Protein Sorting and Targeting

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Objectives

- Introduction
- Sorting
- Targeting
- Intracellular protein traffic
- Clinical aspect
- Summary

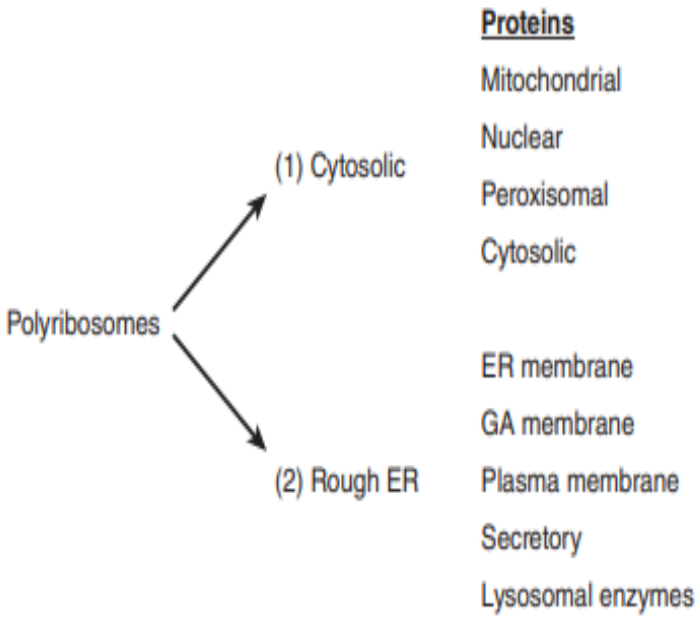
Introduction

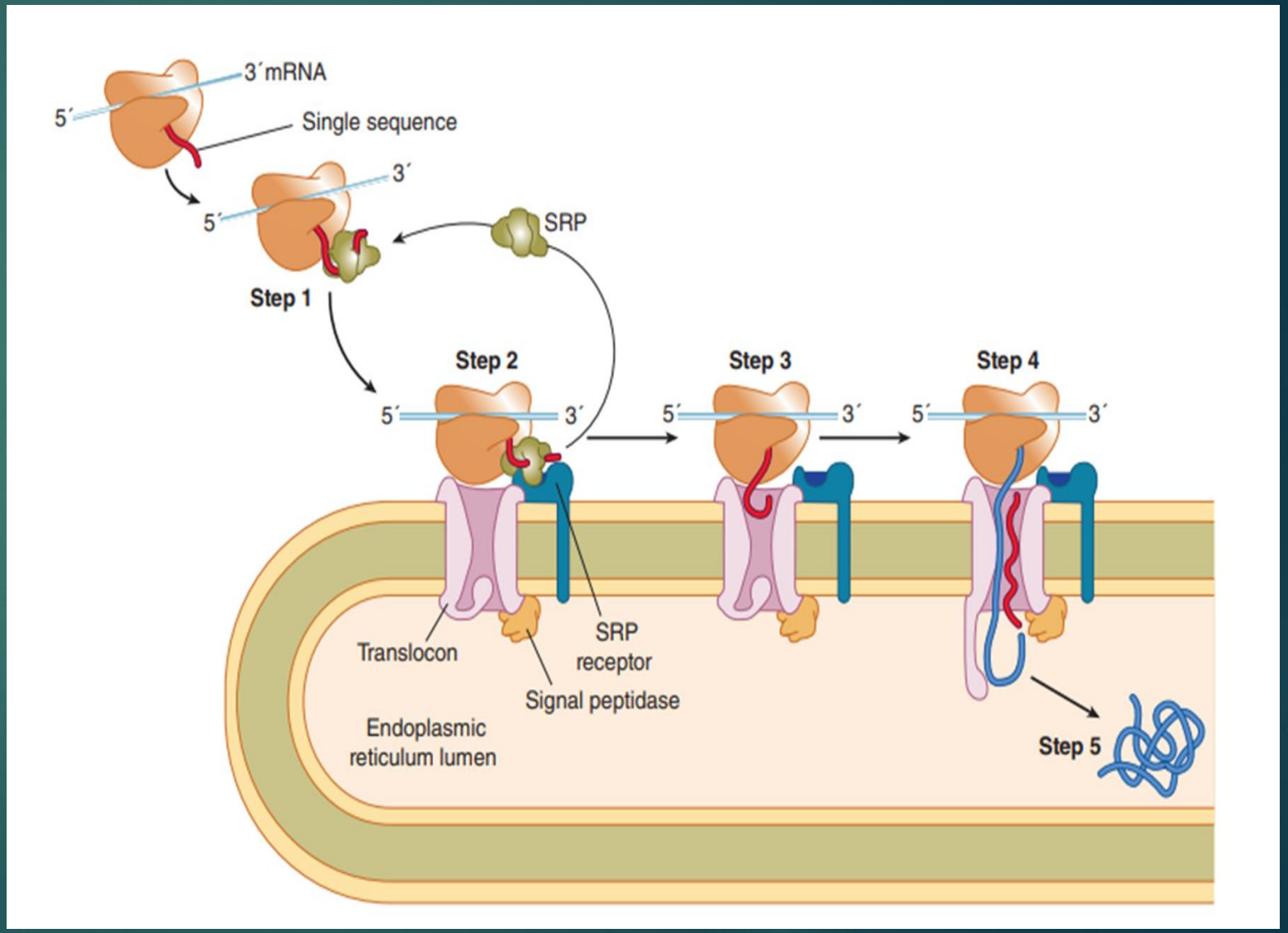
- Protein sorting and targeting is the biological mechanism by which proteins are sorted and targeted simultaneously together to their appropriate destinations within or outside the cell.

- It was first recognized by Blobel in 1970 that for proteins to attain their proper locations, they generally contain information (a signal or coding sequence) that targets them appropriately.

- This led to the identification of a number of the specific signals and it became apparent that certain diseases result from mutations that affect these signals.

Sorting





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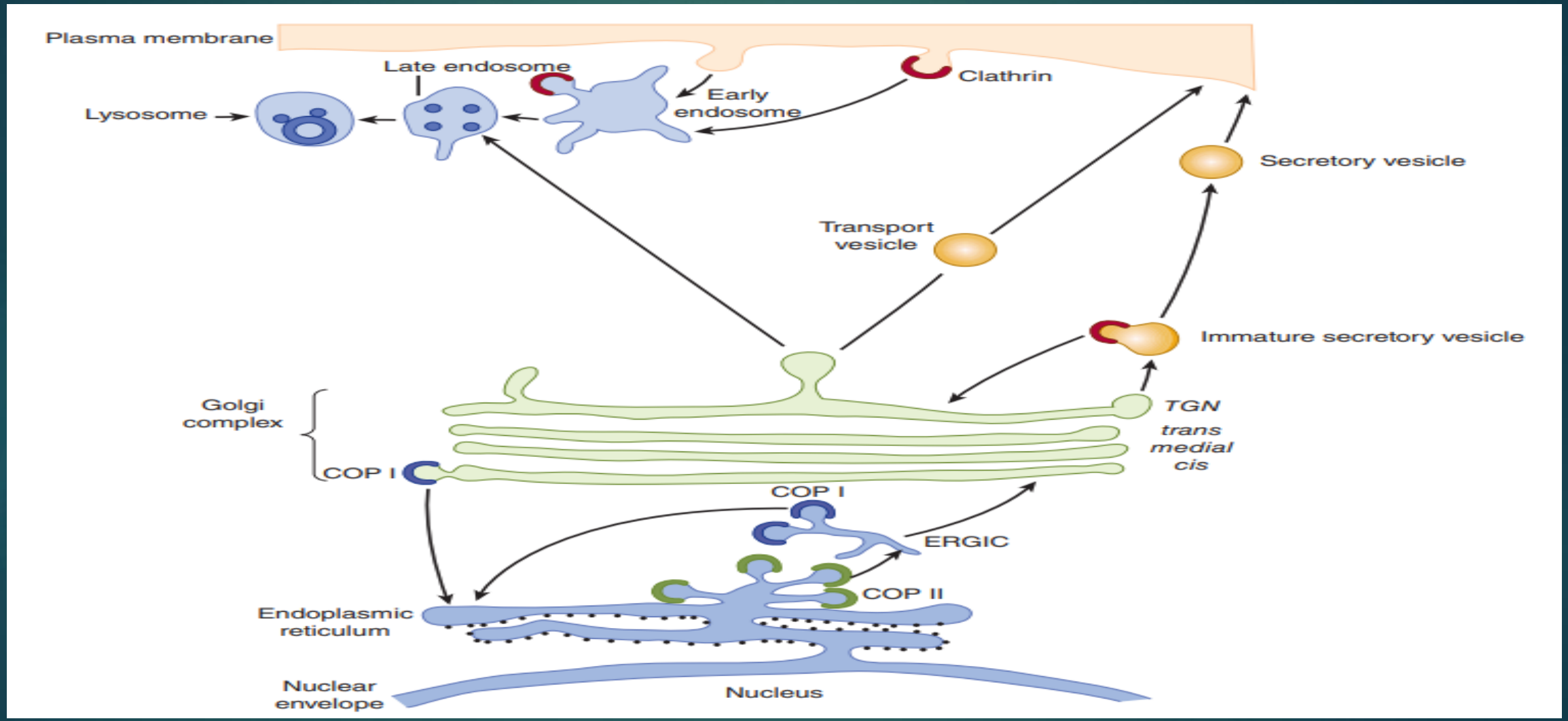
Sorting



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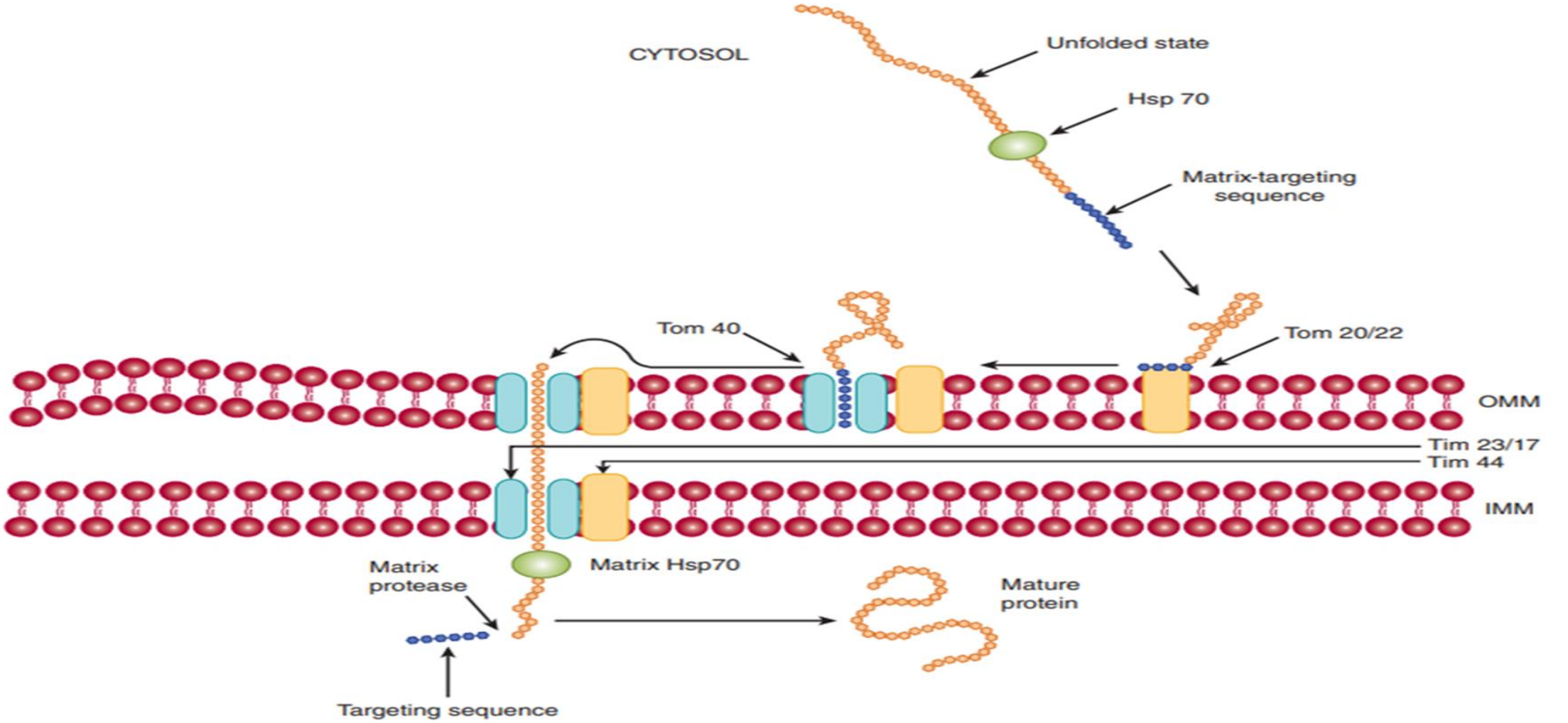
Sequences or Molecules That Direct Proteins to Specific Organelles

Targeting Sequence or Compound	Organelle Targeted
N-terminal signal peptide	ER
Carboxyl-terminal KDEL sequence (Lys-Asp-Glu-Leu) in ER-resident proteins in COPI vesicles	Lumen of ER
Di-acidic sequences (eg, Asp-X-Glu) in membrane proteins in COPII vesicles	Golgi membranes
Amino terminal sequence (20-50 residues)	Mitochondrial matrix
NLS (eg, Pro2 -Lys3 -Arg-Lys-Val)	Nucleus
PTS (eg, Ser-Lys-Leu)	Peroxisome
Mannose 6-phosphate	Lysosome

Targeting of protein into Mitochondrial Membrane



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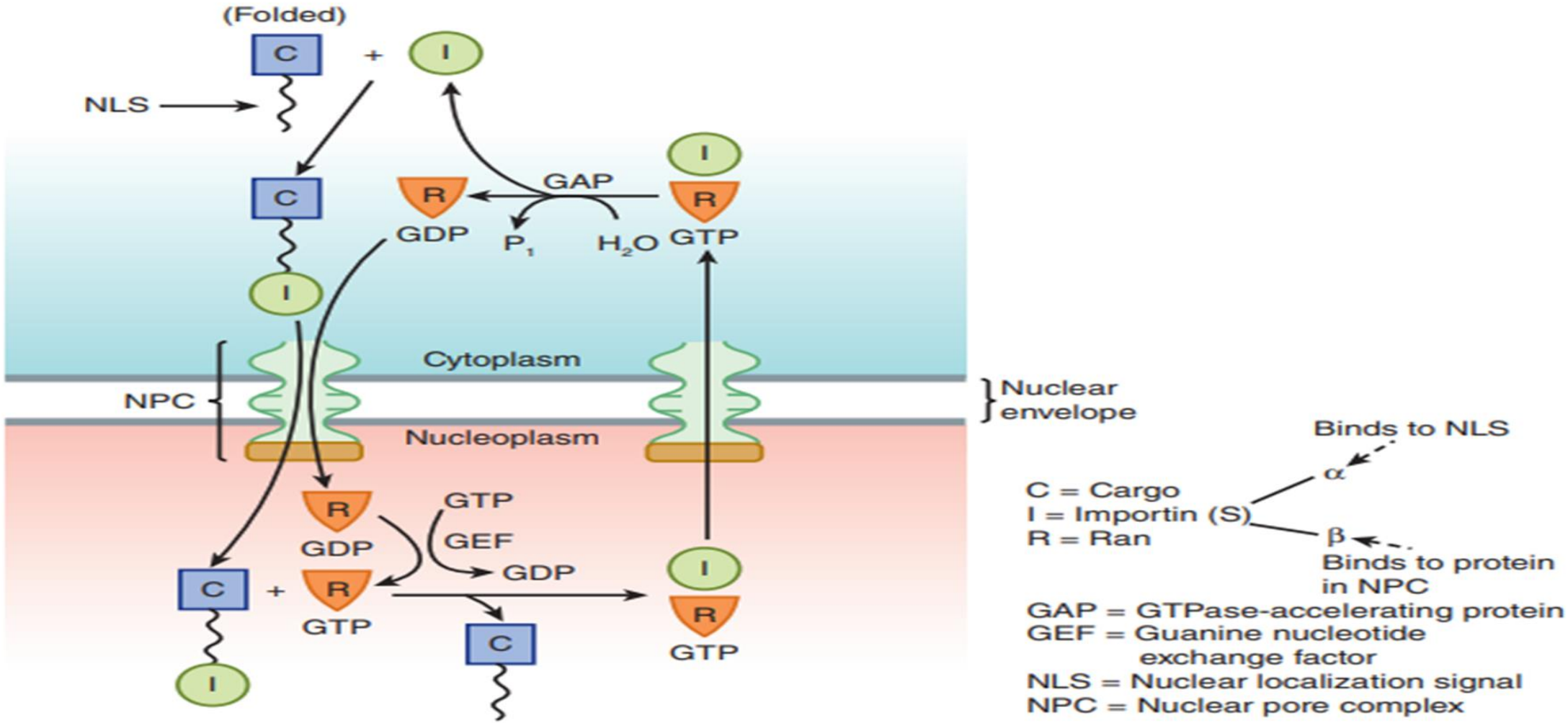
Targeting of protein into nucleus



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Nuclear and Mitochondrial Protein Targeting

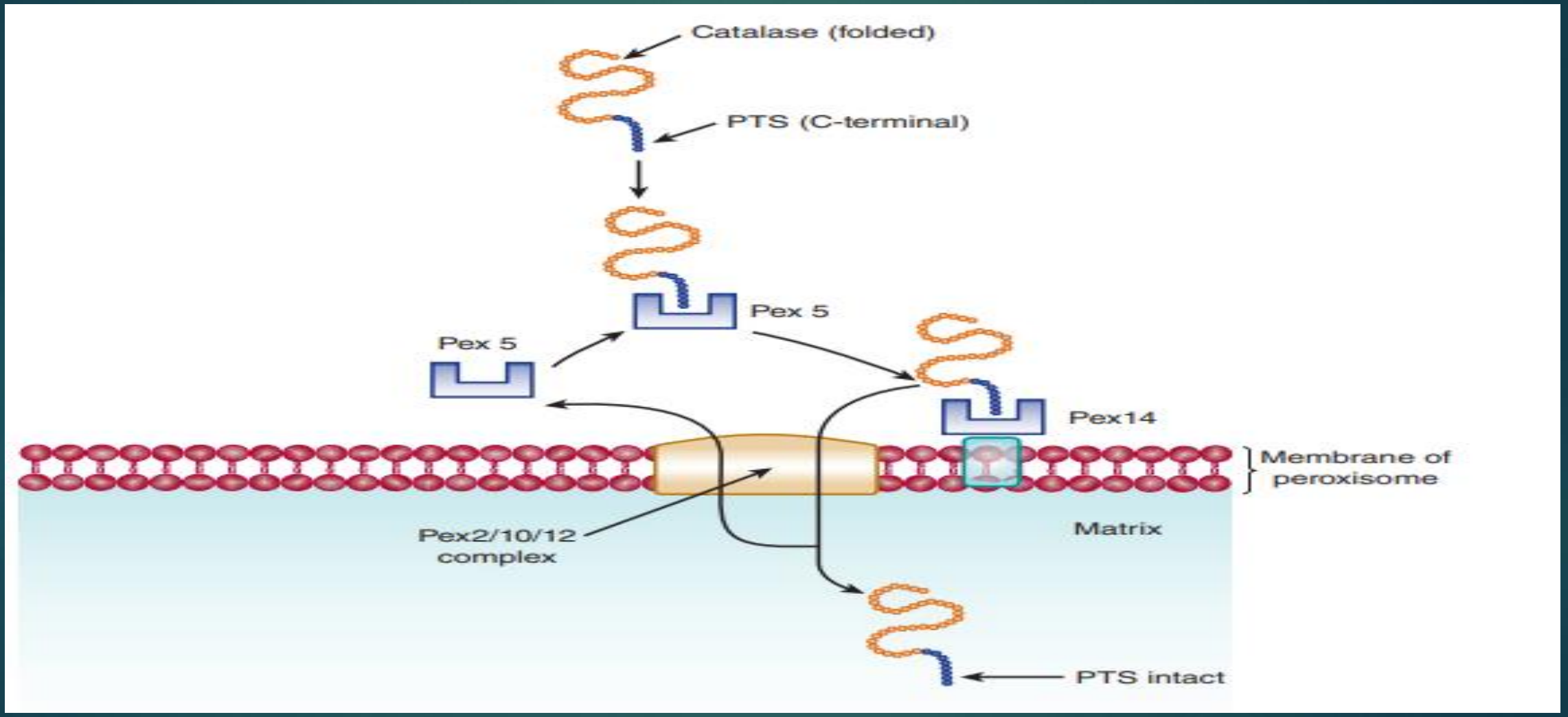
Nuclear proteins

- Translocate into nucleus via special pores.
- Pores are large in size and can easily accommodate transport of proteins in native state.
- So, proteins need not to be unfolded for transport.

Mitochondrial proteins

- A targeting sequence at the amino terminus directs their translocation.
- Proteins need to be in unfolded state
- Chaperones help the protein to be in the unfolded state which is required in the translocation.

Targeting of protein into peroxisome



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Peroxisome

- At least two peroxisomal–matrix targeting sequences (PTSs) have been discovered.
- One, PTS1, is a tripeptide (i.e., Ser-Lys-Leu [SKL], but variations of this sequence have been detected) located at the carboxyl terminal of a number of matrix proteins, including catalase.
- Another, PTS2, is a nine amino acid sequence at the N-terminus and has been found in at least four matrix proteins (e.g., thiolase).
- Neither of these two sequences is cleaved after entry into the matrix.
- Proteins containing PTS1 sequences form complexes with a cytosolic receptor protein (Pex5) and proteins containing PTS2 sequences complex with another receptor protein (Pex7).

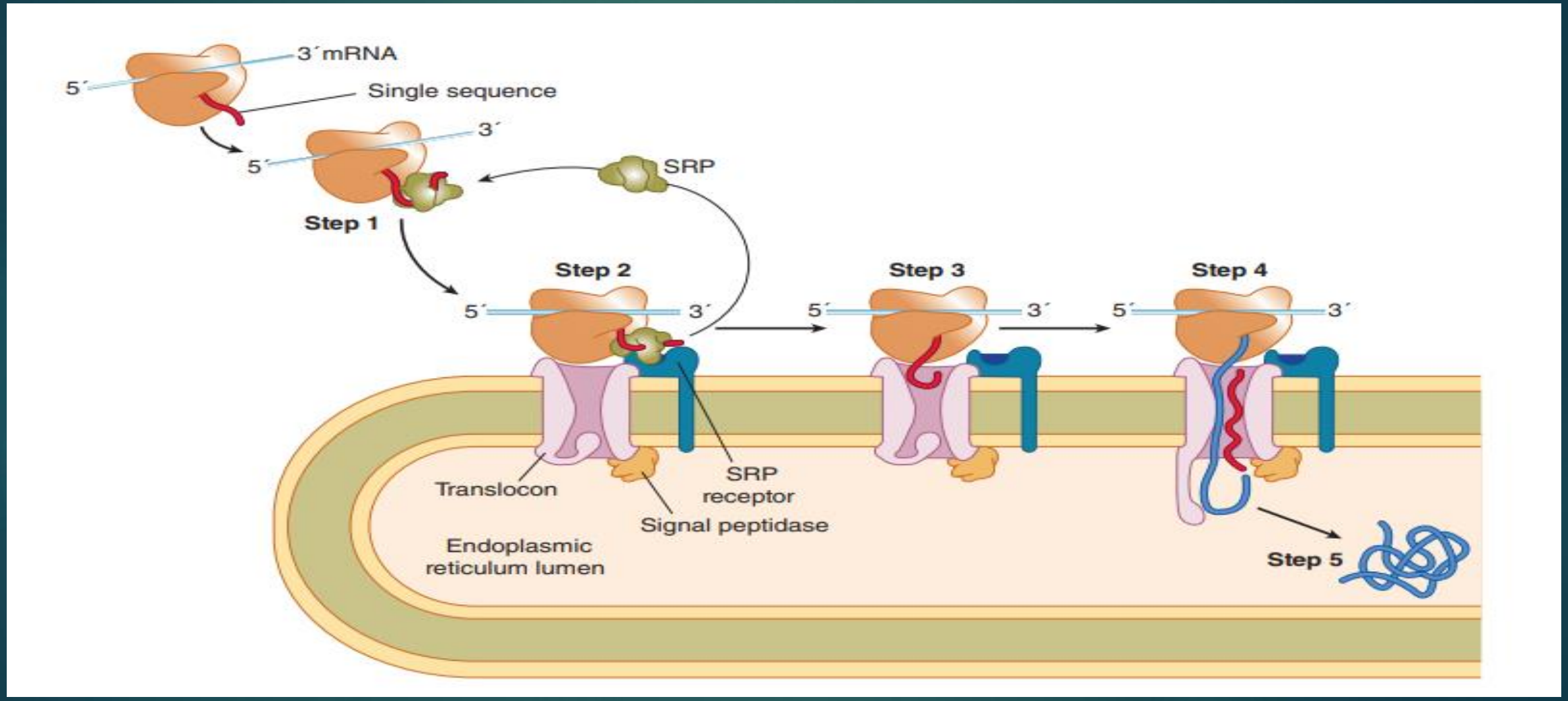
Properties of Signal Peptides Directing Proteins to the ER

- Usually, but not always, located at the amino terminal.
- Contain approximately 12-35 amino acids.
- Methionine is usually the amino terminal amino acid.
- Contain a central cluster (~6-12) of hydrophobic amino acids.
- The region near the N-terminus usually carries a net positive charge.
- The amino acid residue at the cleavage site is variable, but residues −1 and −3 relative to the cleavage site must be small and neutral.

Principal components involved in ER Co-translocation

- N-terminal signal peptide
- Polyribosomes
- SRP, signal recognition particle
- SRP-R, signal recognition particle receptor
- Sec 61, the translocon
- Signal peptidase

Targeting of secretory proteins



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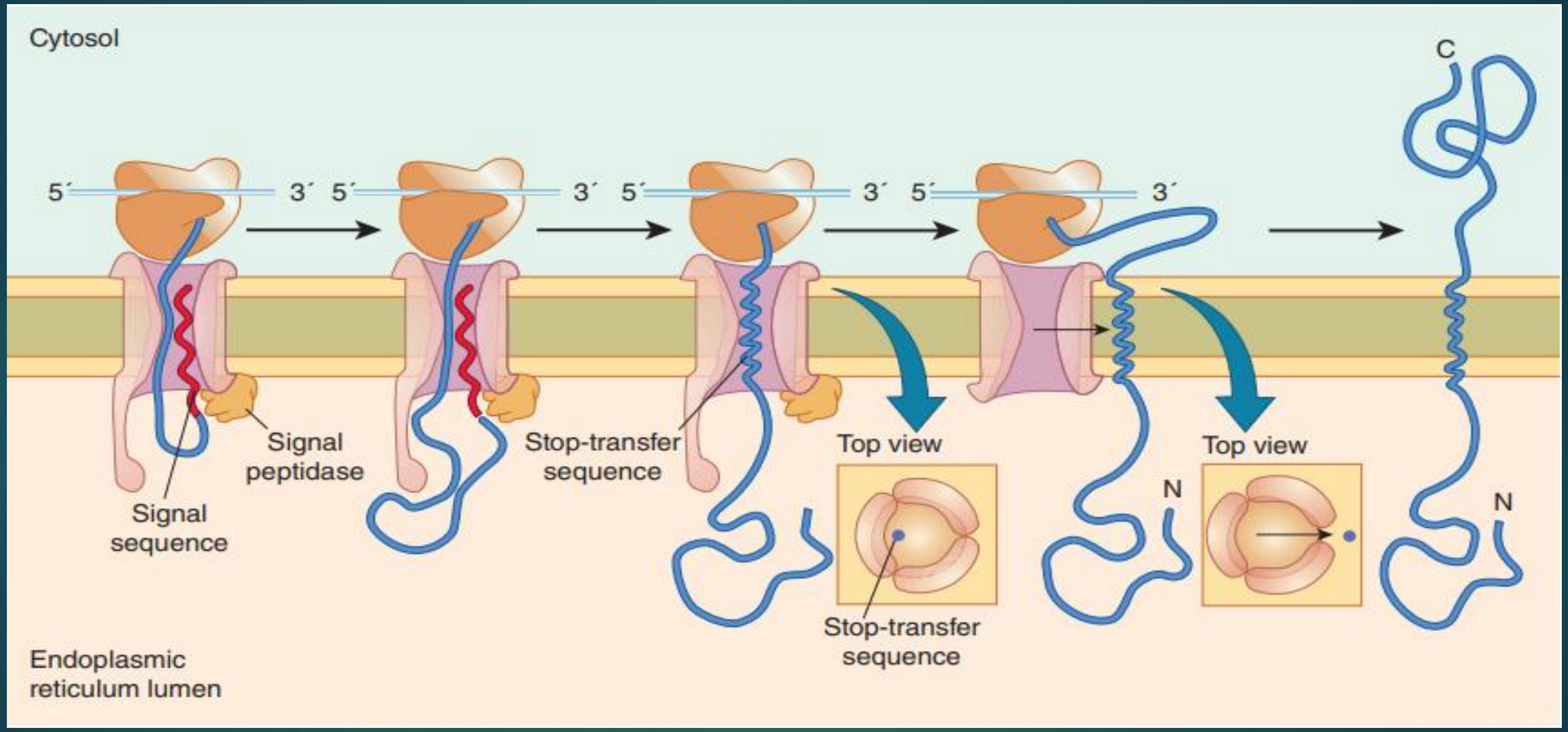
Targeting of Membrane Proteins



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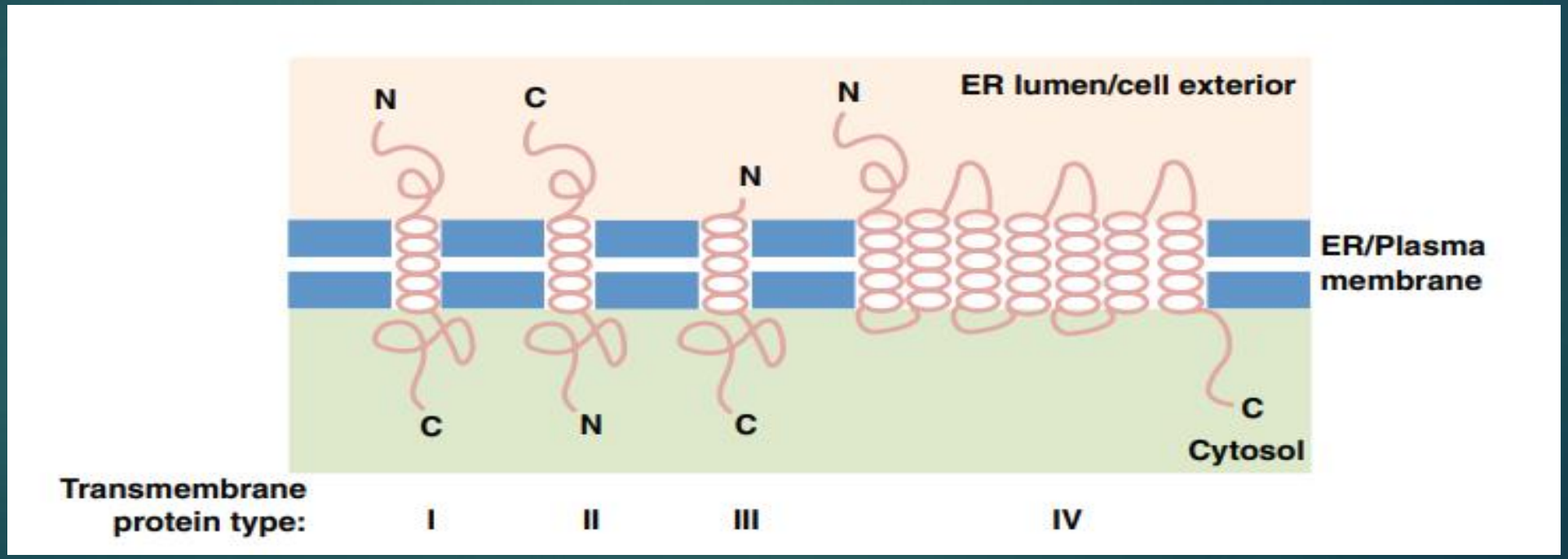
Variations in the way in which proteins are inserted into membranes

- Type 1 : LDL receptor
- Type 2 : The asialoglycoprotein receptor
- Type 3 : Cytochrome P450
- Type 4 : G-protein coupled receptor



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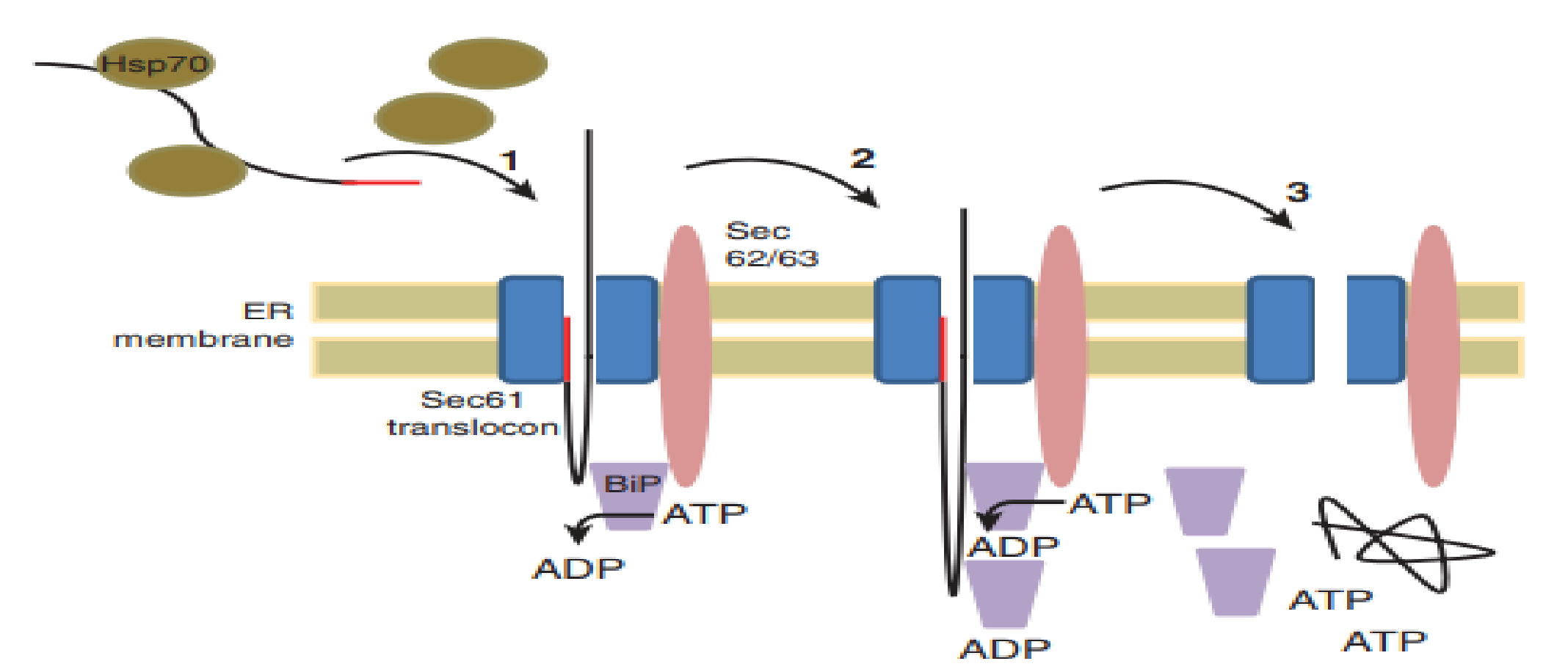
Post translational translocation



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GA routes include retention with retrieval to ER and also retrograde transport

- Anterograde transport–
- Transport from ER to the Golgi apparatus or plasma membrane occurs in COP-II vesicles.

- Retrograde transport –
- Movement of proteins from the Golgi apparatus to the ER occurs in COP-I vesicles.

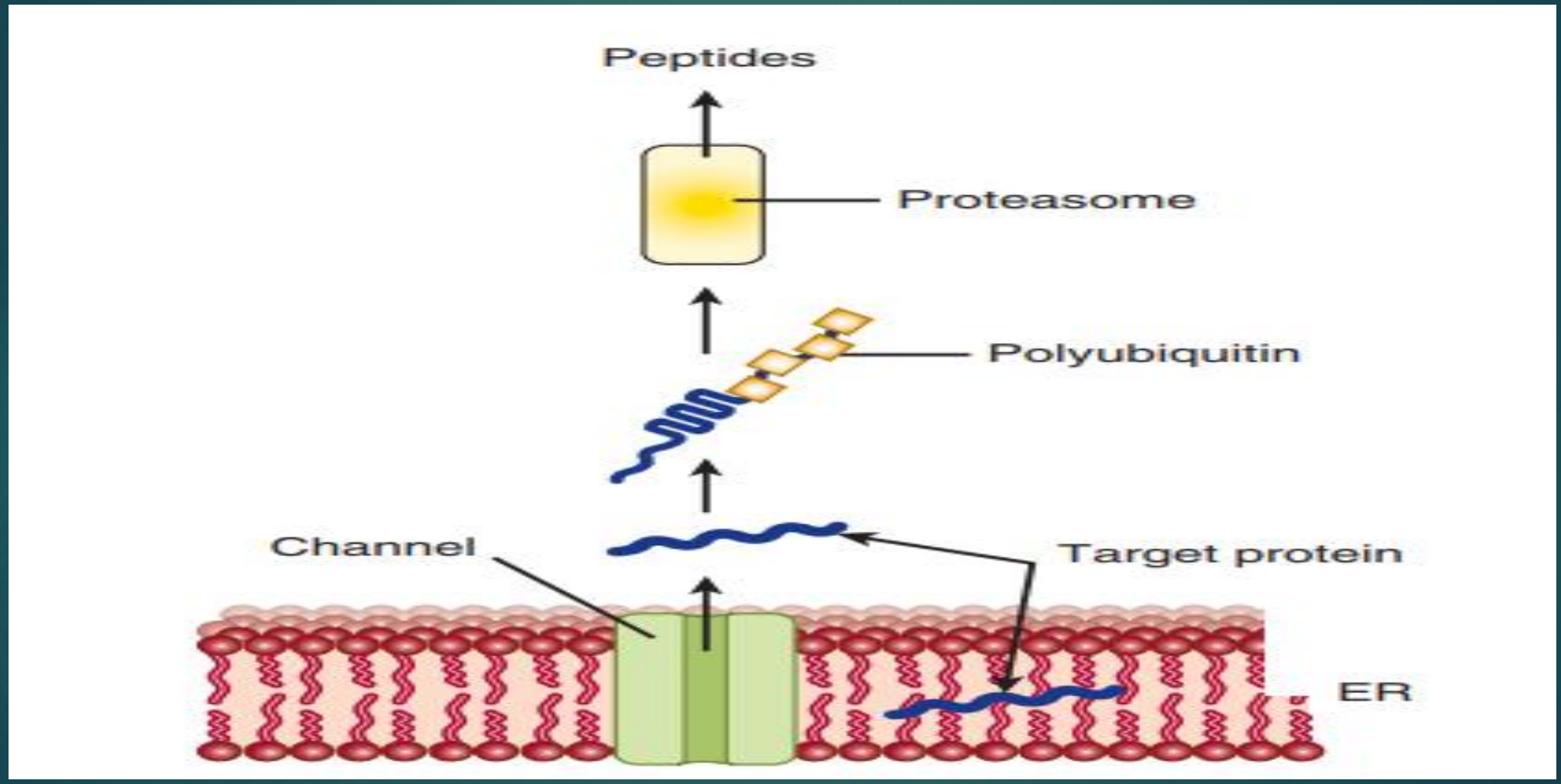
- Movement of proteins through the GA appears mainly by cisternal maturation.

- In the TGN, the exit side of Golgi, proteins are segregated and sorted.
- Secretory proteins accumulate in secretory vesicles, from which they are expelled at plasma membrane.
- Protein destined for plasma membrane or those secreted in constitutive manner are carried up to the cell surface in transport vesicles.
- Clathrin-coated vesicles are involved in endocytosis, carry cargo to late endosomes and to lysosomes.

Some Chaperones and Enzymes Involved in Folding That Are Located in the Rough Endoplasmic Reticulum

- BiP (immunoglobulin heavy chain binding protein)
- GRP94 (glucose-regulated protein)
- Calnexin (calcium binding protein)
- Calreticulin (calcium binding protein)
- PDI (protein disulfide isomerase)
- PPI (peptidyl prolyl cis-trans isomerase)

MISFOLDED PROTEINS UNDERGO ENDOPLASMIC RETICULUM-ASSOCIATED DEGRADATION



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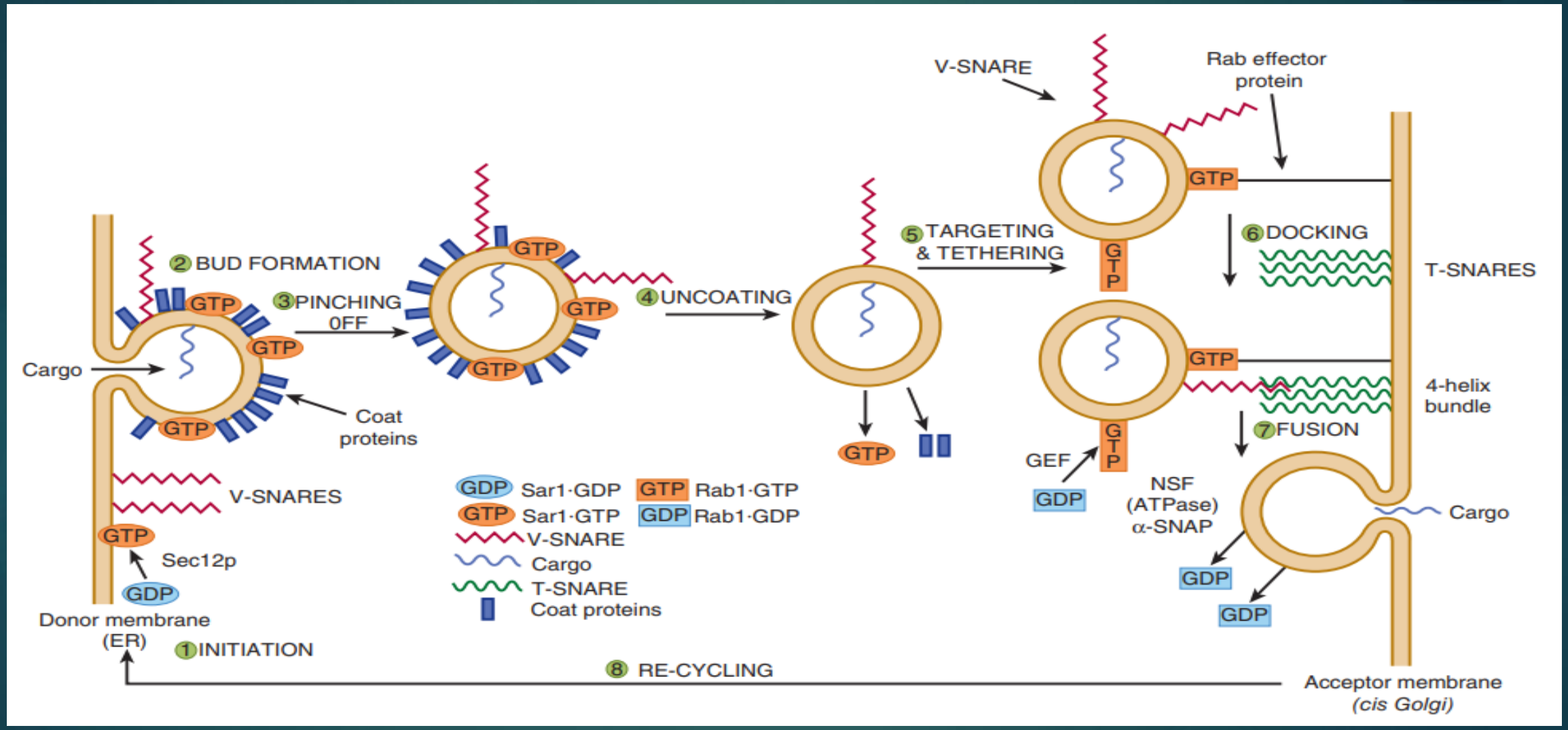
TRANSPORT VESICLES ARE KEY PLAYERS IN INTRACELLULAR PROTEIN TRAFFIC

Vesicles lie at the heart of intracellular transport of many proteins

VESICLE	FUNCTION
COPI	Involved in intra-GA transport and retrograde transport from the GA to the ER
COPII	Involved in export from the ER to either ERGIC or the GA
Clathrin	Involved in transport in post-GA locations including the PM, TGN and endosomes
Secretory vesicles	Involved in regulated secretion from organs such as the pancreas (eg, secretion of insulin)
Vesicles from the TGN to the PM	They carry proteins to the PM and are also involved in constitutive secretion

Transport Vesicles Involves SNAREs & Other Factors



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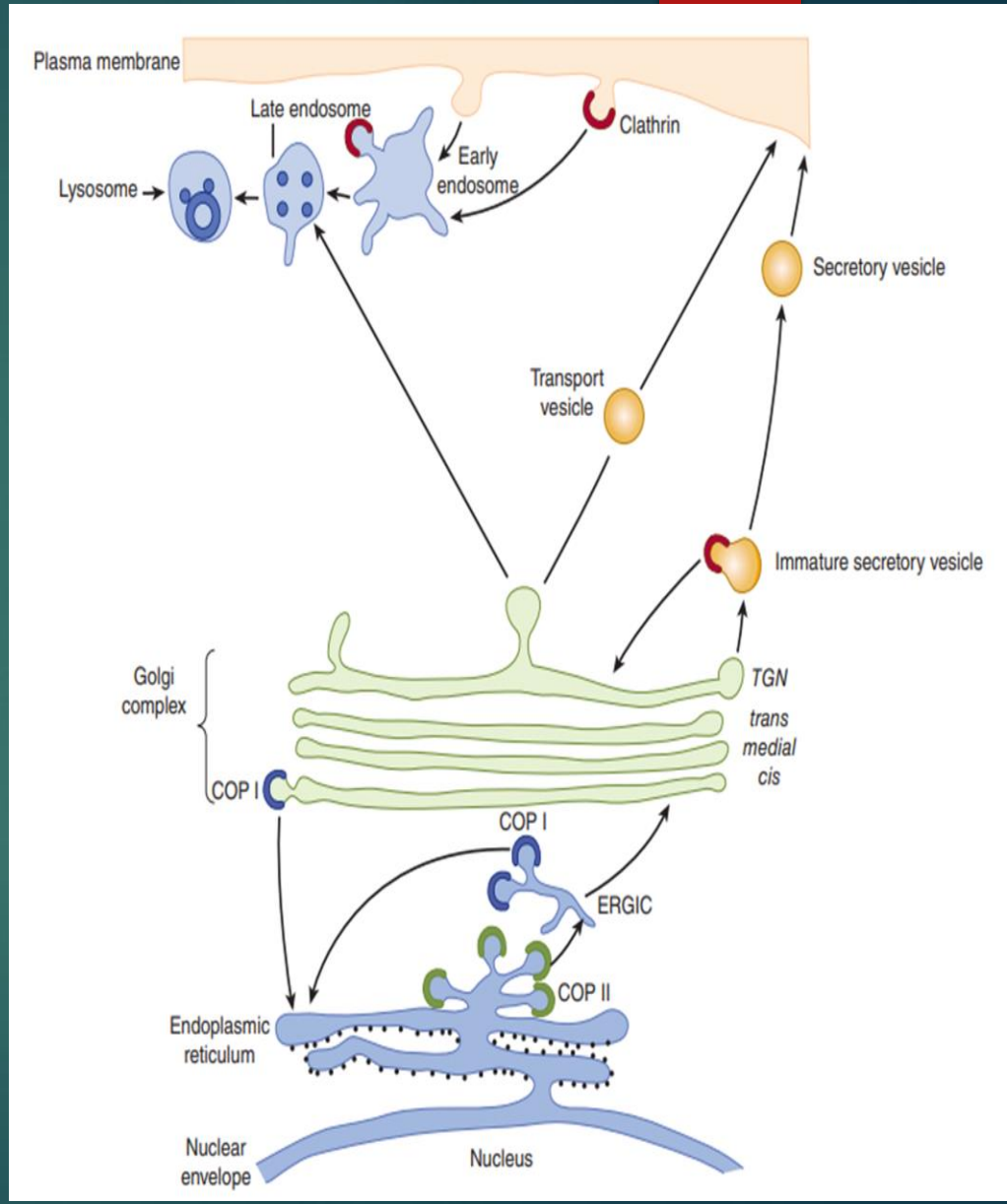
Some Transport Vesicles Travel via the Trans Golgi Network

- Proteins in the apical or basolateral areas of the plasma membranes of polarized epithelial cells can be transported to these sites in transport vesicles budding from the trans Golgi network.
- Different Rab proteins likely direct some vesicles to apical regions and others to basolateral regions.
- In certain cells, proteins are first directed to the basolateral membrane, then endocytosed and transported across the cell by transcytosis to the apical region.

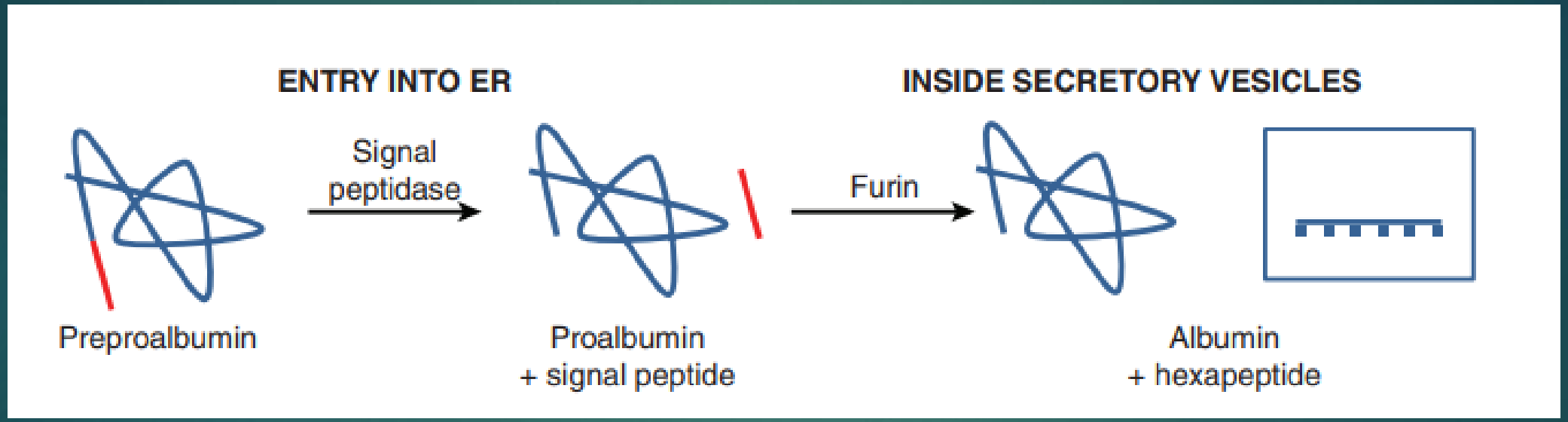
- Once proteins in the secretory pathway reach the cis-Golgi from the ER in vesicles, they can travel through the GA to the trans-Golgi in vesicles, or by a process called cisternal maturation, in which the cisternae move and transform into one another, or perhaps in some cases diffusion via intracisternal connections that have been observed in some cell types.

- In this model, vesicular elements from the ER fuse with one another to help form the cis-Golgi, which in turn can move forward to become the medial Golgi, etc.

- COPI vesicles return Golgi enzymes (e.g., glycosyltransferases) back from distal cisternae of the GA to more proximal (e.g., cis) cisternae.



Some Proteins Undergo Further Processing While Inside Vesicles



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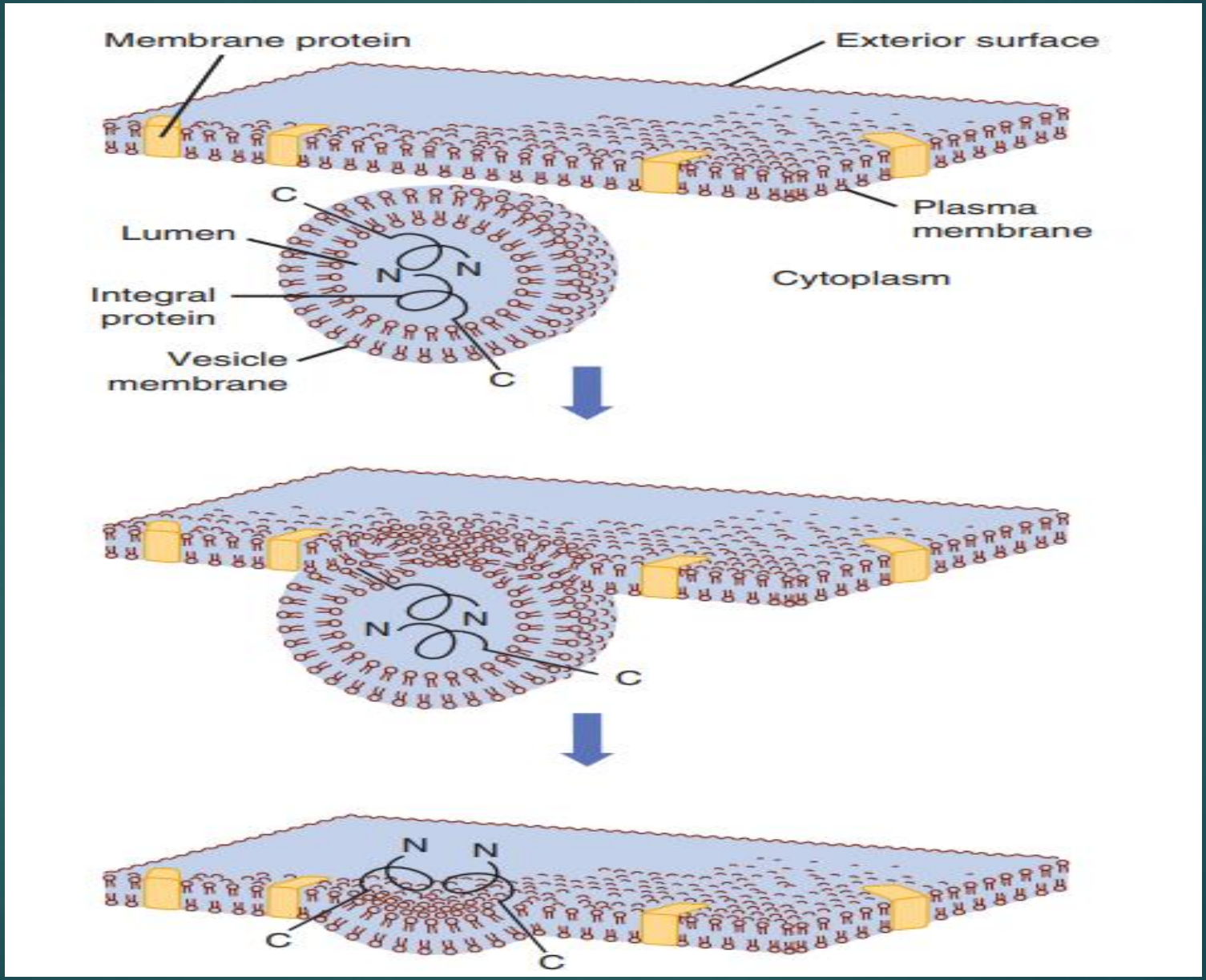
Membrane Assembly



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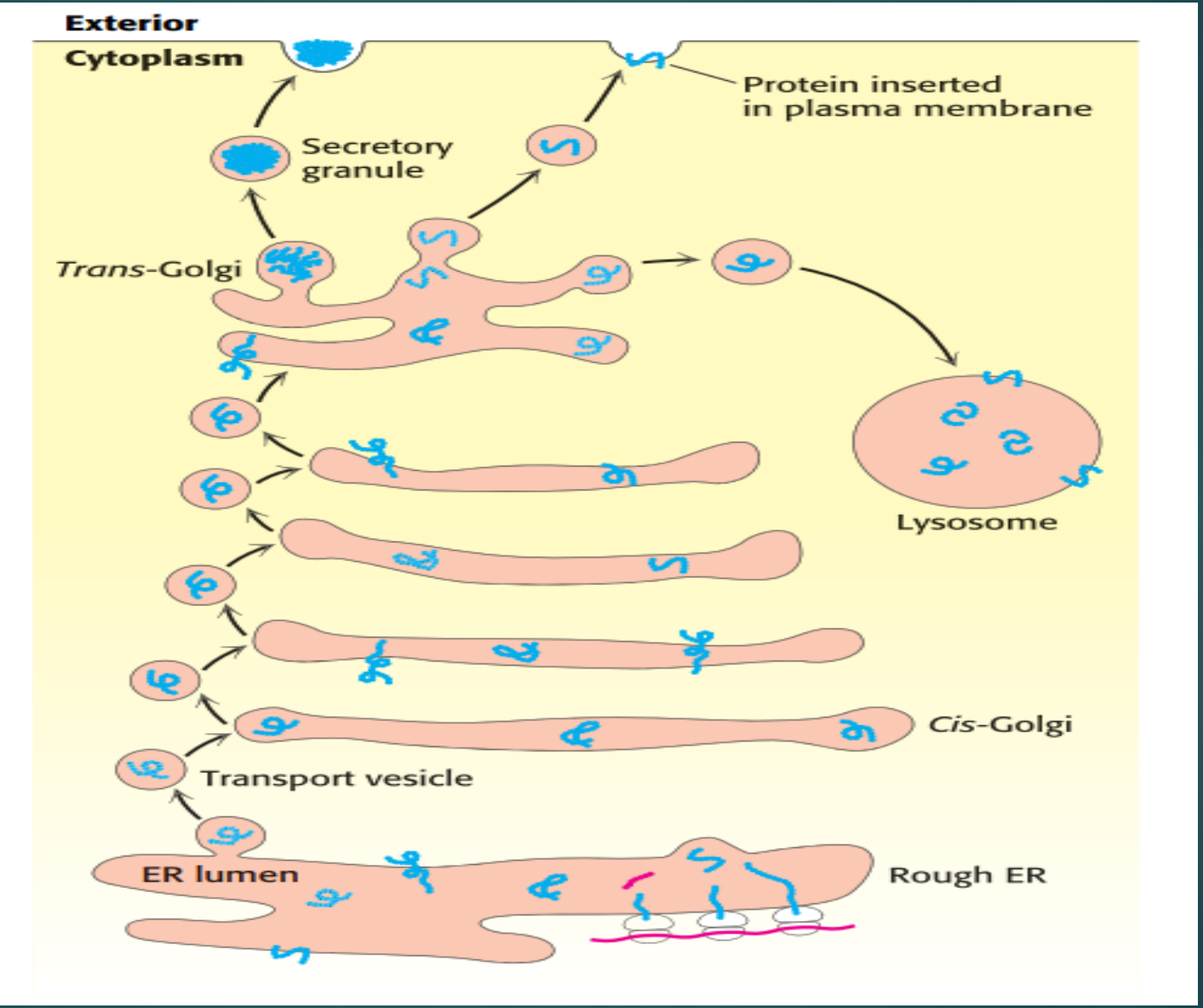
Transport vesicles carry cargo proteins to their final destination



Ref : Biochemistry Jeremy M Berg

Clinical Aspect

Why disorders arises in sorting and targeting of the protein?

1. Mutations that affect protein targeting signals.
2. Mutations affecting intracellular sorting of individual proteins
3. Changes in post translational modification.
4. Deregulation of protein trafficking machinery.
5. Improper folding/ improper maturation of proteins

Clinical Aspect

Disease	Affected protein
a1-Antitrypsin deficiency with liver disease	a1-Antitrypsin (SERPINA 1 gene)
Chediak-Higashi syndrome	Lysosomal trafficking regulator (Lyst gene)
Combined deficiency of factors V and VIII	ERGIC53, a mannose-binding lectin (LMAN1)
Cystic fibrosis	CFTR
Familial hypercholesterolemia, AD	LDL receptor (LDLRAP-1 gene)
Gaucher disease	β-Glucosidase (GBA)
I-cell disease	N-acetylglucosamine 1-phosphotransferase
Tay-Sachs disease	β-Hexosaminidase
von Willebrand disease	von Willebrand factor
Lowe oculocerebrorenal syndrome	PIP25-phosphatase (OCRL)
Hereditary hemochromatosis	HFE
Hemophilia A and B	Factors VIII and IX
Hermansky-Pudlak syndrome	AP-3 adaptor complex β3A subunit (HPS-1 gene)

Clinical Aspect

- Some of the disorders due to peroxisomal abnormalities :

1. Zellweger Syndrome (PEX 5)
2. Neonatal adrenoleukodystrophy (PEX 5)
3. Infantile Refsum disease (PEX 12)
4. Hyperpipecolic acidemia (PEX 1)
5. Rhizomelic chondrodysplasia punctata (PEX 7)
6. Adrenoleukodystrophy (PEX 1)
7. Pseudoneonatal adrenoleukodystrophy (ACOX 1)
8. Pseudo-Zellweger syndrome (HSD17B4)
9. Hyperoxaluria type 1 (AGXT)
10. Acatalasemia (CAT)
11. Glutaryl-CoA oxidase deficiency (C7ORF10)

Clinical Aspect

- ***Zellweger Syndrome :***
- apparent at birth and is characterized by profound neurologic impairment, victims often dying within a year.

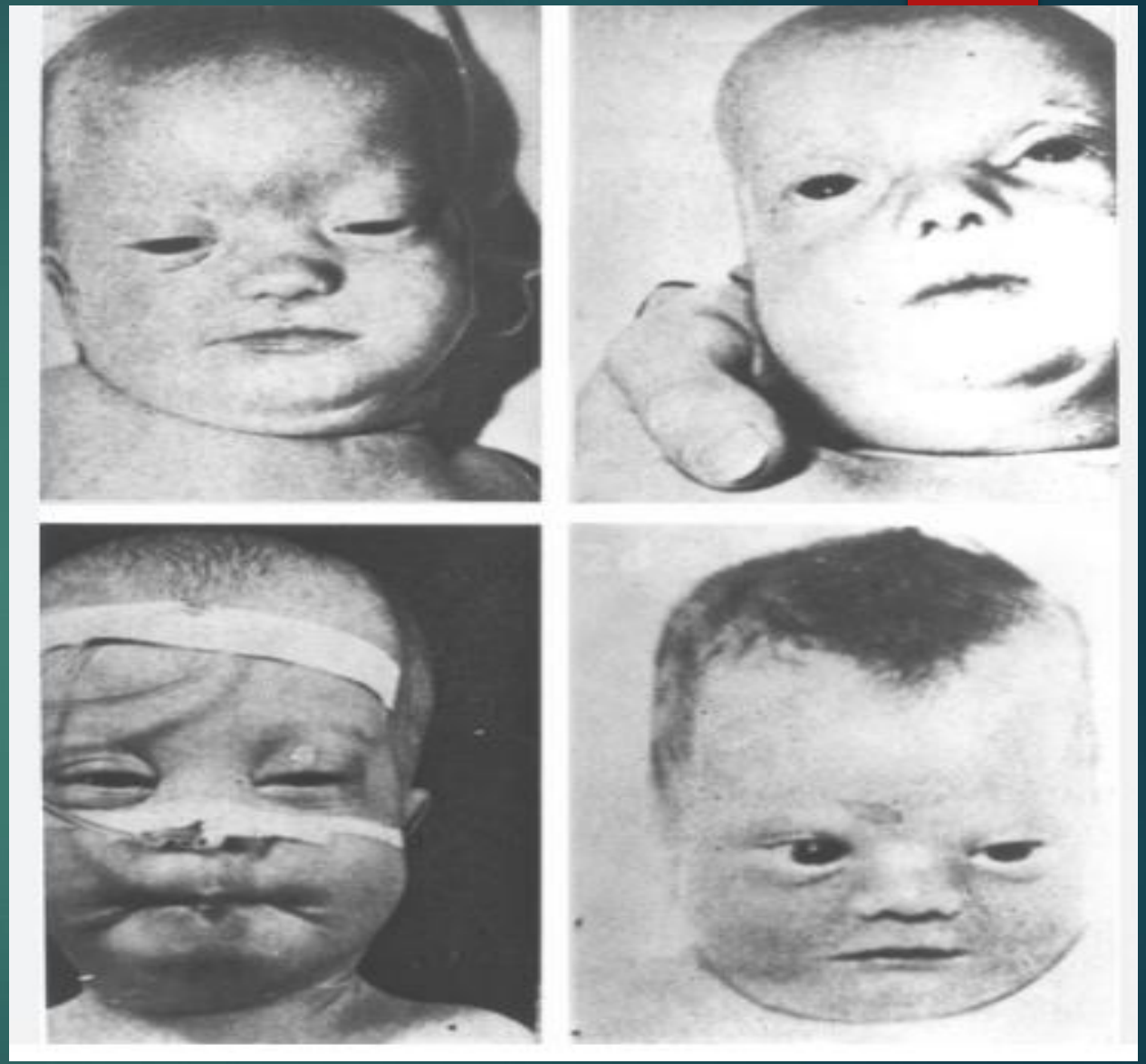
- Biochemical findings include an accumulation of very-long-chain fatty acids, abnormalities of the synthesis of bile acids, and a marked reduction of plasmalogens.

- The condition is usually caused by mutations in genes encoding certain proteins —the PEX family of genes, also called peroxins —involved in various steps of peroxisome biogenesis.

- Two closely related conditions are neonatal adrenoleukodystrophy and infantile Refsum disease.

- Zellweger syndrome and these two conditions represent a spectrum of overlapping features.

- Where Zellweger syndrome being the most severe (many proteins affected) and infantile Refsum disease the least severe (only one or a few proteins affected)

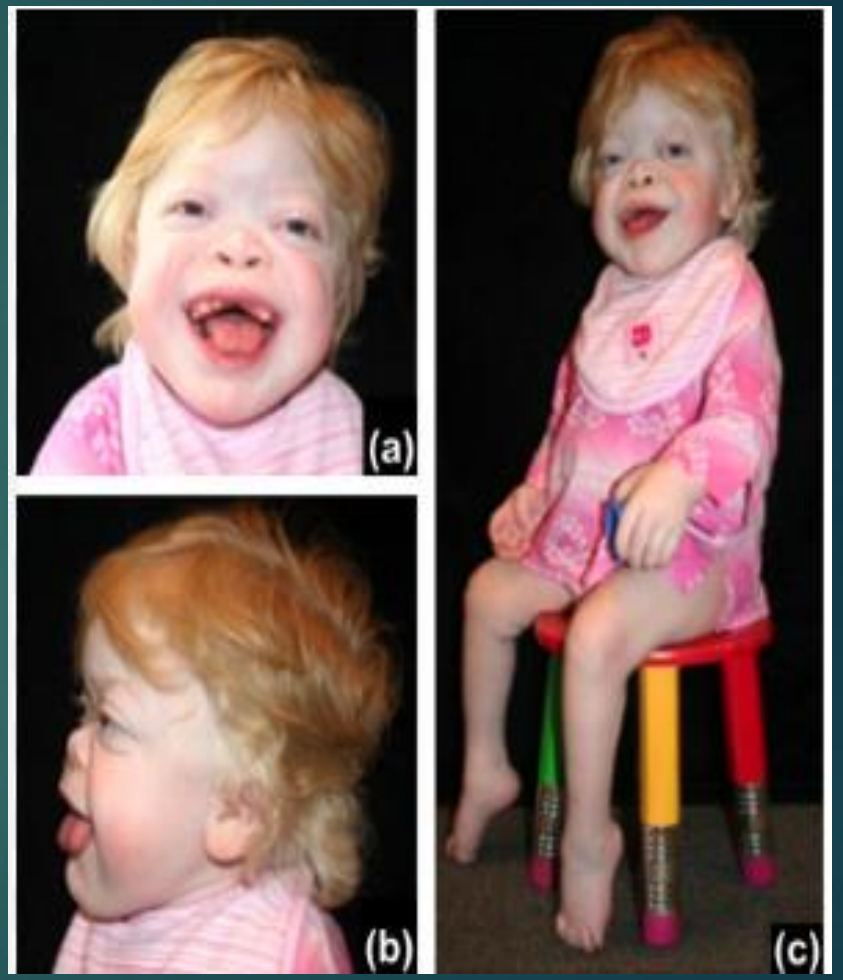


Ref : Scriver CR, Beaudet AL, Sly WS, et al., eds. The Metabolic and Molecular Bases of Inherited Disease. New York, NY: McGraw Hill; 1994:2297.

Clinical Aspect

Mucolipidosis II(I cell disease) : Inclusion cell disease

- Mutations in the gene encoding GlcNAc phosphotransferase .
- Results in the absence of Mannose-6-Phosphate signal for lysosomal localization of certain hydrolases.
- Lysosomes lack in enzymes which are seen in blood.
- Lysosomes accumulate different types of undegraded molecules(oligosaccharides, lipids, GAGs) forming inclusion bodies.
- C/F : Hepatomegaly, splenomegaly, Failure to grow and develop, Recurrent respiratory tract infection, Death from Congestive cardiac failure or Recurrent respiratory tract infection before 7th year of life.

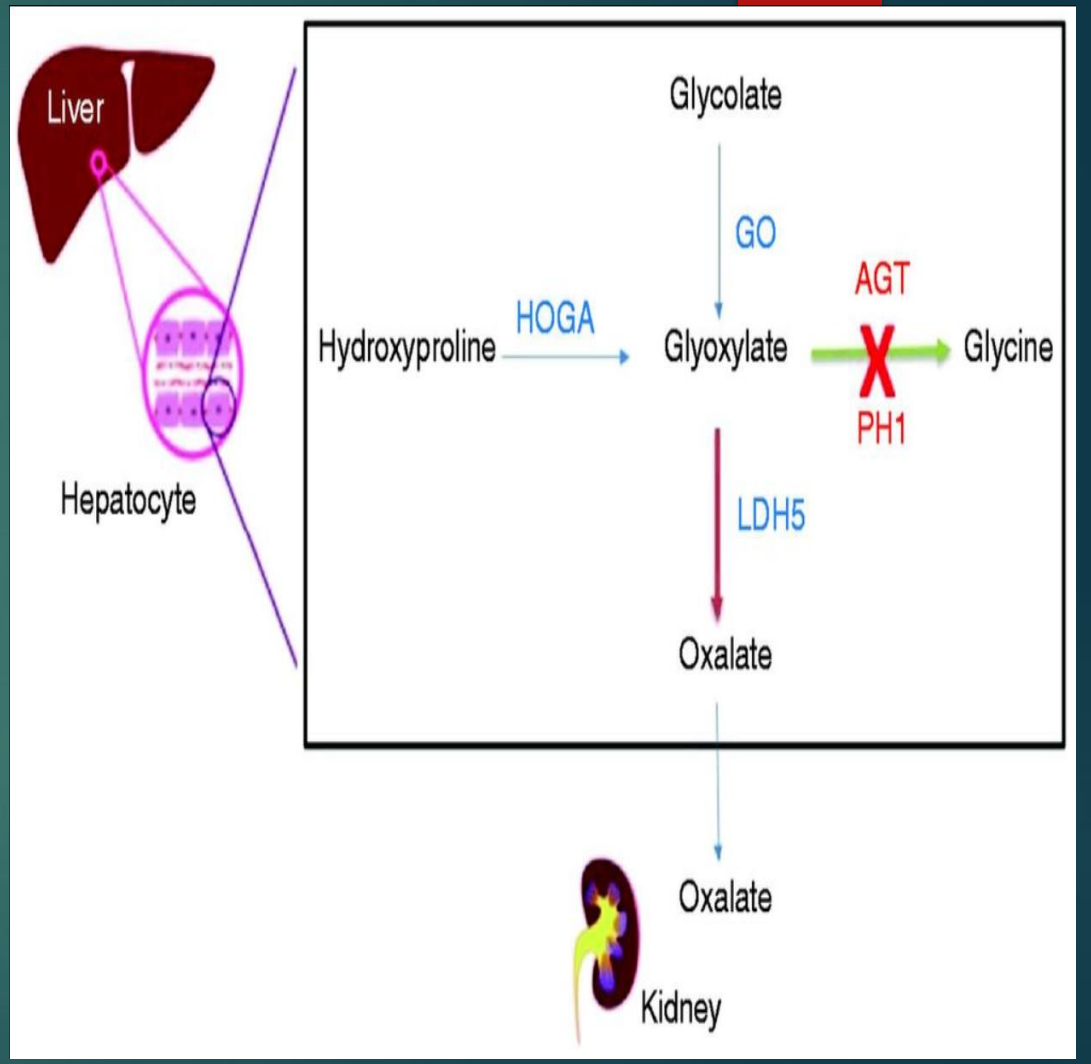


Ref : Jules G Leroy, Chapter 103 - Oligosaccharidoses: Disorders Allied to the Oligosaccharidoses,Editor(s): David Rimoin, Reed Pyeritz, Bruce Korf,Emery and Rimoin's Principles and Practice of Medical Genetics (Sixth Edition),Academic Press,2013,Pages 1-51,ISBN 9780123838346

Clinical Aspect

Primary Hyperoxaluria

- Enzyme Alanine Glyoxalate aminotransferase is seen in mitochondria, instead of its normal peroxisomal location.
- Inactive enzyme results in increased pool size of glyoxalate, and excess production of oxalate.
- Patients suffer from neprolithiasis, renal colic and hematuria.
- Extrarenal oxalosis may be seen in heart, blood vessels, bone.



Ref : Garrelfs, Sander F.; van Harskamp, Dewi; Peters-Sengers, Hessel; van den Akker, Chris H.P.; Wanders, Ronald J.A.; Wijburg, Frits A.; van Goudoever, Johannes B.; Groothoff, Jaap W.; Schierbeek, Henk; Oosterveld, Michiel J.S.. Endogenous Oxalate Production in Primary Hyperoxaluria Type 1 Patients. JASN 32(12):p 3175-3186, December 2021. | DOI: 10.1681/ASN.2021060729

Clinical Aspect

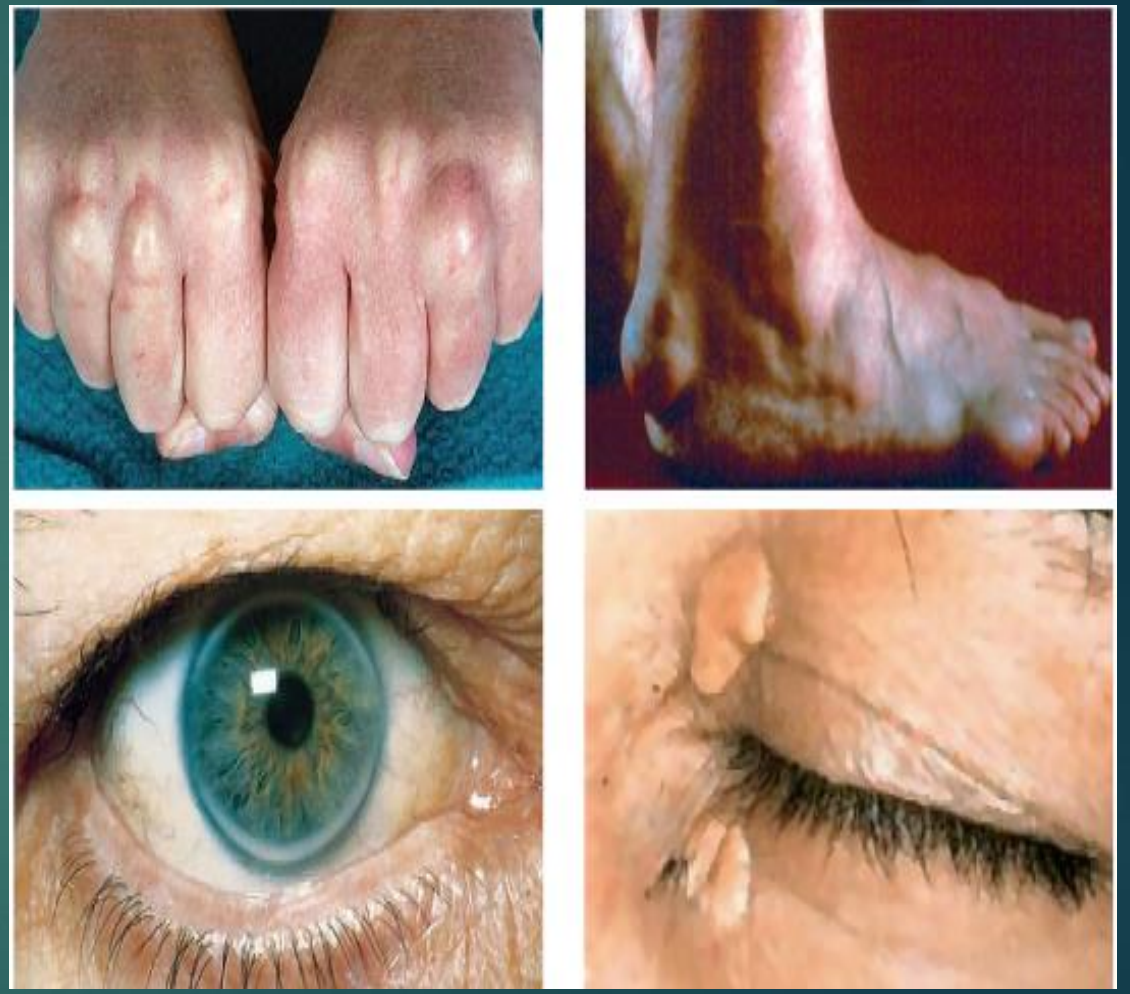
Cystic fibrosis

- Due to mutation of Cystic fibrosis transmembrane regulator(CFTR) gene resulting in improper localization of proteins, AR.
- Affects cells that produce sweat, mucus and digestive juices.
- Mutation disrupts the function of chloride channel, preventing usual flow of chloride and water into and out of the cells.
- Very high Cl levels in sweat (> 60 mmol/L)
- Due to excessive excretion of chloride, body makes thick sticky mucus instead of thin watery mucus.
- The thick mucus clogs and blocks tubes and ducts resulting in damage to the lungs, digestive system and other organs.

Clinical Aspect

Familial Hypercholesterolemia

- Due to deficient transport signals.
- LDL receptor defect results in high LDL levels in plasma.
- Receptor deficiency.
- ***Receptor does not reach target membrane of liver and peripheral tissues.***
- Defective binding of B-100 to LDL receptor.



Ref : Yuan, George & Wang, Jian & Hegele, Robert. (2006). Heterozygous familial hypercholesterolemia: An underrecognized cause of early cardiovascular disease. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 174. 1124-9. 10.1503/cmaj.051313.

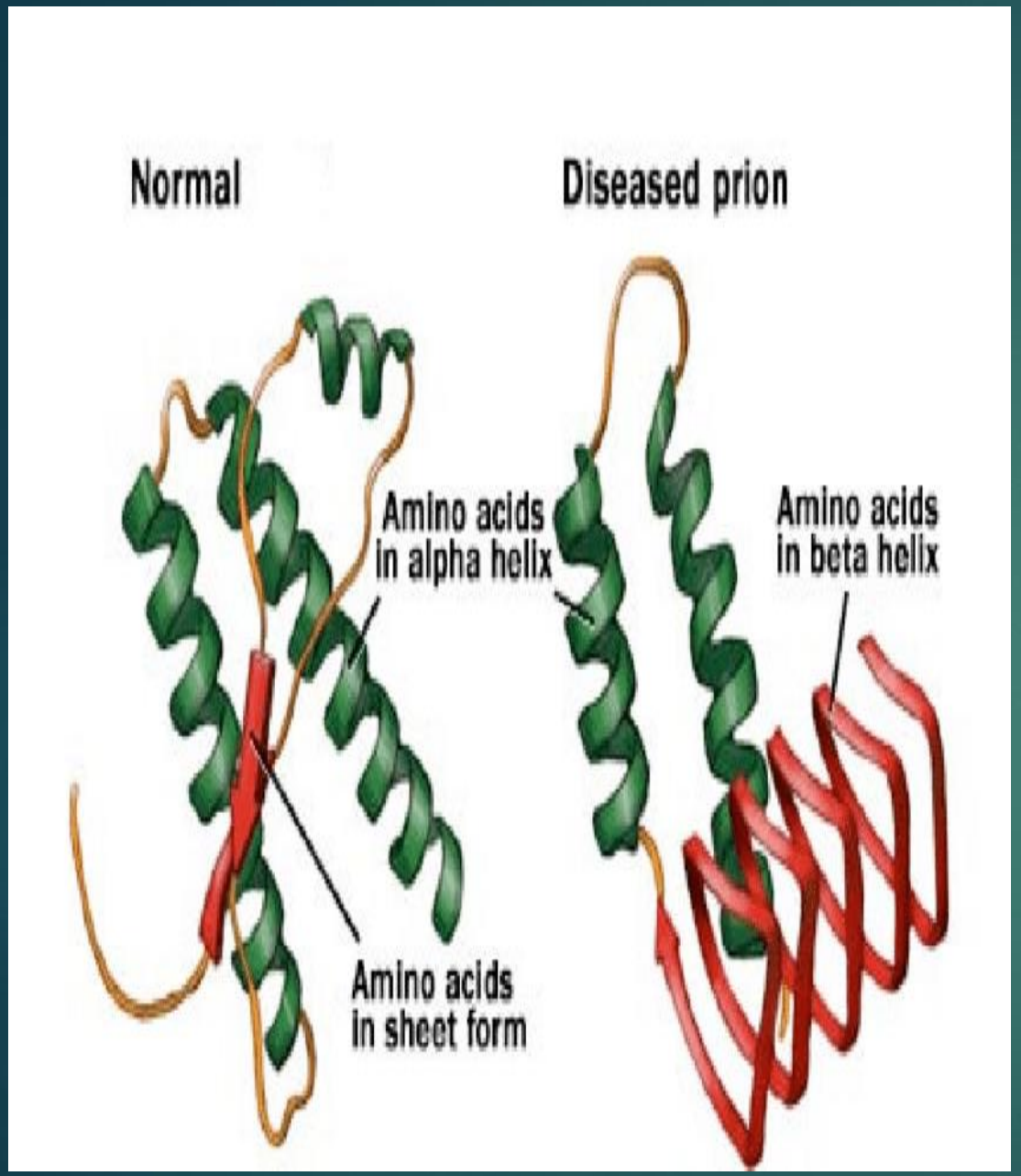
Clinical Aspect

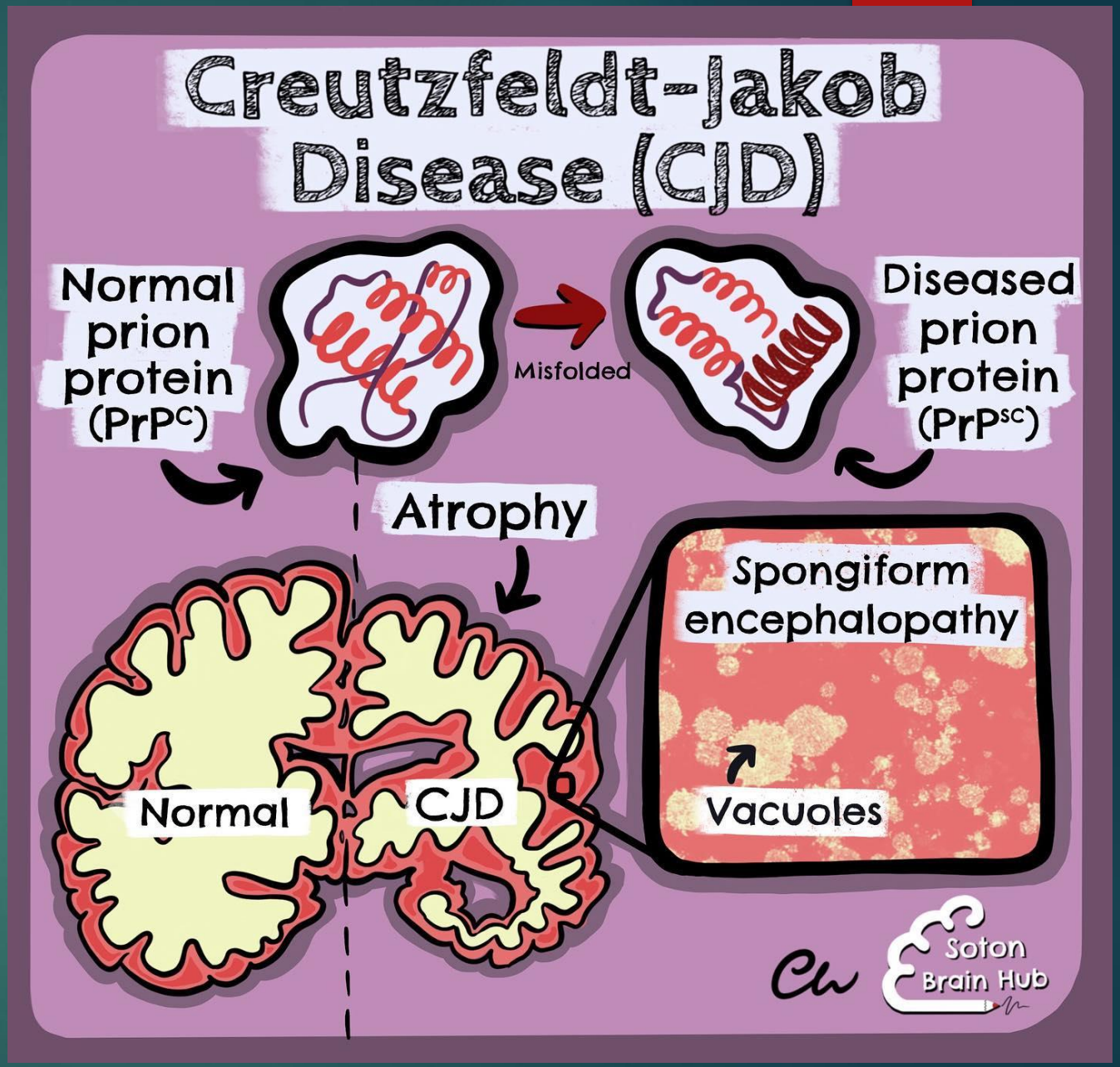
- ***Prion Diseases***
- AKA spongiform encephalopathies, transmissible, fatal neurological degenerative diseases caused by obnoxious protein aggregates.

- Creutzfeldt-Jakob disease and kuru.

- Prion proteins have certain changes in three dimensional structures.
- Major replacement of α-helix by β-sheets.

- Forming an abnormal isoform called prion protein-cellular form (PrPC).
- This abnormal conformation makes it resistant to the action of proteolytic enzymes.
- These proteins are highly infectious in nature.





Ref : Paudyal, Anuja & Gillock, Eric. (2017). FHSU Scholars Repository Polymorphism In The PrPC Prion Protein Gene In Pigs. 10.13140/RG.2.2.12822.86089.

Ref : https://medium.com/@bagwe.aditya05/creutzfeldt-jakob-disease-cjd-125ba6dce1e5

- Many other mutations affecting folding of proteins and their intracellular transport to various organelles have been reported, including neurodegenerative disorders such as Alzheimer disease, Huntington disease and Parkinson disease.
- The elucidation of the causes of these various conformational disorders has contributed significantly to our understanding of molecular pathology.
- The term “diseases of proteostasis deficiency "has also been applied to diseases due to misfolding of proteins.
- Proteostasis is a composite word derived from protein homeostasis.
- Normal proteostasis is due to a balance of many factors, such as synthesis, folding, trafficking, aggregation, and normal degradation.
- If any one of these is disturbed (e.g., by mutation, aging, cell stress, or injury), a variety of disorders can occur, depending on the particular proteins involved.

Summary

Signal sequences : targets the proteins to their specific destination.

Sorted : cytosolic or RER.

Protein are targeted accordingly in the MM, Nucleus, Peroxisome and ER.

Proteins embedded in ER undergoes Co-translational, Post-translational, Anterograde and Retrograde transport.

Misfolded proteins undergo degradation via ERAD pathway.

Transport vesicles, helps from budding to the movement through cytosol, tethering, docking and fusion, also plays a key role in the intracellular traffic of proteins.

Any Proteostatic deficiency due to mutations in gene or other factors causes diseases as mentioned above.

References :

1. Harper's Illustrated Biochemistry 30th edition
2. Biochemistry Jeremy M Berg 8th edition
3. Lehninger Principles of Biochemistry
4. FHSU Scholars Repository Polymorphism In The PrPC Prion Protein Gene In Pigs - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Normal-and-abnormal-form-of-prion-protein-Normal-prion-protein-has-amino-acids-in-alpha_fig1_326330981 [accessed 13 Mar, 2024]
5. Scriver CR, Beaudet AL, Sly WS, et al., eds. The Metabolic and Molecular Bases of Inherited Disease. New York, NY: McGraw Hill; 1994:2297.
6. Jules G Leroy, Chapter 103 - Oligosaccharidoses: Disorders Allied to the Oligosaccharidoses,Editor(s): David Rimoin, Reed Pyeritz, Bruce Korf,Emery and Rimoin's Principles and Practice of Medical Genetics (Sixth Edition),Academic Press,2013,Pages 1-51,ISBN 9780123838346
7. Garrelfs, Sander F.; van Harskamp, Dewi; Peters-Sengers, Hessel; van den Akker, Chris H.P.; Wanders, Ronald J.A.; Wijburg, Frits A.; van Goudoever, Johannes B.; Groothoff, Jaap W.; Schierbeek, Henk; Oosterveld, Michiel J.S.. Endogenous Oxalate Production in Primary Hyperoxaluria Type 1 Patients. JASN 32(12):p 3175-3186, December 2021. | DOI: 10.1681/ASN.2021060729
8. Yuan, George & Wang, Jian & Hegele, Robert. (2006). Heterozygous familial hypercholesterolemia: An underrecognized cause of early cardiovascular disease. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 174. 1124-9. 10.1503/cmaj.051313.
9. Paudyal, Anuja & Gillock, Eric. (2017). FHSU Scholars Repository Polymorphism In The PrPC Prion Protein Gene In Pigs. 10.13140/RG.2.2.12822.86089.
10. https://medium.com/@bagwe.aditya05/creutzfeldt-jakob-disease-cjd-125ba6dce1e5


Thank you