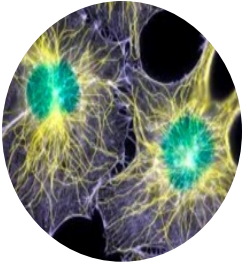


Fisiologia Celular e Molecular

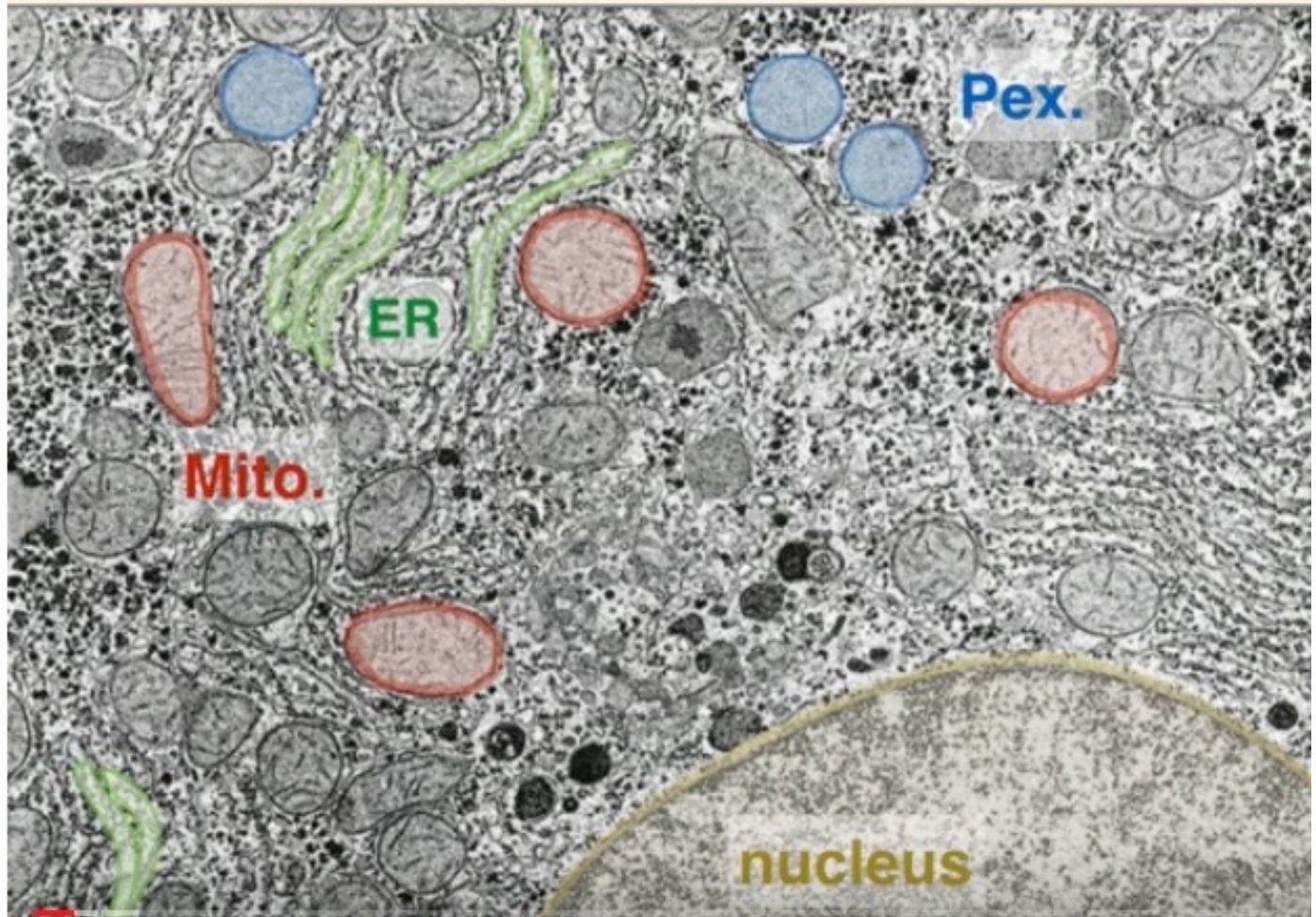
T5 Transporte de proteínas na célula

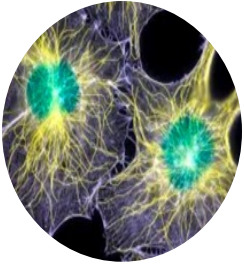
Cristina Cruz



Fisiologia Celular e Molecular

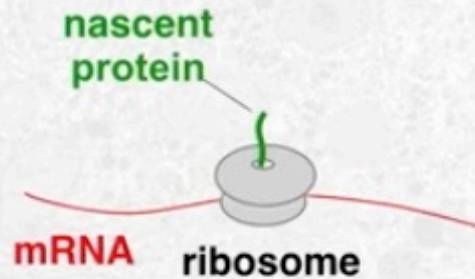
Cells are highly organised into numerous compartments





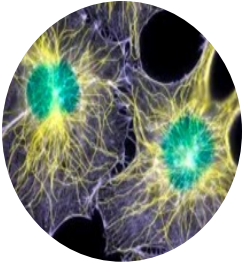
Fisiologia Celular e Molecular

The massive scale of protein synthesis & maturation



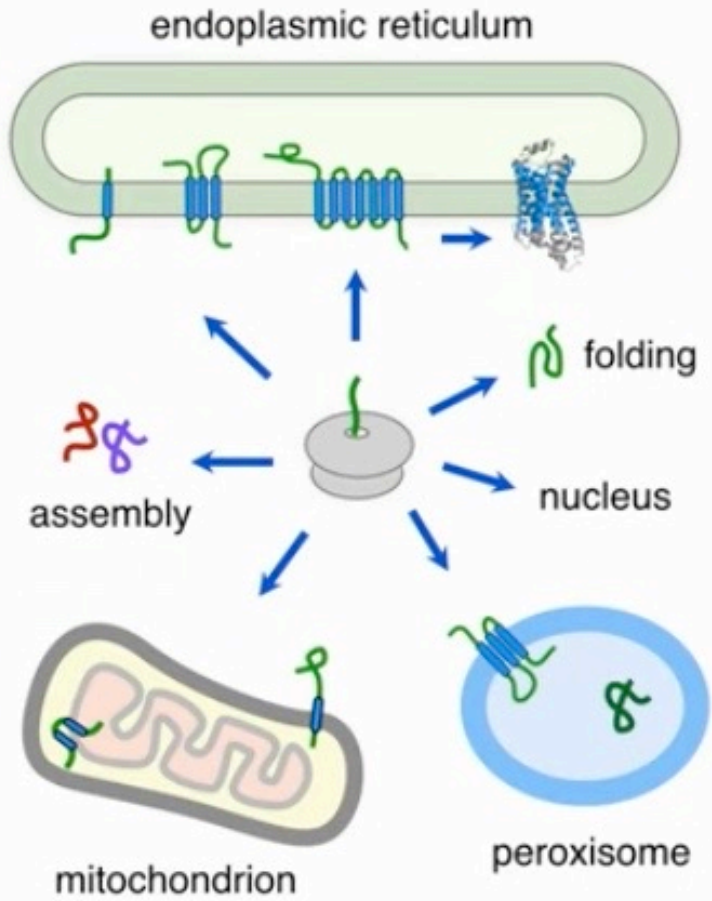
~10 million or more per cell

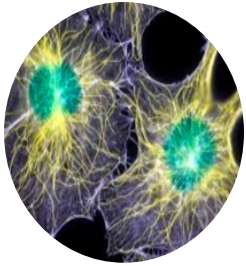
new protein every 1-2 min



Fisiologia Celular e Molecular

The massive scale of protein synthesis & maturation

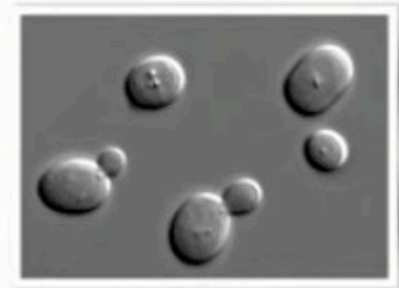




Fisiologia Celular e Molecular

Protein secretion is ubiquitous

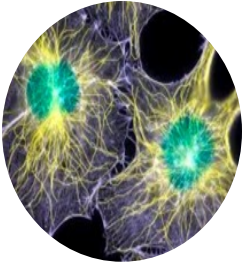
Mammals



Yeast



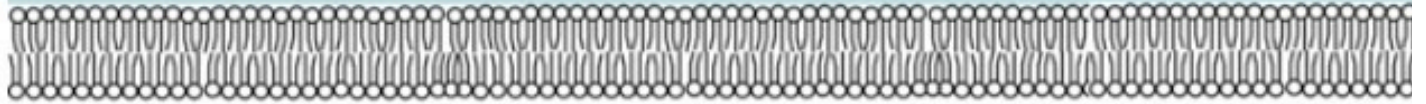
Bacteria



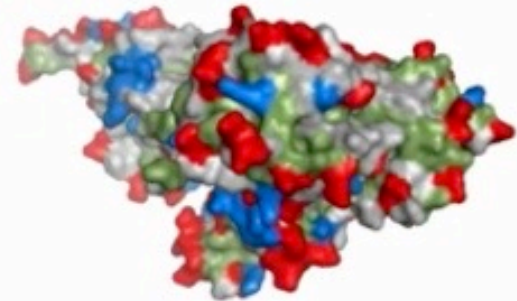
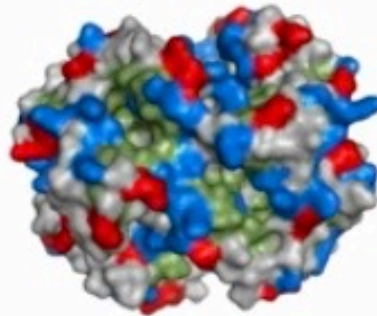
Fisiologia Celular e Molecular

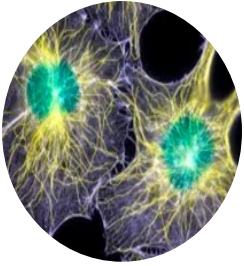
How are proteins moved across membrane barriers?

Outside



Inside





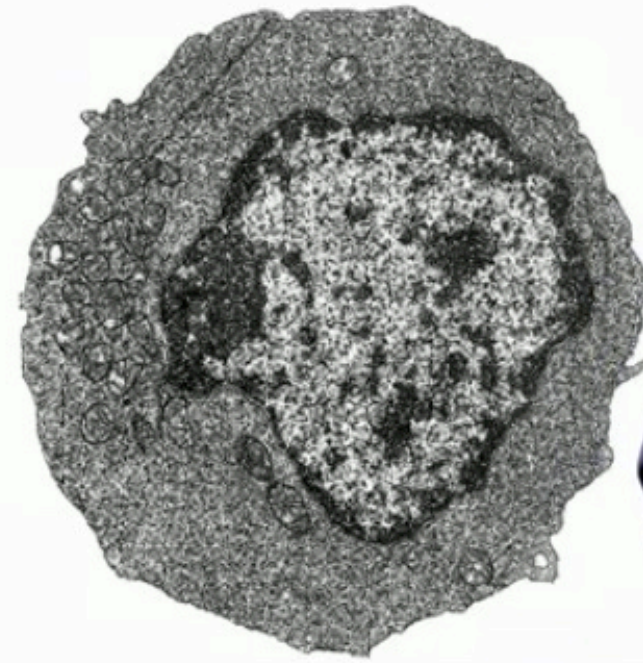
Fisiologia Celular e Molecular

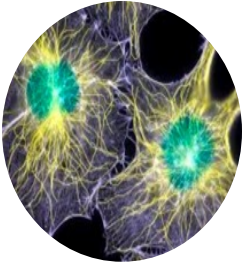
Correlation between rough ER and secretion

Antibody-secreting plasma cell



T-lymphocyte

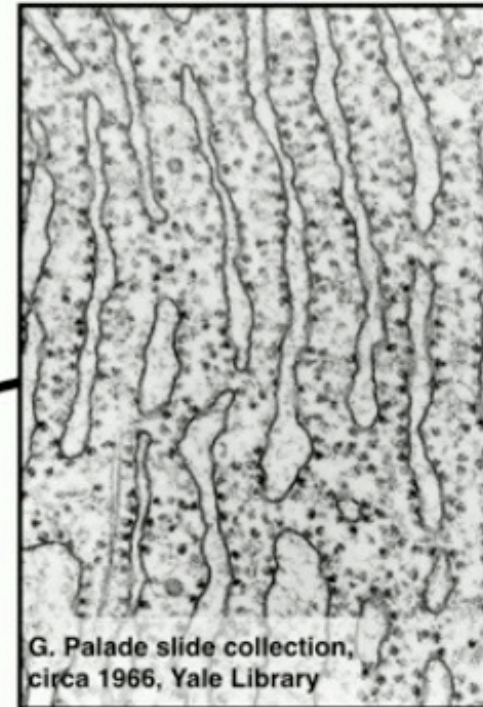




Fisiologia Celular e Molecular

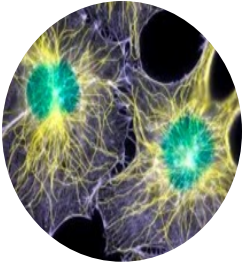
Correlation between rough ER and secretion

Antibody-secreting plasma cell



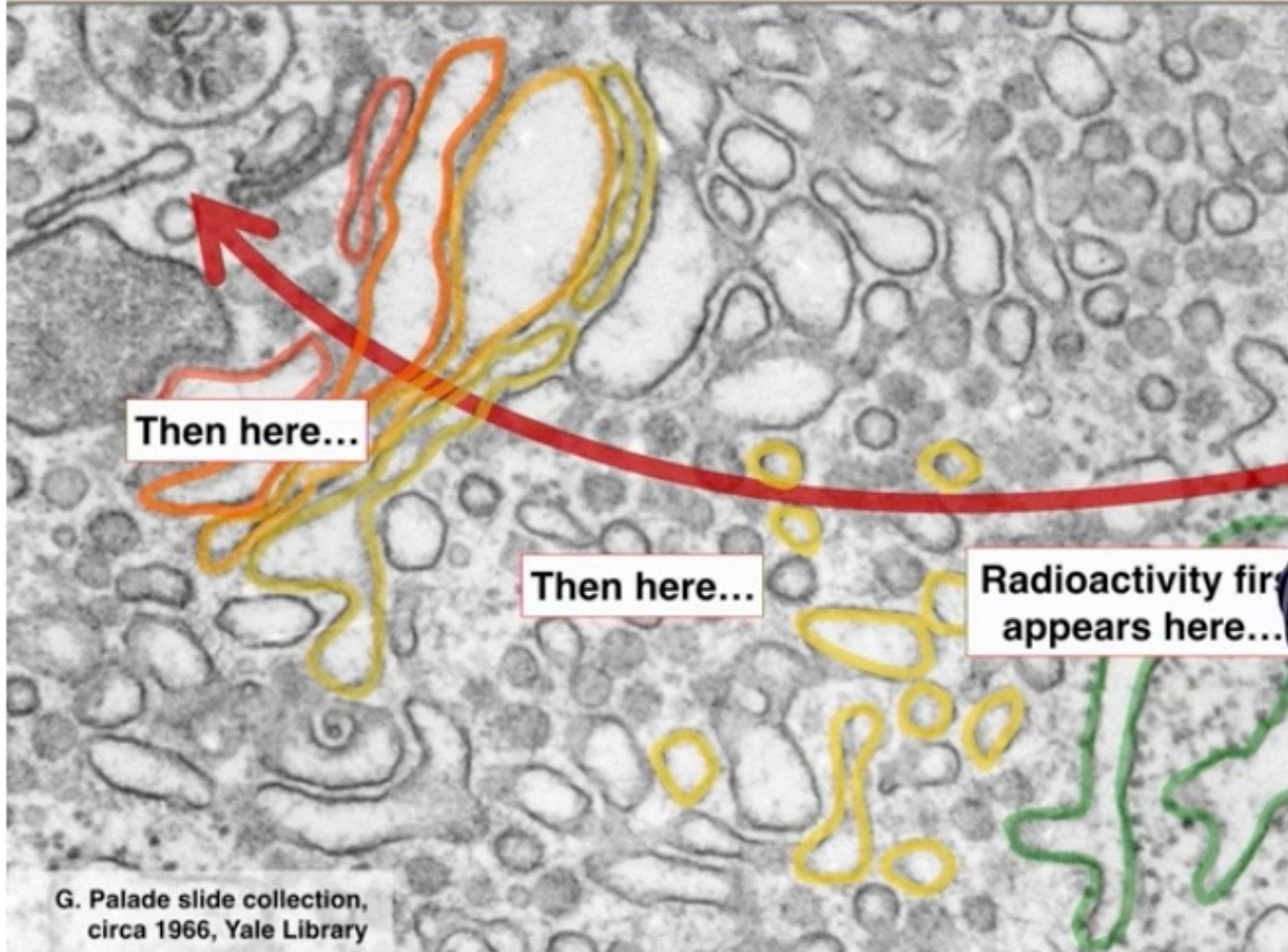
G. Palade slide collection,
circa 1966, Yale Library

the ER of a secretory cell

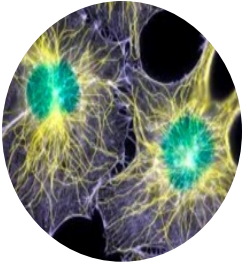


Fisiologia Celular e Molecular

The 'secretory pathway' of eukaryotic cells

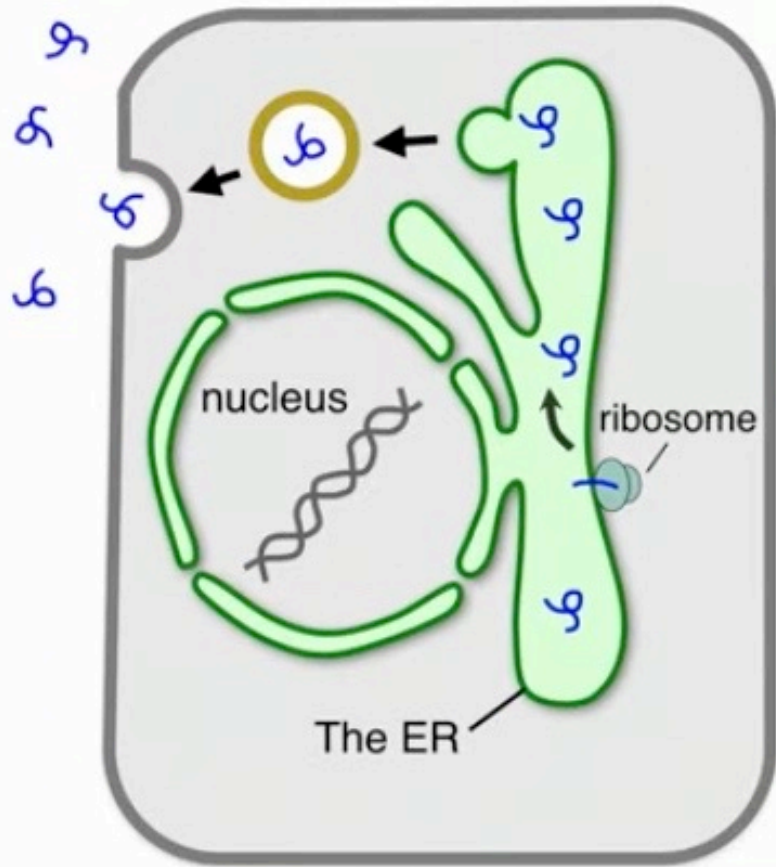


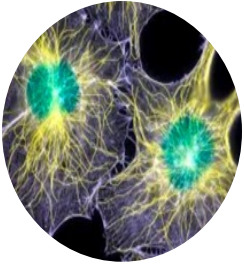
G. Palade slide collection, circa 1966, Yale Library



Fisiologia Celular e Molecular

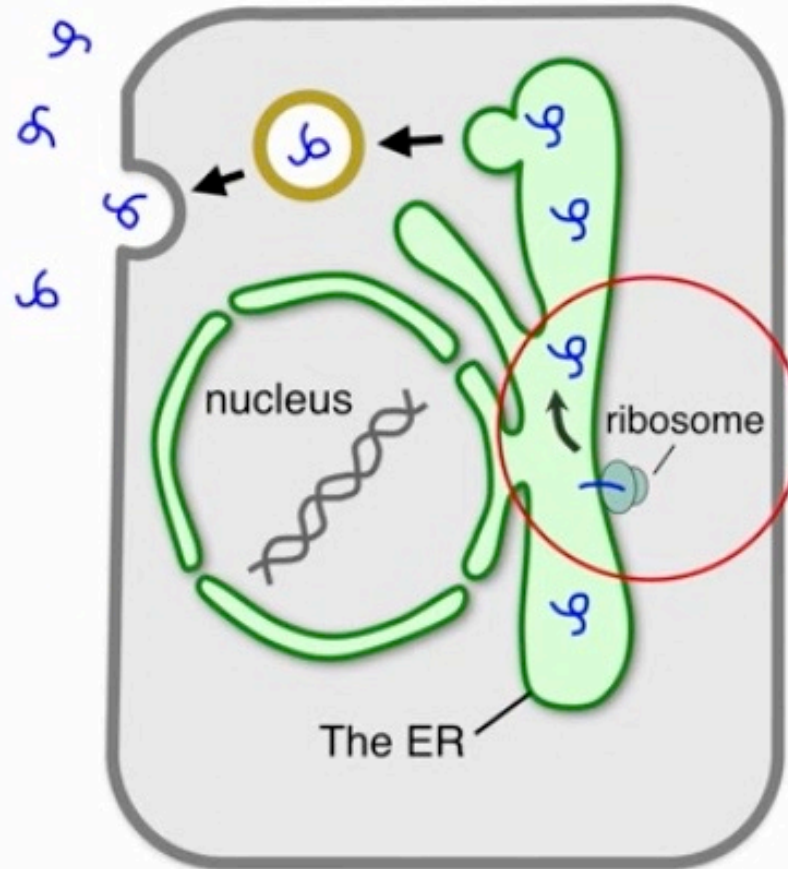
Entry into the ER is the first step of protein secretion



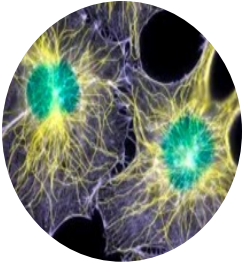


Fisiologia Celular e Molecular

Entry into the ER is the first step of protein secretion

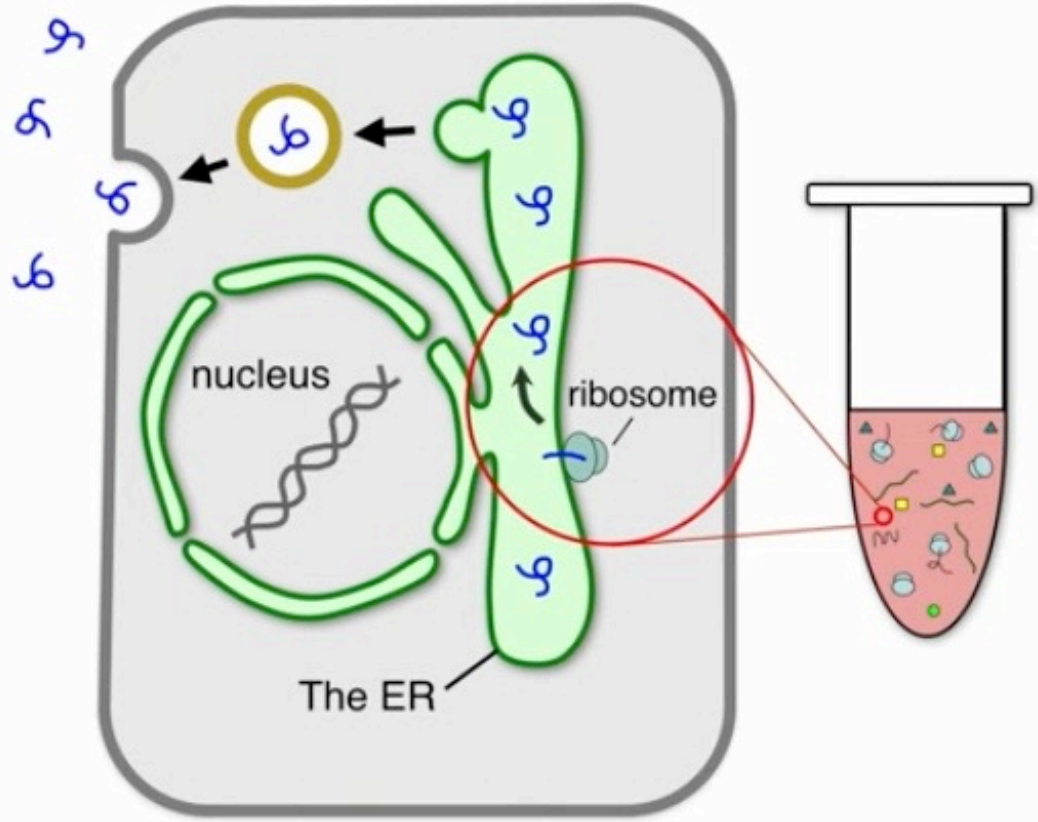


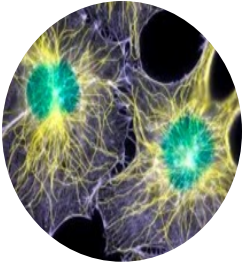
how does a protein get into the ER?



Fisiologia Celular e Molecular

Analysis of protein translocation in a cell-free reaction

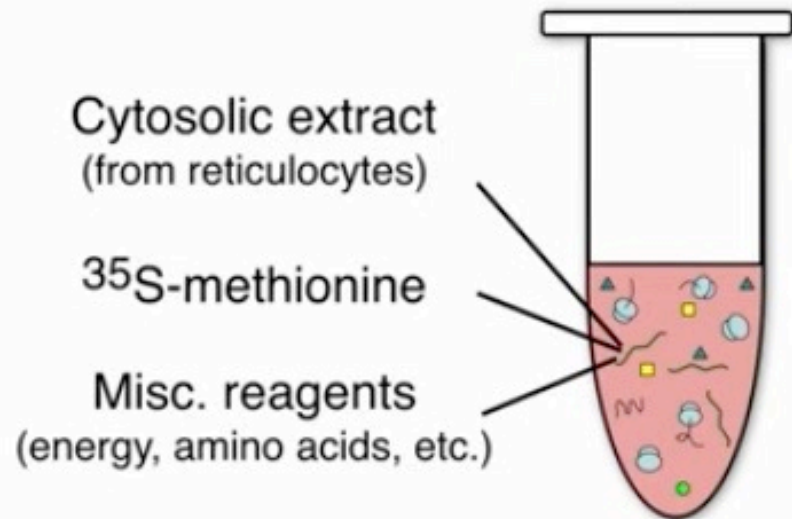


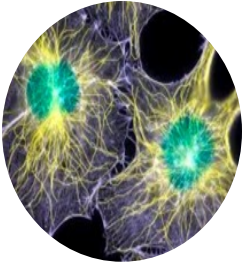


Fisiologia Celular e Molecular

Assembly of a cell-free protein translocation system

Part 1: translation extract





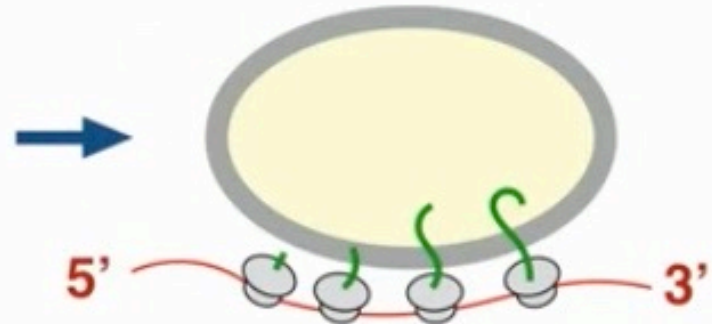
Fisiologia Celular e Molecular

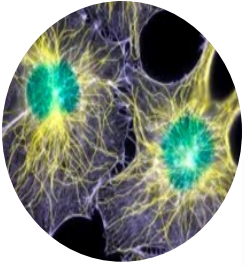
Assembly of a cell-free protein translocation system



highly secretory
cells or tissue

Part 2: ER 'microsomes'



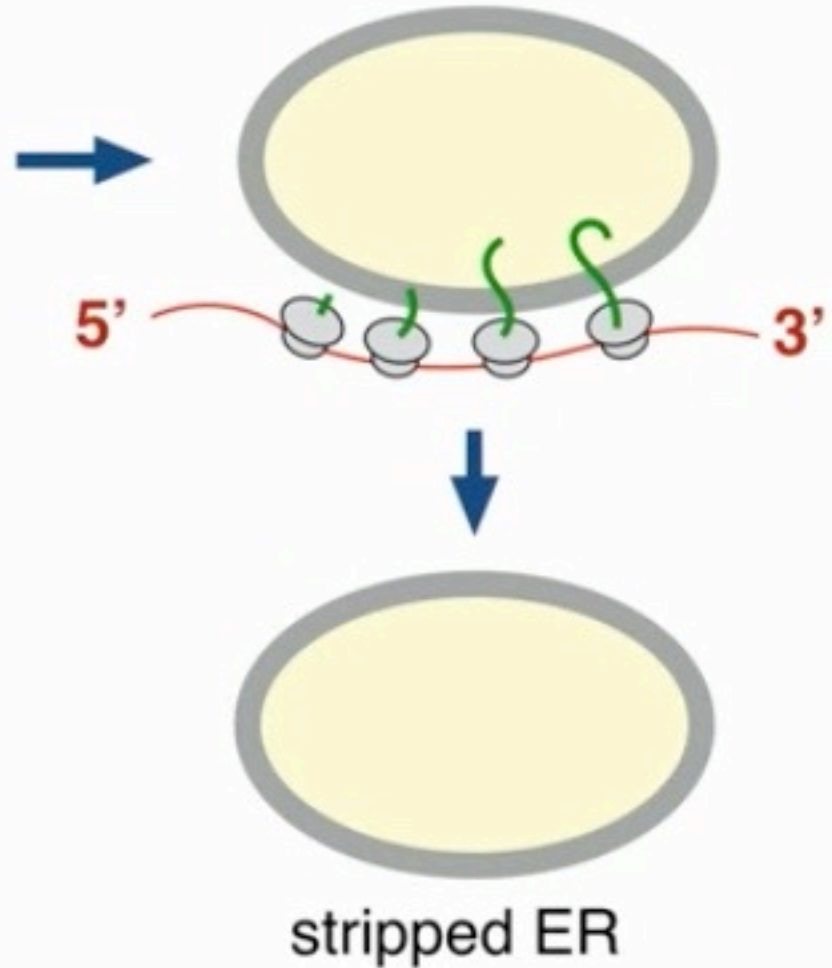


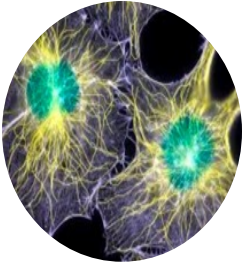
Fisiologia Celular e Molecular



highly secretory
cells or tissue

Part 2: ER 'microsomes'





Fisiologia Celular e Molecular

Assembly of a cell-free protein translocation system

Part 3: mRNA for secreted protein

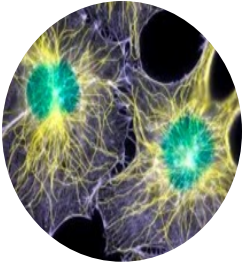


antibody-secreting
tumour cells



total RNA

IgG heavy chain
IgG light chain



Fisiologia Celular e Molecular

Reconstitution of protein translocation from isolated fractions

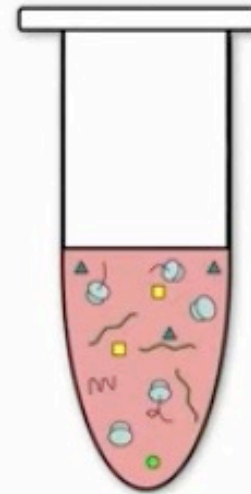
stripped ER
'microsomes'

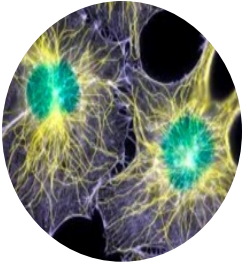


+

IgG
mRNA

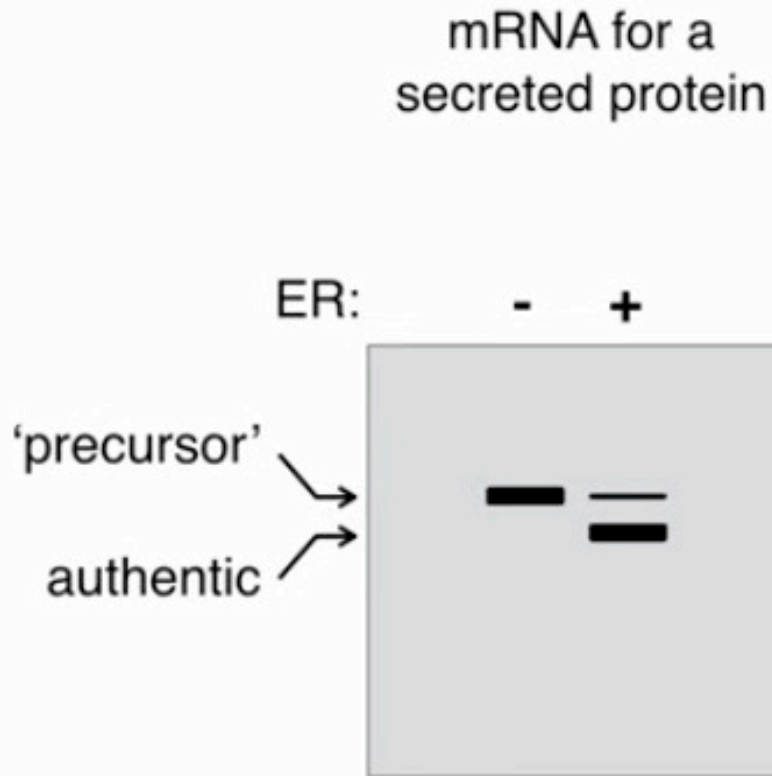
+

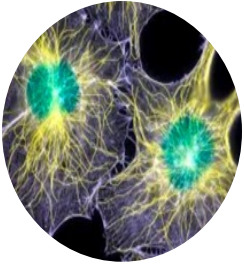




Fisiologia Celular e Molecular

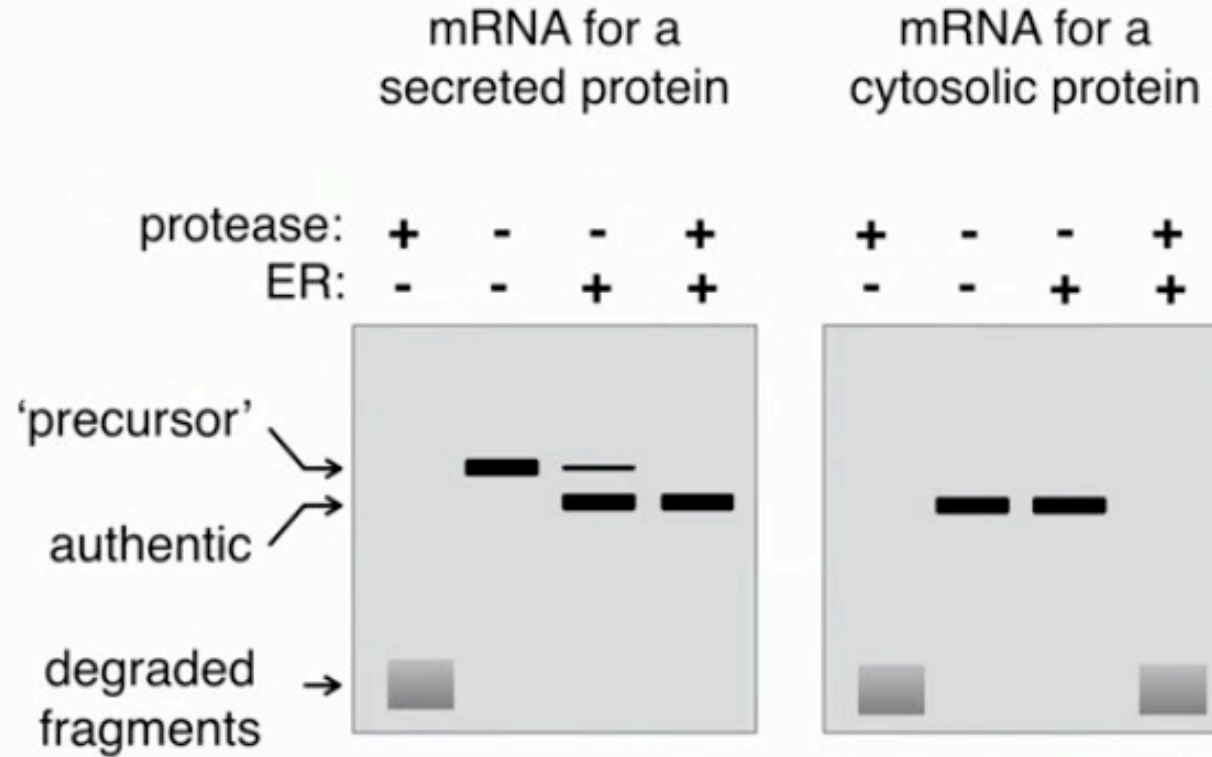
Reconstitution of protein translocation from isolated fractions

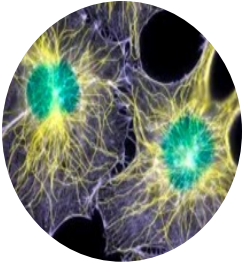




Fisiologia Celular e Molecular

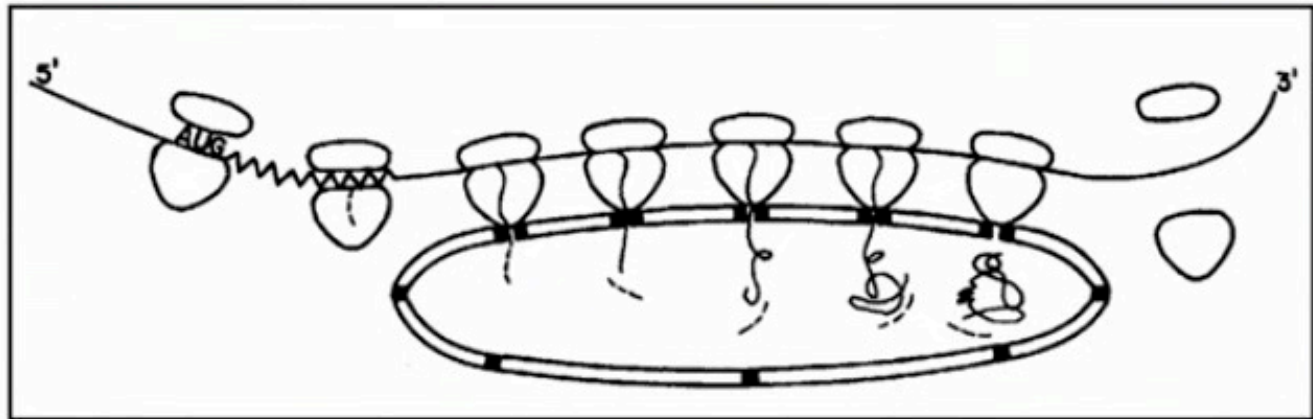
Reconstitution of protein translocation from isolated fractions



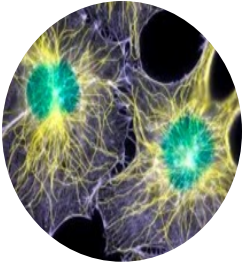


Fisiologia Celular e Molecular

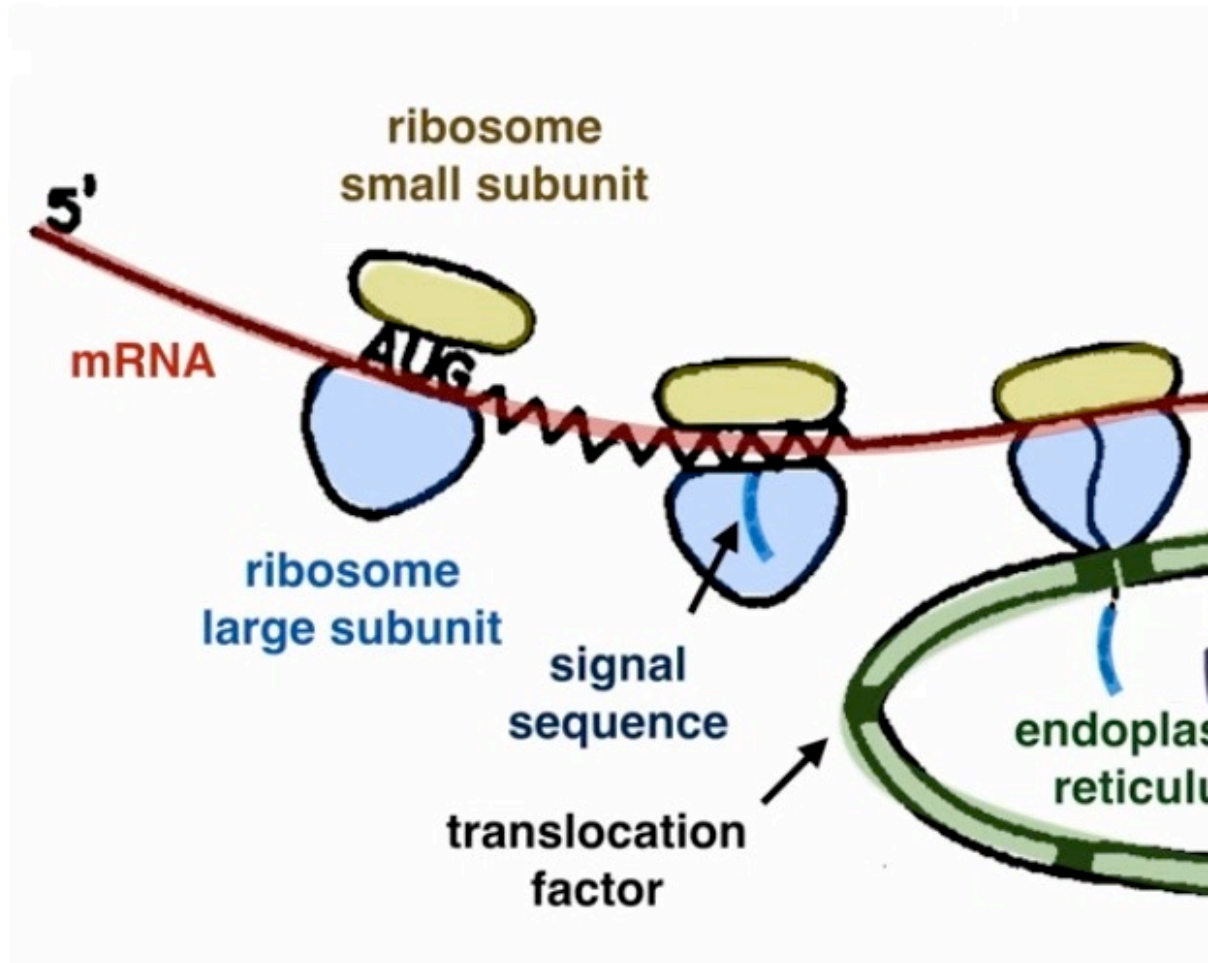
The Signal Hypothesis

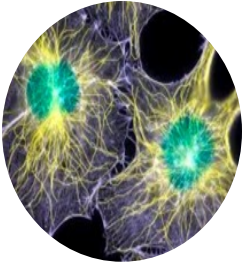


Blobel and Dobberstein (1975) *J. Cell Biol.*

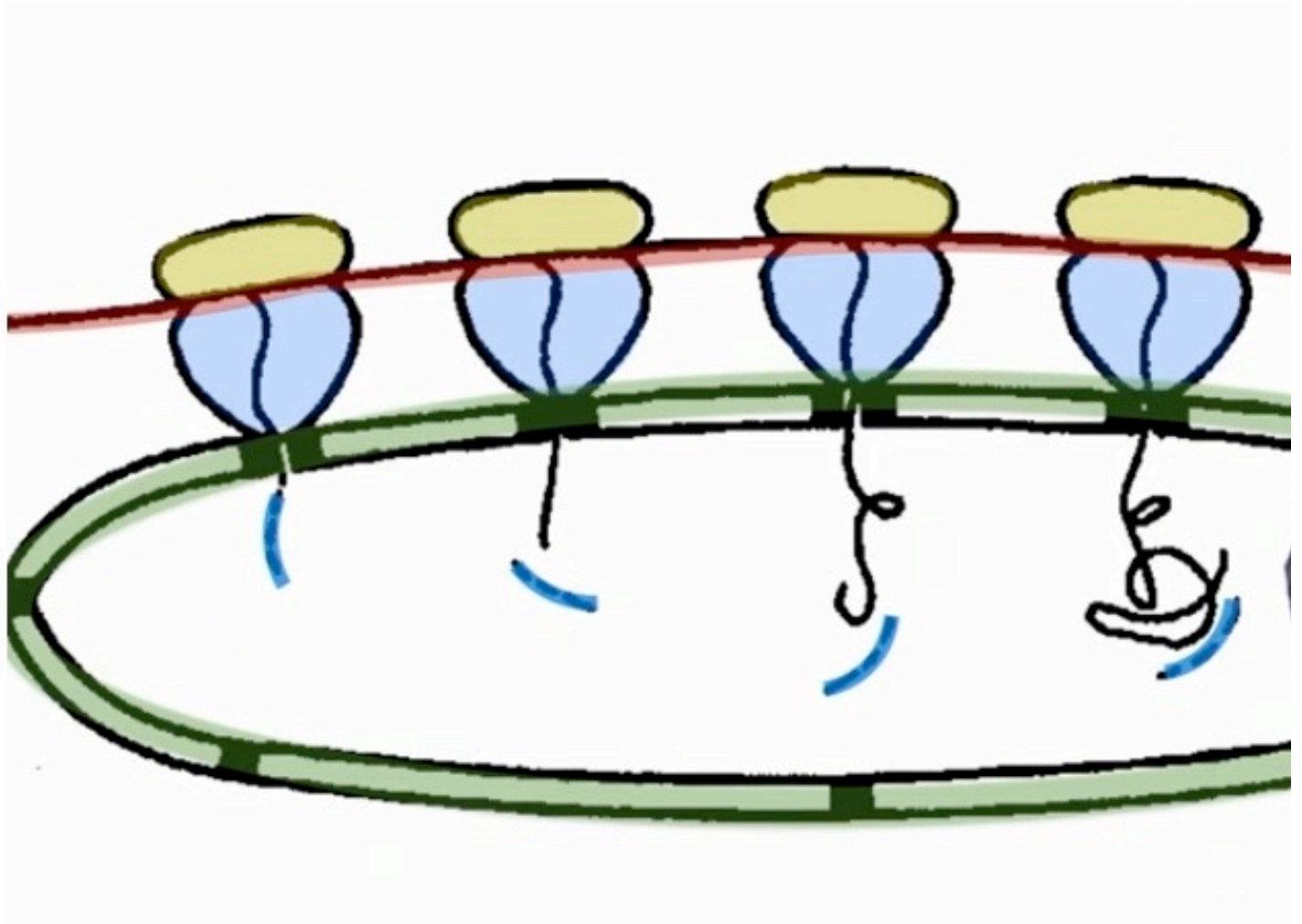


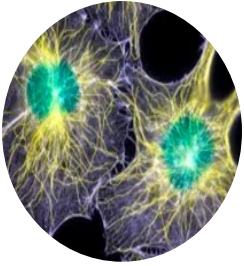
Fisiologia Celular e Molecular



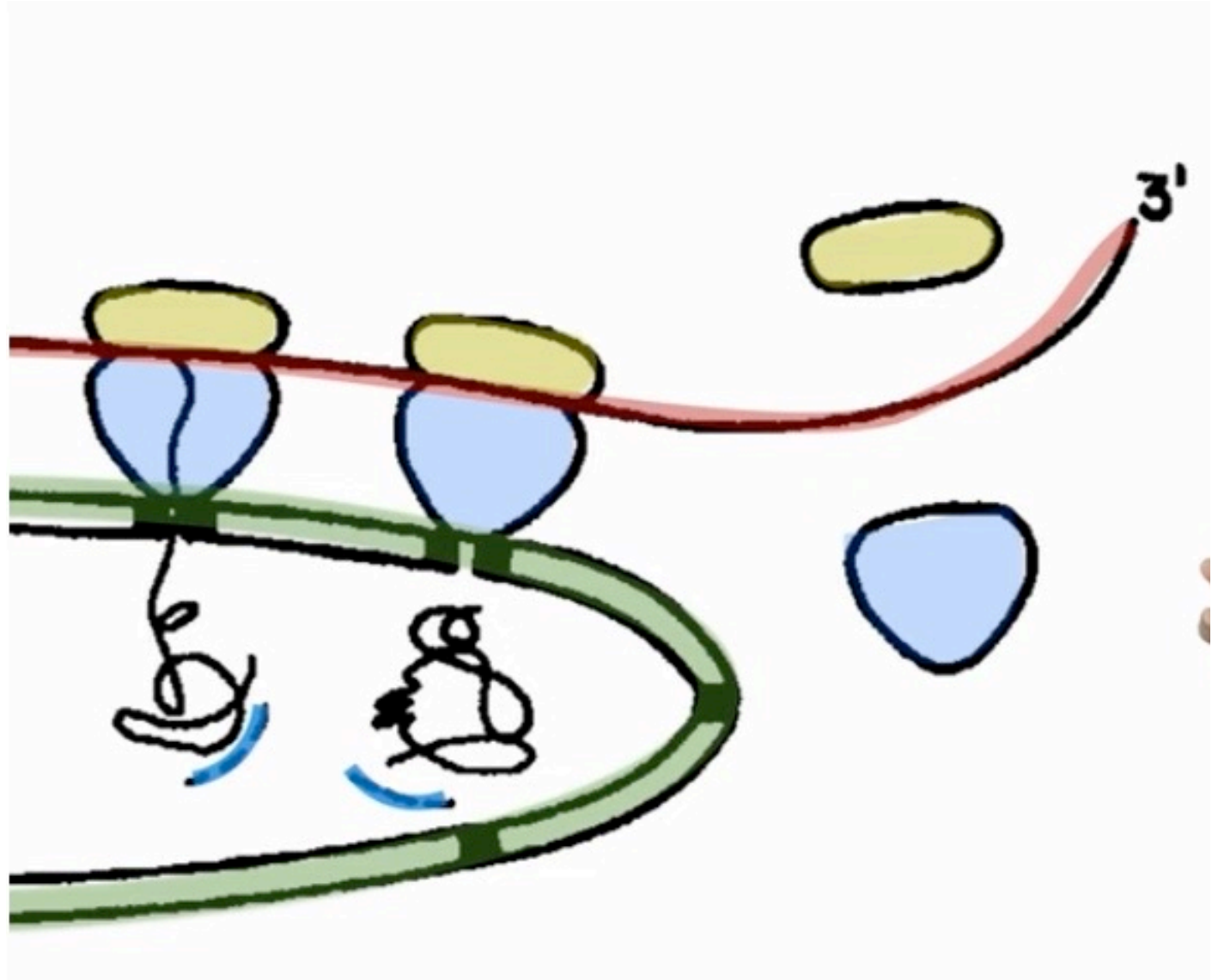


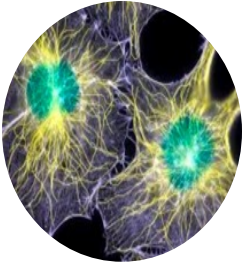
Fisiologia Celular e Molecular





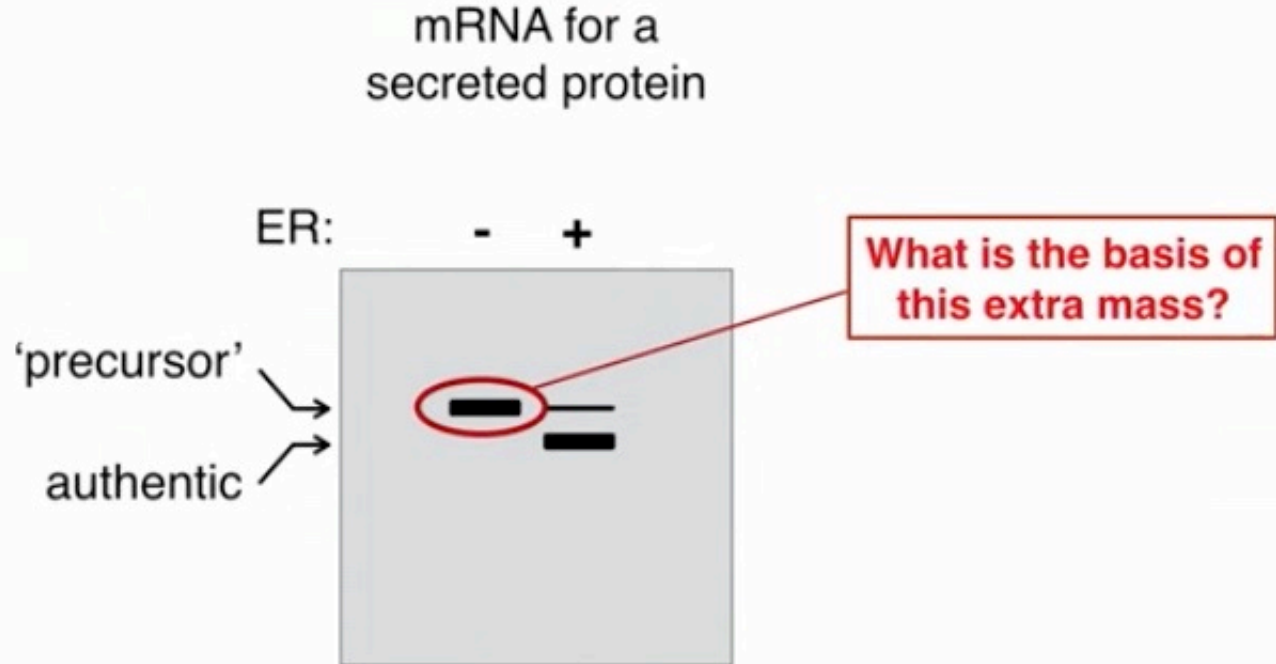
Fisiologia Celular e Molecular

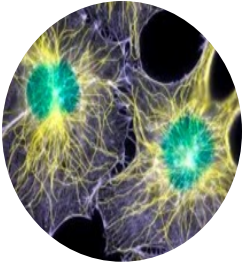




Fisiologia Celular e Molecular

What defines a signal sequence?

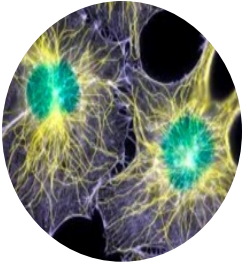




Fisiologia Celular e Molecular

'Radiosequencing' of the signal sequence

Actual sequence	MNIKGSPWKGSLLLLLVSNLLLCQSVAP...
³ HSerine labeled	MNIKGS SPWKGS LLLLLV SNLLLCQ SVAP...

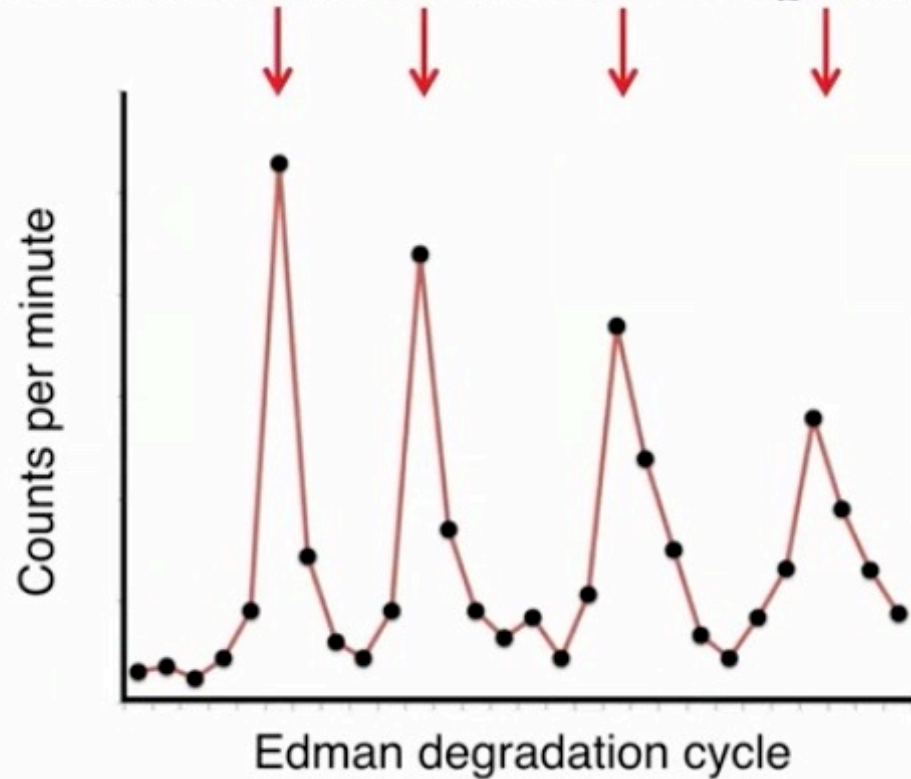


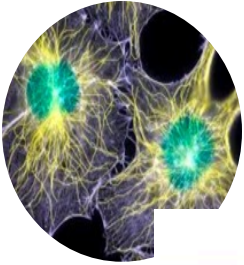
Fisiologia Celular e Molecular

'Radiosequencing' of the signal sequence

Actual sequence **MNIKGS**PWKGSLLLLLVSNLLLCQSVAP...

³HSerine labeled **MNIKGS**PWKGSLLLLLVSNLLLCQSVAP...

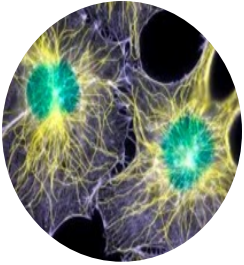




Fisiologia Celular e Molecular

'Radiosequencing' of the signal sequence

Actual sequence	MNIKGS PWKGS LLLLLL VSNLLLCQSVAP...
³ H Serine labeled	MNIKGS S PWKGS S LLLLLL V S NLLLCQ S VAP...
³ H Proline labeled	MNIKGS P WKGS LLLLLL VSNLLLCQSVAP P...
³ H Leucine labeled	MNIKGS PWKGS L LLLLL VSN L L L CQSVAP...
³ H Lysine labeled	MNI K GSPW K GS LLLLLL VSNLLLCQSVAP...



Fisiologia Celular e Molecular

'Radiosequencing' of the signal sequence

Actual sequence	MNIKGS PWKGSLLLLLVSNLLLCQSVAP...
³ HSerine labeled	MNIKGS S PWKGS S LLLLLV S NLLLCQ S VAP...
³ HProline labeled	MNIKGS P WKGSLLLLLVSNLLLCQSVAP P ...
³ HLeucine labeled	MNIKGS P WKGS LLLLL VSN LLL CQSVAP...
³ HLysine labeled	MNI K GS P W K GSLLLLLVSNLLLCQSVAP...

Deduced
sequence

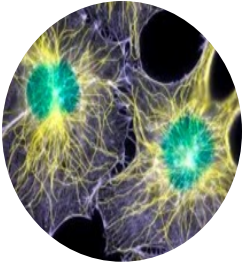
M--K-S--K-SLLLLL-S-LLL--S--P.



stretch of hydrophobic amino acids

total length of ~15 to 30 amino acids

basic residues near the N-terminus



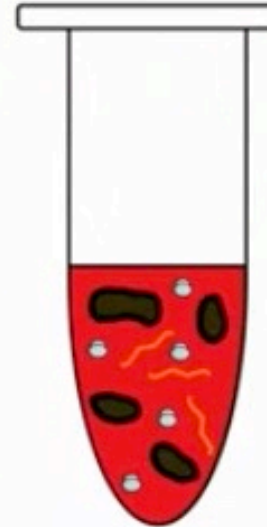
Fisiologia Celular e Molecular

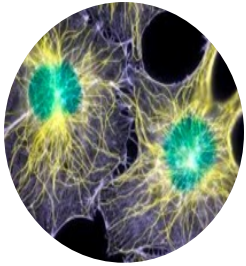
Approaches to identify the machinery of translocation

Genetic
mutants defective
for secretion



Biochemical
Fractionation &
reconstitution

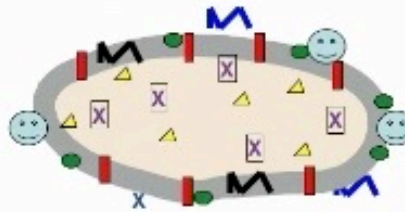




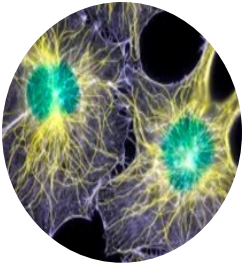
Fisiologia Celular e Molecular

Using biochemistry to identify translocation machinery

**Native ER
microsomes**

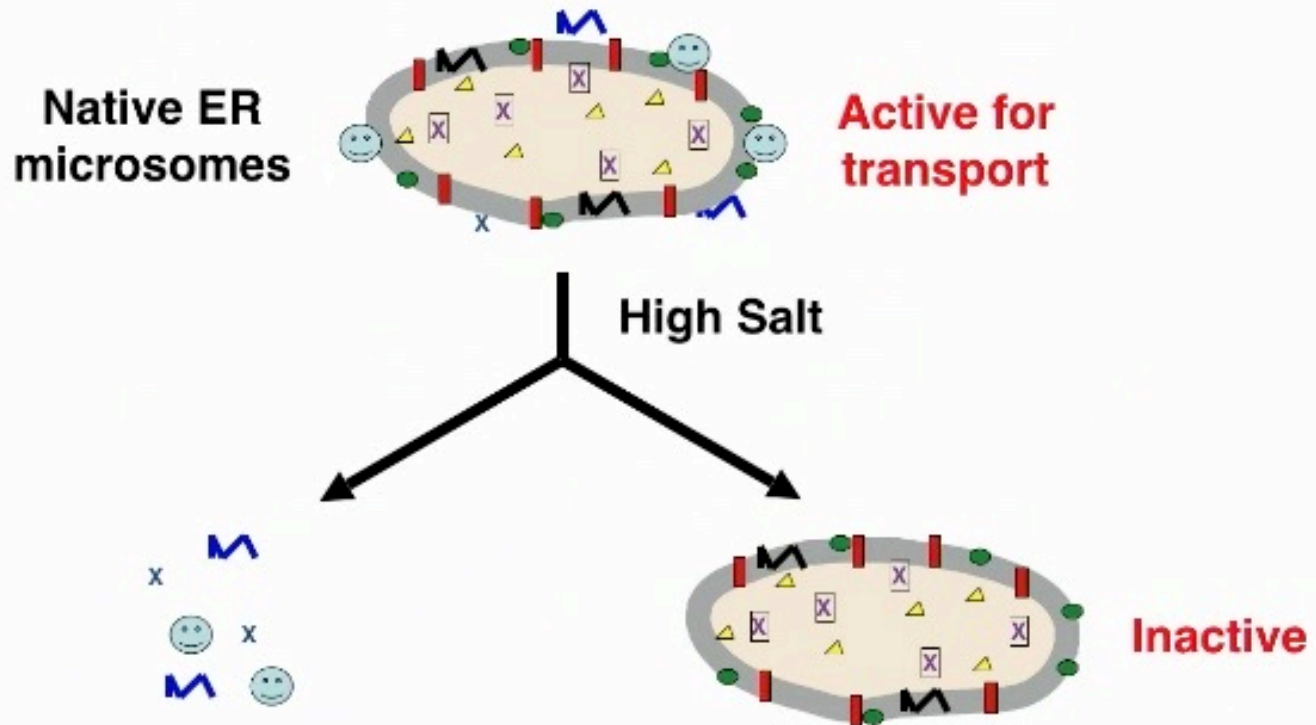


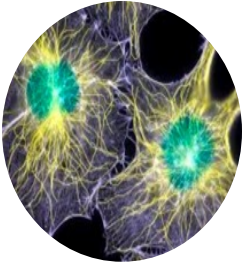
**Active for
transport**



Fisiologia Celular e Molecular

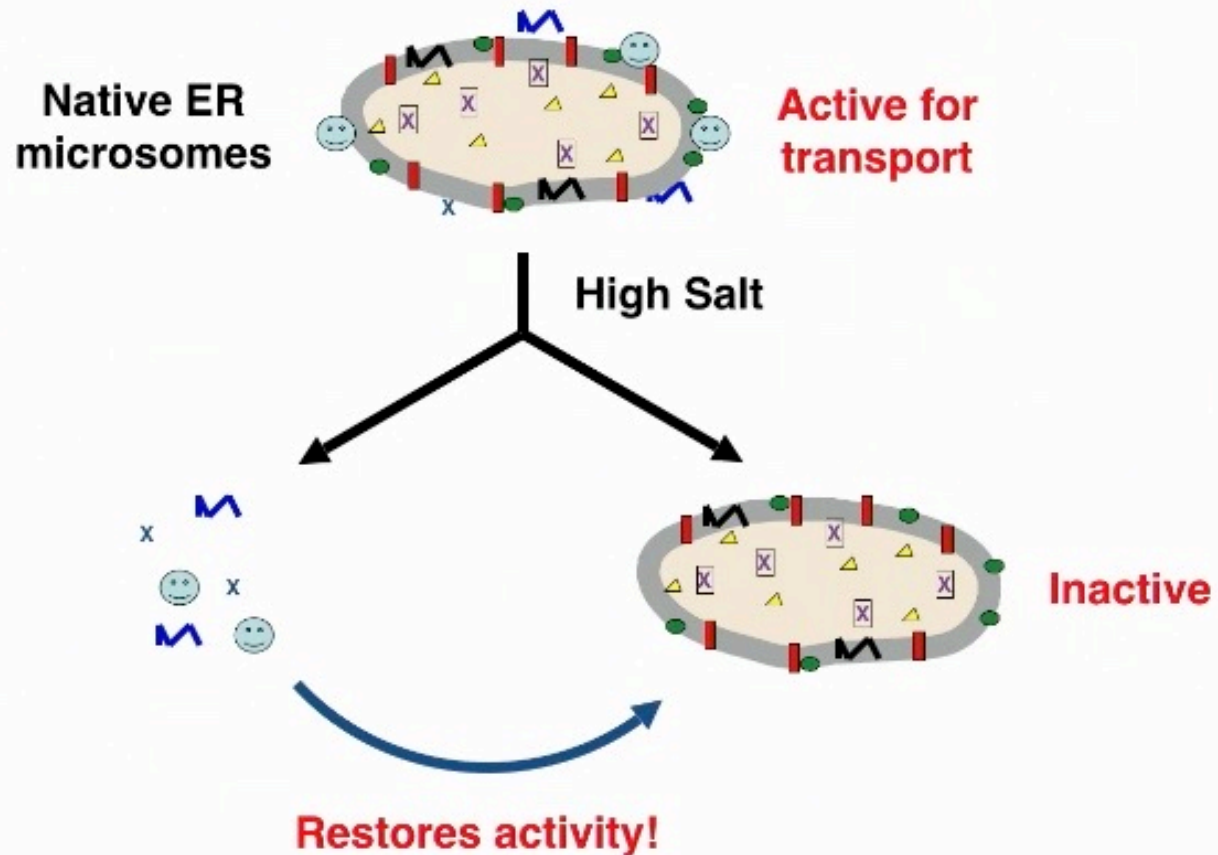
Using biochemistry to identify translocation machinery

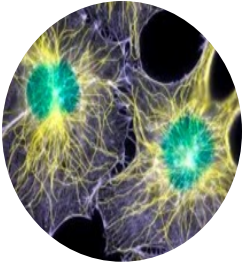




Fisiologia Celular e Molecular

Using biochemistry to identify translocation machinery





Fisiologia Celular e Molecular

Purification of the active fraction from the salt extract

