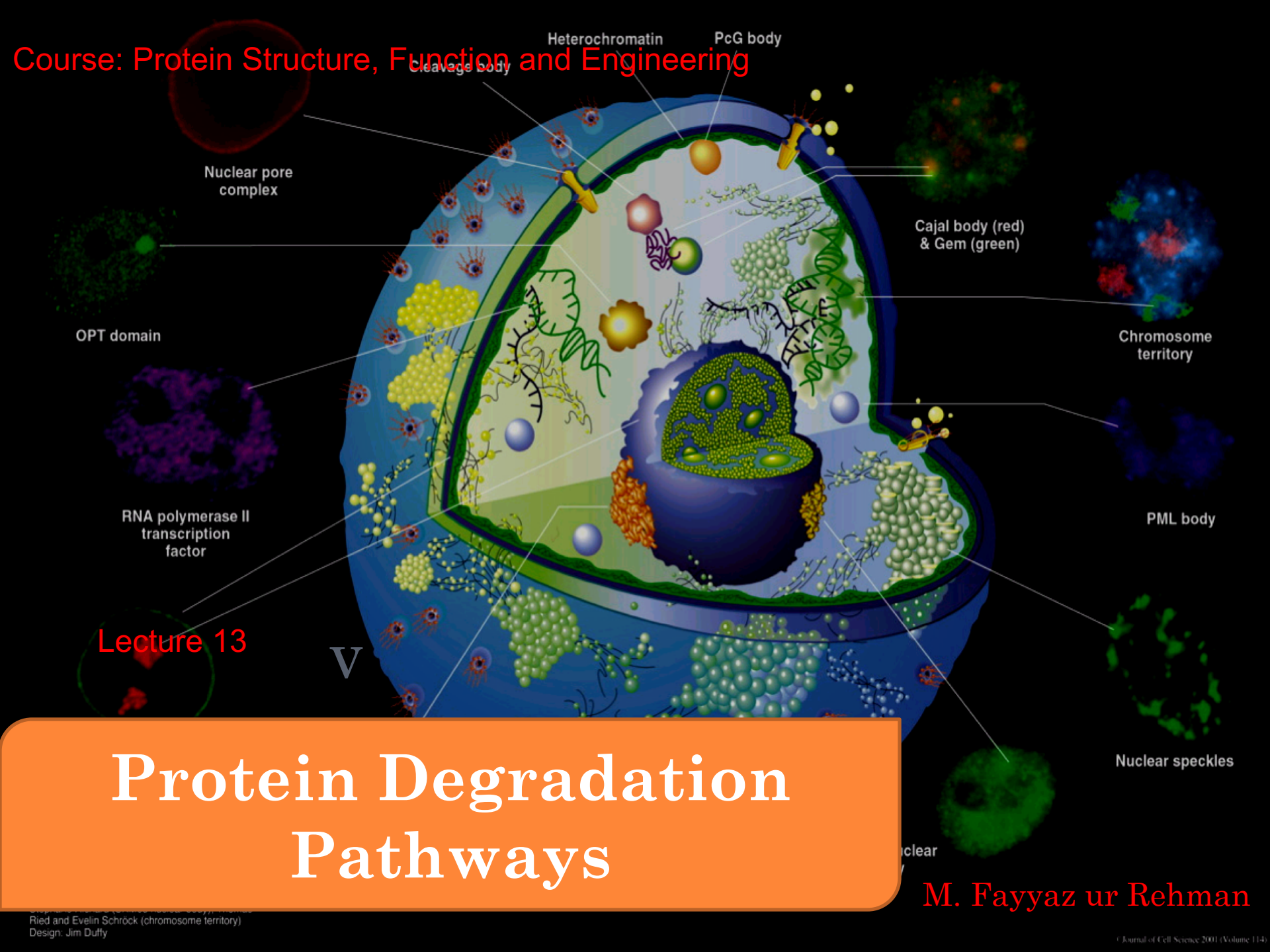
Course: Protein Structure, Function and Engineering

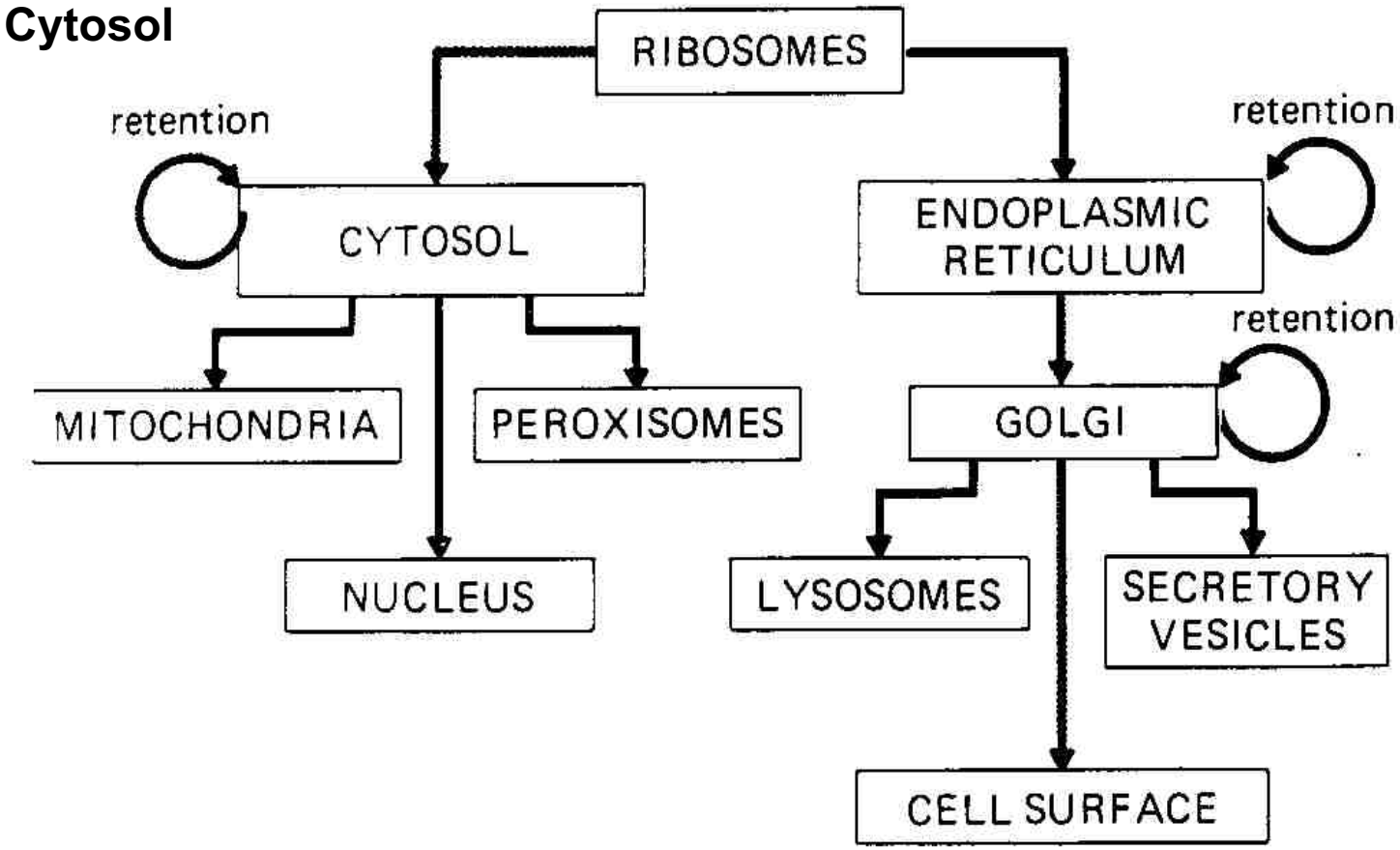


Lecture 13

Protein Degradation Pathways

M. Fayyaz ur Rehman

Compartments for Protein Sorting



Degradation of Misfolded Proteins

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

Which signals lead to ubiquitination?

- Genetic program (amino acids)
  - N—end rule
    - N—terminal amino acid: D,R,L,K,F (< minutes); A,G,M,S,V (>10 hours)
  - Sequence of significant hydrophobicity
  - 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" sequences (sequences rich in Pro, Asp, Glu, Ser and Thr)

Protein turnover; selective degradation/cleavage

- Phosphorylation of Ser and Thr
- Binding to adaptor proteins
- Protein damage
  - Processing
  - Oxidation of Cys and Met
  - Age-dependent modifications of side chains: hydrolsis; deaminations, racemizations, disulfide bond breaks, ketoamines…
  - Wrong folding

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

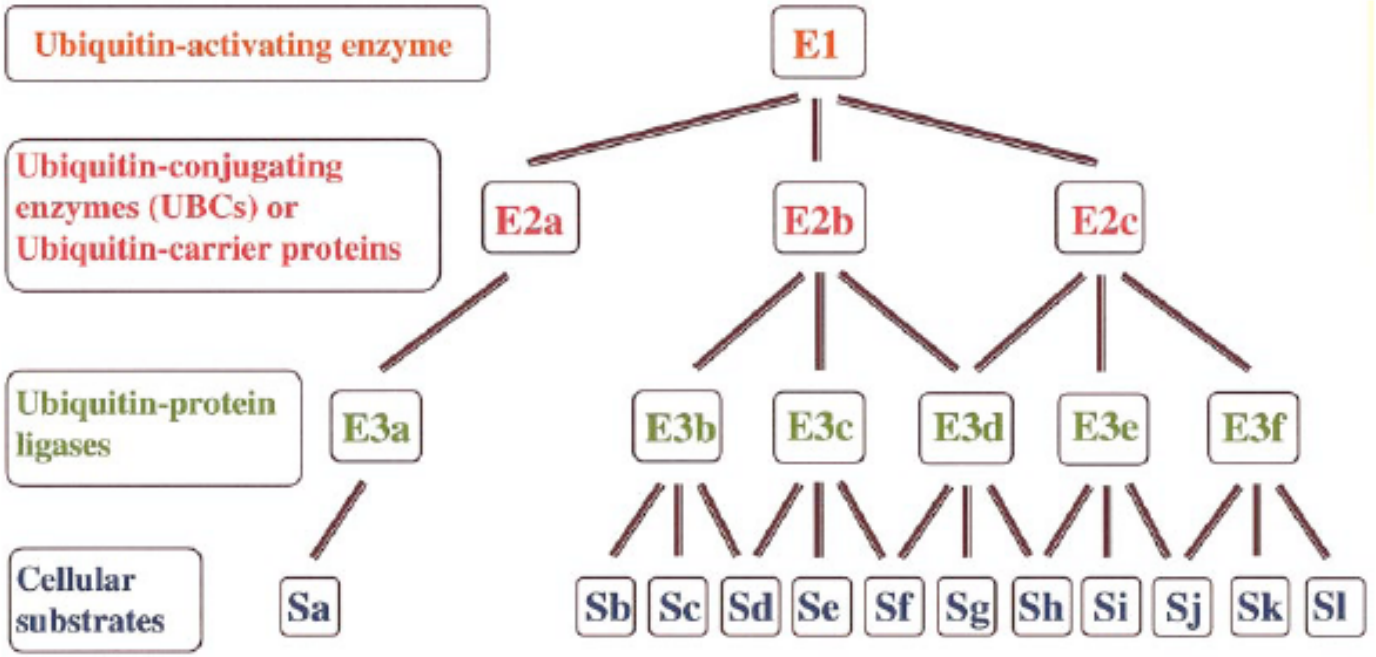
Enzymes of the Ubiquitination

- 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.

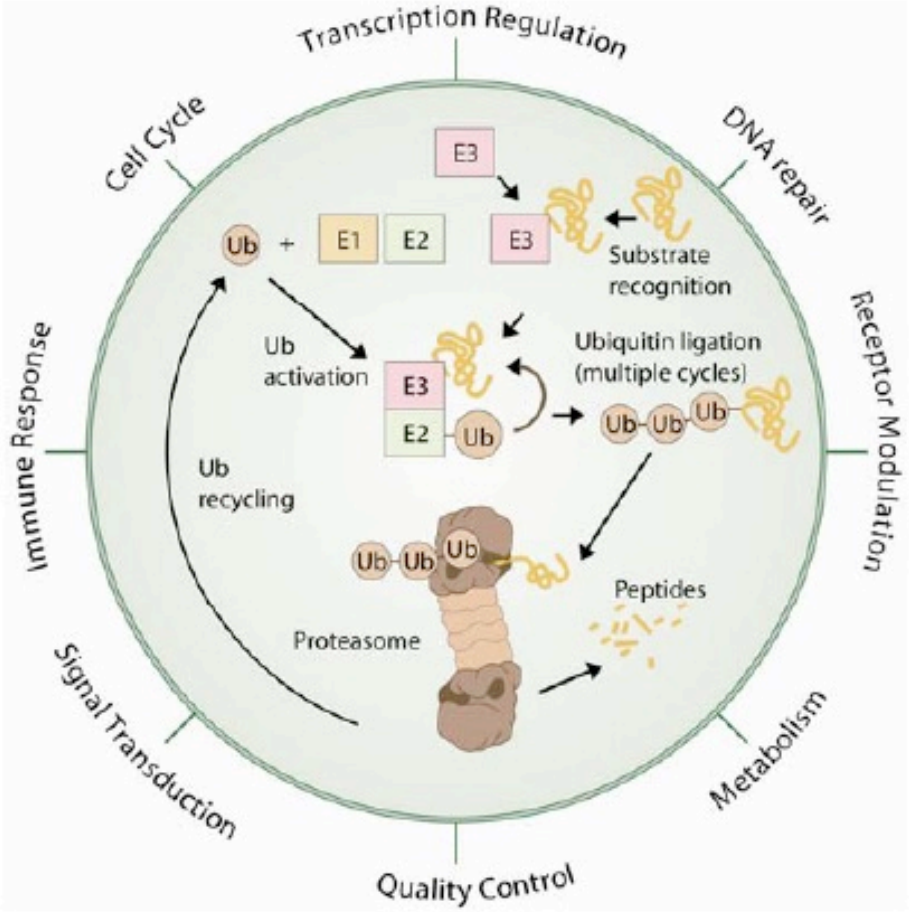
- E4:
  - catalyzes the efficient polymerization of very long polyubiquitin chains, it has been characterized in yeast.

Hierarchical structure

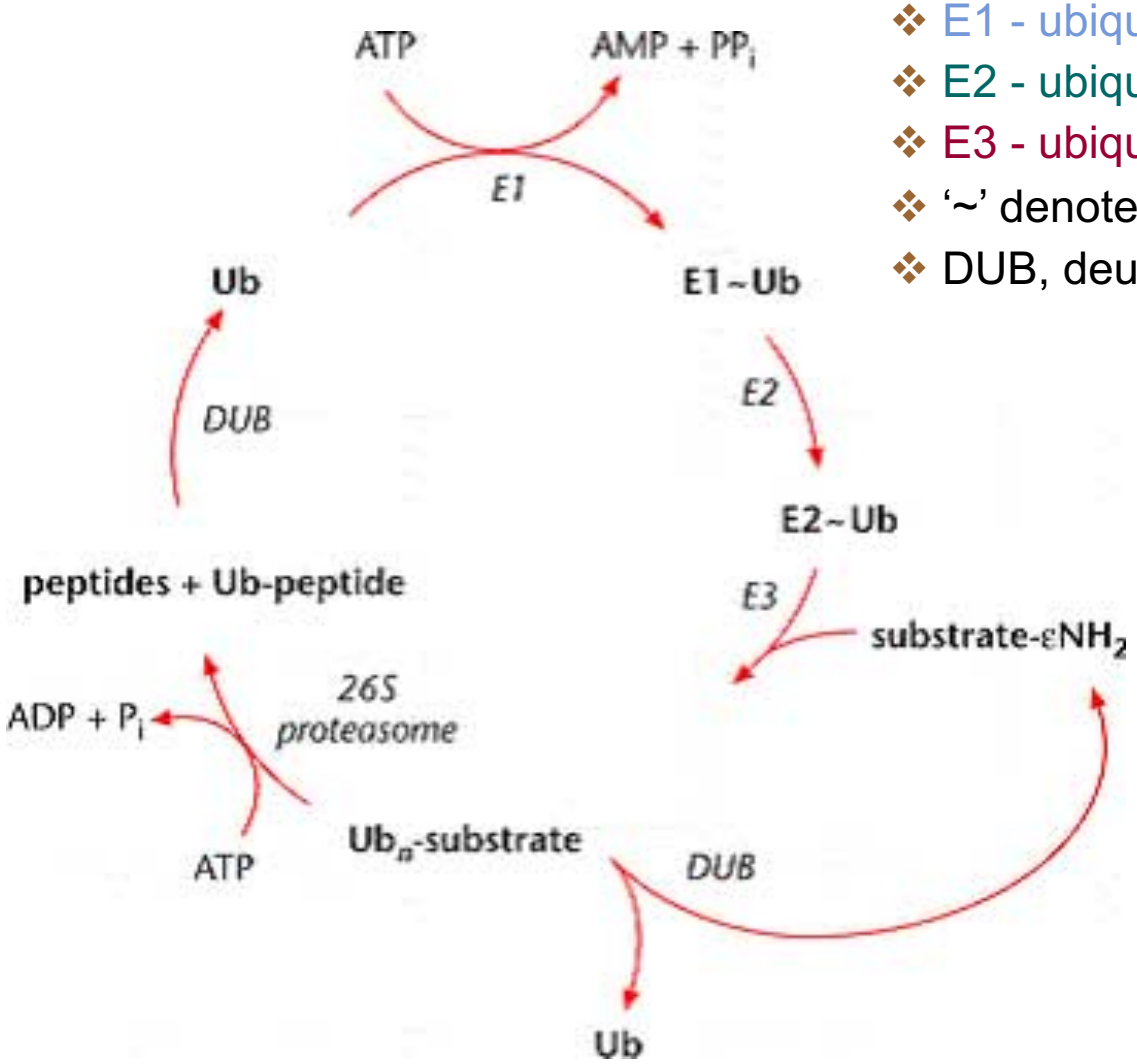


- Several E2 transfer Ub from E1 to E3 to which substrate protein is bound
- E3s catalyze covalent attachment to the substrate and recognize the substrate

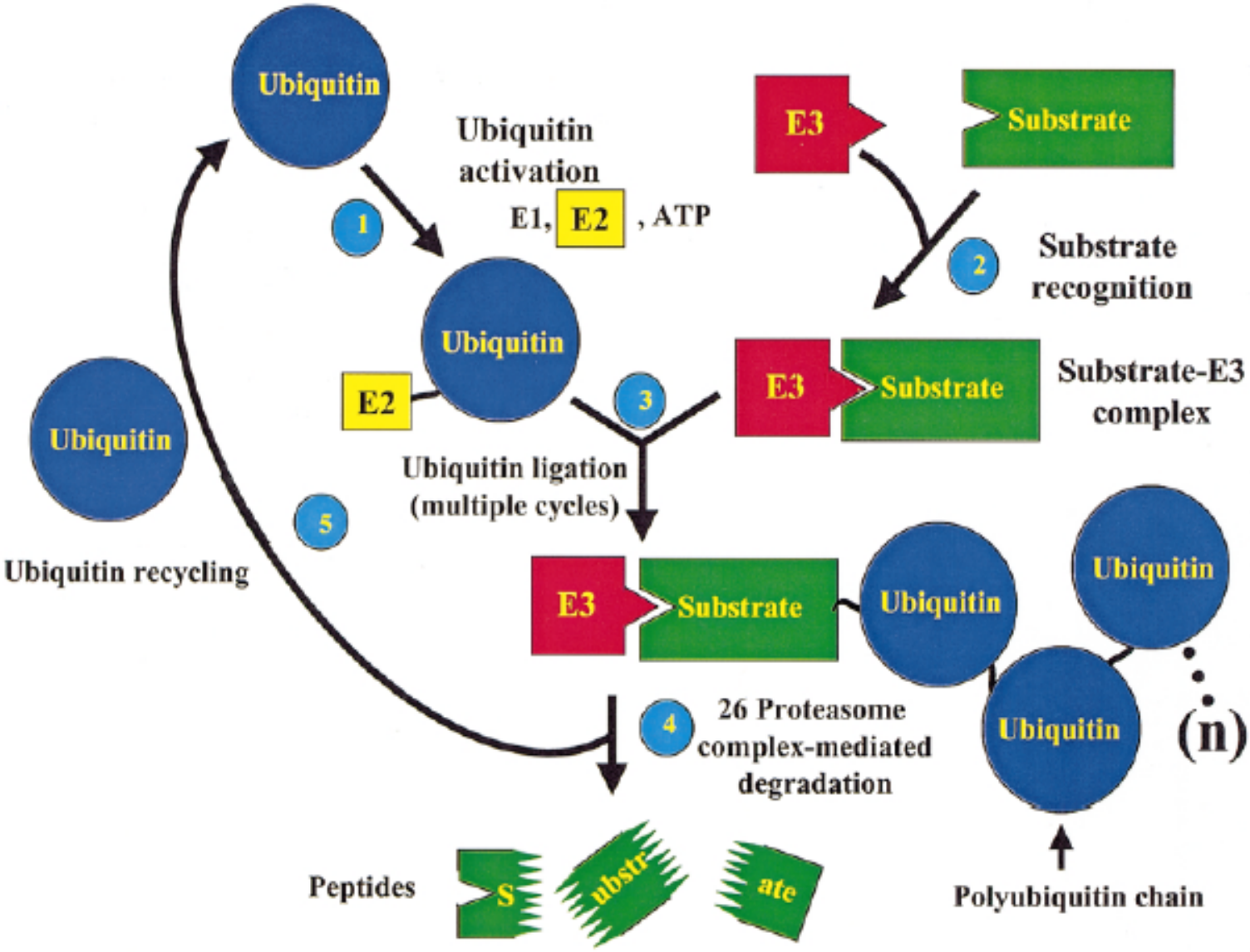
Biological function of Ubiquitin Proteosome pathway



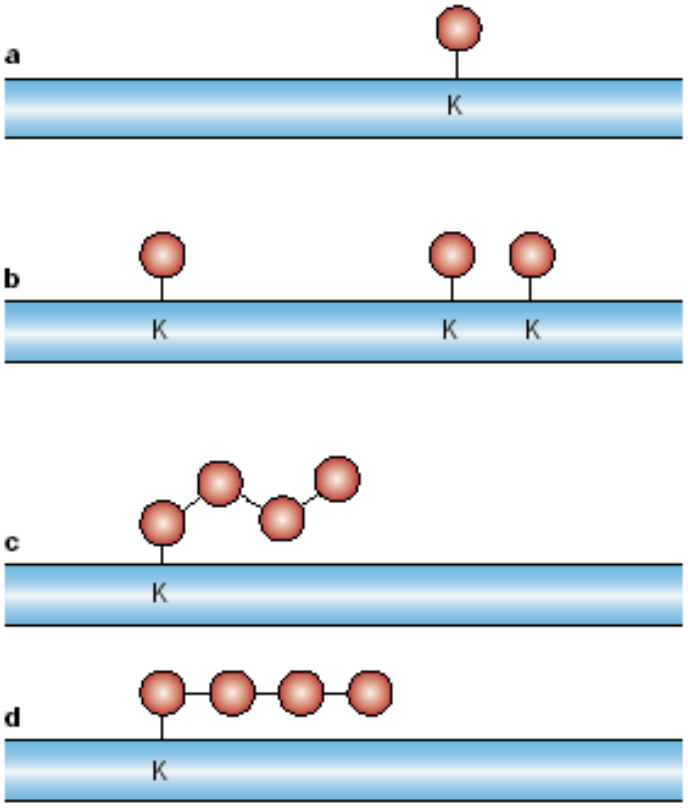
The ubiquitin degradation pathway



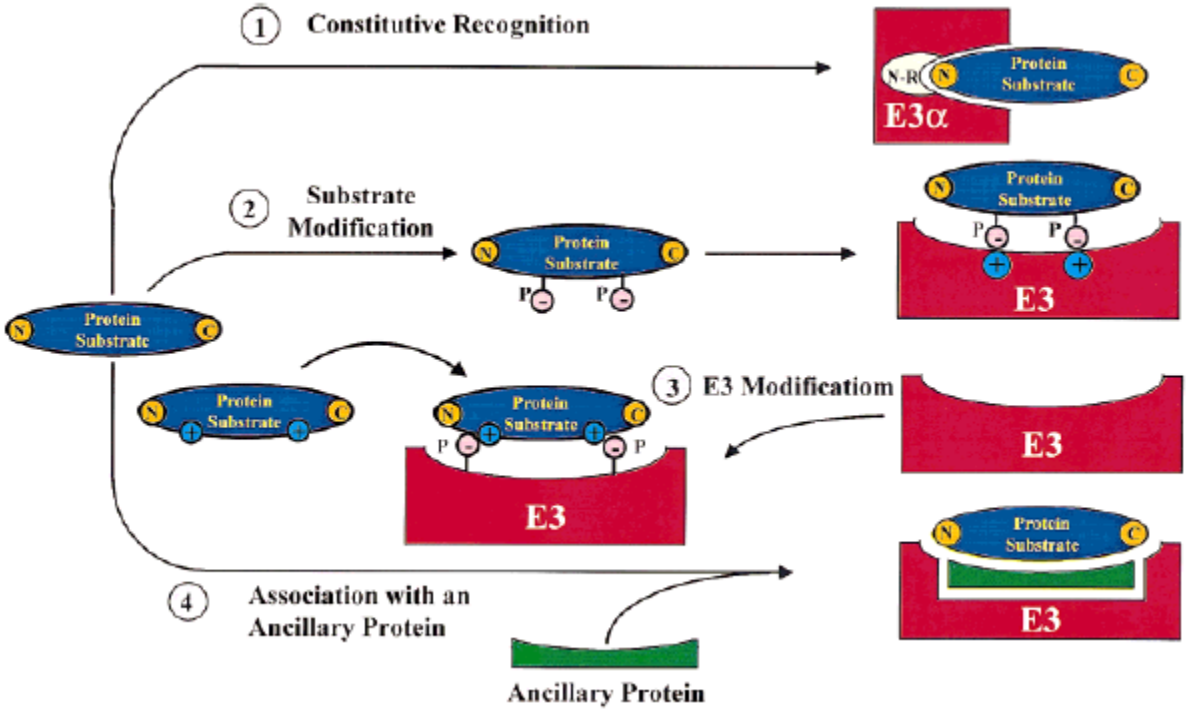
- E1 - ubiquitin activating enzyme
- E2 - ubiquitin conjugating enzyme
- E3 - ubiquitin ligase
- '~' denotes high-energy thioester bond
- DUB, deubiquinating enzyme


Ubiquitin
Ubiquitin activation
E1, E2 , ATP
1
E3
Substrate
2
Substrate recognition
Ubiquitin
E2
3
E3
Substrate
Substrate-E3 complex
Ubiquitin ligation (multiple cycles)
Ubiquitin
5
Ubiquitin recycling
E3
Substrate
Ubiquitin
Ubiquitin
Ubiquitin
(n)
4
26 Proteasome complex-mediated degradation
Peptides
S
ubstr
ate
Polyubiquitin chain

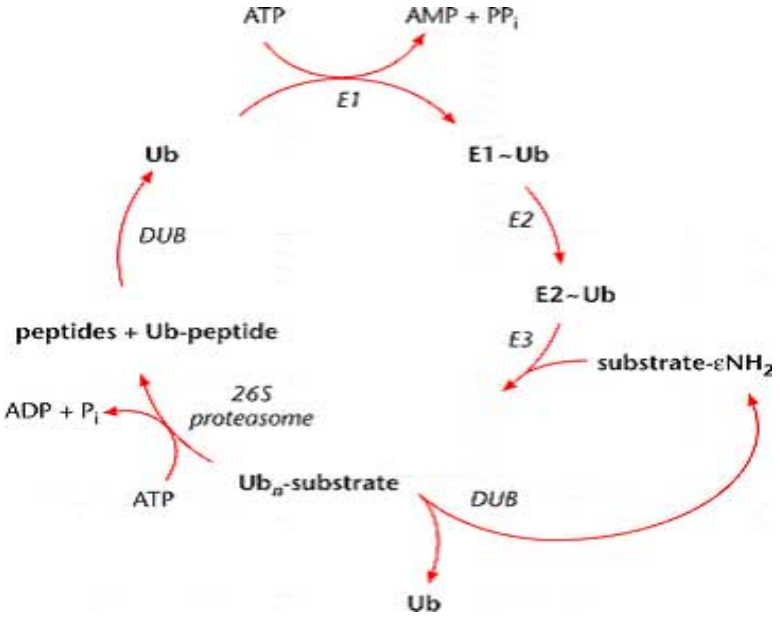
Mono- and multi- ubiquitination

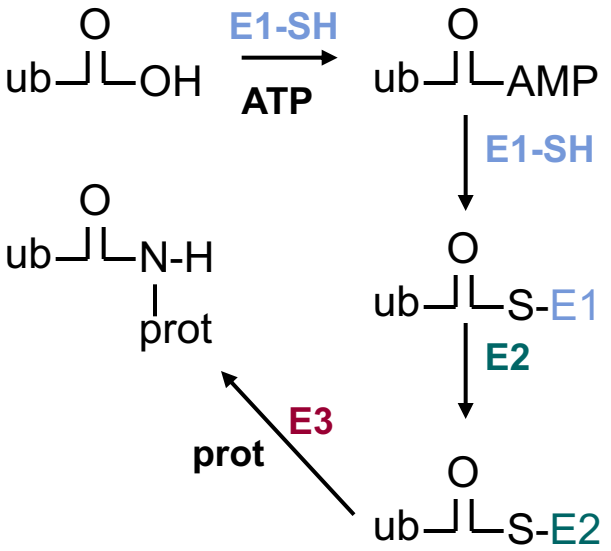


Modes of recognition of protein substrates by the different E3s



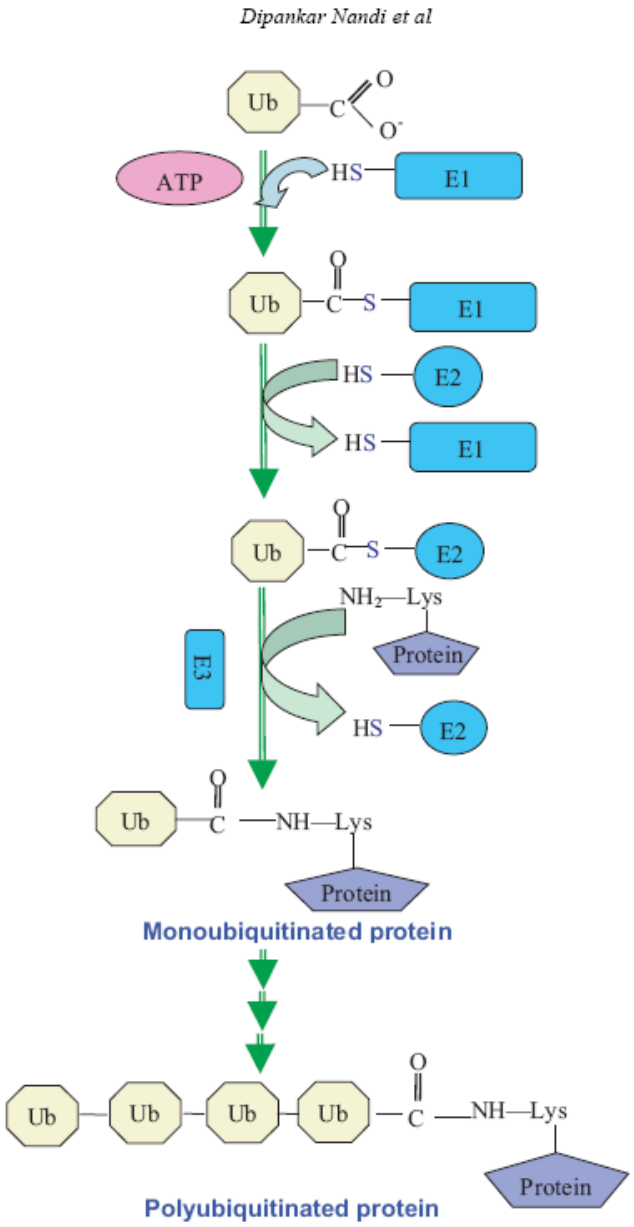
UBIQUITIN-MEDIATED DEGRADATION



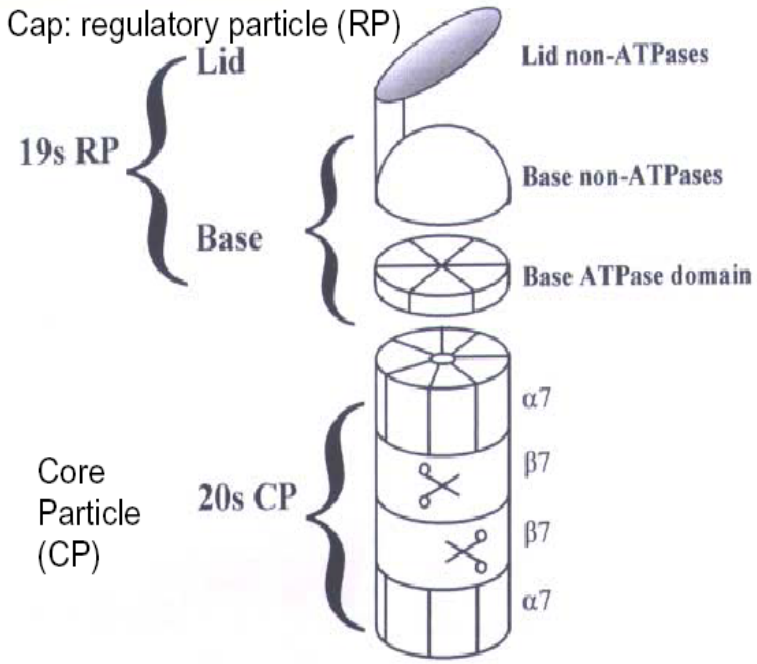


- E1 - ubiquitin activating enzyme
  - uses ATP to activate the carboxyl group of ubiquitin's C-terminal residue (Gly76). The outcome of this reaction is the formation of a thioester between Gly76 of ubiquitin, and a cysteine residue of E1
- E2 - ubiquitin conjugating enzyme
  - accepts the ubiquitin 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

Ubiquitin Pathway



Schematic representation of the eukaryotic



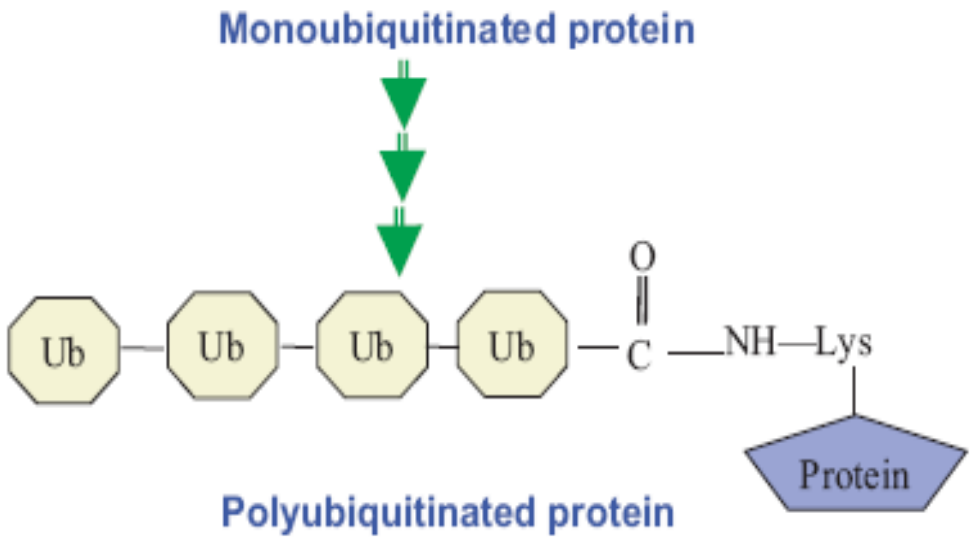
- Core particle is composed of four 7-membered rings.
- Two types of subunits (25 kDa): αand ß, all differ .
- Subunits are similar in structure, different in sequence.
- only only ß subunits are catalytically active .
- Cap region regulates activity, performes the energy dependent steps.

Processing via the proteasome

- Length of produced peptides: 3-23 amino acids
- Average length of peptides: 7-9 amino acids
- Peptide composition of given protein stays constant
- Protein is completely degraded before import of next protein
- Peptides produced by proteasome are further degraded by other roteases and aminopeptidases (Tricorn, Multicorn, Thimet, TPPII)
- Proteasome and immune system function:
  - Peptides of 8-9 amino acids in length are transported to the cell surface via the ER presented on the cell surface via MHC class I – molecules

Polyubiquitination

- Poly Ub chain synthesized by adding Ub moieties to Lys of the previous Ub
- Another enzyme E4 may be catalyzing this step



Deubiquitination

- Thiol proteases
- Ubiquitin processing (UBP) enzymes
  - Removes Ub from polyubiquinated proteins
- Ubiquitin carboxy terminal hydrolases (UBH)
  - Regenerates monomeric Ub

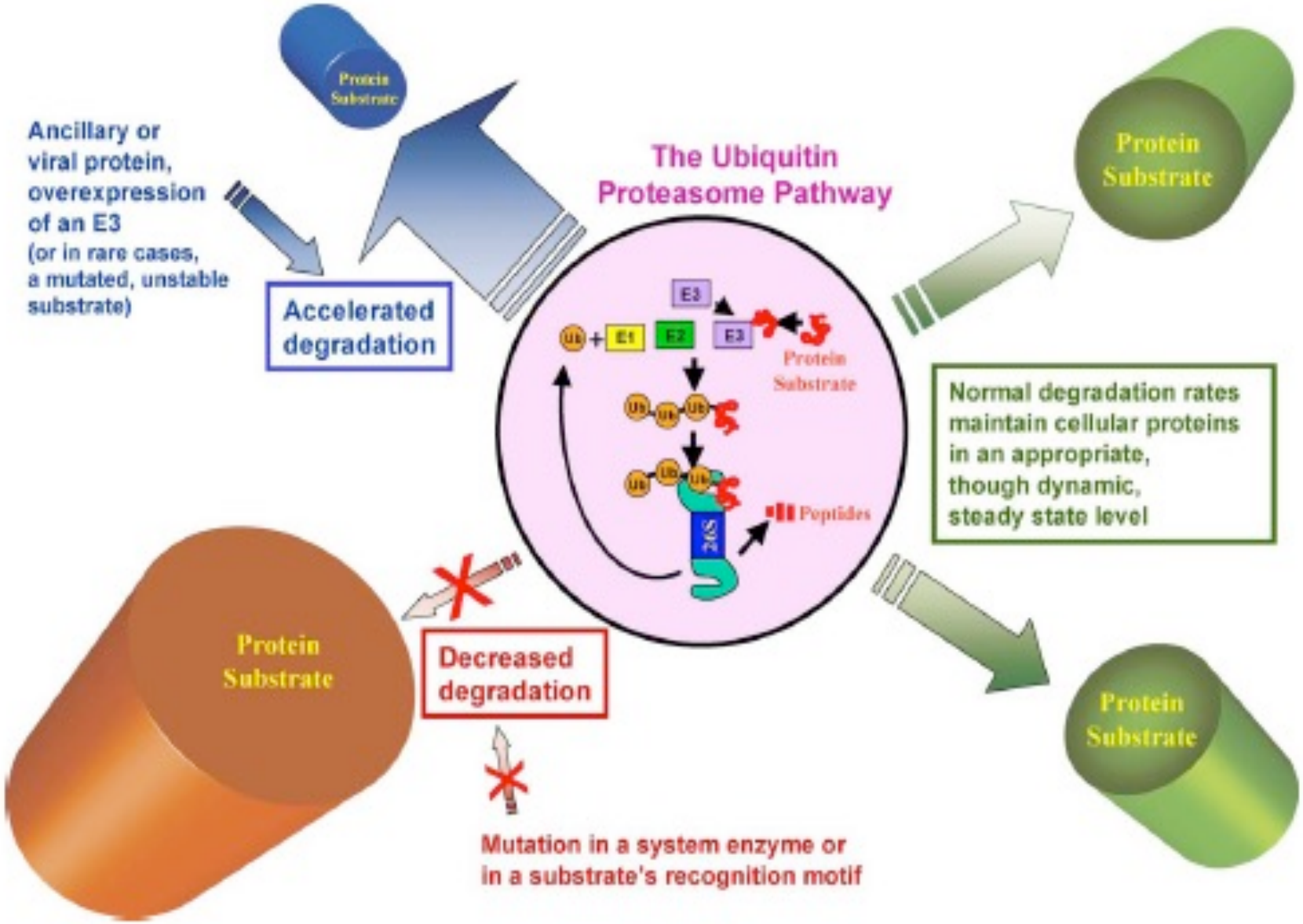
Regulation of ubiquitination:

- Some proteins regulate or facilitate ubiquitin conjugation.
- Regulation by phosphorylation of some target proteins has been observed.
    - E.g., phosphorylation of PEST domains activates ubiquitination of proteins rich in the PEST amino acids.
- Glycosylation of some PEST proteins with GlcNAc has the opposite effect, prolonging half-life of these proteins.
- GlcNAc attachment increases with elevated extracellular glucose, suggesting a role as nutrition sensor.

Regulation by ancillary proteins

- Several viral proteins exploit the ubiquitin system
  - by targeting for degradation cellular substrates which may interfere with propagation of the virus.
- In some instances, the viral protein functions as
  - a bridging‘ element between the E3 and the substrate,
  - thus conferring recognition in trans.
- The prototype of such a protein is the high risk HPV oncoprotein E6 which interacts with an E3 domain, and with the tumor suppressor protein p53.
- This interaction targets p53 for rapid degradation and, thus, most probably prevents stress signal induced apoptosis and ensures further replication propagation of the virus .

Consequences of Defects in Ubiquitination



Pathological Conditions Associated with Ubiquitin Proteosome Pathway

- Malignancies
- Neurodegenerative disorders
- Genetic disease
  - Cystic fibrosis, Angelman's syndrome & Liddle's syndrome
- Immune and inflammatory responses

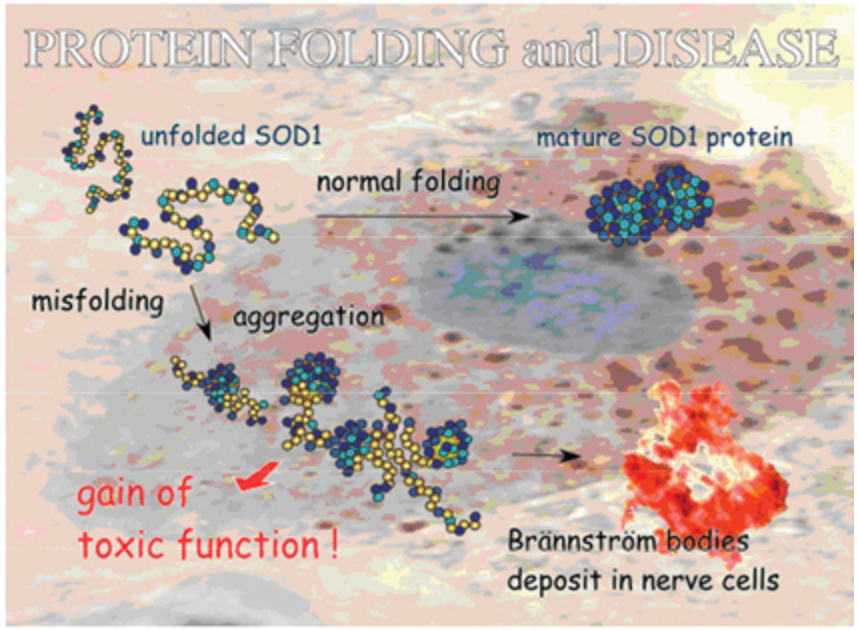
Malignancies

- Oncoproteins like NMyc, c-Myc, c-Fos, are substrates of U-P pathway.
- Destabilization of tumor suppressor genes like p53 and p27.
- Extremely low levels of p53 in uterine cervical carcinoma.

Neurodegenerative disorders

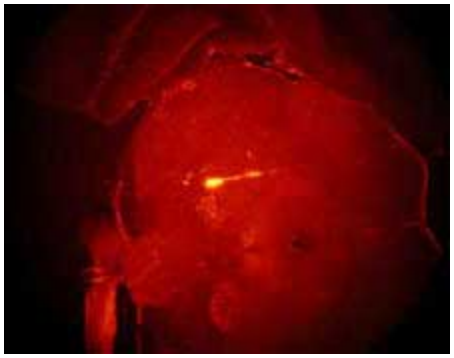
- Alzheimer's disease
- Parkinson's disease
- Huntington's disease
- Spinocerebellar ataxias
- Spinobulbar muscular dystrophy (Kennedy's syndrome)

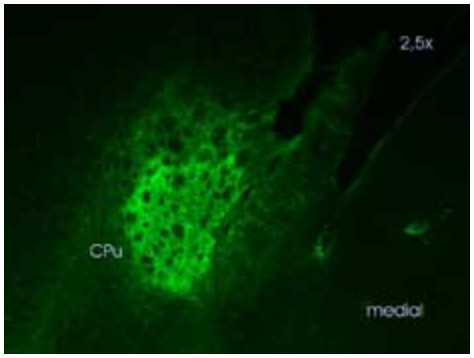
Formation of inclusion bodies



- Accumulation of ubiquitin may be secondary reflecting unsuccessful attempts of ubiquitination.
- Abnormal protein associate with each other forming aggregates.
- Hypothesis: Aggregated proteins inhibit ubiquitin proteosome pathway.

Parkinson's disease and Lewy Bodies





Angleman syndrome

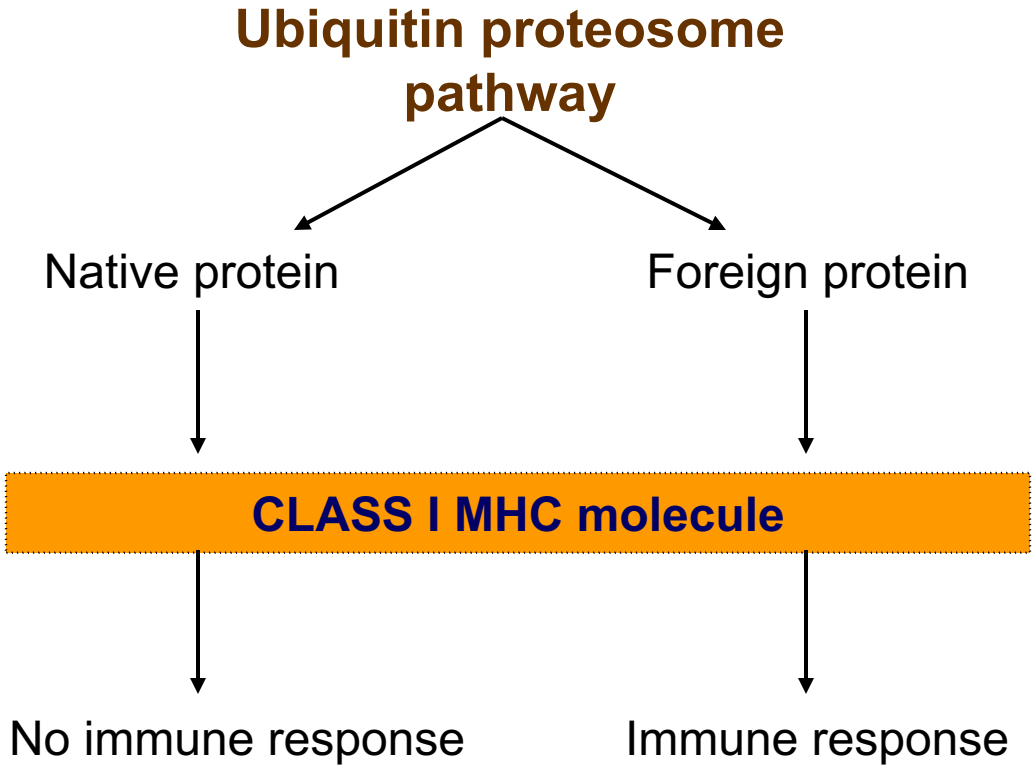
- Ubiquitin system is considered to be involved in brain development.
- Defective synthesis of gene coding for E3 ligase E6-AP
- Characteristic symptoms involve mental retardation, seizures, out of context frequent smiling and laughter.
- Brain proteins that could be stabilized by mutation have not been identified.

Cystic fibrosis

- Gene codes for a protein, CFTR, which is chloride ion channel.
- Small fraction of protein matures to the cell surface.
- Mutation in protein ΔF508, $CFTR^{\Delta F508}$ doesn't reach the cell surface.
- Ubiquitination degrades mutant $CFTR^{\Delta F508}$, resulting in complete lack of cell surface expression.

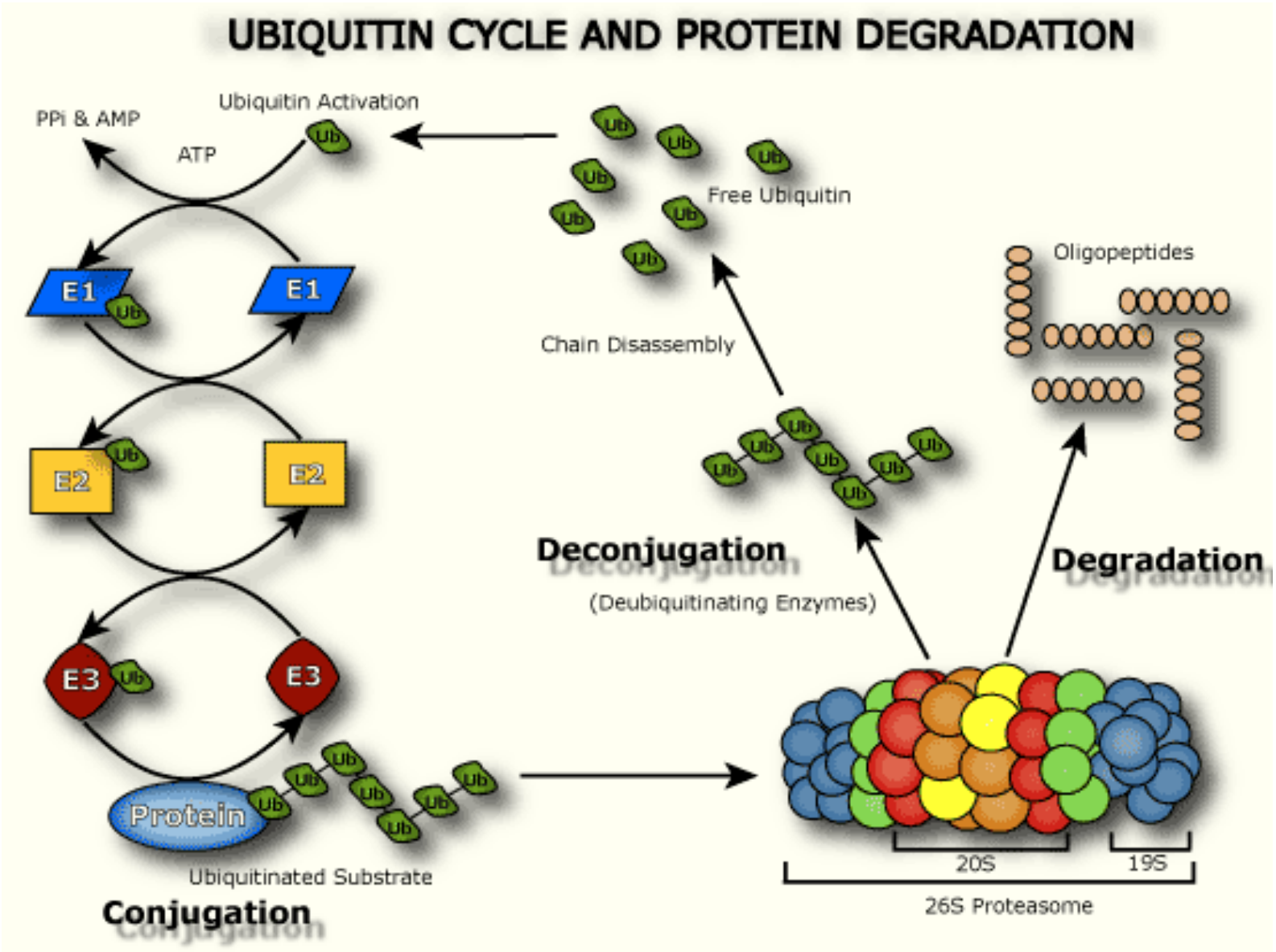
Immune and inflammatory responses

- Ubiqutin proteosome pathway is involved in processing of antigenic proteins.
- Epitopes are presented on class I MHC molecule generating T cell immune response.



Drug Development for Ubiquitin Dysfunction

- Inhibition of enzymes common to entire pathway would target the process non- specifically.
- Narrow window between benefits and toxicity needs to be identified.
- Develop completely specific E3 ligase inhibitors that would affect the pathways of interests.
- Better approach would be development of small molecules that ould be specific for substrates.


UBIQUITIN CYCLE AND PROTEIN DEGRADATION
PPi & AMP
ATP
Ubiquitin Activation
Ub
E1
E2
E3
Protein
Ubiquitinated Substrate
Conjugation
Free Ubiquitin
Chain Disassembly
Deconjugation
(Deubiquitinating Enzymes)
Oligopeptides
Degradation
20S
19S
26S Proteasome

PROTEOLYTIC ENZYMES

- several classes
- **Serine proteases** include
- Digestive enzymes
  - trypsin, chymotrypsin, & elastase.
- Different serine proteases differ in substrate specificity. For example:
  - Chymotrypsin prefers an aromatic side chain on the residue whose carbonyl carbon is part of the peptide bond to be cleaved.
  - Trypsin prefers a positively charged Lys or Arg residue at this position.

Site of intracellular degradation

- Ubiquitin—mediated degradation of cytosolic and membrane proteins occurs in the
  - cytosol and
  - on the cytosolic face of the ER membranes.
- Although components of the system have been localized to the nucleus, conjugation and degradation have not been demonstrated in this organelle.

PROTEOLYTIC ENZYMES

- **Aspartate proteases** include
  - the digestive enzyme pepsin
  - Some proteases found in lysosomes
  - the kidney enzyme renin
  - HIV-protease.
- Two aspartate residues participate in acid/base catalysis at the active site.
  - In the initial reaction, one aspartate accepts a proton from an active site H2O, which attacks the carbonyl carbon of the peptide linkage.
  - Simultaneously, the other aspartate donates a proton to the oxygen of the peptide carbonyl group.

PROTEOLYTIC ENZYMES

- **Zinc Proteases** (metalloproteases) include:
  - digestive enzymes carboxypeptidases
  - matrix metalloproteases (MMPs), secreted by cells
  - one lysosomal protease.
- Some MMPs (e.g., collagenase) are involved in degradation of extracellular matrix during tissue remodeling.
- Some MMPs have roles in cell signaling relating to their ability to release cytokines or growth factors from the cell surface by cleavage of membrane-bound pre-proteins.

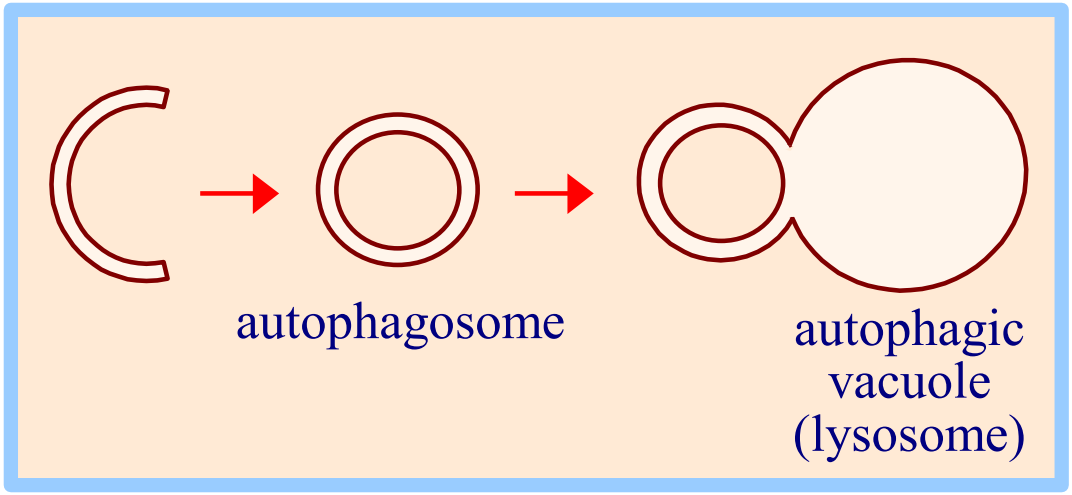
PROTEOLYTIC ENZYMES

- **Cysteine proteases**
  - a cysteine sulfhydryl group.
    - Papain is a well-studied plant cysteine protease.
    - Cathepsins are a large family of lysosomal cysteine proteases, with varied substrate specificities.
    - Caspases are cysteine proteases involved in activation & implementation of apoptosis (programmed cell death).
      - Caspases get their name from the fact that they cleave on the carboxyl side of an aspartate residue.
    - Calpains are Ca++-activated cysteine proteases that cleave intracellular proteins involved in cell motility & adhesion.
      - They regulate processes such as cell migration and wound healing.

- The proteasome hydrolases constitute a unique family of **threonine proteases**. A conserved N-terminal threonine is involved in catalysis at each active site.
- The 3 catalytic b subunits are synthesized as pre-proteins. They are activated when the N-terminus is cleaved off, making threonine the N-terminal residue.
- Catalytic threonines are exposed at the lumenal surface.

- Lysosomes contain a large variety of hydrolytic enzymes that degrade proteins & other substances taken in by endocytosis.
- Materials taken into a cell by inward budding of vesicles from the

- 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.
- **chaperone-mediated autophagy**
  - Cytosolic proteins that include the sequence KFERQ may be selectively taken up by lysosomes .
  - This process is stimulated under conditions of nutritional or oxidative stress, involves interaction of proteins to be degraded with:
    - Cytosolic chaperones that unfold the proteins.
    - A lysosomal membrane receptor (LAMP-2A) that may provide a pathway across the membrane.
    - Chaperones in the lysosomal lumen that may assist with translocation across the membrane.

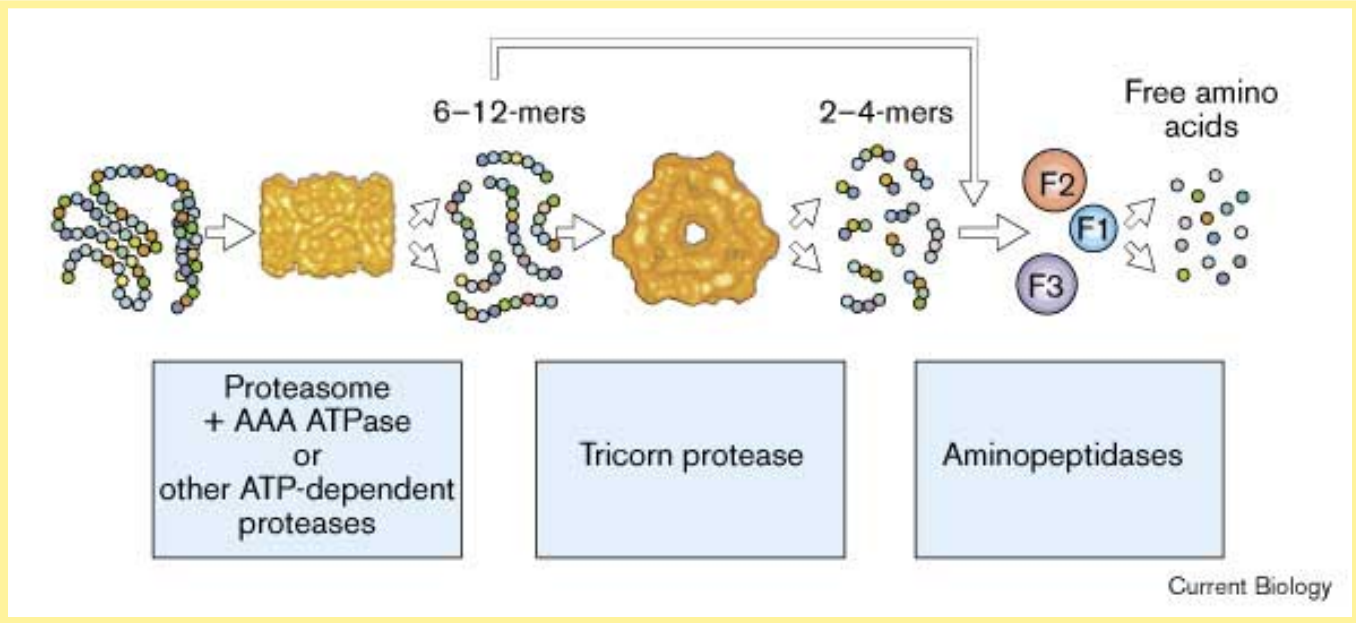
- Proteasomal degradation of particular proteins is an essential mechanism by which cellular processes are regulated, such as cell division, apoptosis, differentiation and development.
  - E.g., progression through the cell cycle is controlled in part through regulated degradation of proteins called cyclins that activate cyclin-dependent kinases.

Protease Inhibitors

- Many inhibitors of proteasome protease activity are known, some of which are natural products and others experimentally produced.
  - TMCs are naturally occurring proteasome inhibitors.
    - They bind with high affinity adjacent to active site threonines within the proteasome core complex.
    - TMCs have a heterocyclic ring structure derived from modified amino acids.
- Proteasome inhibitors cause cell cycle arrest and induction of apoptosis (programmed cell death) when added to rapidly dividing cells.
  - The potential use of proteasome inhibitors in treating cancer is being investigated.

TRICORN PROTEIN DEGRADATION PATHWAY

- Tricorn protease in prokaryotes may be part of a degradation pathway that involves proteasome (in archaea) or other ATP-dependent proteases in archaea/bacteria
- Proteasomes/other oligomeric proteases digest proteins to small peptides
- Tricorn protease then cleaves these to 2-4 mers, which are then degraded down to the level of free amino acids by aminopeptidases



- probably one of many pathways of protein degradation in prokaryotes

Membrane protein degradation

- AAA proteases mediate the degradation of membrane proteins in bacteria, mitochondria and chloroplasts (i.e., compartments of eubacterial origin)
- combine proteolytic and chaperone activities in one system, acting as quality-control machineries

Degradation of foreign proteins

- The immune system is a surveillance mechanism that can
  - recognize foreign proteins
  - degrade them
- An essential feature of this system is the ability to distinguish ‘self’ from ‘non-self’.
- The MHC class I antigen presenting cells display peptide fragments that are derived from the foreign protein, to cytotoxic T cells.
- The generation of these peptides requires the 26S proteasome.

Degradation of regulators:

- Many regulators of cell growth and development are
  - highly unstable proteins,
  - Stability is controlled by the ubiquitin/proteasome pathway
  - Substrates of this pathway include p53, Rb, cyclins, CDK inhibitors, transcription factors, and signal-transducing molecules.
  - Distinct targeting complexes accomplish the recognition of these proteins.

The generation of active proteins

- Enzymes whose activities can be deleterious to the cell are often expressed as precursors that are catalytically inactive.
- The proteolytic cleavage of the precursor generates an active enzyme.
  - For instance, proteases that are present in the digestive tract,
  - those that function in the lysosome, are initially synthesized as precursors.
  - Ubiquitin, and catalytic subunits of the proteasome are also expressed as precursors that are proteolytically processed to yield catalytically active subunits.

The recycling of amino acids:

- Proteases are required for
  - Generation of free amino acids from short peptides
    - Generated by the proteasome
    - intracellular proteases.
- In many microorganisms dipeptidases and other proteases that hydrolyze short amino acid chains
- The availability of free amino acids and di-peptides can allosterically regulate the activity of a specific E3 protein,
  - which in turn controls the levels of a transcription factor that is required for inducing amino acid biosynthetic pathway genes.