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SUBTOPIC NAME	**Protein targeting -1 (Basics of Protein Translocation)**
CONTENT TYPE	**PDF**
SEARCH KEYWORD	**Protein Translocation , protein insertion in membrane**

Protein Targeting: 1. Basics

Objectives:

To acquaint the students about:

i) Concept of targeting newly synthesized proteins to cellular destinations

ii) Secretory pathway and organelle targeting pathway

iii) Post-translational and co-translational translocation

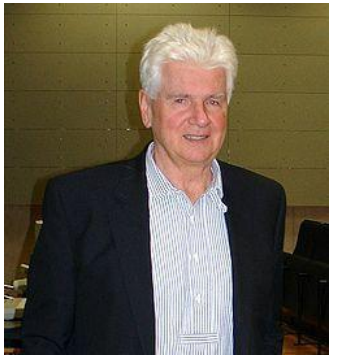
iv)Requirements for protein targeting

v)Types of membrane proteins

vi)Insertion of proteins into membranes.

An integral part of the post transcriptional mechanism within a cell is directing a newly synthesized protein molecule to their proper destination by tagging the proteins and the tag is known as the “Signal Peptide”.

Most lysosomal, membrane, or secreted proteins have an amino-terminal signal sequence that marks them for translocation into the lumen of the ER.
The carboxyl terminus of the signal sequence is defined by a cleavage site, where protease action removes the sequence after the protein is imported into the ER. Signal sequences vary in length from 13 to 36 amino acid residues, but all have : (1) ~10-15 hydrophobic amino acid residues; (2) one or more positively charged residues, usually near the amino terminus, preceding the hydrophobic sequence; and (3) a short sequence at the carboxyl terminus (near the cleavage site) that is relatively polar, typically having amino acid residues with short side chains (especially Ala) at the positions closest to the cleavage site.

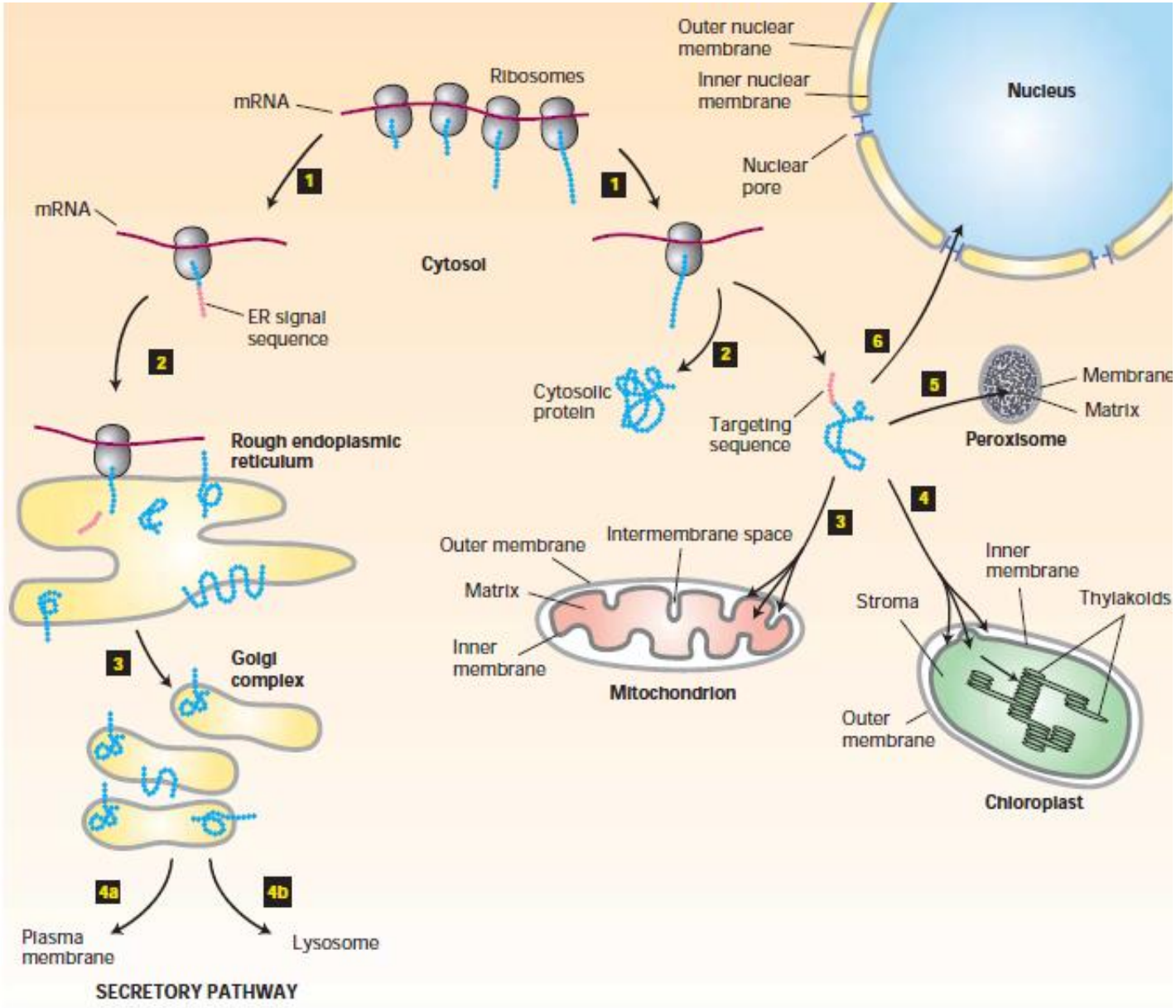


Discovery of signal peptides is attributed to Gunter Blobel, awarded 1999 Nobel Prize in Physiology or Medicine.

George demonstrated proteins with signal sequences are synthesized on ribosomes attached to ER.

Targeting capacity of particular signal sequences has been confirmed by fusing the signal sequence from 1 protein to a 2nd protein and showing that the signal directs the 2nd protein to the location where the 1st protein is normally found.

PROTEIN TARGETING: Moving proteins into membranes and organelles



The delivery of newly synthesized proteins to their proper cellular destinations, usually referred to as *protein targeting* or *protein sorting.*

Protein targeting or *protein sorting* encompasses two very different kinds of processes:

❖ Ist general process involves targeting of a protein to the membrane of an **intracellular organelle** and can occur either during or soon after synthesis of the protein by translation at the ribosome.

- For membrane proteins, targeting leads to insertion of the protein into the lipid bilayer of the membrane, whereas for water-soluble proteins, targeting leads to translocation of the entire protein across the membrane into the aqueous interior of the organelle.
- Proteins are sorted to the endoplasmic reticulum (ER), mitochondria, chloroplasts, peroxisomes, and the nucleus by this general process.

❖IInd sorting process applies to proteins that initially are targeted to the ER membrane, thereby entering the **secretory pathway.**

- The soluble and membrane proteins that reside in the ER itself but also proteins that are secreted from the cell, enzymes and other resident proteins in the lumen of the Golgi complex and lysosomes, and integral proteins in the membranes of these organelles and the plasma membrane.

- Proteins whose final destination is Golgi, lysosome, or cell surface are transported along the secretory pathway by small vesicles that bud from membrane of one organelle and then fuse with the membrane of the next organelle in the pathway

Protein sorting in different organelles are governed by the same basic mechanisms with variations.

Requirements:

- A Signal sequence
- A Receptor for recognition signal sequence
- Translocation channel and how protein pass it
- Energy source for protein translocation

Signal sequence or Uptake-targeting sequence

- Carrying the information for a protein to target to a particular organelle destination.

- Signal sequences are often removed from the mature protein by specific proteases present in the targeting organelles.

- Signal sequence: 16~30 residues with 6-12 hydrophobic residues (the core) and one or more positively charged amino acids adjacent to them.

- Each organelle carries a set of specific receptor proteins that recognize and bind only to specific kinds of signal sequences.

- Translocation channel will allow the protein to pass through the membrane once a protein containing a signal sequence has been recognized by its receptor protein.

- Protein translocation unidirectional process it will required Energy during translocation time.

Translocation of secretory proteins across the ER membrane

- Co-translational translocation of protein.
- Post-translational translocation of protein.
- Co-translational translocation of secretory proteins across the ER membrane: Signal sequence will direct the ribosome to the ER membrane and initiates translocation of the growing peptide across the ER membrane.

Co-translational translocation of secretory proteins across the ER membrane

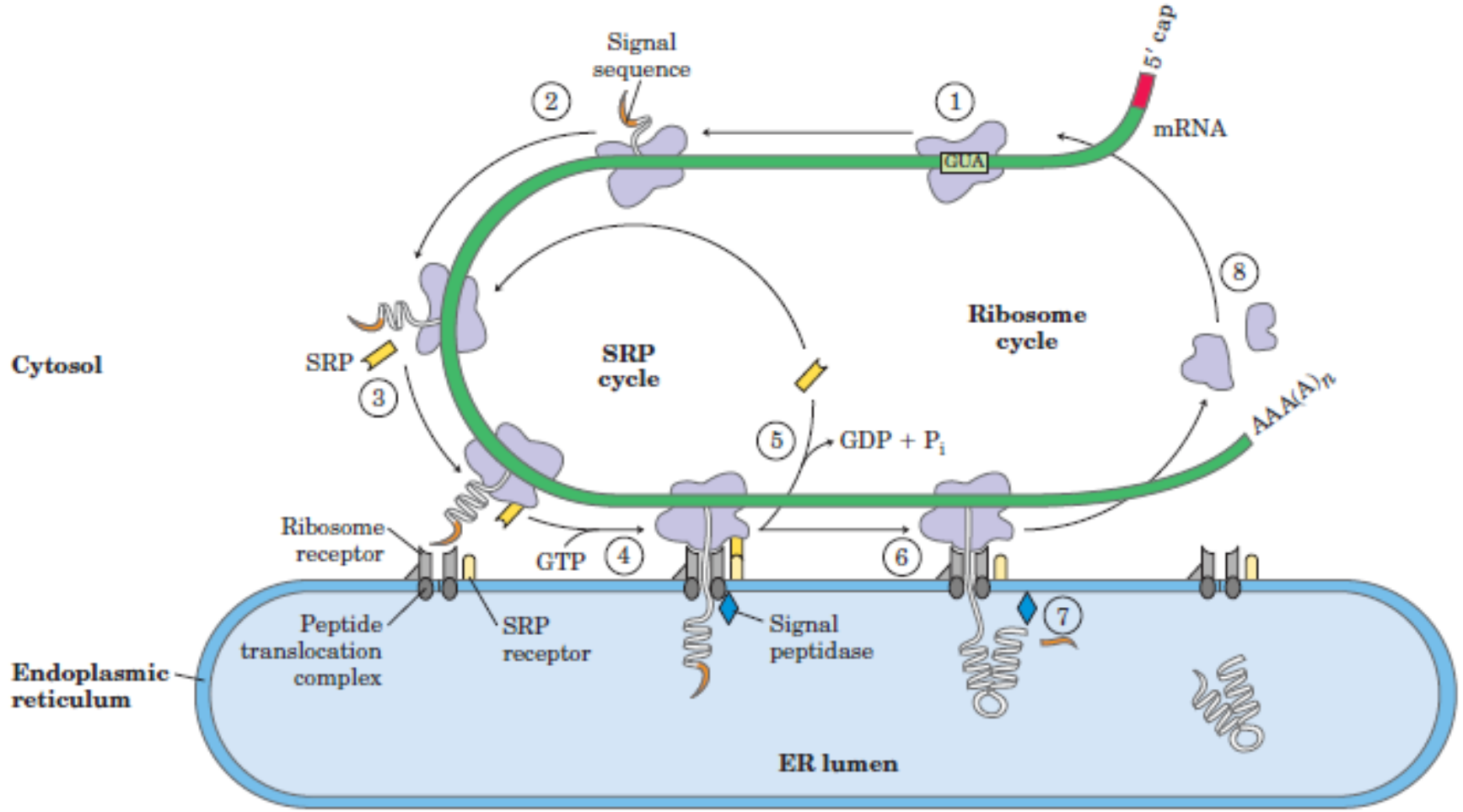
Requisites for translocation:

- ***Signal sequence***: 16-30 residues containing 6-12 residues of hydrophobic core with one or more positively charged residues adjacent to them

- ***Receptor***: SRP and SRP receptor (for recognition of signal sequence)

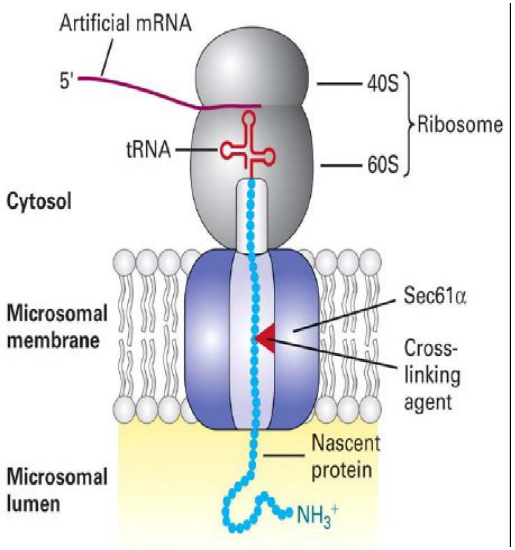
- ***Translocon***: Sec-61 complex

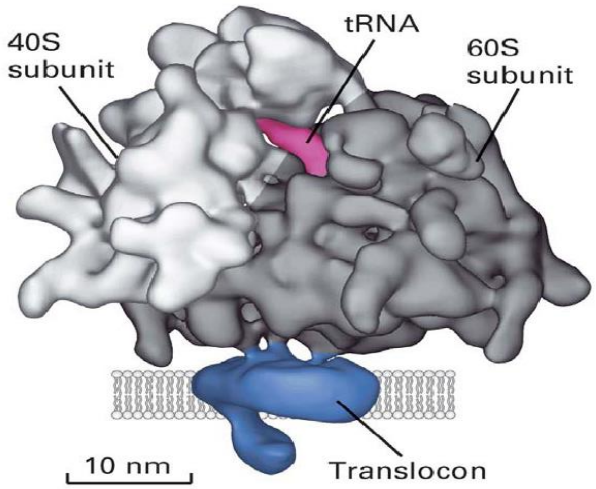
- ***Energy requirement***: GTP hydrolysis - driving force for protein translocation through the ER.



Directing eukaryotic proteins with appropriate signals to the ER. This process involves SRP cycle and translocation and cleavage of nascent polypeptide. One protein subunit of SRP binds directly to signal sequence; inhibing elongation by sterically blocking the entry of amino-acyl tRNAs and inhibiting peptidyl transferase. Another protein subunit hydrolyzes GTP. The SRP receptor is a homodimer of α (M69,000) and β (M 30,000) subunits, both bind and hydrolyze multiple GTP molecules during this process.

Ribosome and protein chain transferred to the ER Translocon





- 60S aligned with pore of membrane.
- ER Translocon Channel: 3-4 Sec61 complexes IMP. pentagonal cylinder

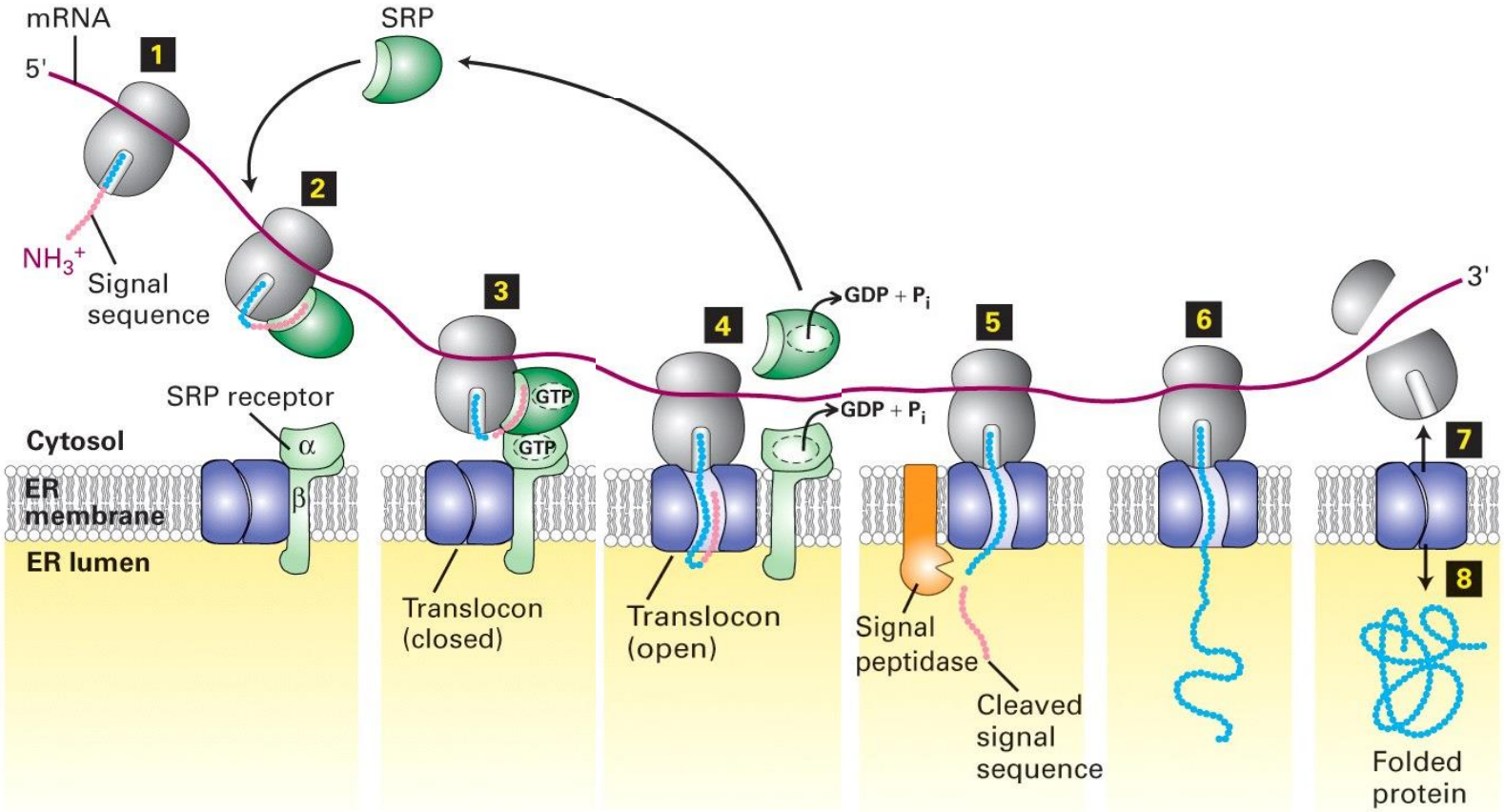
Translocation channel: Sec61 complex (5x8 nm / 2nm pore)

Three subunit, α, β and γ. α is the largest, containing 10 membrane-spanning α helices.

Energy from chain elongation pushes pp chain across membrane

- Opens only when ribosome bound.
- Absence of ribosome: closed channel. ribosome attached: opened gate.

Co-translational translocation of secretory proteins across the ER membrane



•Synthesis starts on free ribosome first. The whole complex must be trans located to ER before the 70th residue was added. Without ER, the synthesis will come to a halt.

•GTP hydrolysis is required to increase the fidelity of this process. If the protein does not have correct signal sequence, GTP hydrolysis will occur and protein will leave the complex. If the protein is correct, GTP hydrolysis will trigger Translocon (Sec-61) to open and protein will enter ER lumen.

•By recognizing its C-terminal sequence, signal peptidase will cleave the ER signal sequence after it enters the ER lumen.

VESICULAR TRAFFIC, SECRETION AND ENDOCYTOSIS

- Mechanisms that allow soluble and membrane proteins synthesized on RER to move to final destinations via the **secretory pathway.**

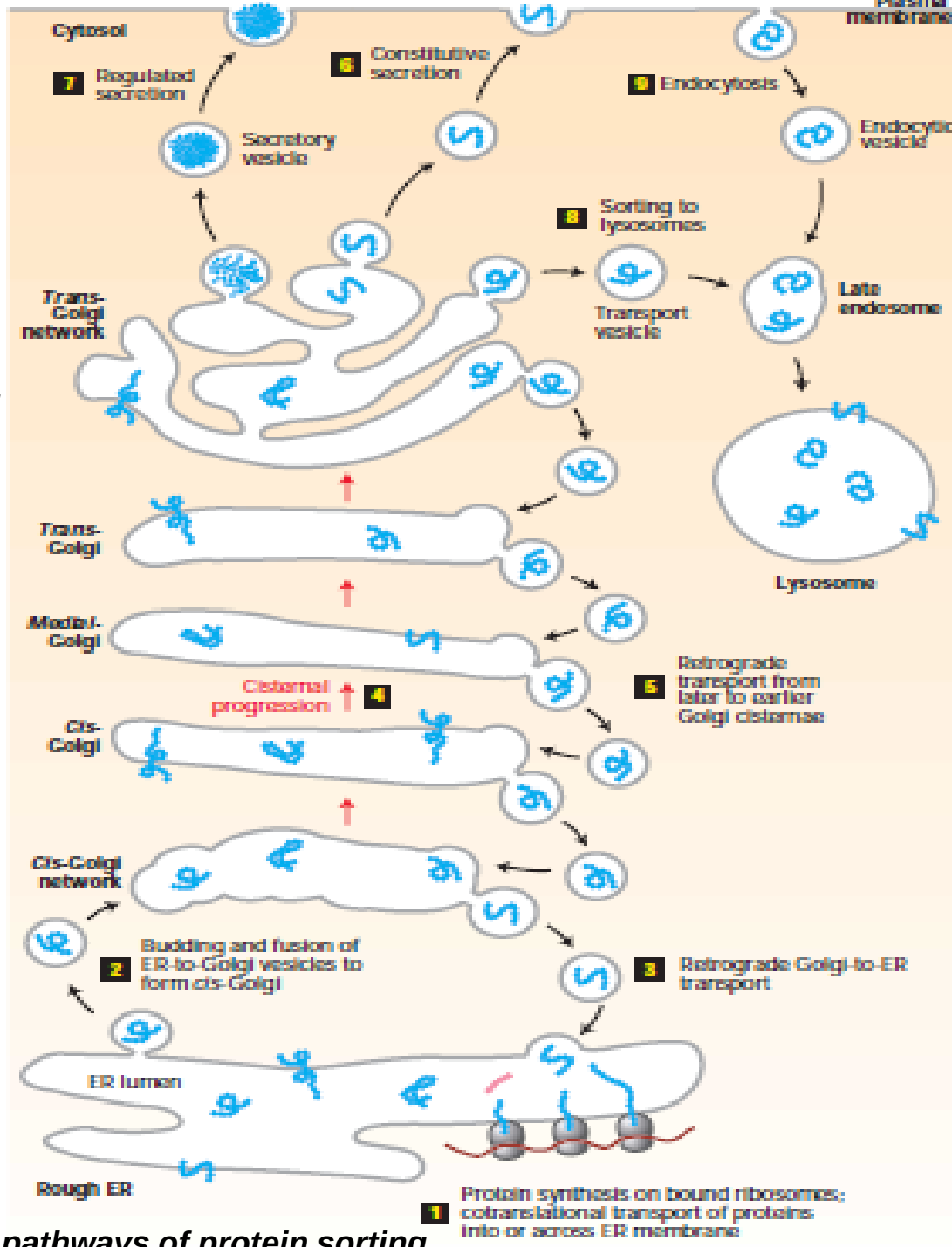
- **Transport vesicles (TV) collect** "*cargo" proteins in buds arising from the membrane of one* compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment.

- As TVs bud from one membrane and fuse with the next, the same face of the membrane remains oriented toward the cytosol.

- Once newly synthesized proteins are incorporated into the ER lumen or membrane, they can be packaged into *anterograde* (forward-moving) transport vesicles which fuse to form a flattened membrane-bounded compartment -*cis-Golgi* ***cisterna.***

- Certain proteins**,** mainly ER-localized proteins, are retrieved from the *cis-Golgi* to the ER via a different set of *retrograde (backward-moving)* transport vesicles.

Secretory pathway**:* Newly made polypeptide chains having an ER signal sequence are inserted into ER membrane or enter lumen (**1**). Some proteins remain in ER, some are packaged into transport vesicles (**2**), budding from ER and fuse together to form new *cis-Golgi* cisternae. Missorted ER-resident proteins and vesicle membrane proteins are retrieved to ER by vesicles (**3**) that bud from *cis-Golgi and* fuse with ER. Each *cis-Golgi* cisterna, physically moves from *cis to trans face of Golgi* complex (**4**) by a nonvesicular process called ***cisternal progression. Retrograde transport vesicles (**5**) move Golgi-resident proteins to proper Golgi compartment. In all cells, certain soluble proteins move to cell surface in transport vesicles (**6**) and are secreted continuously (constitutive secretion). In certain cells, some soluble proteins are stored in secretory vesicles (**7)** and are released only after an appropriate signal (regulated secretion). Lysosome-destined membrane and soluble proteins are transported in vesicles budding from *trans-Golgi* (**8**), first move to late endosome and then to lysosome. ***Endocytic pathway**: Membrane and* soluble extracellular proteins taken up in vesicles that bud from PM (**9**) also move to lysosome via endosome.



Secretory and endocytic pathways of protein sorting

DISCLAIMER

All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline.

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