

Protein engineering

Protein engineering describes the intentional alteration of a protein's amino acid sequence, usually with the aim of achieving either:

- a better understanding of the relationship between a protein's primary and higher-level structure, or its structure and function; or
- the development of a protein variant which, relative to the wild-type protein, displays some enhanced property in the context of its commercial use.

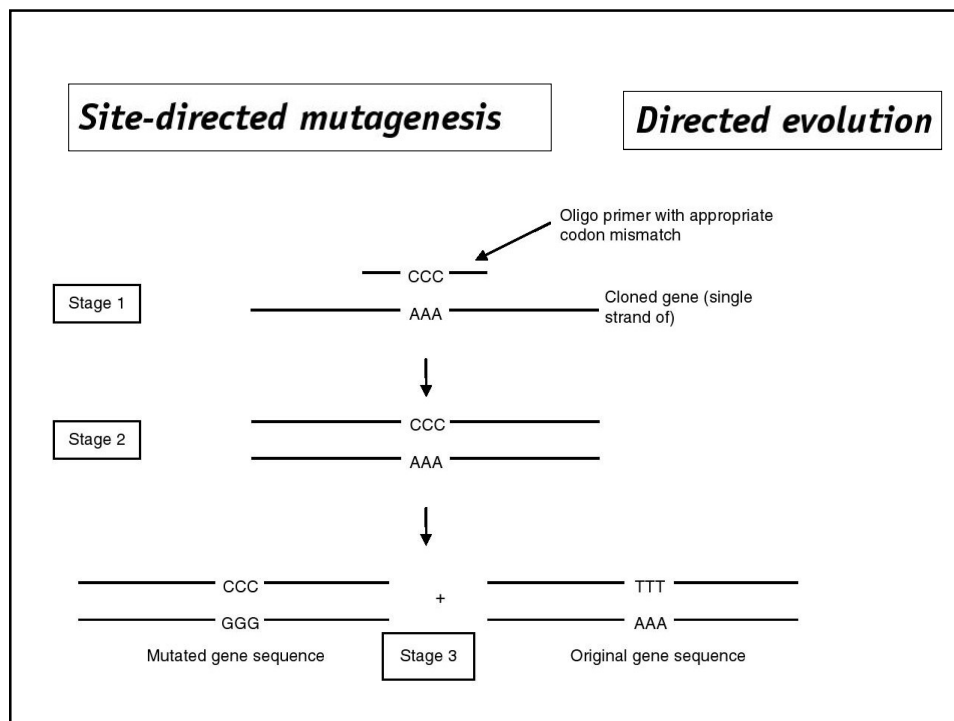
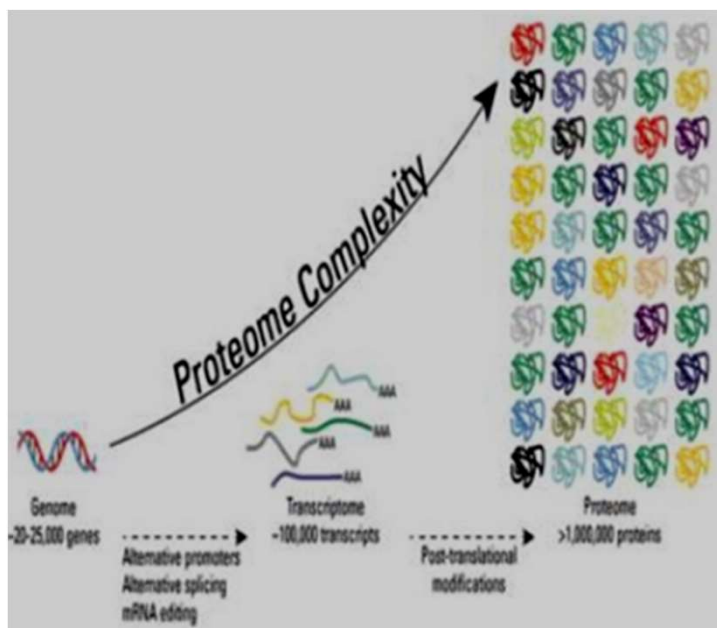
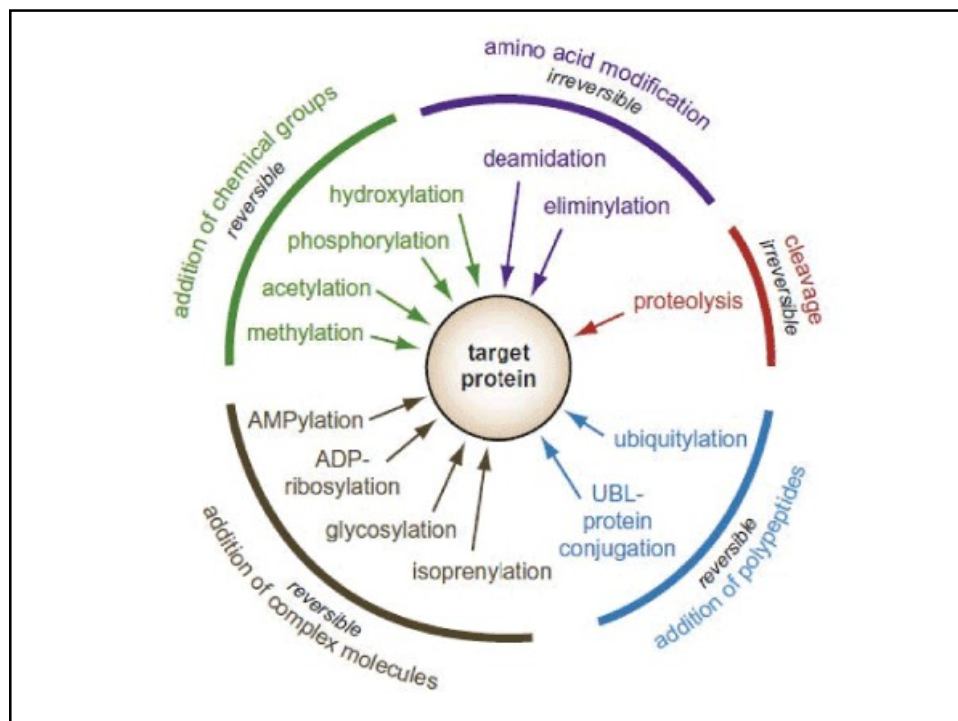


Table 2.6 Representative engineered proteins which are now used commercially. These and other examples are considered in later chapters of this book.

Protein	Use	Engineering detail	Chapter
Tissue plasminogen activator (tPA)	Thrombolytic agent	Various engineered products developed with altered amino acid sequences or with whole domains deleted in order to make clot degradation more efficient or lengthen serum half-life	6
Antibodies	Various, including cancer treatment	Various engineered products developed, including mouse-derived antibodies in which large segments have been replaced by human antibody domains (in order to reduce immunogenicity in humans), or the development of antigen-binding antibody fragments (which could for example penetrate tumours more effectively)	7
Fusion proteins	Various, including treating rheumatoid arthritis and cancer	Generation of novel hybrid proteins by combining one or more domains from two different proteins together. The fusion product 'Enbrel' for example consists of the extracellular domain of the tumour necrosis factor (TNF) receptor (allowing it bind TNF), fused to antibody constant (Fc) domains (which increases its serum half-life)	7
Engineered insulins	Diabetes	The replacement/alteration of amino acids in the insulin backbone in order to make the engineered product either faster-acting or slower-acting than un-engineered insulin	8
Various detergent proteases and amylases	Added to detergents to enhance cleaning	Removal/replacement of oxidation-sensitive amino acid residues, allowing the enzymes to retain activity in the presence of oxidants usually also present in detergents	12
DNA polymerase	PCR reactions	Enhances enzyme's affinity for DNA	13



Post-translational modifications (PTMs)



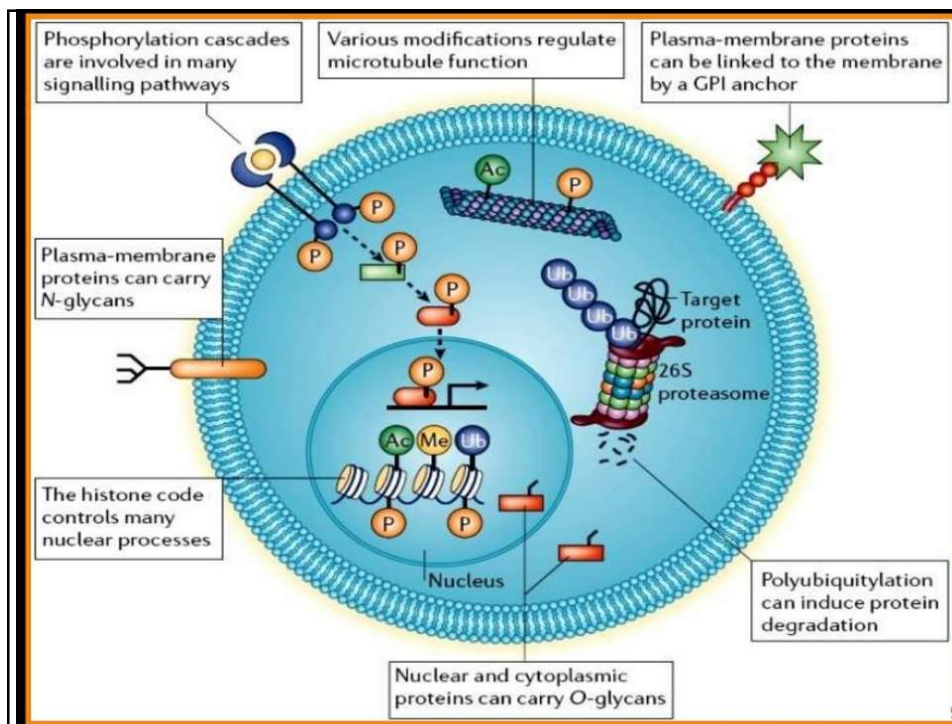
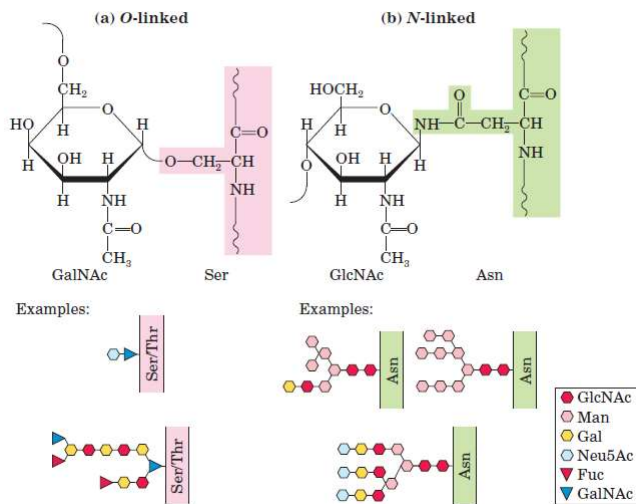


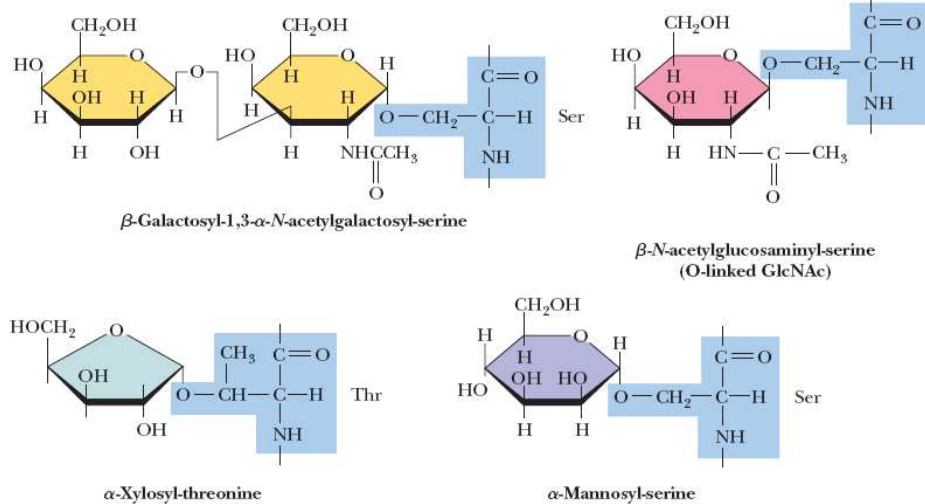
Table 2.7 The more common forms of post-translational modifications that polypeptides may undergo. Refer to text for additional details.

Modification	Comment
Glycosylation	For some proteins glycosylation can increase solubility, influence biological half-life and/or biological activity
Proteolytic processing	Various proteins become biologically active only on their proteolytic cleavage (e.g. some blood factors)
Phosphorylation	Influences/regulates biological activity of various regulatory proteins including polypeptide hormones
Acetylation	Modulation of target protein activity
Acylation	May help some polypeptides interact with/anchor in biological membranes
Amidation	Influences biological activity/stability of some polypeptides
Sulfation	Influences biological activity of some neuropeptides and the proteolytic processing of some polypeptides
Hydroxylation	Important to the structural assembly of certain proteins
γ -Carboxyglutamate formation	Important in allowing some blood proteins to bind calcium
ADP-ribosylation	Regulates biological activity of various proteins
Disulfide bond formation	Helps stabilize conformation of some proteins

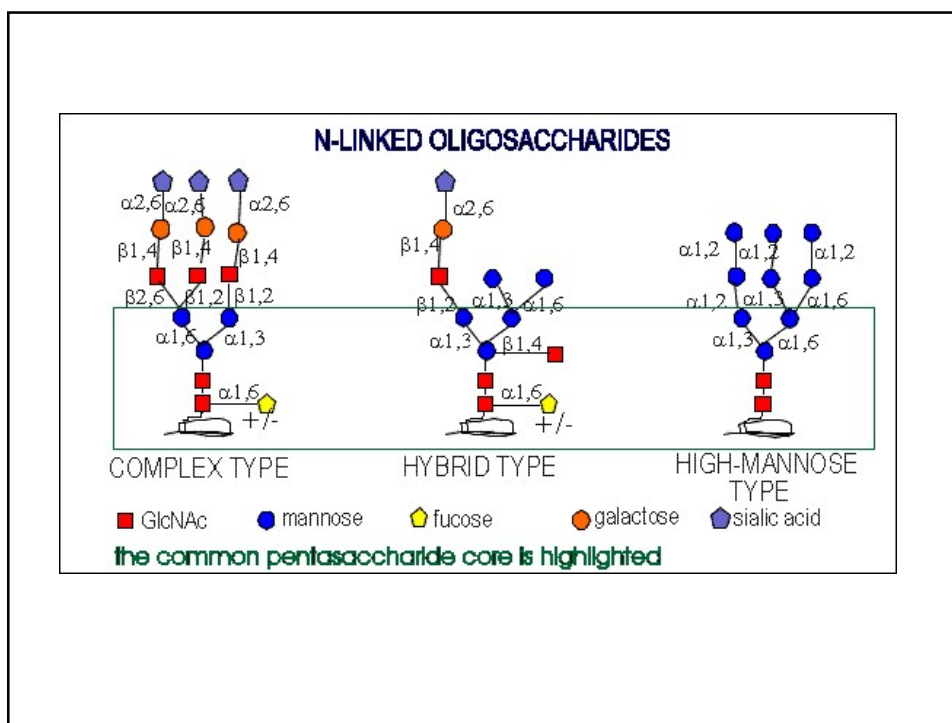
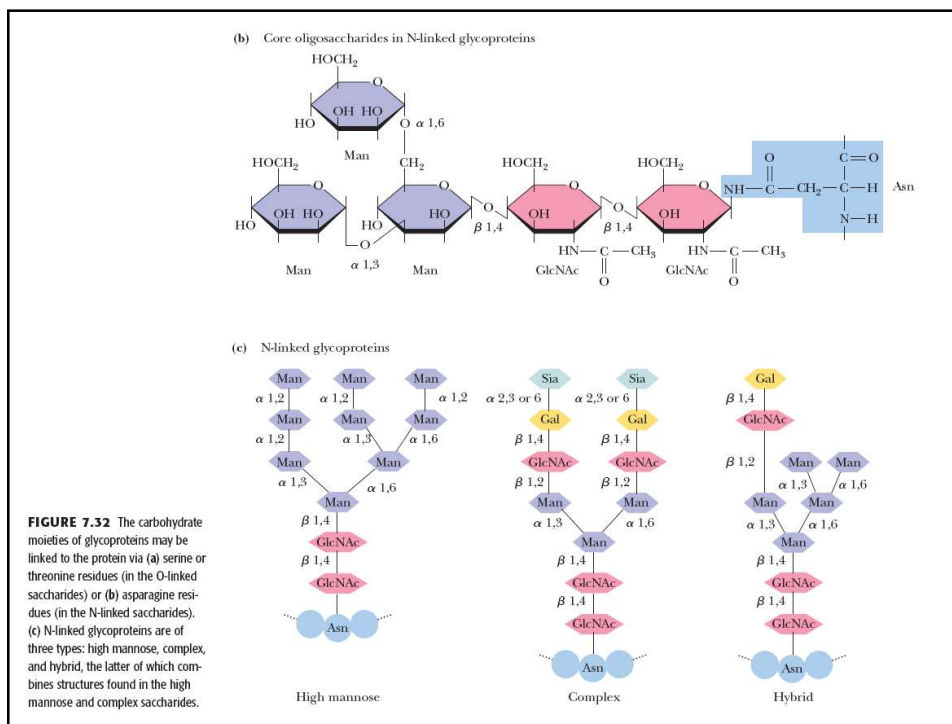
Glycosylation

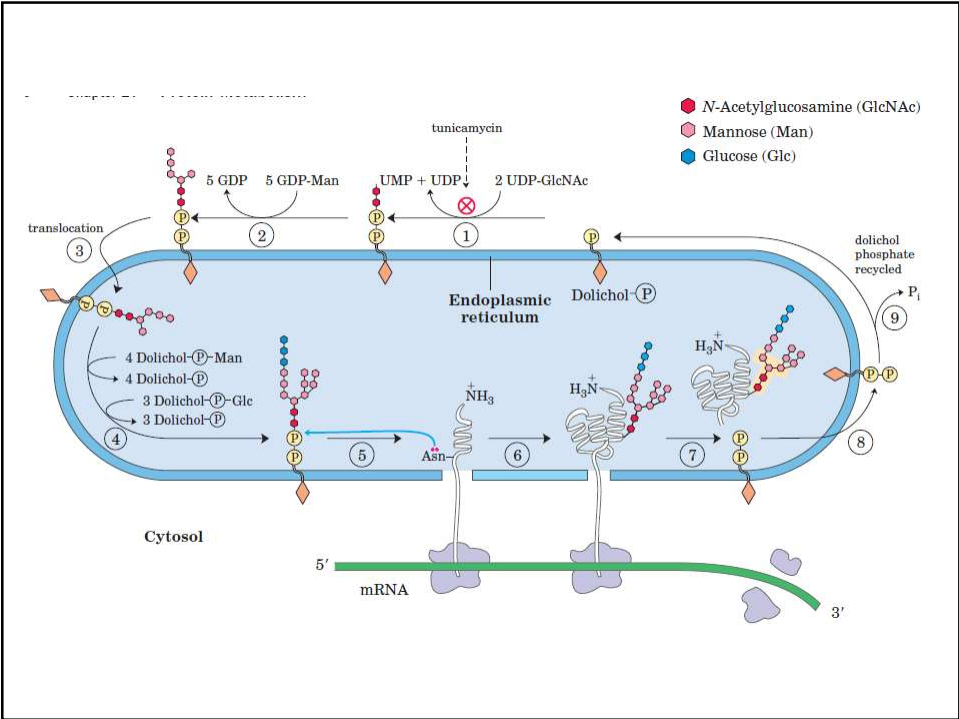
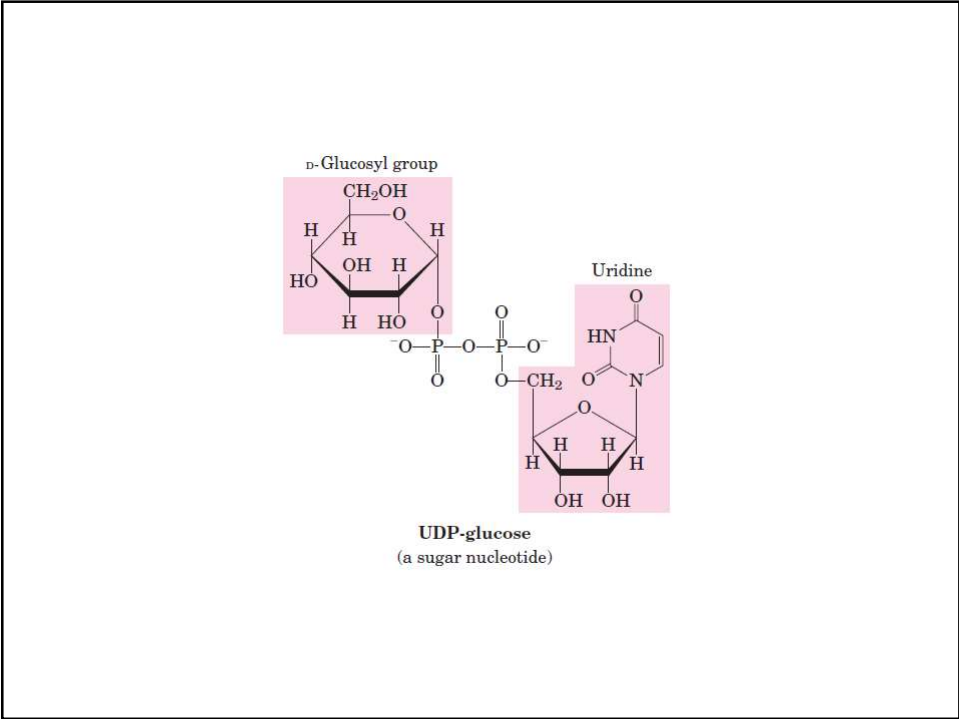


(a) O-linked saccharides

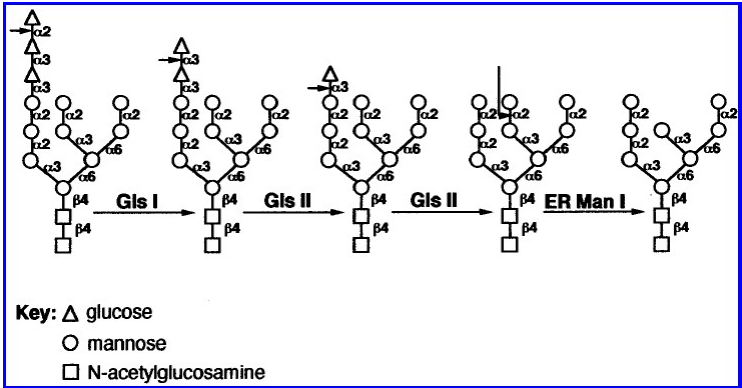


The carbohydrate residue linked to the protein in O-linked saccharides is usually an *N*-acetylgalactosamine, but mannose, galactose, and xylose residues linked to protein hydroxyls are also found.

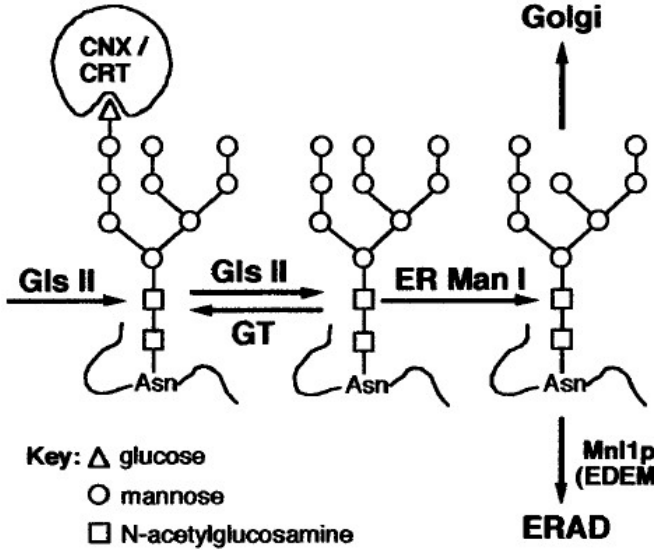




Protein N-glycosylation in ER



Protein quality control



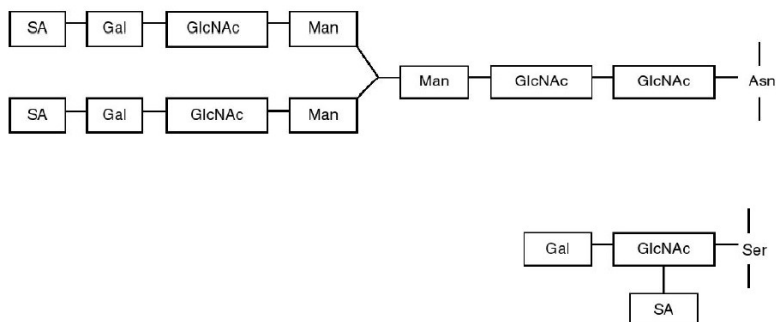


Figure 2.18 Structure of two sample oligosaccharide side chains (one N-linked the other O-linked) found in glycoproteins. Man, mannose; Gal, galactose; SA, sialic acid; GlcNAc, *N*-acetylglucosamine.

Sialic Acid Cleavage Can Serve as a Timing Device for Protein Degradation

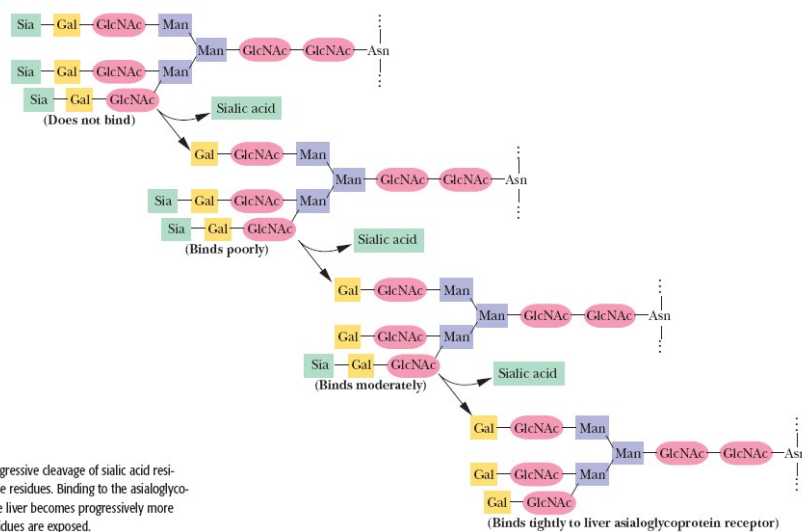
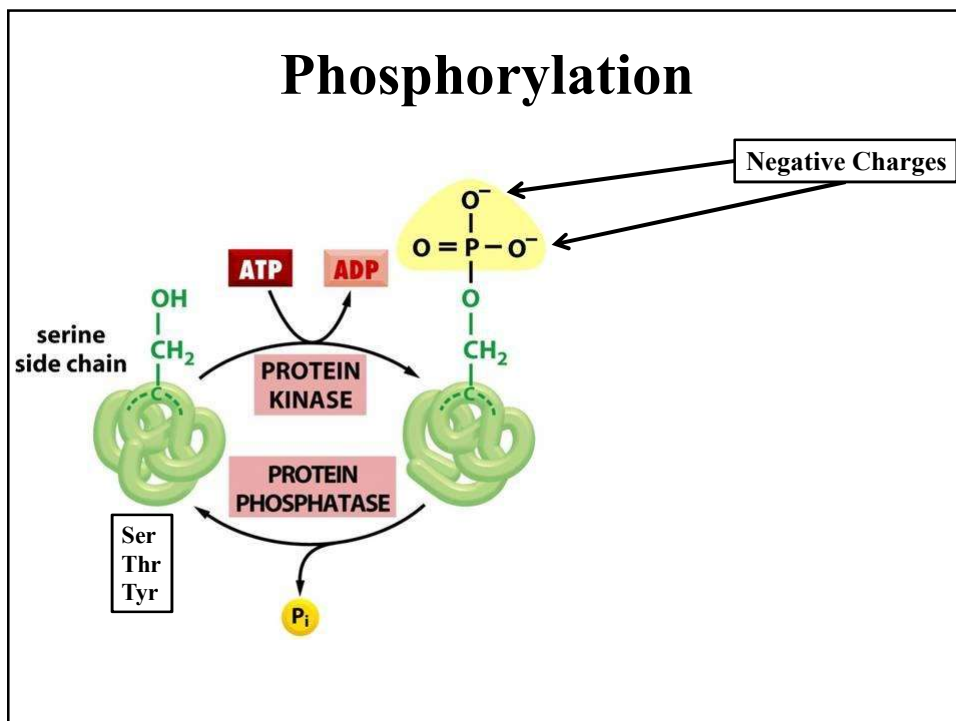
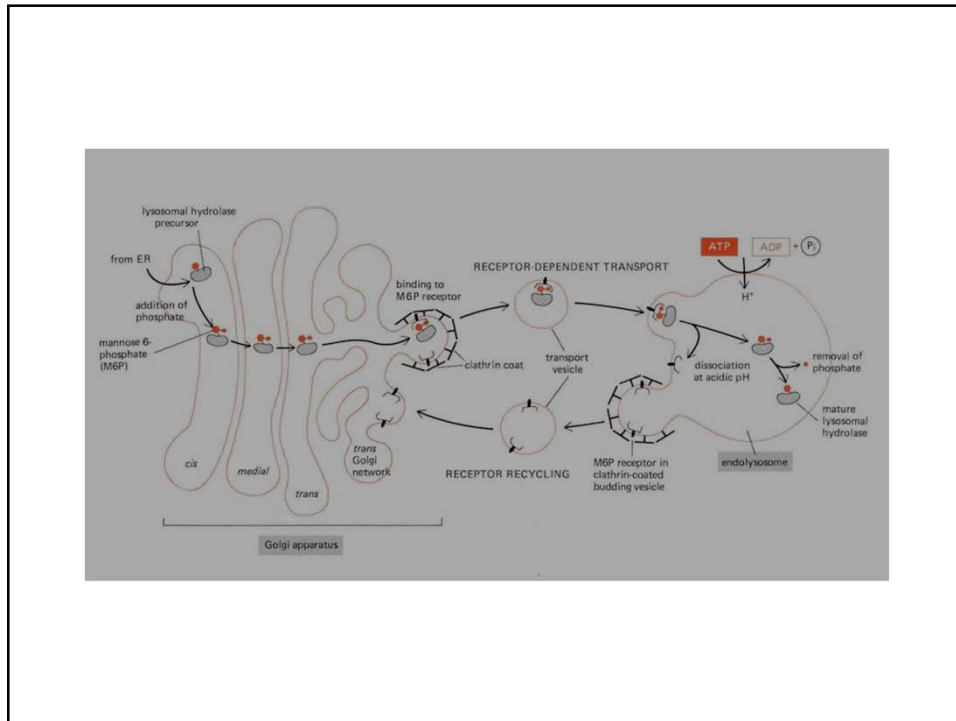
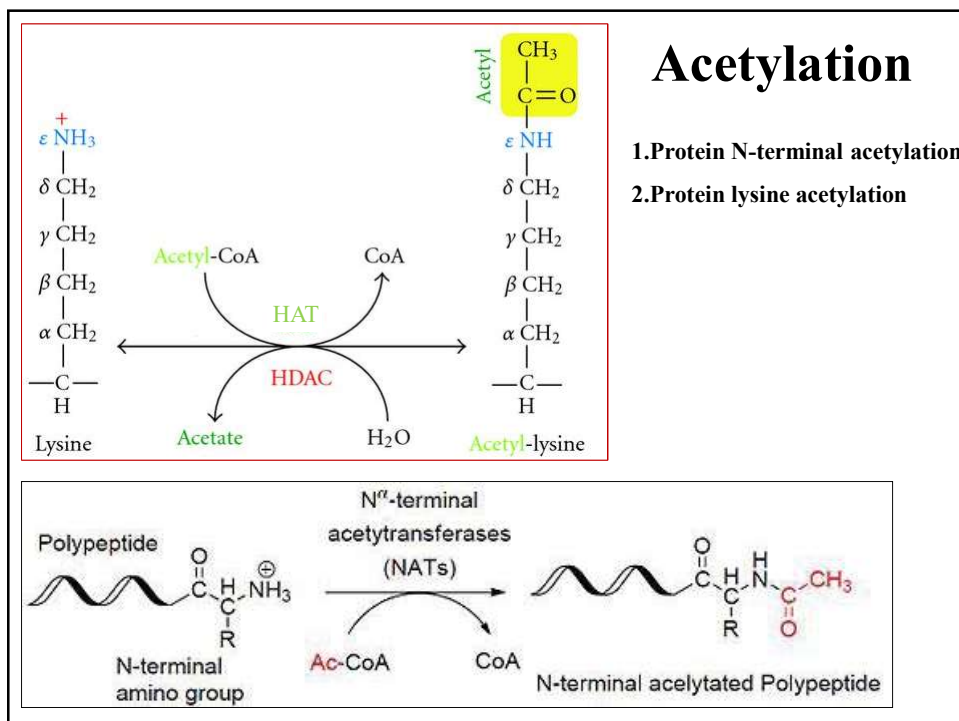
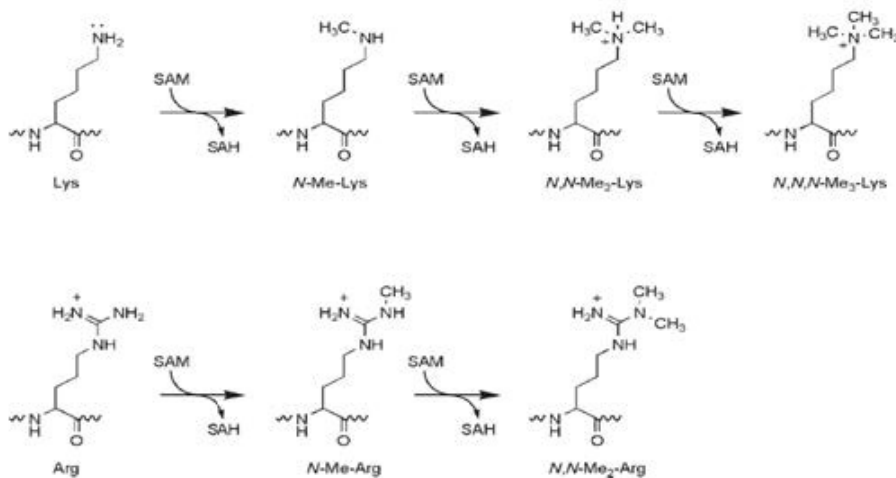


FIGURE 7.35 Progressive cleavage of sialic acid residues exposes galactose residues. Binding to the asialoglycoprotein receptor in the liver becomes progressively more likely as more Gal residues are exposed.



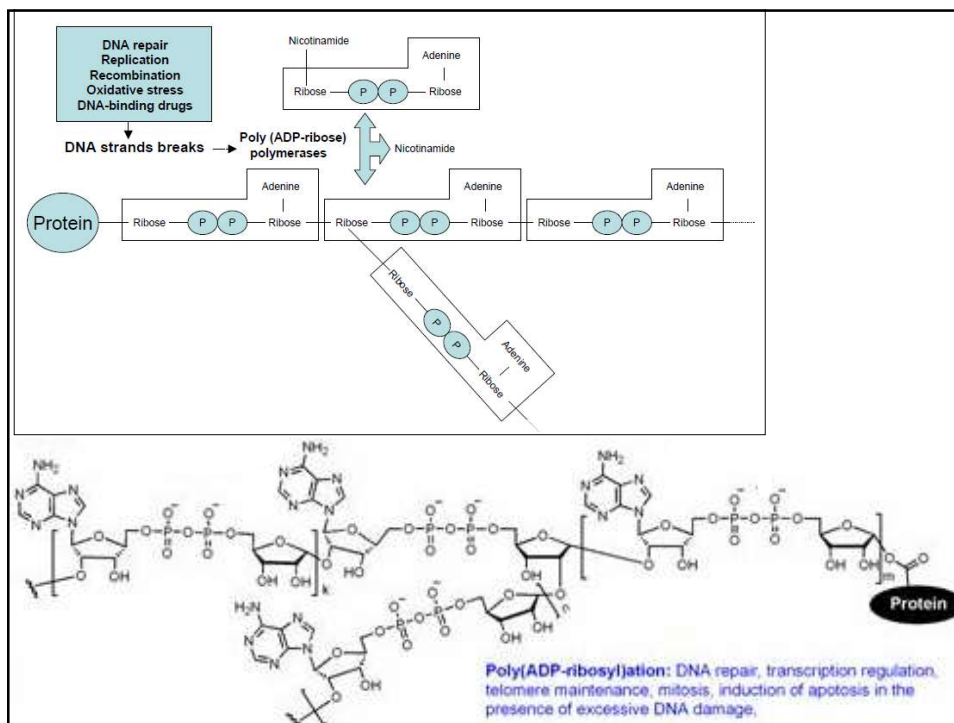
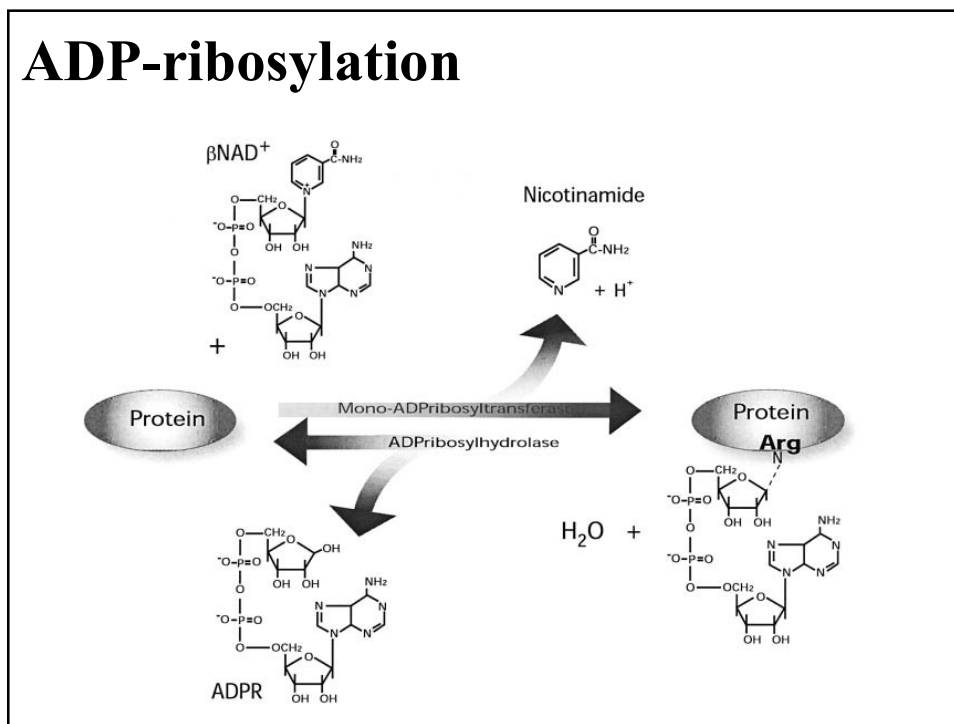


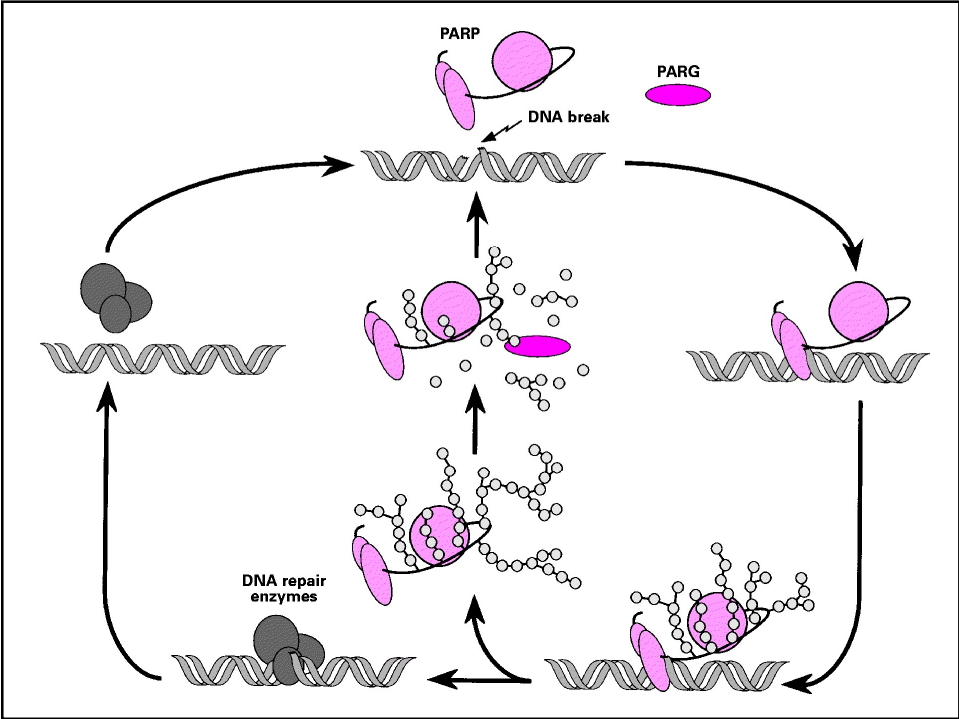
Methylation



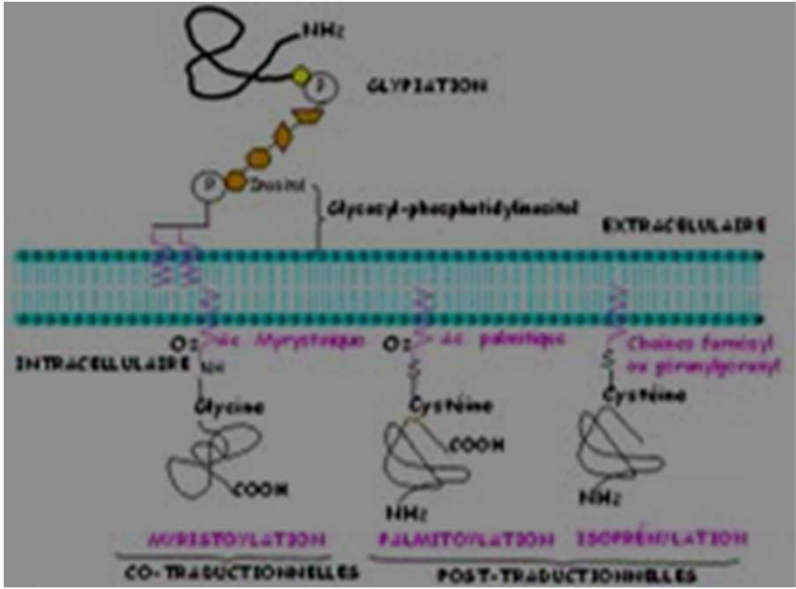
Methylation in arginin or lysine residues is an important PMT in proteins

ADP-ribosylation

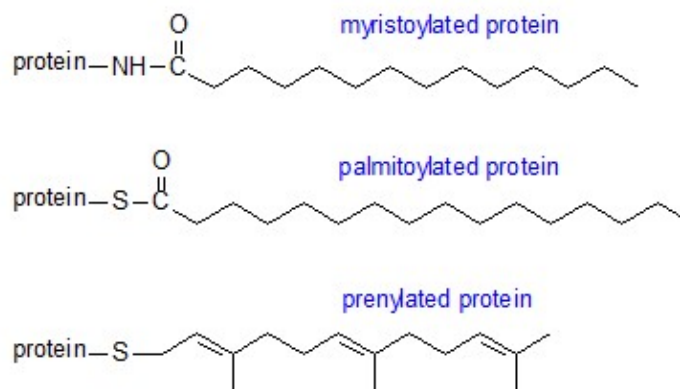




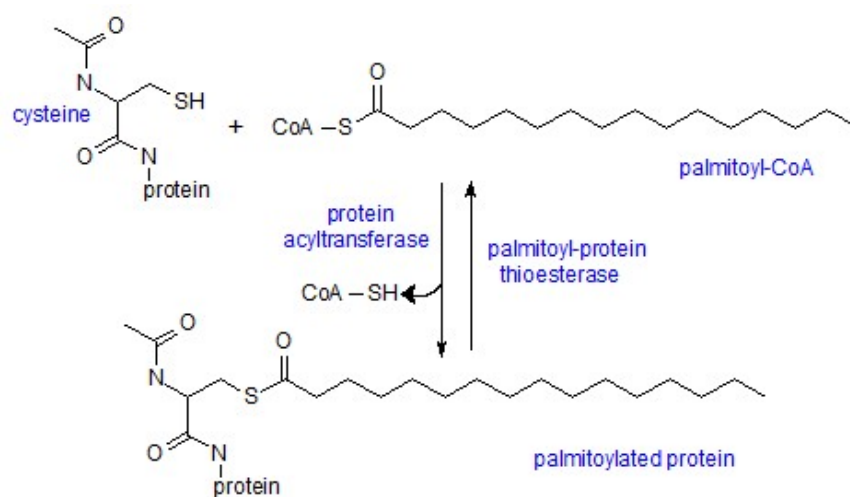
Acylation



Acylation



Palmitoylation



Myristoylation

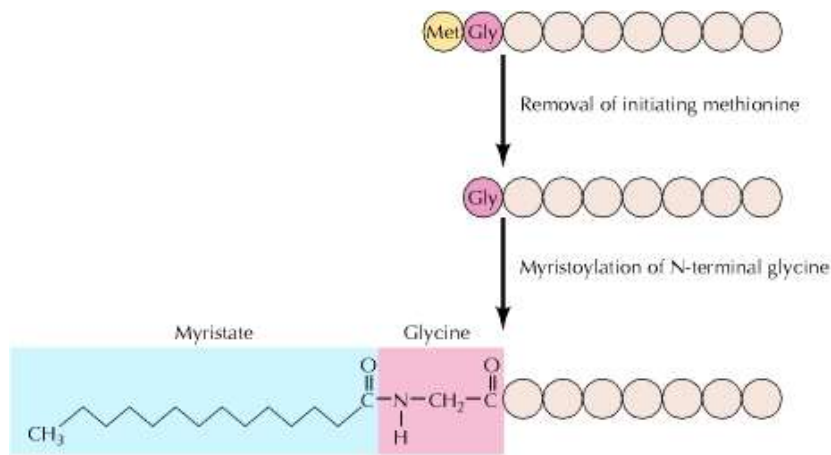
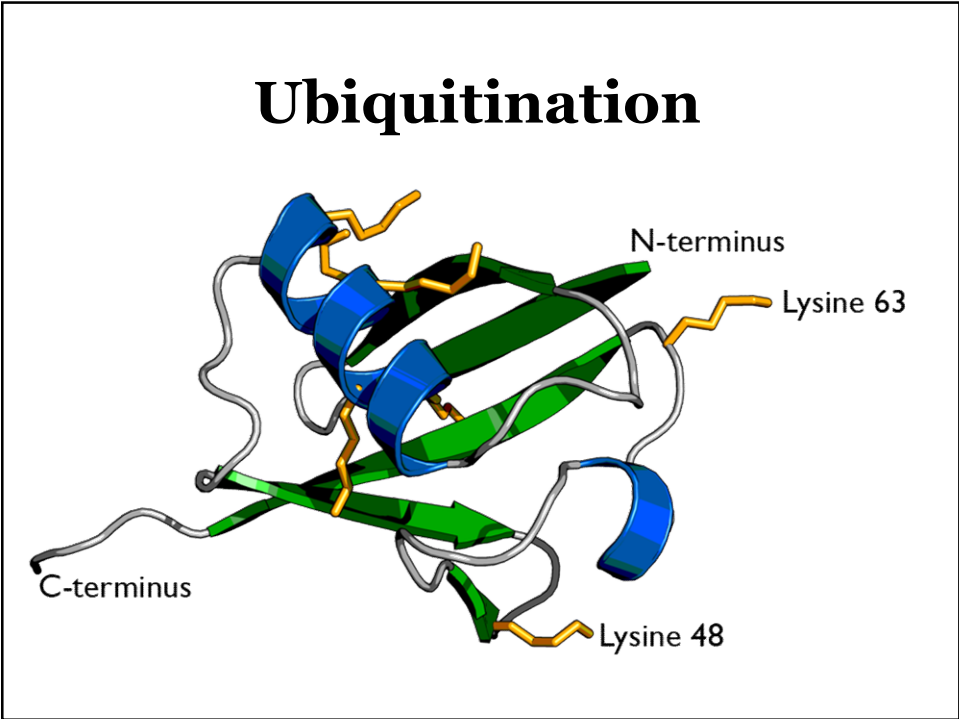
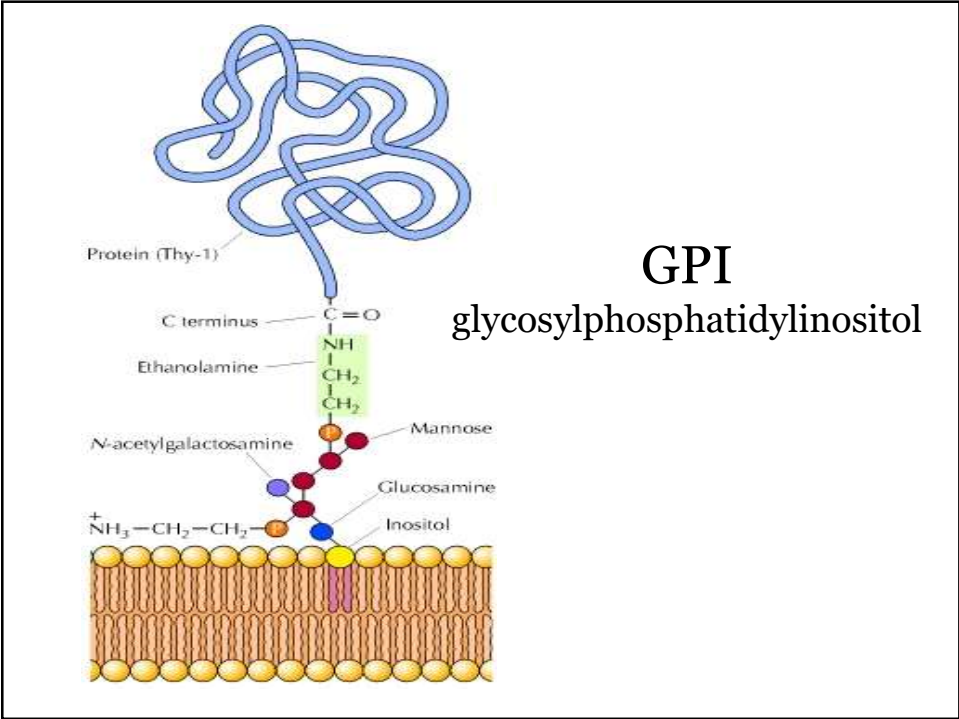
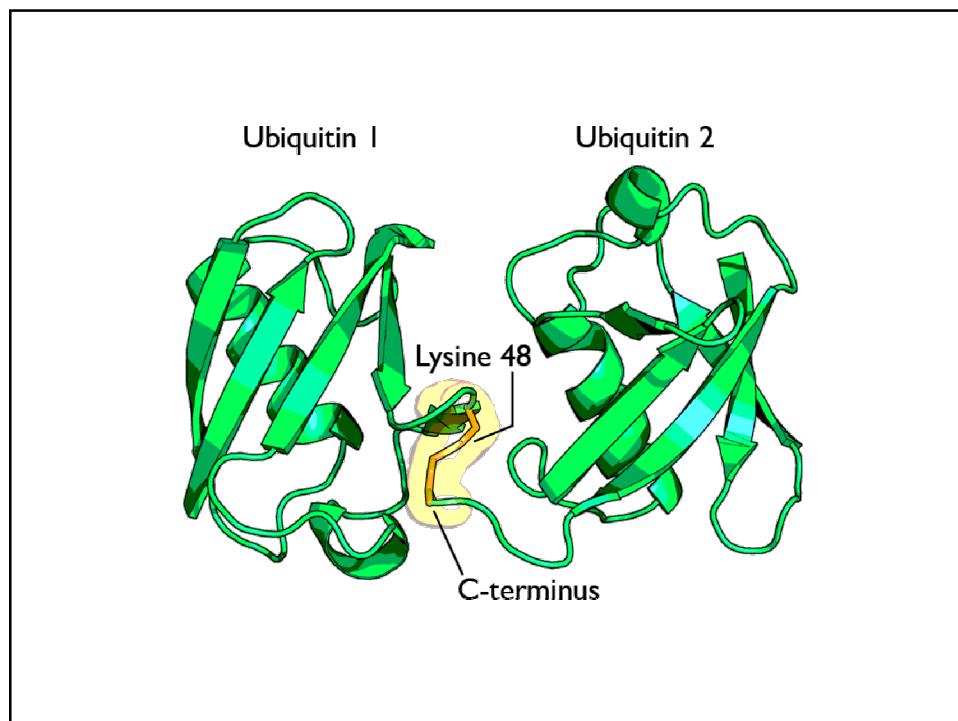
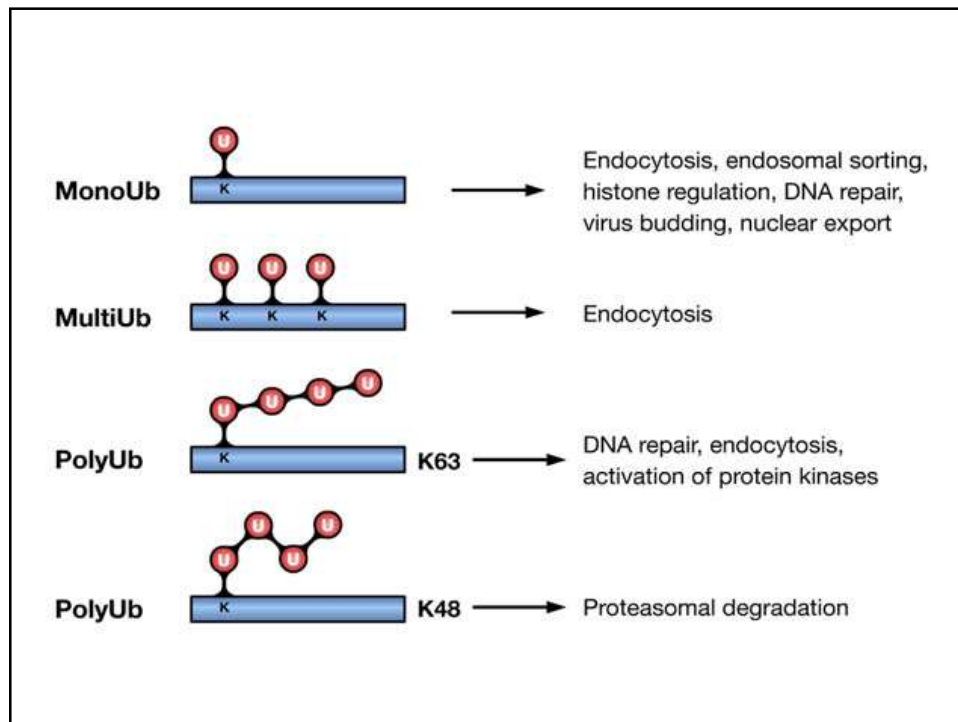
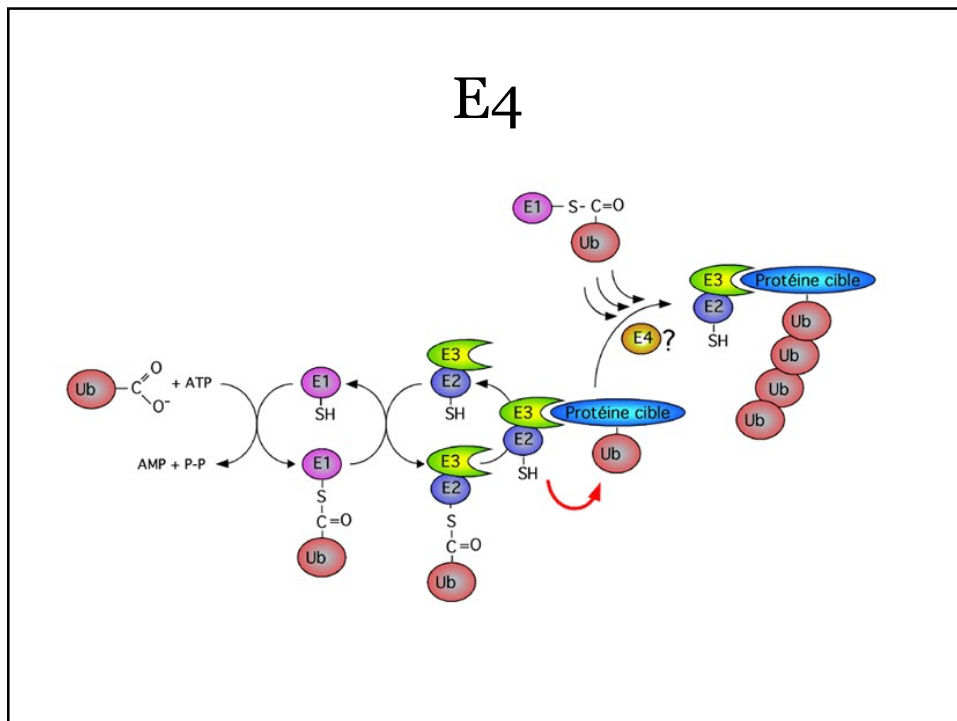
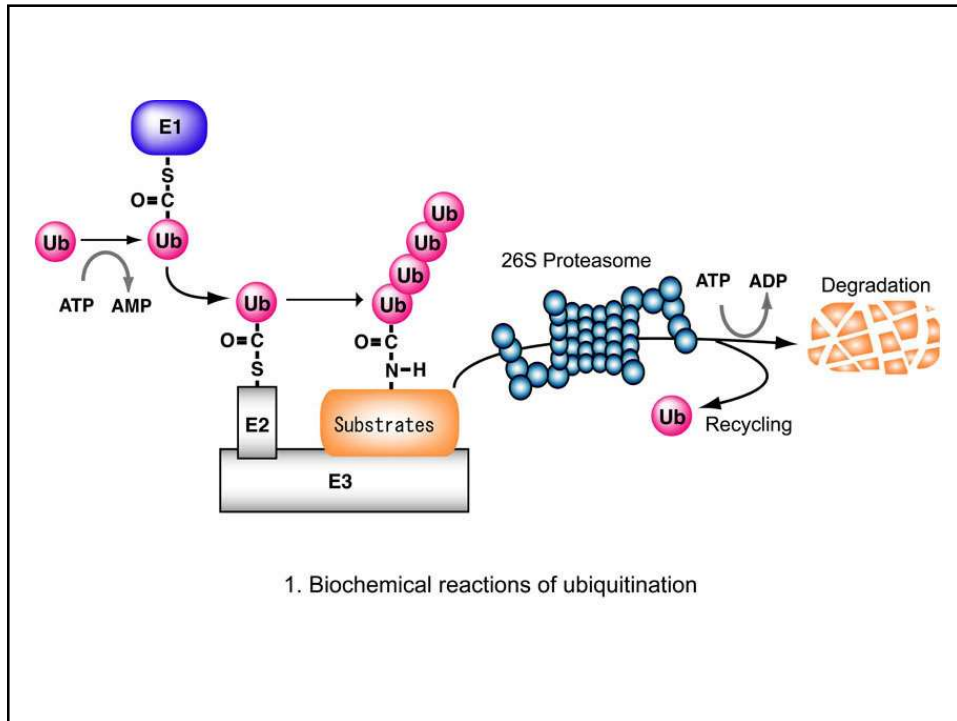


Table 2.8 Representative examples of some acylated proteins. The fatty acid moiety attached and the location of eukaryotic-derived acylated proteins are also listed.

Protein	Cellular location	Fatty acid
cAMP-dependent protein kinase	Cytoplasm	Myristic acid
Cytochrome <i>b₅</i> reductase	ER and mitochondria	Myristic acid
G _s and G _i α-subunits	Plasma membrane	Myristic acid
Insulin receptor	Plasma membrane	Palmitic acid
Interleukin 1 receptor	Plasma membrane	Palmitic acid
Transferrin receptor	Plasma membrane	Palmitic acid
Rhodopsin	Disc membranes in retina	Palmitic acid
P55 and P28	HIV	Myristic acid
VP4	Picornaviruses	Myristic acid
P19 (gag)	HTLV I	Myristic acid
HA	Influenza virus	Palmitic acid
gE	Herpes simplex virus	Palmitic acid







Proteasome

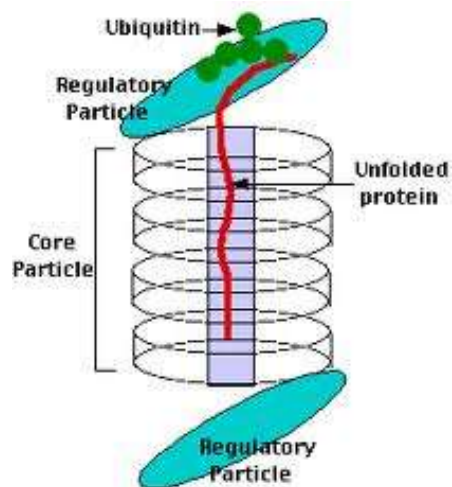


Table 2.9 Some post-translational modifications (PTMs) associated with proteins for therapeutic use. The vast majority of such proteins are (recombinant forms of) native human extracellular proteins, and as such their PTM profile is biased towards PTMs characteristic of extracellular proteins derived from higher eukaryotes. The list is representative only, with specific examples being found throughout Chapters 6–9.

Protein	Therapeutic application	PTM detail
Blood factor VIII	Haemophilia A (Chapter 6)	Glycosylation, disulfide bond formation, sulfation
Hirudin	Anticoagulant (Chapter 6)	Disulfide bond formation, sulfation
Tissue plasminogen activator (tPA)	Thrombolytic (Chapter 6)	Glycosylation, disulfide bond formation, proteolytic processing
α -Galactosidase	Fabry disease (Chapter 6)	Glycosylation, disulfide bond formation
Antibodies	Various (Chapter 7)	Glycosylation, disulfide bond formation
Insulin	Diabetes (Chapter 8)	Disulfide bond formation, proteolytic processing
Human growth hormone (hGH)	Dwarfism (Chapter 8)	Disulfide bond formation, phosphorylation
Erythropoietin (EPO)	Anaemia (Chapter 8)	Disulfide bond formation, glycosylation
Interferon β	Multiple sclerosis	Disulfide bond formation, glycosylation, phosphorylation

