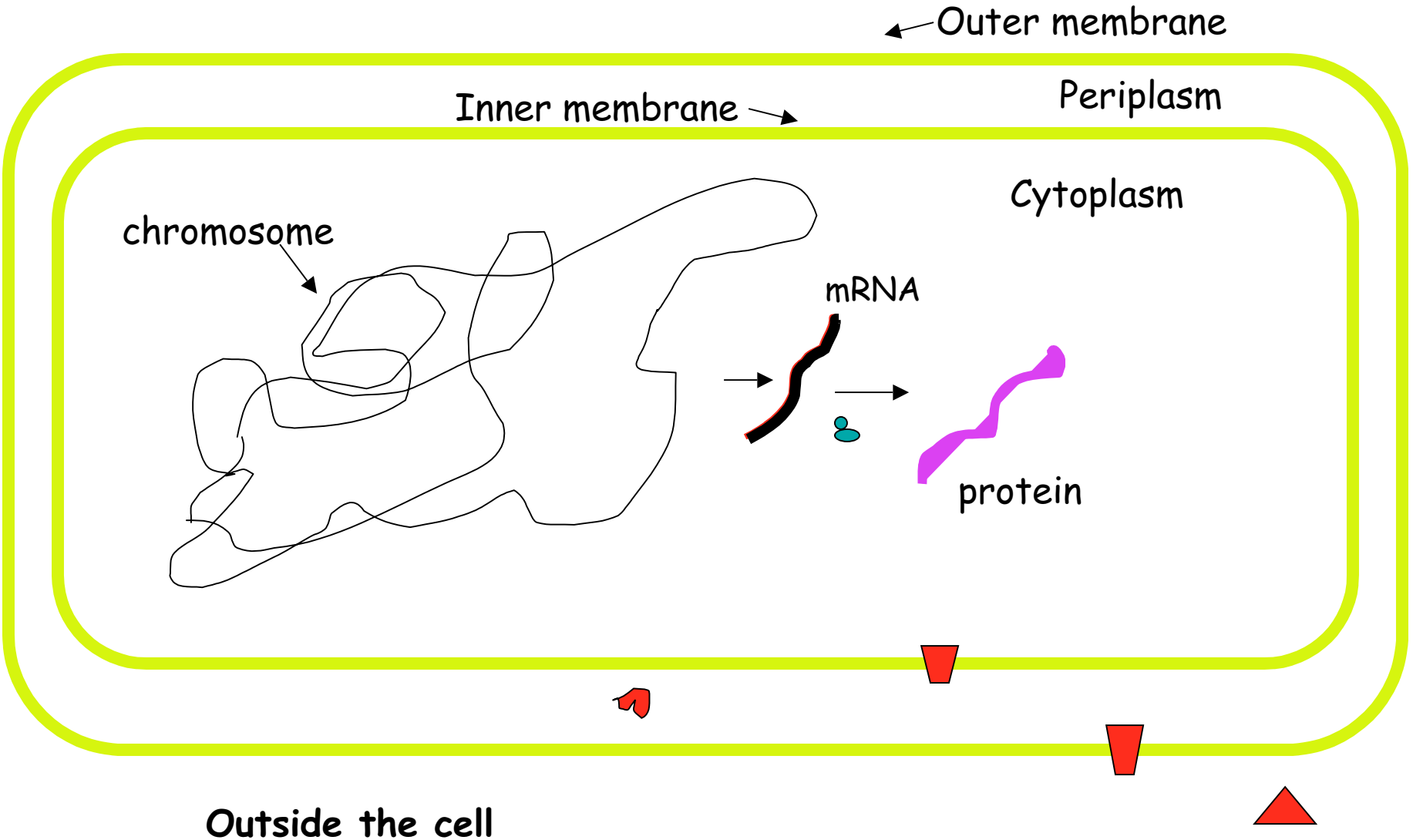
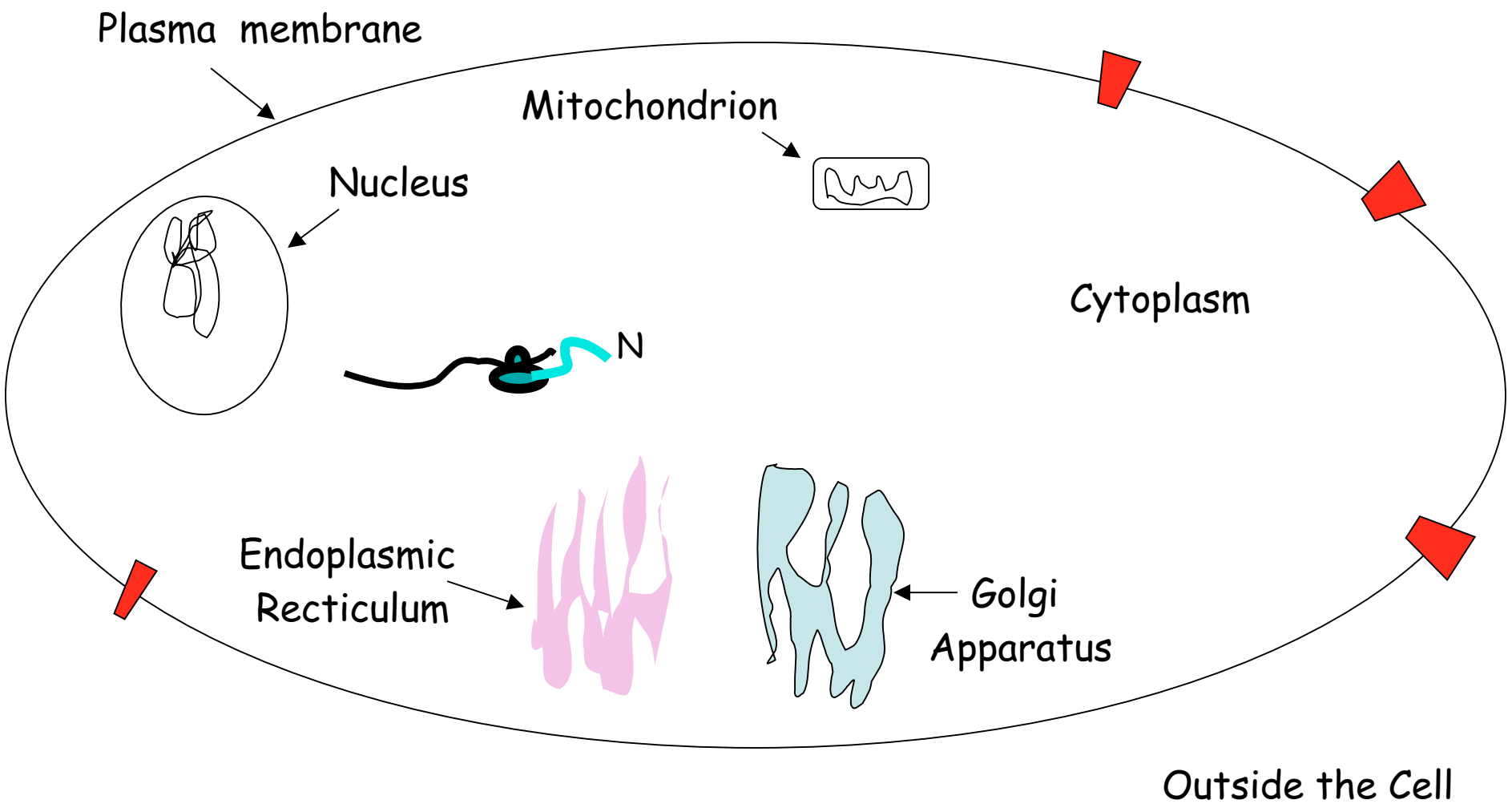


BACTERIAL CELL



EUKARYOTIC CELL

Examples

	Cytoplasmic Protein	Membrane Protein	Fully Secreted Protein (Outside the Cell)
Bacteria	β–galactosidase	Lactose Receptor	Toxin
Eukaryotic Cell	Histidine synthesis Lactase Glycolysis Enzymes Cyclins	Insulin Receptor Growth Factor Receptors	Insulin Growth Factors Antibodies

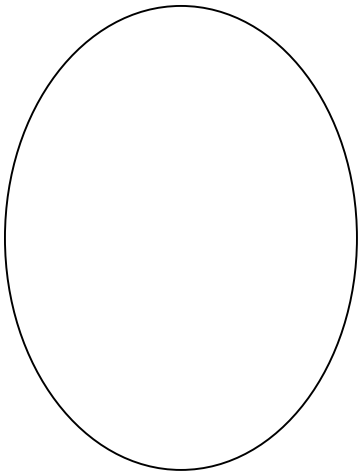
George Palade

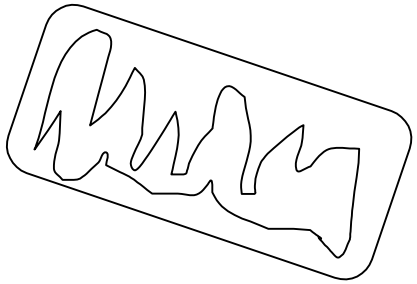
Images removed due to copyright reasons.

Images removed due to copyright reasons.

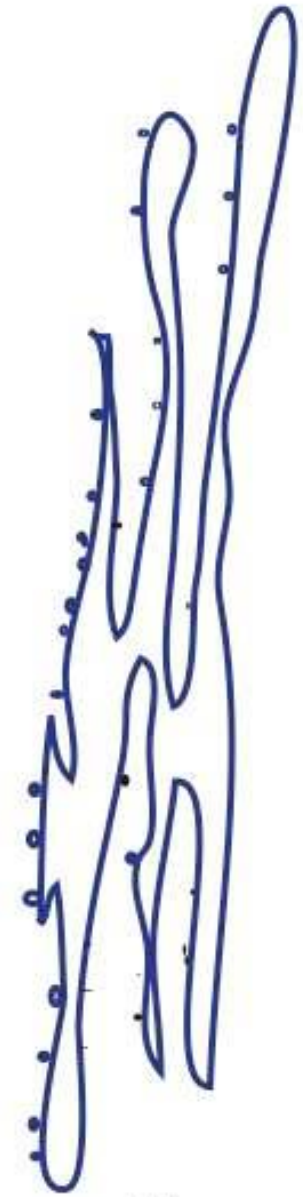
Hamster pancreatic

Nucleus





Mitochondrion

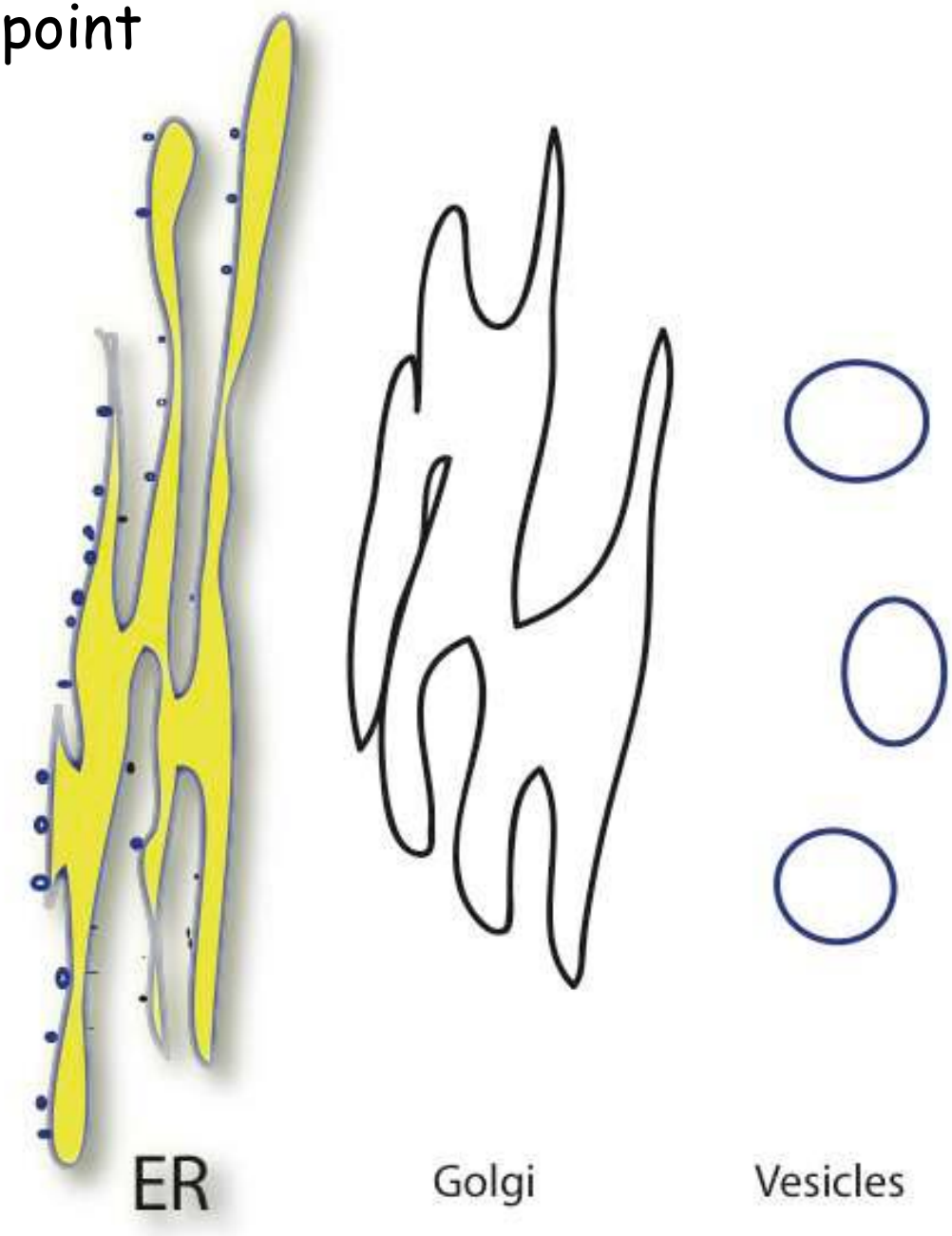


ER

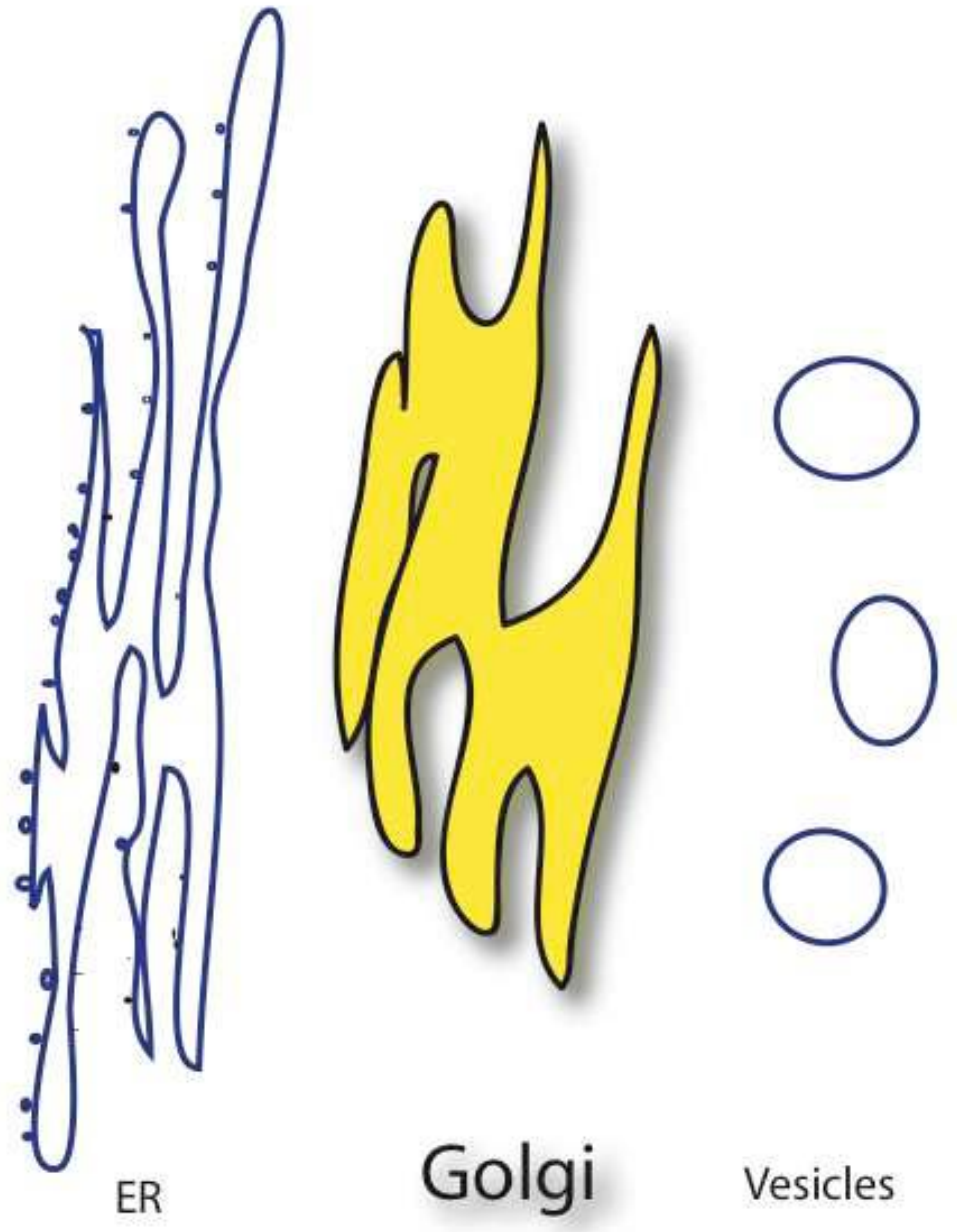
Golgi

Vesicles

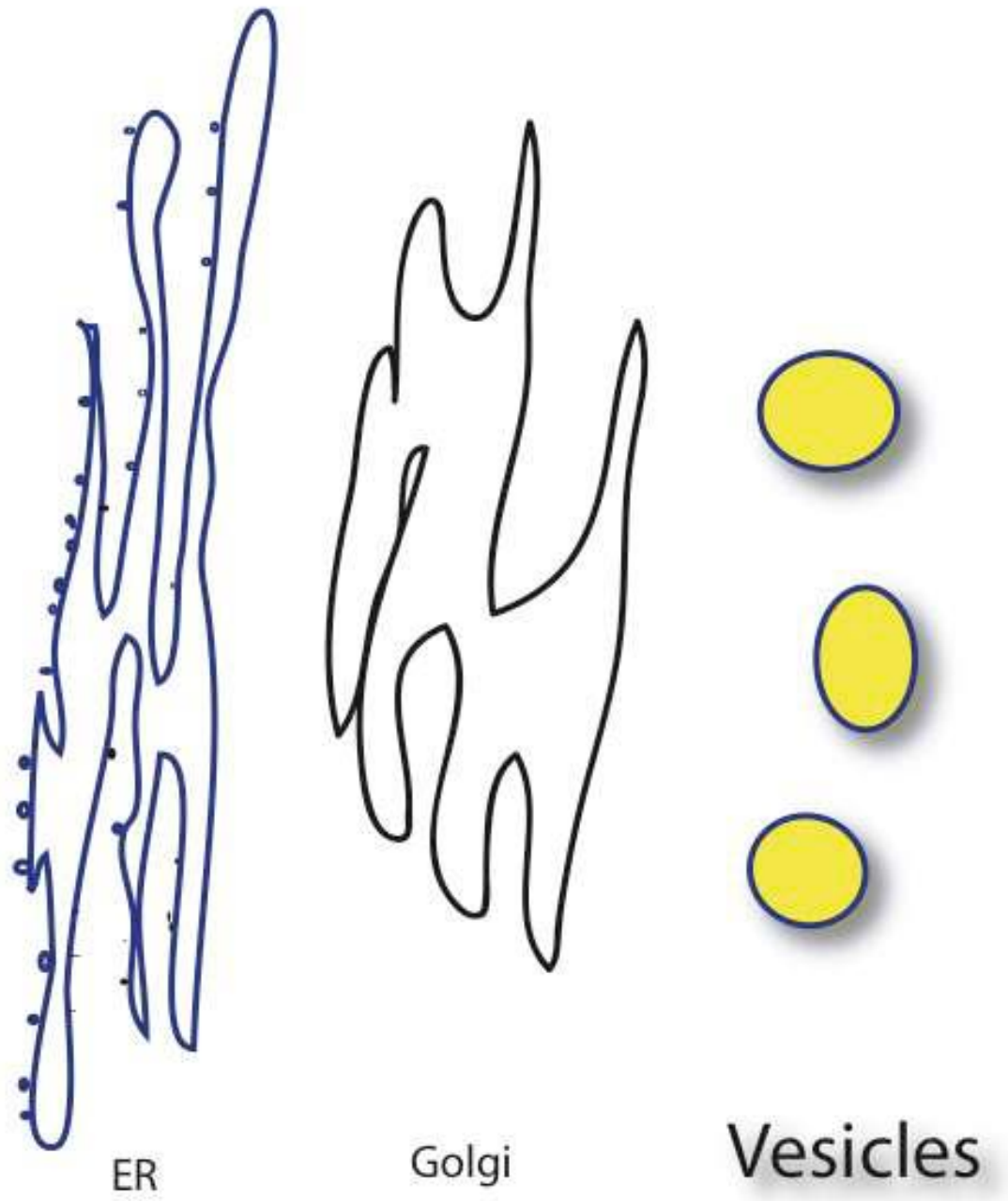
Earliest Time point



Next observed location



Location After Golgi



Millstein

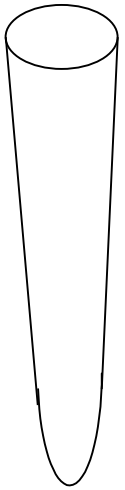
Image removed due to copyright reasons.

FROM NOBEL LECTURE 1984

"in vitro synthesis of immunoglobulin light chains. …
To our delight we ran into the unexpected observation
of the existence of a biosynthetic precursor of
light chains. Further experiments led us to propose
the extra N-terminal sequence was a signal for
vectorial transport across the membrane during
protein synthesis. That was the first evidence which
indicated that the signal for secretion was an N-terminal
segment, rapidly cleaved during protein synthesis."

Blobel

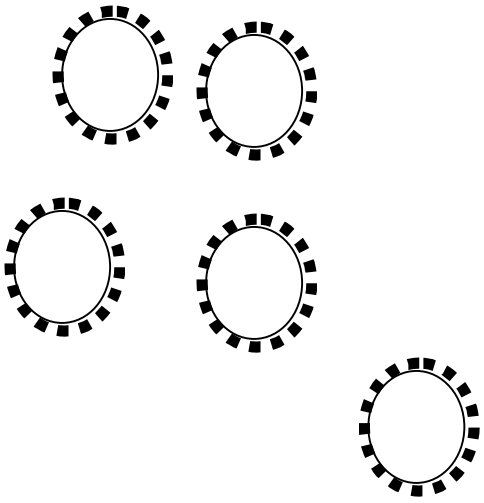
Image removed due to copyright reasons.



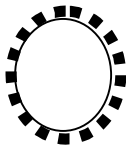
in vitro

Messenger RNA
Ribosomes &
charged tRNAs

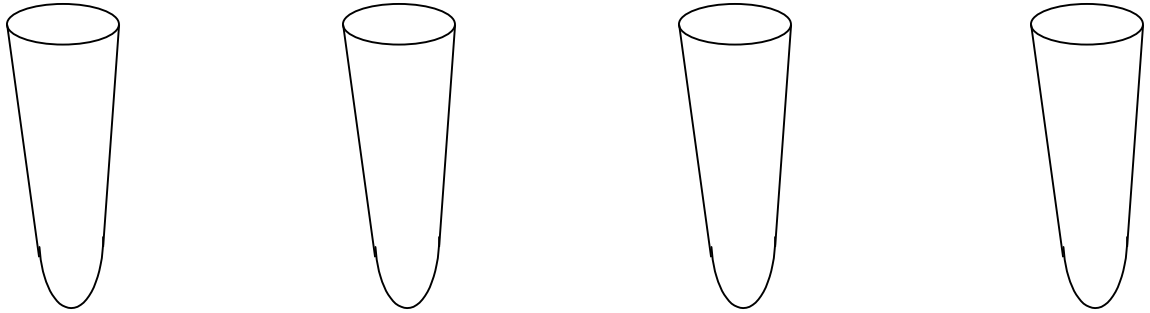
Microsomes
(RER vesicles)



Cytoplasmic
Extracts



Message Ribosomes tRNAs	+	+	+	+
Microsomes	-	+	+	+
Purified extract	-	-	+ added late	+ added early



Protein in supernatent

Protein in supernatent

Protein in supernatent

Protein in lumen of microsomes

Nobel Laureate, 1999

Gunter Blobel

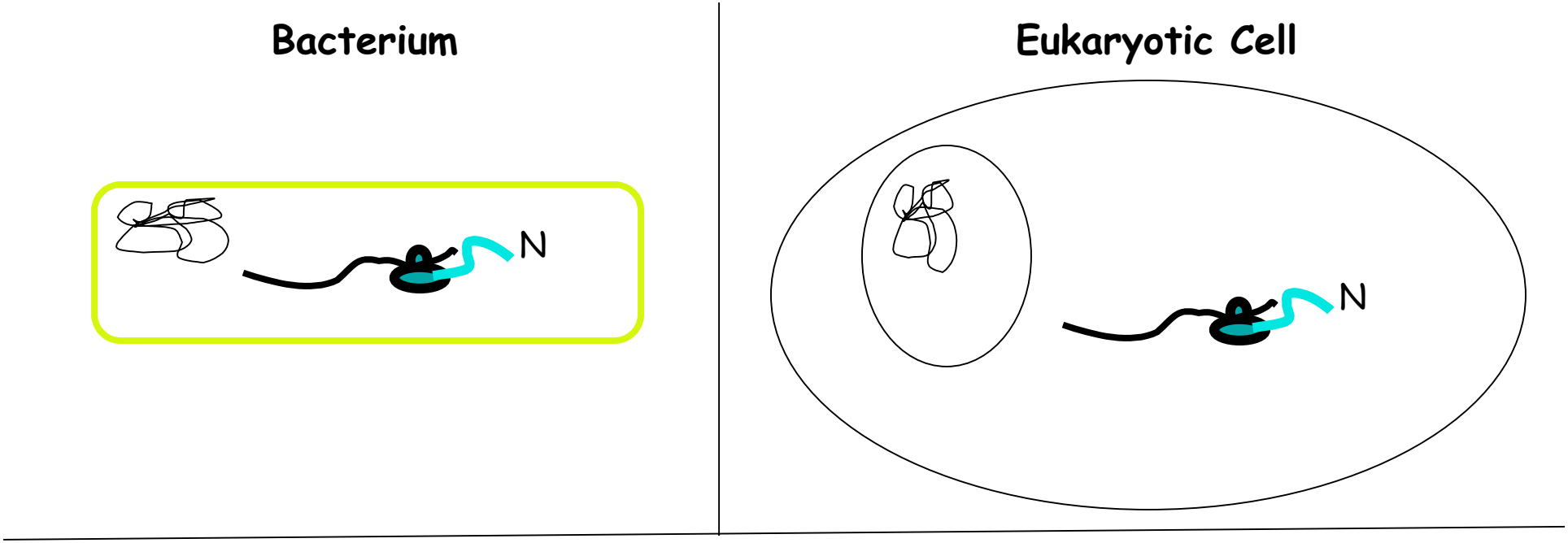
Image removed due to copyright reasons.

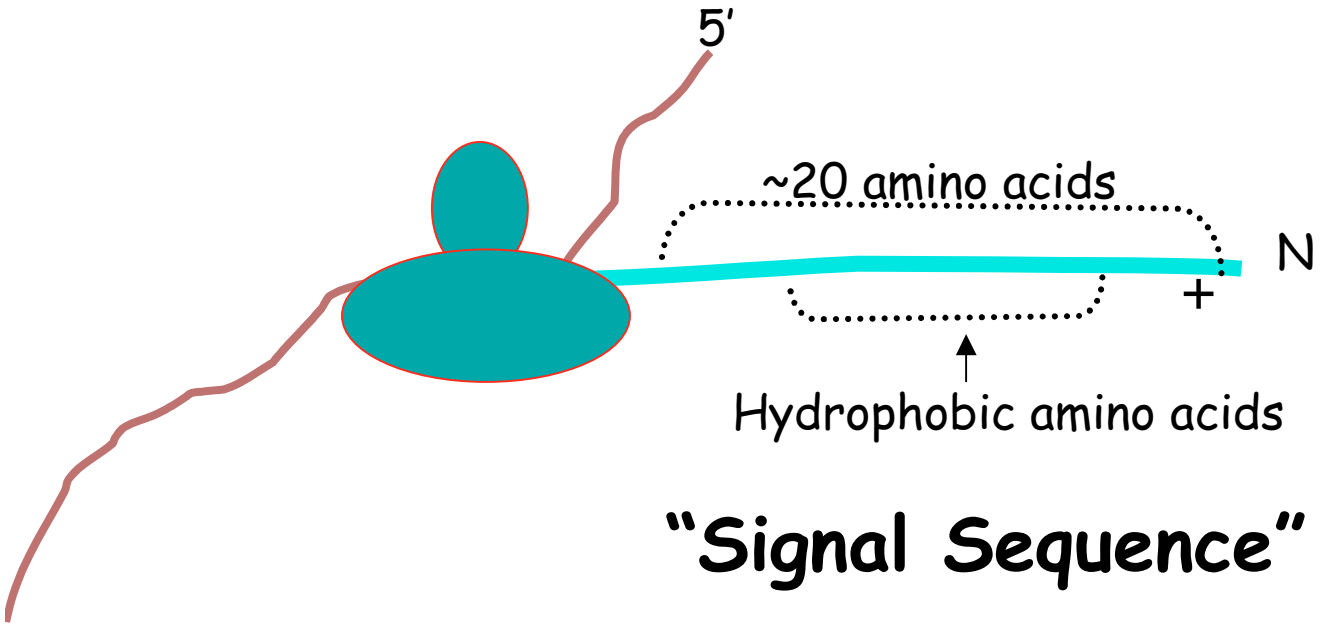
From the previous experiment, Blobel demonstrated that the amino acid sequence at the beginning (N terminus) of exported proteins is recognized by a complex.

This complex is required to get the protein into the lumen of ER.

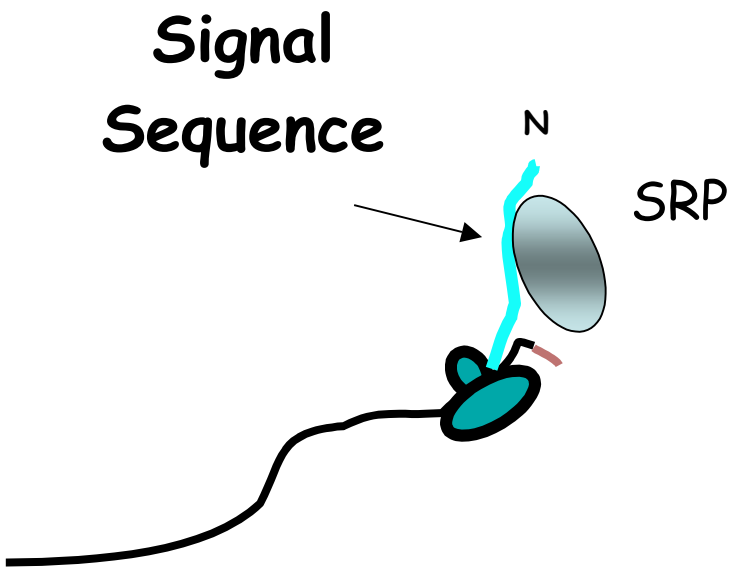
To get into the lumen of the ER the protein has to be just beginning to be translated.

Since not all exported proteins have the same N terminus, Blobel predicted, like Millstein, whatever the sequence was, it would be later cleaved.

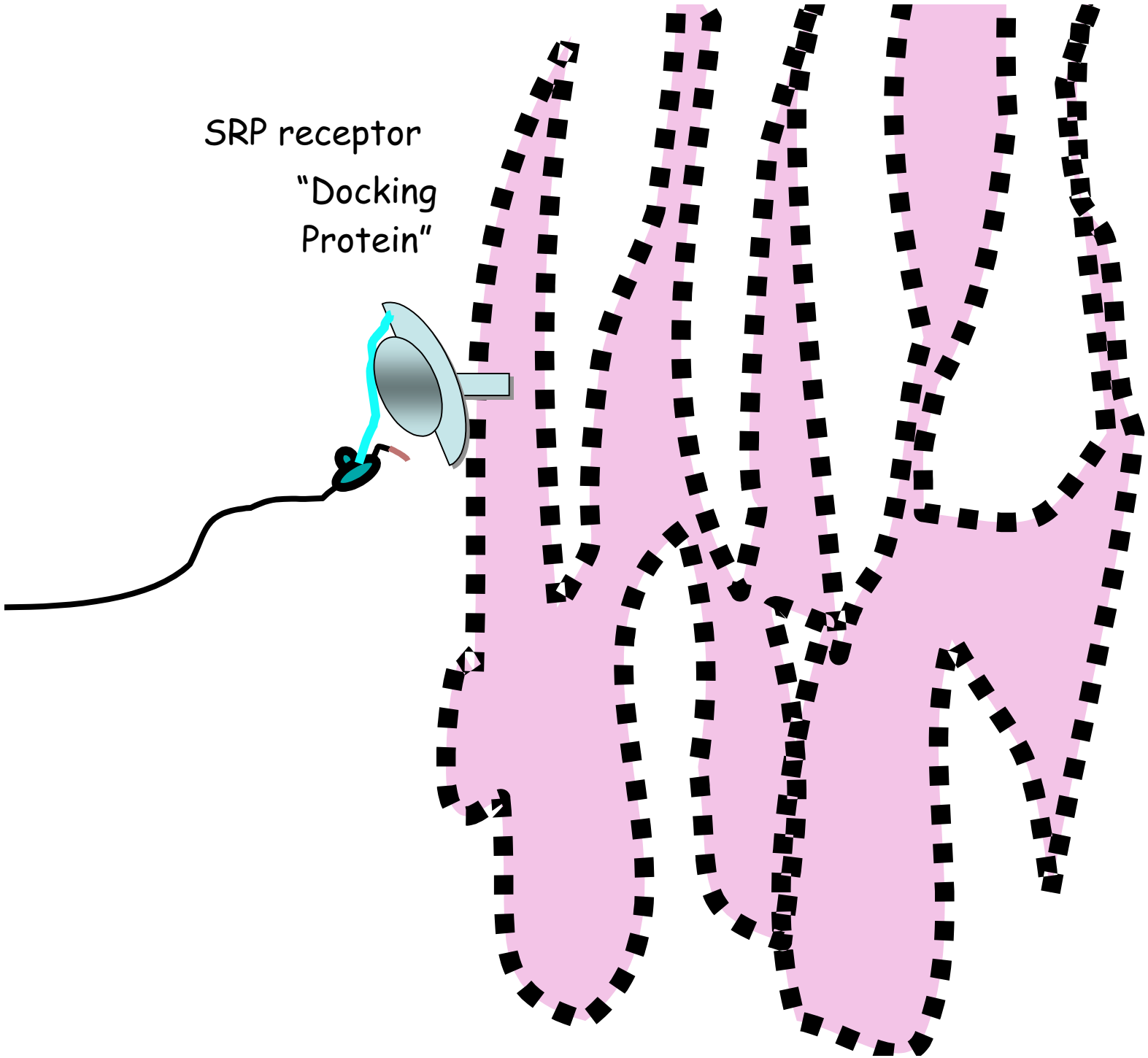


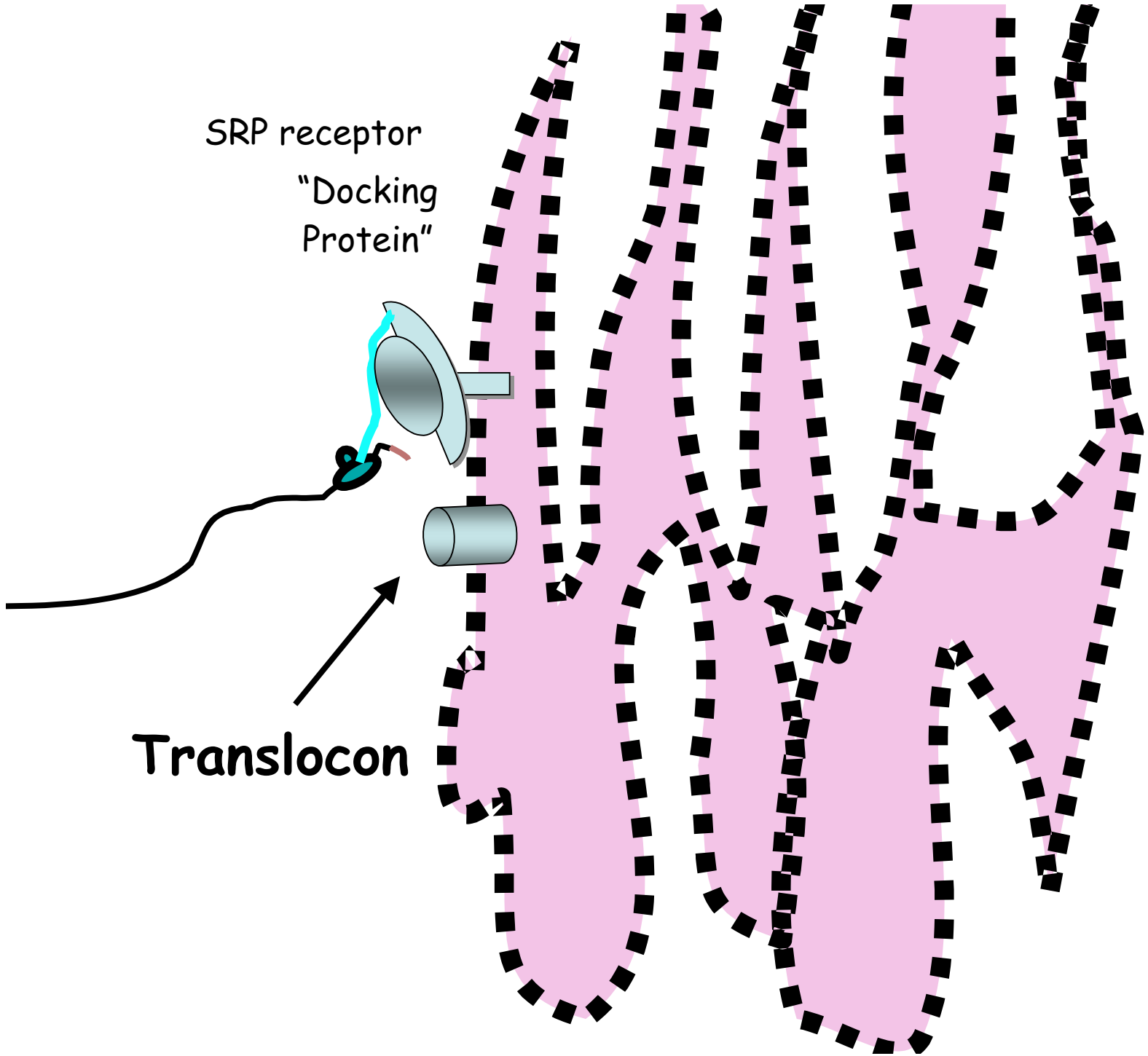


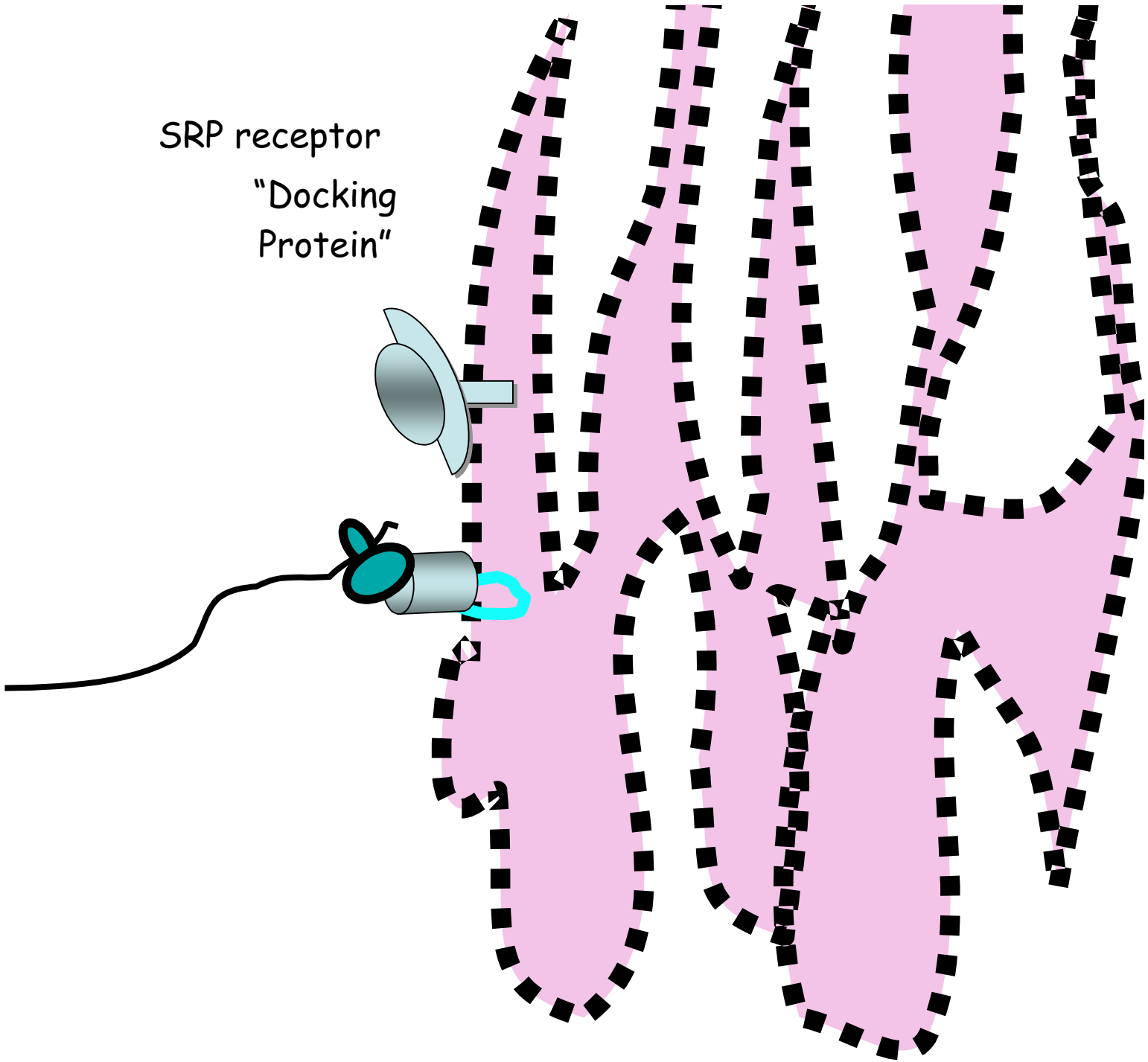
"Signal Sequence"

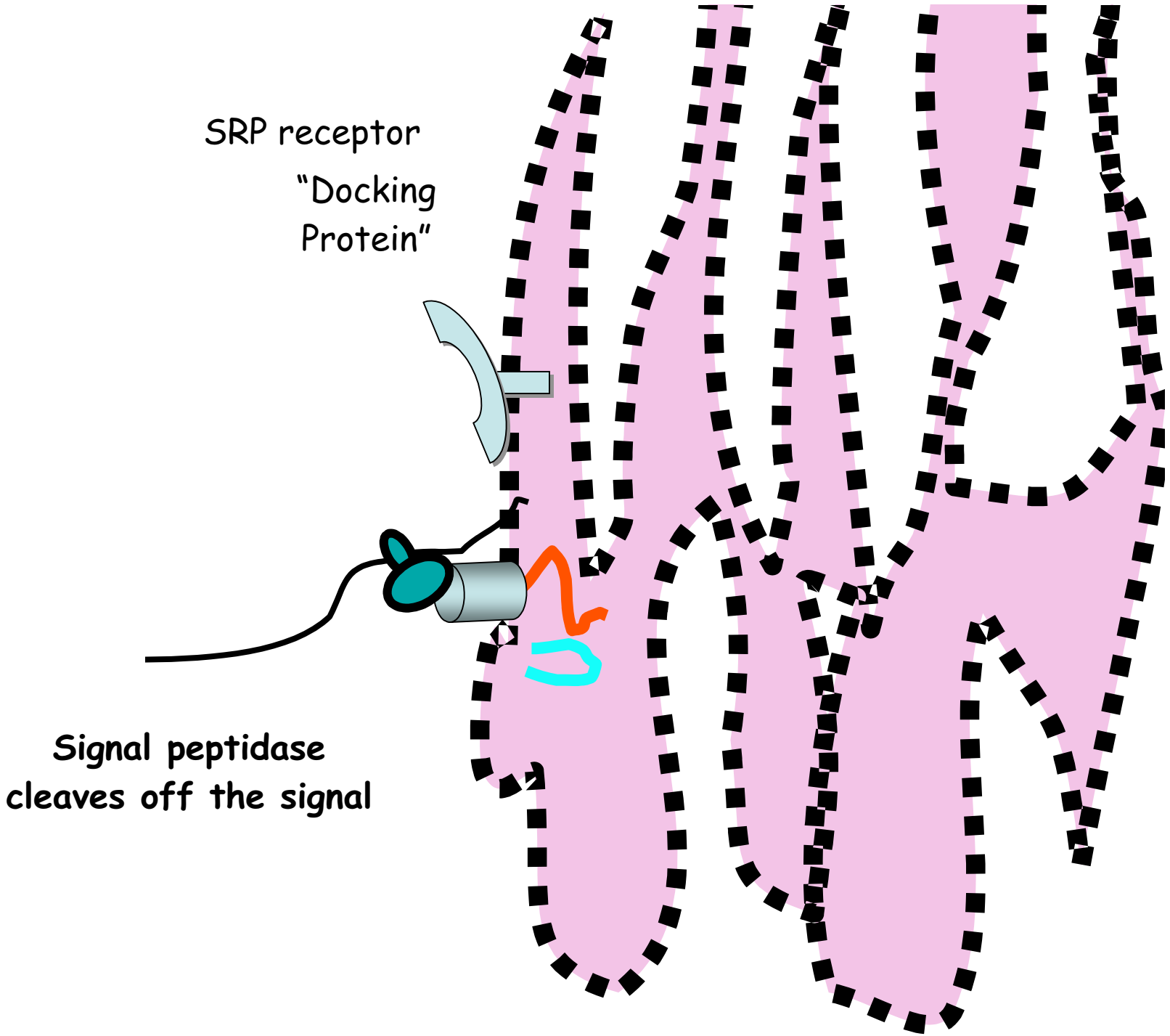


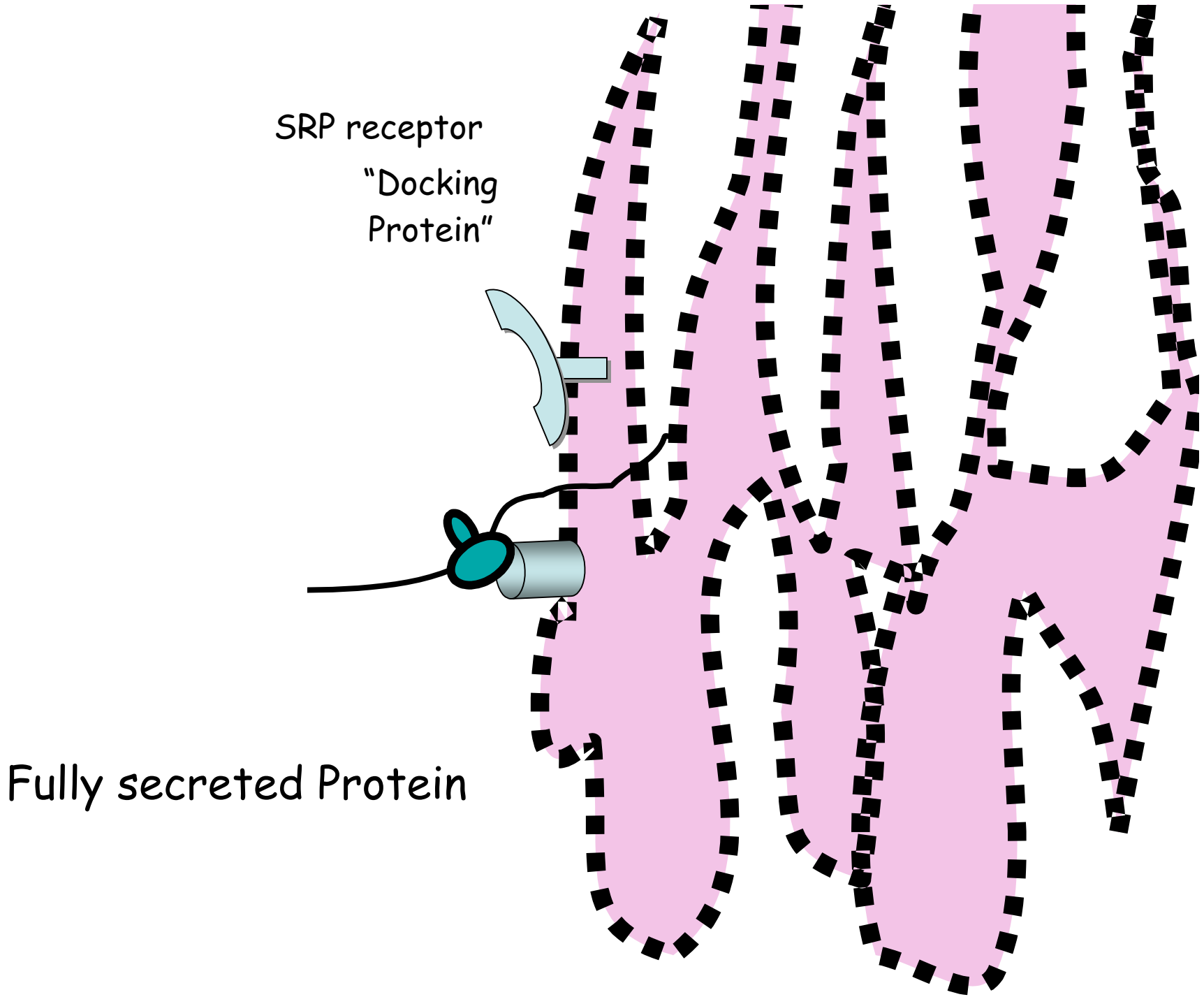
Signal Recognition Particle

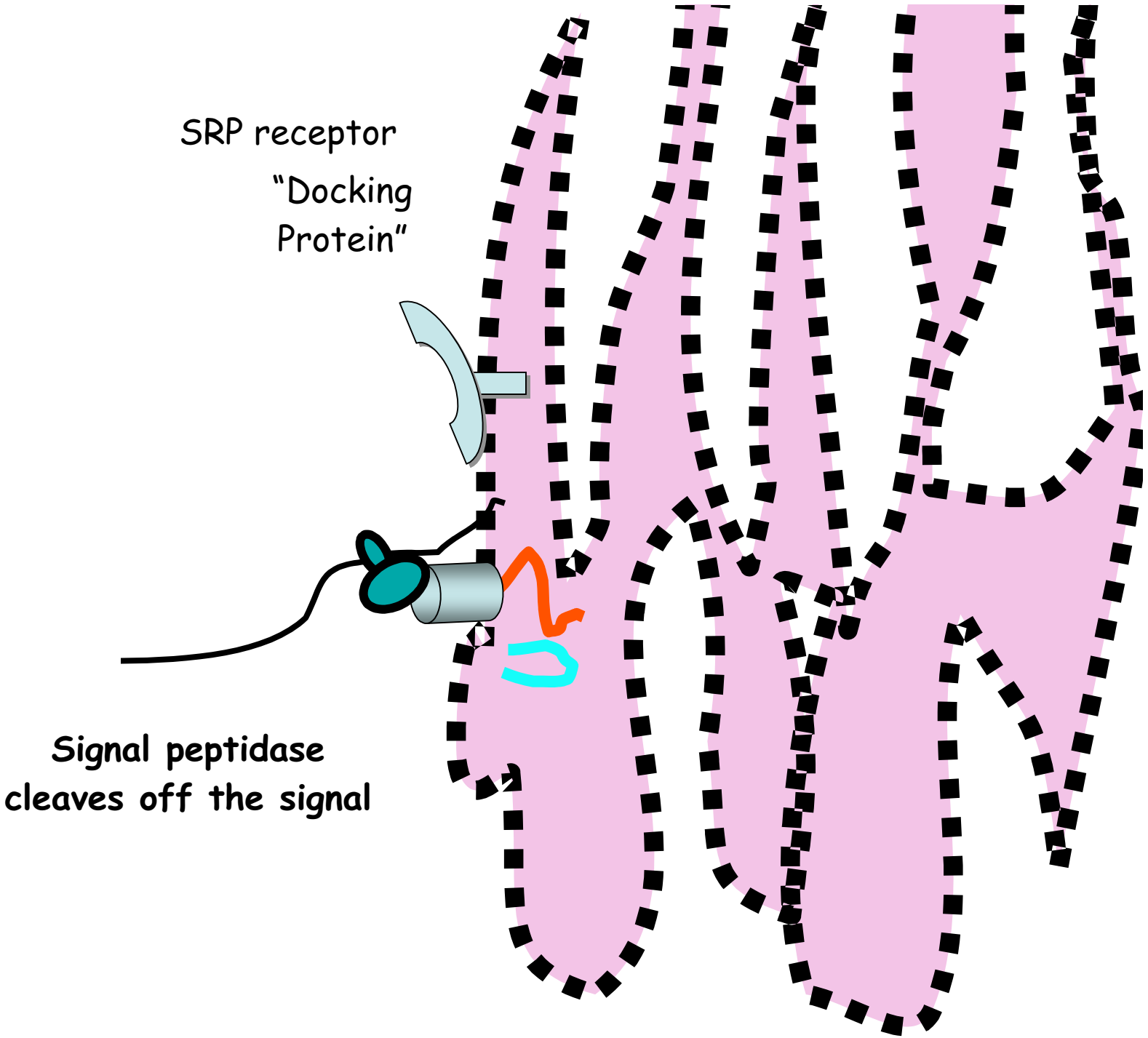

SRP receptor
"Docking
Protein"

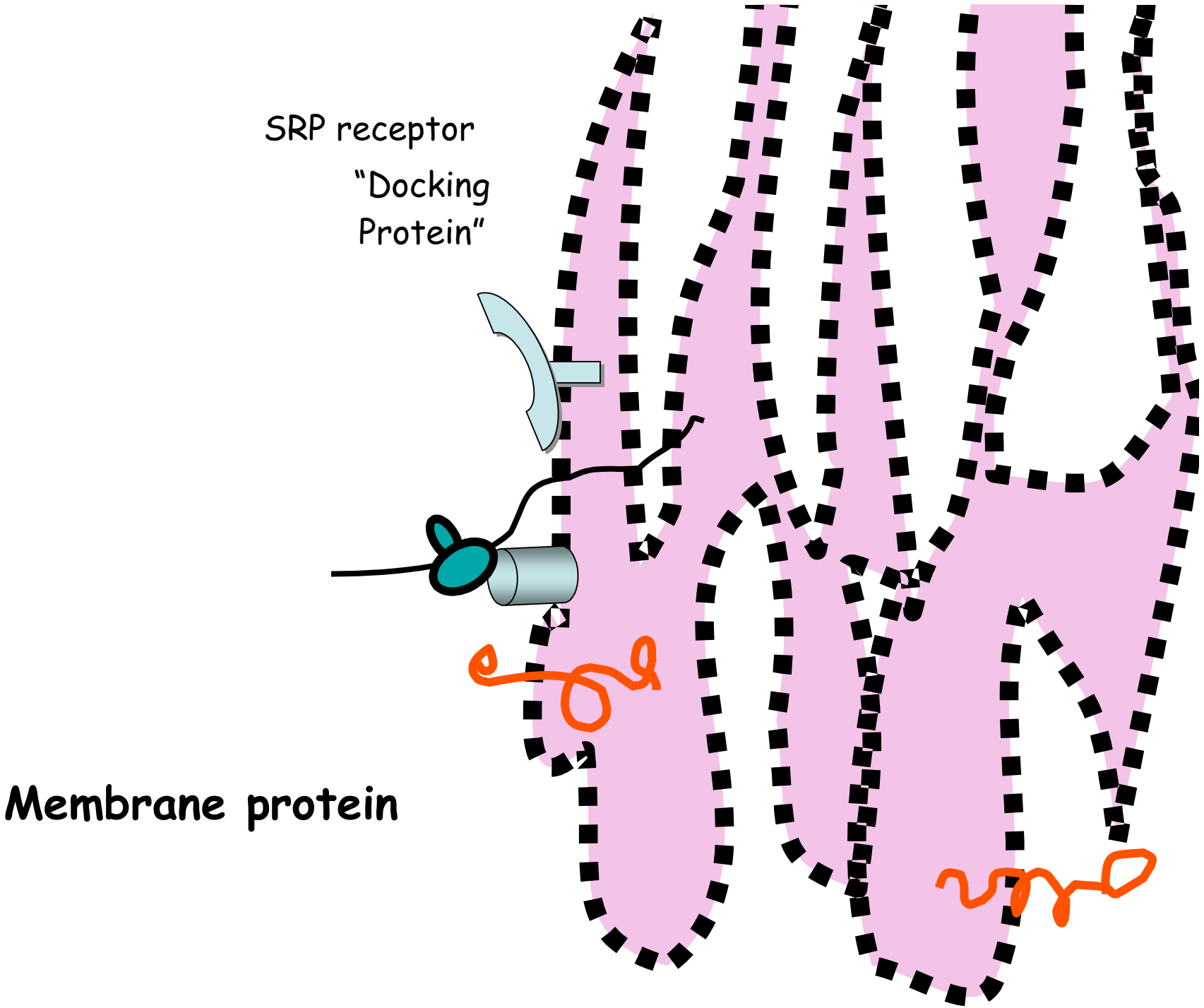

SRP receptor
"Docking Protein"
Translocon

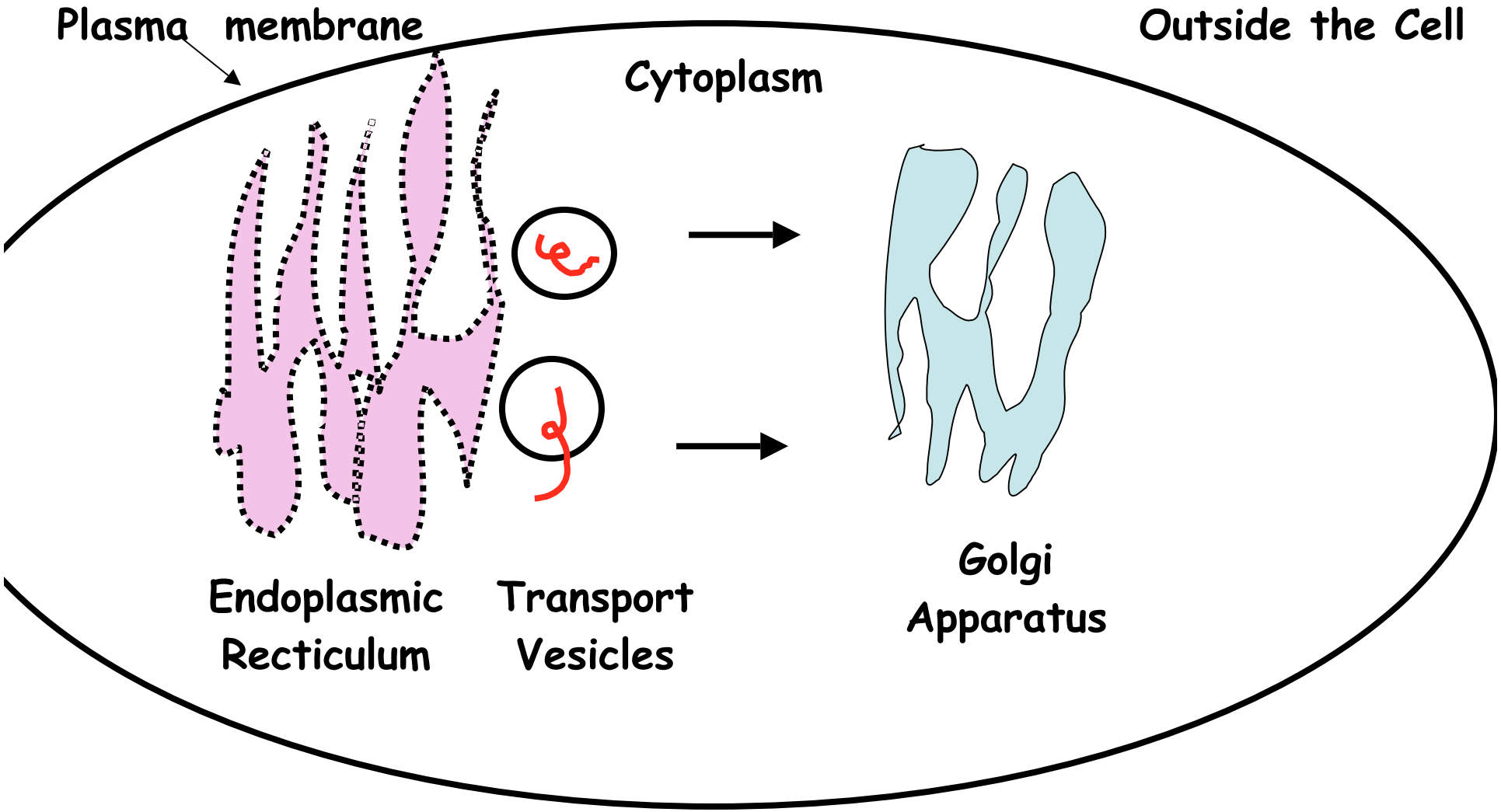

SRP receptor
"Docking
Protein"


SRP receptor
"Docking Protein"
Signal peptidase cleaves off the signal

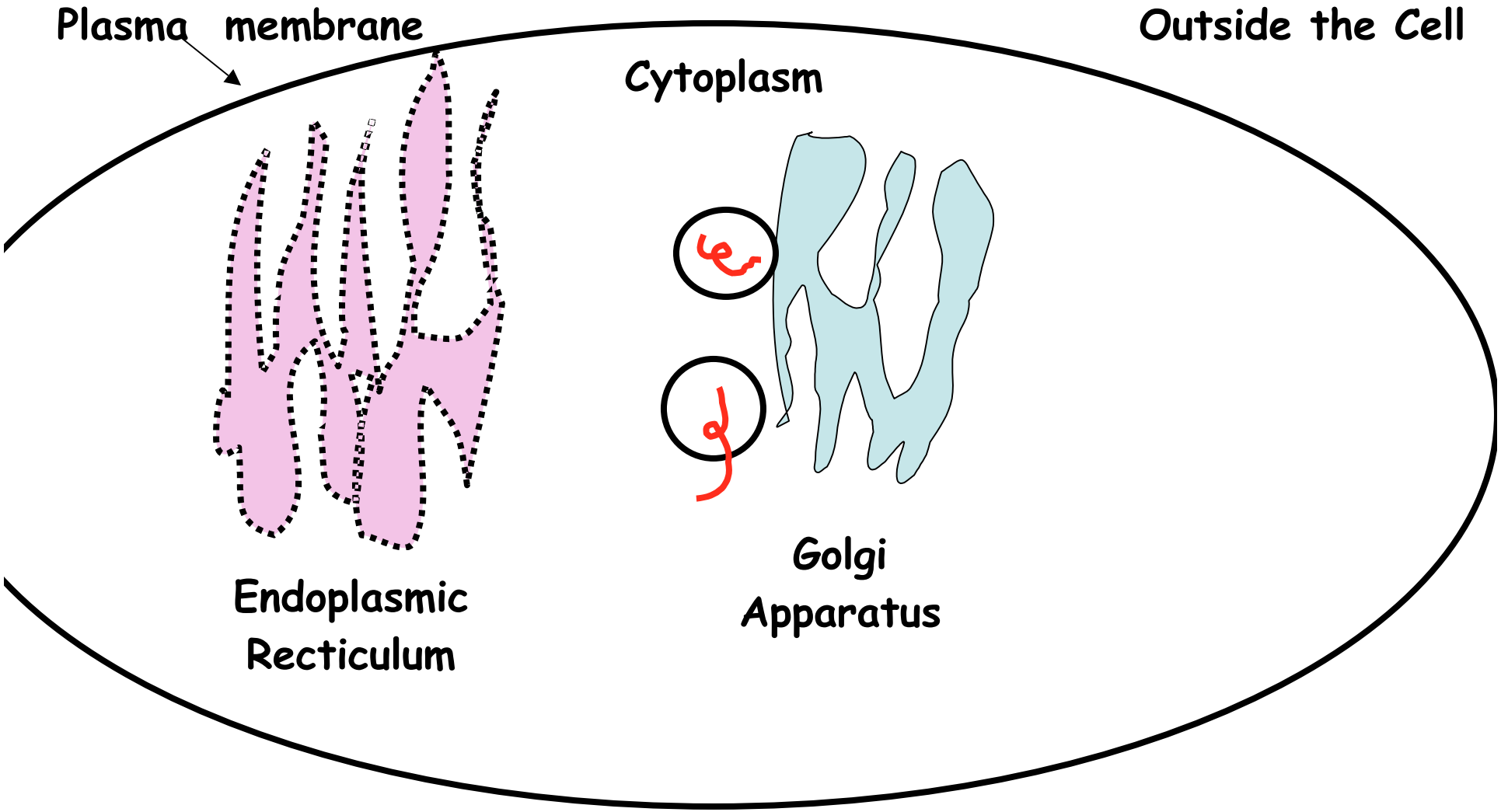

SRP receptor
"Docking
Protein"
Fully secreted Protein


SRP receptor
"Docking Protein"
Signal peptidase cleaves off the signal

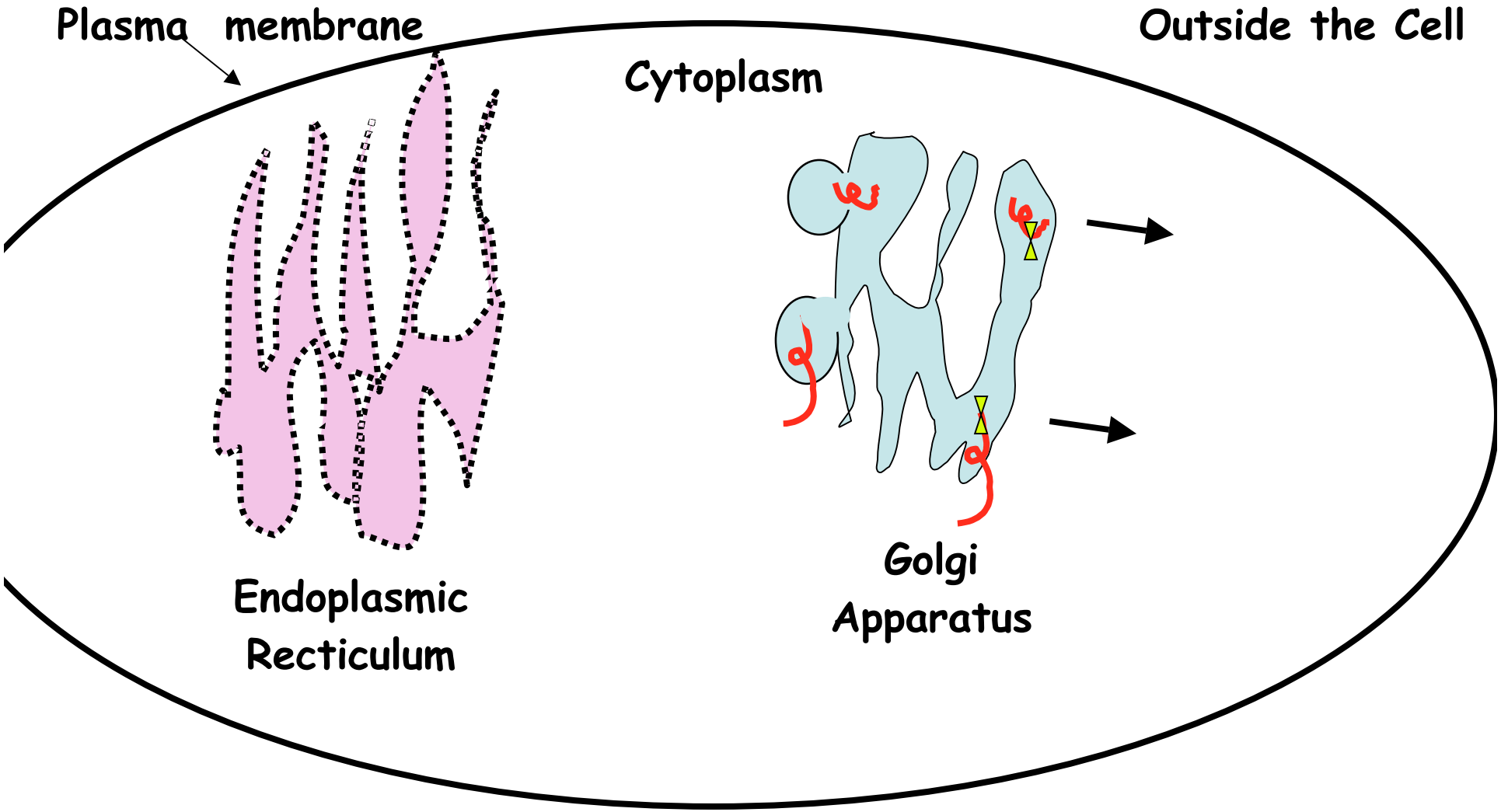

SRP receptor
"Docking
Protein"
Membrane protein



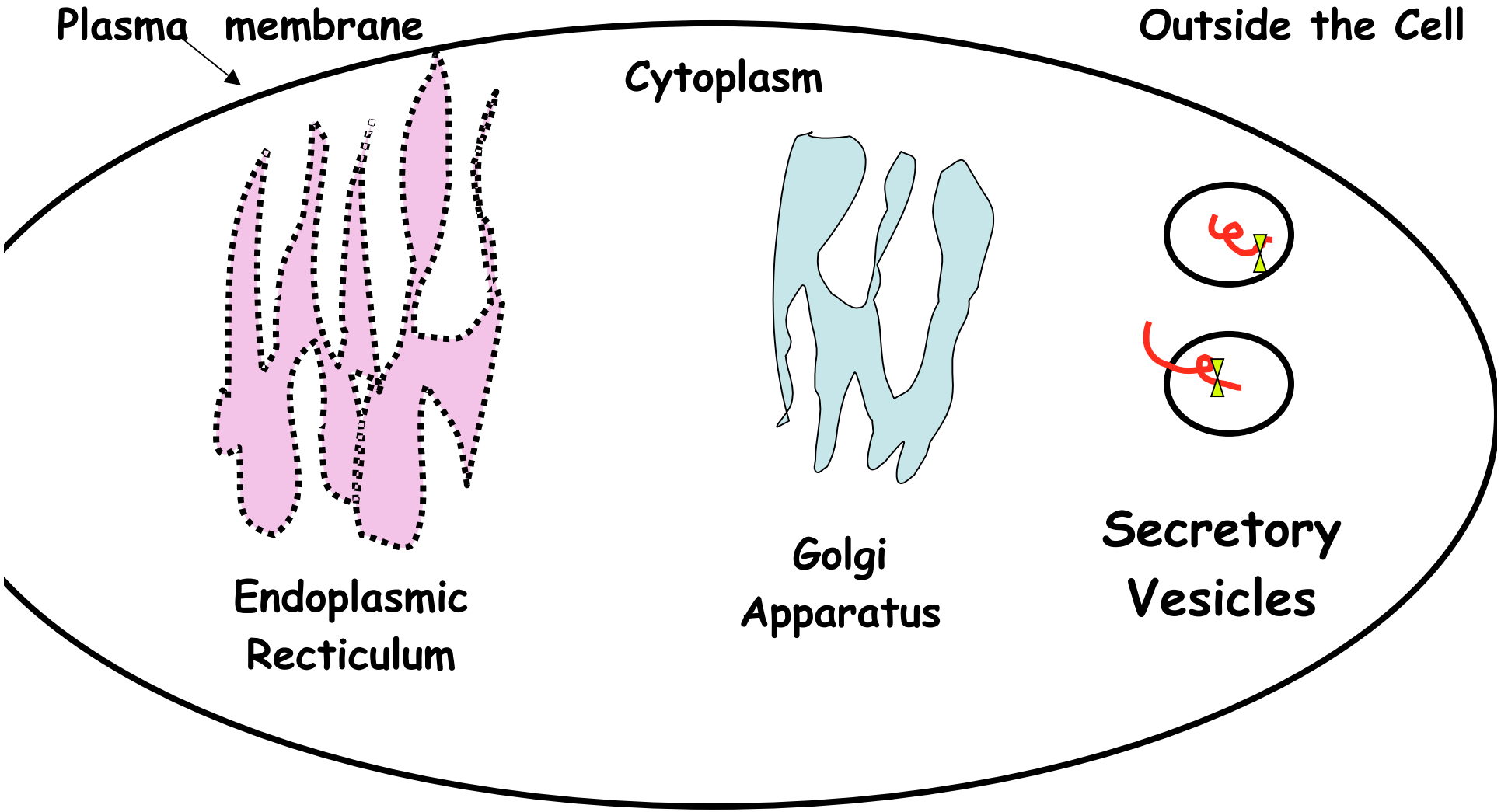
EUKARYOTIC CELL



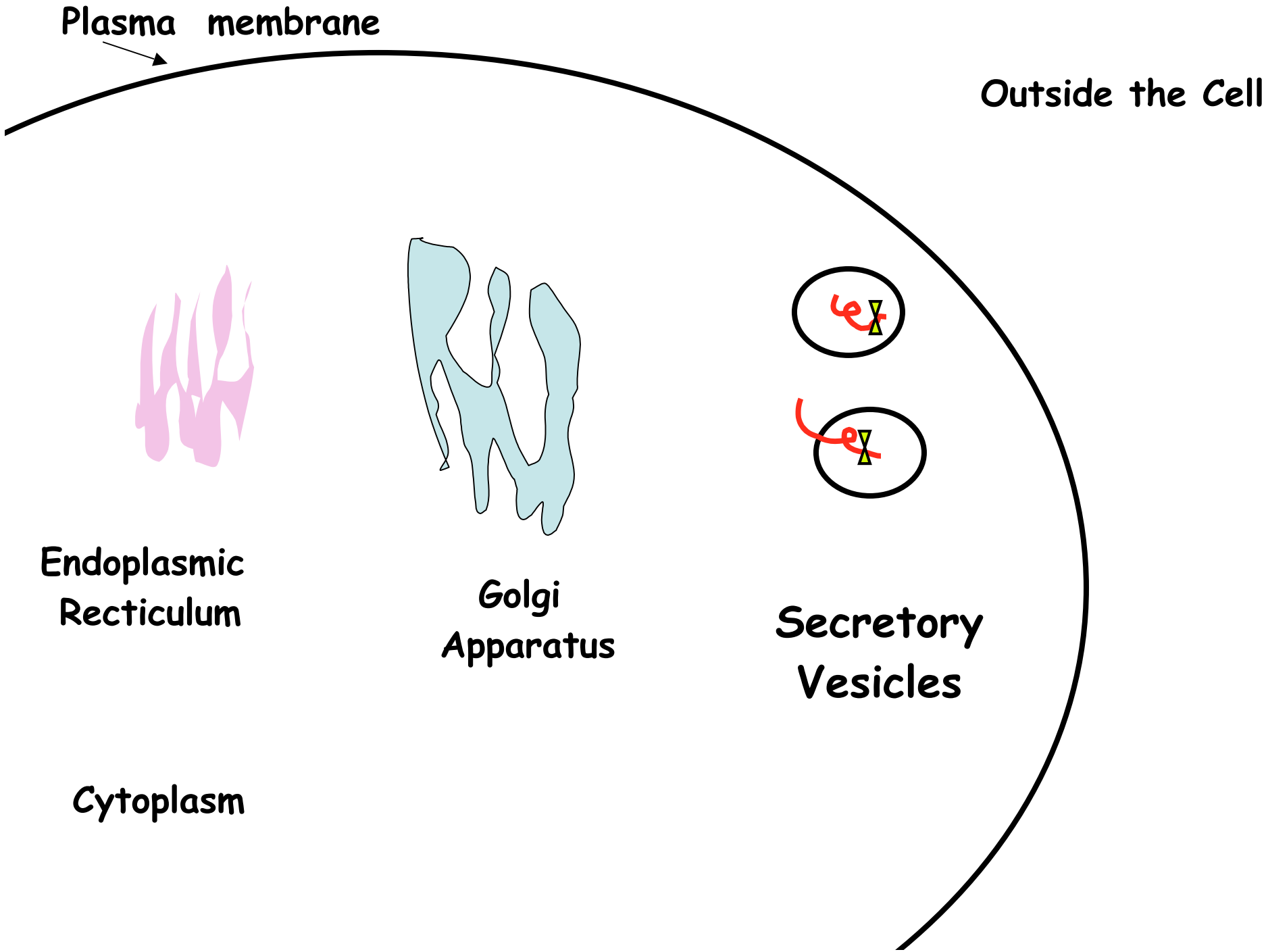
EUKARYOTIC CELL

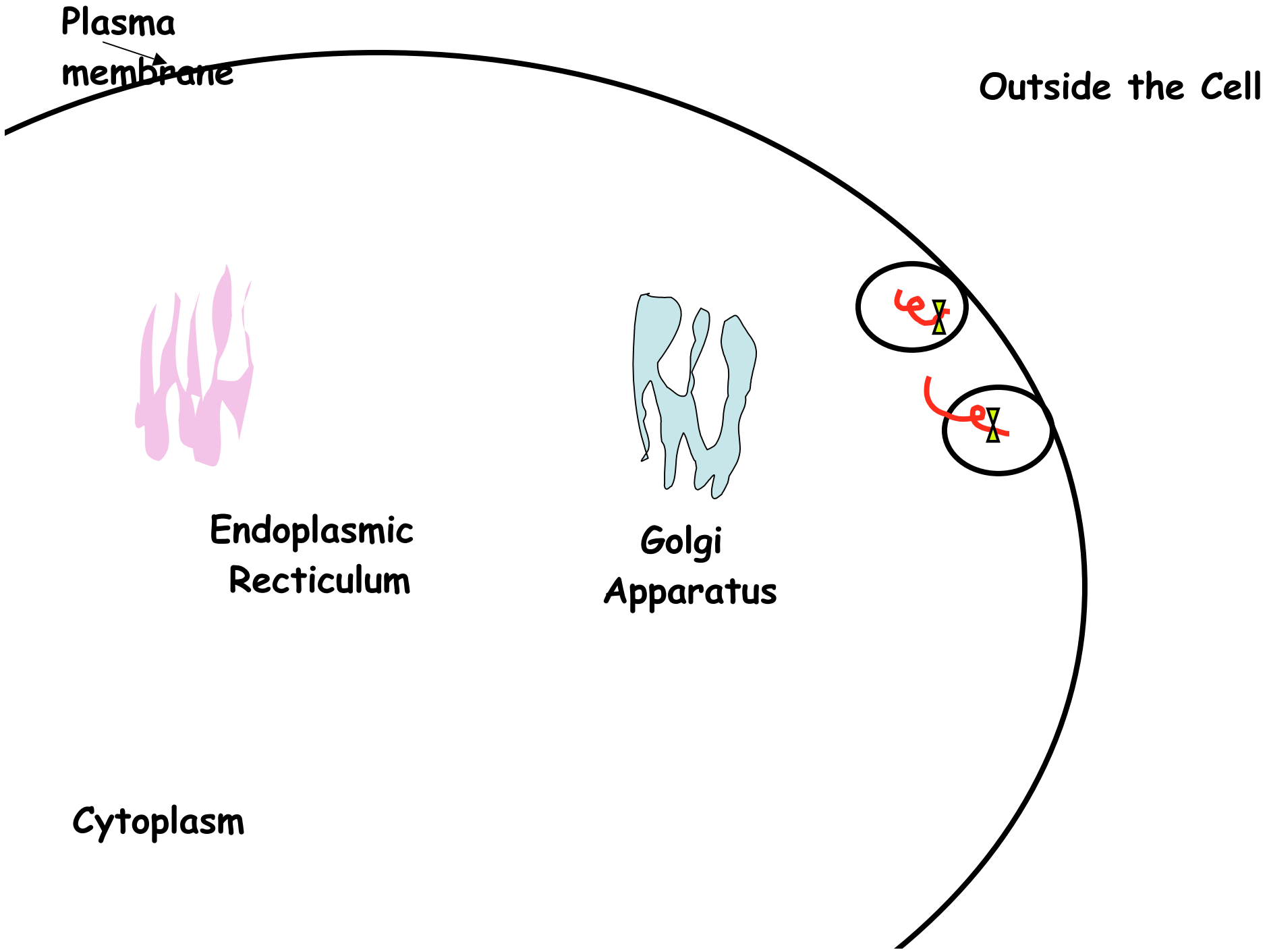


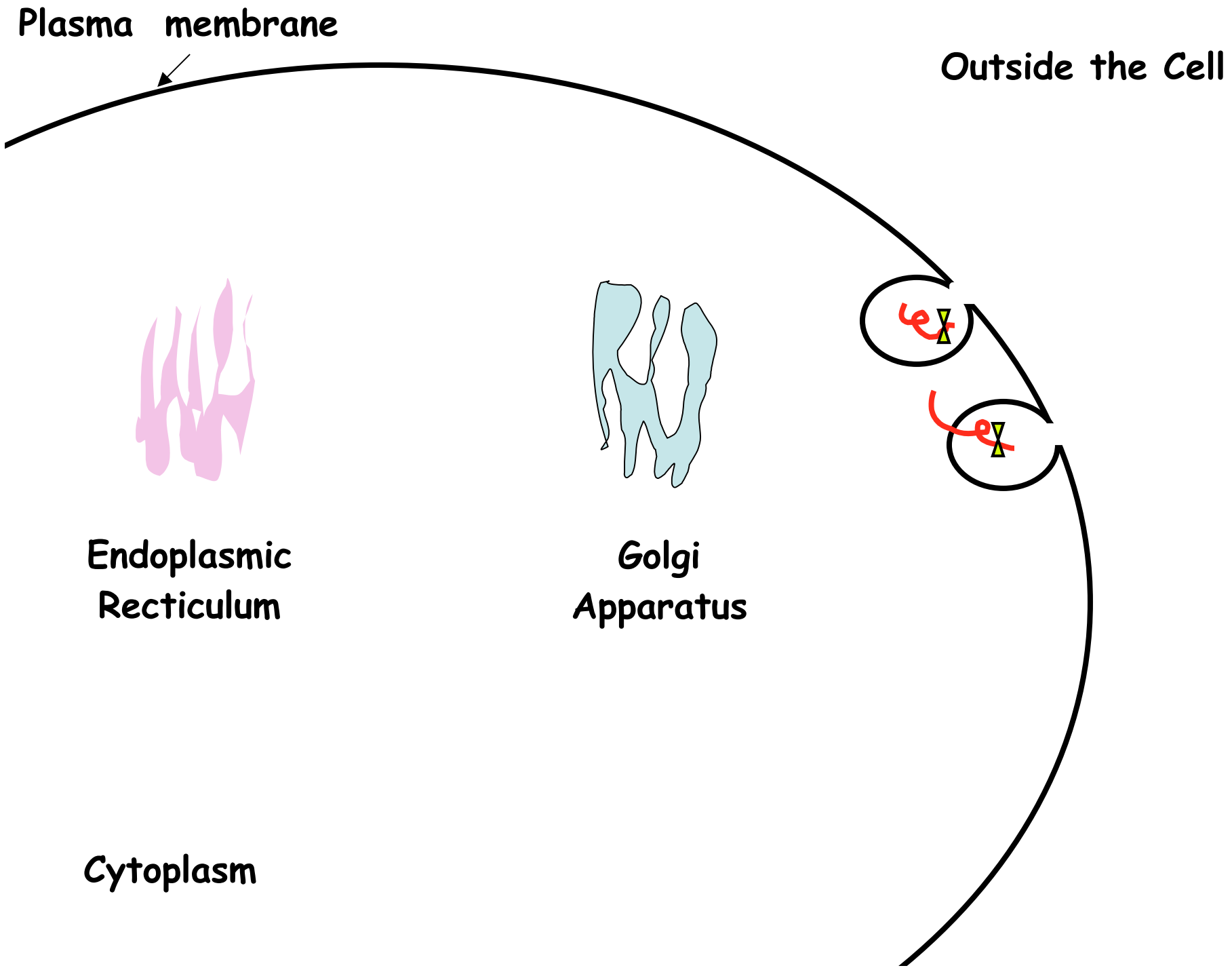
EUKARYOTIC CELL

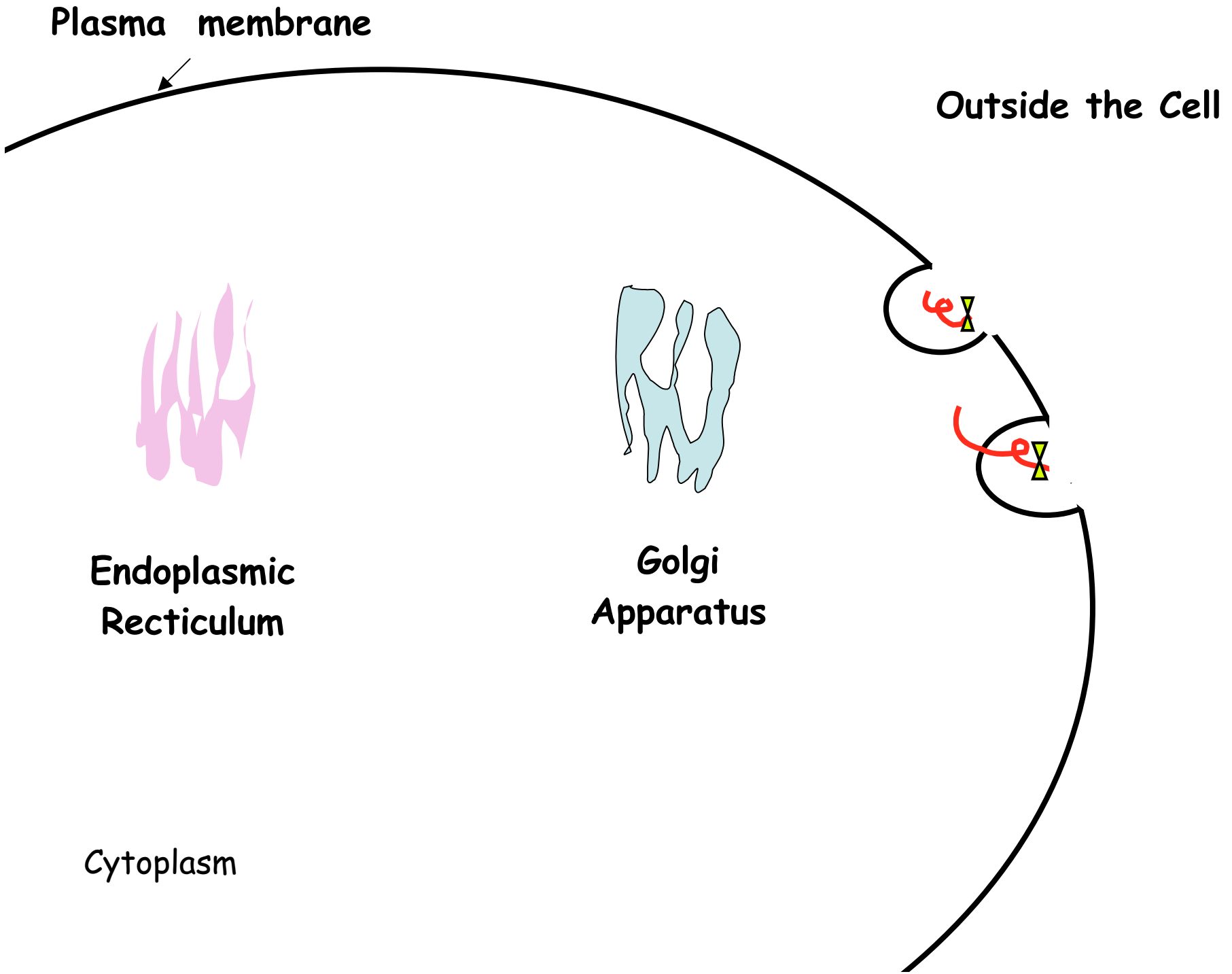


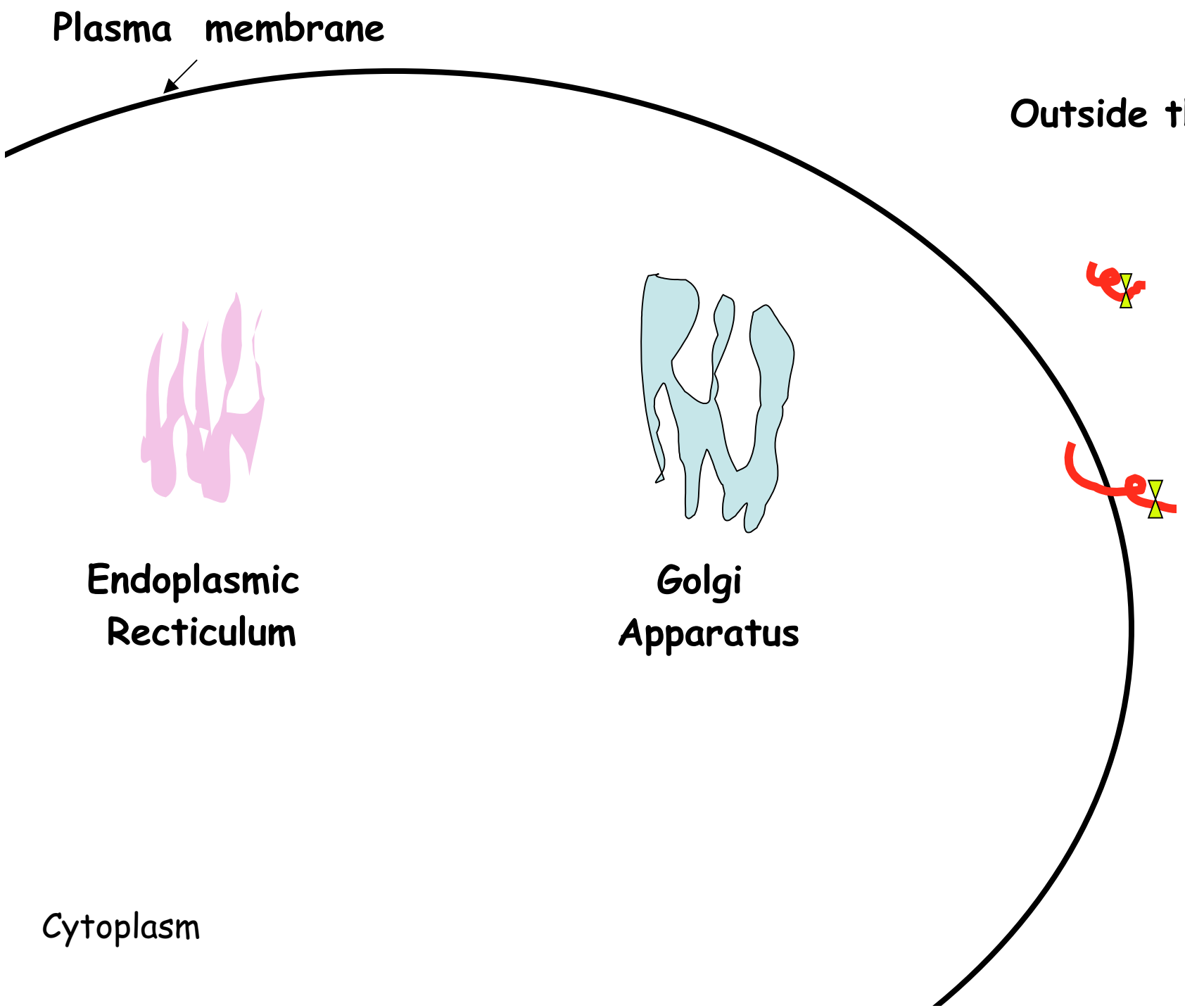
EUKARYOTIC CELL

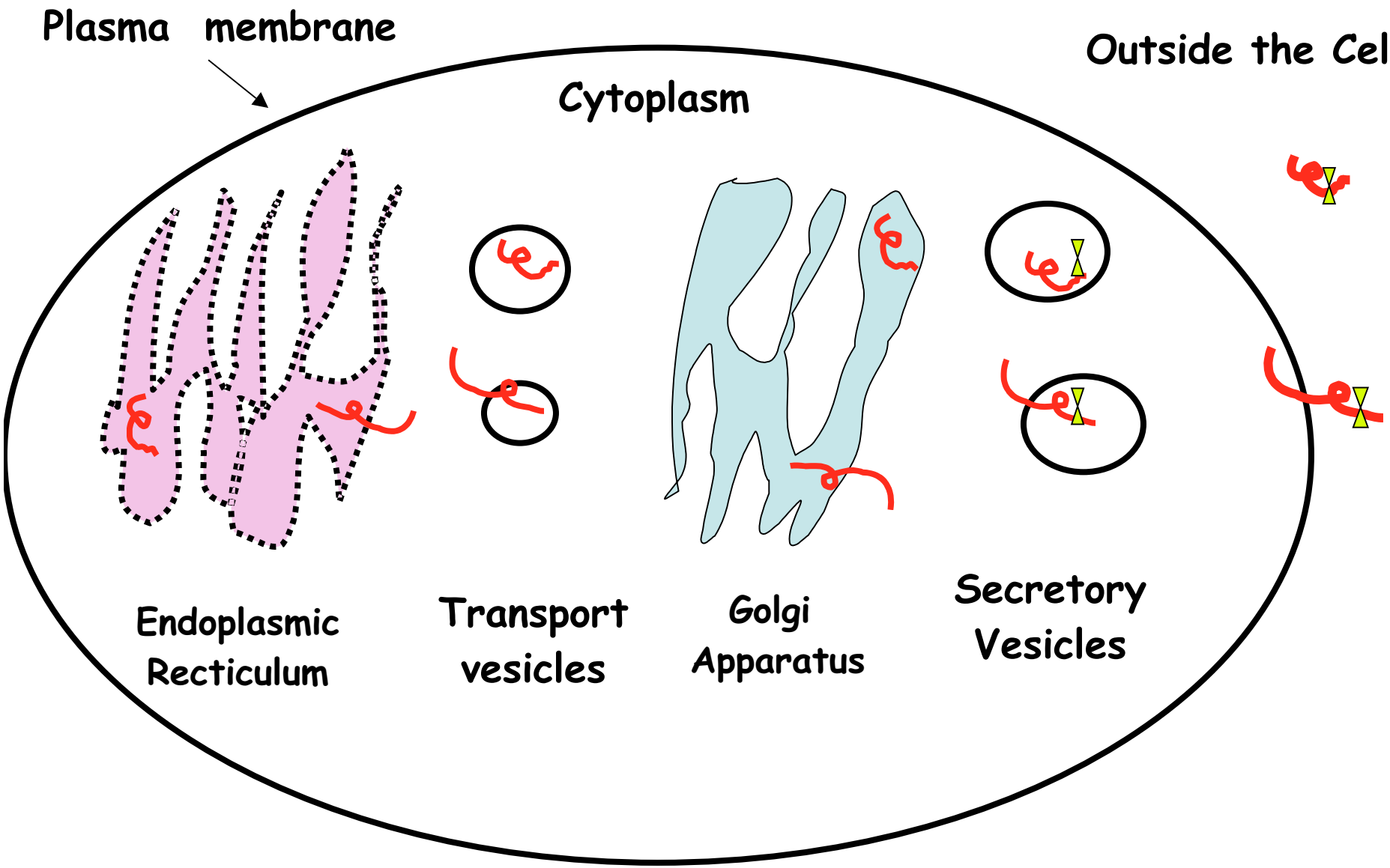

Plasma membrane
Outside the Cell
Endoplasmic Recticulum
Golgi Apparatus
Secretory Vesicles
Cytoplasm

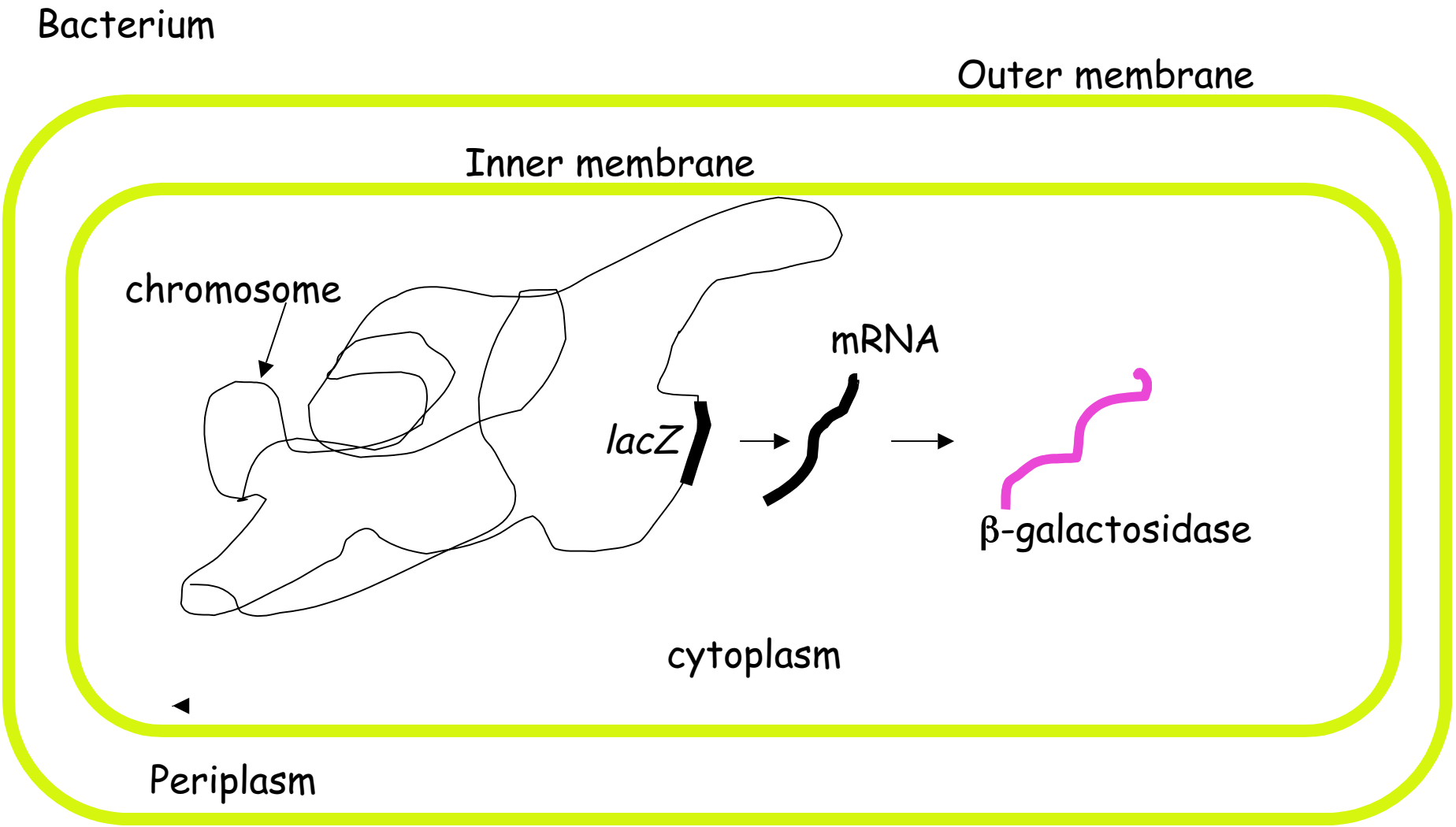

Plasma membrane
Outside the Cell
Endoplasmic Recticulum
Golgi Apparatus
Cytoplasm


Plasma membrane
Outside the Cell
Endoplasmic Recticulum
Golgi Apparatus
Cytoplasm


Plasma membrane
Outside the Cell
Endoplasmic Recticulum
Golgi Apparatus
Cytoplasm

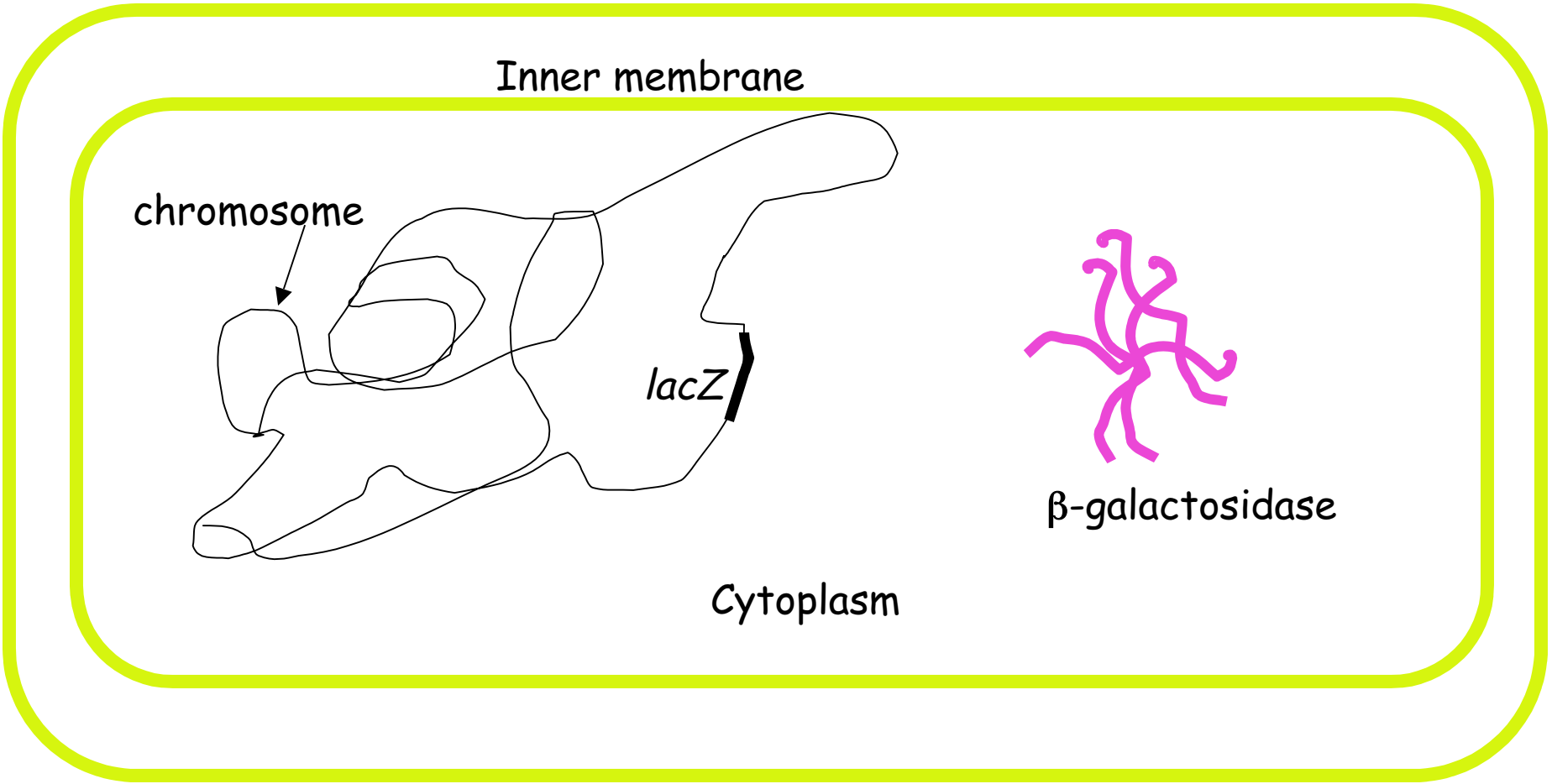

Plasma membrane
Outside the Cell
Endoplasmic Recticulum
Golgi Apparatus
Cytoplasm


Plasma membrane
Outside the Cell
Cytoplasm
Endoplasmic Recticulum
Transport vesicles
Golgi Apparatus
Secretory Vesicles



How were the *Sec* genes identified?

How were the *Sec* genes identified?



Active β-galactosidase is a tetramer.
This cell can utilize lactose as a carbon source. → **LAC+**

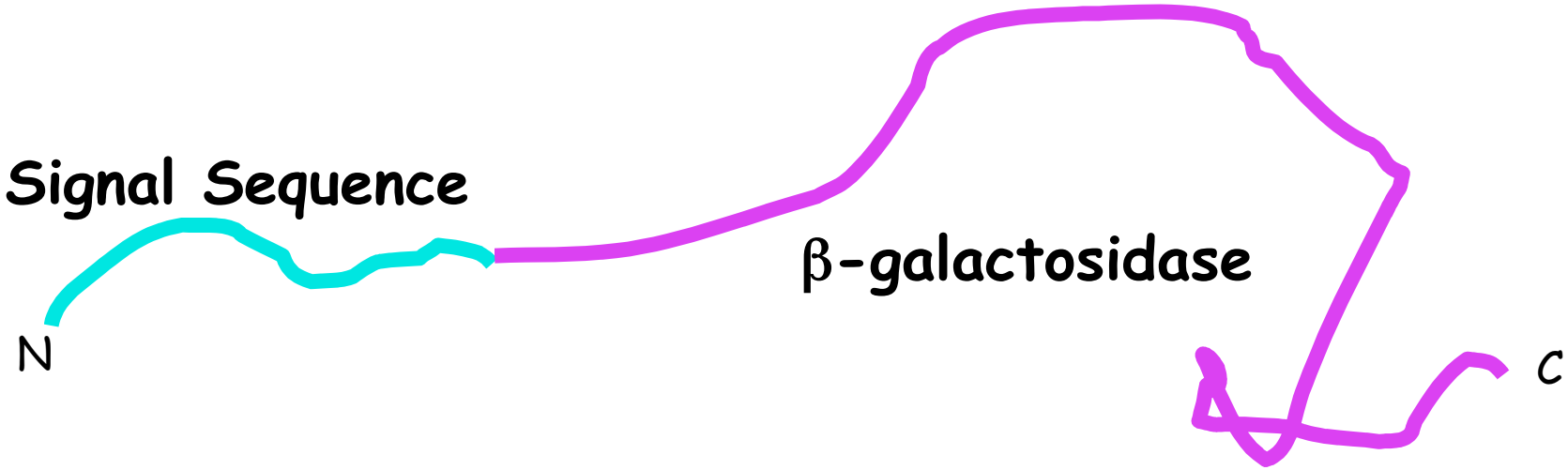
Gene Fusion

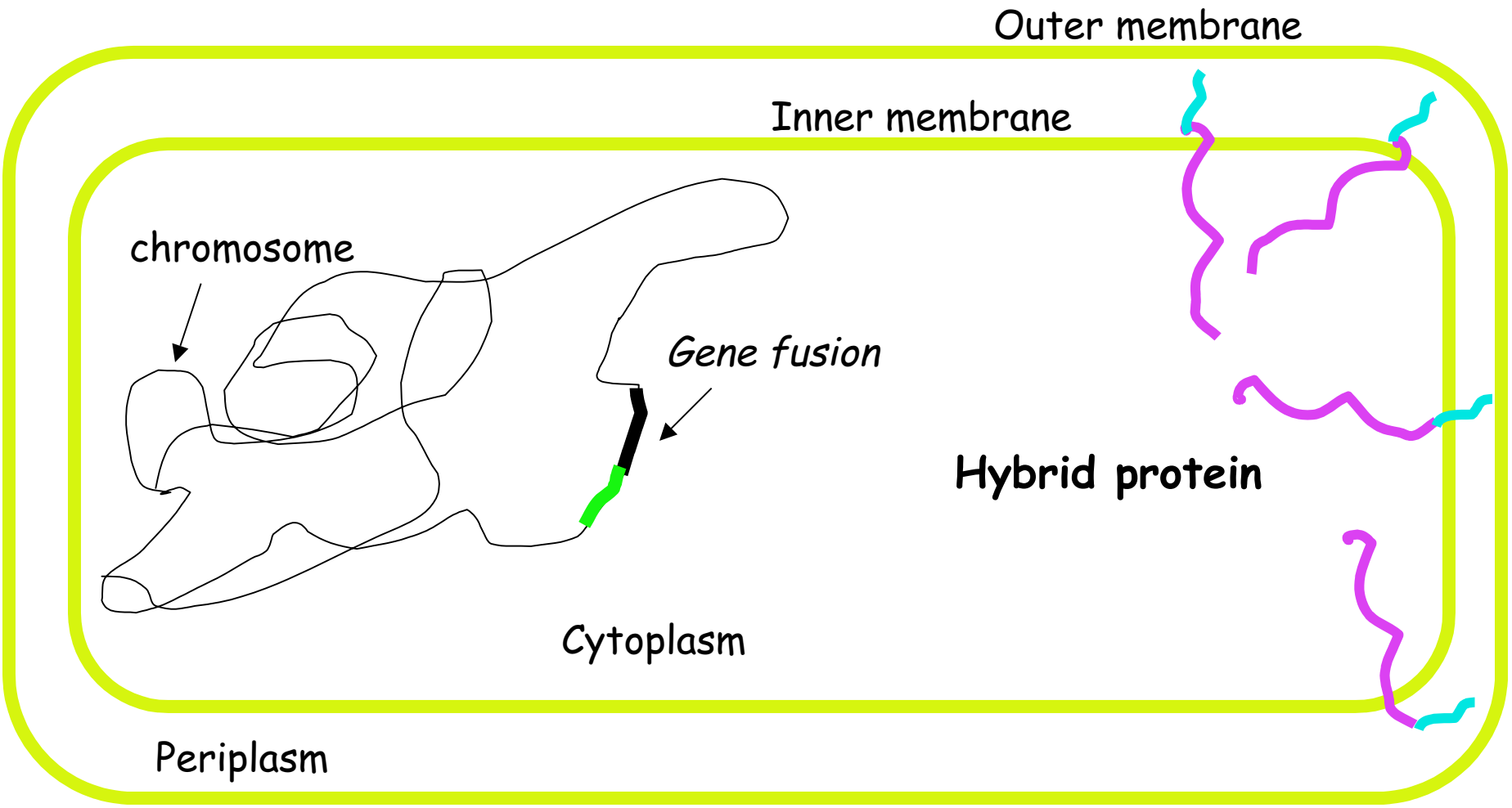
The 5′ end of the coding region of *lac Z* is fused to the 5′ end of a gene encoding an exported protein including the signal sequence.

Gene Fusion

Where the 5′ end of the *lac Z* gene is fused to the 5′ end of a gene encoding an exported protein including the signal sequence.

This gene fusion results in a hybrid protein where the N-terminus of β-Galactosidase is fused with a signal sequence.





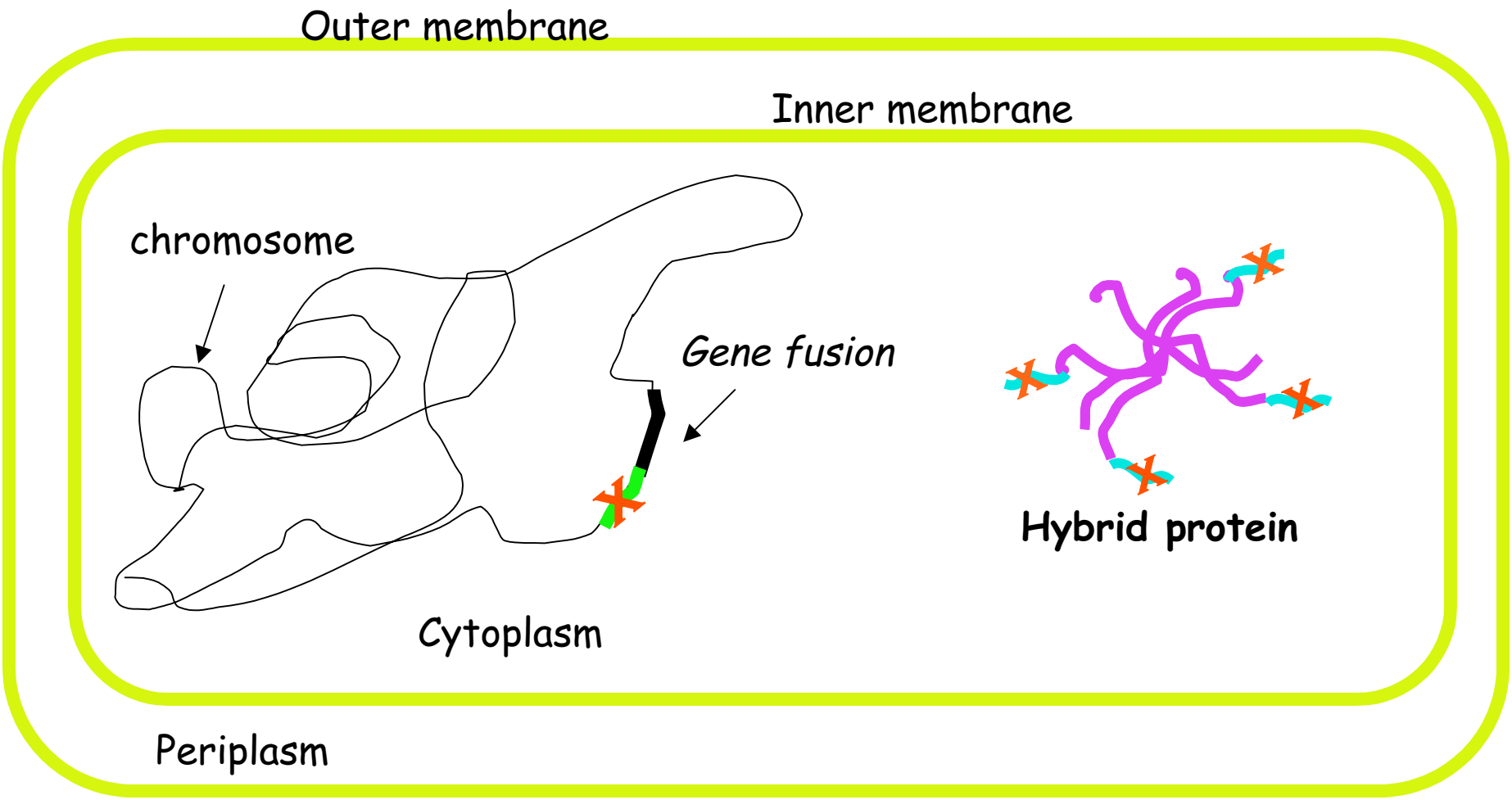
The hybrid protein protein localizes to the membrane.

This cell is unable to utilize Lactose as a Carbon Source.

Cells with this gene fusion are… LAC^-

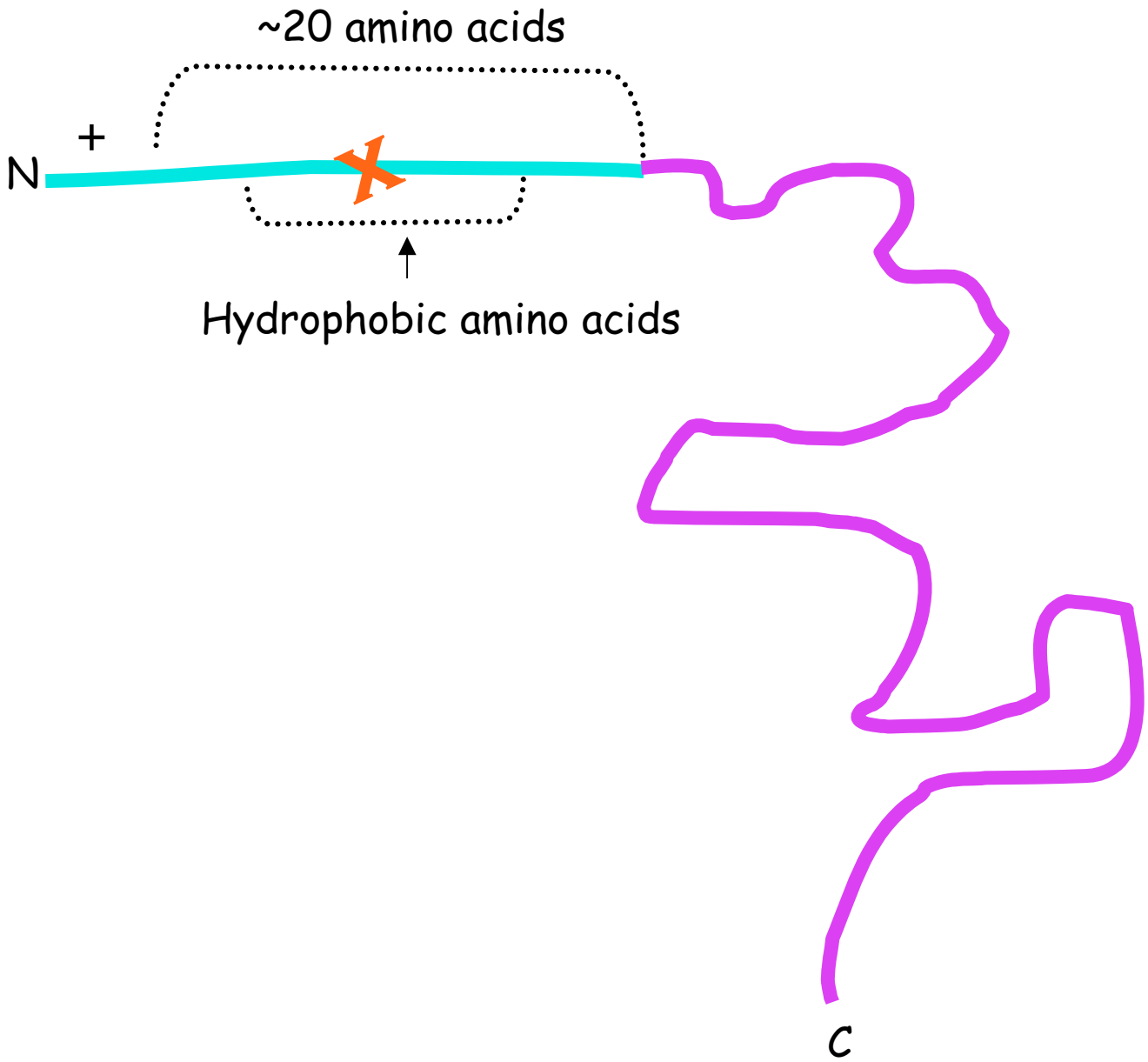
Jon Beckwith

LAC- ⟶ LAC+

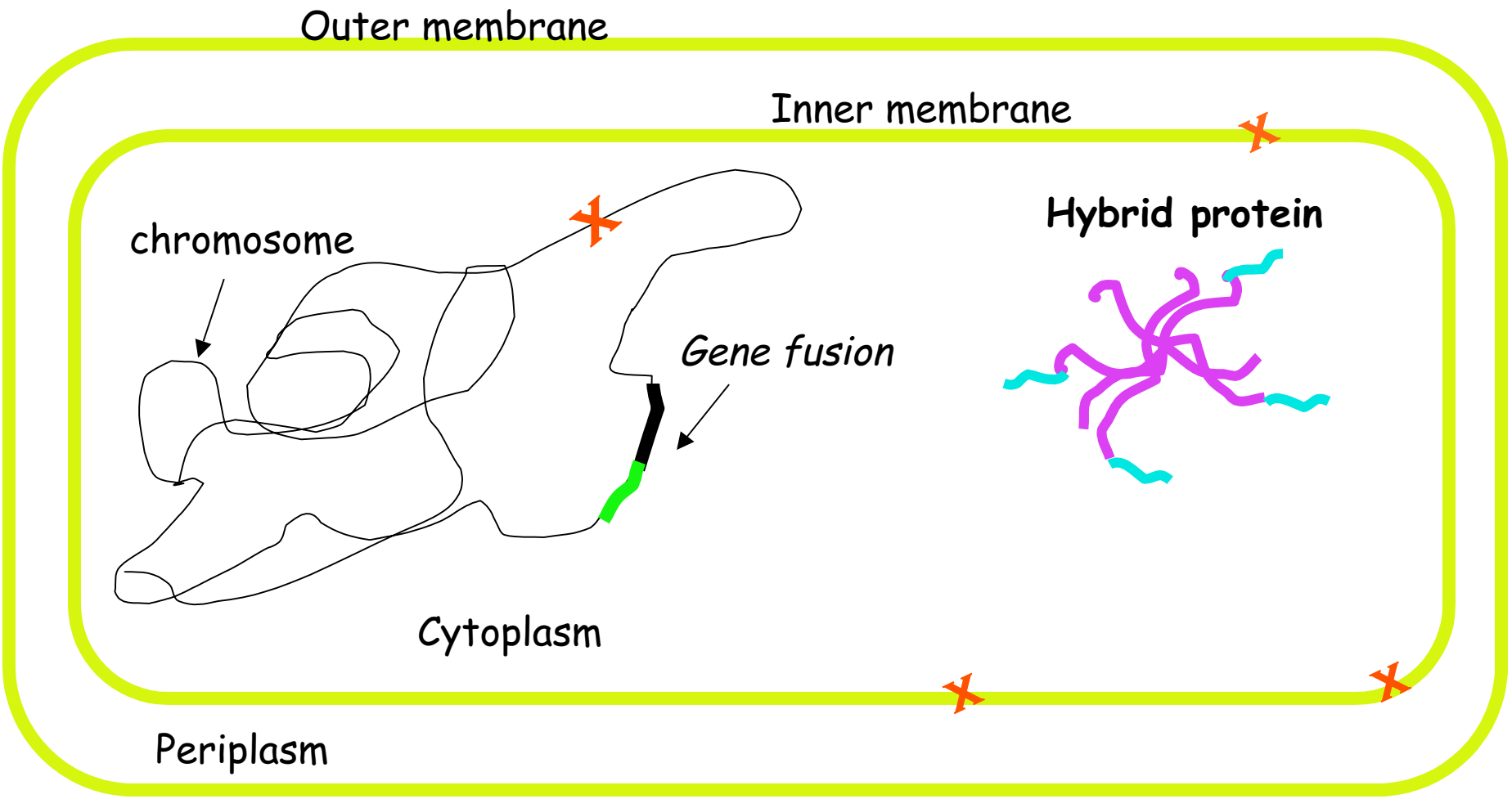


>95% of the Lac+ mutants have mutations .....

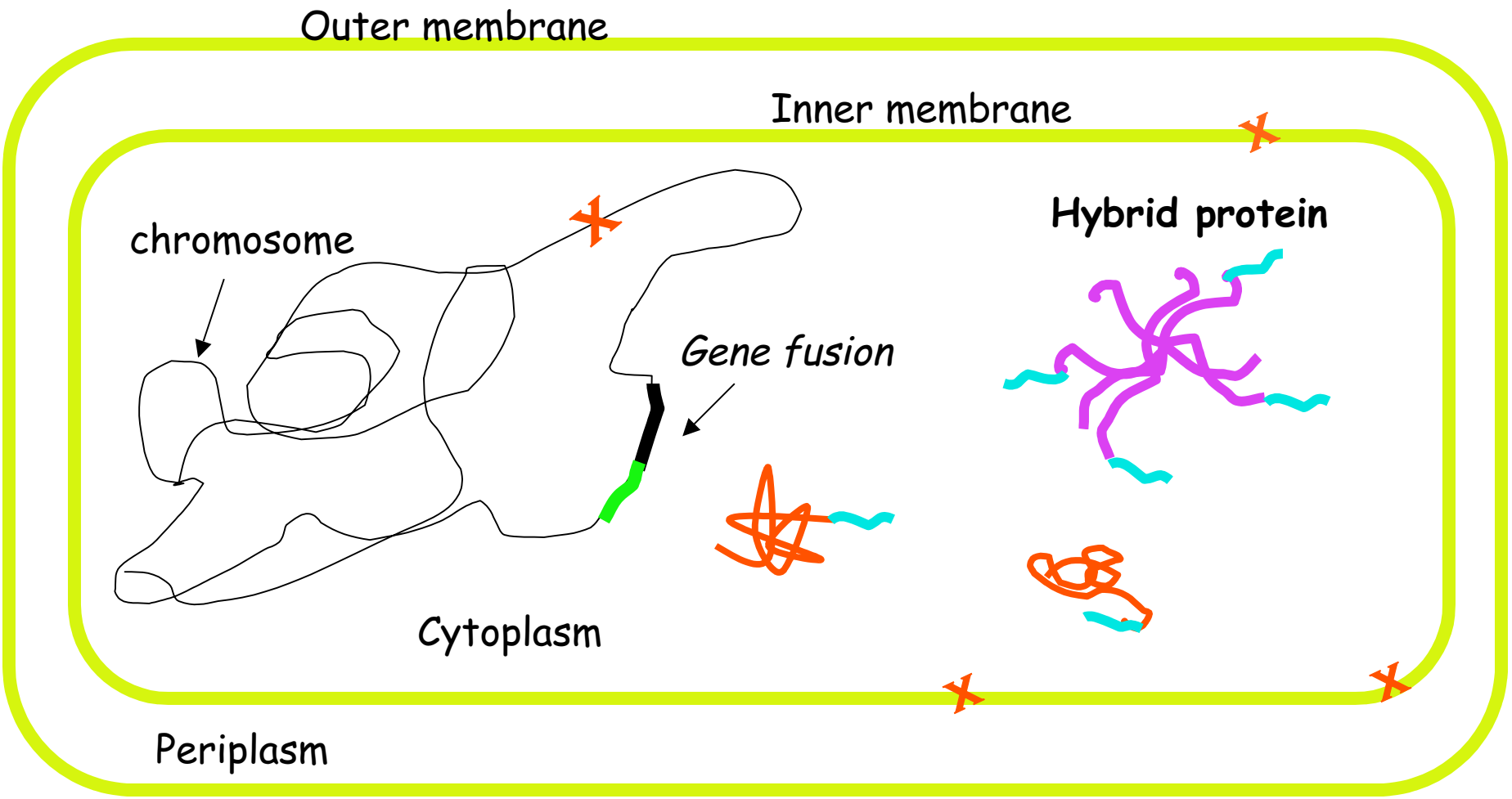
linked to the gene fusion resulting in ....?


~20 amino acids
+
N
Hydrophobic amino acids
C

LAC- ⟶ LAC+

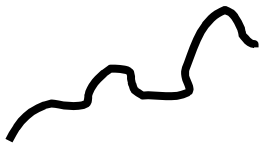

Outer membrane
Inner membrane
chromosome
Gene fusion
Hybrid protein
Cytoplasm
Periplasm

LAC- ⟶ LAC+


Outer membrane
Inner membrane
chromosome
Gene fusion
Hybrid protein
Cytoplasm
Periplasm

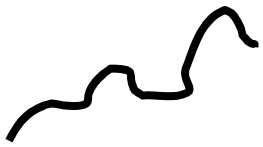
Conditional Lethal

How to get get Mutations in essential genes

	20°C	**37°C**
Temperature- sensitive		
	Active	**Inactive**
Cold-sensitive		
		Active

Conditional Lethal

How to get get Mutations in essential genes

	20°C	**37°C**
Temperature- sensitive		
	Active	**Inactive**
Cold-sensitive		
	Inactive	**Active**

Sec A

Sec B

Sec D

Sec E

Sec G

Sec Y

Destination →	Plasma membrane	Outside the cell	Mitochondrion	Nucleus
Signal	Signal Sequence	Signal Sequence	**N-terminal** Amphipathic Helix **20-50 aa**	Nuclear Localization Signal (NLS) **7aa + charged**
How does the protein cross the membrane?	SRP binds SS SRP binds DP Protein enters channel	SRP binds SS SRP binds DP Protein enters channel	Chaperones bind Protein enters Mito. Channel	Importins deliver to Nuclear Pore Complex (NPC)
Translational state of protein in channel	Cotranslational	Cotranslational	Post-translational	Post-Translational
What is the Energy Source?	Powered by translation	Powered by translation	ATP hydrolysis	GTP hydrolysis
Signal Cleaved?	Yes	Yes	Yes	NO