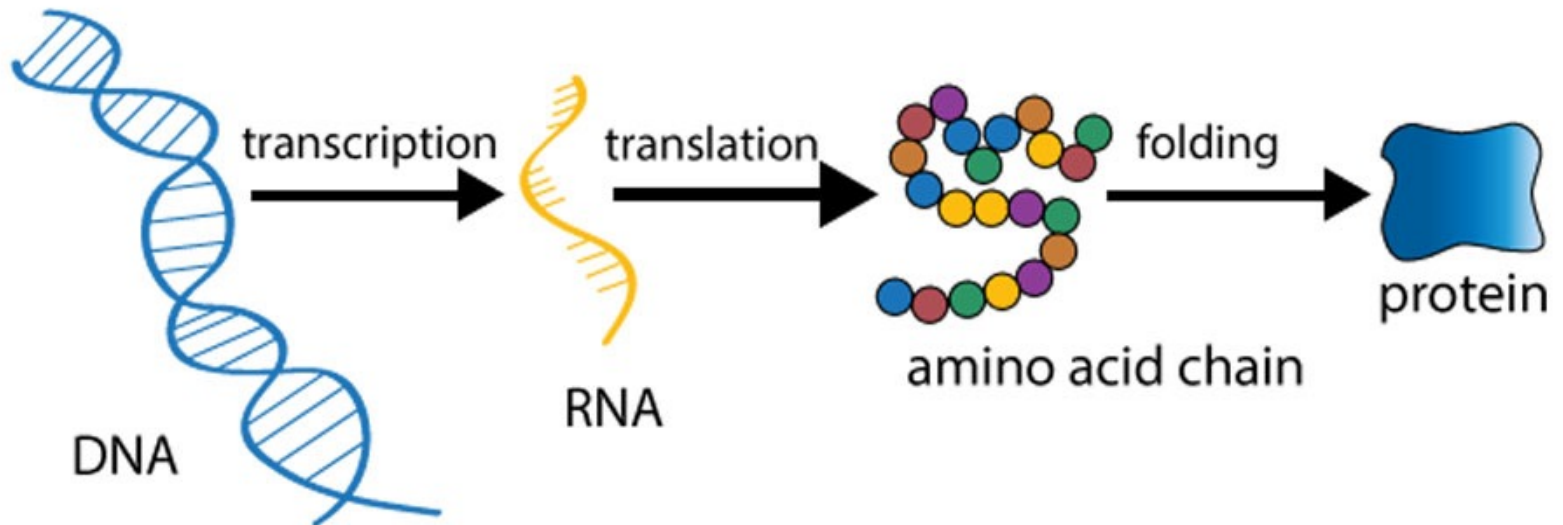




Post Translation Modification

Harmand A. Hama
Molecular Biology-BIO 305
Fall semester
Week Nine
04.12.2023

Gene expression



Objectives:

1. Introduction of PTMs
2. Impacts of PTMs
3. Types of PTM
4. Phosphorylation, Glycosylation, and Ubiquitination.



Objectives:

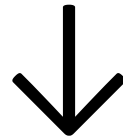
1. To give an overview of PTMs
2. To discuss the main reason of PTMs.
3. To outline the main types of PTMs.



Protein Biosynthesis at Three Levels of Modifications

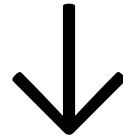
20 Amino acids + 20 tRNAs

Pre-translational
Modifications



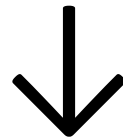
20 aa-tRNAs

Co-translational
Modifications



Nascent polypeptide

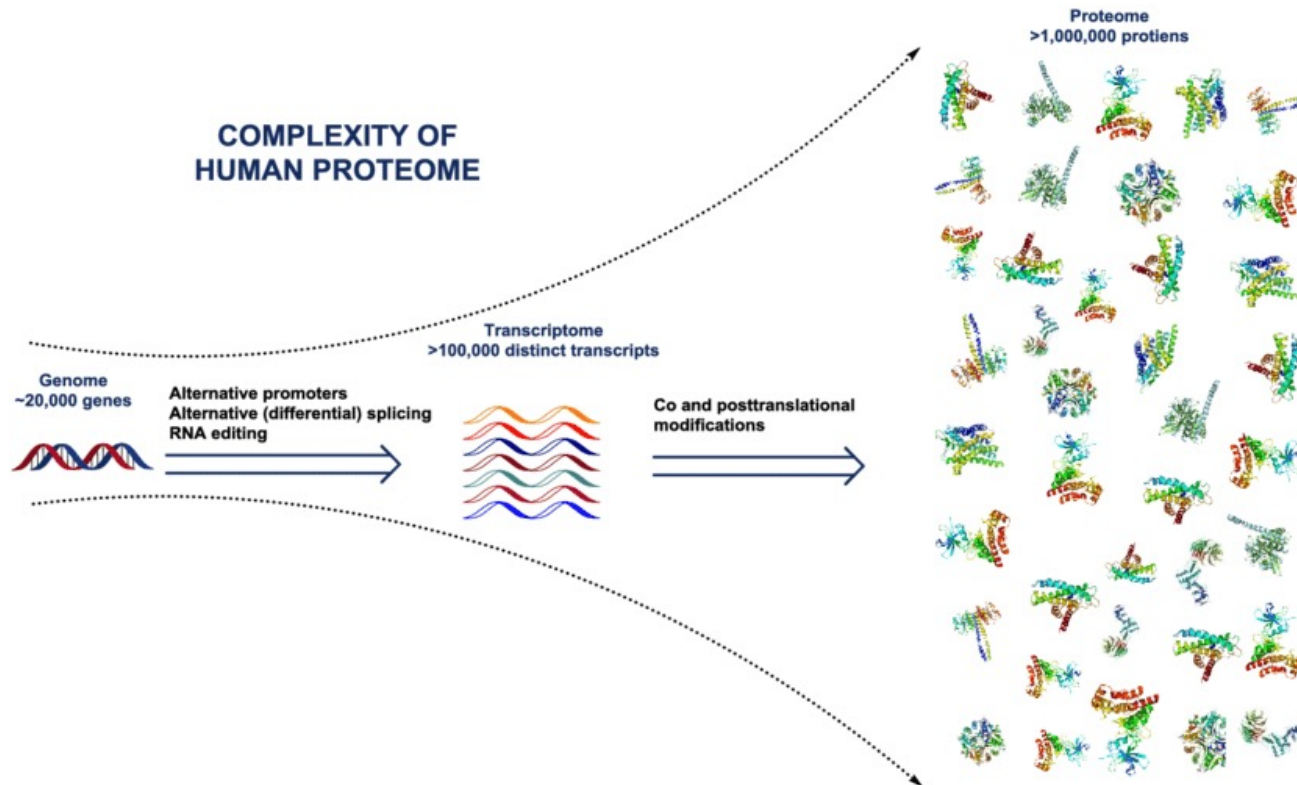
Post-translational
Modifications



Completed polypeptide

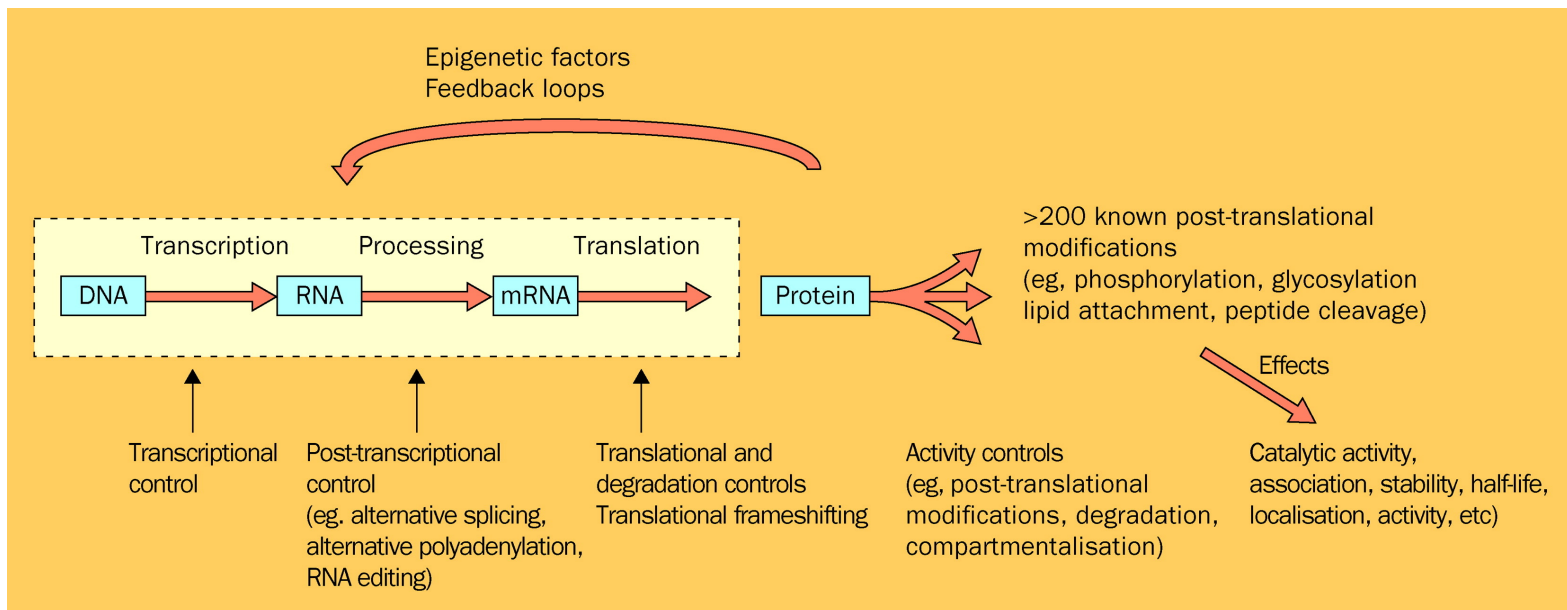
Proteome and Proteomics

- Proteome is **the complete set of proteins expressed by an organism.**
- Proteomic is **the large-scale study of proteomes.**



Post-translational modifications

- Many proteins undergo chemical modifications at certain amino acid residues following translation. These modifications are essential for normal functioning of the protein and are carried out by one or more enzyme catalyzed reactions.
- The chemical modifications that take place at certain amino acid residues after the protein is synthesized by translation are known as post-translational modifications.



Post-translational modifications

Gene encoding region (ORF)

↓ transcription

mRNA

↓ translation

Protein (nascent protein, precursor protein)

↓ protein processing, post-translational modification

Mature protein

↓ folding

Biological active protein

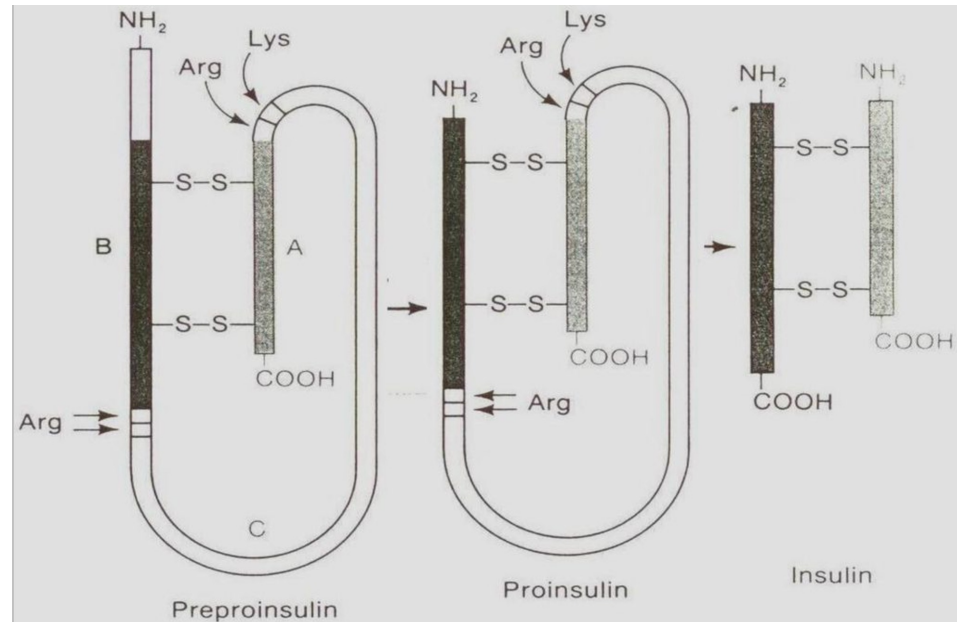
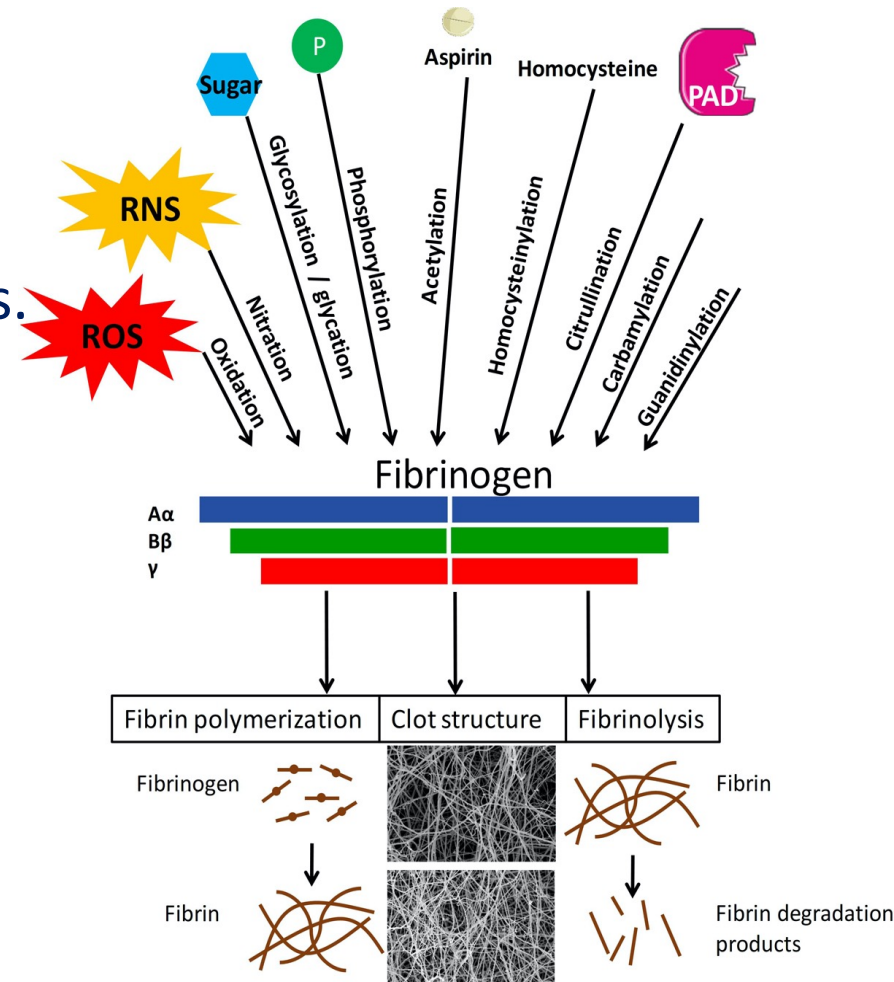


Figure 20-22

Conversion *in vivo* of preproinsulin to proinsulin and then to insulin.

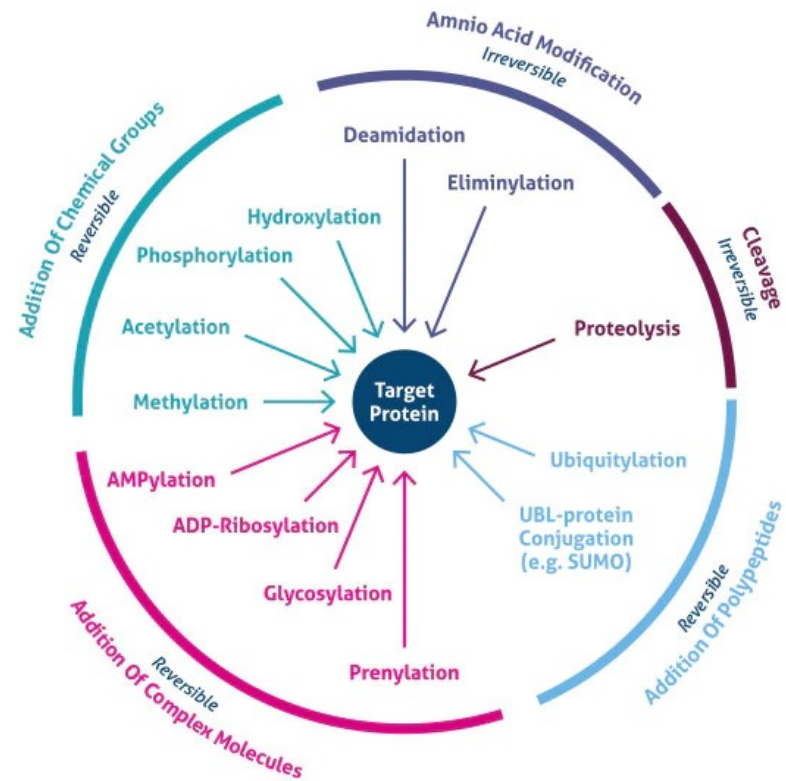
Effects of Post-translational Modification

- Protein stability and localization.
- Interaction with other biomolecules (Nucleic acid, lipids, carbs, proteins).
- Degradation and Recycling of proteins.
- Regulation of protein activity.
- **Increase protein diversity.**



Classification of Post-Translational Modifications

- Addition of functional group.
- Backbone cleavage (proteolysis, protein splicing).
- Change in nature of amino acid as; Deamination, oxidation.
- Attachment with other proteins such us; ubiquitination, sumoylation



Main types of Additions in PTM

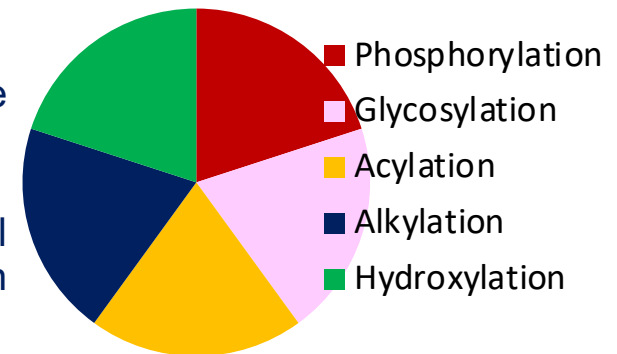
a) Phosphorylation: The process by which a phosphate group is attached to certain amino acid side chains in the protein, most commonly serine, threonine and tyrosine.

b) Glycosylation: The attachment of sugar moieties to nitrogen or oxygen atoms present in the side chains of amino acids like asparagine, serine or threonine.

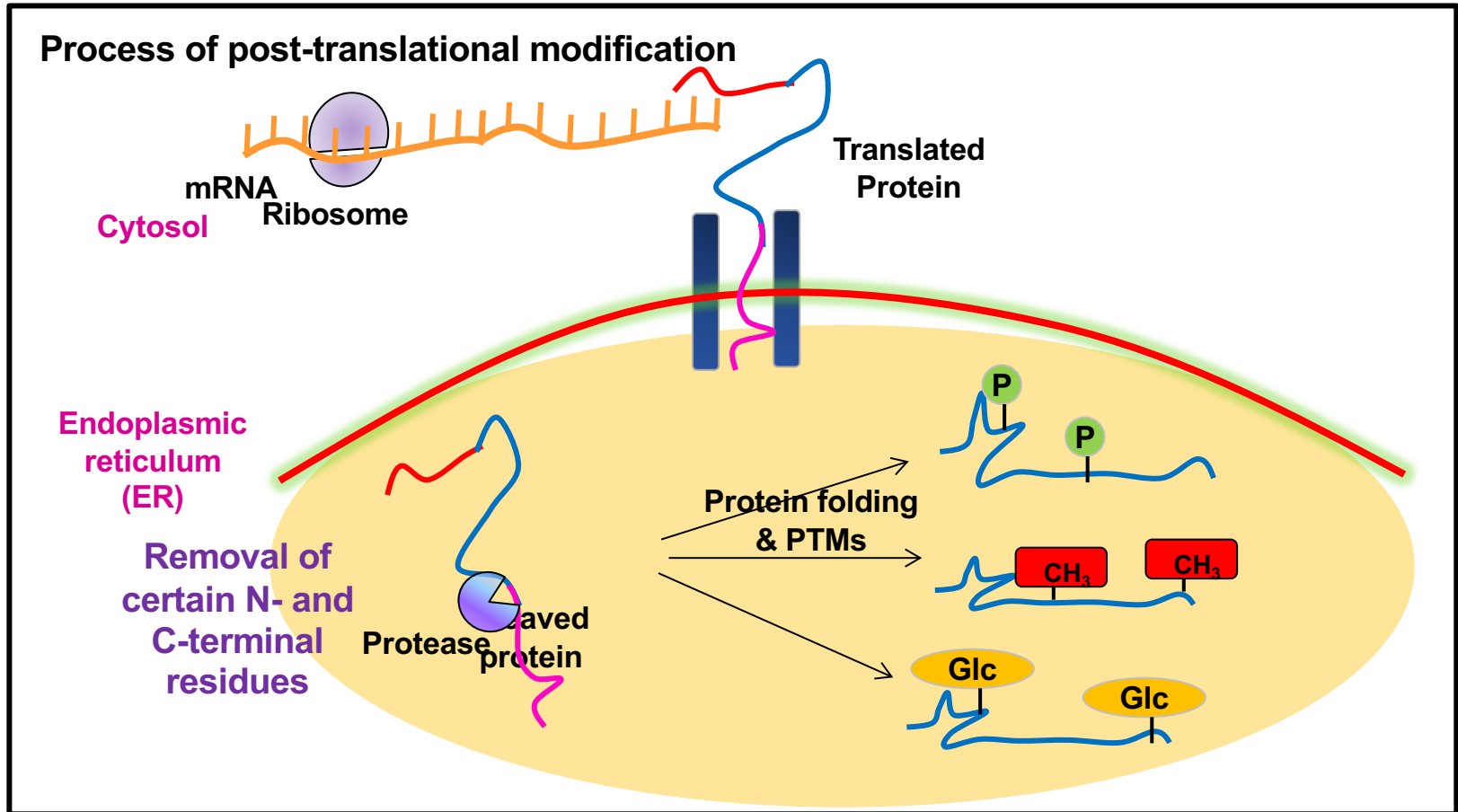
c) Acylation: The process by which an acyl group is linked to the side chain of amino acids like asparagine, glutamine or lysine.

d) Alkylation: Addition of alkyl groups, most commonly a methyl group to amino acids such as lysine or arginine. Other longer chain alkyl groups may also be attached in some cases.

e) Hydroxylation: This PTM is most often found on proline and lysine residues which make up the collagen tissue. It enables crosslinking and therefore strengthening of the muscle fibres.



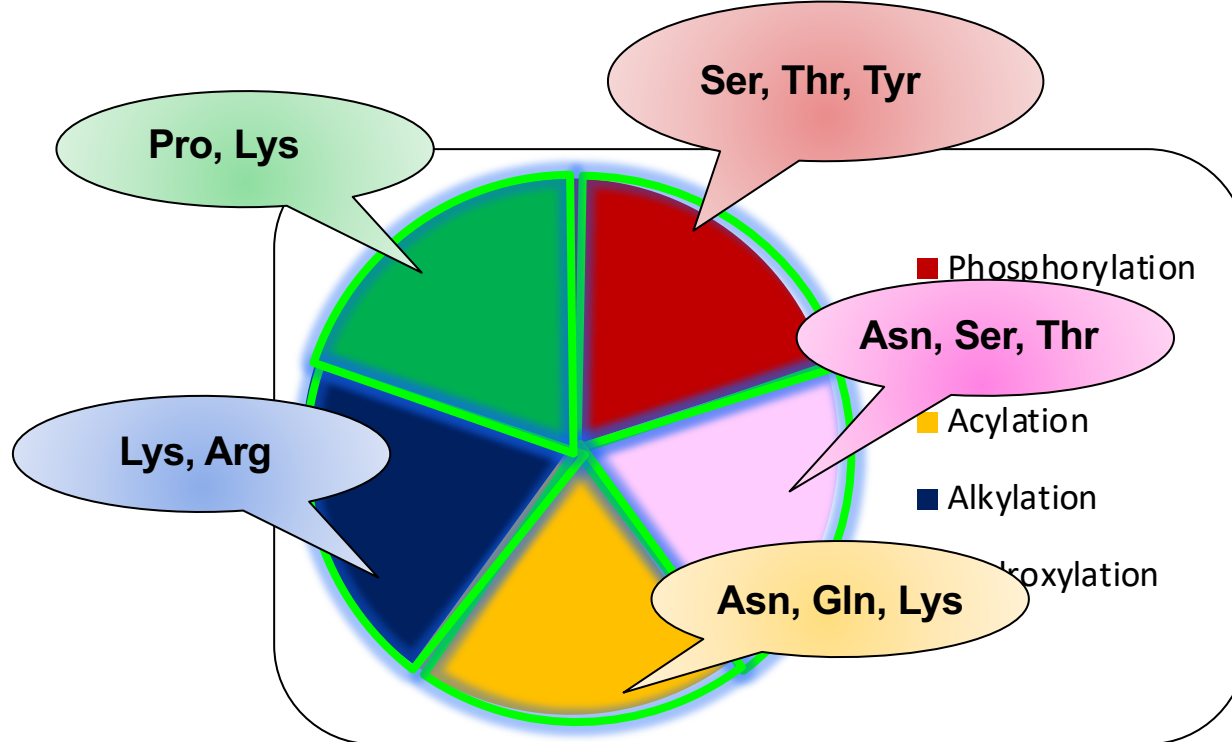
Process of post-translational modification



Action	Description of the action	Audio Narration
As shown in animation.	First the 'mRNA' & 'ribosome' must be shown in the 'cytosol'. The 'ribosome' must move across the mRNA as shown and as it moves, the 'protein' must appear slowly as though it is growing out of the ribosome (not depicted here). Next, this 'protein' must enter the 'ER' through the green channels shown. Next, the pie-shaped 'protease' must appear which must cut the pink strand followed by the red strand followed by appearance of text on the left. Next, the arrows must appear one at a time with their respective figures on the right.	Once the protein has been synthesized by the ribosome from its corresponding mRNA in the cytosol, many proteins get directed towards the endoplasmic reticulum for further modification. Certain N and C terminal sequences are often cleaved in the ER after which they are modified by various enzymes at specific amino acid residues. These modified proteins then undergo proper folding to give the functional protein.

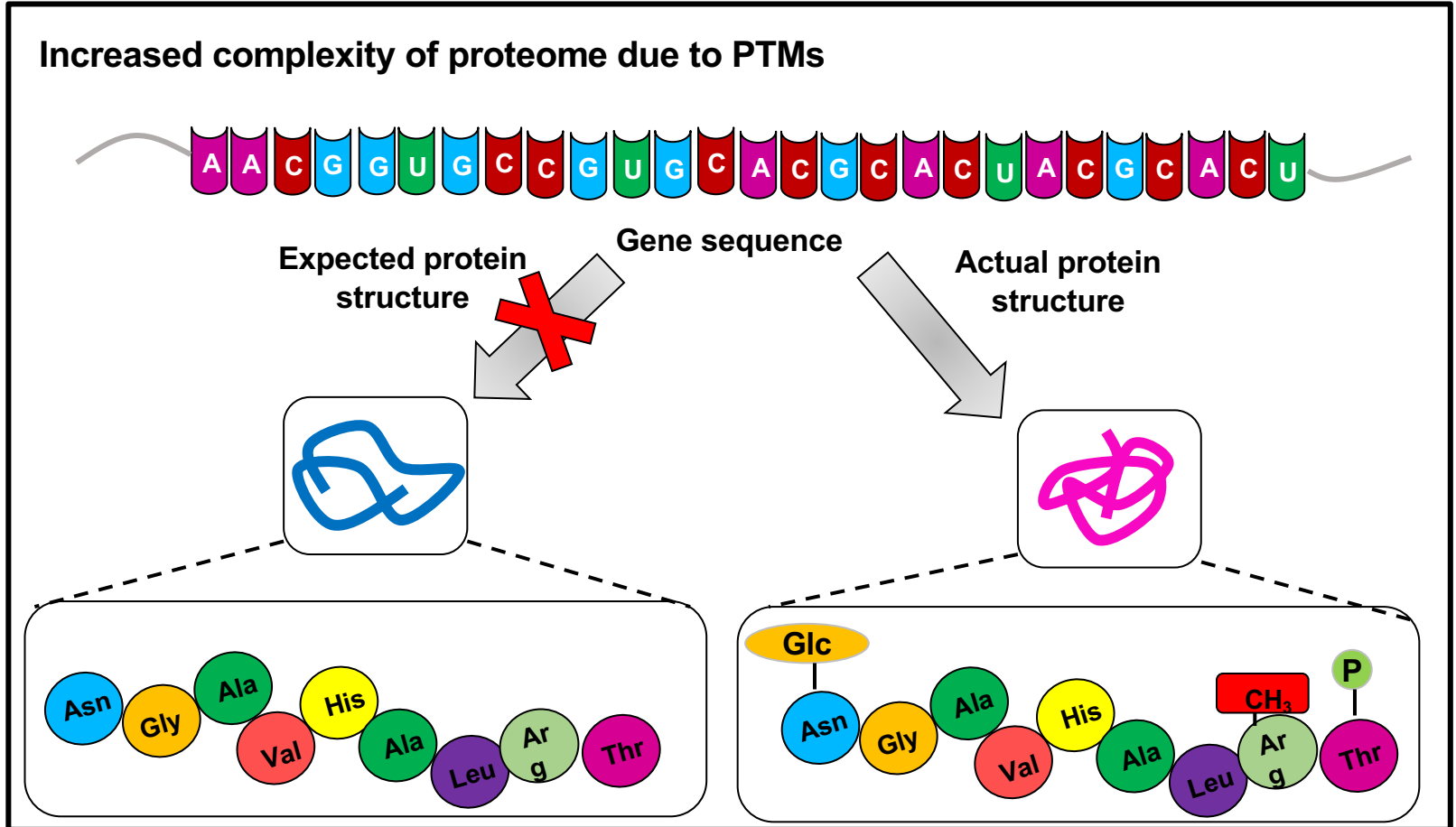
Modification sites of Different PTMs

Different types of PTMs & their modification sites



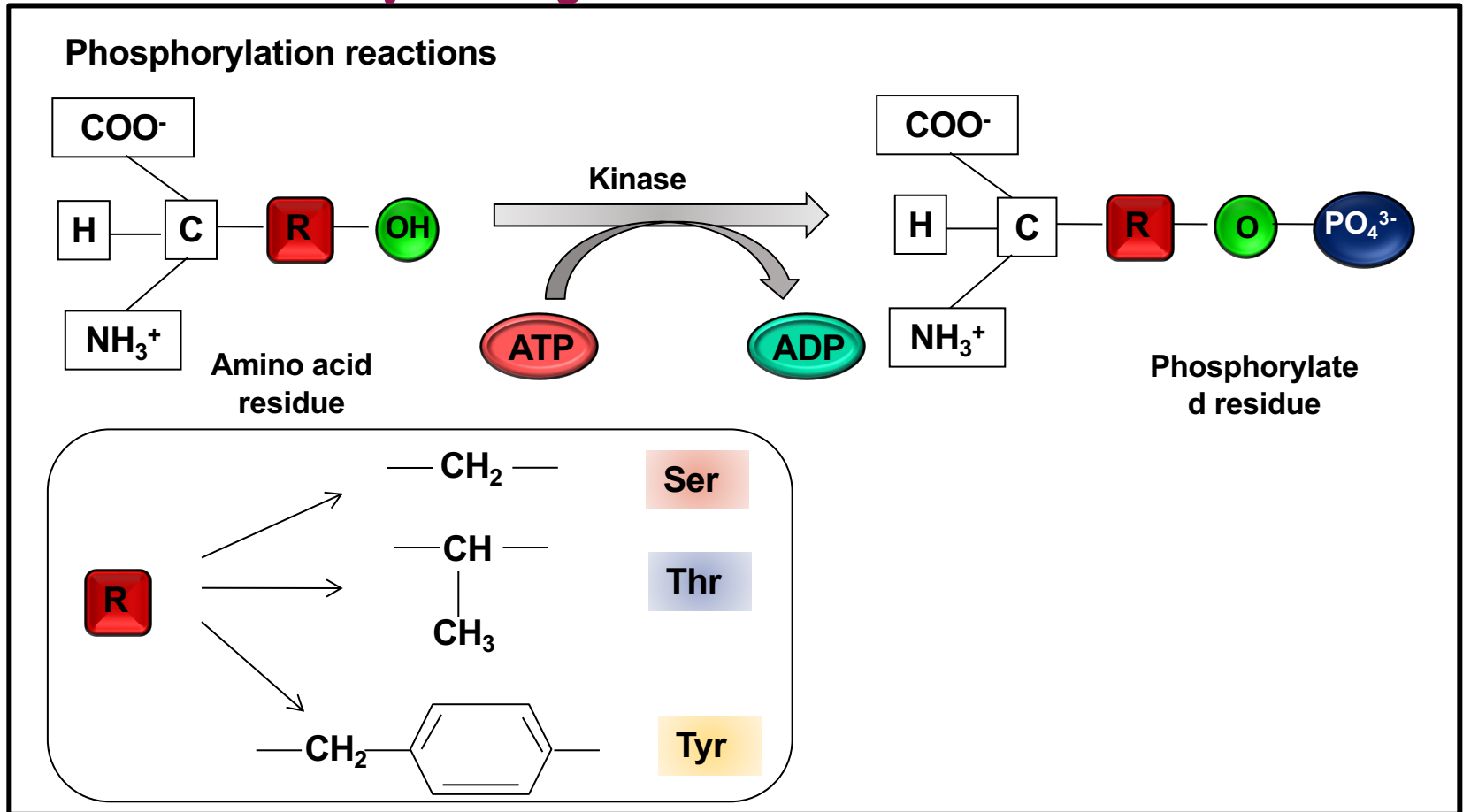
Action	Description of the action	Audio Narration
As shown in animation.	First show the pie chart as depicted. Next, each segment must be highlighted sequentially along with appearance of the corresponding text in the boxes as depicted.	There are several types of post translational modifications that can take place at different amino acid residues. The most commonly observed PTMs include phosphorylation, glycosylation, methylation as well as hydroxylation and acylation. Many of these modifications, particularly phosphorylation, serve as regulatory mechanisms for protein action.

Part 1, Step 3



Action	Description of the action	Audio Narration
As shown in animation.	First show the 'gene sequence' on top followed by the arrow to the left and the blue structure. This must be zoomed into to show the inset below. The red cross must then appear on this arrow. Next, the arrow to the right must appear followed by the pink structure which must again be zoomed into to show the inset below.	The final structure of functional proteins most often does not correlate directly with the corresponding gene sequence. This is due to the PTMs that occur at various amino acid residues in the protein, which cause changes in interactions between the amino acid side chains thereby modifying the protein structure. This further increases the complexity of the proteome as compared to the genome.

Phosphorylation reactions

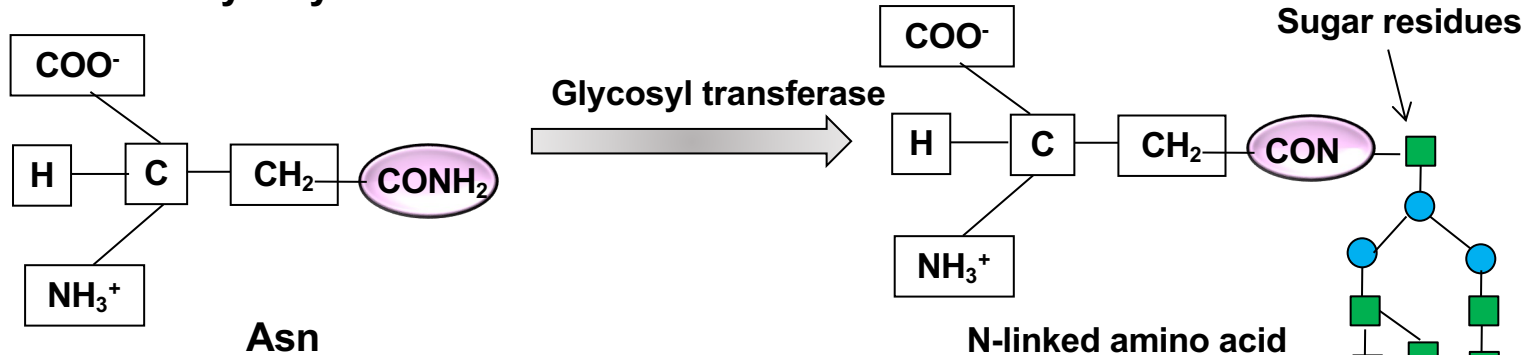


Action	Description of the action	Audio Narration
As shown in animation.	First show the figure on the top left entering followed by the box below having the various three arrows from "R". Next the arrow must ease in along with the curved arrow below. Finally, the figure on the right must appear.	Phosphorylation of amino acid residues is carried out by a class of enzymes known as kinases that most commonly modify side chains of amino acids containing a hydroxyl group. Phosphorylation requires the presence of a phosphate donor molecule such as ATP, GTP or other phosphorylated substrates. Serine is the most commonly phosphorylated residue followed by threonine and tyrosine. Removal of phosphate groups is carried out by the phosphatase enzyme and thus this forms one of the most important mechanisms for regulation of proteins.

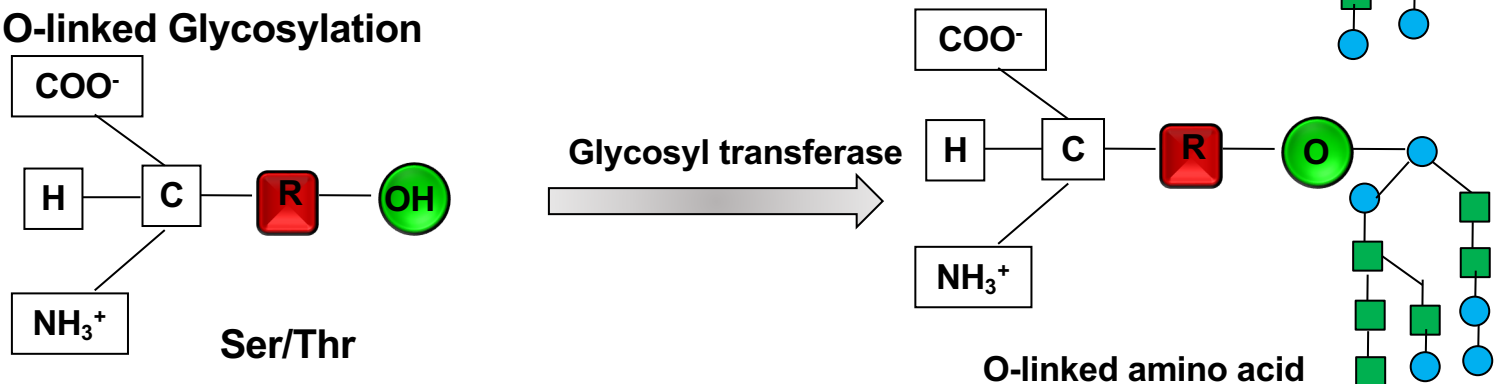
Glycosylation reactions

Glycosylation reactions

N-linked Glycosylation



O-linked Glycosylation



Action

As shown in animation.

Description of the action

First show appearance of the figure on top left with heading followed by arrow and finally product on right. Similar animation must be carried out for the second reactions.

Audio Narration

Glycosylation involves the enzymatic addition of saccharide molecules to amino acid side chains. This can be of two types – N-linked glycosylation, which links sugar residues to the amide group of asparagine and O-linked glycosylation, which links the sugar moieties to the hydroxyl groups of serine or threonine. Suitable glycosyl transferase enzymes catalyze these reactions. Sugar residues that are attached most commonly include galactose, mannose, glucose, N-acetylglucosamine, N-acetylgalactosamine as well as fucose.

Protein Glycosylation

Common in Eukaryotic Proteins

- Occurs on **secreted** proteins & external face of **membrane** proteins
- **HEAVY**: the carbohydrate content can exceed the protein content
- **Roles**

Enhanced solubility, prolonged lifespan of secreted proteins
Cell recognition (extracellular face of membrane proteins)
Structural support (proteoglycans)

•N-linked:

- Attached to specific **Asn** residues
- Modification occurs in **ER**

Consensus: ---NXS---
 T
 C

O-linked:

- Attached to specific **Ser, Thr** residues
- Modification occurs in **Golgi stack**
- Consensus lacking, with few exceptions

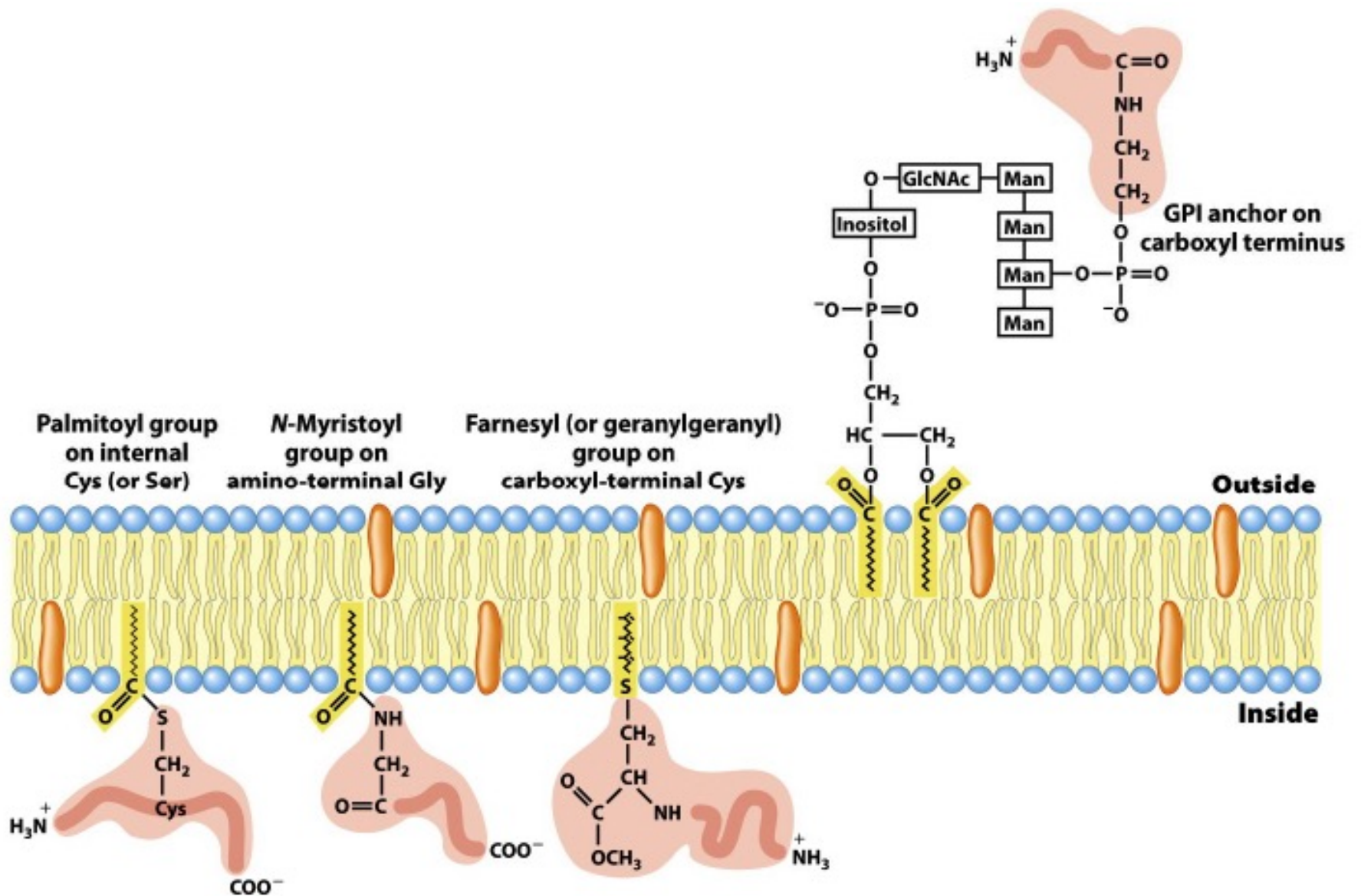


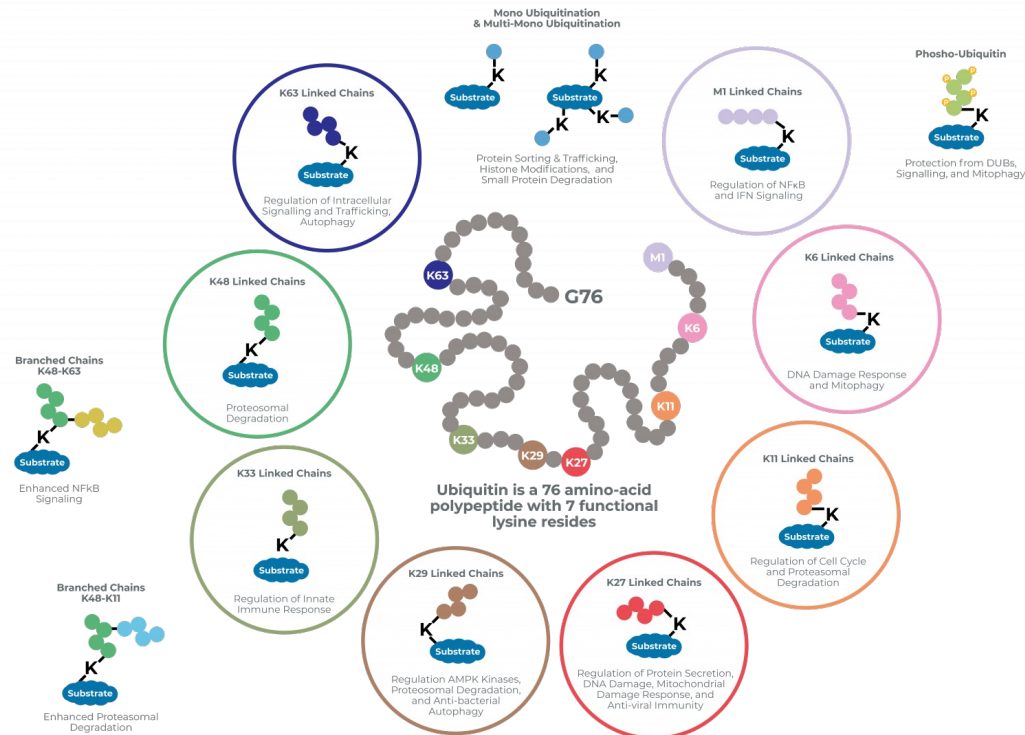
Figure 11-14

Lehninger Principles of Biochemistry, Fifth Edition

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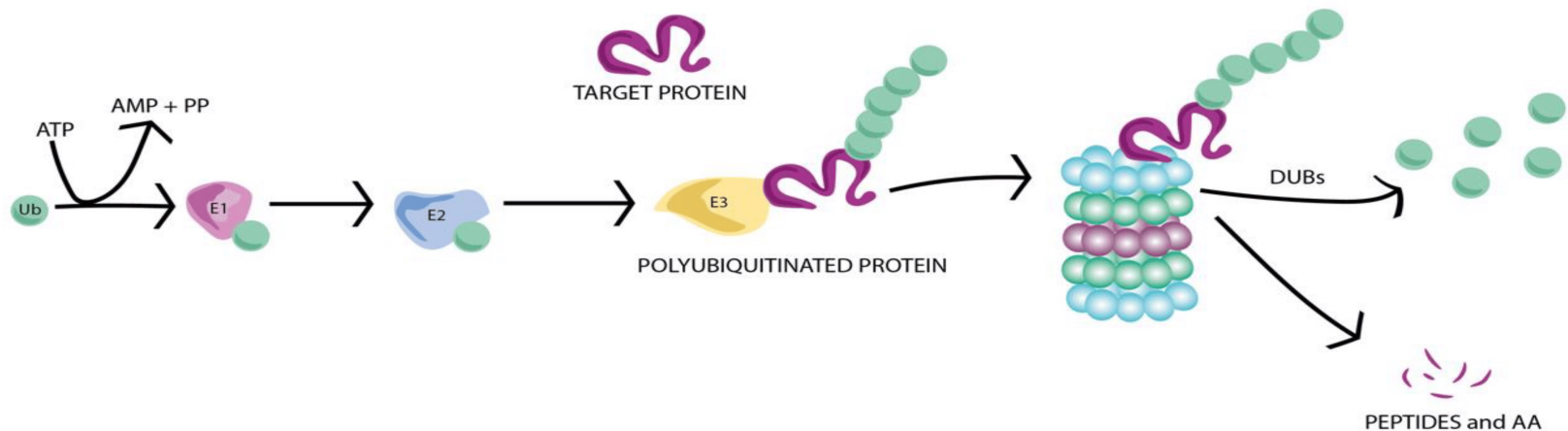
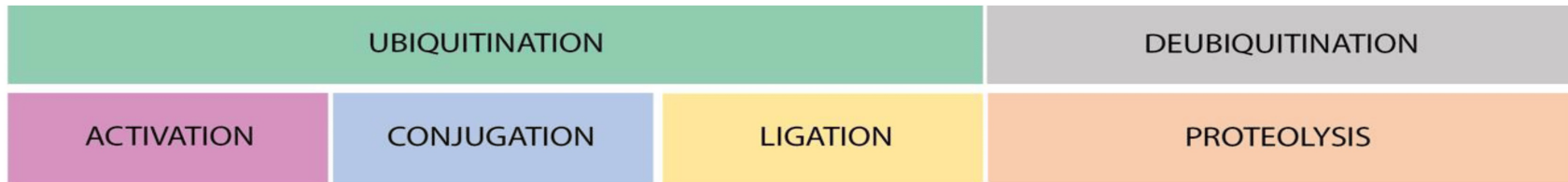
Ubiquitination

- Ubiquitination, an important type of protein posttranslational modification (PTM), plays a crucial role in controlling substrate degradation and subsequently mediates the “quantity” and “quality” of various proteins, serving to ensure cell homeostasis and guarantee life activities.
- Ubiquitination is a tightly regulated, highly specific, and ATP-dependent biological process carried out by a complex cascade of enzymes.

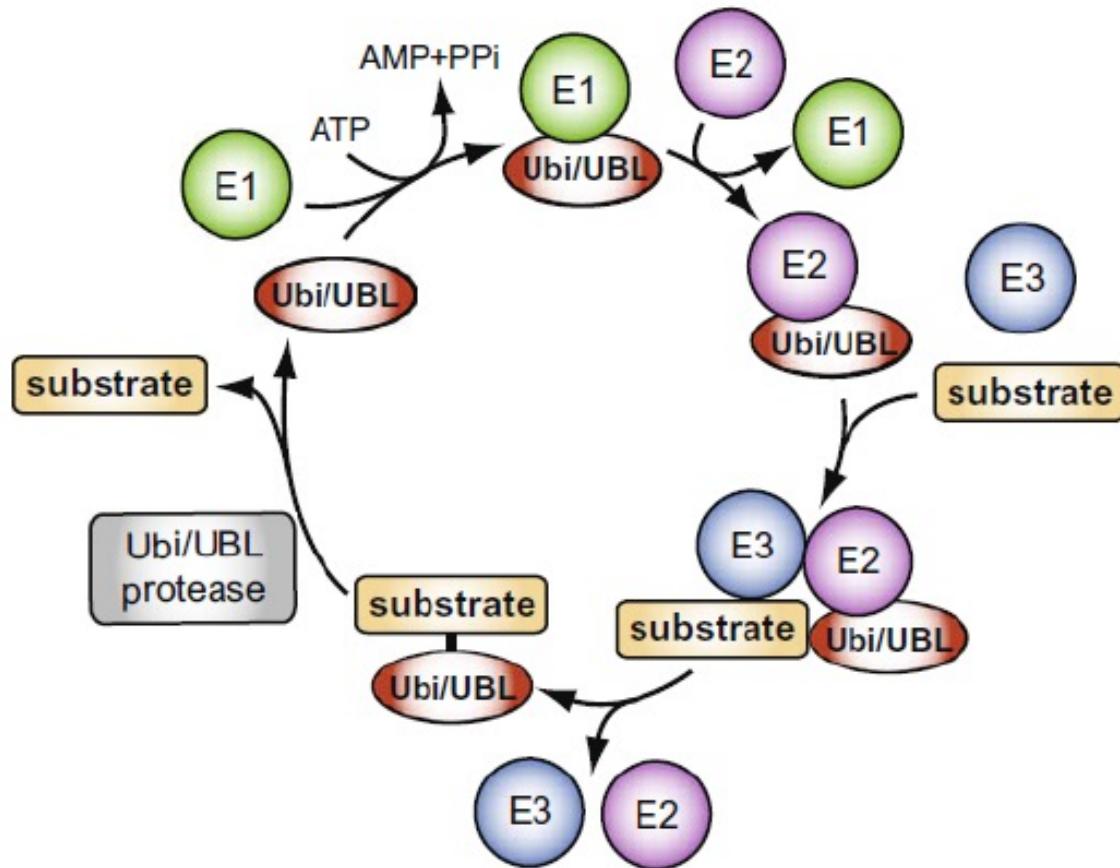


Ubiquitination

Ubiquitin (Ubi) and UBLs are small polypeptides that can be attached to substrate proteins via related enzymatic pathways. These polypeptides are first activated by an E1 enzyme in an ATP-dependent manner, then transferred to an E2 conjugating enzyme and eventually conjugated to a substrate protein with the help of E3 ligases. The set of E1, E2 and E3 enzymes involved is specific of each Ubi/UBL protein. Ubiquitin and UBLs can be deconjugated from target proteins by the action of specific proteases.

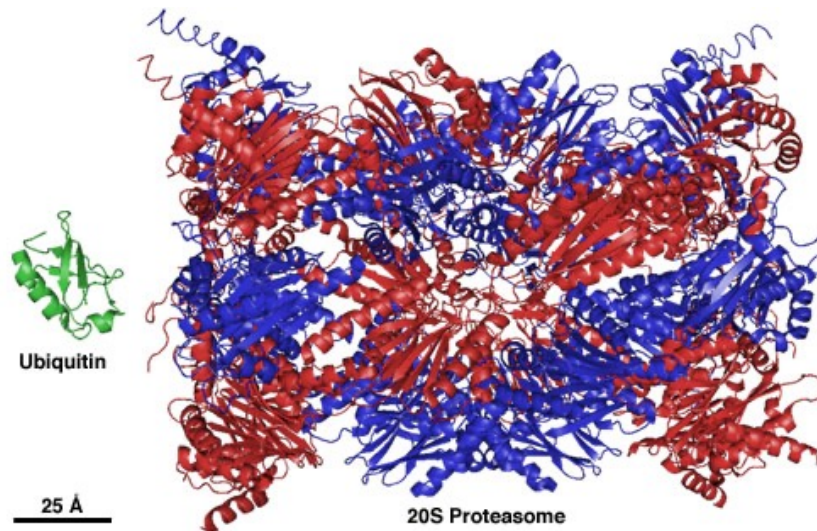
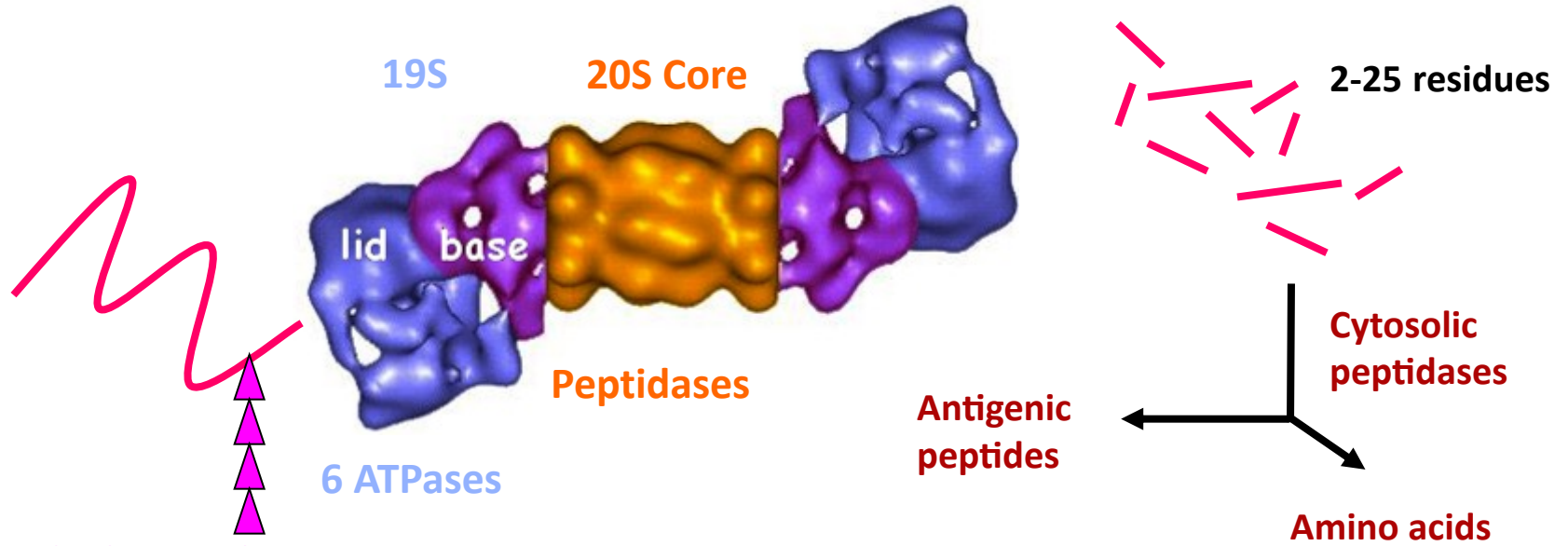


Covalent linkage of small proteins

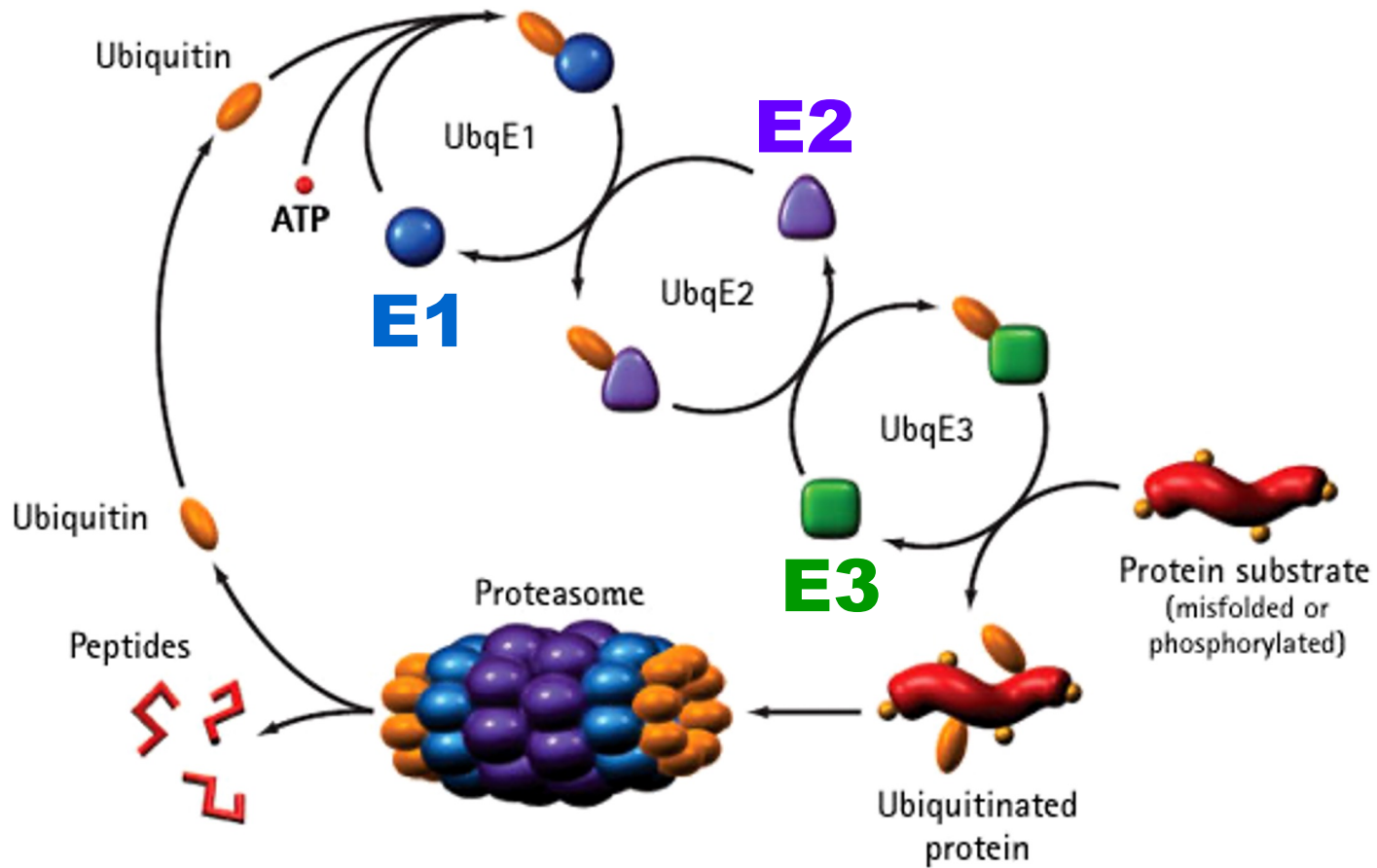


Ubiquitin and ubiquitin-like proteins conjugation machineries.

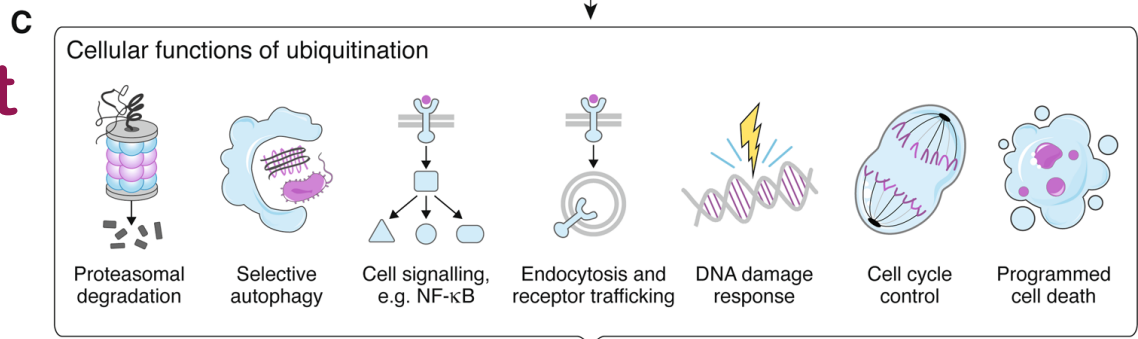
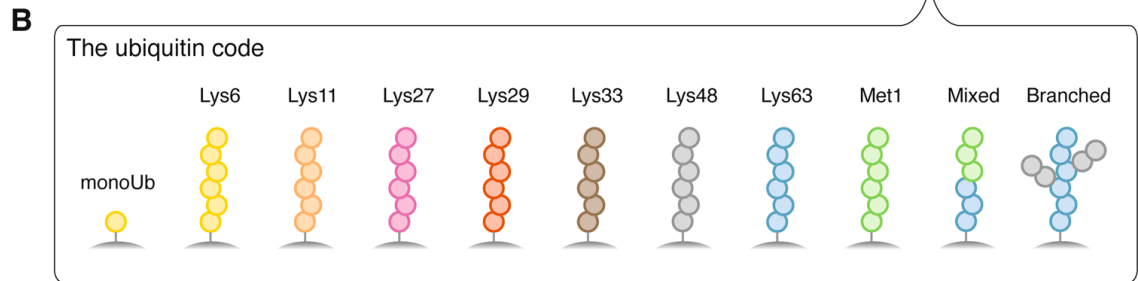
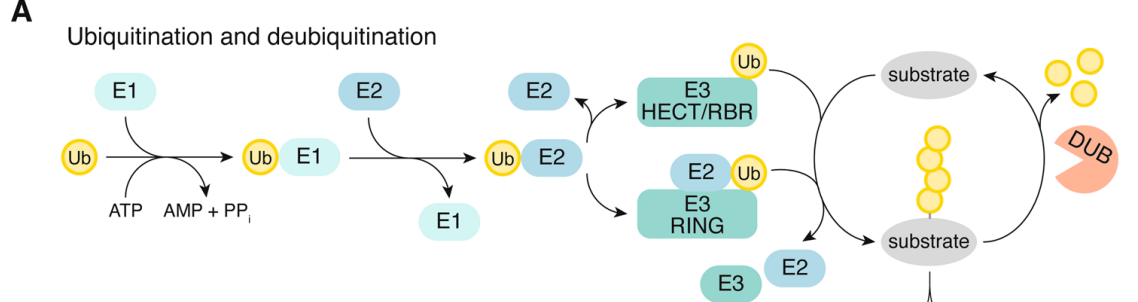
■ Steps involved in protein degradation



The Ubiquitination cascade



Degradation



Normal cellular function and homeostasis

- proteostasis
- normal signalling
- cell survival and robustness
- adaption to stress

Cellular dysfunction and disease

- developmental diseases
- cancer
- immune disorders
- neurodegenerative diseases
- metabolic disorders
- ageing

Ubiquitination Impact

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