4. PROTEIN METABOLISM

chaper 20 Mathews-Van Holde, chapetr 27 Lenhinger

PROTEIN HOMEOSTASIS

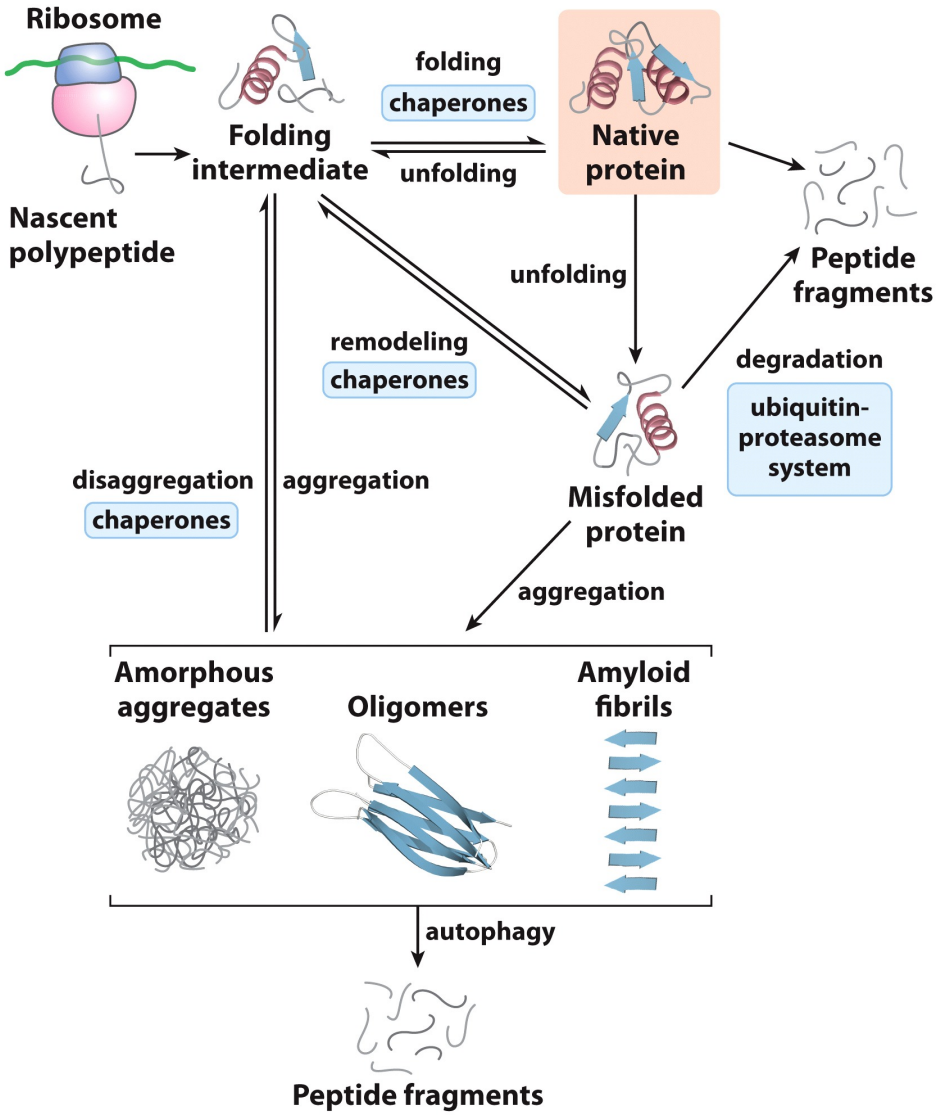

Figure 4-25
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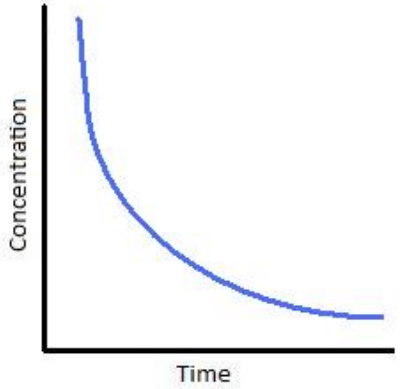
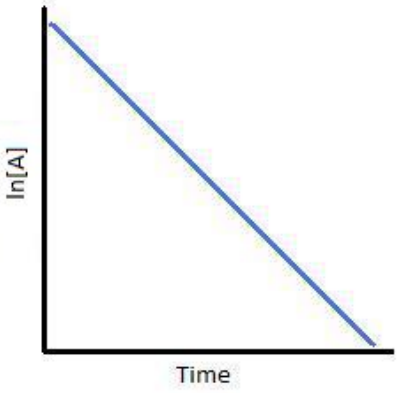
PROTEIN HOMEOSTASIS

- In a typical day, a person who is in nitrogen balance will consume 100 grams of protein, break down 400 grams of bodily protein, resynthesize 400 grams of protein, and excrete/catabolize 100 grams.
- Individual proteins exhibit tremendous variability in their metabolic lifetimes, from a few minutes to a few months.
- Protein degradation obeys first order kinetics. For a particular protein, individual molecules are degraded at random, such that a semilogarithmic plot of isotope remaining in a protein versus time is linear. Thus, we can determine the metabolic half-life of a particular protein. In a rat the average protein has a half-life of 1 or 2 days.
- Proteins in extracellular environments, such as digestive enzymes, polypeptide hormones, and antibodies, turn over quite rapidly, but proteins with predominantly structural roles, such as collagen of connective tissue, are much more stable.
- Enzymes catalyzing rate-determining steps in metabolic pathways are also short-lived. Protein breakdown may thus be an important regulatory mechanism for a protein.
- In cells, protein degradation appears to focus on proteins that have become chemically altered in some way.
- In bacteria, mutant proteins are degraded much more rapidly than their wild-type counterparts.





PROTEIN HOMEOSTASIS

Long-lived proteins might be particularly prone to accumulation of damage and thus have a crucial role in the functional deterioration of key regulatory processes during ageing.

Table 1 | **Known long-lived proteins and molecules**

Protein or molecule*	Age‡	Measure	Organism	Method	Refs
Eye lens crystallin	>70 years	Lifetime	Human	L-/D-Asp racemization	15
Collagen	117 years	Half-life	Human	L-/D-Asp racemization	12
Elastin	>78 years	Lifetime	Human	L-/D-Asp racemization	14
Enamel and dentine	>70 years	Lifetime	Human	L-/D-Asp racemization	16,17
Histones	223 days	Half-life	Mouse	Radio isotope pulse-labelling	77
	117 days	Half-life	Mouse	Radio isotope pulse-labelling	9
	218 days	Half-life	Rat	Radio isotope pulse-labelling	78
Nuclear pore proteins	>1 month	Lifetime	Worm	Radio isotope pulse-labelling	3
	>1 year	Lifetime	Rat	Stable isotope pulse-chase labelling and mass spectrometry	4
Myelin	95 days	Half-life	Rat	Radio isotope pulse-labelling	11
	>100 days	Half-life	Mouse	Radio isotope pulse-labelling	10
Myelin proteolipid protein	>100 days	Half-life	Mouse	Radio isotope pulse-labelling	10
REC8	Weeks	Lifetime	Mouse	Radio isotope pulse-labelling	18
mRNA	Possibly indefinite	Lifetime	Plant seed	Not determined	71
	>2 years	Half-life	Frog oocyte	Radio isotope pulse-labelling	72
Cholesterol	>18 months	Lifetime	Rabbit	Radio isotope pulse-labelling	74
Phospholipids	>192 days	Lifetime	Rabbit	Radio isotope pulse-labelling	73

*Listed are all known long-lived proteins or molecules. ‡Half-lives are listed when determined. Otherwise, the listed age is how old at least a subset of the molecules was confirmed to be (a 'lifetime' measurement).

Toyama, B. H.; Hetzer, M. W. (2013). "Protein homeostasis: Live long, won't prosper". Nature Reviews Molecular Cell Biology 14 (1): 55–61.

PROTEIN SYNTHESIS AND FOLDING

- Before a newly translated polypeptide can be active, it must be folded into the proper three-dimensional structure and it may have to associate with other subunits.
- At the beginning of translation, the first 30 amino acids are protected before they begin to emerge from the ribosome.
- There is good evidence that folding actually begins during translation and in most cases is nearly complete by the time the chain is released.
- This spontaneous folding during translation may be blocked or delayed, however, by chaperone proteins.

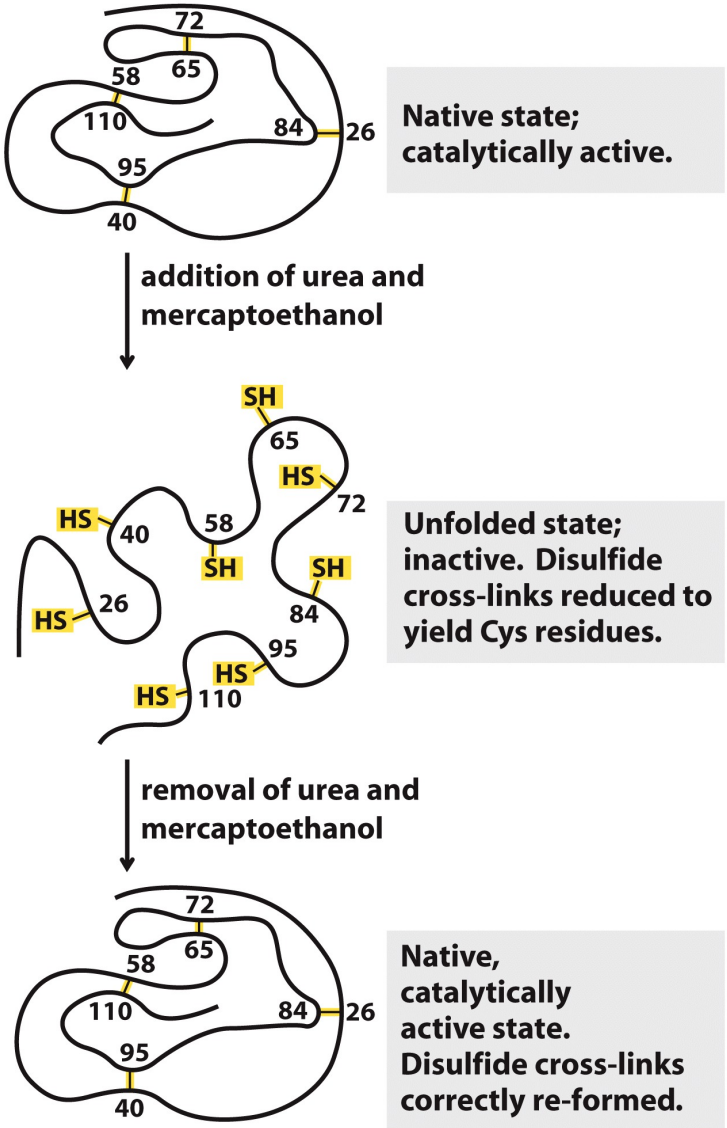


Figure 4-27
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PROTEIN FOLDING

All proteins have to fold under the same set of conditions in a living cell.

These conditions seem to be counterproductive for efficient folding, mainly because of the high temperature and the large number of non-native proteins present.

it seems surprising that cells are usually devoid of aggregated proteins.

1) Aggregation does occur *in vivo*, but its products are rapidly removed by cellular proteases. This would imply that cells waste a lot of energy to produce proteins that never become functional.

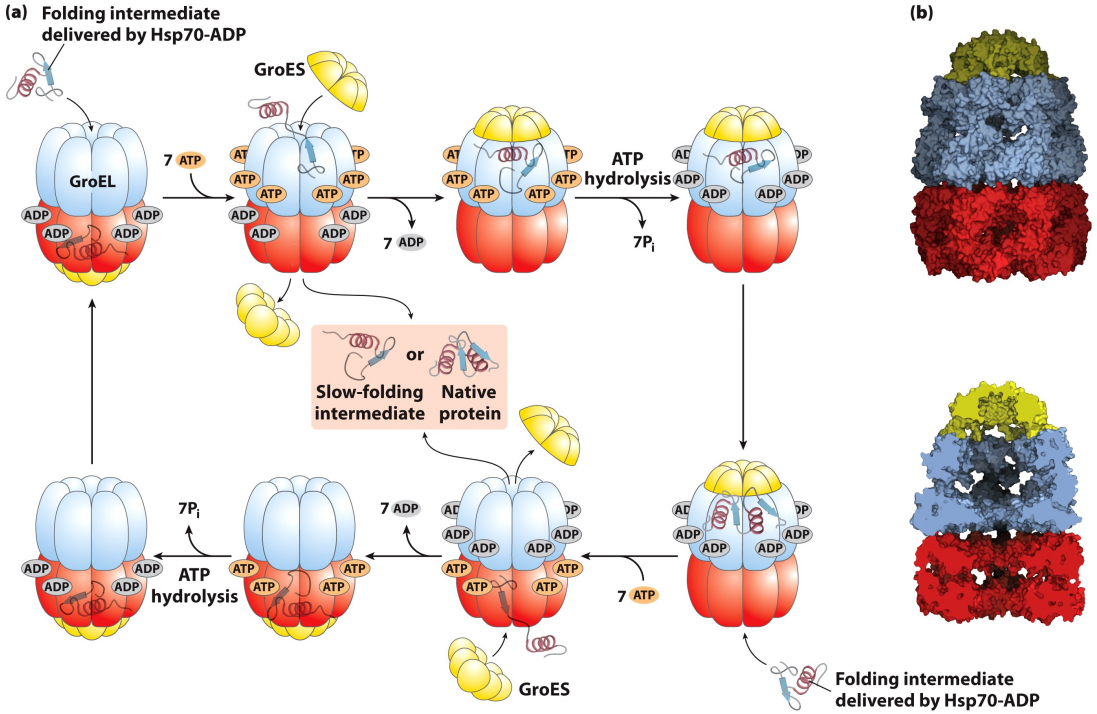
2) Cells have found a strategy of minimizing the aggregation of newly synthesized proteins in the first place. This has been achieved by complex protein machinery, the chaperones, which influence the spontaneous folding reaction of proteins, thus preventing aggregation.

PROTEIN FOLDING: MOLECULAR CHAPERONES

- Chaperonins are special proteins (also called molecular chaperones) that function to keep a newly synthesized protein from either improperly folding or aggregating.

- Aggregation is often a danger because the protein, released from the ribosome in an unfolded state, will have hydrophobic groups exposed. These will be tucked inside in normal folding, but when exposed they stand the chance of making hydrophobic interactions with other polypeptide strands and thereby aggregating.

- It is probable that only a fraction of the proteins made is processed via chaperonins. Some would be too large to be accommodated within the cavity. Others can seemingly fold safely on their own.



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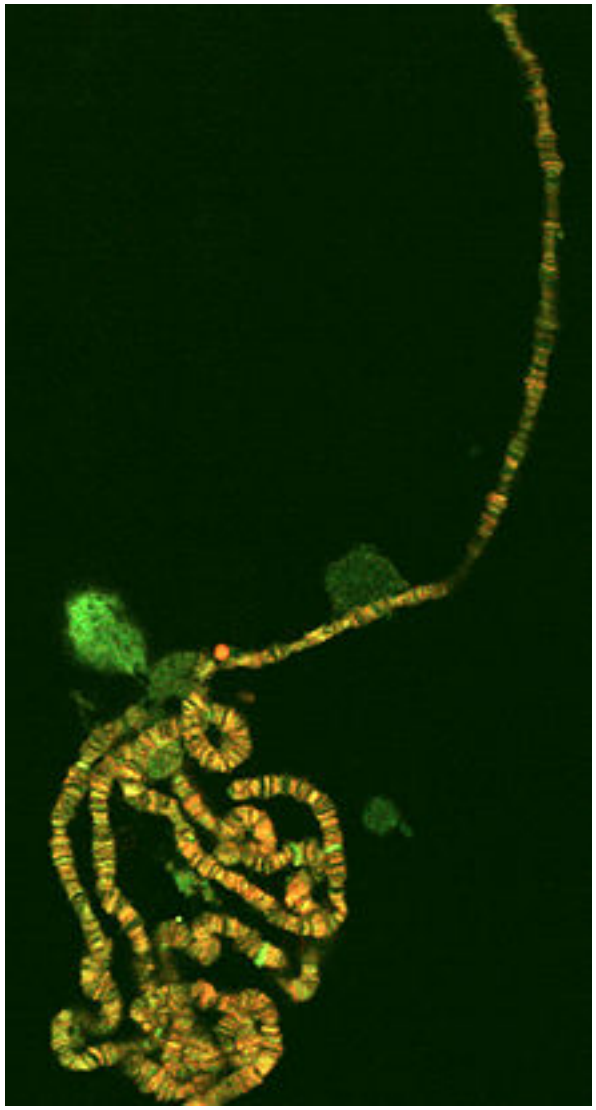
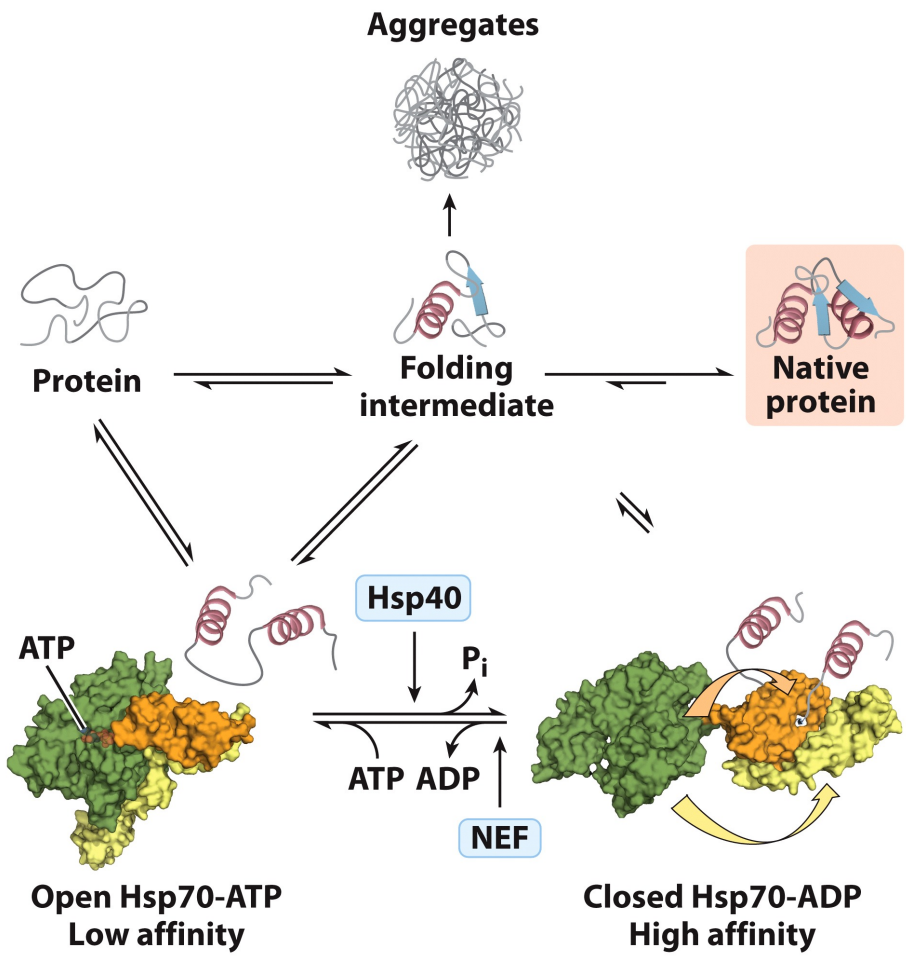
HEAT SHOCK PROTEINS (HSPs)

- In the early 1960s, F. Ritossa first discovered the heat shock (HS) response while observing the salivary cells of Drosophila melanogaster (Ritossa, 1962).
- It was noted that heating these cells induced puffs to form at various regions of the polytene chromosomes. Further analysis revealed that these puffs were actually areas of localized transcription that correlated to the increase of several families of proteins.
- This response was termed "heat shock response" because heat shock was the most common inducer used
- Heat shock proteins are now known to be induced when a cell undergoes various other types of environmental stresses like cold and oxygen deprivation.
- Heat shock proteins assist in the recovery from stress either by repairing damaged proteins (protein refolding) or by degrading them, thus restoring protein homeostasis and promoting cell survival.



PROTEIN FOLDING: MOLECULAR CHAPERONES

- Molecular chaperones are found in all compartments of a cell where folding or, more generally, conformational rearrangements of proteins occur.
- Although protein synthesis is the major source of unfolded polypeptide chains, other processes can generate unfolded proteins as well (high temperatures).
- This would result in loss of function of the affected proteins and in the accumulation of protein aggregates.
- The cell responds to this threat by producing increasing amounts of specific protective proteins, a phenomenon referred to as *heat-shock response or stress response.*
- *Many of these proteins were found to be molecular chaperones.*
- It is important to note that these molecular chaperones do not provide specific steric information for the folding of the target protein, but rather inhibit unproductive interactions and thus allow the protein to fold more efficiently into its native structure.



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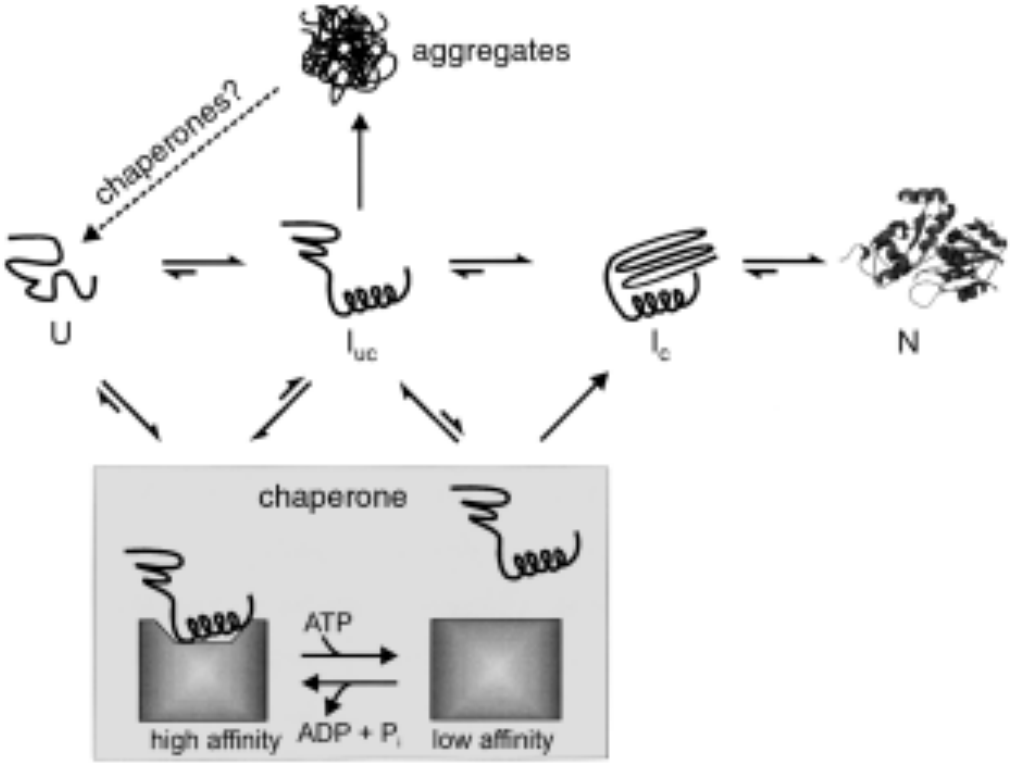
PROTEIN FOLDING: MOLECULAR CHAPERONES

- Protein biosynthesis as well as cellular stress results in the formation of unfolded polypeptides (U). These molecules fold via several intermediates (I_{uc}, I_c) with increasing structure complexity, until they reach the native, functional state (N).

- Some intermediates (I_{uc}) may expose hydrophobic surfaces that render them susceptible to aggregation. This reaction was thought to be irreversible, but recent results indicate that some chaperones may resolubilize aggregates.



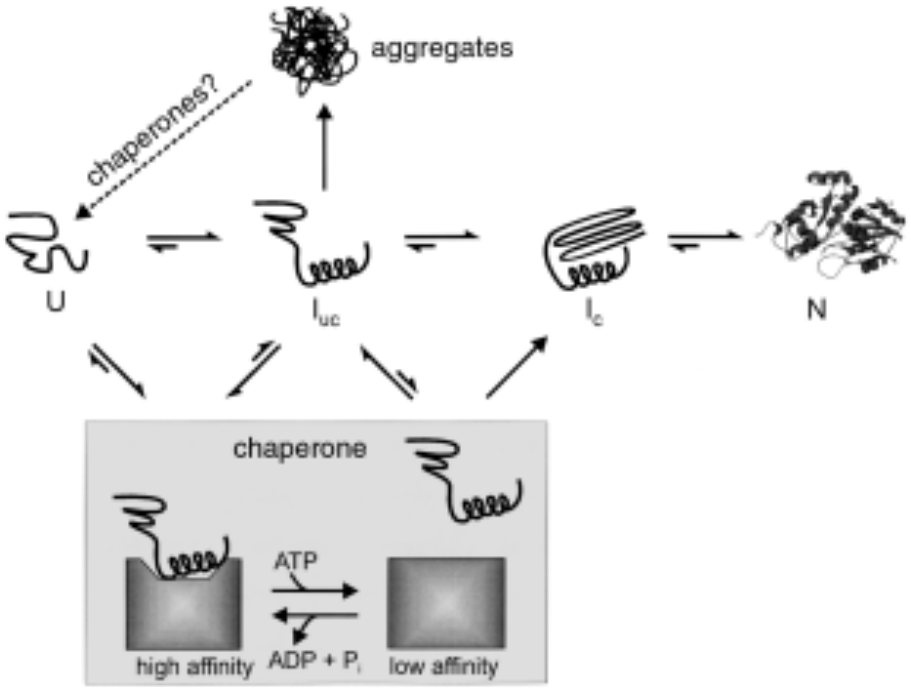
- Molecular chaperones interfere with the deleterious process of aggregation by binding to species I_{uc} and U.

PROTEIN FOLDING: MOLECULAR CHAPERONES

- This association not only blocks the hydrophobic patches on the bound polypeptides, but also decreases the concentration of aggregation-prone molecules, thereby slowing down aggregation.

- In many cases, an ATP-mediated conformation change in the chaperone triggers the dissociation of the bound polypeptide. A fraction of the released molecules may fold into a committed state (Ic), which no longer requires the assistance of the chaperone, whereas the remaining uncommitted (uc) molecules rebind and participate in another chaperone cycle.

- Depending on the type of chaperone, conformation changes in the polypeptide may occur during its association with the chaperone.



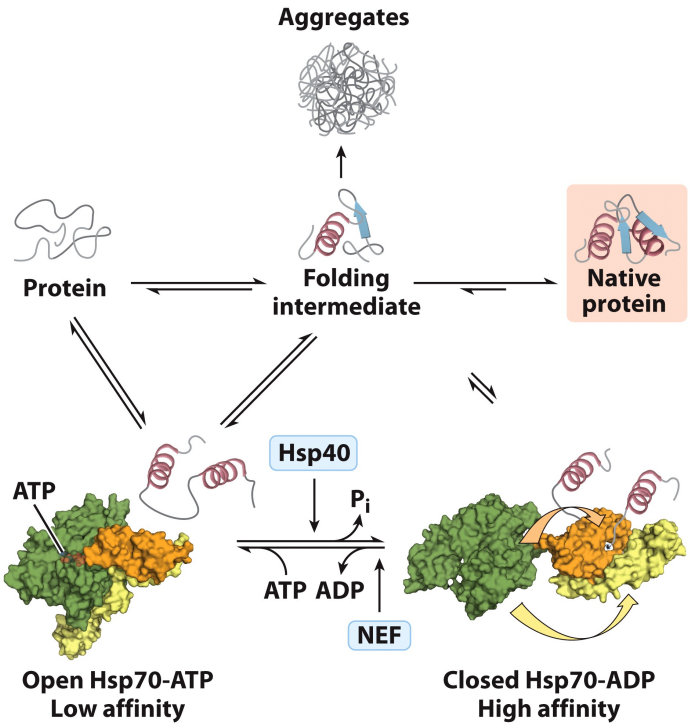


Figure 4-30
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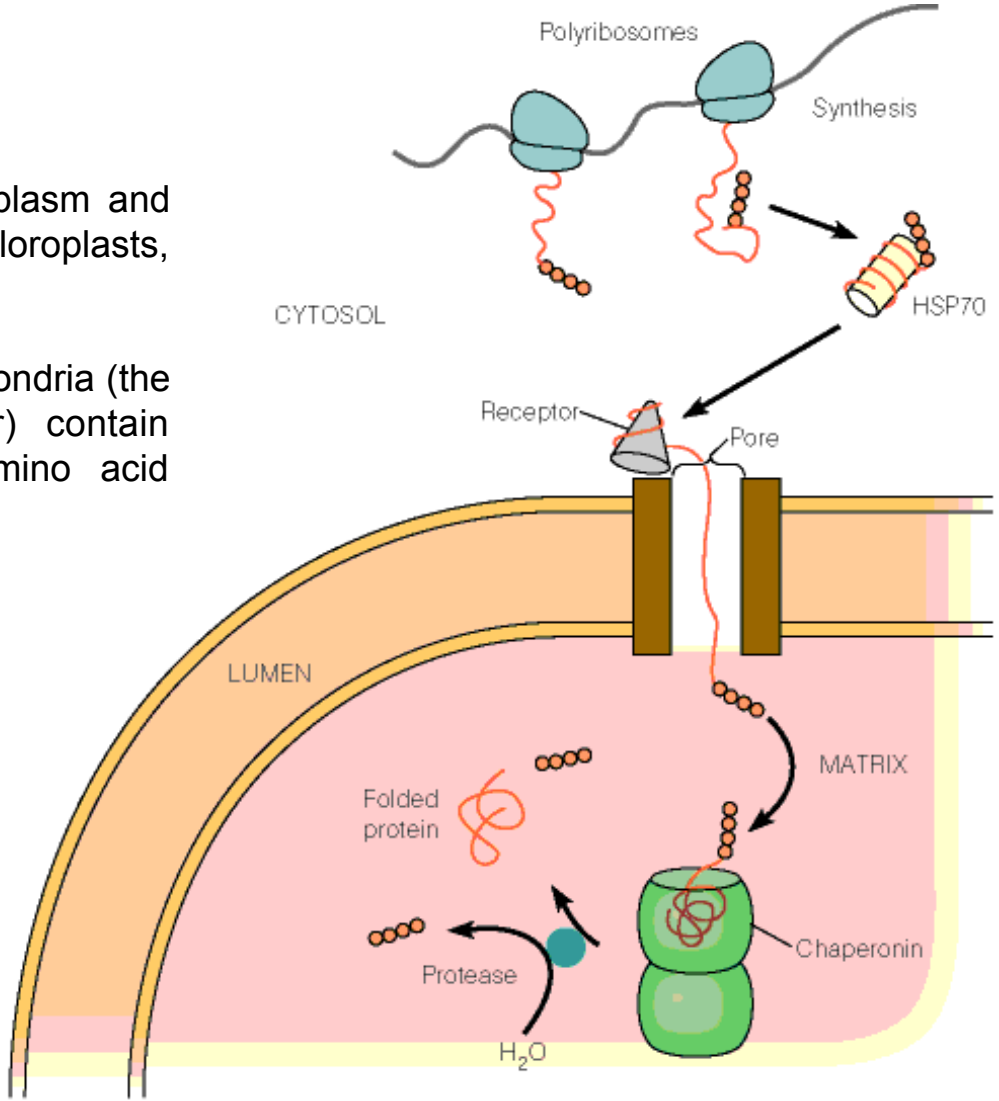
PROTEIN TARGETING

- Eukaryotic cells contain several organelles.
- Each organelle, in turn, requires specific proteins, only a few of which are synthesized within the organelles themselves.

Proteins synthesized in the cytoplasm

- These include proteins destined for the cytoplasm and those to be incorporated into mitochondria, chloroplasts, or nuclei.
- Newly synthesized proteins targeted to mitochondria (the chloroplast mechanism is probably similar) contain specific signal sequences (i.e., specific amino acid sequences) at their N-terminal ends.



A schematic view of steps in the delivery of a protein, synthesized in cytosol, to the matrix of an organelle.

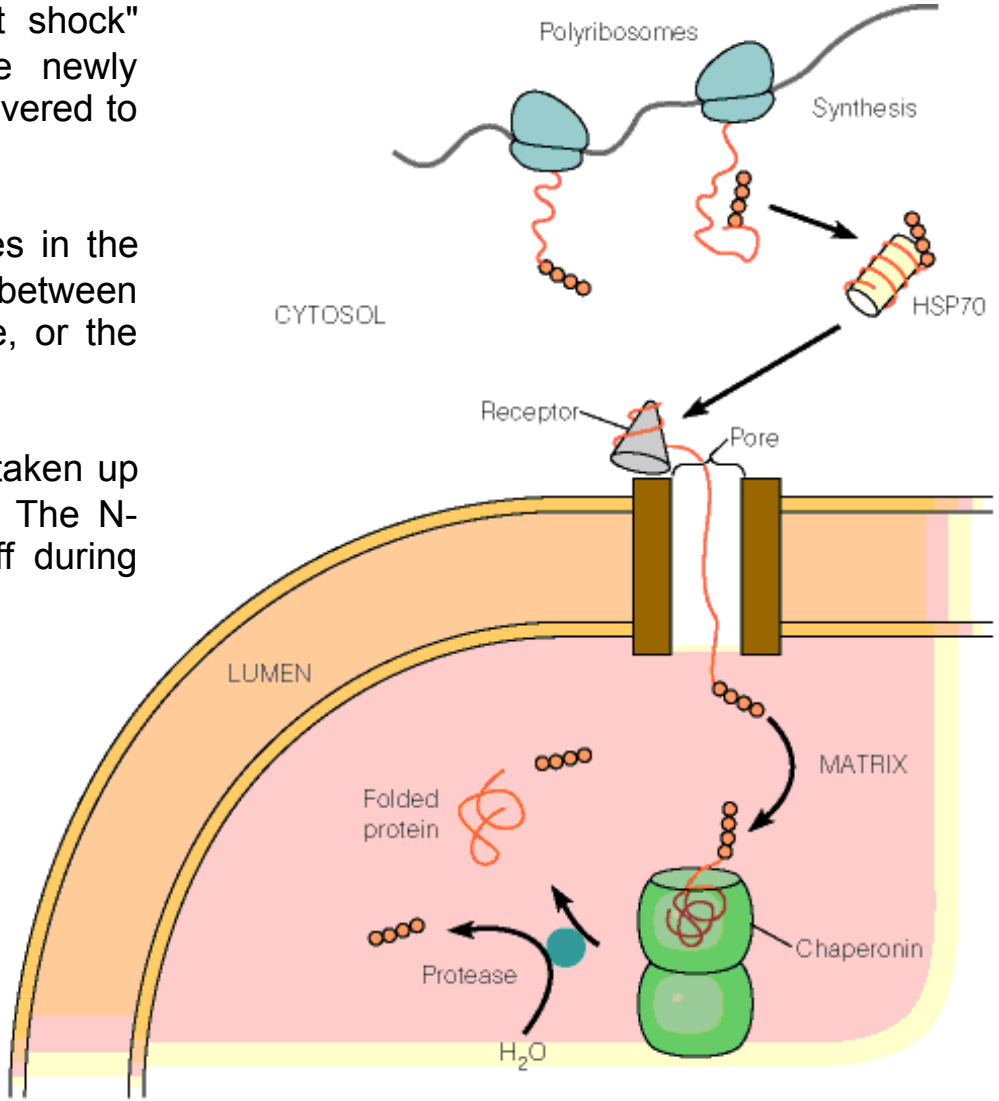
PROTEIN TARGETING

- The signal sequences probably aid in membrane insertions, but they also signal that these polypeptides will interact with a particular class of chaperonins.

- These chaperones are members of the "heat shock" HSP70 family, which act to insure that the newly synthesized protein remains unfolded and is delivered to a receptor site on the organelle membrane.

- The unfolded protein then passes through gates in the inner and outer membranes which discriminate between proteins destined for the lumen, the membrane, or the matrix.

- If it passes into the matrix, the protein may be taken up by intra-organelle chaperonins for final folding. The N-terminal targeting sequence is also cleaved off during this transport



A schematic view of steps in the delivery of a protein, synthesized in cytosol, to the matrix of an organelle.

PROTEIN TARGETING

Proteins Synthesized on the rough endoplasmic reticulum

- Proteins destined for cellular membranes, lysosomes, or extracellular transport use a special distribution system involving the rough endoplasmic reticulum (RER) and the Golgi complex.
- The RER is a network of membrane-enclosed spaces within the cytoplasm, which is heavily coated on the outer, cytosolic surface with polyribosomes, giving the membrane its rough appearance.
- The Golgi complex resembles the RER in that it is a stack of thin, membrane-bound sacs, but the Golgi sacs are not interconnected, nor do they carry polyribosomes on their surfaces.
- The Golgi complex acts as a "switching center" for proteins with various destinations.

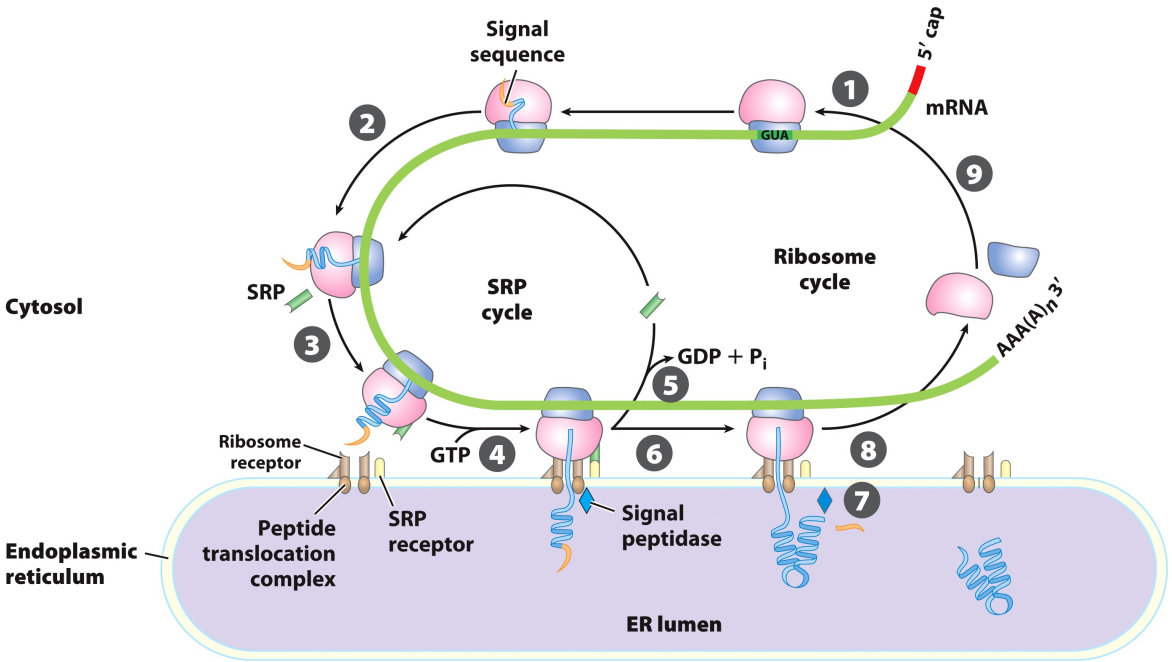


Figure 27-38
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PROTEIN TARGETING

Proteins to be directed to their destinations via the Golgi complex are synthesized by polyribosomes associated with the RER as follows:

1. Synthesis begins when an N-terminal hydrophobic signal sequence is synthesized by ribosomes on cytoplasmic mRNA.

2. Signal sequence is the first to be synthesised as it is at the N-term of the new protein.

3. Signal recognition particles (SRPs), containing several proteins and a small (7SL) RNA, recognize the signal sequences of the appropriate nascent proteins and bind to them as they are being extruded from the ribosomes.
The SRP temporarily halts translation through binding of GTP, so that no more than the N-terminal signal sequence extends from the ribosome and recognizes a docking protein in the RER membrane.

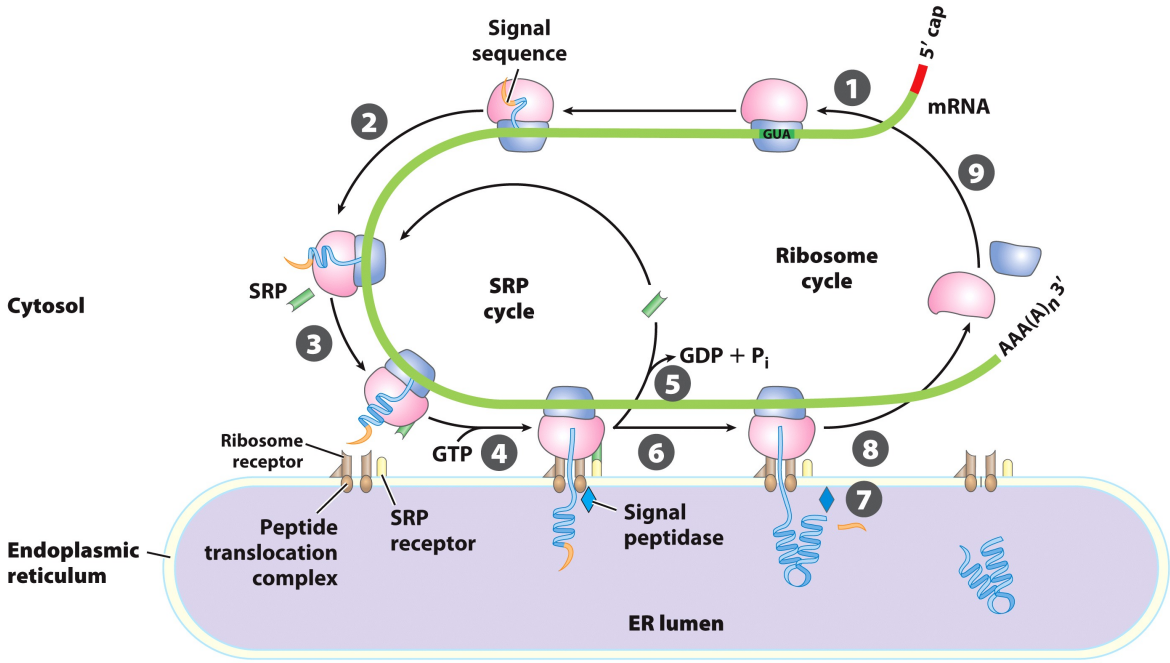

Figure 27-38
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PROTEIN TARGETING

4. The GTP-bound SRP directs the ribosome to the **peptide translocation complex** on the RER, and the signal sequence is inserted into the RER membrane.

5. The SRP is released in a step driven by GTP hydrolysis, allowing translation to resume.

6. The protein being synthesized is actually pulled by the peptide translocation complex through the membrane by an ATP-dependent process.

7. Before translation is complete, signal sequences are cleaved from some proteins by an RER-associated protease. These proteins are released into the lumen of the RER and further transported. Proteins that will remain in the endoplasmic reticulum have resistant signal peptides and thereby remain anchored to the RER membrane.

8. Ribosome dissociates

9. and it is recycled

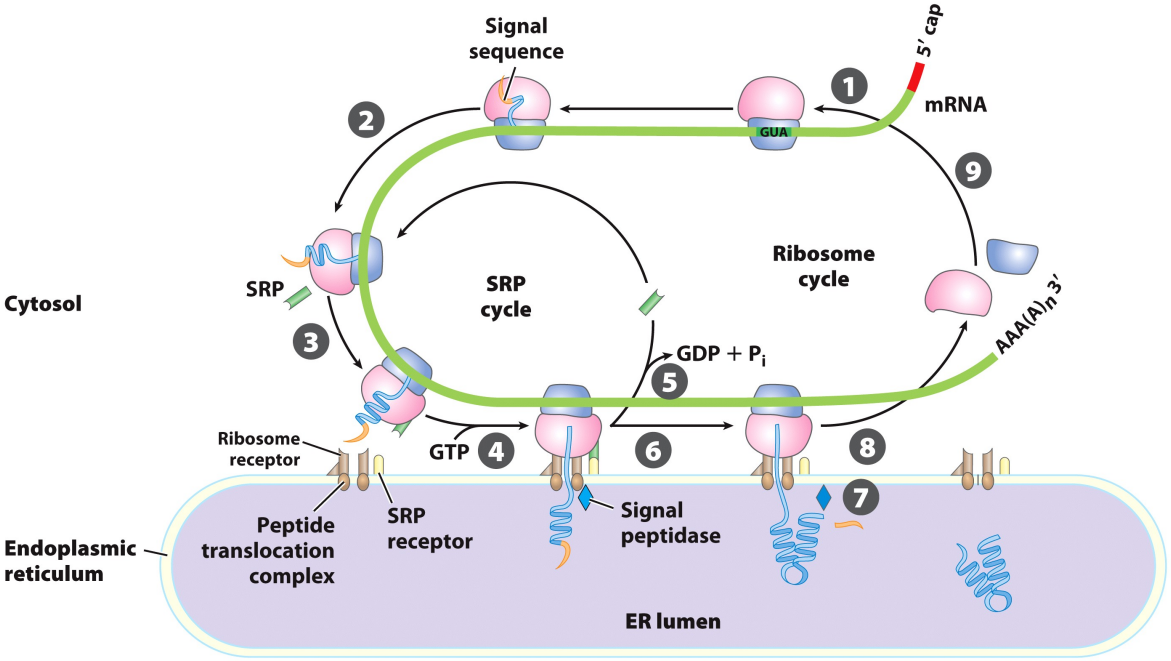

Figure 27-38
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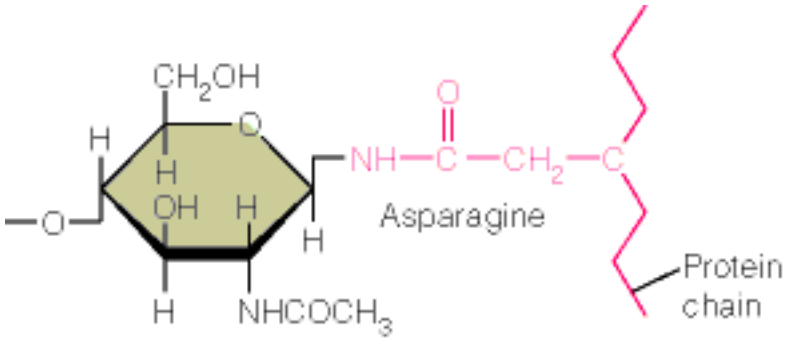
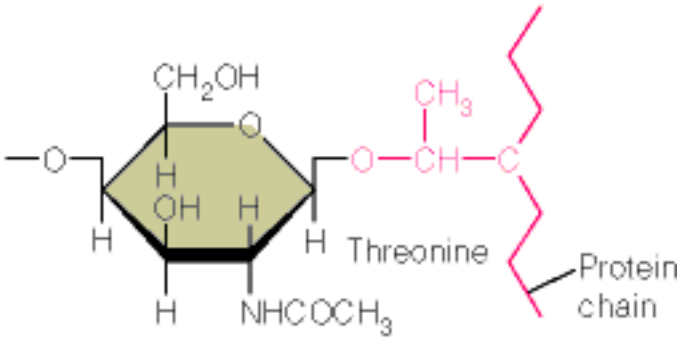
PROTEIN TARGETING

Role of the RER

- In the lumen of the RER, proteins undergo the first stages of glycosylation.
- Covalent binding of carbohydrate to protein or lipid brings about large changes in the physical properties of these substances that allow them to serve specialized biochemical functions.
- Sulfated polysaccharides in glycoproteins, for example, are effective biological lubricants and linking carbohydrates to lipids allows them to be inserted into membranes.
- Mammalian glycoproteins are classified as O-linked or N-linked.
- N-linked glycoproteins contain an N-acetylglucosamine residue linked to the amide nitrogen of an asparagine residue.
- The most common O-linkage involves a terminal N-acetylgalactosamine residue in the oligosaccharide linked to a serine or threonine residue of the protein.



***N*-Acetylglucosamine**



***N*-Acetylgalactosamine**

PROTEIN TARGETING

Role of RER

- In the lumen of the RER, proteins undergo the first stages of glycosylation.

1-2. The first seven sugars are transferred to dolichol phosphate from nucleotide diphosphate sugars, UDP-N-acetylglucosamine and GDP-mannose. Each reaction is catalyzed by a separate glycosyltransferase. The antibiotic, tunicamycin, inhibits synthesis of all N-linked glycoproteins by inhibiting the first enzyme in the process.

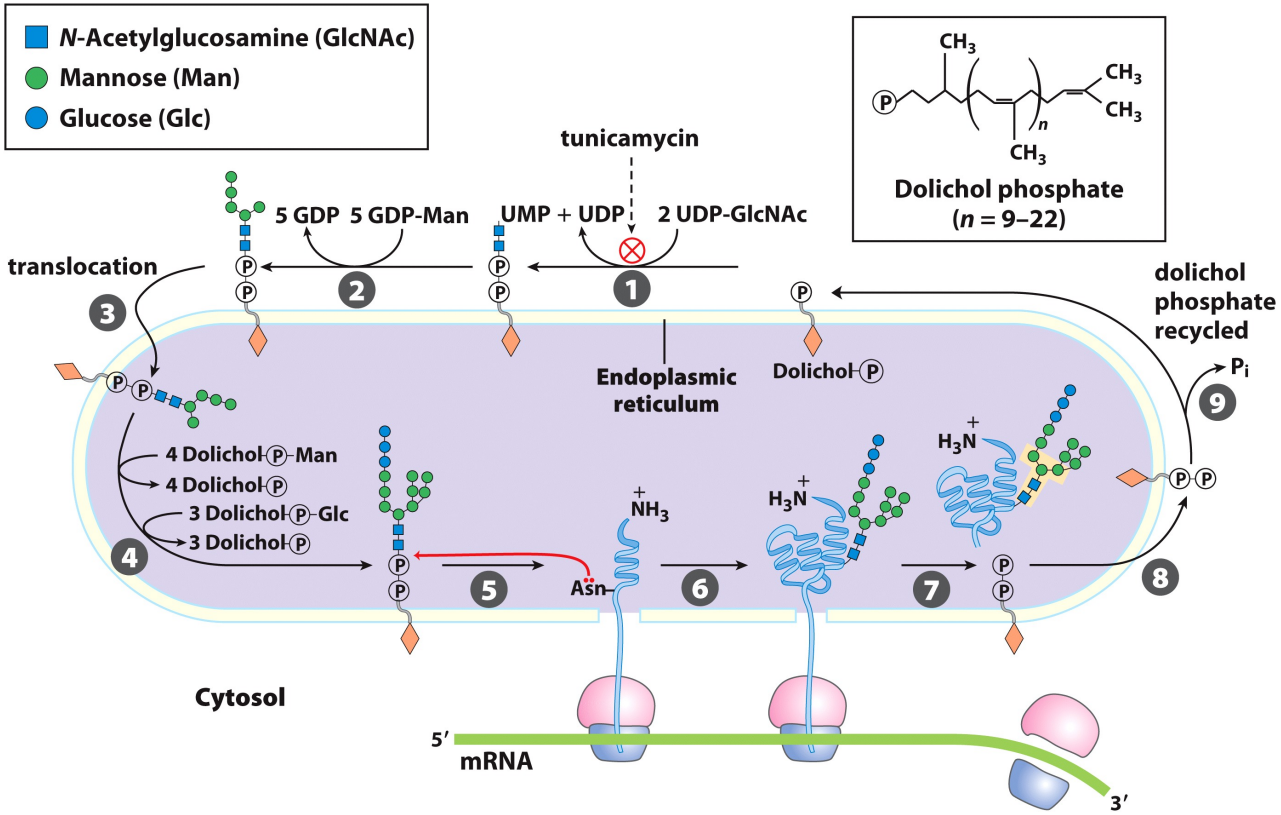


Figure 27-39
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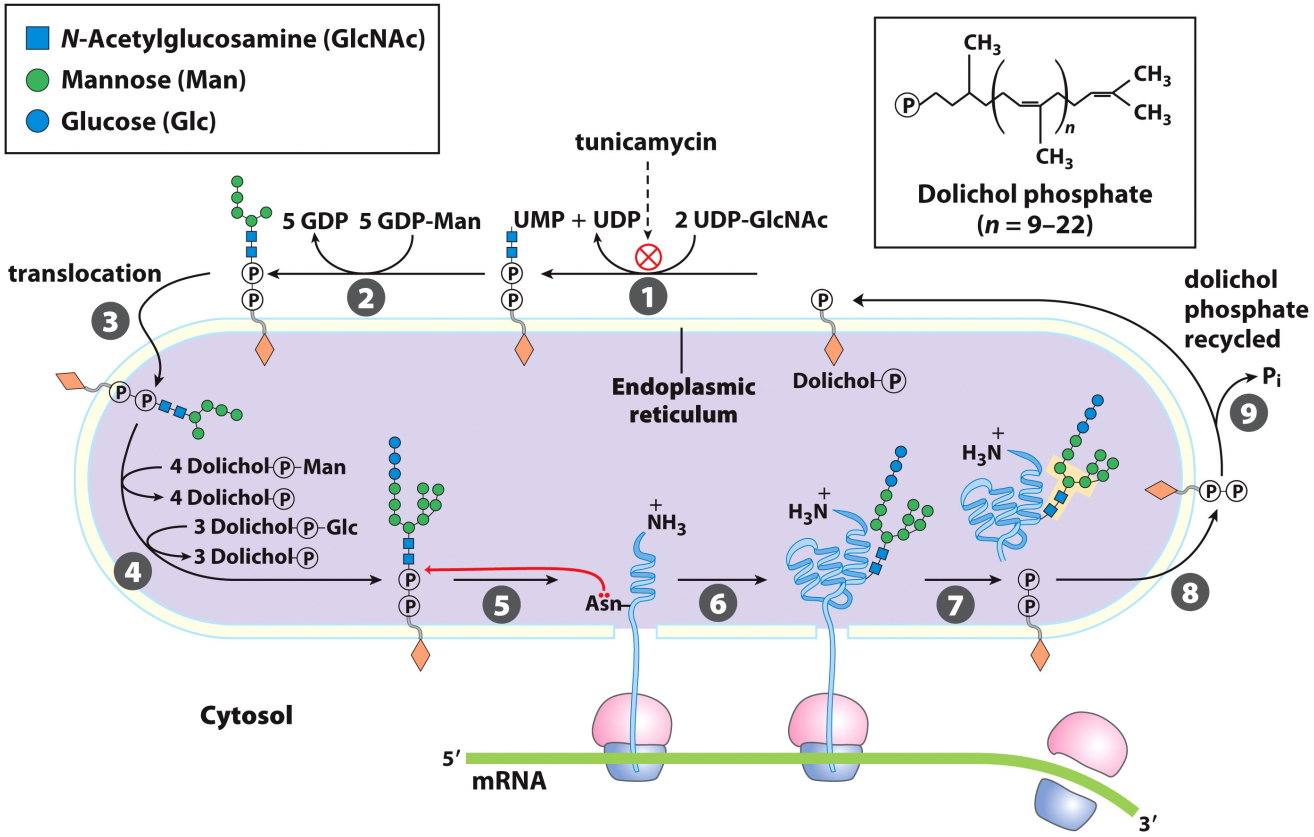
PROTEIN TARGETING

Role of the RER

- In the lumen of the RER, proteins undergo the first stages of glycosylation.

3-4. The next seven reactions utilize dolichol-linked sugars as substrates. These are dol-P-mannose and dol-P-glucose, which are, in turn, synthesized from dol-P plus GDP-mannose and UDP-glucose, respectively. During this stage, the lipid-linked intermediate is translocated from the exterior surface of the ER-membrane to the luminal, or interior side.

5. The oligosaccharide unit is transferred to a polypeptide acceptor in a reaction catalyzed by a specific oligosaccharyltransferase. The acceptor site is an asparagine residue in the sequence Asn-X-Ser/Thr, where X is any amino acid. The acceptor site must be accessible in a loop or a bend in the polypeptide chain.



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PROTEIN TARGETING

Role of RER and Golgi

6-8. Processing of the oligosaccharide-linked polypeptides begins in the lumen of the rough endoplasmic reticulum and continues as the nascent glycoprotein moves into the smooth ER and ultimately through the Golgi apparatus. In virtually all cases, processing begins with removal of the three glycosyl residues in the rough ER, followed by removal of some of the mannosyl residues in the Golgi apparatus. Complex glycoproteins are further processed by addition of N-acetylglucosamine, followed by further trimming of mannosyl residues. Fucose, galactose, and Sialic acid residues are added from appropriate nucleotide-linked sugars by specific glycosyl-transferases.

- Oligosaccharide chains help direct glycoproteins to appropriate intracellular destinations.

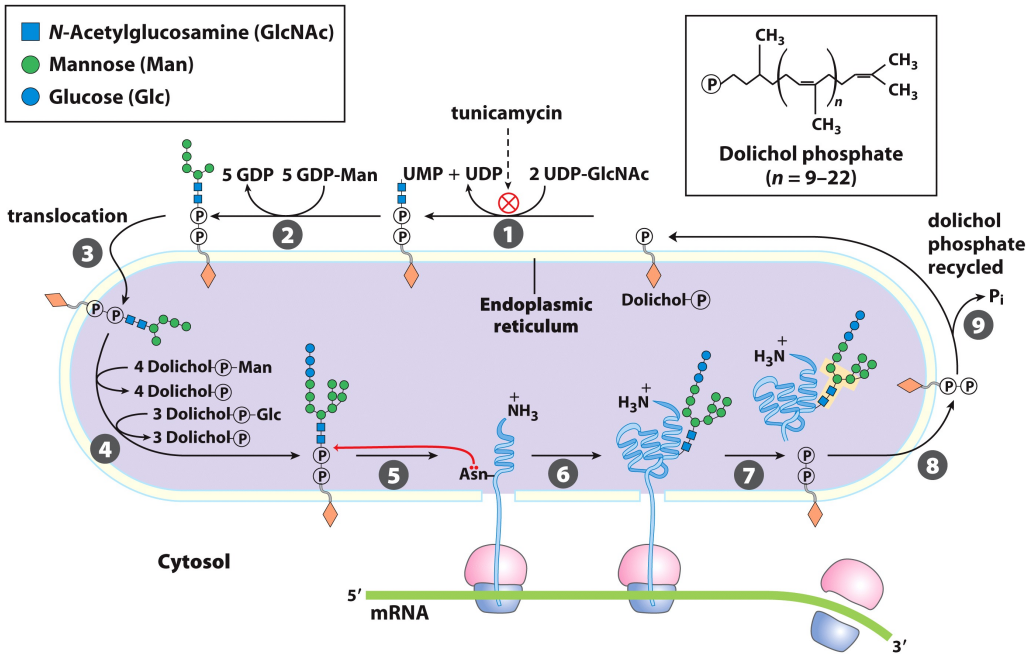

Figure 27-39
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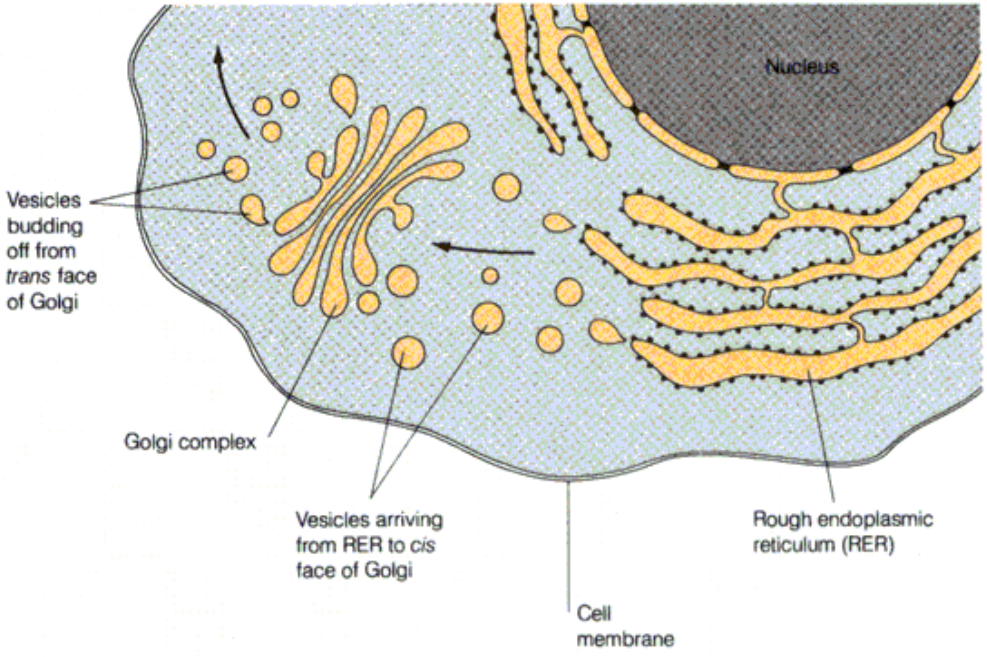
PROTEIN TARGETING

Role of the Golgi complex

- In the lumen of the RER, proteins undergo the first stages of glycosylation.
- Vesicles carrying these proteins bud off the RER and move to the Golgi complex where the carbohydrate moieties of glycoproteins are completed.
- The membrane sacs of the Golgi complex are a multilayer arena for sorting modified proteins.
- Vesicles from the RER enter at the *cis* face of the Golgi complex (that closest to the RER) and fuse with the Golgi membrane.
- Proteins are then passed, again via vesicles, to the intermediate layers.
- Finally, vesicles bud off from the *trans* face of the Golgi complex (that furthest from the RER) to form lysosomes, peroxisomes, or glyoxysomes or to travel to the plasma membrane.



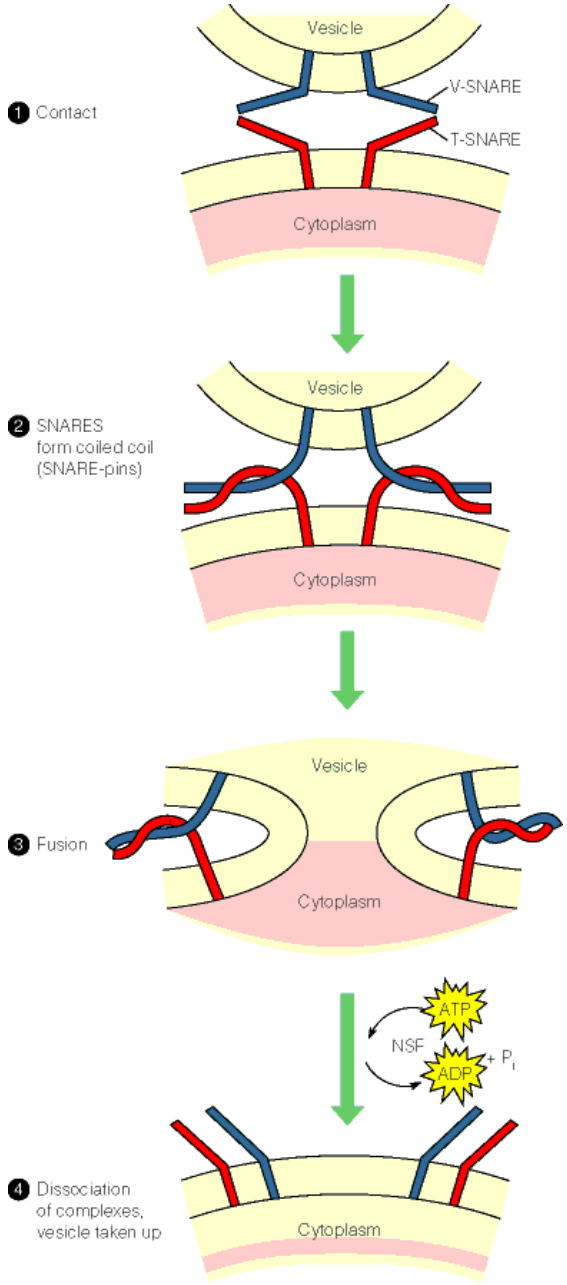
Transfer from the rough endoplasmic reticulum (RER) to the Golgi complex.

PROTEIN TARGETING

Vesicle targeting

- Vesicle transport of proteins from the Golgi complex requires high specificity in targeting, for transport of vesicles to the wrong destinations would cause cellular chaos.

- Each kind of protein carbon packed in a vesicle is marked by specific vesicle membrane proteins. In some cases, target membranes contain complementary proteins (called SNARES), which interact with the vesicle and cause membrane fusion and accurate delivery of the cargo proteins.



A schematic, and somewhat hypothetical, view of SNARE-pin fusion.

PROTEIN TARGETING

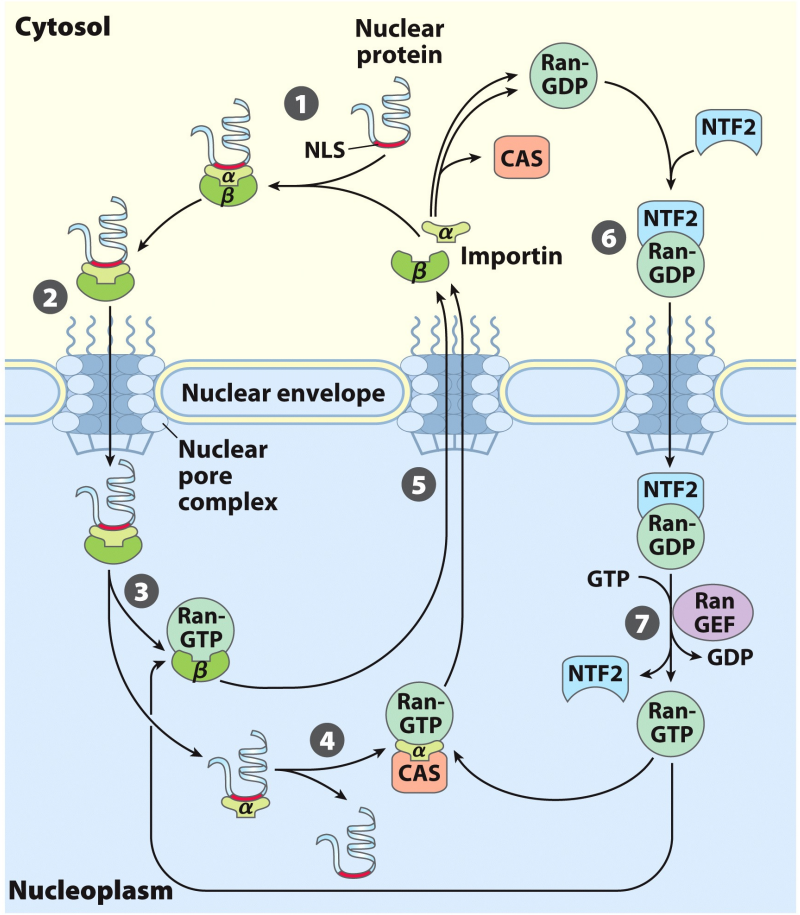


Figure 27-42a
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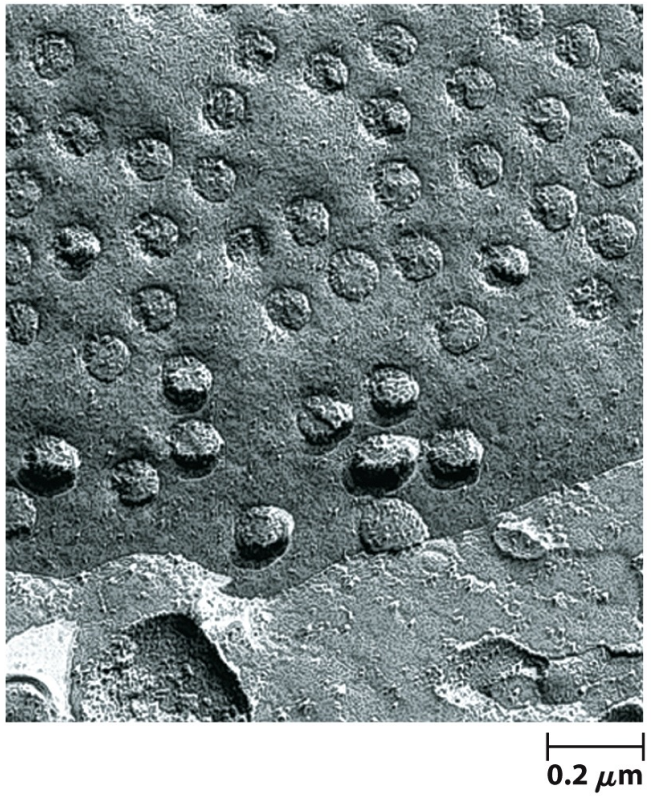

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NLS =nuclear localization signal

Nuclear pore complex is one of the biggest in the cell (Mr ≈ 5 x 10^7), constituted by multiple compies of more than 30 different proteins.

PROTEIN TARGETING

Figure 27-43

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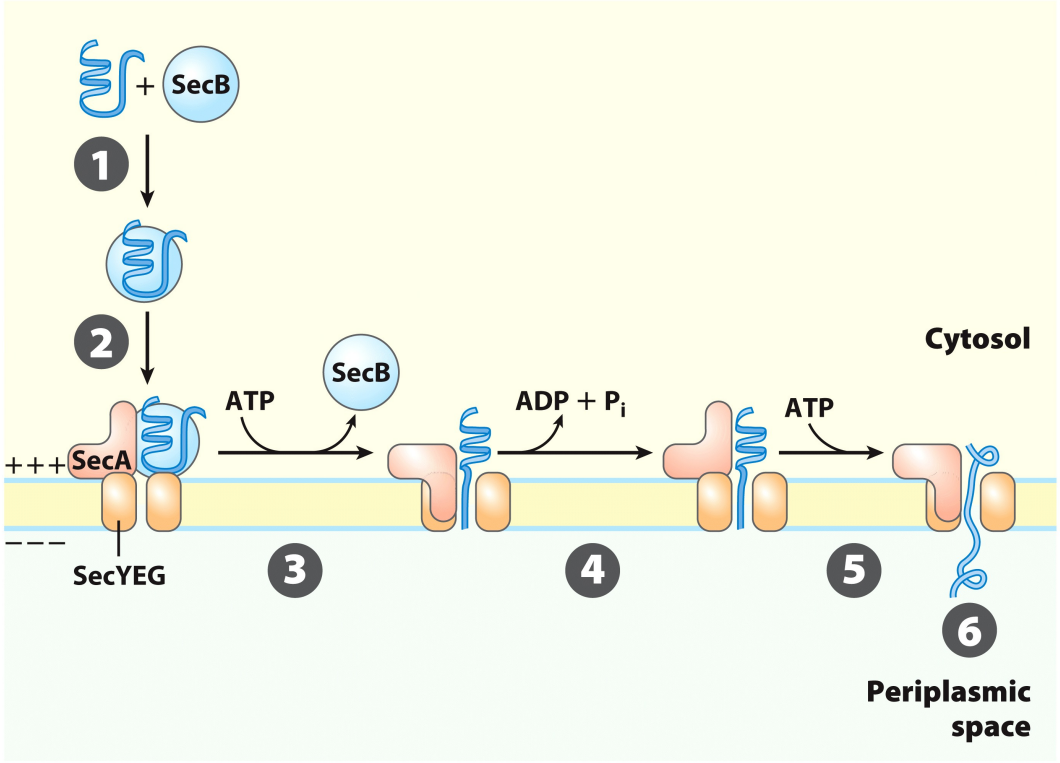
PROTEIN TARGETING



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Protein export model in bacteria. 1) A newly synthesized polypeptide binds to chaperon SecB that 2) trigger it to SecA associated to the translocation complex SecYEG on bacterial membrane. 3) SecB is released and SecA inserted in the membrane forcing 20 aa of the protein to be exported to wedge in the translocation complex. 4) ATP hydrolysis from SecA gives the energy for a conformational change that promotes SecA detachment from the membrane and the release of the polypeptide. 5) SecA binds another ATP molecule and another 20 aa fragmente is pushed through the membrane. 4) and 5) are repeated until 6) the entire protein pass through the membrane.

PROTEIN DEGRADATION

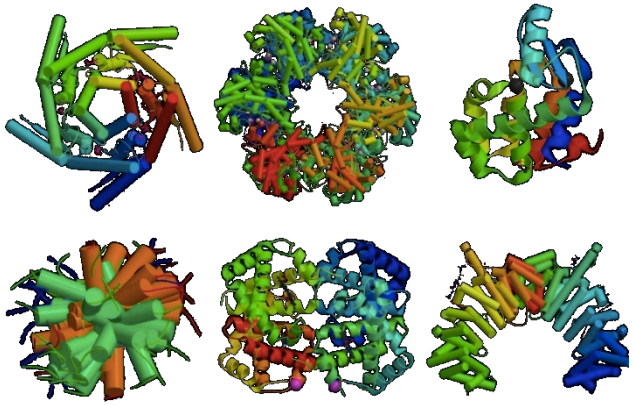
Lysosomal:

- 80-90%
- Most extracellular proteins
- Uptake (autophagy) into lysosomes (secretory vescicles in the cytoplasm);
- Enzyme degradation (hydrolysis)
- Basal degradation – non-selective
- Degradation under starvation

Non-Lysosomal:

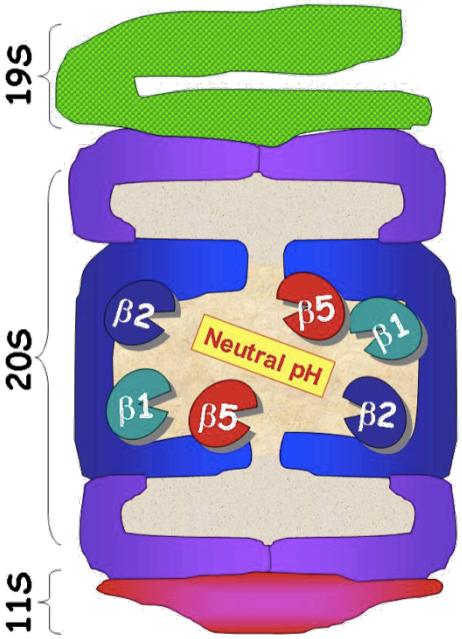
- 10-20%
- Intracellular proteins
- Cell organelles
- Some extracellular proteins
- Tagging of proteins to be degraded
- Recognition of proteolytic system
- Large (26S) multiprotein complex (28 subunits)
- Degrades ubiquitinated proteins

PROTEIN DEGRADATION

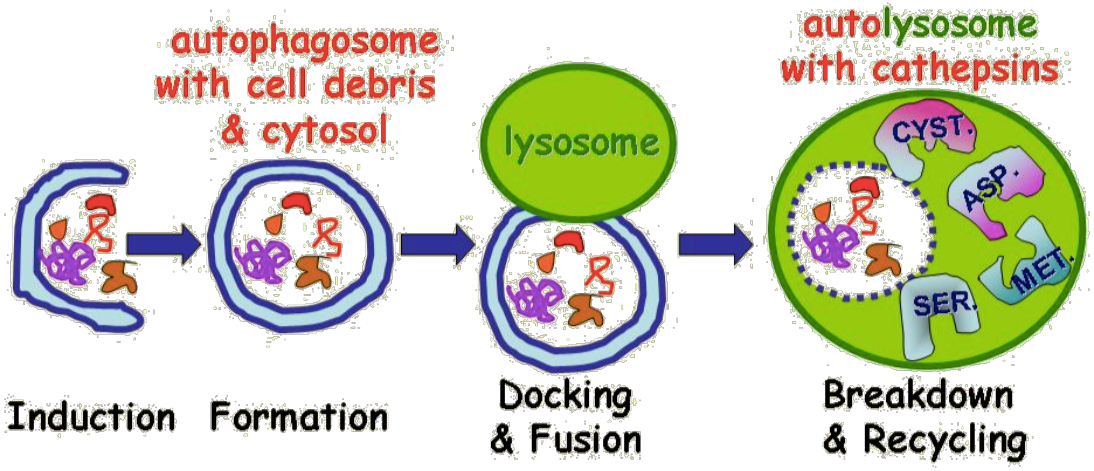


10-20%

80-90%



26S PROTEASOME



AUTOPHAGY/LYSOSOME PATHWAY

LYSOSOMAL DEGRADATION

- Digests
  - ingested materials
  - obsolete cell components
- Degrades macromolecules of all types
  - Proteins
  - Nucleic acids
  - Carbohydrates
  - Lipids

- Lysosomes degrade extracellular proteins that the cell incorporates by endocytosis.

- Lysosomes can also degrade intracellular proteins that are enclosed in other membrane-limited organellas.

- In well-nourished cells, lysosomal protein degradation is *non-selective* (non-regulated).

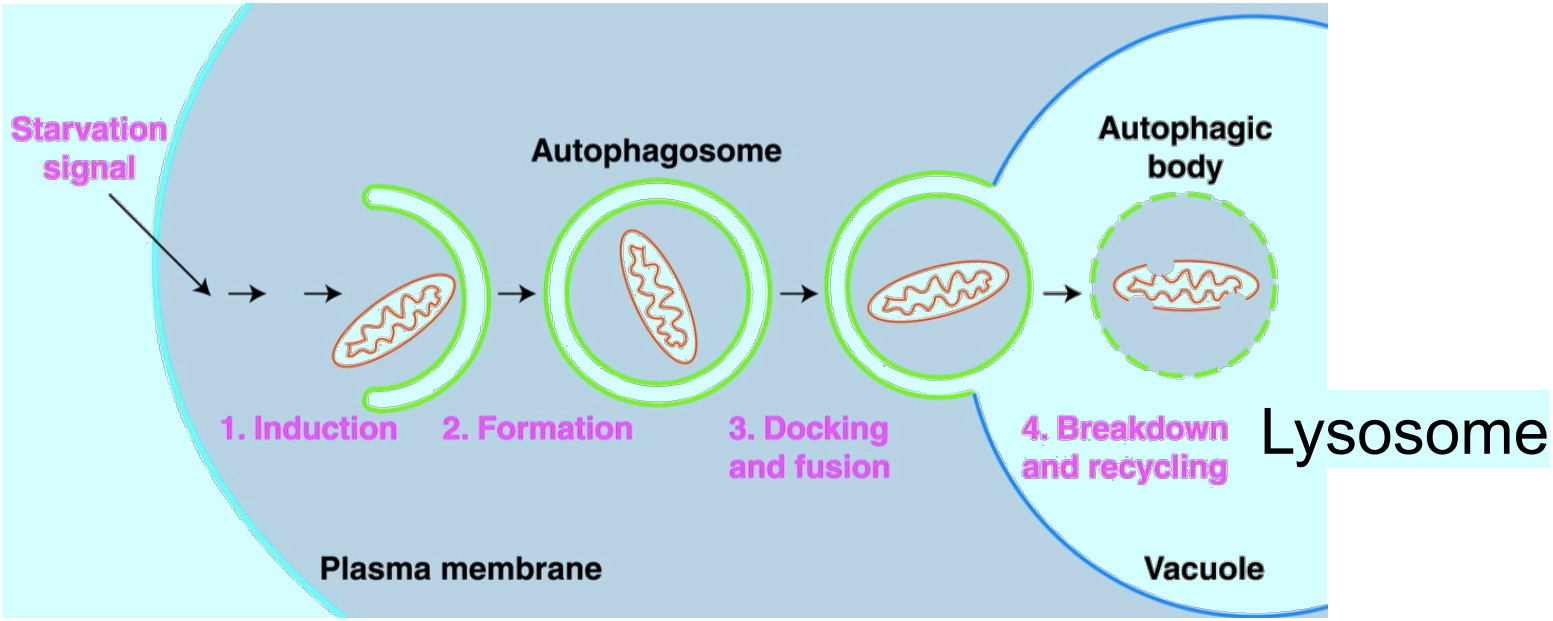
- In starved cells, lysosomes degrade preferentially proteins containing a KFERQ “signal” peptide.

- The regression of the uterus after child birth is mediated largely by lysosomal protein degradation

LYSOSOMAL DEGRADATION

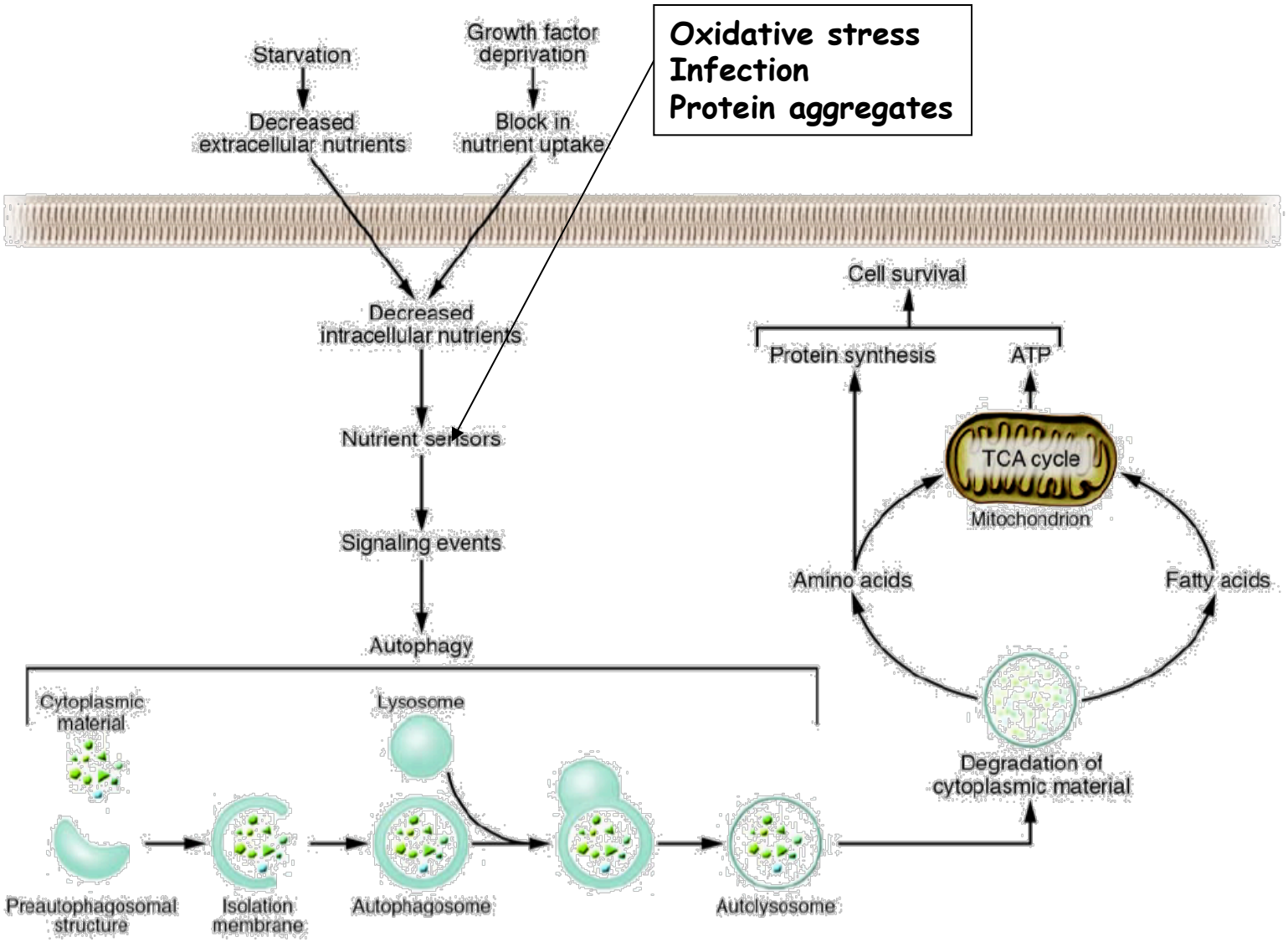
AUTOPHAGY (MACRO) PATHWAY

- In **autophagy**, part of the cytoplasm may become surrounded by two concentric membranes.
- Fusion of the outer membrane of this **autophagosome** with a lysosomal vesicle results in degradation of enclosed cytoplasmic structures and macromolecules.
- Genetic studies in yeast have identified **unique proteins** involved in autophagosome formation.
- Most autophagy is not a mechanism for selective degradation of individual macromolecules.
- However, cytosolic proteins that include the sequence KFERQ may be selectively taken up by lysosomes in a process called chaperone-mediated autophagy.



LYSOSOMAL DEGRADATION

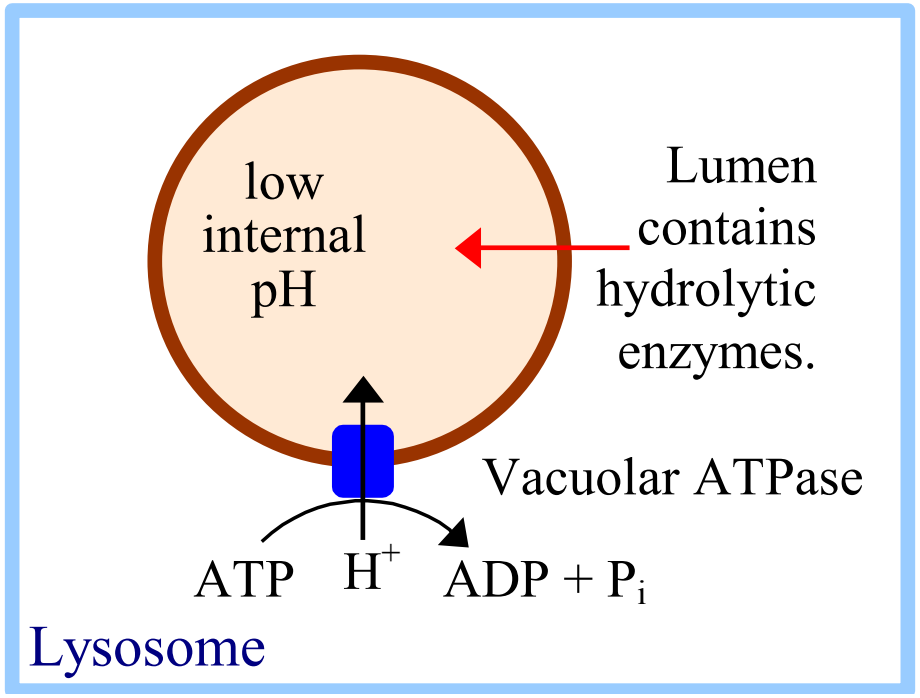
AUTOPHAGY (MACRO) PATHWAY



LYSOSOMAL DEGRADATION

- Lysosomes contain a large variety of hydrolytic enzymes that degrade proteins and other substances taken in by endocytosis.
- Materials taken into a cell by inward budding of vesicles from the plasma membrane may be processed first in an endosomal compartment and then delivered into the lumen of a lysosome by fusion of a transport vesicle.
- Solute transporters embedded in the lysosomal membrane catalyze exit of products of lysosomal digestion (e.g., amino acids, sugars, cholesterol) to the cytosol.

- Lysosomes have a low internal pH due to vacuolar ATPase, a H^+ pump homologous to mitochondrial F1Fo ATPase.
- All intra-lysosomal hydrolases exhibit acidic pH optima.
- Lysosomal proteases include many cathepsins (cysteine proteases), some aspartate proteases and one zinc protease.
- Activation of lysosomal proteases by cleavage may be catalyzed by other lysosomal enzymes or be autocatalytic, promoted by the internal acidic pH.



PROTEASES

Intracellular Proteases

- Proteolytic enzymes are found throught the cell.
- Several proteases are present in the eukaryotic cytosol: two Ca^{2+} activated proteases called calpains, a large multisubunit neutral protease, and a still larger ATP-dependent protease called the proteasome.
- Lysosomal proteases, called cathepsins, are designed to function in an acidic milieu.
- Lysosomes form by budding from the Golgi complex and are bags of digestive enzymes containing proteases, nucleases, lipases, and carbohydrate cleaving enzymes.
- Lysosomes are involved in secretion of digestive enzymes, digestion of organelles destined for destruction, digestion of food particles or bacteria engulfed by phagocytosis, and intracellular release of enyzmes followed by autolysis-digestion and death of the cell.

Nomenclature			Chromosome	
Preferred name	**Other names**	**Gene symbol**	**Location**	**Aberration**
Cathepsin B		*CTSB*	8p22	A novel amplicon that results in amplification and overexpression of cathepsin B; a region of LOH
Cathepsin C	Dipeptidyl peptidase I	*CTSC*	11q14.1	
Cathepsin F		*CTSF*	11q13	A region of gene amplification
Cathepsin H		*CTSH*	15q24	
Cathepsin L		*CTSL*	9q21	A region of unidentified loss
Cathepsin K	O*, O2*, X*	*CTSK*	1q21	An amplicon; a region of LOH
Cathepsin O		*CTSO*	4q31	
Cathepsin S		*CTSS*	1q21	An amplicon; a region of LOH
Cathepsin V	L2, U	*CTSL2*	9q22	
Cathepsin W		*CTSW*	11q13.1	A region of gene amplification
Cathepsin X	Y, Z*	*CTSZ*	20q13	A region of gene amplification and copy-number abnormalities

*Indicates obsolete name[1] (also see MEROPS — The Peptidase Database). LOH, loss of heterozygosity.

Nature Reviews Cancer **6**, 764-775

DEGRADATION: PROTEASOME

Four structural features are currently thought to be determinants of turnover rate:

- Ubiquitination - Ubiquitin is a 76-amino acid residue heat-stable protein found in all eukaryotic cells. An ATP-dependent reaction with proteins links ubiquitin's C-terminal glycine to lysine amino groups in the target protein. Proteins modified in this way are degraded soon afterward.

- Oxidation of amino acid residues - Conditions that generate oxygen radicals cause many proteins to undergo mixed-function oxidation of particular residues. Conditions require Fe^{2+} and hydroxyl radical, and the amino acids most susceptible to oxidation are lysine, arginine, and proline.

- *E. coli* and rat liver each contain a protease that cleaves oxidized glutamine synthetase *in vitro*, but does not attack the native enzyme. Presumably, other oxidized proteins are also targets for this enzyme.

- PEST sequences - Virtually all short-lived proteins (i.e., half-lives less than 2 hours) contain one or more regions rich in proline, glutamate, serine, and threonine. These regions are called PEST sequences because the one-letter codes for these amino acids are P,E,S, and T, respectively. Very few longer-lived proteins contain these sequences. Furthermore, insertion of these sequences into long-lived proteins increases their metabolic lability.

- N-terminal amino acid residue - An N-terminal protein residue of Phe, Leu, Tyr, Trp, Lys, or Arg is correlated with short metabolic lifetimes. Proteins with other termini are far longer-lived. Thus, the intracellular half-life of a particular protein depends on the identity of its N-terminal amino acid residue.

DEGRADATION: PROTEASOME

- N-end rule: On average, a protein's half-life correlates with its N-terminal residue.
- Proteins with N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours.
- Proteins with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less.
- PEST proteins having domains rich in Pro (P), Glu (E), Ser (S), Thr (T), are more rapidly degraded than other proteins.

TABLE 27–9 **Relationship between Protein Half-Life and Amino-Terminal Amino Acid Residue**

Amino-terminal residue	Half-life*
Stabilizing	
Ala, Gly, Met, Ser, Thr, Val	>20 h
Destabilizing	
Gln, Ile	<30 min
Glu, Tyr	<10 min
Pro	<7 min
Asp, Leu, Lys, Phe	<3 min
Arg	<2 min

Source: Modified from Bachmair, A., Finley, D., & Varshavsky, A. (1986) In vivo half-life of a protein is a function of its amino-terminal residue. *Science* 234, 179–186.

*Half-lives were measured in yeast for the β-galactosidase protein modified so that in each experiment it had a different amino-terminal residue. Half-lives may vary for different proteins and in different organisms, but this general pattern appears to hold for all organisms.

Table 27-9
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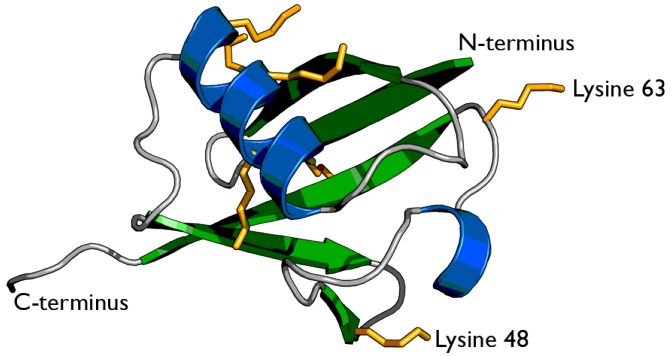
TABLE 15–1 **Average Half-Life of Proteins in Mammalian Tissues**

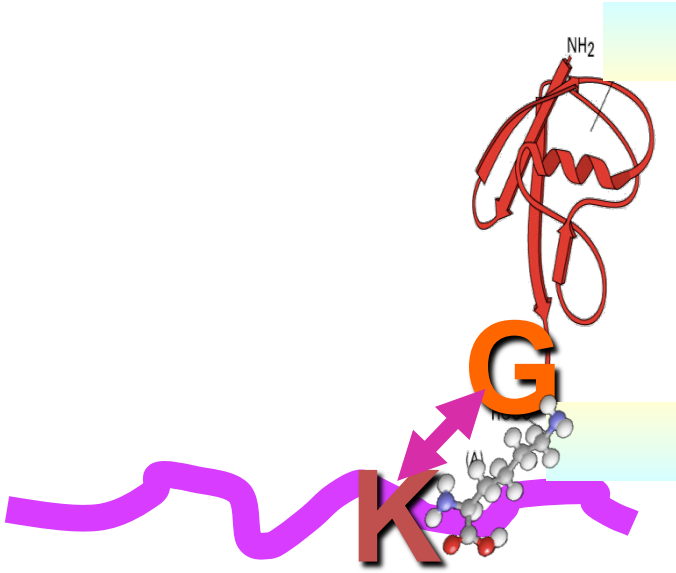
Tissue	Average half-life (days)
Liver	0.9
Kidney	1.7
Heart	4.1
Brain	4.6
Muscle	10.7

Table 15-1
Lehninger Principles of Biochemistry, Sixth Edition

DEGRADATION: UBIQUITINATION

- Ubiquitin is a small (76-residue), heat-stable protein found in all eukaryotic cells.
- It derives its name from its widespread (ubiquitous) distribution.
- Ubiquitin undergoes an ATP-dependent reaction with proteins, which condenses C-terminal glycine residues of ubiquitin with lysine amino groups on the target protein.
- Such modified proteins are degraded soon afterward, by a large protease complex whose assembly requires more ATP and that recognizes the ubiquitin marker.
- Mono-ubiquitination
  - Receptor internalization
  - Endocytosis – lysosome
  - Transcription regulation
- Poly-ubiquitination
  - Targets proteins from cytoplasm, nucleus and ER for degradation by the PROTEASOME
  - DNA repair





DEGRADATION: UBIQUITINATION

- First, Ubiquitin is activated by forming a link to "enzyme 1" **(E1).**
- Then, ubiquitin is transferred to one of several types of "enzyme 2" **(E2).**
- Then, "enzyme 3" **(E3)** catalizes the transfer of ubiquitin from E2 to a Lys e-amino group of the "condemned" protein.
- Lastly, molecules of Ubiquitin are commonly conjugated to the protein to be degraded by **E3s and E4s**

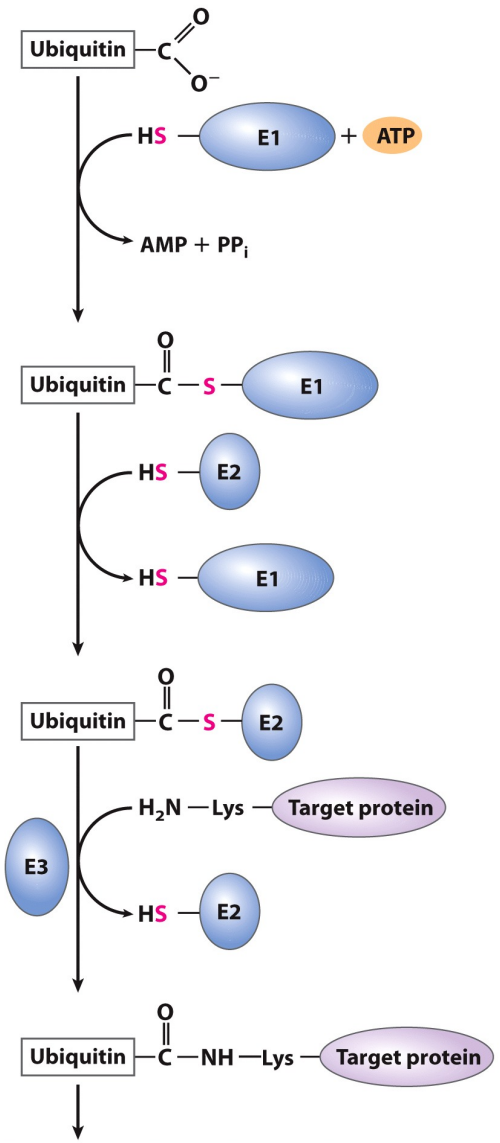


Figure 27-47
Lehninger Principles of Biochemistry, Sixth Edition

DEGRADATION: UBIQUITINATION

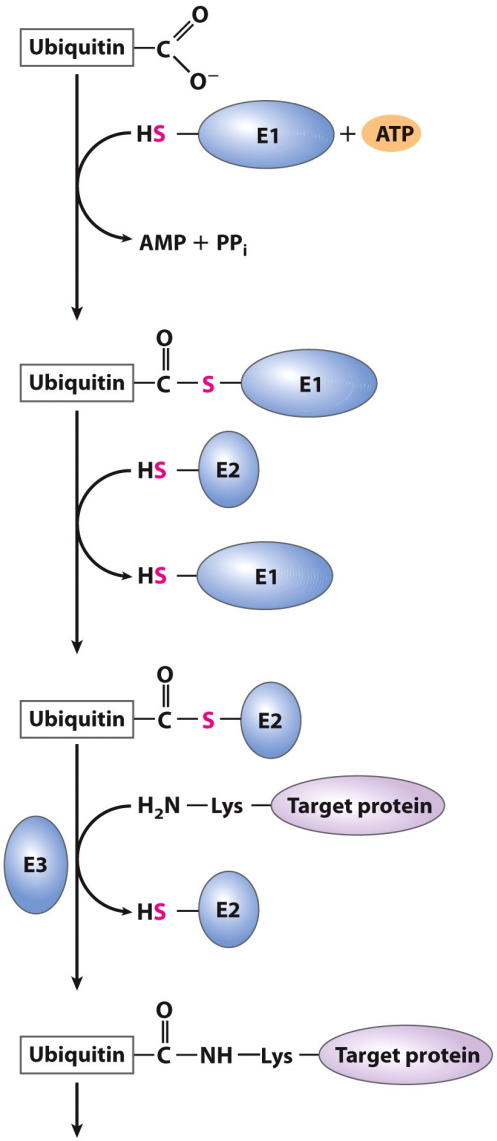
Enzymes of the Ubiquitination

- E1:
  - ubiquitin-activating enzyme.
  - exists as two isoforms of 110- and 117-kDa, which derive from a single gene and are found in both the nucleus and cytosol. Inactivation of this gene is lethal.
  - In mammals there is a single E1.

- E2:
  - Ubiquitin-conjugating enzymes.
  - E2s are a superfamily of related proteins. There are eleven E2s in yeast, and 20-30 E2s in mammals.

- E3s: Ubiquitin-protein ligases.
  - E3s play a key role in the ubiquitin pathway, as they are responsible for the selective recognition of protein substrates.
  - E3 ligases can be subdivided into at least six subtypes.

- E4:
  - catalyzes the efficient polymerization of very long polyubiquitin chains, it has been characterized in yeast.



Repeated cycles lead to attachment of additional ubiquitin.

Figure 27-47
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DEGRADATION: PROTEASOME

Proteasome evolution:

Proteasomes are considered very old.

They are in Archea bacteria, but not most Eubacteria, although Eubacteria have alternative protein-degrading complexes.

- The **archeabacterial proteasome** has just **2** proteins, α and β, with 14 copies of each.
- The **eukaryotic proteasome** has evolved **14** distinct proteins that occupy unique positions within the proteasome (7 α-type and 7 β-type).

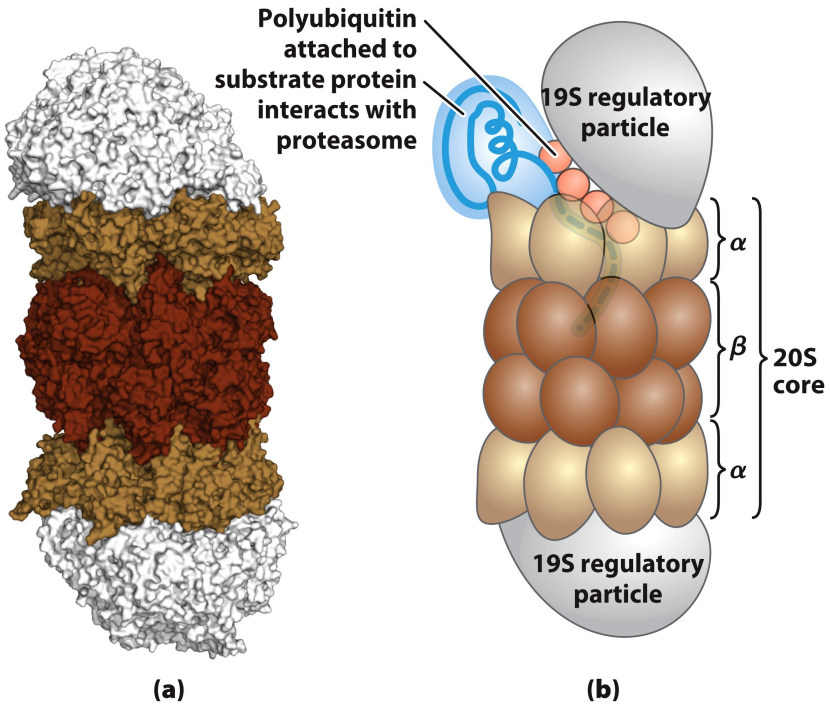


Figure 27-48
Lehninger Principles of Biochemistry, Sixth Edition

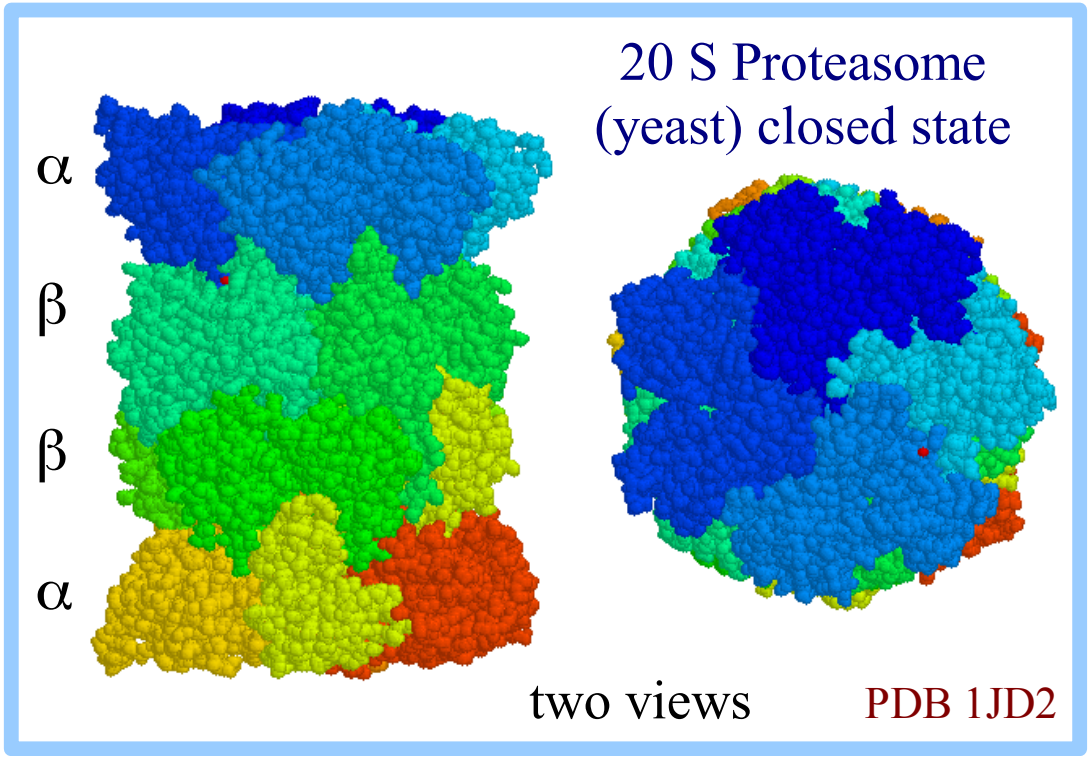
Courtesy of W. Baumeister from A. Lupas, J. M. Flanagan, T. Tamura, and W. Baumeister, Trends Biochem. Sci. (1997) 22:399-404, with permission from Elsevier Science.

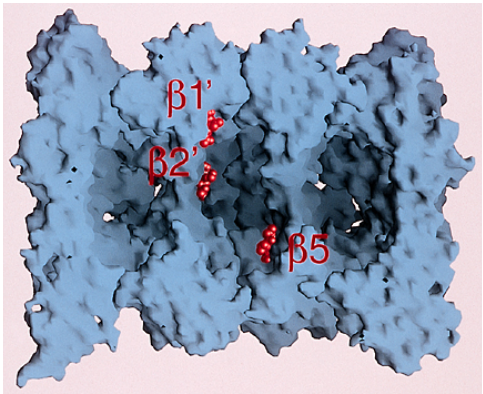
DEGRADATION: PROTEASOME

Proteasomes: Selective protein degradation occurs in the proteasome, a large protein complex in the nucleus & cytosol of eukaryotic cells.

The proteasome core complex, with a 20S sedimentation coefficient, contains 2 each of 14 different polypeptides.

- 7 α-type proteins form each of the two a rings, at the ends of the cylindrical structure.
- 7 β-type proteins form each of the 2 central b rings.
- The 20S proteasome core complex encloses a cavity with 3 compartments joined by narrow passageways.
- Protease activities are associated with 3 of the β subunits, each having different substrate specificity.

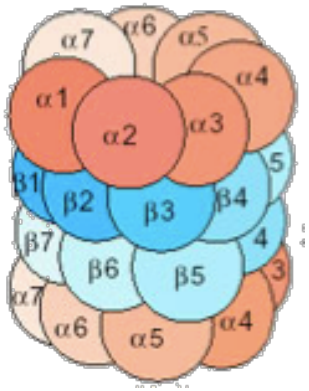




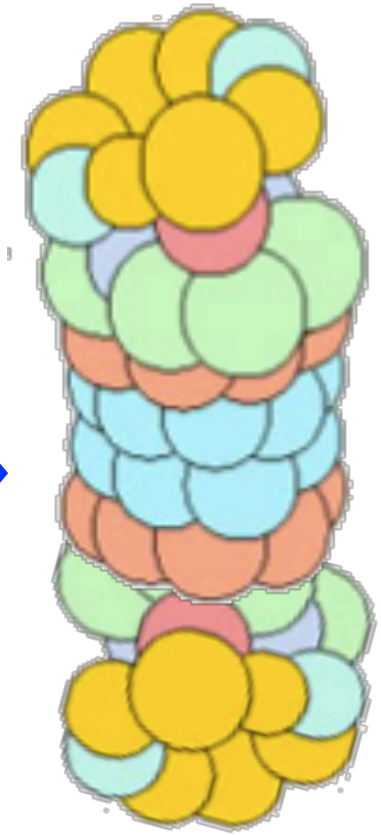
PROTEASOME COMPONENTS

20S
Proteasome

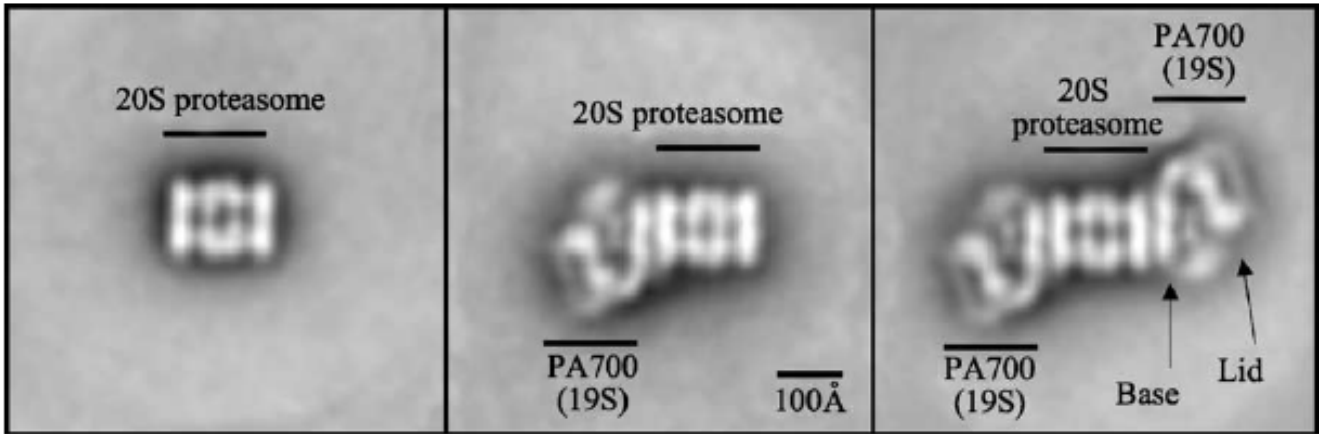
19S
Particle



ATP



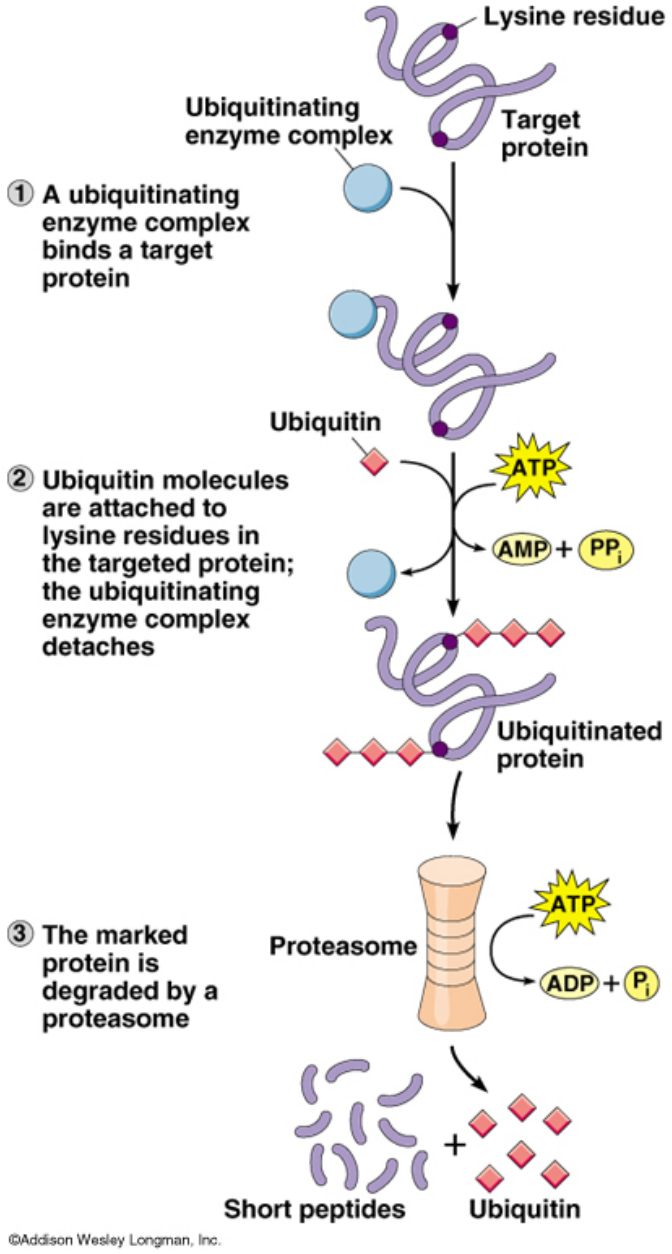
26S
Proteasome



DEGRADATION: PROTEASOME

- Ubiquitinated proteins are degraded in the cytoplasm and nucleus by the proteasome.

- Proteasomal protein degradation ***consumes*** ATP.
- The proteasome degrades the proteins to ~8 amino-acid peptides.
- Access of proteins into the proteasome is tightly regulated.
- The peptides resulting from the proteasome activity diffuse out of the proteasome freely.



DISEASES RELATED TO PROTEIN TURNOVER

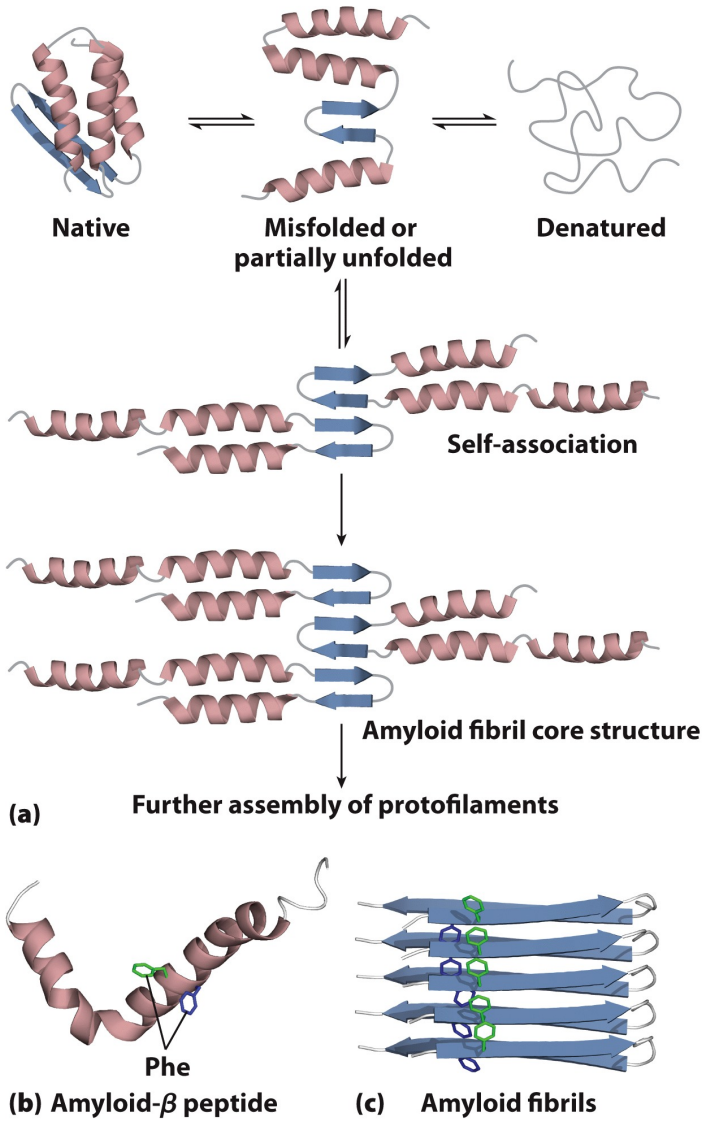


Figure 4-32
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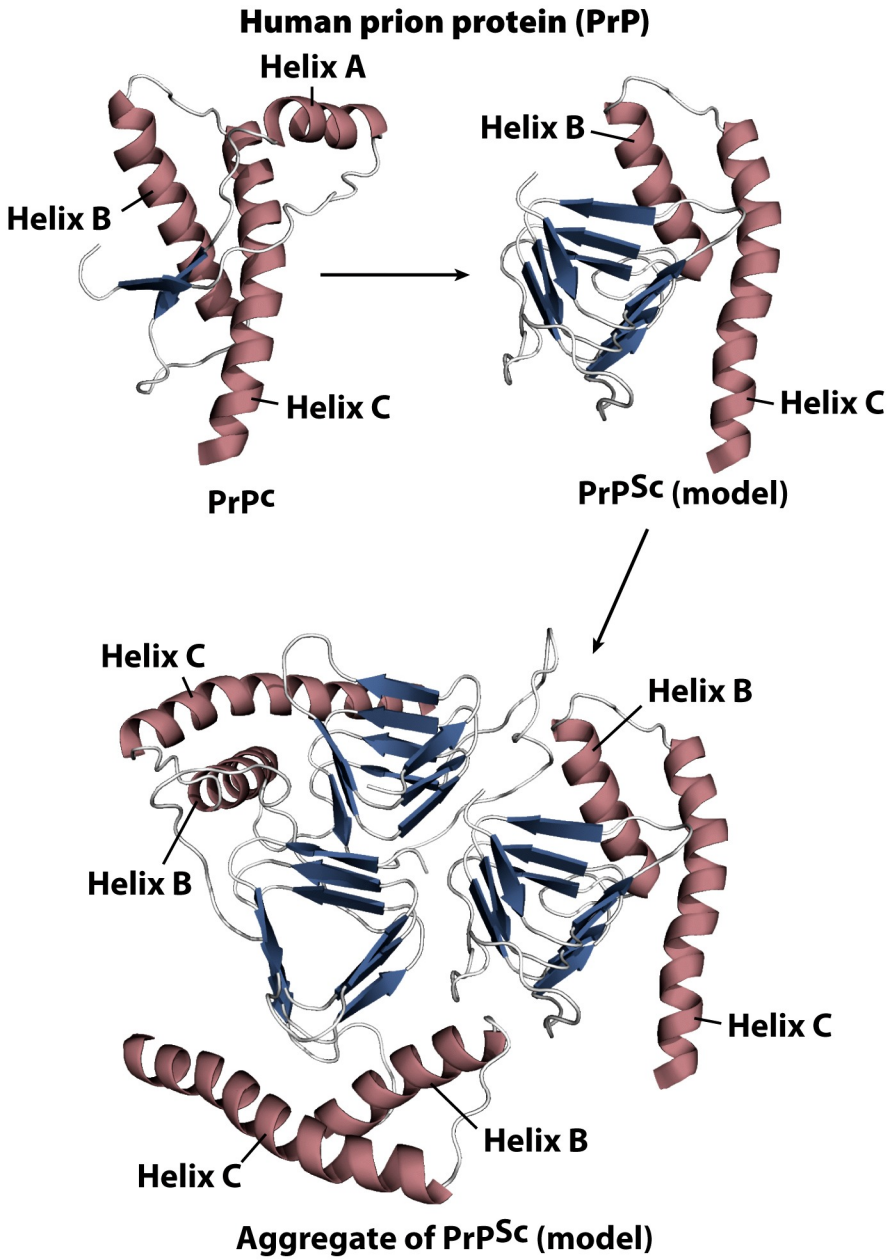

Box 4-6 figure 2

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Neurodegenerative Disorders

Disease

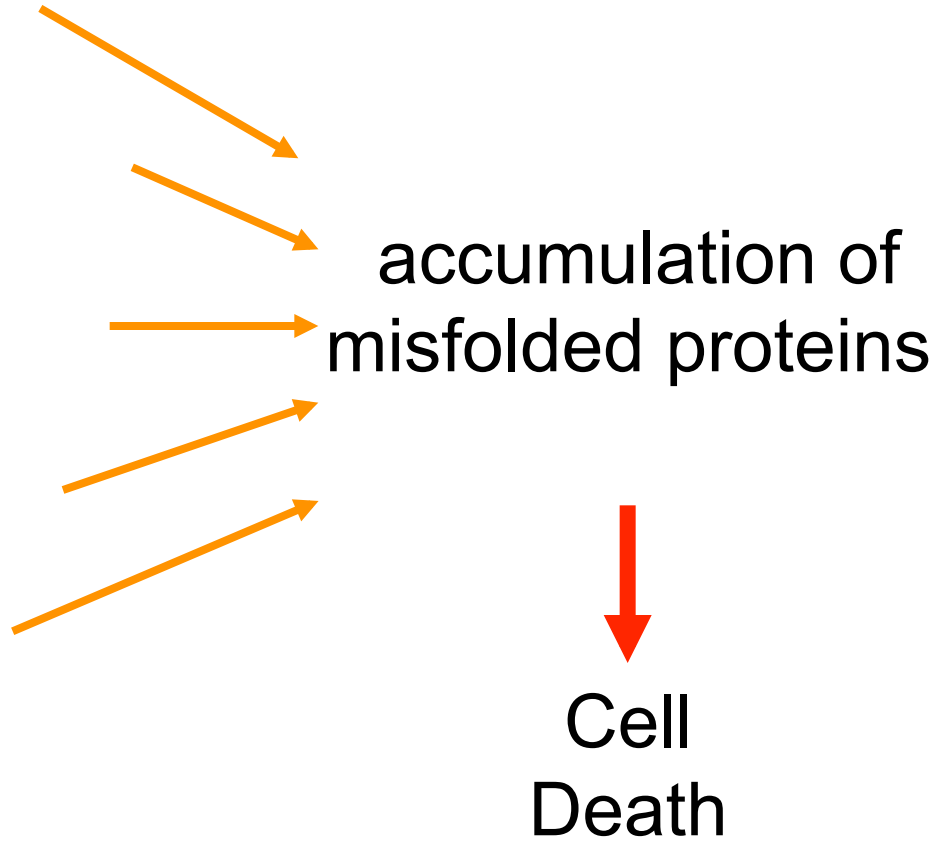
Parkinson's Disease

Alzheimer's Disease

Huntington's Disease

Amyotrophic lateral sclerosis

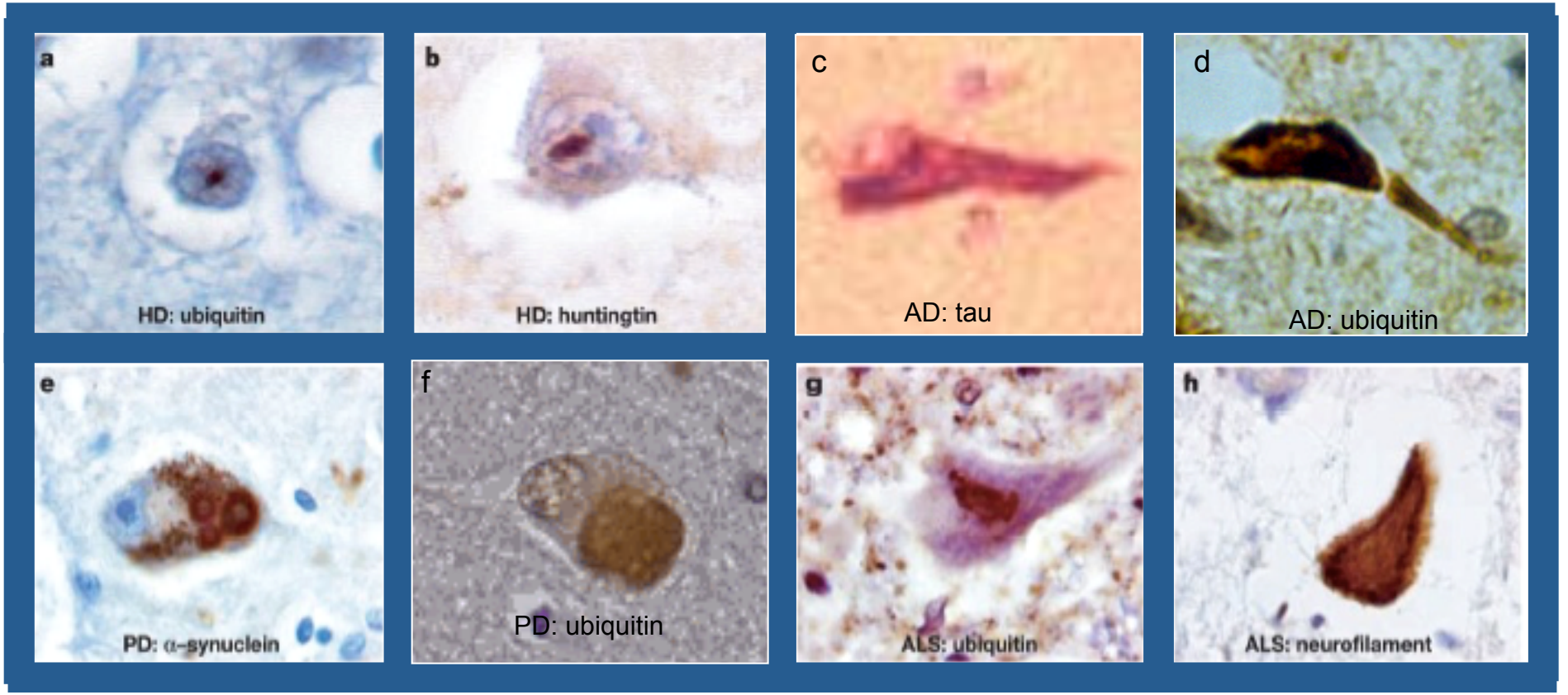
Spinocerebellar Ataxia



Ubiquitin-Protein Aggregates

HUNTINGTON'S

ALZHEIMER'S



PARKINSON'S

LOU GEHRIG'S