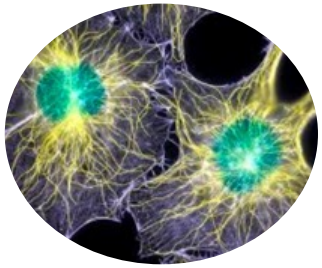
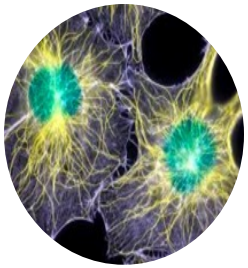


Fisiologia Celular e Molecular

T7 Transporte de proteinas na célula

Cristina Cruz

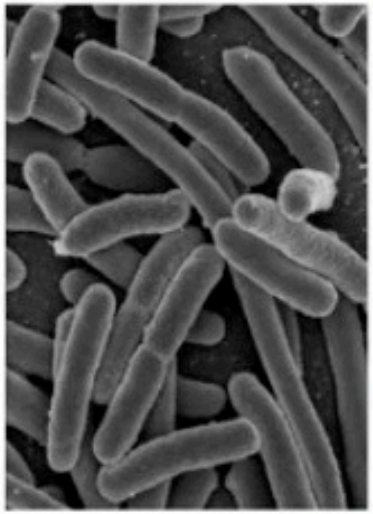


Fisiologia Celular e Molecular

Approaches to identify the machinery of translocation

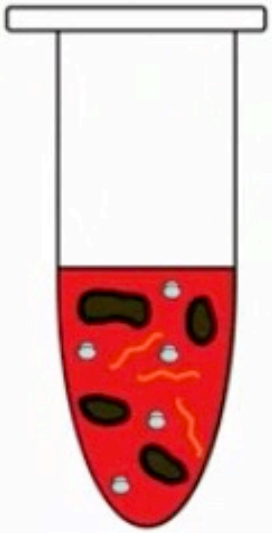
Genetic

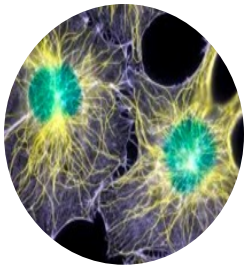
mutants defective for secretion



Biochemical

Fractionation & reconstitution

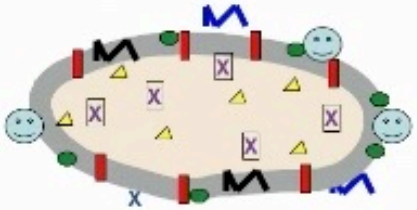




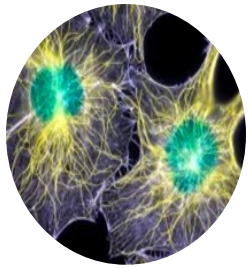
Fisiologia Celular e Molecular

Using biochemistry to identify translocation machinery

Native ER microsomes

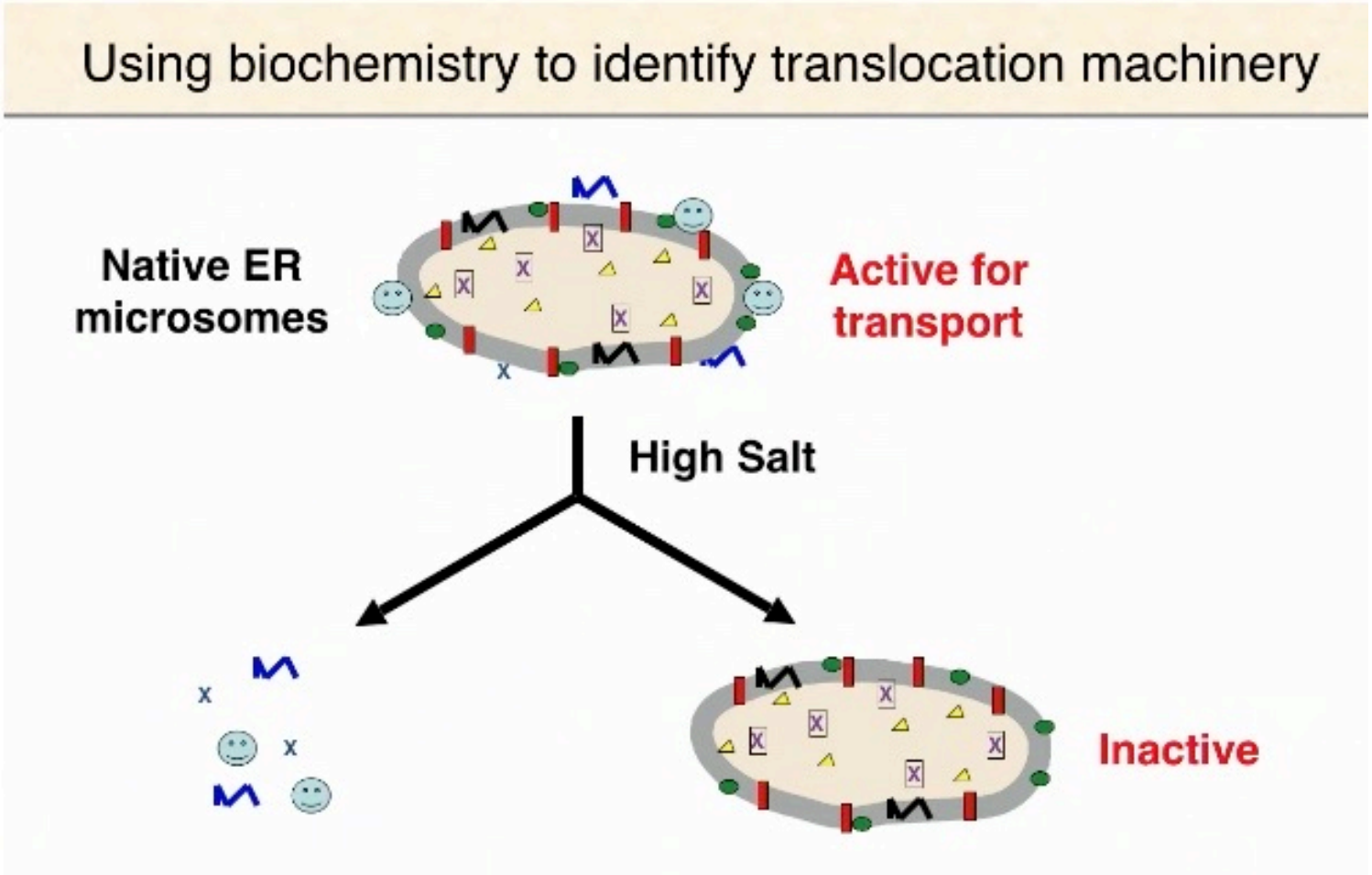


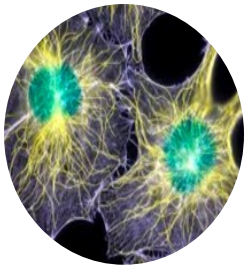
Active for transport



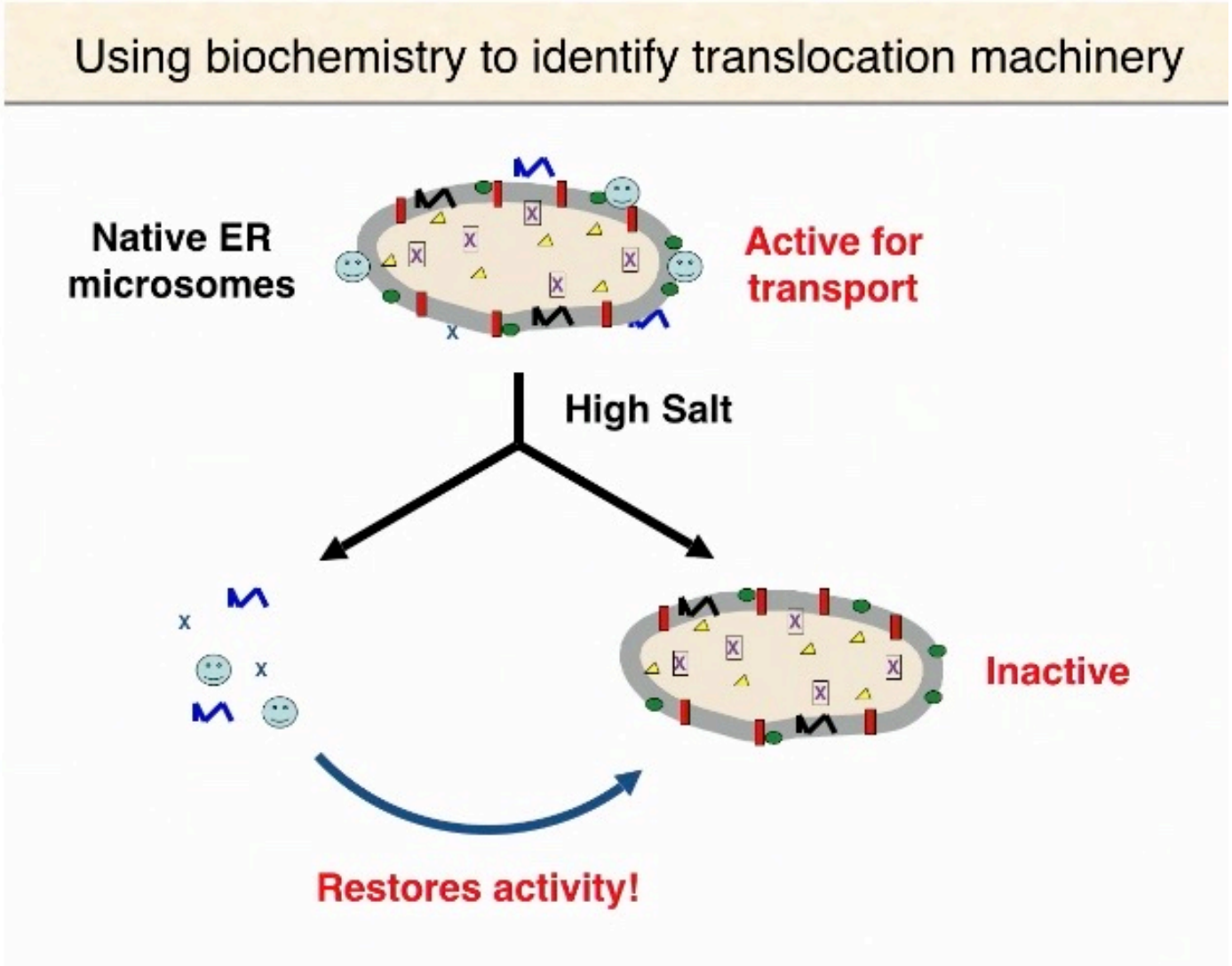
Fisiologia Celular e Molecular

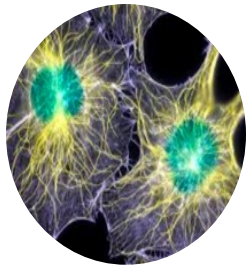
Using biochemistry to identify translocation machinery





Fisiologia Celular e Molecular





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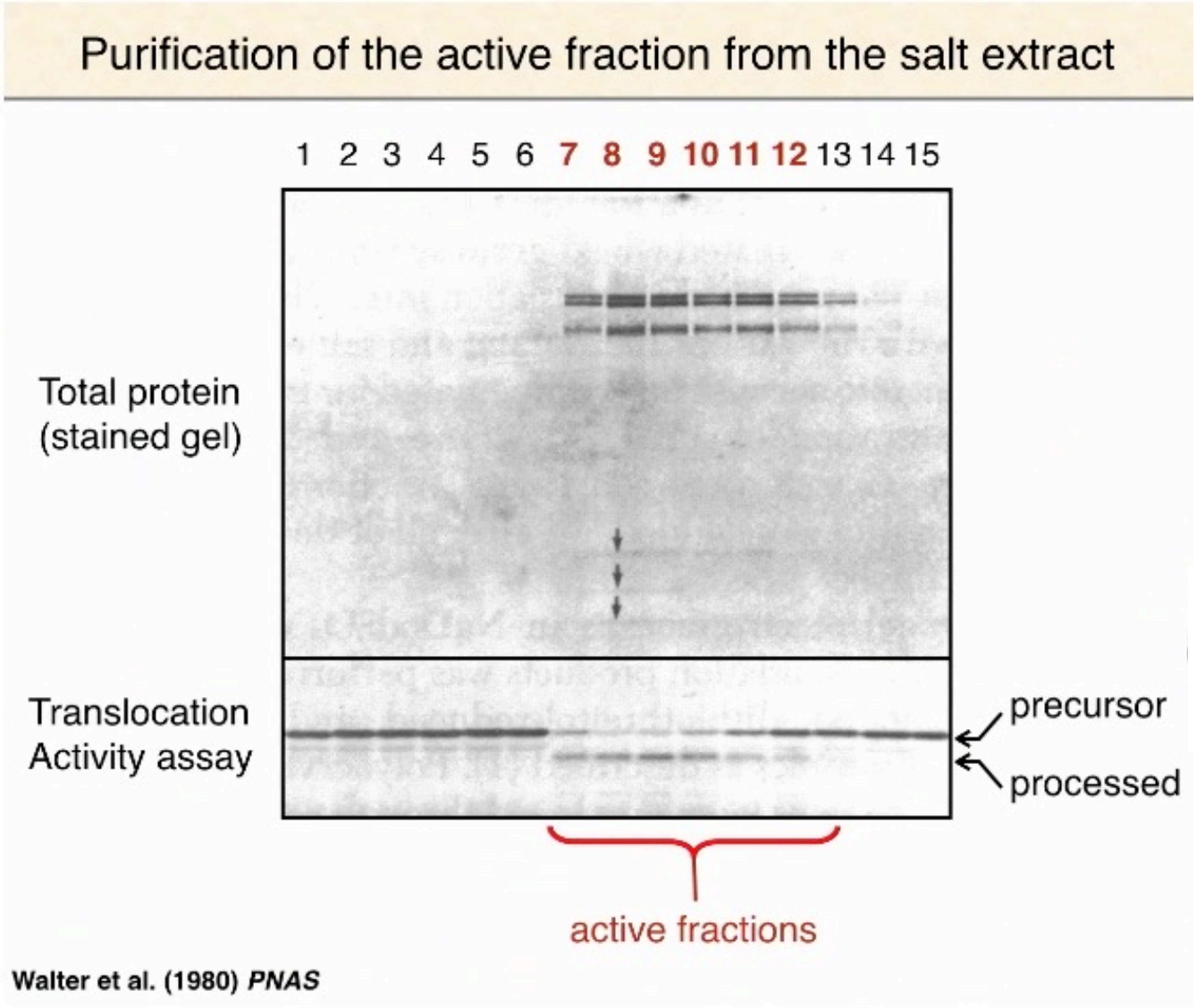
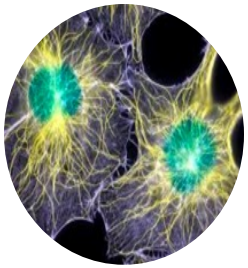
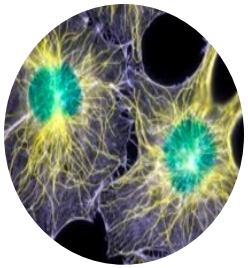




Fisiologia Celular e Molecular

Key features of the new factor: 'signal recognition protein'

Walter…Blobel
***J. Cell Biology*, 1981**
(series of three papers)



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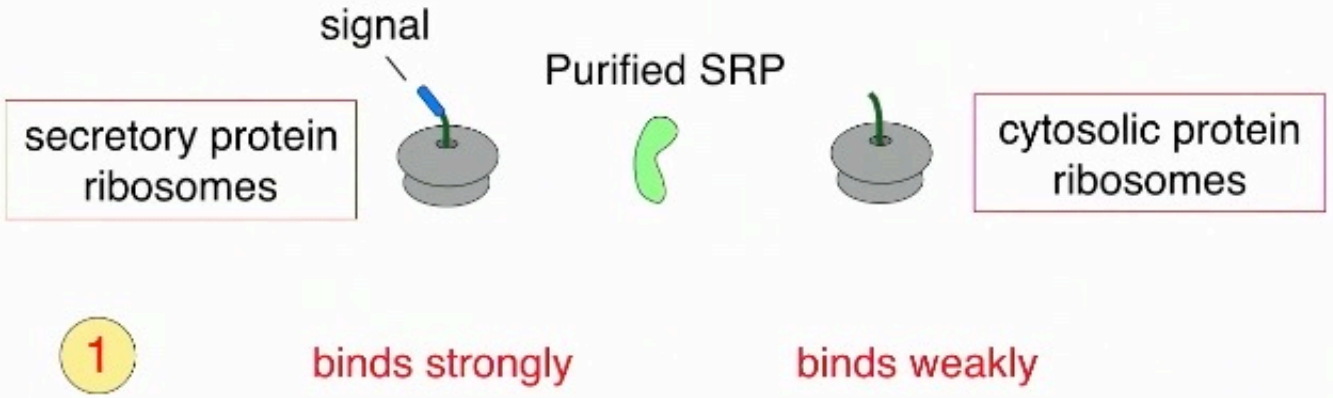

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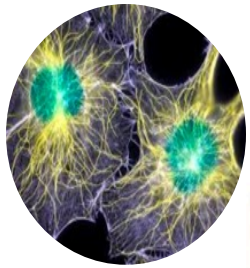
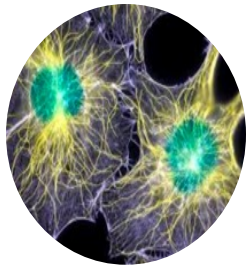


Fisiologia Celular e Molecular

Key features of the new factor: 'signal recognition protein'

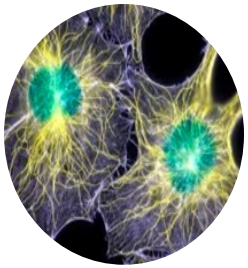
1	binds strongly	binds weakly
2	mediates binding to ER membranes	no binding to ER membranes



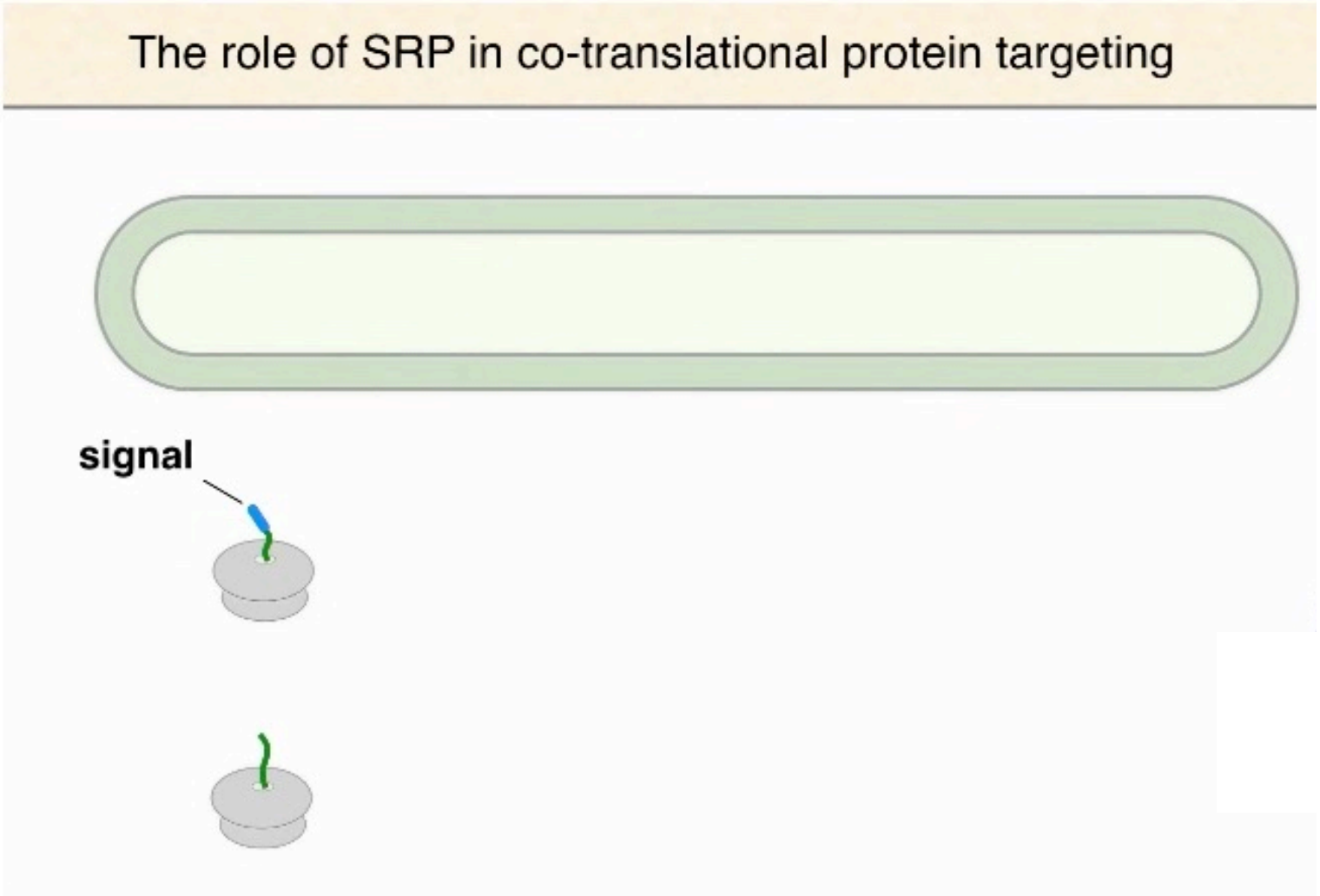
Fisiologia Celular e Molecular

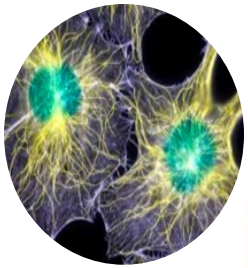
Key features of the new factor: 'signal recognition protein'

1	binds strongly	binds weakly
2	mediates binding to ER membranes	no binding to ER membranes
3	'arrests' translation	no effect on translation

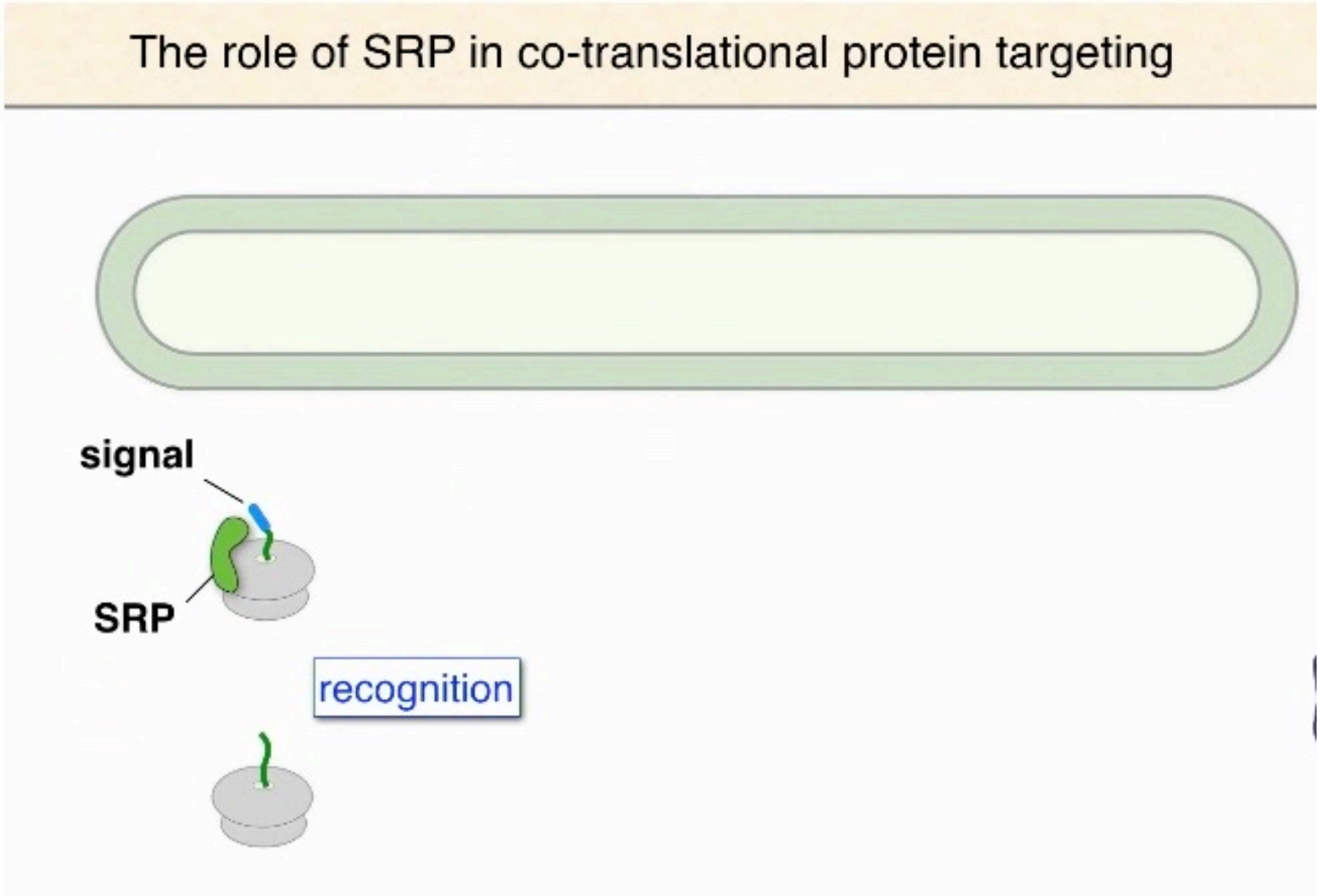


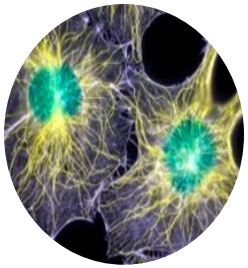
Fisiologia Celular e Molecular





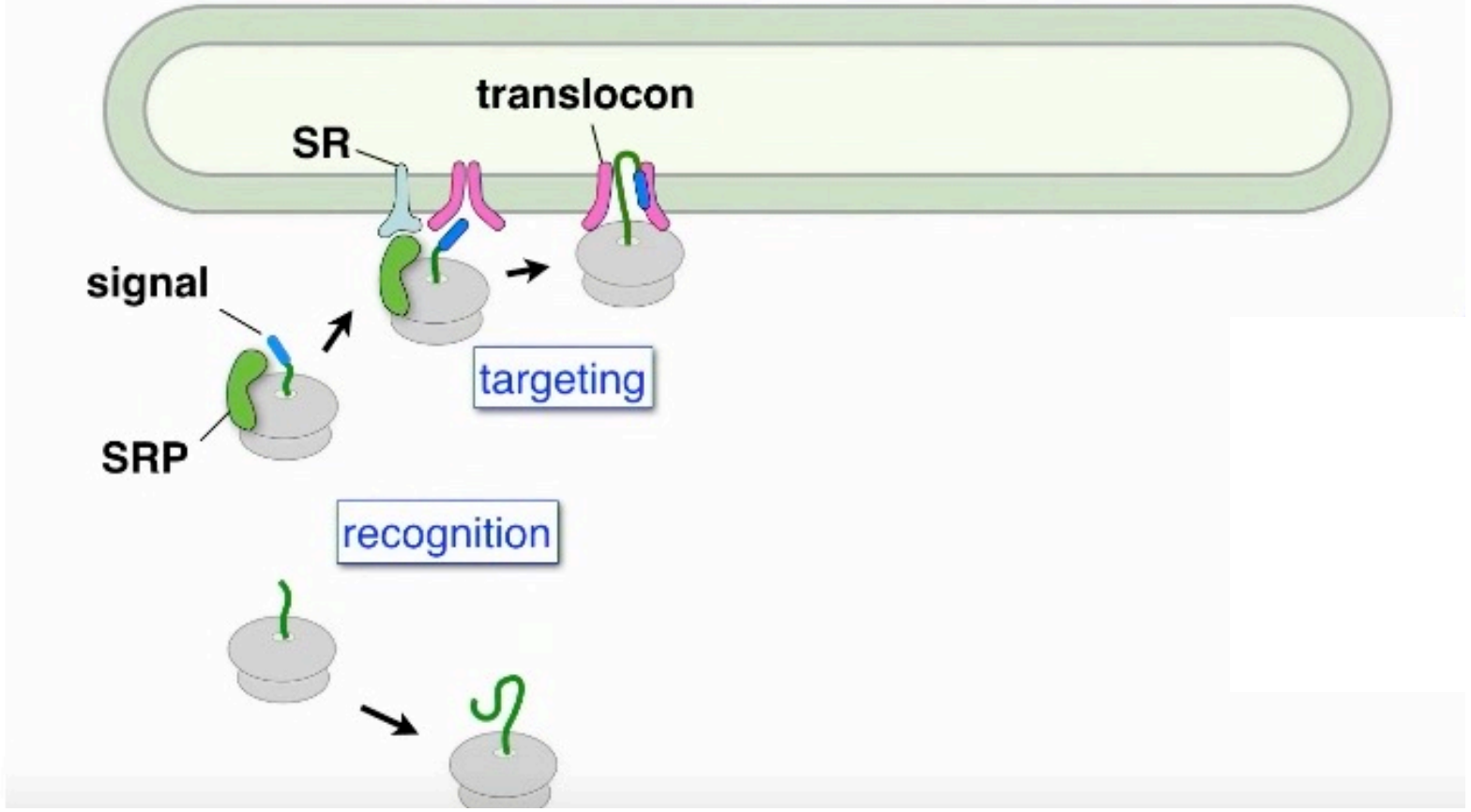
Fisiologia Celular e Molecular

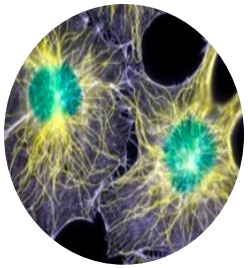




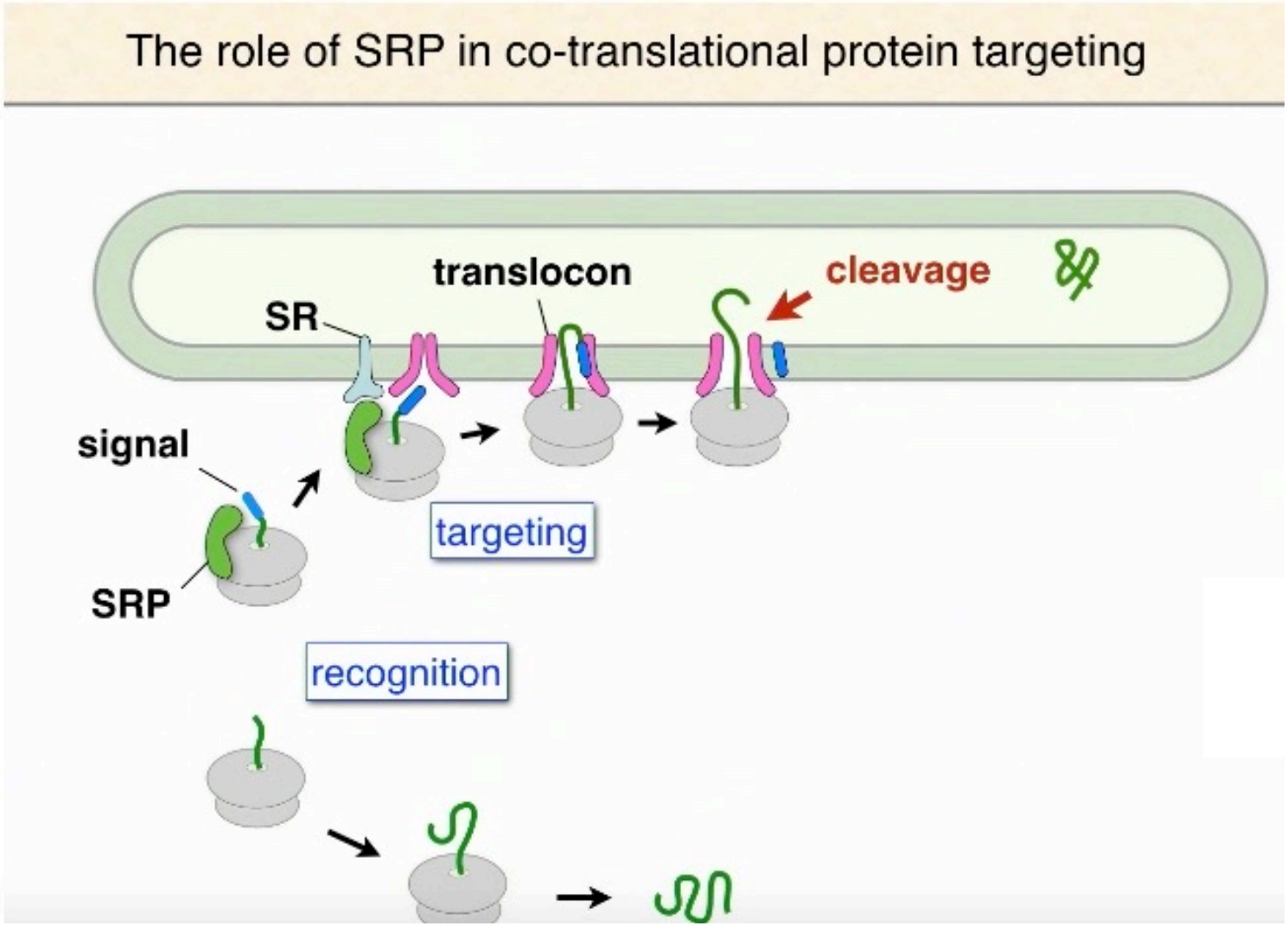
Fisiologia Celular e Molecular

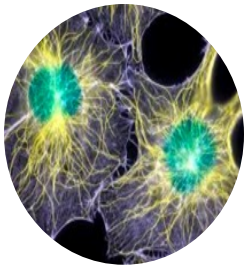
The role of SRP in co-translational protein targeting



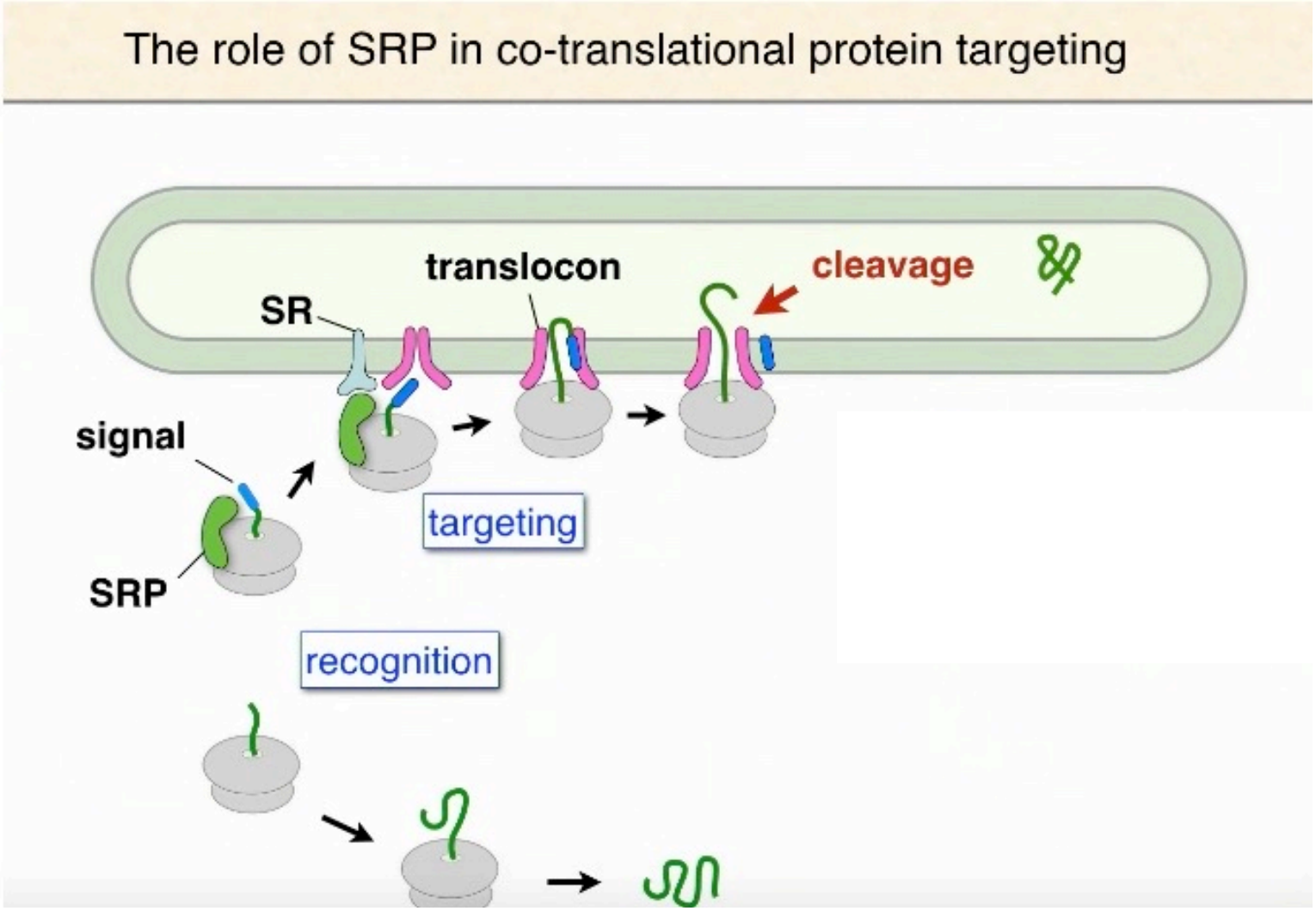


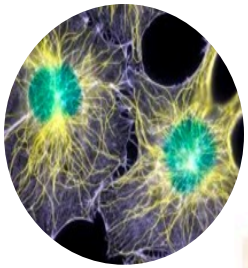
Fisiologia Celular e Molecular





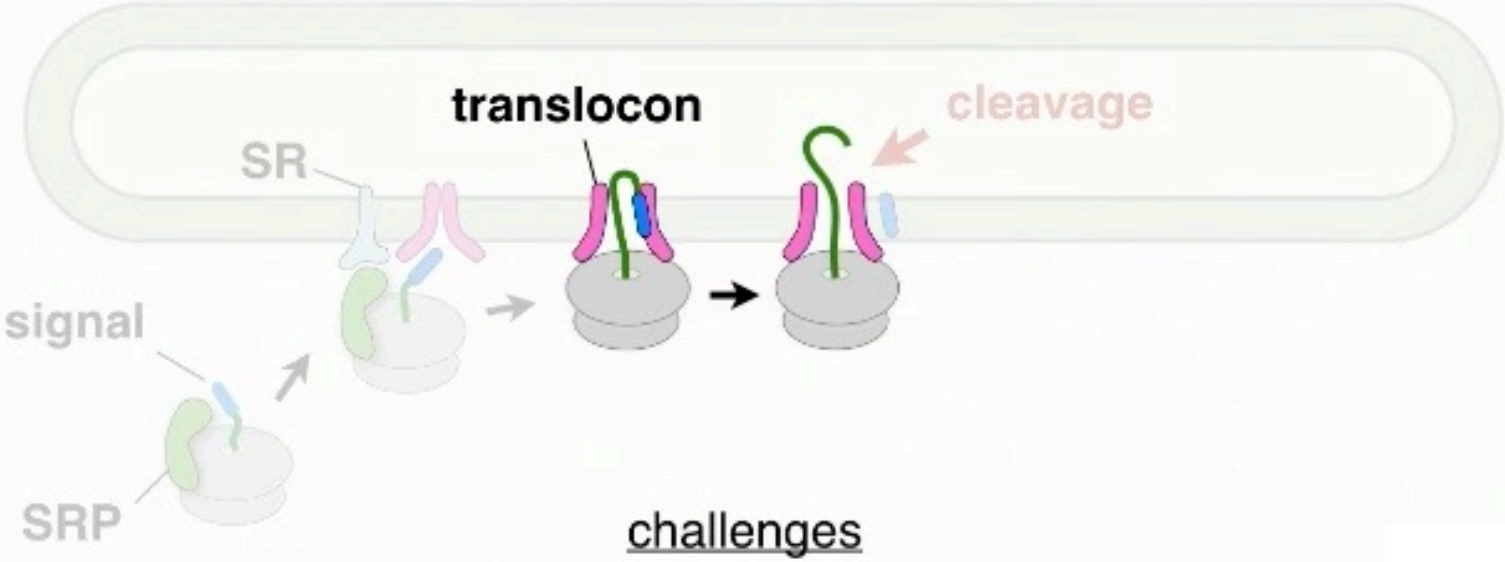
Fisiologia Celular e Molecular





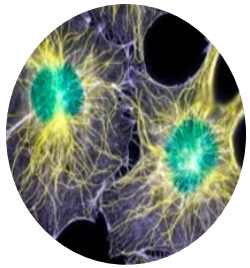
Fisiologia Celular e Molecular

What is the identity of the putative translocation channel?



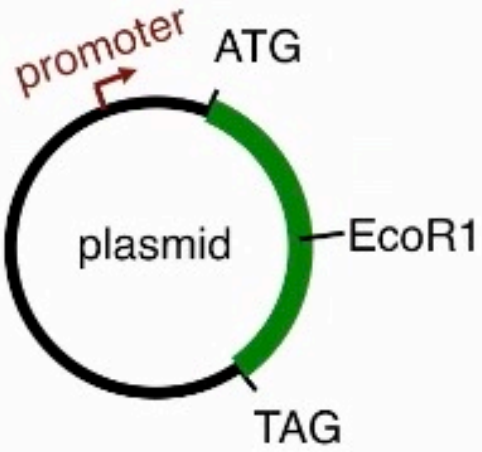
challenges

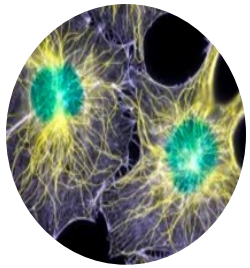
membrane embedded
no assayable 'activity'
transiently engaged



Fisiologia Celular e Molecular

Generating ‘stalled’ intermediates in vitro





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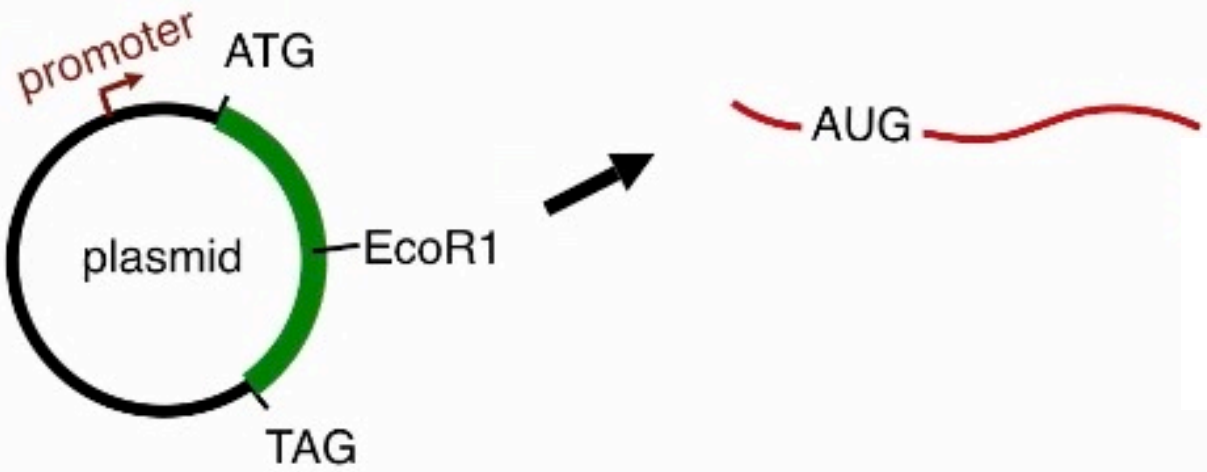

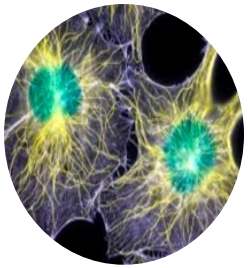

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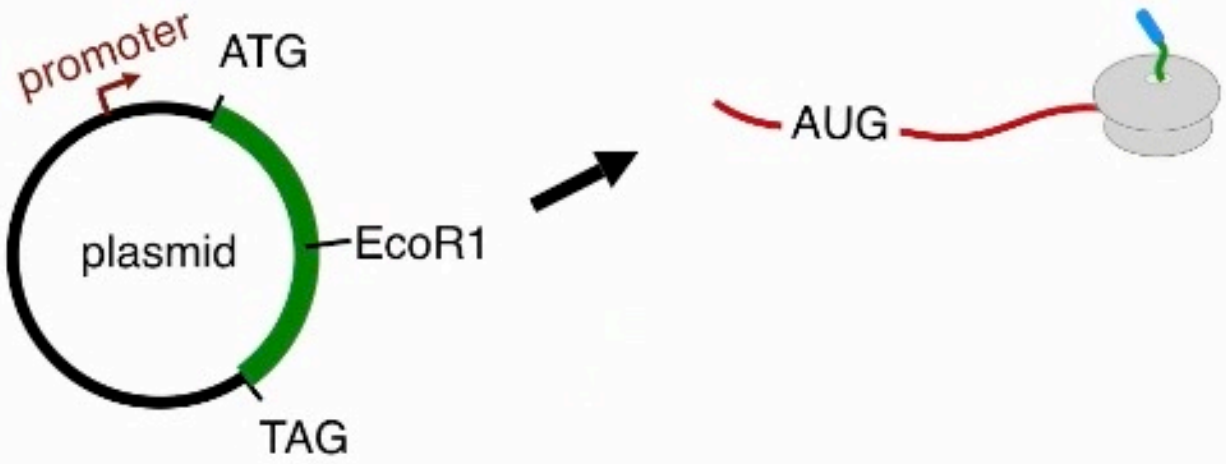

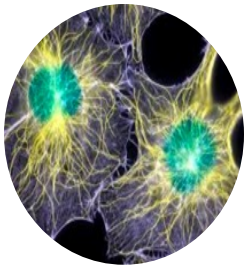

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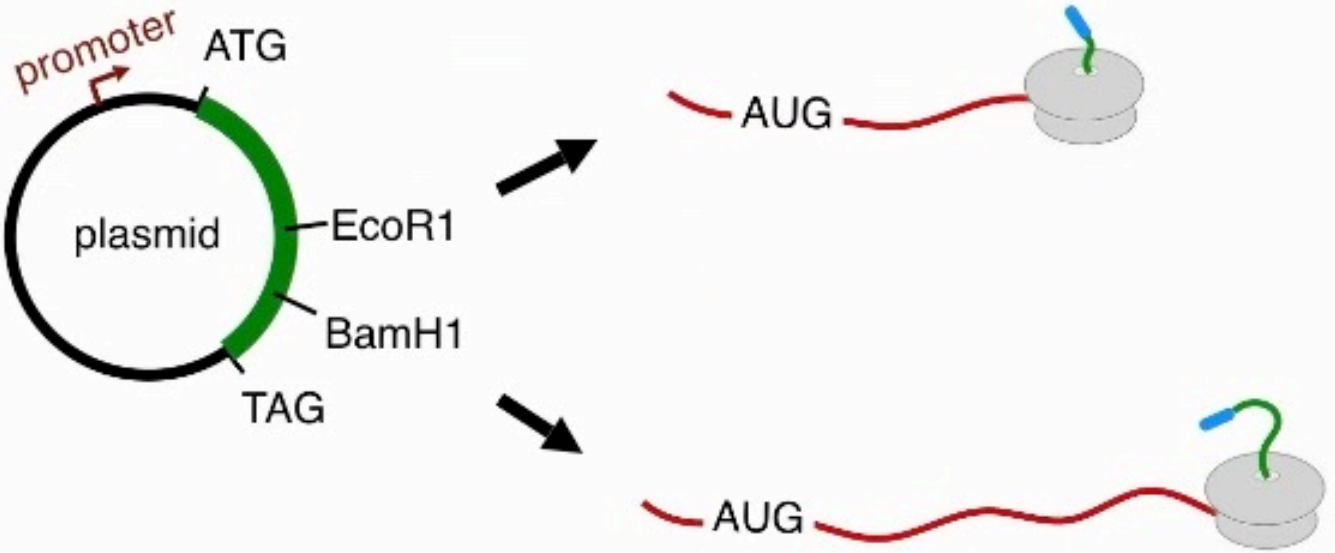
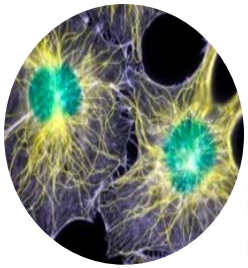
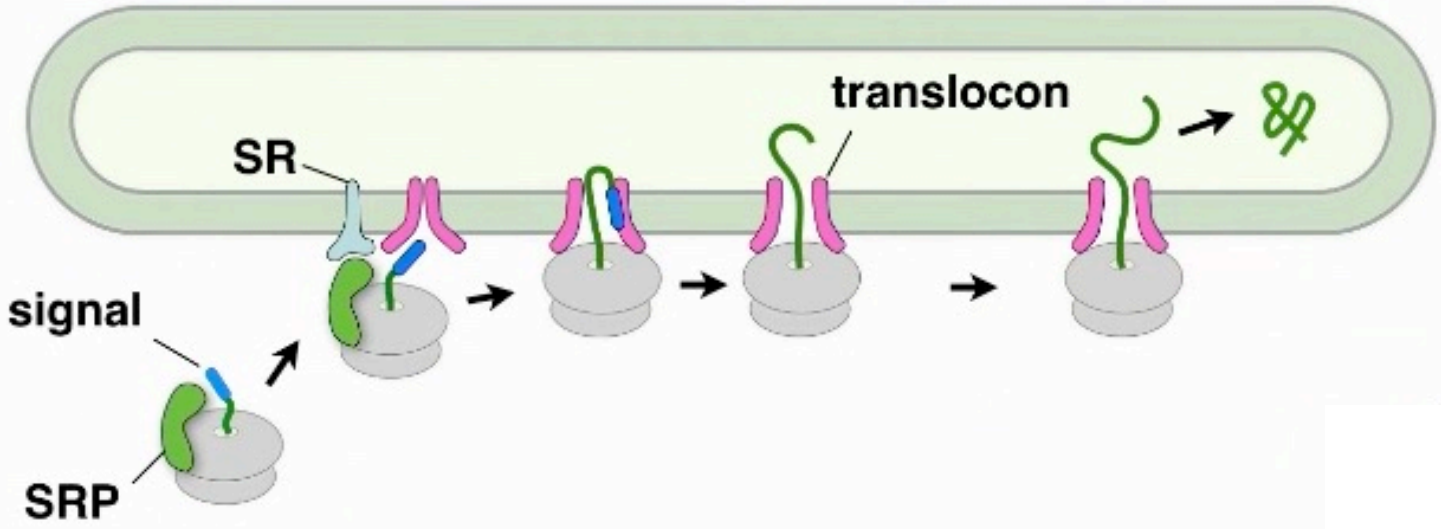
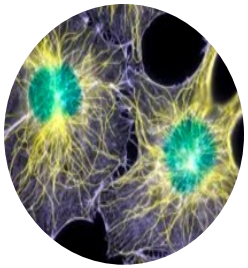




Fisiologia Celular e Molecular

Separating a co-translational process into discrete steps





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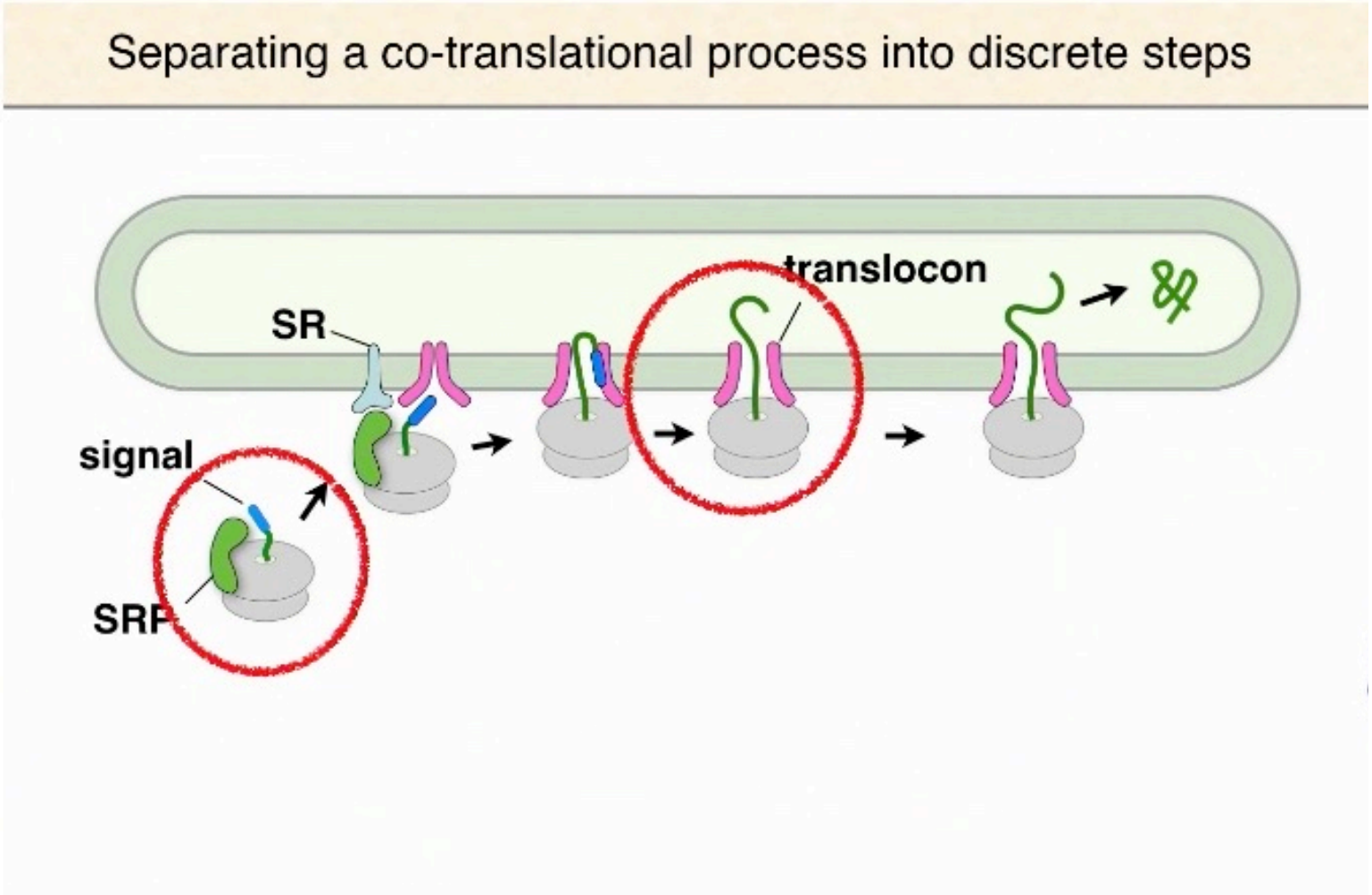
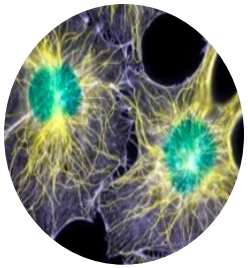
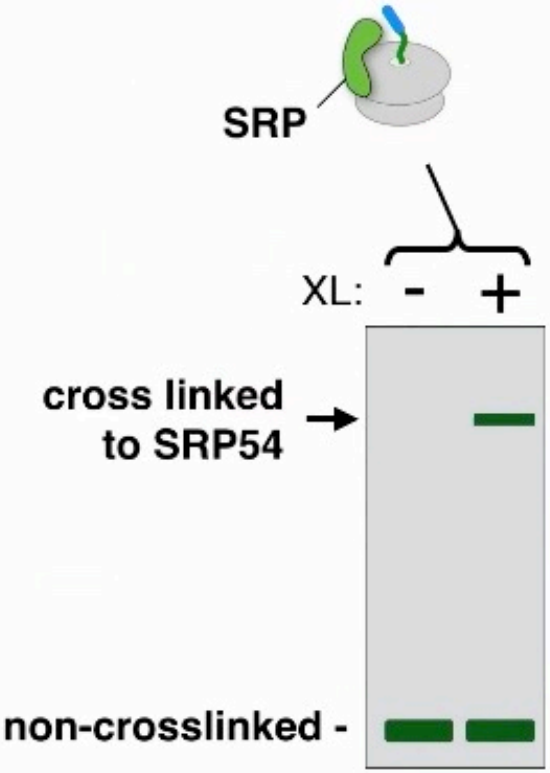
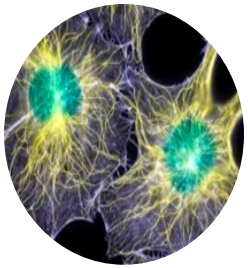




Fisiologia Celular e Molecular

Probing the environment at different stages of translocation





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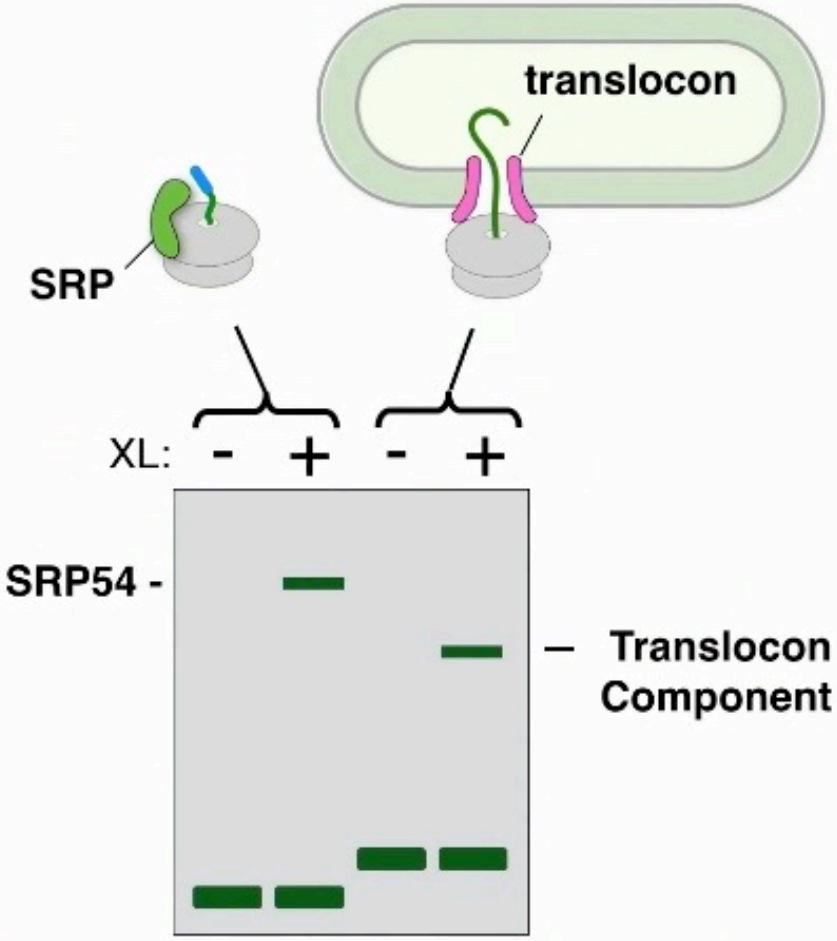
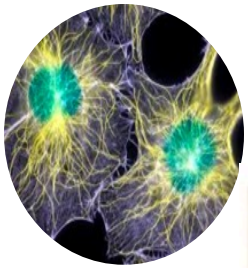
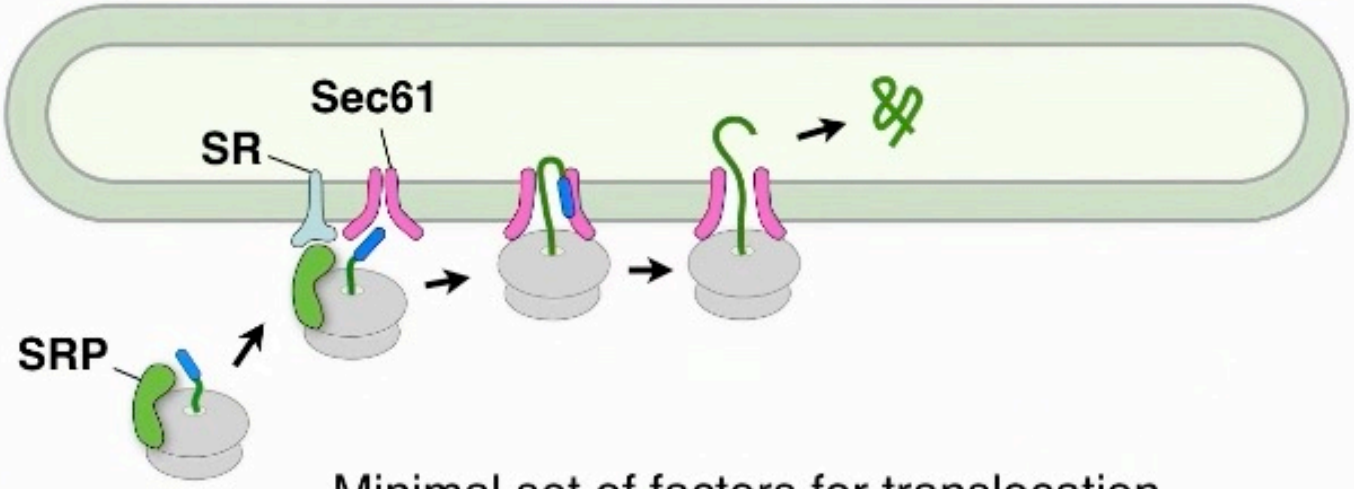




Fisiologia Celular e Molecular

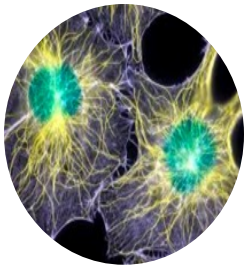
Co-translational protein targeting, translocation, & insertion



Minimal set of factors for translocation

All organisms contain SRP, SR, Sec61

Also used for many membrane proteins



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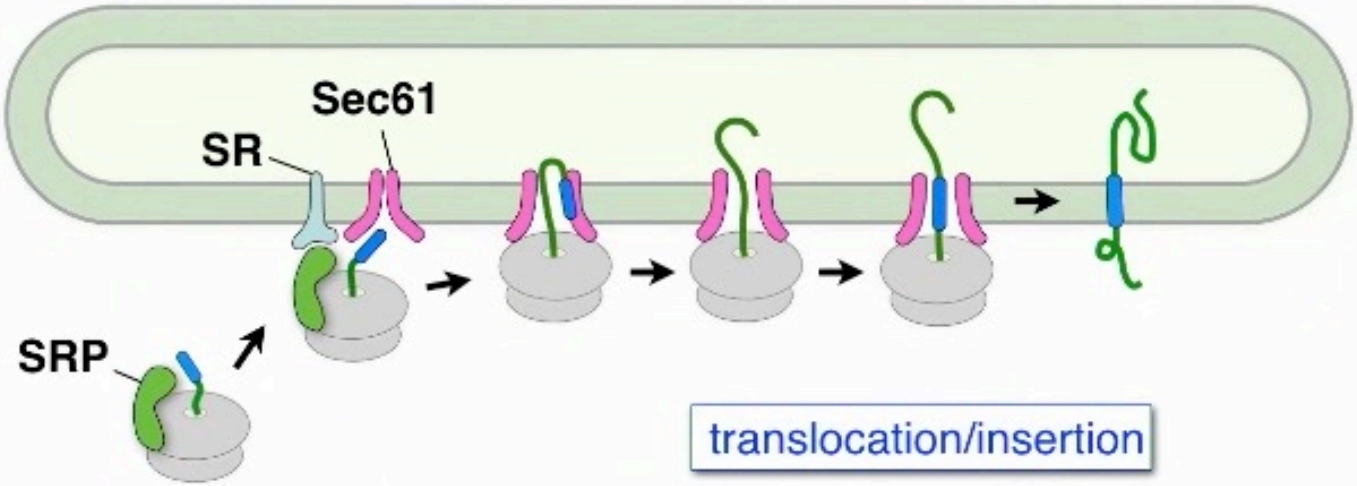
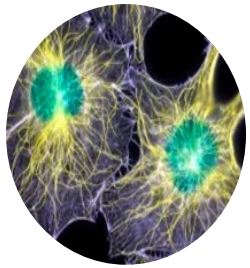




Fisiologia Celular e Molecular

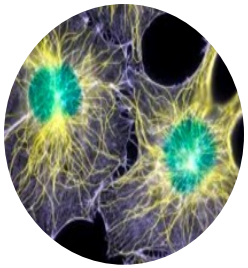
What next?

pathways are still being discovered

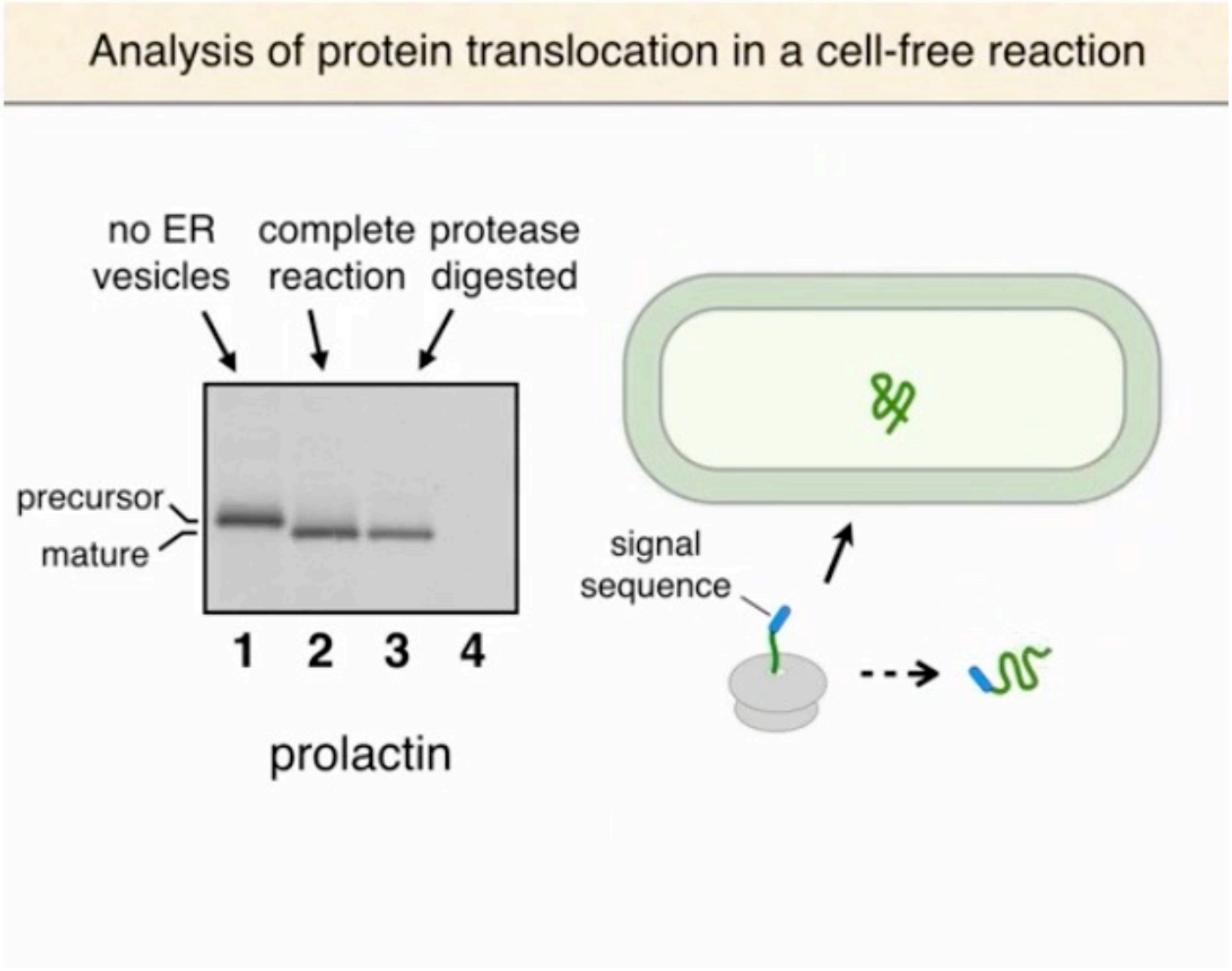
mechanisms of translocation reaction

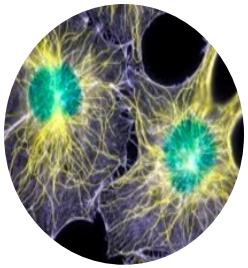
folding & assembly of membrane proteins

mechanisms of fidelity and quality control



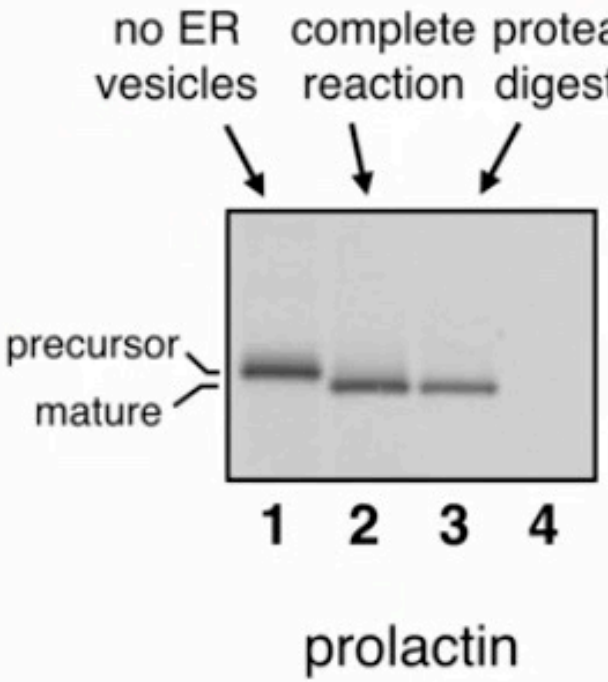
Fisiologia Celular e Molecular

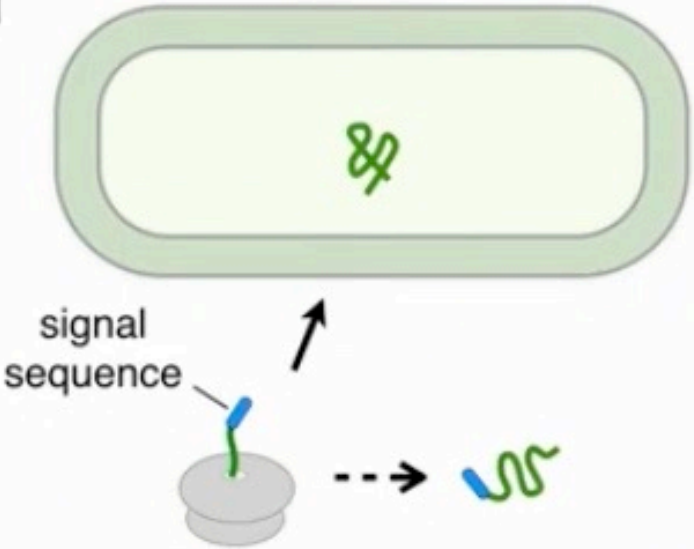


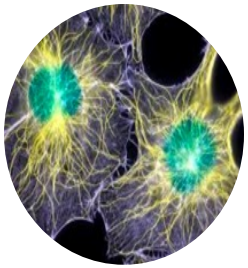


Fisiologia Celular e Molecular

Analysis of protein translocation in a cell-free reaction

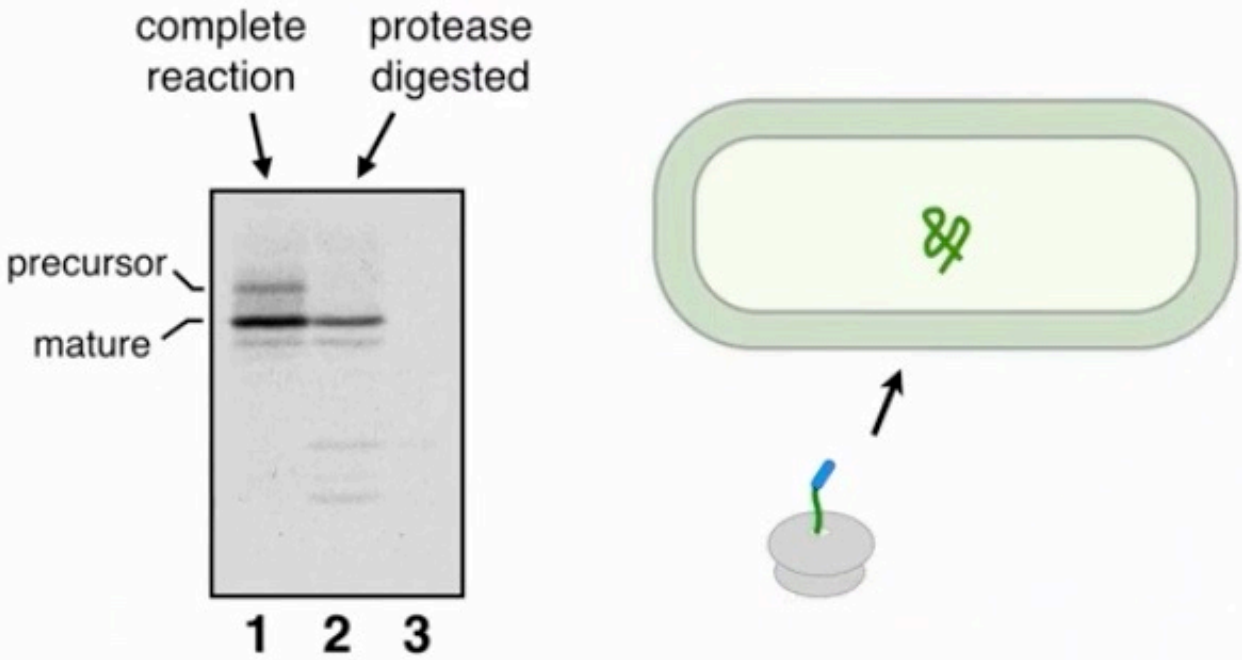


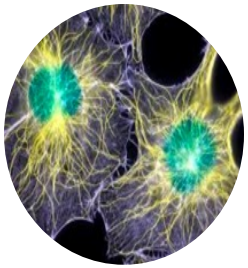




Fisiologia Celular e Molecular

Analysis of Prion protein (PrP) translocation *in vitro*





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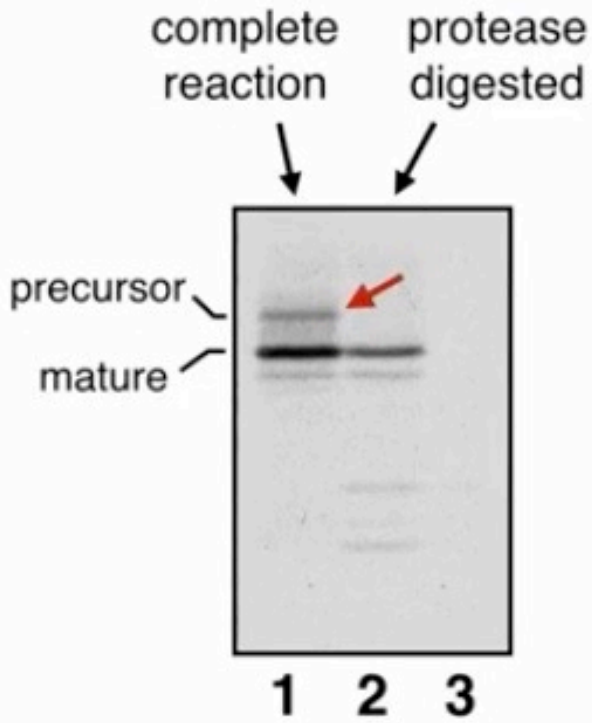

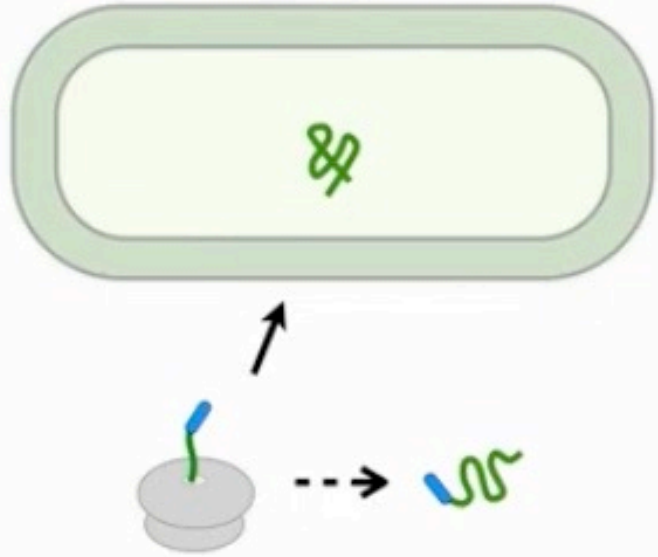

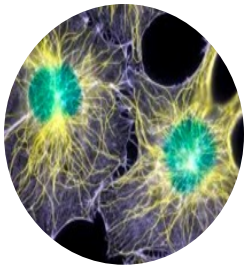

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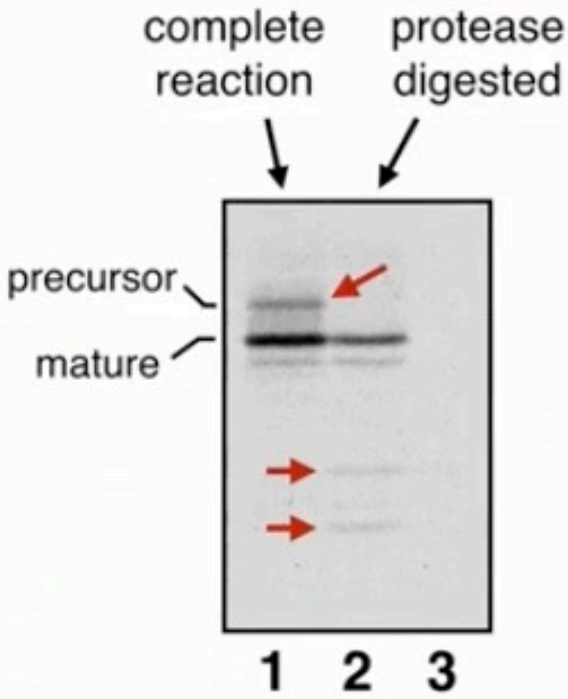
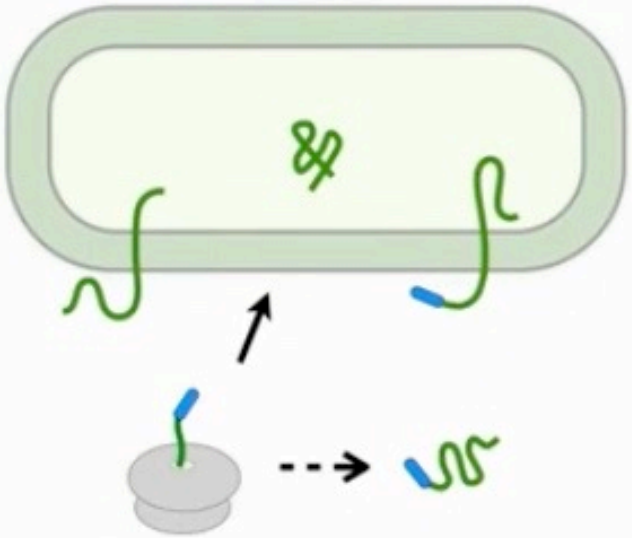
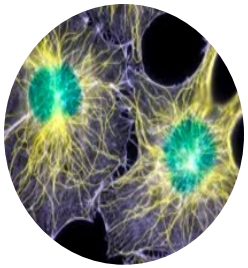




minor 'mislocalized' forms of PrP



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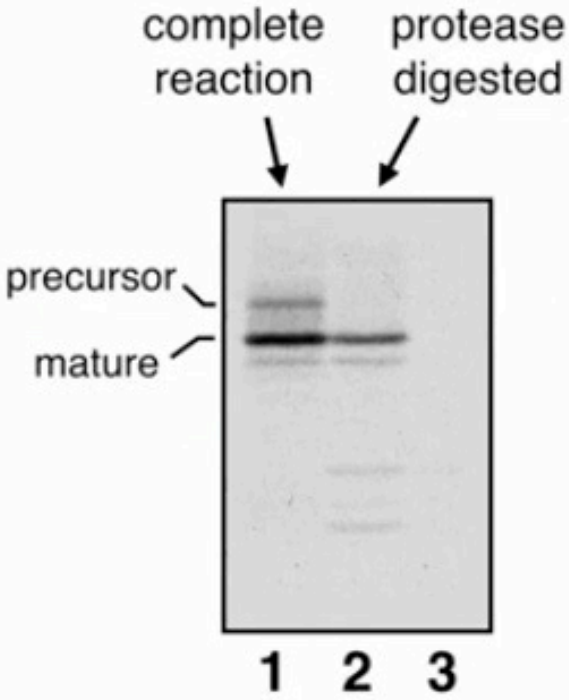
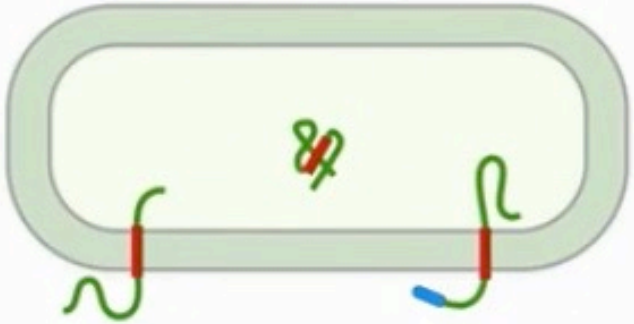
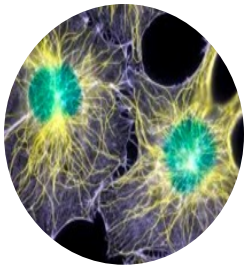




Central hydrophobic region

...NMKHMAGAAAAGAVVGGLGGYMLGSAMSR...



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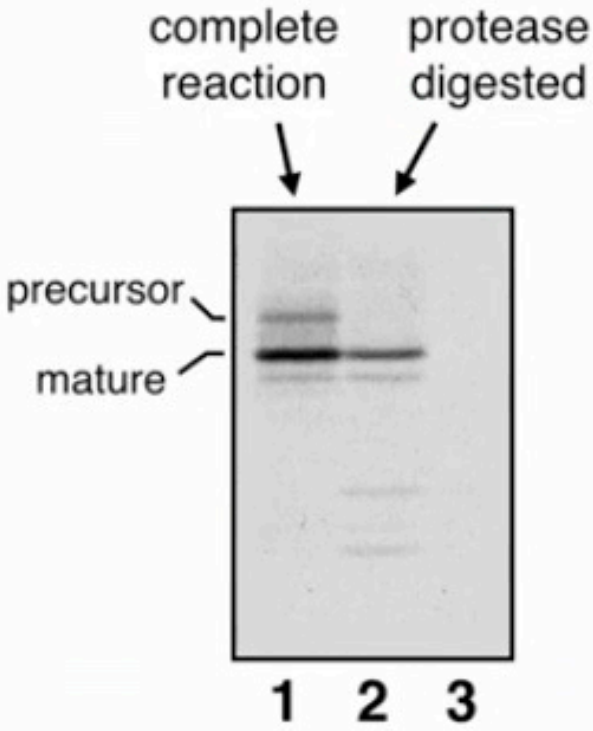

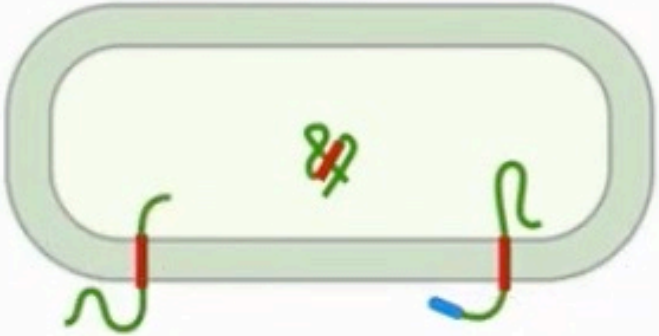

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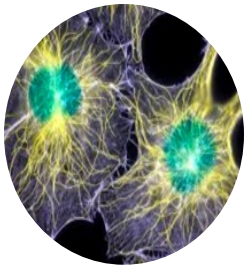
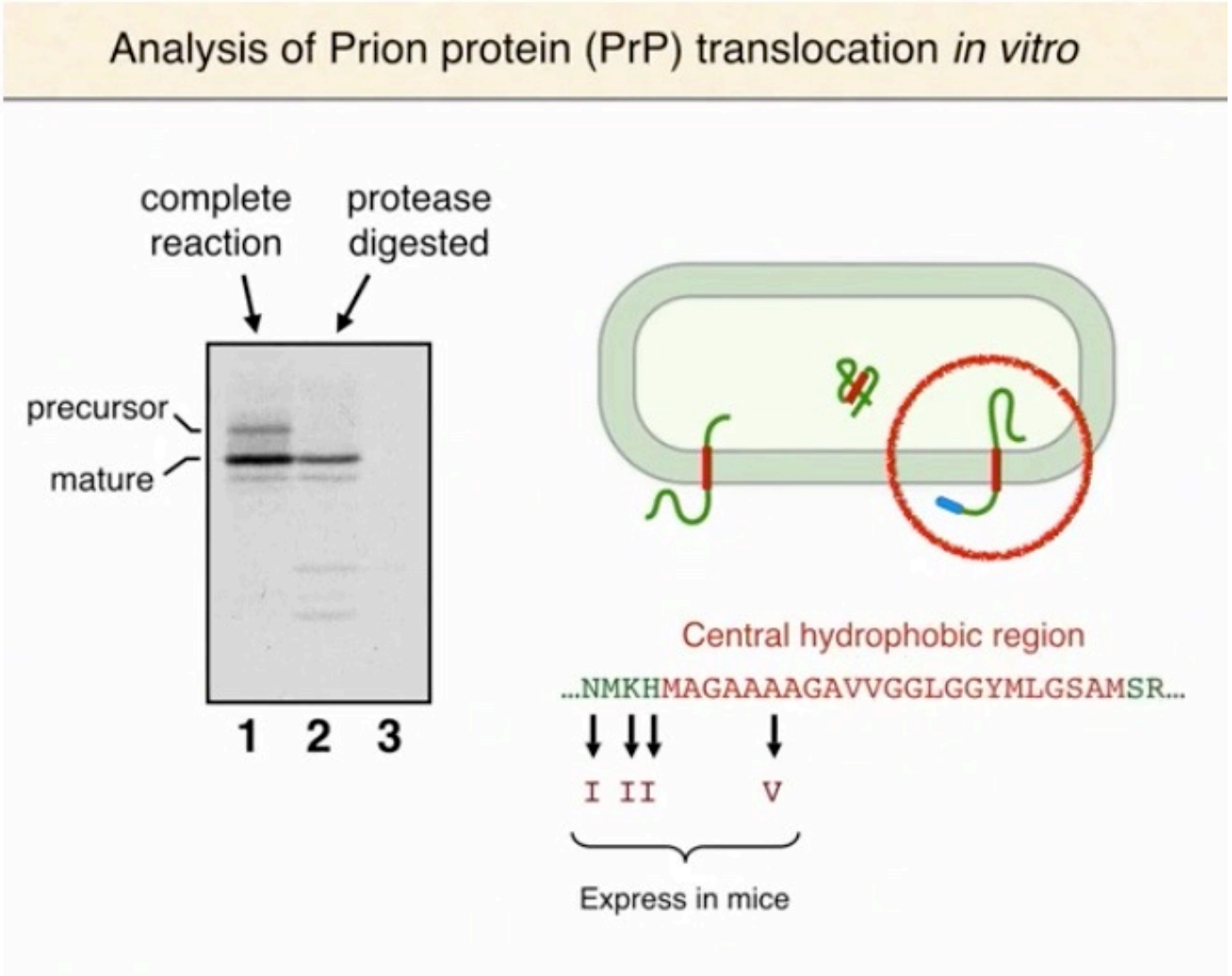
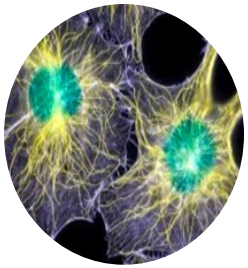
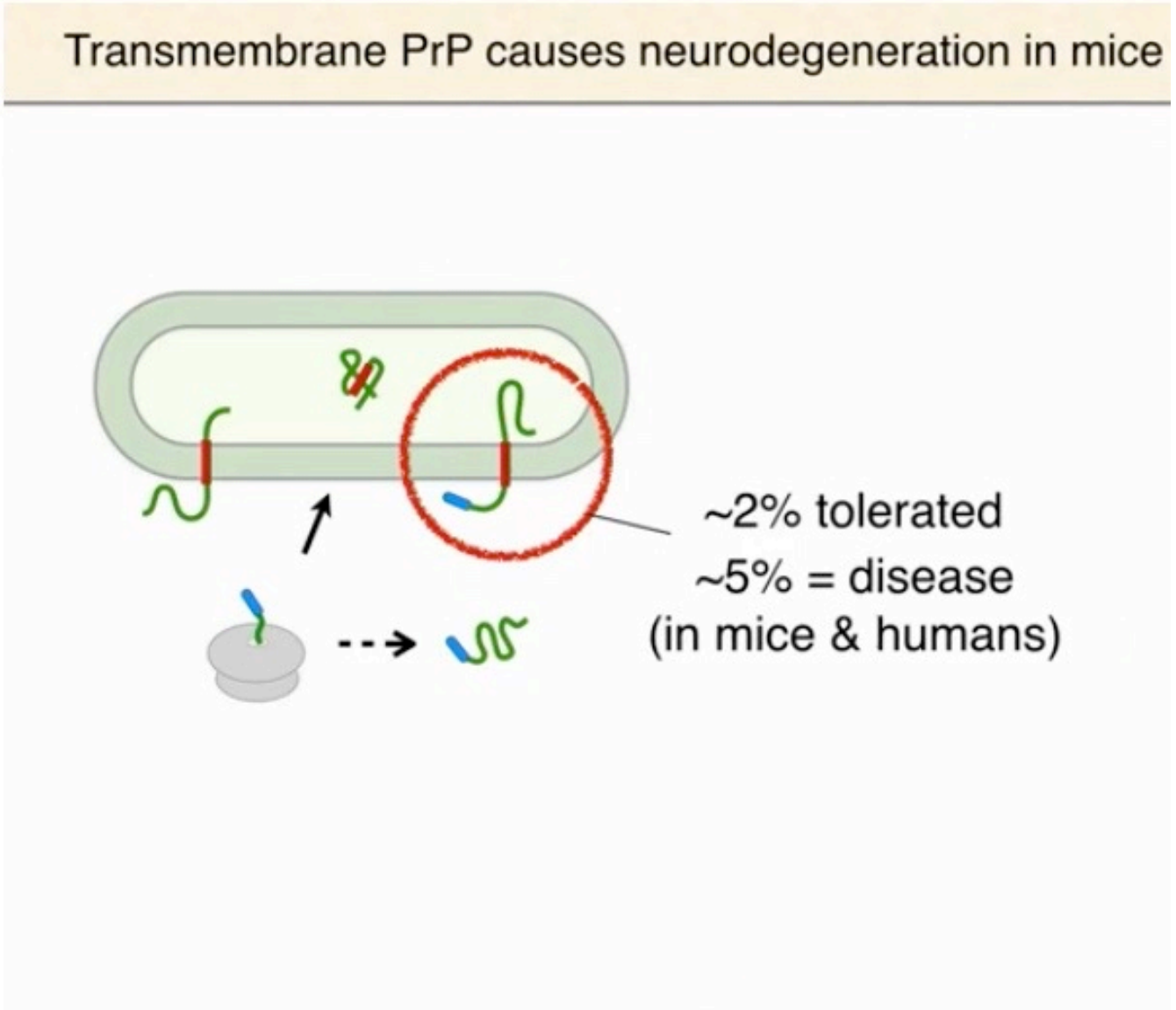


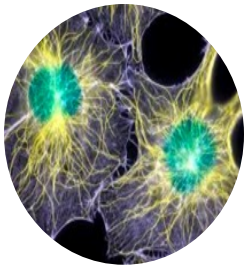
Fisiologia Celular e Molecular





Fisiologia Celular e Molecular





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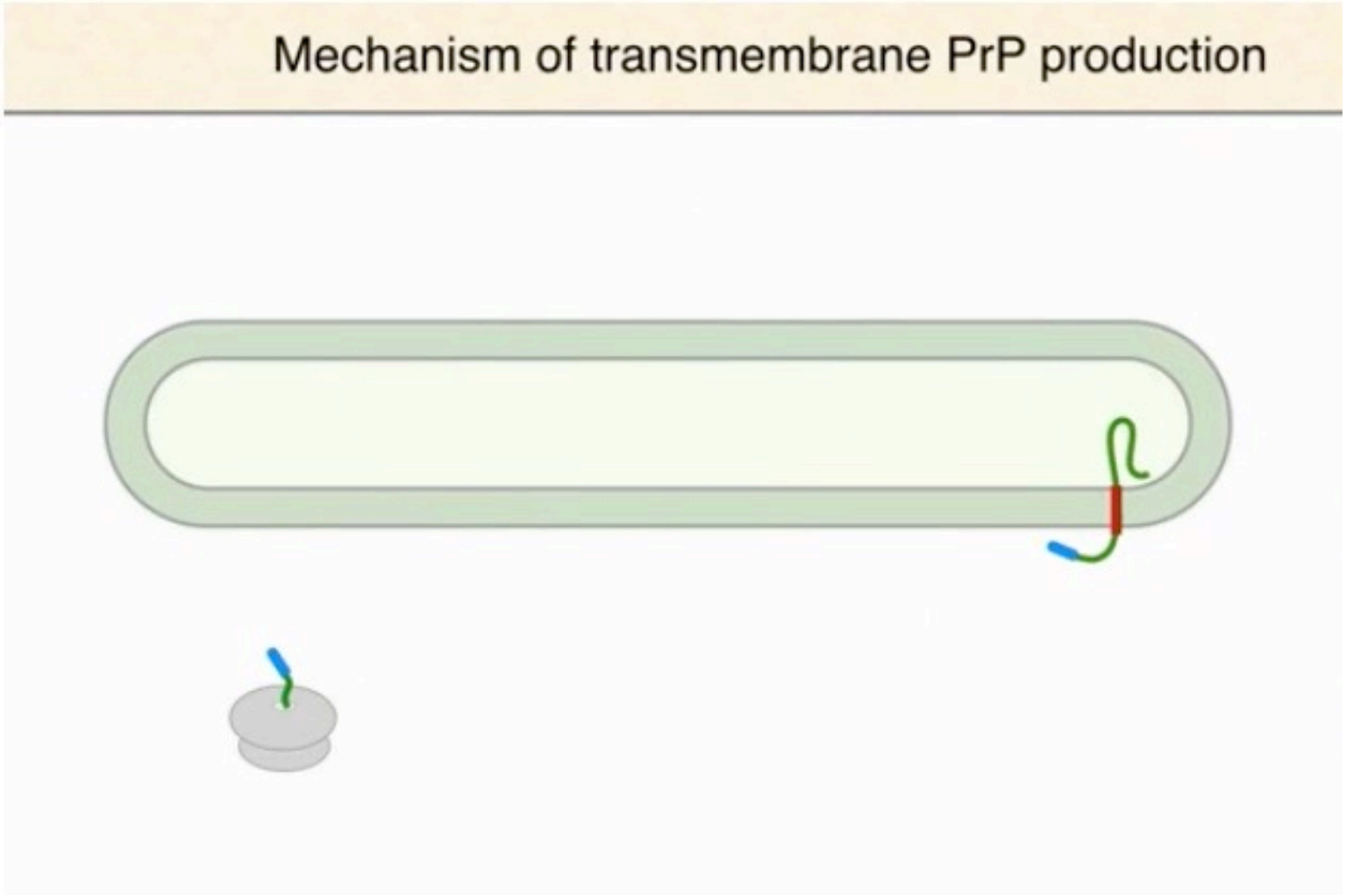
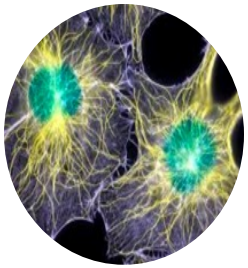
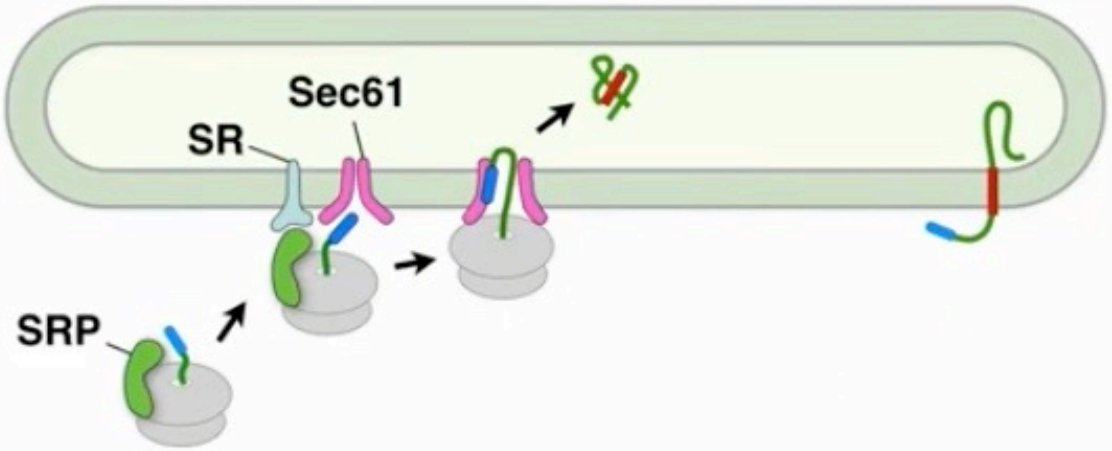


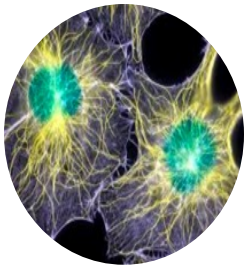


Fisiologia Celular e Molecular

Mechanism of transmembrane PrP production



Kim et al. (2002) *Dev. Cell*
Kim et al. (2002) *MBoC*



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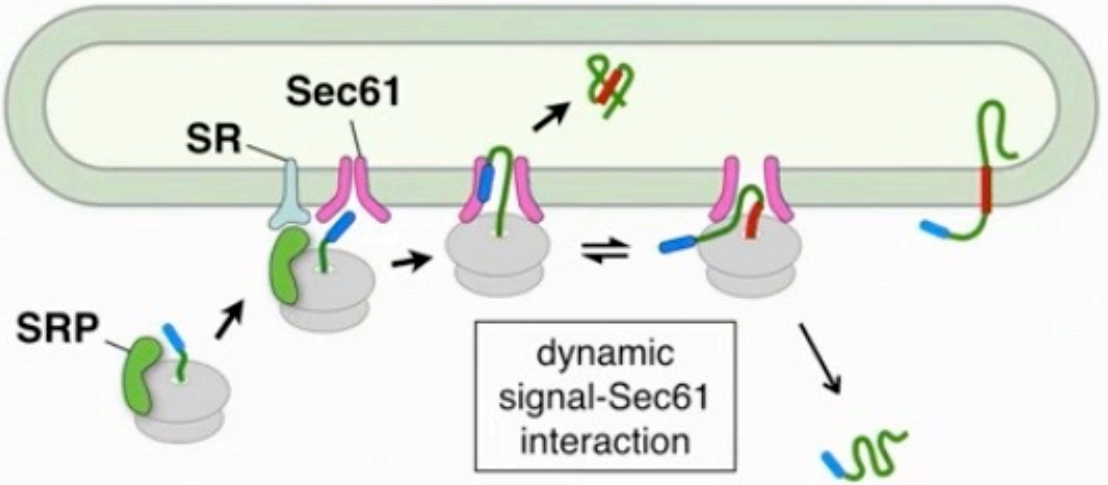

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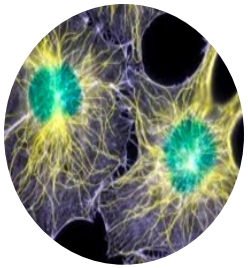

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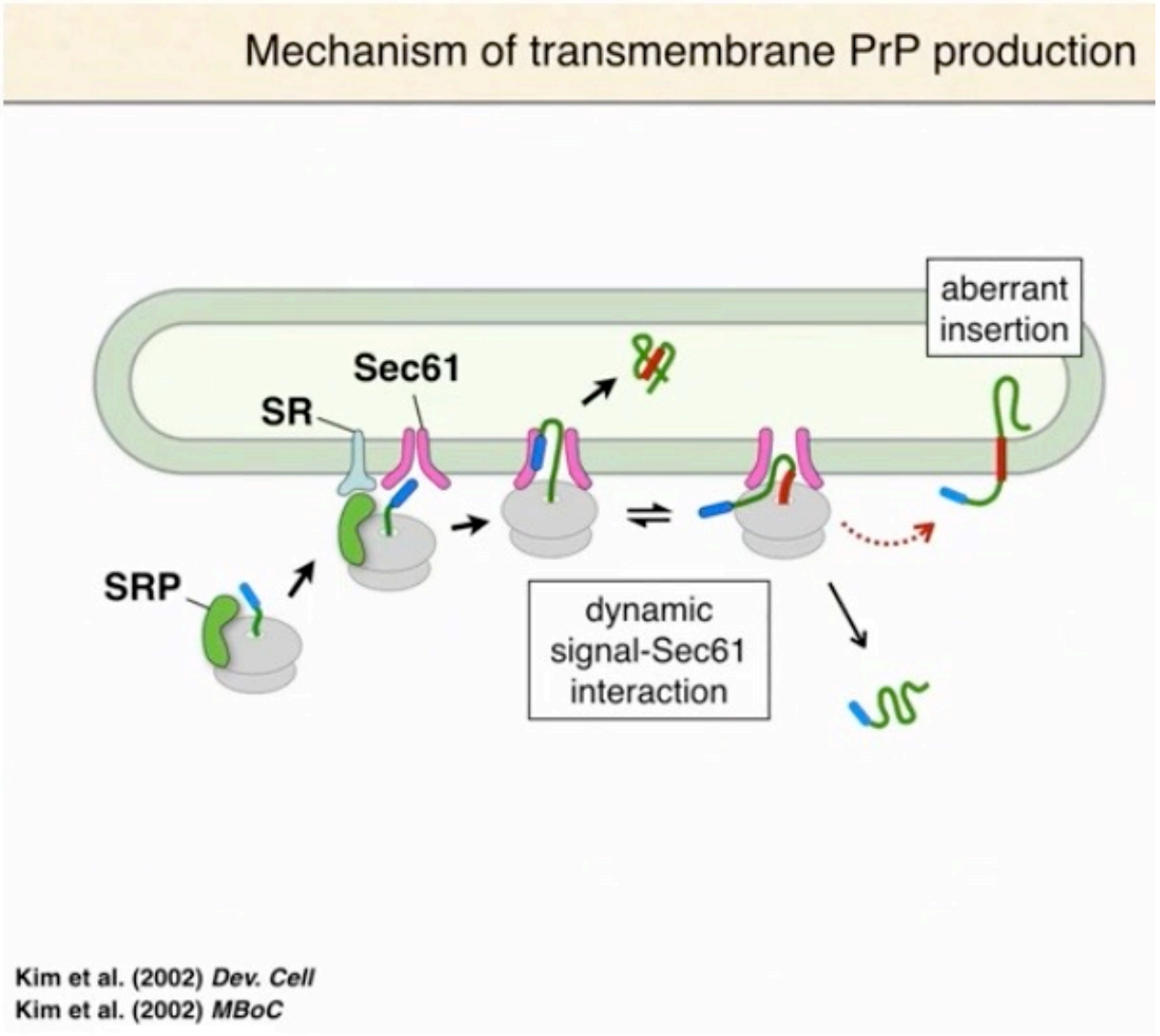

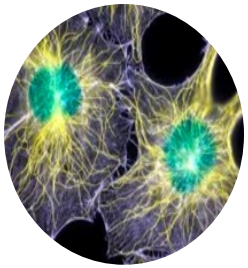

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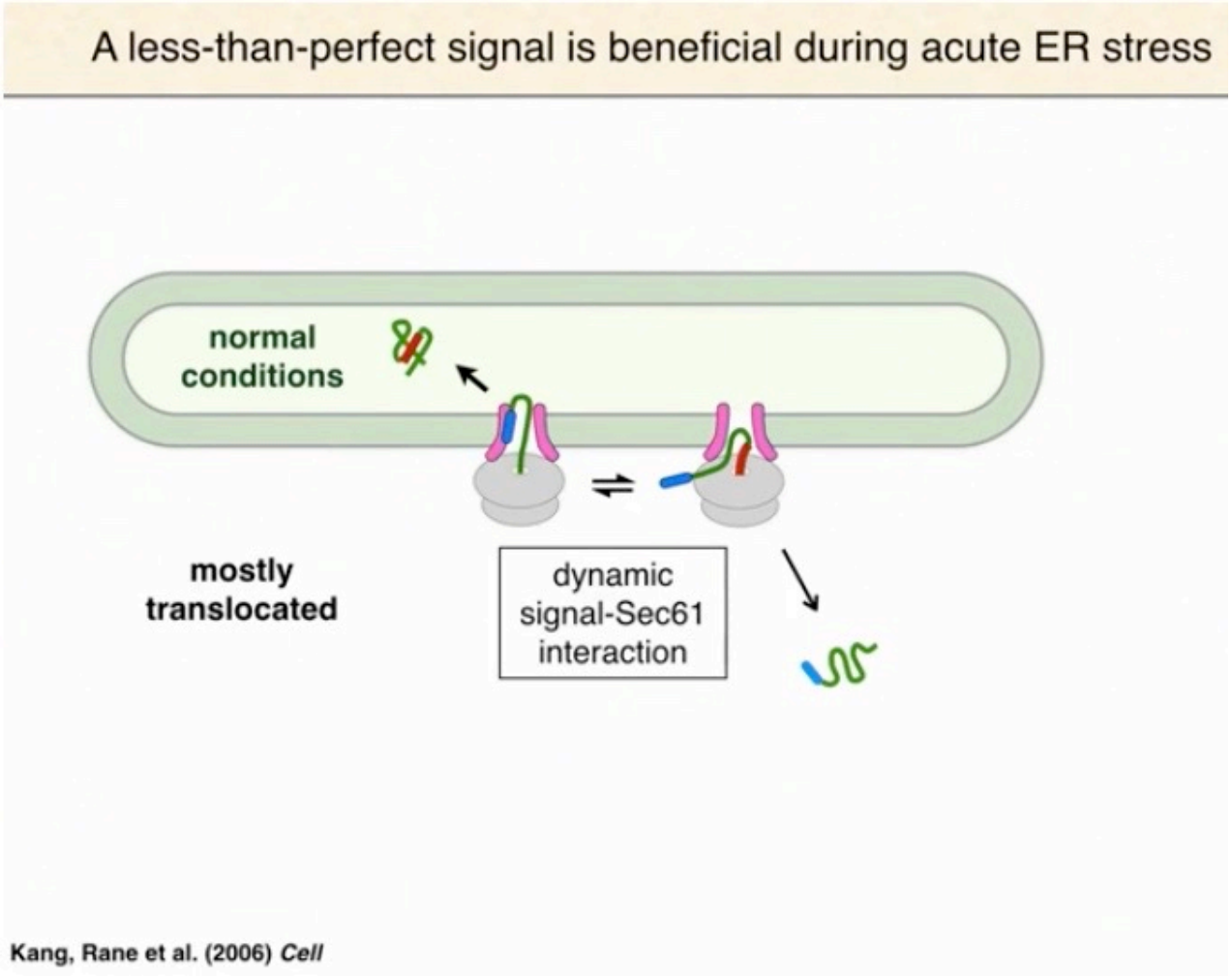
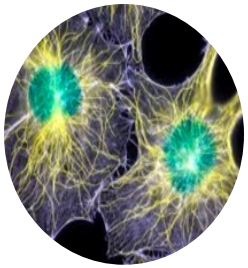
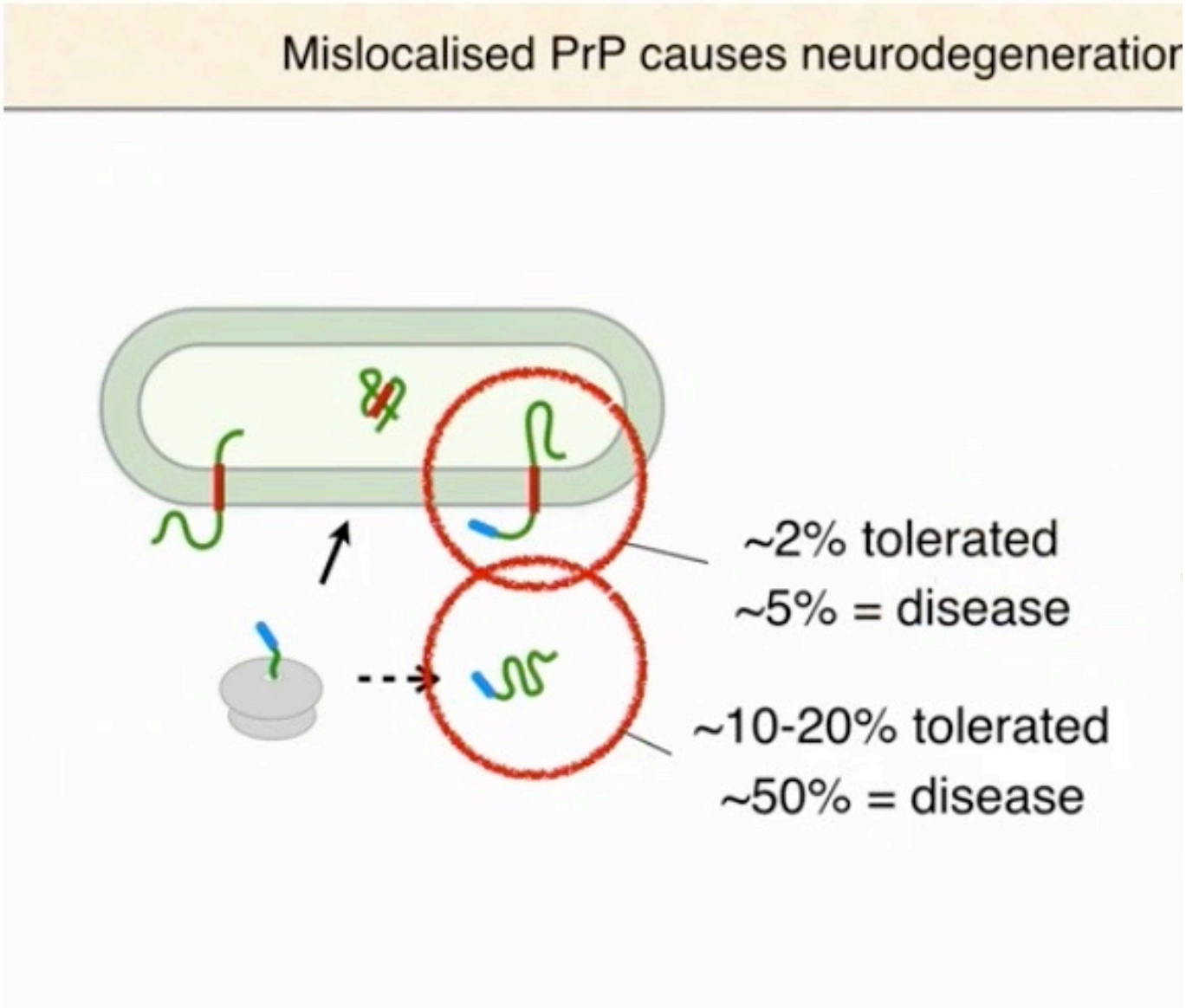
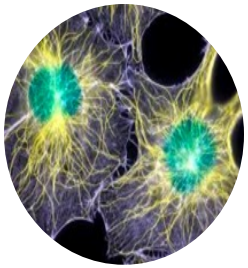




Fisiologia Celular e Molecular





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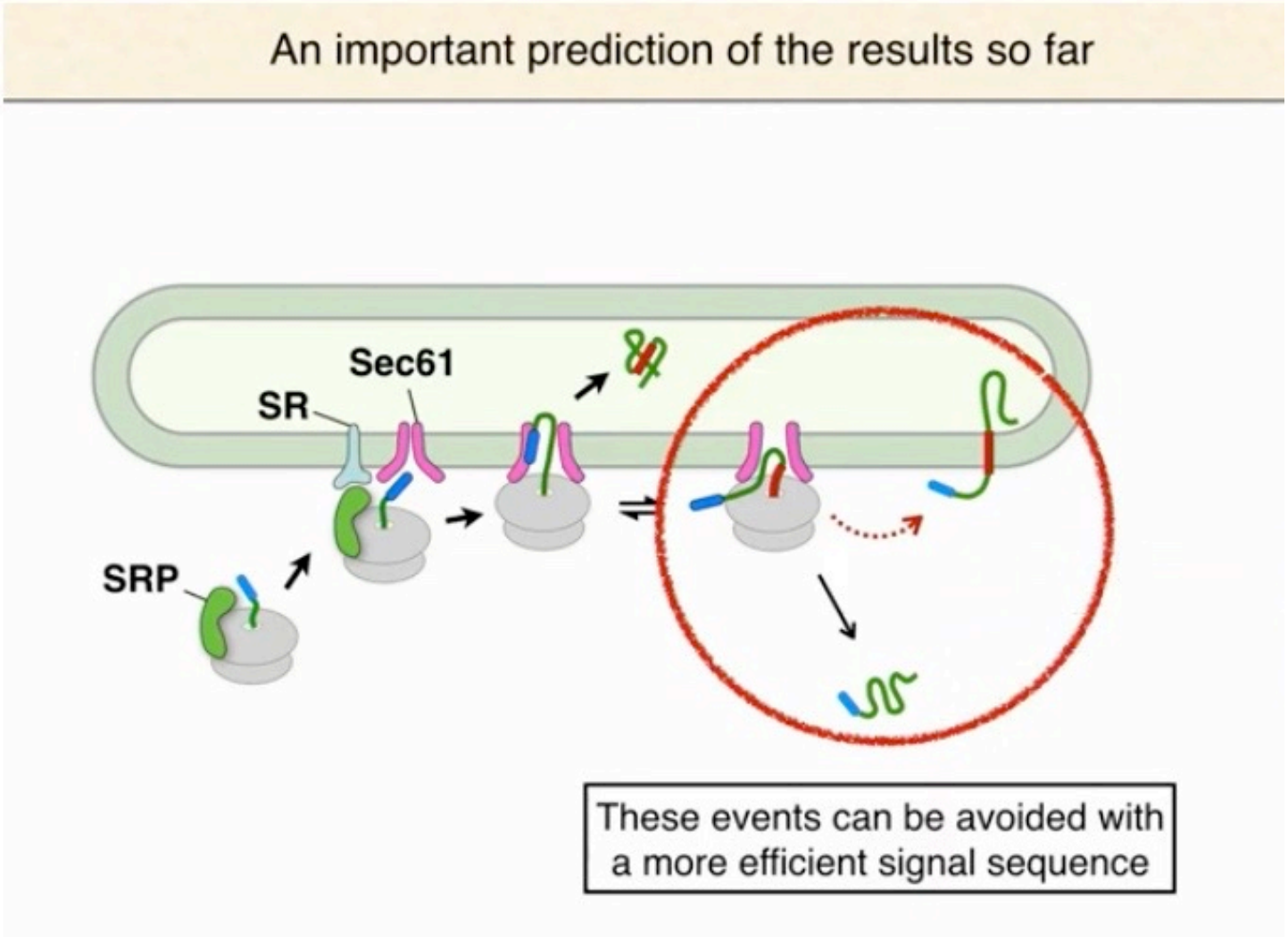
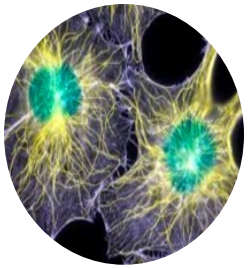
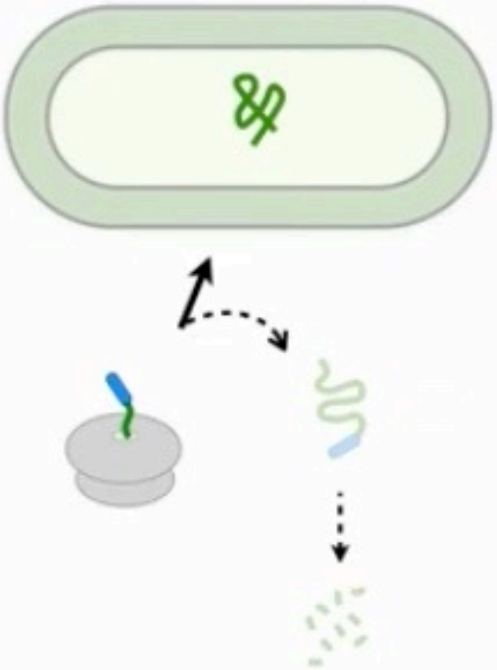




Fisiologia Celular e Molecular

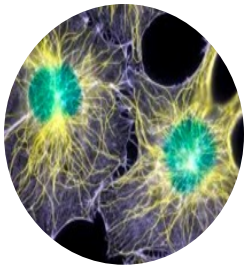
What did we learn from these experiments?



Errors during biosynthesis are pervasive, but 'invisible'

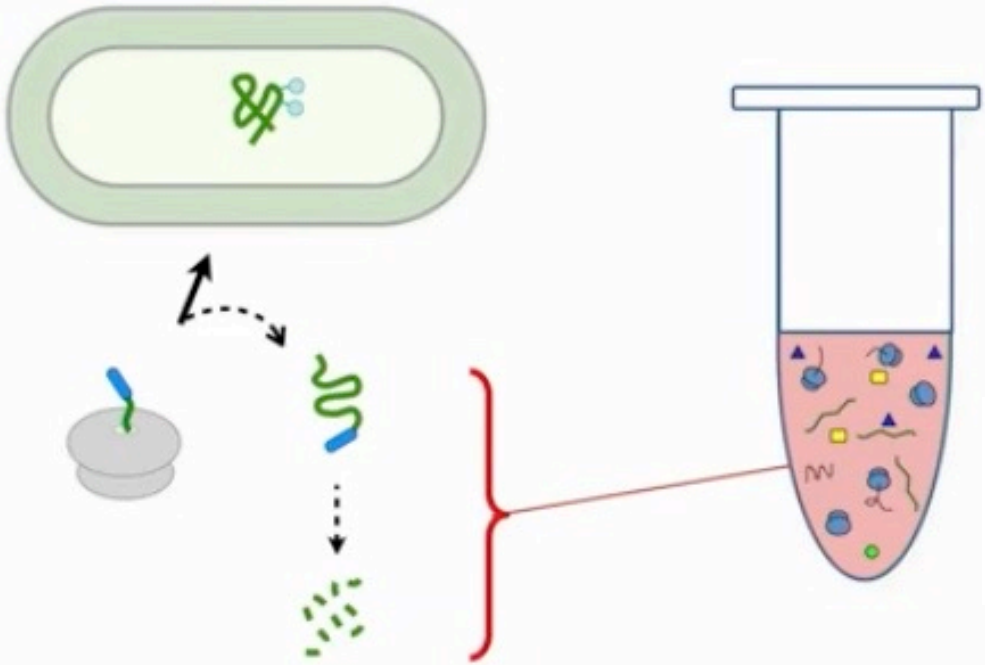
Subtle excess of errors can lead to disease over time

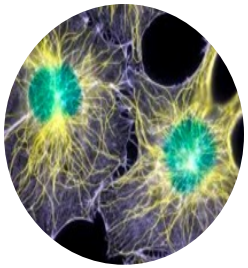
Not unique to prion protein



Fisiologia Celular e Molecular

Does mislocalised protein degradation work *in vitro*?





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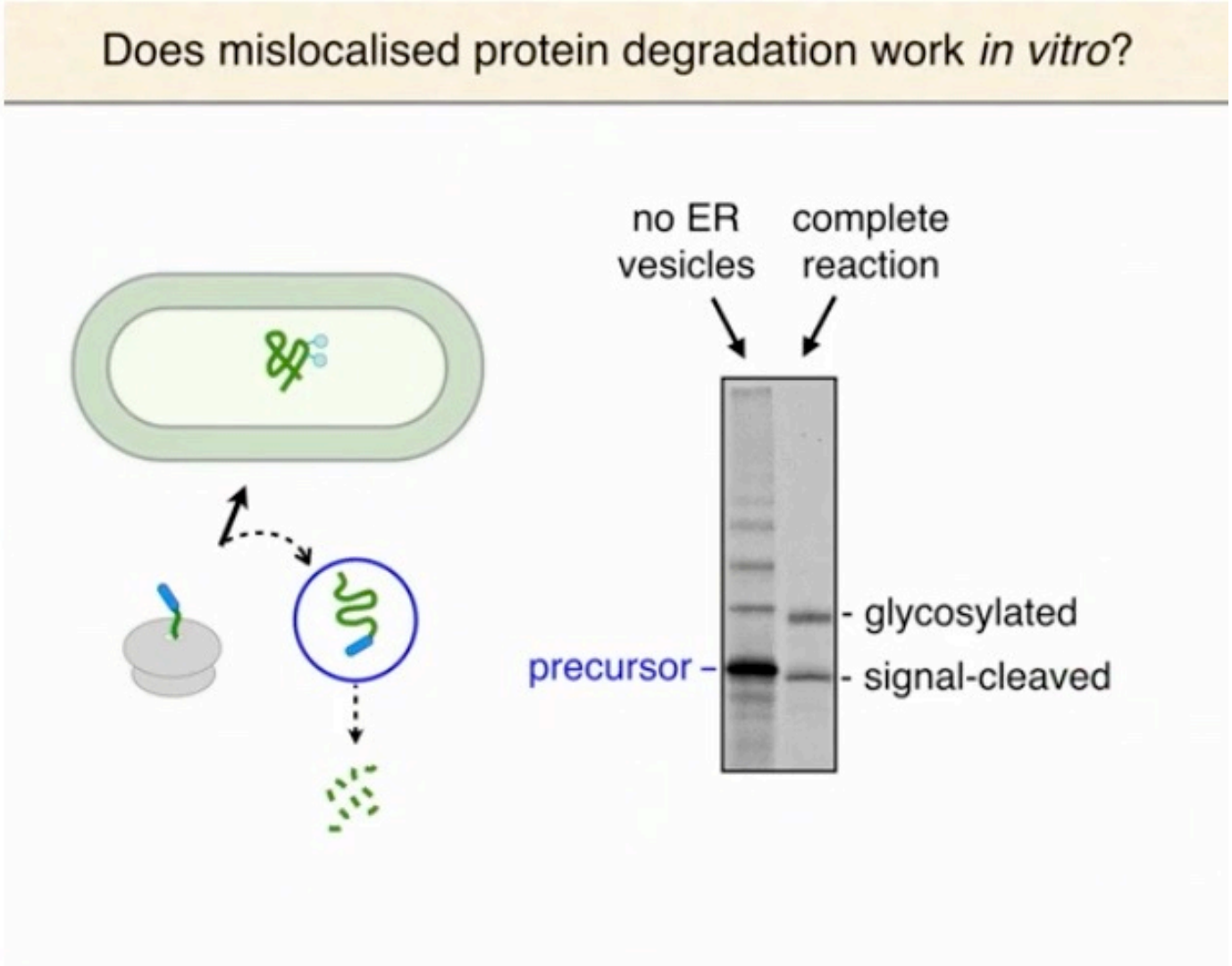

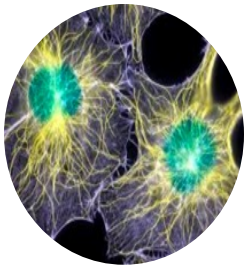

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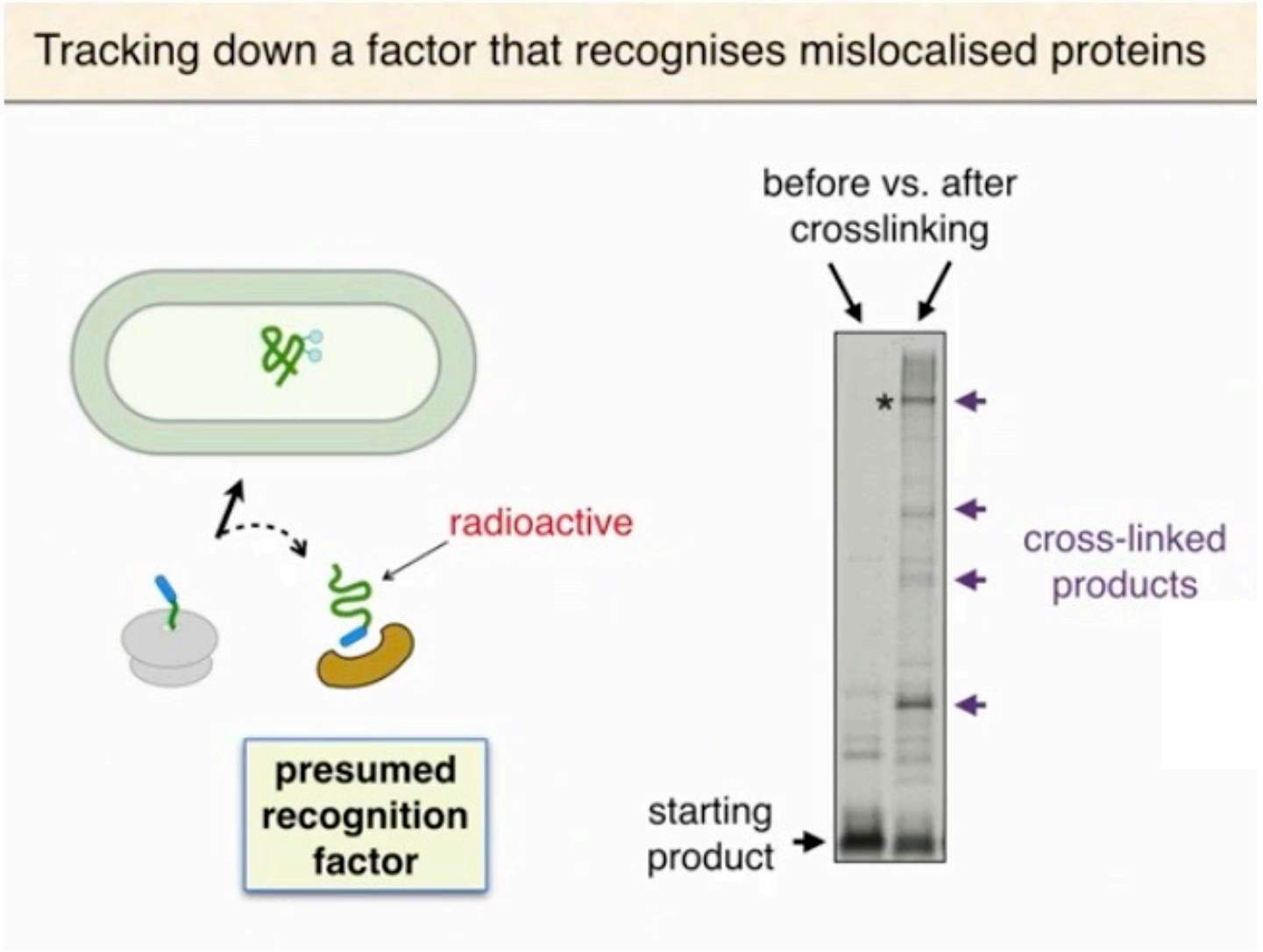
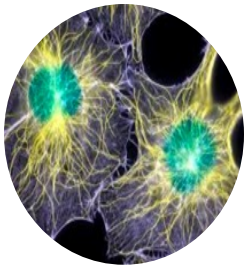
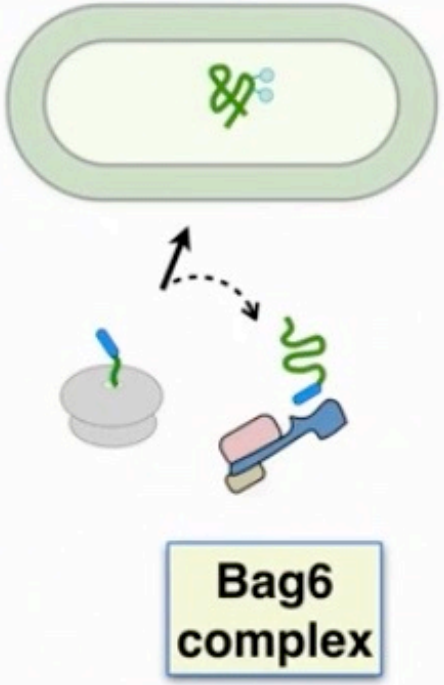




Fisiologia Celular e Molecular

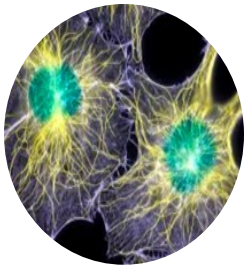
Bag6 is a factor that recognises mislocalised proteins



Features of the Bag6 complex

- composed of three subunits
- function not understood
- recognises many unrelated mislocalised proteins

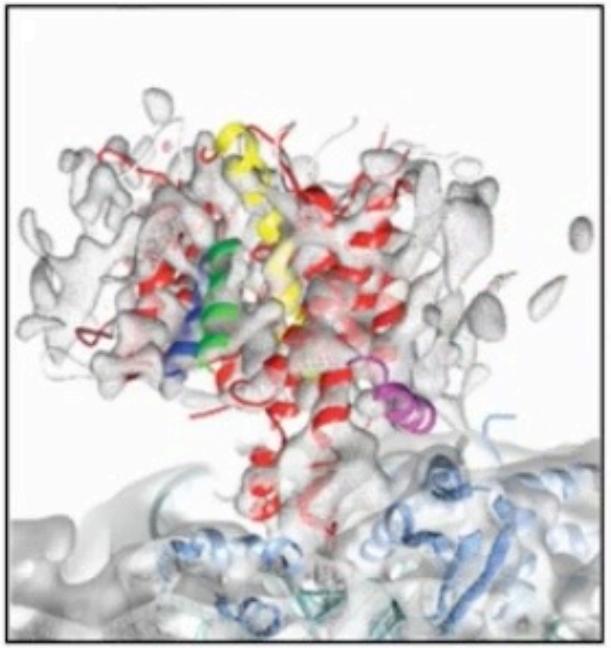
Hessa et al. (2011) *Nature*



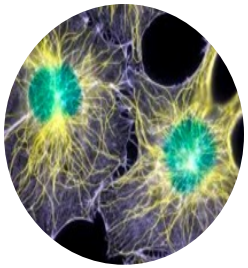
Fisiologia Celular e Molecular

Recognition events during protein targeting and insertion

Halic et al. (2004) *Nature*, **427**:808
(Beckmann lab)



Gogala et al. (2014) *Nature*, **506**:107
(Beckmann lab)



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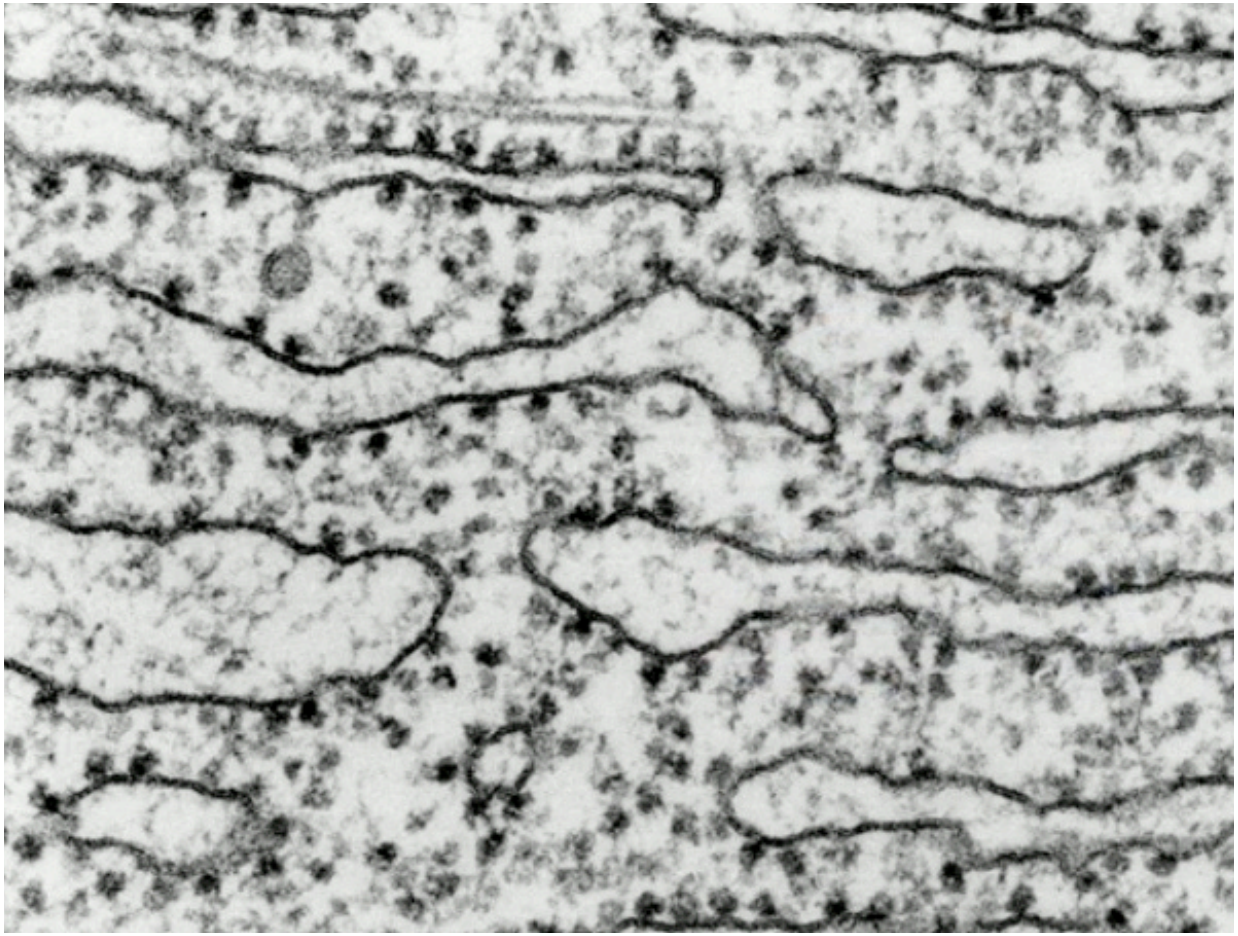
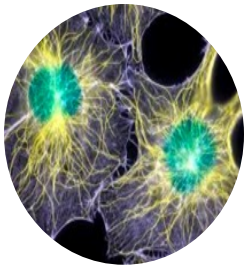
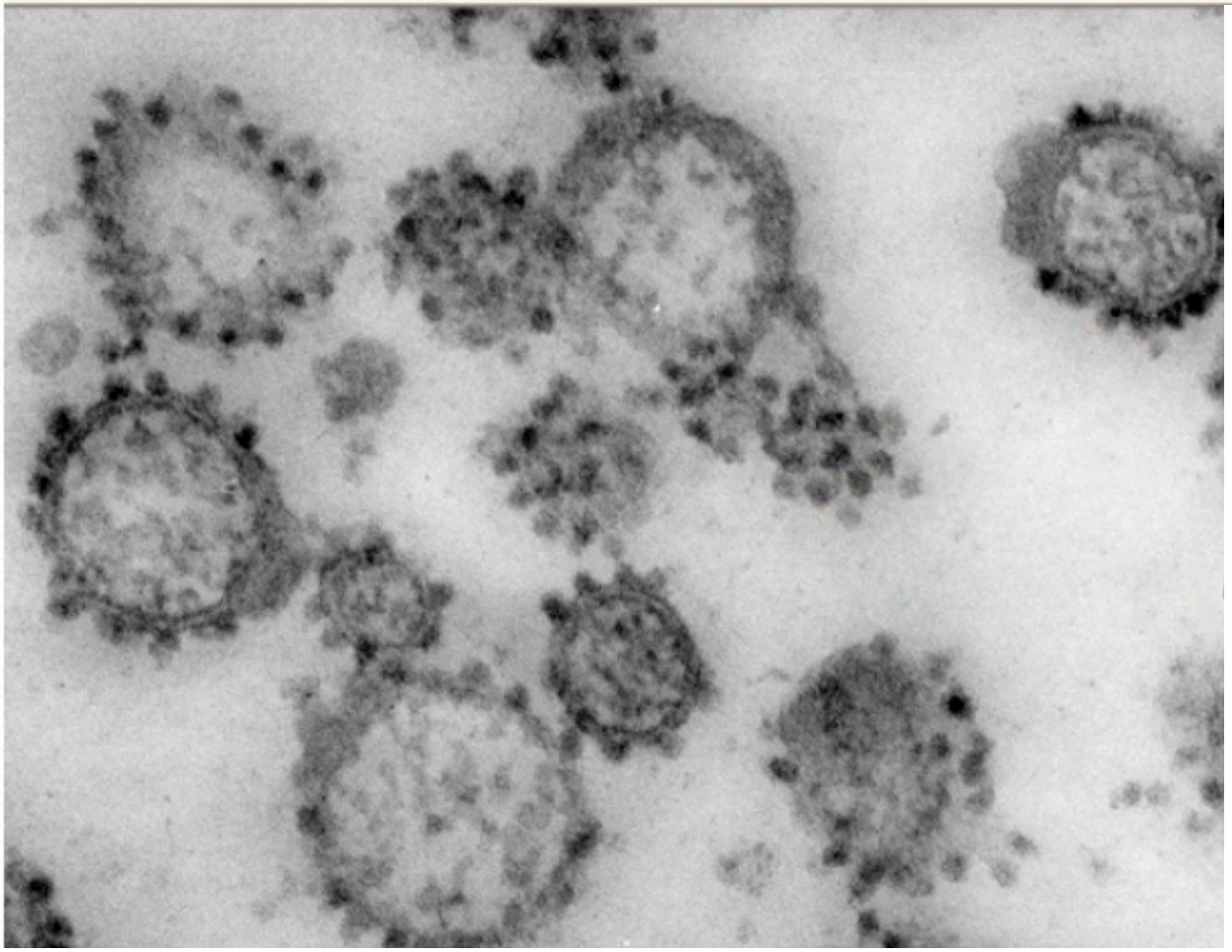
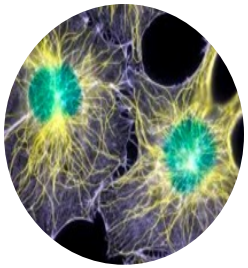




Fisiologia Celular e Molecular

Benchmarking cryo-EM methods using native ER complexes





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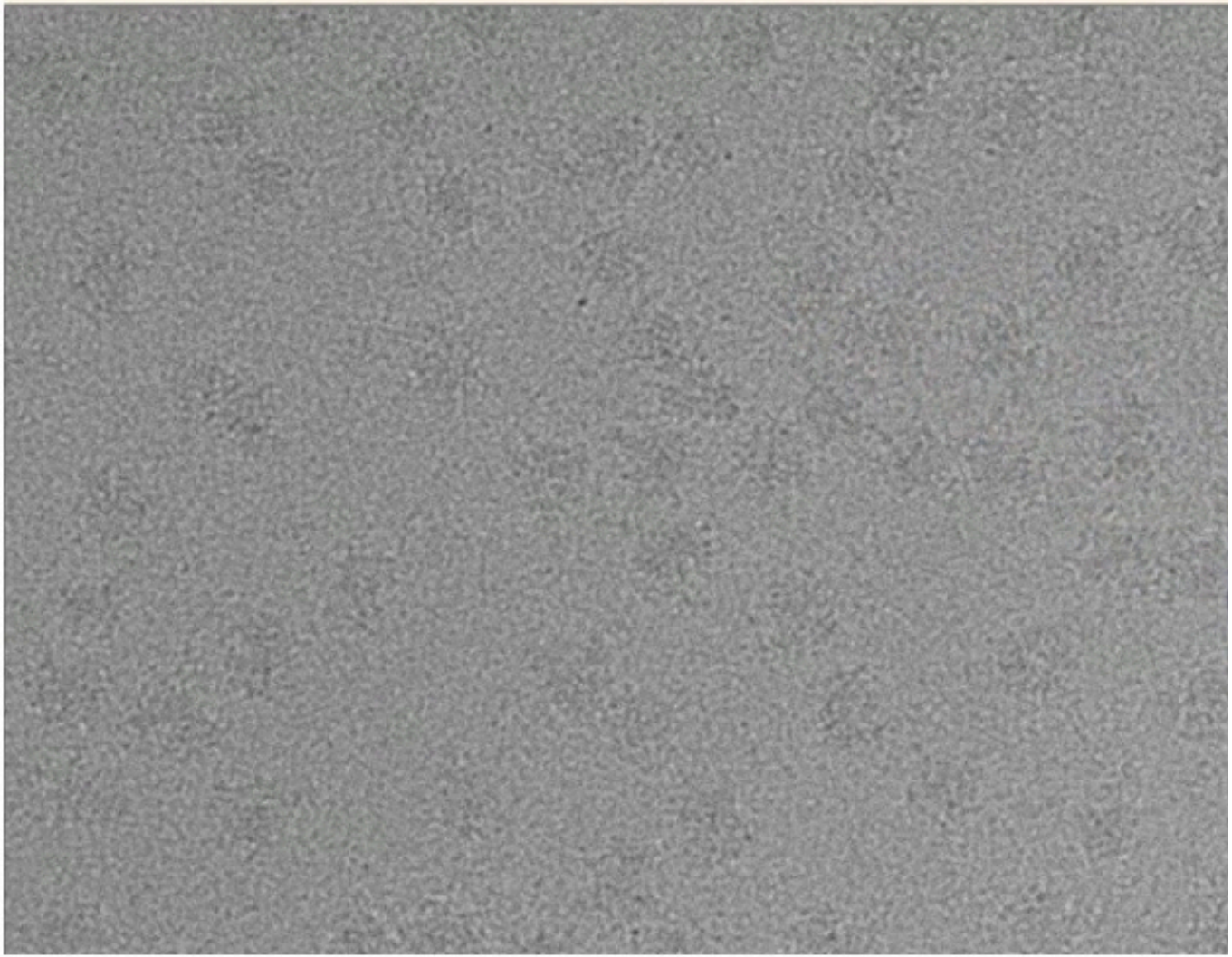

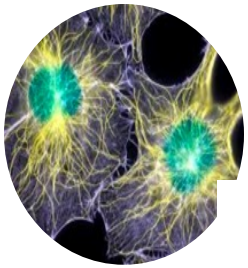

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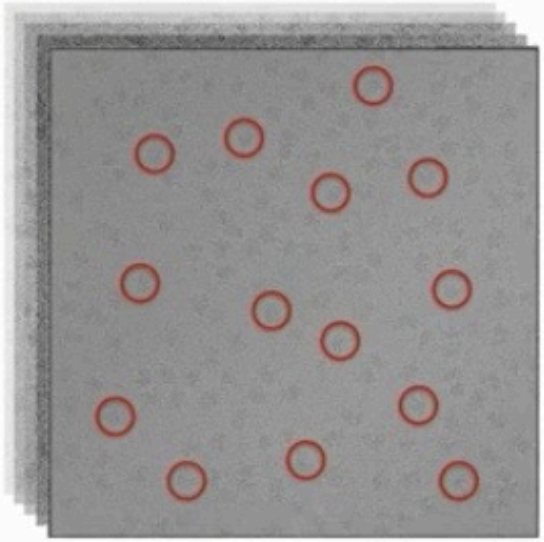

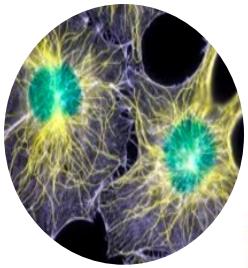

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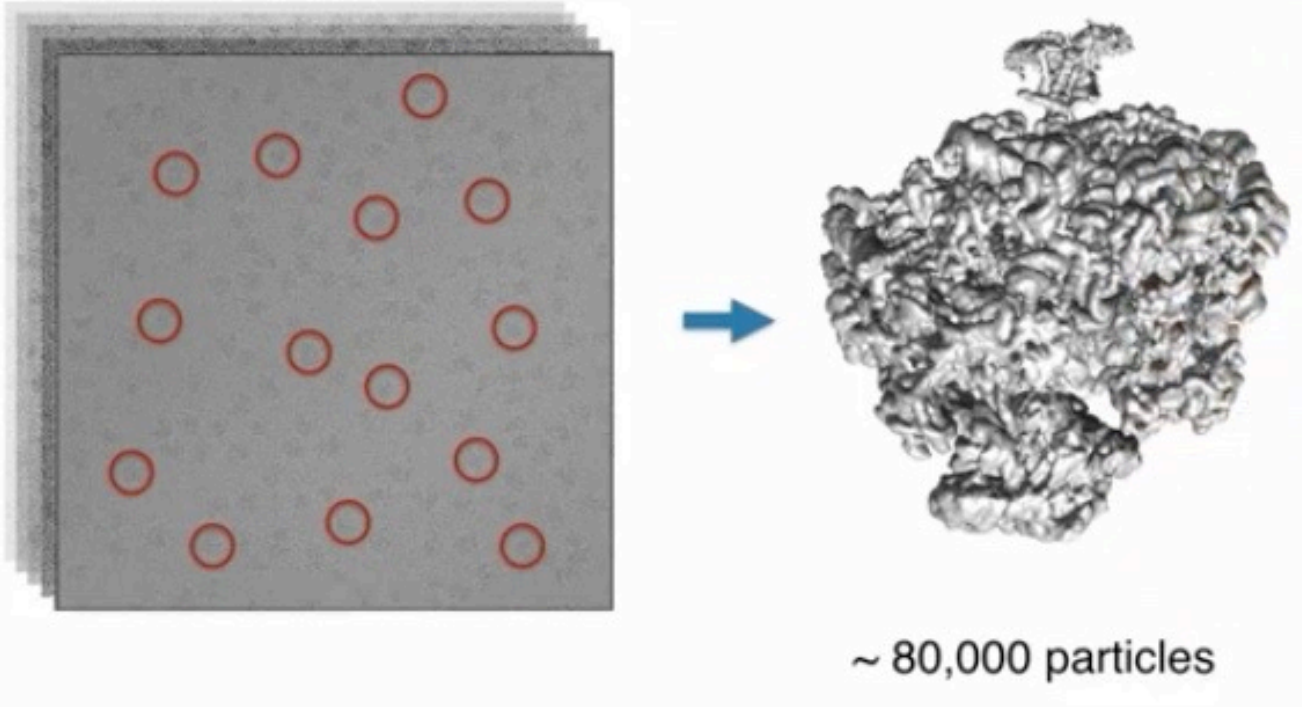

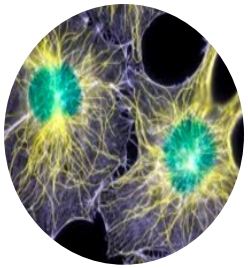

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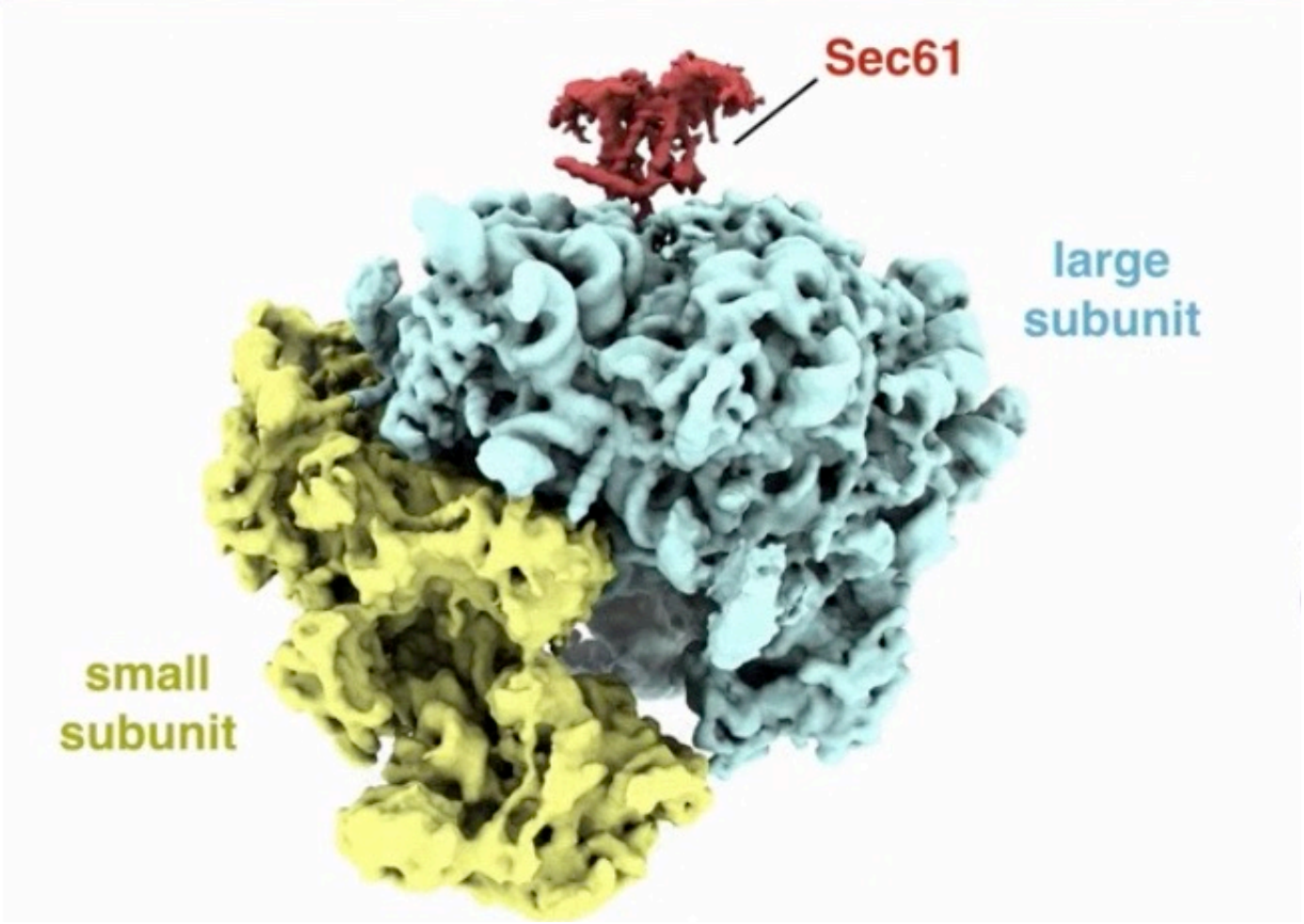
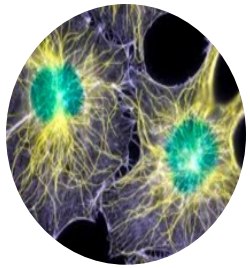
The initial results of our 'practice' sample





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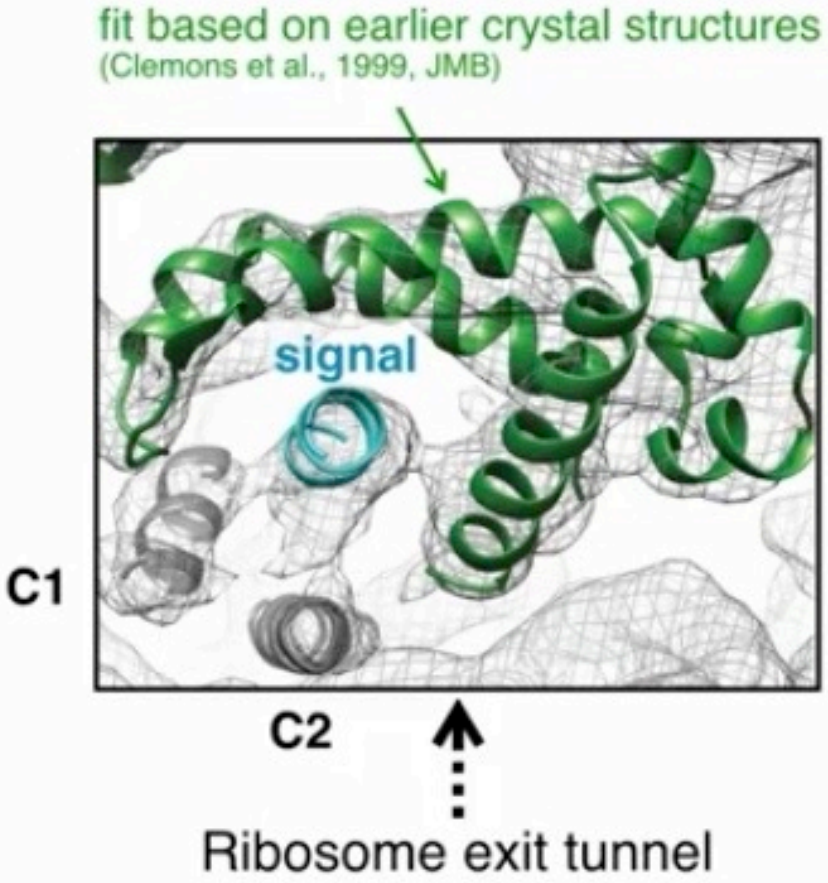
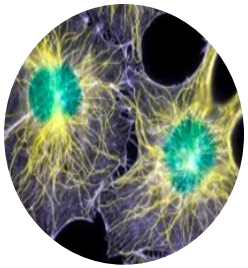
Signal recognition and shielding by SRP





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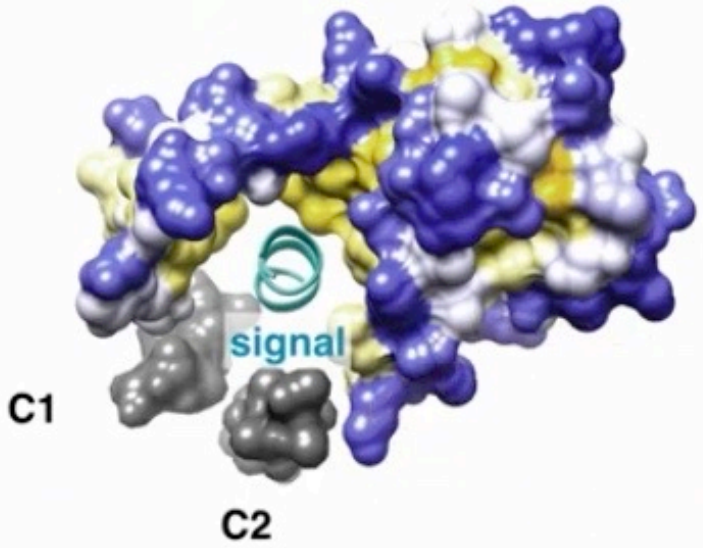
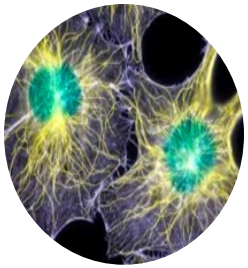


C1 and C2 helices form a 'lid' to fully shield the hydrophobic signal



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Conformation changes that accompany signal engagement