Learning outcomes 14

HOW PROTEINS FROM THE DIET ARE DEGRADED TO AMINO ACIDS

Essential amino acids:

- Amino acids that cannot be synthesized and must be provided from the diet
- Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine

Digestion of dietary proteins begins in the stomach and is completed in the intestine

- Acidic environment in the stomach favors denaturation of proteins into random coils
- Denatured proteins are more accessible for proteolysis
- *Pepsin* is the primary proteolytic enzyme in the stomach
    - Nonspecific protease
    - Maximally active at pH 2
    - Can function in highly acidic environment of the stomach
- Degradation continues in the lumen of the intestine
    - Pancreas secretes variety of proteolytic enzymes into intestinal lumen as inactive zymogens that are converted into active enzymes.
    - These enzymes are specific, and so the substrates are degraded into free amino acids as well as di- and tripeptides.
- Digestion is further enhanced by proteolytic enzymes such as aminopeptidase N
    - Located in the plasma membrane of the intestinal cells
    - Aminopeptidases digest proteins from the amino-terminal end
- Single amino acids, as well as di- and tripeptides, are transported into the intestinal cells from the lumen and subsequently released into the blood for absorption by other tissues

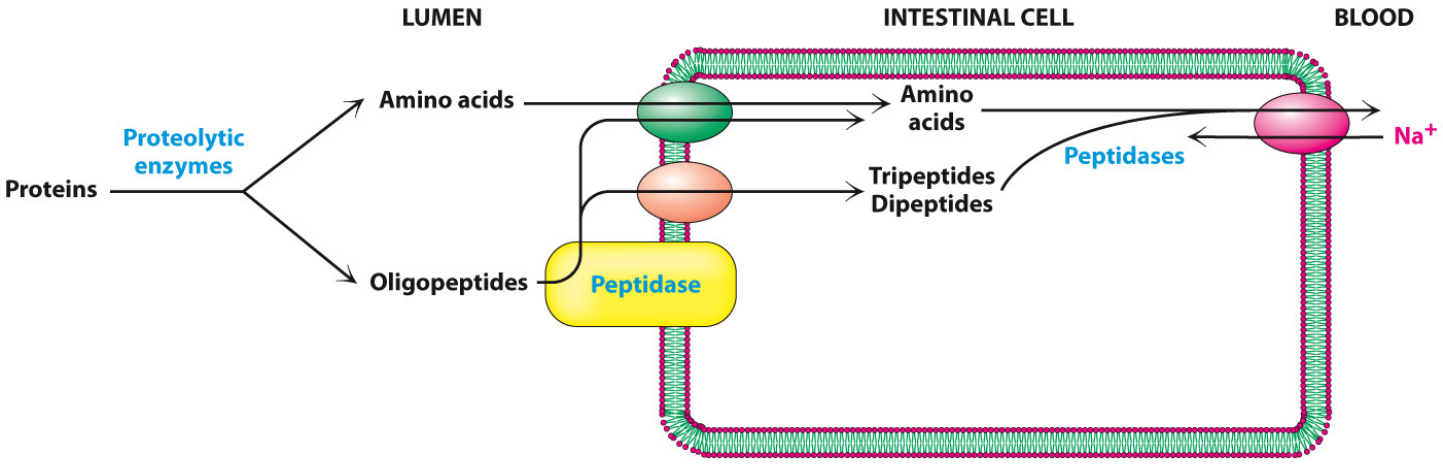
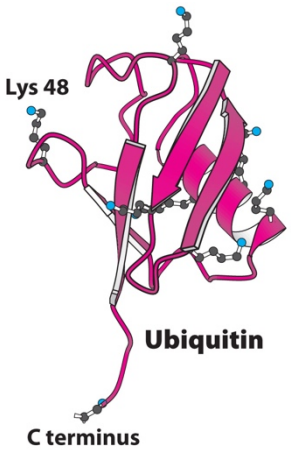


TABLE 10.3 Gastric and pancreatic zymogens

Site of synthesis	Zymogen	Active enzyme
Stomach	Pepsinogen	Pepsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Trypsinogen	Trypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase

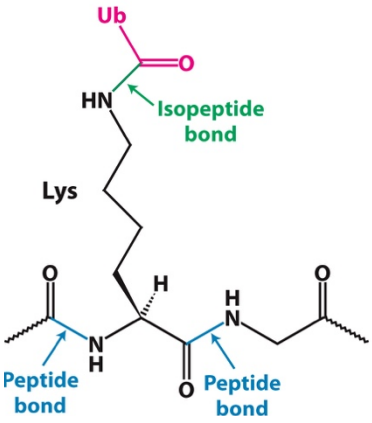
WHAT IS UBIQUITIN

- Ubiquitin is a small protein present in all eukaryotic cells
- It is a tag that marks proteins for destruction
- Highly conservative in eukaryotes



HOW CELLULAR PROTEINS ARE TAGGED WITH UBIQUITIN

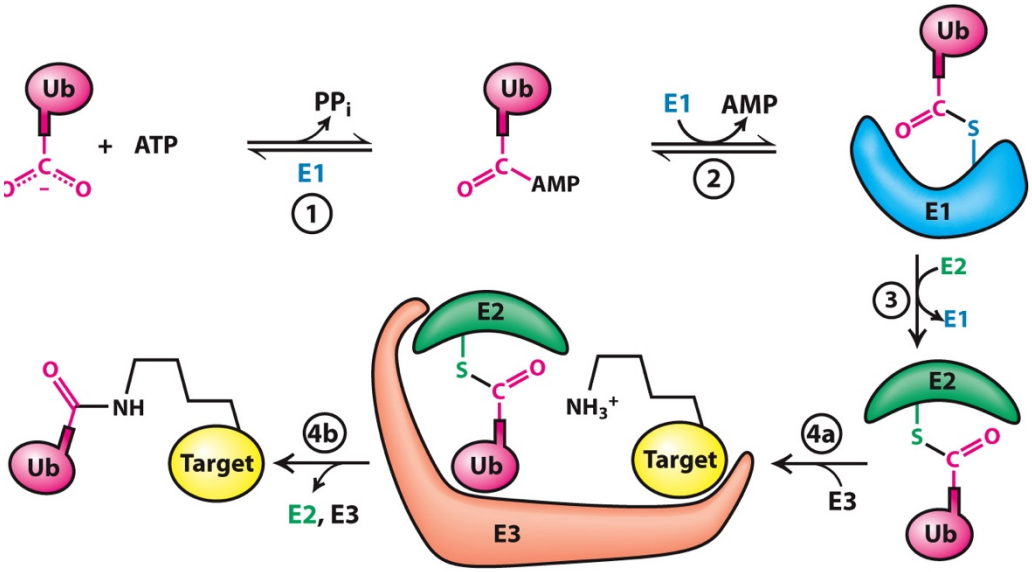
- The carboxyl-terminal glycine residue of ubiquitin becomes covalently attached to the ε-amino groups of several lysine residues on a protein destined to be degraded



  - Isopeptide bonds (iso because ε- rather than α-amino groups are targeted)
  - The energy of formation of the bonds come from ATP hydrolysis
- Three enzymes participate in the attachment of ubiquitin to a protein
  - Ubiquitin-activating enzyme (E1)
  - Ubiquitin-conjugating enzyme (E2)
  - Ubiquitin-protein ligase (E3)

MECHANISM

- C-terminal carboxylate group of ubiquitin becomes linked to a sulfhydryl group of E1 by *thioester bond*
  - ATP driven reaction, reminiscent of fatty acid activation
  - ATP is linked to the C-terminal carboxylate of ubiquitin with the release of pyrophosphate, and the ubiquitin is transferred to a sulfhydryl group of a key cysteine residue in E1
- Activated ubiquitin is then shuttled to a sulfhydryl group of E2, reaction catalyzed by E2 itself.
- E3 catalyzes the transfer of ubiquitin from E2 to an ε-amino group on the target protein.



The ubiquitin-activating enzyme E1 adenylates ubiquitin (Ub) (1) and transfers the ubiquitin to one of its own cysteine residues (2). Ubiquitin is then transferred to a cysteine residue in the ubiquitin-conjugating enzyme E2 by the E2 enzyme. (3). Finally, the ubiquitin–protein ligase E3 transfers the ubiquitin to a lysine residue on the target protein (4a and 4b).

What determines whether a protein becomes ubiquitinated?

- Specific amino sequence
    - Degron
- Half-life of a cytoplasmic protein is determined to a large extent by its amino-terminal residue
    - N-terminal rule or N-terminal degron
    - Destabilized N-terminal equals rapid ubiquination
- Sometimes N-terminal degron is only exposed after the protein is proteolytically cleaved
    - Pro-N-terminal degrons
- E3 enzymes are the readers of N-terminal residues

COMPONENTS OF THE PROTEOSAME AND THEIR FUNCTION

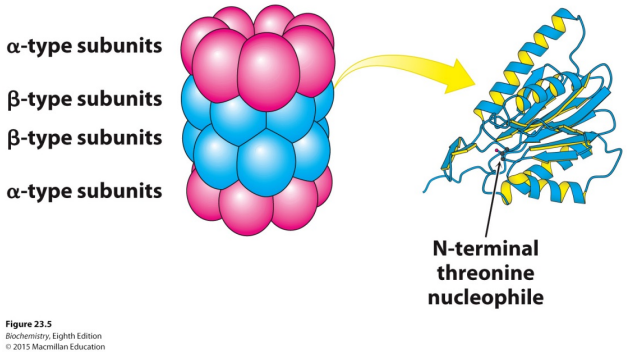


Figure 23.5
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- The proteasome is a protease complex also called the 26S proteasome
    - Made of 20S catalytic unit
        - 28 homologous subunits encoded by 14 genes, arranged in 4 heteroheptomeric rings, each of 7 subunits
        - Some of the beta subunits include protease active sites at their amino termini
        - Access to the interior is controlled by the 19S regulatory unit
    - 19S regulatory unit
        - Two 19S complexes bind to the 20S proteasome core, one at each end, to form the complete 26S proteasome
        - 19S has 3 functions
            - some components of the complex are ubiquitin receptors that bind specifically to polyubiquitin chains, ensuring only ubiquitinated proteins are degraded
            - Isopeptidase in the unit cleaves off intact ubiquitin molecules from proteins so they can be reused
            - Protein is unfolded and directed in the catalytic core (ATPase)
        - Key components are: 6 ATPases of the type AAA class (ATPase associated with various cellular activities)
        - ATP hydrolysis assists the 29S complex to unfold the substrate and induce conformational change in the 20S catalytic core so that the substrate can be passed into the centre of the complex
        - Proteolytic active sites are sequestered in the interior of the barrel to protect potential substrates until they are directed in the barrel

HOW UBIQUINATED PROTEINS ARE DEGRADED IN THE PROTEASOME

- Beta subunits have 3 types of active site, each with a different specificity but all use an N-terminal threonine
  - Hydroxyl group of threonine is converted into a nucleophile that attacks the carbonyl groups of peptide bonds to form acyl-enzyme intermediates
  - Substrates are degraded in a possessive manner without the release of degradation intermediates until substrate is reduced to peptide ranging in length from 7 to 9 residues
  - The peptides are released from proteasome and degraded to amino acids

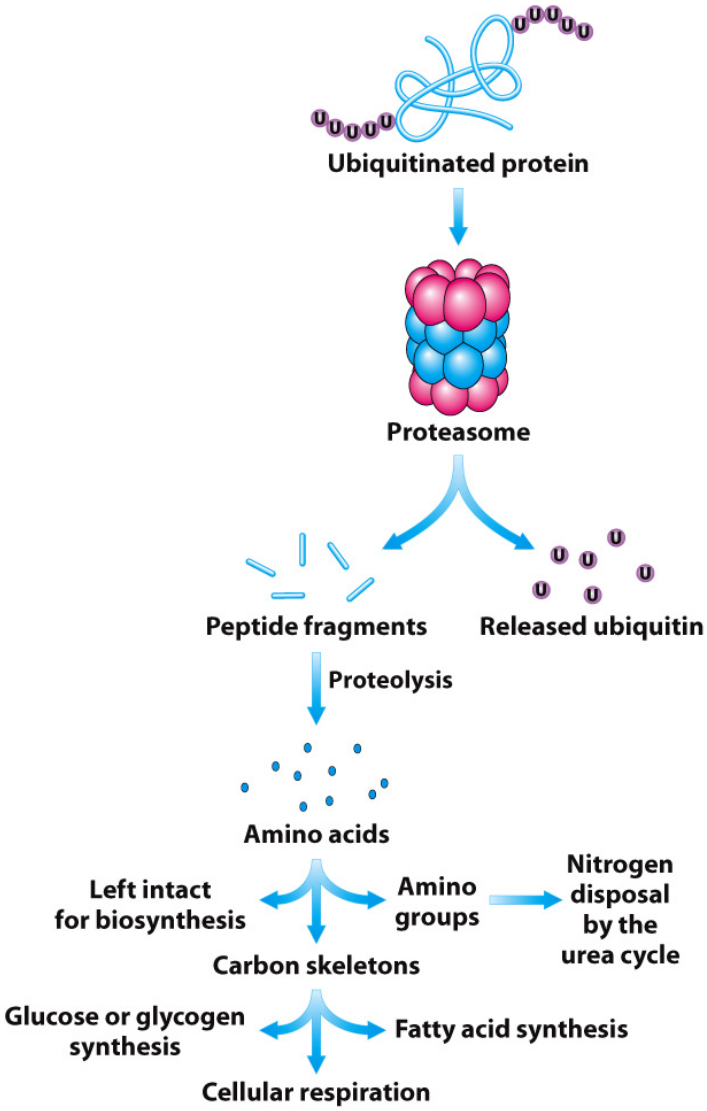

Figure 23.7
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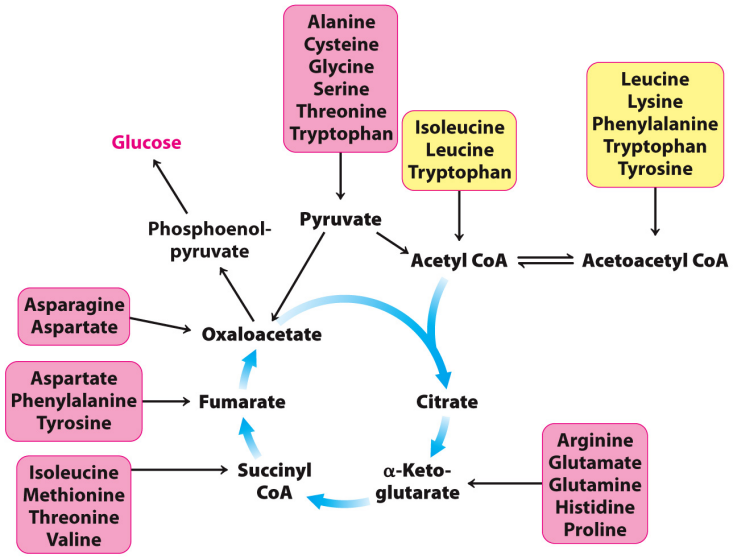

Figure 23.20
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