

Lecture 19: Protein Modifications and Proteomics

Many times proteins are modified after synthesis. These modifications refer to any alteration in the amino acid sequence of the protein or modification of amino acid side chain or terminal amino or carboxyl group. Generally, these post translational modifications influence the structure, stability, activity, cellular localization or substrate specificity of the protein. Post translational modification provides complexity to proteome for diverse function with limited number of genes (Fig. 1).

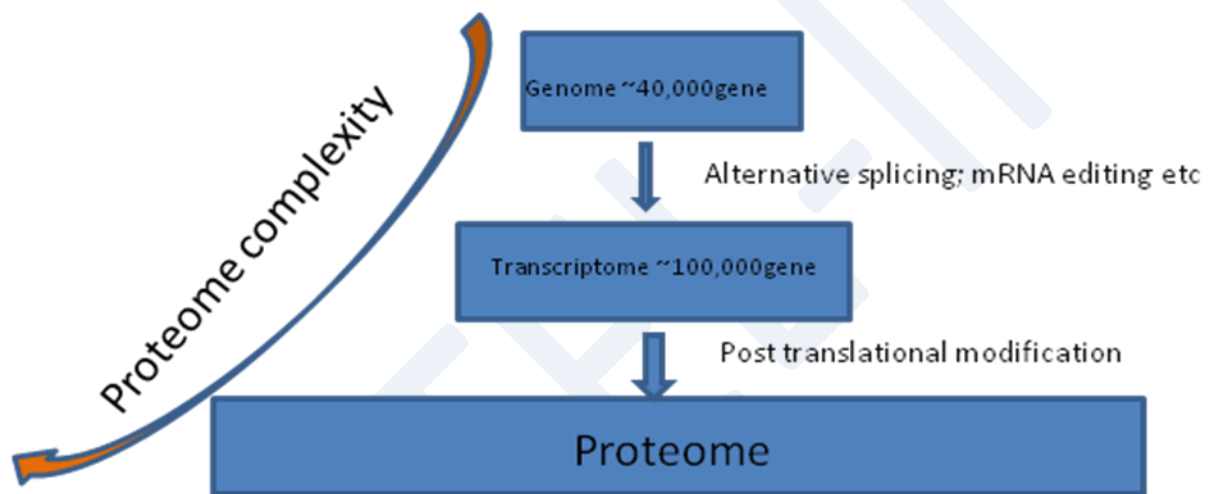


Figure 1: Complexity to proteome for diverse function with limited number of genes

Post-translational modifications (PTMs) mainly occur in the endoplasmic reticulum of the cell but sometimes continue in the golgibody as well. Though there are different types of PTMs, but the most predominant among them are:-

- Proteolysis
- Glycosylation
- Ubiquitinylation

1) *Proteolysis*

During translation, the signal peptide is synthesized first, which is a 20 hydrophobic residue long peptide at the N terminal end of the protein. Once synthesized, it is recognized by the signal recognition particle (SRP) and translation is temporarily stopped. The signal peptide-SRP complex binds to SPR receptor on the ER membrane.

This results in opening of a trans-membrane pore (translocon). Now translation resumes and peptide is directed into the ER. As the newly synthesized protein is released in the lumen of the ER, signal peptidases cleave peptide sequence. This explains why despite having AUG as start codon which codes for methionine, all the proteins do not have methionine as the N terminal amino acid. Apart from signal peptide, some polypeptide sequence of the protein is also cleaved resulting in the final sequence. For example, in case of insulin it is released in the ER lumen as preproinsulin. After removal of signal sequence it is called proinsulin, which is a further cleaved form residue 31-60 (C Chain) to form mature insulin (Fig. 2).

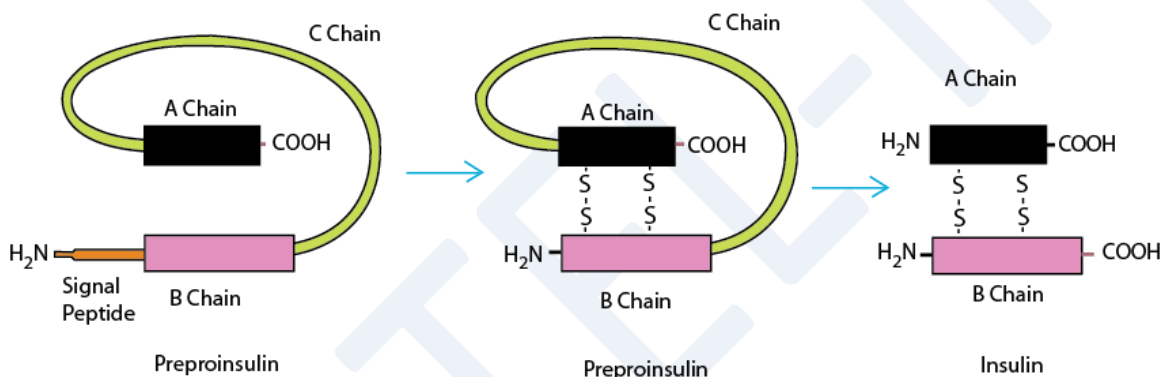


Figure 2: Activation of insulin by proteolysis

2) Glycosylation

Glycosylation is addition of carbohydrate moieties to the polypeptide sequence. Mainly there are two types of glycosylation (Fig. 3)

- i) N-linked glycosylation
- ii) O-linked glycosylation.

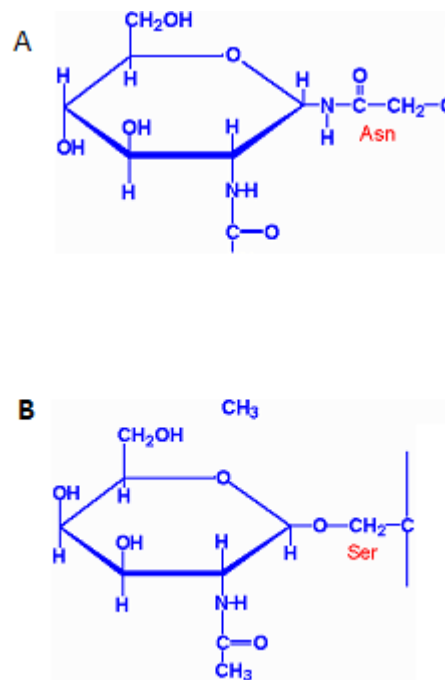


Figure 3: Two types of glycosylation (A) N-linked glycosylation (B) O-linked glycosylation

i) N-linked glycosylation- It takes place in the ER and continues in the golgi body. Here the carbohydrate moiety is attached to the amide group of the asparagine residue when it is present in the sequence NXS/T where, N is asparagine, X is any amino acid other than proline, S/T stands for serine/threonine residue. N-acetyl glucosamine (NAG) is the first residue transferred to the protein with the aid of an enzyme called N-acetyl glucosamine transferase (Fig. 4). Based on the structure of the carbohydrate moiety attached, N-linked glycosylation can be classified into three groups –

- a) High mannose- Where beyond the core structure of carbohydrates which is common to all the three groups, mostly mannose residues are present.
- b) Hybrid type- Here beyond the core structure along with mannose other residues are present like glucose, galactose and N acetyl glucosamine.
- c) Complex- Beyond the core structure few complex carbohydrate residues are present such as sialic acid.

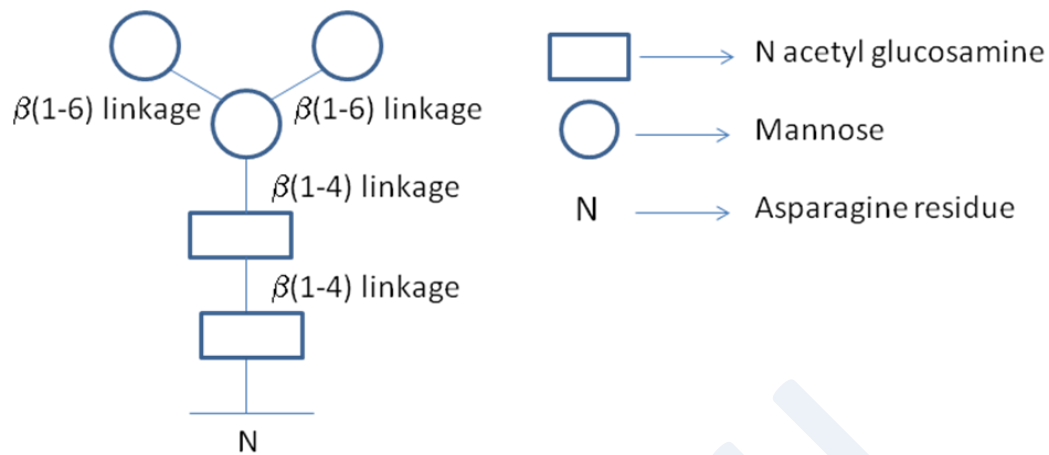


Figure 4: Core structure of carbohydrate moiety in N-linked glycosylation.

- iii) O-linked glycosylation- Unlike N-linked glycosylation, O-linked glycosylation takes place exclusively in the golgi body. Here the carbohydrate moiety is attached to the hydroxyl group of serine, threonine or hydroxylysine residue. Unlike N-linked, it does not require any sequence and occur randomly. There is also no specific core structure of carbohydrates in O-linked glycosylation, any carbohydrate groups can be attached.

3) Ubiquitinylation

Proteins are usually modified for selective destruction by cellular proteolytic complexes called proteasomes by attachment of ubiquitin. Ubiquitinylation involves attachment of a small 76 amino acid protein called ubiquitin to the polypeptide chain following which it is degraded by the cellular proteasome machinery. Ubiquitin binds to the lysine residue of the target protein, present in a specific sequence. The tagged protein is then recognized by the proteasomes complex and it gets degraded.

Apart from the above mentioned PTMs there are other types of PTMs as well which are –

- a) SUMOylation- SUMO (small ubiquitin related modifier) proteins are 100 amino acid residue proteins which bind to the target protein in same way

as ubiquitin, except it also confers the transcription regulatory activity of the protein. It also helps in transport of the target protein from cytosol to the nucleus.

- b) Phosphorylation: Involved in Signal transduction, regulation of enzyme activity.
- c) Disulfide bond formation: Stabilizes protein structure and involved in redox processes.
- d) Lipidylation.
- e) Sulfation: Signalling and protein localization; also involved in protein–protein interactions.
- f) Hydroxylation: Structural stability (collagens).
- g) Acetylation
- h) Methylation
- i) Prenylation and so on.

PTMs have significant biological functions which are:-

- Aids in proper protein folding – few lectin molecules called calnexin binds to glycosylated proteins and assist in its folding.
- Confers stability to the protein- glycosylation can modify the stability of the protein by increasing protein half life. It protects the protein against cleavage by proteolytic enzyme by blocking the cleavage sites.
- Protein sorting or translocation- If phosphorylated mannose residues are present in the protein it always goes to lysosome.
- It regulates protein activity and function- phosphorylation of protein is a reversible PTM which activates the protein.
- It significantly increases the diversity and complexity in the proteome.

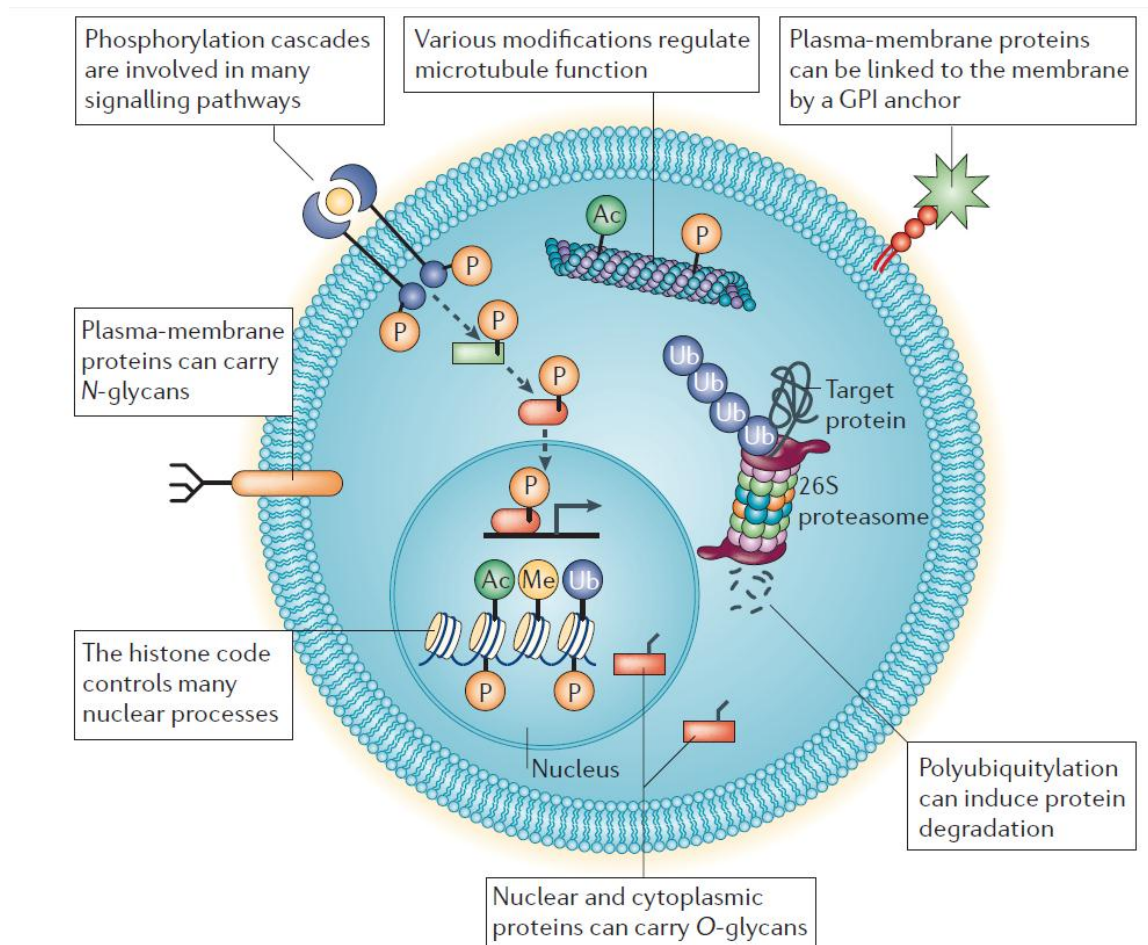


Figure and figure legend Source: Nature Reviews Mol Cell Bio. 7, 2006, 391-403
Figure: Cellular post-translational modifications. This schematic figure shows the location and role of a selection of some of the most important of more than 200 types of post-translational modification (PTM). PTMs are found on all types of protein, from nuclear transcription factors to metabolic enzymes, structural proteins and plasmamembrane receptors. PTMs affect the physicochemical properties of proteins, which provides a mechanism for the dynamic regulation of molecular self-assembly and catalytic processes through the reversible molecular recognition of proteins, nucleic acids, metabolites, carbohydrates and phospholipids. Ac, acetyl group; GPI, glycosylphosphatidylinositol; Me, methyl group; P, phosphoryl group; Ub, ubiquitin (Nature Reviews Molecular Biology, 2006, 7, 391-403)

Following article is highly recommended for reading

Ole N. Jensen. Interpreting the protein language using proteomics. Nature Reviews Mol Cell Bio. 2006, 7, 391-403
<http://www.ncbi.nlm.nih.gov/pubmed/16723975>

Another highly recommended article for further reading

<http://www.ncbi.nlm.nih.gov/pubmed/16267872>

Reviews

C. T. Walsh et al.

Protein Chemistry

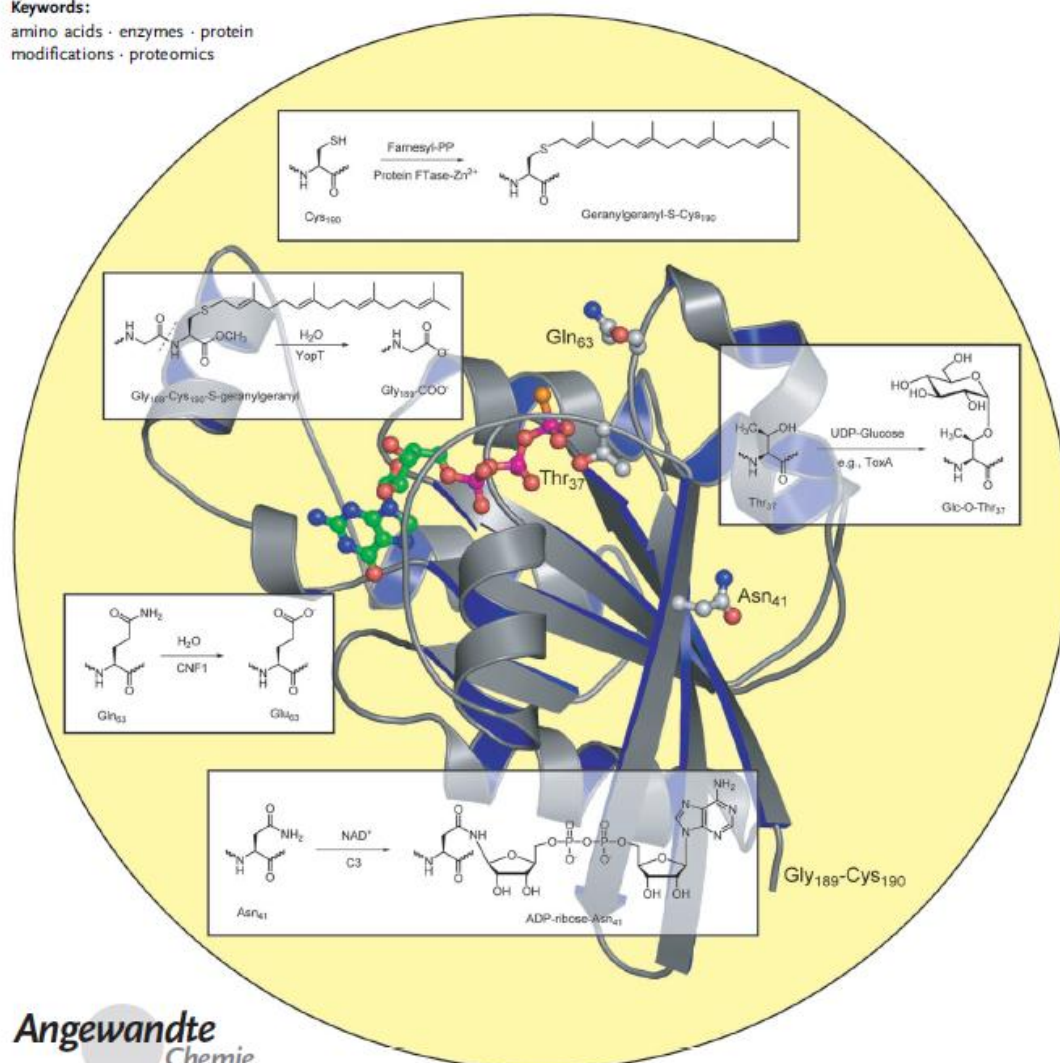
DOI: 10.1002/anie.200501023

Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications

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Keywords:

amino acids · enzymes · protein modifications · proteomics



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Home Assignment

Explain how mass spectrometry is can be used for study of post translational?

Hint:

- ✓ Addition or removal of phosphate groups or acetyl groups results in mass changes of 80 Da) and 42 Da, respectively.
- ✓ Modification may be identified by analysis of intact protein. Further, protein may be cleaved by proteases and fragments may be analyzed to exact site of modification.
- ✓ With this hints explain your answer.

Submit your assignment to course developer by e-mail; you will get an e-mail reply with grading and feedback in a week time.