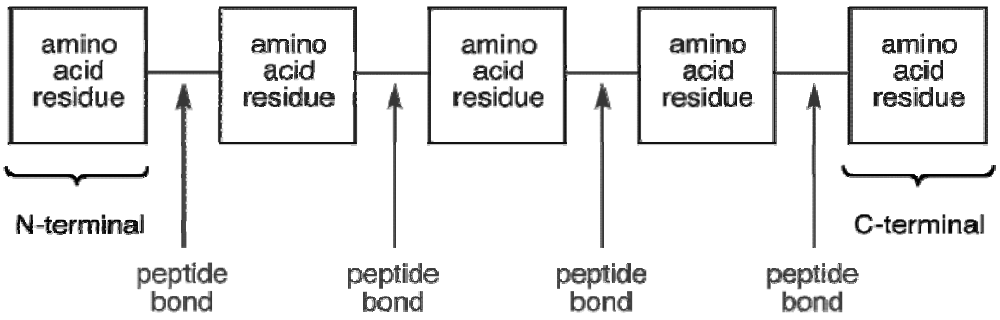
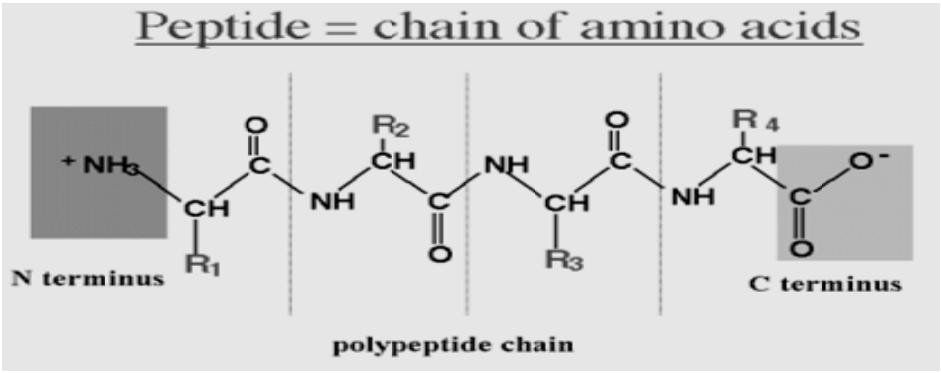
**Post-translational modification (PTM)**

1. Post-translational modification (PTM) refers to the covalent and enzymatic modification of proteins.
2. Proteins are synthesized by ribosome, translating mRNA into polypeptide chains.
3. Polypeptides then undergo PTM to form the mature protein product.
4. PTMs are important components in cell signaling, (eg.: pro-hormones are converted to hormones).
5. Post-translational modifications can occur on the amino acid side chains or at the protein's C- or N- termini.
6. Polypeptide chains have an end with an unbound carboxyl group, the C-terminus, and an end with an unbound amine group, the N-terminus.
7. Proteins are naturally synthesized starting from the N-terminus and ending at the C-terminus.




**Post translational modifications**

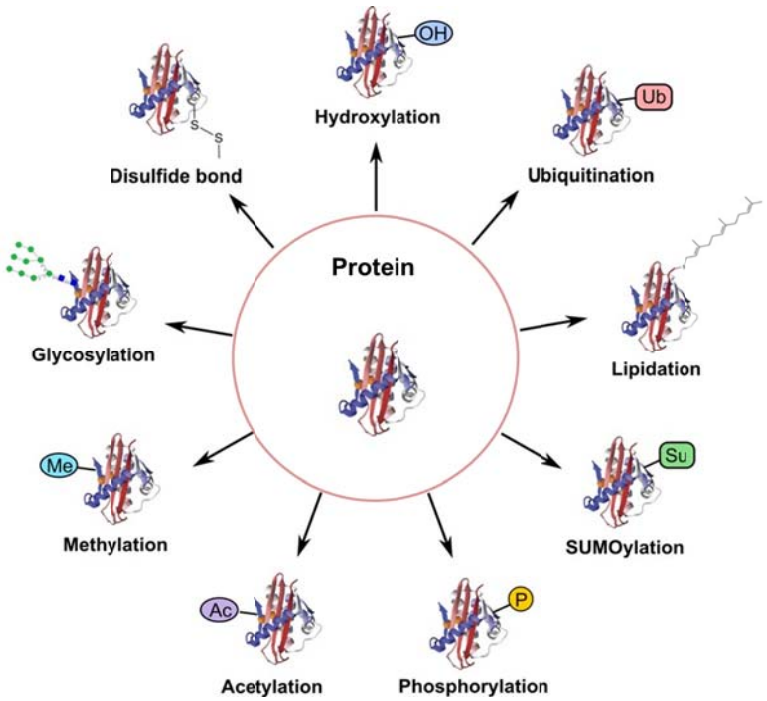
1. Post translational modifications refer to any alteration in the amino acid sequence of the protein after its synthesis.
2. It may involve the modification of the amino acid side chain, terminal amino or carboxyl group by means of covalent or enzymatic means following protein biosynthesis.

3. Generally, these modifications influence the structure, stability, activity, cellular localization or substrate specificity of the protein.
4. Post translational modification provides complexity to proteome (The proteome is the entire set of proteins that can be expressed by a genome) for diverse function with limited number of genes.

**Examples of Three Levels of Protein Modifications**

| Levels | Examples |
|---|---|
| 1. Pre-translational | a) Selenocysteine t-RNA<br>b) Nonnatural amino acid t-RNA |
| 2. Co-translational | a) Signal sequence cleavage<br>b) N-Glycosylation |
| 3. Post-translational | a) O-Glycosylation<br>b) Peptide bond cleavage<br>c) Protein splicing<br>d) Lipidation<br>e) Disulfide bond formation<br>f) Ubiquitination, Sumoylation |

**Various types of Post-translational modifications**




After synthesis is completed, proteins can be modified by various methods such as phosphorylation, glycosylation, ADP ribosylation, hydroxylation, and addition of other groups.

1. **Proteolysis:** As the newly synthesized protein is released in the lumen of the ER, signal peptidases cleave peptide sequence. Apart from signal peptide, some polypeptide sequence of the protein is also cleaved resulting in the final

sequence. Example: Insulin is synthesized in the cells in its inactive form which cannot perform its function. Post translational modifications ensure proper function which involves the removal of the part of protein to convert it into a three dimensional and fully active form.

2. **Phosphorylation:** Phosphoryalation is the addition of one or more phosphate groups to the protein. Post Translational Phosphorylation is one of the most common protein modifications that occur in animal cells. Majority of phosphorylation occurs as a mechanism to regulate the biological activity of a protein. In animal cells Serine, tyrosine and thereonine are the amino acids that subjected to the phosphorylation.

3. **Glycosylation:** Glycosylation is the addition of carbohydrate molecules to the polypeptide chain and modifying it into glycoproteins. Many of the proteins that are destined to become a part of plasma membrane or to be secreted from the cell, have carbohydrate chains attached to the amide nitrogen of asparagine(N linked) or the hydroxyl groups of serine, threonine(O linked). N glycosylation occurs in ER and O glycosylation occurs in the golgi complex.

4. **Sulfation:** Sulfate modidication takes place by the addition of sulphate molecules and these modifications of proteins occurs at tyrosine residues. Tyrosine sulfation accomplished via the activity of tyrosylproteinsulfotransferases (TPST) which are membrane associated enzymes of trans-Golgi network. There are two known TPSTs. TPST-1 TPST-2. The universal phosphate donor is 3'-phosphoadenosyl- 5'-phosphosulphate (PSPA).

5. **Methylation:** The transfer of one-carbon methyl groups to nitrogen or oxygen to amino acid side chains increases the hydrophobicity of the protein. This can neutralize a negative amino acid charge when bound to carboxylic acids. Methylation is mediated by methyltransferases and S-adenosyl methionine (SAM) is the primary methyl group donor.

6. **Hydroxylation** The biological process of addition of a hydroxy group to a protein amino acid is called Hydroxylation. Protein hydroxylation is one type of PTM that involves the conversion of –CH group into –COH group and these hydroxylated amino acids are involved in the regulation of some important factors called transcription factors. Among the 20 amino acids, the two amino acids regulated by this method are proline and lysine.

7. **Ubiquitination** / ubiquitylation, is a type of protein post-translational modification by adding **ubiquitin** to protein sequence. Ubiquitin is a universal regulatory protein that has been found in also all tissues.

8. **Lipidylation** The covalent binding of a lipid group to a peptide chain, also known as *lipidation*, can affect the activity of the *protein* and/or alter its subcellular location.

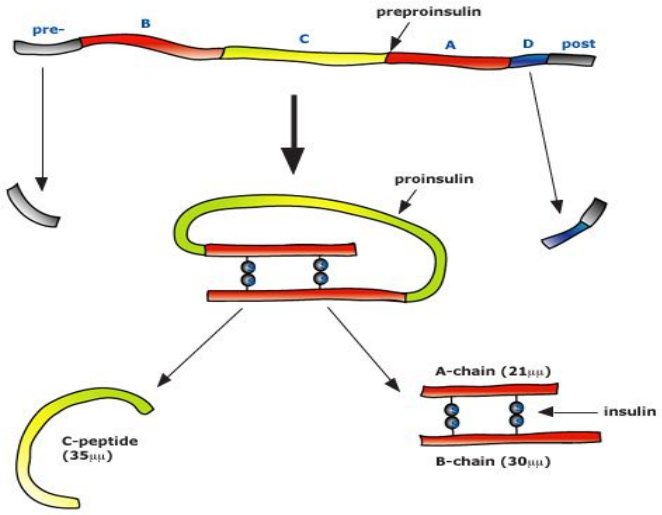
**9. Acetylation** is a chemical reaction that is called ethanoylation in the IUPAC nomenclature. It describes a reaction that introduces an acetyl functional group

into a chemical compound. The opposite chemical reaction is called deacetylation – it is the removal of the acetyl group.

10. **Prenylation/Lipidation** Prenylation (also known as isoprenylation or lipidation) is the addition of hydrophobic molecules to a protein or chemical compound. It is usually assumed that prenyl groups (3-methylbut-2-en-1-yl) facilitate attachment to cell membranes, similar to lipid anchors like the GPI anchor, though direct evidence of this has not been observed. Prenyl groups have been shown to be important for protein–protein binding through specialized prenyl-binding domains.

**Example: Post-translational modification of insulin**

1.At the top, the ribosome translates a mRNA sequence into a protein, insulin.
2. Insulin passes the protein through the endoplasmic reticulum, where it is cut, folded and held in shape by disulfide (-S-S-) bonds.
3. Then the protein passes through the golgi apparatus, where it is packaged into a vesicle. In the vesicle, more parts are cut off, and it turns into mature insulin.




**Significance:**

PTMs have significant biological functions, they are:

1. Helps in proper folding of protein
2. Confers stability to the protein by increasing protein life
3. Protects the protein against cleavage by enzymes
4. Regulates protein activity and function
5. Regulations diverse functions
6. Influences cellular homeostasis
7. Forms important components in cell signalling
8. Increases the diversity and complexity in the protein types.

**Table 1.** Various types of Post-translational modifications

| S. no. | Process | Post translational Modification |
|---|---|---|
| 1. | Proteolysis | Signal peptidases cleave signal peptide sequence of the newly synthesized protein.<br>Some polypeptide sequence of the protein is also cleaved resulting in the final sequence. |
| 2. | Phosphorylation | Addition of one or more phosphate groups to the protein. |
| 3. | Glycosylation | Addition of carbohydrate molecules to the polypeptide chain and modifying it into glycoproteins. |
| 4. | Lipidylation | The covalent binding of a lipid group to a peptide chain. |
| 5. | Acetylation | Addition of acetyl functional group |
| 6. | Amidation | Addition of an amide group to the end of the polypeptide chain.<br>**Amide** groups have the general chemical **formula** CO-NH. They may be produced by the interaction of an amine ($NH_2$) group and a carboxyl ($CO_2H$) group, |
| 7. | Hydroxylation | Addition of a hydroxy group to a protein, it involves the conversion of –CH group into –COH group |
| 8. | Methylation | The transfer of one-carbon methyl groups to nitrogen or oxygen to amino acid side chains that increases the hydrophobicity of the protein. |
| 9. | Ubiquitylation | Adding ubiquitin to protein sequence |
| 10. | Sulfation | Addition of sulphate molecules and the modifications of proteins occurs at tyrosine residues. |