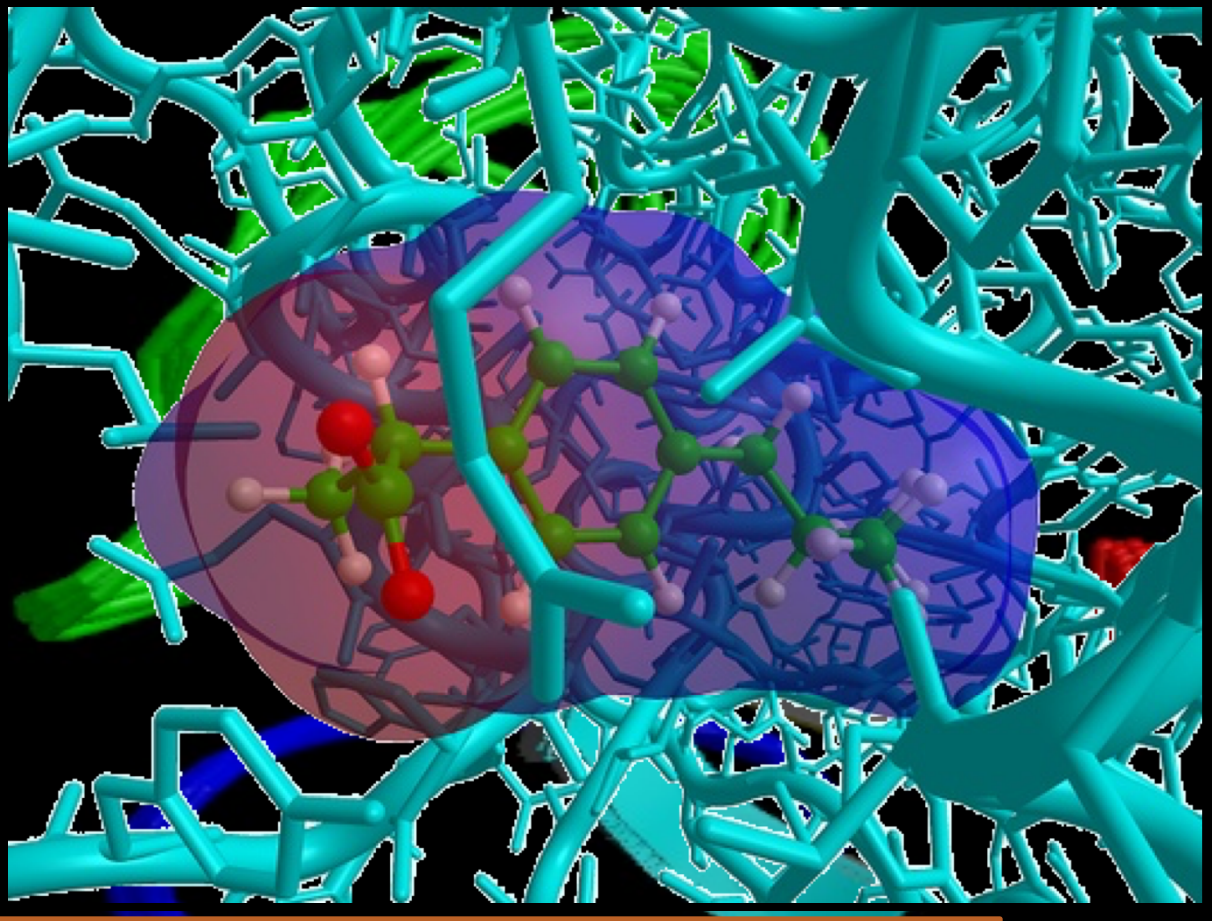
Course: Protein Structure, Function and Engineering



Lecture 10

Post Translational Modifications

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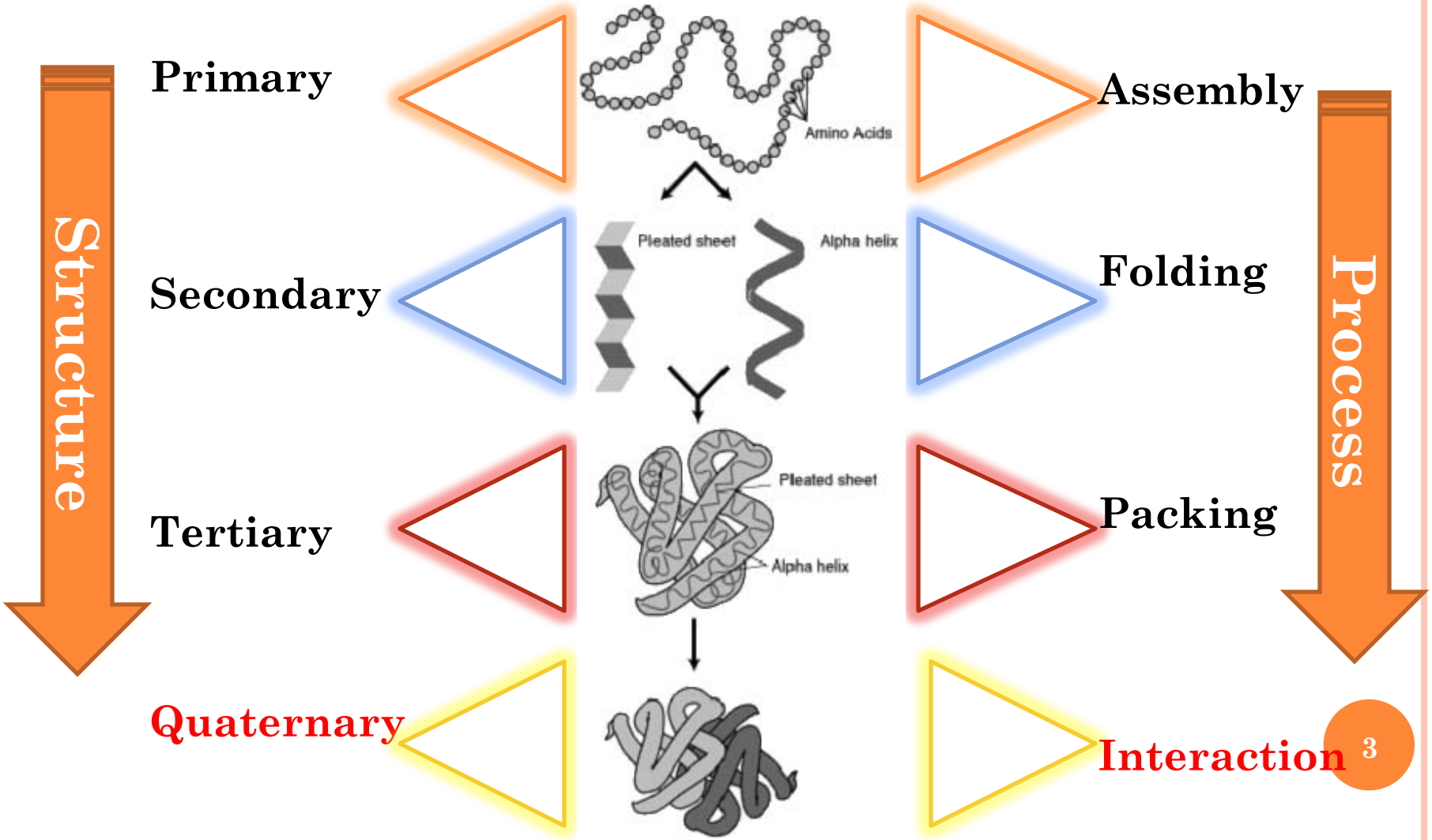
Last Lecture

- Quaternary Structure
  - Hemoglobin Structure and Ionic forces
- Protein domains
  - Classification
- How proteins are made by domains

Protein Structures



Synthesis of protein

Involve the following steps;

- Activation of amino acid.
- Initiation of polypeptide chain.
- Elongation of polypeptide chain.
- Termination and release.
- Folding and processing.

Posttranslational Modification

- Post-translational processing controls *folding*, *targeting*, *activation* and *stability* of proteins

Forms of post-translational modifications

1- Modification of individual amino acid
2- Glycosylation (**N-linked & O-linked)**
3. Proteolytic processing
4. Prosthetic group addition
5. Disulphide bond formation
6.Lipophilic modification
7.Selenoproteins
8.Vit.K dependent modification
9.Sulfation

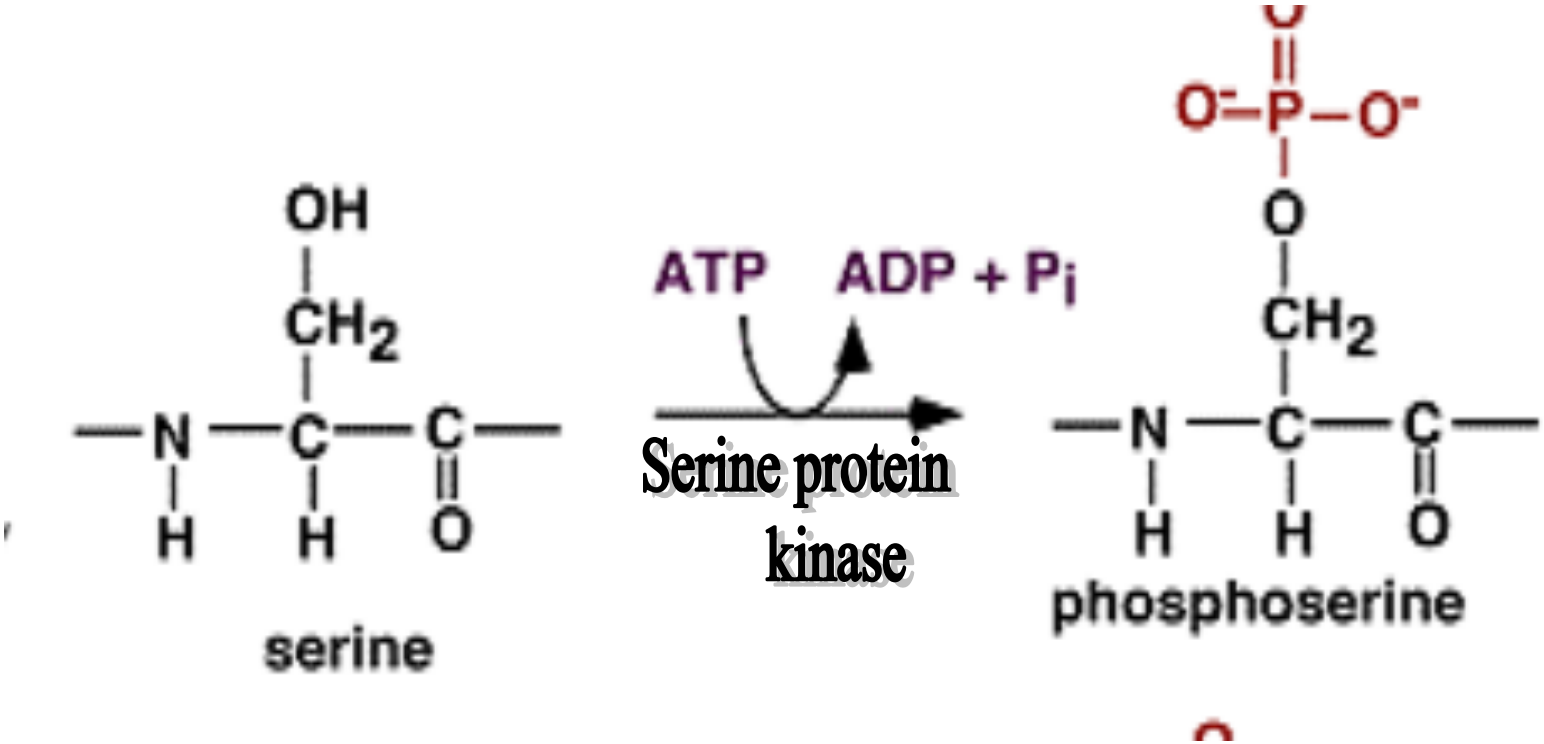
1. Modification of individual amino acid

- Phosphorylation
- Hydroxylation
- Carboxylation
- Methylation

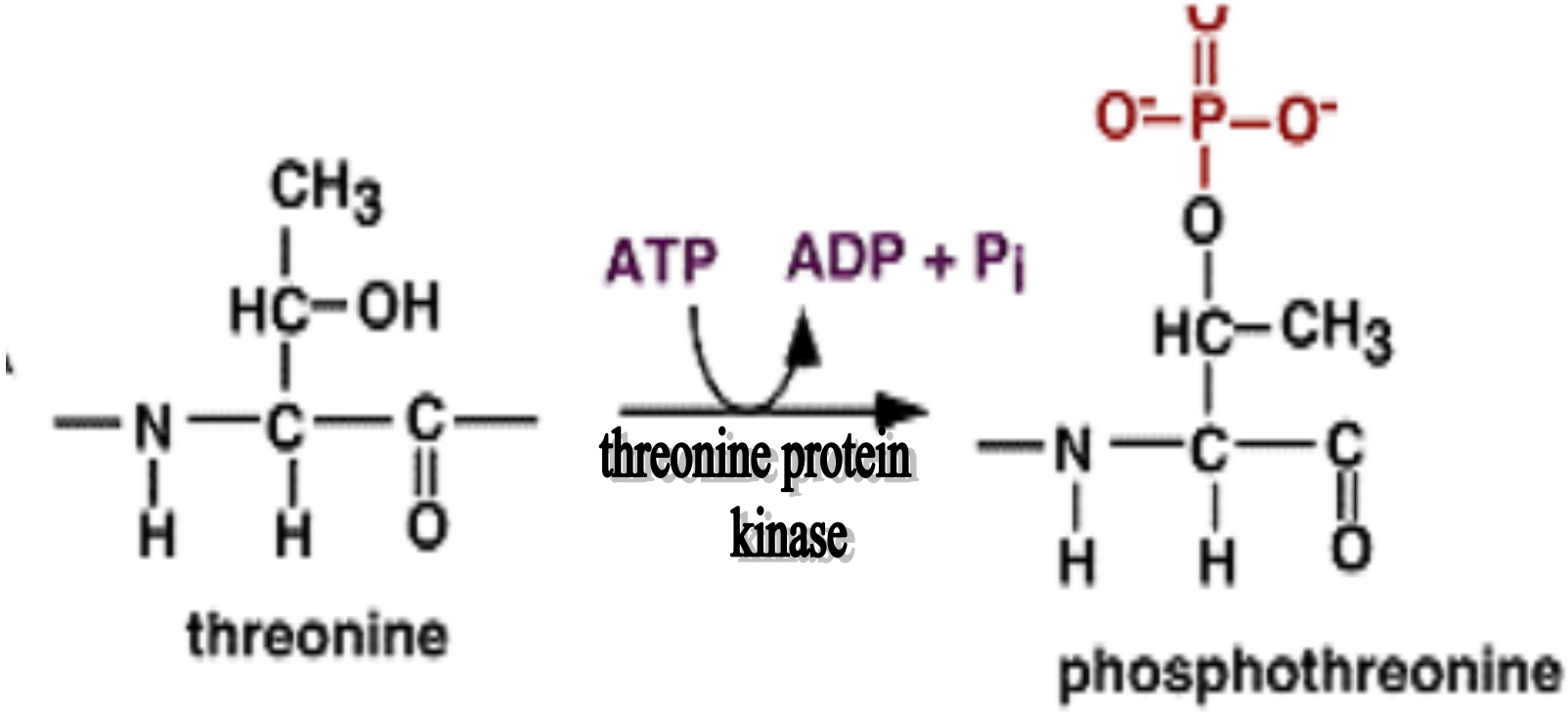
1.1.Phosphorylation

- Addition of phosphate group to specific amino acid
  - play a critical role in protein-protein interaction
  - Autophosphorylation of tyrosine residue in PDGF receptor apparently results in the subsequent binding of certain cytoplasmic proteins
- Phosphorylation of OH group of serine, threonine,tyrosine e.g;Casein
- His Phosphorlyation and Catalysis

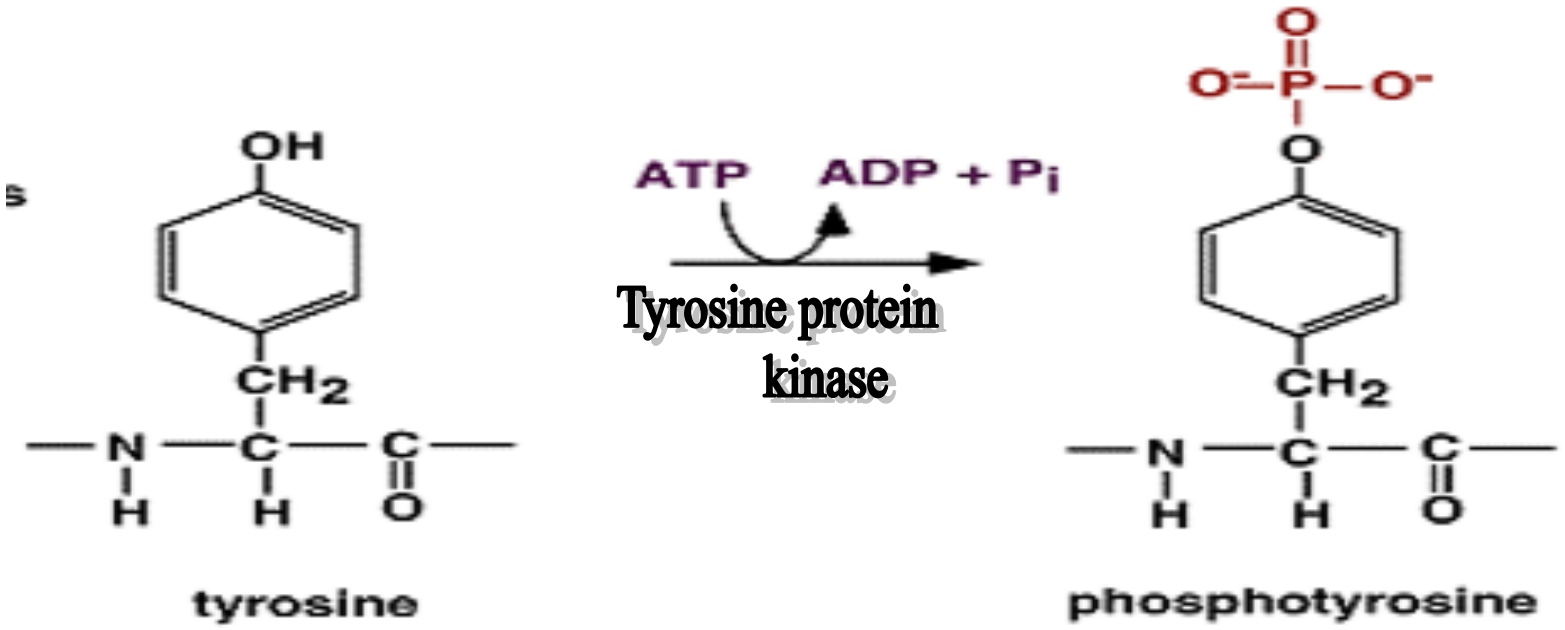
Phosphorylation of hydroxyls by kinases at serine



Phosphorylation of hydroxyls by kinases at threonine



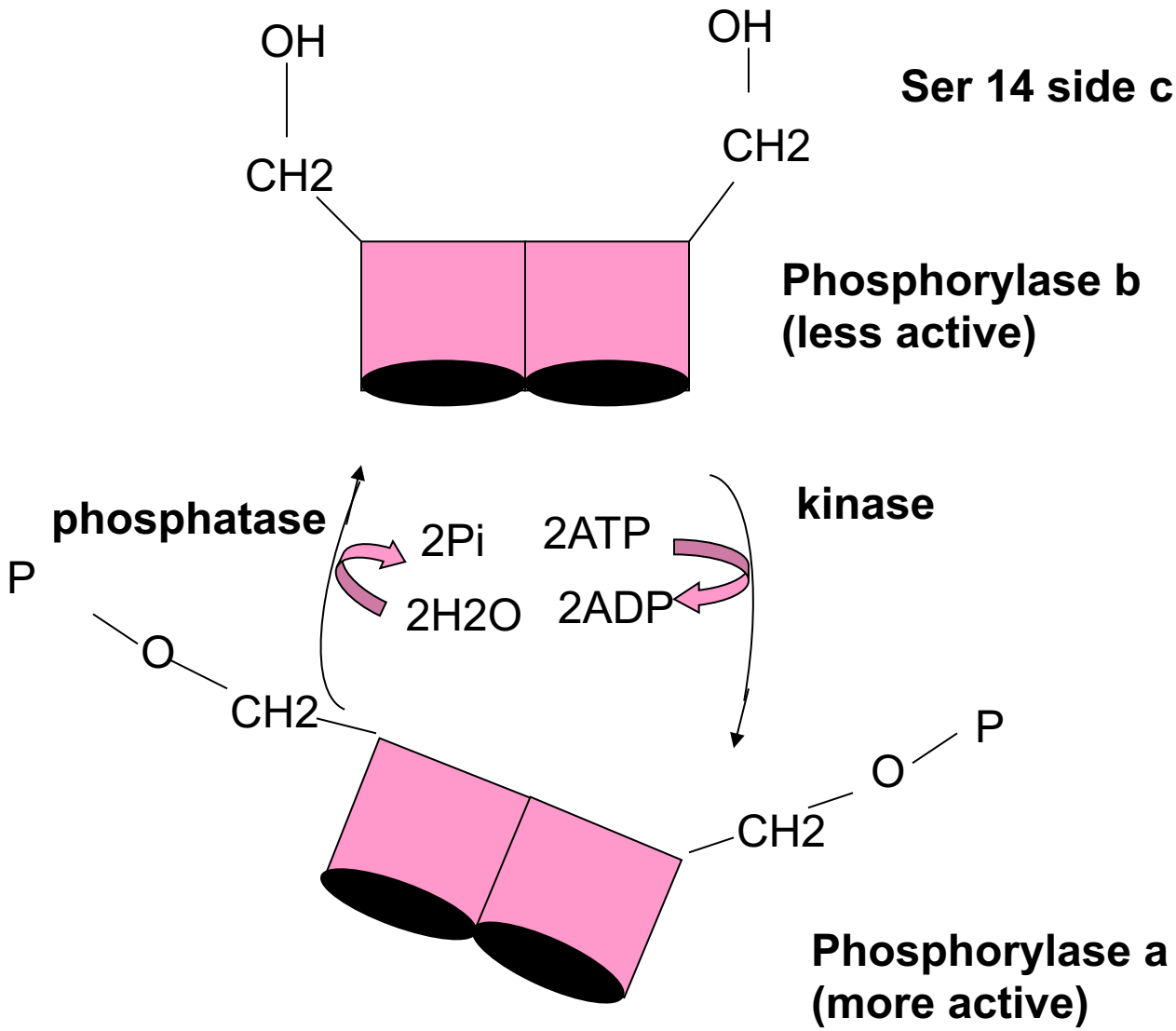
Phosphorylation of hydroxyls by kinases at tyrosine



Glycogen phosphorylase

Glycogen -*Glycogen phosphorylase* → G-1-P

Phosphorylation of phosphorylase occurs on serine-14 and converts the inactive *phosphorylase b* to the active *phosphorylase a*.


OH
OH
CH2
CH2
Ser 14 side chain
Phosphorylase b
(less active)
phosphatase
kinase
2Pi
2ATP
2H2O
2ADP
P
O
CH2
CH2
O
P
Phosphorylase a
(more active)

1.2.Hydroxylation

- Hydroxylation of amino acid proline and lysine
  - Required for the structural integrity of the connective tissue proteins
    - collagen and elastin.
- Three mixed functions of oxygenase (prolyl-4-hydroxylase,prolyl-3-hydroxylase and lysyl hydroxylase) located in RER are responsible for the hydroxylation of certain proline and lysine residues.
- Substrate required is highly specific.

1.2.Hydroxylation

- Prolyl-4-hydroxylase hydroxylates only proline residues in the Y position of peptide containig Gly-X-Y sequences.
- Prolyl-3-hydroxylase requires Gly-X-Hyp
- Lysyl hydroxylase acts on the Y residue of the Gly-X-Y.

1.3.Carboxylation

- Addition of carboxyl group to asparagine and glutamate residue activate the protein functionally e.g.prothrombin

1.4.Methylation

- A group of enzyme reffered to as the protein methyltransferases
  - utilize s-adenosyl methionine to methylate certain proteins.
- The methylation of altered aspartate residue by specific type of methyl transferase
  - promotes either the repair or the degradation of damaged proteins.
- Other methyltransferases catalyze reactions that alter the cellular roles of certain proteins

1.4 Methylation

- Methylated lysine residue have been found in such disparate proteins
  - Ribulose-2,3-biphosphate carboxylase calmodulin, histones, some ribosomal proteins and cytrochrome c.
- Other methylated amino acid residue include histidine
  - Histones and rhodopsin and
- Arginine
  - Heat shock proteins and ribosomal proteins

2.Glycosylation

- Most abundant form of modification
- Covalent attachment of oligosaccharide to proteins
- Covalently attached to the polypeptide as oligosaccharide chains containing 4 to 15 sugars.
- Sugars frequently comprise 50% or more of the total molecular weight of a glycoprotein .
- Most glycosylated proteins are either *secreted* or *remain membrane-bound* .

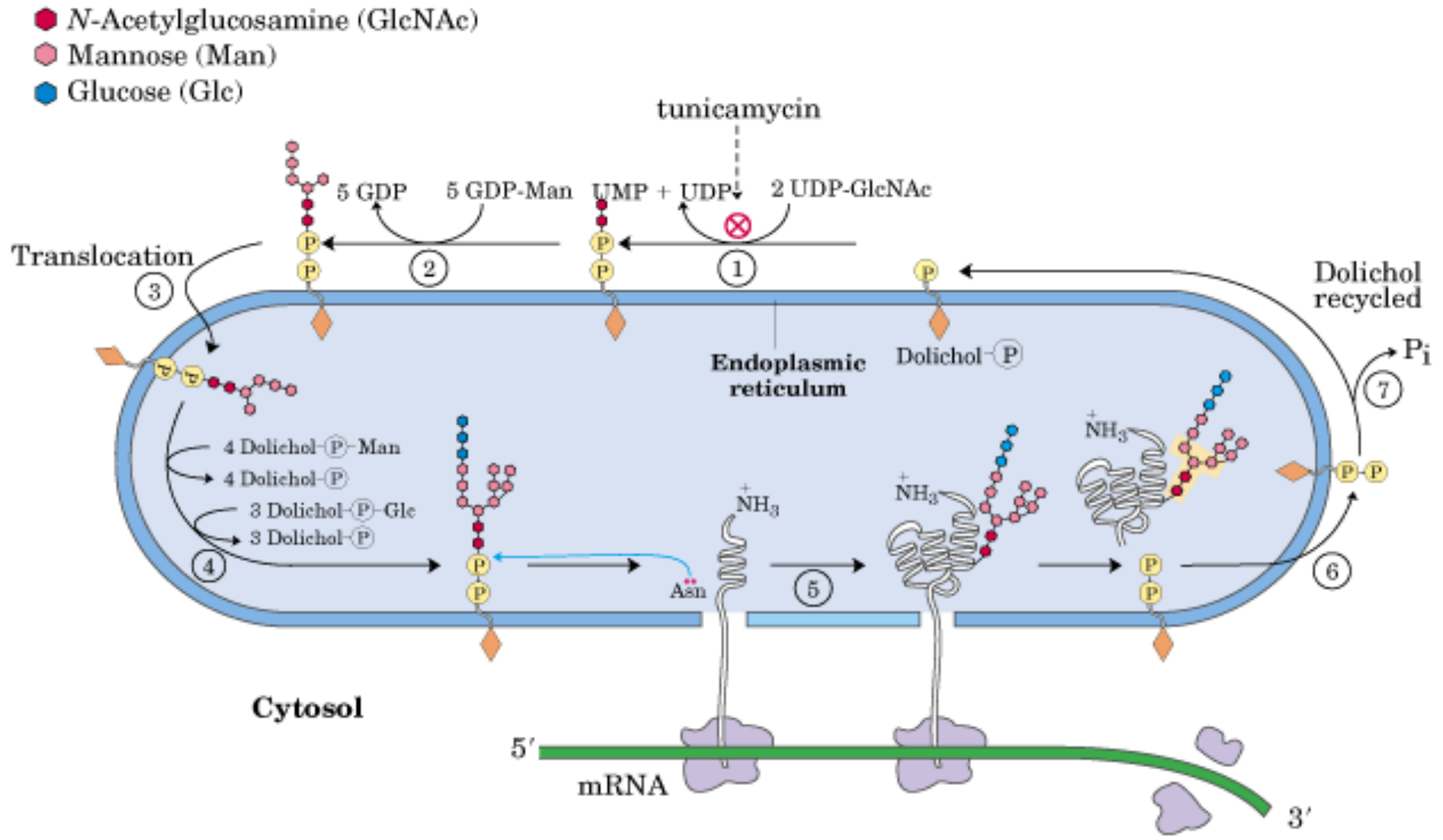
2.1.N-LINKED GLYCOSYLATION

- N-linked glycosylation on asparagine (Asn) side chains.
- An alkali-stable bond between the amide nitrogen of asparagine and the C-1 of an amino sugar residue .
- Occurs co-translationally in the endoplasmic reticulum (ER) during synthesis.
- Lipid-linked oligosaccharide complex is transferred to polypeptide by *oligosaccharyl transferase*.

2.1.N-LINKED GLYCOSYLATION

- *Target sequence* or *consensus site* on protein is Asn-X-Ser/Thr.
- Further processing in golgi apparatus.
- Examples
  a) Heavy chain of immunoglobulin G (IgG).
  b) Hen ovalbumin .
  c) Ribonuclease B.

N-LINKED GLYCOSYLATION



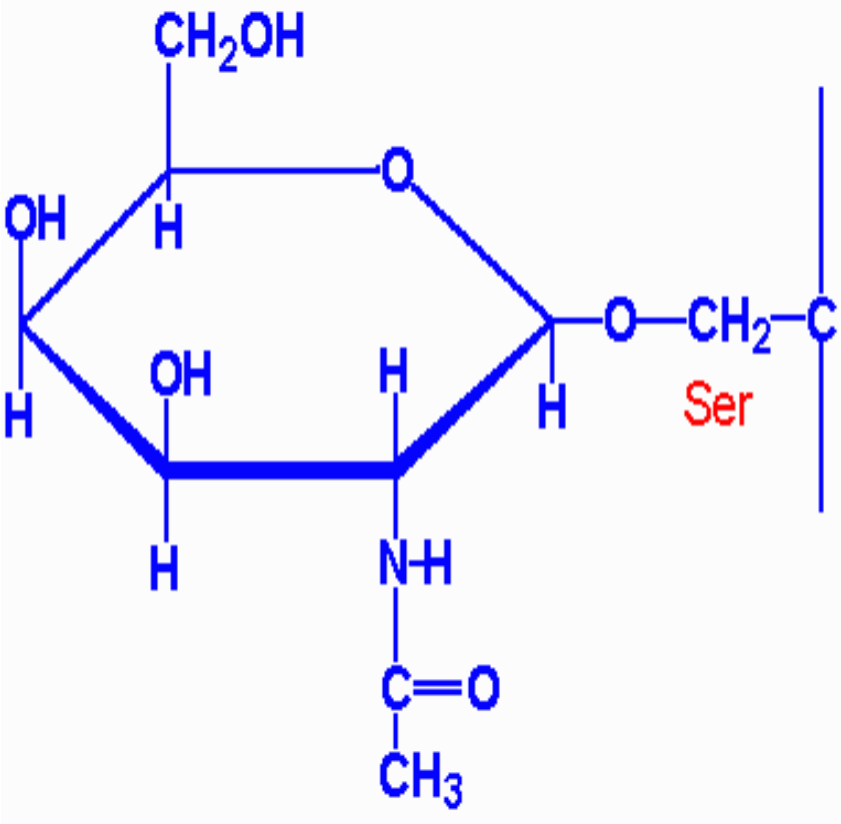
2.2.O-LINKED GLYCOSYLATION

- O-linked glycosylation on serine (Ser) or threonine (Thr) side chains.
- An alkali-stable bond between the hydroxyl group of serine or threonine and an amino sugar.
- Carried out by enzymes called ***glycosyl transferases*** which reside in the ER or the golgi apparatus

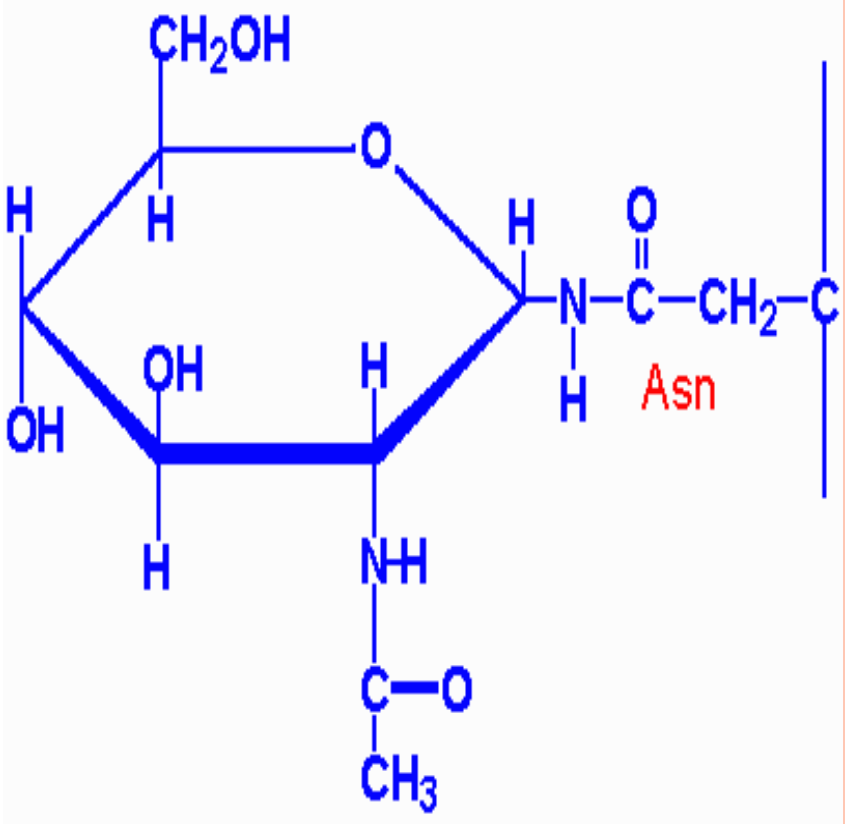
2.2.O-LINKED GLYCOSYLATION

- Example: Blood group antigens on erythrocyte surface:
  - The *A antigen* and *B antigen* are pentasaccharides which differ in composition of the 5th sugar residue .
  - The *O substance* is a tetrasaccharide which is missing the 5th residue and does not elicit an antibody response (non-antigenic).

O-LINKED



N-LINKED



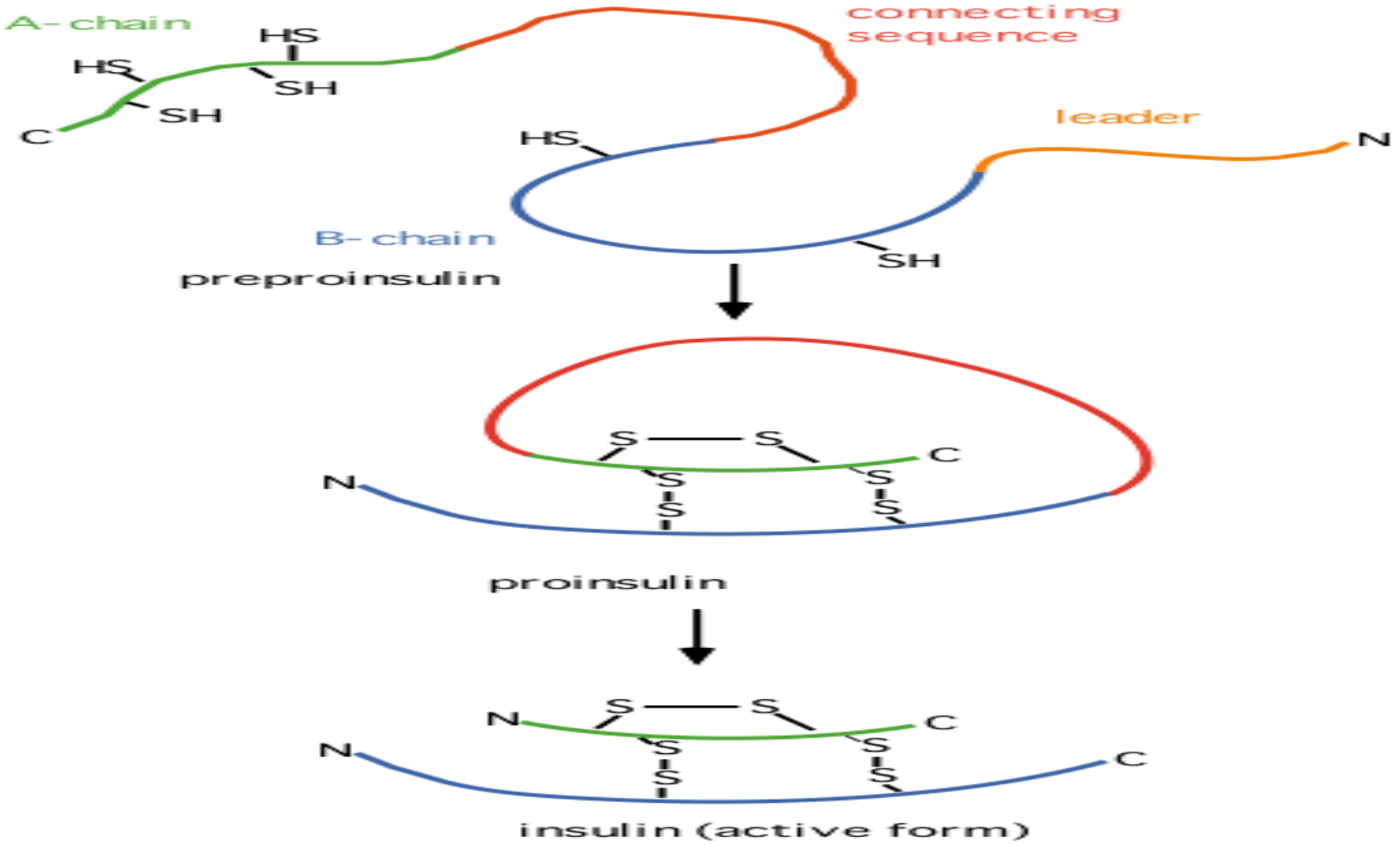
3.Proteolytic Processing

- Most of the proteins involved in a wide variety of biological processes are synthesized as inactive precursors
- Inactive proteins that are activated by removal of polypeptides are termed as proproteins where as the excised polypeptides are termed propeptides
- Many transemembrane proteins that are destined to be secreted are synthesized with an N- terminal signal peptide of 13 to 36 a.a

3.Proteolytic Processing

- Proteins bearing signal peptide are known as preproteins if they also contain propeptide as preproproteins
- Both insulin and collagen are secreted proteins and are therefore synthesised with leading signal peptides in the form of preproinsulin and preprocollagen

Processing of pre-pro-insulin to active insulin



4.Lipophilic Modification

- The covalent attachement of lipid moieties to proteins improves membrane binding capacity and /or certain protein-protein interaction
- Among the most common lipophilic modification are acylation and prenylation

4.1.Acylation

- The attachment of fatty acids to proteins
- Myristoylation is one of the most common form of acylation
  - N-myristoylation
    - i.e the covalent attachement of myristate by the way of an amide bond to a polypeptide amino glycine residue
    - increase the affinity of the alpha subunit of certain G protein for membrane bound beta and gamma subunits

4.2.Prenylation

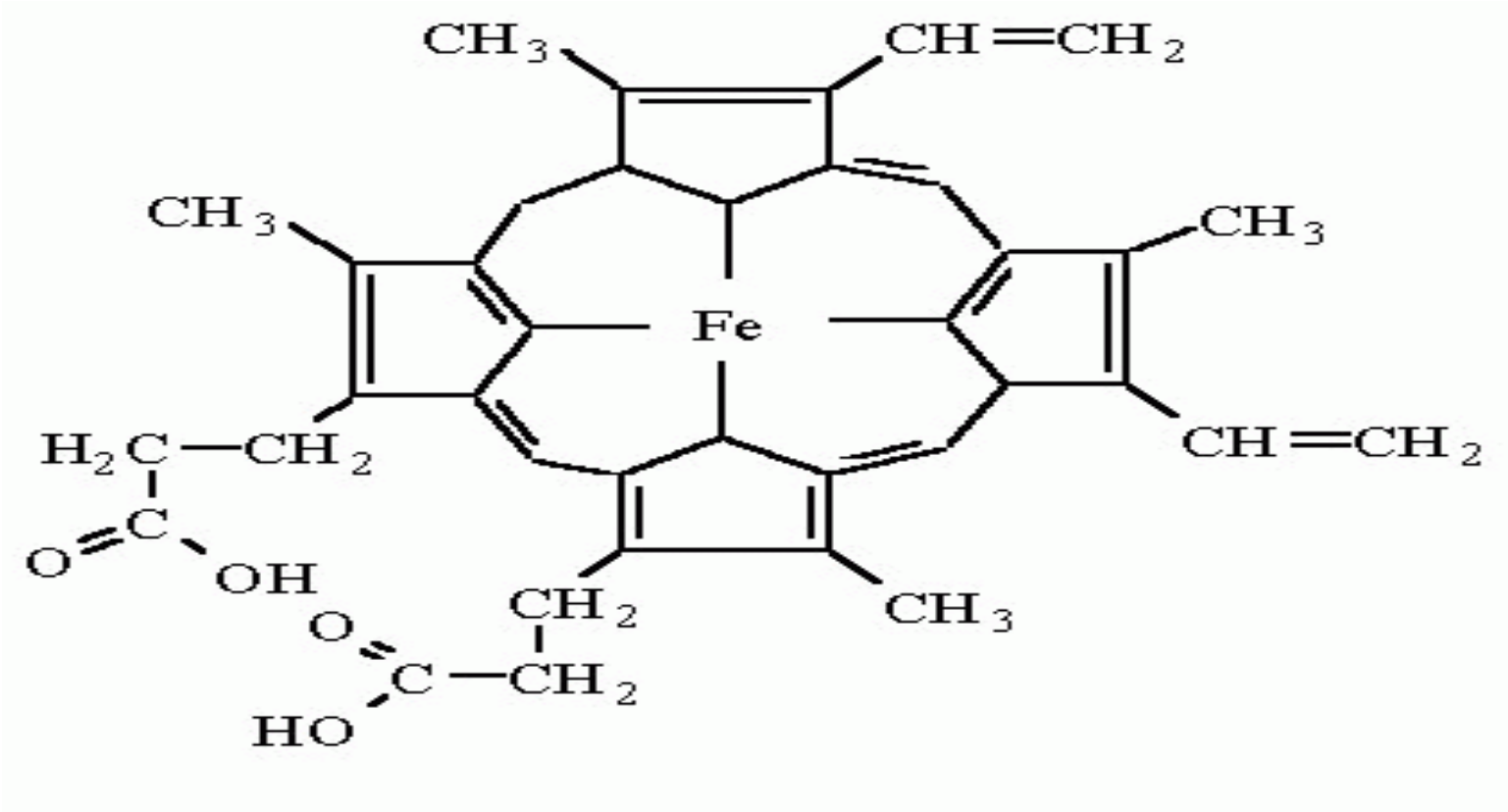
- A variety of proteins in eukaryotic cells are covalently attached to prenyl group after their biosynthesis on ribosome
- The prenyl group that are most often involved in this process referred to as prenylation are farnesyl and geranylgeranyl groups
- Products of ras oncogene and proto-oncogene and G-protein are modified in this way.

5. Prosthetic groups addition

- Addition of metal ions and co-factors (heme, retinal etc.)
- Nearly 50% of all proteins contain metal ions
- Metal ions play regulatory as well as structural roles

Prosthetic group attachment (heme, retinal etc.)

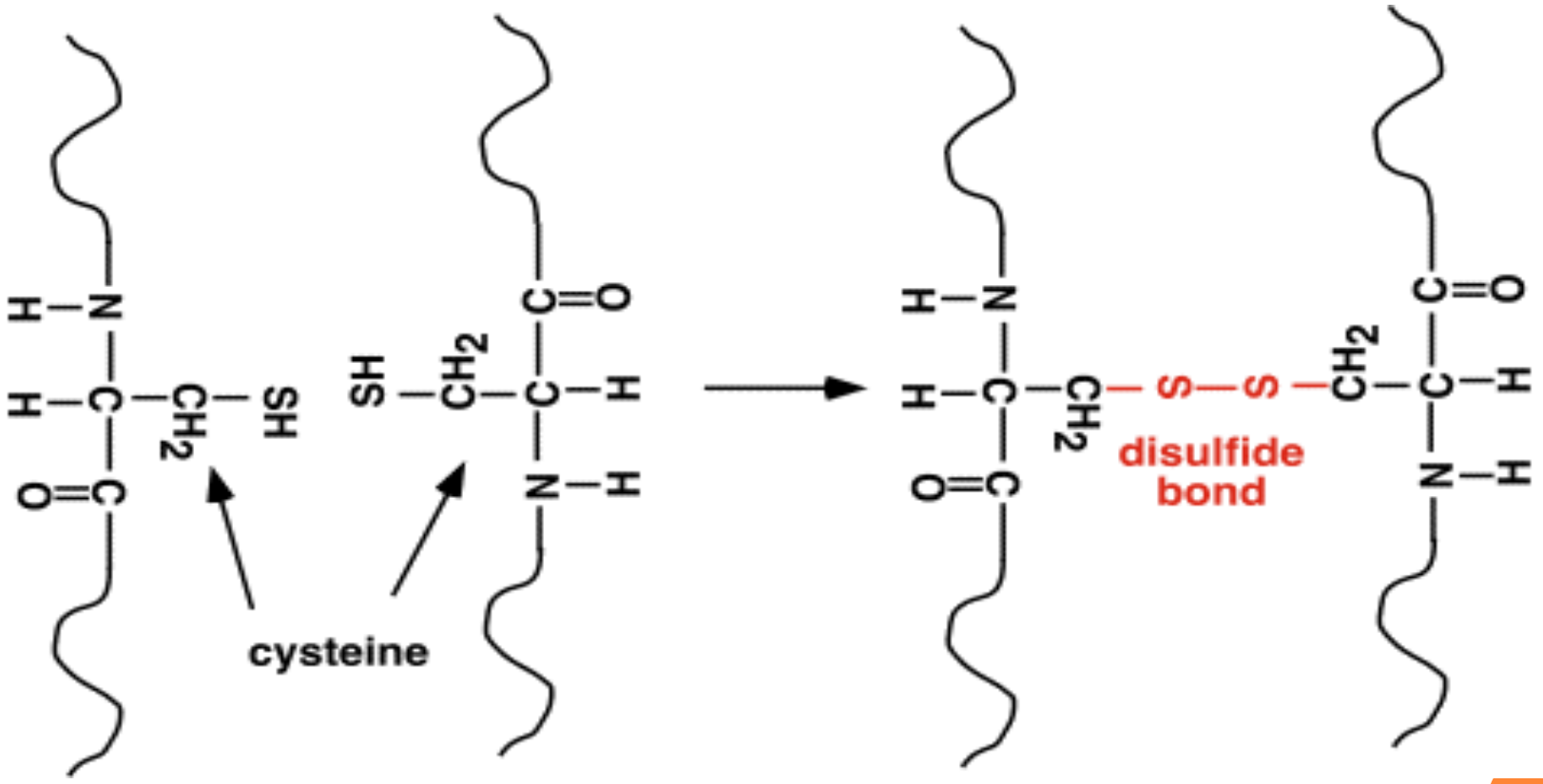
- histidine side chain via Fe.



6.Formation of disulfide bonds

- Formation of intrachain and interchain disulphide bridges between cys residues.
- Role in protecting the conformation of protein.

6.Disulfide cross-linking



7. Vitamin K-Dependent Modifications

- Vitamin K is a cofactor in the carboxylation of glutamic acid residues.
- The result of this type of reaction is the formation of a γ-carboxyglutamate, referred to as a gla residue.

8.SELENOPROTEINS

- **Selenium** is a trace element and is found as a component of several prokaryotic and eukaryotic enzymes that are involved in redox reactions.
  - The selenium in these selenoproteins is incorporated as a unique amino acid, **selenocysteine**, during translation.
  - A particularly important eukaryotic selenoenzyme is ***glutathione peroxidase***.
  - This enzyme is required during the oxidation of glutathione by hydrogen peroxide (H_2O_2) and organic hydroperoxides.

9. Sulfation

- Sulfate modification of proteins occurs at tyrosine residues such as in fibrinogen and in some secreted proteins (e.g. gastrin).
  - The universal sulfate donor is 3'-phosphoadenosyl-5'-phosphosulphate (PAPS).