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Bi4025en
Molecular Biology

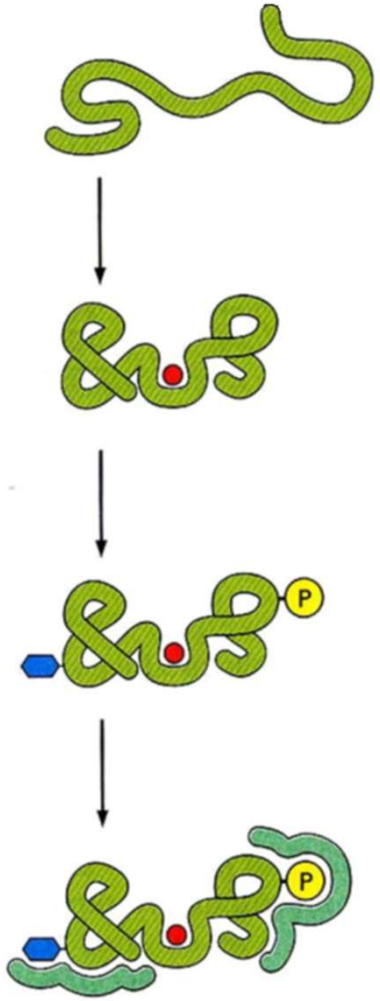
Mgr. Jiří Kohoutek, Ph.D.

Lecture 6

- Posttranslational processing of proteins.

Generation of maturated functuional protein

- Newly synthetized propeptide chain.
- Folding.
  - Noncovalent binding of cofactors.
- Covalent modifications
  - Glycolsylation, phosphorylation, acetylation, etc.
- Assembly
  - Noncovalent binding of other protein subunits/partners.
  - Maturated functional protein.



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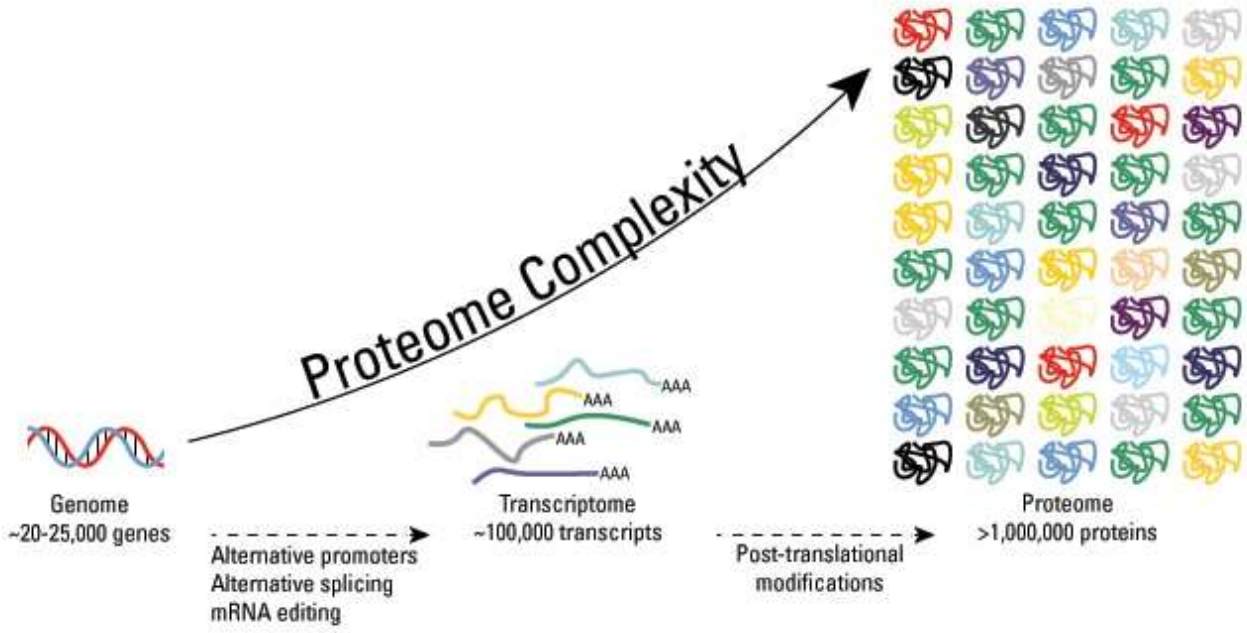
Post-translation modifications are key to proteome diversity

- Genome comprises 20,000 to 25,000 genes.
- Changes at the transcriptional and mRNA levels increase the size of the transcriptome relative to the genome.
- Myriad of different post-translational modifications exponentially increases the complexity of the proteome relative to both the transcriptome and genome .
- The proteome is estimated to encompass over 1 million proteins.



https://www.thermofisher.com/cz/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-post-translational-modification.html

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Interactions in an Organism Compose the Interactome

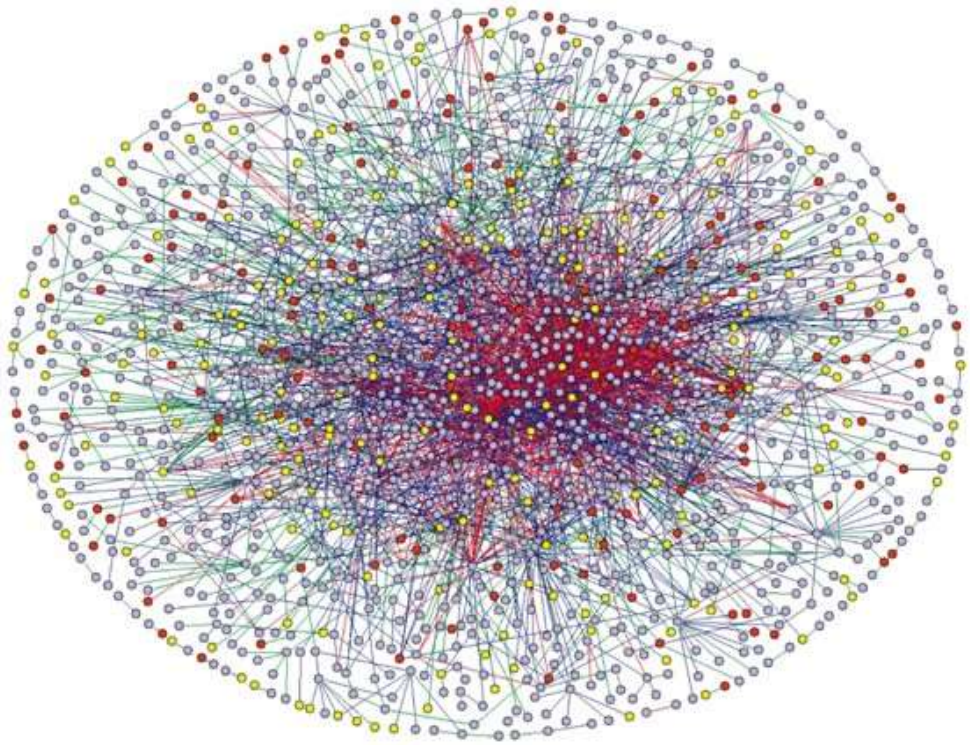


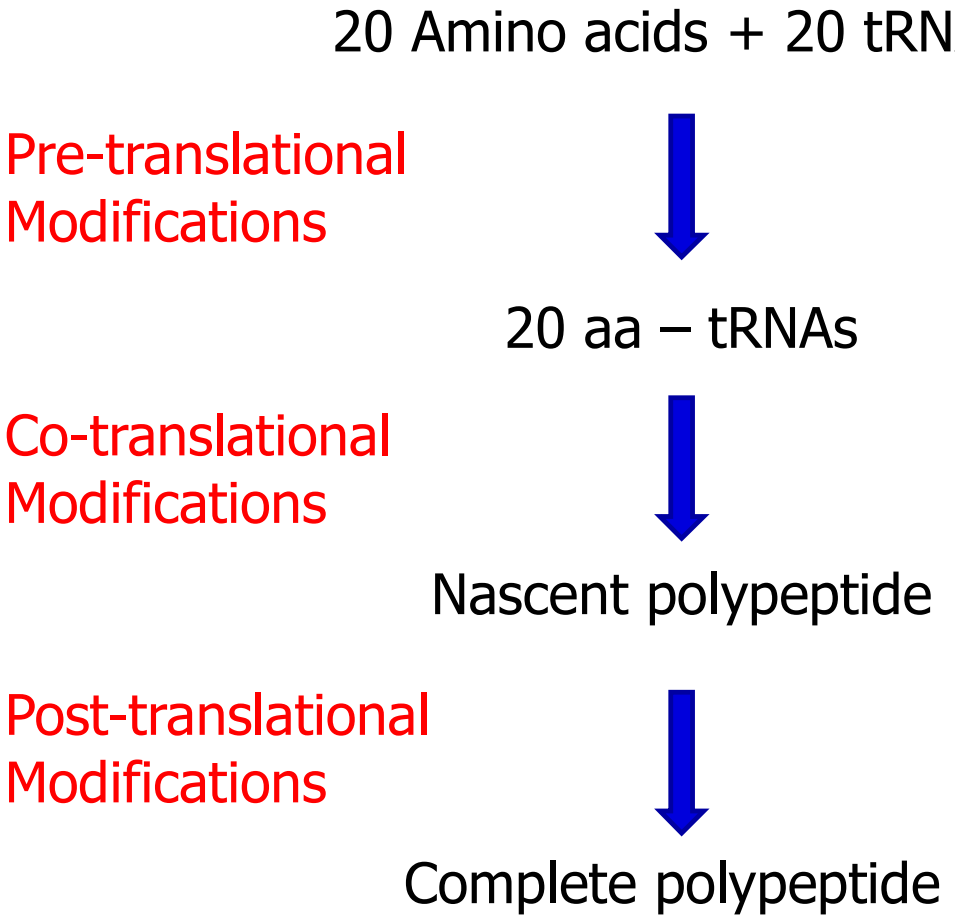
Figure 9-21
Introduction to Genetic Analysis, Tenth Edition
© 2012 W. H. Freeman and Company

Proteome:
- Complete set of proteins produced by genetic material of an organism.

Interactome:
- Complete set of protein interactions in an organism.

https://slideplayer.com/slide/5746723/

Protein synthesis in three levels of modifications



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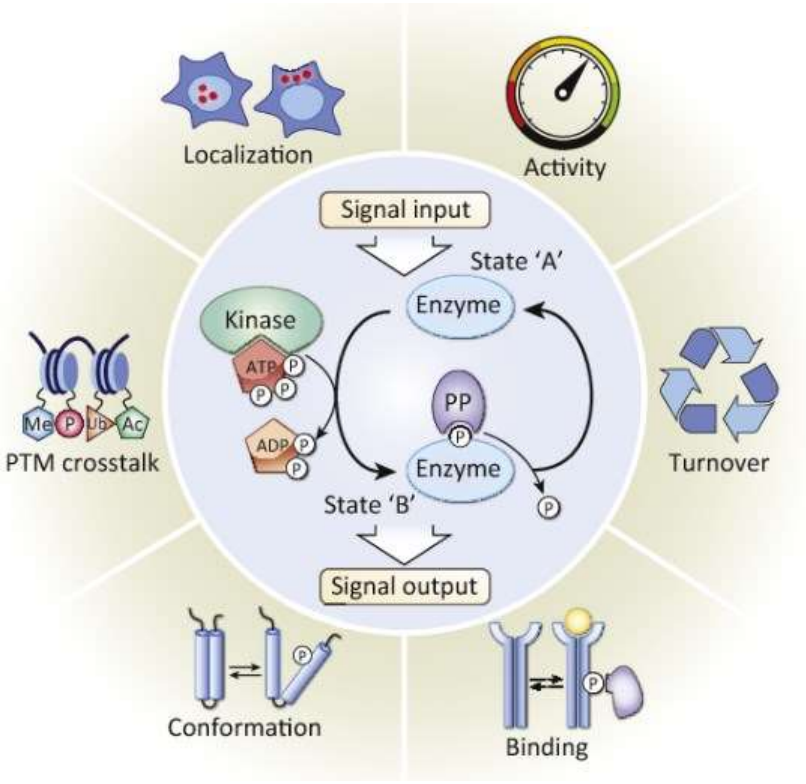
Co-translational and Post-translational modifications

- Post-translational modifications influence protein:
  - Charge
  - Conformation
  - Size

- Effects of post-translational modifications on protein:
  - Stability
  - Biochemical activity
  - Protein targeting (localization)
  - Protein signaling (protein-protein interaction)



CELL, VOLUME 108, ISSUE 4, P557-572, FEBRUARY 22, 2002

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Protein synthesis in three levels of modifications

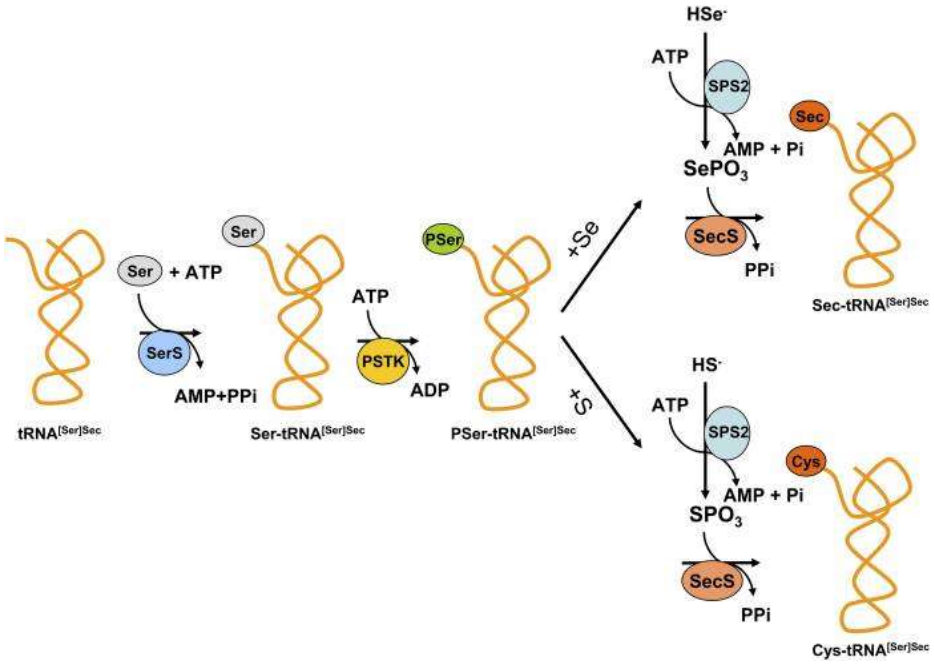
Levels	Modifications
1. Pre-translational	a) Selenocysteine tRNA b) Non-standard/natural amino acid tRNA - dansylalanine (fluorescent reporter) - phosphoserine, phosphothreonine, and phosphotyrosine (production of translational proteins in *E. coli* with Eukaryotic post-translational modifications)
2. Co-translational	a) Signal sequence celavage b) N-Glycosylation
3. Post-translational	a) Phosphorylation b) Acetylation c) O-Glycosylation d) Methylation e) Lipidation f) Proteolytic cleavage g) Protein splicing h) Ubiquitination, Sumoylation

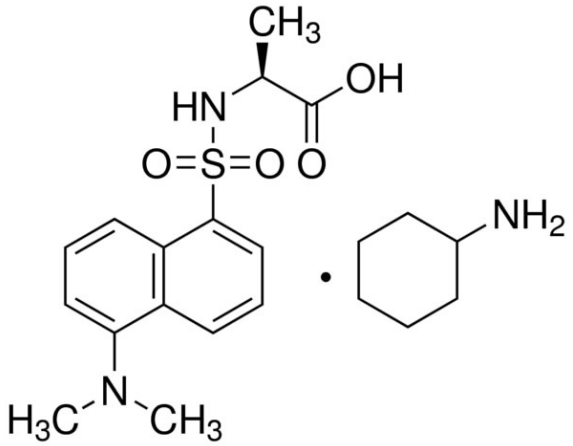
https://slideplayer.com/slide/12777240/

- Pre-translational modifications

Pre-translational modifications

- Selenocysteine-tRNA is initially charged with serine. Then the attached serine is enzymatically modified to form selenocysteine.





- Alanine - conjugated to the fluorophore 5-(dimethylamino)naphthalene-1-sulfonyl is Dansyl-L-alanine. The unnatural amino acid is incorporated into the proteins by use of a mutated aminoacyl-tRNA synthetase specific for dansyl-L-alanine.

Adv Nutr. 2011 Mar; 2(2): 122–128.

https://www.sigmaaldrich.com/CZ/en/product/sigma/d0125

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- Co-translational modifications

N-terminal signal sequence

N-terminal signal sequences

- N-terminal signal sequence mediates targeting of nascent secretory and membrane proteins to the endoplasmic reticulum (ER) in a signal recognition particle (SRP)-dependent manner.
- Signal sequences have a tripartite structure, consisting of a hydrophobic core region (h-region) flanked by an n- and c-region.
- The c-region contains the signal peptidase (SPase) consensus cleavage site. Usually, signal sequences are cleaved off co-translationally.

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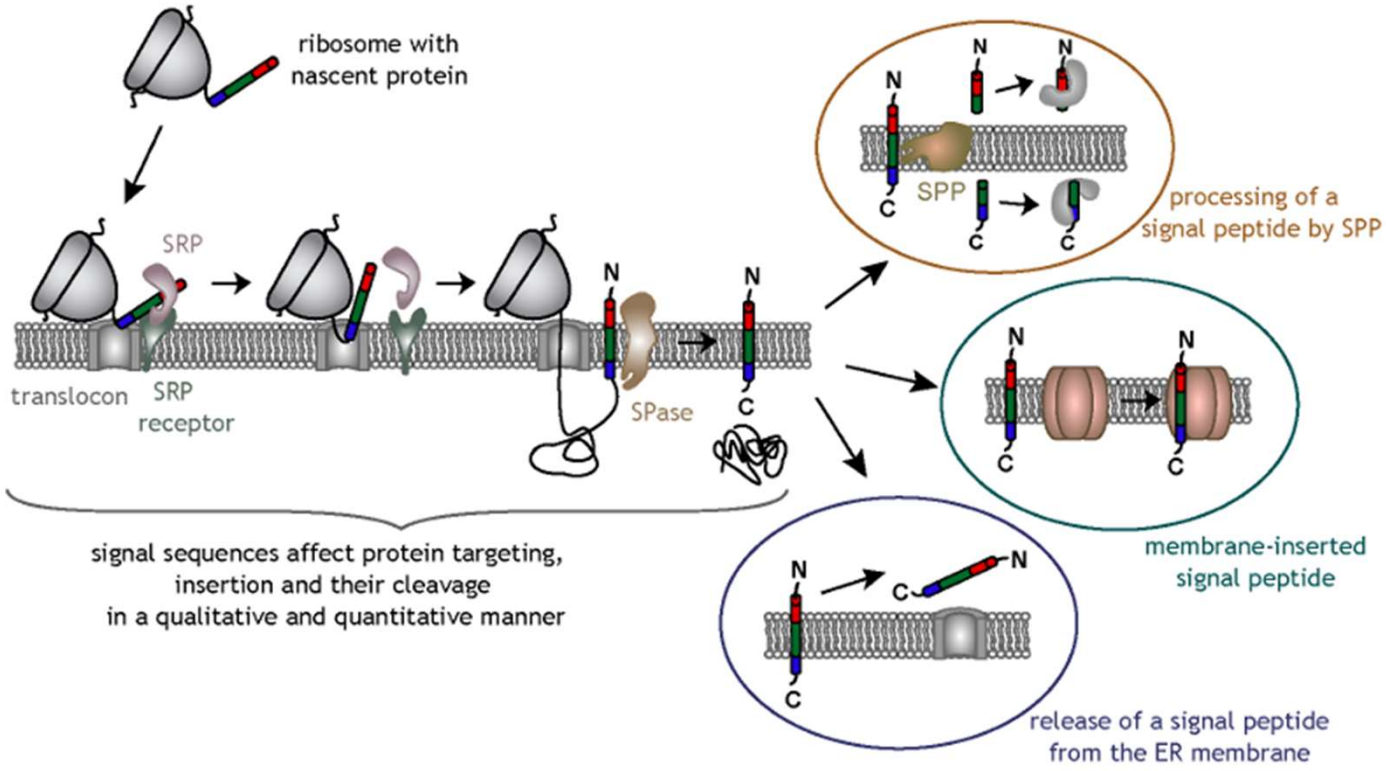
N-terminal signal sequence



http://www.signalpeptide.de/

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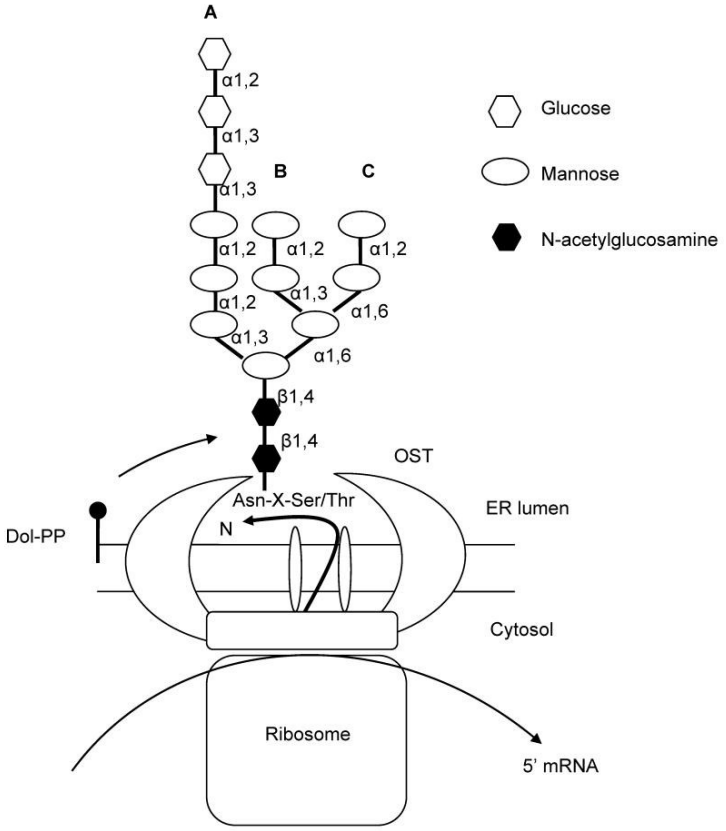
Protein glycosylation

Protein glycosylation

- One of the major post-translational modifications of many cell surface and secreted proteins.
- Significant effects on protein folding, conformation, distribution, stability and activity.
- Selection of sugar ranges from simple monosaccharide to highly complex branched polysaccharide changes.
- The sugar residues are often used as molecular flags or recognition signals to other cells than come in contact with them.



https://www.thermofisher.com/cz/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-post-translational-modification.html

Adv Neurobiol. 2014; 9: 47–70.

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Protein glycosylation

- Two types of glycosylation:
  - N-linked - occurs on asparagine (N) residues within an N-X-S or N-X-T sequence – co-translational.
  - O-linked occurs on the side chain hydroxyl oxygen of either serine or threonine residues determined not by surrounding sequence, but by secondary and tertiary structure– post-translational.

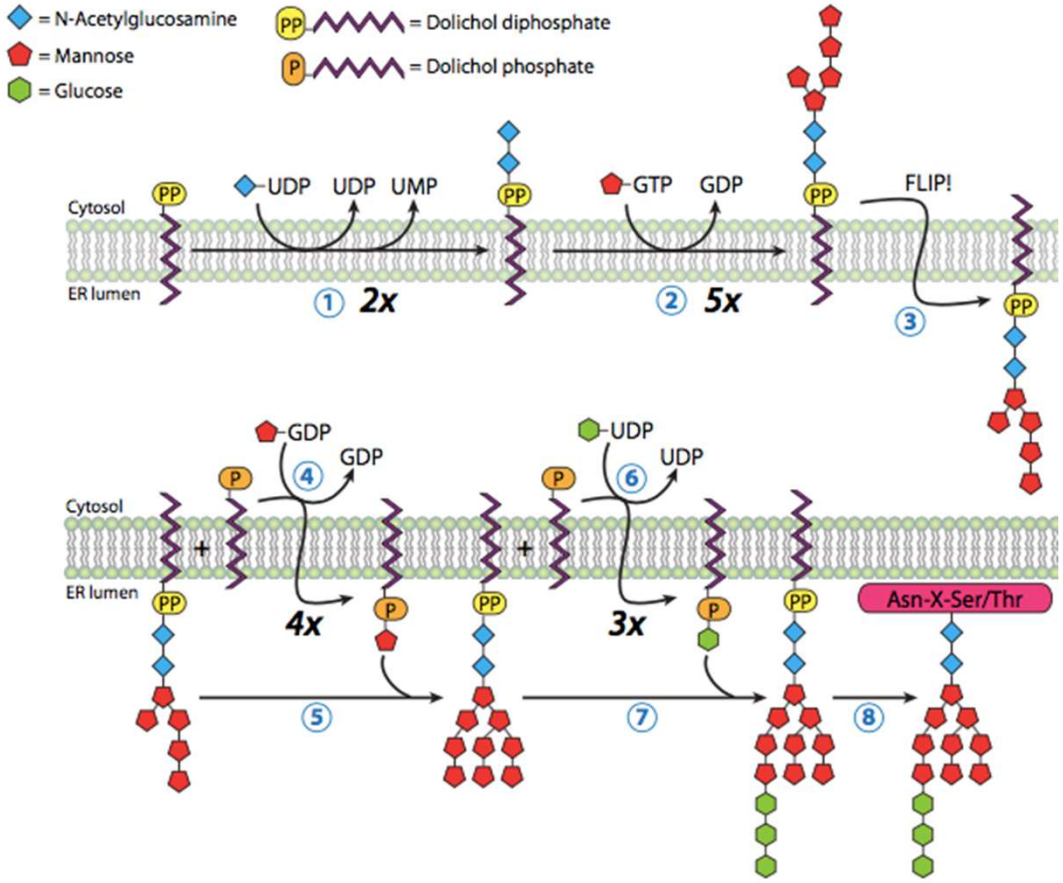
- N-linked glycosylation begins with a "tree" of 14 specific sugar residues that is then pruned and remodeled, but remains fairly large.

- O-linked glycosylation is based on sequential addition of individual sugars, and does not usually extend beyond a few residues.

- Glycosylation, both of which require import of the target polypeptide into first in the ER, but O-linked glycosylation is achieved in the Golgi apparatus.

https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

N-glycosylation



- N-glycosylation begins before a protein translation, as the dolichol pyrophosphate oligosaccharide, in the ER without being triggered by translation or protein entry.
- Lipid-glycan are bound Asp by the multisubunit oligosaccharyltransferase (OST).
- Glycosyltransferase catalyzes each step.
- Sugar substrates are sugar nucleotides, not isolated sugar molecules.

https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

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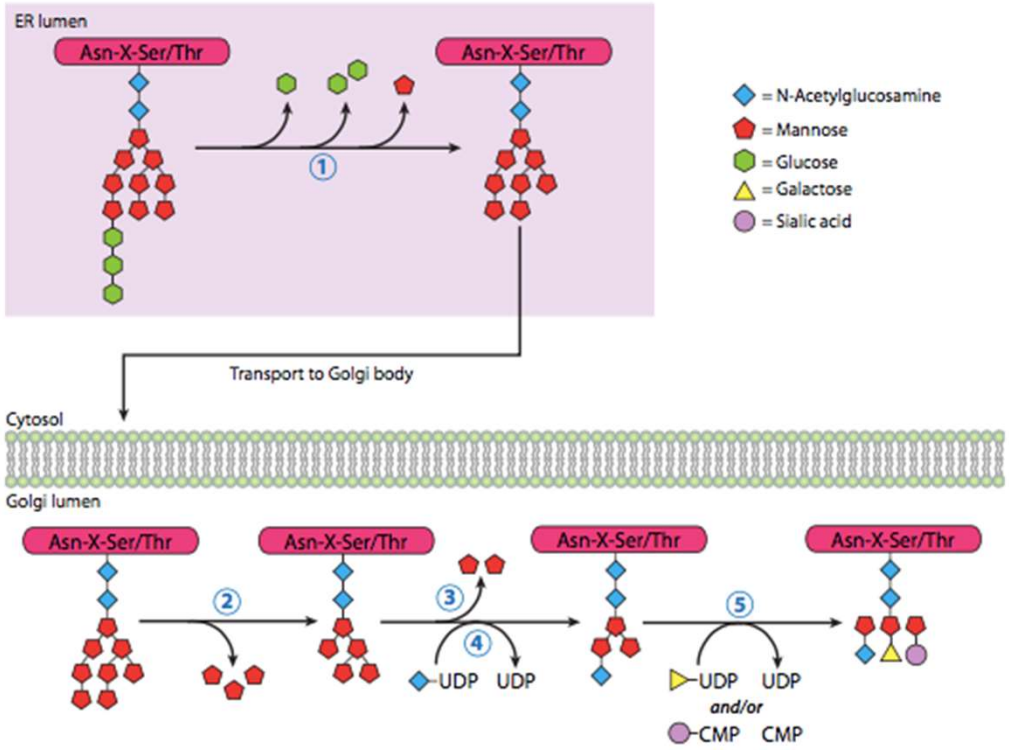
N-glycosylation



- The glucose residues are sequentially removed by two α-glucosidases (α-Glc I–II) and an initial Man residue is removed by the ER α-mannosidase (ER α-Man).
- After a quality-control checkpoint, the glycoprotein moves to the Golgi apparatus for additional trimming by α-mannosidase I and II (α-Man I–II) and further glycan modifications.
- A cis-to-trans distribution of glycosidases and transferases facilitates further processing by these carbohydrate-modifying enzymes to create a plethora of N-glycoforms that often terminate with sialic acid moieties.

Nature Reviews Nephrology, 2019, volume 15, pages 346–366.
https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

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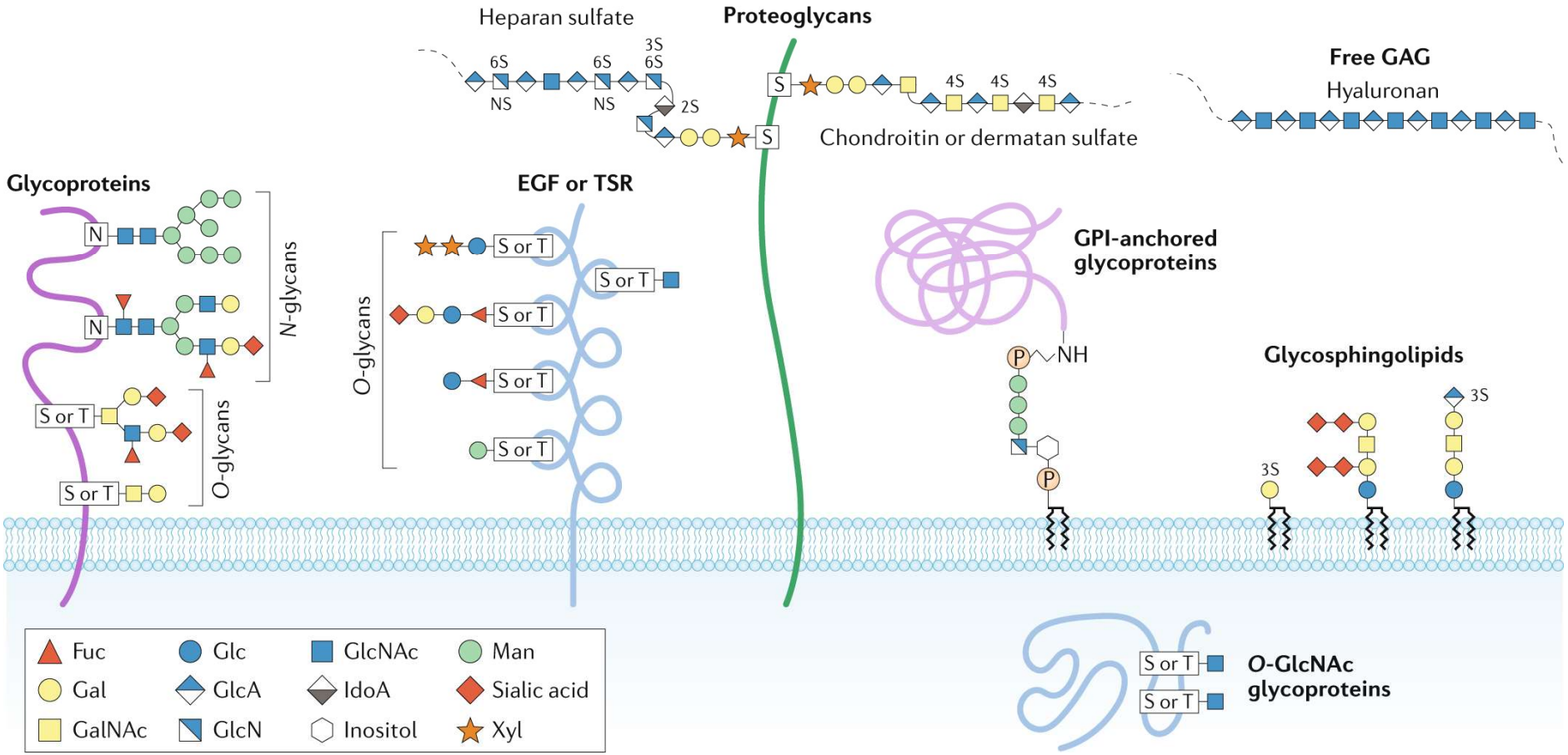
Glycosyalation



Nature Reviews Nephrology, 2019, volume 15, pages 346–366.

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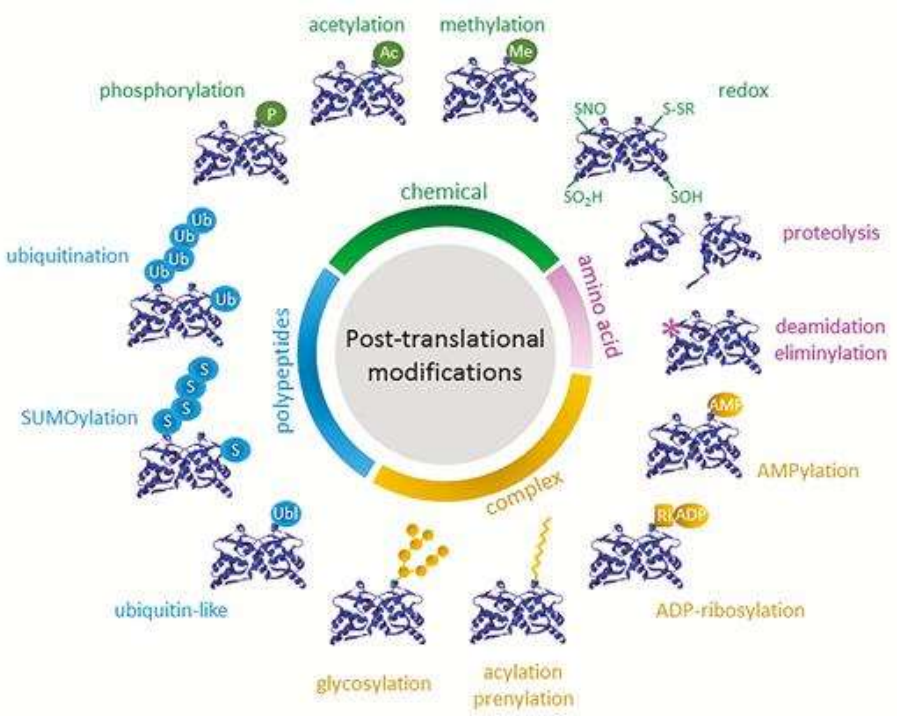
- Post-translational modifications

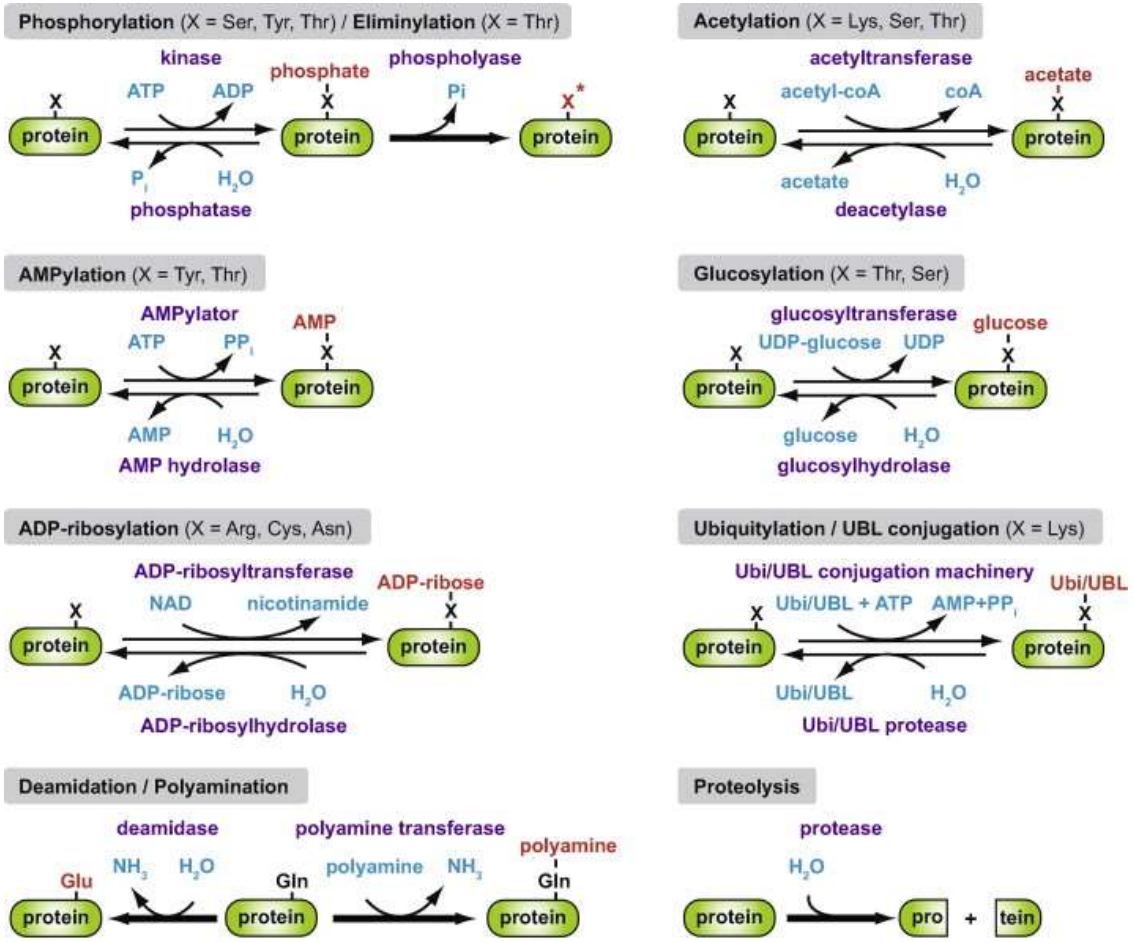
Post-translational modifications – PTMs

- More than 300 PTMs are currently known.
- Addition of chemical groups (e.g. phosphate or acetate).
- Addition of complex molecules (e.g. carbohydrates or lipids).
- The covalent linkage of small proteins (like ubiquitin and ubiquitin-like proteins (UBLs)).
- Cleavage and Splicing.
- Modification of specific amino acids (like deamidation or eliminylation).



Journal of Experimental Botany, Volume 69, Issue 19, 31 August 2018, Pages 4499–4503

Post-translational modification are carried out by enzymes



FEBS Letters, Volume 584, Issue 13, 2 July 2010, Pages 2748-2758

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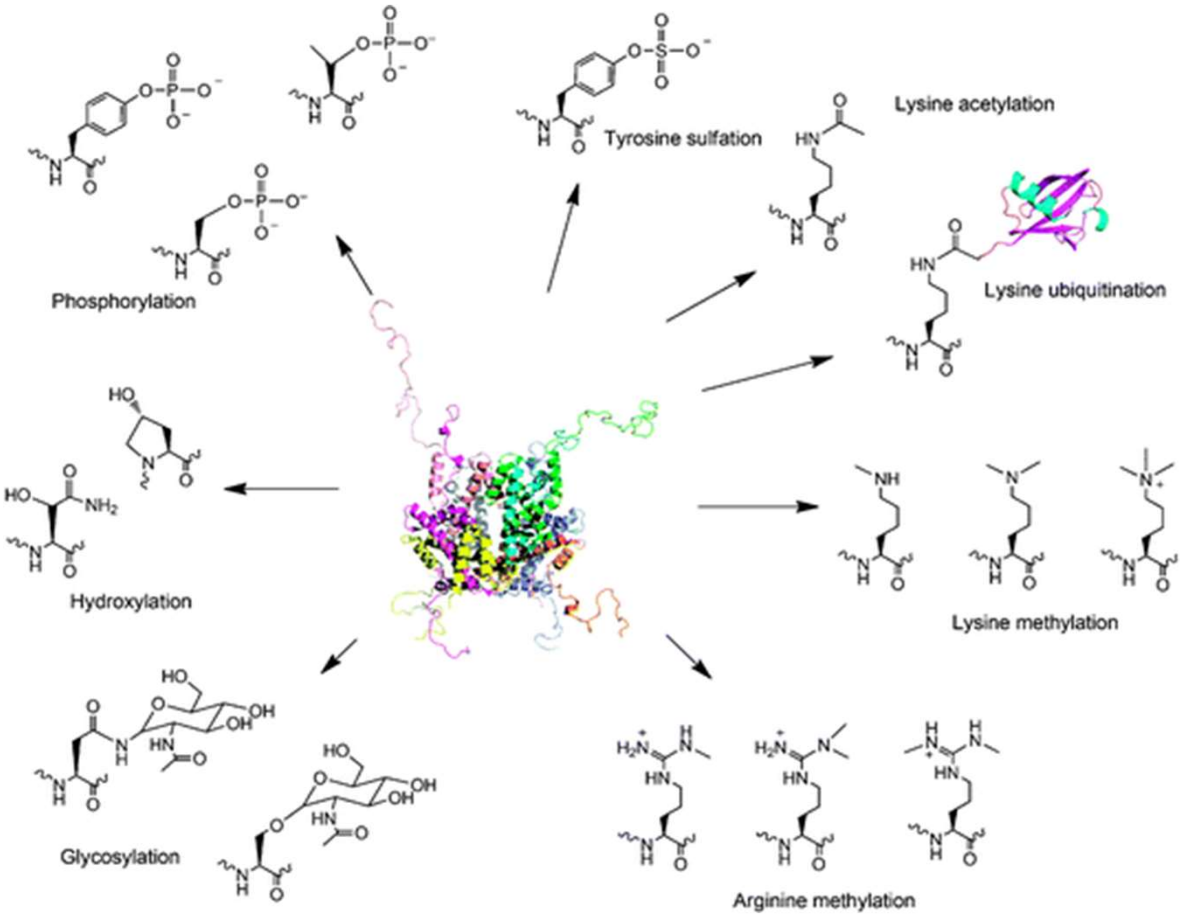
Post-translational modification are carried out by enzymes



- Phospho kinase
- Tyrosine phosphatase
- Ubiquitin ligase
- Deubiquitylase/deneddylase
- AMPylator
- ADP-ribosyl transferase
- Acetyltransferase
- Deacetylases
- Methyl transferase
- Demethylase
- Etc…

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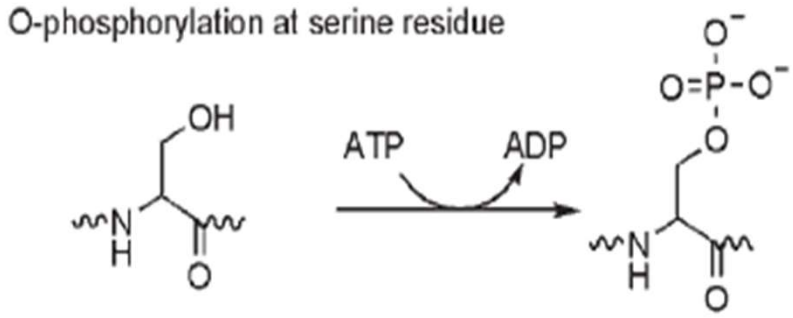
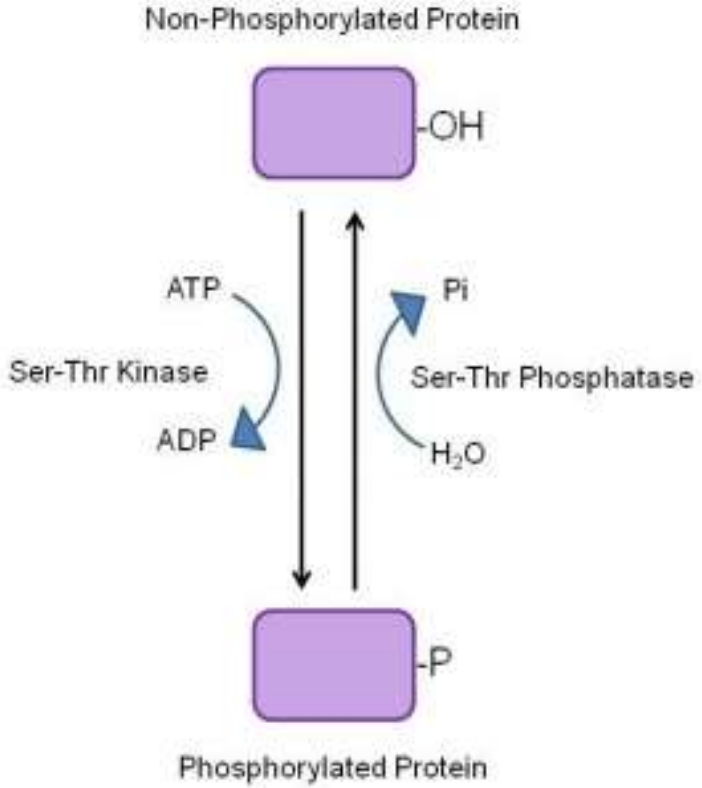
Phosphorylation

- Principally on serine, threonine or tyrosine residues.
- Also known as Phospho regulation.
- Critical role in cell cycle, growth, apoptosis and signal transduction pathways.





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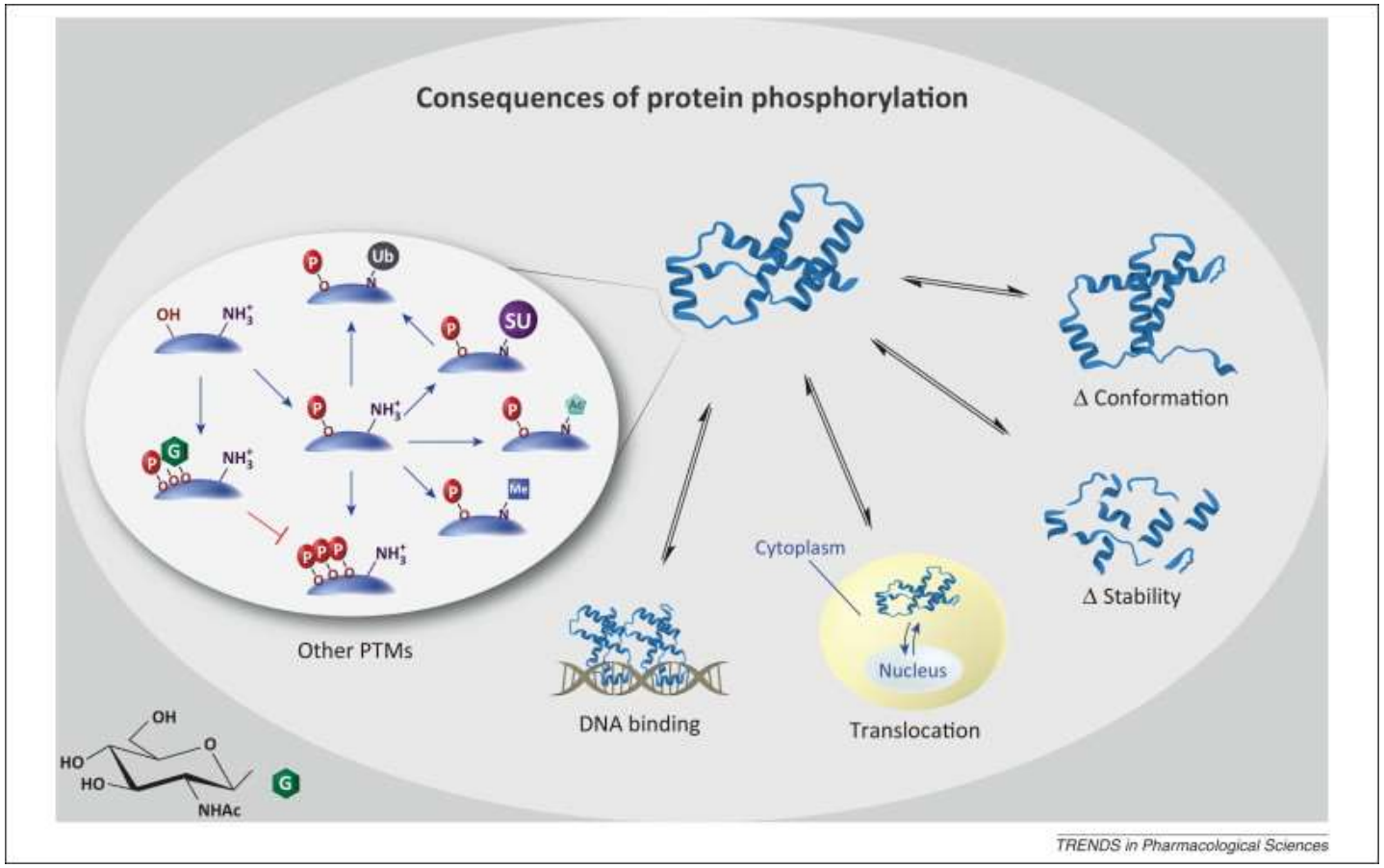
Phosphorylation affects protein capability



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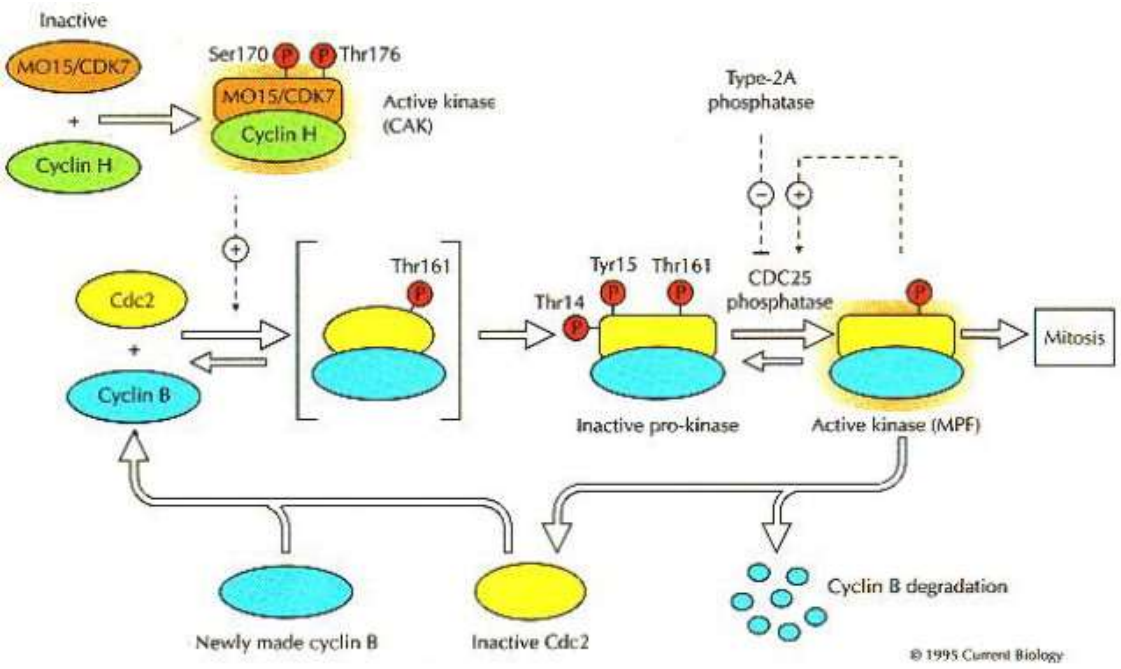
Phosphorylation



- As cyclin B is synthesized during S and G2 phases of the cell cycle, it associates with Cdc2.
- Active CDK-activating kinase (CAK) phosphorylates Cdc2 at threonine 161, stabilizing its association with cyclin B
- Wee1 and Myt1 phosphorylates inhibitory sites, threonine 14 and tyrosine 15.
- Final activation is triggered by dephosphorylation of Thr14 and Tyr15 by CDC25 phosphatase.

Current Biology, Volume 5, Issue 1, January 1995, Pages 40-42

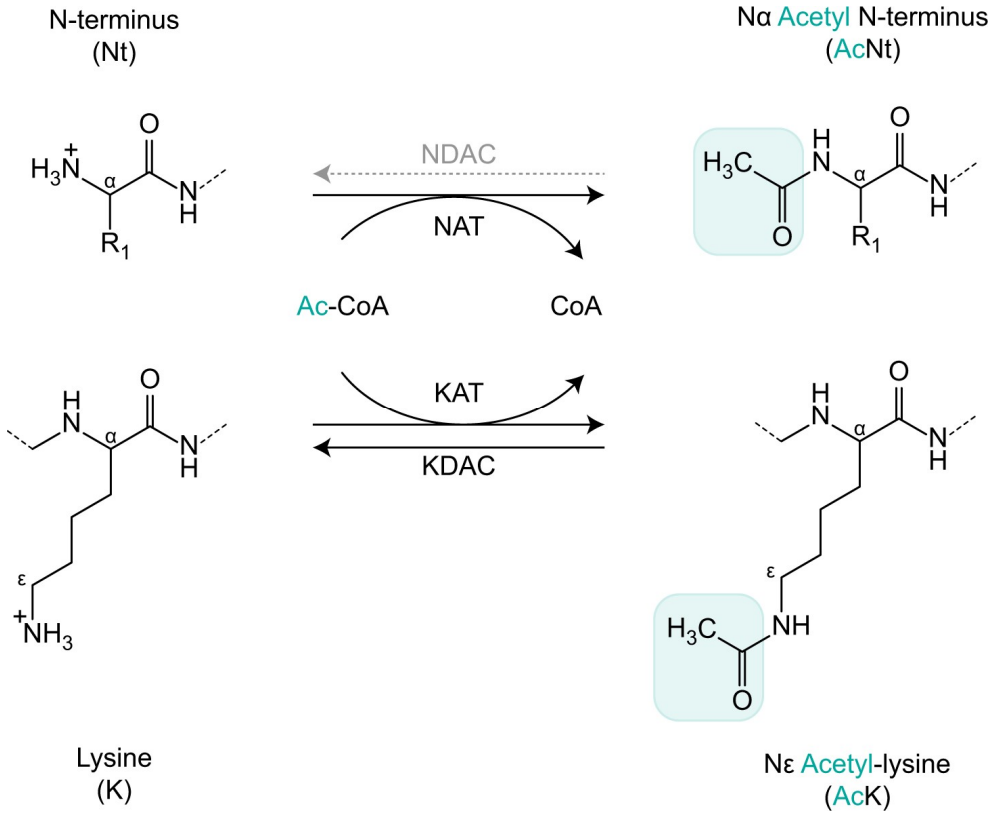
Acetylation

- Acetylation is one of the major post-translational protein modifications in the cell, with manifold effects on the protein and the metabolome level.
- Covalent attachment of an acetyl group eliminates the positive charge (+) of the amino group, thus affecting local electrostatic properties.
- These reactions are catalyzed by various N-terminal and lysine acetyltransferases.
- Involved in regulation of transcription factors, histones, effector proteins, molecular chaperons and cytoskeletal proteins.

Experimental & Molecular Medicine, 2018, volume 50, pages1–13.

Acetylation

- N-terminal acetyltransferases (NAT) transfer an acetyl group (CH_3O) to an α-amino group of protein N-termini.
- Acetyltransferases (KATs) catalyze the transfer of an acetyl group (CH_3O) to the ε-amino group of lysine (K) side chains.
- NATs and KATs use acetyl-CoA (Ac-CoA) as a donor of acetyl group.
- In the case of lysine acetylation, the acetyl moiety may be removed by lysine deacetyltransferases (KDACs), making it a reversible protein modification – deacetylation.



Experimental & Molecular Medicine, 2018, volume 50, pages1–13.

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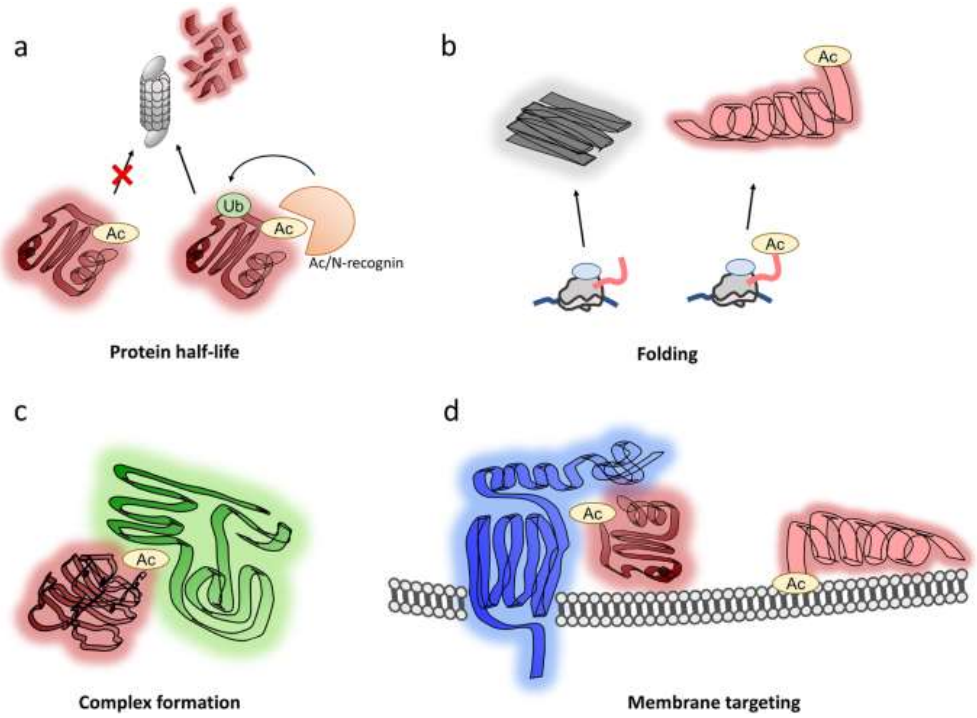
N-terminal Acetylation

- N-terminal acetylation (Nt-acetylation) is a common protein modification, affecting an estimated 80% of all human protein species to a varying extent.
- Nt-acetylation has many functions in the cell.
  - Targets proteins for polyubiquitination and proteasomal degradation or protects against such degradation.
  - Proper folding of some proteins.
  - Protein–protein interactions.
  - Targets some proteins for membranes.



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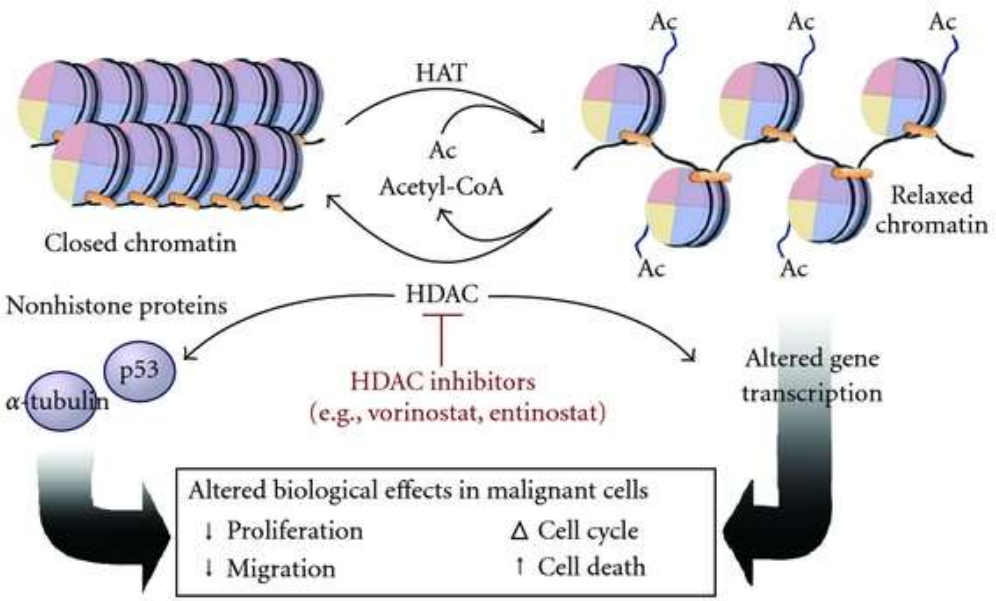
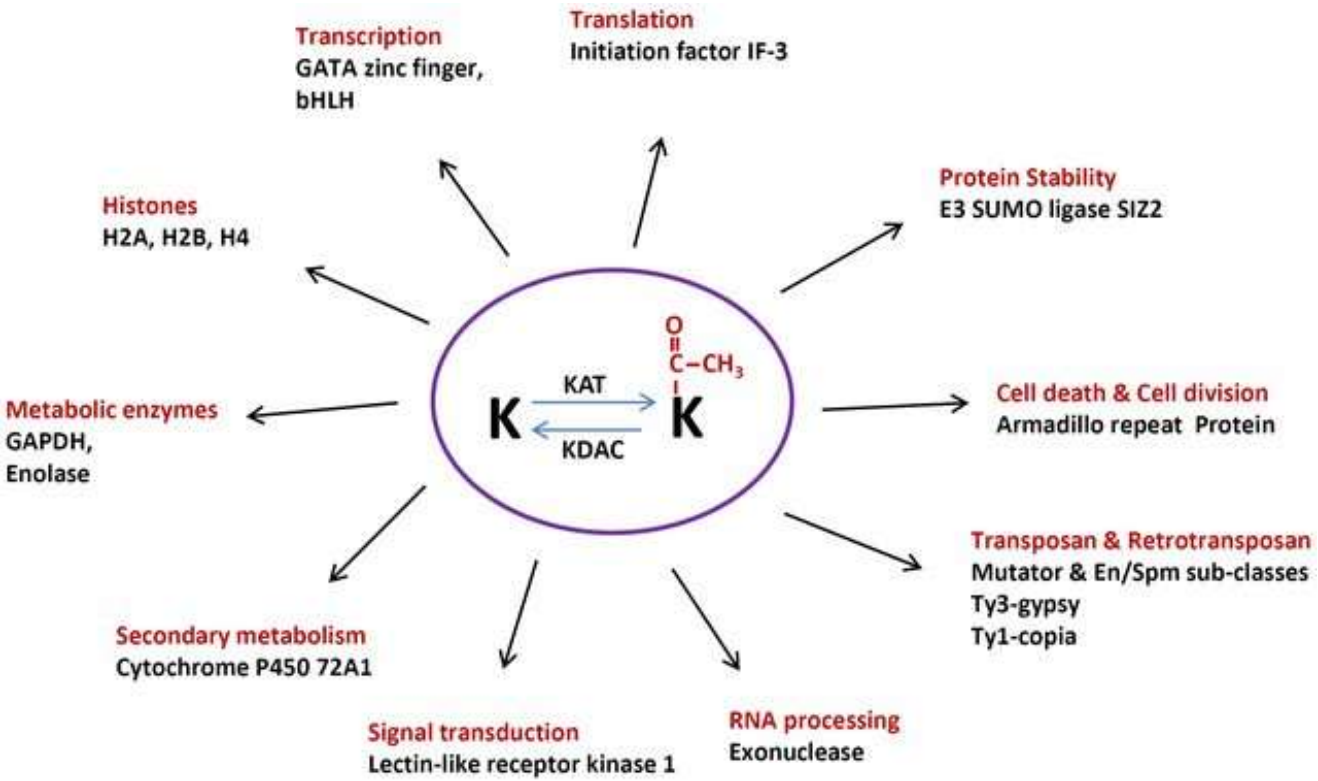
Lysine Acetylation



- Histone acetylation and deacetylation are essential parts of gene regulation.
- These reactions are typically catalyzed by enzymes with "histone acetyltransferase" (HAT) or "histone deacetylase" (HDAC) activity.
- Acetylation of histones alters accessibility of chromatin and allows DNA binding proteins to interact with exposed sites to activate gene transcription and downstream cellular functions.

https://www.wikiwand.com/en/Histone_acetylation_and_deacetylation

Lysine Acetylation



PLoS ONE 9(2): e89283. doi:10.1371/journal.pone.0089283

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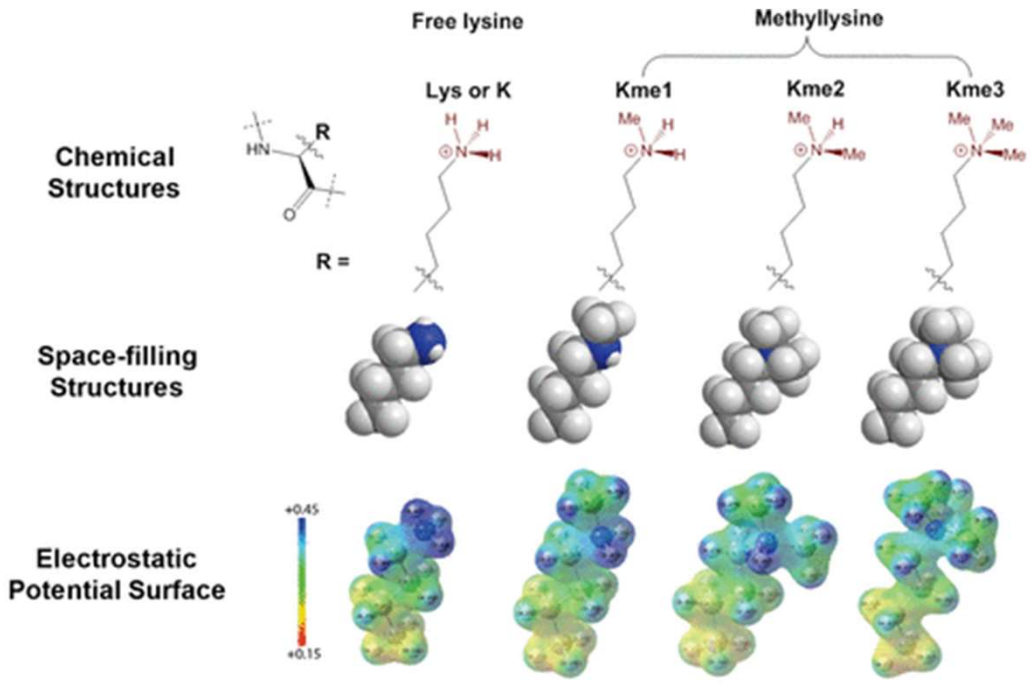
Methylation

- Addition of methyl group to a protein.
- Usually at lysine or arginine residues, also histidine.
- Lysine contains a primary ε-amine.
- Methyl donor is S-adenosylmethionine (SAM).
- Enzyme for this is methyltransferase.



Chem. Rev. 2018, 118, 14, 6656–6705

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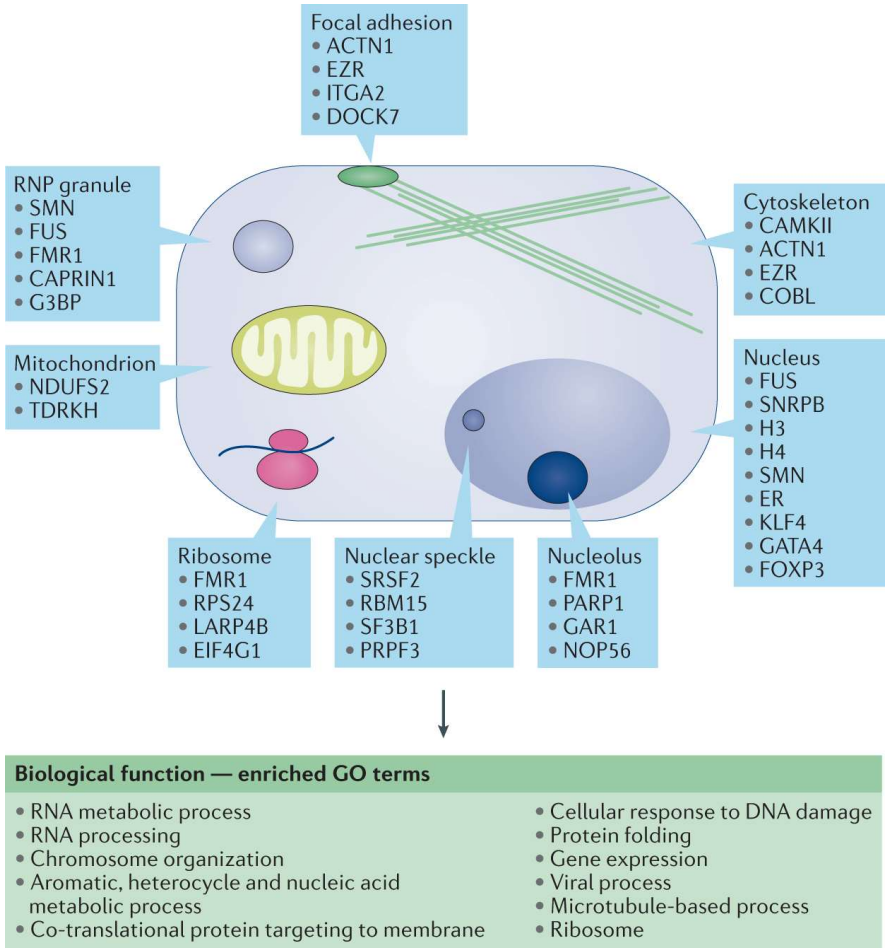
Methylation



Protein, lysine and arginine, methylation function in:

- Epigenetic regulation.
- DNA damage response.
- Signaling pathways.
- Membrane less organelles by arginine methylation.

Nature Reviews Drug Discovery volume 20, pages509–530 (2021)

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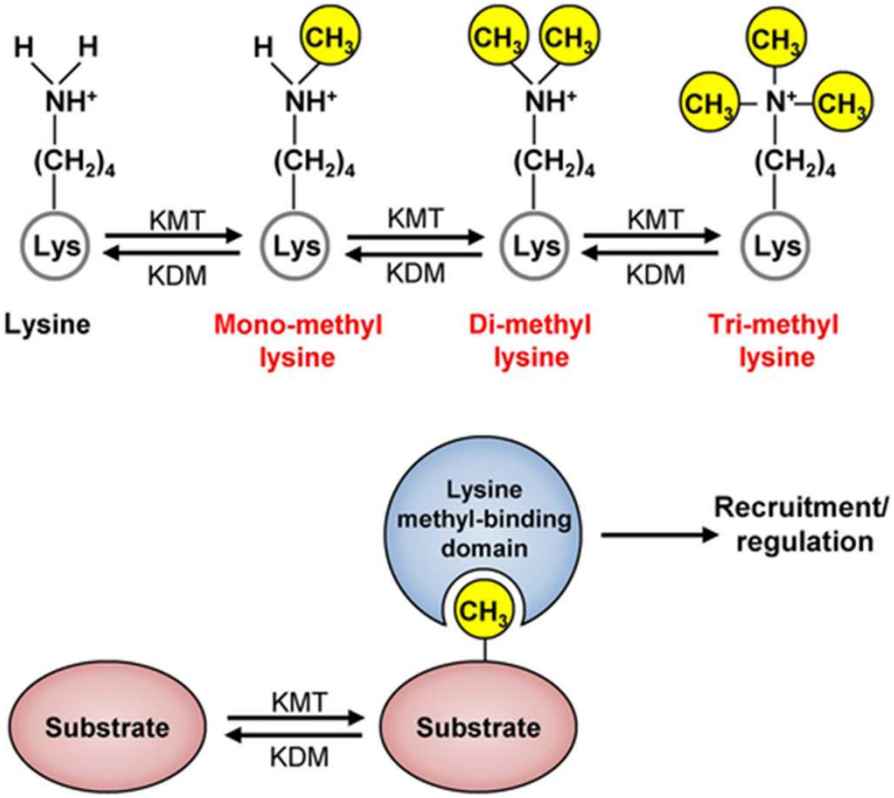
Methylation – Lysine



- Lysine can be methylated like mono-, di- or trimethylated (Kme1, 2 or 3) through the addition of a methyl group to its terminal side-chain ε-amine.
- Lysine methylation dynamics are controlled by the regulated action of KMTs to add and KDMs to remove the methyl.
- Once the methyl modification has been made to the lysine, it can result in either the recruitment of binding proteins or direct effects to regulate protein function.

FEBS Journal, 2017, Vol. 284, 2732–2744.

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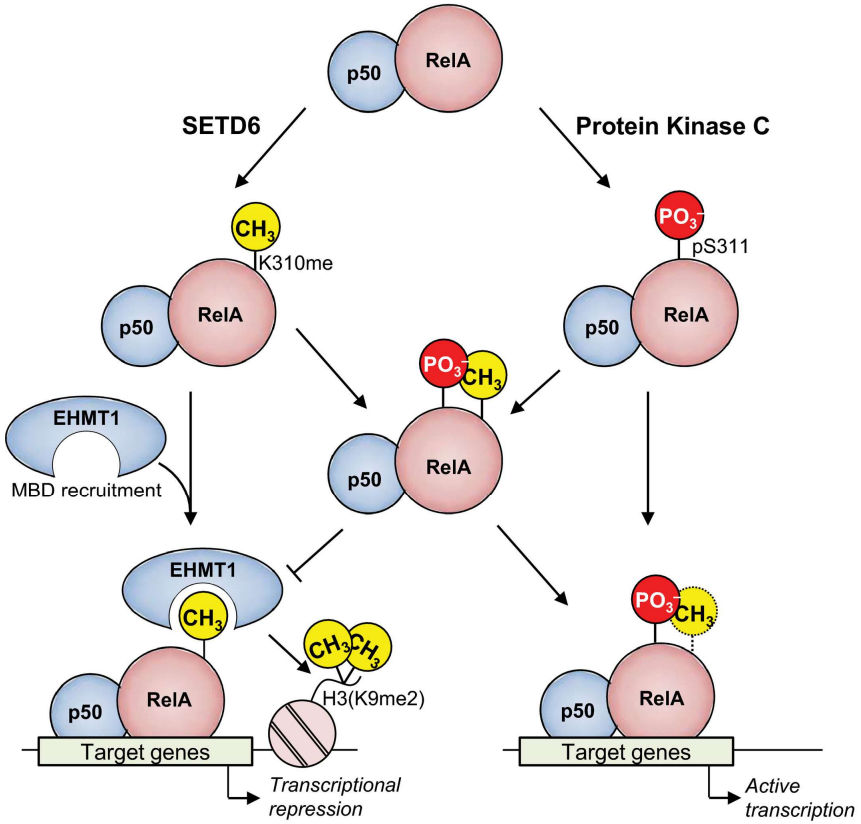
Methylation – Lysine

- Methylation of K^{310} leads to translational repression of NF-κB through the recruitment of the EHMT1 (Euchromatic Histone Lysine Methyltransferase 1).
- Recruitment of EHMT1 increases the localized methylation of H3(K9me2), resulting in the transcriptional repression of NF-κB target genes.
- Serine phosphorylation of S^{311} blocks the binding of EHMT1 to K^{310}, relieving the methylation-induced translational repression.



FEBS Journal, 2017, Vol. 284, 2732–2744.

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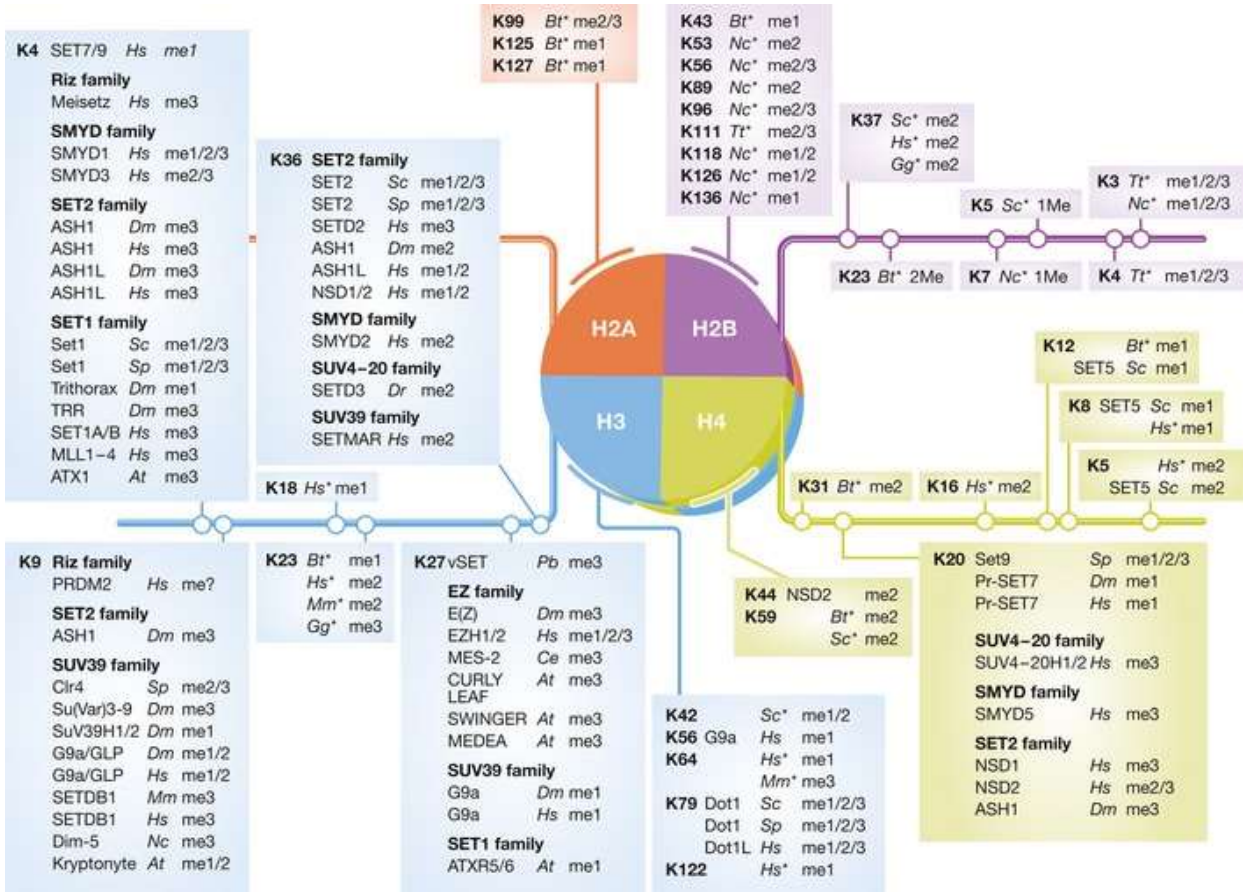
Methylation – Lysine – Epigenetic regulation



Mol Syst Biol. 2014 Apr 4; 10(4): 724.

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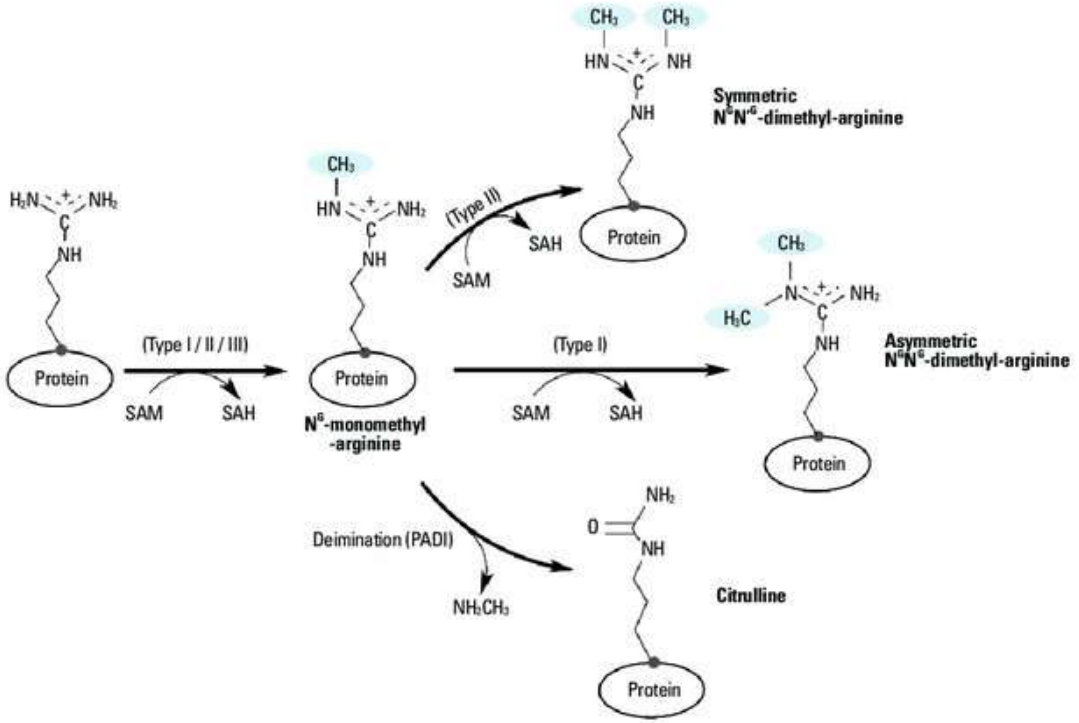
Methylation – Arginine

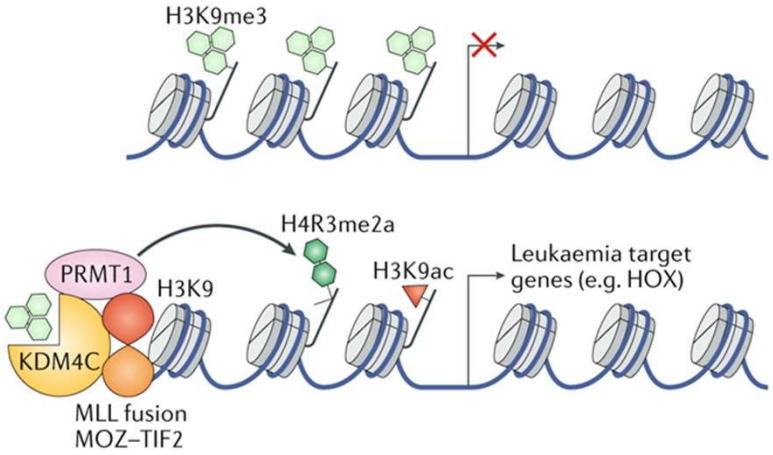
- Arginine can be methylated like mono- or dimethylation (Rme1 or 2) by the addition of a methyl group to its terminal side-chain ε-amine.
- PRMTs can produce
  - Symetrical dimethylation.
  - Asymetrical di-methylation.
- Arginine methylation dynamics are controlled by the regulated action of PRMTs, existence of PRDMs is controversial.
- Arginine methylation is involved in regulation of many cellular processes.



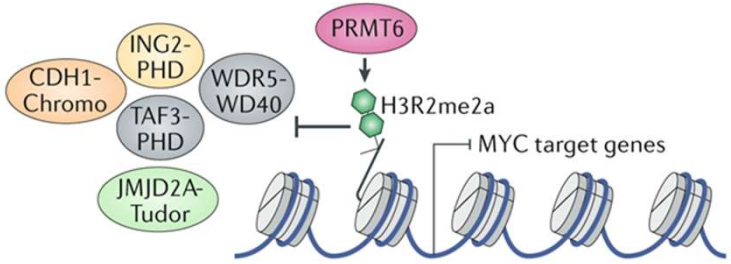
Yonsei Medical Journal, 2014, Vol. 55(2), p. 92 - 303

Methylation – Arginine - Epigenetic regulation

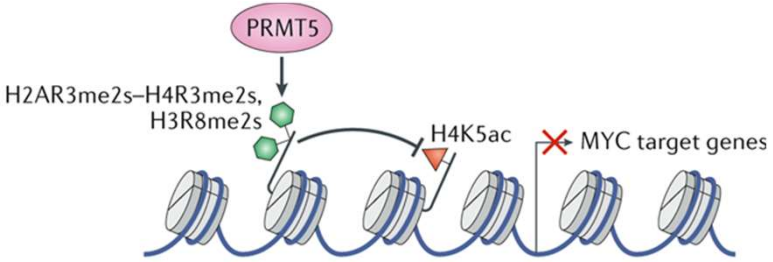
- Activation of acute myeloid leukemia genes.



- Inhibition of transcription by preventing the binding of several readers of methylated H3K4.



- Repression of transcription by reducing the levels of H4K5ac.



Nature Reviews Molecular Cell Biology, 2019, volume 20, pages 642–657.

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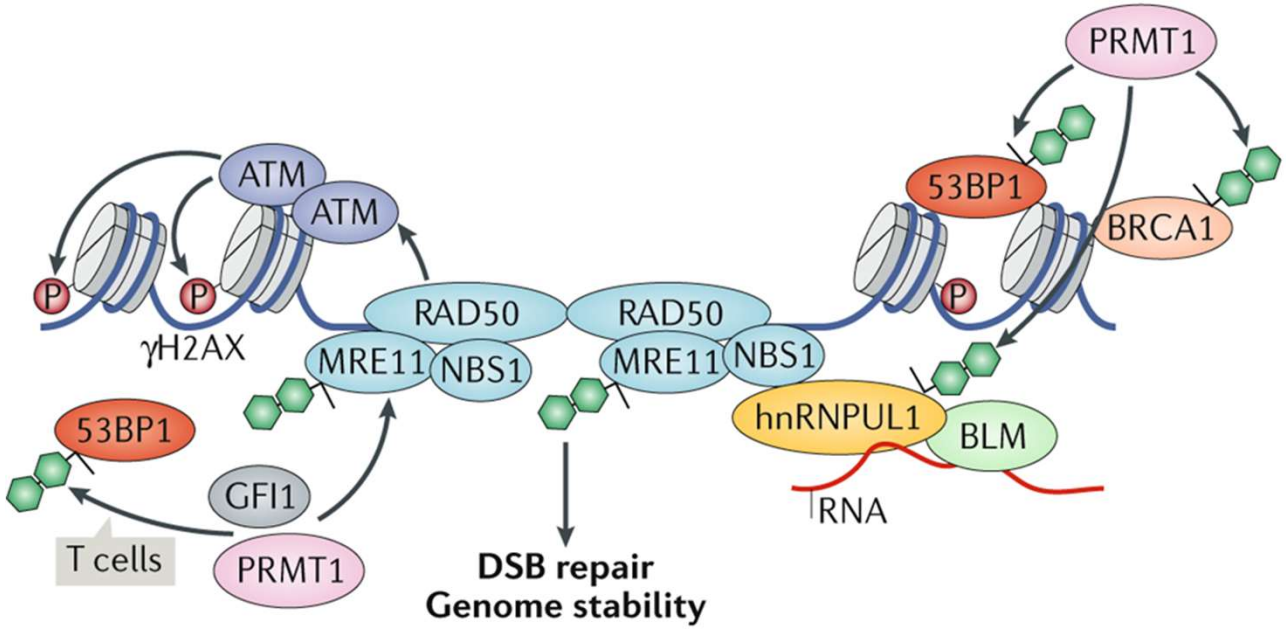
Methylation – Arginine – DNA-damage response

- Methylation of MRE11 by protein arginine methyltransferase 1 (PRMT1) is required for the exonuclease and resection activities of MRE11.



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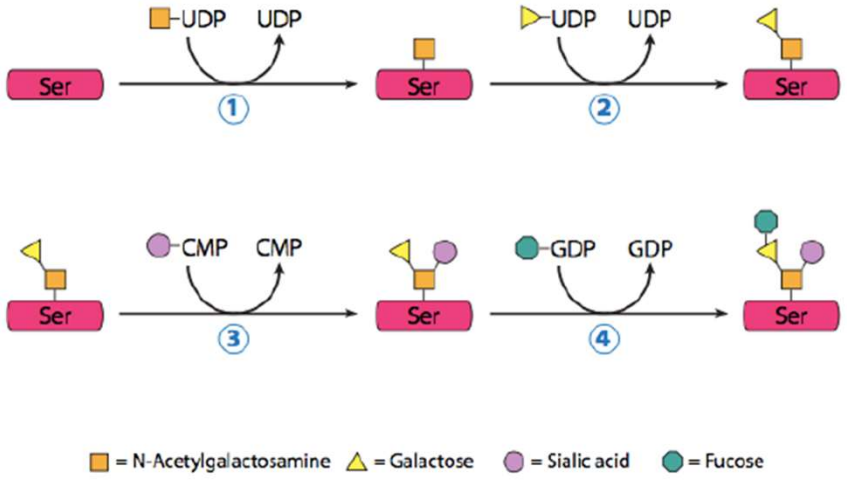
O-glycosylation

- O-linked glycosylation – acetylgalactosamine (GalNAc) transferase attaches GalNAc to the -OH group of a Ser or Thr in the Golgi apparatus.
- Determination of which residue is glycosylated depends on the secondary and tertiary structure.
- Usually combined oligosaccharide chains attached to an O-linked glycoprotein can contribute over 50% of the mass of a glycoprotein.



Two examples of O-linked glycoproteins:

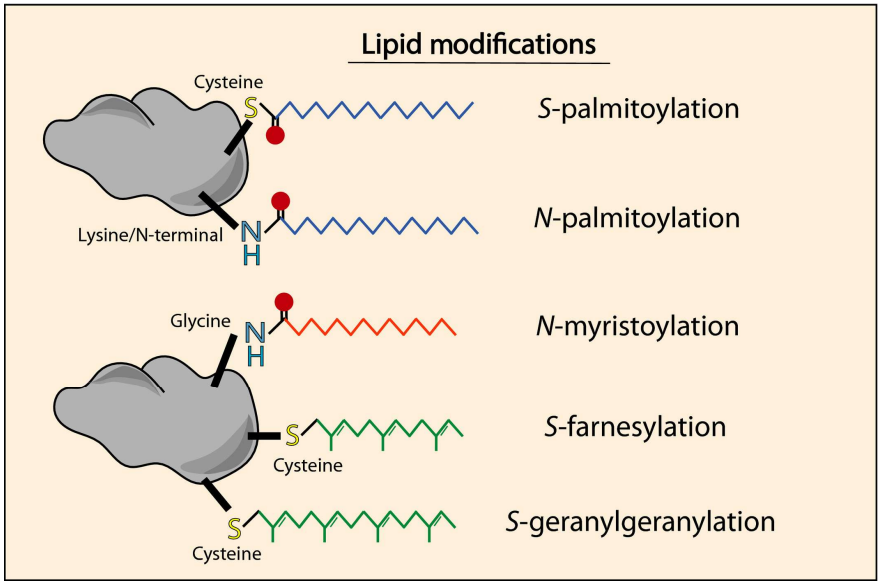
- mucin, a component of saliva.
- ZP3, a component of the zona pellucida (which protects egg cells), if glycosylated ZP3 also acts as a sperm receptor.

https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

MUNI SCI

Lipidation

- Lipidation attachment of a lipid group, such as a fatty acid, covalently to a protein.
- In general, lipidation helps in cellular localization and targeting signals, membrane tethering and as mediator of protein-protein interactions.

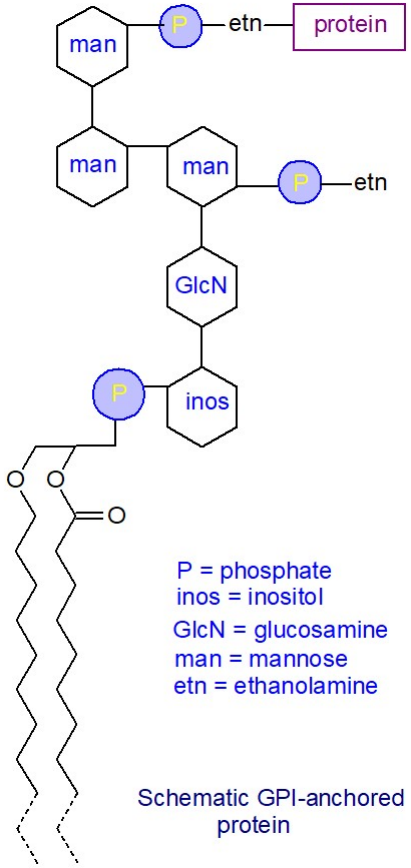


- C-terminal glycosyl phosphatidylinositol (GPI) anchor
- N-terminal myristoylation
- S- and N-palmitoylation
- S-prenylation

The FEBS Journal, 2022, Vol. 289, 861–882.

C-terminal glycosylphosphatidylinositol anchor

- C-terminal glycosylphosphatidylinositol (GPI) in short, is a phosphoglyceride that can be attached to the C-terminus of a protein during posttranslational modification.
- GPI anchors tether cell surface proteins to the plasma membrane often to cholesterol- and sphingolipid-rich lipids, which act as signaling platforms on the plasma membrane.
- These protein-lipid complexes are ubiquitous in fungi, protozoans, plants, insects and animals.
- The resulting GPI-anchored proteins play key roles in a wide variety of biological processes.



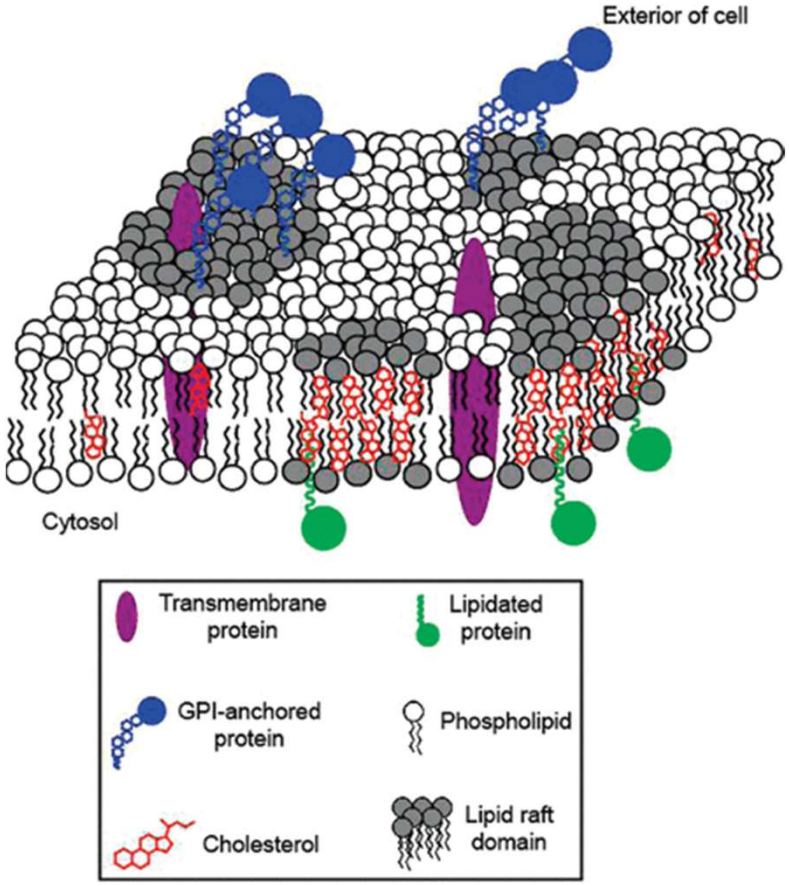
Schematic GPI-anchored protein

https://en.wikipedia.org/wiki/Glycosylphosphatidylinositol

https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/complex/GPIanchor/index.htm

MUNI SCI

C-terminal glycosylphosphatidylinositol anchor



- GPI anchors proteins to the plasma membrane are often tethered to cholesterol- and sphingolipid-rich regions called „lipid rafts“.

GPI-anchored proteins function as:

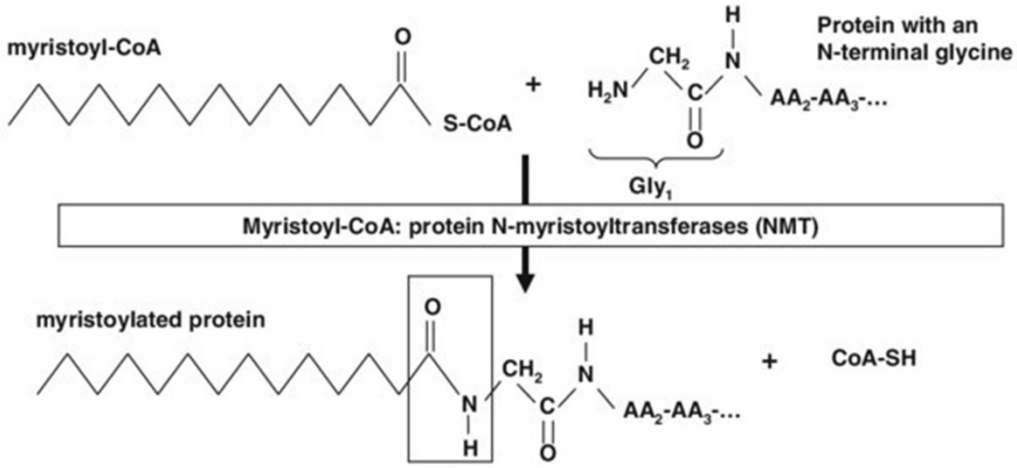
- receptors, transporters, and adhesion molecules
- essential for the fertility of mouse sperm and egg
- coordinated growth during embryonic development.
- signaling molecules to mediate cell–cell communication
- sorting signal for transport of GPI-anchored proteins in the secretory and endocytic pathways.

Biochemistry 2008 47 (27), 6991-7000
https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/complex/GPIanchor/index.htm

MUNI SCI

N-terminal myristoylation

- N-myristoylation consists of the addition of the 14-carbon fatty acid, myristate, to the N-terminal glycine residue of a protein via a covalent amide bond.
- In rare cases, myristic acid is attached to a lysine (Ras GTPases and TNF) lysine myristoylation.
- N-myristoylation is catalyzed by the enzyme N-myristoyltransferase (NMT).
- Lower eukaryotes have only one isoform of NMT, whereas most mammals express two isozymes (NMT1 and NMT2).



Lipids, Oct. 2020, Vol. 45(10), pages 941 – 6.
Cellular & Molecular Immunology, 2021, Vol. 18, pages 878–888.

MUNI SCI

N-terminal myristoylation

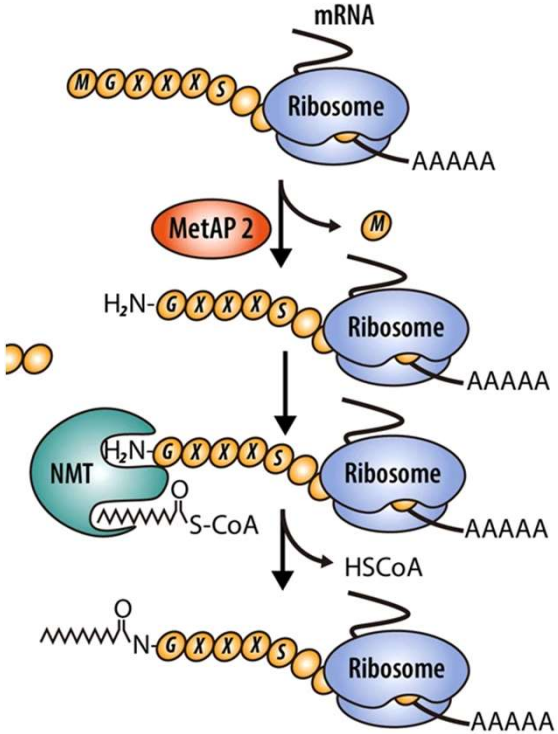
- N-myristoyl moiety in the protein affects, changing protein stability, influencing protein–protein interactions and enhancing subcellular targeting to organelles or the plasma membrane and so on.
- In humans, NMT isozymes, NMT1 and NMT2, are expressed in most tissues and are essential for cell survival, regulation of immune responses, and HIV-1 infection.
- N-myristoylation is considered a co-translational modification with the most accurate step occurring after the removal of the methionine initiator by methionine aminopeptidase (MetAP).
- N-myristoylation can also occur post-translationally on an internal glycine exposed by caspase cleavage during apoptosis.

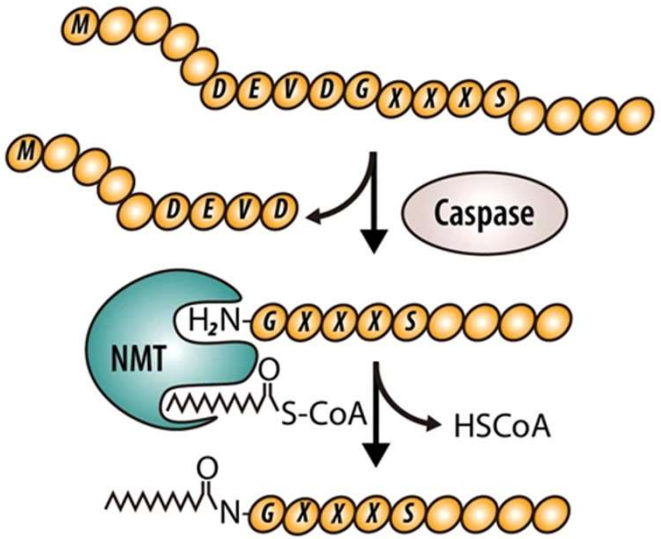
Acta Pharmacologica Sinica, 2020, Vol. 41, pages 1005–1015.

N-terminal myristoylation

Co-translational modification:

- Removal of methionine by methionine aminopeptidase (MetAP).





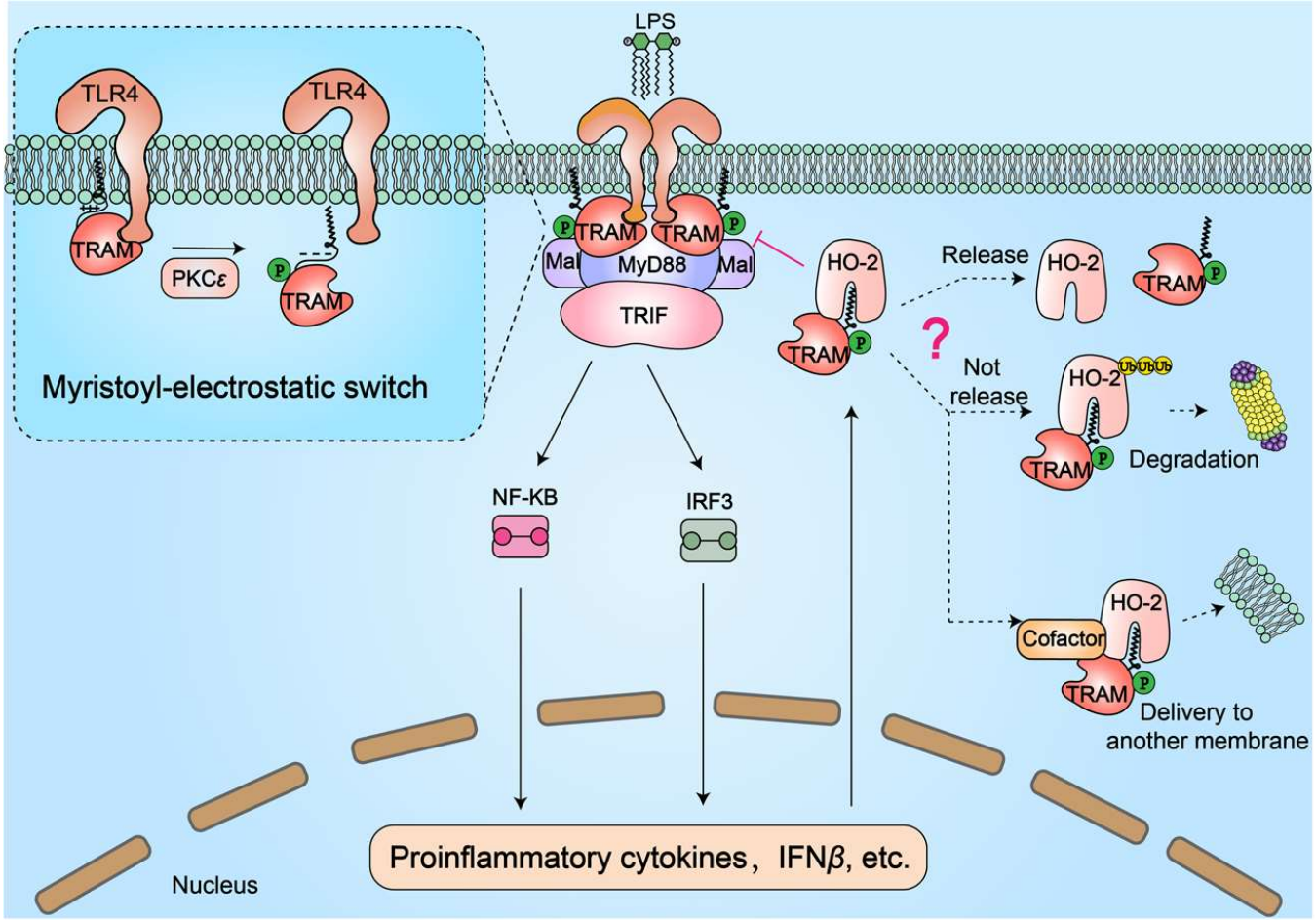
Post-translational modifiction:

- Internal glycine is first exposed by caspase cleavage during apoptosis.

Acta Pharmacologica Sinica, 2020, Vol. 41, pages 1005–1015.

MUNI SCI

N-terminal myristoylation

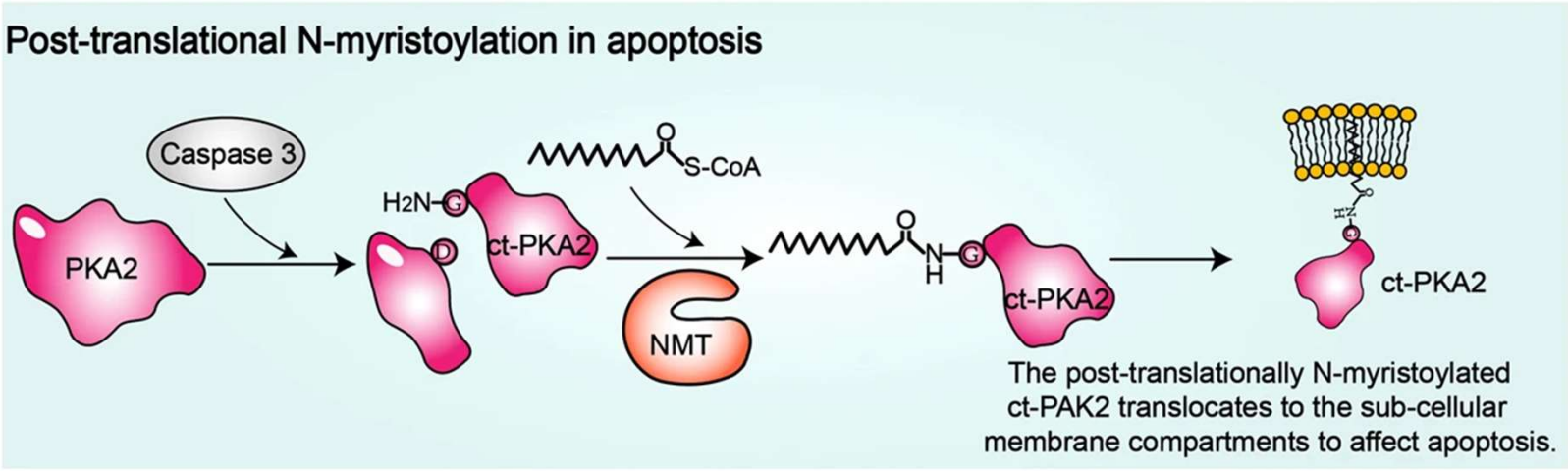


- LPS-induced TLR4 inflammatory responses.
- TRAM is myristoylated and anchored to the plasma membrane.
- After LPS stimulation, TLR4 dimerizes and TRAM is transiently phosphorylated by PKCε on Ser-16, which is near its N-terminus.
- TRAM dissociation from the membrane and binds to heme oxygenase-2 (HO-2), which inhibits TRAM and negatively regulating TLR4 signaling.

Cellular & Molecular Immunology, 2021, Vol. 18, pages 878–888.

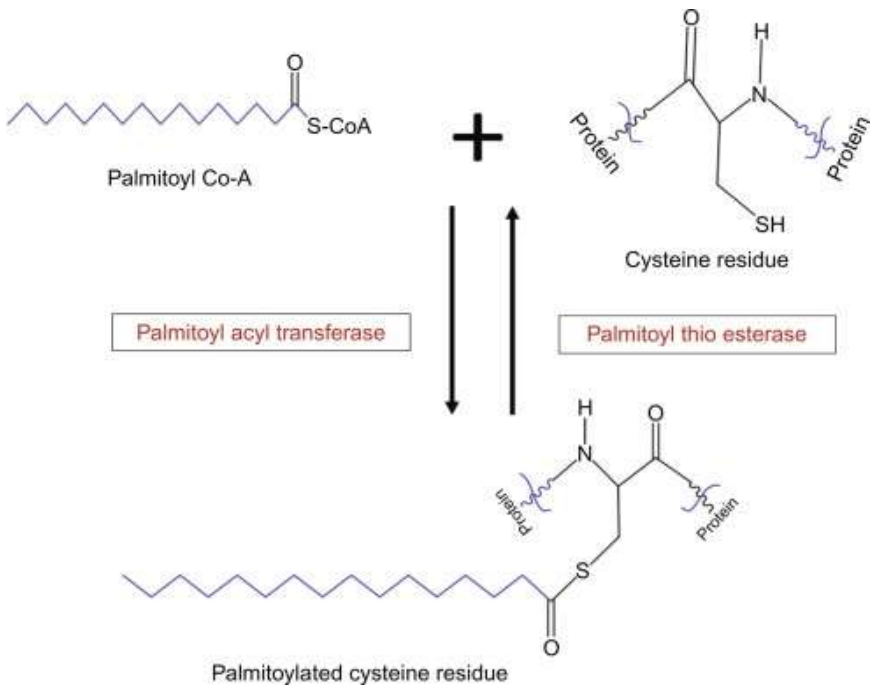
N-terminal myristoylation

- P21-activated kinase 2 (PAK2) is cleaved by caspase 3 to produce caspase-truncated PAK2 (ctPAK2), which has a newly exposed glycine residue at the N-terminus.
- Then, NMT catalyzes the covalent attachment of myristic acid to the glycine residue of ctPAK2. Post-translationally myristoylated ctPAK2 translocates to subcellular membrane compartments to induce apoptosis.



Cellular & Molecular Immunology, 2021, Vol. 18, pages 878–888.

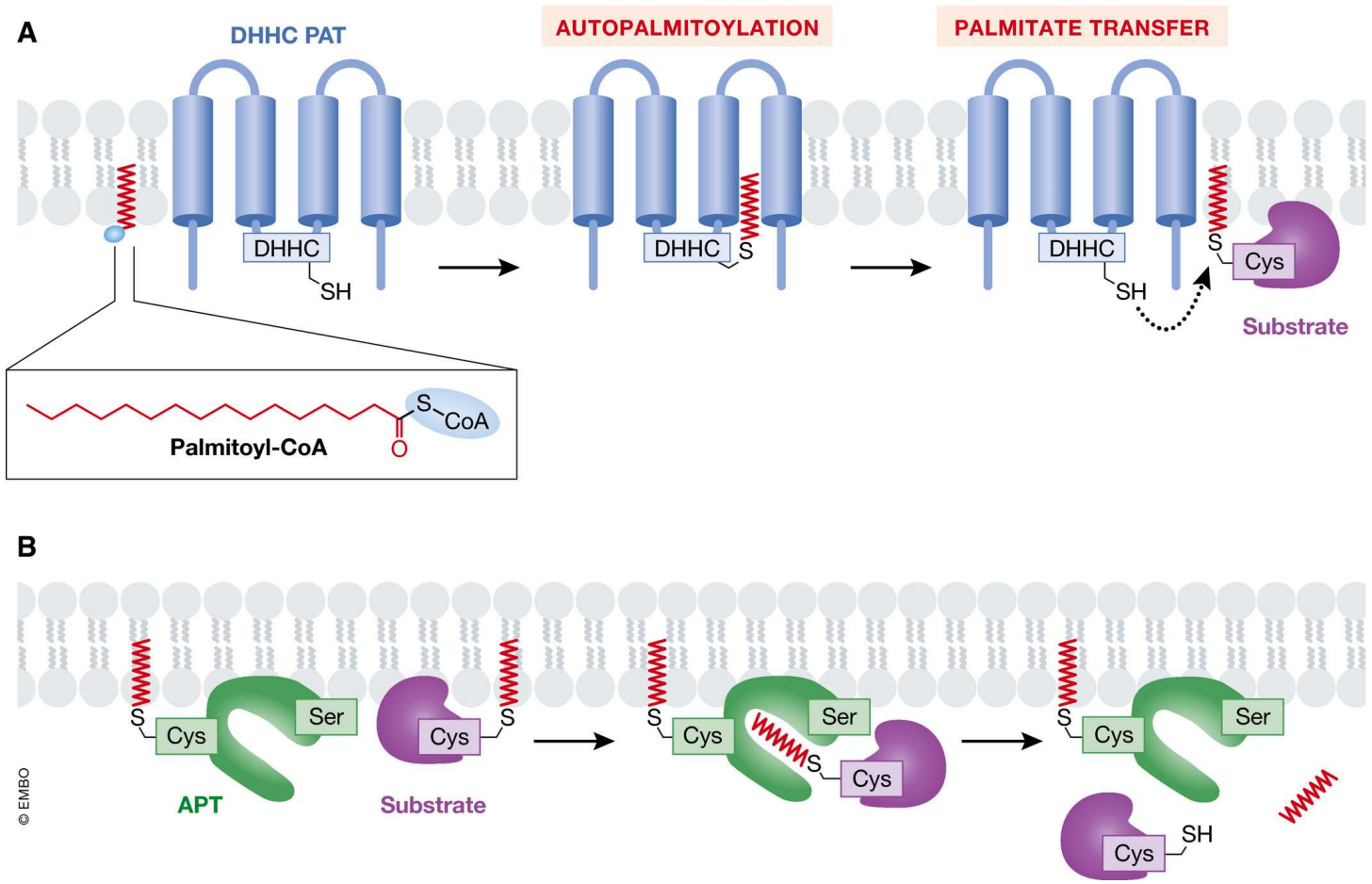
S-palmitoylation



- Palmitoylation is post-translational attachment of the saturated 16-carbon palmitate from its lipid donor, palmitoyl-coenzyme A ester.
- Protein acyl Transferases (PATs) are enzymes responsible for catalyzing the addition of palmitate to the substrate.
- Romoving of palmitol by Palmitol-thio-esterase.
- It is caried out on membranes. Palmitoylation is a reversible process, and several cellular proteins undergo dynamic palmitoylation.

https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/palmitoylation

S-palmitoylation



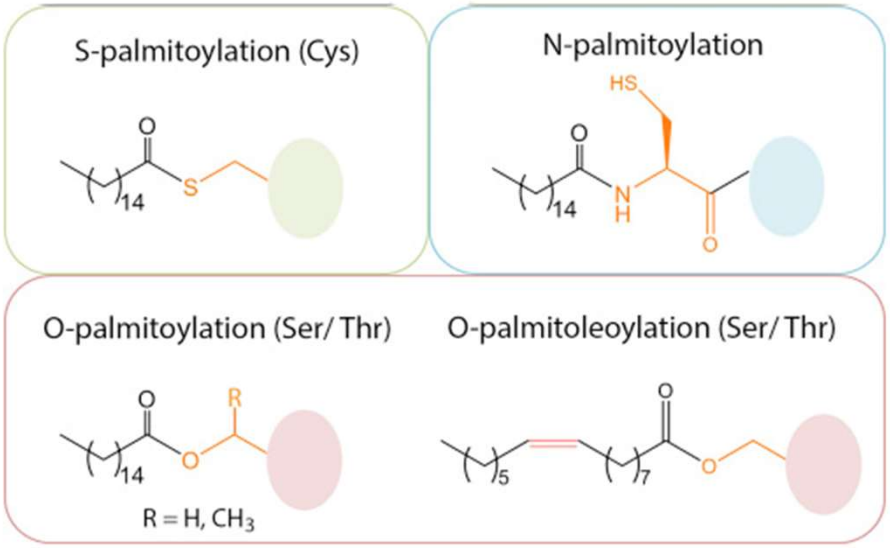
EMBO Reports (2018)19:e46666

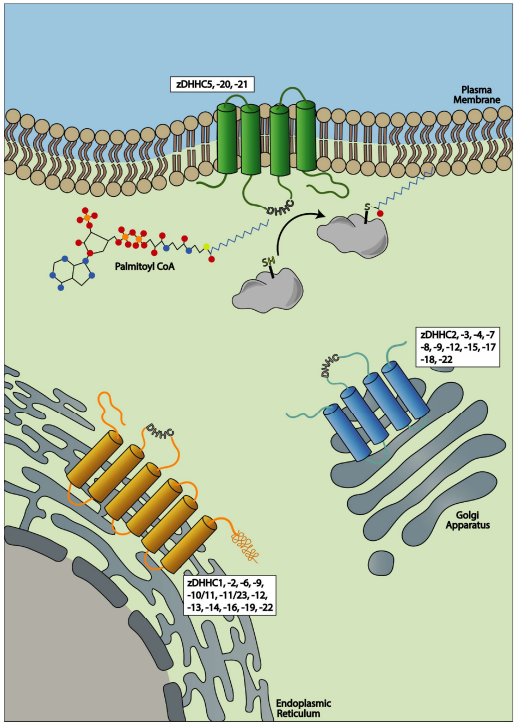
MUNI SCI

S-palmitoylation

- S-Palmitoylation occurs at cysteine.
- N-palmitoylation occurs at amino-terminal cysteine.
- O-Palmitoylation and O-palmitoleoylation occur at serine/threonine.

- Protein acyl transferases are located throughout the secretory pathway in the endoplasmic reticulum, Golgi apparatus and plasma membrane.



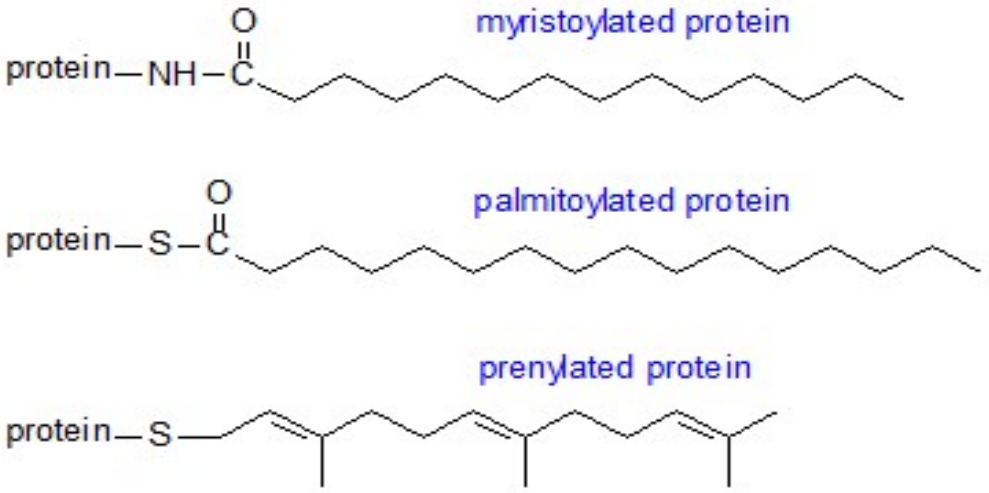


Cell Chem Biol, Volume 25, Issue 3, 15 March 2018, Pages 236-246.
The FEBS Journal, 2022, Vol. 289, 861–882.

MUNI SCI

S-prenylation

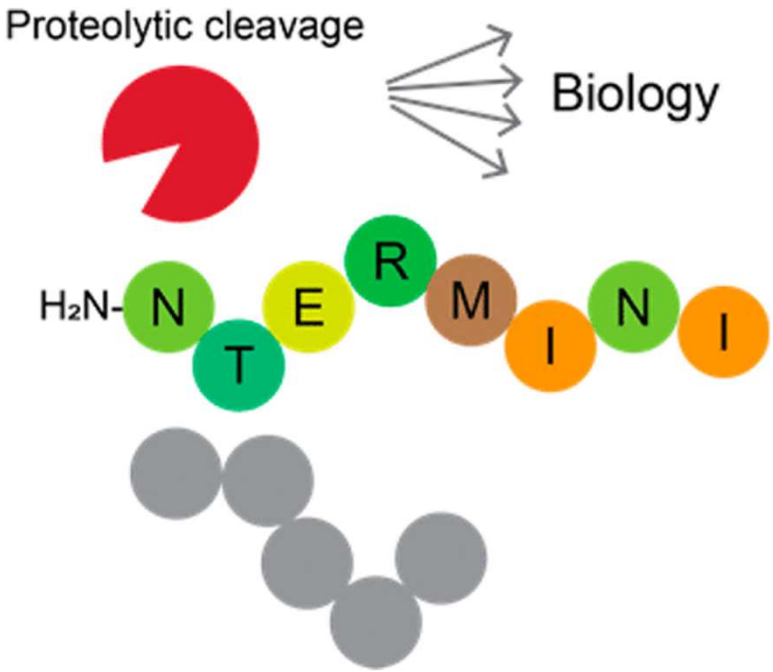
- S-prenylation, similarly to S-palmitoylation, provides a hydrophobic, membrane attracted C terminus through the enzymatic addition of farnesyl (C15) or geranylgeranyl (C20) to a cysteine residue.
- Enzyme involved in this reaction is farnesyl transferase (FT) or geranylgeranyl transferases (GGT I and II).
- Dysregulated S-prenylation is implicated in several diseases including cancer.



MUNI SCI

Proteolytic cleavage

- Proteolytic cleavage – very common irreversible post-translational modification of the protein's structure and biological function.
- Also, first amino acid methionine of a newly synthesized polypeptide is very often cleaved off (also true for some prokaryotic f-Met).
- The precursor protein is termed a proprotein, and the peptide that is cleaved off of it to activate the protein is called the propeptide.
- Classical examples of proproteins are the hormone insulin, the cell death protein family of caspases, collagen and the Alzheimer-associated neural protein β-amyloid.



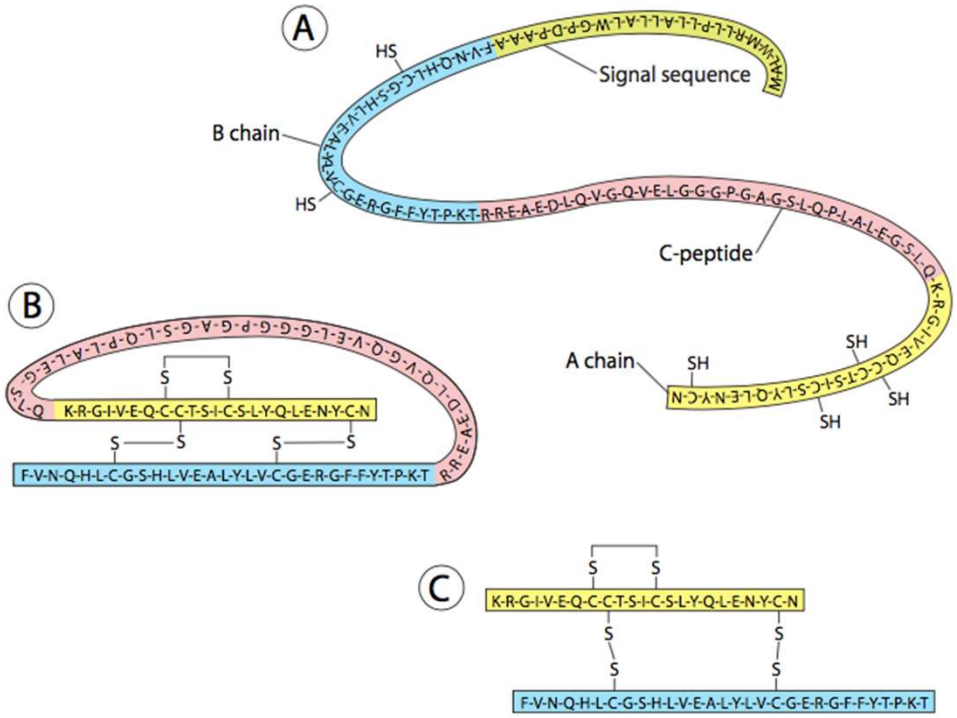
https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

Chem. Rev. 2018, 118, 3, 1137–1168.

MUNI SCI

Proteolytic cleavage

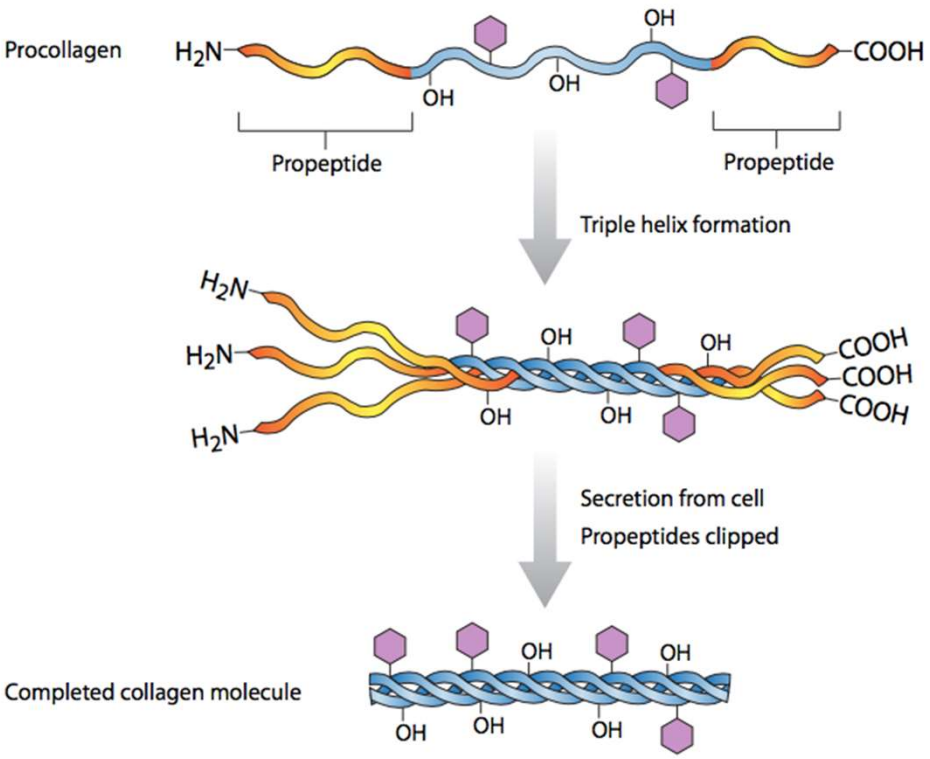
- In mammals - preproinsulin (inactive as a hormone) is first translated from the insulin mRNA.
- Proteolytic processing is necessary to make biologically active insulin.
- (A) The linear protein contains a signal sequence, which is cleaved after the protein enters the ER, an A chain, a B chain, and a C-peptide.
- (B) Inside the ER, the proinsulin (insulin precursor) folds and disulfide bonds form between cysteines.
- (C) Finally, two cleavages release the C peptide, which leaves the A and B chains attached by the disulfide bonds. This is now active insulin.



https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

MUNI SCI

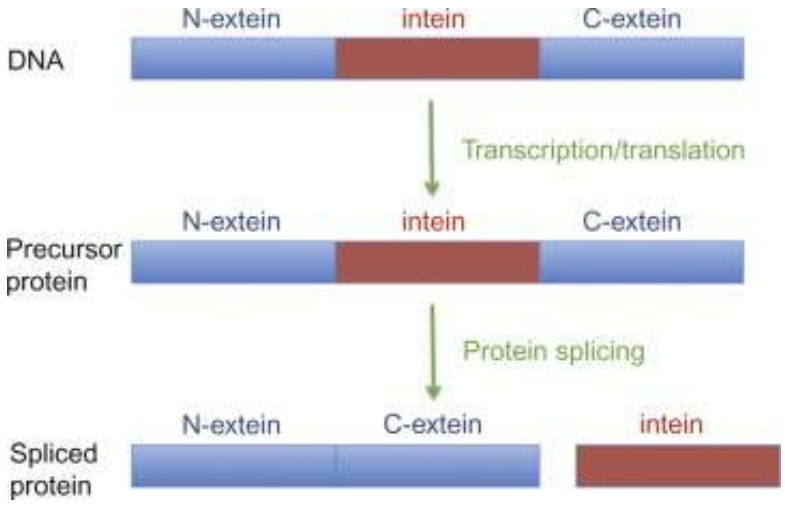
Proteolytic cleavage

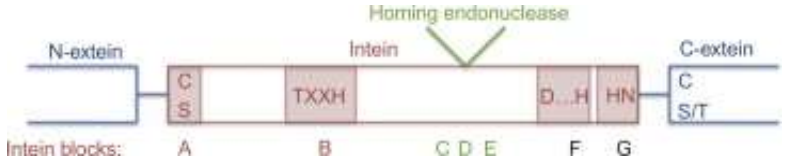


- Collagen is a very large secreted protein, twisted triple-helix of three subunit, that provides structure and shock absorbance for the extracellular matrix in animals.
- The collagen subunits are made as procollagen, and propeptides are lopped off of both N- and C-termini to generate the final protein.
- However, they are not cleaved off until after the three subunits assemble around one another.
- The propeptide sequences are clearly necessary for efficient assembly of the final protein complex.

https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

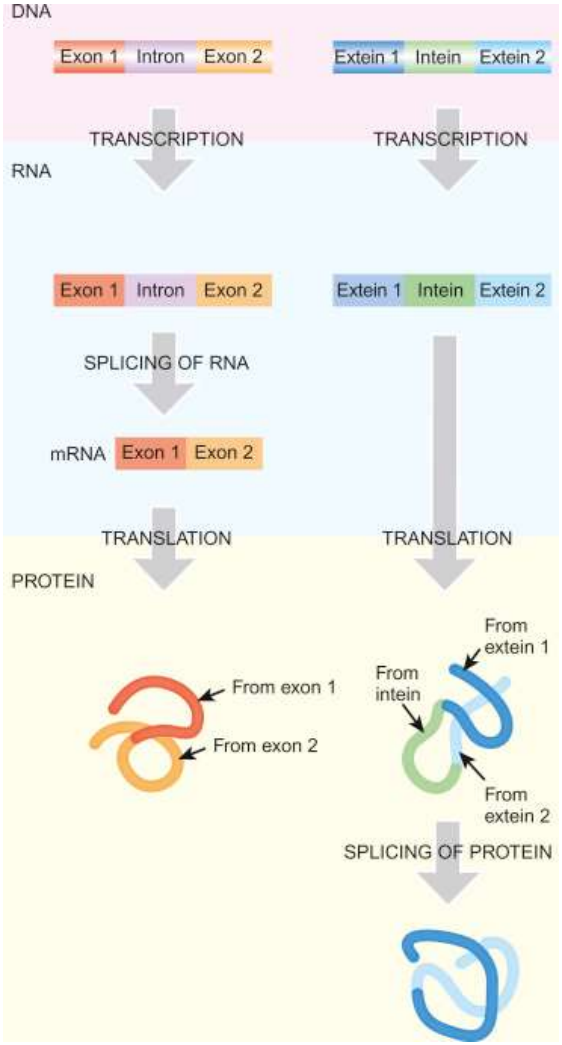
Protein splicing





- Protein splicing is a post-translational process facilitated by an intervening polypeptide, called an intein (for internal protein).
- The intein interrupts flanking polypeptides called exteins.
- The intein is responsible for catalyzing its excision from the exteins, concomitant with extein ligation.
- Inteins have four conserved sequence blocks. Block C, D and E where the split sites of naturally occurring split inteins and homing endonuclease domain (HED) are located.

Handbook of Proteolytic Enzymes, Volume 1, 2013, Pages 315-321.



Protein splicing

- The protein splicing is rare PMT.
- Inteins and exteins are the protein analogs of the introns and exons found in the DNA and RNA. In other words, inteins are intervening sequences in proteins that are present when the protein is first made, but are later spliced out.
- The final protein is made of the exteins that are now joined together.
- Inteins have been found in yeasts, algae, bacteria, and archaea (archaebacteria), such as VMA1 in a precursor of a vacuolar H+-ATPase enzyme.

https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-splicing

MUNI SCI

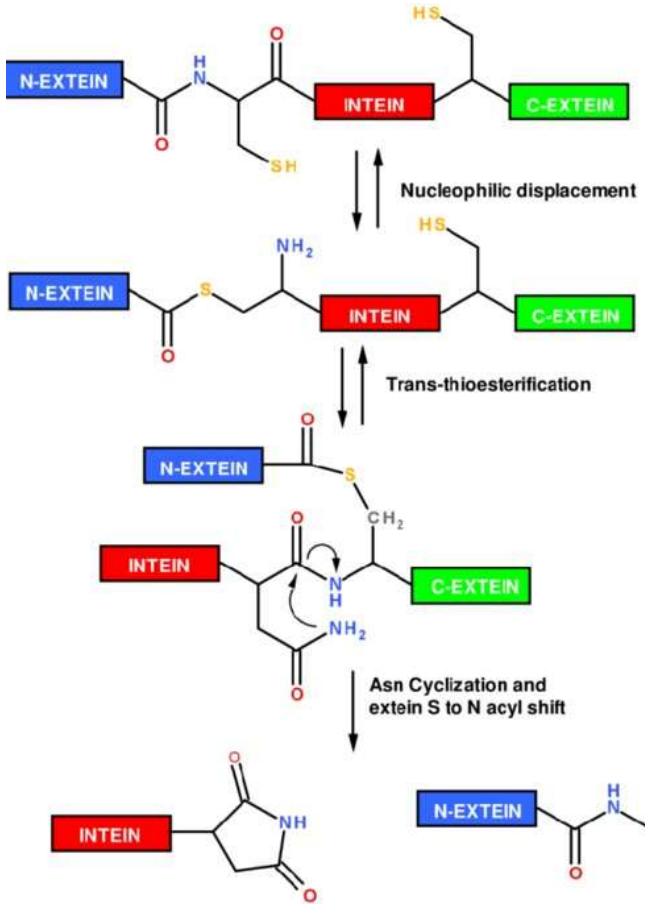
Protein splicing

- Protein splicing is four-step process, which is achieved through structural conformational change and chemical bonds shifting on junction sites between intein and exteins.

- 1. A nucleophilic displacement converts the peptide bonding to an ester or thioester group.

- 2. Transesterification - transfers the to the first residue of C-extein, forming a branched intermediate.
- 3. The Asn cyclization leads to intein peptide bond cleavage and exteins ligation.

- 4. Rapid conversion from the ester bond to the amide bond occurs to form the final peptide.



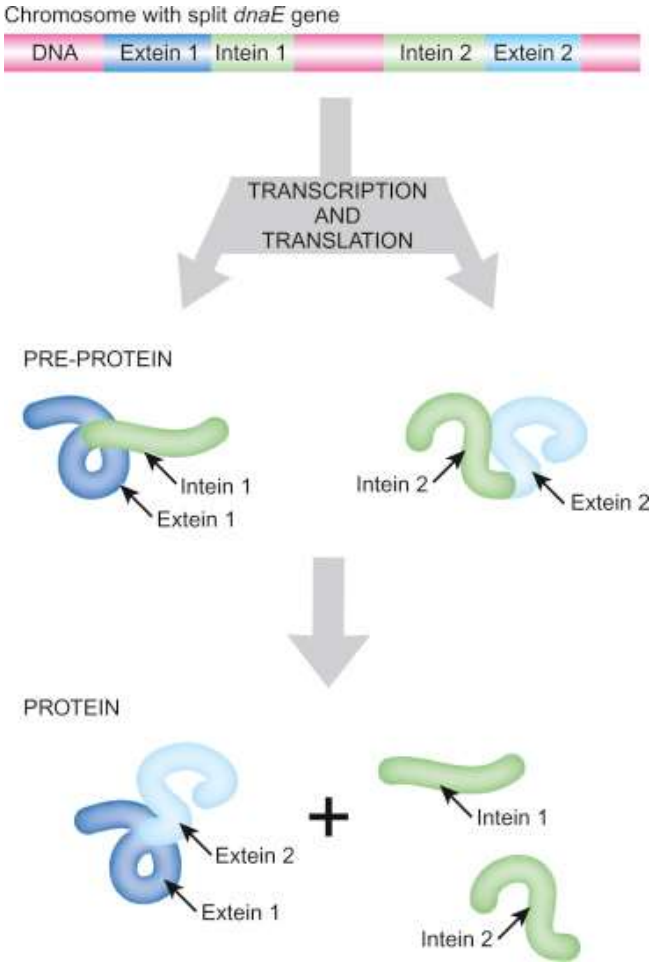
April 2013Molecules 18(1):440-65

Front. Bioeng. Biotechnol., 2022, Protein Splicing of Inteins: A Powerful Tool in Synthetic Biology., 10:810180

MUNI SCI

Protein splicing

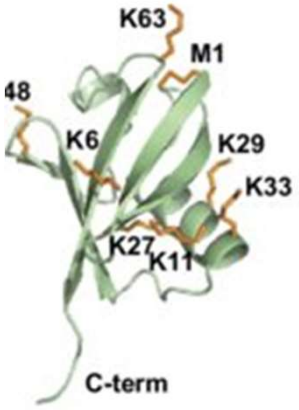
- The DNA coding for the DnaE protein of *Synechocystis* is transcribed and translated into two separate proteins, each containing an intein and an extein.
- The exteins of the two proteins are spliced together by the inteins. During splicing both inteins are lost.
- Also, DnaE, the catalytic subunit α of DNA polymerase III, is encoded by two separate genes, dnaE-n and dnaE-c.

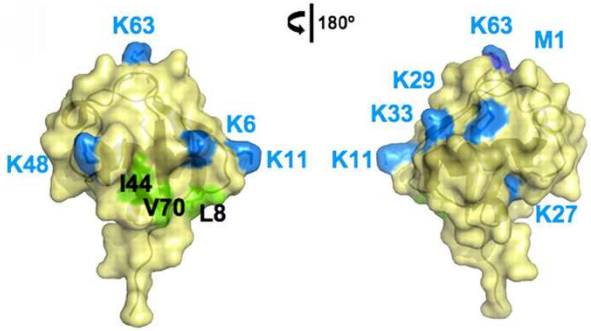


https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/protein-splicing

MUNI SCI

Post-translational Ubiquitination



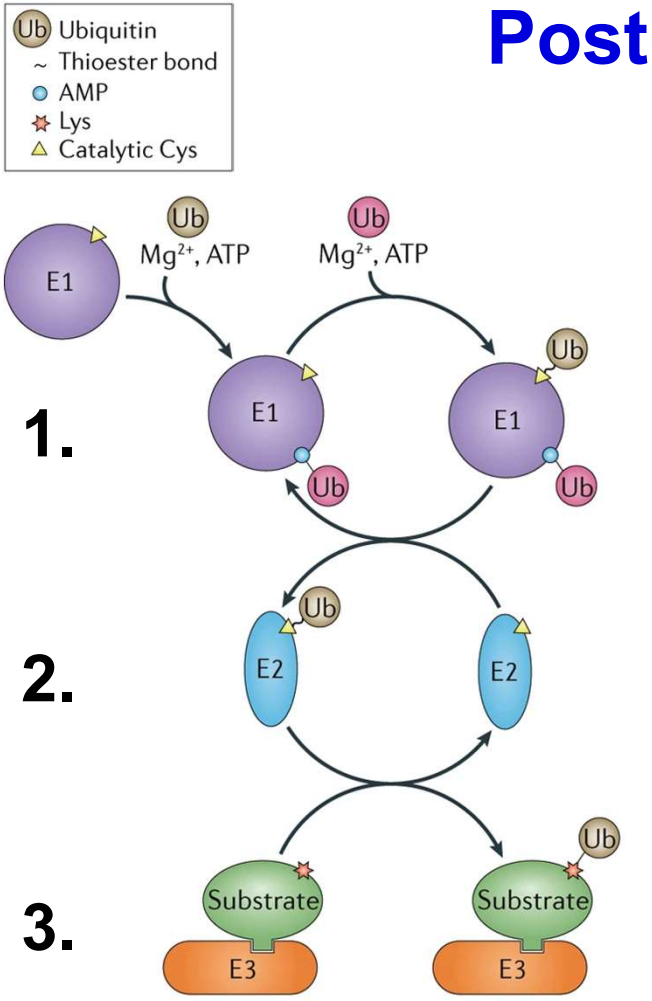


- Ubiquitin (Ub), a highly conserved regulatory protein containing 76 amino acids, can be covalently tagged to target proteins.
- Ubiquitin is attached to the Lysine residue in polypeptide.
- Ubiquitin provides eight types of polyubiquitin linkages (K6, K11, K27, K29, K33, K48, K63 and Met1) with specific functions.
- The ubiquitin ligase adds ubiquitin to the substrate and deubiquitilating enzyme removes Ub from the substrate. Thus ubiquitination is reversible process.

Mater methods, 2014;4:827
Physiological Reviews. Jul 2013, 93(3):1289-315.

MUNI SCI

Post-translational Ubiquitination

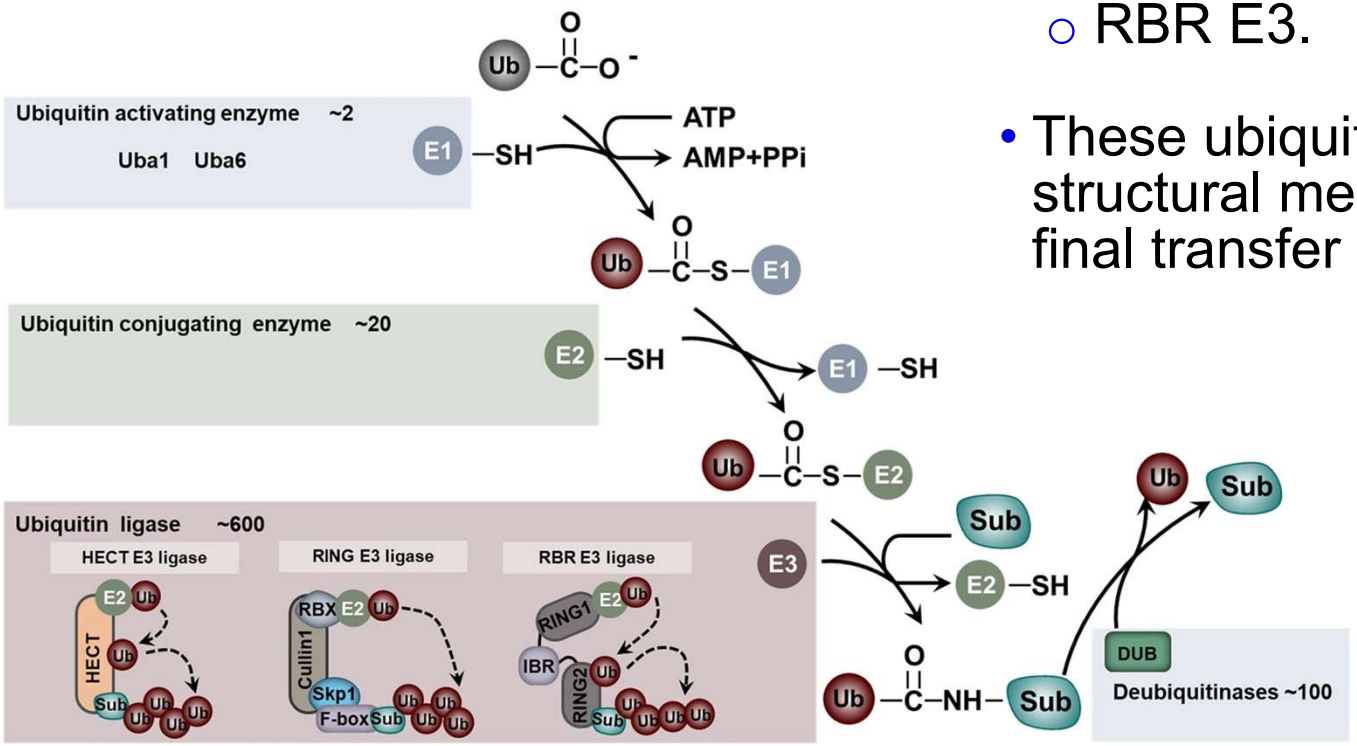


- Ubiquitin can be covalently tagged to target proteins via a cascade of enzymatic reactions.
- **1.** A ubiquitin-activating enzyme (E1) catalyzes binding to one ubiquitin by a thioester.
- **2.** E1 then binds a ubiquitin-conjugating enzyme (E2) and transfers ubiquitin from its catalytic cysteine to the catalytic cysteine of E2 to form E2~ubiquitin (~ indicates a thioester bond).
- **3.** A ubiquitin ligase (E3) recruits E2~ubiquitin and a substrate to catalyze ubiquitin transfer to a lysine on the substrate.

Nature Reviews Molecular Cell Biology, 2016, Volume 17, pages 626–642.

MUNI SCI

Post-translational Ubiquitination



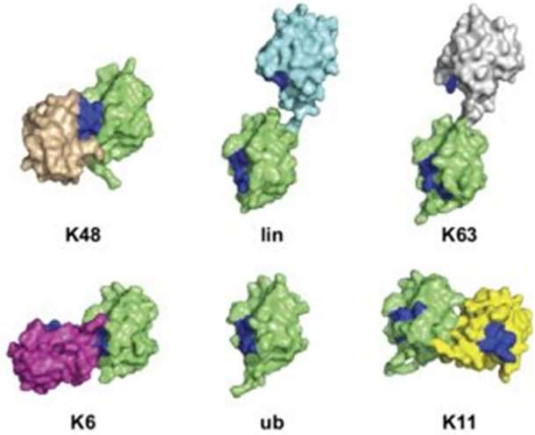
- Three distinct classes of ubiquitin ligases.
  - HECT E3
  - RING/U-box E3
  - RBR E3.
- These ubiquitin ligases utilize different structural mechanisms for mediating the final transfer of ubiquitin onto substrates.

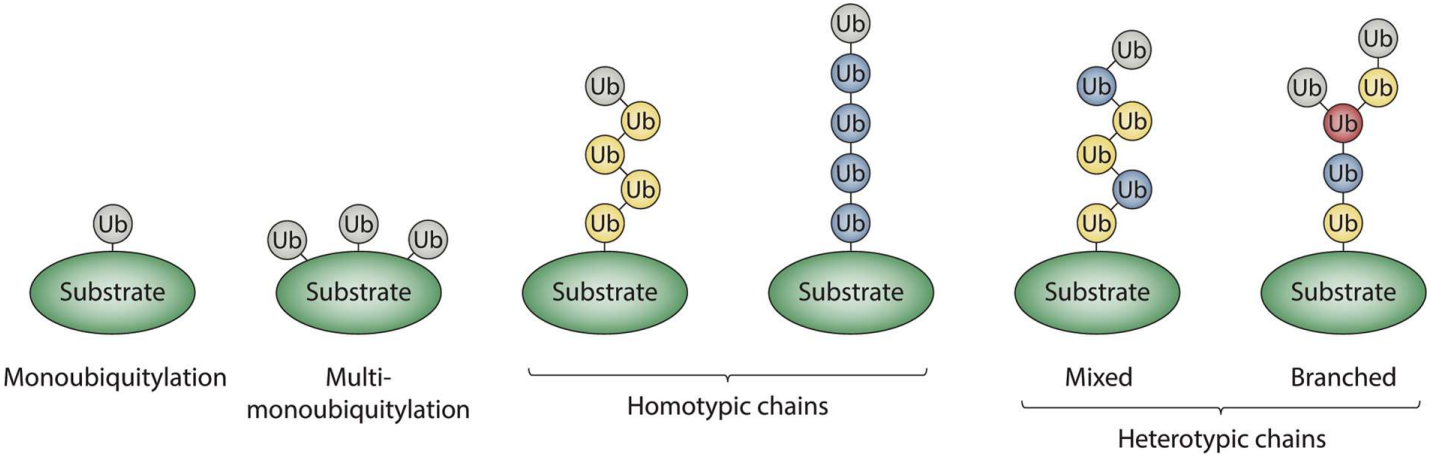
Signal Transduction and Targeted Therapy (2020) 5:11.

MUNI SCI

Post-translational Ubiquitination

- Protein could be:
  - Monoubiquitylated.
  - Multi-monoubiquitylated.
  - Polyubiquitylated.
  - Branched ubiquitin chain.



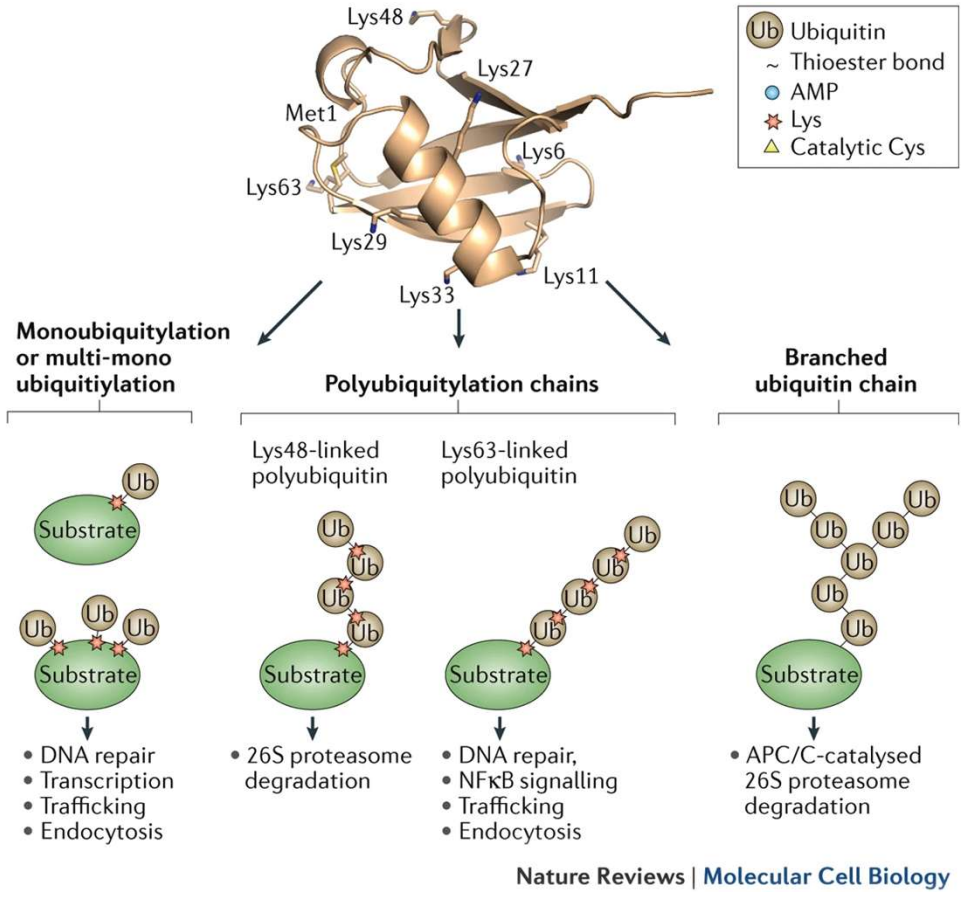


Physiological Reviews. Jul 2013, 93(3):1289-315.
Cell Discovery volume 7, Article number: 6 (2021)

MUNI SCI

Post-translational Ubiquitination

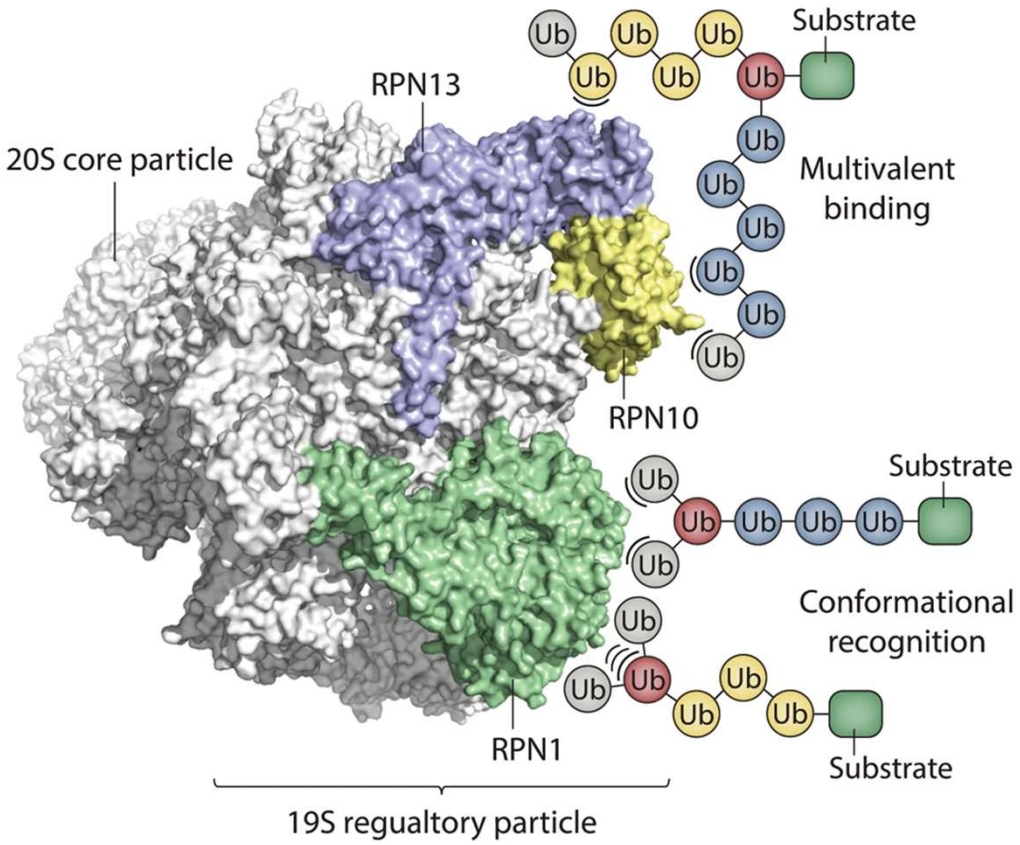
- Branched ubiquitin chains of different topologies are specialized for different cellular functions and control the stability, activity, interaction properties, and localization of many different proteins.
- Branched ubiquitin chains regulate cell signaling and protein degradation pathways.
- Branched ubiquitin chains are remarkably diverse in terms of their chemical linkages, structures, and the biological information they transmit.



Nature Reviews Molecular Cell Biology, 2016, Volume 17, pages 626–642.

MUNI SCI

Post-translational Ubiquitination

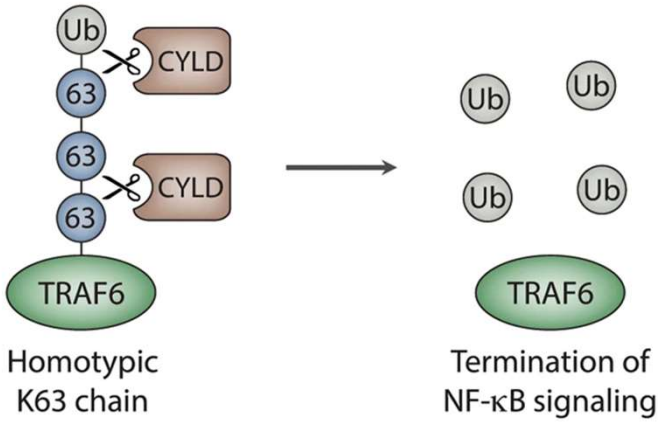


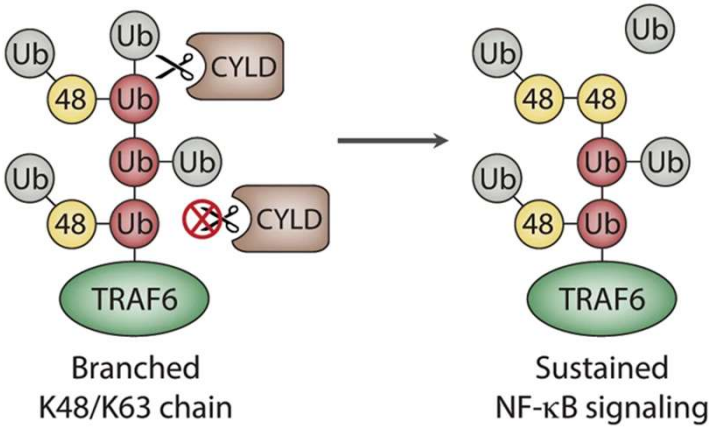
- The enhanced binding of branched chains to the 19S regulatory particle of proteasome as a result of an increase in the local concentration or “density” of ubiquitin subunits surrounding the substrate is illustrated by the multivalent-binding model.
- Enhanced binding due to the recognition of novel interaction surfaces created by branching or recognition of the branch point itself is represented by the conformational recognition model.

MUNI SCI

Post-translational Ubiquitination

- Model for the role of branched K48/K63 chains in the activation of NF-κB signaling.
- Homotypic K63-linked chains are efficiently disassembled by CYLD, resulting in the removal of K63 linkages from TRAF6 and the termination of NF-κB signaling.

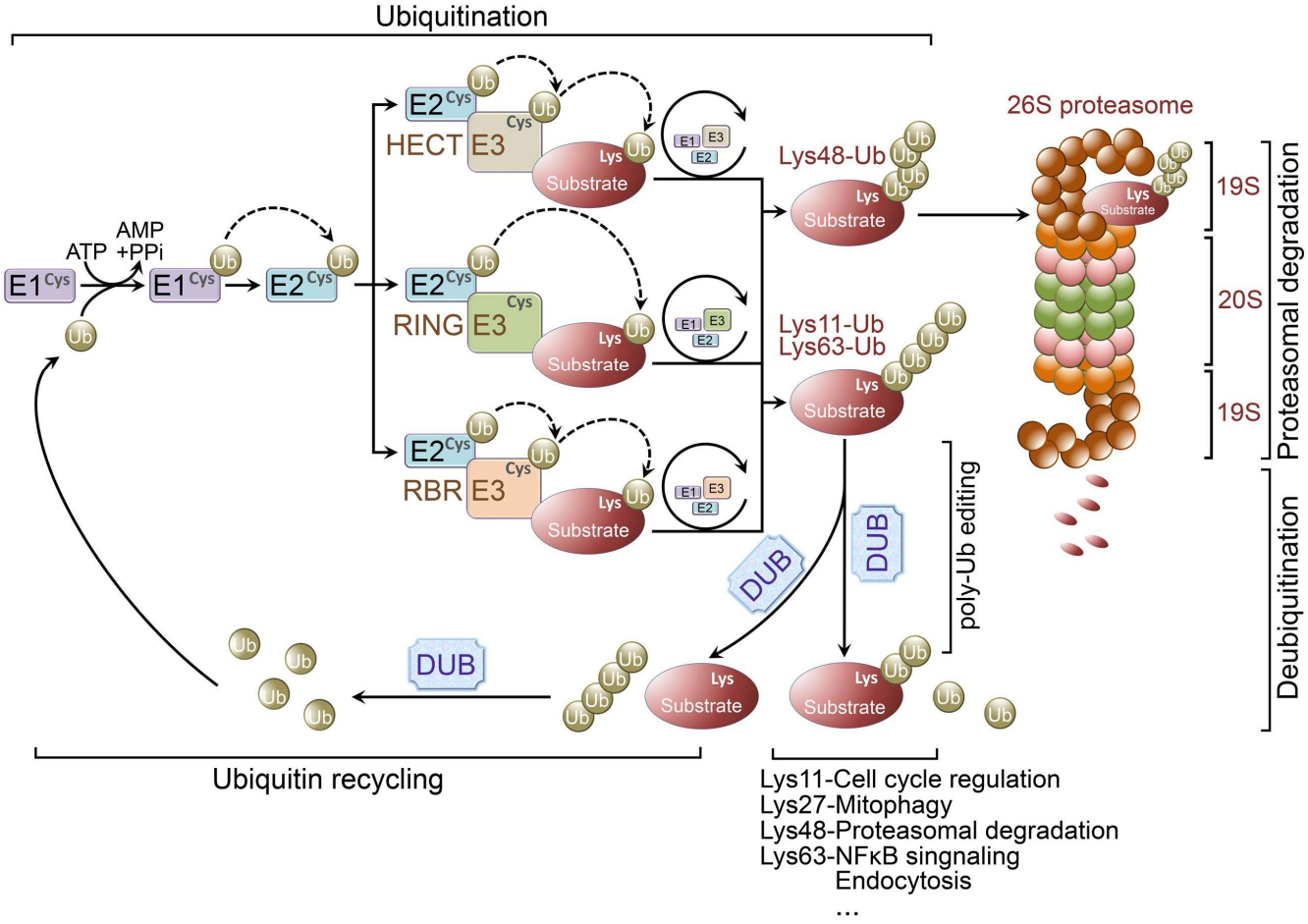




- Branched K48/K63 chains are resistant to CYLD cleavage, resulting in the persistence of K63 linkages on TRAF6 and sustained activation of NF-κB signalling.

Cell Discovery volume 7, Article number: 6 (2021)

Ubiquitin-proteasome system



- The overall system of ubiquitination and proteasomal degradation is known as the ubiquitin–proteasome system.
- Ubiquitination is covalently conjugated to a Lysine residue of the substrate proteins.
- Lys48-linked polyubiquitin chains usually target proteins for proteasomal degradation.

https://www.antibody-creativebiolabs.com/post-translational-modification-ptm.htm

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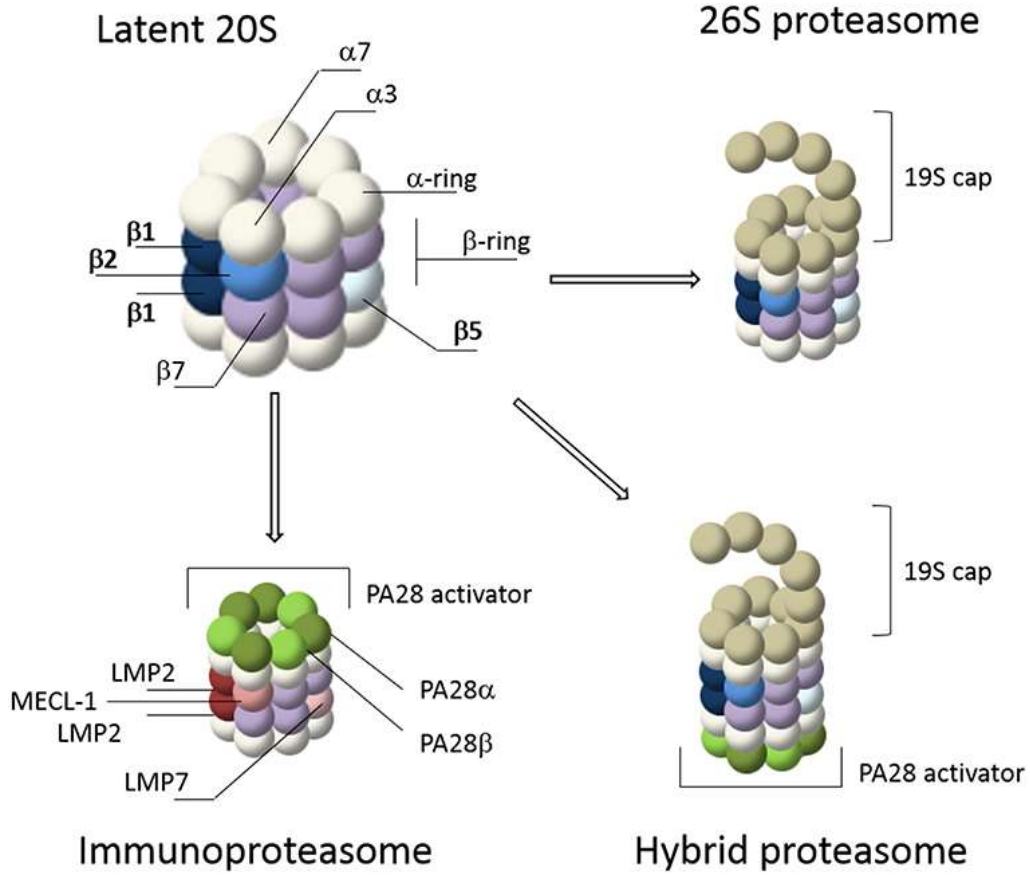
Ubiquitin-proteasome system

- The proteasome is a multi-catalytic molecular machine that plays a key role in the degradation of many cytoplasmic and nuclear proteins.
- The core of the proteasome consists of a symmetrical cylinder-shaped structure composed of four stacked rings, each containing 7 different subunits and is called the 20S proteasome.
- Gate opening of the 20S core occurs via capping by proteasome activators such as the 19S cap or PA28.
- The 19S cap is the most abundant activator and it forms the 26S proteasome together with the 20S core.



Front. Mol. Biosci. 6:56., doi: 10.3389/fmolb.2019.00056

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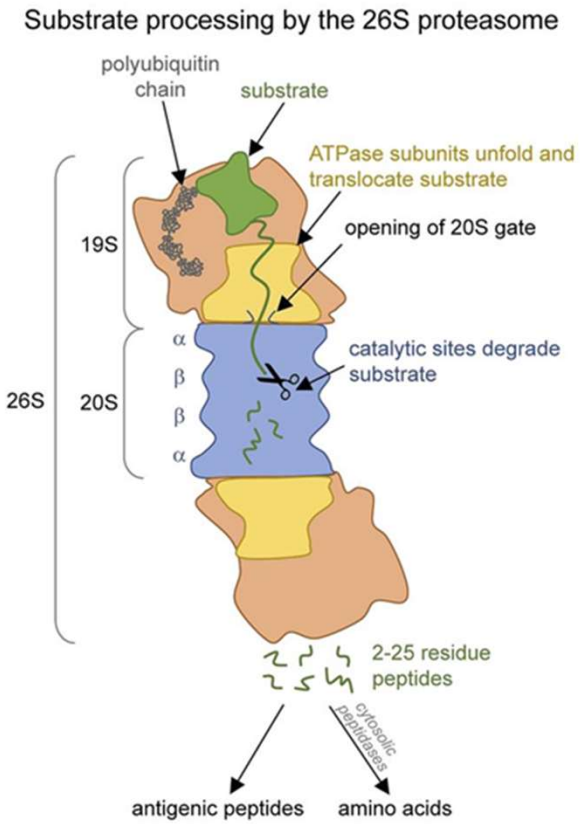
Ubiquitin-proteasome system



- The 26S proteasome is a 2.4-MDa molecular machine that makes up nearly 2% of total cellular protein.
- It is composed of a 20S proteasome core particle capped on one or both ends by the 19S regulatory particle.
- It degrades proteins by a multistep process; the 19S regulatory particle binds ubiquitinated substrates, opens a substrate entry gate in 20S and unfolds its substrates by linearly translocating them into the 20S catalytic chamber, where they are degraded to peptides.

Pharmacological Reviews April 2019, 71 (2) 170-197

Sumolyation

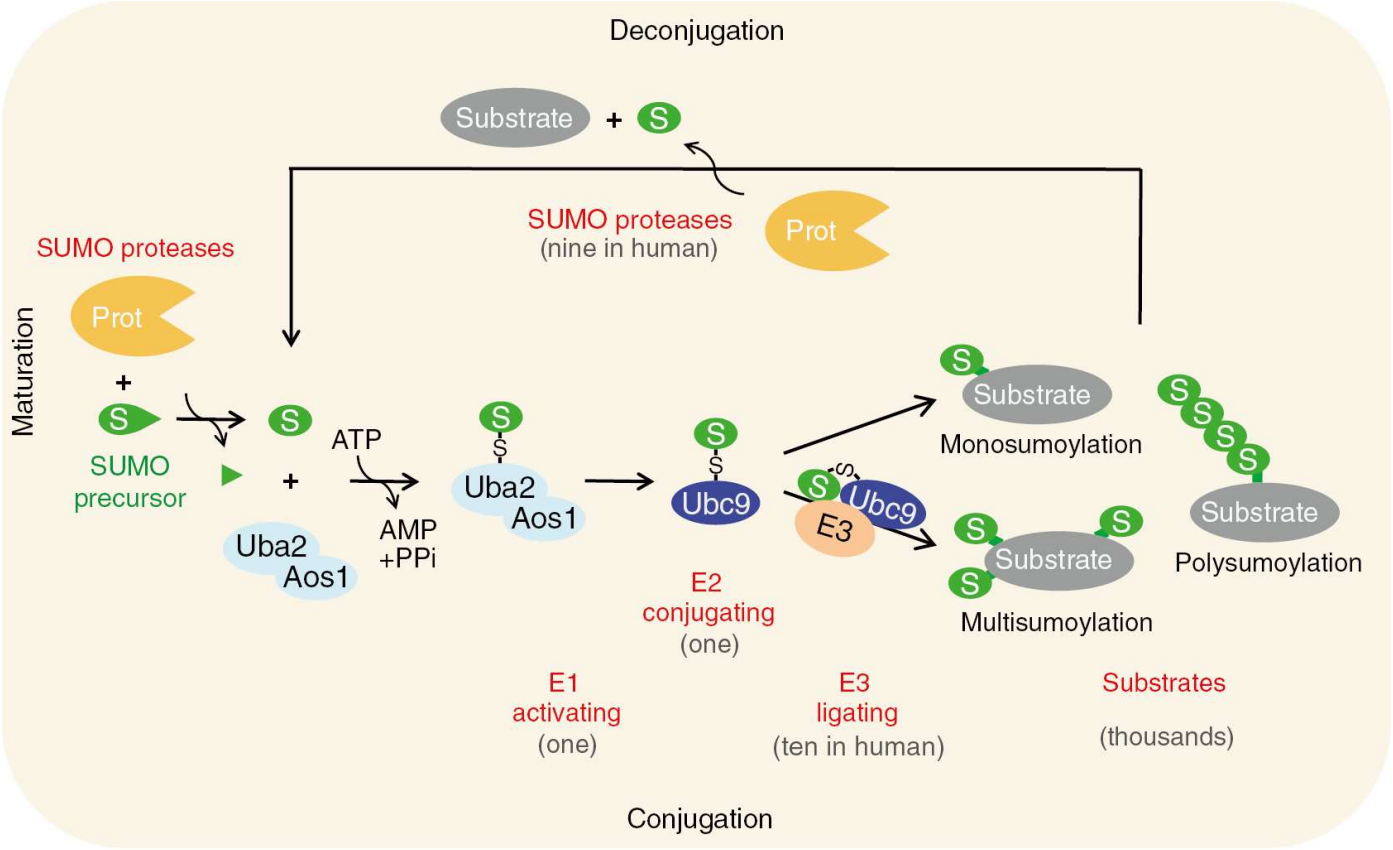
- Sumoylation is a post-translational modification, Small Ubiquitin-like Modifier (or SUMO) proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function.
- Sumoylation is reversed by the action of desumoylating enzymes.
- Here are 4 confirmed SUMO isoforms in humans; SUMO-1, SUMO-2, SUMO-3 and SUMO-4.
- SUMO proteins are small; most are around 100 amino acids in length and 12 kDa in mass.
- SUMO protein has a unique N-terminal extension of 10-25 amino acids which other ubiquitin-like proteins do not have.

https://en.wikipedia.org/wiki/SUMO_protein

Sumolyation

- First, SUMO (S) is matured by SUMO specific proteases (Prot), enabling it to become activated in an ATP-consuming reaction, to form a thioester bond (-S-) with the heterodimeric E1 (Aos1/Uba2).
- SUMO is then transferred to the E2 (Ubc9), resulting in a thioester bond.
- Finally, SUMO is conjugated directly or with the help of an E3 ligase to its substrate, forming an isopeptide bond.
- Sumoylation is reversed by SUMO specific proteases that cleave SUMO from the substrate.

Sumolyation



Enzymes in sumolyation:

- E1 – 1
- E2 – 1
- E3 – 10

Substrates can be:

- Monosumoylation
- Multisumoylation
- Polysumoylation.

BioMol Concepts 2017; 8(1): 13–36.

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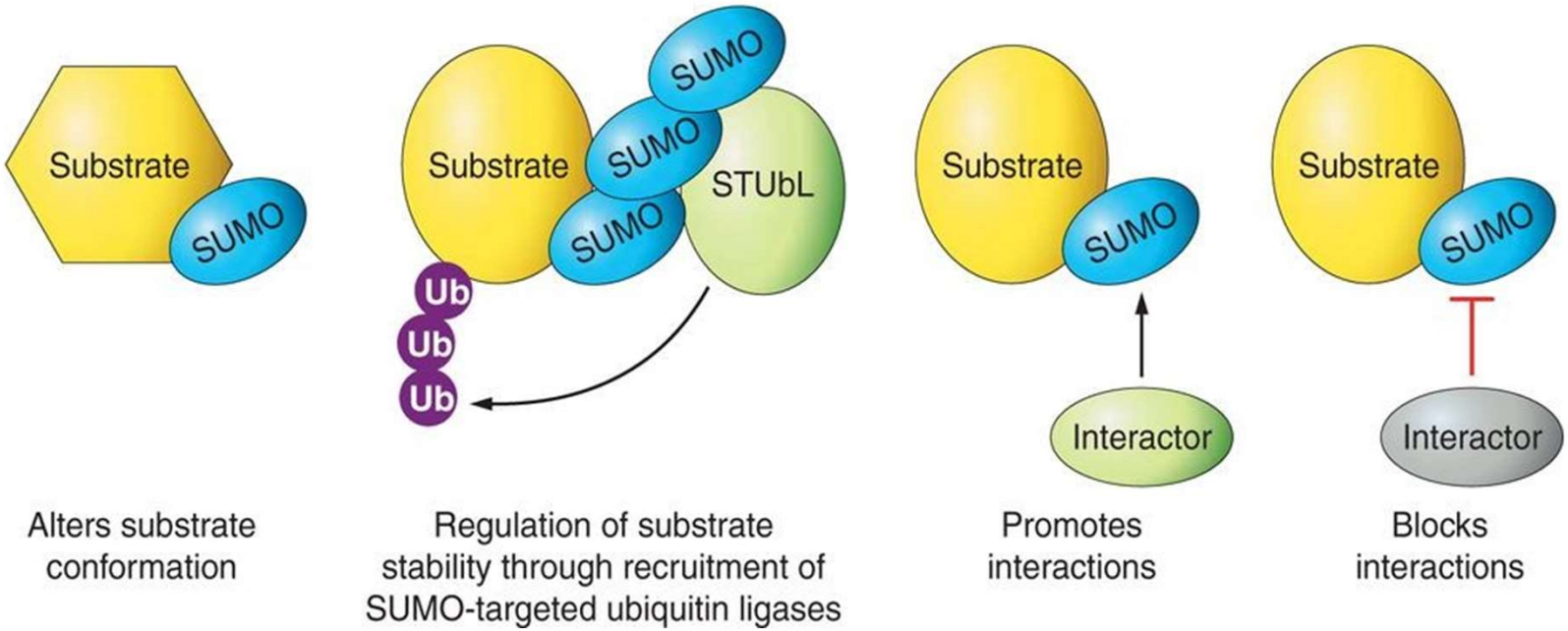
Sumolyation

- Sumoylation is involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle by one of these mechanisms:



Physiol Rev94: 1249 –1285, 2014doi:10.1152/physrev.00008.2014

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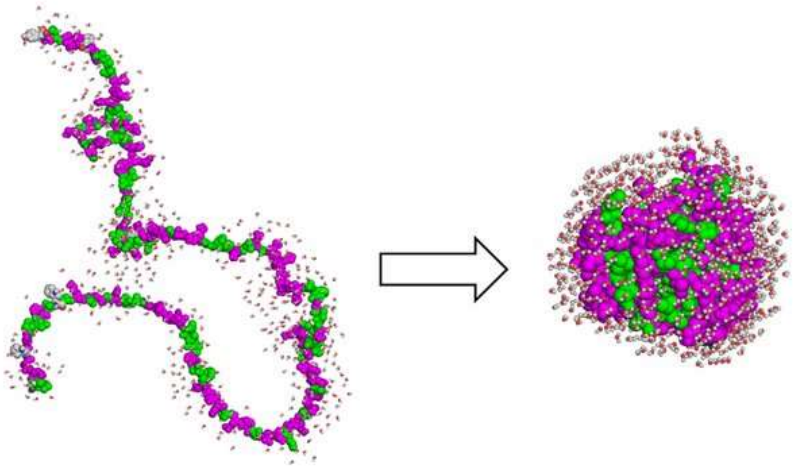
- Protein folding and Quality control

Protein folding

- Protein folding is the physical process by which a linear polypeptide folds into its characteristic and functional three-dimensional structure.
- The final folded configuration, or shape, of a protein is determined by its amino acid sequence.
- Protein folding is also strongly influenced by the solubility of the AA R-groups in water.
- Protein can reach its 3D conformation either alone, co-translational folding, or with help of other factors, chaperones.



https://slidetodoc.com/protein-folding-the-production-of-a-mature-protein/

Conference: 2016 IEEE 2nd International Forum on Research and Technologies for Society and Industry Leveraging a better tomorrow (RTSI)

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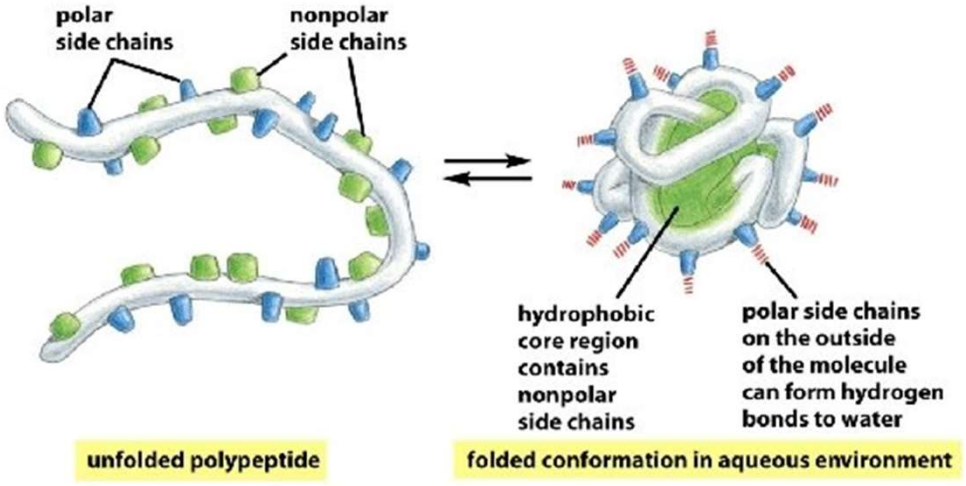
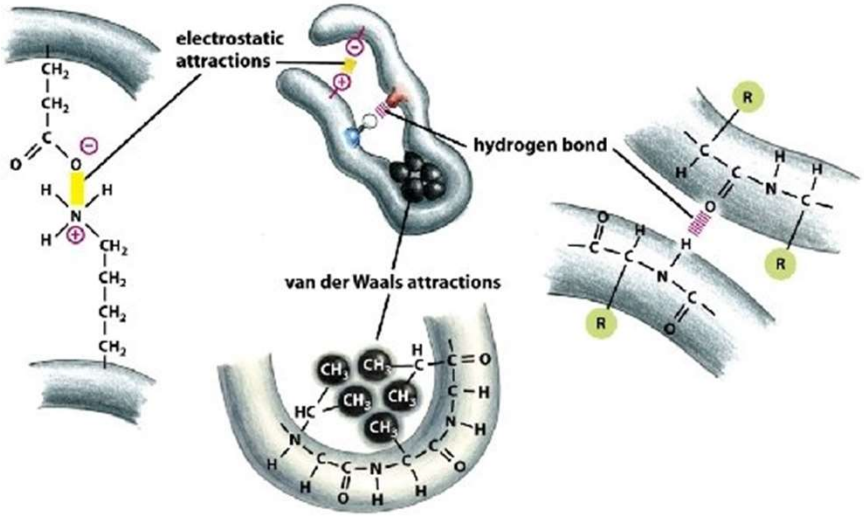
Protein folding

- How a protein folds?



- Three types of noncovalent bonds help proteins to fold.



https://slidetodoc.com/protein-folding-the-production-of-a-mature-protein/

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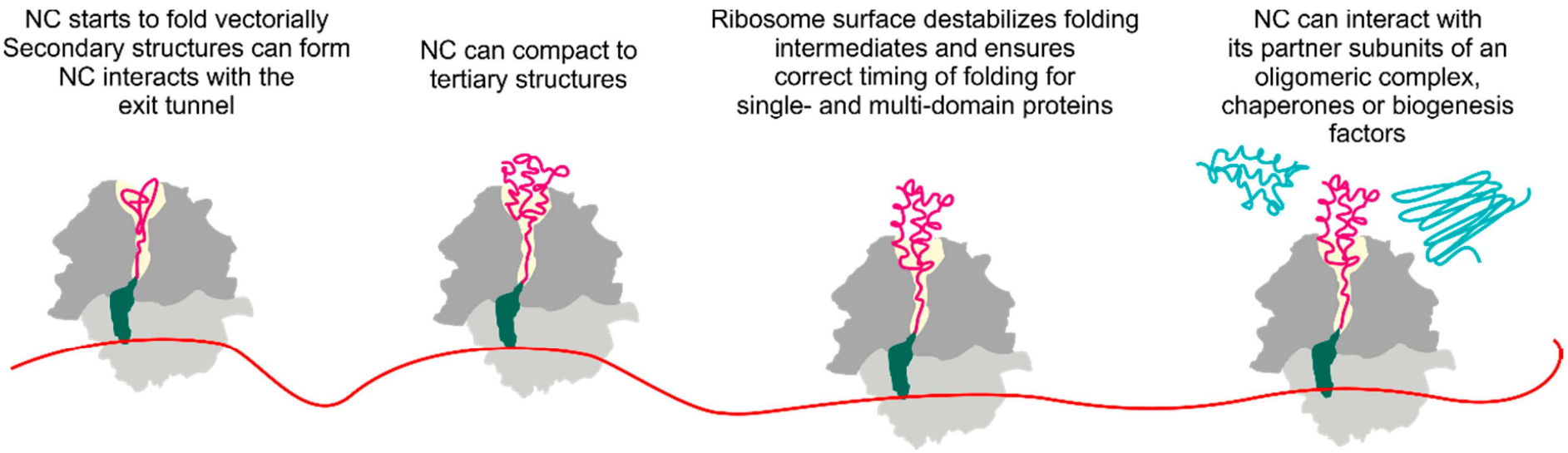
Co-translational protein folding

- Protein domains can fold into stable tertiary structures while they are synthesized by the ribosome in a process known as co-translational folding. Folding begins early inside the polypeptide exit tunnel.

- The nascent chain (NC) emerging from the ribosome can interact with chaperones, biogenesis factors, or other proteins.



Biomolecules 2020, Cotranslational Folding of Proteins on the Ribosome, 10(1), 97.

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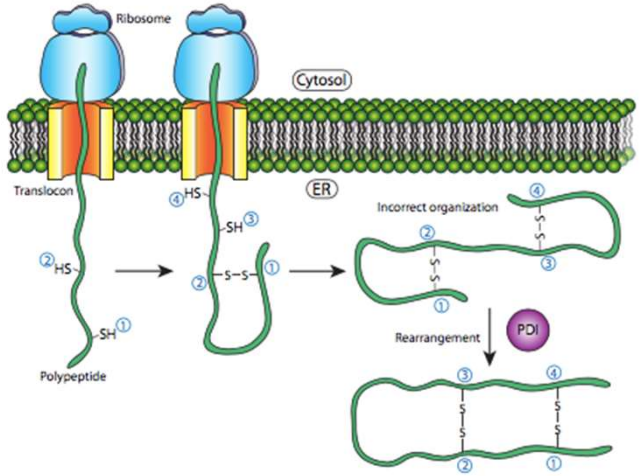
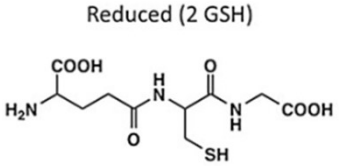
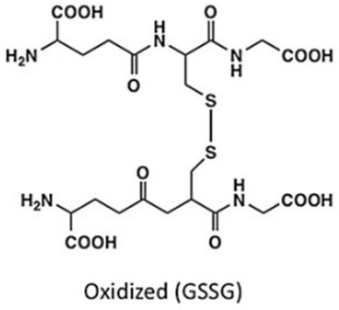
Protein folding

- The endoplasmic reticulum (ER) lumen plays four major protein processing roles:
  - folding/refolding of the polypeptide,
  - glycosylation of the protein,
  - assembly of multi-subunit proteins, and
  - packaging of proteins into vesicles.







- The internal redox environment of the endoplasmic reticulum, is significantly more oxidative than that in the cytoplasm.
- This is largely determined by glutathione, which is found in a 30:1 GSH:GSSG ratio or higher in the cytoplasm but at nearly 1:1 ratio in the ER lumen.

https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/N-linked_Protein_Glycosylation_Begins_in_the_ER

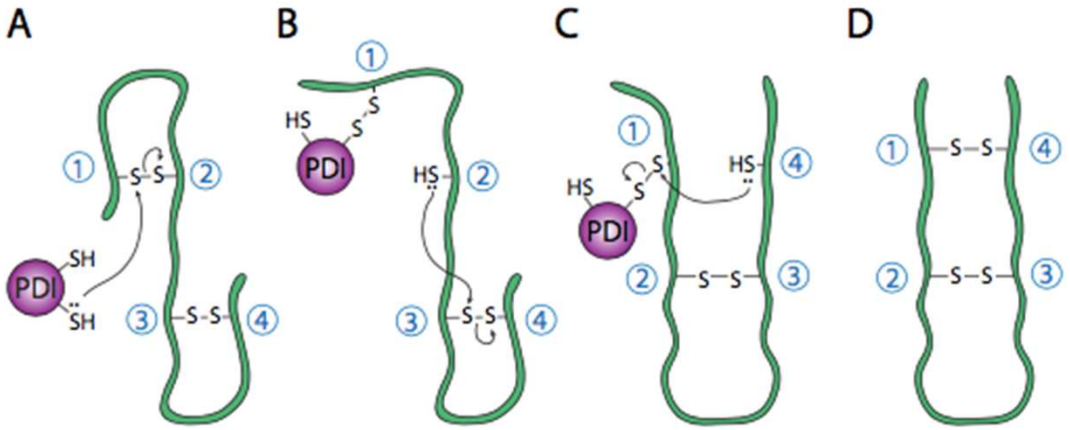
https://commons.wikimedia.org/wiki/File:Shine-Dalgarno_sequence-es.png

Protein folding

- If the there is non correct bound between cysteines, and the more stable cysteines bond in the context of the whole protein, than the exchange of disulfide bonding is catalyzed by protein disulfide isomerase (PDI).

- This enzyme uses a sulfhydryl group of a cysteine residue as temporary bonding partner in order to break disulfide bonds on the target protein and allow for new ones to form.

- Note that the formation of a new bond is not directed by PDI, but is instead a stochastic process in which a stronger binding partner displaces the PDI —SH.



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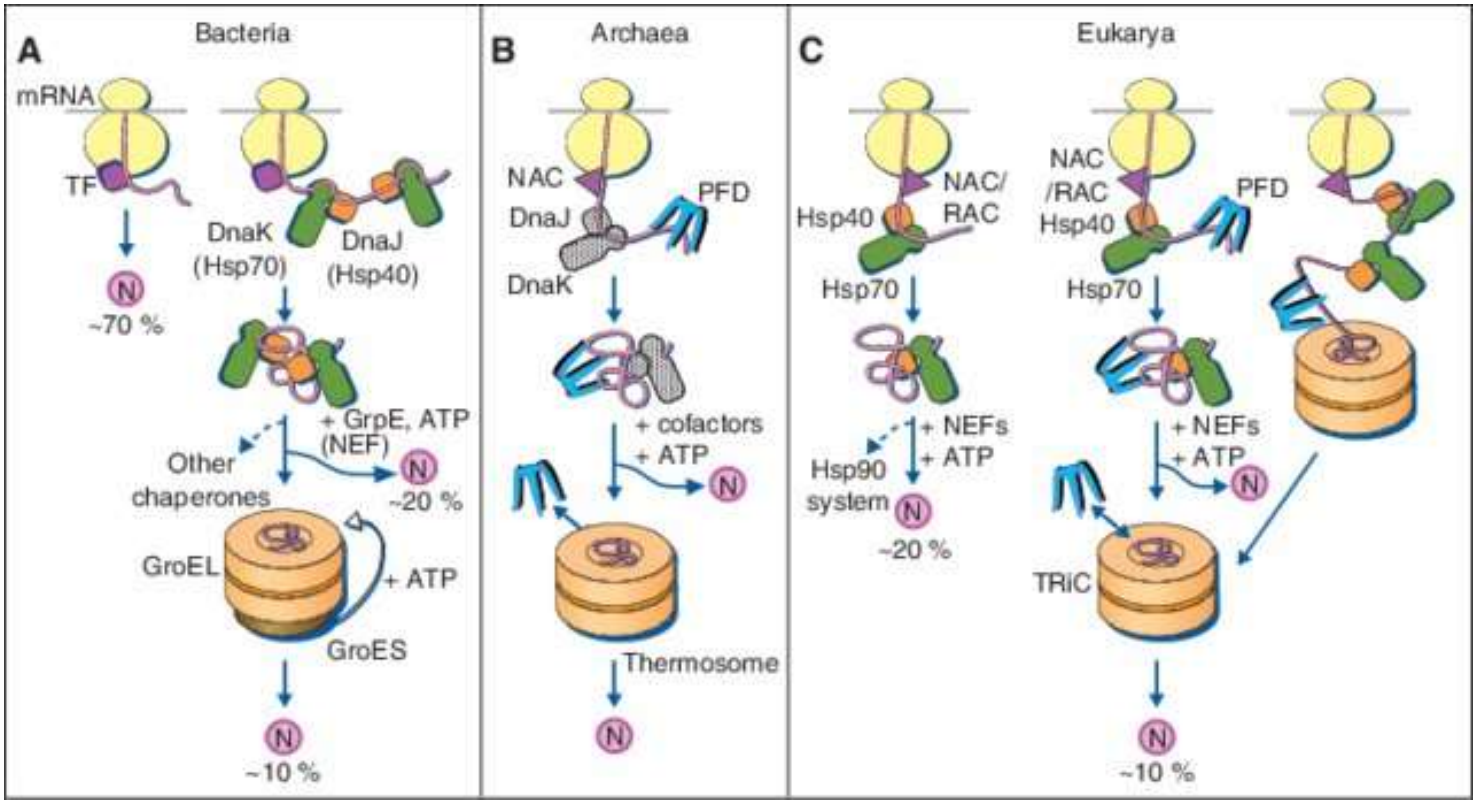
Chaperones

- Protein folding take place in the cytosol.
- Most proteins require the assistance of molecular chaperones.



Cold Spring Harb Perspect Biol 2010;2:a004390

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Chaperones

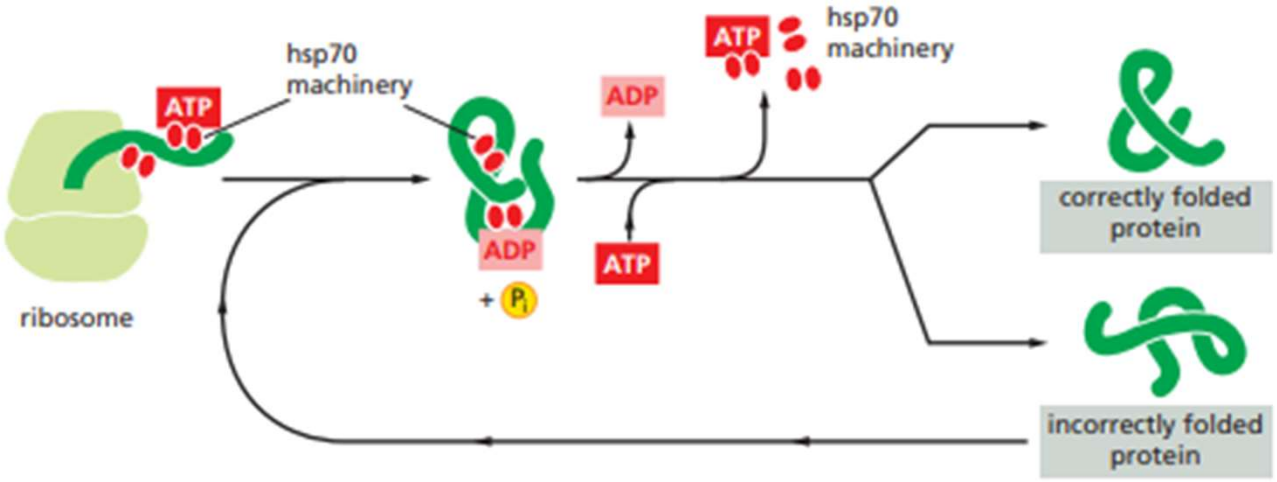
- The heat-shock proteins (Hsp) are a family of molecular chaperones, which collectively form a network that is critical for the maintenance of protein homeostasis.
- Most proteins require the assistance of molecular chaperones, like Hsp 70 & Hsp 60, to reach their final folded form.
- These molecular chaperones bind to exposed hydrophobic patches on the surface of incompletely folded proteins.
- Hsp 70 and Hsp 60 act sequentially on proteins to help them achieve their correct folded state.
- Proteins that fail to be properly folded are ultimately targeted for destruction.

Molecular Biology, 430 (22), 4525-4546

https://slidetodoc.com/protein-folding-the-production-of-a-mature-protein/

Chaperones

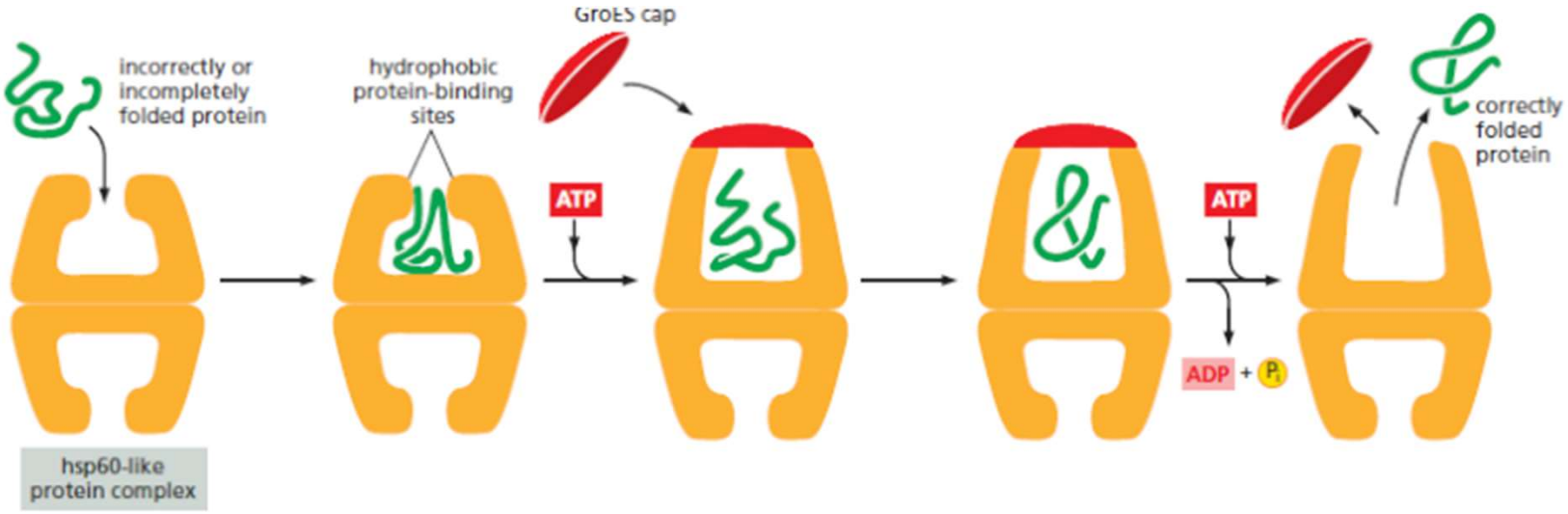
- Molecular chaperones, like those in the hsp 60 and hsp 70 families, help guide the folding of most newly-synthesized proteins.
- Hsp chaperones bind to hydrophobic patches that are exposed on incompletely folded proteins.
- Repeated cycles of ATP binding and hydrolysis are generally required for the proper folding of a polypeptide chain.



https://slidetodoc.com/protein-folding-the-production-of-a-mature-protein/

Chaperones

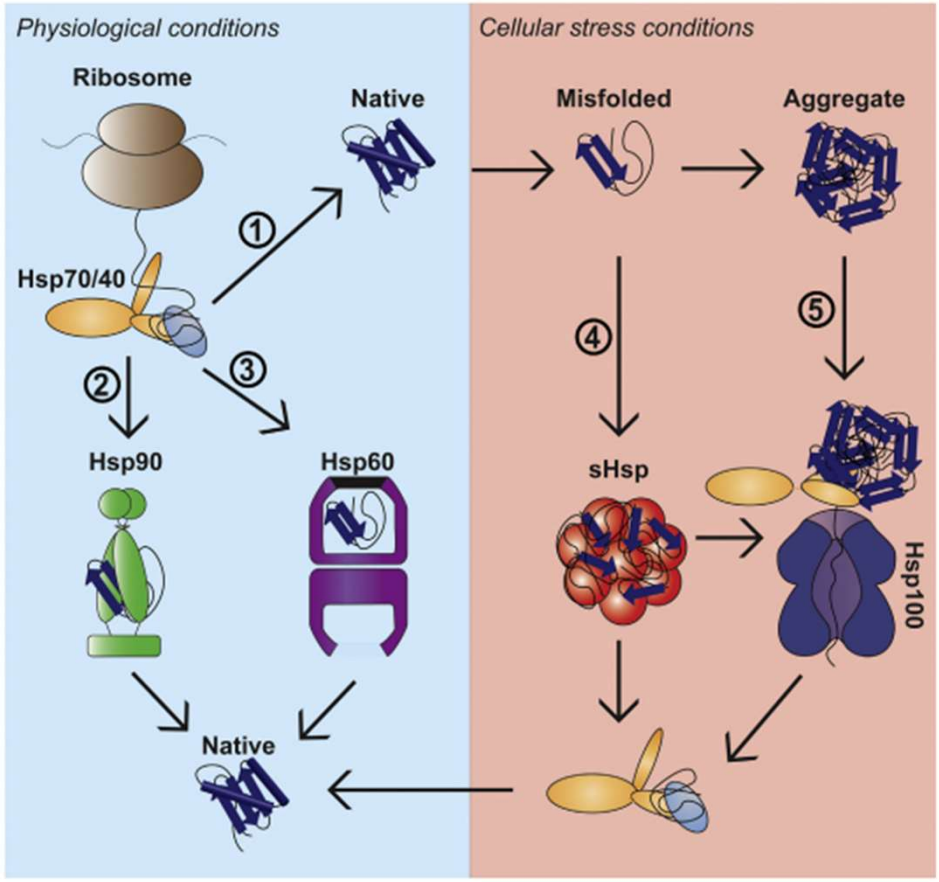
- Hsp 60 acts on fully-synthesized proteins that have not yet achieved their final folded form.
- The presence of incompletely folded proteins can lead to the formation of aggregates that may have dangerous consequences for the cell.



https://slidetodoc.com/protein-folding-the-production-of-a-mature-protein/

Chaperones

- Hsp70/40 mediates the initial stages of protein folding and acts as a central hub in the Hsp chaperone network.
- If Hsp70/40 is only able to partially fold the protein, it can be transferred to either the Hsp90 or Hsp60 systems in order to acquire a folded functional conformation.
- Cellular stress conditions can cause proteins to misfold and associate into insoluble, toxic aggregates.



Molecular Biology, 430 (22), 4525-4546

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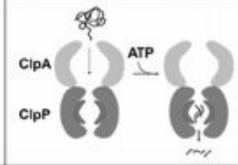
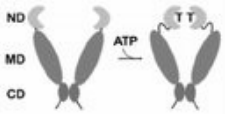
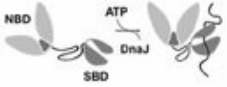
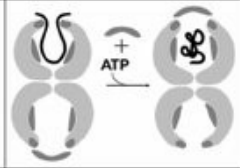
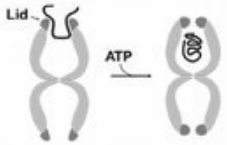
Chaperones

Chaperone Family	Topology of Binding	Co-chaperone	Known Function
Hsp100		ClpP, SspB, Hsp70, Hsp40	• Works with DnaK in ATP-dependent disaggregation and proteolysis • Prevents aggregation, degradation and turnover of unassembled mitochondrial proteins • Reactivates heat-damaged proteins • Establishes and maintains prion phenotype in yeast
Hsp90		Hop, Hip, Hsp70, Immunophilins, Grp78	• Refolds proteins in stressed cells. Probable secretory chaperone in prokaryotes • Major cytosolic chaperone in eukaryotes. • Cytoprotection and intracellular signaling • In ER, controls protein homeostasis, folding and assembly of secretory proteins
Hsp70		Hsp40, GrpE	• Ubiquitous Principal folding chaperone • Works with ClpB as disaggregase • Folding of newly synthesized proteins • Protein transport into ER and mitochondria
Hsp60 (Group I)		Hsp10	• Major chaperone for protein folding in prokaryotes • Stabilizes proteins during heat stress • Promotes folding of over-produced proteins • Major chaperone in mitochondria and chloroplast
Hsp60 (Group II)		Prefoldin/GimC	• Promotes folding of a cytosolic proteins in eukaryotes • Refolding of unfolded polypeptides in vitro
sHsps		-	• Stabilizes unfolded polypeptides • Prevents aggregation • Works with Hsp70 in protein refolding • Structural protein of eye lens

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Quality control of protein synthesis

- Because proteins are structurally dynamic, constant surveillance of the proteome by an integrated network of chaperones and protein degradation machineries is required to maintain protein homeostasis (proteostasis).

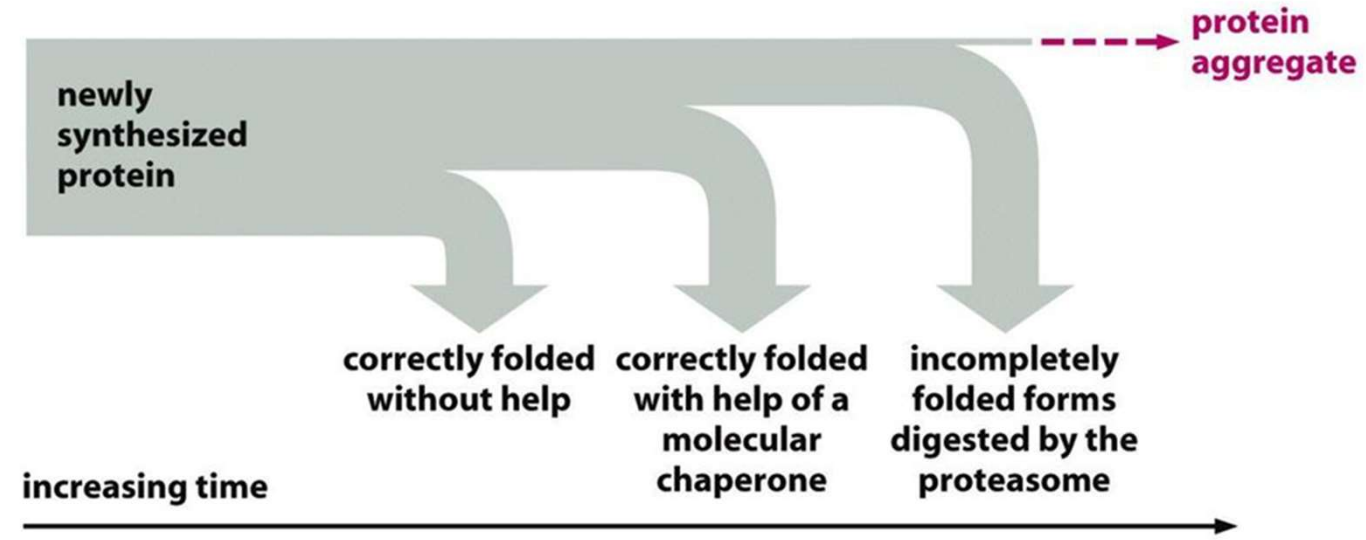
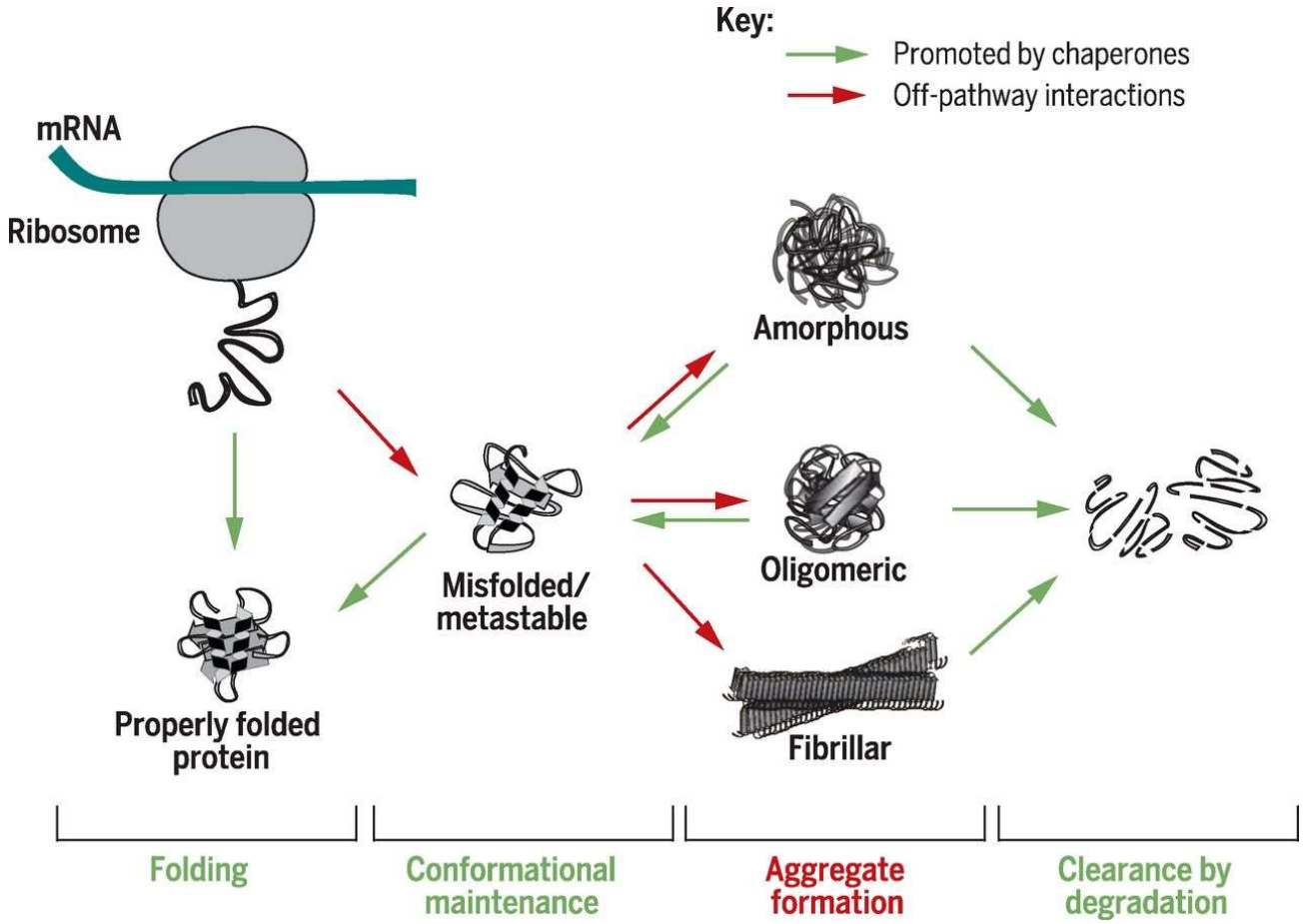


Figure 6-88 *Molecular Biology of the Cell* (© Garland Science 2008)

https://slidetodoc.com/protein-folding-the-production-of-a-mature-protein/

Quality control of protein synthesis

- Chaperones also cooperate with other components of the proteostasis network, such as the proteasome system and autophagy, in the removal of terminally misfolded and aggregated proteins through proteolytic degradation.



Science, Jul 2016 , Vol. 353, issue 6294.

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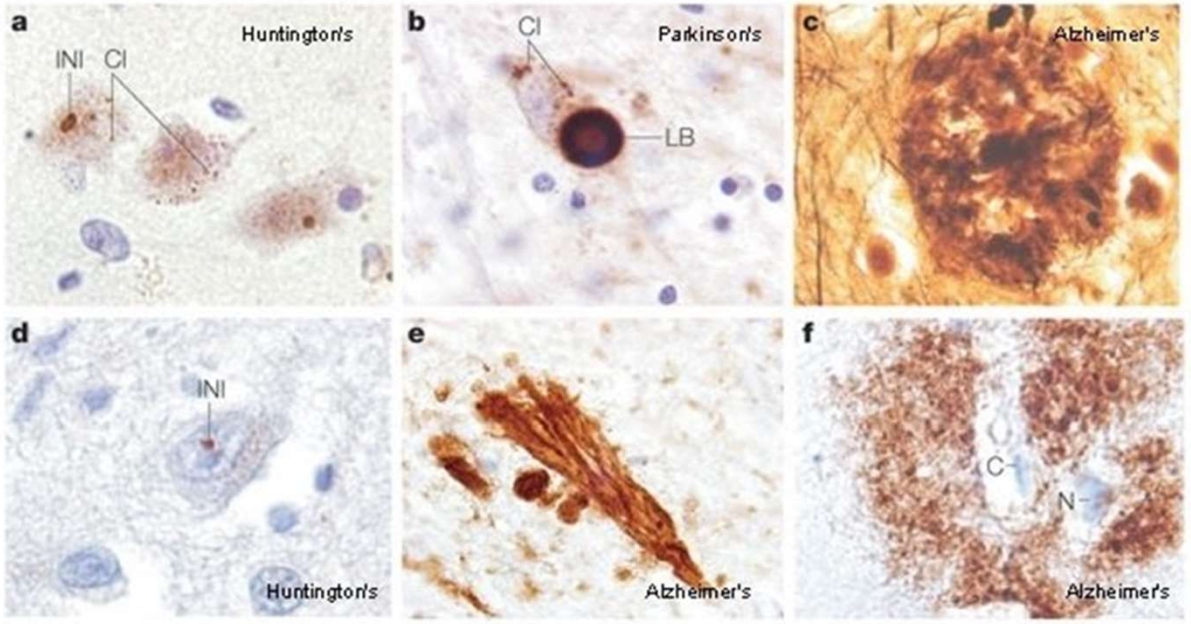
Protein Misfolding Diseases

- Many inherited diseases result from mutant proteins that evade quality control processes, fold abnormally and ultimately form aggregates.

- The gradual decline of protein quality controls with age can also lead to disease by permitting normal proteins to form misfolded protein aggregates that can impair cellular functions.

<u>Protein misfolding diseases:</u>
- Huntington's
- Alzheimer's
- Parkinson´s
- aggregates in patients can be intracellular or extracellular.



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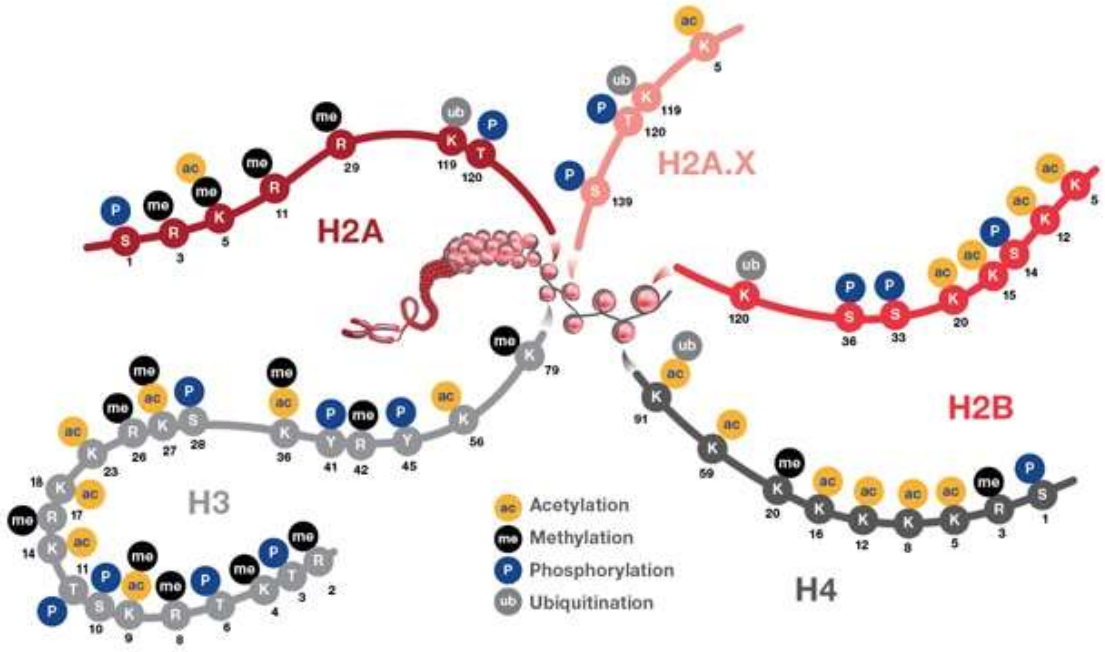
Post-translational modifications in histone function

- Post-translational modifications associated with histone particles.
- PTM events mediate diverse biological functions such as transcriptional activation and inactivation, chromosome packaging, and DNA damage and repair processes.



https://www.thermofisher.com/cz/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-post-translational-modification.html

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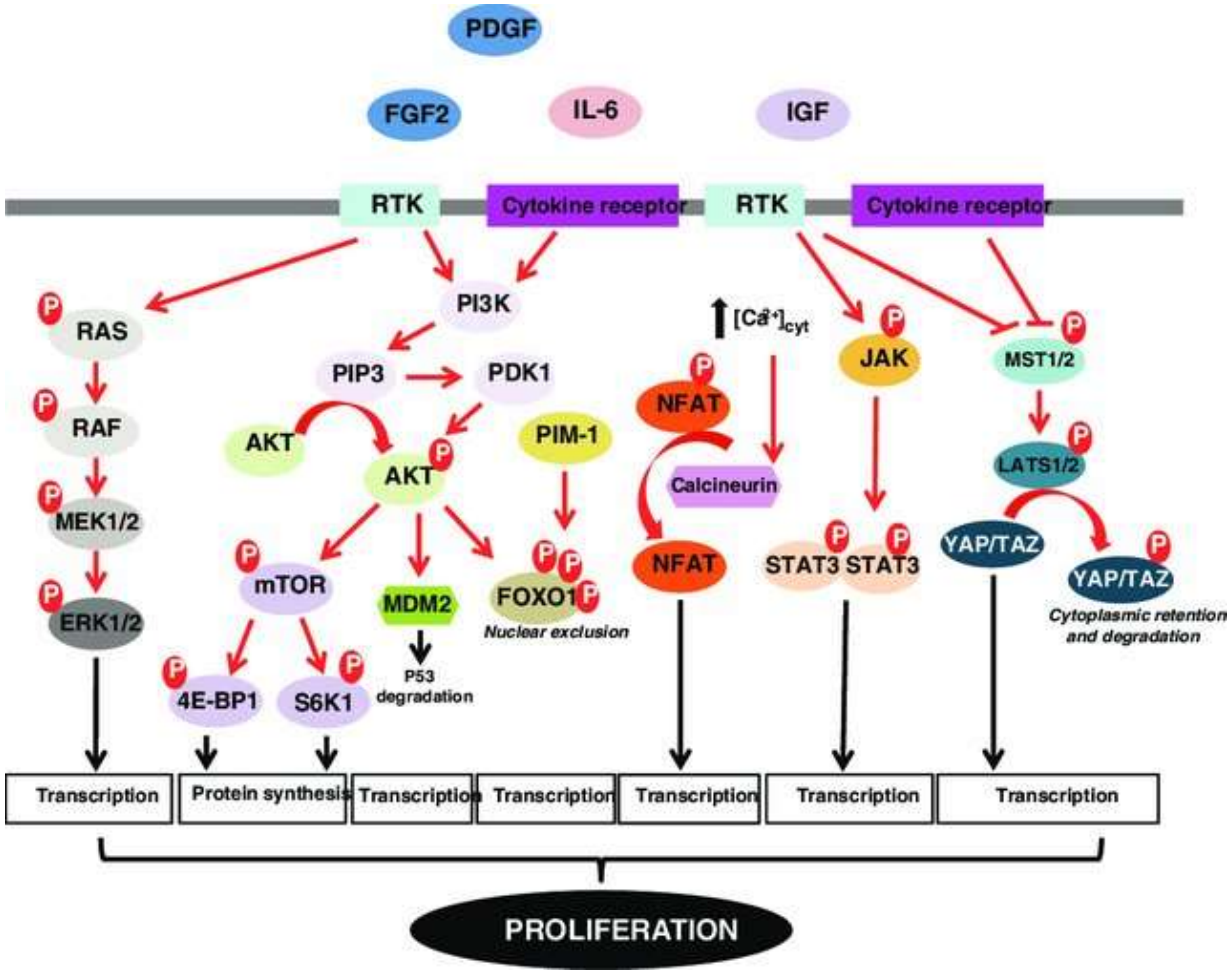
Post-translational modifications in cell signaling



March 2017Pulmonary Circulation 7(2):204589321770143

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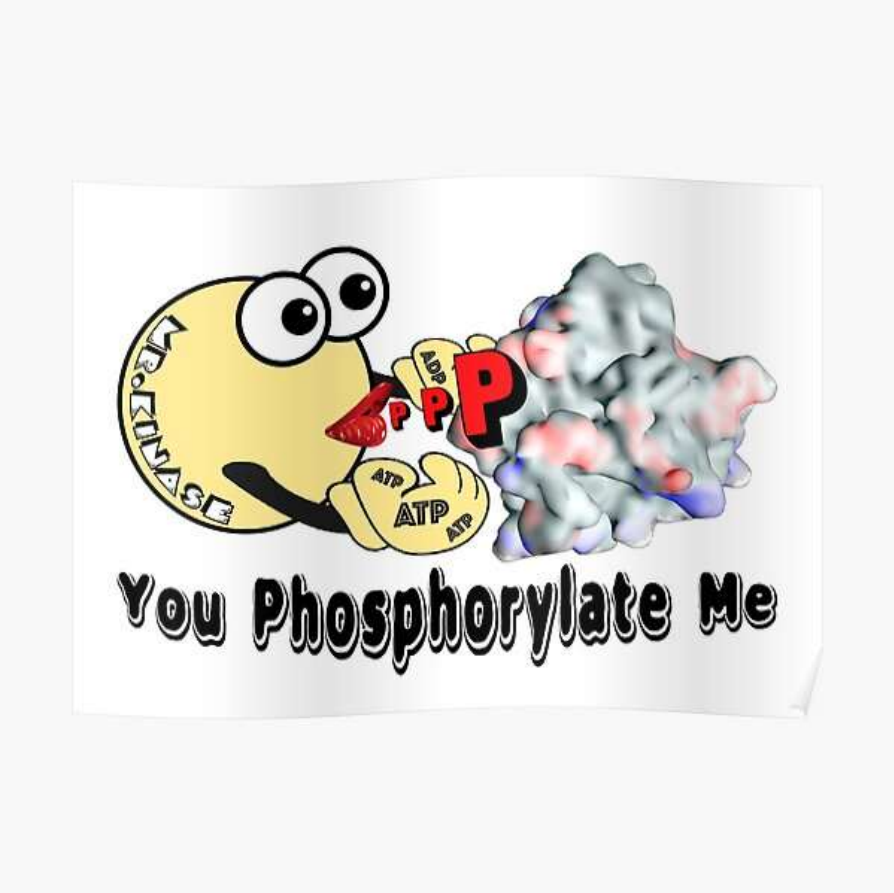
THANK YOU FOR YOUR ATTENTION



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