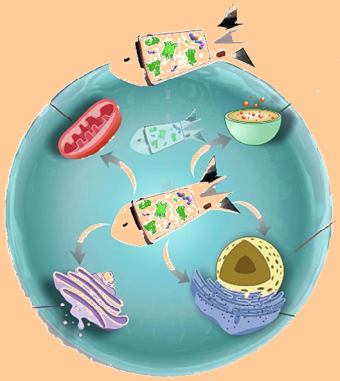
Cellular Biochemistry [918SM]



Module 3

Protein Sorting Pathways I

3_1 Targeting of proteins to the RER

3_2 Targeting membrane proteins

3_3 Folding and PTM of proteins in the ER

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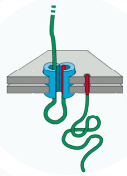
AA 2026-2027

Cellular Biochemistry [918SM]



Module 3_1

Targeting of proteins to the RER

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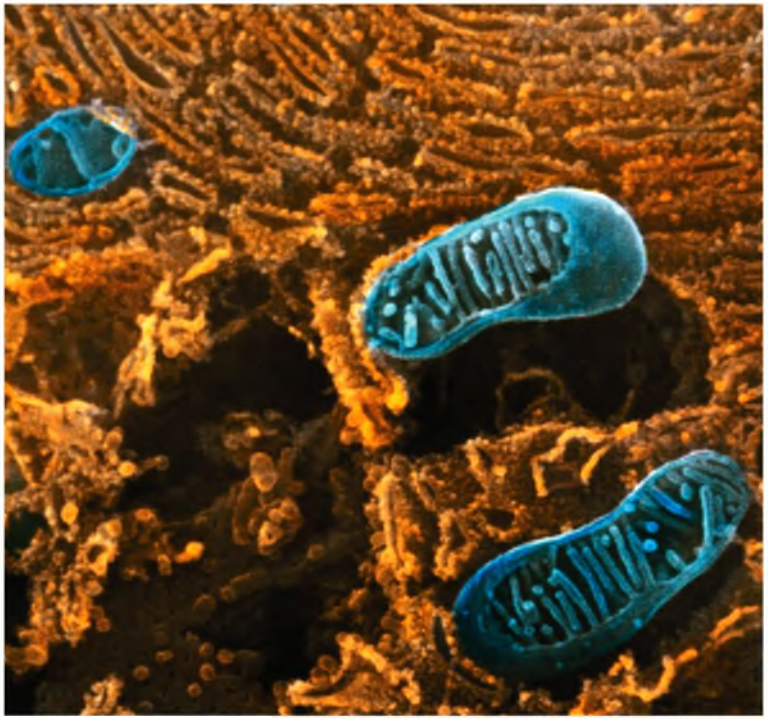
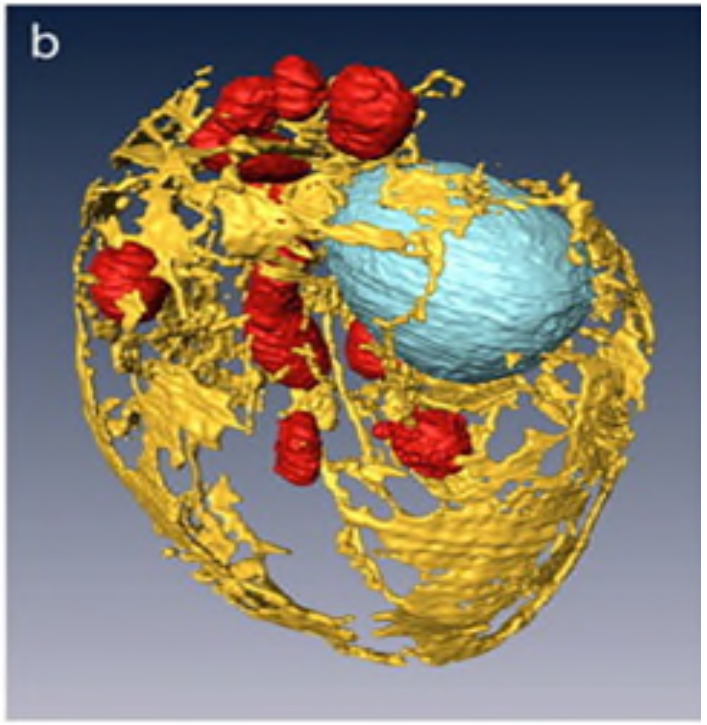
AA 2026-2027

Cells are organised in discrete organelles

- Mitochondria and nuclei are well defined discrete units
- The Endoplasmic reticulum (ER) instead is a large, dynamic structure formed by an extended and interconnected network of flattened sacs or tubules



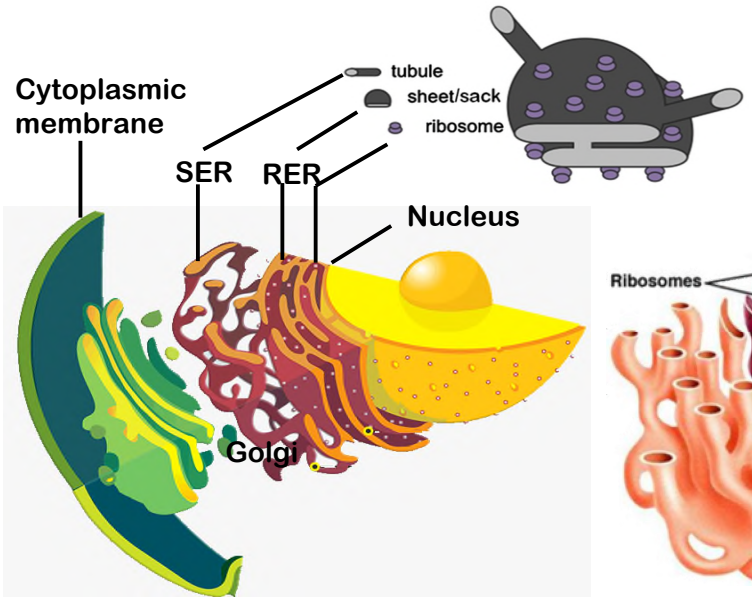
3D reconstruction of the **internal membranes** of a yeast cell using scanning EM. The cell wall and cytoplasmic membrane have been removed and the internal organelles are highlighted with false colours

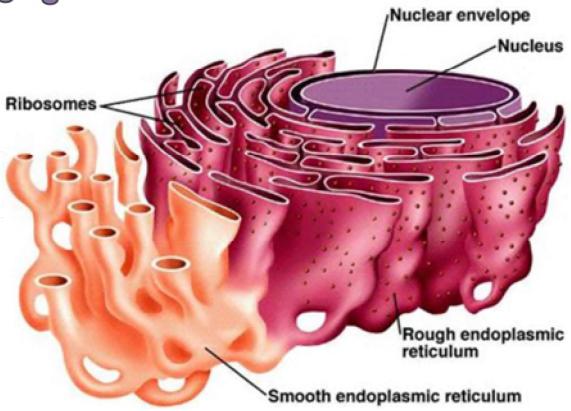


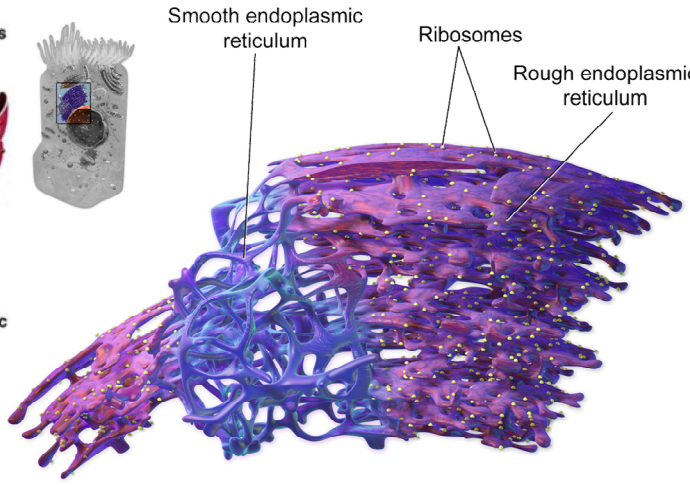
3D segmentation of ER (yellow), mitochondria (red) and nucleus (blue) of a whole yeast cell at 5 nm isotropic voxels. Cell diameter 3.5 µm.

Role of the endoplasmic reticulum (ER)

- Serves many cellular roles: Ca^{2+} storage , protein maturation & sorting, lipid metabolism
- Has distinct domains; tubules in the Smooth Endoplasmic Reticulum (SER)
  sacs/sheets in the Rough Endoplasmic Reticulum (RER)
- RER is connected to the nuclear envelope, its lumen is continuous with the perinuclear space
- The ER interacts with other organelles, the Golgi complex, such as mitochondria, peroxisome, lysosomes and vacuoles, all with specific functions, via vesicular traffic, membrane contact or budding

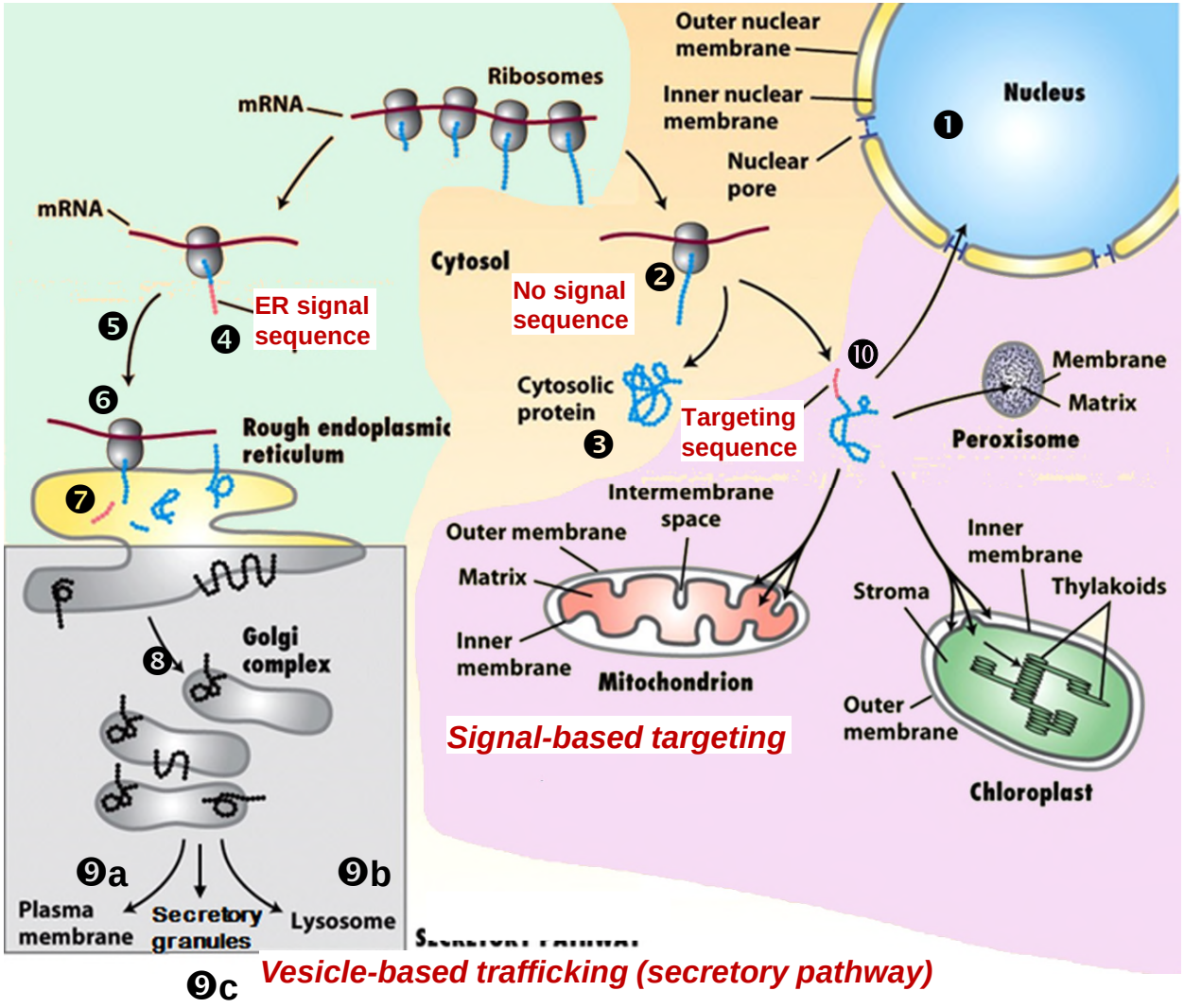






Overview of major protein-sorting pathways in eukaryotes

❶ mRNA exits from nucleus through nuclear pore, and ❷ binds to ribosomes; ❸ cytosolic proteins, with **no signal remain in cytosol**; ❹ plasma membrane proteins, secreted proteins and proteins destined to lysosomes have specific **signal sequences**, ❺ which guide ribosomes to the RER



❻ the translated proteins are translocated into the ER lumen;

❼ in the ER proteins are processed (folded, disulfide bonds formed, sugars added) ❽ and passed to the Golgi, which traffics them to the PM ❾**a**, lysosomes ❾**b** or secretory granules ❾**c** *(vesicle based trafficking)*

❿ Proteins destined to other organelles have specific **targeting sequences**, and are imported by distinct machinery (*Signal-based targeting*)

30-50% of proteins produced in a cell are delivered outside the cytoplasm

PROTEIN TARGETING

Fundamental aspects of protein-targeting events

1) The nature of the targeting sequence

no sequence → cytoplasm
ER signal seq → ER lumen, Golgi, cytoplasmic membrane, lysosomes
Targeting seq → nucleus, mitochondria, peroxisomes

2) The nature of translation

no sequence → cytoplasmic ribosome → cytoplasm
targeting sequence → cytoplasmic ribosome → nucleus, mitochondria, peroxisomes
ER signal seq → RER-bound ribosome → vesicular traffick

3) The receptor for the targeting sequence (protein machinery that guides and delivers nascent protein chains or folded proteins to the translocation machinery)

4) The transport system (translocation channels that allow transfer of proteins across the membrane bilayer)

5) The nature of the protein (if it translocates in a folded or unfolded form)

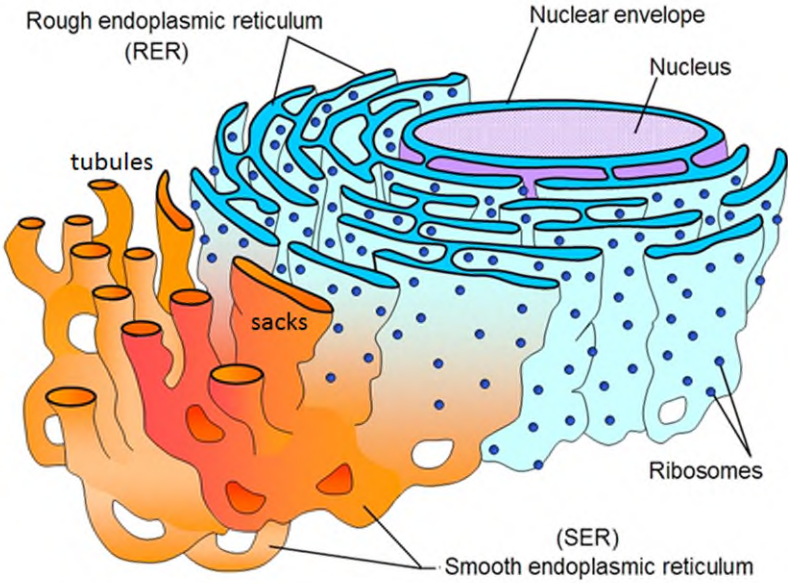
6) The energy source (required to drive unidirectional transfer across the membrane)

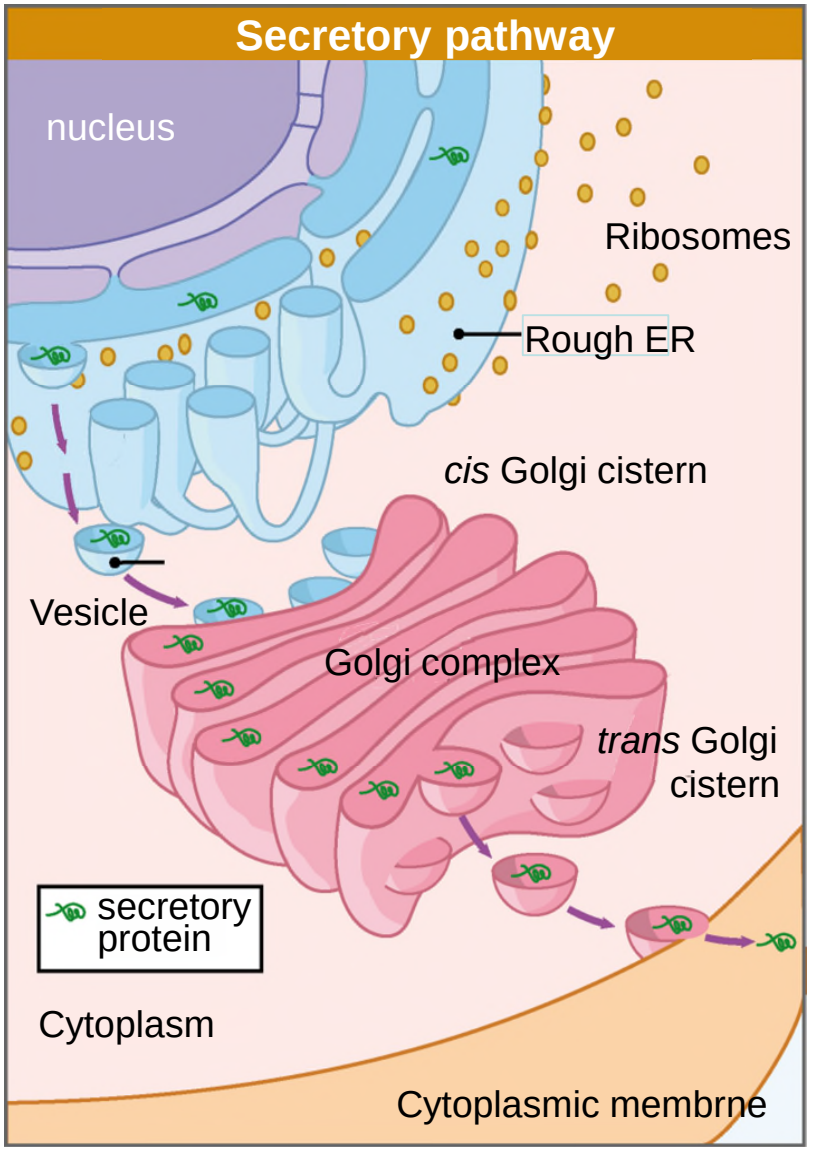
General process

Signal recognition → Guidance & delivery → Translocation
↓
Quality control ← Processing & folding

The ER is an essential component for protein trafficking through the secretory pathway

- it collaborates with the **Golgi complex**, with which it communicates via **vesicles** (Modulo 4)
- **protein processing** & **PTM begin in the ER**
- they **continue in Golgi cisternae** and **secretory vesicles**

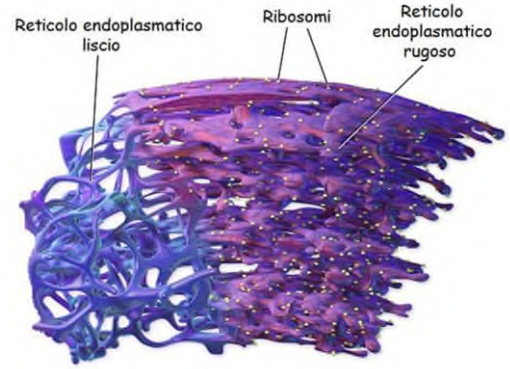


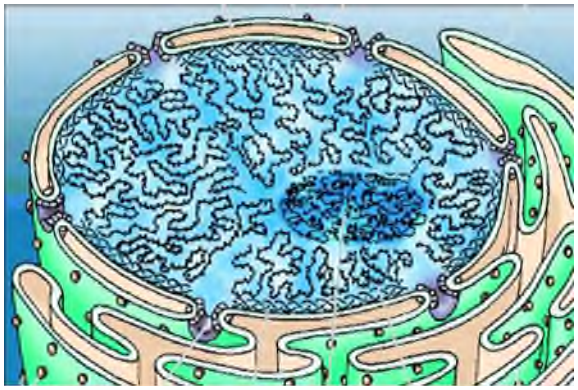


PROTEIN TRAFICKING THROUGH THE ER

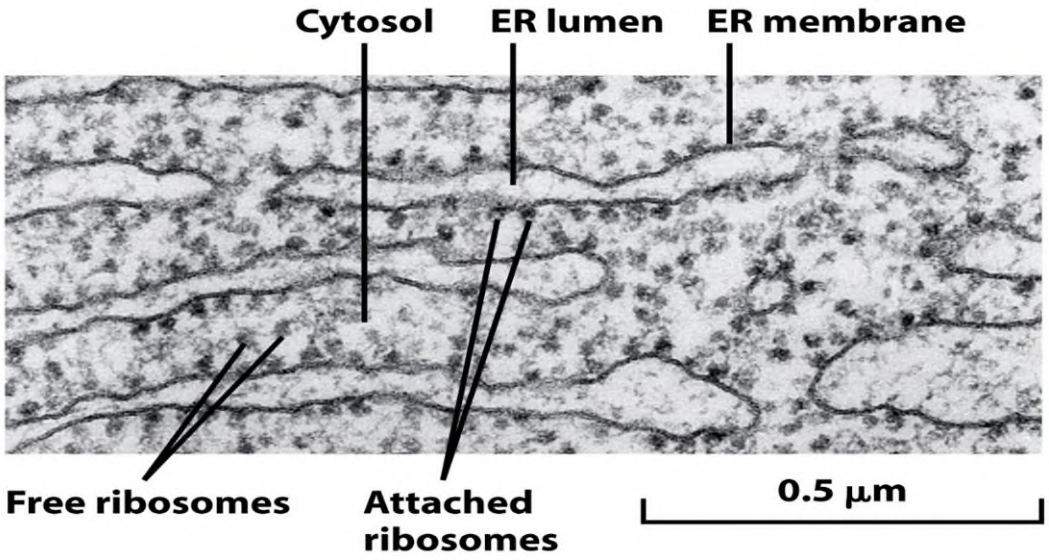
Stages of protein trafficking

- **mRNA** exits the nucleus through the **nuclear pore**, into the **cytoplasm**
- **proteins destined for the secretory pathway** are translated by ribosomes associated with the **cytoplasmic side of the RER membrane** and translocated directly **into the lumen**
- **ribosomes attached to the RER** membrane confer the ROUGH aspect



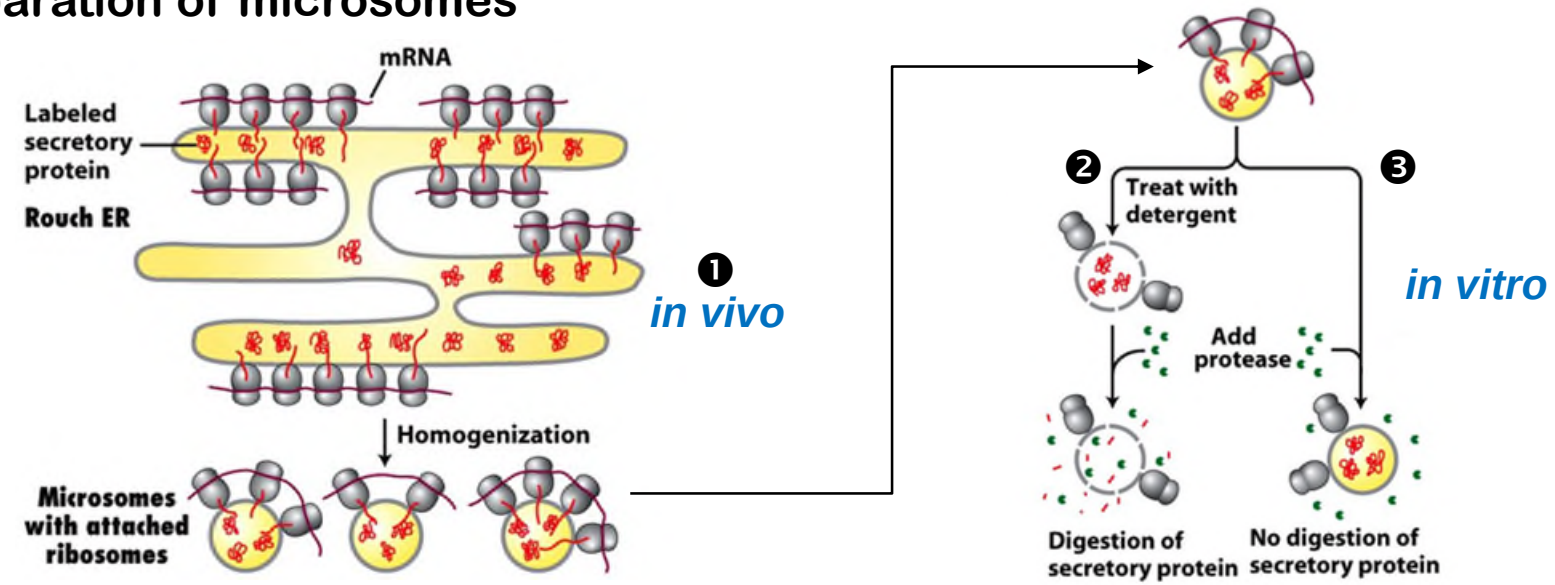


rough ER in a pancreatic acinar cell

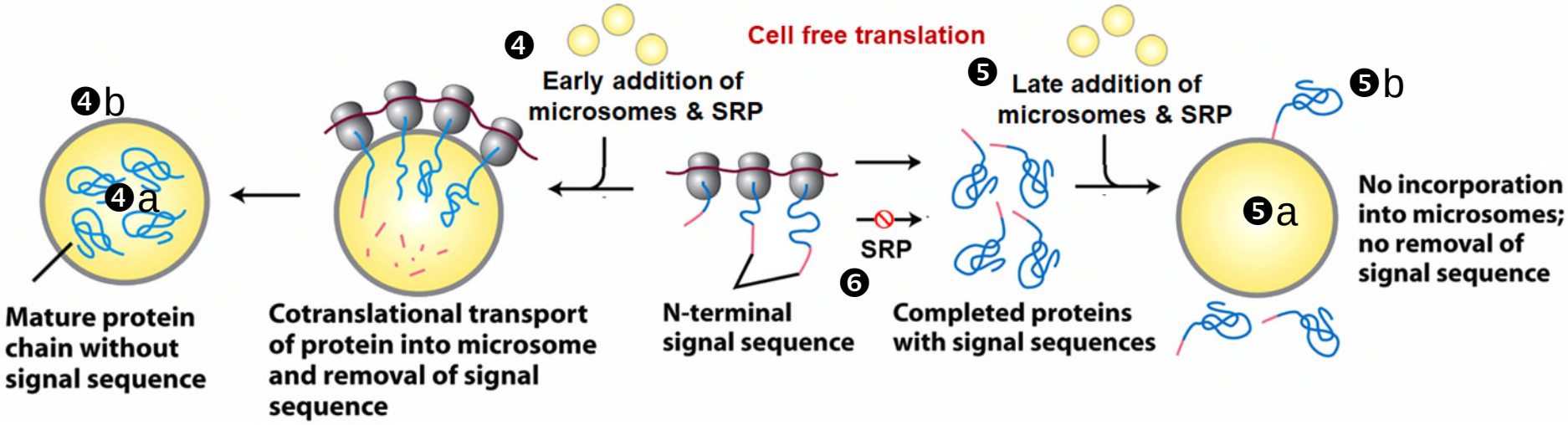


EXPERIMENTAL VERIFICATION OF TRAFFICKING THROUGH THE ER

1) Preparation of microsomes



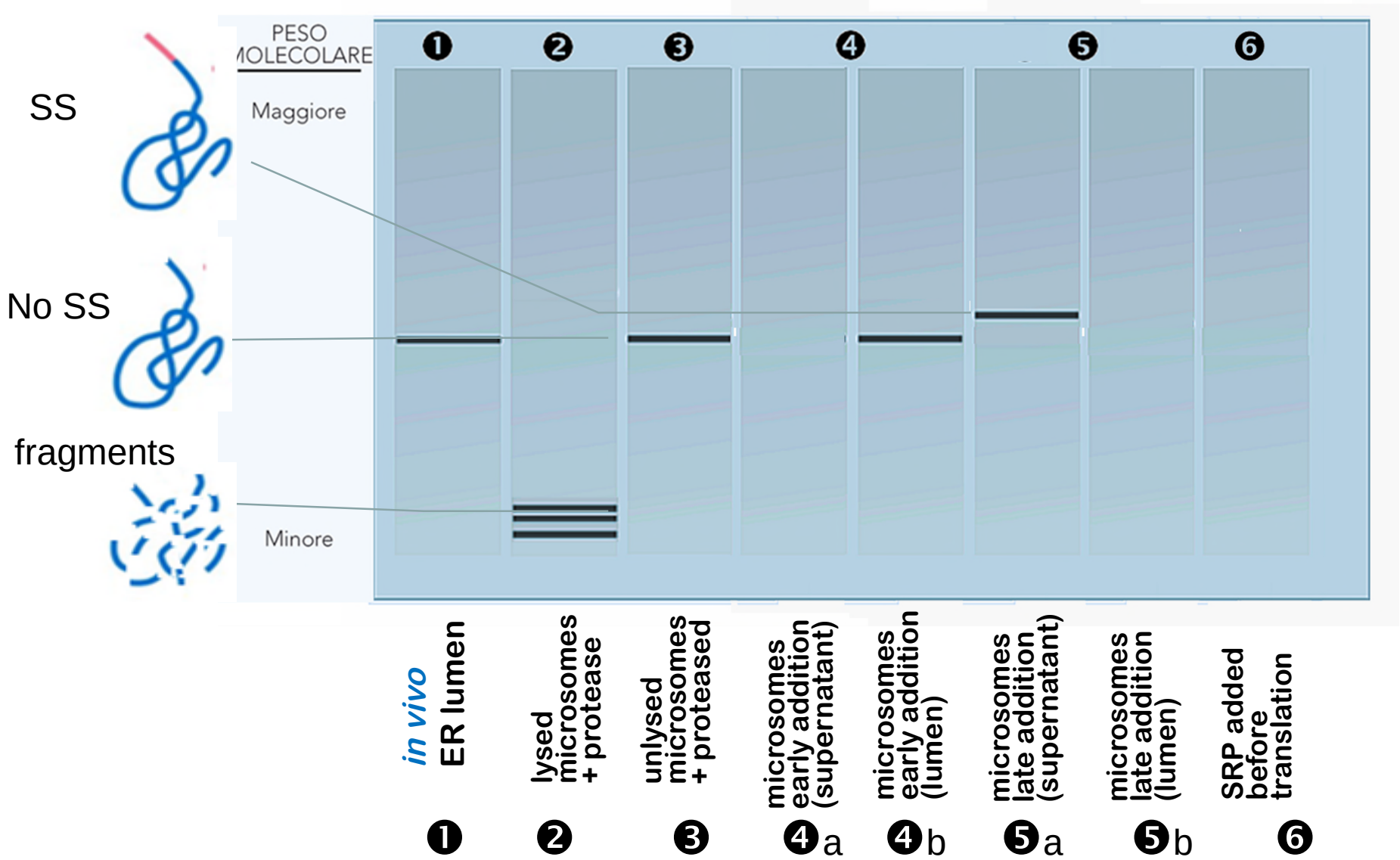
2) Cell-free protein synthesis with and without microsomes present



RER insertion is COTRANSLATIONAL: SS **essential** but **not suffucient**

EXPERIMENTAL RESULT: TRANSLATION & TRANSLOCATION SIMULTANEOUSLY

3) Gel electrophoresis confirms translation into ER (❶❷❸❹a), presence of a removed signal sequence (❺a vs ❶❸❹b), cotranslational nature (❺b, ❻)



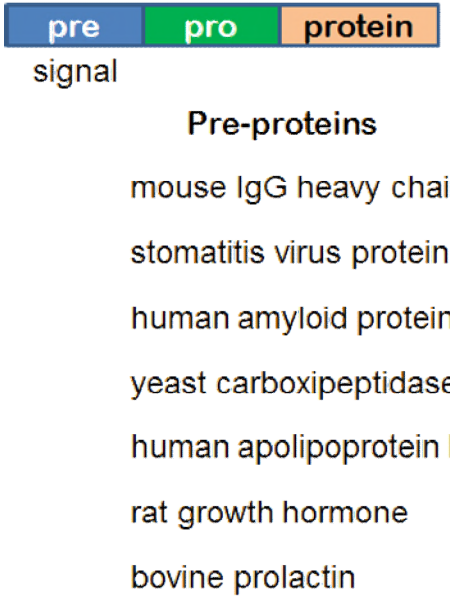
ER translocation signal sequences

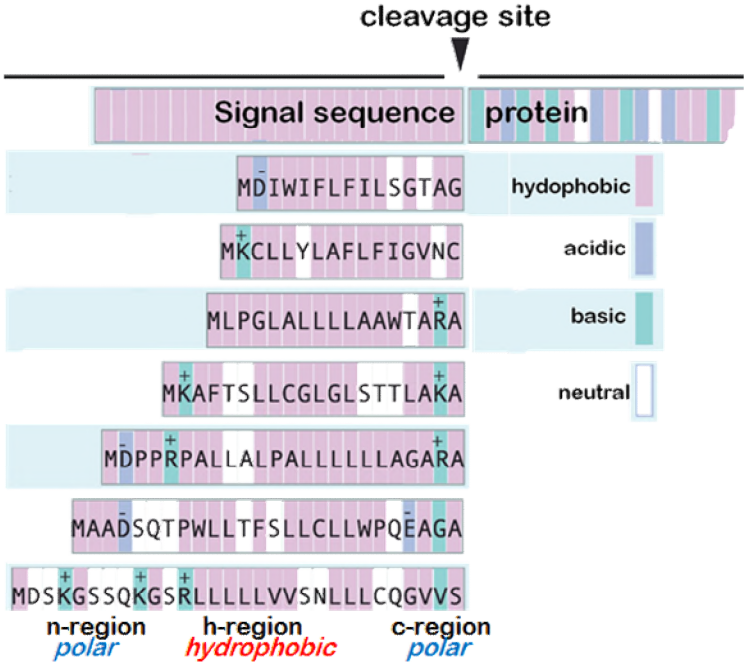
- **FOR soluble proteins**

– **SS with helical, hydrophobic core**; deletion/mutation of these AA abolishes signal function.

– **cytosolic proteins with added N-terminal SS** (rDNA techniques) → ER lumen.

– **SS removed** after use





- **FOR soluble proteins**

- Some MP have N-terminal SS, removed after use
- For other MP, a transmembrane sequence can act as signal sequences (Module 3.2)

MECHANISM OF PROTEIN TRAFFICKING TO ER

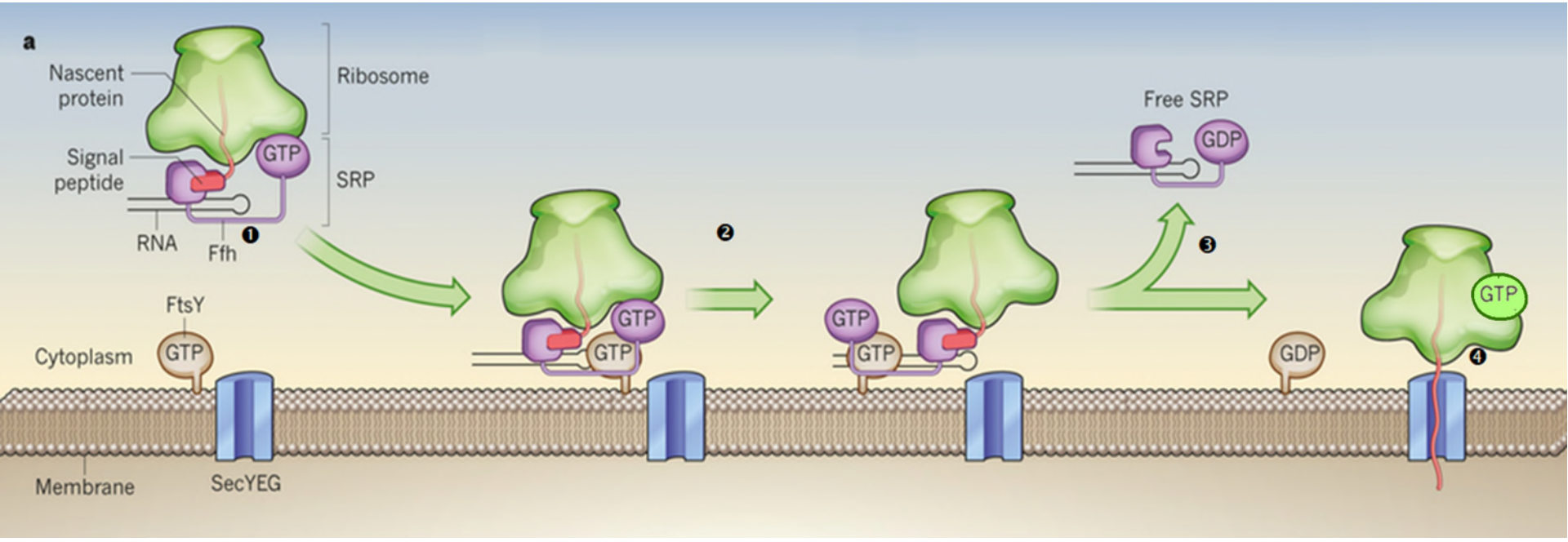
The ER translocation SS is recognised by the Signal Recognition Particle (SRP) - a ribonucleoprotein

❶ **SRP binds to the SS** of the **nascent peptide chain** (**NC**), forming the **Ribosome-NC complex** (**RNC-SRP);** this **process requires GTP** and **arrests translation**

❷ SRP guides the RNC to the **Sec translocase (translocon)**, docking with the help of the **SRP receptor** (e.g. FtsY in *E. coli*). This process also requires **GTP binding**

❸ the SS inserts into the translocation channel, **SRP** and **SRPR** detach in a process energized by GTP hydrolysis, and **translation resumes,** pushing NC into ER lumen

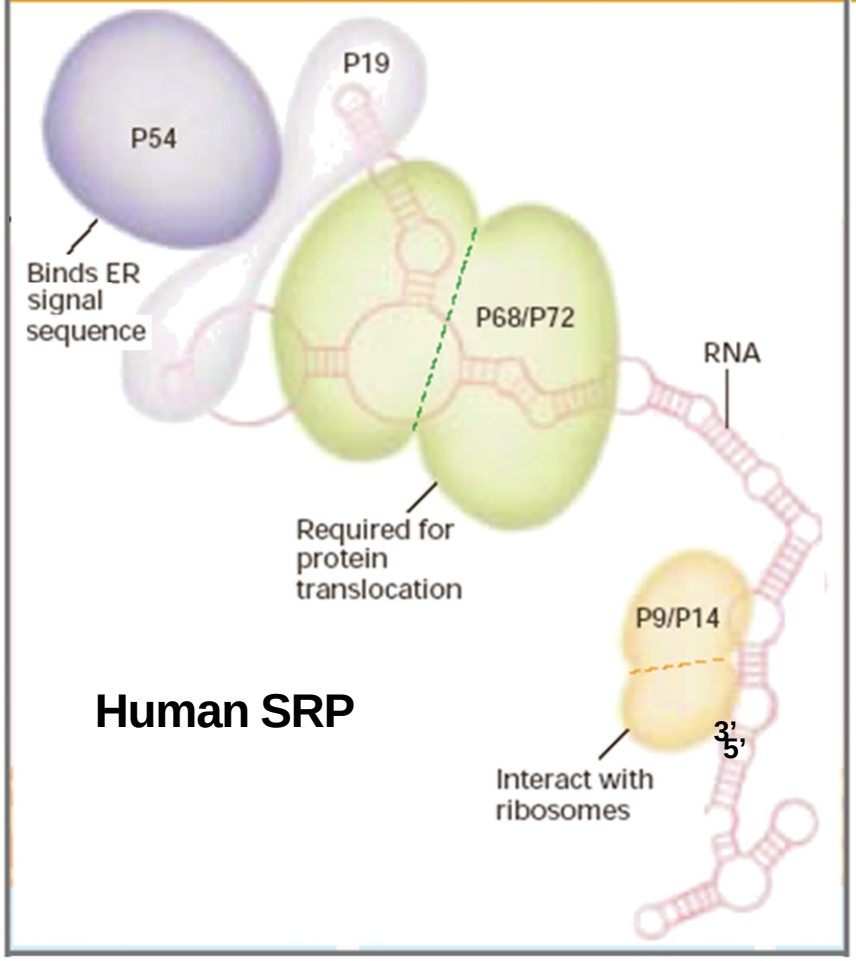
❹ translocation is powered by GTP used by ribosome for translation



STRUCTURE AND FUNCTION OF THE SIGNAL RECOGNITION PARTICLE (SRP)

- SRP is an **abundant**, **cytosolic**, **universally conserved ribonucleoprotein** that targets specific proteins **to the ER in eukaryotes**, by recognizing the SS
- **In prokaryotes** it targets proteins with SS to the plasma membrane

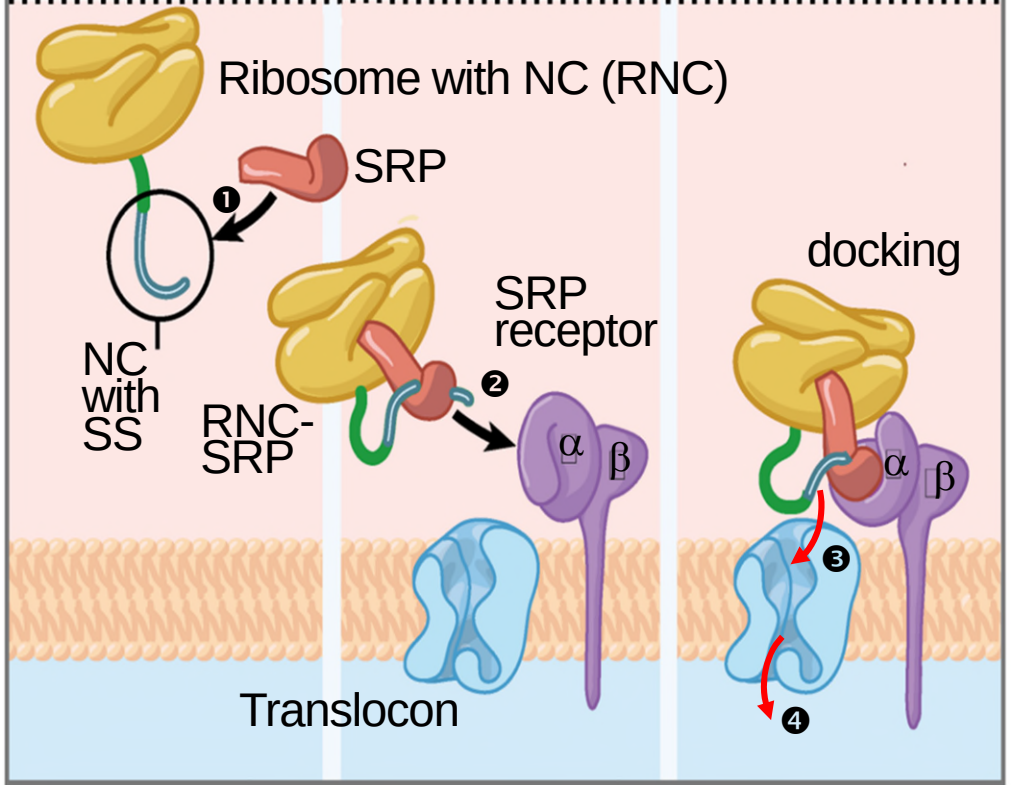
Structure



Human SRP

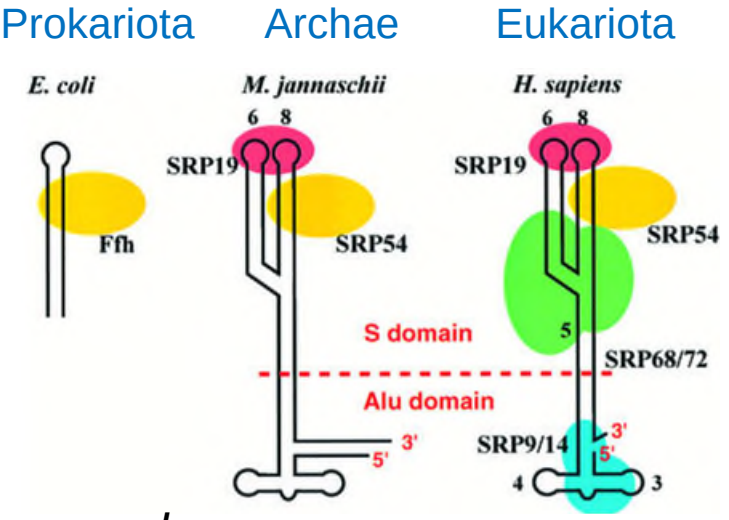
Function

❶ binds to SS in NC
❷ stops translation and docks RNC to SRP receptor
❸ SRPR transfers SS to translocon ❹ translation resumes

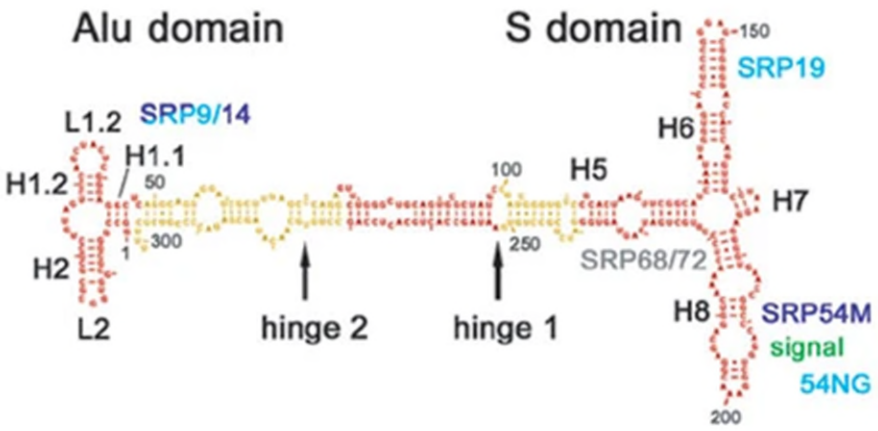


SRP STRUCTURE AND EVOLUTION

- The ribonucleoprotein structure of SRP has become **more complex in higher organisms**
- In mammals it consists of **1 7S RNA** and **6 protein subunits** (SRP9/14, SRP19, SRP54 & SRP68/72) with a **crescent-shaped structure**
- **7S RNA** folds into a roughly **Y-shaped topology** with mostly **double-stranded secondary structure**
- It can be separated into two functional domains by *micrococcal nuclease*; a smaller **Alu-domain** and larger **S-domain**

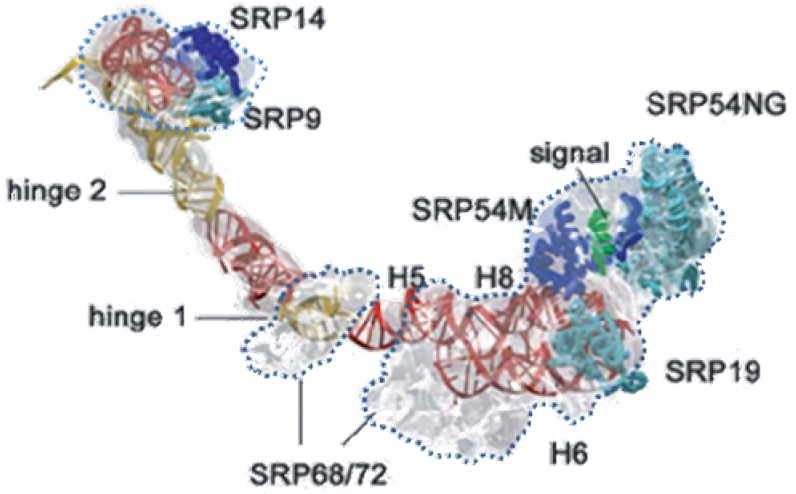


Topology



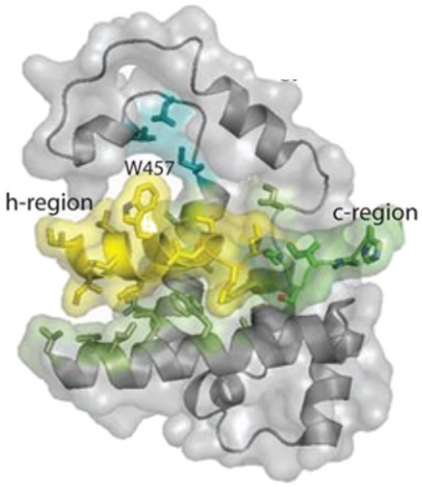
H = RNA helix L = loop

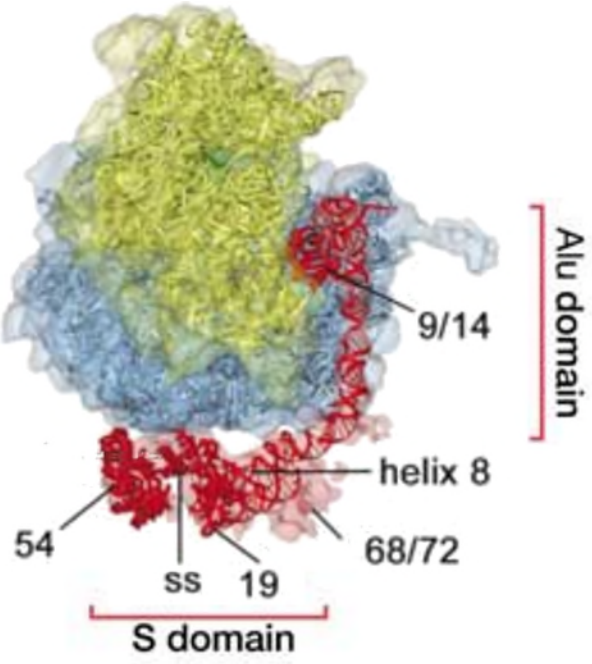
Structure

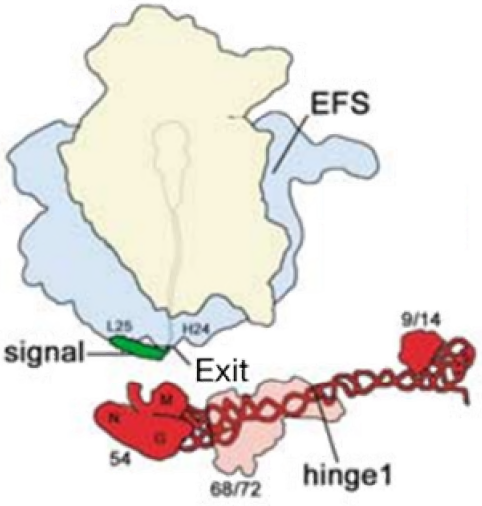


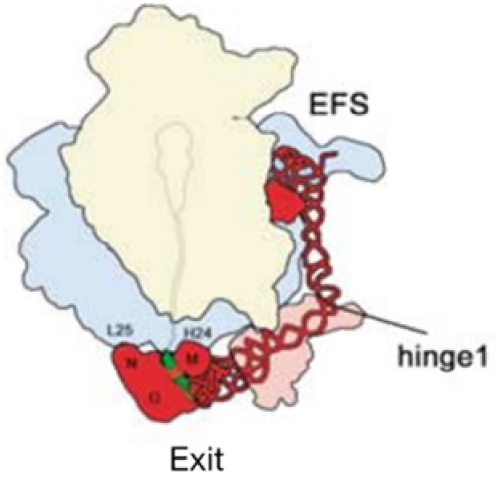
SRP STRUCTURE AND EVOLUTION (CONT.)

- In SRP, **SRP54** in the S-domain **binds to the SS** of Membrane Proteins (MP) or Secretory Pproteins emerging from the ribosome and forms a **R**ibosome **N**ascent **C**hain-**SRP** complex (RNC-SRP)
- In eukaryotes, this causes a **transient arrest of polypeptide chain elongation**, through **interaction of SRP9/14** in the Alu-domain **with the tRNA and elongation factor binding site** (EFS) at the interface between the small and large ribosomal subunits







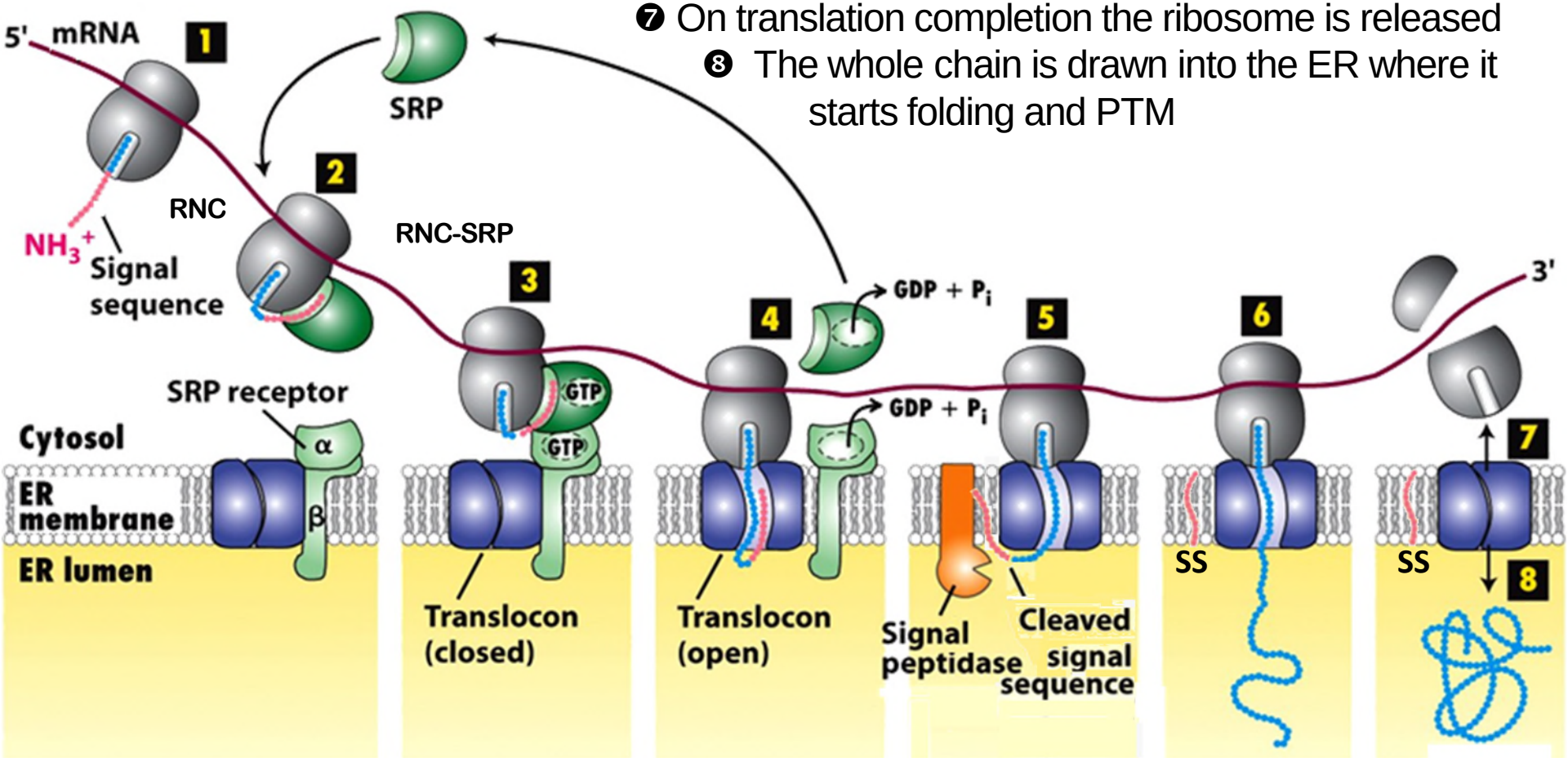


SRP FUNCTION IN CO-TRANSLATIONAL TRANSLOCATION

❶❷ SS emerges from the ribosome and binds to **SRP54**. ❸ **RNC-SRP complex** binds to the **SRC receptor** at the ER membrane primarily via P54 (**both require GTP**); ❹ Transfer of NC to **Translocon (Sec61)** leads to channel opening to admit NC; SS binds to a hydrophobic site next to the central pore. Both SRP & SRP receptor hydrolyse GTP and release the translocon, ready for a new cycle; ❺ The SS enters the membrane via a lateral gate in the translocon, and is cleaved by a ***Signal peptidase*** (ER membrane protein); ❻ The NC **co-translationally translocates** into ER, **driven by ribosome chain elongation energy (GTP)** .

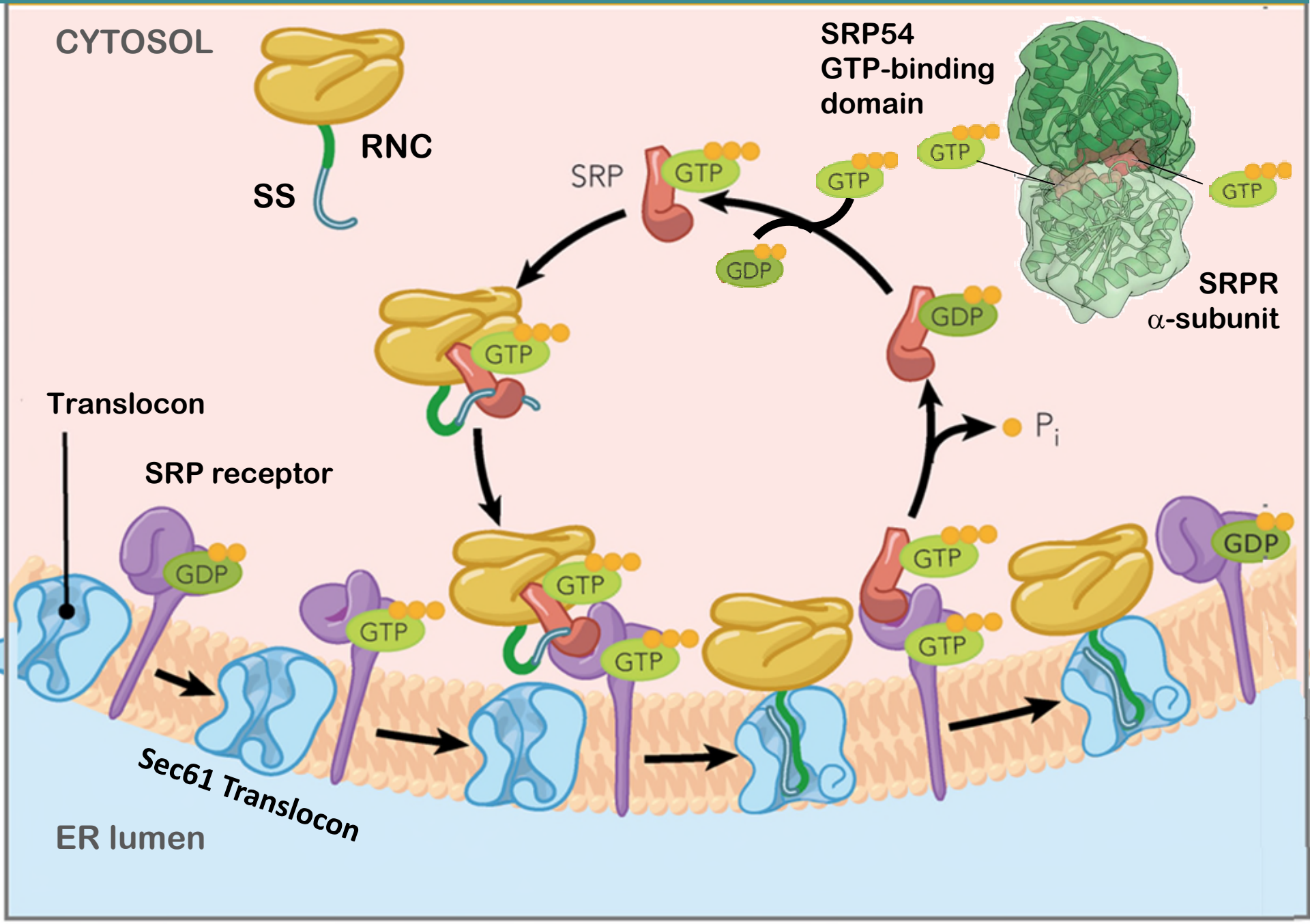
❼ On translation completion the ribosome is released

❽ The whole chain is drawn into the ER where it starts folding and PTM



https://app.jove.com/science-education/v/12125/concepts/directing-proteins-to-the-rough-endoplasmic-reticulum

SRP/SRPR GTPase CYCLE

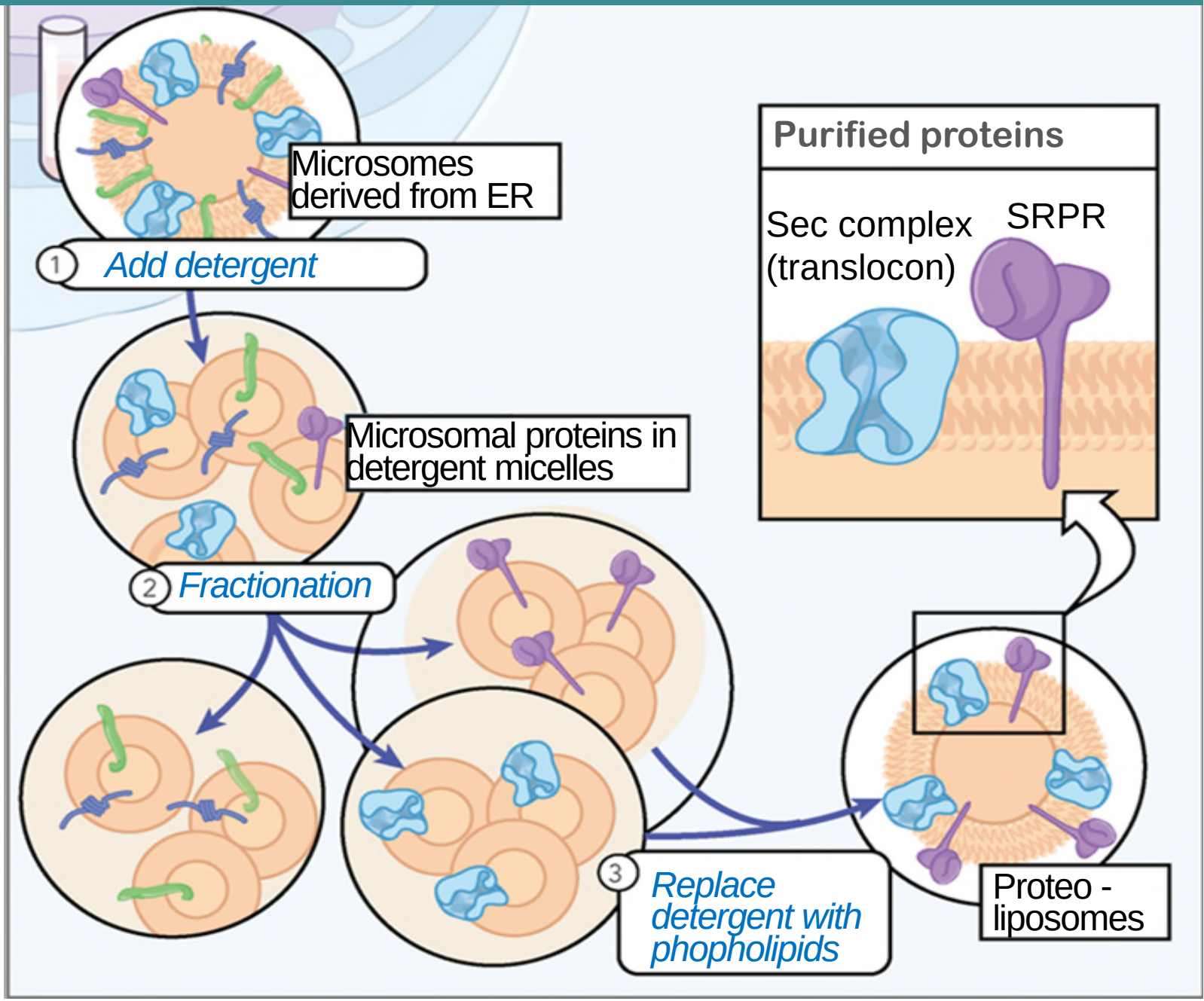


PURIFICATION OF RE TRANSLOCATION PROTEINS

Translocon

SRP receptor

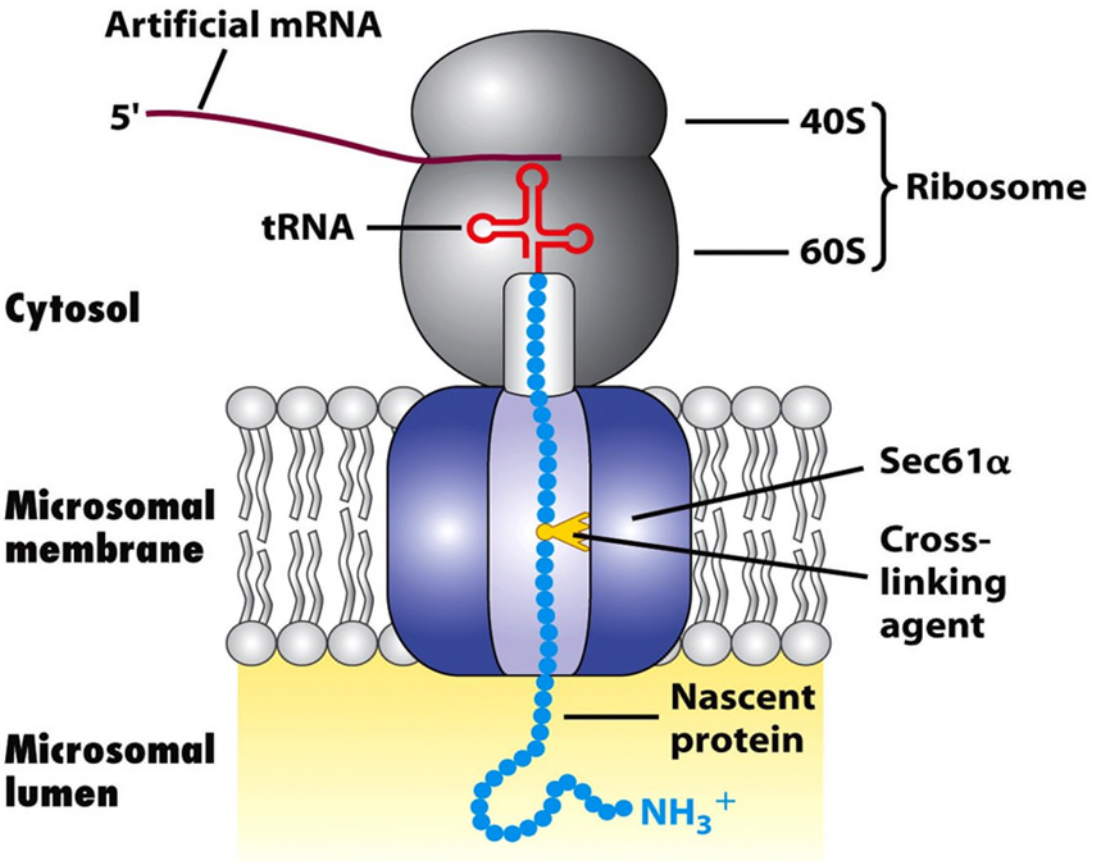
Accessory proteins



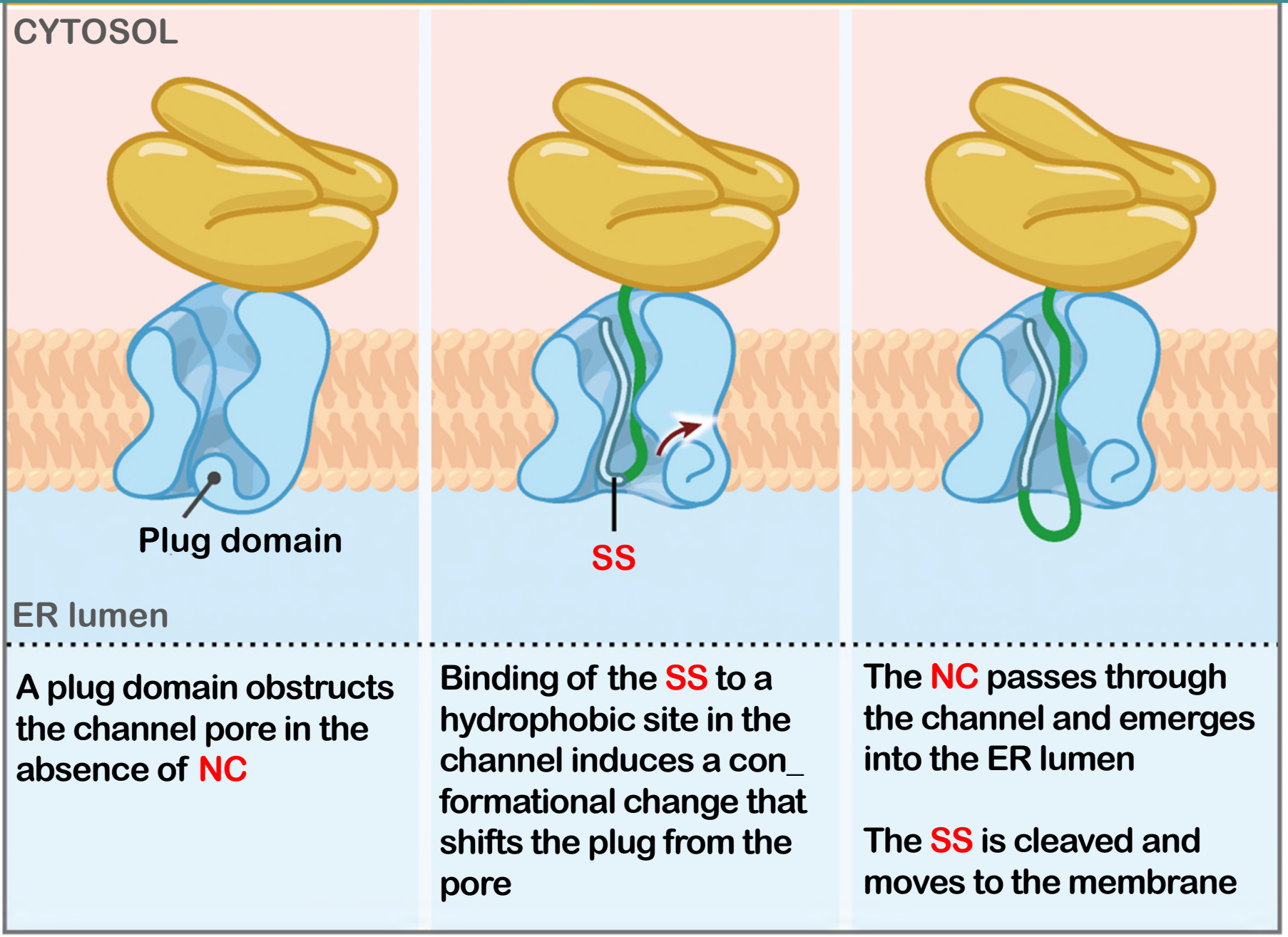
TRANSLOCATION FUNCTION

Translocon function is confirmed by a cross-linking experiment

- Prolactin mRNA encoding the 70 N-terminal AA but lacking a stop codon was translated in a cell-free system containing microsomes with the core translocon component Sec61
- If translation termination is prevented the ribosome cannot disassemble
- A photochemical crosslinking agent (modified Lys) is inserted into the NC
- Crosslinking to Sec61α subunit occurs only if the NC is stuck and Lys is positioned in the channel



MODEL FOR TRANSLOCON CHANNEL OPENING



A plug domain obstructs the channel pore in the absence of NC

Binding of the SS to a hydrophobic site in the channel induces a con_ formational change that shifts the plug from the pore

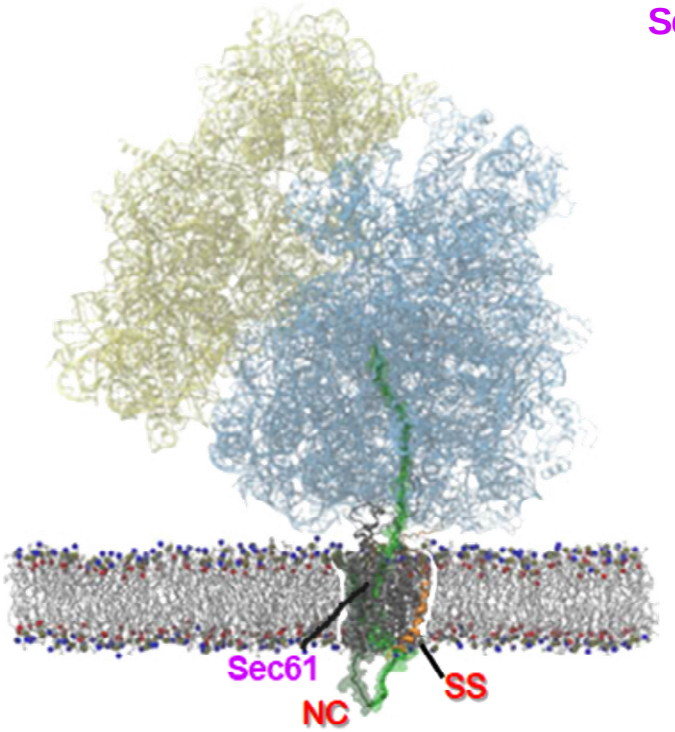
The NC passes through the channel and emerges into the ER lumen

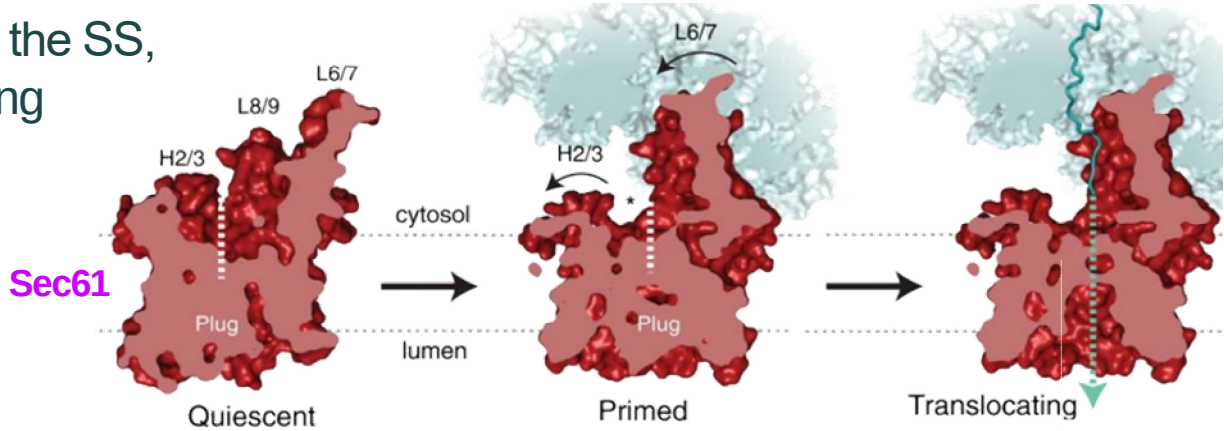
The SS is cleaved and moves to the membrane

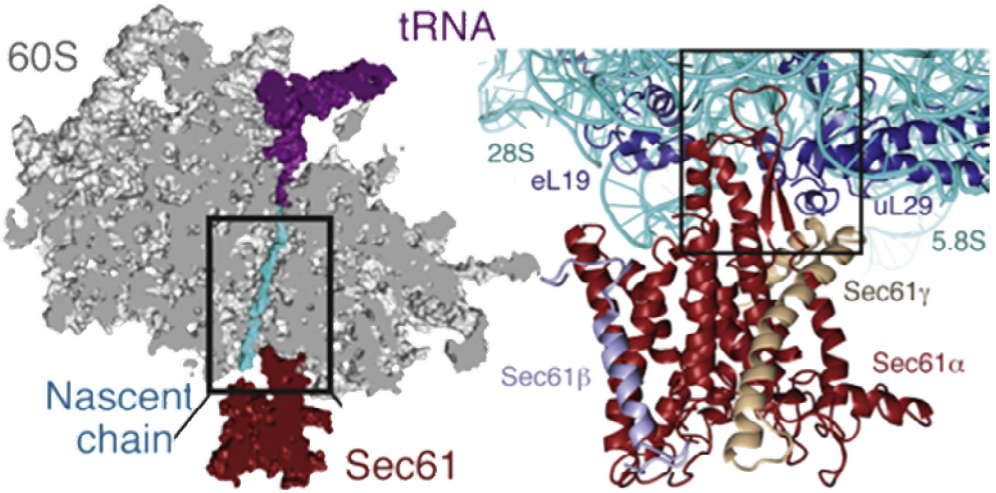
MODEL FOR TRANSLOCON CHANNEL OPENING (cont.)

Interaction of the ribosome with the Translocon

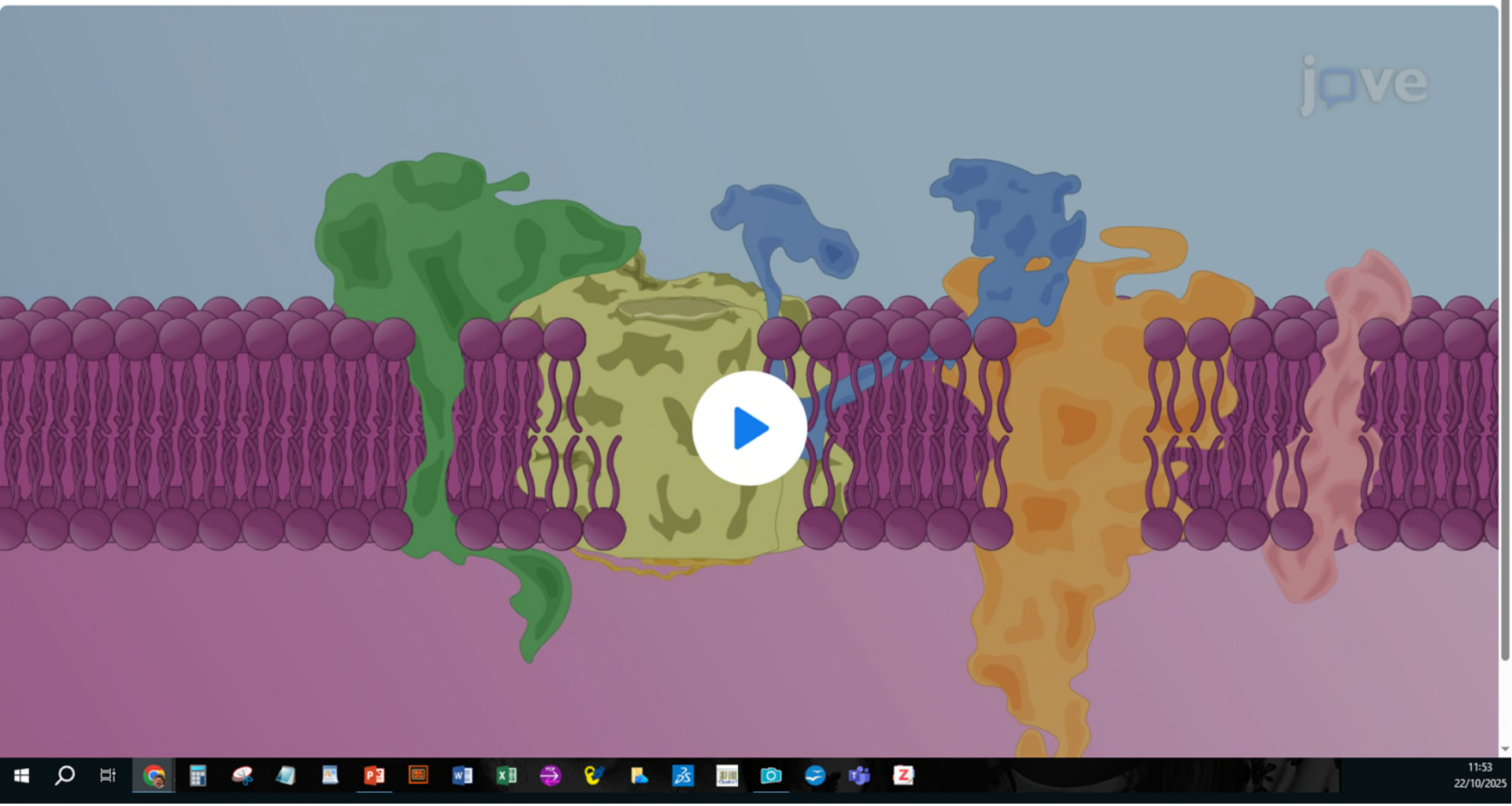
- **Sec61** interacts with the ribosome through the evolutionarily conserved loops in the Sec61α *subunit* and a helix in the Sec61γ subunit
- Ribosome binding primes the Translocon to accept the incoming NC; the loops and helix move and this causes a rearrangement from quiescent to primed structures.
- This creates a binding site for the SS, opening the tunnel and allowing translocation to initiate







Protein Translocation Machinery on the ER Membrane



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FUNCTION OF ARCHEAL TRANSLOCON

- ***M. jannaschii Sec61* has 10 TM**

❶ Side and❷ top views showing 1) plug* helix ; 2) pore ring (a hydrophobic gasket§ of Ile residues) and 3) lateral exit

❸ Binding of Sec61 to ribosome exposes to a hydrophobic site at the tunnel entrance for binding of the SS with the N-terminus facing the cytosol

❹ This causes the small plug helix in the tunnel to move away (see ❶❷ left) allowing insertion of the NC, doubling back on the SS, through the pore ring

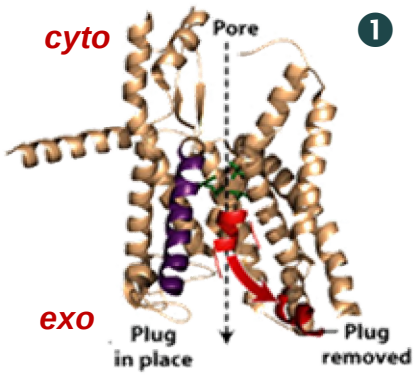
❶- ❹ A ring if Ile residues at the centre of the tunnel (pore ring) acts as a gasket that prevents leakage of small polar species (e.g. ATP, Ca^{2+}) around the translocating NC

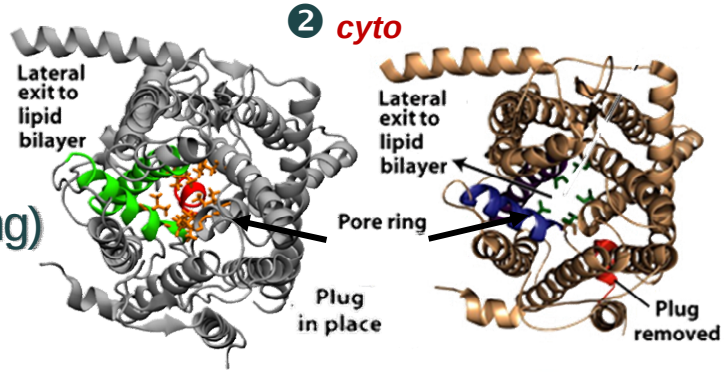
❺ The translocon also has a lateral exit to the tunnel that allows hydrophobic TM sequences to move to the membrane (including SS)

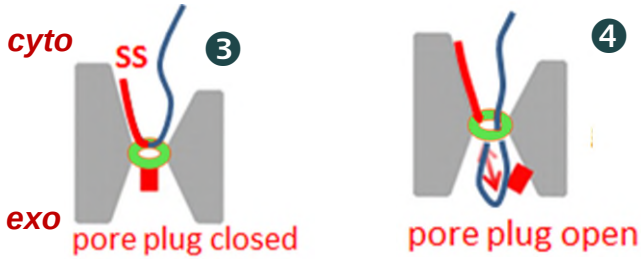
– NC moves into the ER lumen, and a *Signal peptidase* on the luminal side of Sec61 releases the SS from the NC.

– NC leaves the tunnel, the helix plug closes the pore ring and the SS moves through lateral exit into the membrane

* plug = tappo; § gasket = guarnizione







PROTEINS THAT ASSIST THE SEC61 TRANSLOCON

- **"Weak" SS require accessory components for efficient Sec61 gating.**

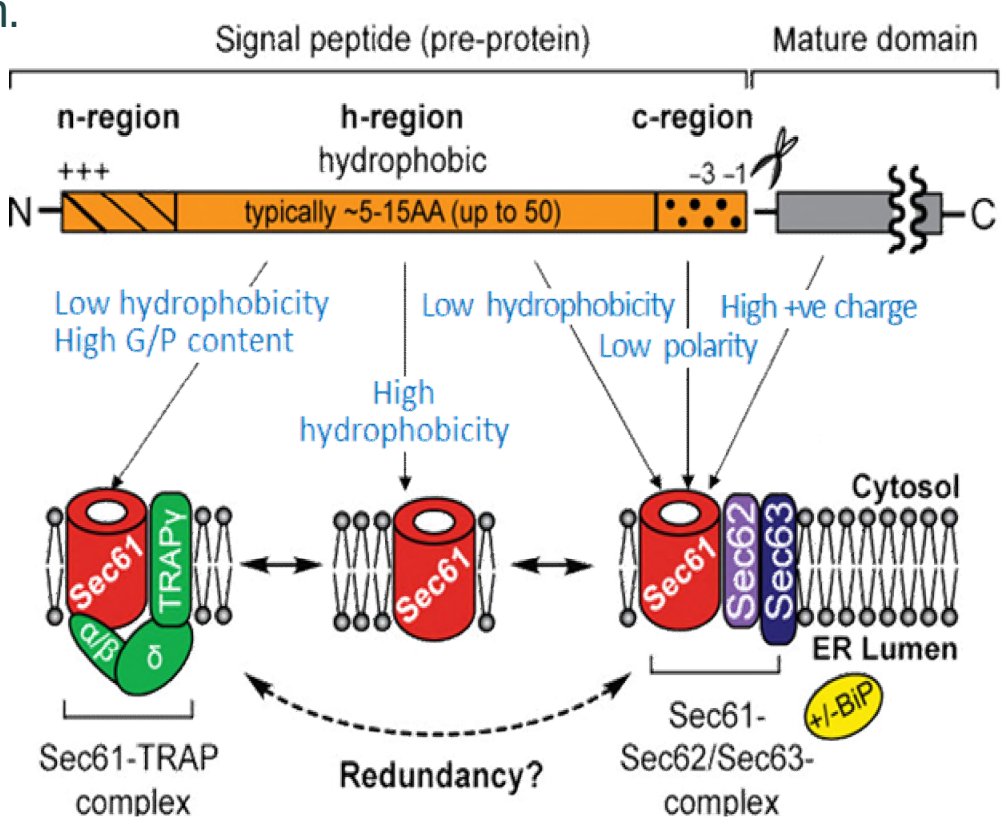
– Strong SS: long, hydrophobic helical stretch (h-region) flanked by polar n- and c-regions

– TRAP complex assists translocation for SS less hydrophobic h-regions and/or a high Gly/Pro content (Weak SS)

– Sec62/Sec63 assists translocation, together with the luminal chaperone ***BiP*** (*) for proteins with Weak SS having long and less hydrophobic h-regions and less polar c-regions and with +ve residue clusters in the mature domain.

– TRAP and Sec62/Sec63 have different client proteins but are functionally redundant, and can compensate for each other in cotranslational translocation

– Sec62/Sec63 can also assists SRP-independent , Post-Translational translocation of small secretory proteins (important in yeast, minor in mammals)



(*) *HSP70* family chaperone, aka *HSPA5*

PROTEINS THAT ASSIST THE SEC61 TRANSLOCON (cont.)

Role of TRAP complex and OST

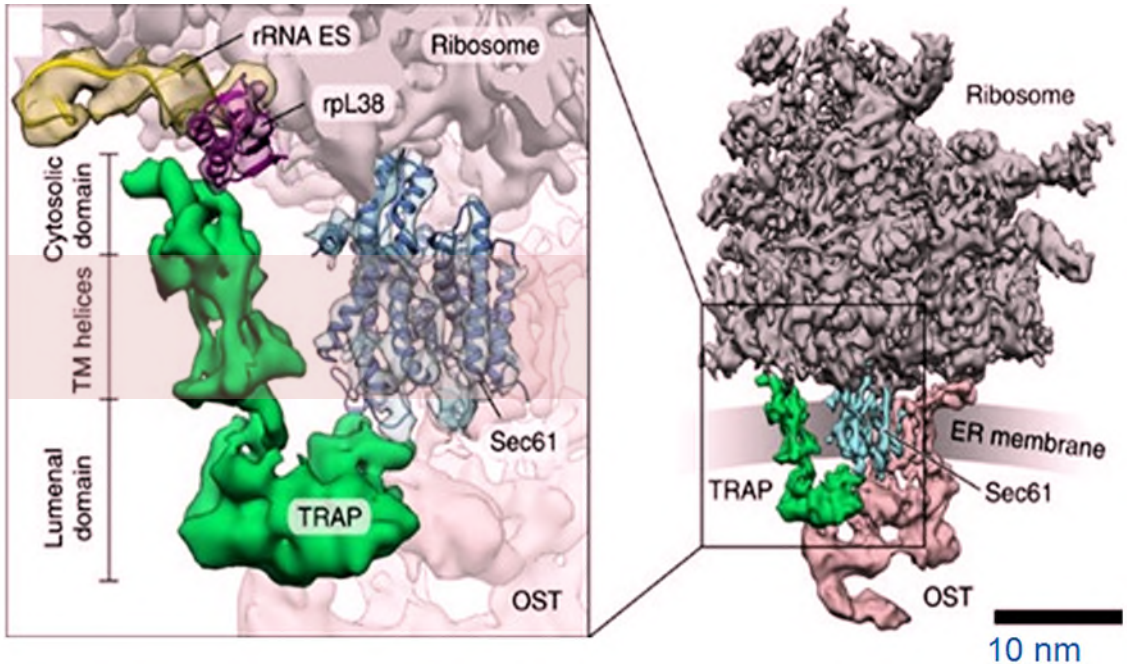
- The TRanslocon-Associated Protein (TRAP) complex is an integral translocon component in mammals, assisting Sec61 by regulating SS and TM helix insertion in a substrate-dependent manner. It has 4 subunits, TRAPα-γ
- *Oligosaccharyl-transferase* (*OST*) (see Module 3_3) carries out cotranslational N-glycosylation, with different isoforms sequentially scanning NC for sugar acceptor sites and glycosylating different positions as it enters the ER lumen, ensuring maximal N-glycosylation efficiency.
- TRAP stabilizes Sec61 interaction with the ribosome and stimulates translocation of proteins with a weak SS
- it also assists NC N-glycosylation and can affect the topology of TM helix insertion for integral membrane proteins

TRAP: translocon-associated protein complex

OST: oligosaccharyl–transferase

rpL38: ribosomal protein L38

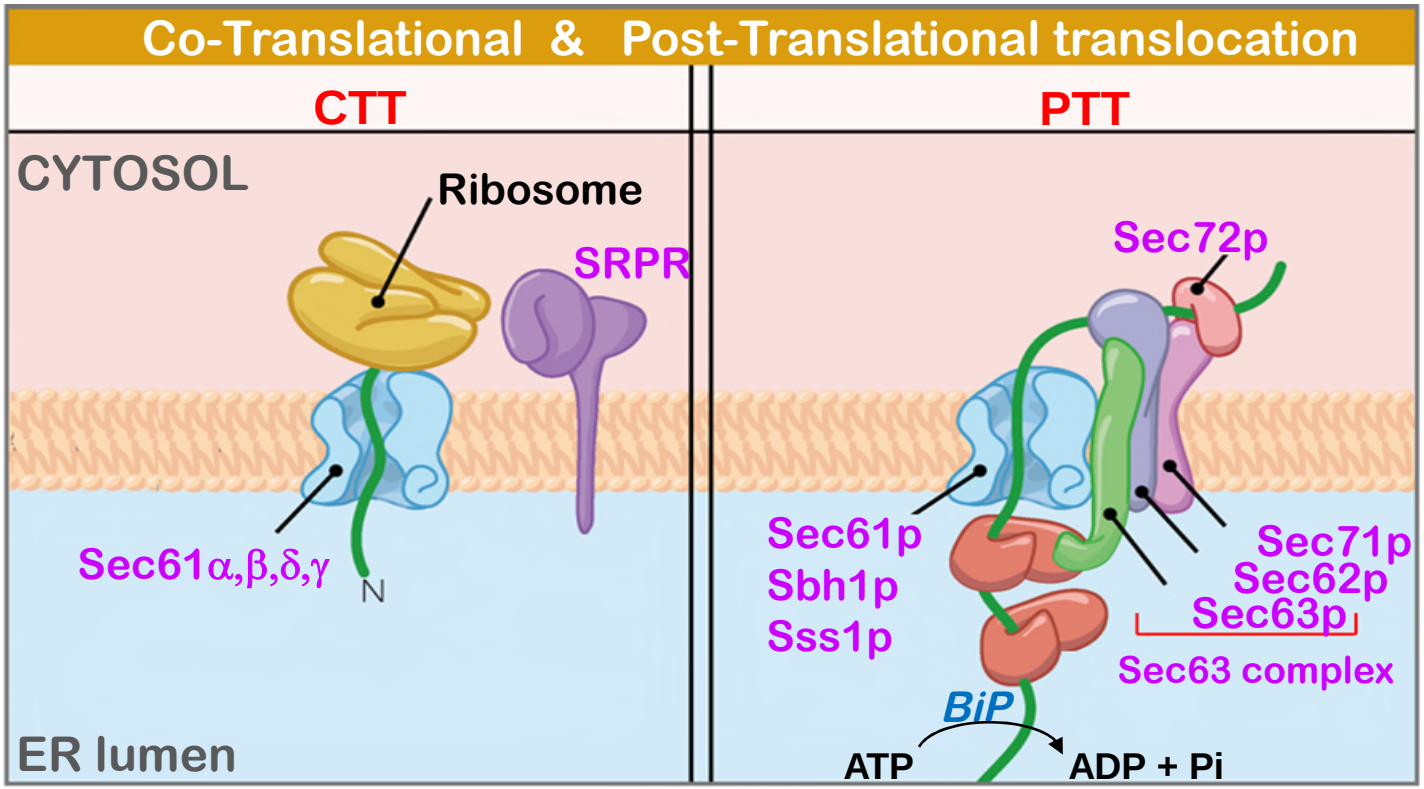
rRNA ES: ribosomal RNA



POST-TRANSLATIONAL TRANSLOCATION

Yeast Sec63 complex allows Post-Translational protein Translocation

- primarily implicated in SRP-independent, Post-Translational Translocation (PTT) of small secretory proteins in yeast (less important in mammals but does occur)
- collaborates with Sec61, which acts in both co-translational translocation (CTT) and PTT
- secretory proteins are targeted to a Sec61,62,63 complex by cytoplasmic chaperones, which dock to Sec71/72. Translocation is assisted by *BiP* chaperone (an *ATPase*)
- the hydrolysis of ATP ensures that PTT is unidirectional into the ER



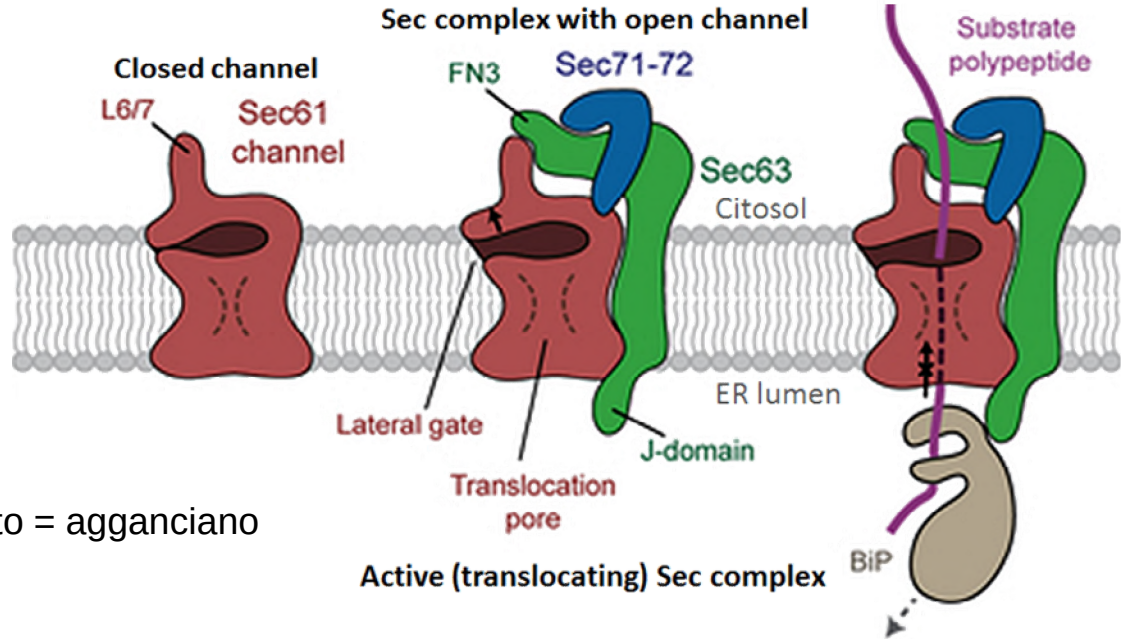
Mammalian translocon
Sec61α,β,δ,γ

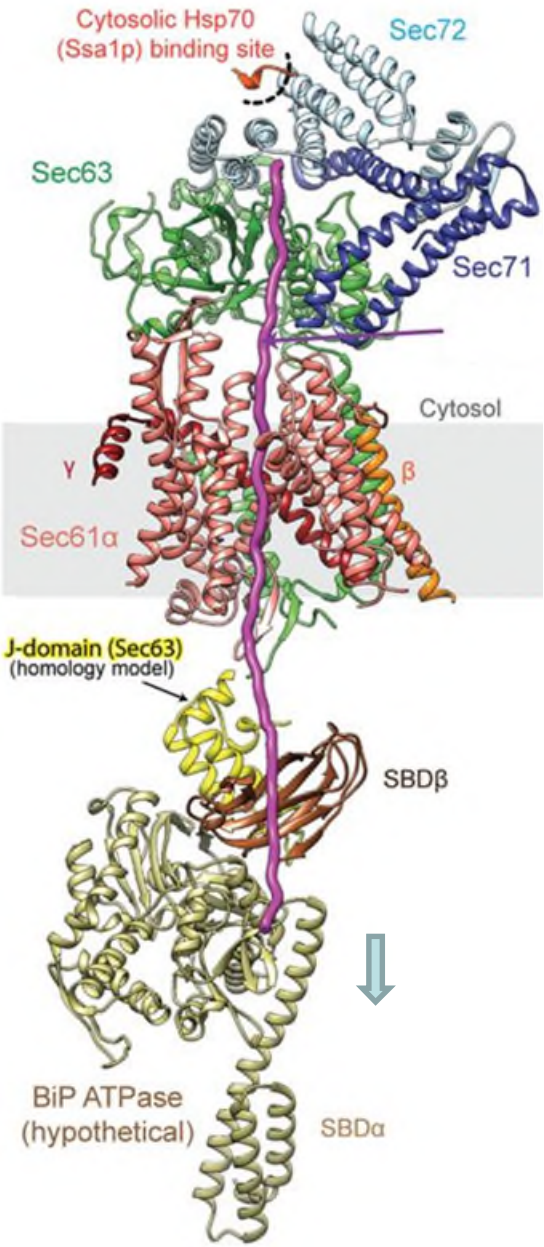
Yeast ortholog
Sec61p, Sbh1p, Sss1p

POST-TRANSLATIONAL TRANSLOCATION (cont.)

Yeast Sec63 complex Mode-of-Action

- Conserved loops in Sec61 interact with a FN3 domain of Sec63
- Sec71/72 clamp on to* Sec63 and bind to a cytosolic (*HSP70*) chaperone that carries a fully translated but unfolded protein chain
- The Sec61 loops shift, opening the translocation channel for the protein chain
- On the luminal side, the J-domain of Sec63 positions the chaperone *BiP* (an *ATPase*) to receive the emerging chain
- *BiP* assists chain translocation and ATP hydrolysis ensures it is unidirectional into the ER lumen





* clamp on to = agganciano

POST-TRANSLATIONAL TRANSLOCATION (cont.)

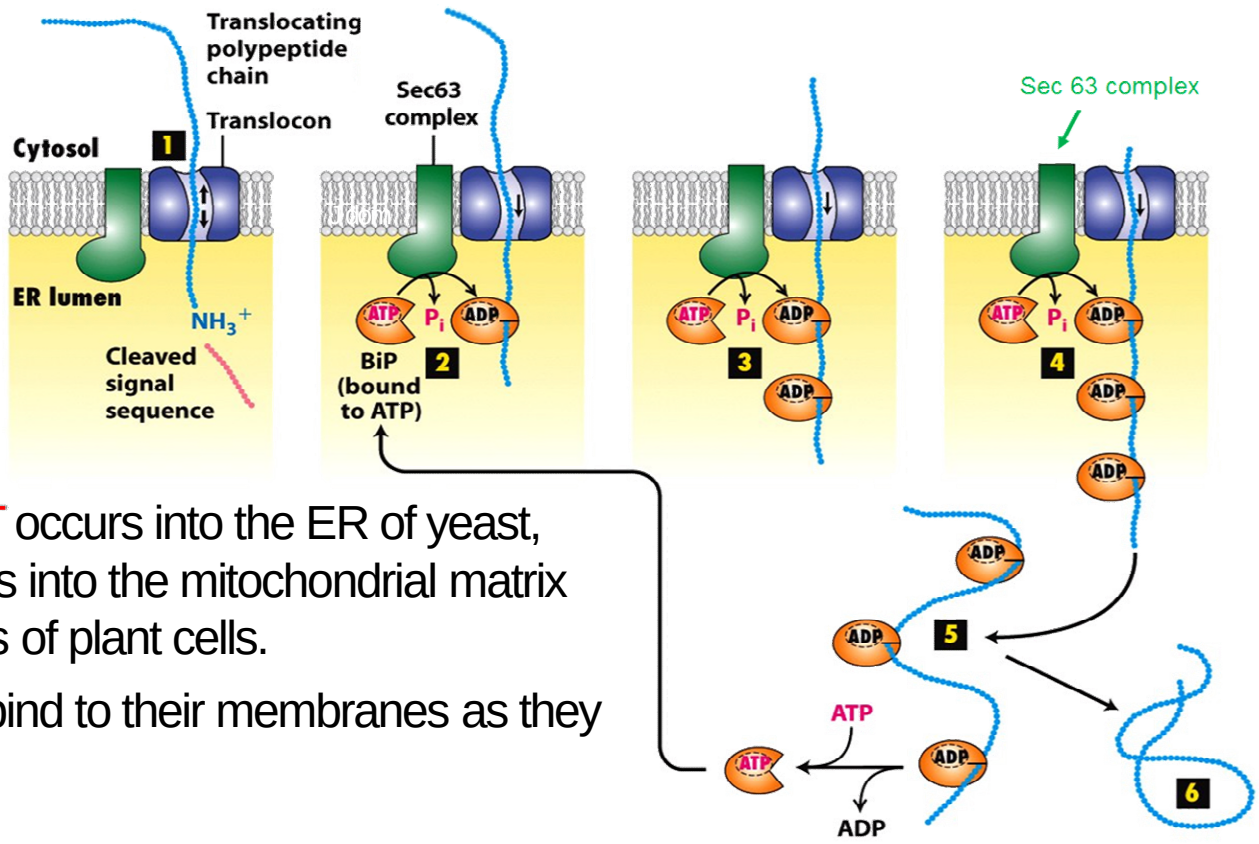
ATP cycle of yeast Sec63 complex in PTT

❶ The protein carried by *HSP70* binds to Sec63 via its SS and is translocated through Sec61 into the ER, where the SS is cleaved; ❷ *BiP* with ATP binds to the J domain on the luminal side of Sec63, causing ATP hydrolysis. *BiP*/ADP now has a high affinity for hydrophobic segments of the emerging protein, preventing it from sliding back to cytoplasm (unidirectional movement)

❸,❹ *BiP*/ADP bind to successive segments of the protein chain, ratcheting it into the ER until it has fully entered ❺ [ratcheting = azione a cremagliera]

❻ Exchange of ADP for ATP in *BiP* is slow and eventually:

- releases the chain into the ER lumen where it can complete its folding and PTM
- recycles Bip/ATP to back to Sec63



- This type of ATP-powered PTT occurs into the ER of yeast, but a similar type of PTT occurs into the mitochondrial matrix of animal cells and chloroplasts of plant cells.
- This is why ribosomes do not bind to their membranes as they do to the RER membrane