

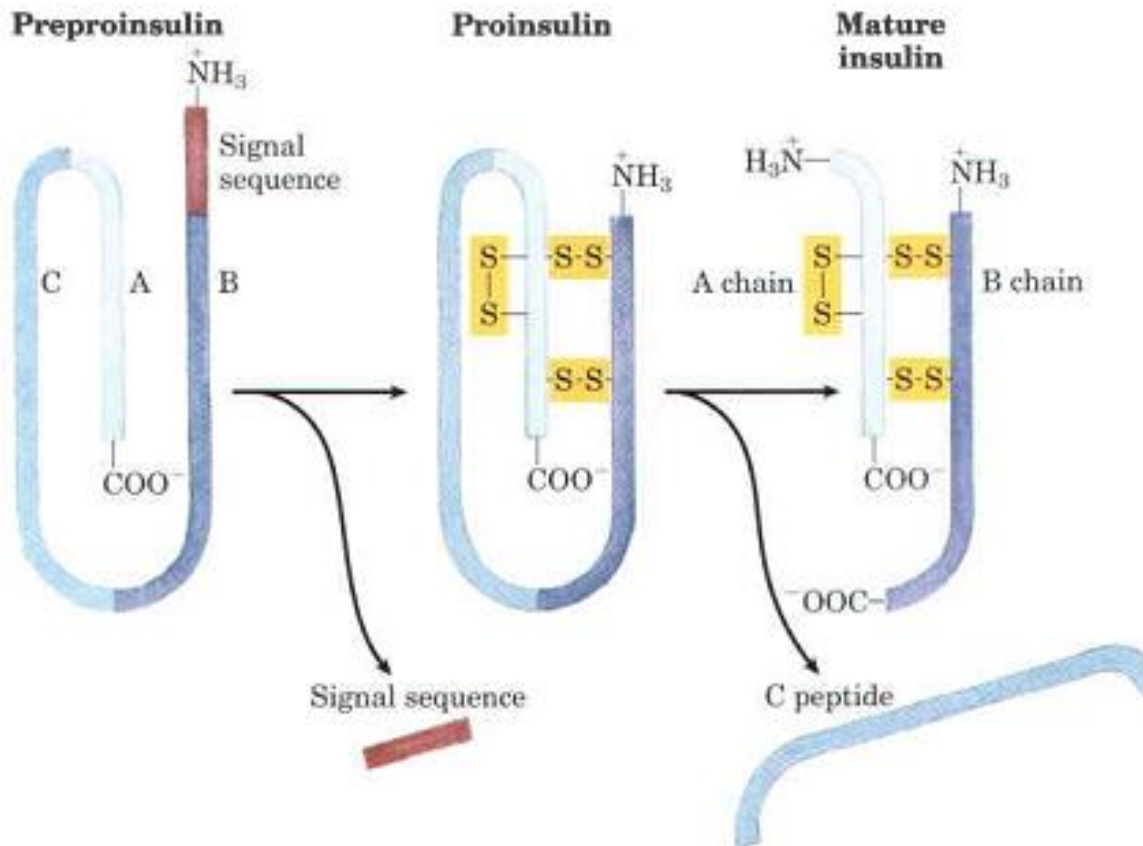
# MOLECULAR BIOLOGY: POSTTRANSLATIONAL MODIFICATION

## Lecture 5

## Protein activation by proteolytic cleavage

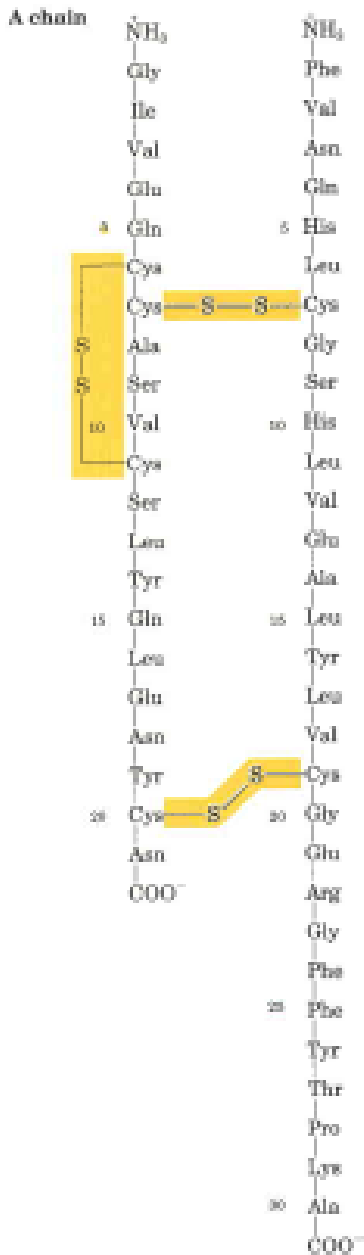
- The simplest form of cleavage is the removal of methionine at the start of the newly synthesized polypeptide.
- Many proteins are synthesized as inactive precursors (proproteins) that are activated under proper physiological conditions by limited proteolytic cleavage
- Proteins that are synthesized with signal peptide for targeting are called preproteins and require further proteolytic cleavage to be functional. An example of protein synthesized as a preprotein is Insulin.
- Other examples of inactive precursors: Several blood clotting factors are synthesized as zymogens which are activated by proteolytic cleavage.

# Proteolytic processing of insulin from its precursor preproinsulin

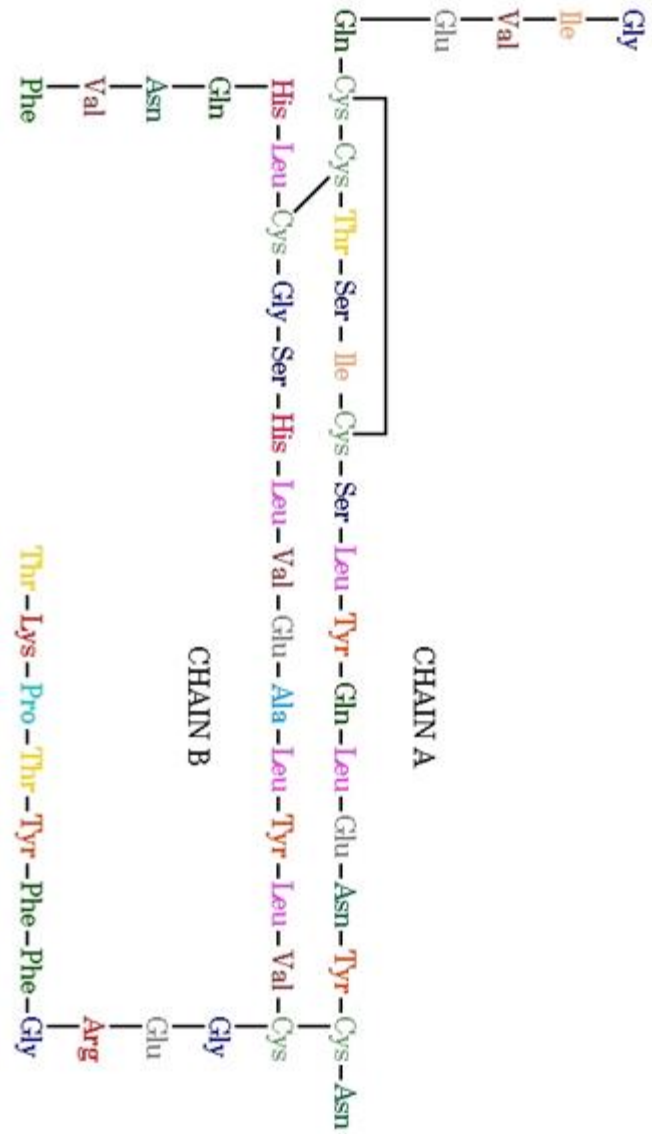


Mature insulin is formed from its larger precursor preproinsulin by proteolytic processing. Removal of a 23 amino acid segment (the signal sequence) at the amino terminus of preproinsulin and formation of three disulfide bonds produces proinsulin. Further proteolytic cuts remove the C peptide from proinsulin to produce mature insulin, composed of A and B chains. Amino acid sequences of bovine and human insulin are shown in the Figures below.

- Chemically, insulin is a small, simple protein. It consists of 51 amino acid, 21 of which comprise chain A and 30 of which constitute polypeptide chain B.
- The two chains are linked by disulfide bonds.
- The gene for insulin is found at the top of the short arm of chromosome 11.



Amino acid sequence of bovine insulin



Amino acid sequence of human insulin

## Protein methylation

- Post-translational methylation of proteins occurs on nitrogens and oxygens.
- The activated methyl donor is S-adenosylmethionine (SAM)
- The most common methylations are on lysine and arginine.
- Methylation of lysine residues in histones is an important regulator of chromatin structure and transcriptional activity

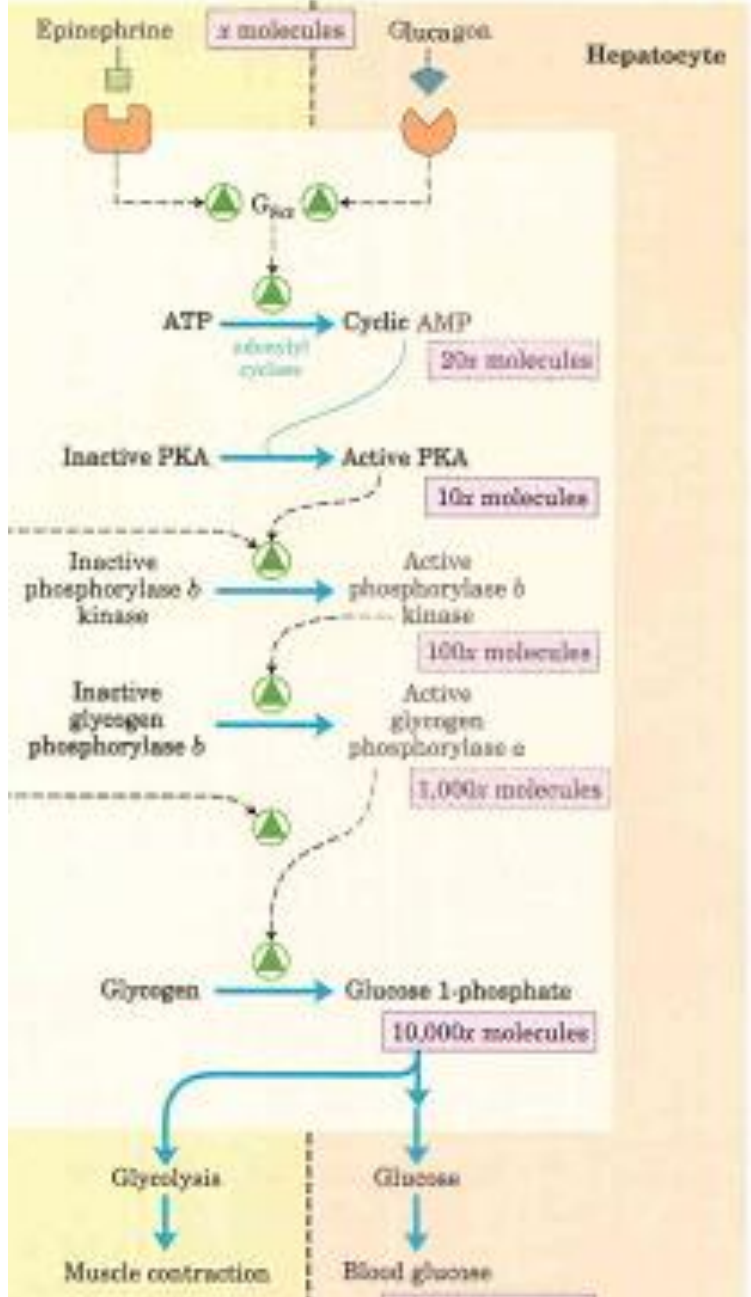
## Protein acetylation

- The most common acetylations are on lysine residues
- The activated acetyl donor for the lysine (K) acetyltransferases (KAT enzymes) is acetyl-CoA
- N-terminal acetylation creates a specific degradation signal for the protein

## Protein phosphorylation

- Post-translational phosphorylation is one of the most common reversible protein modifications that occurs in animal cells
- A phosphate is added by a specific kinase and later removed by a specific phosphatase
- Examples are the phosphorylations that occur in glycogen synthase and glycogen phosphorylase in hepatocytes in response to glucagon release from the pancreas.

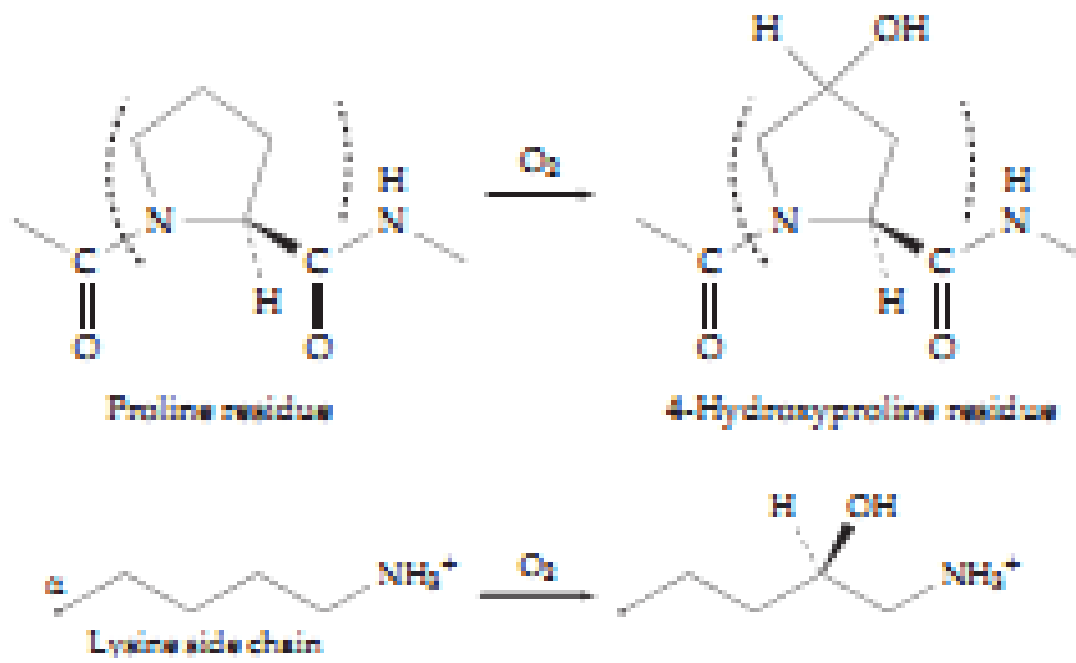
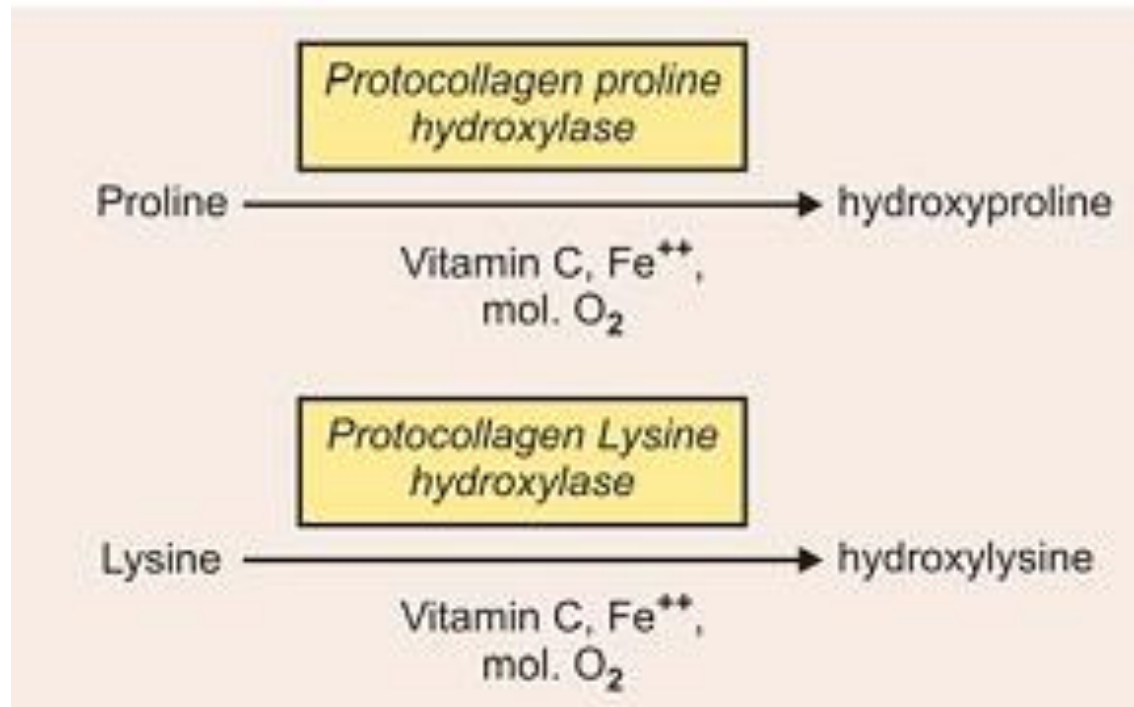
# Cascade mechanism of glucagon action



## Vitamin C-dependent protein modifications

- Modifications of proteins that depend upon vitamin C as a cofactor include proline and lysine hydroxylations by prolyl hydroxylases and lysyl hydroxylases, respectively.
- The most important hydroxylated proteins are the collagens
- Hydroxyproline and hydroxylysine are important constituents of mature collagen fibres. Procollagen molecules contain the amino acids proline and lysine.
- They are hydroxylated by corresponding hydroxylases in presence of vitamin C,  $\text{Fe}^{2+}$  and molecular  $\text{O}_2$ .
- Hydroxylases localized in the membranous vesicles of the ER convert some of the proline and lysine residues of the procollagen chains into 4-hydroxyproline and hydroxylysine.





# Scurvy

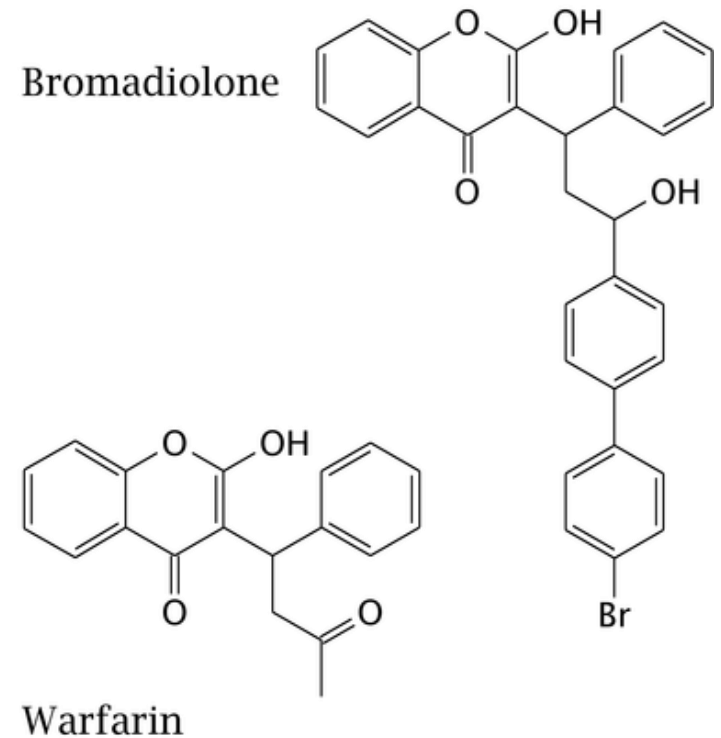
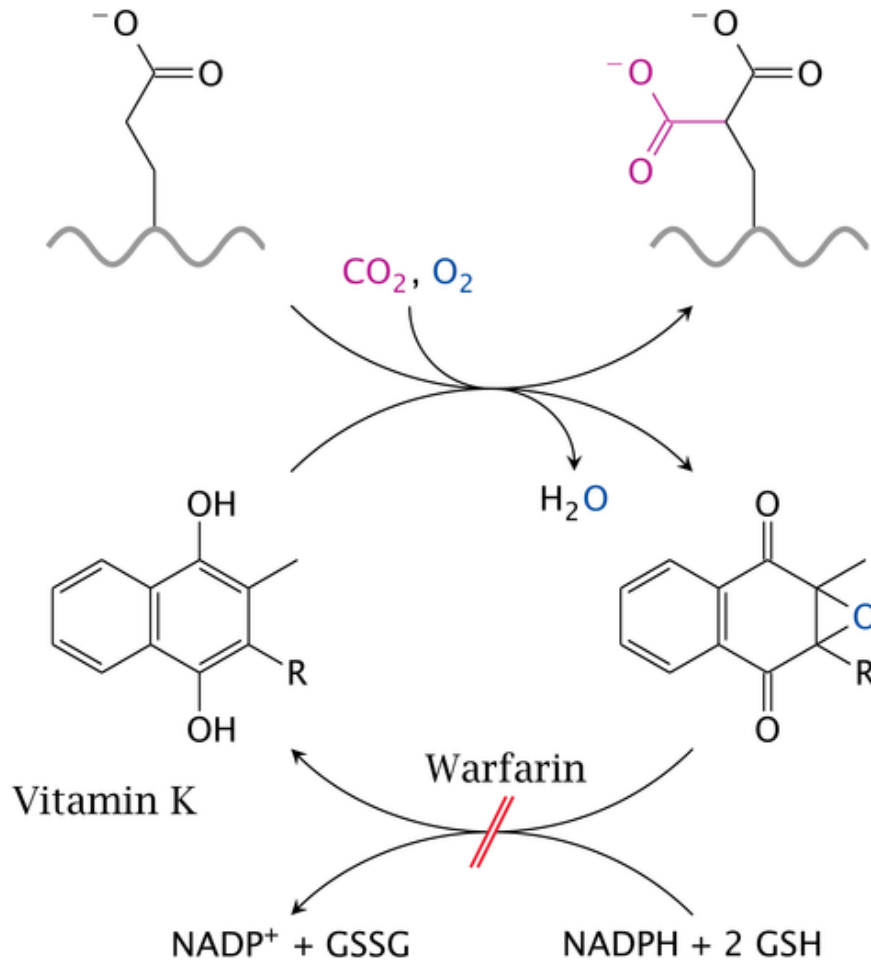
- Formation of hydroxyproline from proline is dependent on Vitamin C hence lack of this vitamin leads to lower amounts of hydroxyproline and that leads to poorly deposited collagen which is weak and highly susceptible to damage and this results in scurvy.
- The addition of hydroxyl groups to many proline residues stabilizes fibers of newly synthesized collagen, a fibrous protein found in connective tissue. Deficiency of vitamin C results in insufficient hydroxylation of collagen and the abnormal collagen fibers that result are unable to maintain normal tissue strength.

Hydroxyproline stabilizes the collagen triple helix by forming interstrand hydrogen bonds. The abnormal fibers formed by insufficiently hydroxylated collagen contribute to the skin lesions and blood-vessel fragility seen in Scurvy.

## Vitamin K-dependent protein modifications

- Several blood coagulation factors (II, VII, IX, and X) require posttranslational modification of glutamate residues to  $\gamma$ -carboxyglutamate.
- The carboxylation of glutamic acid residues is catalyzed by the enzyme  $\gamma$ -glutamyl carboxylase which requires reduced vitamin K as a cosubstrate/cofactor and converts it to an epoxide.
- The epoxide formed is reduced again by vitamin K epoxide reductase in the presence of NADPH and reduced glutathione (GSH).
- Vitamin K epoxide reductase is inhibited by the drug warfarin
- Complete inhibition of vitamin K reductase is deleterious, and indeed is used to kill rats with rodenticide such as bromadiolone. Warfarin is also a rat poison. Therefore, this type of treatment has a very low therapeutic index and requires careful monitoring.

# Posttranslational modification of glutamate residues in coagulation proteins



In the structures of vitamin K and its epoxide, R represents a side chain containing several isoprenoid residues