Function and Analysis of Post-translational Protein Modifications

Vorlesung 4610

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Posttranslational Modifications of Proteins

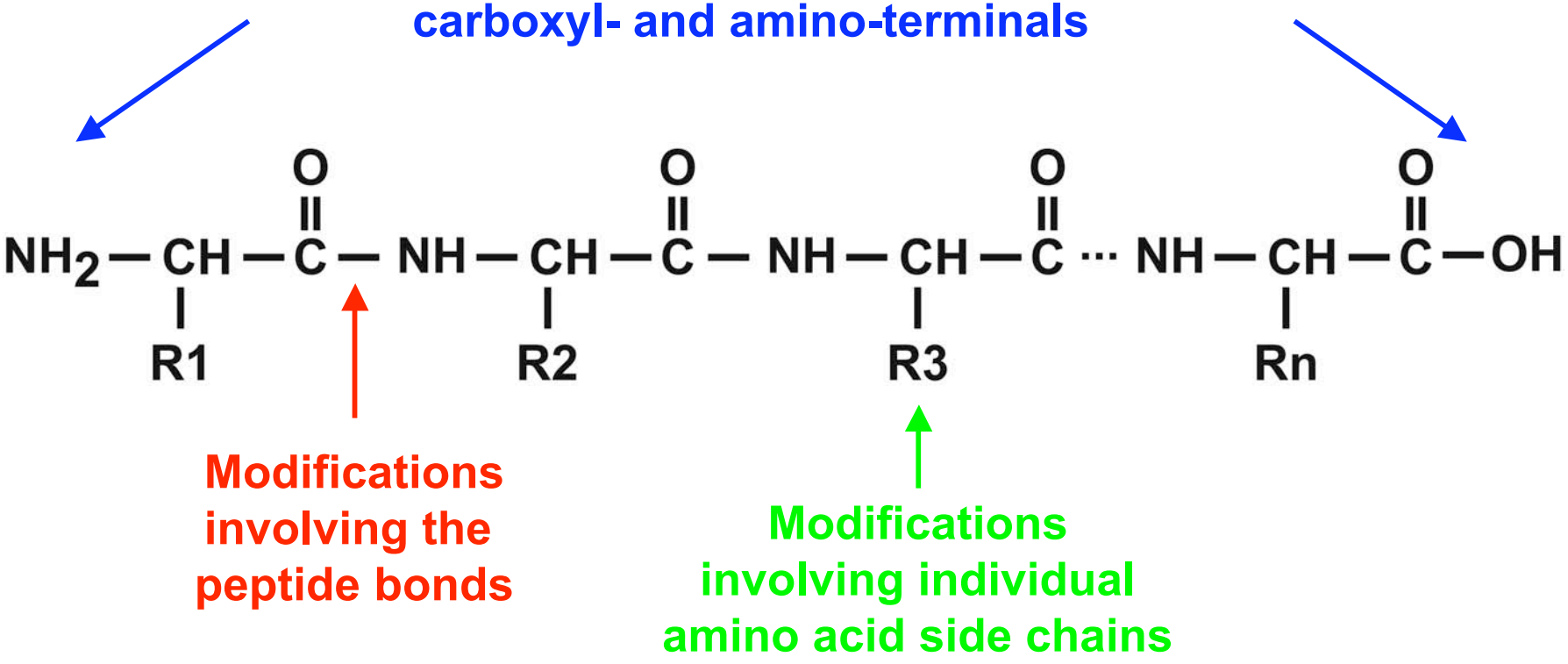
http://www.abrf.org/index.cfm/dm.home?AvgMass=all

Approx. naturally occuring 260 PTMs listed!

Posttranslational Modifications of Proteins

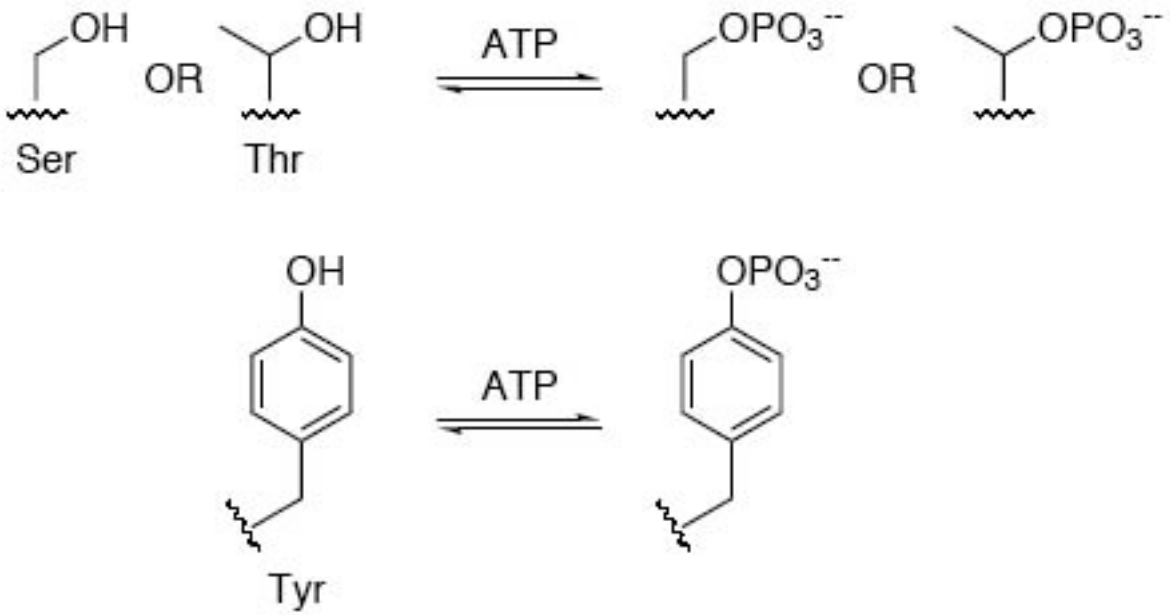
- Covalent attachment of chemical groups to a protein
- Some modifications occur while the protein is translated and exit from the ribosome
- Other modifications occur only after protein translation
- Require dedicated enzymatic catalysis

Possible Sites of Modifications in Proteins



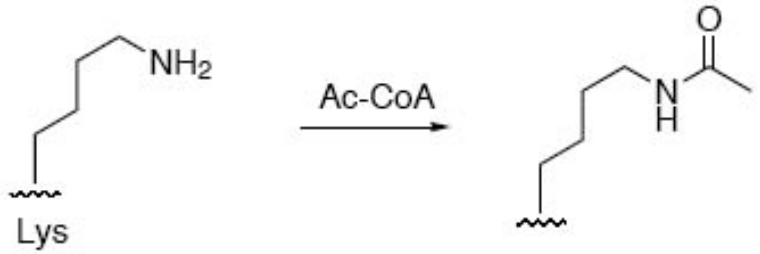
Some of the Side Chains Enzymatically Modified by PTMs

- Ser, Thr, Tyr

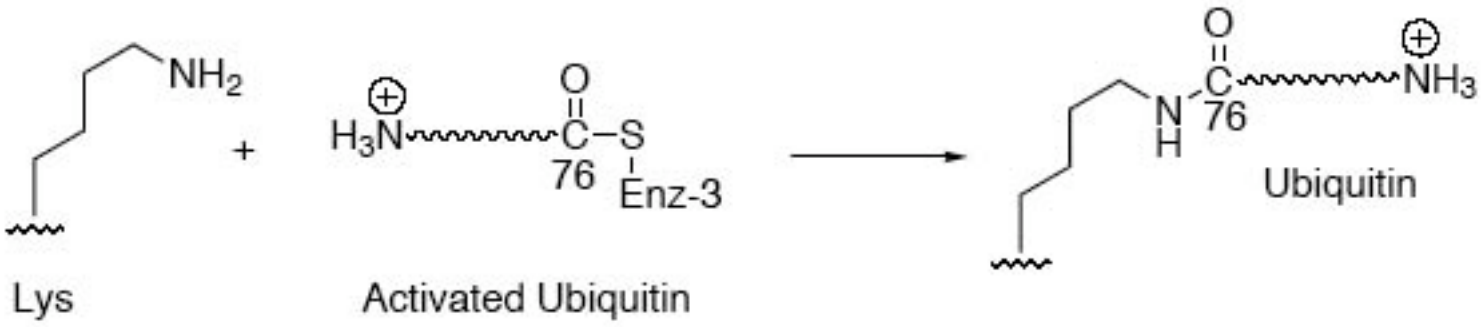


Some of the Side Chains Enzymatically Modified by PTMs

- Lys acetylation

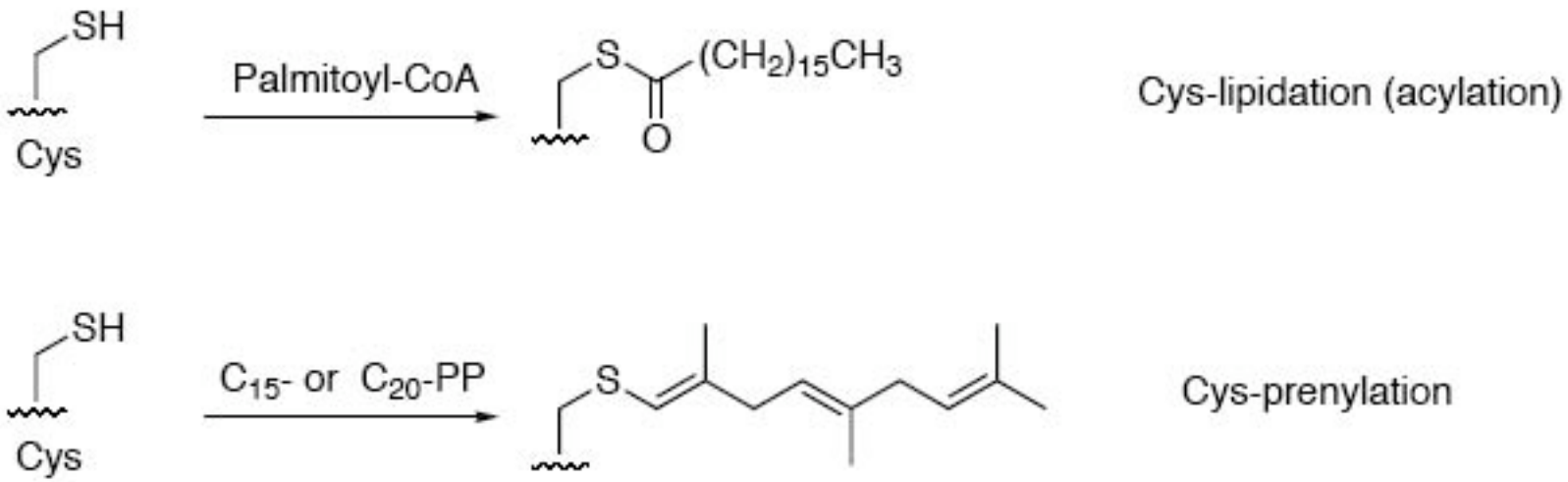


- Lys ubiquitination



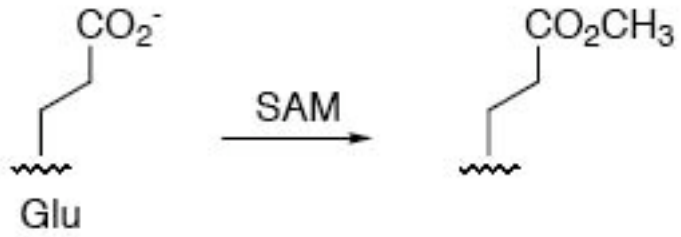
Some of the Side Chains Enzymatically Modified by PTMs

- Cys lipidation
- Acylation by long chain fatty acyl CoAs (C_{14}, C_{16})
- Prenylation by C_{15} (farnesylation)

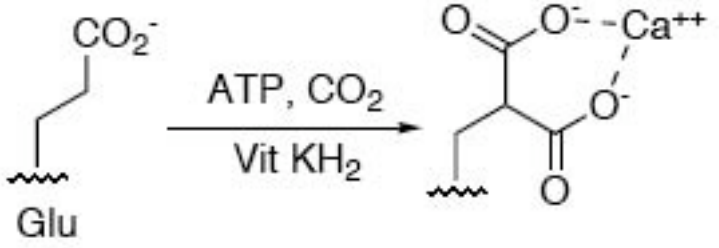


Some of the Side Chains Enzymatically Modified by PTMs

- Glu methylation



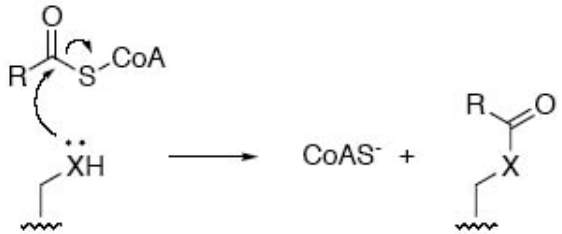
- Glu carboxylation (blood coagulation cascades)



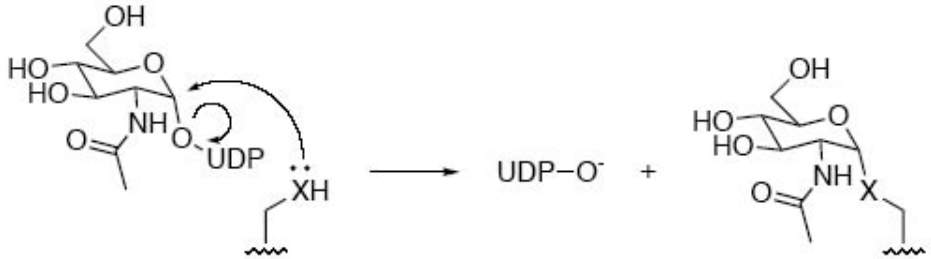
General Reaction Scheme for PTM of Proteins

- ATP ➔ phosphoproteins

- AcylCoAs ➔ acylated lipoproteins

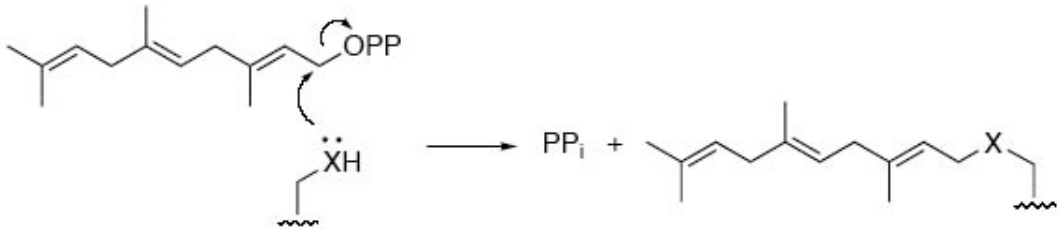


- UDPGlcNAc ➔ glycoproteins

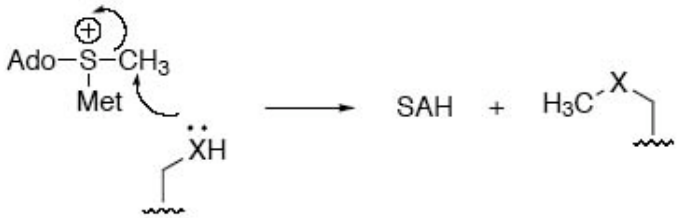


General Reaction Scheme for PTM of Proteins

- Farnesyl-PP, Geranylgeranyl-PP ➔ prenylated lipoproteins



- SAM ➔ Methylated proteins



Cellular Sites of Major Post-translational Modifications

Site	Modification
Cytoplasm	Removal of initiating Met
	Acetylation of N-terminus
	Myristoylation of N-terminus
	O-Glycosylation with GlcNac
	Addition of palmitoyl groups

Cellular Sites of Major PTM's

Site	Modification
Mitochondria/Chloro-plasts	Cleavage of Signal Peptides
Golgi Apparatus	Modification of N-glycosyl groups, O-glycosylation with GalNAc Amidation of C-terminus
Secretory Vesicles/Granules	Proteolytic processing of some precursors

Cellular Sites of Major PTM's

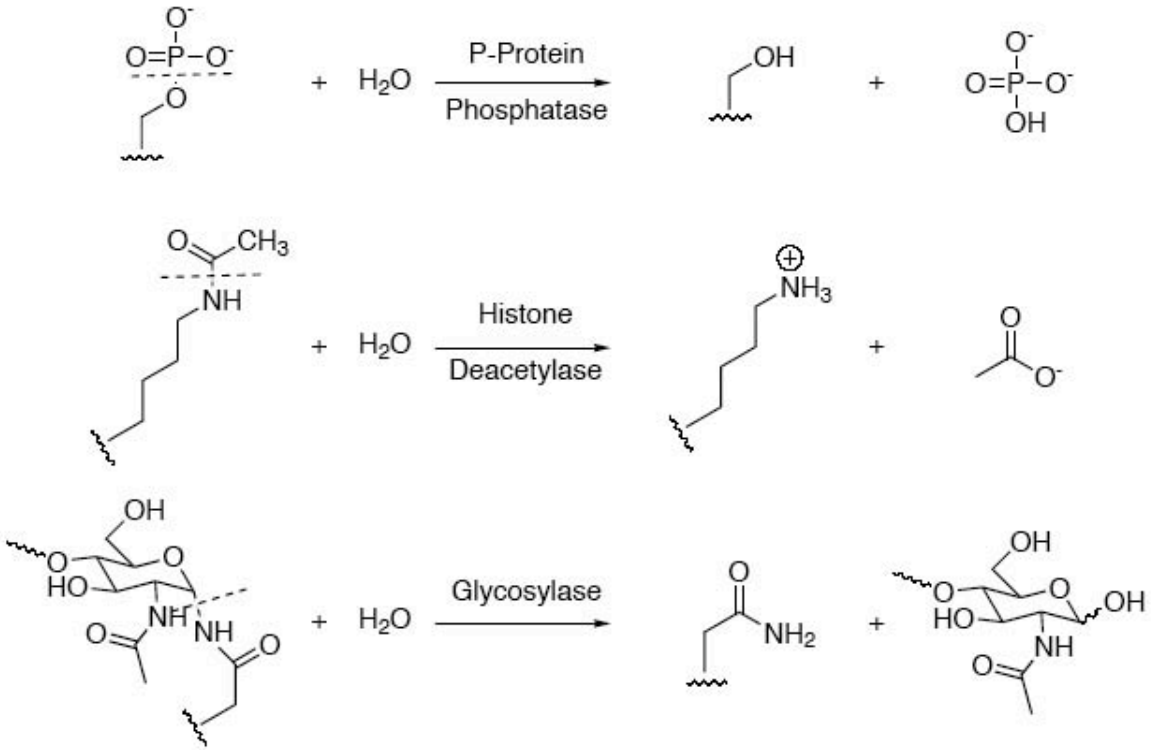
Site	Modification
ER	Cleavage of signal peptides
	Core glycosylation of Asn residues
	Addition of palmitoyl and glycosyl-phosphatidylinositol
	Hydroxylation of Pro/Lys in procollagen
	Disulfide bond formation

Functions Enabled by Posttranslational Modification

- Alterations in local folding of proteins
  - E.g. transitions between unstructured and structured regions
  - Generation of charge pairs
- Marking proteins for degradation
  - Proteasome targeting
- Marking chromatin for transcriptional regulation
- Changing the intracellular or extracellular adresses of proteins
  - Signal peptides direct proteins to …
  - Plasma membrane
  - Secretory pathway
  - Mitochondria
  - Cytosol
- Inactive apo to active holo forms of enzymes

Sets of Enzymes

- PTMs are generally stable modifications
- Reversal of modification requires a distinct set of enzymes



Modifications Involving the α-amino Group

- Nα-acylation
  - formyl
  - acetyl
  - pyruvoyl
  - α-ketobutyryl
  - glucuronyl
  - pyroglutamate
  - murein

Formylation of the α-Amino Group

- Nα-formyl-Met
  - forms the start of the nascent protein chain in prokaryotes
  - Eukaryotic start signal is Met
- Deformylase may remove the CHO group
- Aminopeptidase later removes Met from some, but not all chains
- Nα-formyl-Gly
  - occurs in honey bee melittin
- Murein derivatives
  - link *E. coli* peptidoglycan and membrane lipoprotein

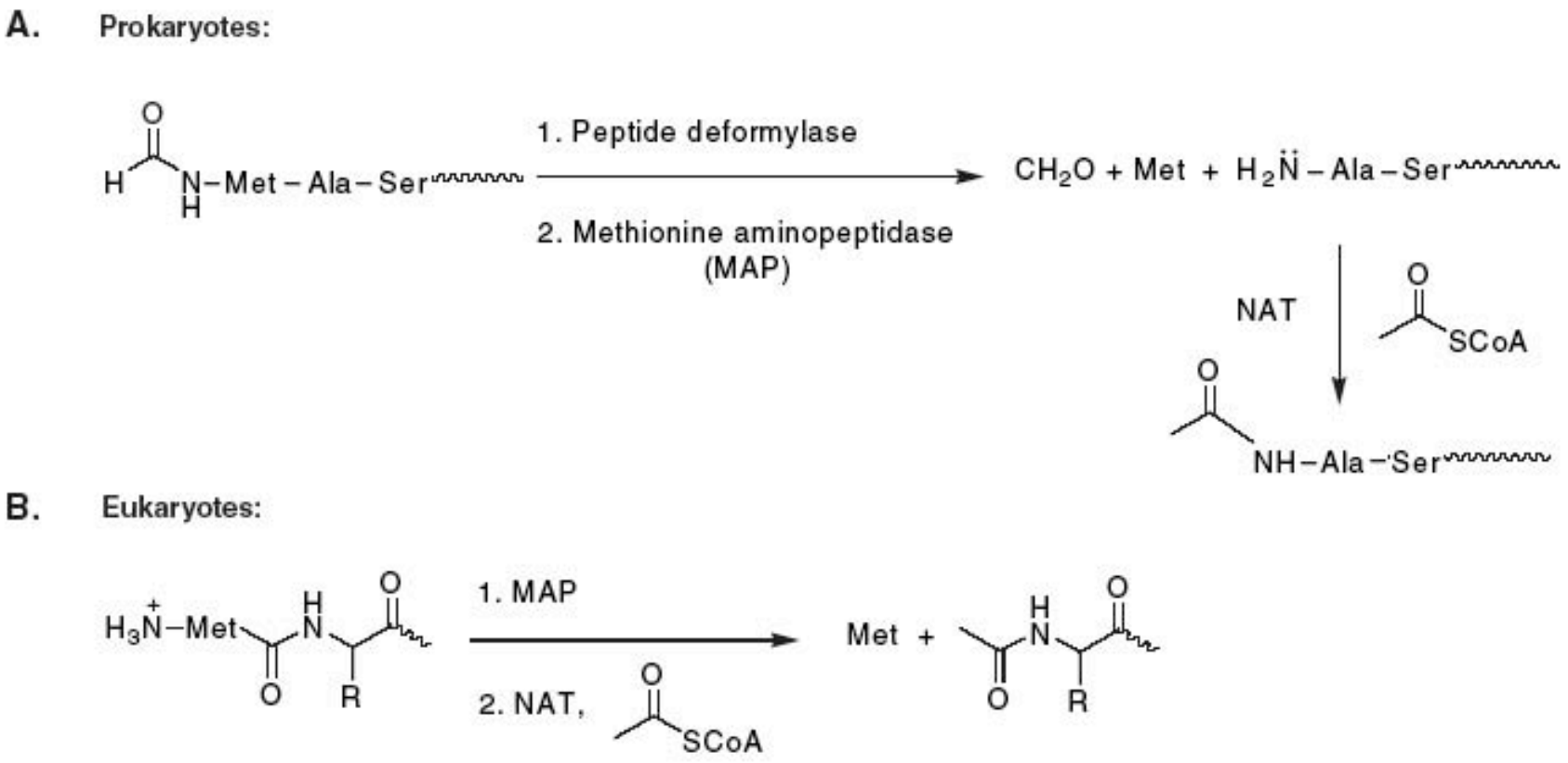
Protein Acetylation

Function and Analysis of Post-translational Protein Modifications

Protein N-Acetylation

- In about 50% of yeast proteins and about 80-90% of higher eukaryotic proteins
- Very rare in *E. coli*
- S5 (N-Ac-Ala-Arg-…), S18 (N-Ac-Ala-His-…), L12 (N-Ac-Ser-Ile-…)

Protein N-Acetylation



Specificity of Protein N-Acetylation

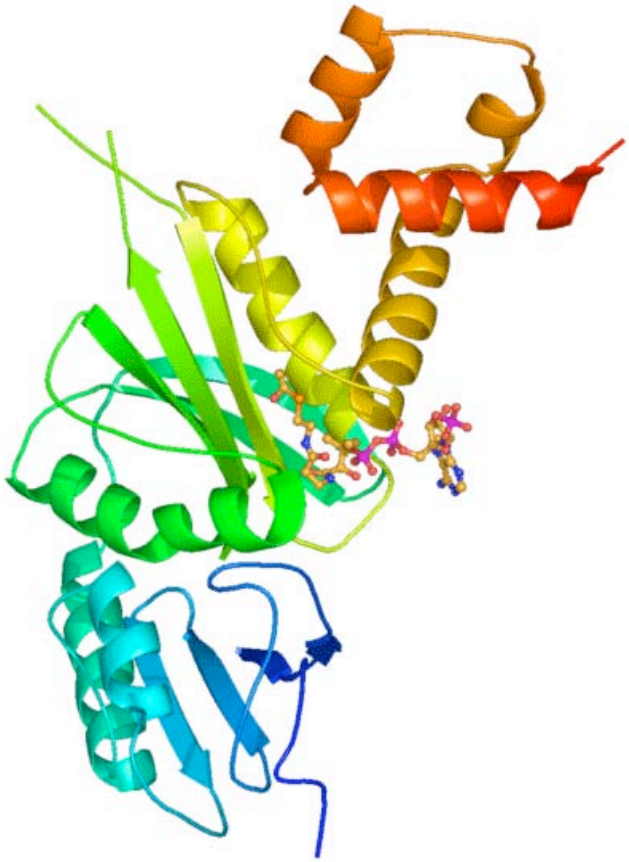
- Lies in the specificity of MAP
- MAP cleaves preferentially
  - Met-Gly
  - Met-Ala
  - Met-Ser
  - Met-Cys
  - Met-Thr
  - Met-Pro
  - Met-Val
- Uncovered Ser, Ala, Gly, Thr, get acetylated

N-acetyl Transferases (NATs)

- In yeast, 3 NATs (NAT A, B, and C)
- NATB and NATC acetylate proteins with Met_1 still in place
- NATB recognises M-E, M-D, M-Q, M-M
- NATC recognises M-I, M-L, M-W, M-F
- NATA acetylates S, A, G, T

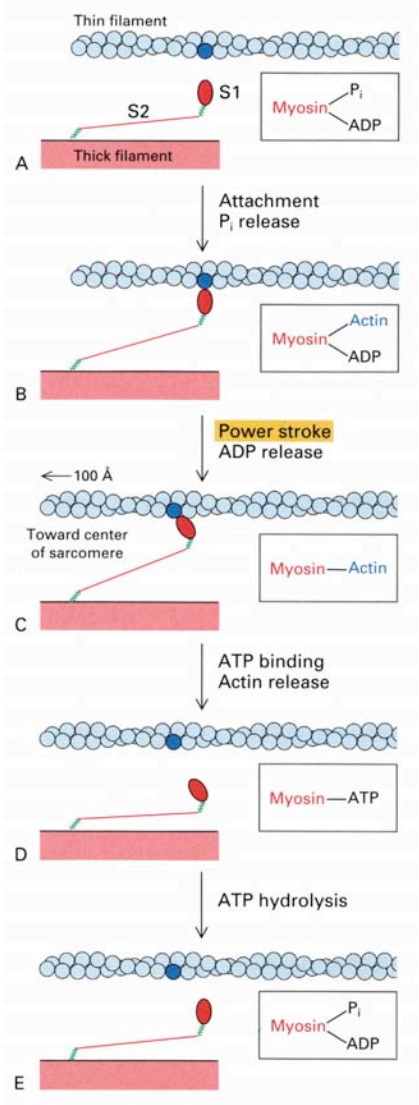
N-acetyl Transferases

- In eukaryotes, hundreds of NATs exist
- Involved in K acetylation of histones
- Control
  - -transcriptional activation
  - -Chromatin assembly
  - -DNA replication
  - -Involved in N-acetylation of amino-glycoside antibiotics, results in decreased affinity of the drug for its target



Biological Significance of N-terminal Acetylation

- Unclear in eukaryotes!
- Functions only detected on a case-by-case study
- Actins are known to be acetylated at N-terminus
  - N-terminus is M-E…
  - E exposed by aminopeptidases
  - Further acetylation to yield mature N-acetyl-E
  - Strengthens interaction between actin and myosin

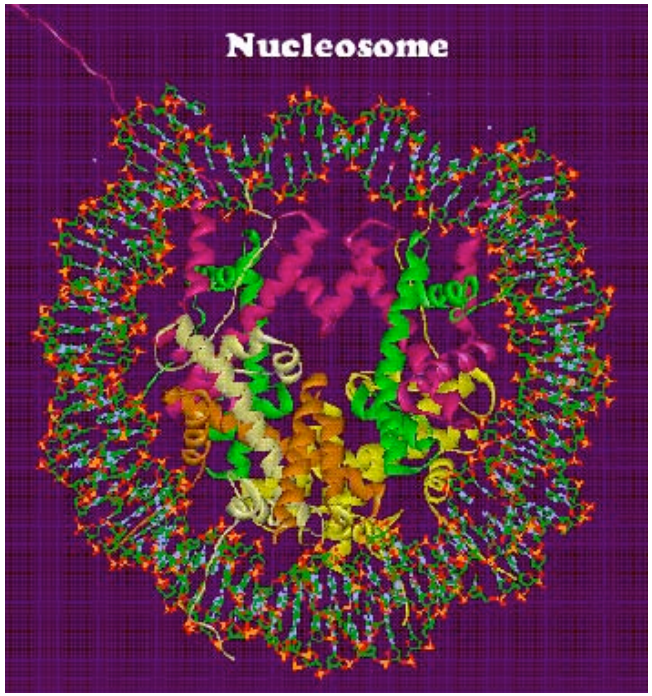


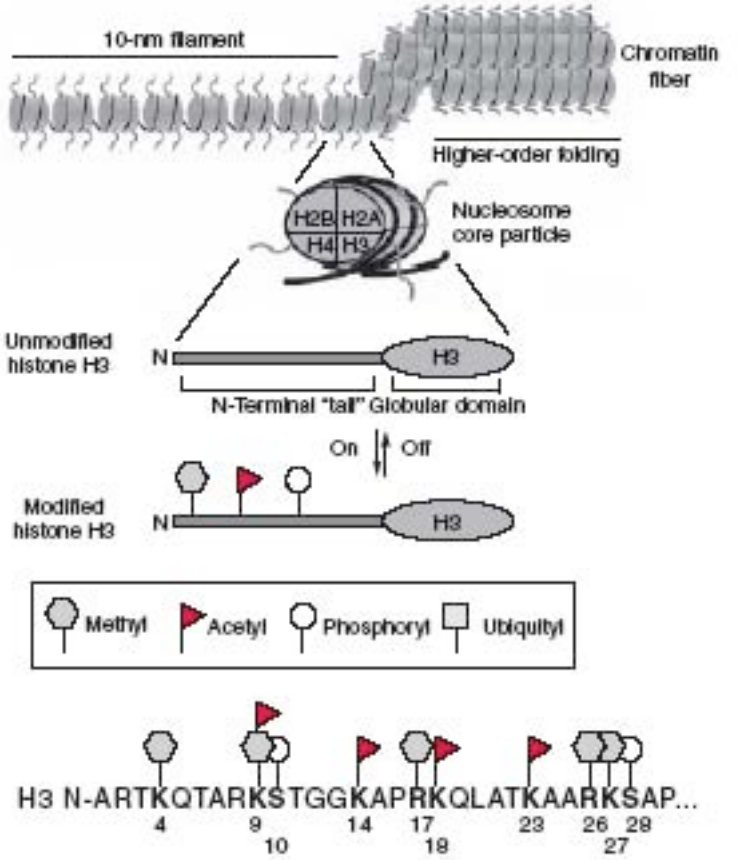
N-Acetylation of Lysine-ε-NH_2 Side Chains

- Interest in protein N-acetylation is on regiospecific modification of K side chains
- Large number of PTMs detected on histones
- Transcriptional coactivators and corepressors turned out to be HATs and HDACs
- N-terminal regions of histones are flexible and amenable to PTMs

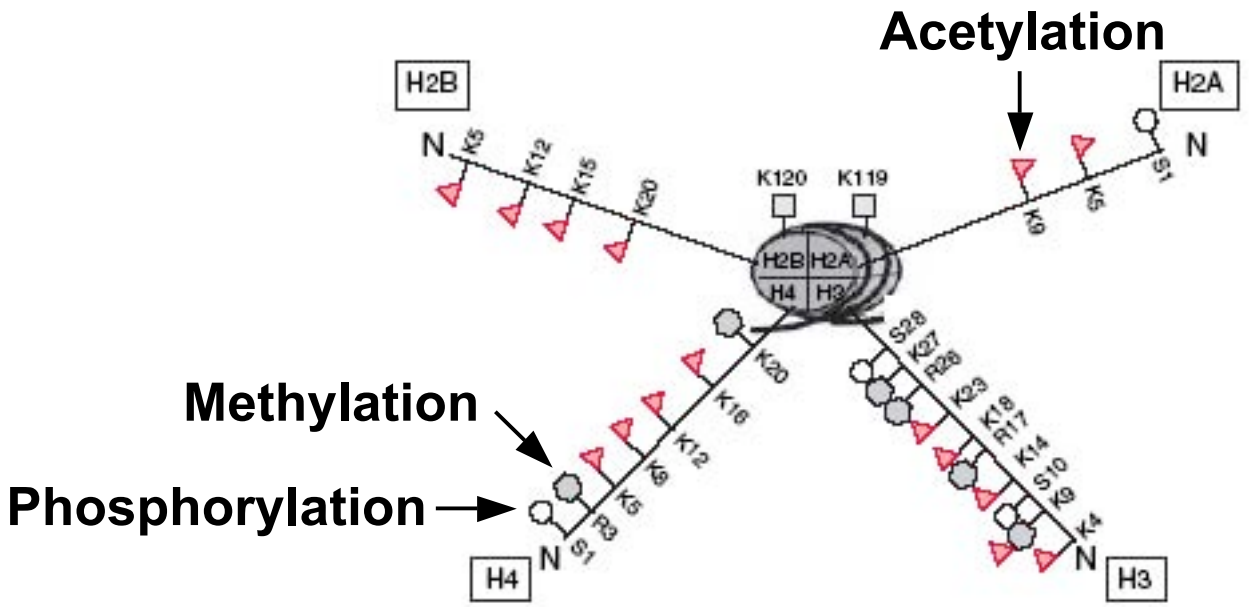
N-Acetylation of Lysine-ε-NH_2 Side Chains

- Chromatin contains H2A, H2B, H3 and H4
- Histone core $(H2A)_2(H2B)_2(H3)_2(H4)_2$
- 145-147 bps around core





Histone Acetylation

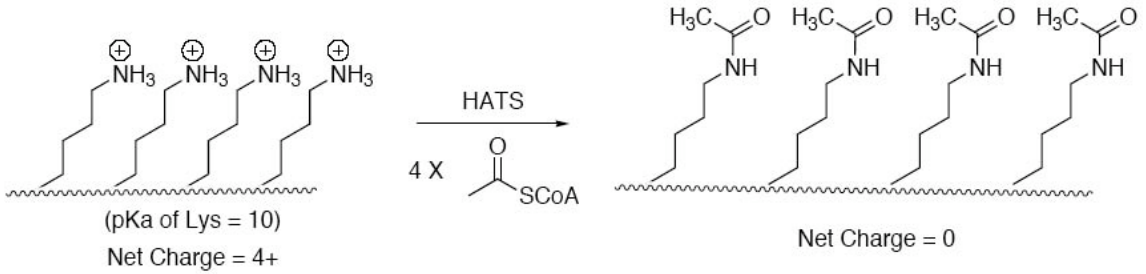


Histone Acetylation

- MS analysis revealed acetylation on…
- H2A (K_5 and K_9)
- H2B (K_5, K_{12}, K_{15}, K_{20})
- H3 (K_9, K_{14}, K_{18}, K_{23})
- H4 (K_5, K_8, K_{12}, K_{16})
- For two copies each of histone: 28 potential acetylation sites
- Yeast: 13 acetylation sites found/octamer
- 50% posttranslational utilization
- Immense combinatorial possibilities

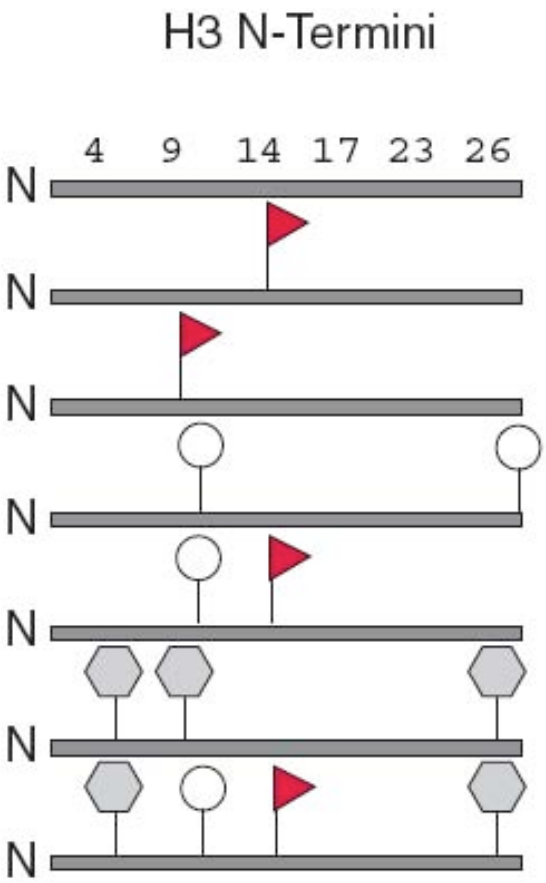
Consequences of Histone Acetylation

- K's cationic at physiological pH
- N-acetylation quenches positive charges



- Electrostatic weakening of histone/DNA interactions
- Opening of the chromatin
- Allows TFs to bind to promoter regions

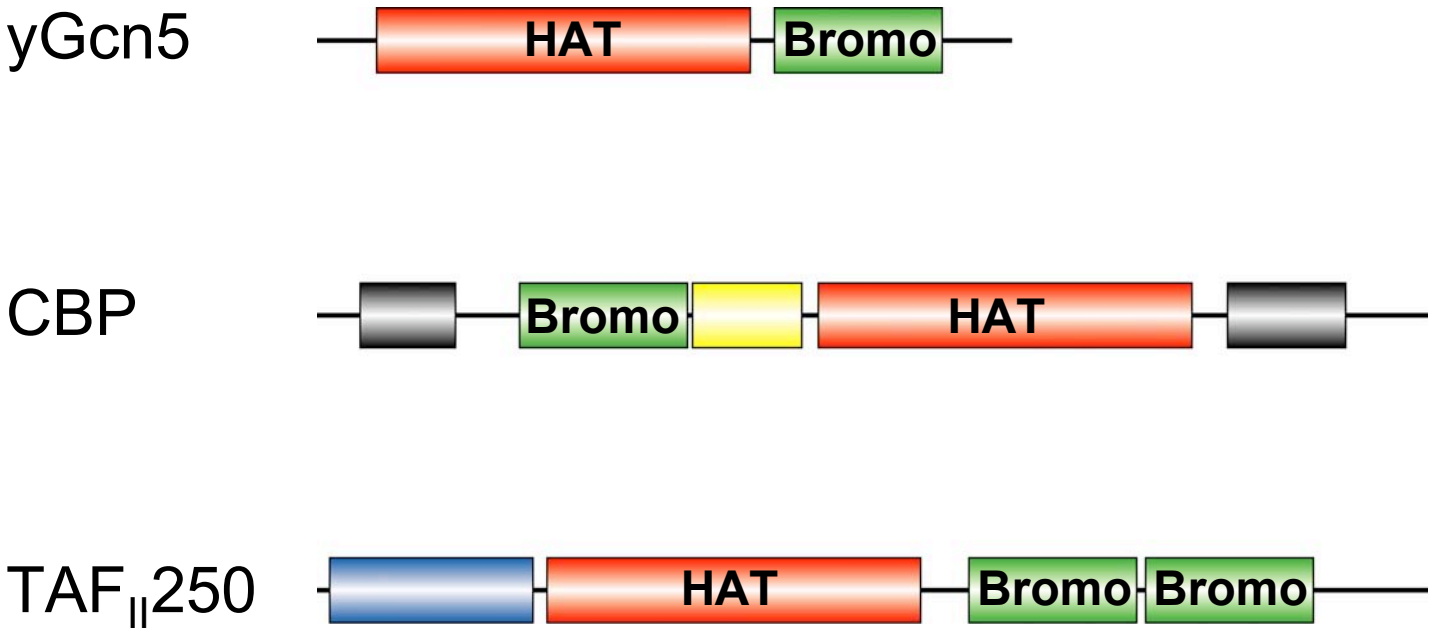
Consequences of Histone Acetylation



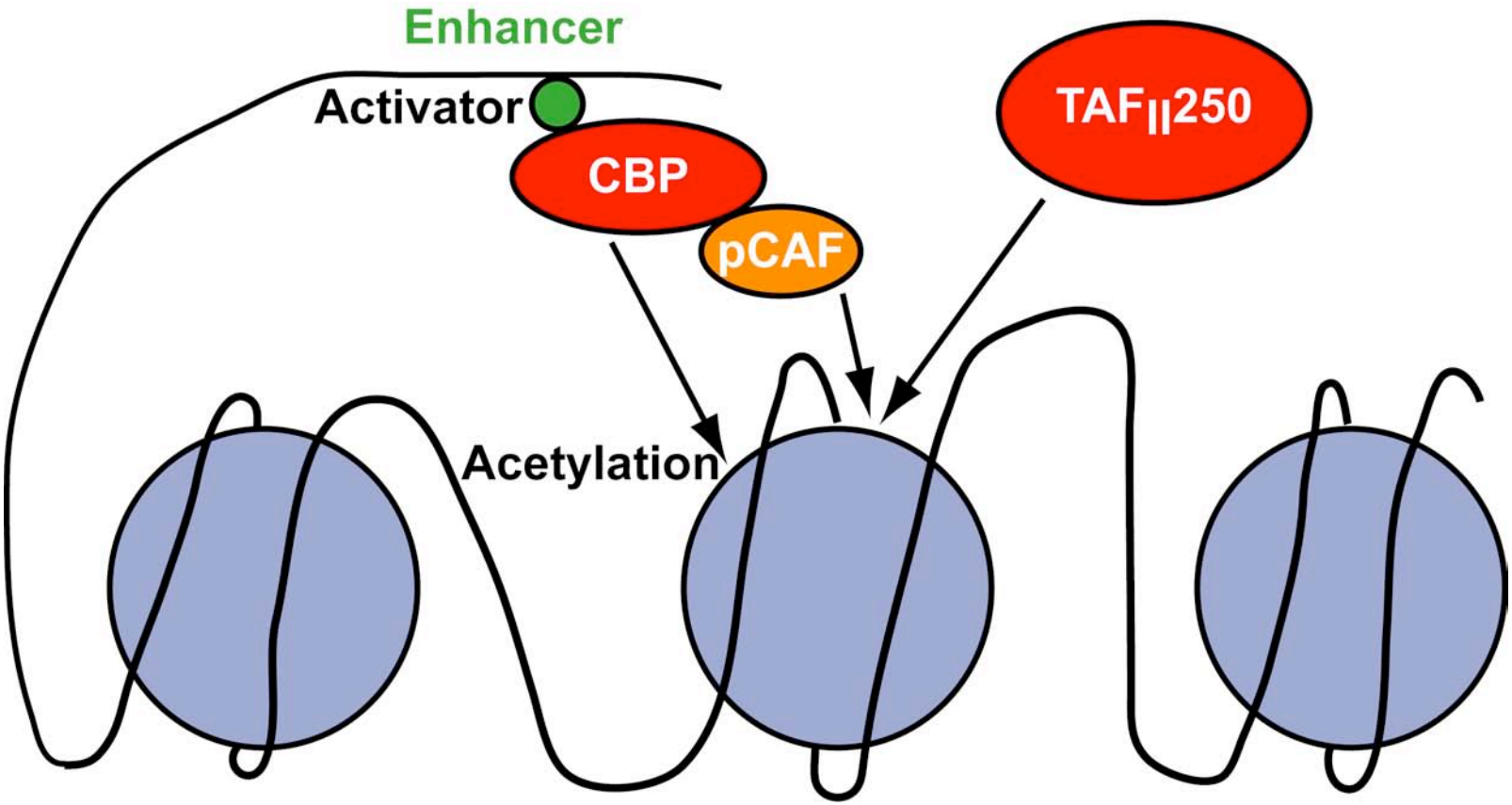
Modification	Function
Unmodified	Silencing
Acetylated	Transcription
Acetylated	Histone deposition?
Phosphorylated	Mitosis/Meiosis
Phos/Acetyl	Transcription
Methylated	Transcription?
Higher-order combinations	?

Histone Acetyltransferases - A Family

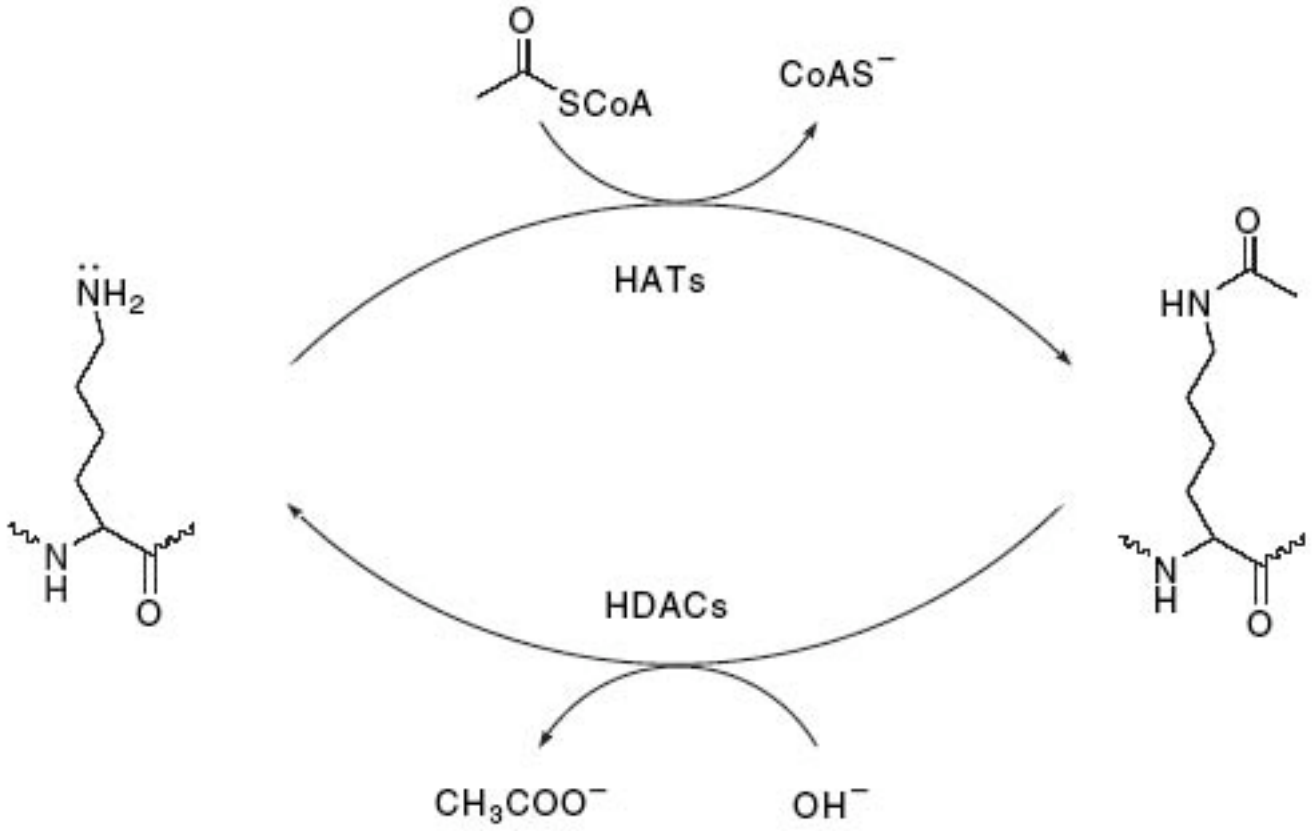
- Family of GNATs (Gcn5-related N-acetyltransferases)



Transcriptional Activation by HATs



Histone Deacetylases (HDACS)

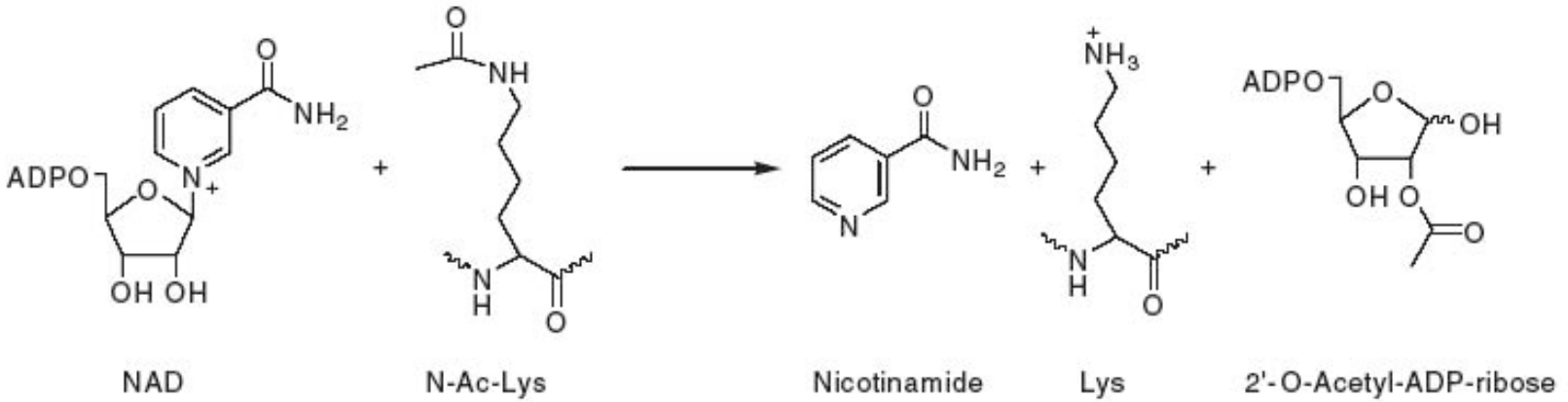


Histone Deacetylases (HDACS)

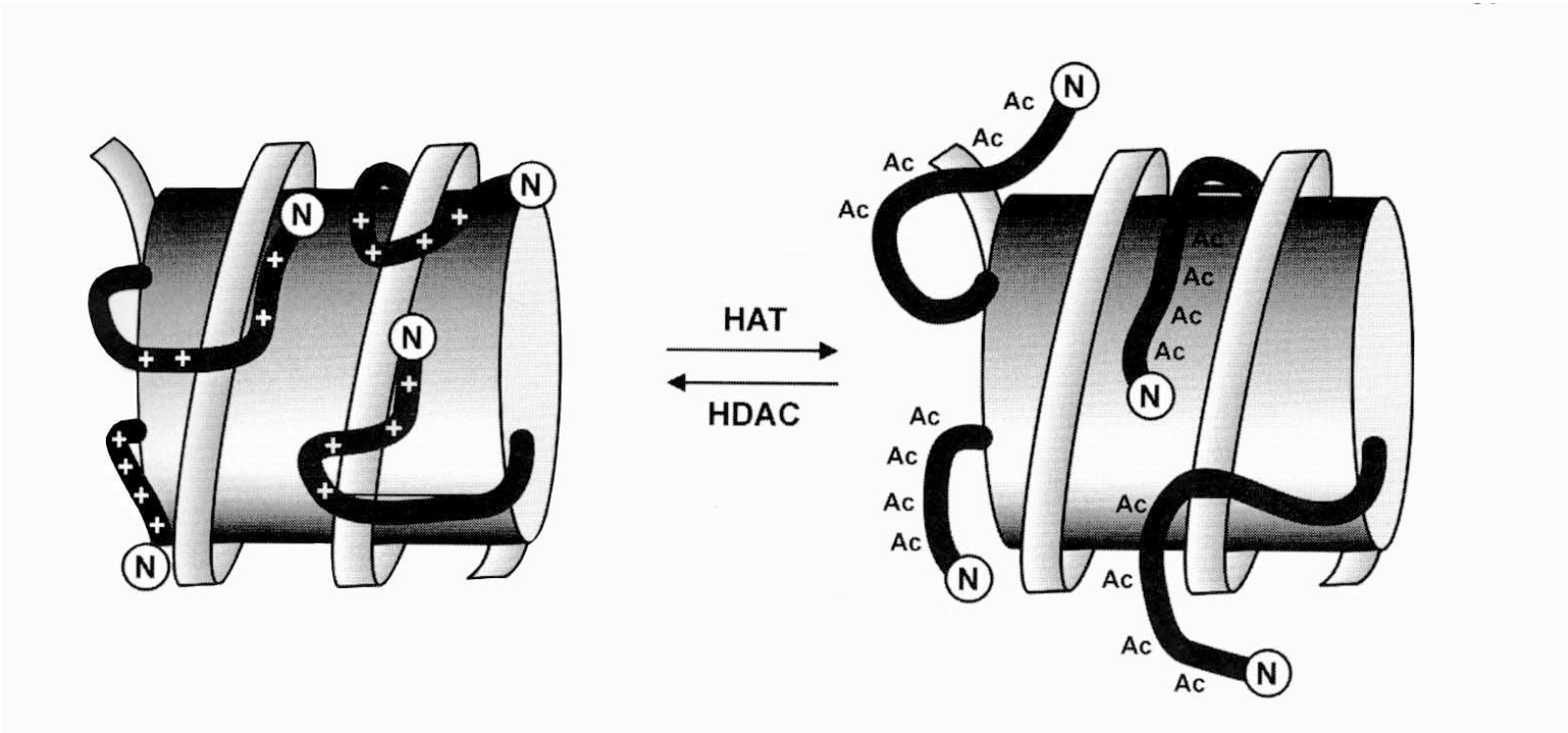
- HDACS are corepressors of transcription
- Maintain histone tails in hypoacetylated state
- Leads to chromosome condensation
- Silence promoters
- 2 distinct families
  - HDACs that release the acetyl moiety as acetate
  - Sirtuins (silent information regulator)

Sirtuin HDACS

Sirtuin reaction stoichiometry:



Influence of Histone Acetylation and Deacetylation on Nucleosome Structure



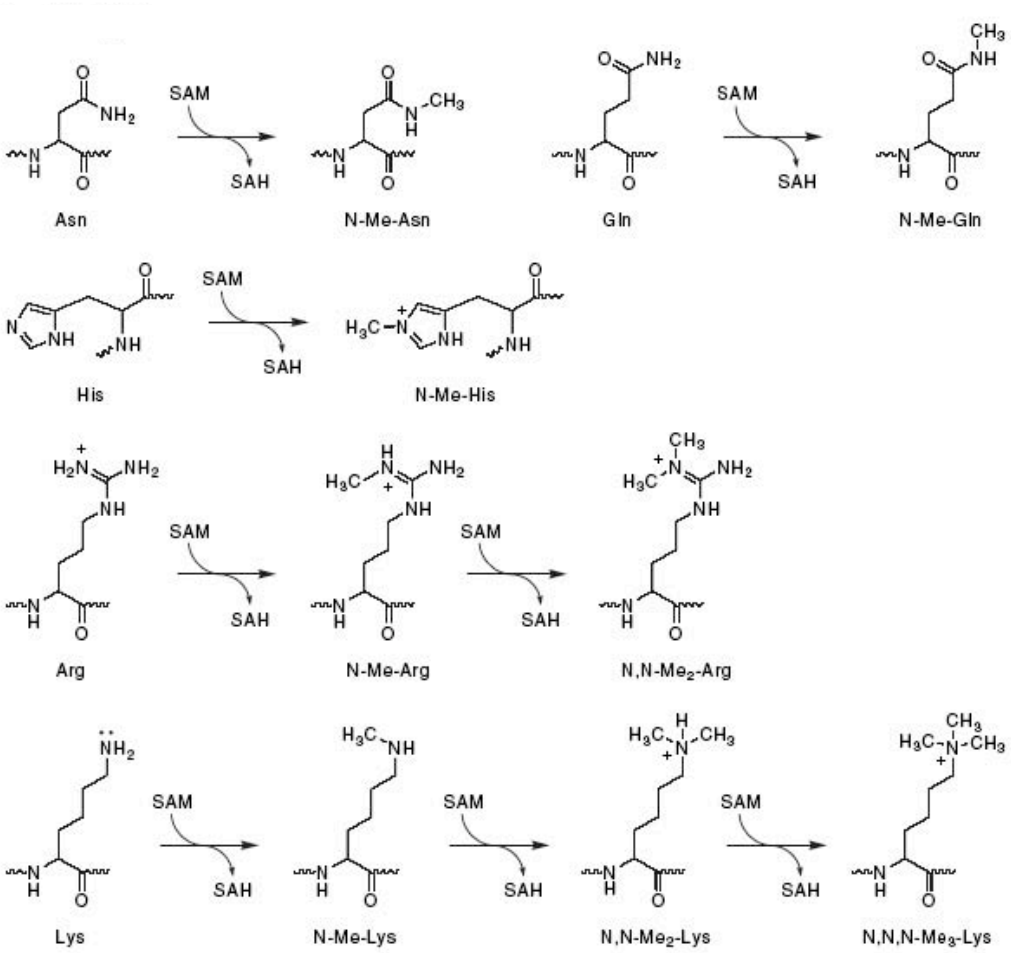
Protein Methylation

Function and Analysis of Post-translational Protein Modifications

Protein Methylation

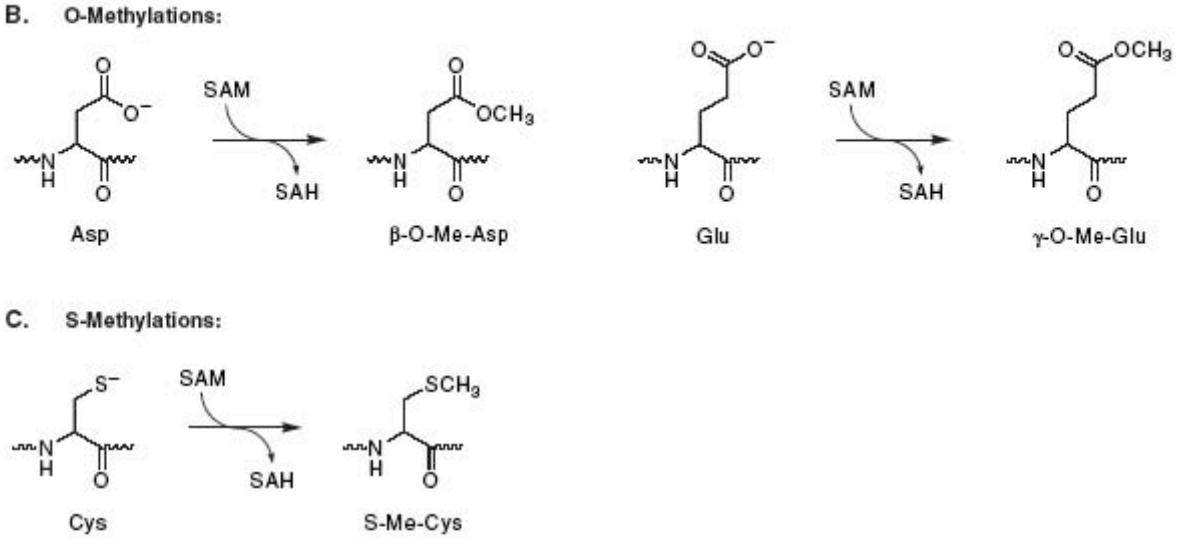
- Occurs on N- or O-atoms
- Methylation of -COO^- covers up negative charge
- N-methylation of Ks does not alter charge, increases hydrophobicity
- Di- and trimethylation of Ks increases both hydrophobicity and steric bulk
- Affects protein-protein interactions
- Occurs on ε-amino group of K, imidazole ring of H, guanidino group of R, amides of Q and N
- N-methylation irreversible

N-Methylations

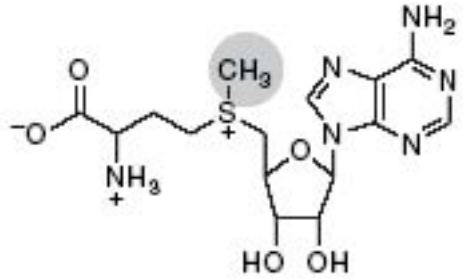


Function and Analysis of Post-translational Protein Modifications

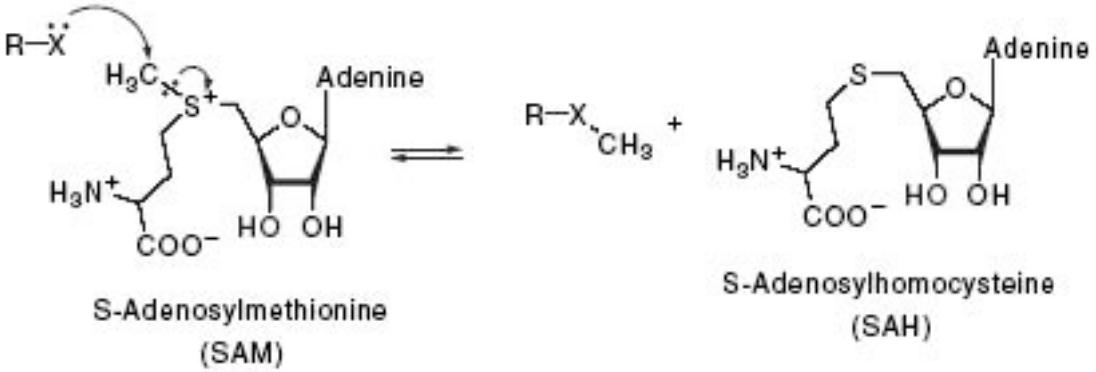
O-, S-, and C-Methylations



One Carbon Donor



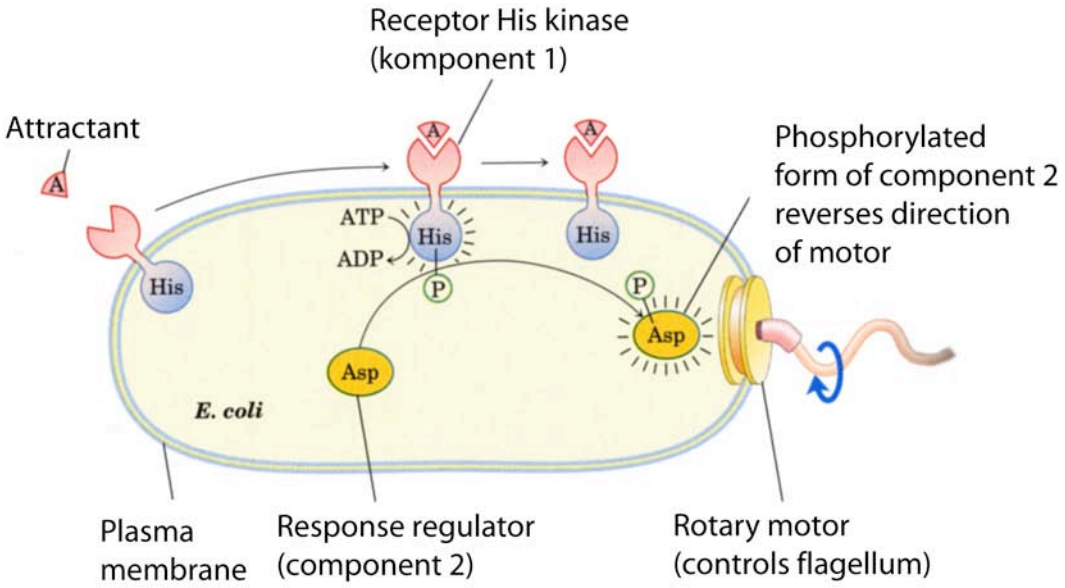
S-Adenosylmethionine (SAM)



S-Adenosylmethionine (SAM)

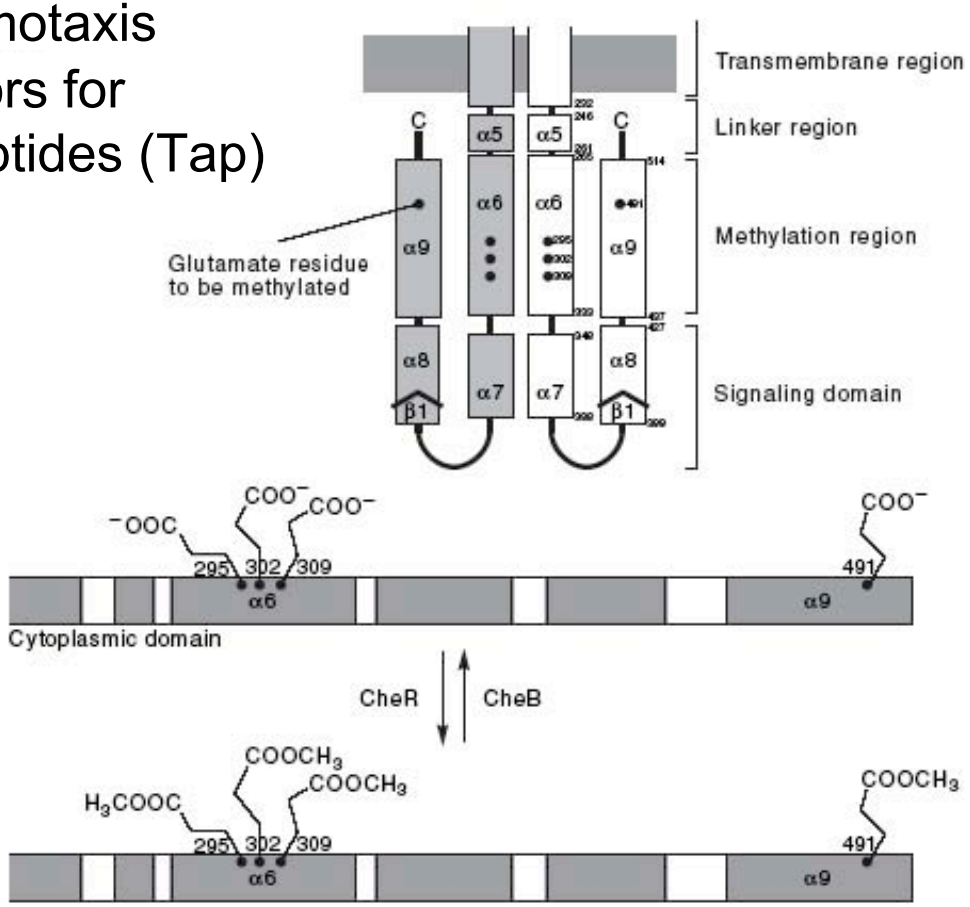
S-Adenosylhomocysteine (SAH)

Protein O-Methylation Occurs in Bacterial Chemotaxis

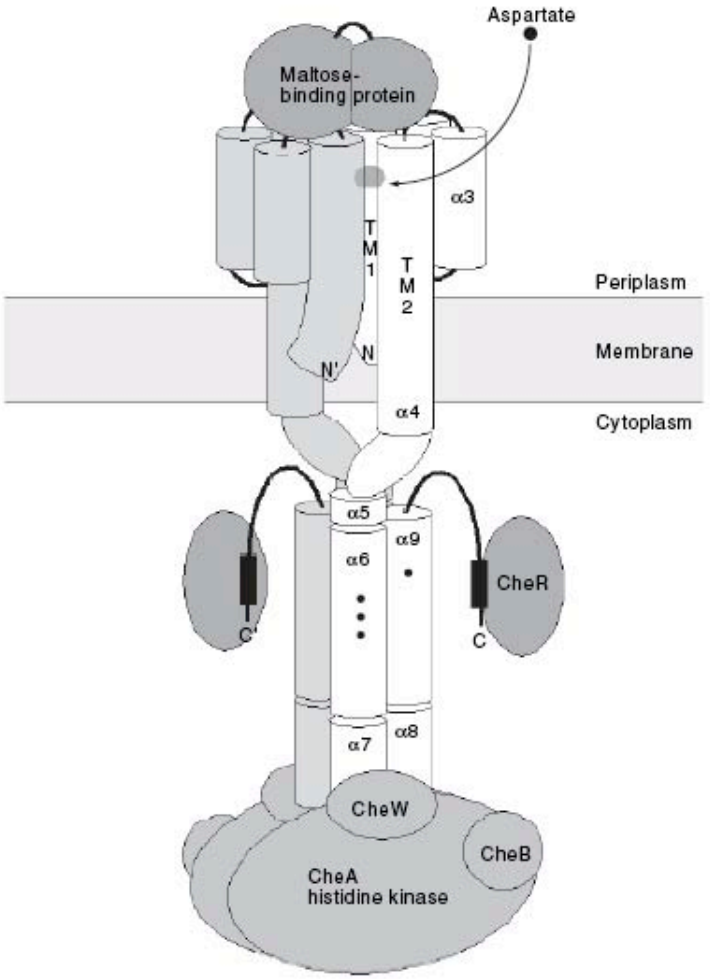


Protein O-Methylation

- Occurs in bacterial chemotaxis Transmembrane receptors for
- Asp (Tar), Ser (Tsr), Peptides (Tap)

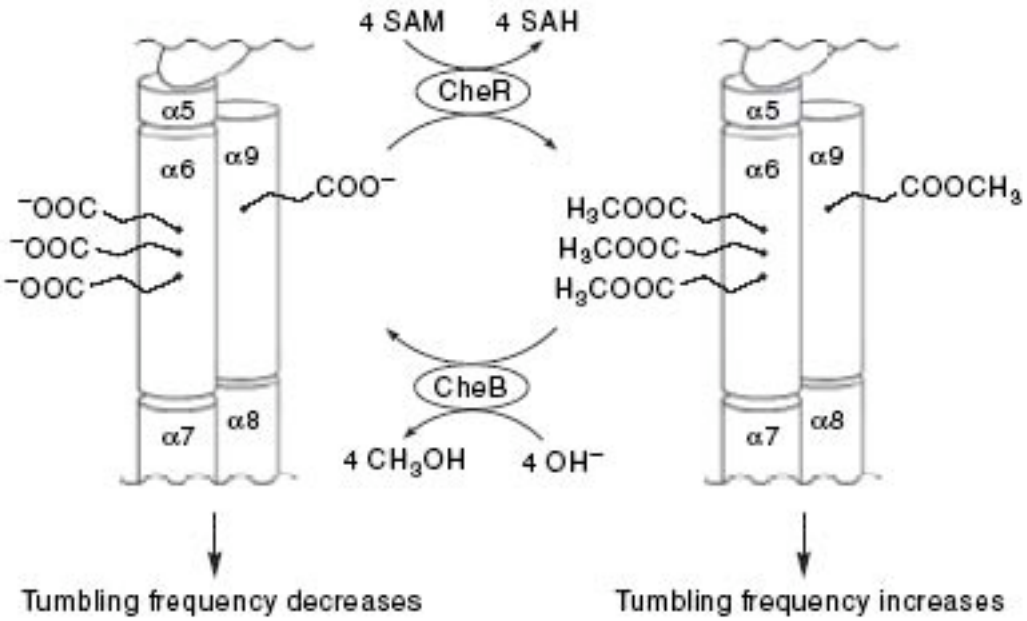


Protein O-Methylation

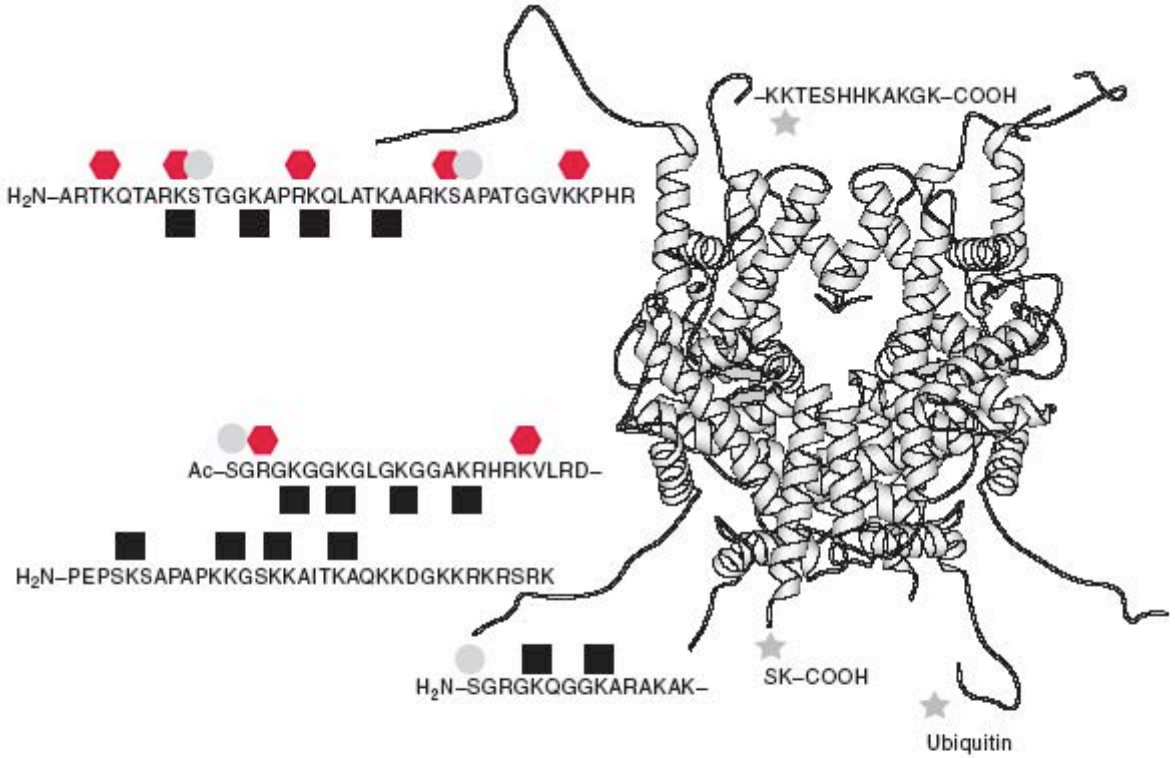


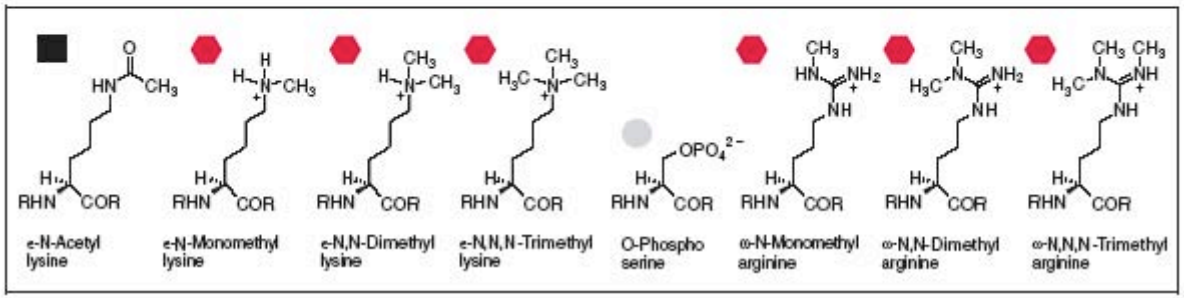
Function and Analysis of Post-translational Protein Modifications

Protein O-Methylation



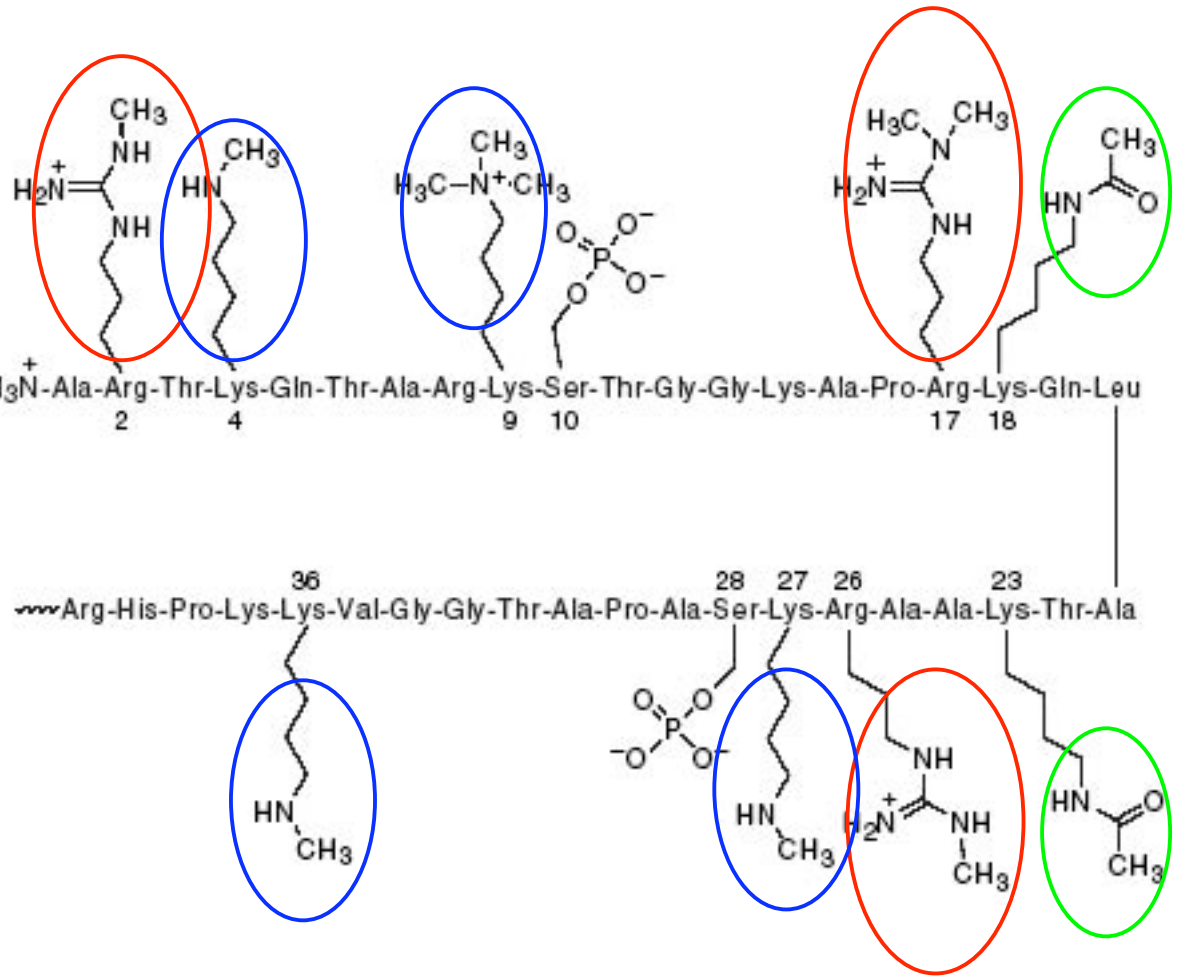
Histone Methylation





Function and Analysis of Post-translational Protein Modifications

Histone Methylation in H3



Histone Modification

- H3 modification
  - 3 Rs methylated
  - 4 Ks methylated
  - 2 Ss phosphorylated
- Results in over 110,000 combinations of possible modifications
- approx. 10^7 possible nucleosomes

Protein Phosphorylation

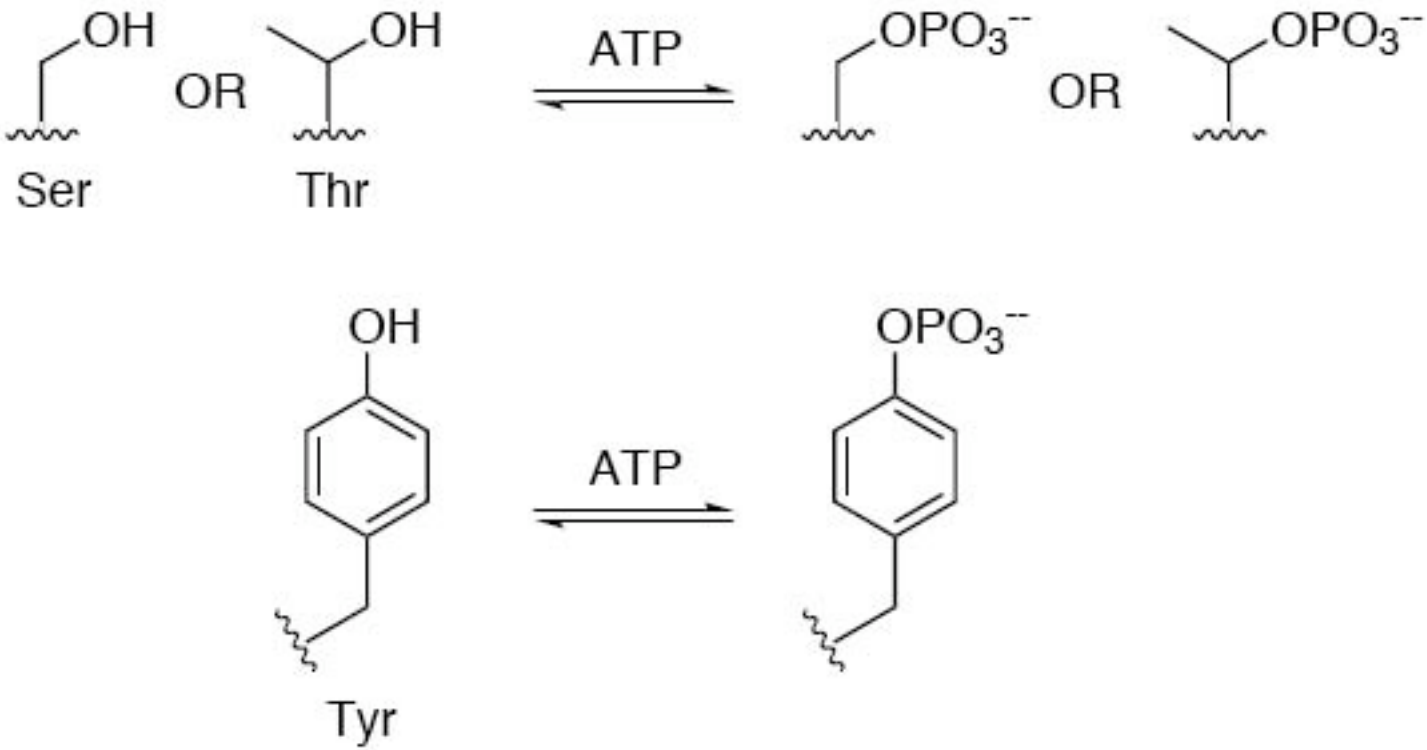
Function and Analysis of Post-translational Protein Modifications

Protein Phosphorylation

- One of the most common PTMs
- Estimate that approx. 30% of all eukaryotic proteins become phosphorylated
- Human genome codes for approx. 2,000 protein kinases
- Transient
- Regulate a vast number of biological processes
  - Enzymatic activity
  - Metabolism
  - Motility
  - Signal transduction
  - Cell division
  - Cell growth
  - Apoptosis

Protein Phosphorylation

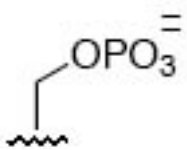
- Ser, Thr, Tyr phosphorylation by protein kinases



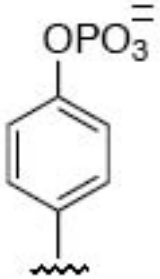
Protein Phosphorylation

- Protein kinase variants defined by the type of protein side chain modification

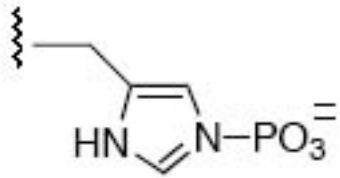
a) Ser/Thr protein kinases (e.g. cAMP-dependent PK)



b) Tyr protein kinases (e.g. insulin receptor kinase)

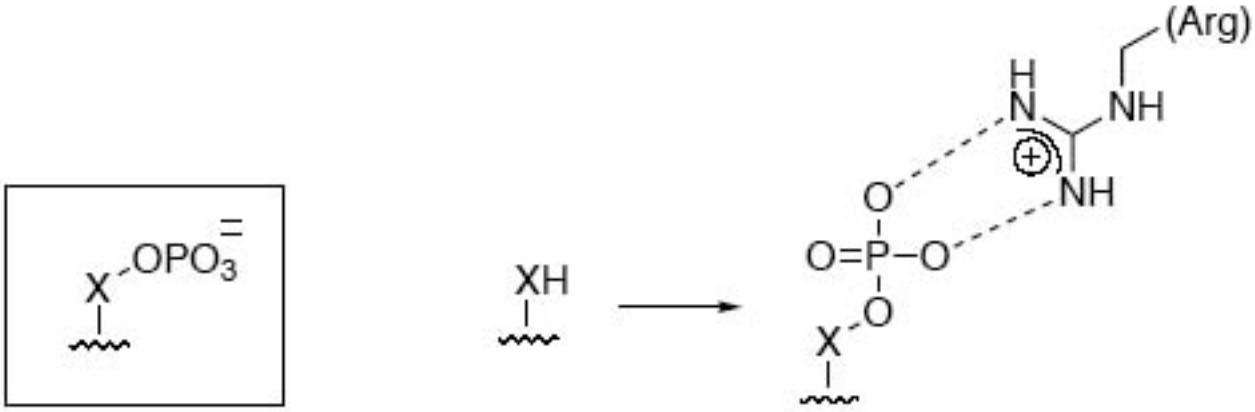


c) His protein kinases (e.g. bacterial two component sensor/respons regulators)



Protein Phosphorylation

- What is the purpose/effect of protein side chain phosphorylations?
  - Protein conformational switching
  - Introduction of a dianionic phosphate group (- PO_3^{--}) induces electrostatic reorganization of local regions, loops of proteins
  - Reorganization often occurs via charge pairing with a cationic Arg side chain

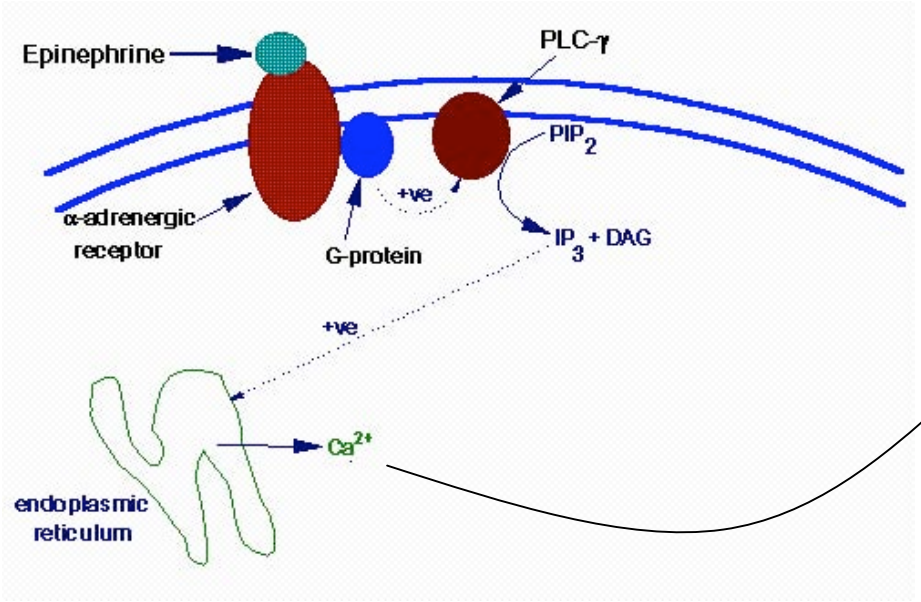


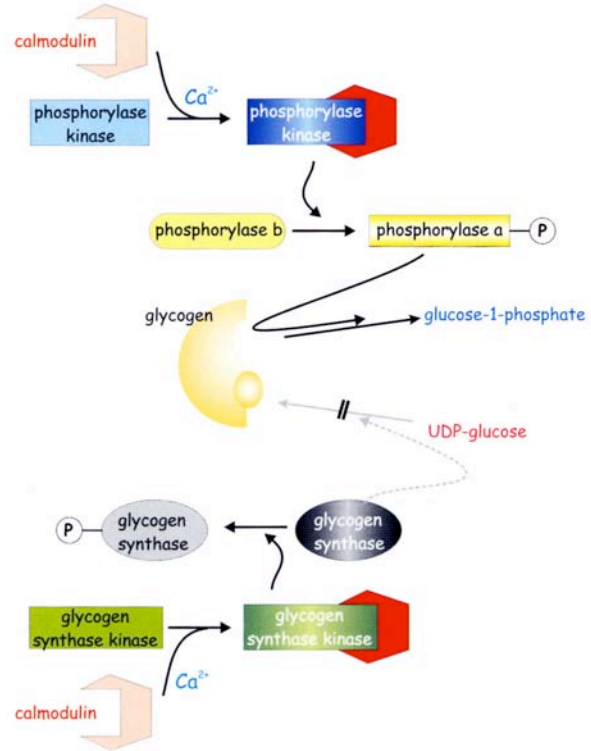
Protein Phosphorylation

- What governs specificity of phosphorylation?
  - PKs recognise certain motifs

Protein Kinase	Recognition Motifs[a]	Phosphorylation Sites[b]	Protein Substrate (reference)
cAMP-dependent Protein Kinase (PKA, cAPK)	R-X-S/T[c] R-R/K-X-S/T	Y_7LRRASLAQLT F_1RRLSIST A_{29}GARRKASGPP	pyruvate kinase (2) phosphorylase kinase, α chain (2) histone H1, bovine (2)
Casein Kinase I (CKI, CK-1)	S(*P*)-X-X-S/T	R_4TLS(*P*)VSSLPGL D_{43}IGS(*P*)ES(*P*)TEDQ	glycogen synthase, rabbit muscle (4) α_{s1}-casein (4)
Casein Kinase II (CKII, CK-2)	S/T-X-X-E	A_{72}DSESEDEED L_{37}ESEEEGVPST E_{26}DNSEDEISNL	PKA regulatory subunit, R_{II} (2) p34^{cdc2}, human (5) acetyl-CoA carboxylase (2)
Glycogen Synthase Kinase 3 (GSK-3)	S-X-X-X-S(*P*)	S_{641}VPPSPSLS(*P*) S_{641}VPPS(*P*)PSLS(*P*)	glycogen synthase, human (site 3b) (6,2) glycogen synthase, human (site 3a) (6,2)
Cdc2 Protein Kinase; CDK2-cyclin A	S/T-P-X-R/K[c]	P_{13}AKTPVK H_{122}STPPKKKRK	histone H1, calf thymus (2) large T antigen (2)
Calmodulin-dependent Protein Kinase II (CaMK II)	R-X-X-S/T R-X-X-S/T-V	N_2YLRRRLSDSN K_{191}MARVFSVLR	synapsin (site 1) (2) calcineurin (2)
Mitogen-activated Protein Kinase (Extracellular Signal-regulated Kinase) (MAPK, Erk)	P-X-S/T-P[d] X-X-S/T-P	P_{244}LSP P_{92}SSP V_{420}LSP	c-Jun (7) cyclin B (7) Elk-1 (7)
Abl Tyrosine Kinase	I/V/L-Y-X-X-P/F[e]		

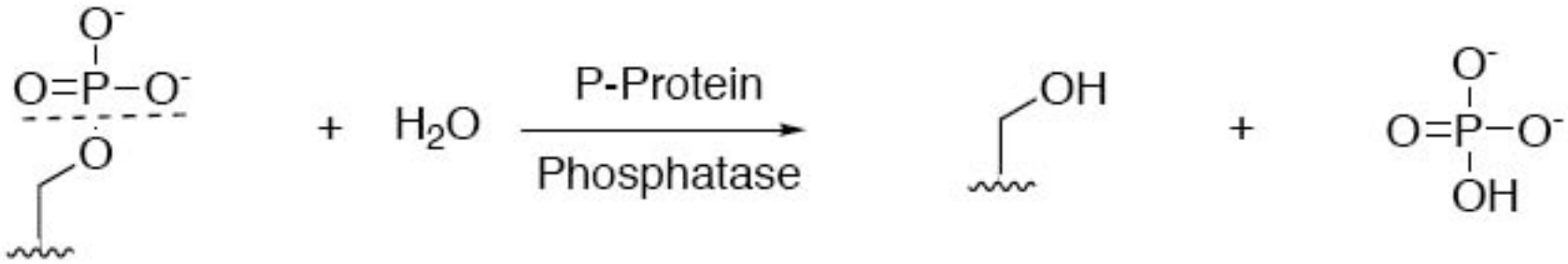
Physiological Importance of Protein Phosphorylation





Protein Dephosphorylation

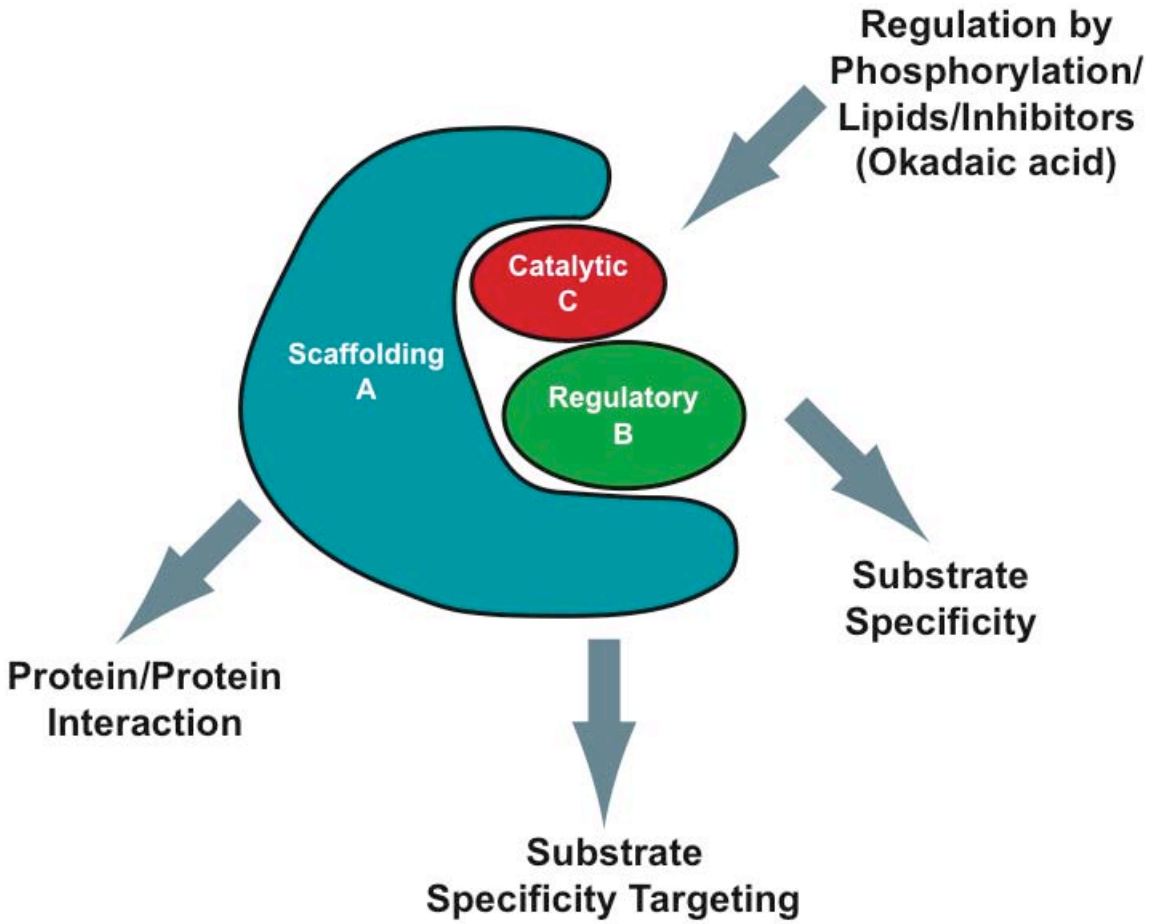
- Phosphoproteins are dephosphorylated by P-protein phosphatases
- Phosphatases counteract protein kinases
- Human genome encodes approx. 500 protein phosphatases
- Ser/Thr- and Tyr-specific phosphatases



Protein Phosphatases

- Phosphatase 1
  - Ser/Thr-specific phosphatase
  - Regulates cell-cycle progression
  - Muscle contraction
  - Carbohydrate metabolism
- Protein Phosphatase 2A
  - Controls numerous cellular processes
  - Oligomeric enzymes

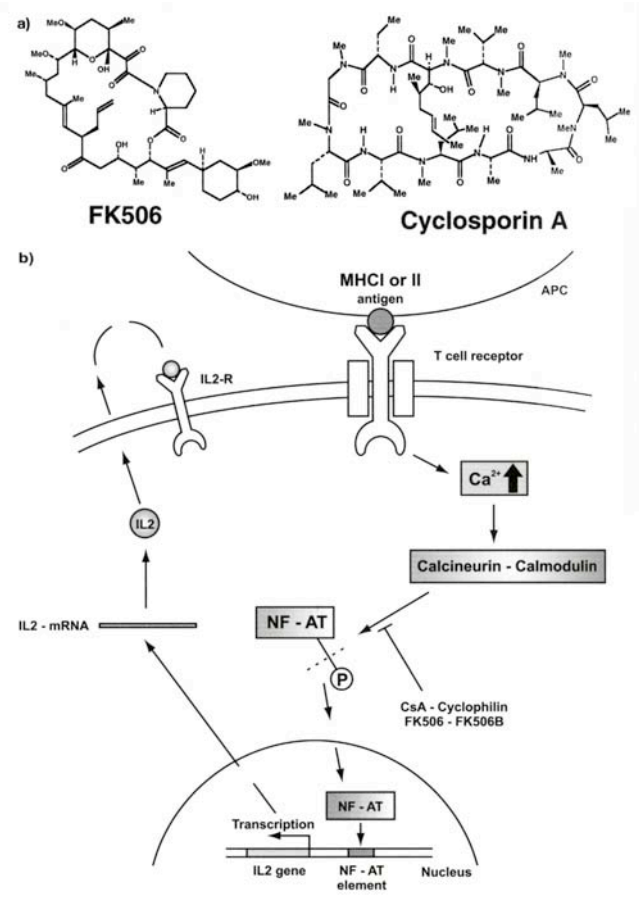
Protein Phosphatase 2A



Function and Analysis of Post-translational Protein Modifications

Protein Phosphatase 2B, Calcineurin

- Ser/Thr phosphatase
- Controlled by cellular Ca^{2+}
- Multimeric enzyme, binding to Ca^{2+}/calmodulin required for active enzyme

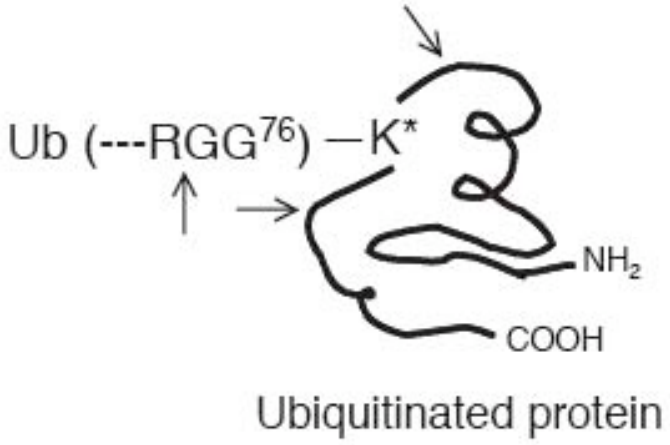


Ubiquitination

Function and Analysis of Post-translational Protein Modifications

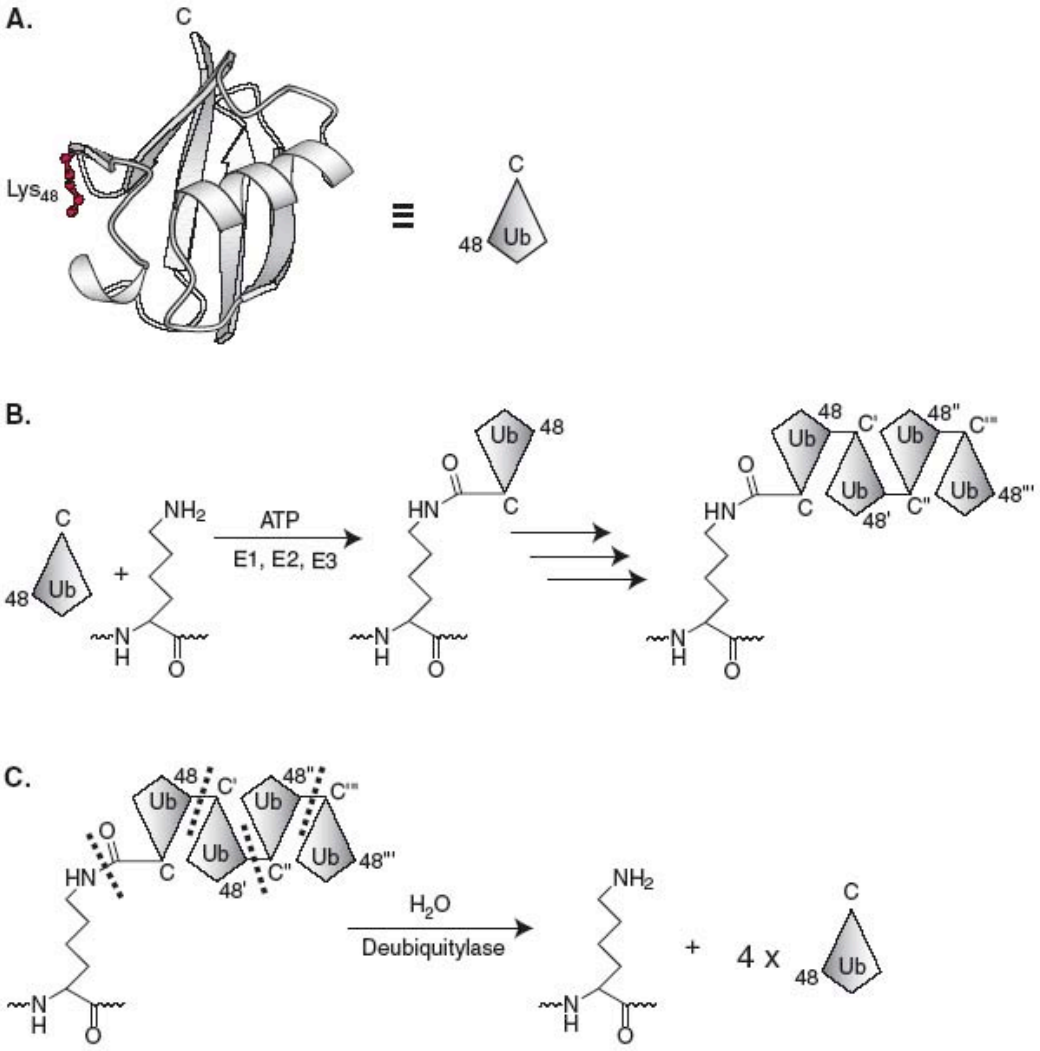
Ubiquitination

- Occurs in eukaryotes, not in prokaryotes
- Used for proteolytic destruction of specific proteins
- Ubiquitin is a small 76 residue protein
- Covalent attachment of multiple ubiquitin molecules to a protein substrate
- Degradation of the tagged protein by the 26S proteasome (ubiquitin is recycled)



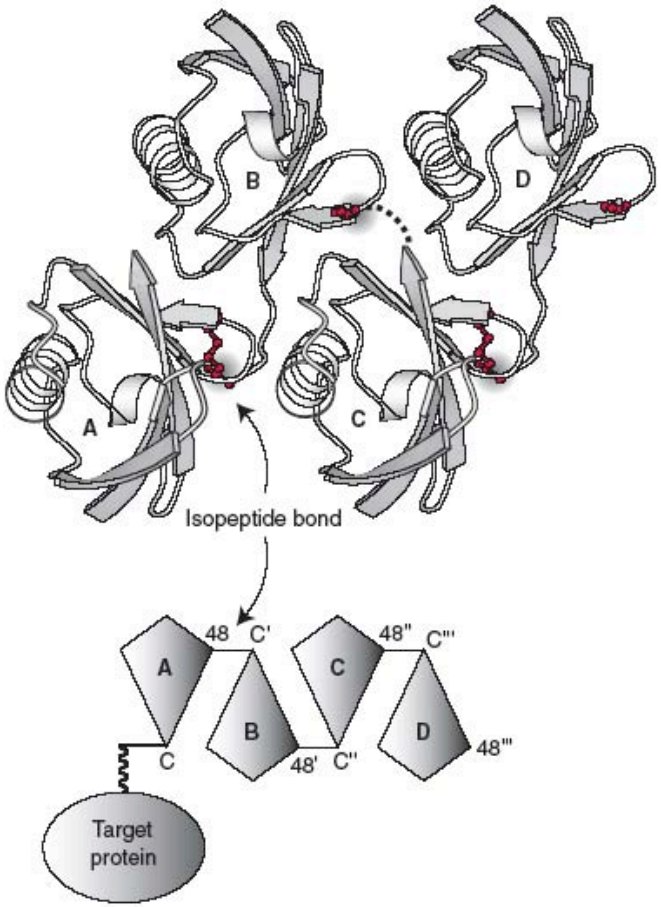
Ubiquitinated protein

Ubiquitination



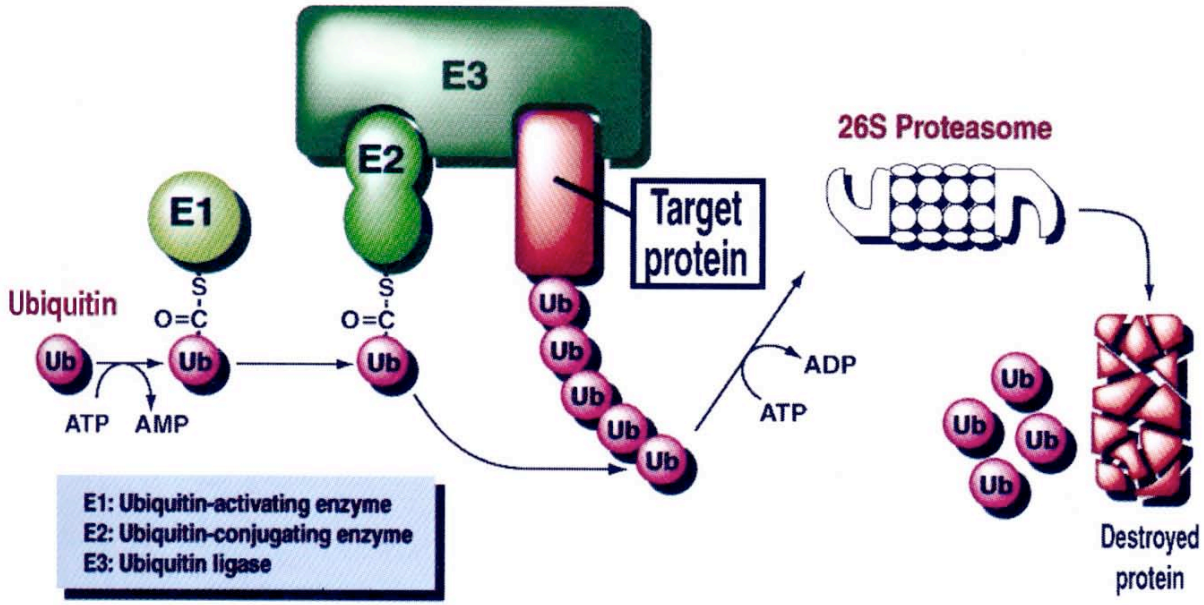
Function and Analysis of Post-translational Protein Modifications

Ubiquitination



Function and Analysis of Post-translational Protein Modifications

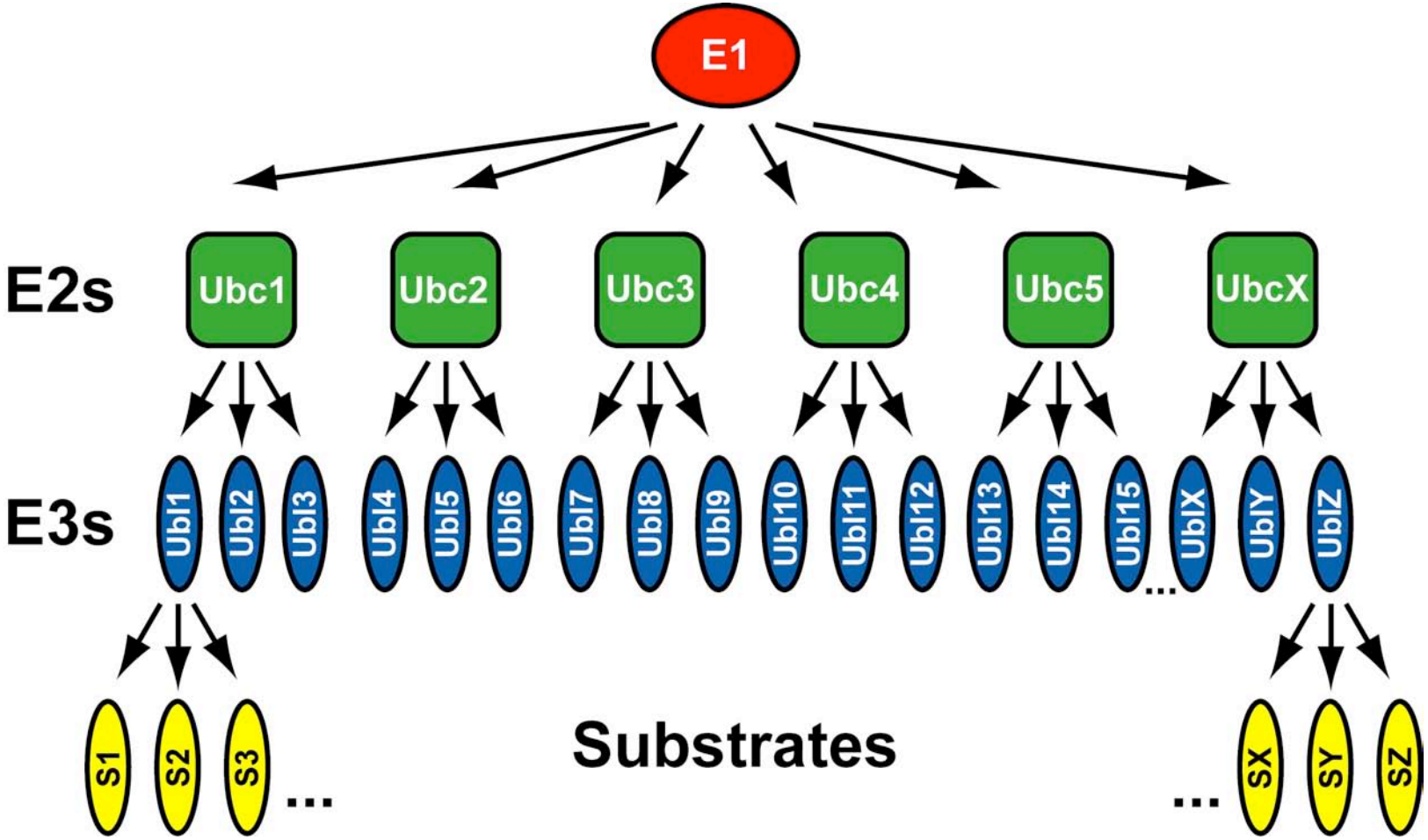
Ubiquitination Reaction Mechanism



Enzymes Involved in the Ubiquitination Process

- **E1:** only one known, consists of one or two polypeptidic chains, with M.W. 105 kDa per chain, very conservative protein
- **E2:** 5-12 proteins of this type known (depending on literature), homologous family
- **E3:** many and structurally unrelated, characterized by ultimate biological specificity, M.W. about 250 kDa
- **E4:** not known yet, but postulated as existing

Ubiquitination



SUMO (Small Ubiquitin-Related Modifier)

- SUMO does not have the Lys-48 found in ubiquitin
- SUMO does not make multi-chain forms
- SUMO-1,2,3 are the mammalian form
- SUMO-1: 101 amino acids, C-terminal Gly, 18% identical to ubiquitin

SUMO Substrates

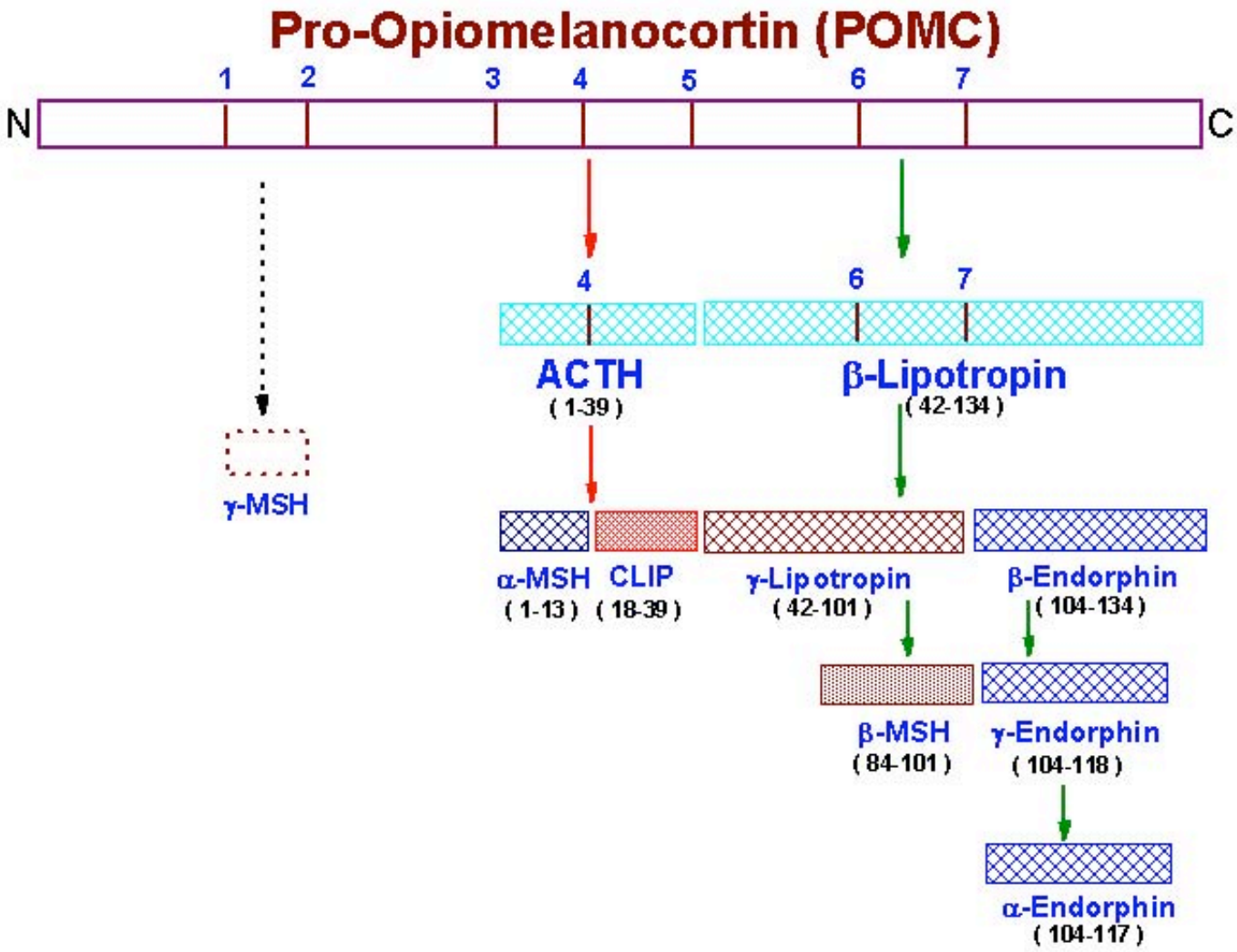
- Many of the known sumoylation substrates are nuclear proteins
  - p53
  - CREB
  - STAT1/4
  - GATA2, etc

Proteolytic Cleavage

Proteolytic Cleavage

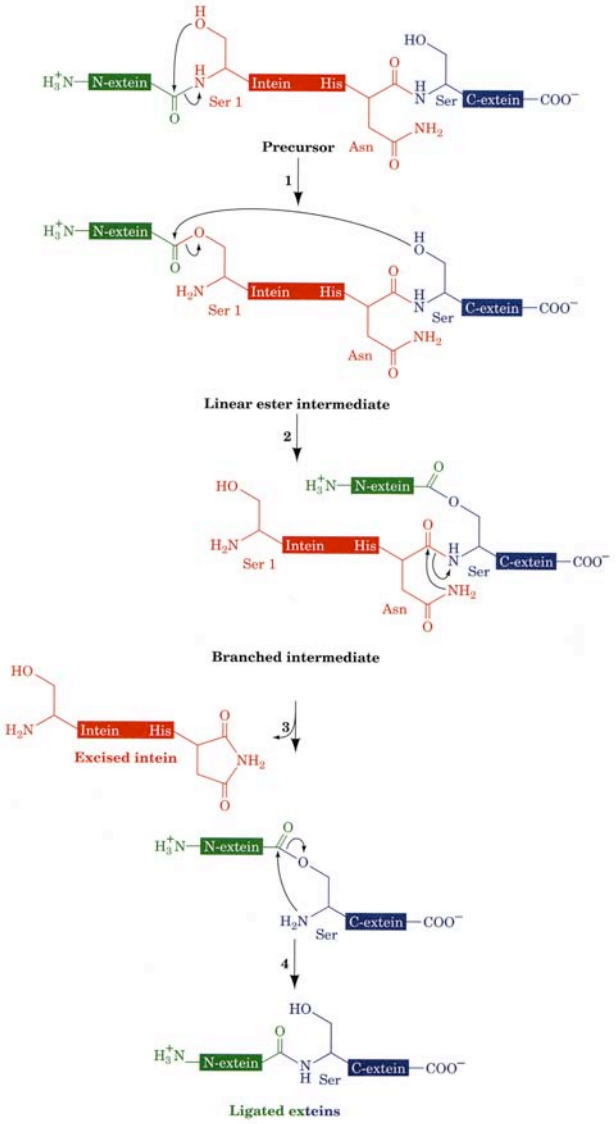
- Following translation, most proteins undergo proteolytic cleavage
- Removal of the initiation Met
- Many proteins are synthesised as inactive precursors
- E.g. pancreatic enzymes, enzymes involved in blood clotting = proproteins
- Activation occurs via removal of polypeptides

Peptide Preprohormone



Protein Splicing

- Internal protein sequence (intein)
  Excises itself from a surrounding
- External protein
- N- and C-terminal exteins are ligated
- Protein splicing occurs in bacteria and single-celled eukaryotes
- Exteins have no sequence similarity
- Inteins have conserved splice junctions:
  - Ser/Thr/Cys at the N-terminus
  - His-Asn/Gly dipeptide at the C-terminus
- inteins encode endonucleases that copy the intein gene into extein sequences
- Intein genes propagate themselves



Glycoproteins

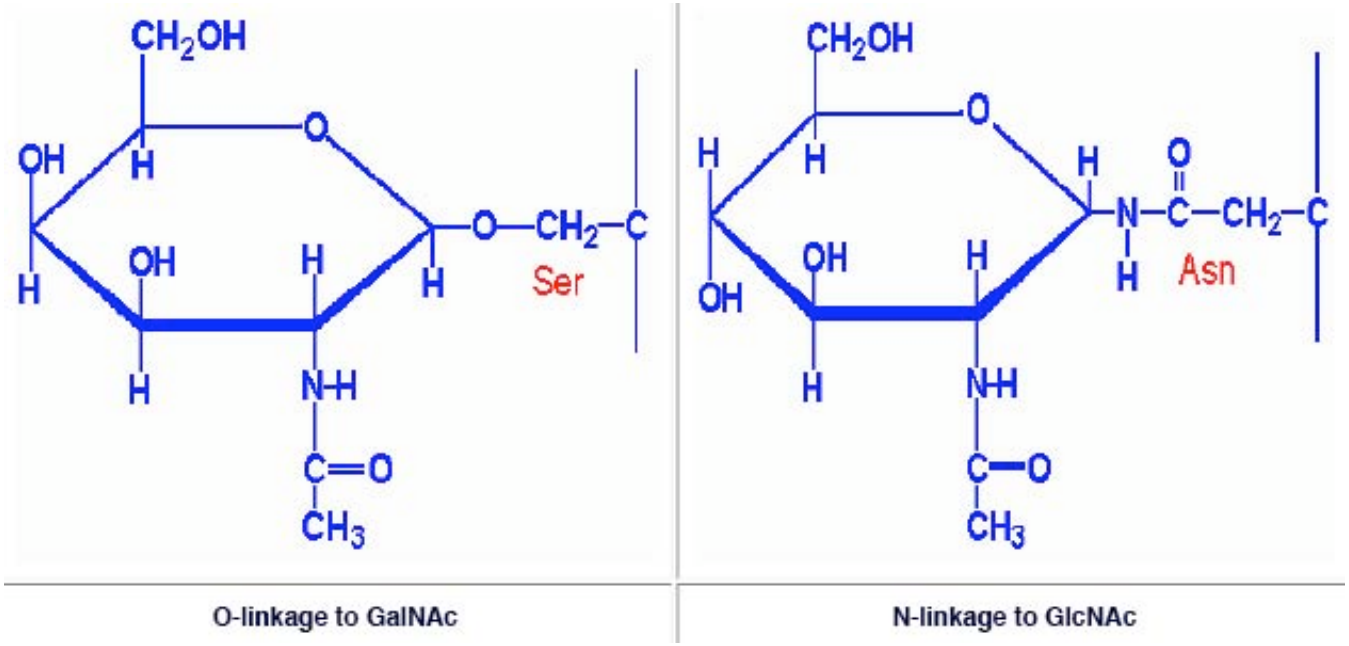
Function and Analysis of Post-translational Protein Modifications

Glycoproteins

- Most secreted, or plasma membrane bound proteins are glycosylated
- Extracellular part modified
- Cytosolic and/or nuclear proteins also found to be glycosylated
- Predominant sugars found in glycoproteins are
  - Glucose, galactose, mannose, fucose, GalNAc, GlcNAc, NANA

Glycoproteins

- Carbohydrates attached either O- or N-glycosidacally
- N-glycosidic linkage through amide of Asn
  - Carbohydrate attachment within consensus sequence
    N-X-S(T)
- O-glycosidic linkage is through -OH of Ser, Thr, or OH-Lys

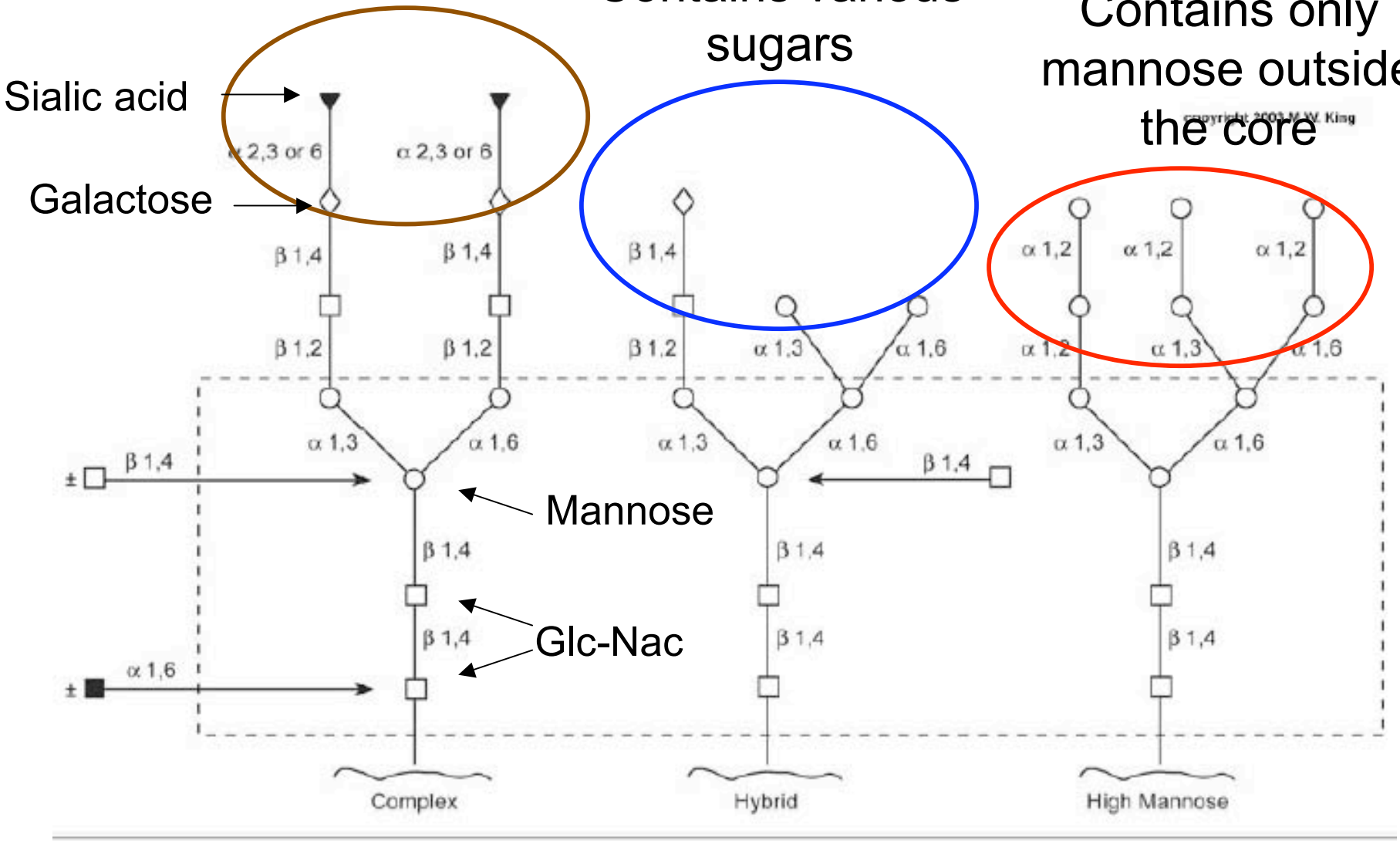


Glycoproteins

Similar to hybrid, contains sialic acid

Contains various sugars

Contains only mannose outside the core

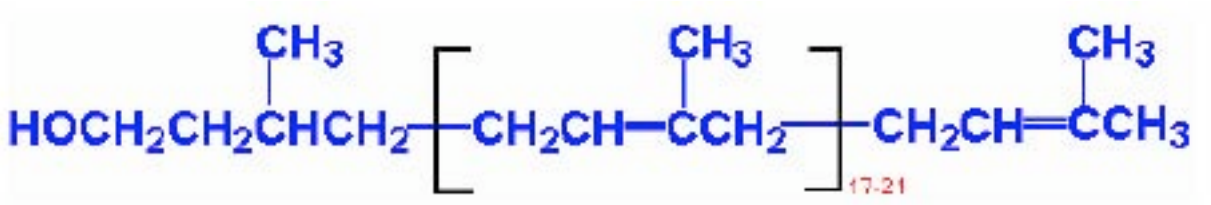


Glycoproteins

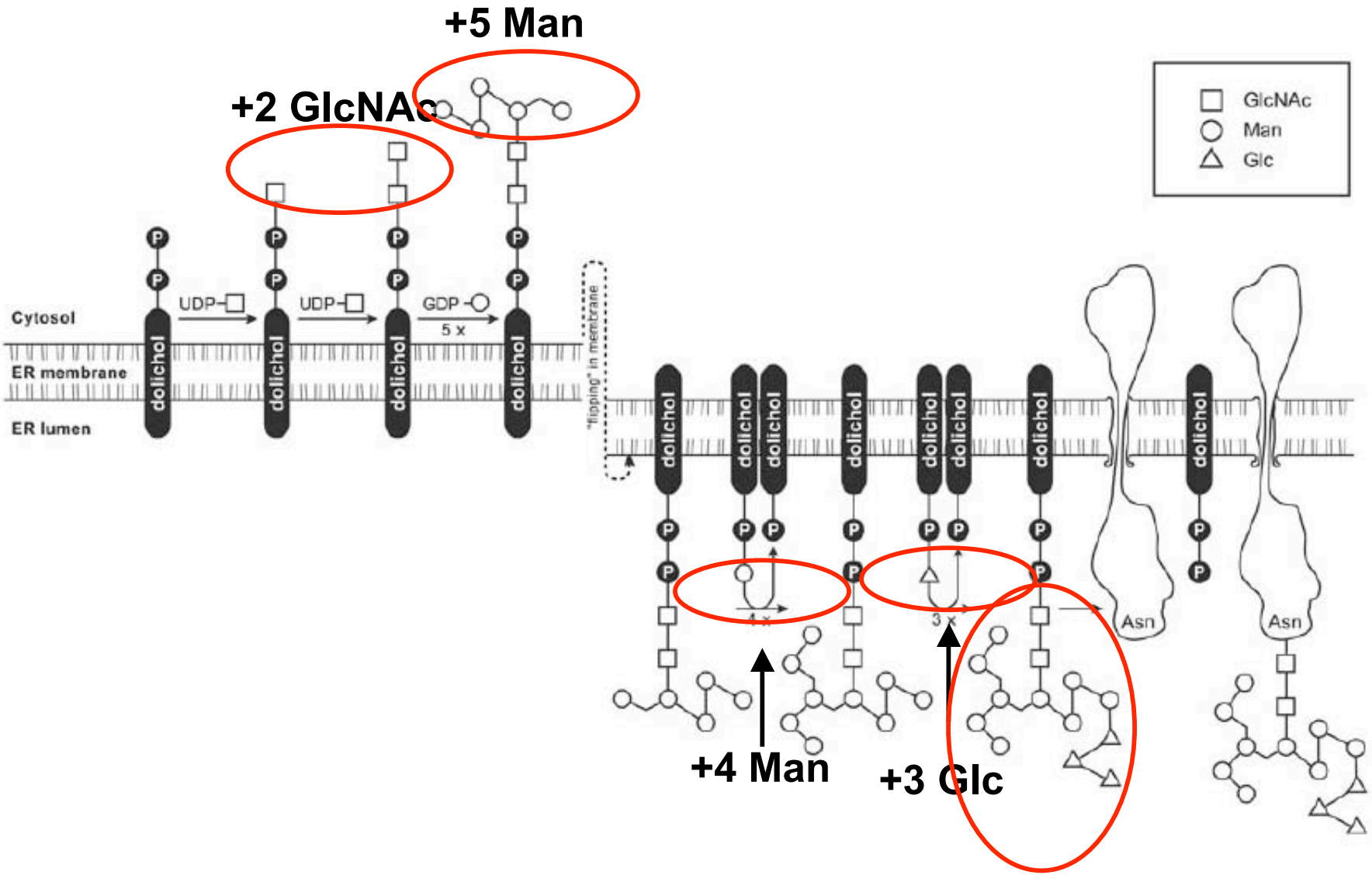
- Glycoproteins synthesised at rER
- Sugar attachment cotranslationally in the lumen of ER and continues in the Golgi for N-linked sugars
- O-linked sugars are attached post-translationally in the Golgi
- Sugars are activated by coupling to nucleotides
- Glc and GlcNAc are coupled to UDP
- Mannose is coupled to GDP

Glycoprotein Synthesis

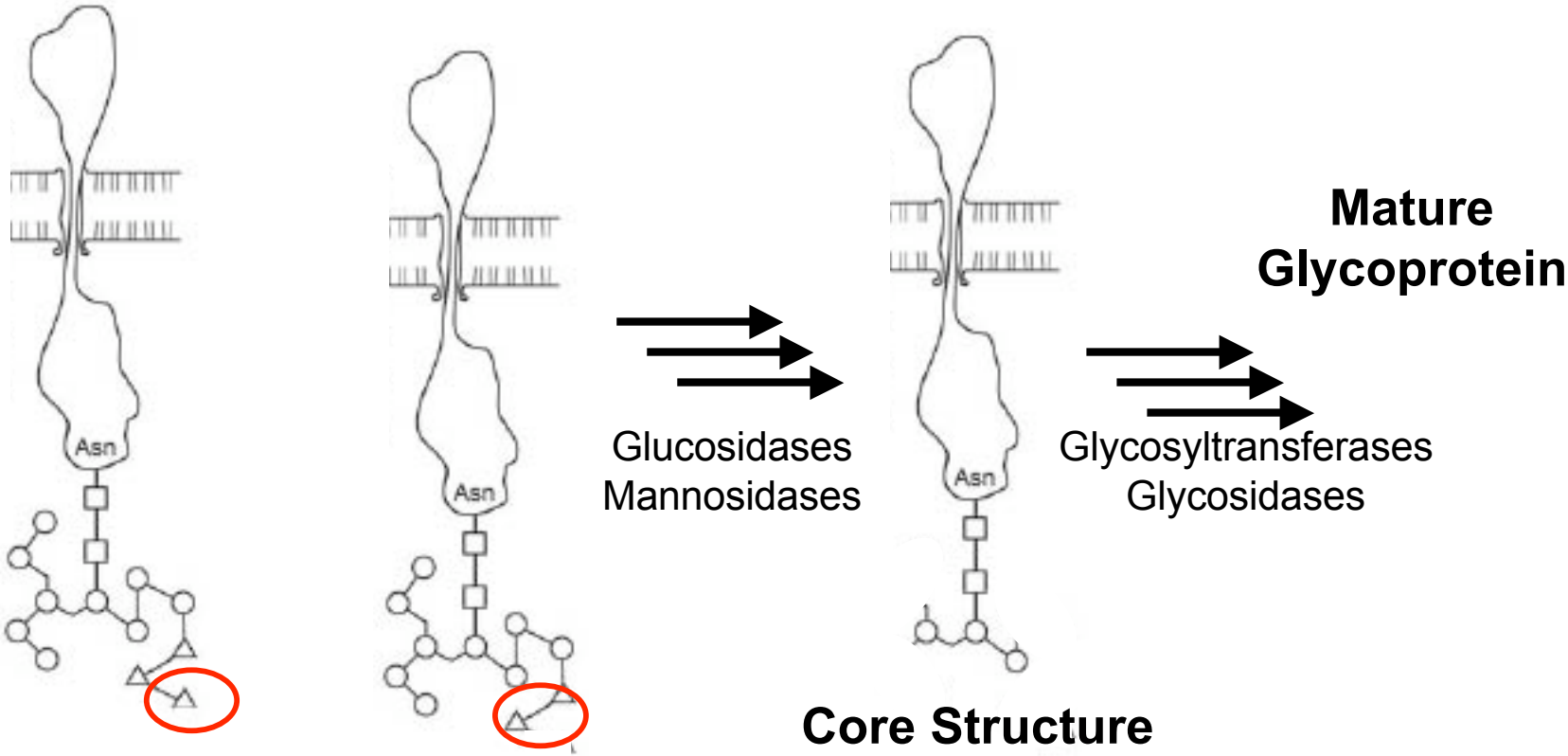
- N-linked glycoprotein synthesis requires the lipid intermediate dolichol phosphate



Glycoprotein Synthesis



Sugar Trimming



Clinical Significance of Glycoproteins

- AB0 blood group antigens
  - AB0 carbohydrates linked to lipids
  - AB0 associated with proteins occur in the serum = secreted form
  - Some individuals produce secreted AB0
  - Used in forensic medicine
- Dystroglycan
  - Laminin receptor
  - alpha-distroglycan serves as receptors for *Mycobacterium leprae* and other pathogens
- *Helicobacter pylori* attaches Lewis blood group antigen on the surface of gastric mucose
- Etc. …

Further Reading

- Walsh, C.T. (2005) Posttranslational Modifications of Proteins. Expanding Nature's Inventory. Roberts and Company Publishers.
- Krishna, R. G. and F. Wold (1998). Posttranslational Modifications. Proteins - Analysis and Design. R. H. Angeletti. San Diego, Academic Press. 1: 121-206.
- Wold, F., (1981) In vivo chemical modification of proteins (post-translational modification) Ann. Rev. Biochem. 50,, 783-814.