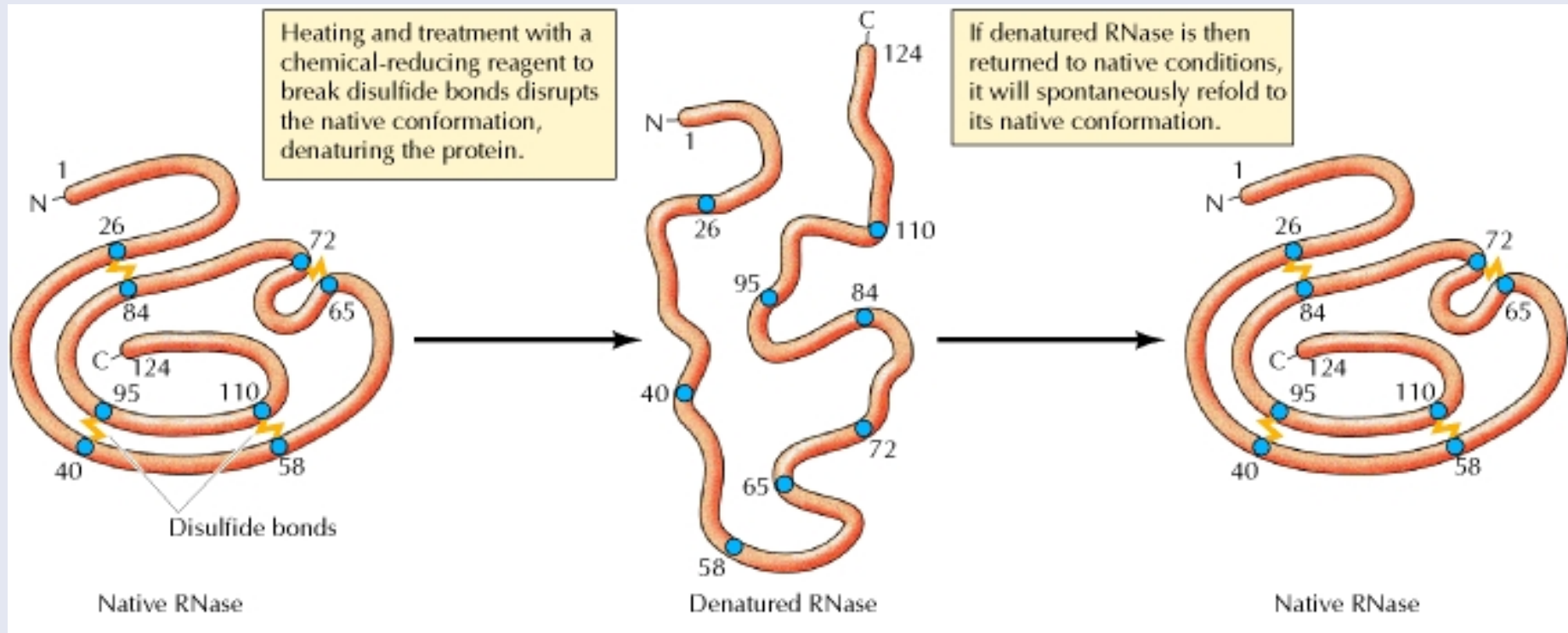
- The newly produced proteins are not yet functional. They need to undergo **folding**, **processing**, and **modification** to become matured and functional
- At the end of their usefulness, damaged or partially denatured proteins are subject to **degradation**.
- Degradation is part of **regulatory programs**

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?

RNase

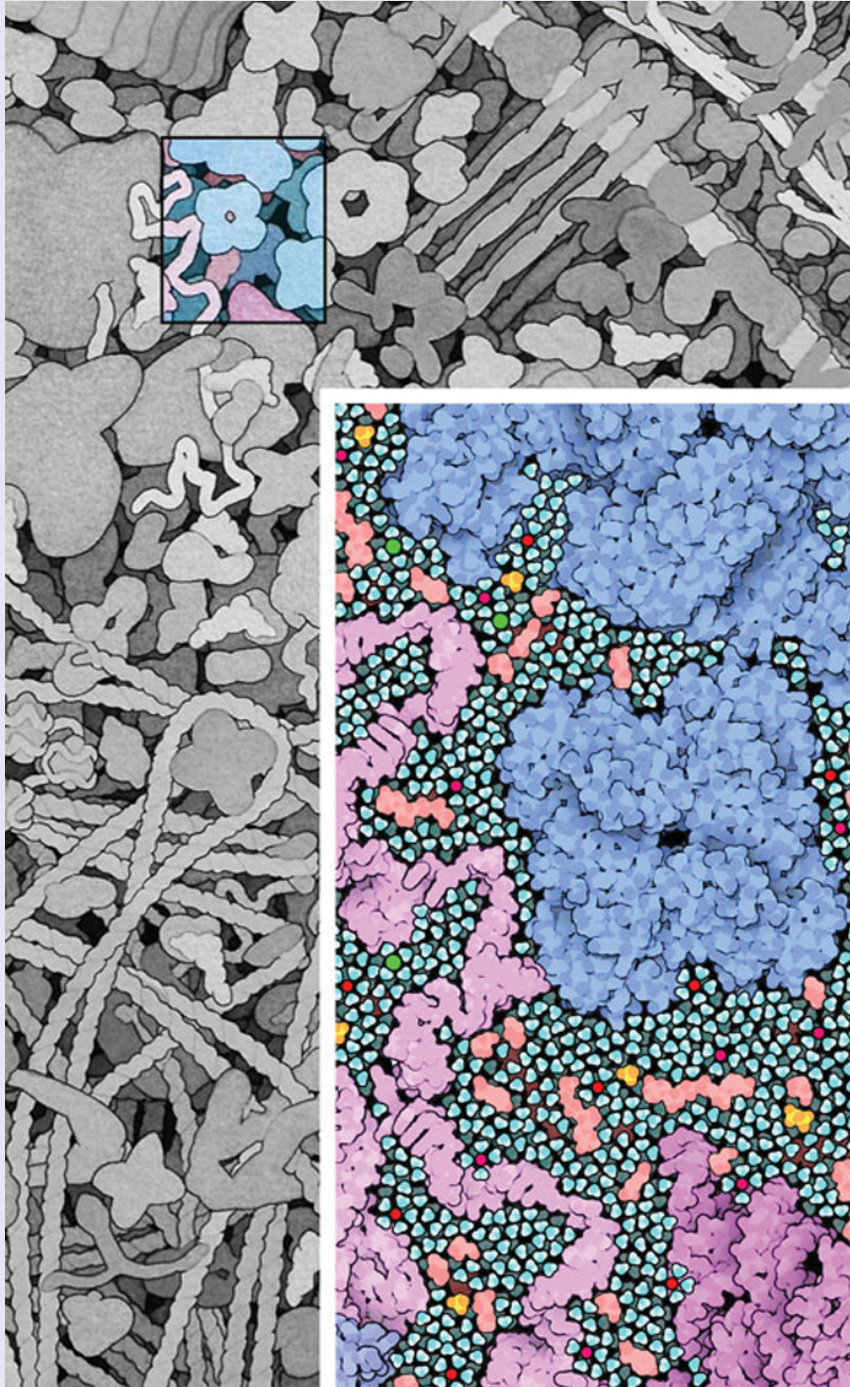


The information within the primary structure is sufficient to determine the tertiary structure

31.1 How Do Newly Synthesized Proteins Fold?

- **The information for folding resides within the amino acid sequence**; protein begin to fold even before their synthesis by ribosomes is completed
- A folded protein has a **hydrophilic surface** and a **buried hydrophobic core**.
- In crowded cytosol, **folding proteins are very likely to associate and aggregate with each other** through hydrophobic interaction
- To prevent this problem, folding of a protein is often assisted by a family of helper proteins known as **chaperones**

To-scale drawing of *E. coli* cytosol



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Biology
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Year published:
(2011)

Functions of chaperones

- Assist protein folding by preventing non-specific association with other proteins
- Escort proteins to their cellular destinations
- Rescue partially unfolded mature proteins by chaperone-assisted refolding

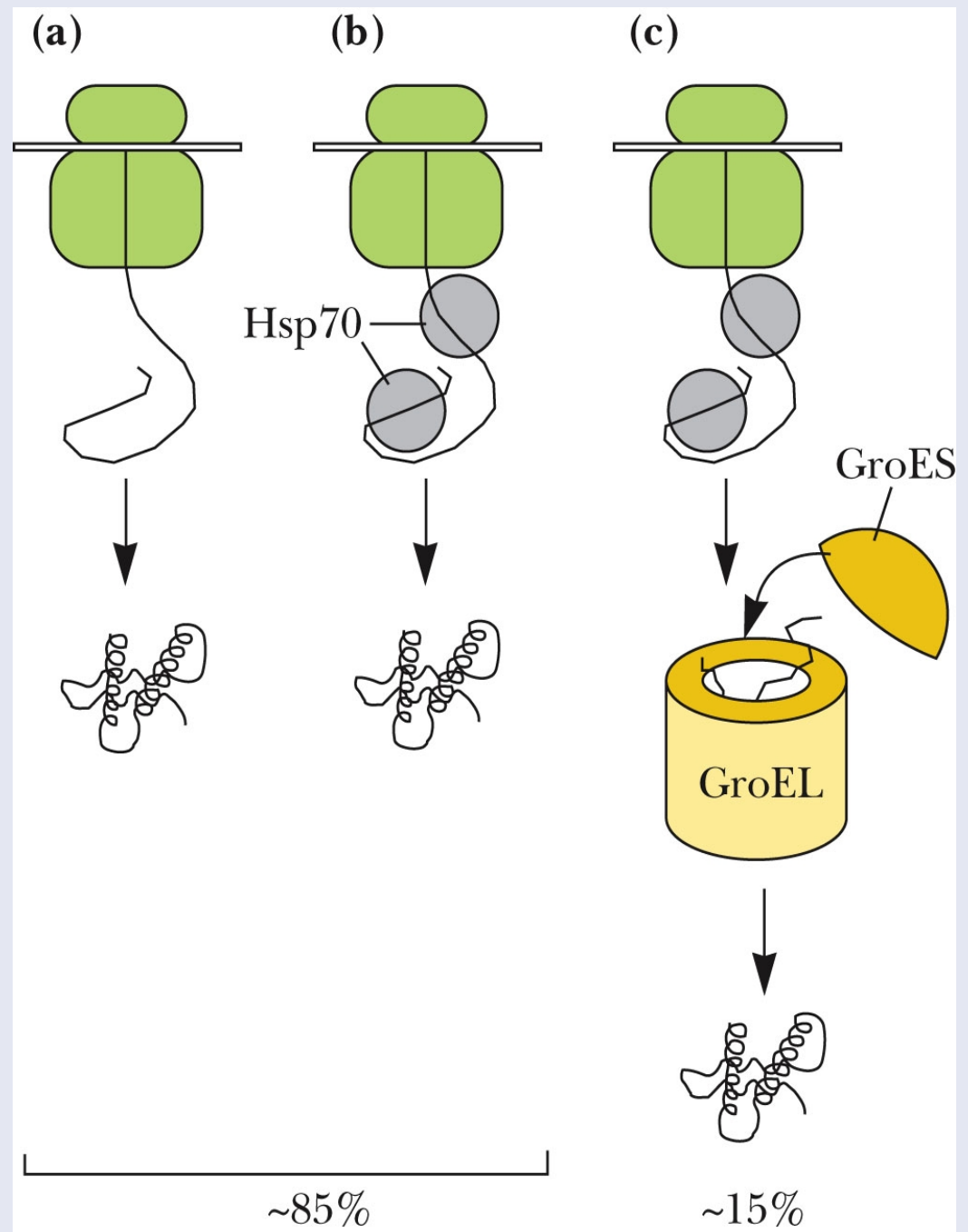
Chaperones Help Some Proteins Fold

- Several chaperone systems are found in all cells
- Many of these are designated **heat-shock proteins (HSPs)**
- The principal chaperones are **Hsp70**, **Hsp60** (the **chaperonins**), and **Hsp90**
- Nascent proteins emerging from the ribosome are met by **ribosome-associated chaperones**, including:
 - **Trigger factor (TF)** in *E. coli*
 - **Nascent chain-associated complex (NAC)** in eukaryotes

Protein folding pathways

- Ribosome-associated chaperones:
 - **Trigger factor (TF)** in *E. coli*
 - **Nascent chain-associated complex (NAC)** in eukaryotes
- TF docks to the 50S subunit L23, which is situated at the peptide exit tunnel.
- TF bind the emerging polypeptide at hydrophobic region to prevent aggregation and to help it folding
- TF and NAC will transfer the nascent polypeptide chain to Hsp70, if necessary
- Then, if needed, the Hsp70 will pass the polypeptide to Hsp60

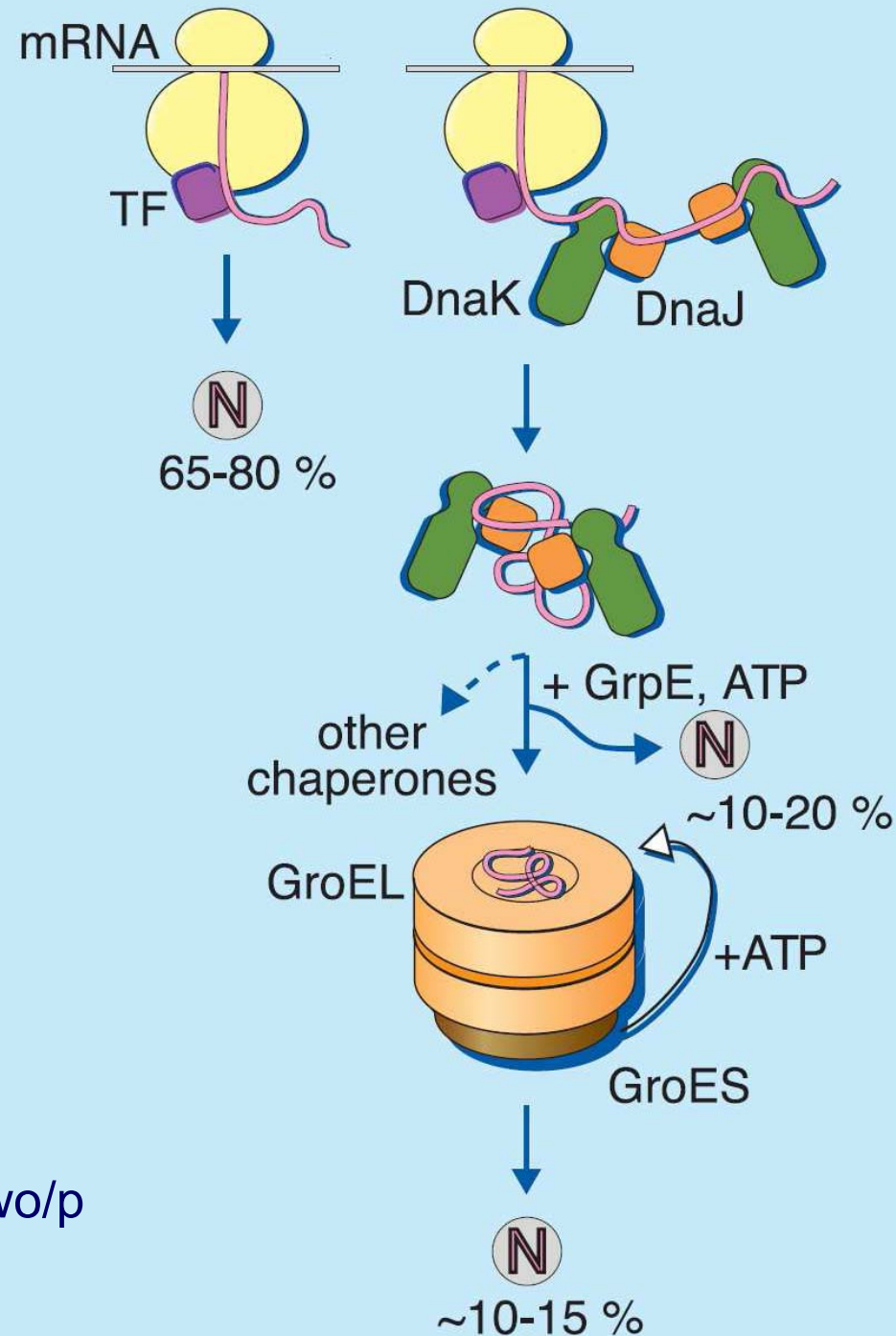
Figure 31.1 Protein folding pathways (a) Chaperone-independent folding. (b) Hsp70-assisted protein folding. (c) Folding assisted by Hsp70 and chaperonin complexes.



Protein folding pathways

TF: trigger factor
Hsp70: DnaK
Hsp60: GroEL
GroES

<http://people.csail.mit.edu/cwo/projects/788proj.pdf>



Hsp70 Chaperones

- Bind to nascent polypeptides while they are still on ribosomes
- Recognizes exposed, extended regions of peptides rich in hydrophobic residues
- In this way, Hsp70 prevents aggregation and keeps the peptide unfolded until productive folding interactions can occur
- Completion of folding requires release of the protein from Hsp70, which needs the energy from ATP

DnaK (*E. coli* Hsp70) consists of two domains:

- 44-kD N-terminal ATP-binding domain
- 18-kD central domain that binds peptides with exposed hydrophobic regions

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

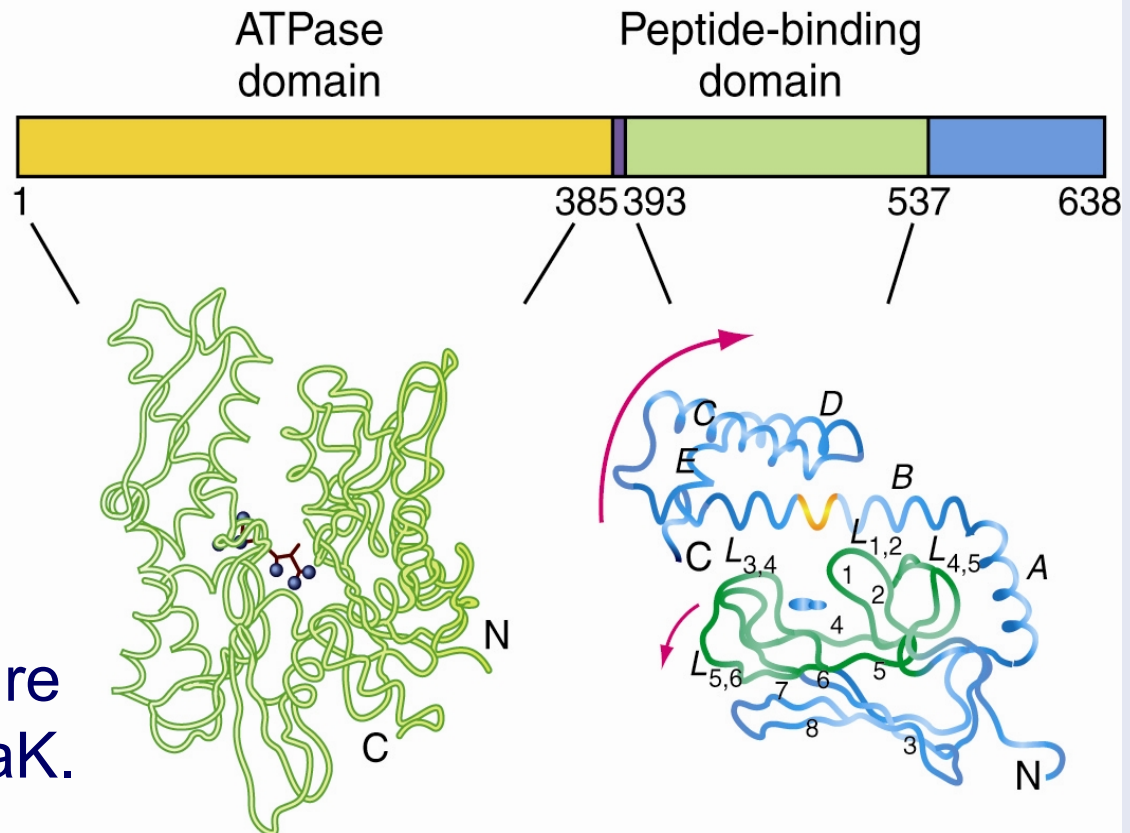


Figure 31.2 Structure and function of DnaK.

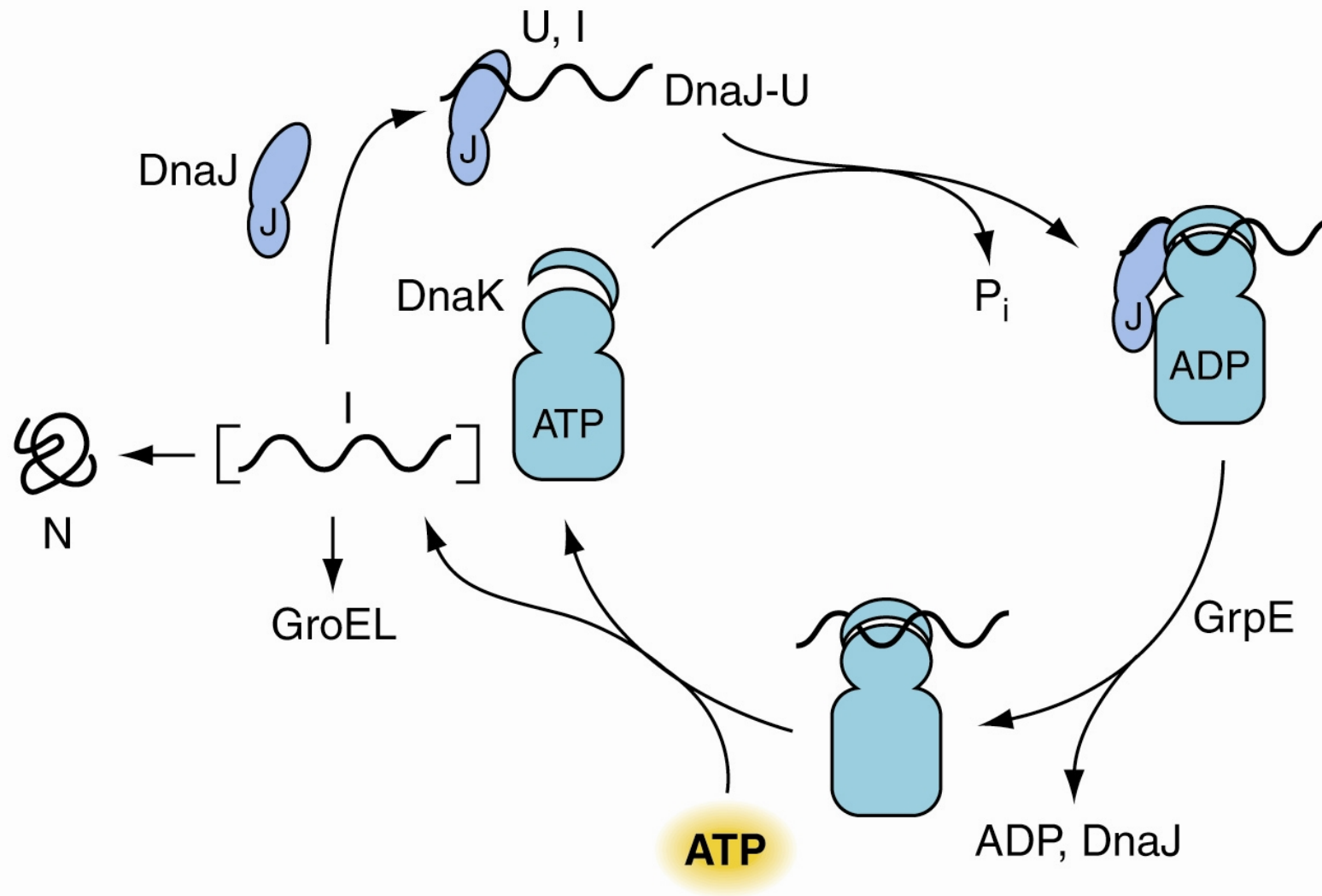
DnaK mechanism of action

DnaK, DnaJ (Hsp40), GrpE

- **DnaK:ATP** receives an unfolded polypeptide from **DnaJ**
- ATPase activity is triggered; DnaK:ADP forms stable complex with unfolded polypeptide
- GrpE exchange ADP on DnaK with ATP; DnaK:ATP forms, releasing polypeptide

DnaK mechanism of action

- Released polypeptide has the opportunity to fold
- Multiple cycles of interaction with DnaK give rise to partially or completed folded polypeptide
- The partially folded intermediates may be passed along to the **Hsp60** for completing folding



•Figure 31.2 (b) DnaK mechanism of action. U:unfolded protein; I: partially folded intermediate

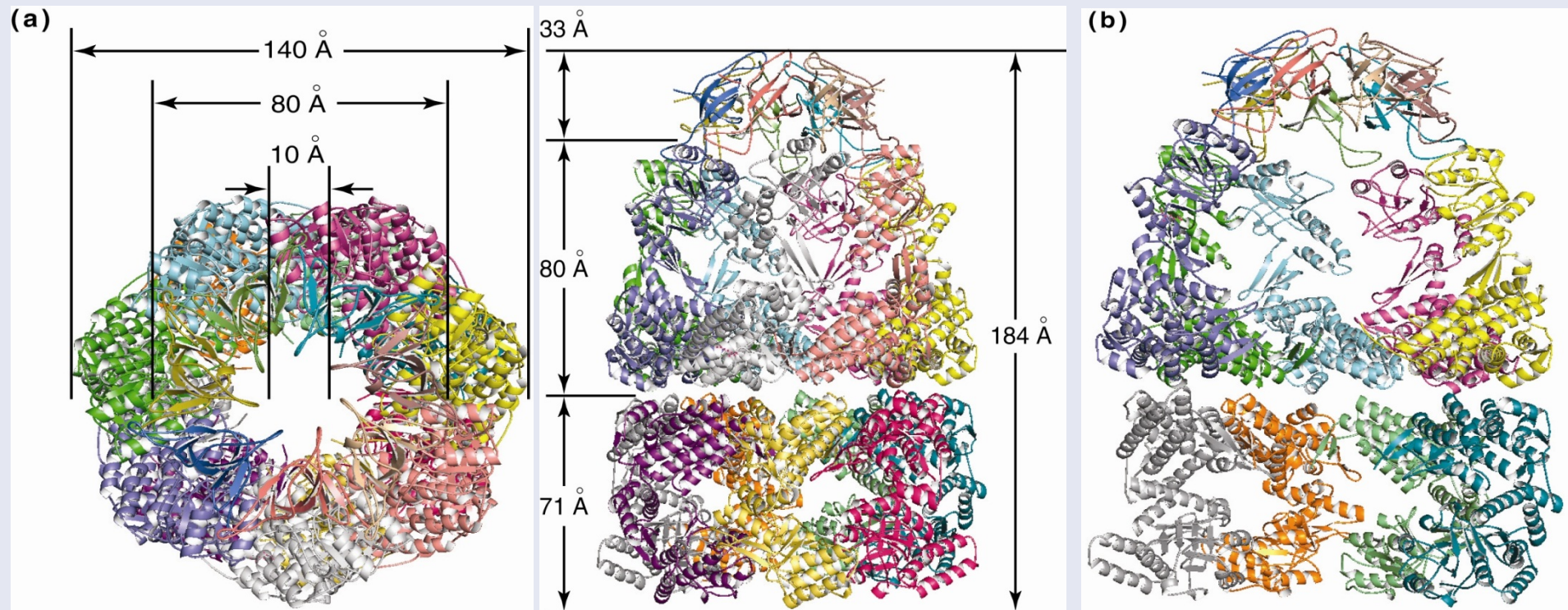
Hsp60 Chaperonin

- Hsp60 chaperones (known as chaperonins) assist some protein to complete folding
- Chaperonins are large, cylindrical protein complexes formed from two stacked rings of subunits
- “**Anfinsen cage**” in chaperonin provides the enclosed space for protein to **fold spontaneously**, free from aggregating with other proteins.
- The proteins in the “cage” undergo forced **unfolding** followed by folding processes that produce the native form

E. coli Hsp60 Chaperonin GroES-GroEL

- Two groups: group I in bacteria, group II in archaea and eukaryotes
- The *E. coli* chaperonin is **GroES-GroEL complex**
- GroEL: two stacked rings with 7 subunits of 60-kD, forming a cylindrical α_{14} oligomer. A central cavity in each ring.
- GroES: co-chaperonin, a single ring with 7 subunits of 10 kD, like a dome on one end of GroEL

Figure 31.3 (a) Structure and overall dimensions of GroES-GroEL. (b) Section through the center of the complex to reveal the central cavity



GroEL subunit has two structural domains:

- Apical domain: has hydrophobic residues, interacting with hydrophobic regions on partially folded proteins; also with GroES
- Equatorial domain: binds ATP, interacts with neighbors in the other α_7 ring

GroES-GroEL mechanism of action

- Apical domain bind the unfolded (or partially folded) protein and deliver it to the central cavity
- ATP binding causes upper α_7 subunit to denature the substrate protein, GroES caps, and upper α_7 subunits transforms to create a hydrophilic cavity
- GroES promote ATP hydrolysis
- GroEL:ADP:GroES complex dissociates when ATP binds to lower α_7 subunits
- Folded (partially or completely) protein releases from GroEL

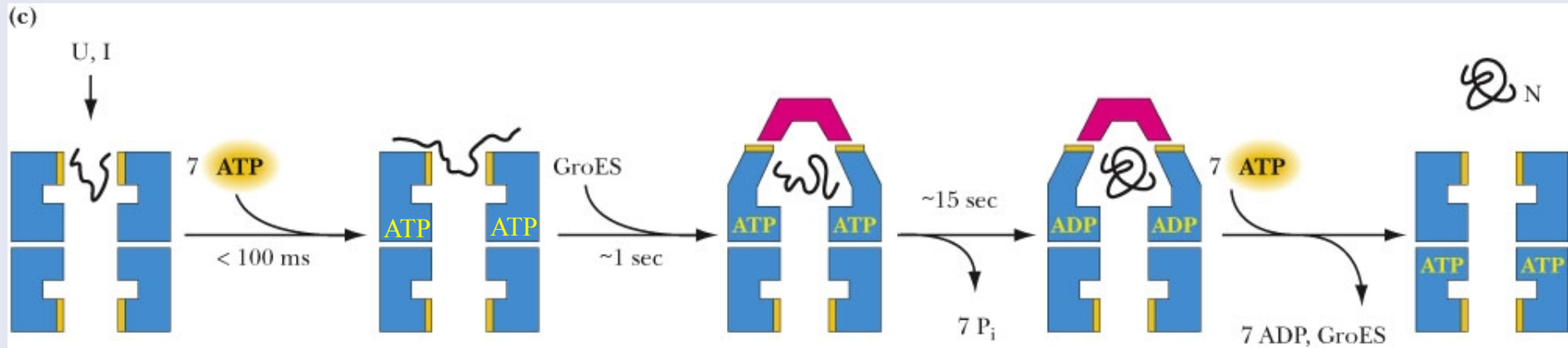
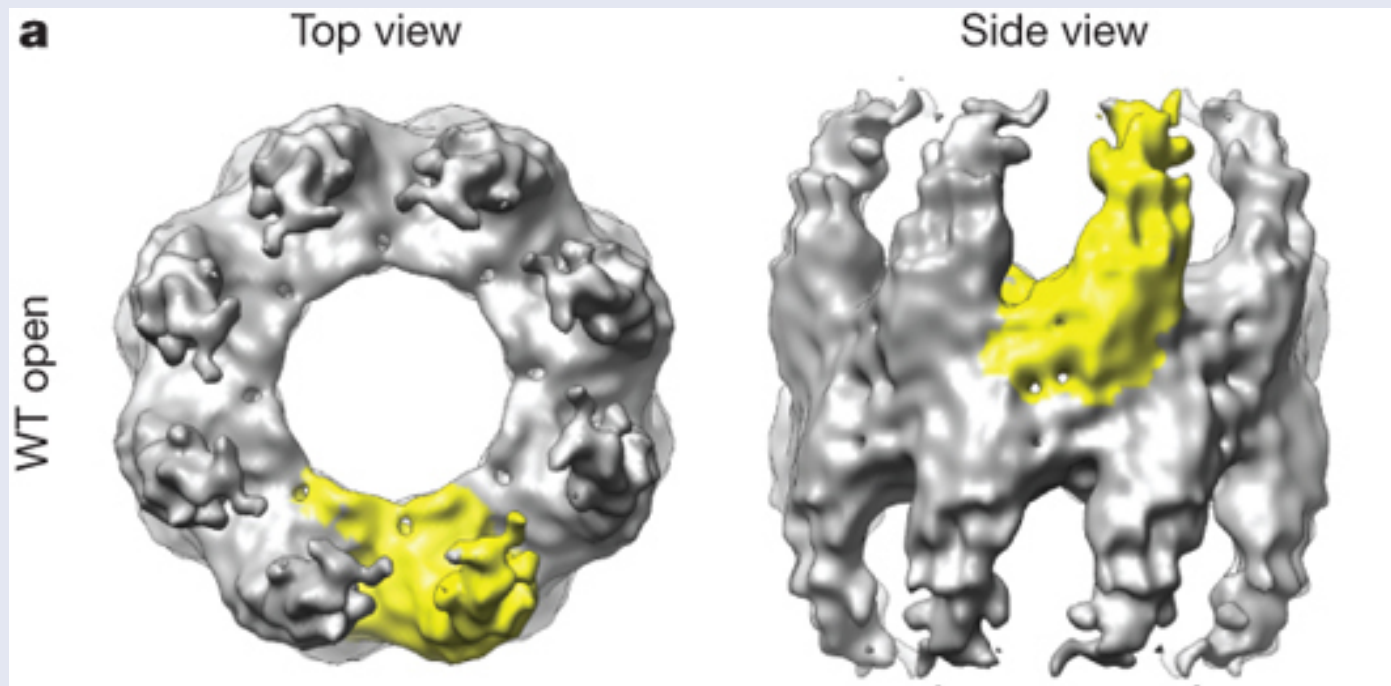


Figure 31.3 (c) Model of the GroEL cylinder (blue) in action. An unfolded (U) or partially folded (I) polypeptide binds to hydrophobic patches on the apical ring of α_7 -subunits, followed by ATP binding, forced protein unfolding, and GroES (red) association.

The eukaryotic group II chaperonin

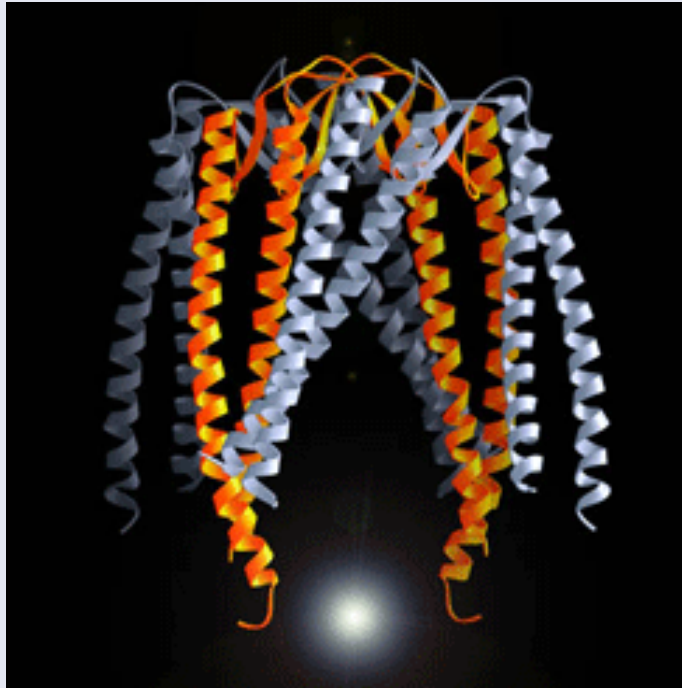
- CCT (TriC): eukaryotic analog of GroEL
- Double-ring structure, each ring consisting of 8 different subunits
- Prefoldin, a hexameric proteins with 2 different proteins, serves as co-chaperone for CCT
- Prefoldin also acts like Hsp70, binds unfolded polypeptide chains emerging from ribosomes and delivers them to CCT
- Prefoldin has 6 tentacles with hydrophobic ends for binding with unfolded proteins

Group II chaperonin

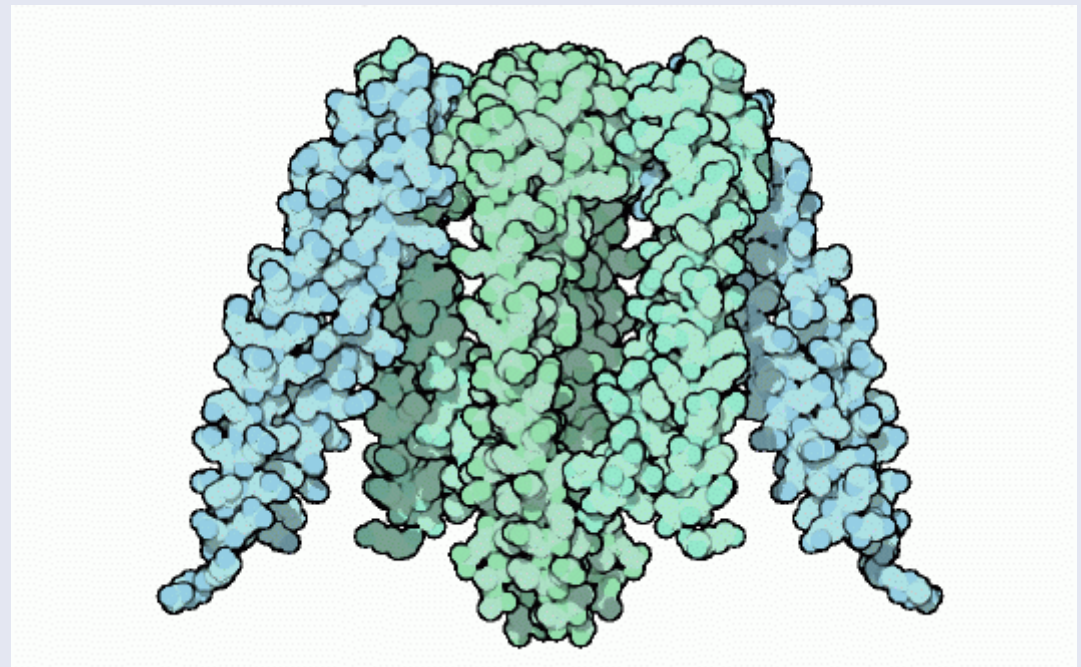


Nature. 2010. 463(7279):379-83.

Prefoldin structure



Siegert et al., 2000, Cell
103, 621-632



<http://www.rcsb.org/pdb/101/motm.do?momID=32>

Figure 31.3 (d)

Depiction of a protein folding reaction in the cytoplasm of an *E. coli* cell, emphasizing macromolecular crowding.

Orange: new protein undergoing folding

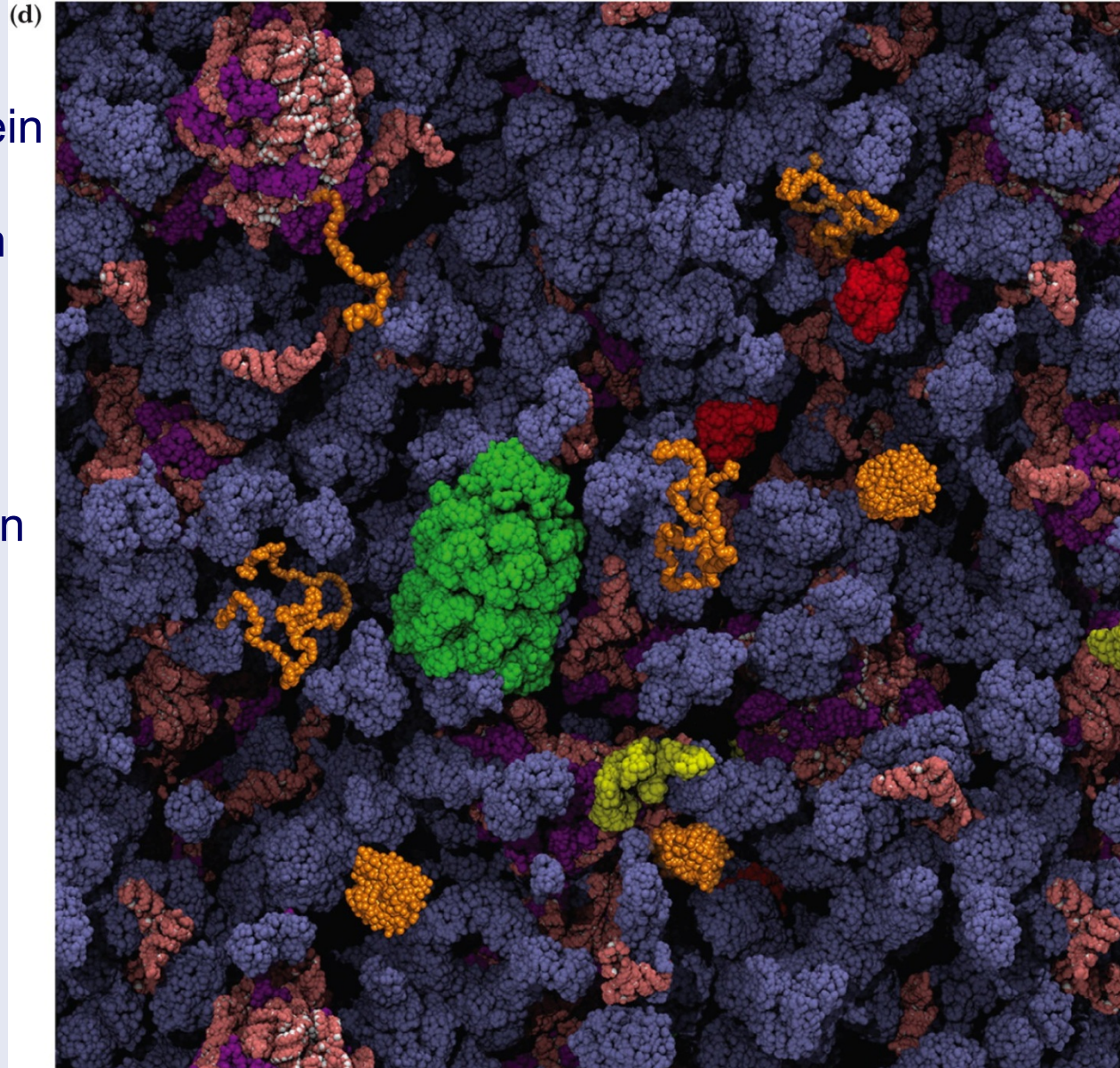
Green: GroES-GroEL

Red: DnaK

Yellow: Trigger Factor

Purple: ribosomes

Salmon: RNA



The eukaryotic Hsp90 Chaperones Act on Proteins of Signal Transduction Pathways

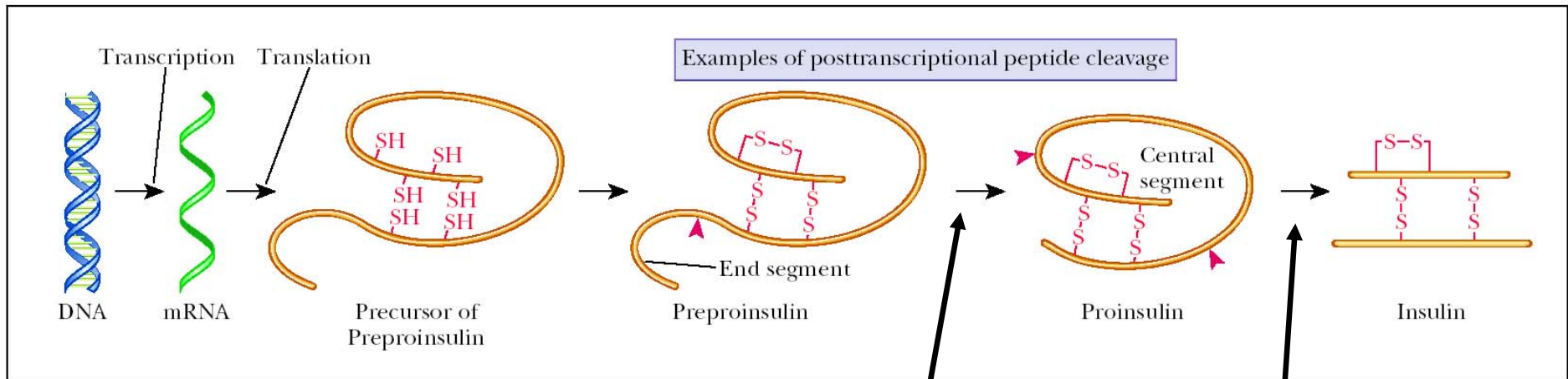
- Hsp90 constitutes 1% to 2% of total cytosolic proteins of eukaryotes, suggesting its importance
- Its action depends on ATP-binding and hydrolysis
- Conformational regulation of signal transduction molecules
- Receptor tyrosine kinase, soluble tyrosine kinase, steroid hormone receptor...Hsp90 “client proteins”
- Foldosome: hsp70 + hsp90 + other proteins ...
- Telomerase, nitric oxide synthase,.. are Hsp90-dependent

31.2 How Are Proteins Processed Following Translation?

- After translation, many proteins are subject to covalent alterations to become functional
- In **post-translational modifications (PTM)**, the primary structure of a protein may be altered, and/or novel derivations may be introduced into its side chains
- Hundreds of different post-translational modifications are known, glycosylation, phosphorylation, methylation, acetylation

Proteolytic cleavage is the most common form of post-translational modification

- Introducing diversity at N-terminal; Met-aminopeptidase
- Activation mechanism for certain proteins, such as enzymes or hormones, synthesized as pro-proteins; N terminal sequence might help folding of active site
- Protein targeting to proper cellular locations



Signal peptide; in ER

In the secretory vesicle, maturation

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

- Proteins are targeted to their proper cellular locations by **signal sequences**
- **Leader peptide** at N-terminal for secretory proteins or for proteins in membrane organelles
- Once the protein is routed to its destination, the signal sequences are often clipped by **signal peptidase**

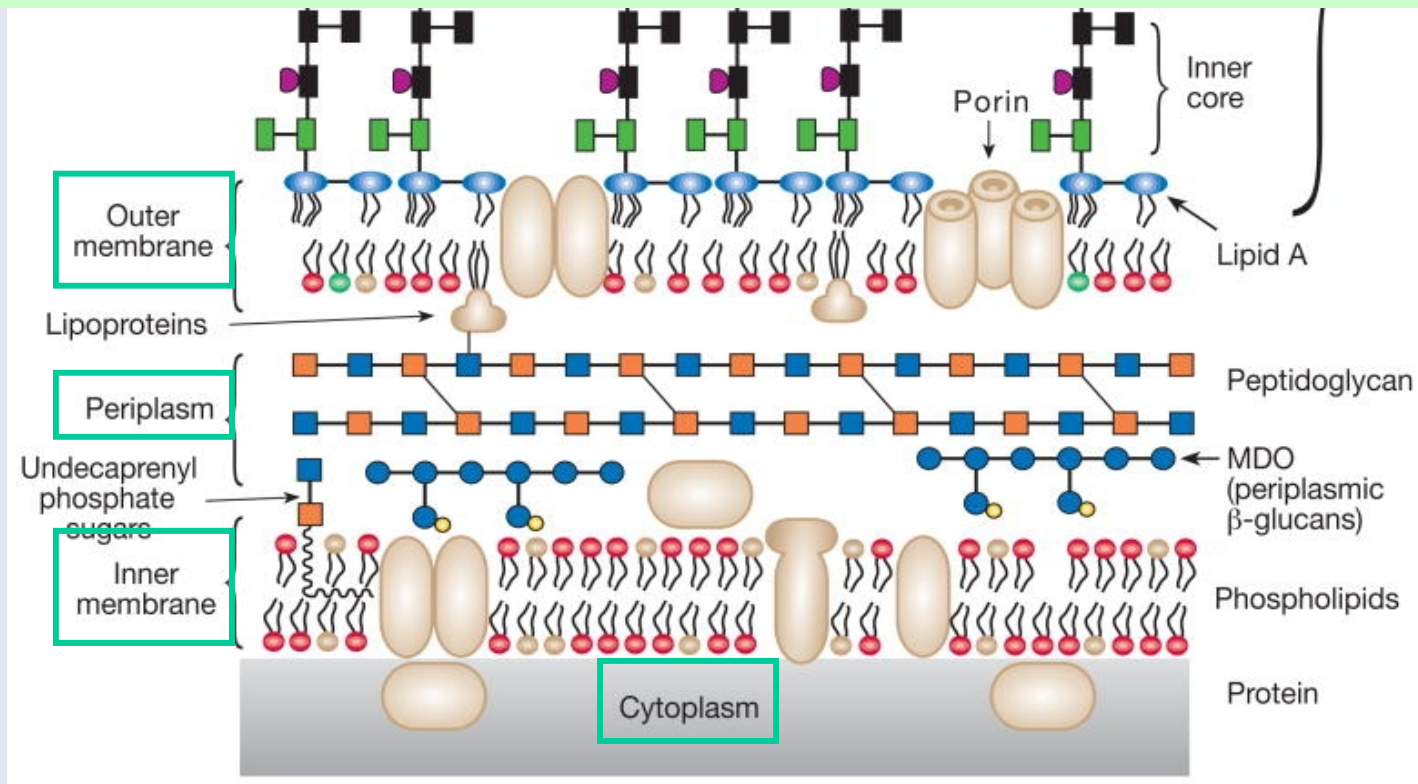
Proteins are delivered to their proper cell compartment by translocation

- **Protein translocation:** the process whereby proteins are inserted into membranes or delivered across membranes
- Translocations occurs at plasma membrane in prokaryotes; ER, nucleus, mitochondria, chloroplasts, peroxisomes in eukaryotes
- **Translocons** are selectively permeable channels that catalyze movement of protein across the membrane; energy is needed.

- Proteins to be translocated are made as preproteins containing signal sequences
- Preprotein are maintained in a loosely folded, translocation-competent conformation through interaction with chaperones
- Eukaryotic **chaperones within membrane compartment** are usually associated with entering polypeptide and provide the energy for translocation
- Membrane proteins has **stop-transfer signals**, allowing diffusion of transmembrane segment into the bilayer

The four compartments in Gram (-) bacteria

Most proteins destined for any location other than cytoplasm are synthesized with amino-terminal leader sequences 16 to 26 a.a.



Prokaryotic proteins destined for translocation are synthesized as preproteins

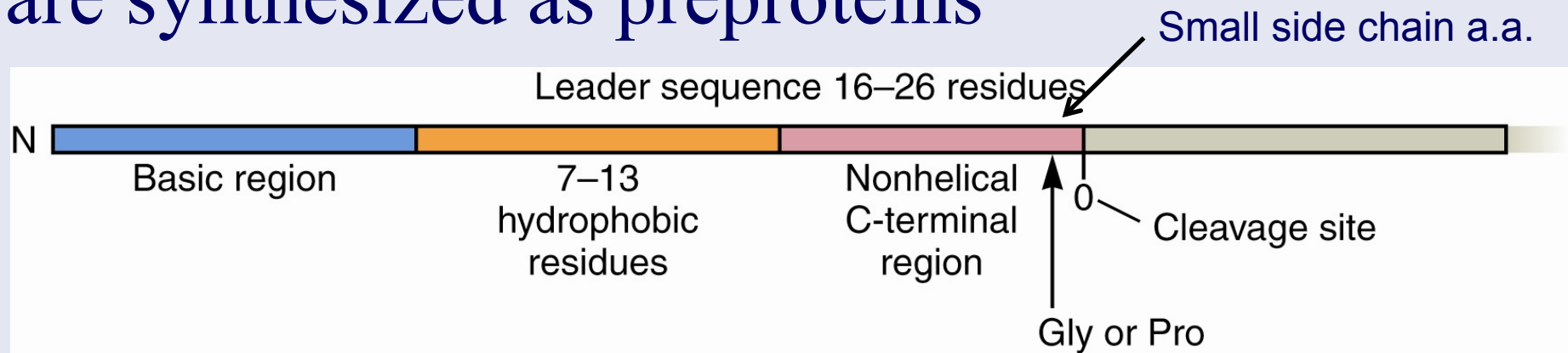


Figure 31.4 General features of the N-terminal signal sequences on *E. coli* proteins destined for translocation: a basic N-terminal region, a central apolar domain, and a nonhelical C-terminal region. The C-terminal region is signals for leader peptidase.

- The exact amino acid sequence is not important.
- The leader peptides retard the folding of the preprotein so that chaperones can interact with it and provide recognition signals for the translocation machinery and leader peptidase

Eukaryotic Proteins Are Delivered to Locations by Protein Sorting and Translocation

- Eukaryotic cells have many membrane compartment
- Signal sequences to compartments may be located at N-terminus or internal region
- A N-terminal signal may or may not be cleaved by specialized proteases, signal peptidase
- Signal sequences for each compartment show only general features, such as charge distribution, relative polarity, secondary structure

Recognition by the ER:

N-terminal sequence with one or more basic amino acids followed by 6 – 12 hydrophobic amino acids

Serum albumin: MKWVTFLLLLFISGSAFSR

↑
Signal peptidase

Synthesis of Secretory Proteins and Many Membrane Proteins is Coupled to Translocation

- As the N-terminal sequence of a synthesizing protein emerges from ribosome, the **signal sequence** is detected by a **signal recognition particle (SRP)**.
- SRP: 325 kD, 6 polypeptides + 7S RNA
- **SRP54**, a G protein, recognizes and binds to the signal sequence, ceasing the translation
- **RNC-SRP**= ribosome nascent chain:SRP complex

Interaction between the RNC-SRP and the SR Delivers the RNC to the membrane

- RNC-SRP is directed to the cytosolic face of the ER, and binds to the **signal receptor (SR)**
- SR: $\alpha\beta$ heterodimer, α is anchored to membrane by transmembrane β subunit; both subunits are G-proteins and have bound GTP
- **SRP54 docks with SR α** , stimulating each other's GTPase activity. GTP hydrolysis causes the dissociation of SRP from SR and **transfer of the RNC to the translocon.**

Ribosome and Translocon Form a Common Conduit for Nascent Protein Transfer

- Through interaction with translocon, the ribosome **resumes protein synthesis**
- The tunnel of ribosome and the channel of translocon form a continuous conduit, through which the growing polypeptide is delivered
- As the protein is threaded through the channel, **BiP**, an ER Hsp70, binds to the protein, and mediates its **proper folding**
- The ATP-dependent folding exerted by BiP provides the **driving forces for translocation** of the protein into the lumen

- After entering ER, the signal peptide is clipped off by membrane-bound **signal peptidase** (leader peptidase)
- Secretory proteins enter ER completely
- Membrane proteins have **stop-transfer** sequences that arrest the passage across ER
- Stop-transfer: 20-residue stretch of hydrophobic amino acid
- When the ribosome dissociates from the translocon, **BiP plugs the channel**, preventing ions and other substances moving between ER and cytosol

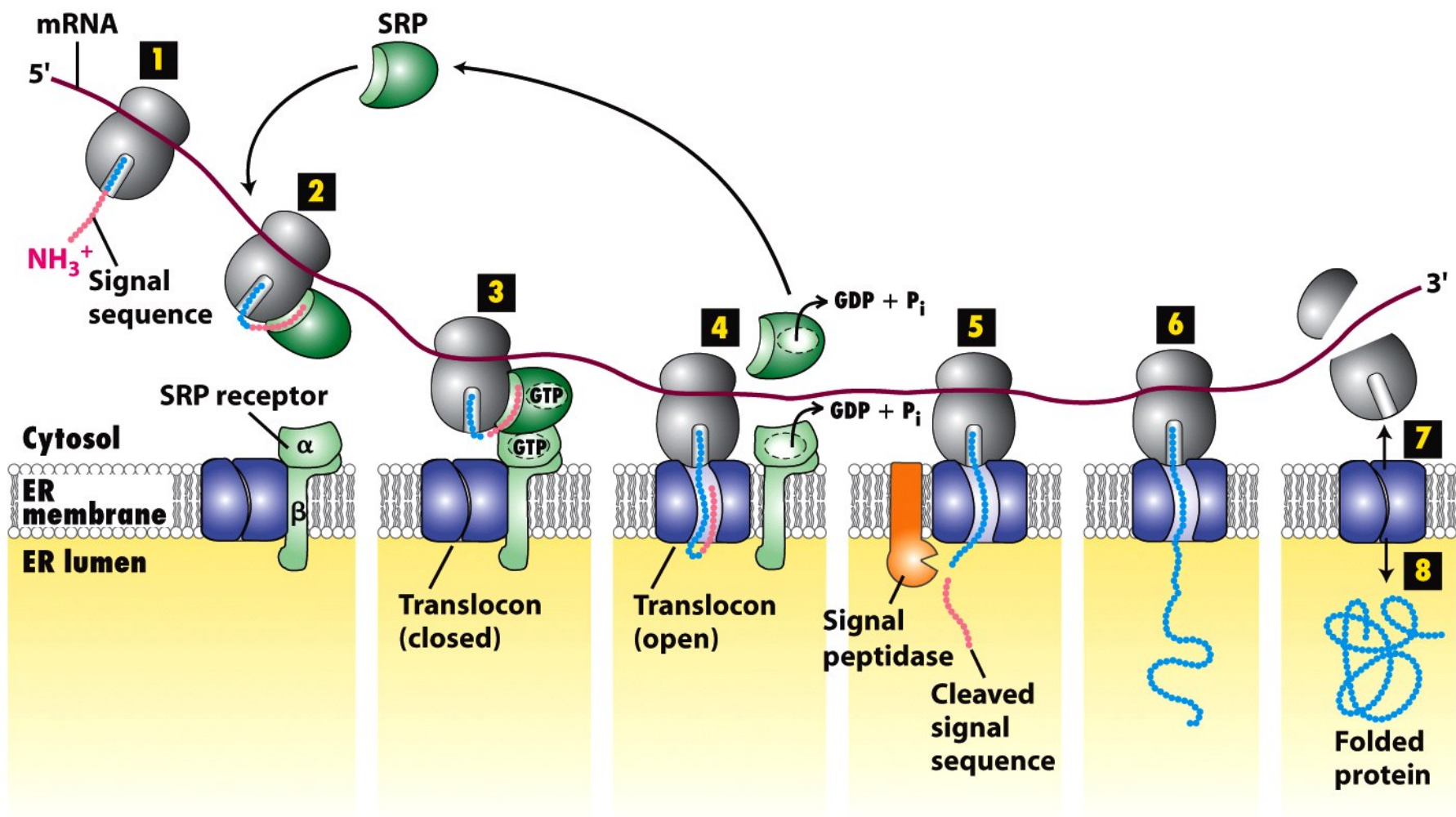
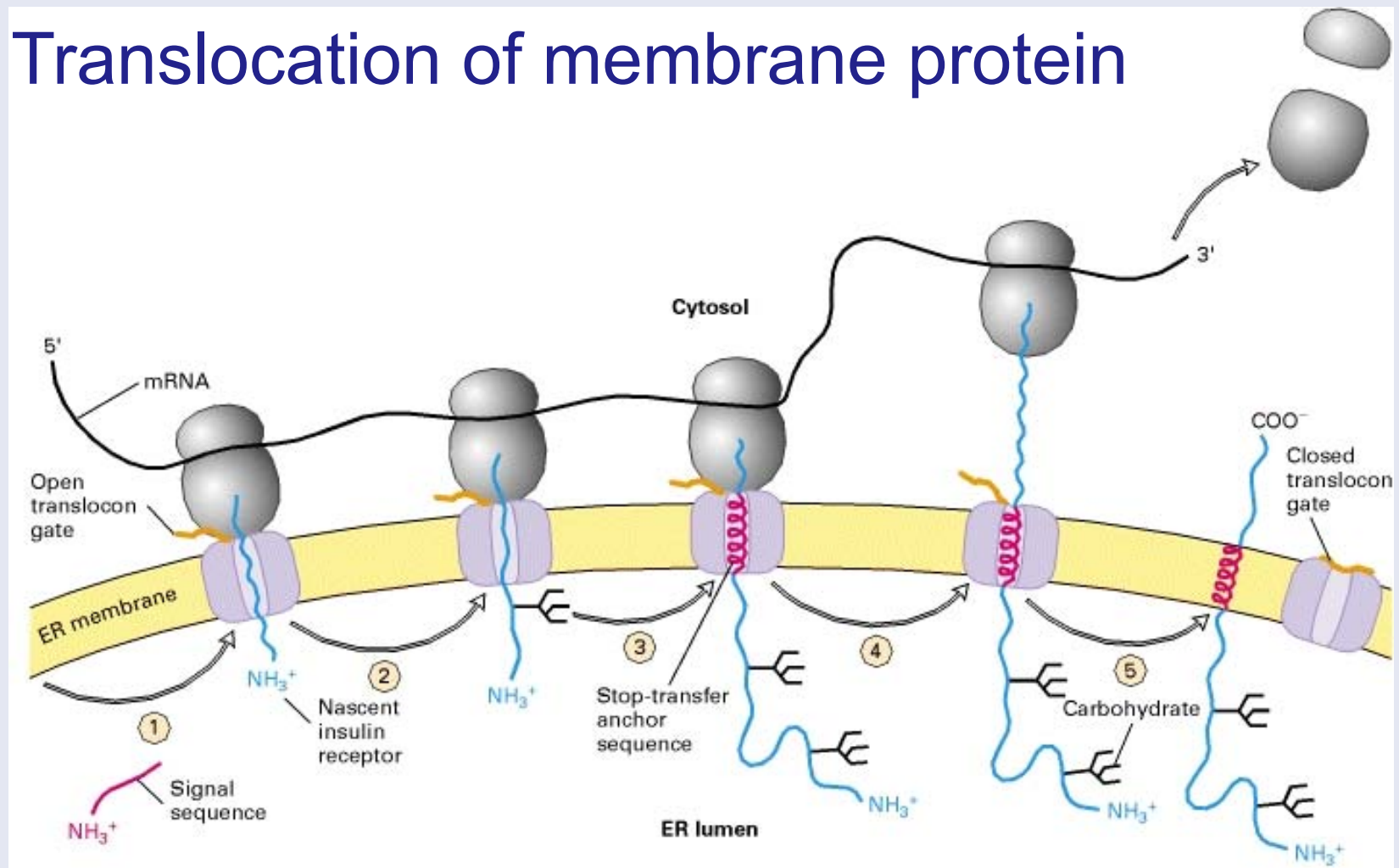


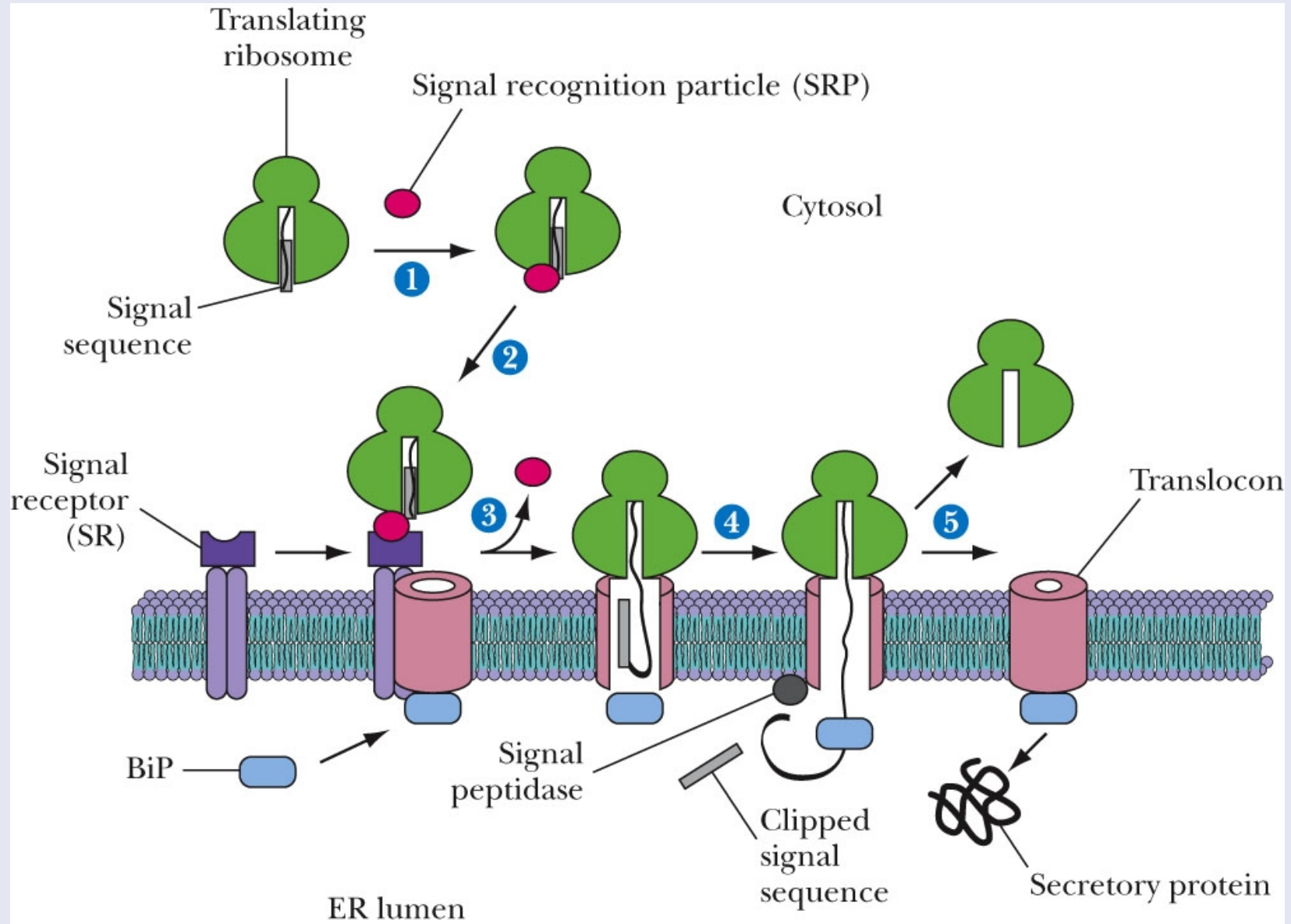
Figure 13-6
Molecular Cell Biology, Sixth Edition
 © 2008 W. H. Freeman and Company

Translocation of membrane protein



<http://www.ncbi.nlm.nih.gov/books/NBK21731/>

Figure 31.5 Synthesis of a eukaryotic secretory protein and its translocation into the ER

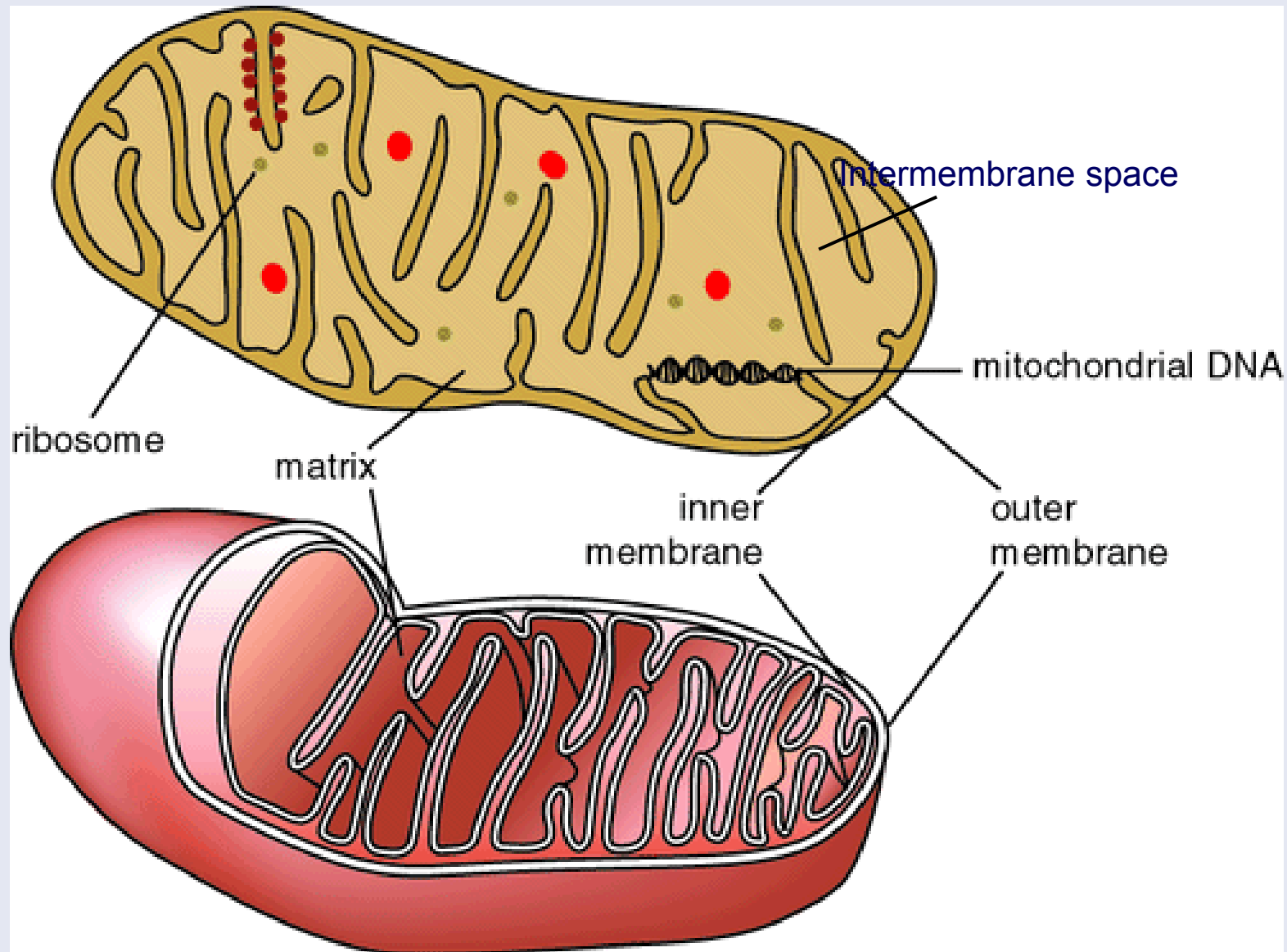


- Other post-translational modifications occurs, glycosylation
- Mammalian translocon: Sec61 complex (α , β , γ) + TRAM
- Sec61 complex forms the channel; dynamic pore size, 0.6 – 6 nm
- TRAM inserts the nascent integrate membrane proteins into membrane.

Retrograde Translocation Prevents Secretion of Damaged Proteins and Recycles Old ER Proteins

- To prevent secretion of inappropriate proteins, damaged or misfolded secretory proteins or ER membrane **proteins are passed from ER back into cytosol via Sec61p**
- The sent-back proteins are subsequently destroyed by proteasome

Mitochondrial Protein Import

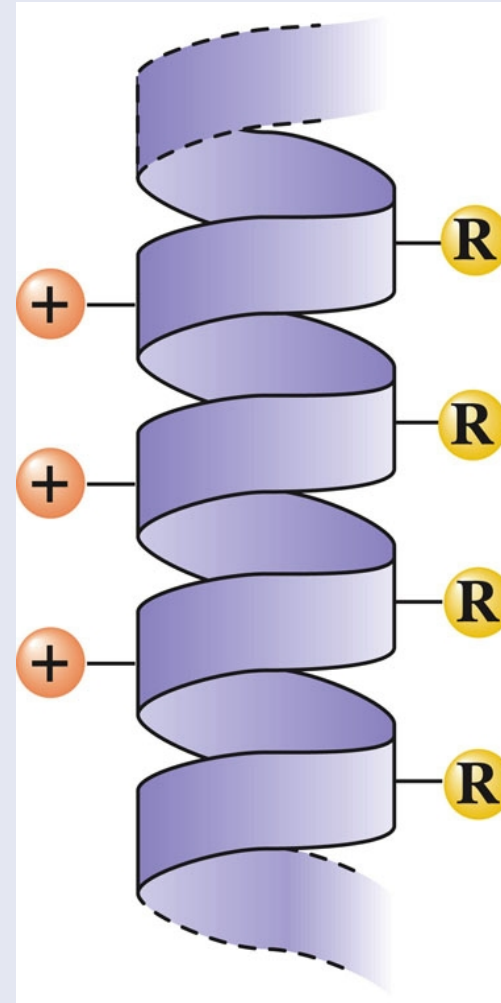


Mitochondrial Protein Import

- Most mitochondrial proteins are encoded by the nuclear genome and synthesized on cytosolic ribosomes; thus mitochondrial proteins must be imported into mitochondria
- Mitochondria possess multiple preprotein **translocons** and **chaperones**
- Signal sequences for mitochondria are N-terminal cleavable presequences 10 to 70 residues long
- These presequences have positively charged , hydroxy residues on one side and hydrophobic residues on the other, forming **amphiphilic α -helices**

Mitochondrial Presequences Contain Positively Charged Amphipathic α -Helices

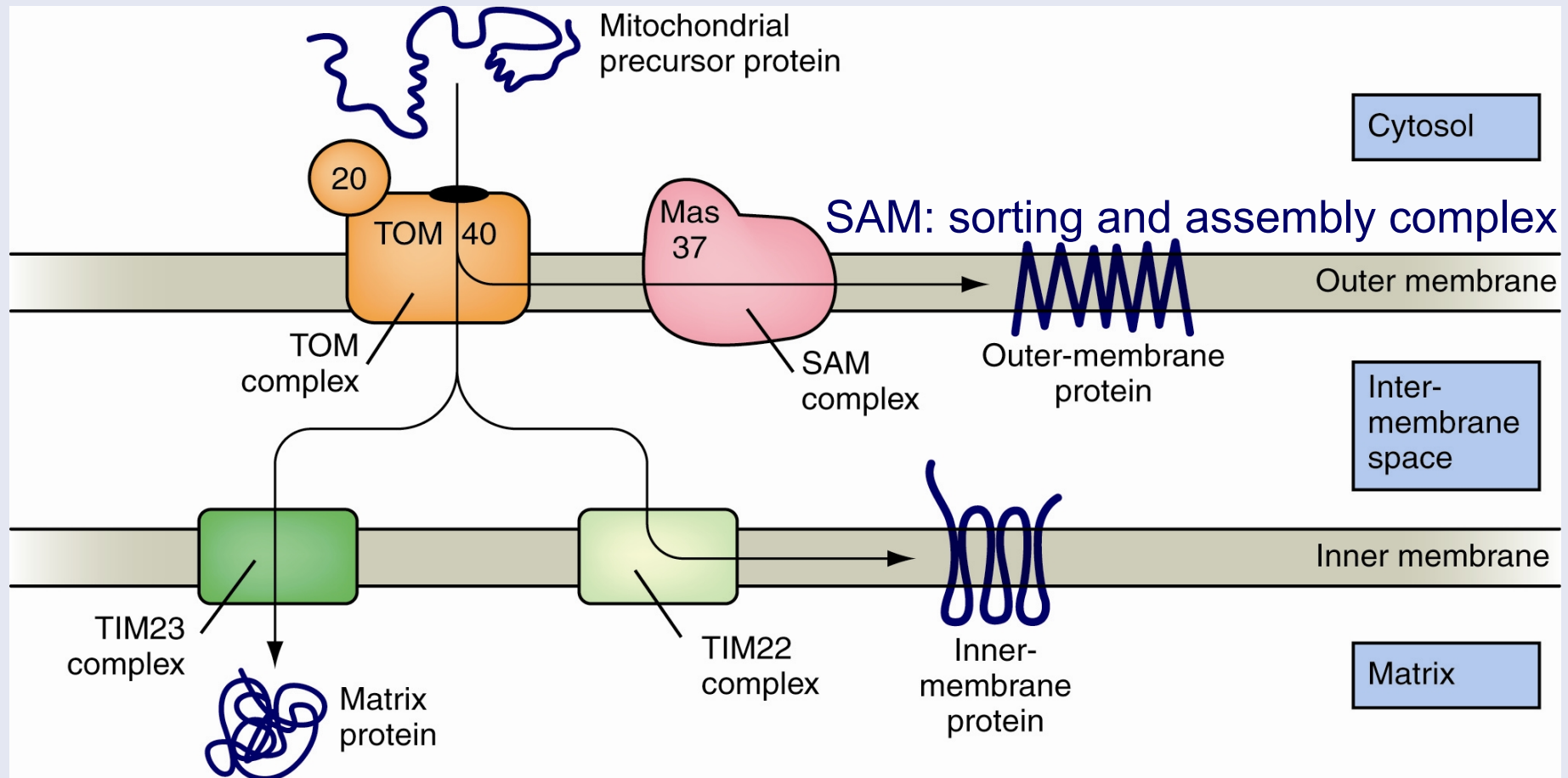
Figure 31.6 Structure of an amphipathic α -helix having basic (+) residues on one side and uncharged and hydrophobic (R) residues on the other. Mitochondrial presequences contain positively charged amphipathic α -helices.



Translocation of Mitochondrial Preproteins Involves Distinct Translocons

- Once synthesized, mito preproteins are associated with Hsp70 and retained in an **unfolded state with exposed target sequences**
- **TOM**: Translocase of the outer mitochondrial membrane; **mitochondrial outer membrane translocon**
- **TIM**: Translocase of the inner mitochondrial membrane; **mitochondrial inner membrane translocon**; TIM22 for inner mem. protein, TIM23 for matrix protein

Figure 31.7 All mitochondrial proteins must interact with the outer mitochondrial membrane (TOM). They can be passed to the SAM complex or enter the intermembrane space.



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

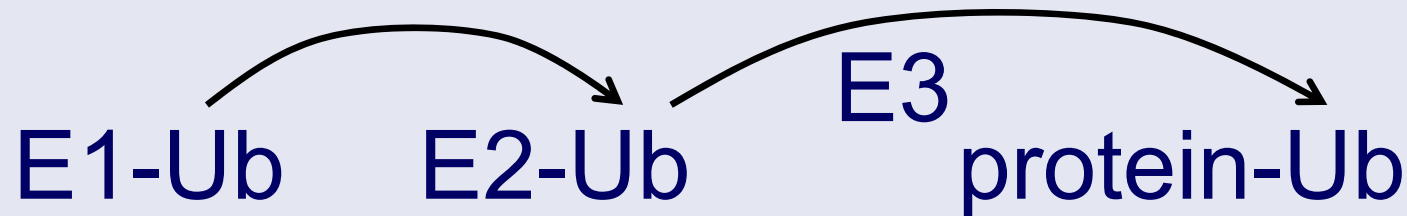
- Abnormal, damaged proteins need to be degraded.
- Transcriptional regulation, translational regulation and protein degradation control protein levels
- Protein degradation is hazard to cell, need to be confined: lysosomes or proteasomes
- Lysosome: eukaryote only, selective uptake, nonspecific degradation
- Proteasome: both pro- and eukaryotes; selective degradation; ribosome counterpart

Eukaryotic proteins are targeted for proteasome destruction by ubiquitination



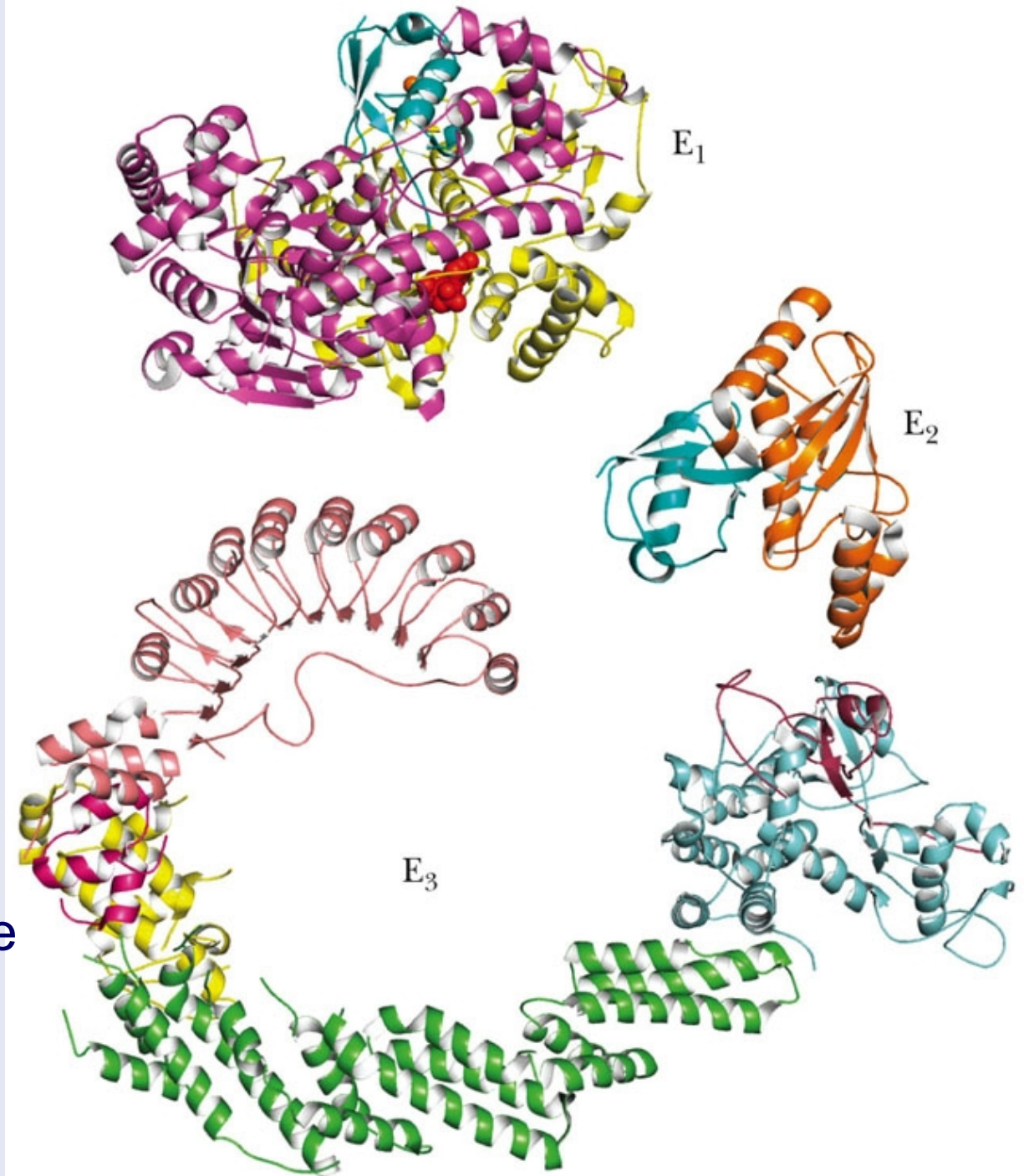
- **Ubiquitination:** post translational modification (PTM)
- **Ubiquitin** is a highly conserved, 76-residue protein found widely in eukaryotes
- Proteins subjected to degradation are ligated to ubiquitin

The three proteins involved in ubiquitination E_1 , E_2 , and E_3



- E_1 : **ubiquitin-activating enzyme**; attached via a thioester bond to the C-terminal **Gly** of ubiquitin requiring ATP as energy
- E_2 : **ubiquitin-carrier protein**; ubiquitin is transferred from E_1 to an SH group on E_2
- E_3 : **ubiquitin-protein ligase**; ubiquitin is transferred from E_2 to free amino groups (**Lys** ϵ - N_2H) on proteins selected by E_3

(Top) A ubiquitin: E_1 heterodimer complex (ubiquitin in blue);
(middle) A ubiquitin: E_2 complex (E_2 in orange, ubiquitin in blue)
(bottom) The clamp-shaped E_3 heteromultimer. The target protein is bound between the jaws of the clamp.



Enzymatic Reactions in the Ligation of Ubiquitin to Proteins

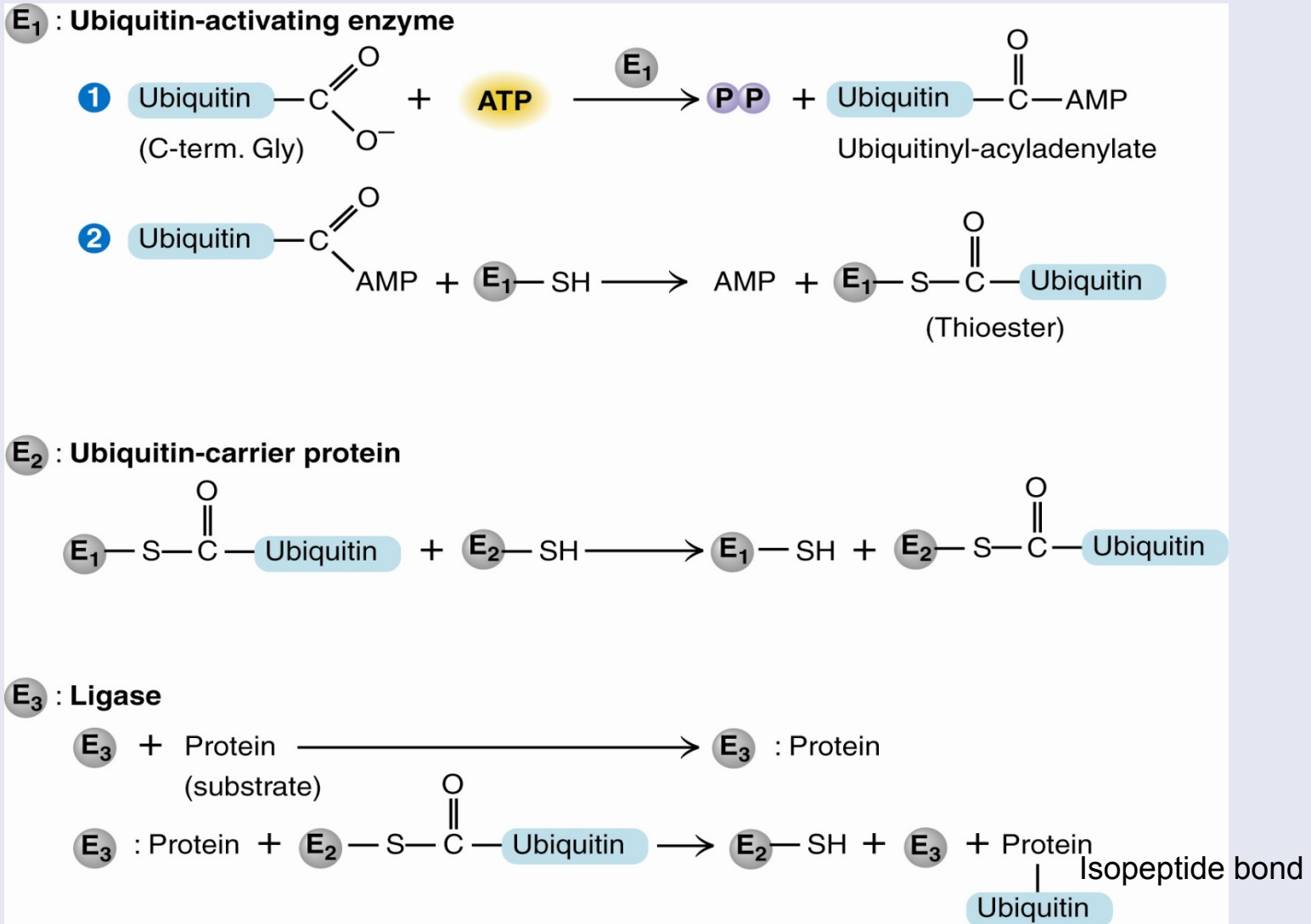
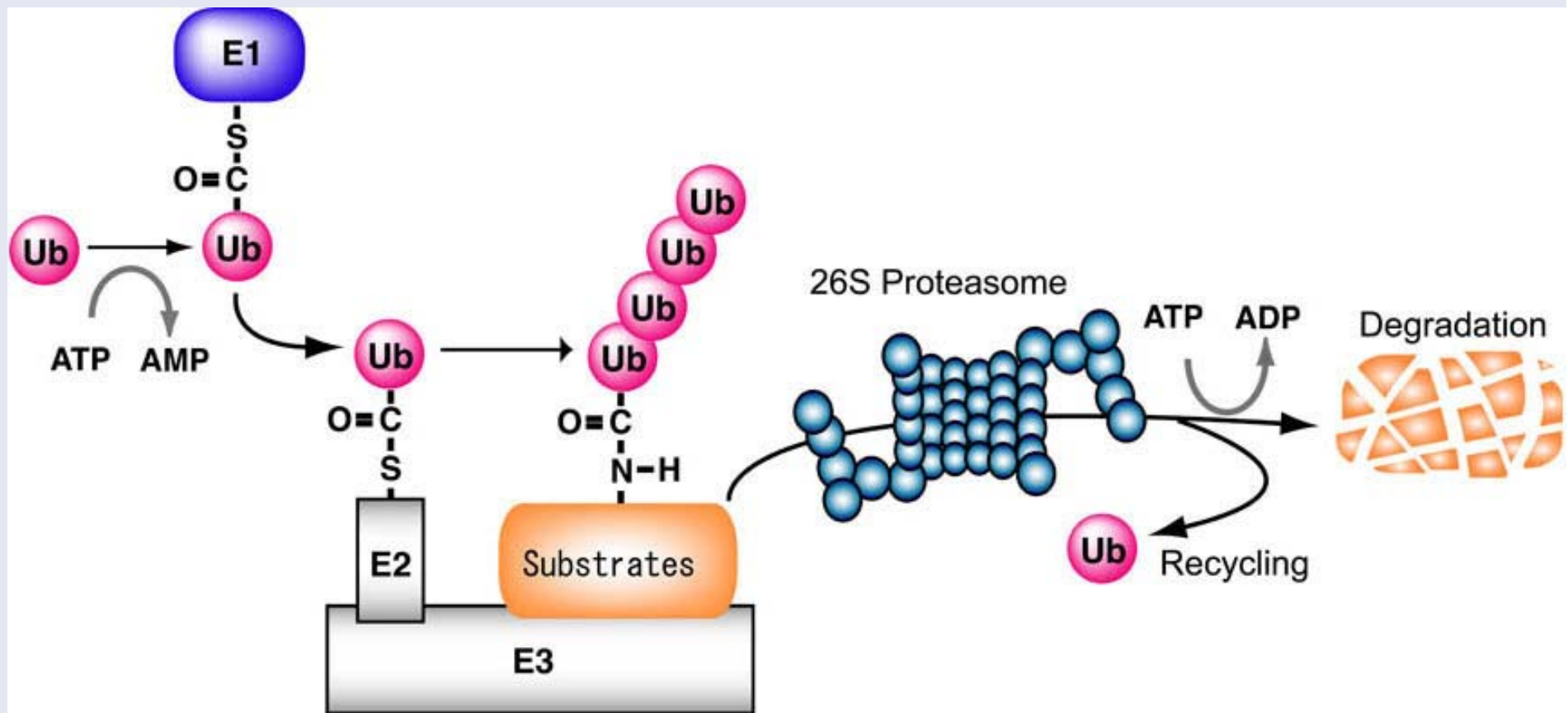


Figure 31.8



More than one ubiquitin is attached; chains of ubiquitins also occur via isopeptide bond between Gly and Lys of different Ub

<http://www.med.hokudai.ac.jp/en/dept/outline/mc/ubiquitination.jpg>

E3 recognizes and selects protein for degradation

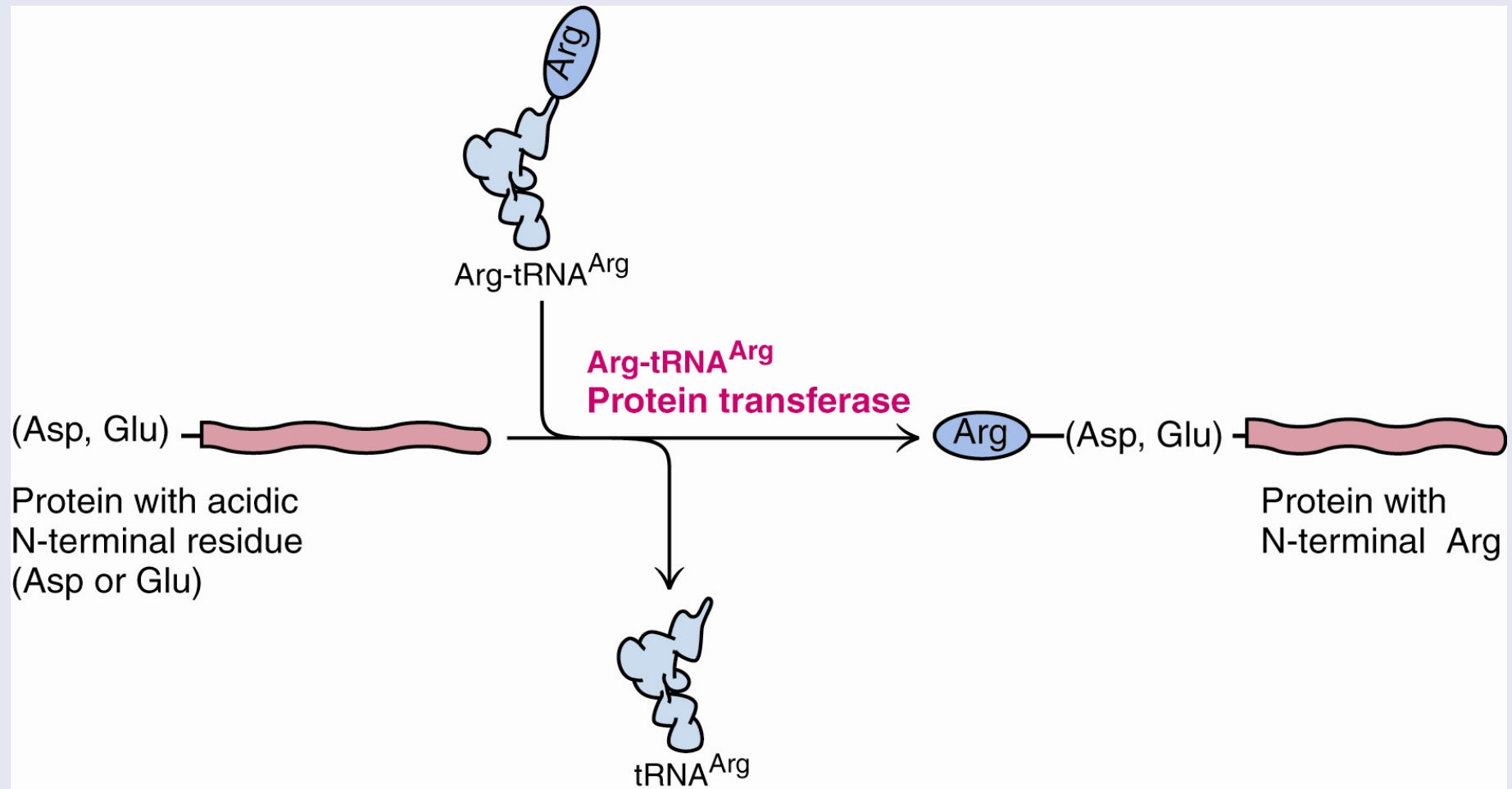
- N-terminal amino acid with free α -amino group
- PEST sequence

PEST: short, highly conserved sequence elements rich in proline (P), glutamate (E), Serine (S), threonine (T)

E3 recognizing N-terminal amino acid

- Arg, Lys, His, Phe, Tyr, Trp, Leu, Asn, Gln, Asp, Glu at N-termini with half-lives of only 2 to 30 min
- Met, Ser, Ala, Thr, Val, Gly, Cys at N-termini are resistant
- Proteins with acidic N-termini (Asp or Glu) need a tRNA for degradation: Arg is transferred from **Arg-tRNA** to N-terminus to become basic
- Most proteins with susceptible N-terminal sequences are secreted proteins; removing invading foreign or secreted proteins

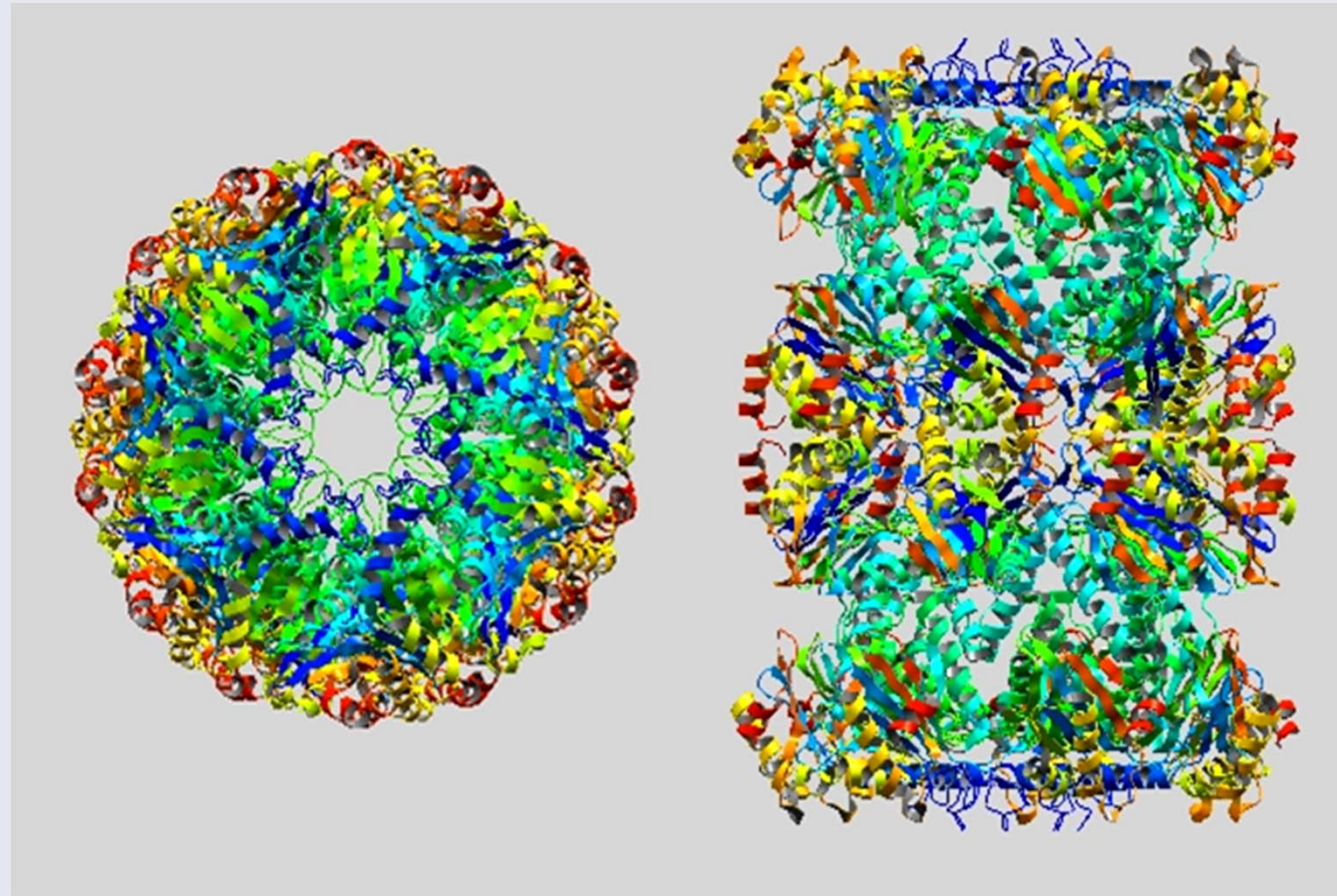
Figure 31.9 Arginyl-tRNA^{Arg}:protein transferase transfers Arg to the free α -NH₂ of proteins with Asp or Glu N-termini. **Arg-tRNA^{Arg}:protein transferase serves as part of the protein degradation recognition system.**



Proteins Targeted for Destruction Are Degraded by Proteasomes

- Proteasomes are large oligomeric structures enclosing a central cavity where proteolysis takes place
- Archaeon *Thermoplasma acidophilum* 20S proteasome: consists of a core barrel structure with a four stacked rings of $\alpha_7\beta_7\beta_7\alpha_7$ subunits
- The outer α rings are thought to unfold proteins and transport them into the central cavity of β subunits, which possess the proteolytic activity
- The N-terminal Thr side-chain O attacks carbonyl-C of a peptide bond in target protein; 7-9 residues long.

Thermoplasma acidophilum 20S proteasome



α_7 : unfolding

β_7 : proteolytic

β_7

α_7

15 nm height, 11 nm diameter, 1.3 nm opening

http://www.pdbj.org/pdb_images/3c91.jpg

Eukaryotic Cells Contain Two Forms of Proteasomes

- Eukaryotic cells contain two forms of proteasomes: **20S proteasomes** and **26S proteasomes**
- The **26S** proteasome is a 45 nm-long structure composed of a **20S proteasome** plus **two** additional structures known as **19S regulators (caps)**
- **20S**: 2 copies of 14 proteins: 7 different α + 7 different β ; similar to archaea; degradation cavity; 3 β has the active sites
- **19S**: 2 copies of 18 – 20 proteins, select and deliver ubiquitinated proteins into cavity

The 26S Proteasome is Composed of a 20S Proteasome Plus 19S Regulator Caps

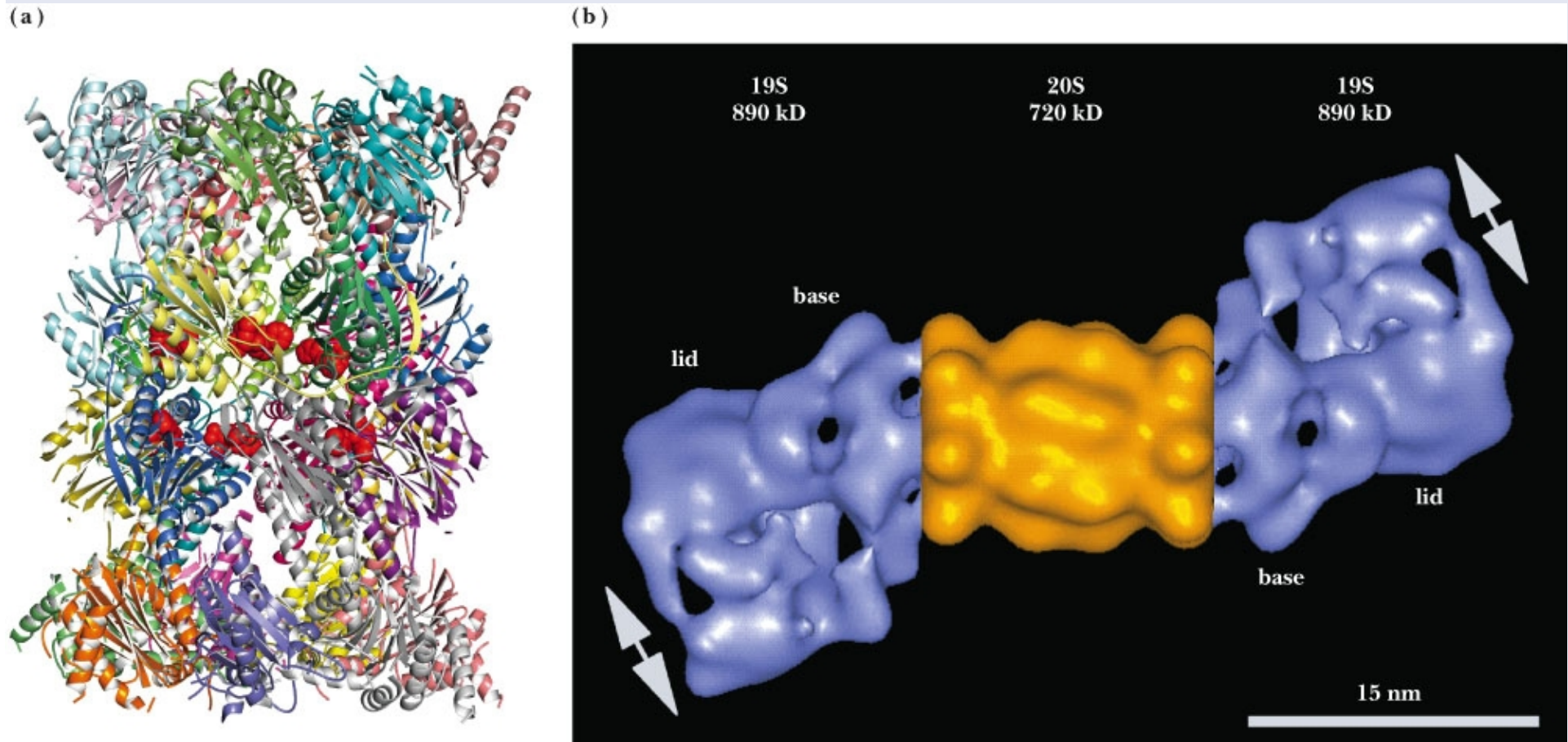


Figure 31.10 The structure of the 26S proteasome. (a) the yeast 20S proteasome core with bortezomib bound (red). (b) Composite model of the 26S proteasome.

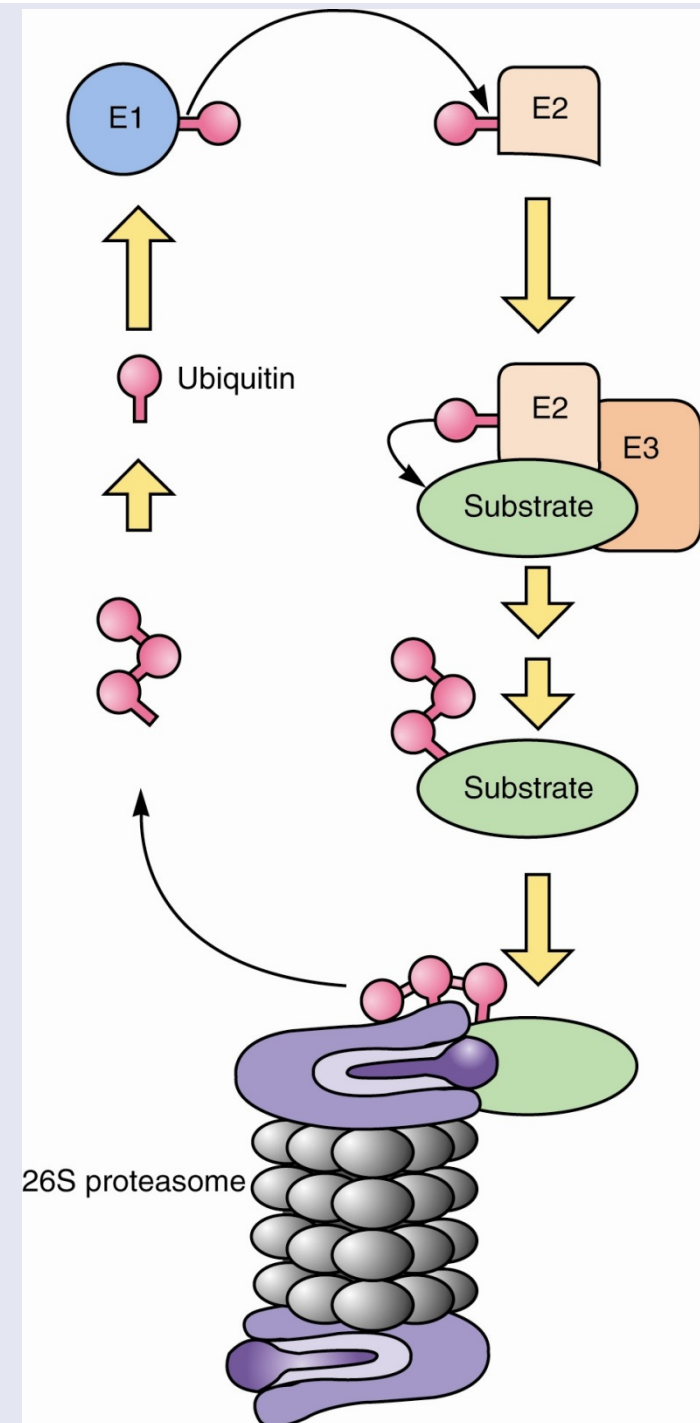
19S regulator: lid + base

- Lid: recognizes and processes ubiquitin-chain of the substrates
- Base: hexameric ring of ATPase, with loops that that move up (ATP) and down (ADP) to unfold and transport the substrates down to the cavity of 20S proteasome

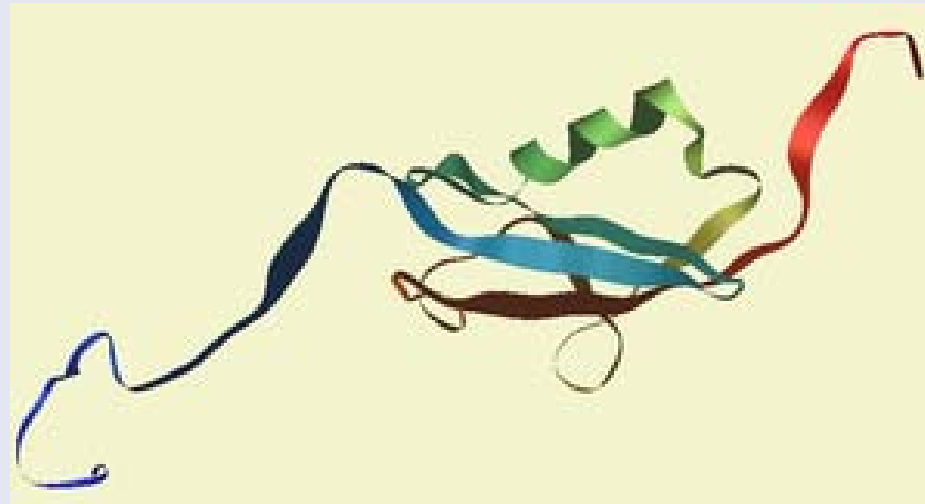
The Ubiquitin-Proteasome Degradation Pathway

Figure 31.11 Diagram of the ubiquitin-proteasome degradation pathway. Pink “lollipop” structures symbolize ubiquitin molecules.

Monoubiquitination:
chromatin remodeling
DNA repair
Transcription
Signal transduction
Endocytosis
Protein sorting..



Small Ubiquitin-Like Protein Modifiers Are Post-Translational Regulators



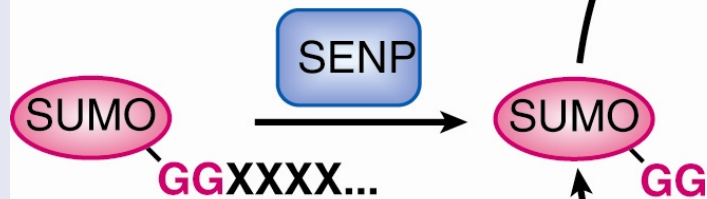
absoluteastronomy.com

- **Small ubiquitin-like protein modifiers (SUMOs)** are a highly conserved family of proteins found in all eukaryotic cells, about 100 a.a.
- **Sumoylation** alters the ability of the modified protein to interact with other proteins
- SUMOs are covalently ligated to lysine residues in target proteins by a three-enzyme conjugation system

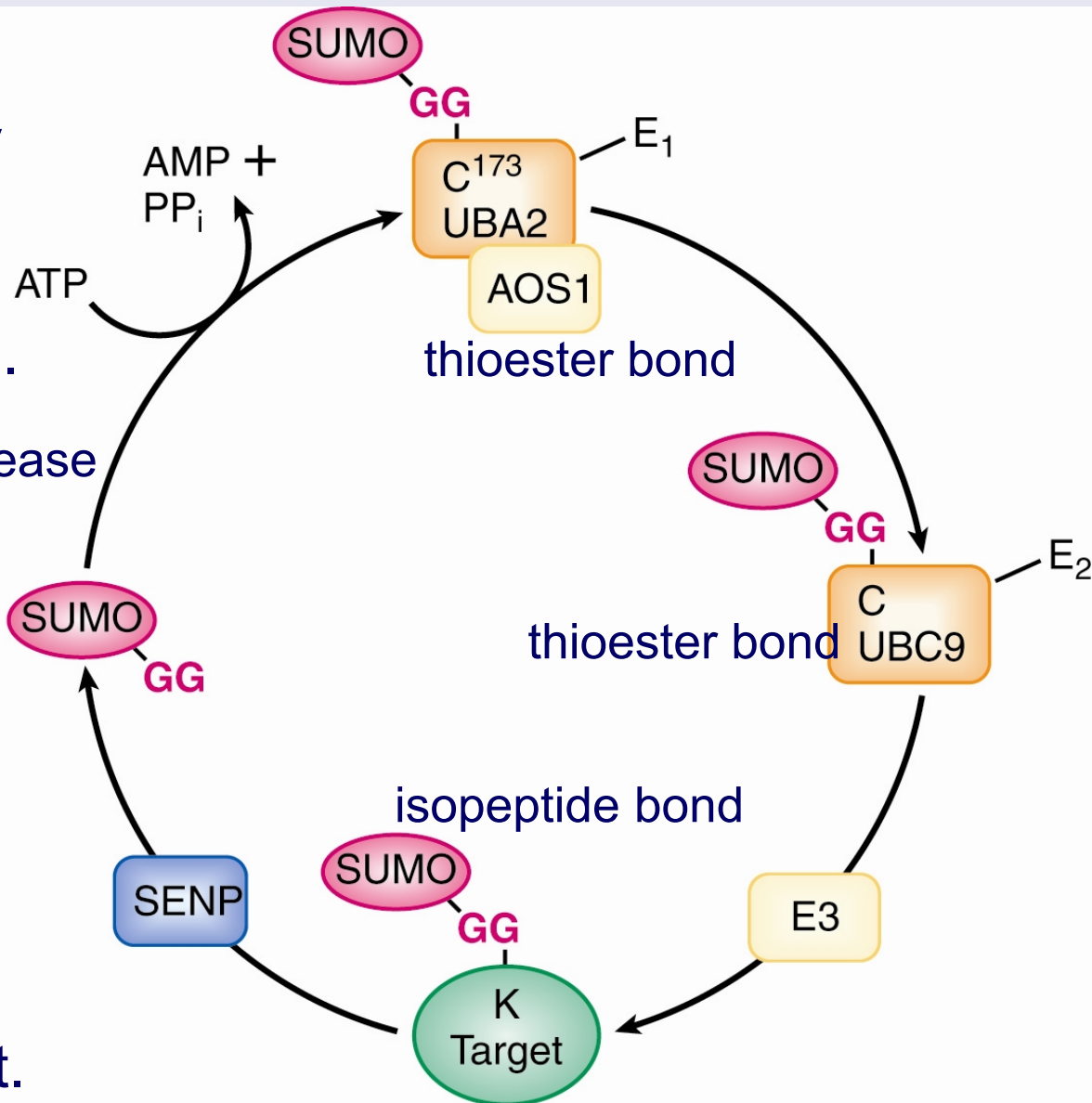
The Mechanism of Reversible Sumoylation

Figure 31.12 SUMOs are first proteolytically processed, then activated by E1, and then transferred to E2.

SENp: SUMO-specific protease



Finally, the SUMO is linked to the target protein by E3. SENp can remove the SUMO from the target.



The Molecular Consequences of Sumoylation

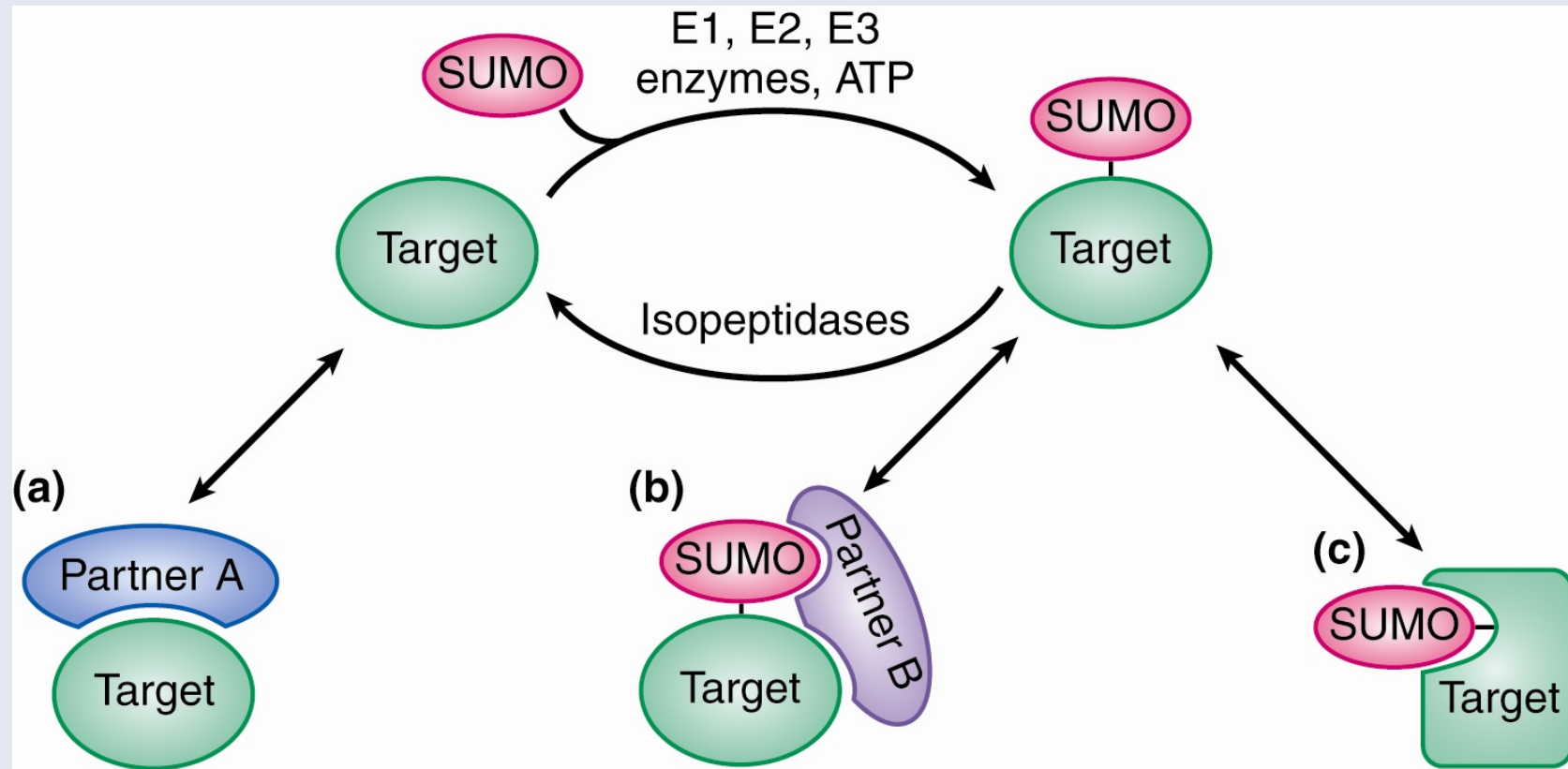


Figure 31.13 The sumoylation process can affect a modified protein in three ways. (a) binding interference (b) creating binding site (c) conformational change

- Ubc9 is the only known SUMO E2 enzyme, which recognize Ψ KXD/E

Ψ (psi) : an aliphatic branched amino acid (Leu)

K: the lysine to which SUMO is conjugated

X: any amino acid

- The recognition site should be in a relative unstructured part of a target protein or in an extended loop
- Sumoylation is involved in transcriptional regulation, chromosome organization, nuclear transport, and signal transduction.

The Structure of an E2 Enzyme:Target Protein Complex

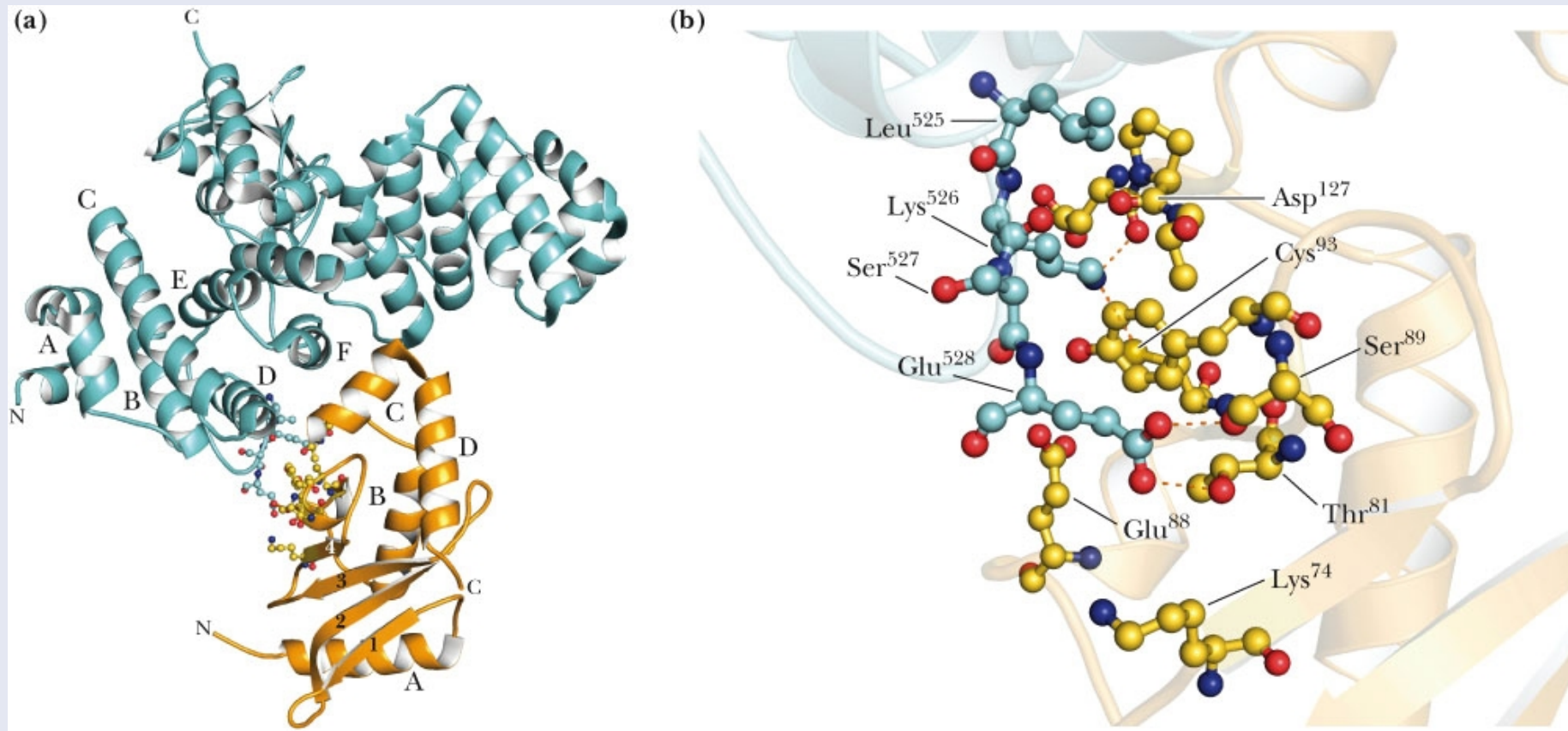


Figure 31.14 (a) The complex formed by the E2 enzyme, Ubc9 (yellow), and a target protein, RanGAP1 (blue). (b) In the Ubc9-RanGAP1 complex, the exposed loop of RanGAP1 lies in the binding pocket of Ubc9.

HtrA Proteases Also Function in Protein Quality Control

- **HtrA proteases** are a class of proteins involved in quality control that combine the dual functions of chaperones and proteasomes
- HtrA proteases are the only known protein quality control factor that is not ATP-dependent
- Htr: High temperature requirement; *E. coli* mutants do not grow at elevated T.

- Prokaryotic HtrA proteases act as chaperones at low temperatures (20°C), but as temperature rises they become a protease to remove misfolded or unfolded proteins
- **DegP** (from *E. coli*) is the best characterized HtrA protease
- DegP is localized in periplasm to oversee the quality of envelope protein. It has a central Serine protease domain (Asp-His-Ser catalytic triad) and two C-terminal PDZ domains.
- PDZ domains are involved in protein-protein interaction, binds to C-terminal 3 or 4 residues of target proteins.

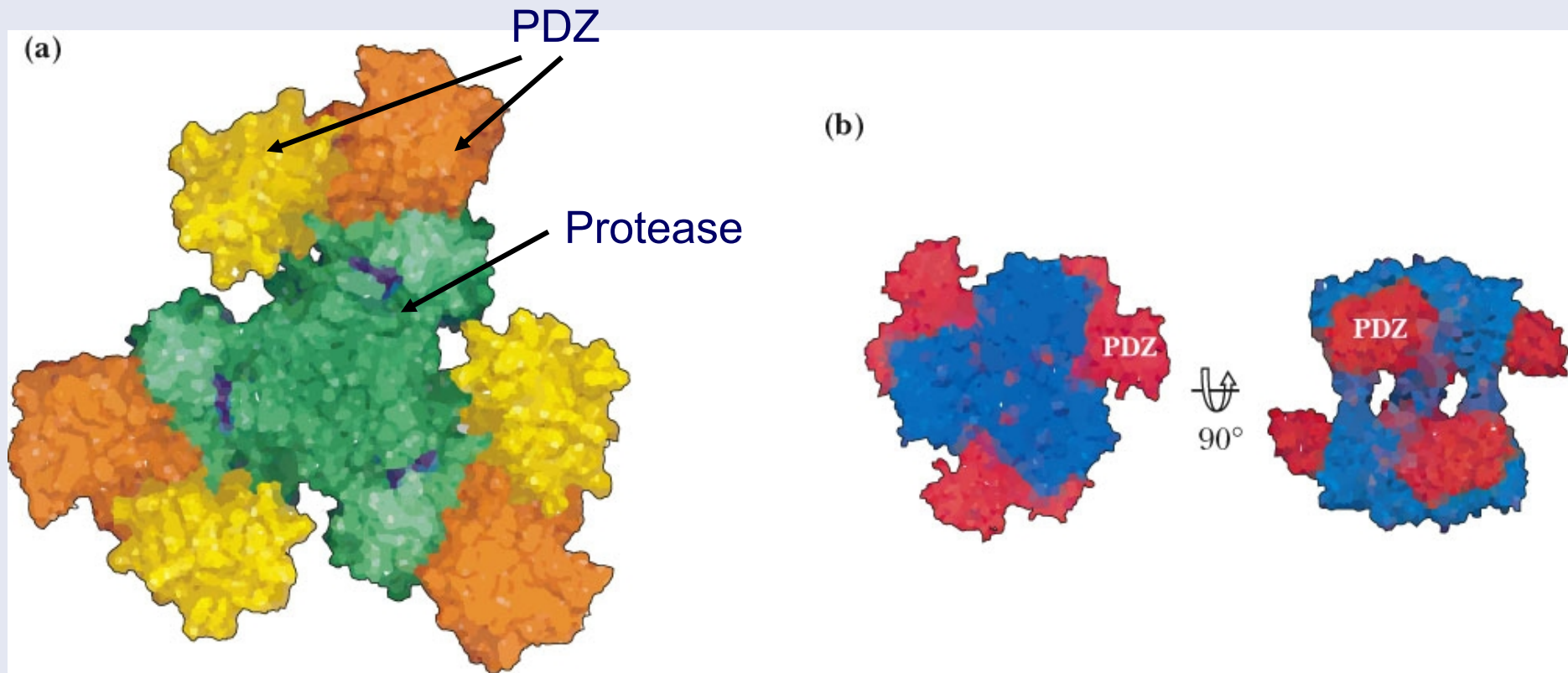
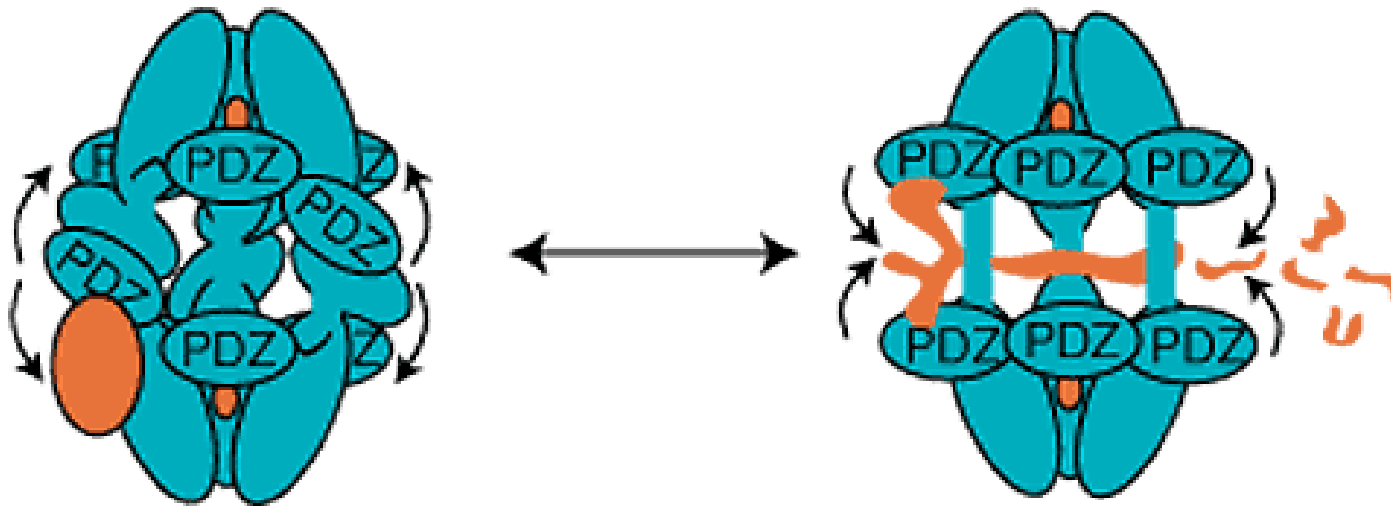


Figure 31.15 The HtrA protease structure. (a) **A trimer of DegP subunits** represents the HtrA functional unit. (b) **Two HtrA trimers** come together to form a hexameric structure in which the two protease domains form a rigid molecular cage (blue) and the six PDZ domains are like tentacles (red) that bind targets and control access into the protease cavity.

Protein unfolding and degradation by *E. coli* HtrA/DegP



PDZ domains first bind substrates tightly. Conformational change of PDZ unfolds the protein and transfers the extended polypeptide into the chamber (1.5 nm height). There the protein is degraded, but when the protease is in an inactive state at low temperature, the unfolded protein is extruded instead and can refold. *Nature Structural Biology* **9**, 410 - 412 (2002)