BIOL 266 – CELL BIOLOGY

Lecture 8:

Protein sorting to organelles (I)

- **A typical mammalian cell contains up to 10,000 *different kinds of proteins***
- **For a cell to function properly, each of its numerous proteins must be localized *to the correct membrane-bound organelle*:**

Proteins:	***Must go to***:
Na+/K+ ATPase →	Plasma membrane
RNA polymerase →	Nucleus
Proteases →	Lysosomes
Catalase →	Peroxisomes
ATP synthase →	Mitochondria
Hormones →	Extracellular space

Proteins synthesized in eukaryotic cells

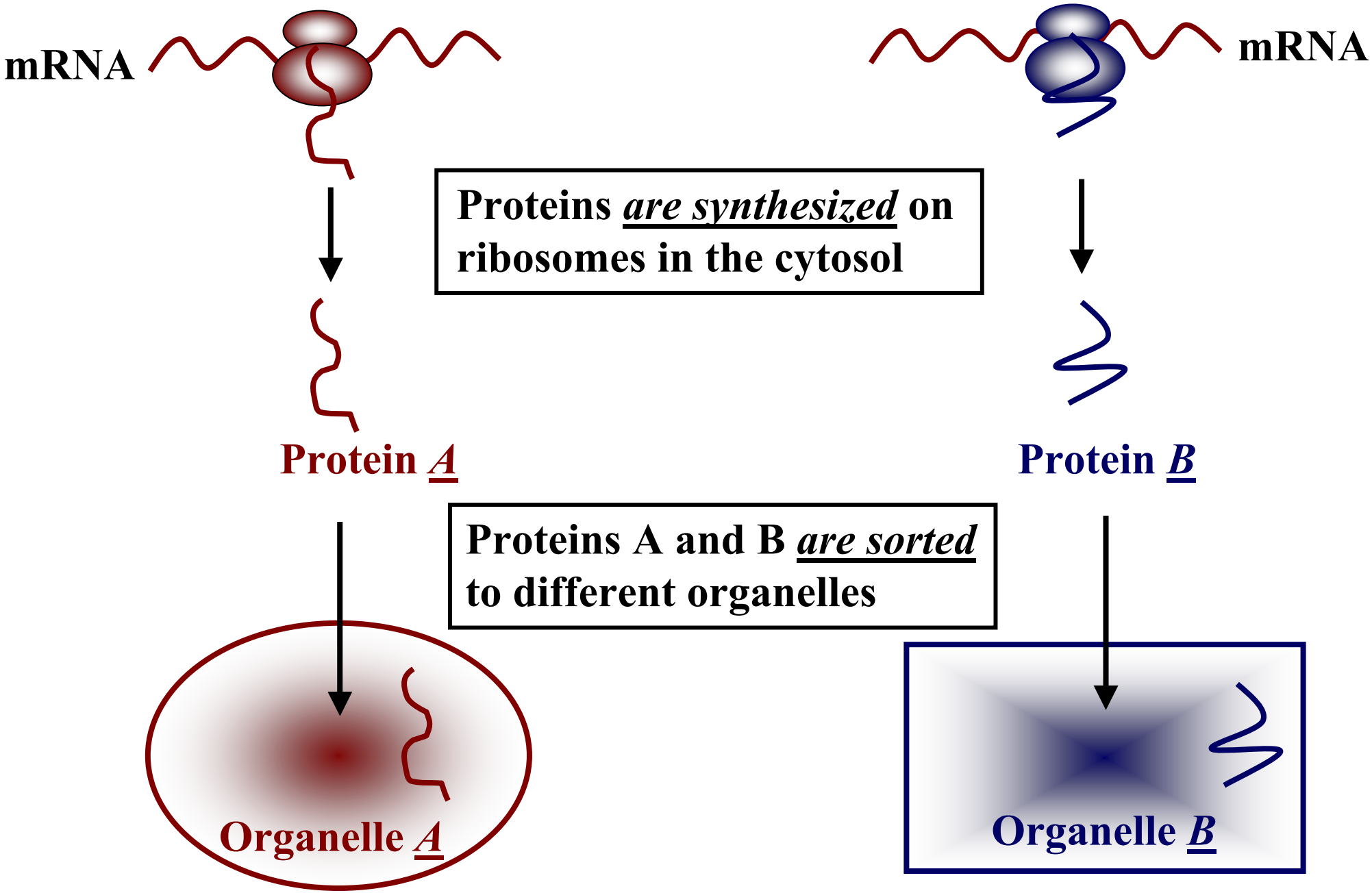
A few proteins:

- Are encoded by the DNA present in mitochondria and chloroplasts
- Are synthesized on ribosomes inside mitochondria and chloroplasts
- Are incorporated directly into compartments within mitochondria and chloroplasts

Most of the proteins:

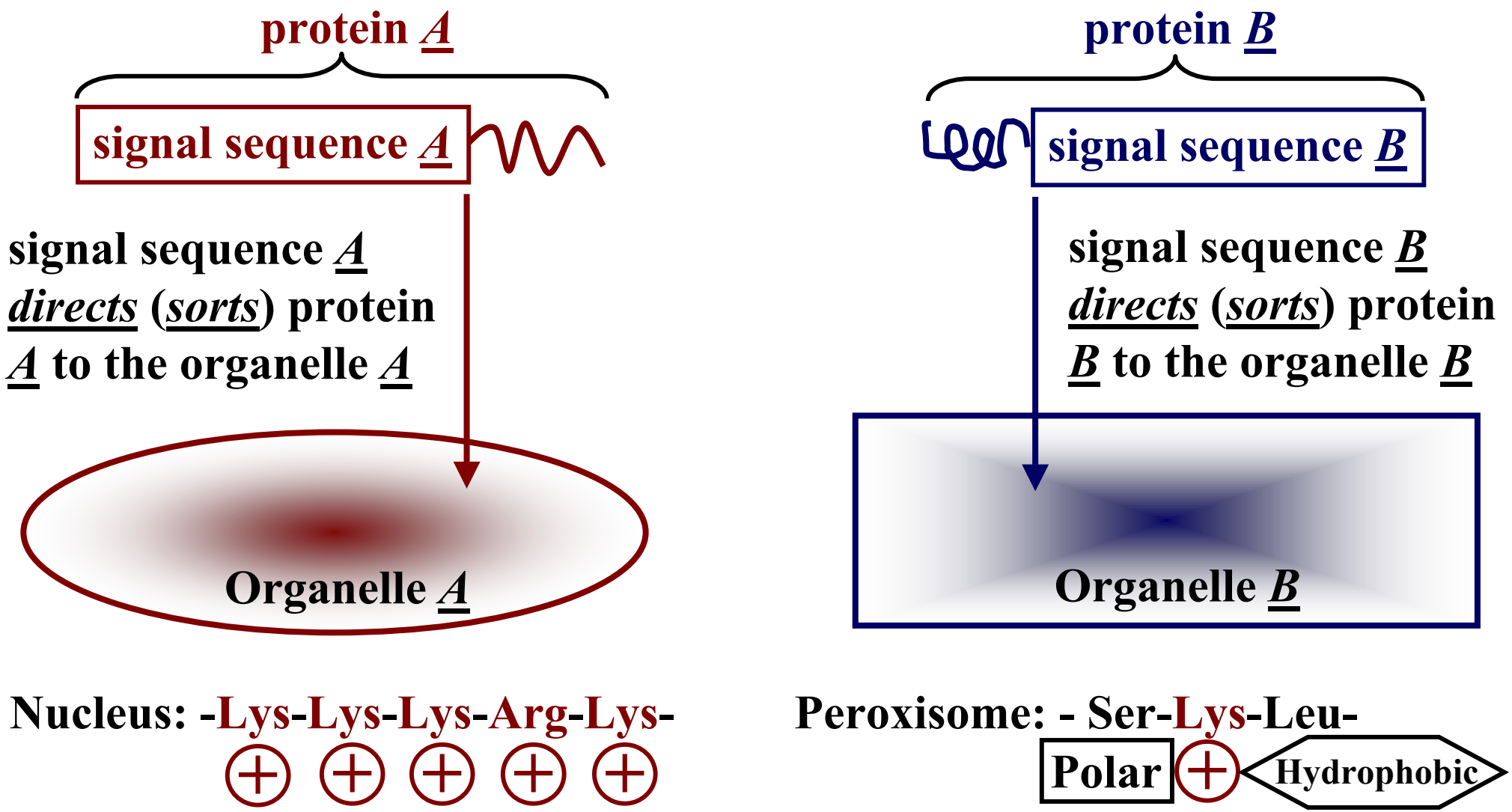
- Are encoded by nuclear DNA
- Are synthesized on ribosomes in the cytosol
- Are delivered to the organelle of destination from the cytosol

Protein sorting **– the process of directing each newly made protein to a particular membrane-bounded organelle**



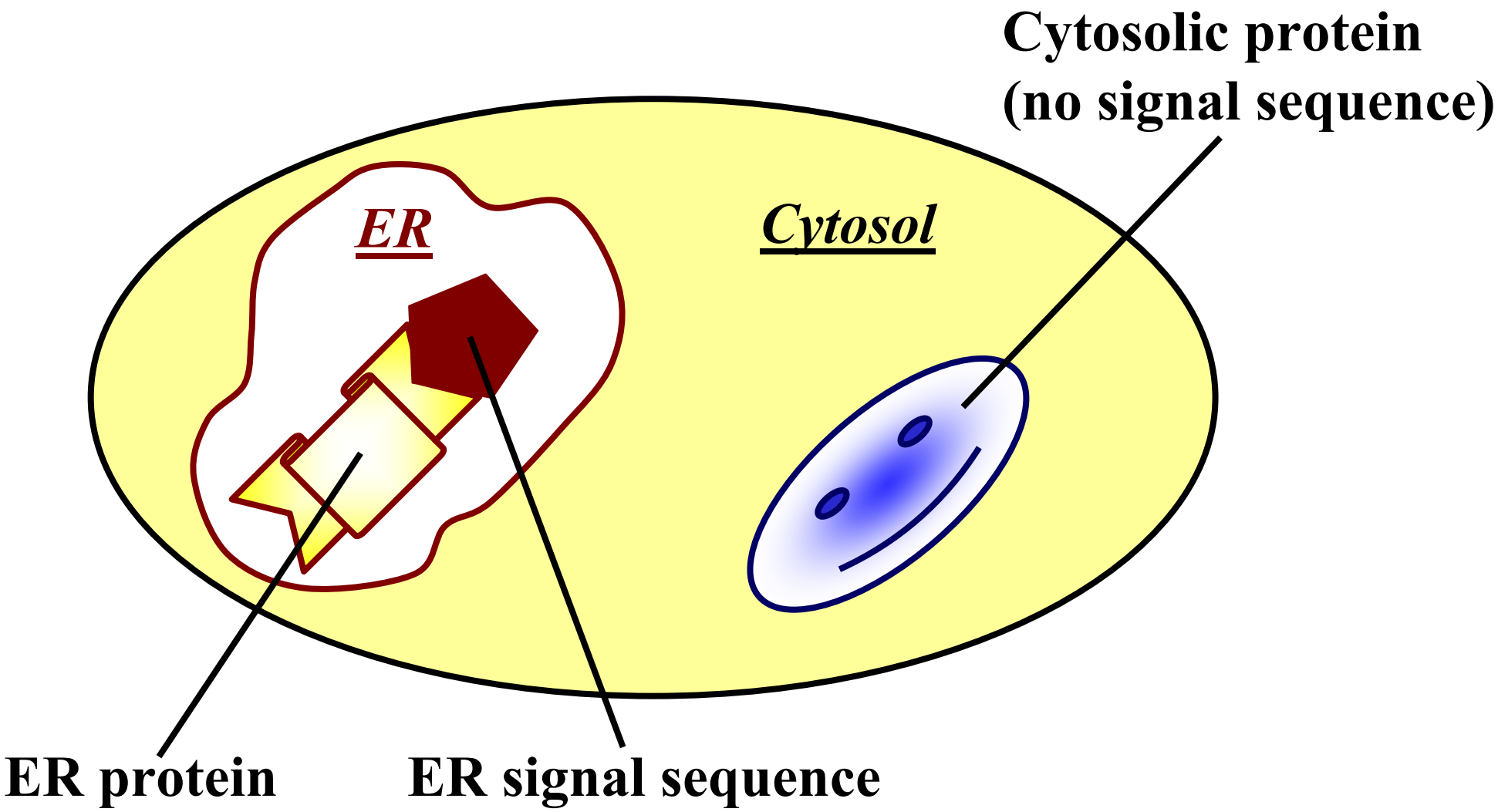
- ***Sorting signal*** – a continuous stretch of 3-60 amino acids that directs a protein to the organelle in which it is required

- Sorting signal = ***signal sequence***



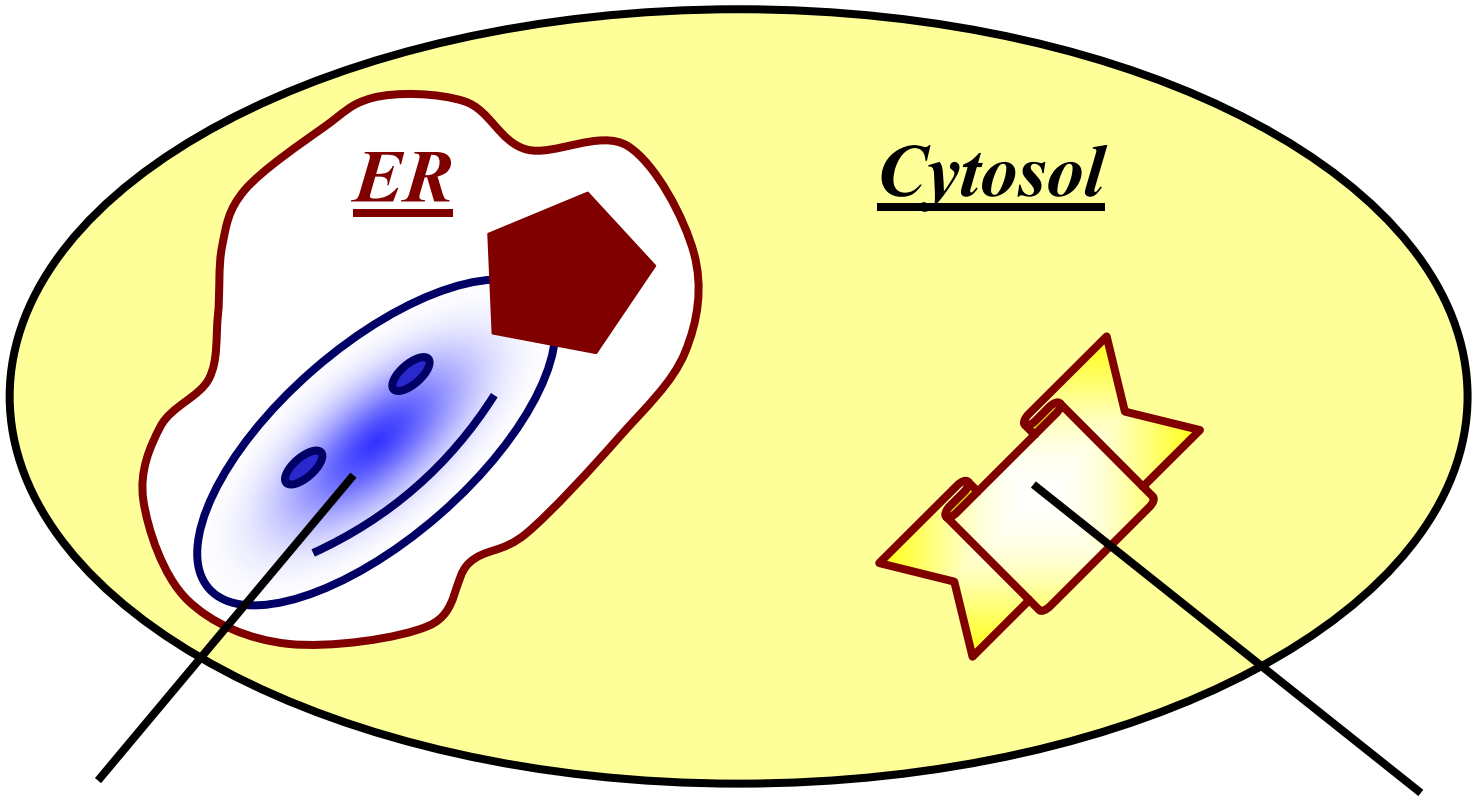
Role of signal sequences in protein sorting

- ***Proteins destined for the ER*** **possess an amino-terminal signal sequence that directs them to that organelle**
- ***Proteins destined to remain in the cytosol*** **lack this sequence**



Role of signal sequences in protein sorting

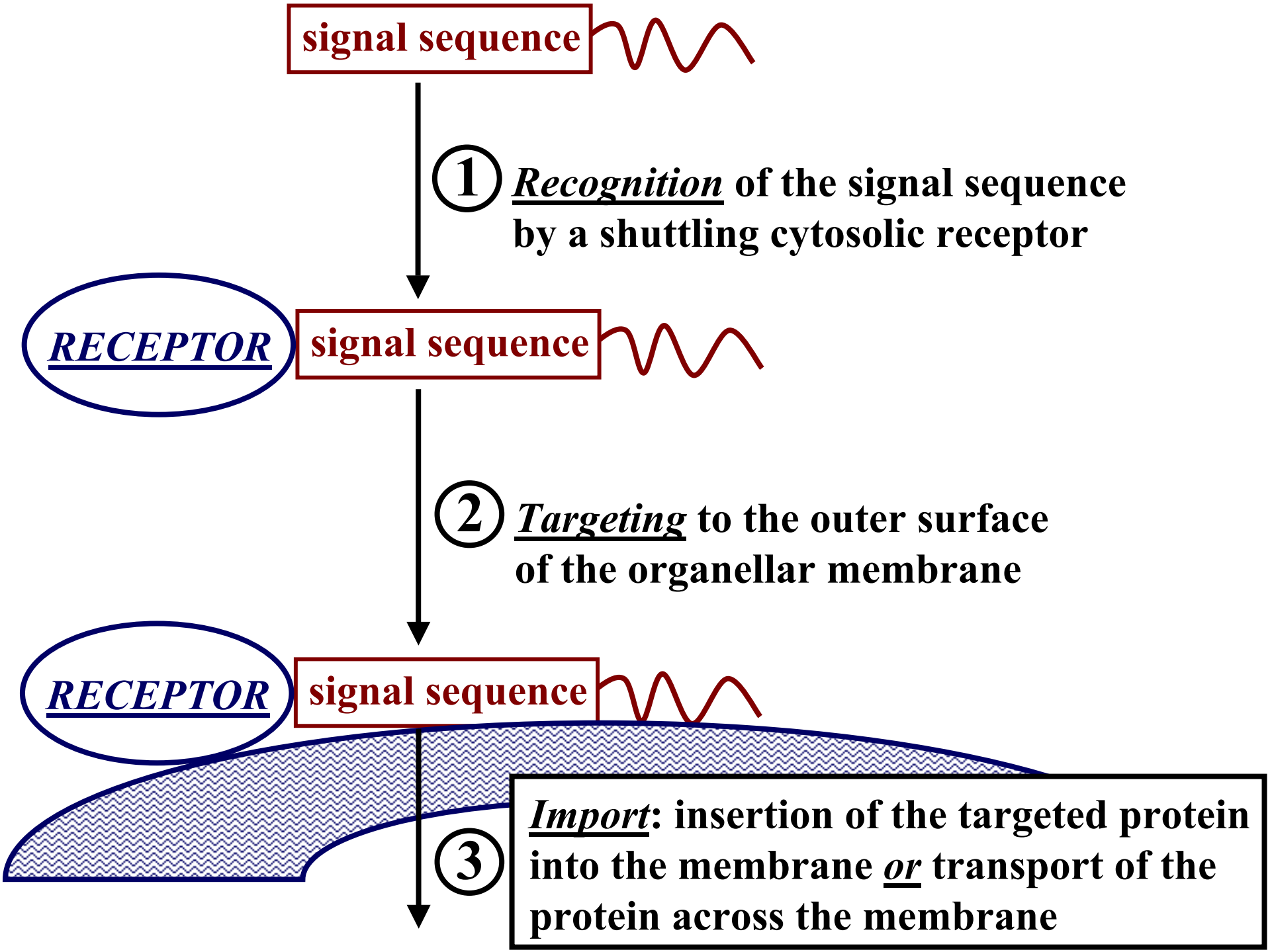
- An ER signal sequence *is attached to a cytosolic protein*
- The signal sequence *is removed from an ER protein*
- *Result*: in each case the altered protein ends up in an abnormal location in the cell
- *Conclusion*: the ER signal sequence is both *necessary and sufficient* to direct a protein to the ER



Cytosolic protein with ER signal sequence

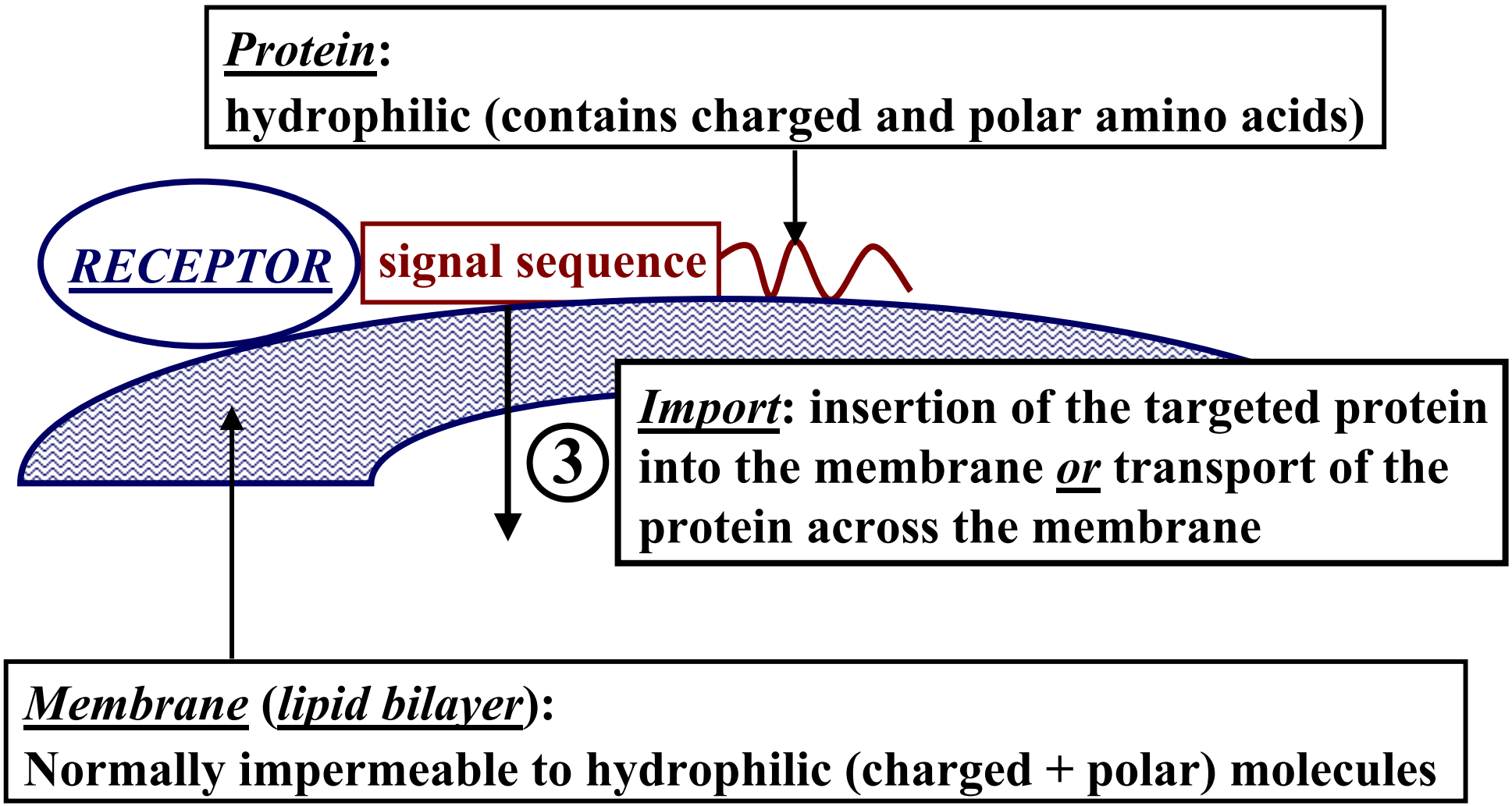
ER protein with signal sequence removed

3 consecutive steps in protein sorting:

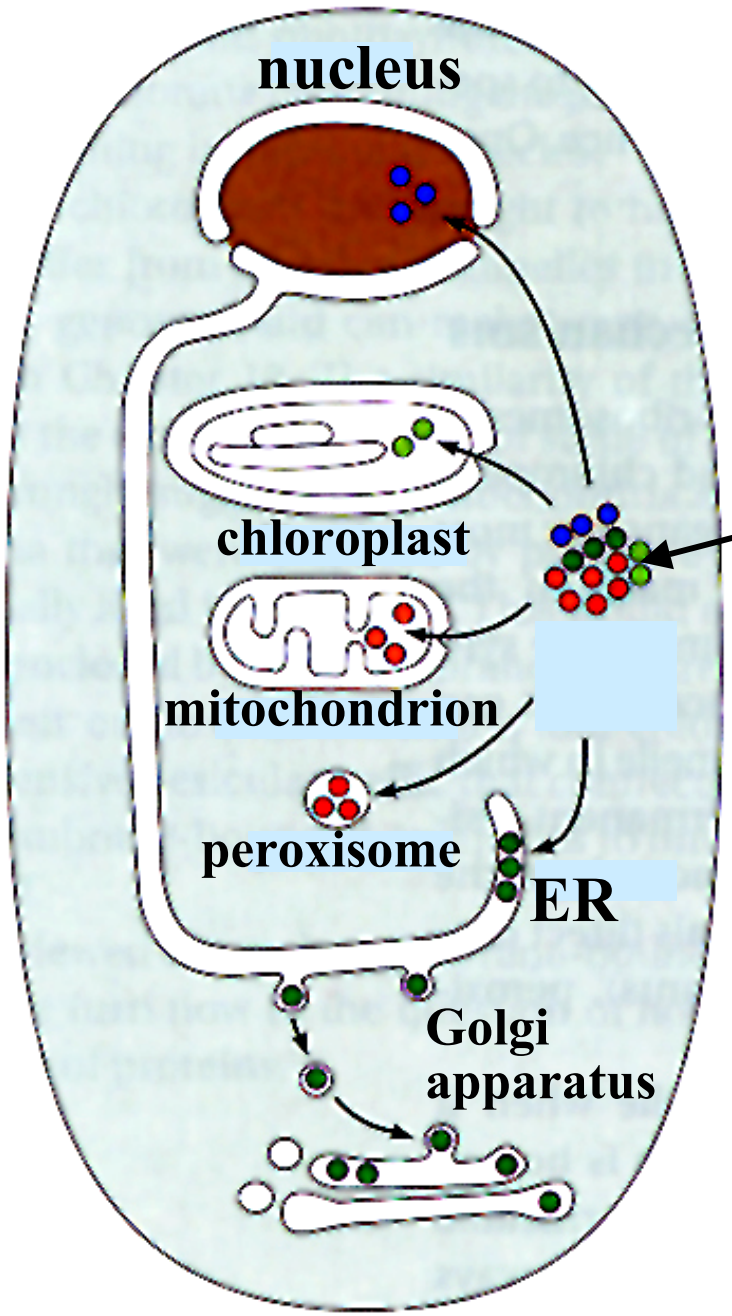


A general problem for protein import into organelles:

How to transport the protein across membranes that are normally impermeable to hydrophilic molecules



Three main mechanisms by which membrane-bounded organelles import proteins

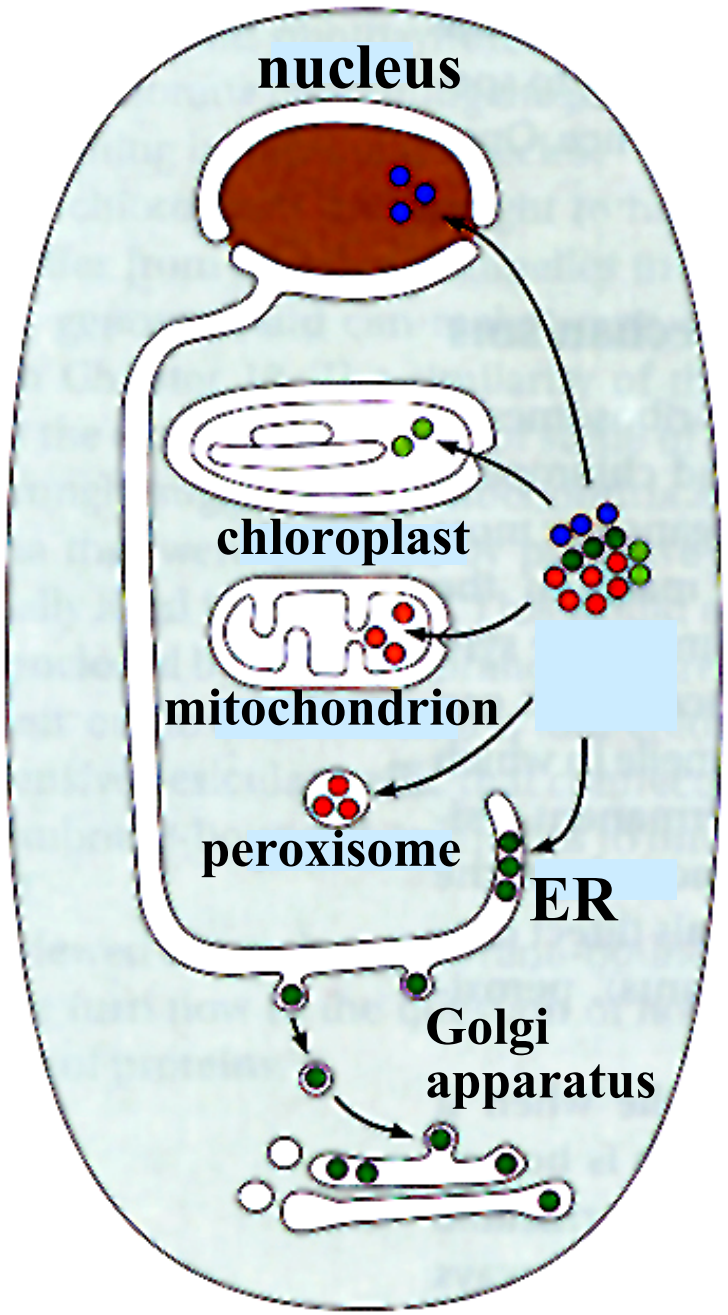


① Transport through *nuclear pores*

Proteins synthesized on ribosomes in the cytosol

② Transport *across membranes*

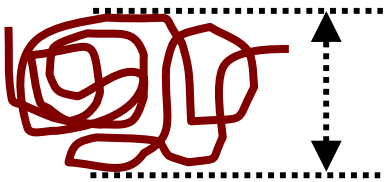
③ Transport *by vesicles*

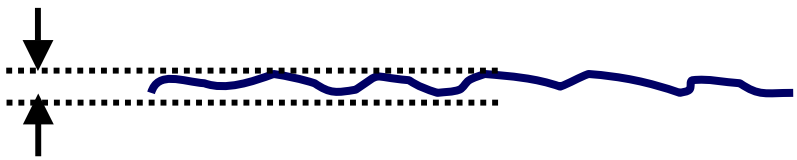


① **Transport through *nuclear pores***

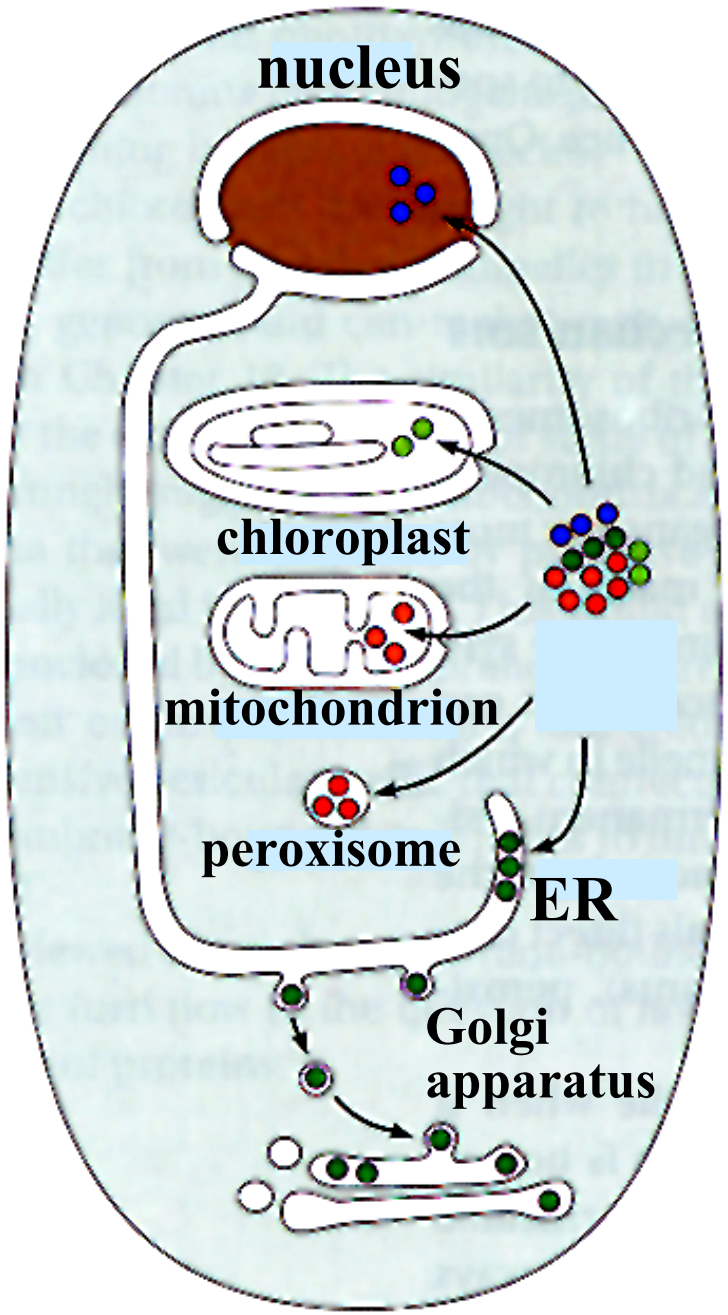
- **Proteins are sorted from the cytosol to the nucleus**
- **Proteins are transported through the nuclear pores that function as selective gates, which actively *transport only specific proteins***
- **Proteins remain *folded* during the transport**

folded





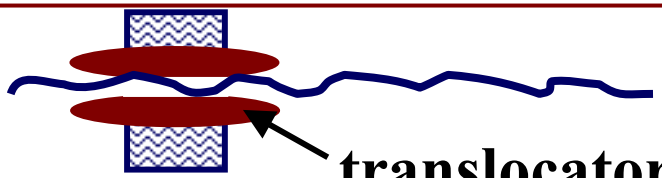
unfolded



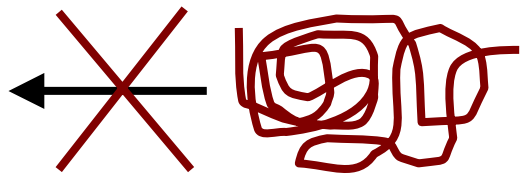
- Proteins are sorted from the cytosol to the ER, mitochondria, chloroplasts or peroxisomes
- Proteins are transported across the organelle membrane by ***protein translocators*** located in the membrane

2 Transport ***across membranes***

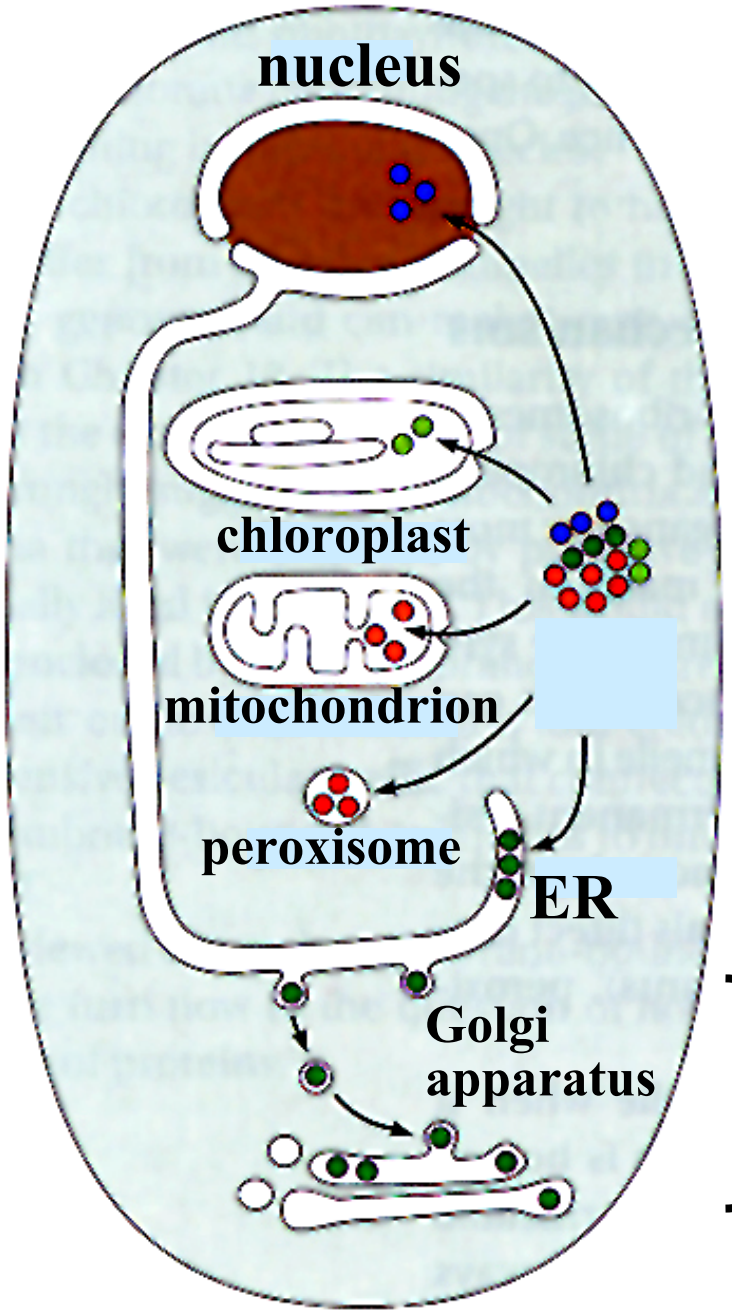
- The transported protein must ***unfold*** in order to snake through the membrane



unfolded



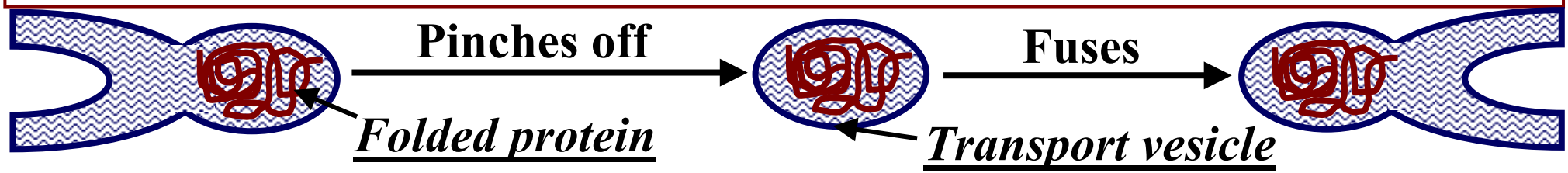
folded

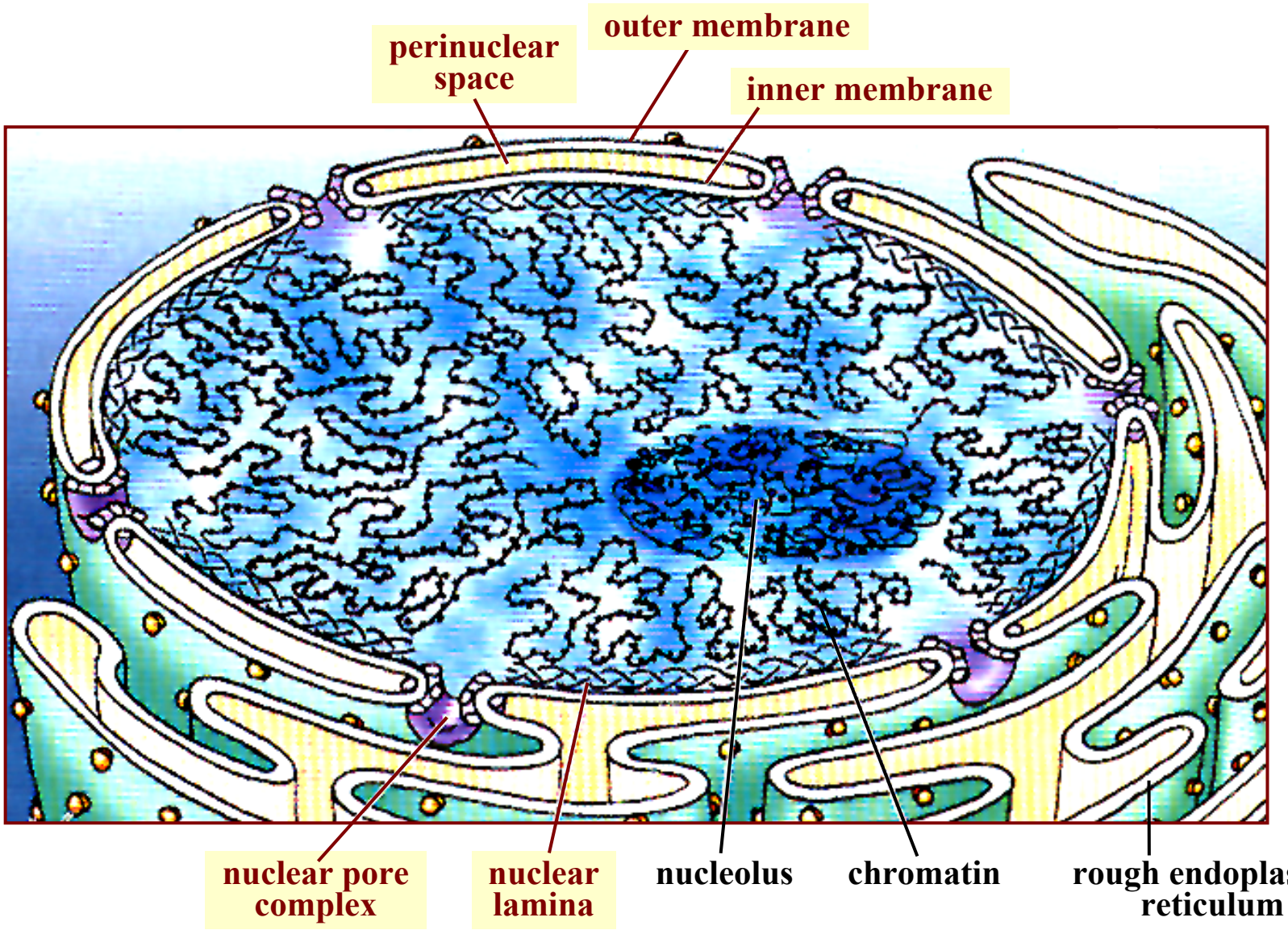


- Proteins are sorted from the ER onward and from one compartment of the ***endomembrane system*** to another
- Proteins are transported by ***transport vesicles***
- Transport vesicles become loaded with a cargo of proteins from the interior space (lumen) of one compartment, as they ***pinch off*** from its membrane
- These transport vesicles subsequently discharge their cargo into a second compartment by ***fusing*** with its membrane

③ Transport ***by vesicles***

- Proteins remain ***folded*** during the transport





The *nuclear envelope* consists of:

- Two concentric membranes, called the inner and outer nuclear membranes
- The nuclear lamina, a fibrous meshwork that provides structural support to the nucleus
- The nuclear pore complexes, the only channels through which molecules are able to travel between the nucleus and the cytoplasm

The nuclear pore complex (NPC) selectively transports macromolecules across the nuclear envelope

Large polar molecules **(amino acids, nucleotides, sugars)**

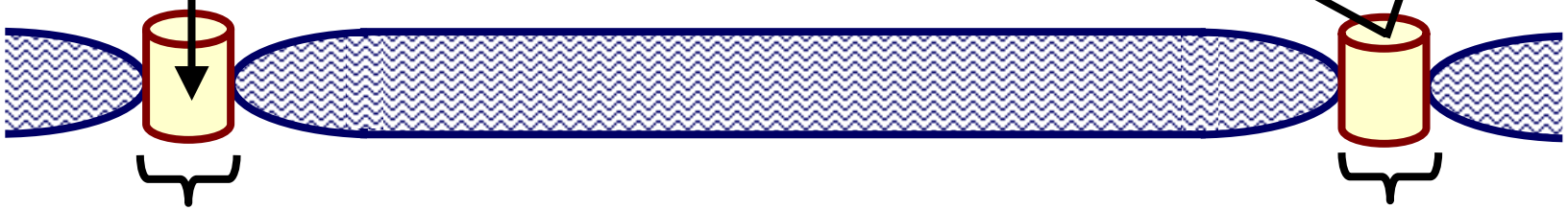
Ions

Globular proteins **up to 60 kDa (~ 1 nm in diameter)**

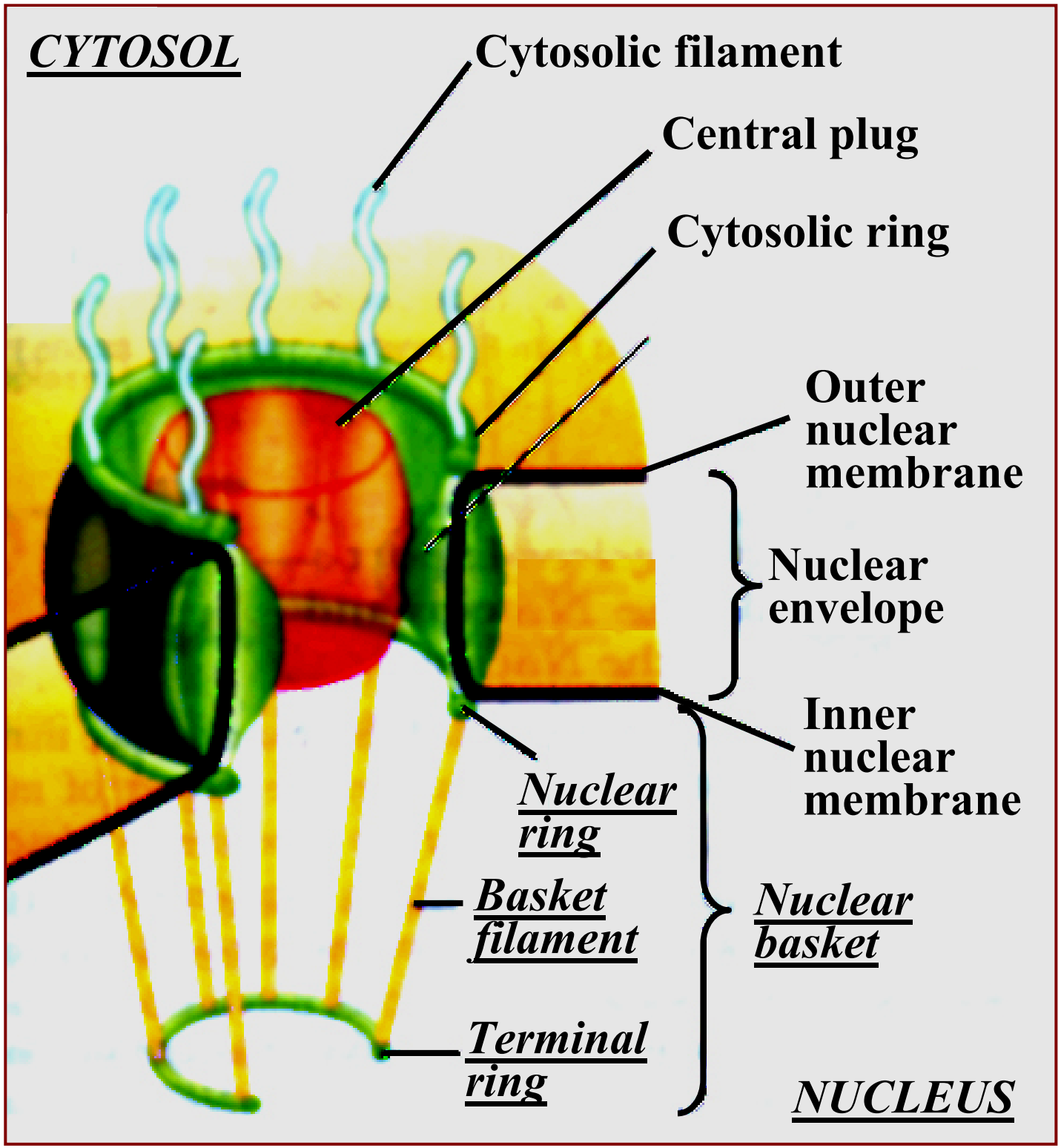
Large proteins **(> 60 kDa, 1-25 nm in diameter)**

Diffuse through water-filled channels in the NPC

Do not Diffuse

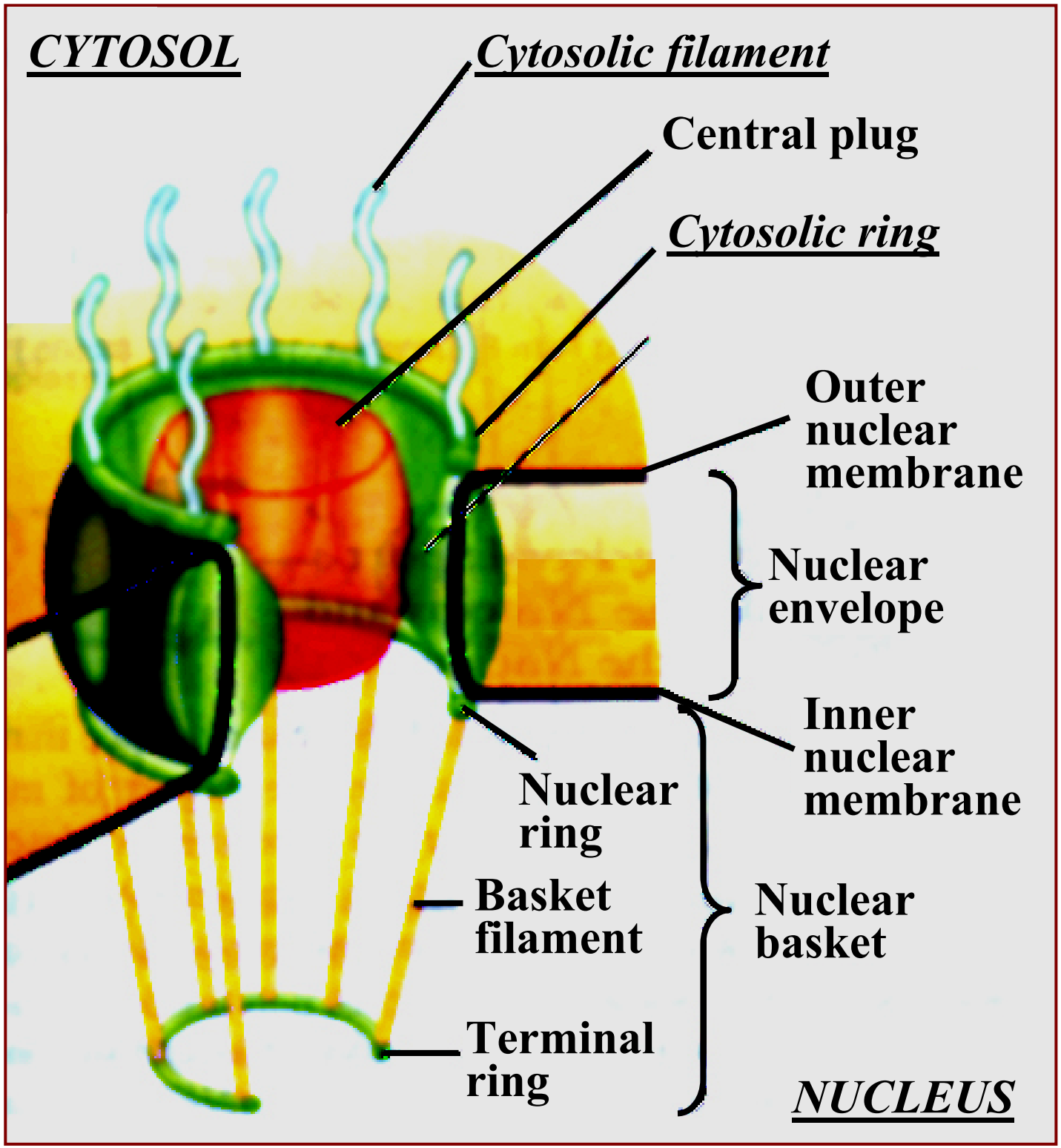


Nuclear pore complex**: made up of multiple copies of ~ 100 different proteins**



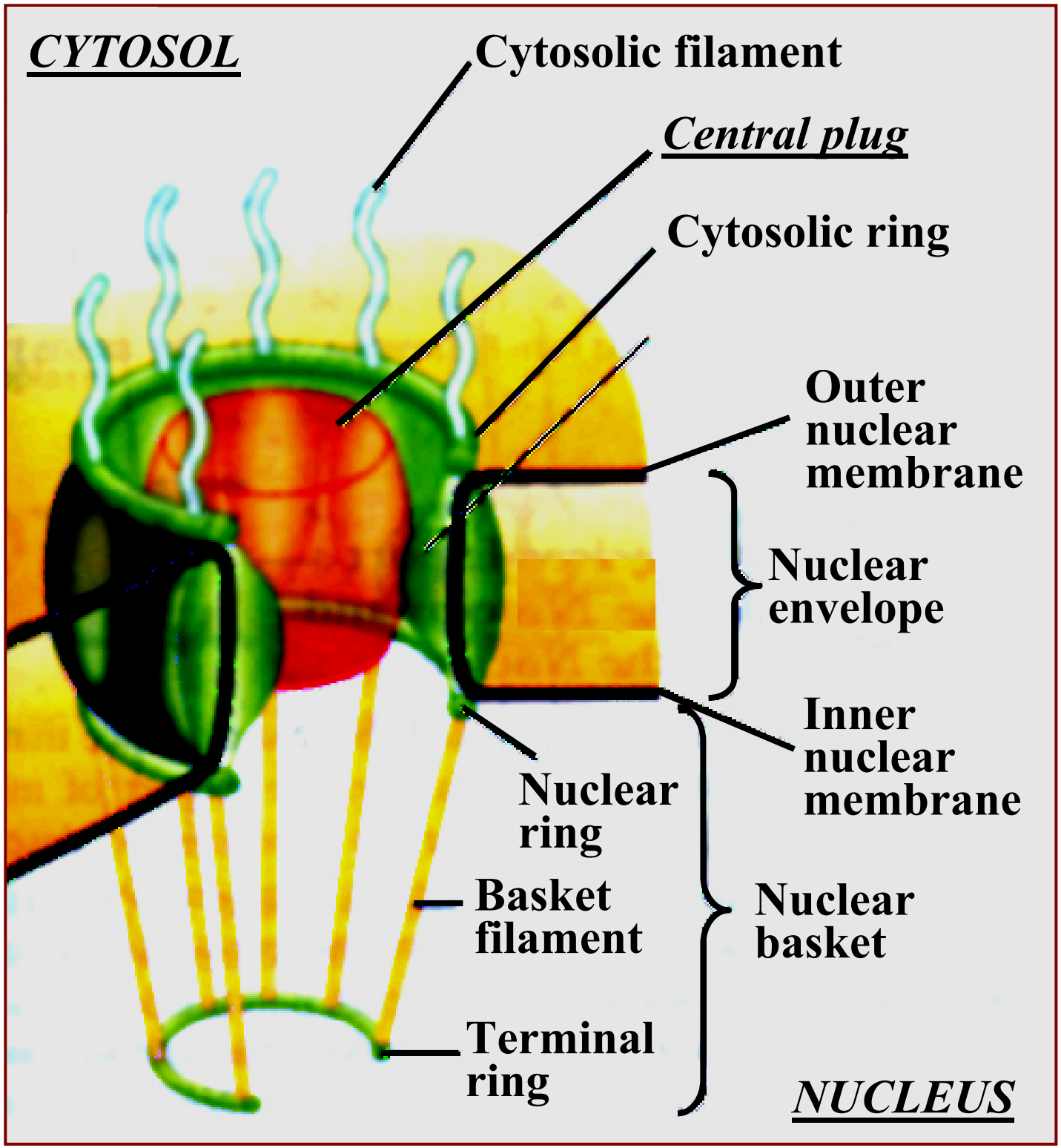
The nuclear pore complex (*NPC*):

The NPC nuclear ring supports eight ~ 100 nm-long basket filaments whose distal ends are joined by the terminal ring, forming a structure called the *nuclear basket*



The nuclear pore complex (NPC):

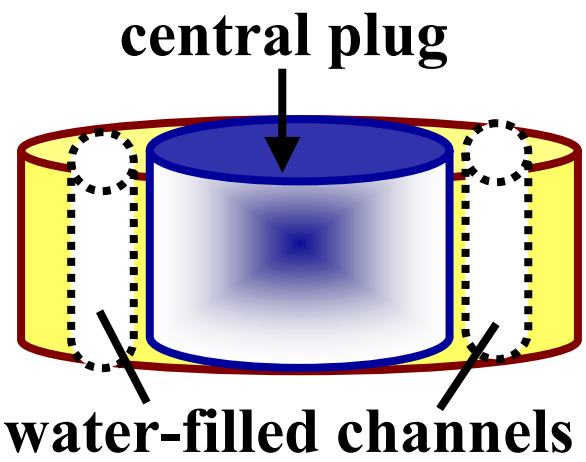
The cytosolic ring supports eight ~ 50 nm-long cytosolic filaments



The nuclear pore complex (NPC):

Large proteins (up to 25 nm in diameter) cannot diffuse through the water-filled channels (~ 1 nm in diameter) in the NPC

These proteins are actively transported through the *central plug* of the NPC



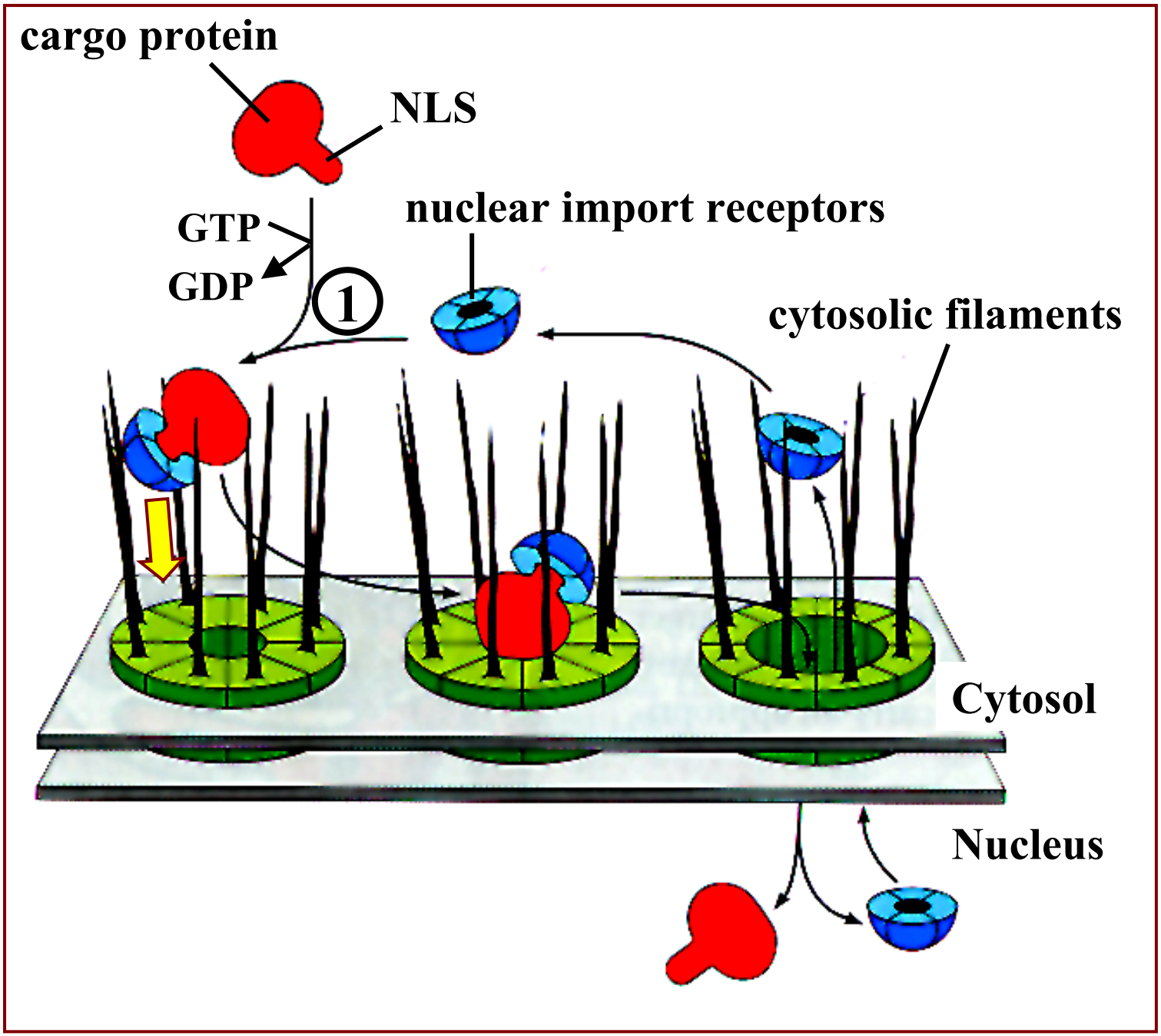
- **Large proteins cannot pass through the central plug of the NPC unless they carry an *appropriate signal sequence***

- **This signal sequence, called a *nuclear localization signal* (*NLS*), directs a protein from the cytosol into the nucleus**

NLS: -Lys-Lys-Lys-Arg-Lys-

⊕ ⊕ ⊕ ⊕ ⊕

The mechanism of active protein transport through the NPC

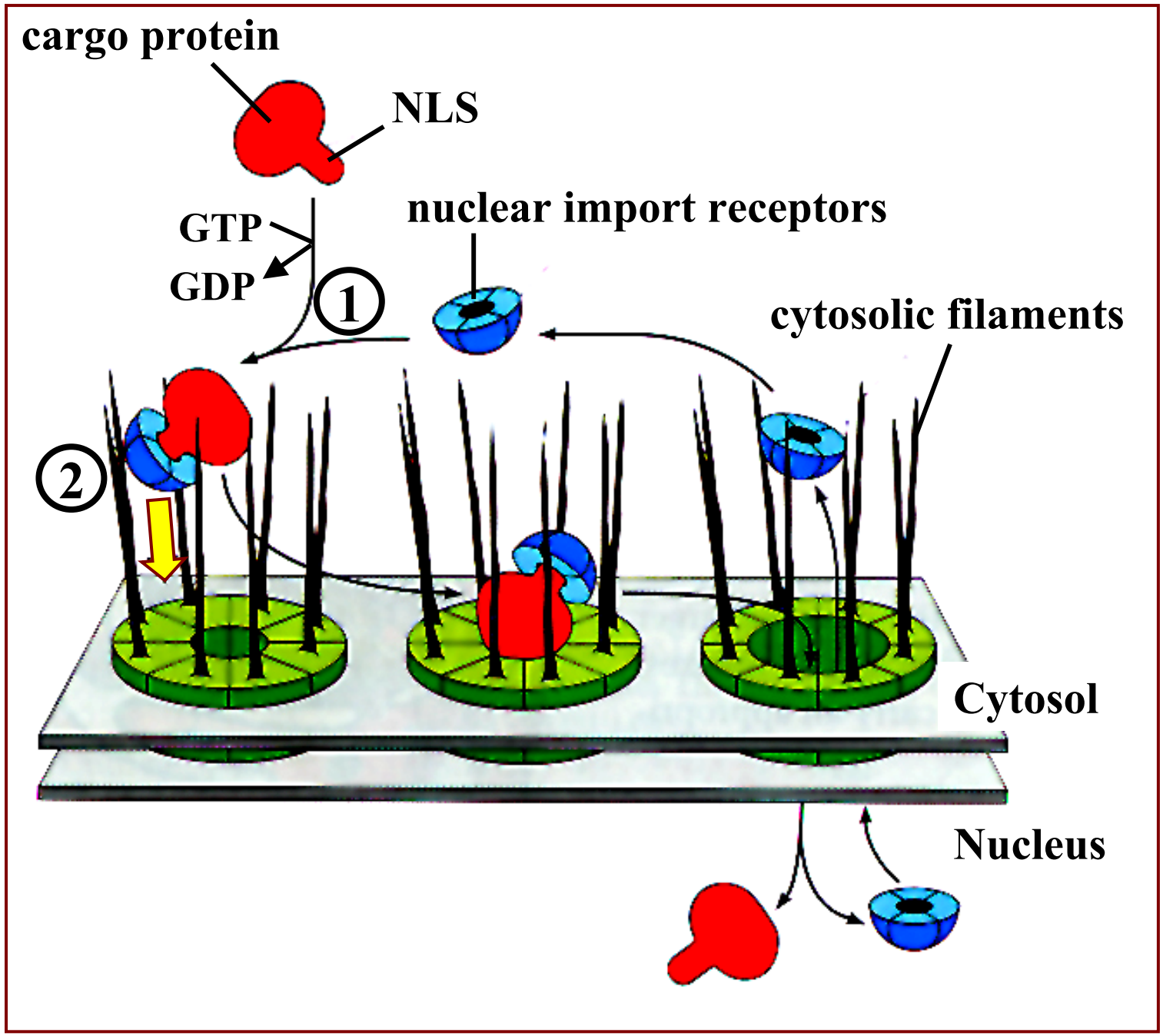


STEP 1

In the cytosol, *nuclear import receptors* (*importins*) bind to the NLS of the cargo protein to be transported

This binding is driven by the energy provided by *GTP hydrolysis*

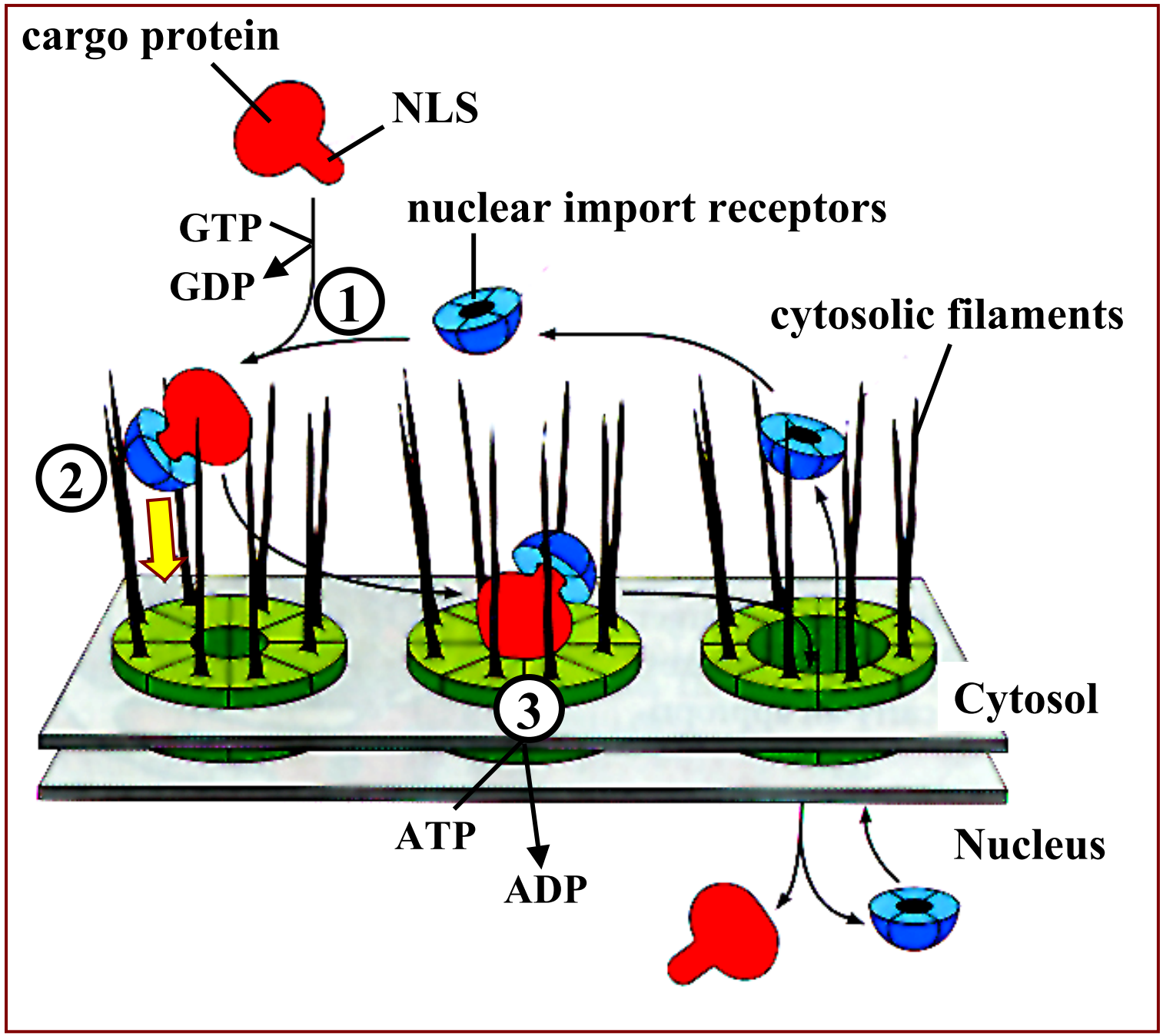
The mechanism of active protein transport through the NPC



STEP ②

Importins help direct the cargo protein to the NPC by *interacting with the cytosolic filaments* of the NPC

The mechanism of active protein transport through the NPC

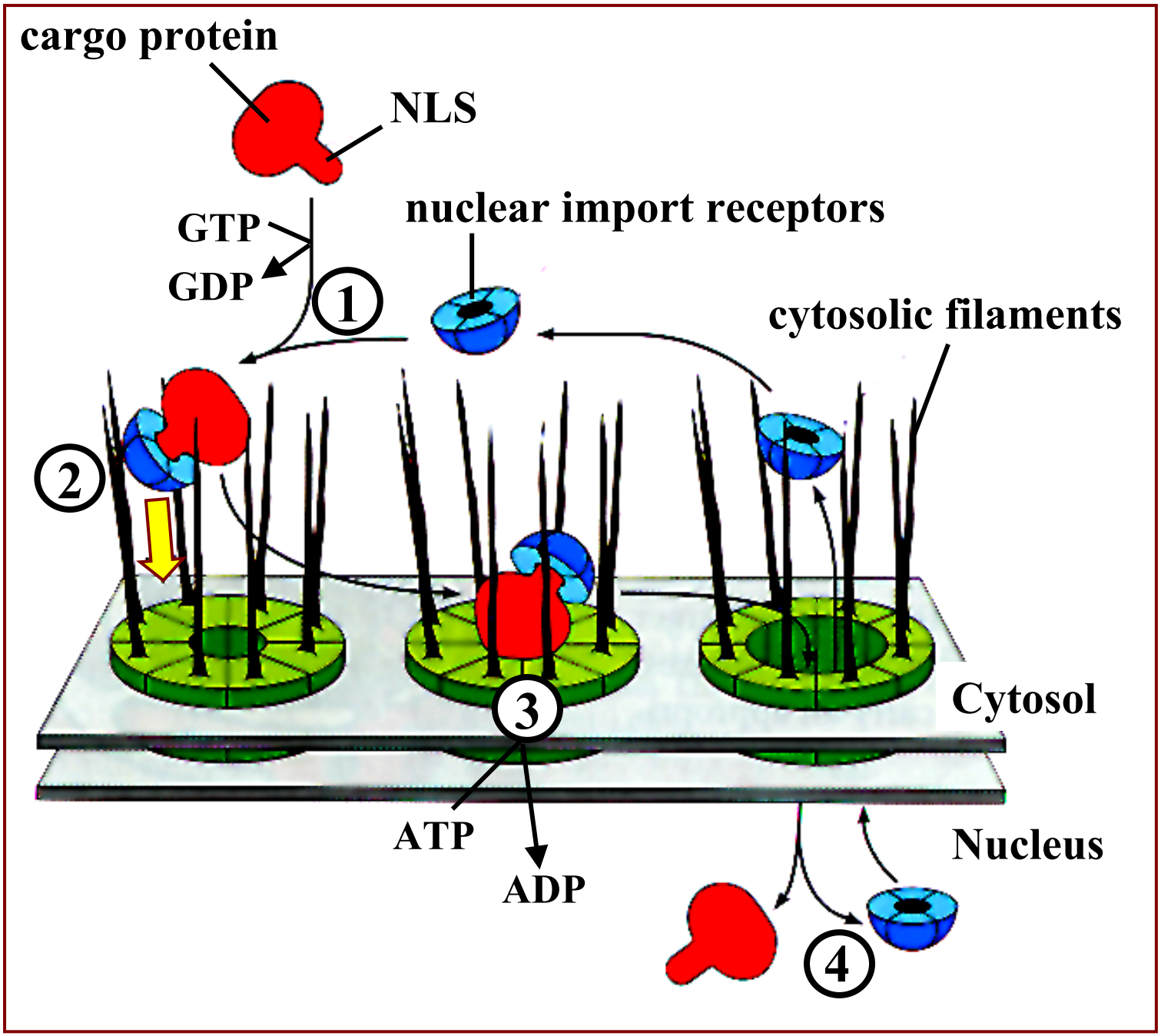


STEP ③

The binding of the cargo protein to the NPC *opens the pore*, and the cargo protein, with its bound importins *is transported into the nucleus*

This transport process is driven by the energy provided by *ATP hydrolysis*

The mechanism of active protein transport through the NPC



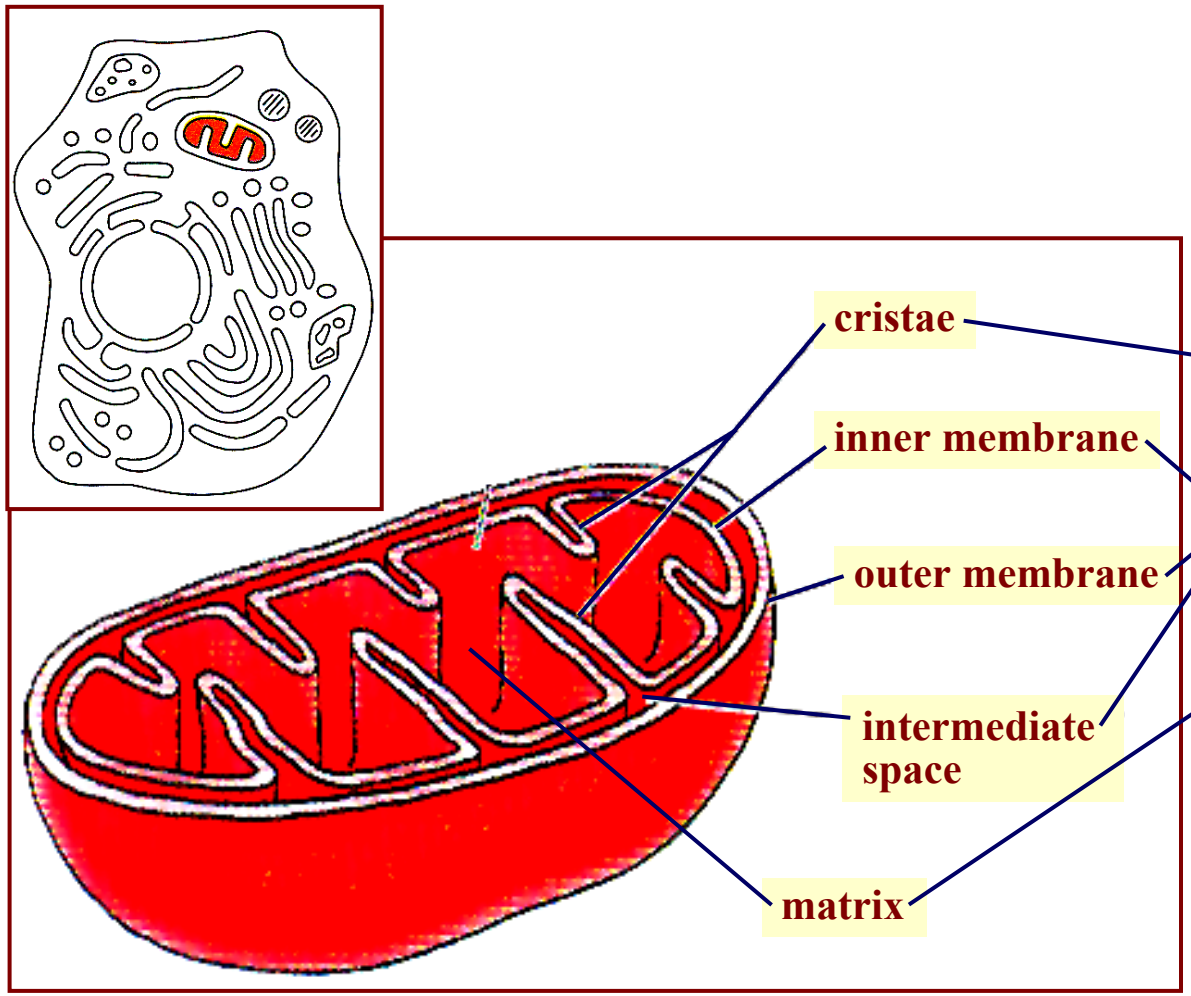
STEP ④

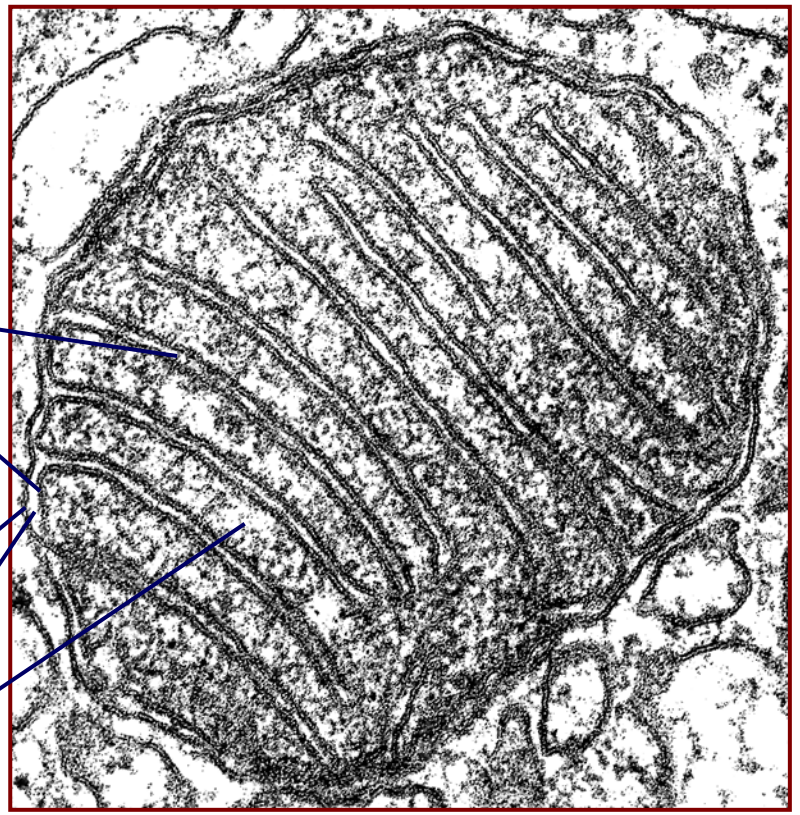
In the nucleus, the "importins-cargo protein" complex *dissociates*

The importins are then *exported back* through the NPC into the cytosol for reuse

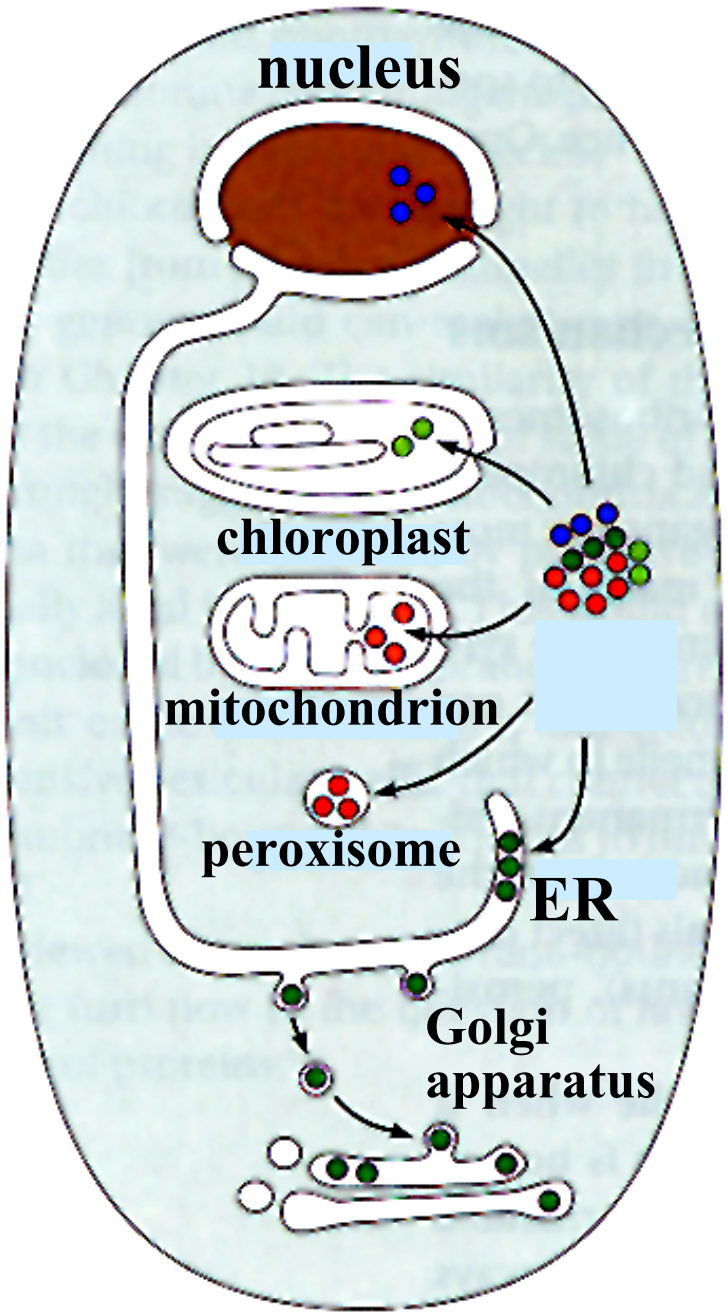
Organization of mitochondria

- Mitochondria are surrounded by a double-membrane system, consisting of ***inner*** and ***outer*** mitochondrial membranes separated by an ***intermembrane space***
- The inner membrane forms numerous folds (***cristae***), which extend into the interior (or ***matrix***) of the organelle. Its *surface area* is substantially increased by its folding into cristae





1 µm



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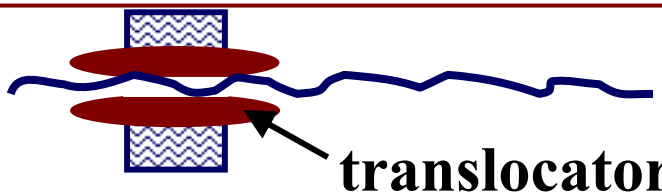
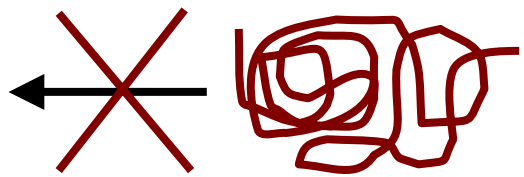
2 Transport ***across membranes***

The transported protein must ***unfold*** in order to snake through the membrane

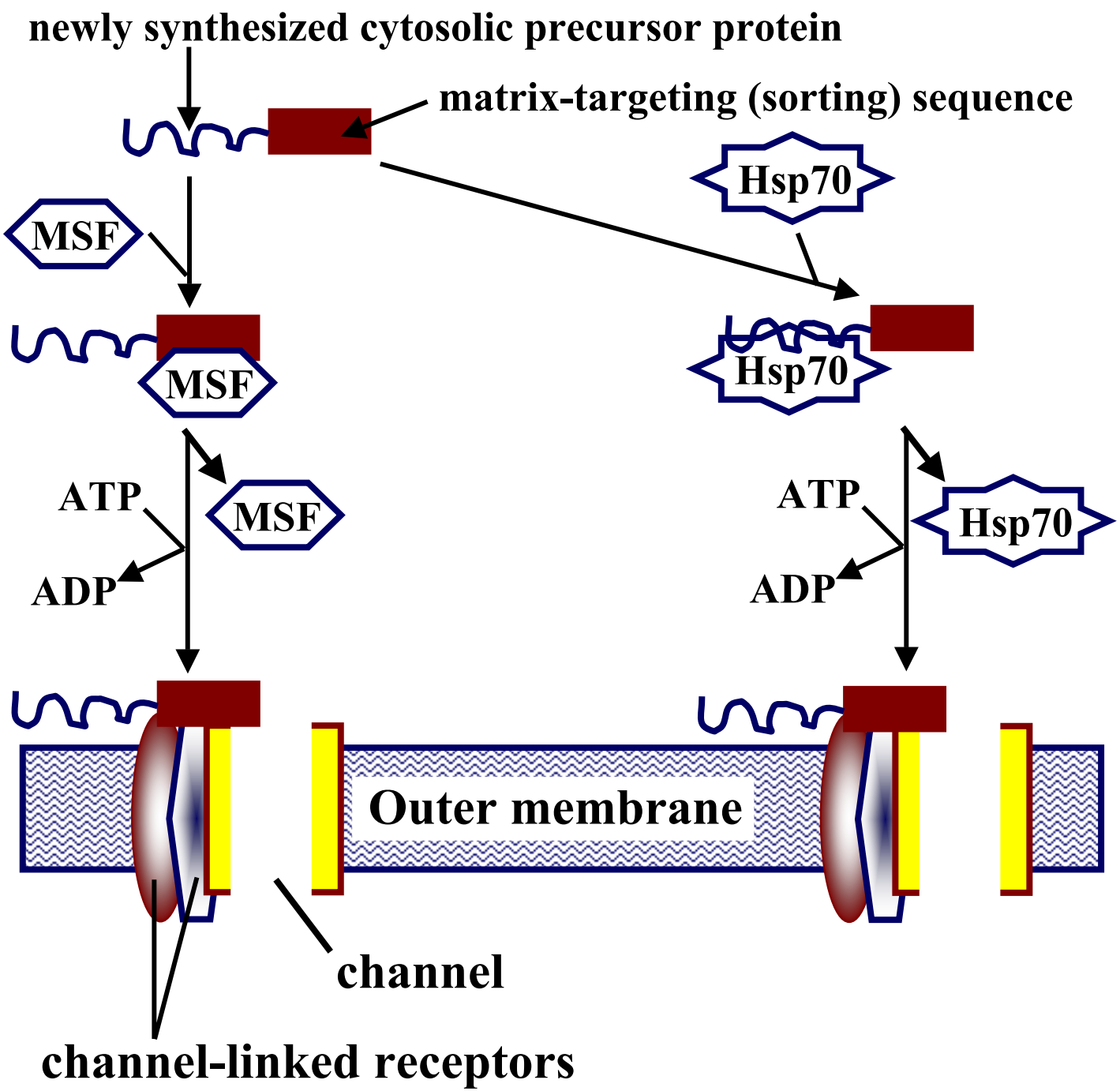


unfolded



folded

Protein import into the mitochondrial matrix



STEP ①

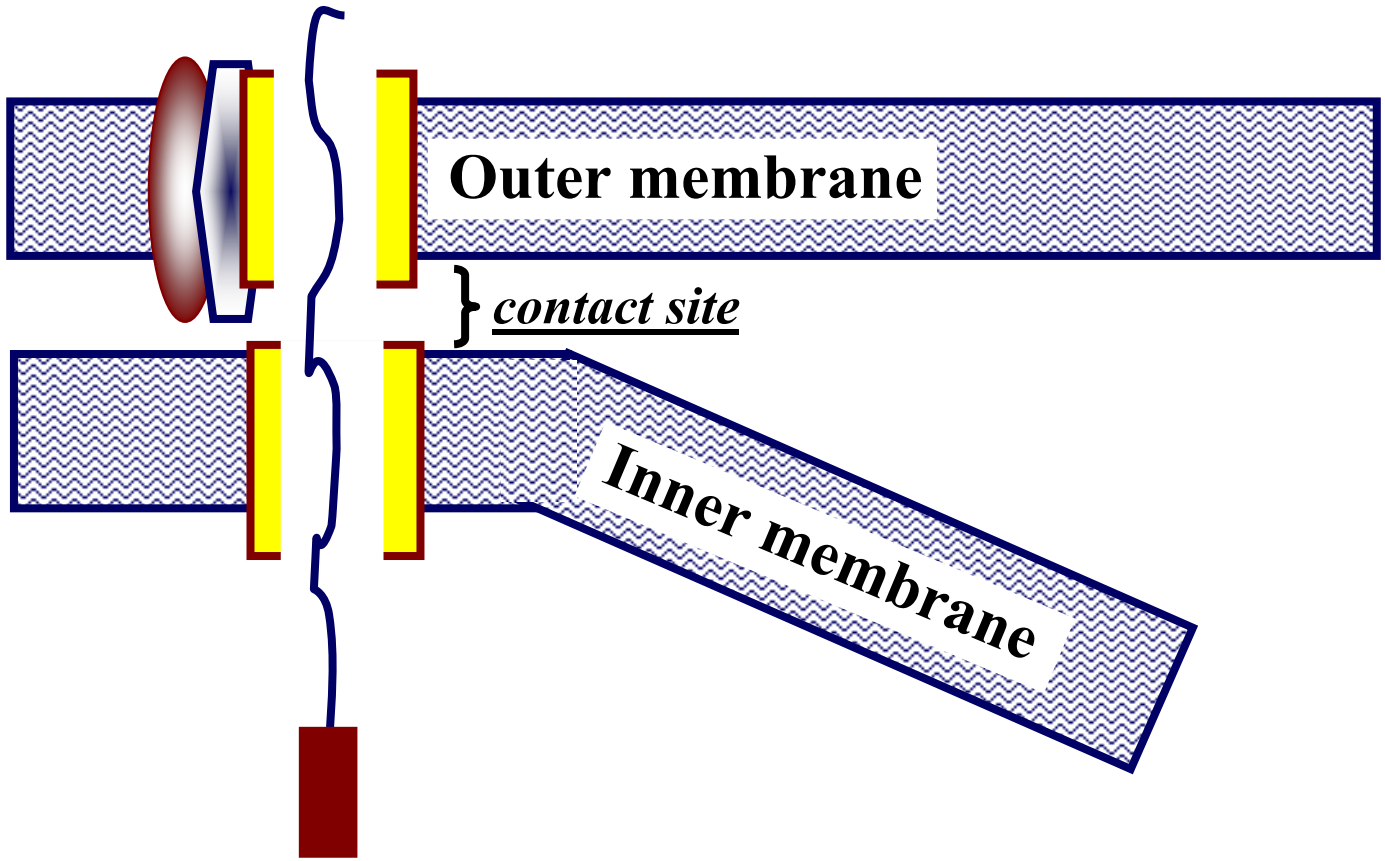
Cytosolic chaperones

MSF (mitochondrial-import stimulating factor) and Hsp70 (heat-shock protein 70):

(1) Use the energy of ATP hydrolysis to keep newly synthesized cytosolic proteins in an *unfolded, import-competent state*

(2) Deliver unfolded precursor proteins to *channel-linked receptors* in the outer mitochondrial membrane

Protein import into the mitochondrial matrix

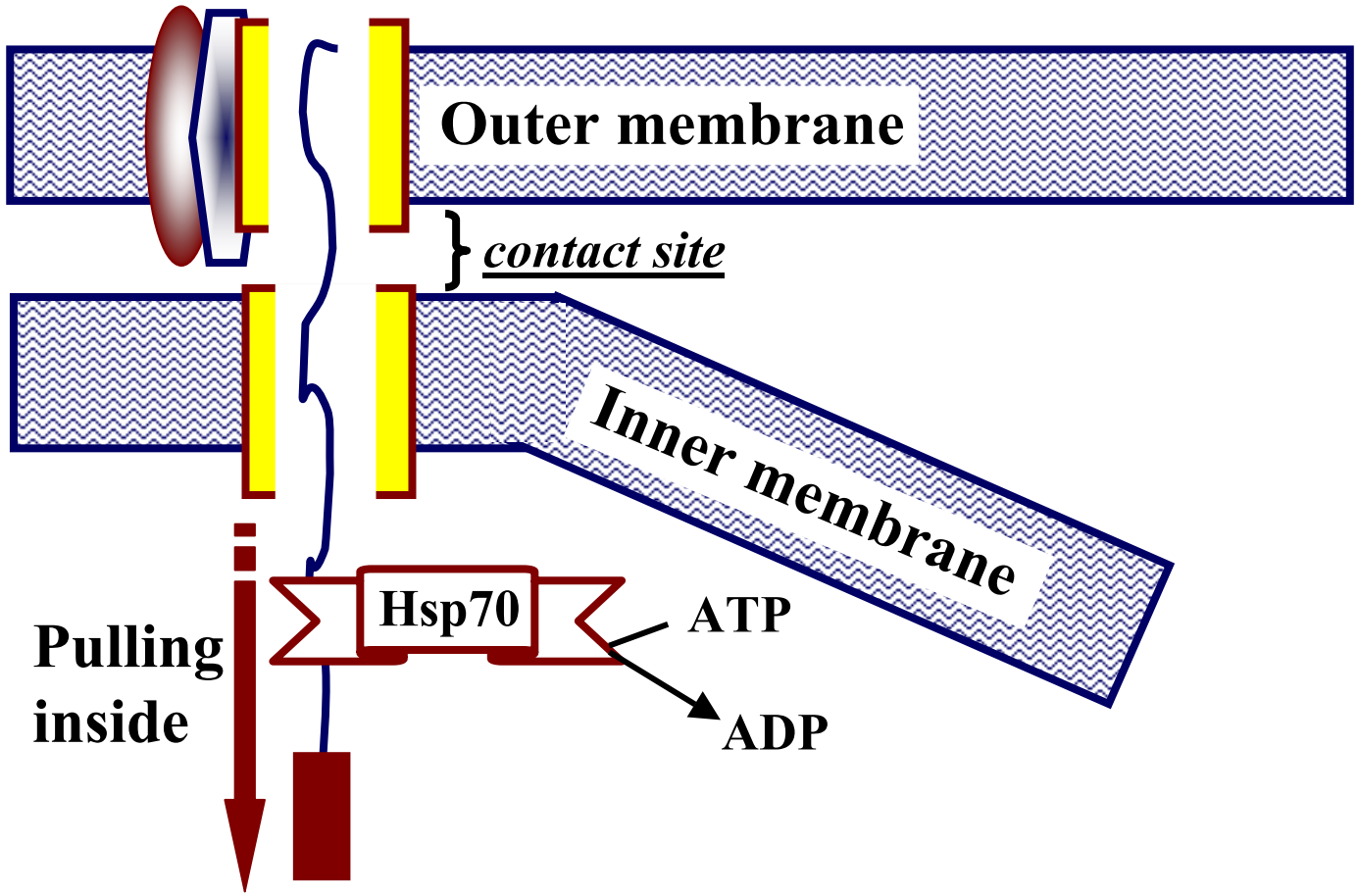


STEP ②

(1) After chaperones are released, a precursor protein *passes* through the channel in the outer membrane and another channel in the inner membrane

(2) Translocation into the matrix occurs only at "*contact sites*" where the outer and inner membranes are in close proximity

Protein import into the mitochondrial matrix

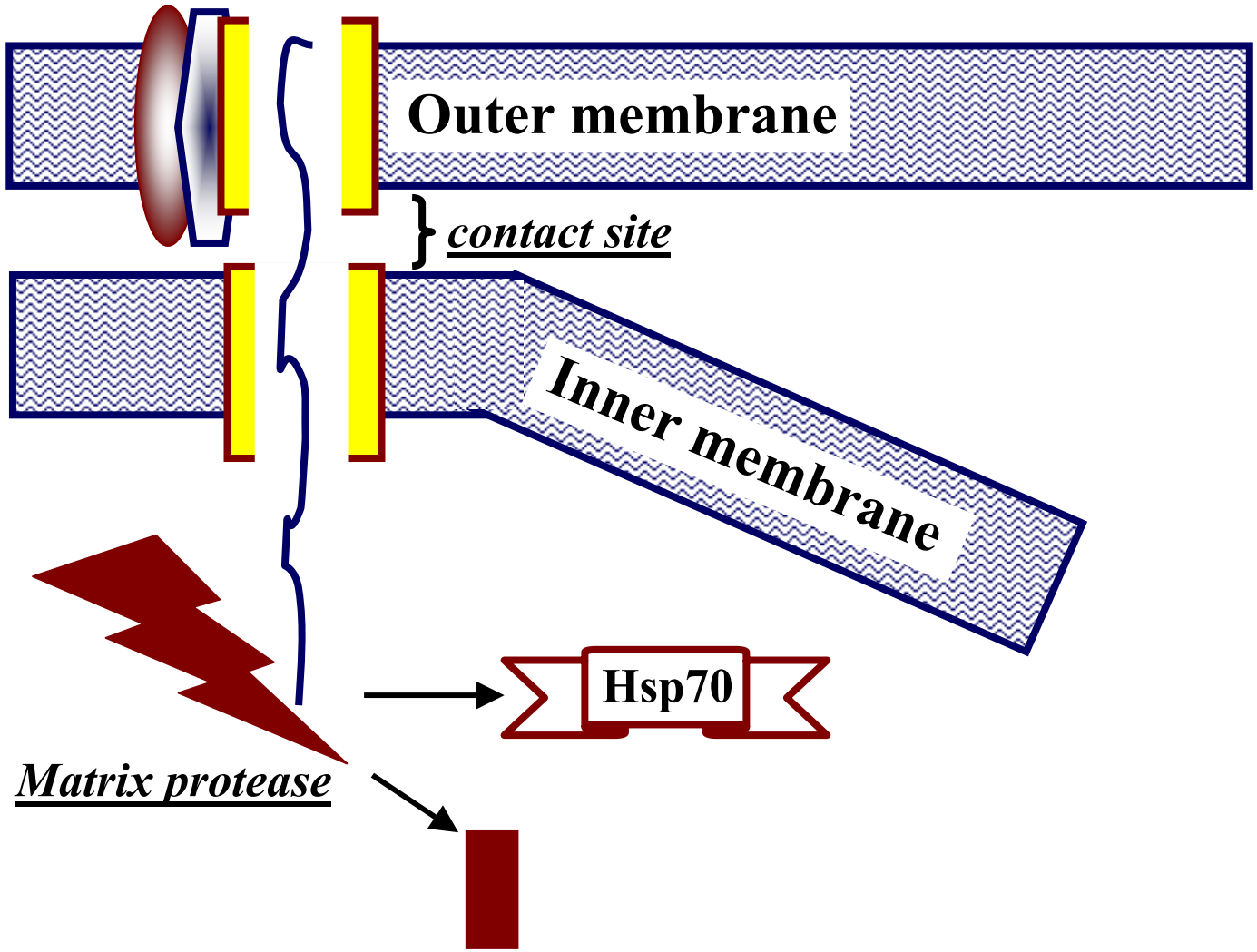


STEP ③

(1) The newly imported protein binds to the *matrix chaperone Hsp70*

(2) Hsp70 uses the energy of ATP hydrolysis *to assist import* (*to pull the protein inside*) into the matrix and to prevent aggregation or premature folding

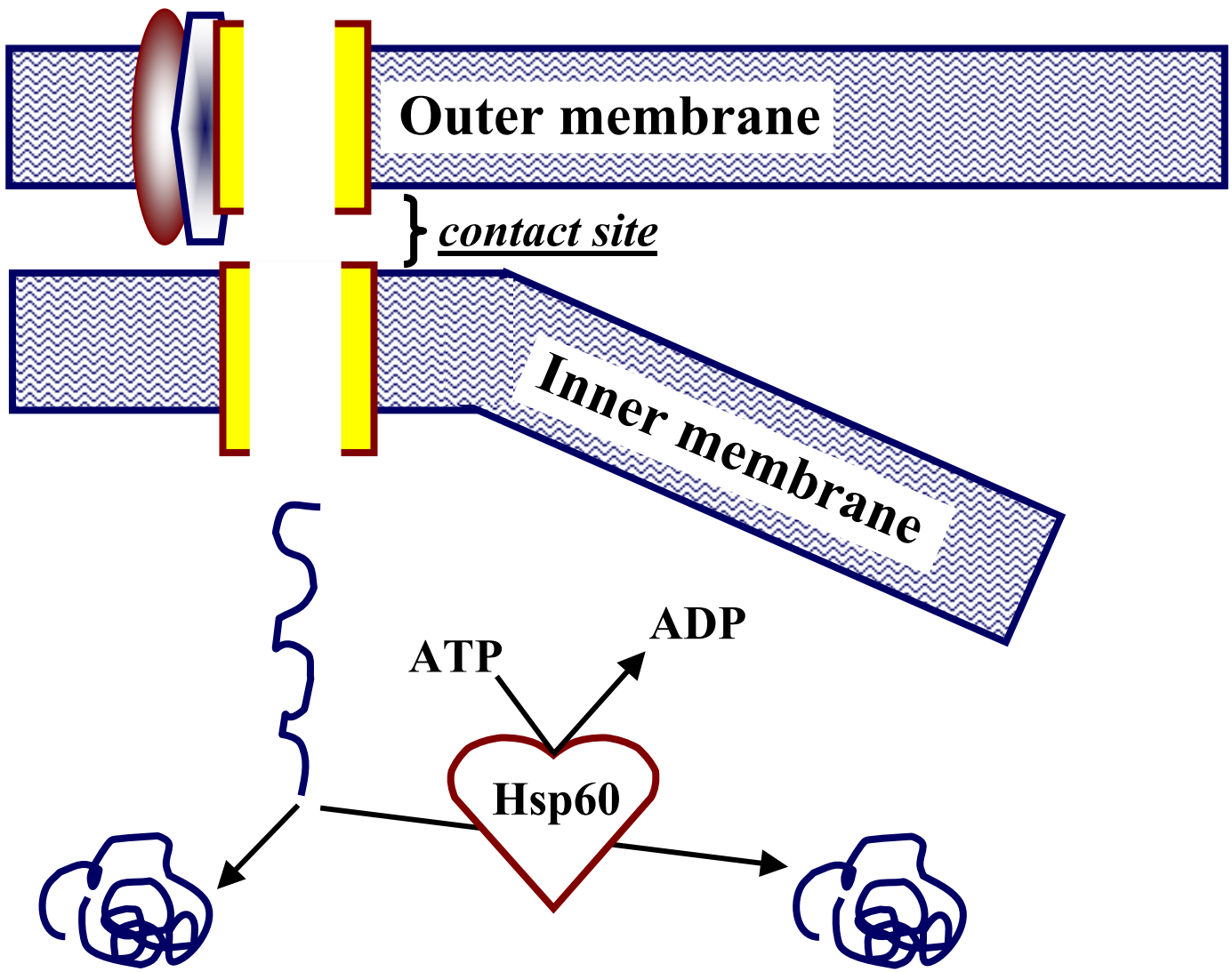
Protein import into the mitochondrial matrix



STEP ④

After matrix Hsp70 is released, the matrix-targeting (sorting) sequence is removed by a *matrix protease*

Protein import into the mitochondrial matrix



Folded, functionally active proteins

STEP ⑤

Within the matrix:

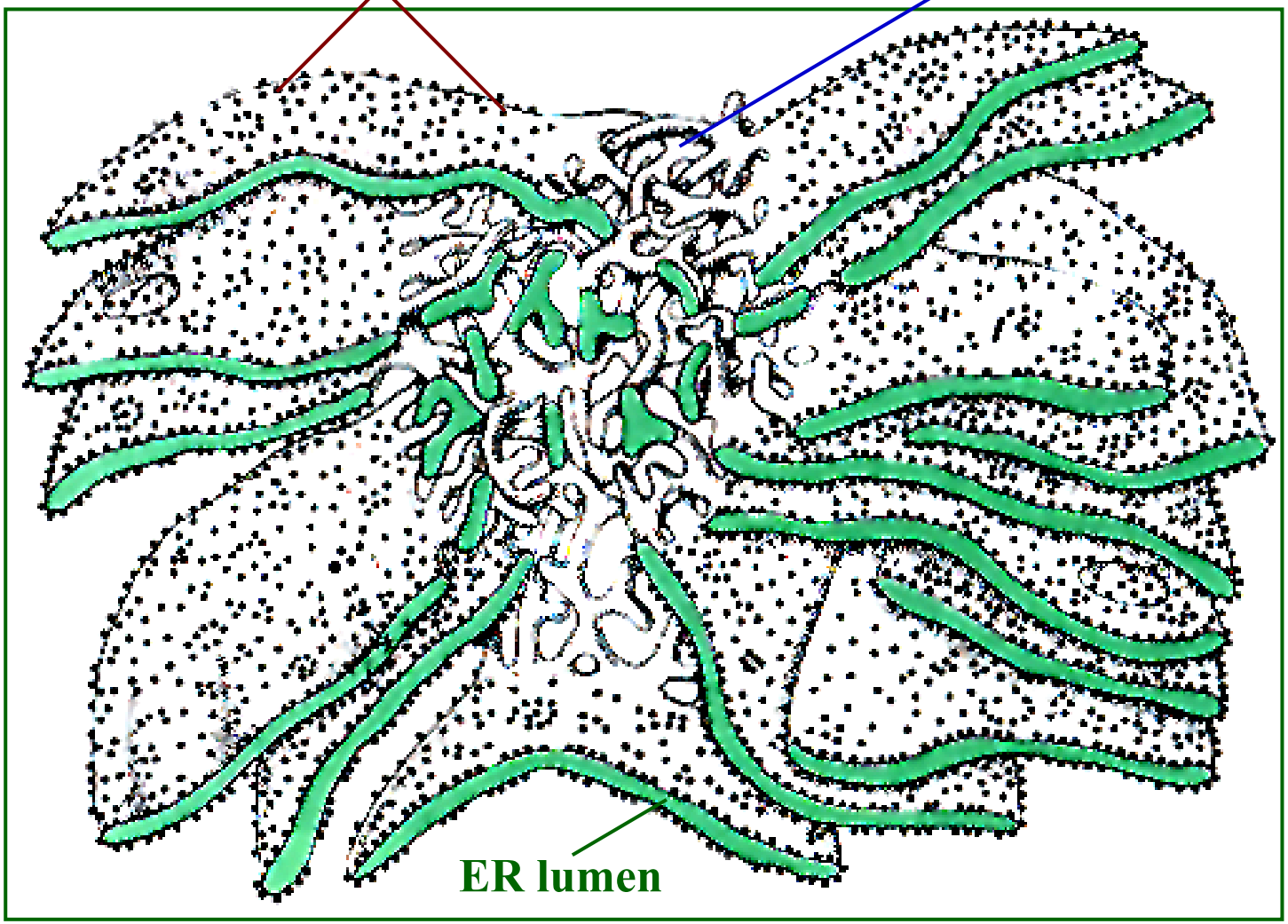
(1) Some proteins *fold* into their mature, active conformation without the aid of a chaperone

(2) Other proteins bind to the *chaperone Hsp60* (heat-shock protein 60), which assists in the final folding in a process that requires energy derived from ATP hydrolysis

Three-dimensional reconstruction of a region of the smooth and rough ER

Ribosomes **are attached to the cytosolic surface of the rough ER**

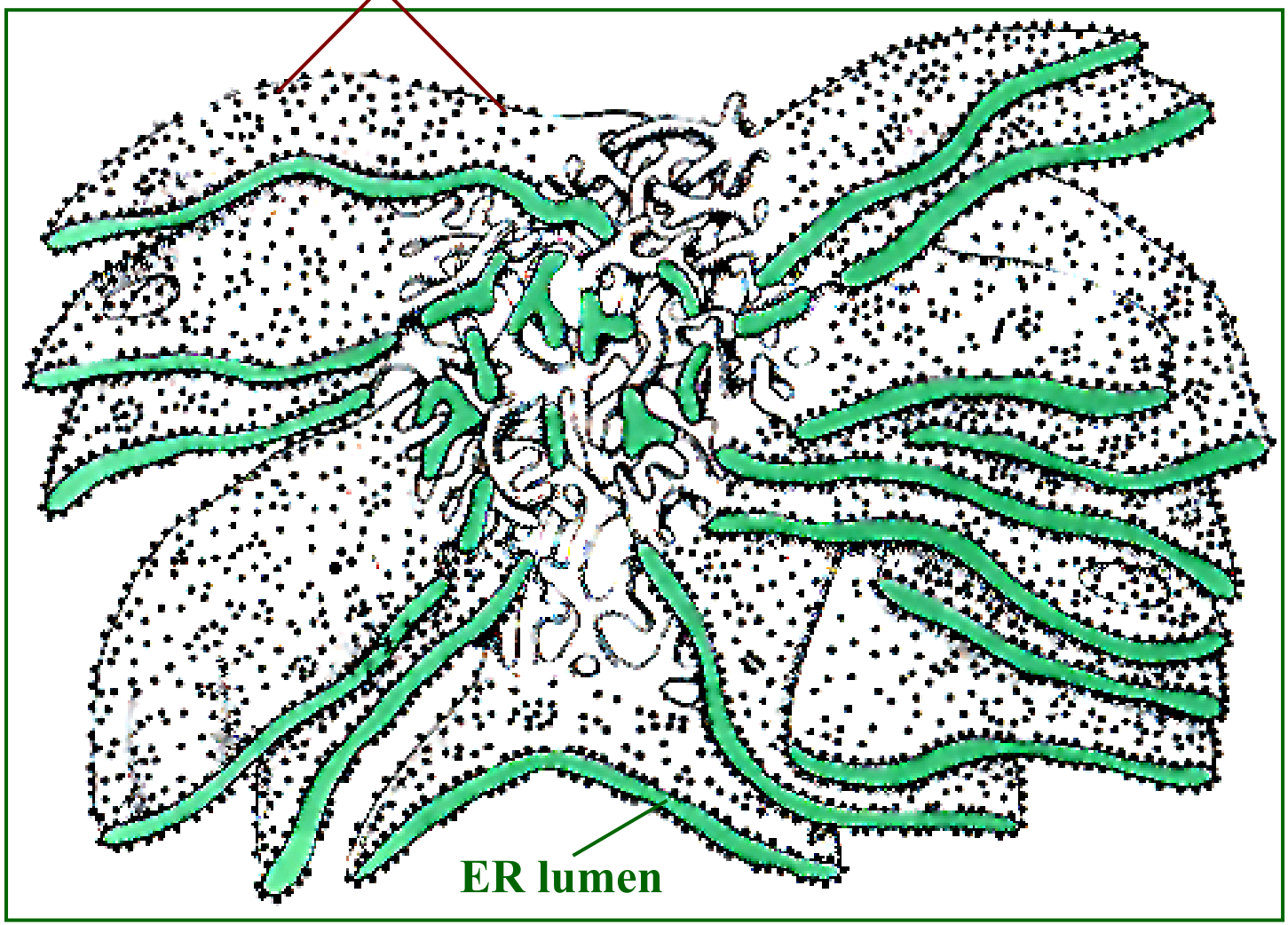
smooth ER



- **The rough ER forms oriented stacks of flattened *cisternae***
- **The smooth ER membrane *is connected to these cisternae* and forms a fine network of *tubules***

- **Unlike the proteins that enter the nucleus, mitochondria, chloroplasts and peroxisomes, most of the proteins that enter the ER begin to be translocated (transported) across the ER membrane *before the protein is completely synthesized***
- **This requires that the ribosome synthesizing the protein *is attached to the ER membrane***

Ribosomes are attached to the cytosolic surface of the rough ER

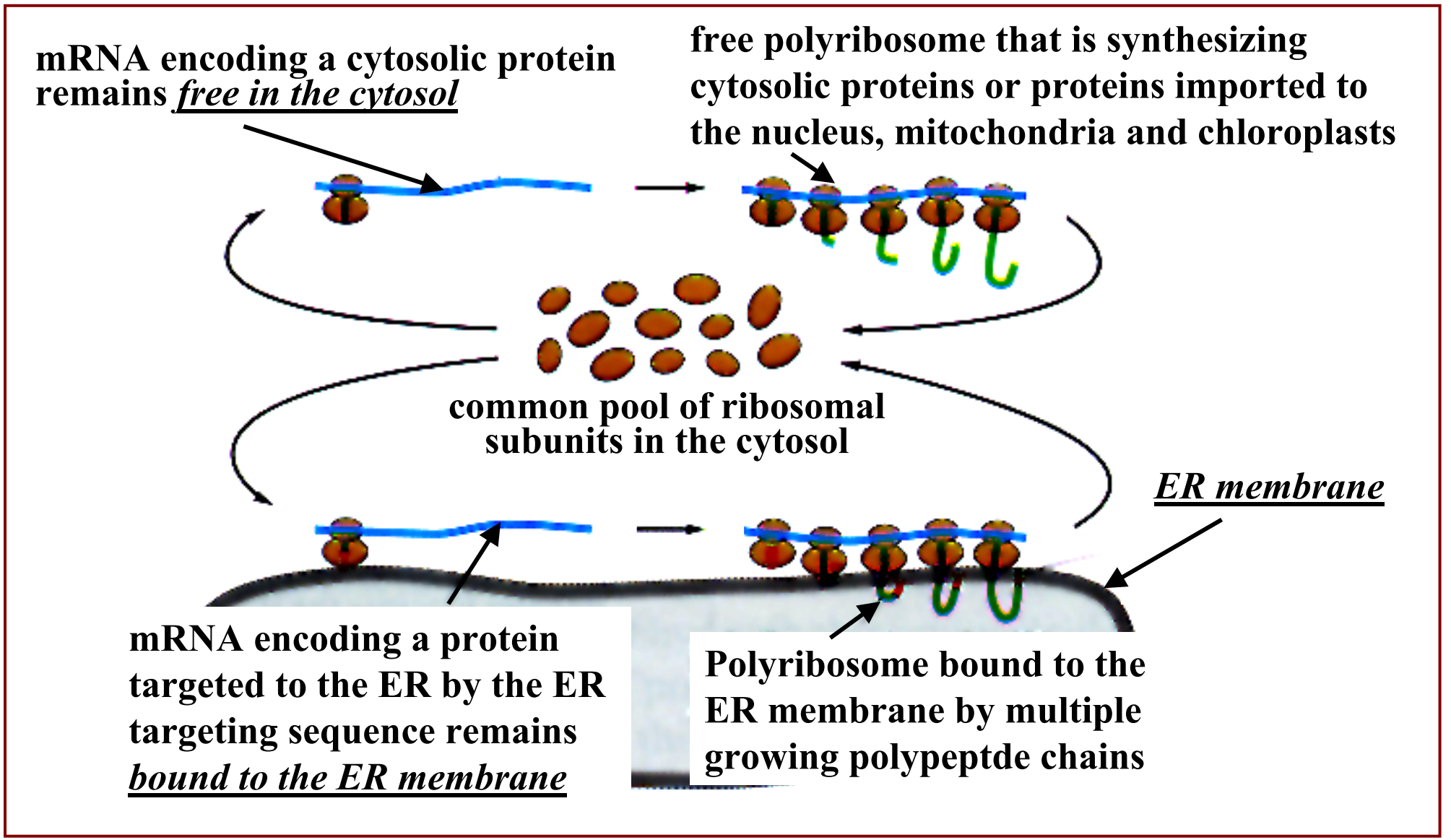


- **These membrane-bound ribosomes coat the surface of the ER, creating regions termed *rough ER* because the characteristic beaded appearance when viewed in an electron microscope**

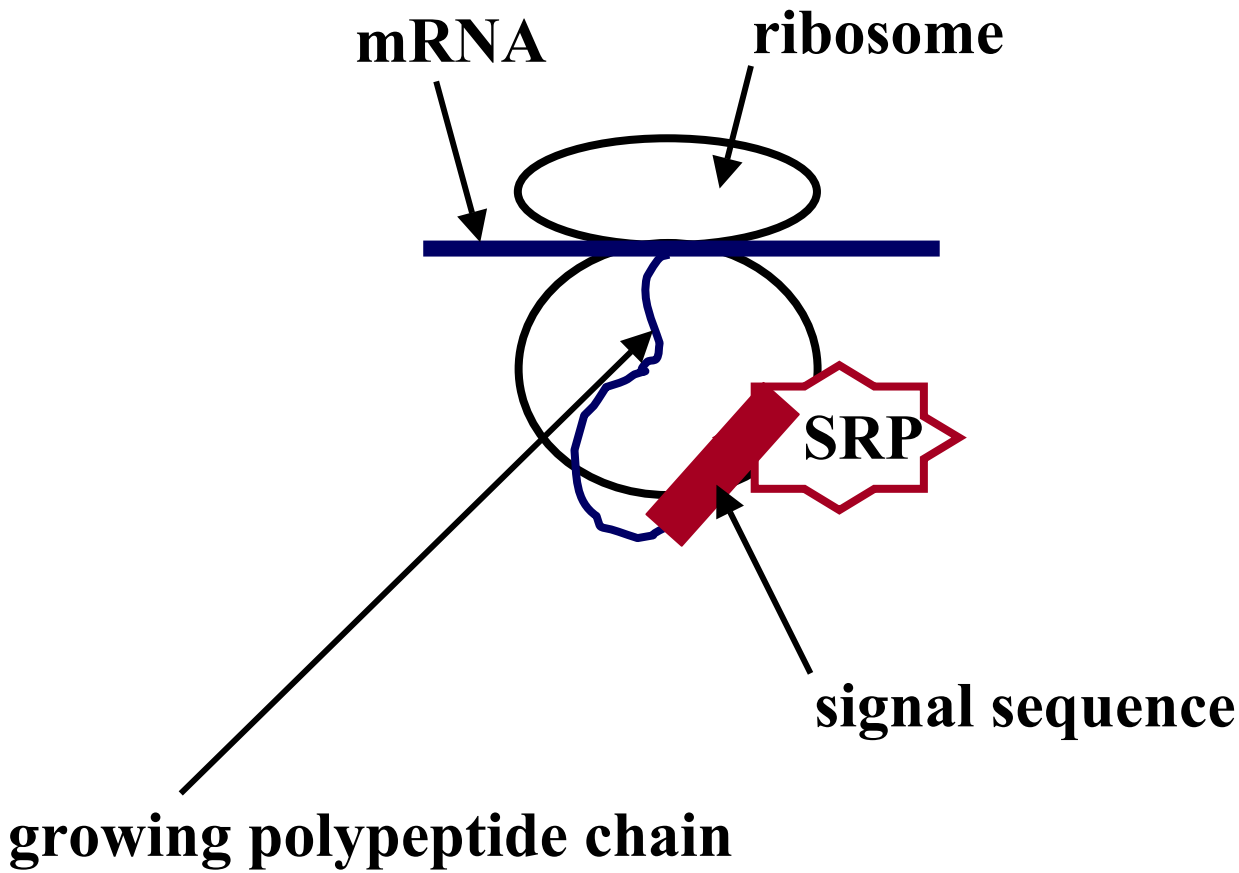
***There are two separate populations of ribosomes in the cytosol*:**

(1) *membrane-bound ribosomes* are attached to the cytosolic surface of the ER membrane and are synthesizing proteins that are translocated into the ER

(2) *free ribosomes* are unattached to any membrane and are synthesizing all of the other proteins



The translocation of a soluble protein across the ER membrane into the lumen



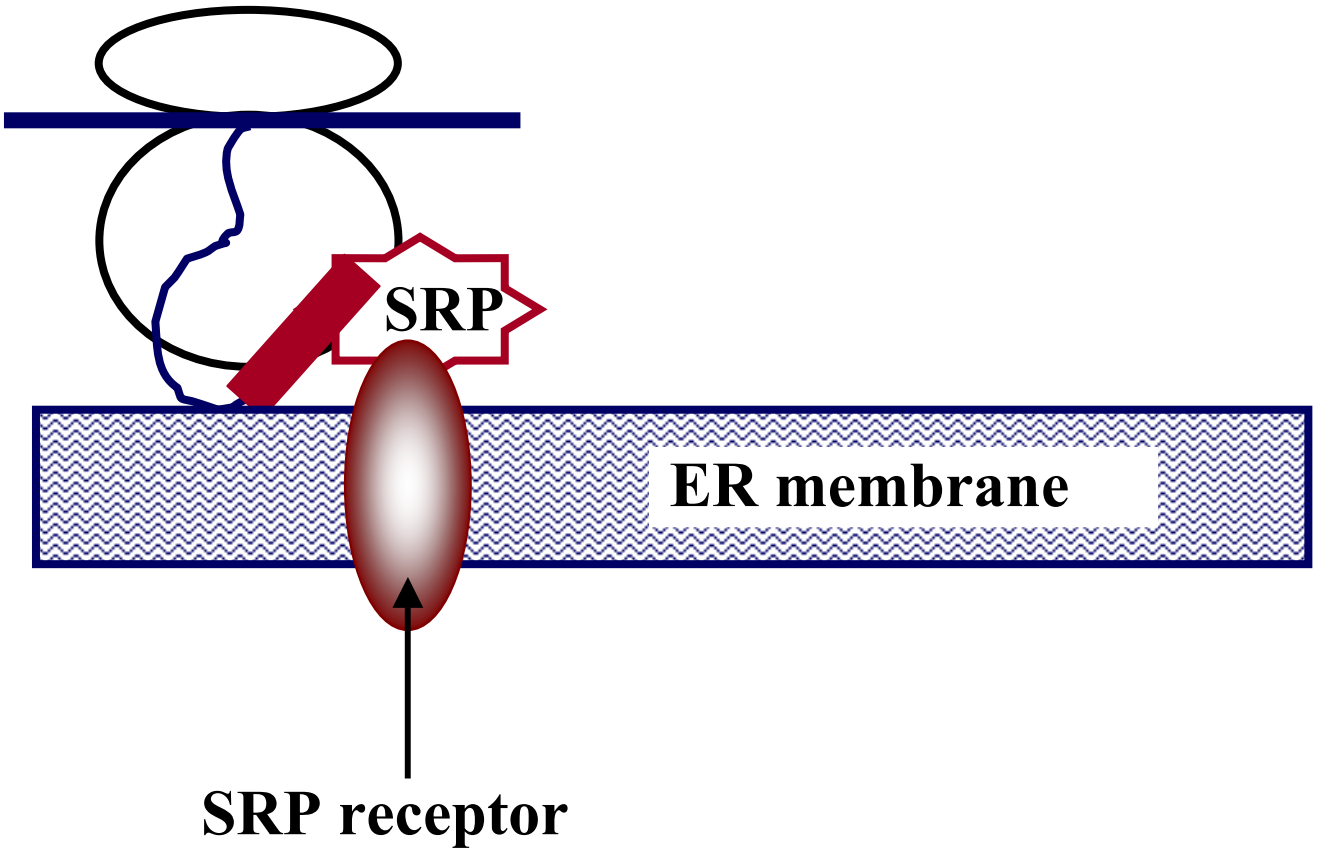
STEP ①

A cytosolic protein, the *signal recognition particle* (*SRP*), binds to the exposed ER signal sequence and to the ribosome, thereby slowing protein synthesis by the ribosome

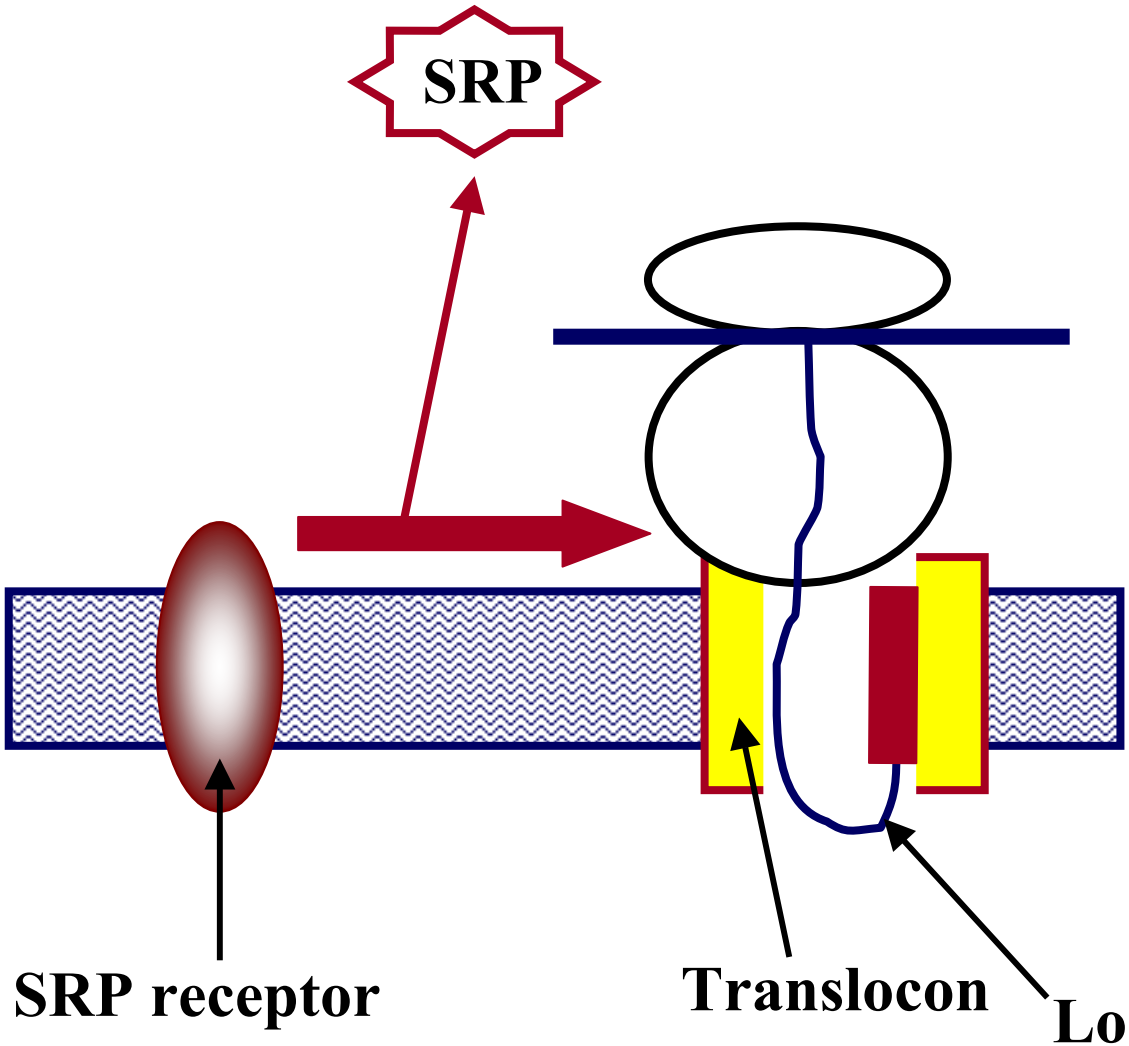
The translocation of a soluble protein across the ER membrane into the lumen

STEP ②

The SRP-ribosome complex then binds to an *SRP receptor* in the ER membrane



The translocation of a soluble protein across the ER membrane into the lumen



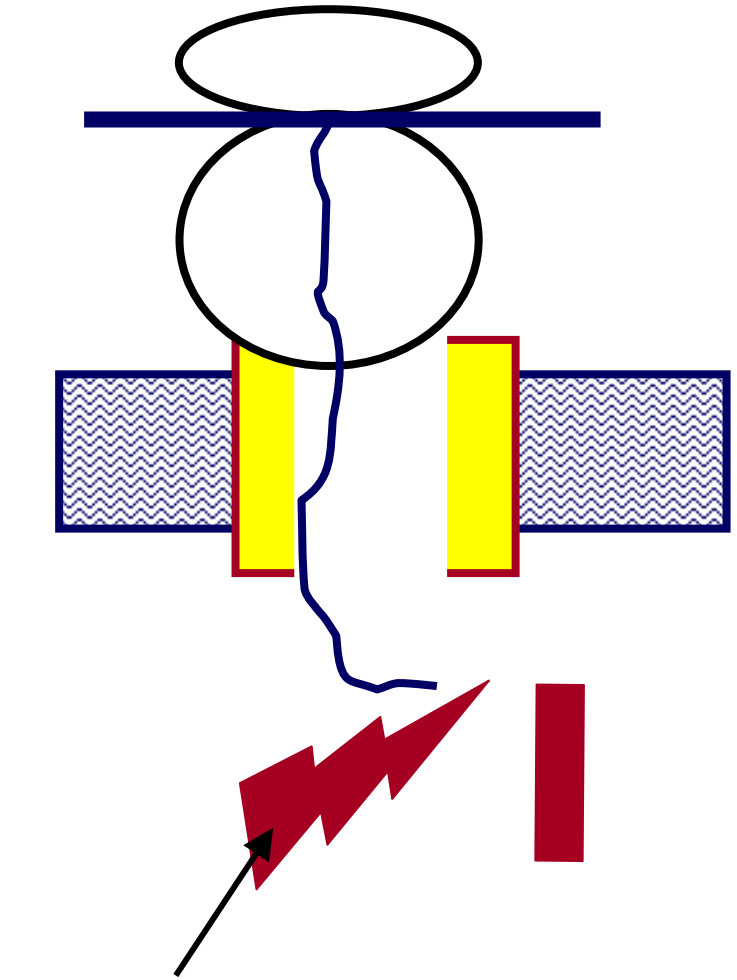
STEP ③

As SRP and its receptor *dissociate* from the ribosome-bound polypeptide chain:

(1) The ER signal sequence binds to the *translocon*, a protein translocation channel in the ER membrane

(2) The ER signal sequence and the adjacent segment of the growing polypeptide chain then insert as a *loop* into the central cavity of the translocon

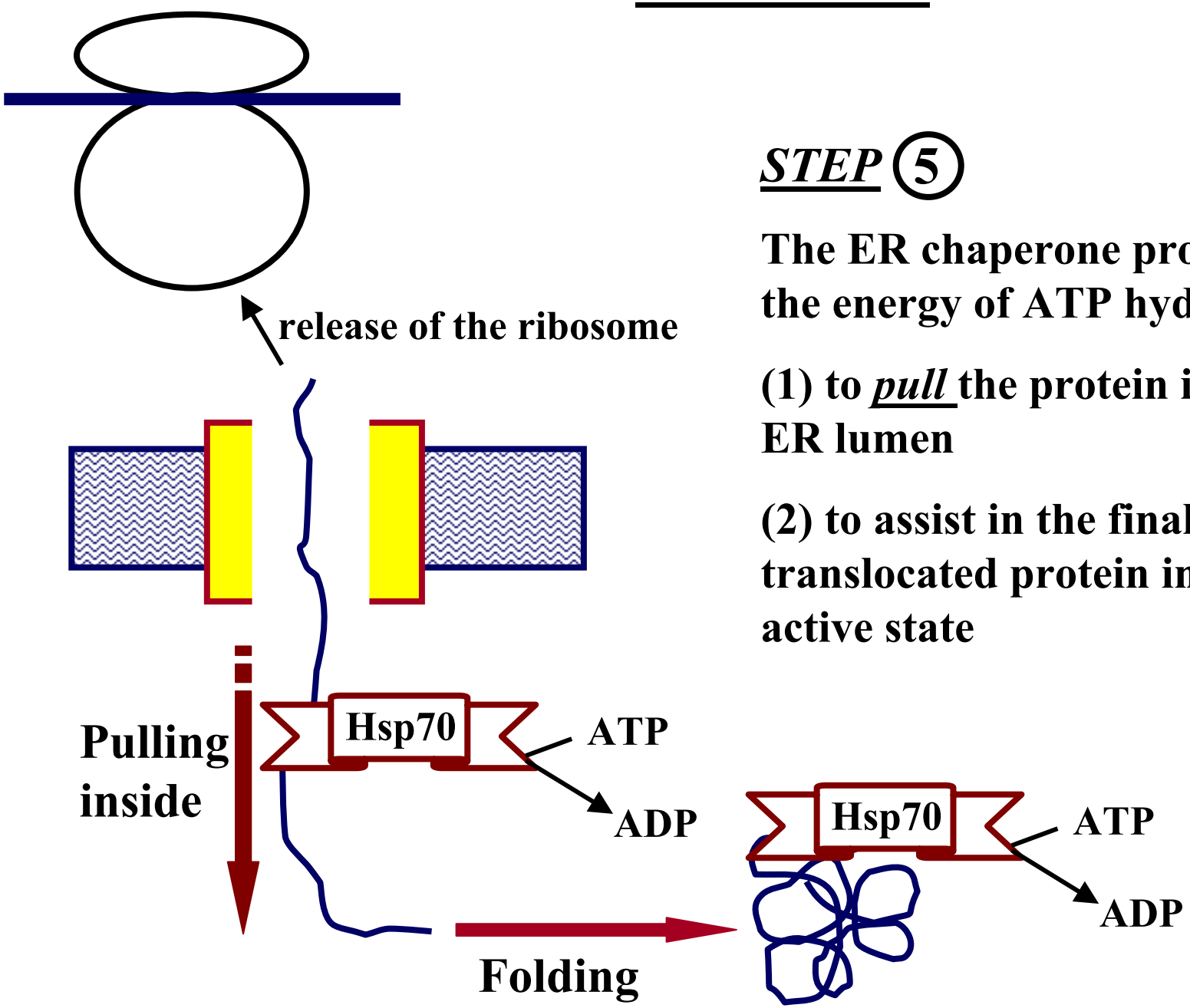
The translocation of a soluble protein across the ER membrane into the lumen



STEP ④

The ER signal sequence is cleaved by a *signal peptidase* in the ER lumen

The translocation of a soluble protein across the ER membrane into the lumen



STEP ⑤

The ER chaperone protein Hsp70 uses the energy of ATP hydrolysis:

(1) to *pull* the protein inside, into the ER lumen

(2) to assist in the final *folding* of the translocated protein into its mature, active state