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FACULTY NAME	**SCIENCE**
DEPARTMENT NAME	**LIFE SCIENCES & BIOTECHNOLOGY**
SUBJECT NAME	**CELL SIGNALLING – PART-4**
PROGRAM	**FOR Ph.D. STUDENTS**
SUBTOPIC NAME	**Protein degradation**
CONTENT TYPE	**PDF**
SEARCH KEYWORDS	**Protein degradation, Ubiquitinylation, Proteasome**

Protein degradation

Objectives:

To acquaint the students about:

i) components of cell signaling

ii) types of cell signaling

iii) cell signaling molecules

iv) nuclear receptors

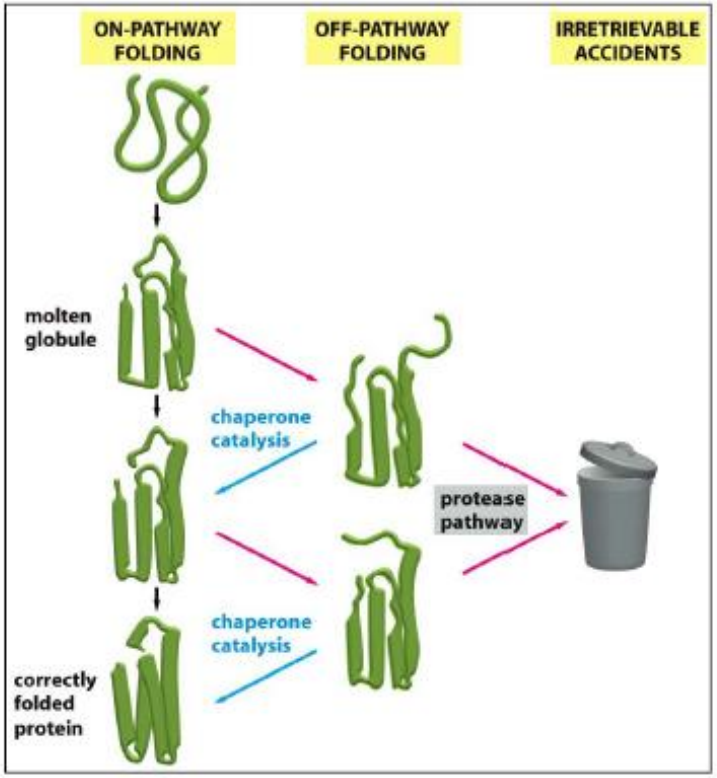
v) G-Protein coupled receptors

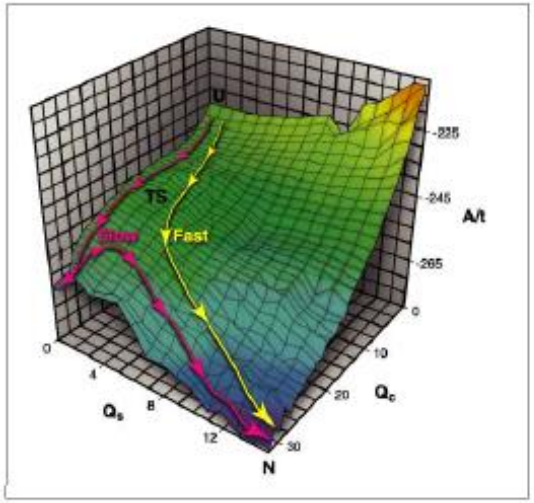
vi) Signal transduction by G-proteins

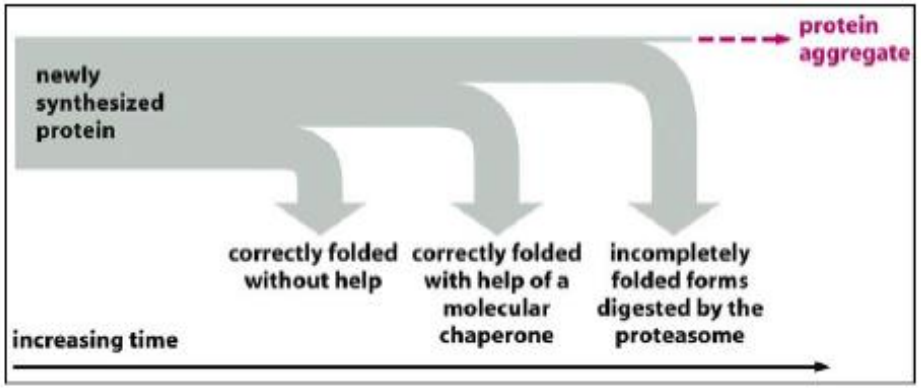
vii) Adenylate cyclase signaling and

viii) Phospholipase C Signaling.

Different Outcomes of Newly Synthesize Proteins







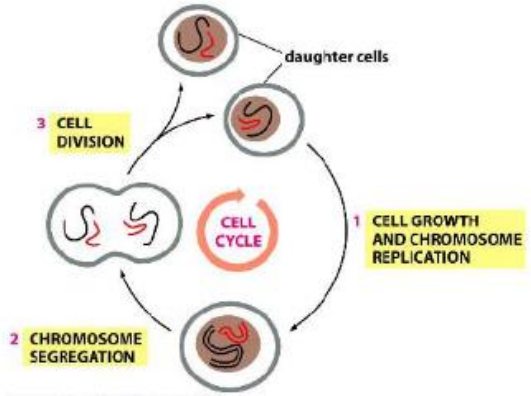
Protein Degradation

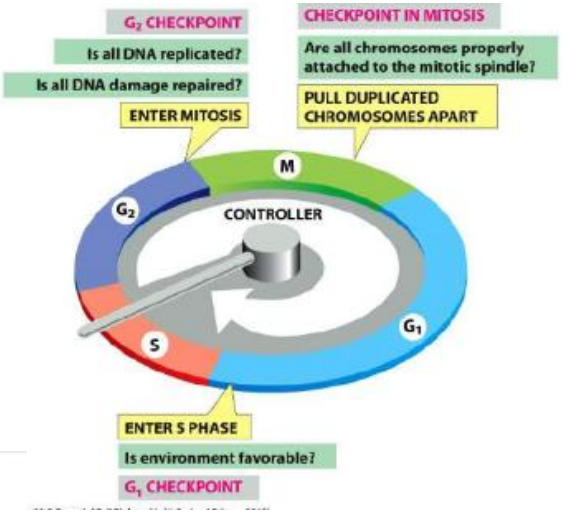
- Prevents build up of abnormal, damaged or unwanted proteins
- Recycles amino acids for protein synthesis for energy
- Uses specialized protein degrading systems
  - ATP dependent pathways in the cytosol
    - proteasome in eukaryotes and prokaryotes
    - Lon protease in prokaryotes
  - Lysosomes acidic, membrane bound organelle containing proteolytic enzymes.
- Essential role in health and disease

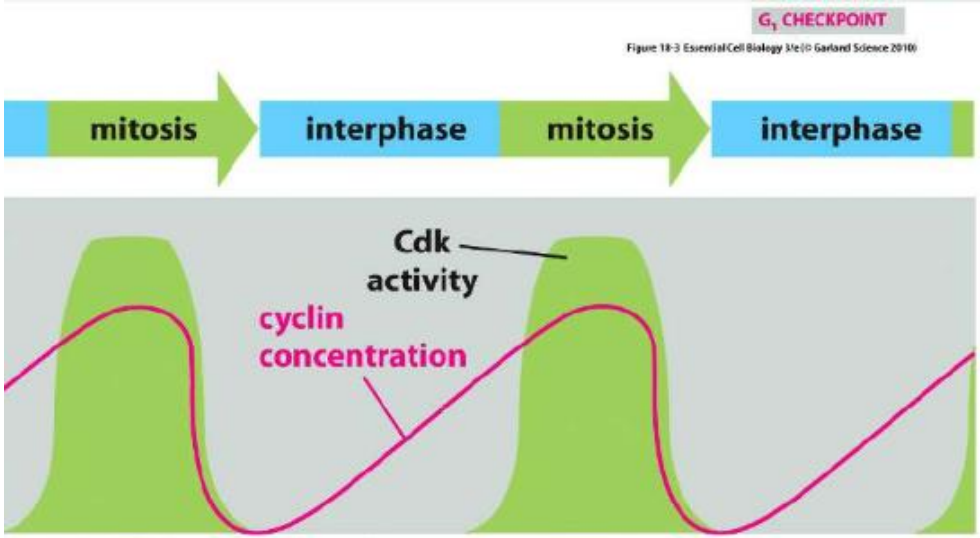
Importance of Protein Degradation

- Abnormal proteins
  - abnormal gene
    - mutation in gene
      - producing mutant protein
    - abnormal regulation of gene
      - abnormal (over)production of protein
  - translation errors
    - mutation in protein incorrectly folding
  - viral proteins
- Damaged proteins
  - environmental damage
    - reactive oxygen species
      - eg. superoxide, peroxide, and hydroxyl radicals

- ‘Unwanted’ proteins
  - those damaged or abnormal proteins that have no function, altered function, or pathological function
  - proteins whose ‘job is done’ for example
    - transcription factors in gene regulation
    - cyclin proteins in cell cycle regulation
    - signal transduction (hormone-receptor complexes)



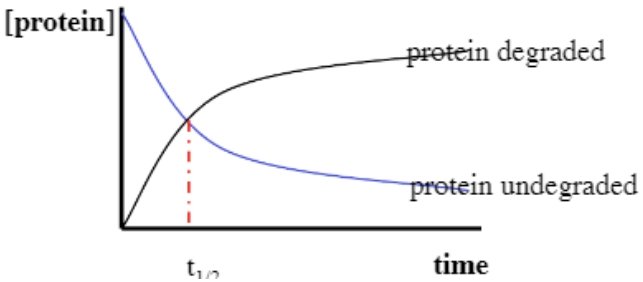




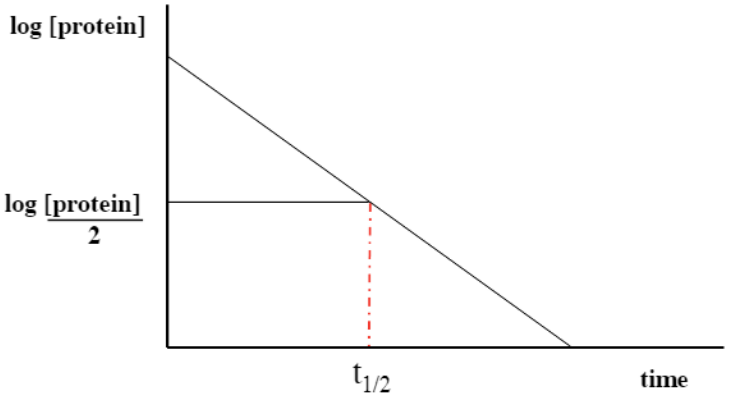
Protein Turnover

- protein degradation part of the concept of 'protein turnover'
  - a dynamic state where proteins are synthesised and then degraded in the cell at a particular rate
- 'steady-state' of protein turnover
  - where concentration of protein in a cell is unchanged with ti
    - this steady-state is maintained such that synthesis is equal to degradation
- protein turnover different from protein stability
  - proteins can vary widely in their turnover but be very similar in stability
    - protein stability measured by denaturation

- Half-life
  - time taken to degrade half of the total amount of protein
  - measured from pulse-chase experiments on total protein degradation
    - protein degradation is first order process



Protein Turnover - Half Life



$t_{1/2}$ = half-life. time required to degrade half [protein]

Protein Turnover - Half Life

- Short half life (minutes to hours)
  - extracellular proteins
    - digestive enzymes, polypeptide hormones, antibodies
  - regulatory proteins
    - enzymes catalyzing rate determining steps
- Longer half life (days to months)
  - structural proteins
    - collagen, tubulin
  - non-regulatory proteins (inside cell)
    - eg. haemoglobin 120 days

Death of proteins

- Chemical ageing: changes in amino acid structure and side chains
- Deamidation (removal of an amide functional group) of glutamine and asparagine
- Oxidation of sulphur-containing residues
- Racemisation about the alpha carbons which is slow
- Peptide bond hydrolysis or proteolysis (adjacent to aspartate residues)
- Ubiquitination/Sumolyation

This is followed by regulated and targeted degradation

- Endosome/Lysosome pathway
- Ubiquitin/Proteosome pathway

Proteins Degradation Rates

Rapid turnover proteins: usually rate-limiting enzymes of metabolic pathways

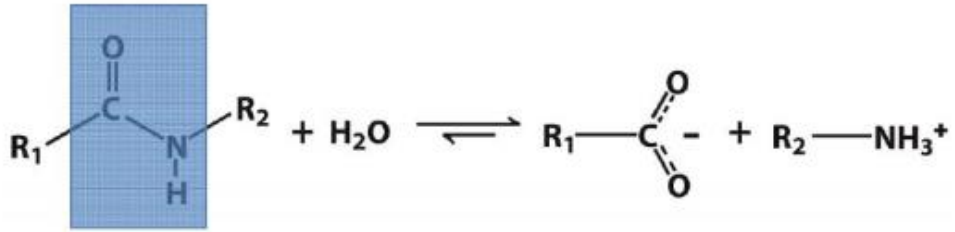
Slow turnover proteins: usually structural proteins

Protein	Half-Life
RNA polymerase	90 minutes
Hexokinase	24 hours
Actin	1 week
Myosin	1 month
Haemoglobin	?
Lens cristallins	> 80 years

Proteases

cleave proteins by hydrolysis.

Protein hydrolysis is exergonic but kinetically very slow.



Serine proteases

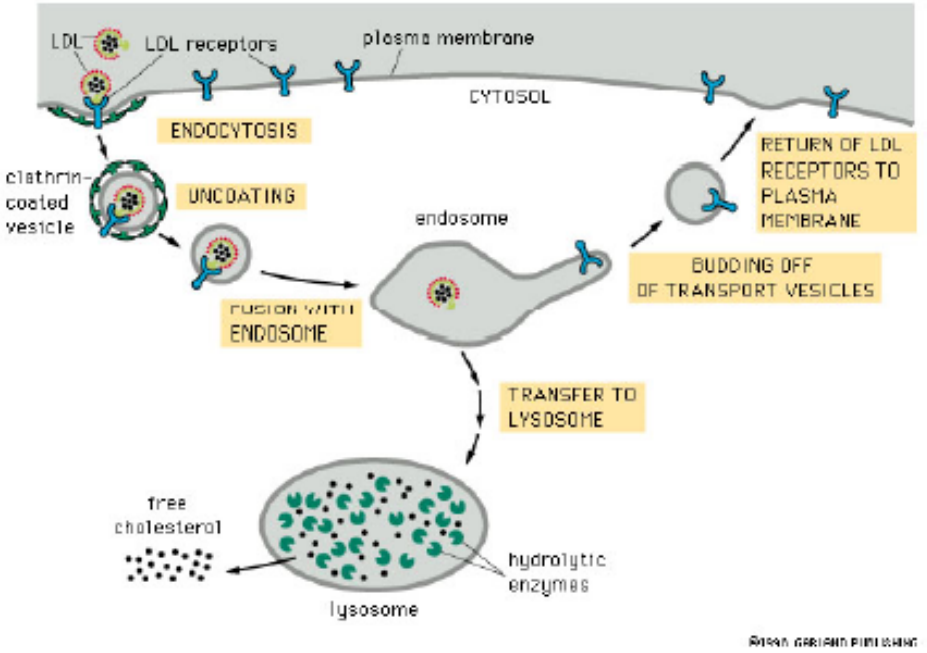
include digestive enzymes **trypsin**, **chymotrypsin**, & **elastase**.

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.

Protein Degradation Pathways

- Approximately 1/3 of newly synthesized proteins are degraded.
- Lysosome degrades proteins and lipids taken in by endocytosis.
- Proteasome is present in both nucleus and cytoplasm.
- Proteasome degrades both cytoplasmic and nuclear proteins after they are marked through conjugation with ubiquitin.

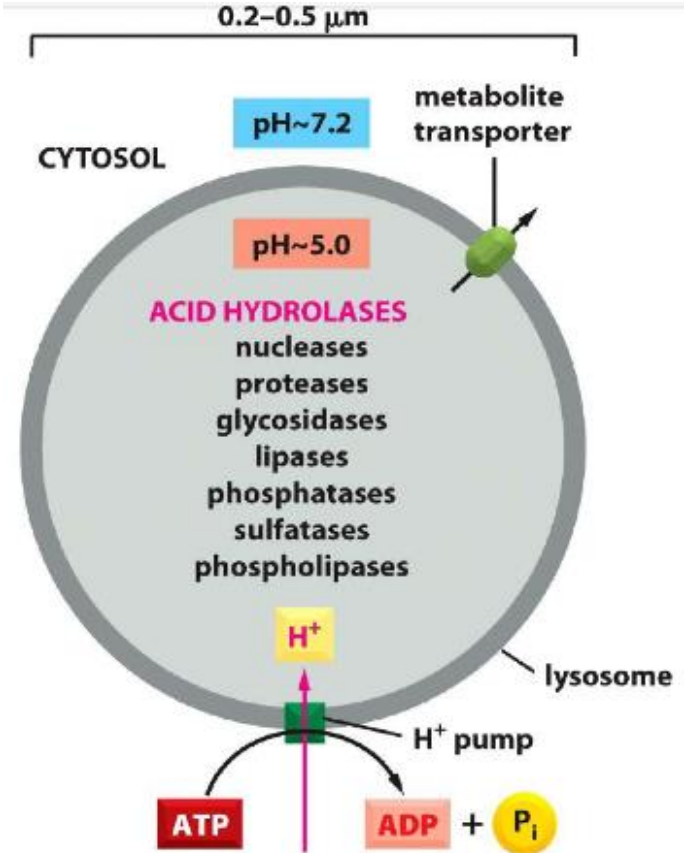


Sites of Cellular Protein Degradation

- Lysosomes
- Cytosol
  - Proteasomes
  - Cytosolic proteases

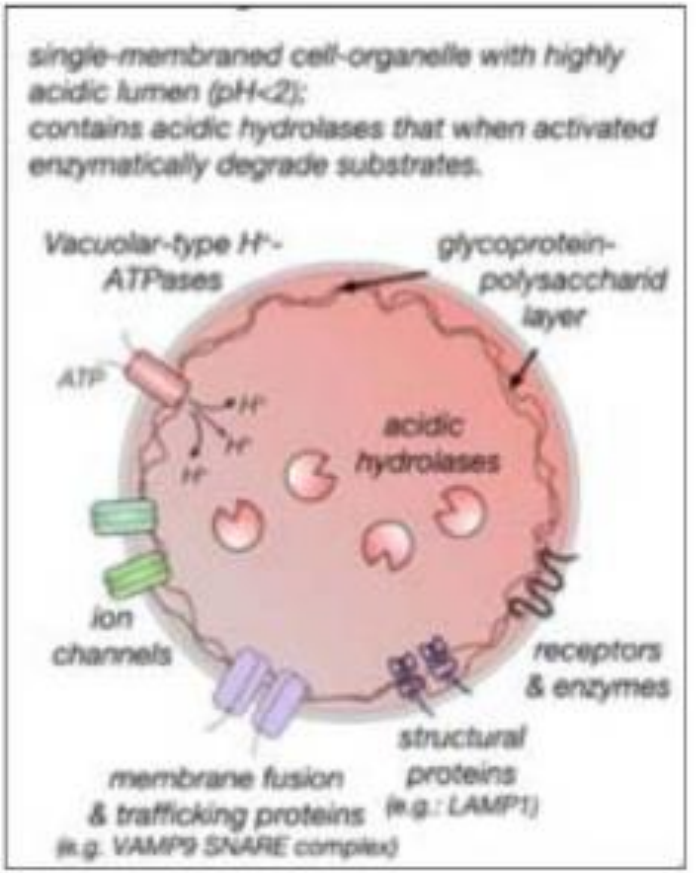
Lysosome Protein Degradation

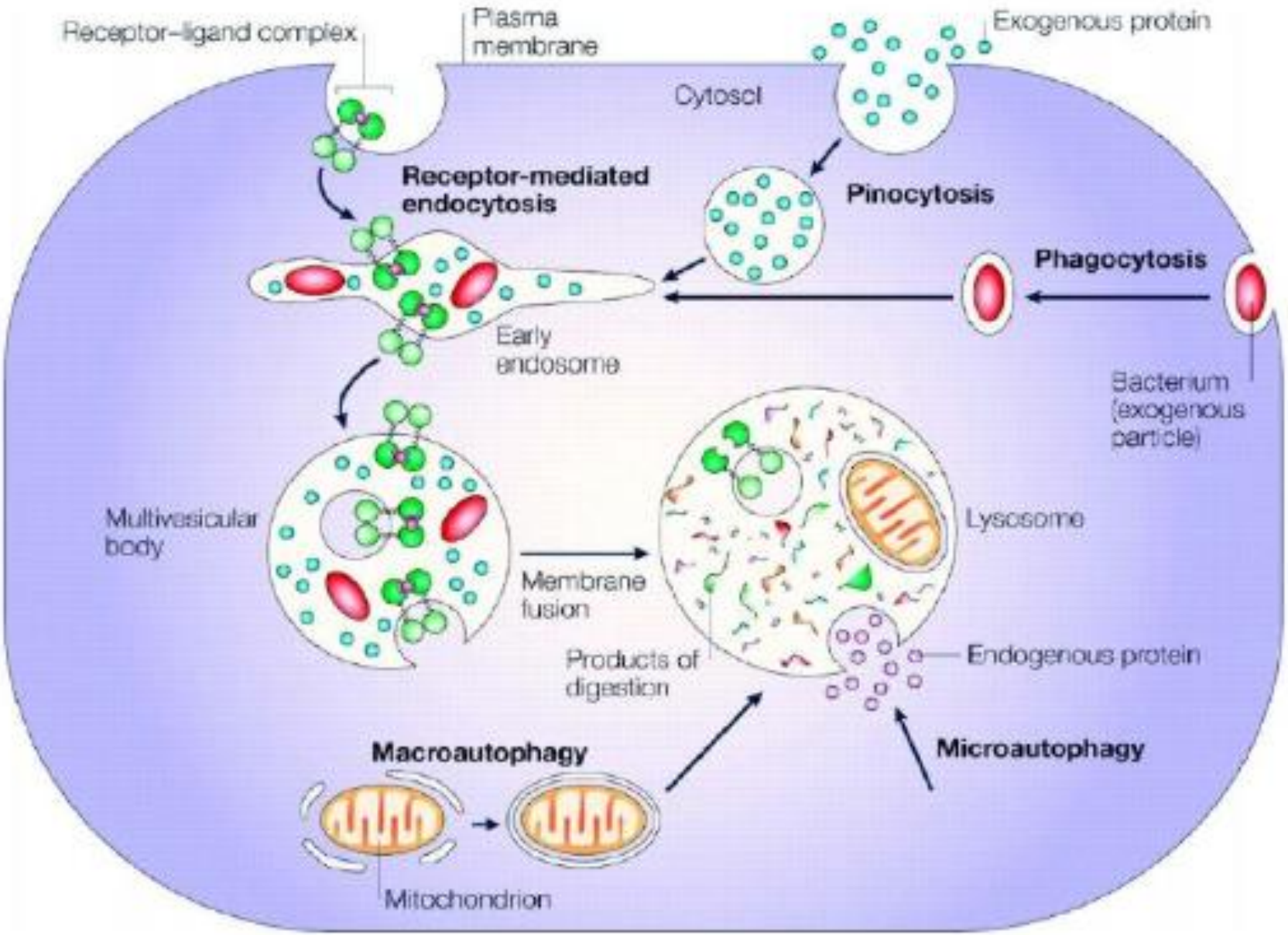
- Lysosomes are membrane bound organelles
  - formed by budding from Golgi
  - contain various proteolytic enzymes in an acidic environment
    - eg. cathepsins
  - digest extracellular or cell surface proteins,
    - extracellular proteins taken into the cell by
      - endocytosis
      - phagocytosis
      - receptor-mediated endocytosis
  - digest intracellular proteins
    - digestion of organelles
  - release proteolytic enzymes
    - extracellularly - exocytosis
    - intracellularly - autolysis (destruction of whole cell)



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
- account for 1-15% of cell volume (most abundant in liver and kidney)
- 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. This enzyme transfers N-acetylglucosamine-1-phosphate to one of more mannose residues. A glucosaminidase next removes the glucosamine to generate the M6P.




Receptor–ligand complex
Plasma membrane
Exogenous protein
Cytosol
Receptor-mediated endocytosis
Pinocytosis
Phagocytosis
Early endosome
Bacterium (exogenous particle)
Multivesicular body
Membrane fusion
Lysosome
Products of digestion
Endogenous protein
Macroautophagy
Microautophagy
Mitochondrion

Cytosolic Protein Degradation

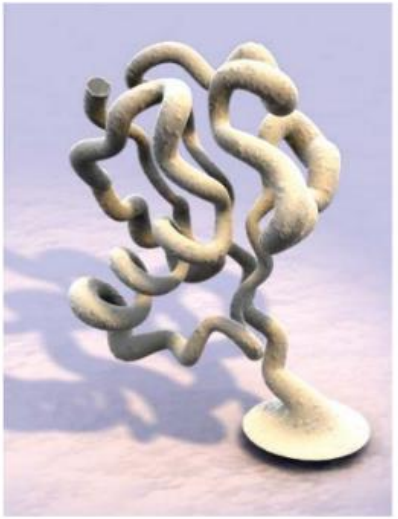
- Proteins degraded to short peptides
  - 'limited proteolysis'
    - 4 to 20 amino acids long
  - not complete digestion to individual amino acids
- Two main proteolytic processes in cytosol
  - Proteasome (also spelt proteosome)
    - large multienzyme, multiprotein, multifunction complex
    - supramolecular complex or 'aggregate'
    - principal role for targeted , or 'labelled' , protein degradation
  - cytosolic proteases
    - Calpain (calcium activated)
    - Neutral protease
      - lage 700 Kda multisubunit

Targeted Degradation

- Oxidation of proteins
  - oxidation of lysine, arginine, and proline
  - Fe^{2+} and hydroxyl radical $OH\cdot$
- PEST sequences
  - regions, 12 to 60 residues long, in proteins with predominance of
    - proline (P), glutamate (E), serine (S), and threonine (T)
  - this pattern found in short lived proteins ($t_{1/2} < 2$hours)
- N-terminal amino acids
  - the identity of N-terminal amino acids is related to the half life of a protein
  - may also play a role in the ubiquitination process
- SUMOylation
  - Small ubiquitin-related modifiers
  - Peptides attached similar to ubiquitin

Targeted Degradation - Ubiquitination

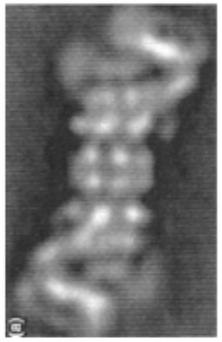
Targeted Degradation - Ubiquitination



- Ubiquitin (ubiquitous in all cells)
  - 76 residue protein
  - forms covalent bond with proteins at lysine residue
  - catalysed by two enzymes
  - requires ATP - ie. an energy requiring process
  - targets protein for degradation by proteasome

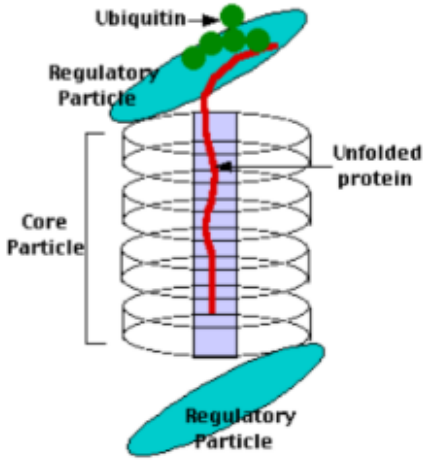
Proteasome

Electron micrograph of 26S proteasome



Baumeister et al. (1997)

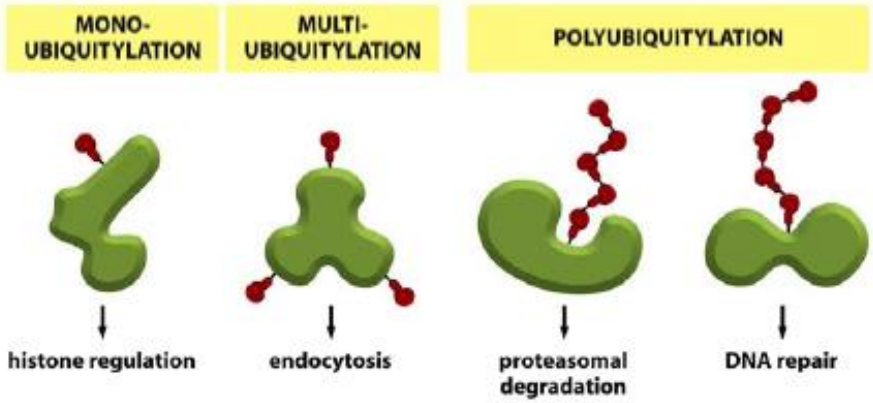
Idealised diagram of 26S proteasome

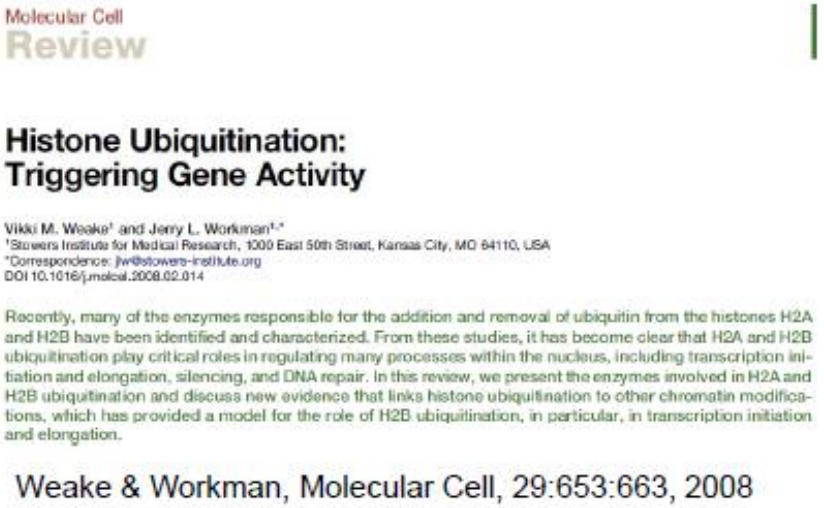


Kimball (2002)

Different Ubiquitylation Related Pathways

- A ubiquitin contains 76 amino acids.
- Ubiquitin conjugation requires a elaborate system of hundres of protein factors.




Molecular Cell
Review

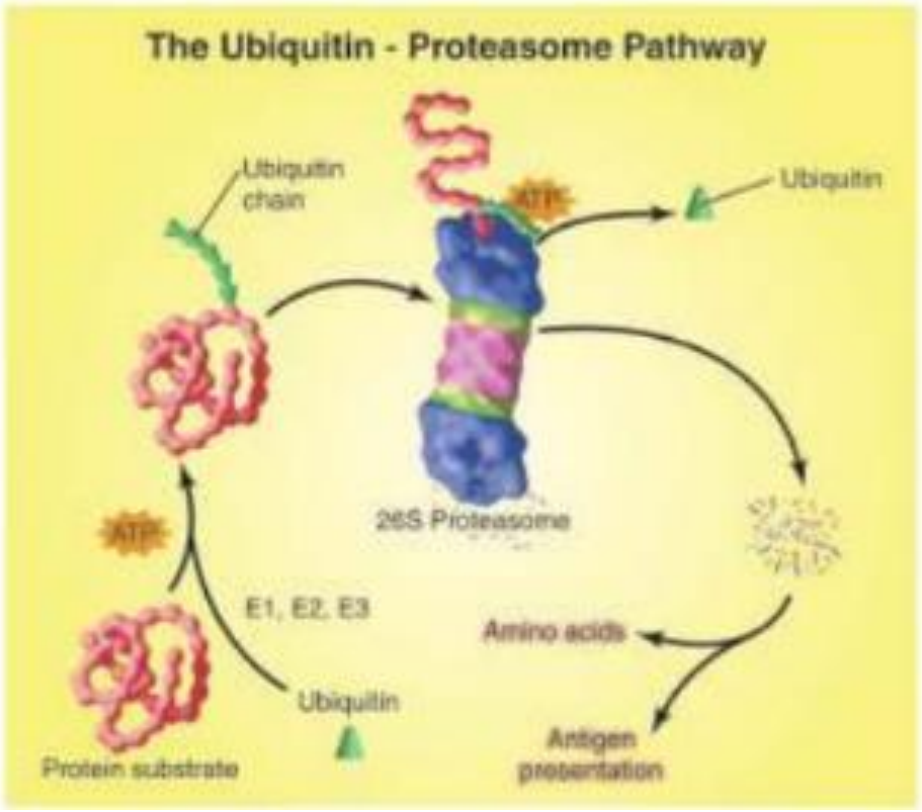
Histone Ubiquitination: Triggering Gene Activity

Vikki M. Weake[1] and Jerry L. Workman[1,*]
[1]Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA
*Correspondence: jlw@stowers-institute.org
DOI 10.1016/j.molcel.2008.02.014

Recently, many of the enzymes responsible for the addition and removal of ubiquitin from the histones H2A and H2B have been identified and characterized. From these studies, it has become clear that H2A and H2B ubiquitination play critical roles in regulating many processes within the nucleus, including transcription initiation and elongation, silencing, and DNA repair. In this review, we present the enzymes involved in H2A and H2B ubiquitination and discuss new evidence that links histone ubiquitination to other chromatin modifications, which has provided a model for the role of H2B ubiquitination, in particular, in transcription initiation and elongation.

Weake & Workman, Molecular Cell, 29:653:663, 2008

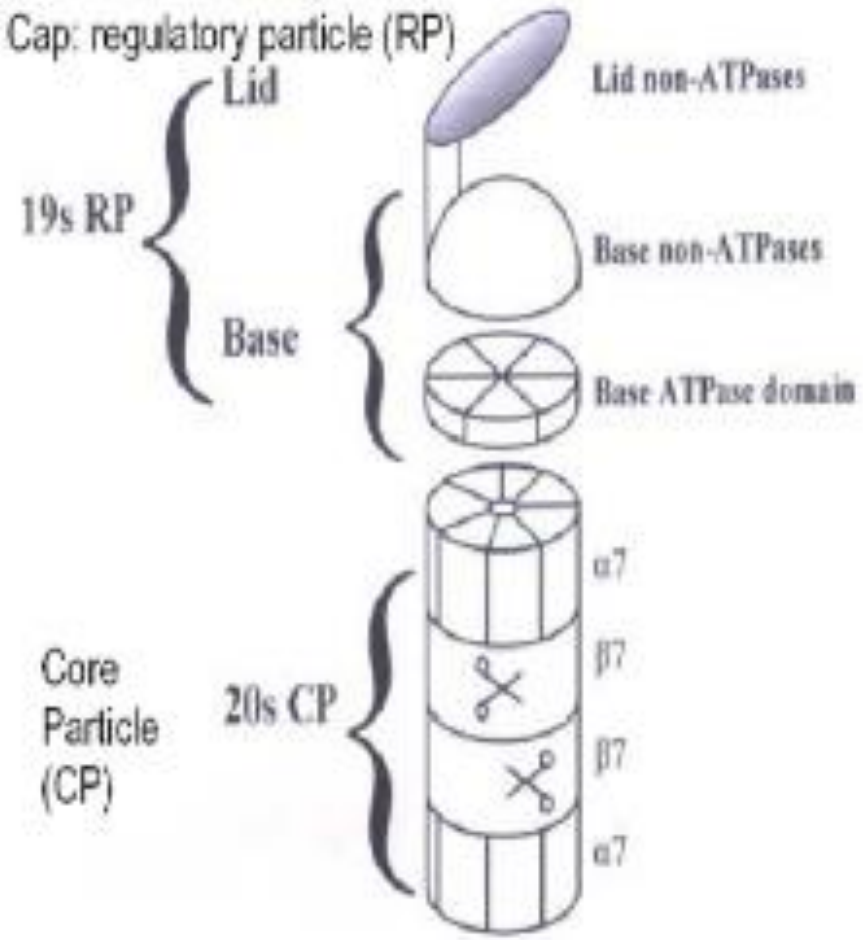
Ubiquitin-Proteasome degradation



Basic features of proteasome

- Essential and ubiquitous intracellular protease
- Degrades most of cytoplasmatic, nuclear and membrane , nuclear and membrane proteins (> 90 %)
- Virtually all target proteins are marked by ubiquitin first
- Ubiquitin is recycled, not cleaved
- Central processes with proteasome involvement are mitosis, antigen presentation, activation and degradation of transcription factors and regulation of developmental processes.
- Eukaryotic proteasomes are large protein complexes of ~ 2000 kDa, consisting of a "core" and a "cap" region
- Prokaryotes lack ubiquitin system and possess no cap region

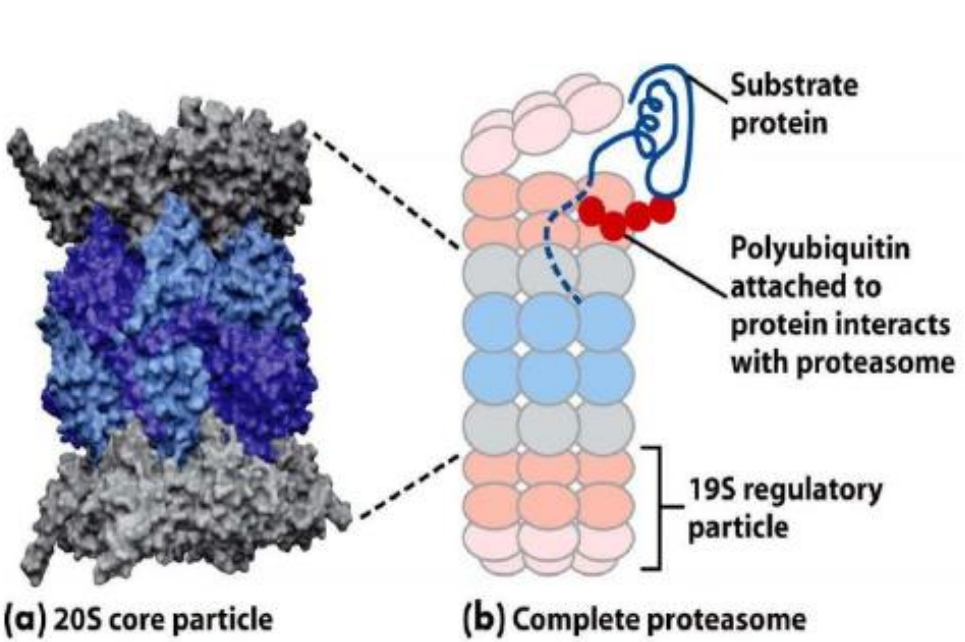
Schematic representation of the eukaryotic

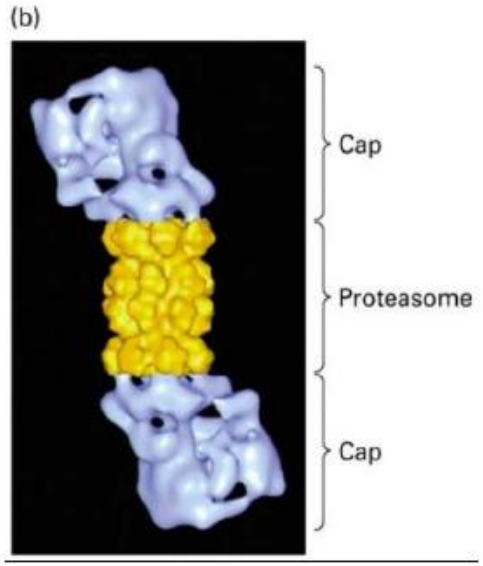


- **Core particle is composed of four 7-membered rings.**
- **Two types of subunits (25 kDa): αand β, all differ .**
- **Subunits are similar in structure, different in sequence.**
- **only only β subunits are catalytically active .**
- **Cap region regulates activity, performes the energy dependent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

Eukaryotic Proteosome

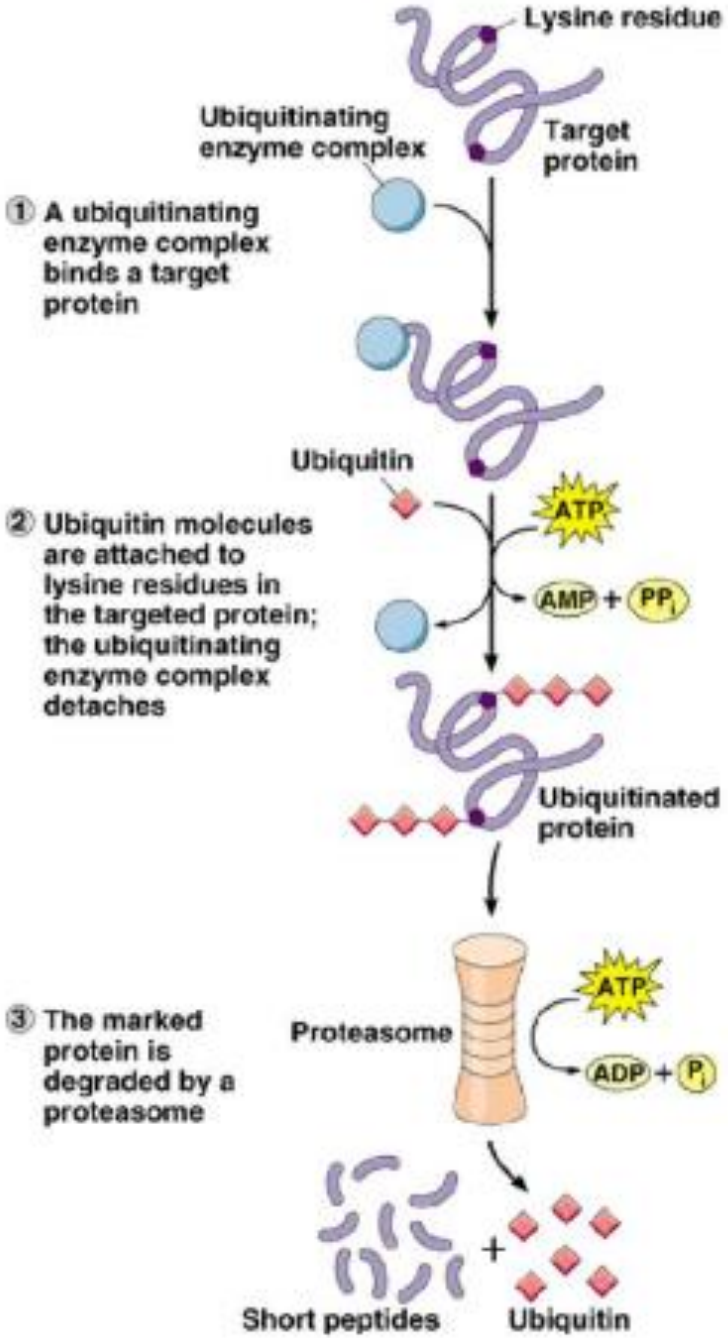
The structure of proteasome

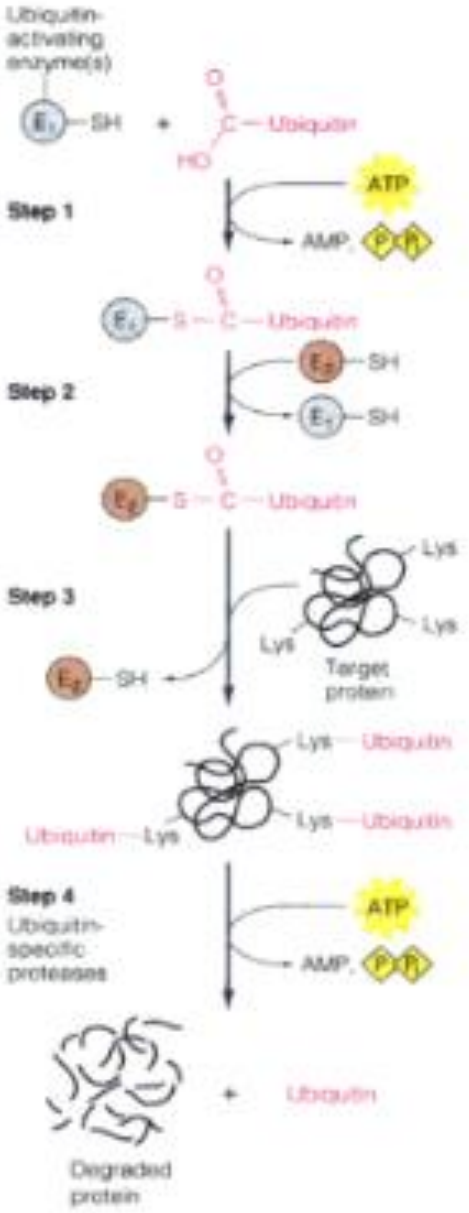




Processing via the proteasome

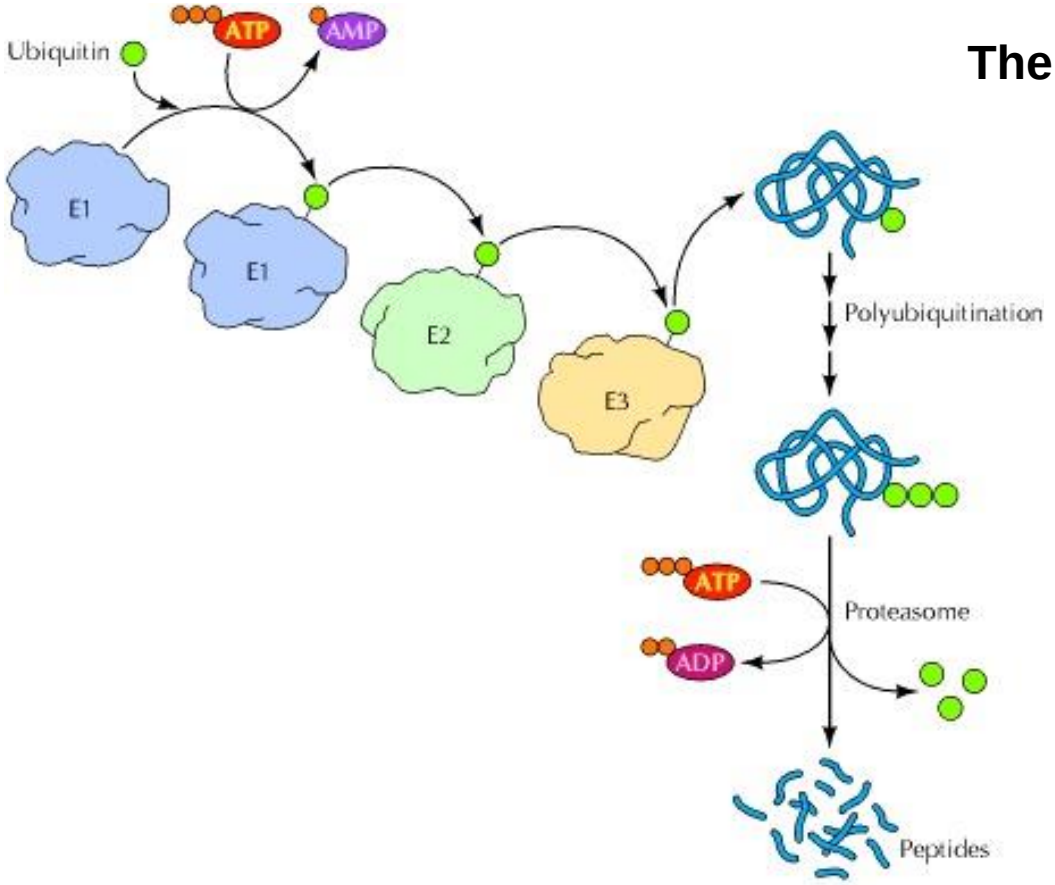
- Length of produced peptides: 3-23 amino acids
- Average length of peptides: 7-9 amino acids
- Peptide composition of given protein stays constant
- Protein is completely degraded before import of next protein
- Peptides produced by proteasome are further degraded by other proteases and aminopeptidases (Tricorn, Multicorn, Thimet, TPPII)





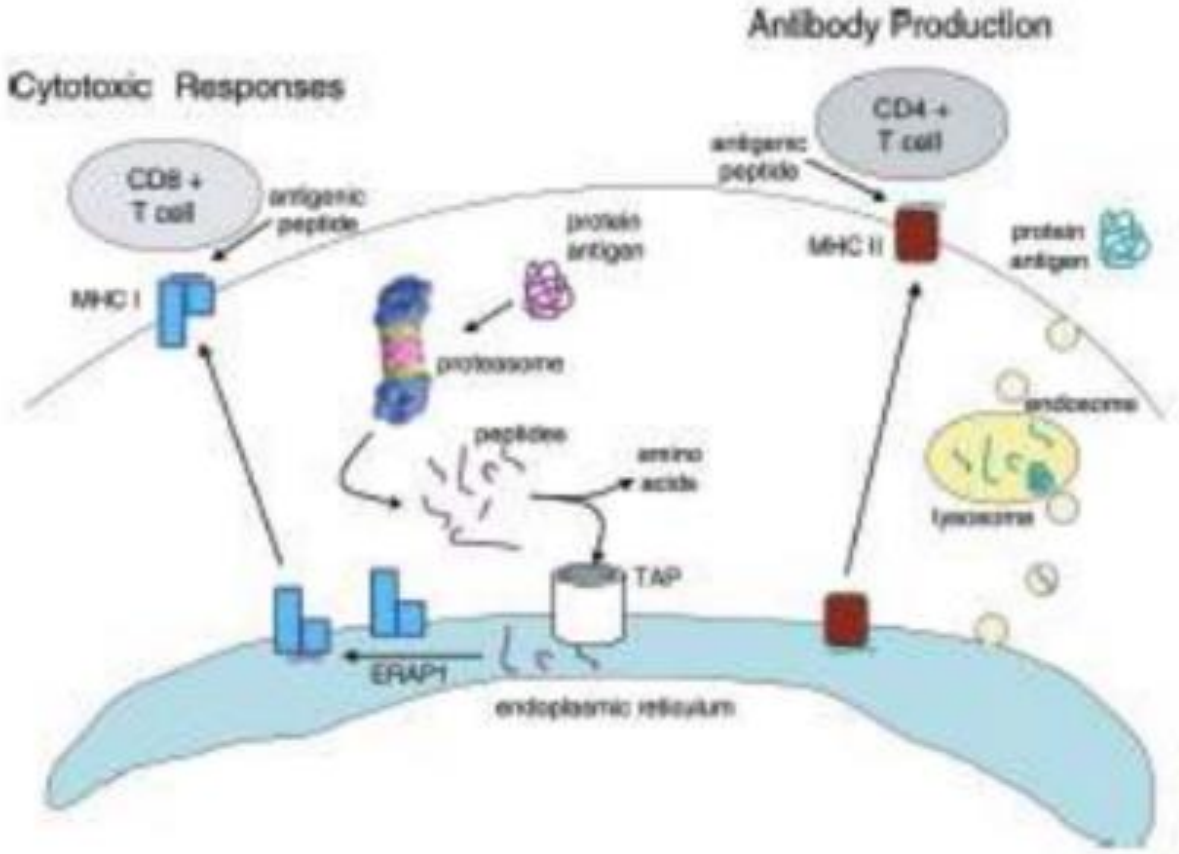
Mathews et al 2000

The ubiquitin - proteasome pathway



Ubiquitination is a multistep process. Proteins are marked for rapid degradation by the covalent attachment of several molecules of ubiquitin. Ubiquitin is first activated by the enzyme E1. Activated ubiquitin is then transferred to one of several different ubiquitin-conjugating enzymes (E2). In most cases, the ubiquitin is then transferred to a ubiquitin ligase (E3) and then to a specific target protein. Multiple ubiquitins are then added, and the polyubiquinated proteins are degraded by a protease complex (the proteasome).

Ubiquitin – Proteasome degradation



Lecker et al. 2006

TAP = transporter associated with antigen processing ERP1 = endoplasmic reticulum aminopeptidase 1. This protein

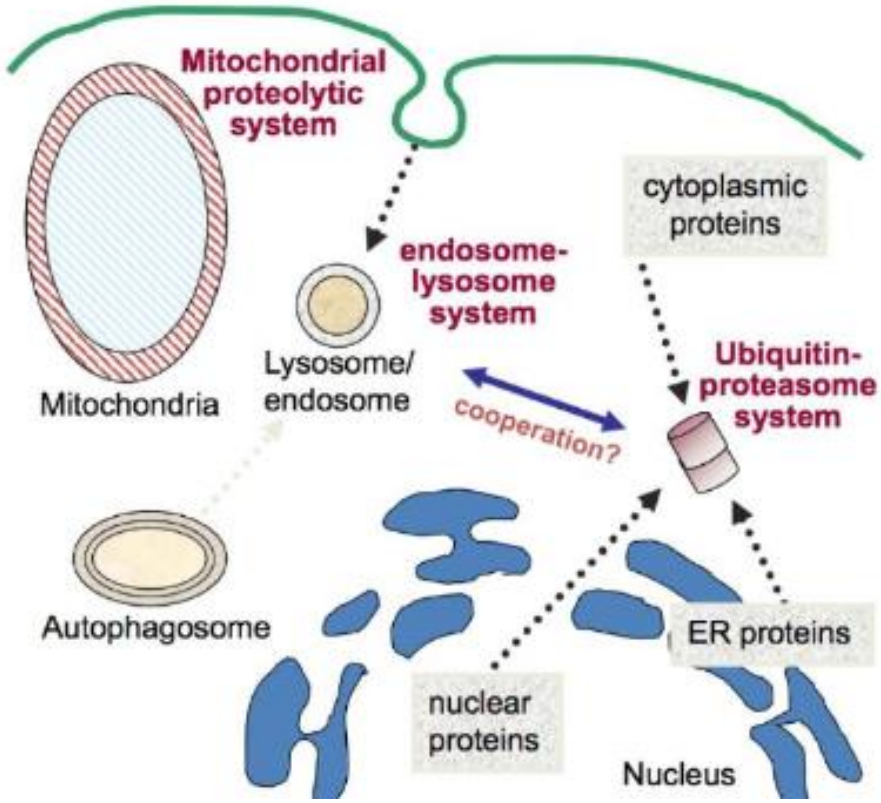
Site of intracellular degradation

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.

Summary

- Lysosomal Pathway to degrade membrane proteins and endocytosed proteins
- Proteosomal Pathway to degrade intracellular proteins
- Mode of action of different proteases found extracellular and inside lysosomes
- Protease Complex as part of proteasome is a multi domain degradation machine with tight regulation
- The 26S proteasome is not an absolute ubiquitin-dependent proteolytic enzyme, as it also degrades non-ubiquitinated substrates.

Main proteolytic pathways



- **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 of bacterial origin

(Note: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

References

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- Carnegie Mellon University Notes : BME 42-620 Engineering Molecular Cell Biology, BME_42_620_Lecture_19_2011 PROTEIN degradation, cell signaling