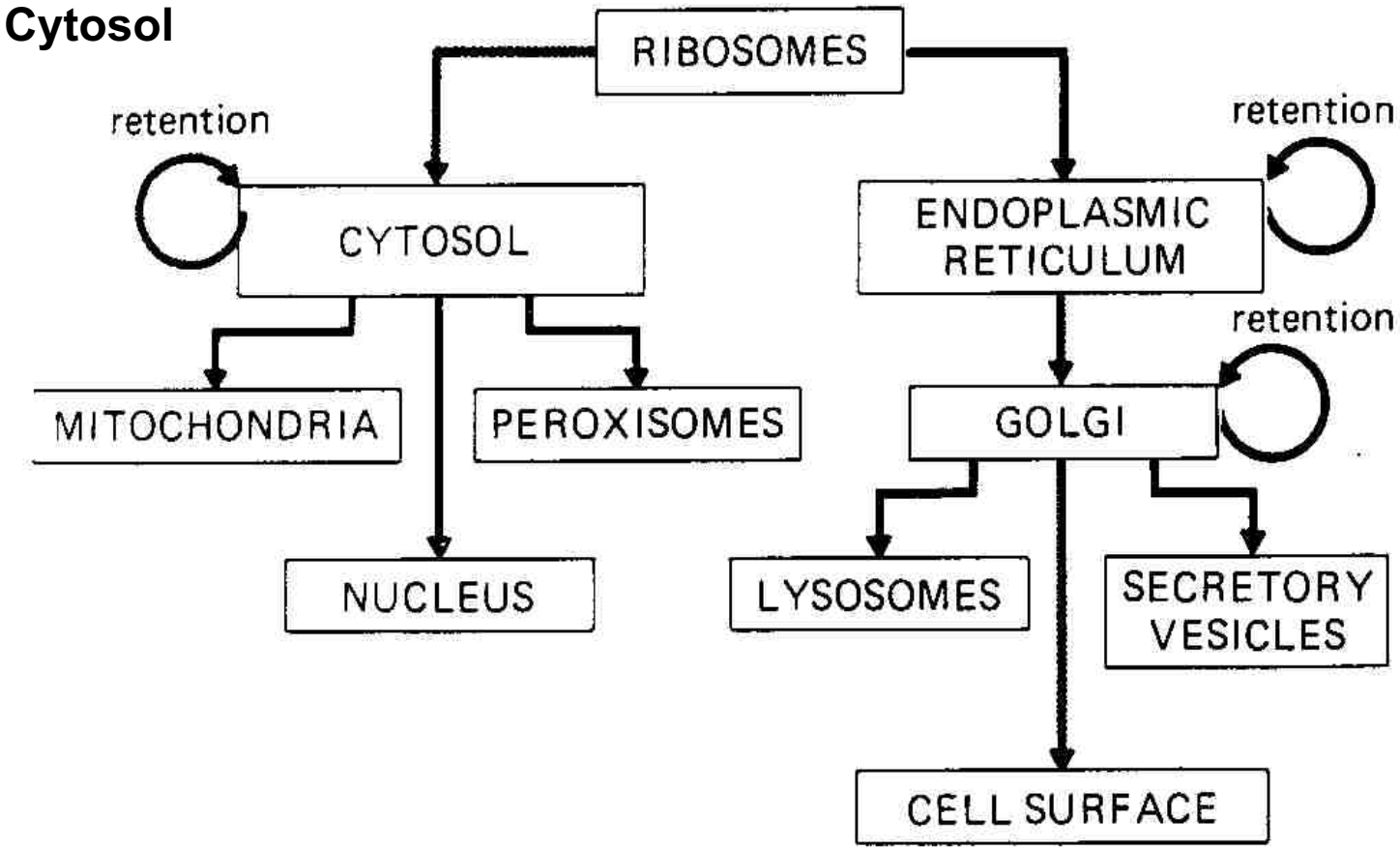
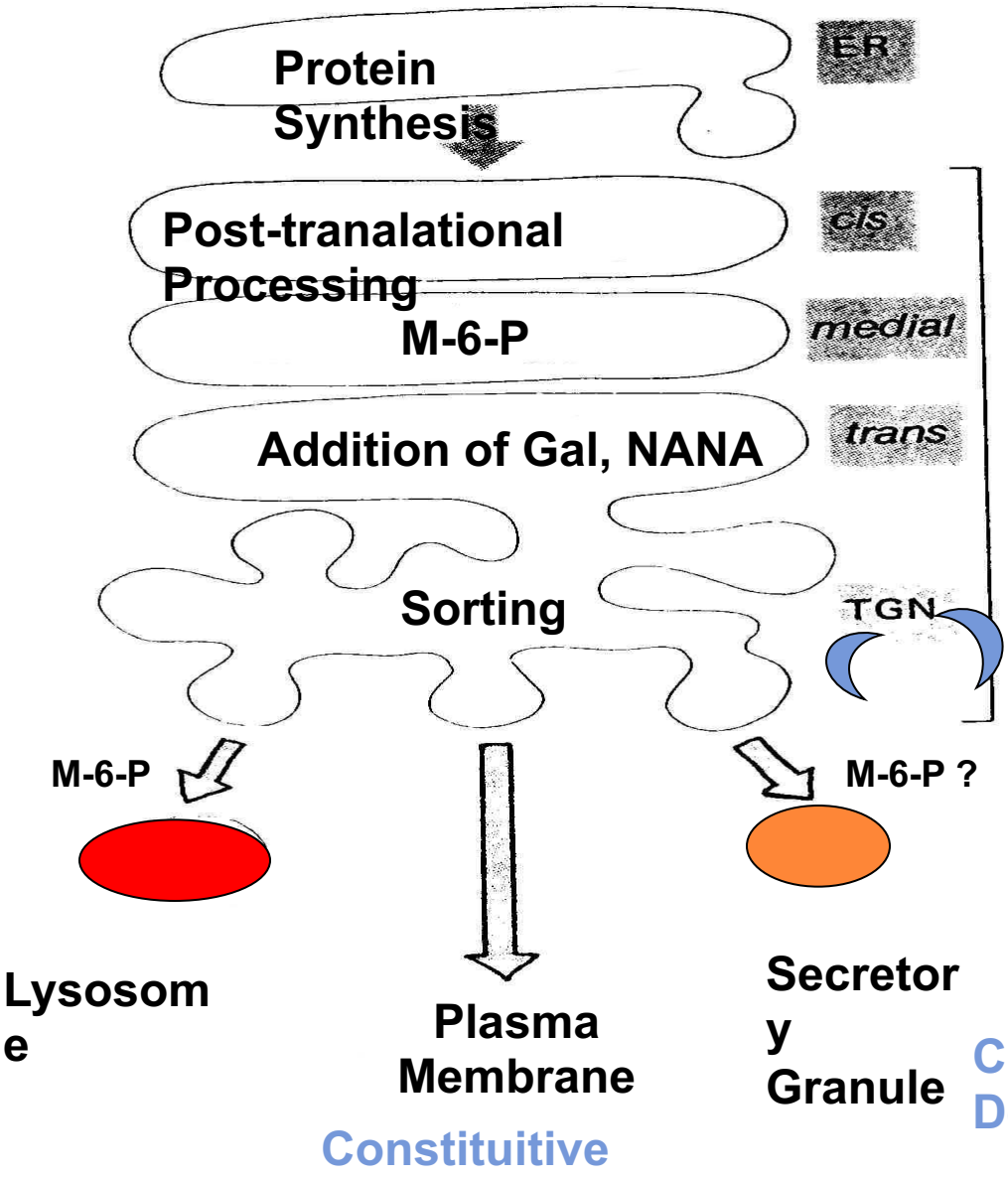
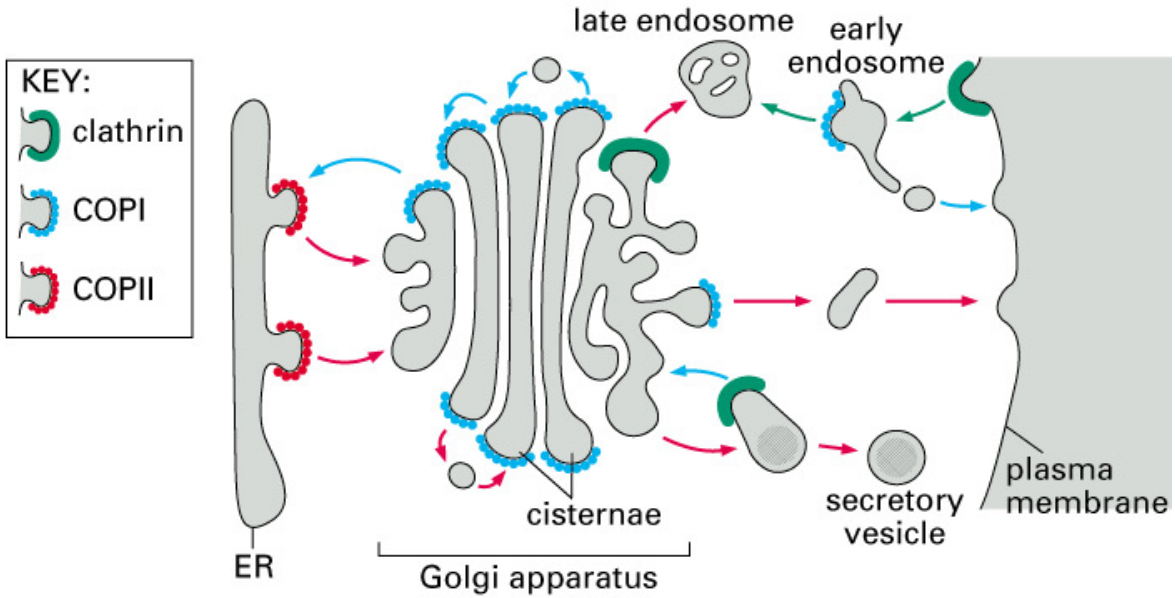
Compartments for Protein Sorting



Golgi Sorting



Vesicle Budding Utilizes Several Coat Protein Complexes



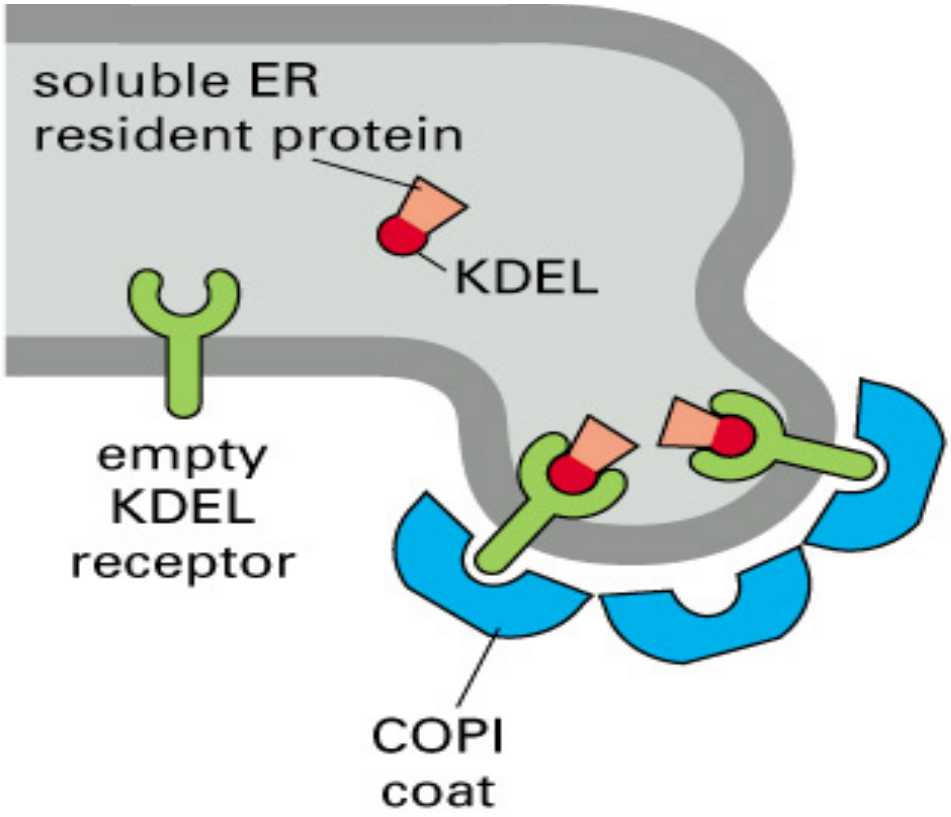
- COPII mediates ER to Golgi transport
- COPI mediates Golgi to ER transport
- Clathrin mediates Golgi to Endosome transport, PM to Endosome transport, and perhaps other transport steps.

Mechanisms of Golgi Sorting

- **The Mannose-6-Phosphate (M-6-P) Receptor.**
- **Example: Acid hydrolase (pH optimal ~ 6.0)**
- **M-6-P receptor affinity is pH dependent – release at 6.0**
- **Glucosamine acetyl transferase – puts M-6-P on**
  - **if defective: hydrolase found in blood not lysosome**

Elimination of Mis-sorted Proteins

- **Ubiquination: n-terminal amino acids**
- **Constitutive Secretion**


soluble ER
resident protein
KDEL
empty
KDEL
receptor
COPI
coat

Molecular mechanisms of vesicular trafficking

-Transport vesicles originate from *coated* regions of membranes

-Coat functions:

- **Concentrates transport proteins into patches**
- **Induce patches to vesicularize**

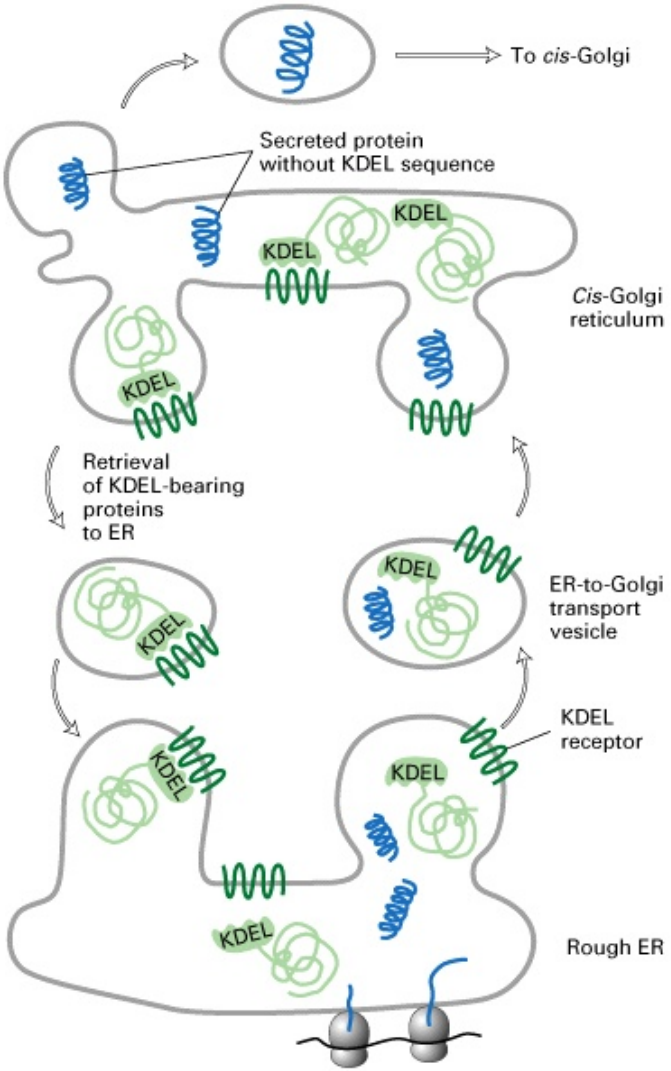
-Types coats:

- **COPII = ER to Golgi**
- **COPI = Golgi to Golgi, Golgi back to ER**
- **Clathrin = Trans-Golgi Network (TGN), endocytosis**

-Proteins essential to vesicle function

- **Monomeric GTPases control coat assembly**
  - **EXAMPLE: Coat-recruitment GTPases**
    - **= ARFs (responsible for COPI and clathrin coat assembly)**
    - **= Sar1 (responsible for COPII coat assembly)**
- **SNARE proteins guide vesicles (mediate vesicle fusion?)**
- **Rab proteins ensure specificity of vesicle docking**

ER-RESIDENT PROTEINS OFTEN ARE RETRIEVED FROM THE *CIS*-GOLGI



Lysosomal degradation of proteins

- lysosomes are cellular vesicles containing proteolytic enzymes
  - (e.g., papain-like cysteine protease, serine proteases, aspartic proteinases, etc., which are typically monomeric
- pH maintained at ~5.5 by proton-pumping ATPase
- Most lysosomal enzymes are transported to lysosomes through recognition by receptors for mannose-6-phosphate.
- Lysosomal enzymes are synthesized like proteins destined to be secreted or for residence on the plasma membrane but are recognized by a phosphotransferase enzyme shortly after leaving the ER.
- a mutation in the transferase leads to disease (I-cell disease); other so-called lysosomal storage diseases are the Tay-Sachs syndrome (ganglioside accumulates due to beta-Hexosaminidase deficiency), Pompe disease (accumulation of glycogen due to lack of α-Glucosidase), etc. (6 others!)

TABLE 17-1 Coated Vesicles Involved in Protein Trafficking

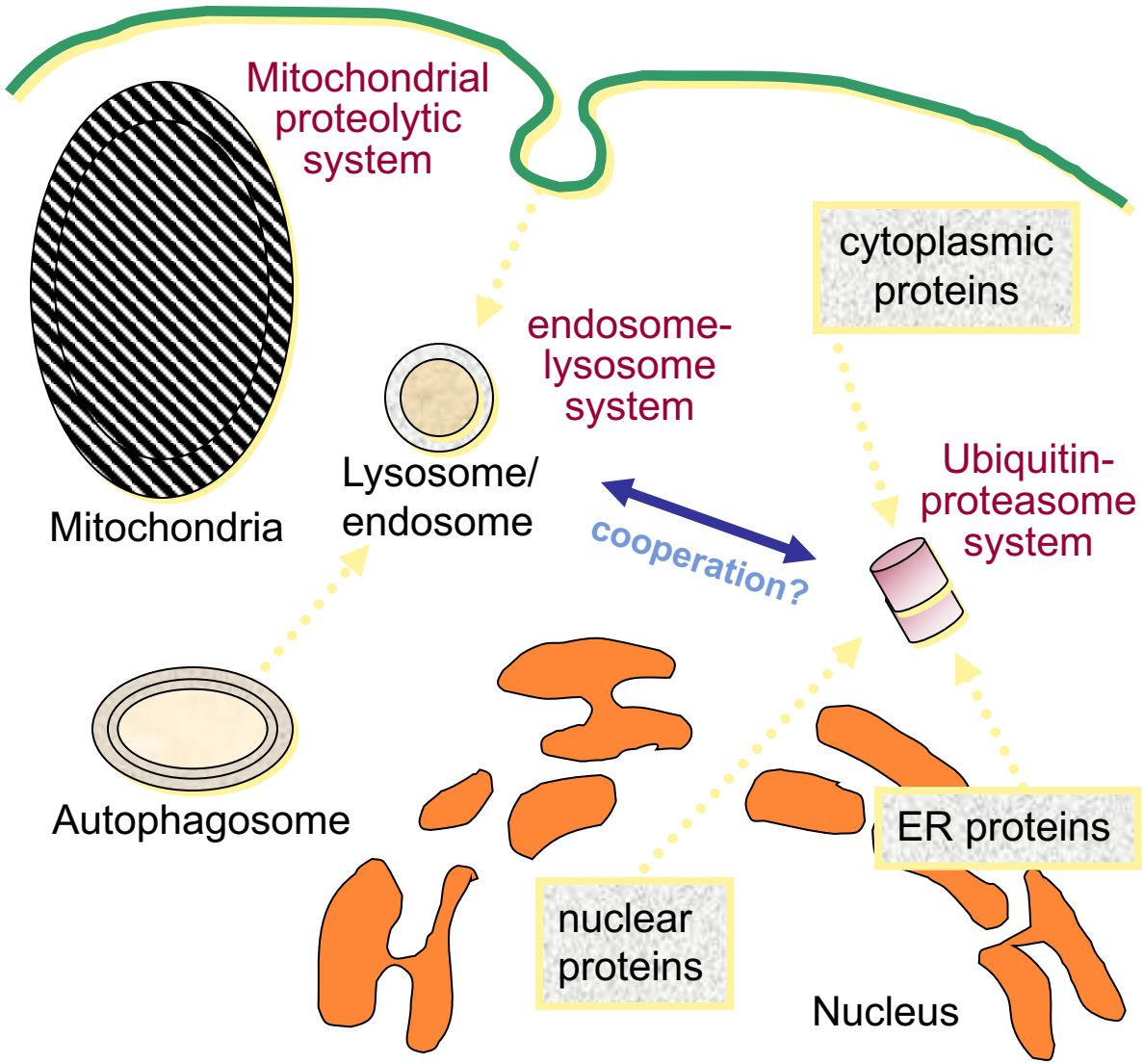
Vesicle Type	Coat Proteins	Associated GTPase	Transport Step Mediated
COPII	Sec23/Sec24 and Sec13/Sec31 complexes, Sec16	Sar1	ER to *cis*-Golgi
COPI	Coatomers containing seven different COP subunits	ARF	*cis*-Golgi to ER Later to earlier Golgi cisternae
Clathrin and adapter proteins*	Clathrin + AP1 complexes	ARF	*trans*-Golgi to endosome
	Clathrin + GGA	ARF	*trans*-Golgi to endosome
	Clathrin + AP2 complexes	ARF	Plasma membrane to endosome
	AP3 complexes	ARF	Golgi to lysosome, melanosome, or platelet vesicles

*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

Degradation of Misfolded Proteins

- Lysosomal (extracellular) protein degradation
  - Protein degraded by lysosomal enzymes
- Cytosolic (intracellular) protein degradation
  - The Ubiquitin Proteosome pathway

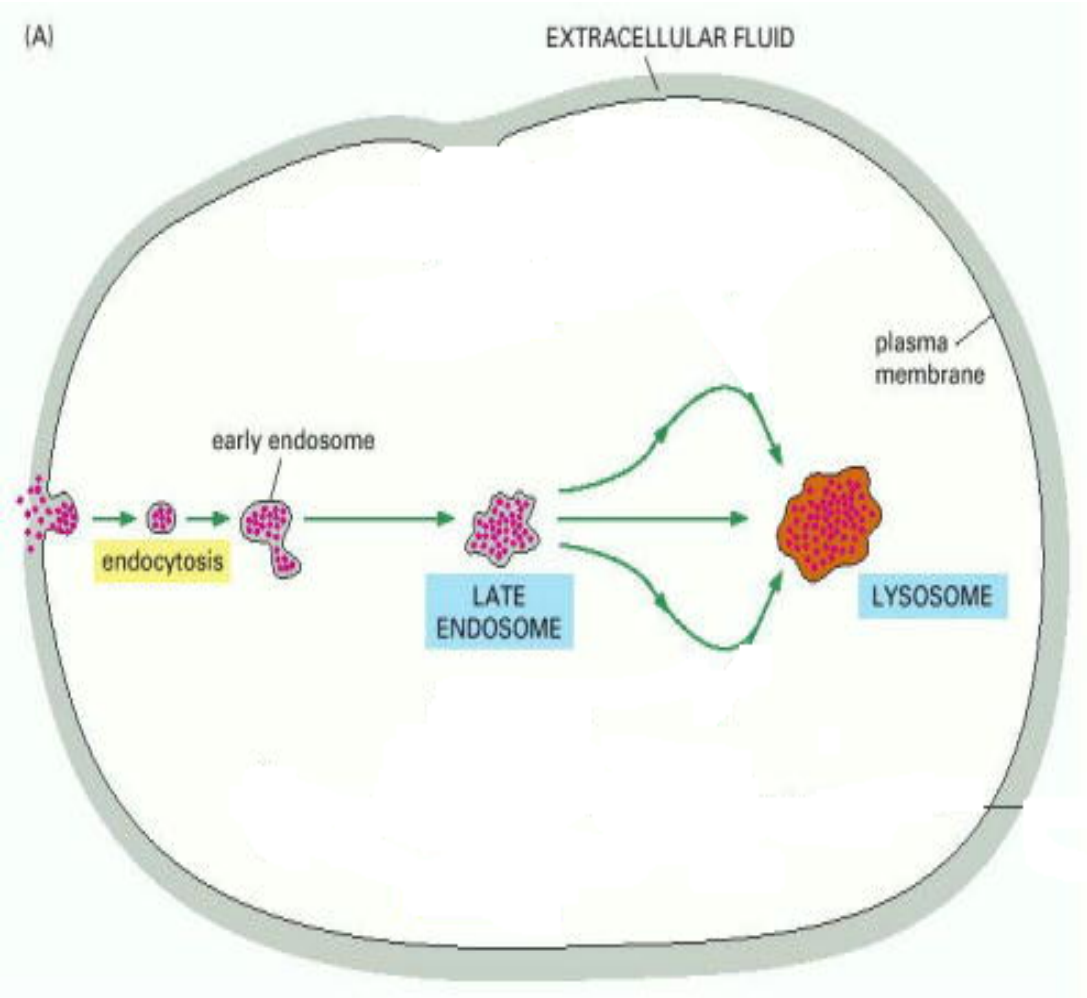
Main proteolytic pathways in eukaryotes



- endosome-lysosome pathway degrades extracellular and cell-surface proteins
- ubiquitin-proteasome pathway degrades proteins from the cytoplasm, nucleus and ER
- mitochondria (and chloroplasts) have their own proteolytic system that are of bacterial origin

Lysosomal degradation

- Proteins delivered via endocytosis
- Lysosomes
  - The cellular dust-bins
  - Contain many hydrolytic enzymes
    - Proteases
    - Lipases
    - Glycosidases


(A)
EXTRACELLULAR FLUID
plasma membrane
early endosome
endocytosis
LATE ENDOSOME
LYSOSOME

LYSOSOMAL DEGRADATION OF PROTEINS

- macroautophagy is the equivalent of forming intracellular endosomes (phagosomes) that fuse to the lysosome and result in the breakdown of its contents
- Hsc73 (constitutively-expressed Hsp70 chaperone) is involved in one pathway of lysosome-mediated degradation

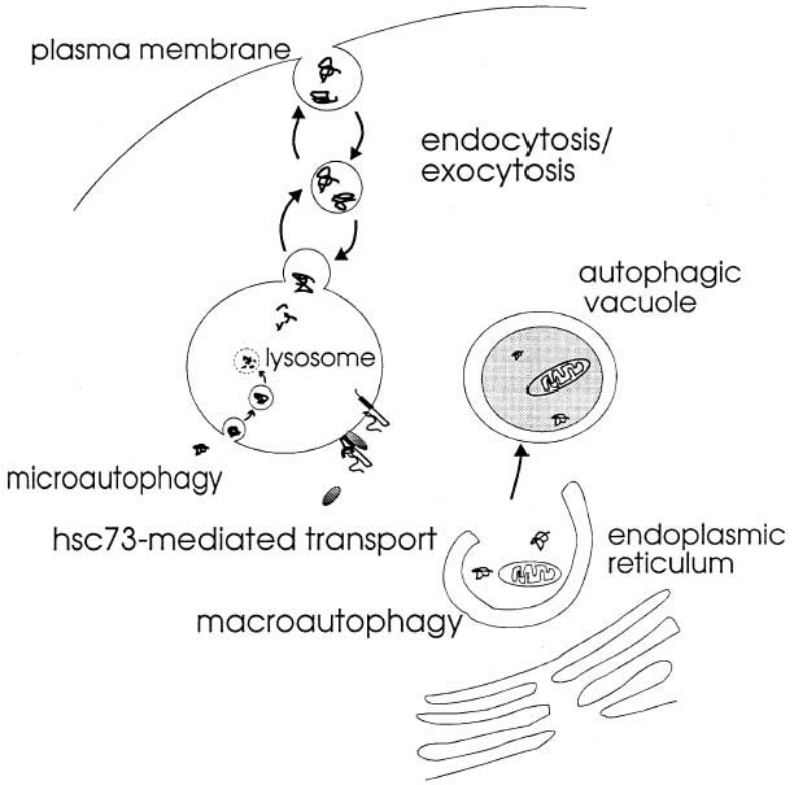
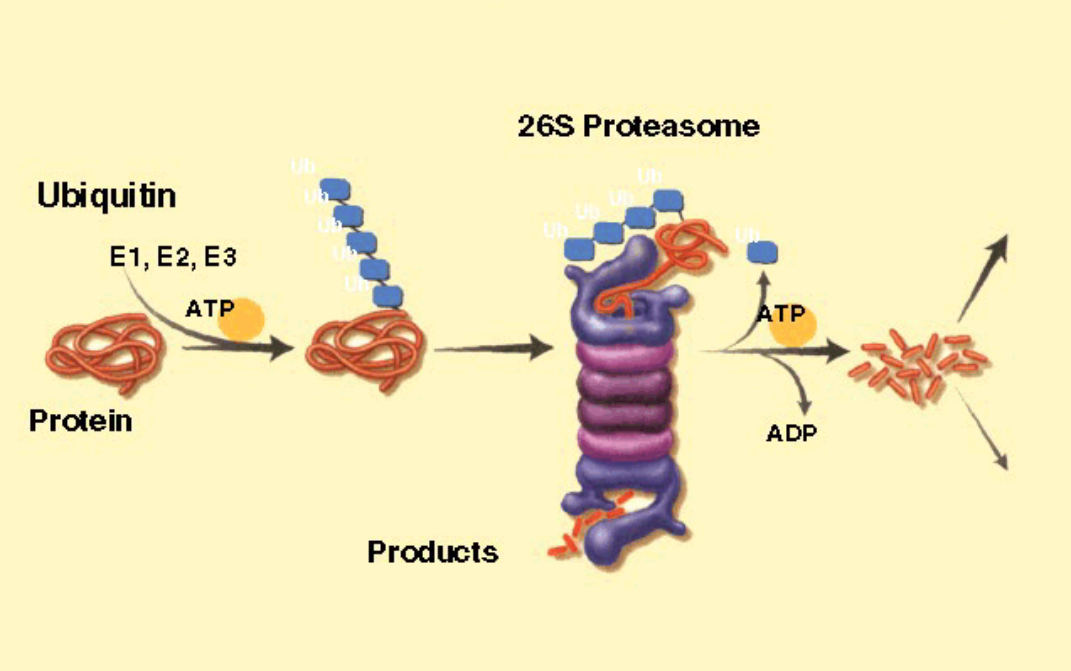


Fig. 1 Pathways of protein degradation in lysosomes. Lysosomes are able to degrade intra- and extracellular proteins following different mechanisms

Cytosolic protein degradation

- The Ubiquitin Proteosome Pathway



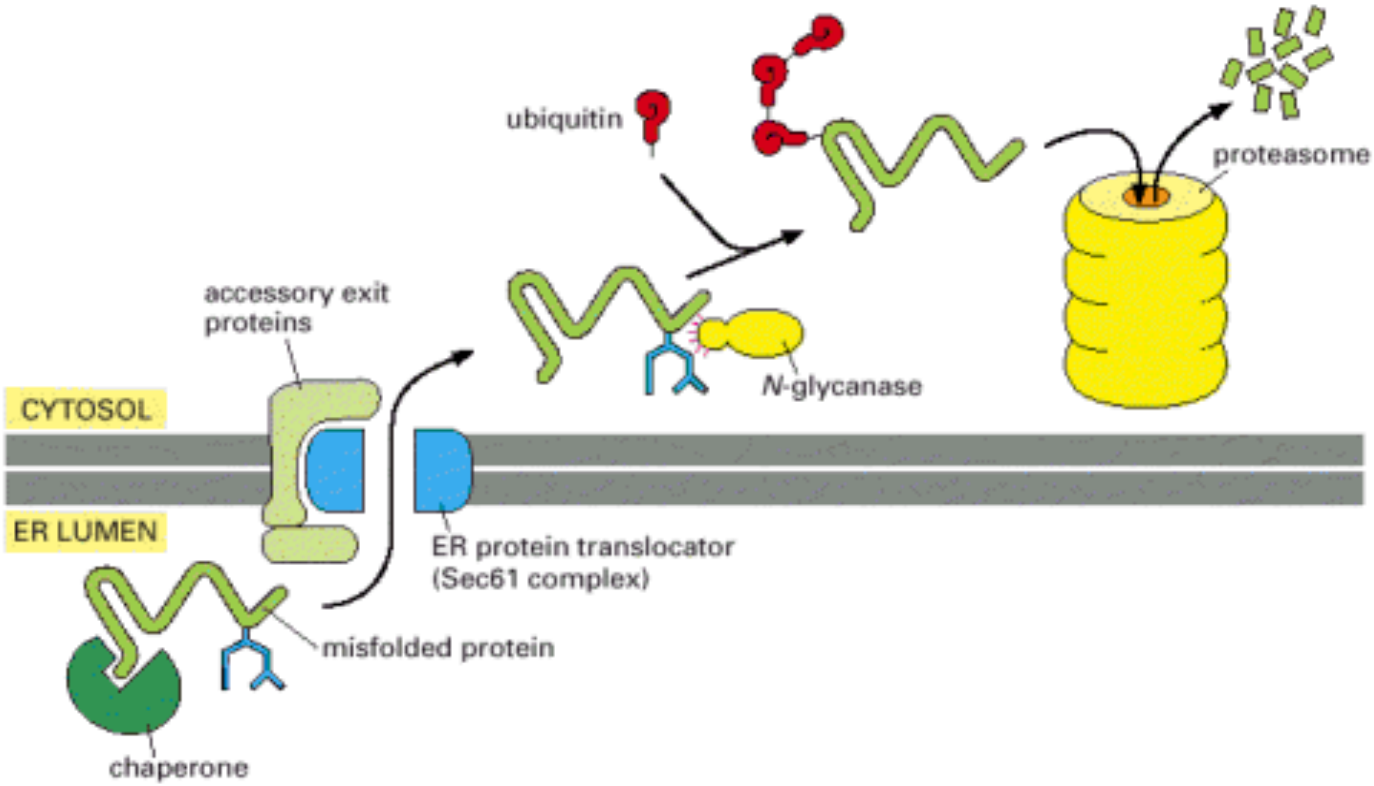
2004 Nobel Prize in Chemistry

- The discovery of ubiquitin-mediated protein degradation
  - Aaron Ciechanover
  - Avram Hershko
  - Irwin Rose
- Cells give a chemical "kiss of death" to proteins that need to be destroyed.

Targeting by Ubiquitin

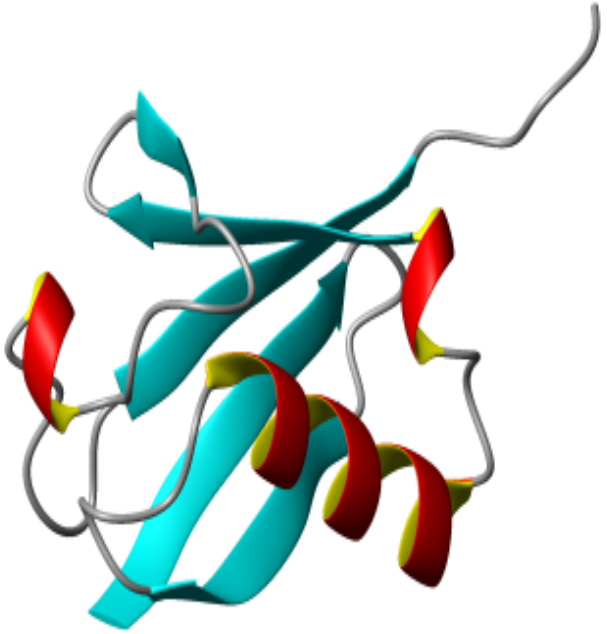
- Despite help from chaperones, more than 80% fold incorrectly
- Proteins are dislocated back into the cytosol
  - Oligosaccharides are removed
  - Deglycosylation is catalyzed by N-glycanase
- One third of the newly made polypeptide chains are selected for degradation

The Export of Misfolded Proteins



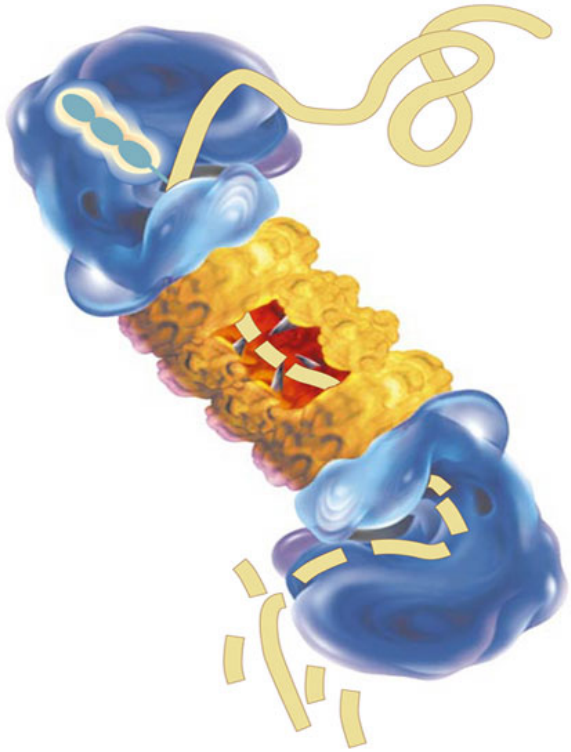
Ubiquitin

- 76 amino acids, 8.5 kDa protein
- Heat stable
- Folds into a compact globular structure
- Found throughout the cell
- Found in all eukaryotic cells
- Human and yeast ubiquitin share 96% sequence identity
- Involved in many cellular processes



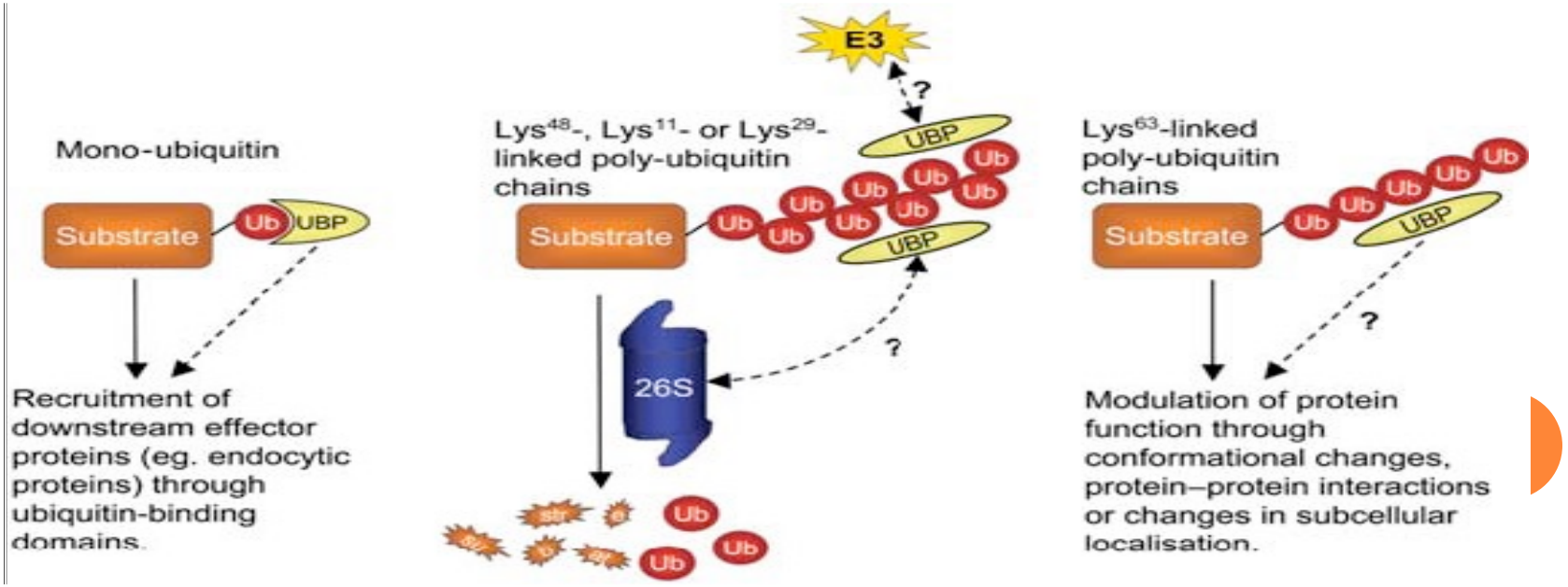
The Proteosome

- Professional protein degrading organelles
- An abundant ATP-dependent protease
- Constitutes nearly 1% of cellular protein
- Present in many copies throughout the cytosol and the nucleus
- Consists of a central hollow cylinder (20S)
- Ends of the cylinder are associated with the 19S cap



Types of Ubiquitination

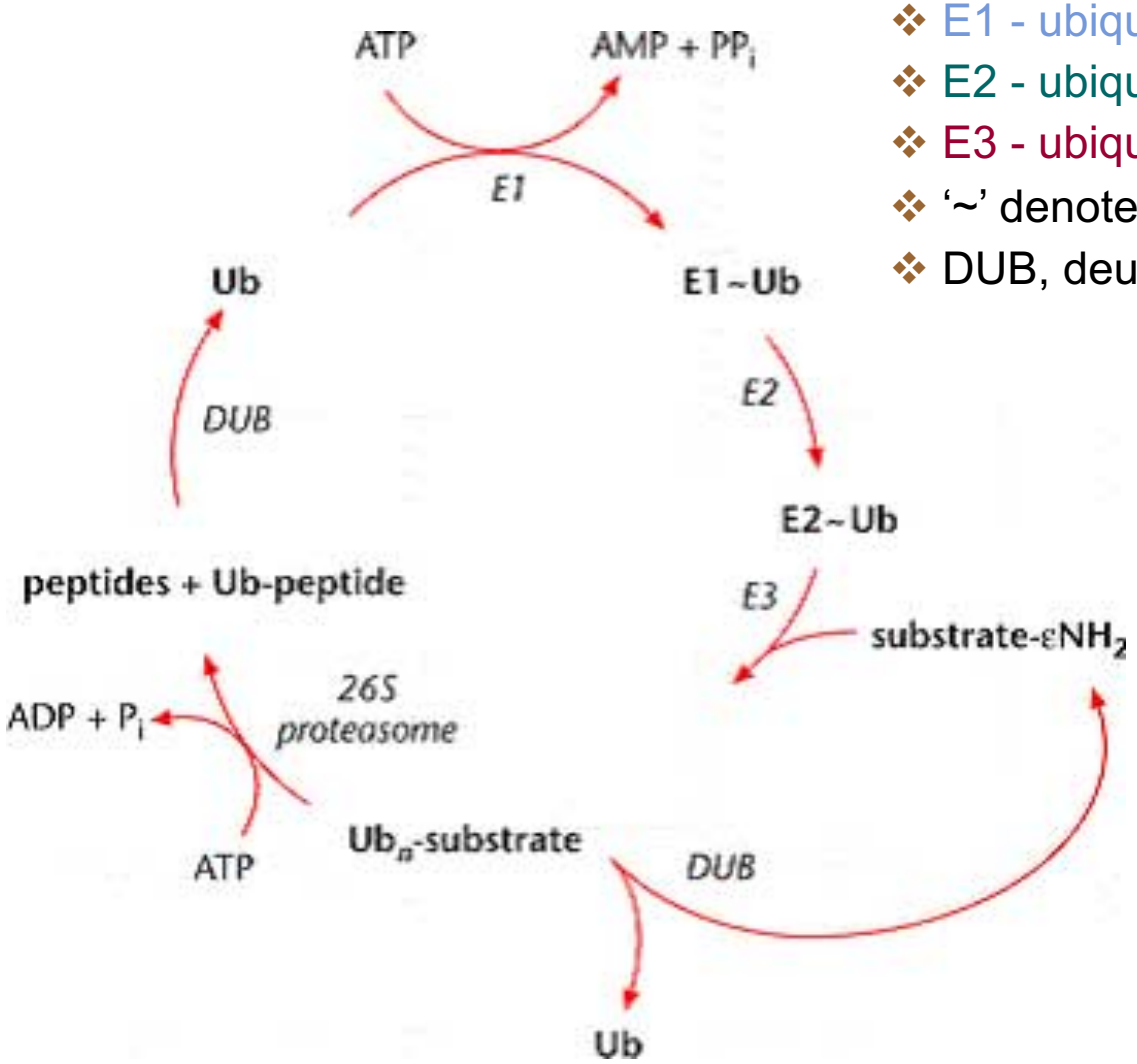
- Mono-ubiquitination
  - Transcription, histone function, endocytosis and membrane trafficking
- Lys48, Lys11 or Lys29 linked poly ubiquitination
  - Target proteins to the proteasome
- Lys63 linked poly ubiquitination
  - Signaling, DNA repair, stress response, endocytosis and signal transduction



UBIQUITIN PATHWAY

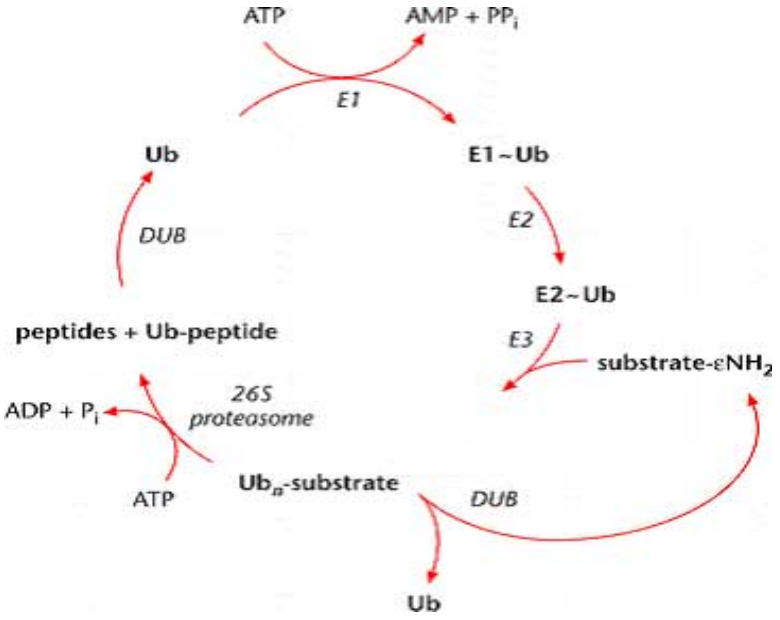
- Covalent Attachment of multiple ubiquitin molecules
- Degradation of the tagged protein
- 3 Enzymes :
  - Ub – Activating enzyme E1
  - Ub – Conjugating enzyme E2
  - Ub – Ligases E3

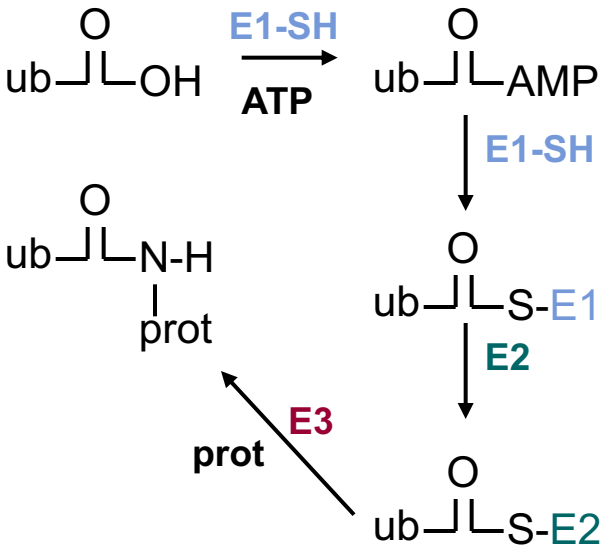
The ubiquitin degradation pathway



- E1 - ubiquitin activating enzyme
- E2 - ubiquitin conjugating enzyme
- E3 - ubiquitin ligase
- '~' denotes high-energy thioester bond
- DUB, deubiquinating enzyme

UBIQUITIN-MEDIATED DEGRADATION





- E1 - ubiquitin activating enzyme
  - uses ATP to activate the carboxyl group of ubiquitin's C-terminal residue (Gly76). The outcome of this reaction is the formation of a thioester between Gly76 of ubiquitin, and a cysteine residue of E1
- E2 - ubiquitin conjugating enzyme
  - accepts the ubiquitin from the E1 through a thioester linkage with a cysteine
- E3 - ubiquitin ligase
  - transfers the ubiquitin molecule to the epsilon NH_2 group of lysine on the substrate
- ubiquitin molecules are then added in succession to the Lysine 48 residue to form a multiubiquitin chain
- the DUB enzyme 'recycles' ubiquitin
- the 26S proteasome degrades the substrate to peptides