Self-organization and dynamics of the secretory pathway

Benjamin Glick
The University of Chicago

Prologue

A Cautionary Tale:

The Perils of Evolutionary Cell Biology

Mitochondria are derived from a bacterial e

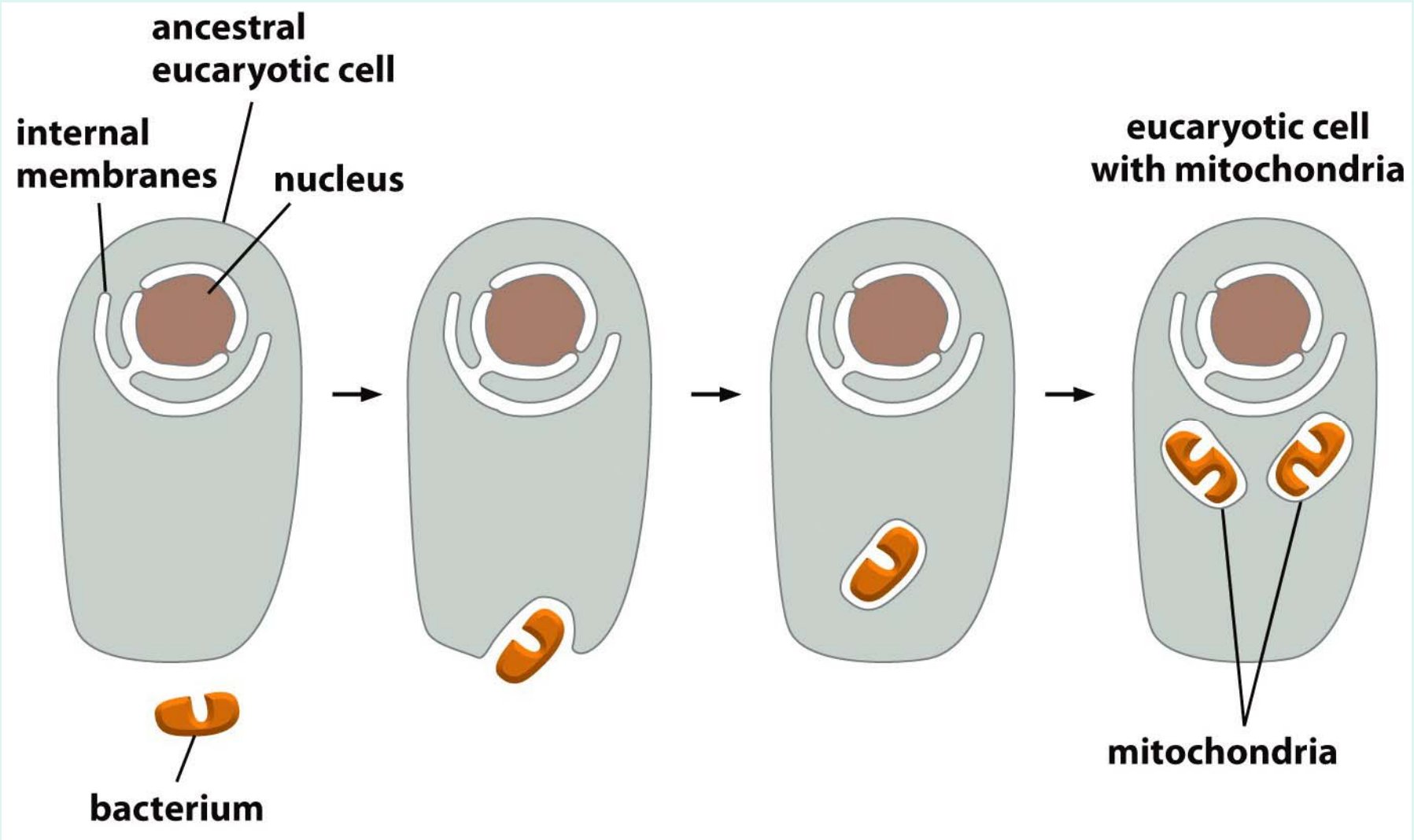

Figure 1-19 *Essential Cell Biology* (© Garland Science 2010)

Mitochondria import and sort protein

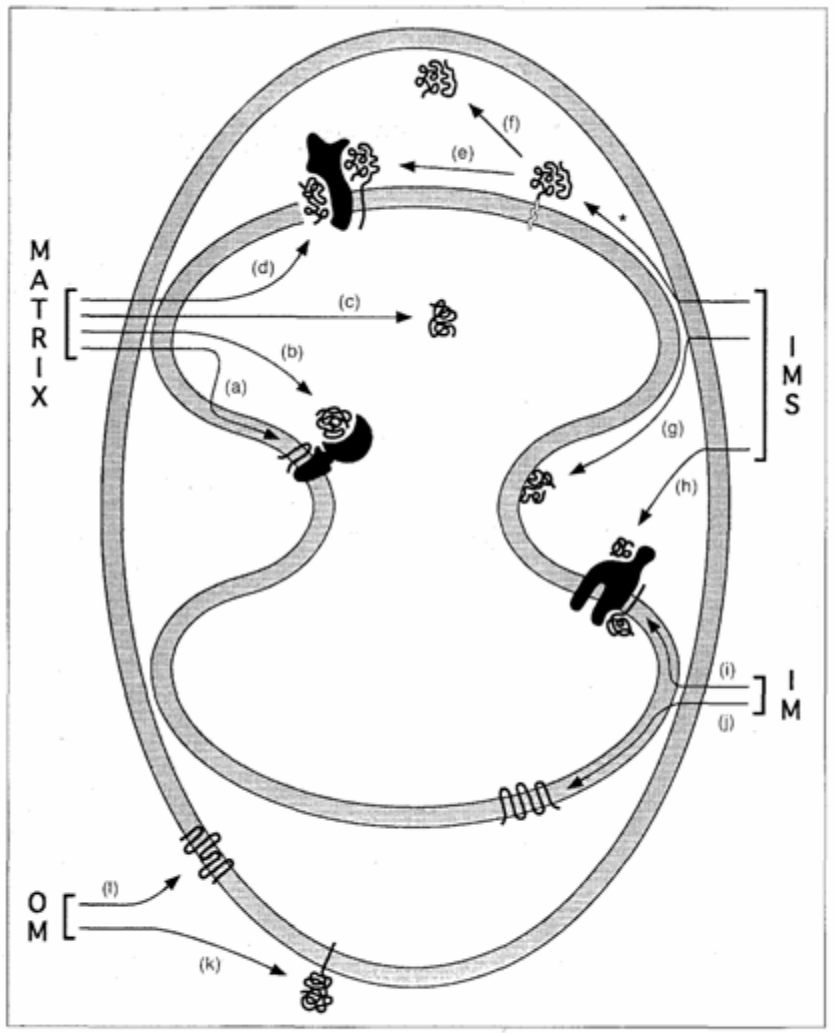

Figure 1

Sorting pathways of imported mitochondrial proteins. Proteins are grouped according to the compartment to which they are initially targeted. Unless otherwise stated, the diagram refers to yeast mitochondria and omits post-translational modifications such as proteolytic cleavage and the formation of homooligomers. OM, outer membrane; IM, inner membrane; IMS, intermembrane space.

Import into the matrix is determined by pr

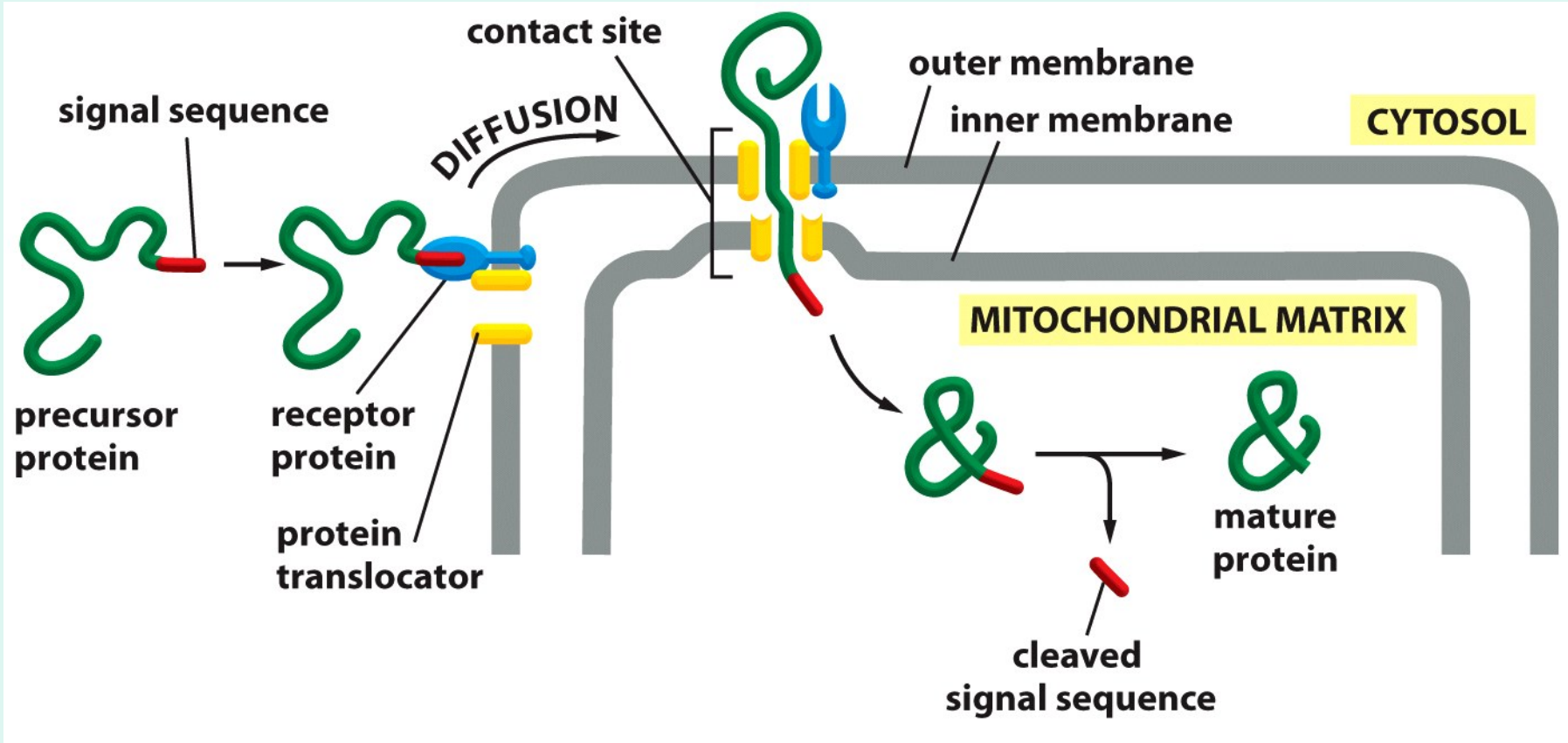

Figure 15-11 *Essential Cell Biology* (© Garland Science 2010)

How do proteins reach the intermembrane

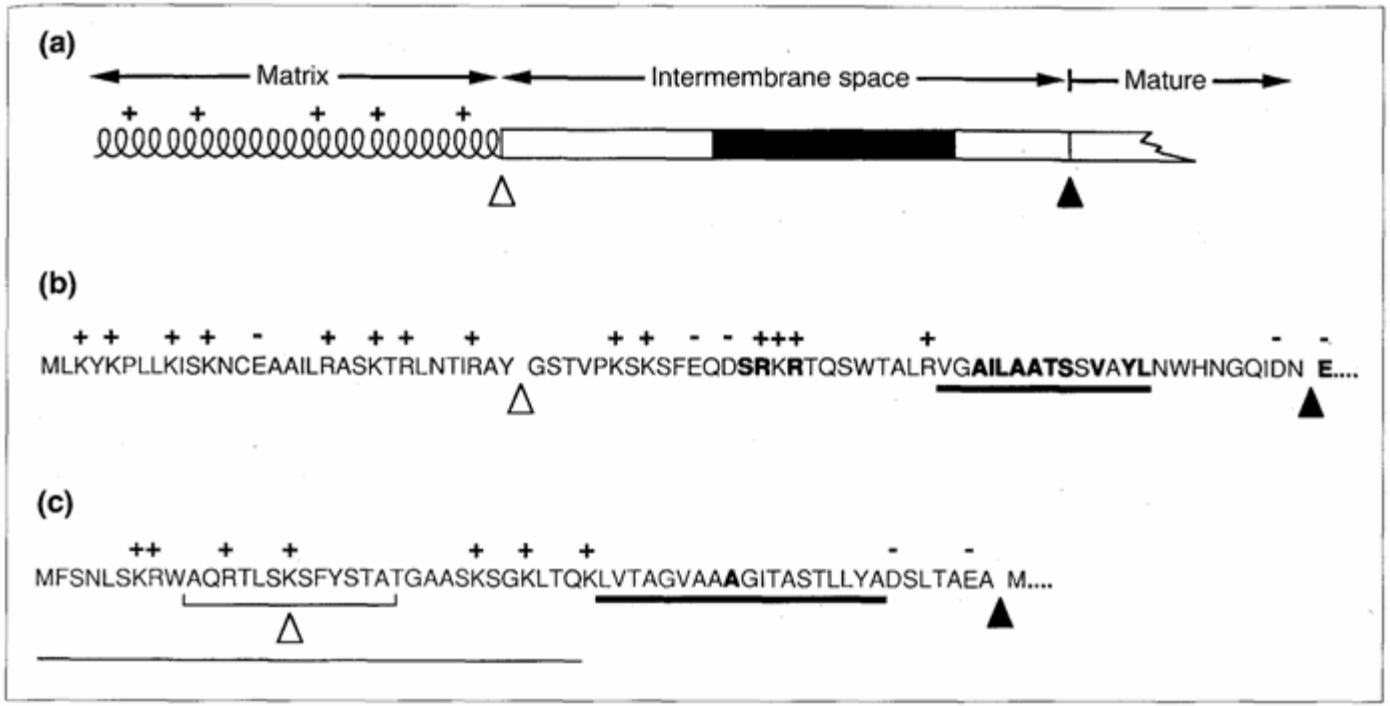

Figure 2

The presequences of cytochromes b_2 and c_1 contain bipartite targeting signals. **(a)** The general structure of a presequence with intermembrane space targeting information. The amino-terminal portion of the presequence resembles a matrix-targeting signal; the carboxy-terminal portion contains a hydrophobic stretch (represented by the black box) that is necessary for targeting to the intermembrane space. The presequence is cleaved twice, first by the soluble matrix processing protease (△) and then by a protease facing the intermembrane space (▲). **(b)** Yeast cytochrome b_2; **(c)** yeast cytochrome c_1; both cytochromes conform to the scheme outlined in (a). The hydrophobic stretch in each presequence is underlined, and charged amino acids are indicated. The first cleavage site for cytochrome c_1 has not been identified, but it is probably within the region marked by the bracket. For the amino acids shown in bold, mutations have been isolated that weaken or inactivate the intermembrane space targeting signals (Ref. 29; E. Beasley, unpublished). In the cytochrome b_2 presequence, this targeting signal has been shown to include residues outside of the hydrophobic stretch.

How do proteins reach the intermembrane

Science, New Series, Vol. 247, No. 4945 (Feb. 23, 1990), pp. 930-938

Protein Sorting to Mitochondria: Evolutionary Conservations of Folding and Assembly

FRANZ-ULRICH HARTL* AND WALTER NEUPERT

According to the endosymbiont hypothesis, mitochondria have lost the autonomy of their prokaryotic ancestors. They have to import most of their proteins from the cytosol because the mitochondrial genome codes for only a small percentage of the polypeptides that reside in the organelle. Recent findings show that the sorting of proteins into the mitochondrial subcompartments and their folding and assembly follow principles already developed in prokaryotes. The components involved may have structural and functional equivalents in bacteria.

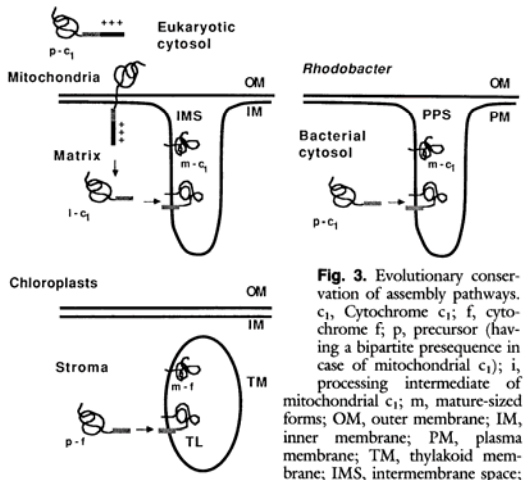

Fig. 3. Evolutionary conservation of assembly pathways. c_1, Cytochrome c_1; f, cytochrome f; p, precursor (having a bipartite presequence in case of mitochondrial c_1); i, processing intermediate of mitochondrial c_1; m, mature-sized forms; OM, outer membrane; IM, inner membrane; PM, plasma membrane; TM, thylakoid membrane; IMS, intermembrane space; PPS, periplasmic space; TL, thylakoid lumen.

How do proteins reach the intermembrane

Cell, Vol. 69, 809–822, May 29, 1992, Copyright © 1992 by Cell Press

Cytochromes c_1 and b_2 Are Sorted to the Intermembrane Space of Yeast Mitochondria by a Stop-Transfer Mechanism

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Biocenter
University of Basel
CH-4056 Basel
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Summary

The pathway by which cytochromes c_1 and b_2 reach the mitochondrial intermembrane space has been controversial. According to the "conservative sorting" hypothesis, these proteins are first imported across both outer and inner membranes into the matrix, and then are retranslocated across the inner membrane. Our data argue against this model: import intermediates of cytochromes c_1 and b_2 were found only outside the inner membrane; maturation of these proteins was independent of the matrix-localized hsp60 chaperone; and dihydrofolate reductase linked to the presequence of either cytochrome was imported to the intermembrane space in the absence of ATP. We conclude that cytochromes c_1 and b_2 are sorted by a mechanism in which translocation through the inner membrane is arrested by a "stop-transfer" signal in the presequence. The arrested intermediates may be associated with a proteinaceous channel in the inner membrane.

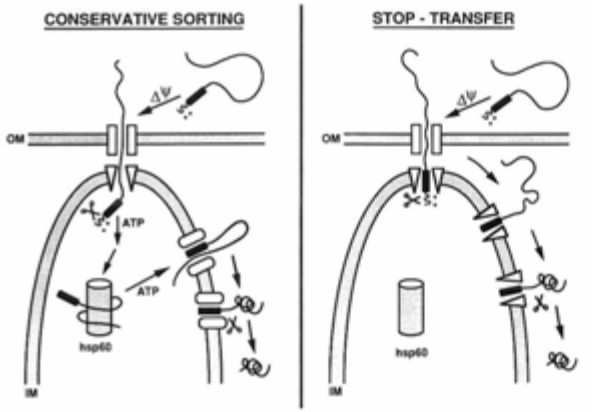


Figure 1. Two Models for Protein Import to the Mitochondrial Intermembrane Space

Left: the conservative sorting or reexport model. A precursor protein is translocated across both membranes into the matrix, where the amino-terminal matrix-targeting sequence is cleaved off. The intermediate-sized form associates with hsp60. The sorting sequence is then recognized by a bacterial-type export machinery, which translocates the polypeptide back across the inner membrane. A second proteolytic cleavage generates the mature protein in the intermembrane space. Both import into the matrix and release from hsp60 would require ATP hydrolysis. Right: the stop-transfer model. Translocation through the inner membrane is arrested by the sorting sequence. Only the amino-terminal part of the presequence reaches the matrix, where it is cleaved by the processing protease. The remainder of the polypeptide continues to cross the outer membrane into the intermembrane space. Proteolytic removal of the sorting sequence releases the mature protein. Hsp60 should not be required for this process, and the import of some proteins to the intermembrane space might proceed in the absence of ATP. The wavy line with "+" signs represents a matrix-targeting signal, and the solid rectangle indicates an intermembrane space sorting sequence. OM: outer membrane. IM: inner membrane. Open rectangles: import channels in the outer membrane. Triangles: import channels in the inner membrane. Ellipses: putative bacterial-type export machinery. Scissors: proteolytic cleavages of the presequence. $\Delta\psi$: electrochemical potential across the inner membrane.

Take-Home Message

Evolutionary arguments are very seductive, but can be misleading.

A lot has happened in the past two billion years.

“No cellular organelle has been the subject of as many, as long-lasting, or as diverse polemics as the Golgi apparatus.”

Whaley, 1975

The trouble begins...

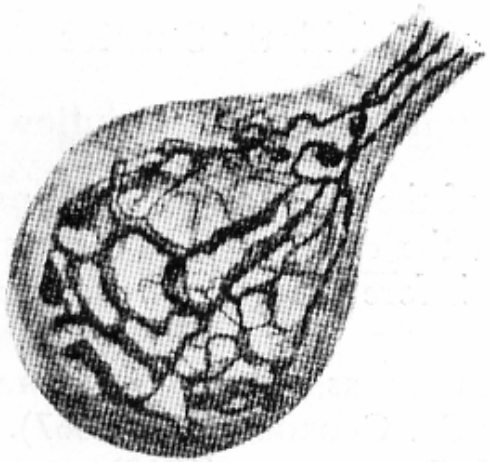
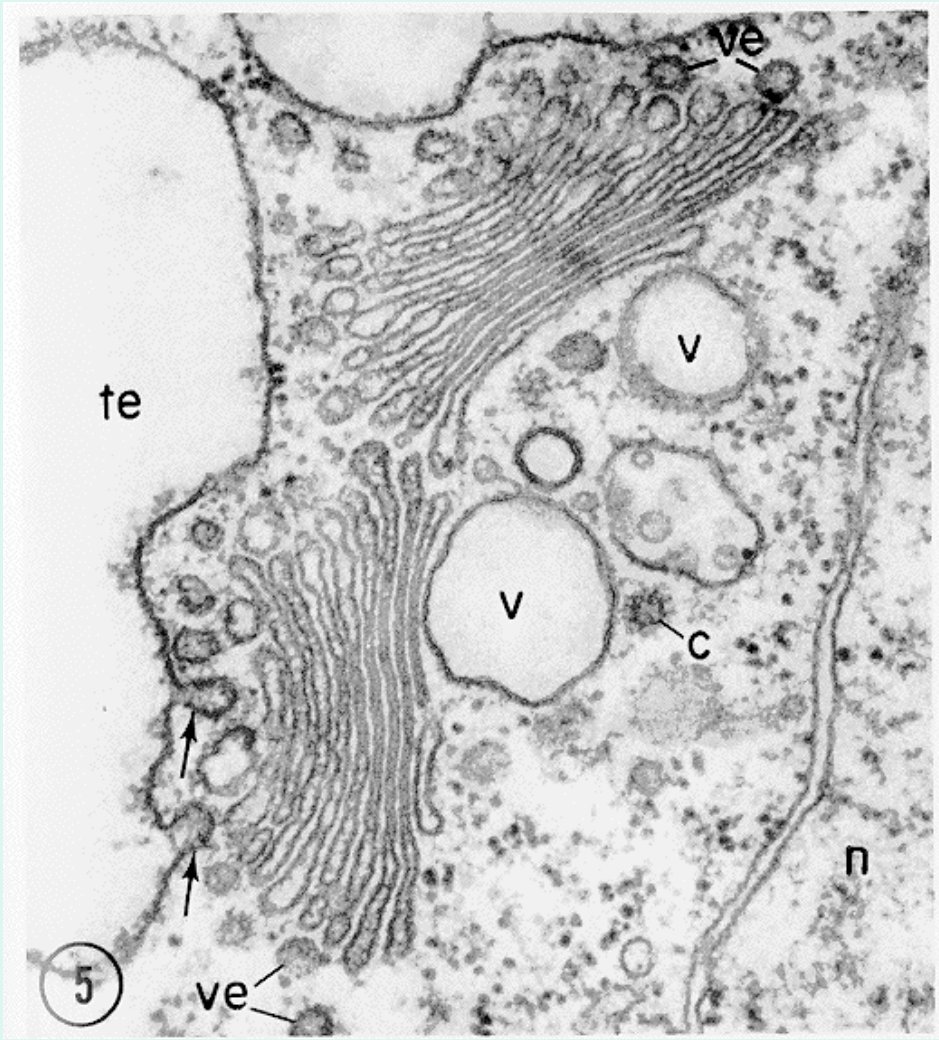
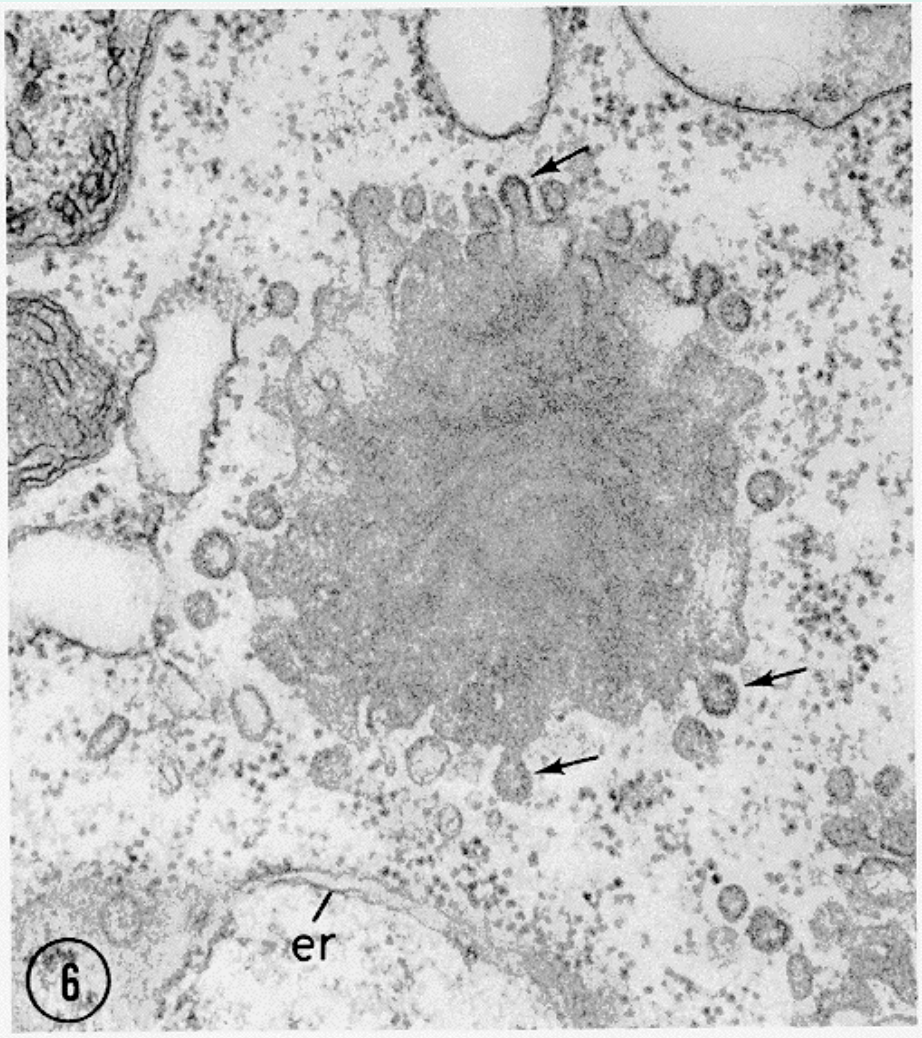


Fig. 1. One of Golgi's original drawings of the internal reticular apparatus as seen in a Purkinje cell of a barn owl. From Arch. ital. Biol. **30**, 1898.

The Golgi in *Chlamydomonas*





Farquhar and Palade, 1981

The mammalian Golgi is a ribbon

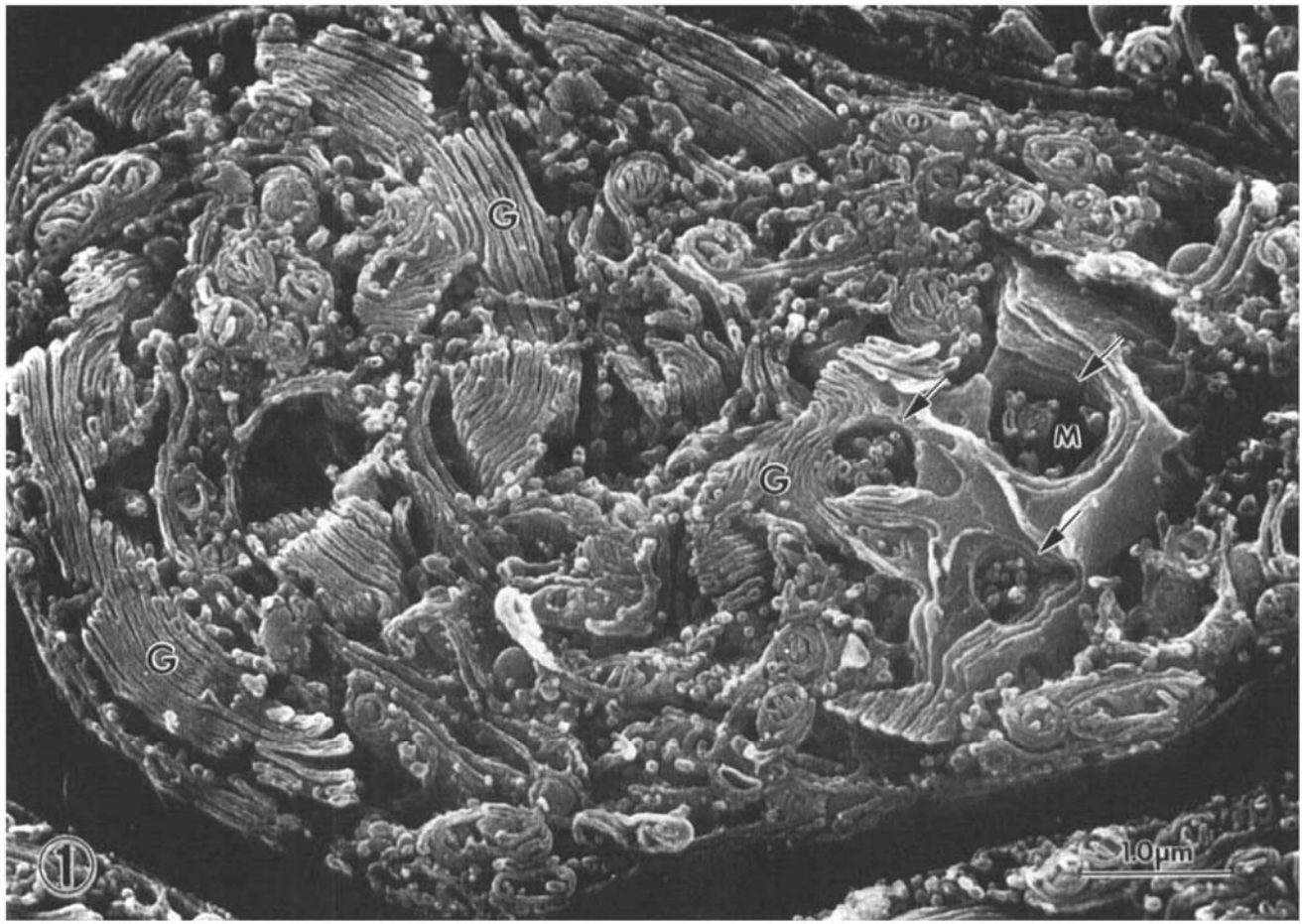
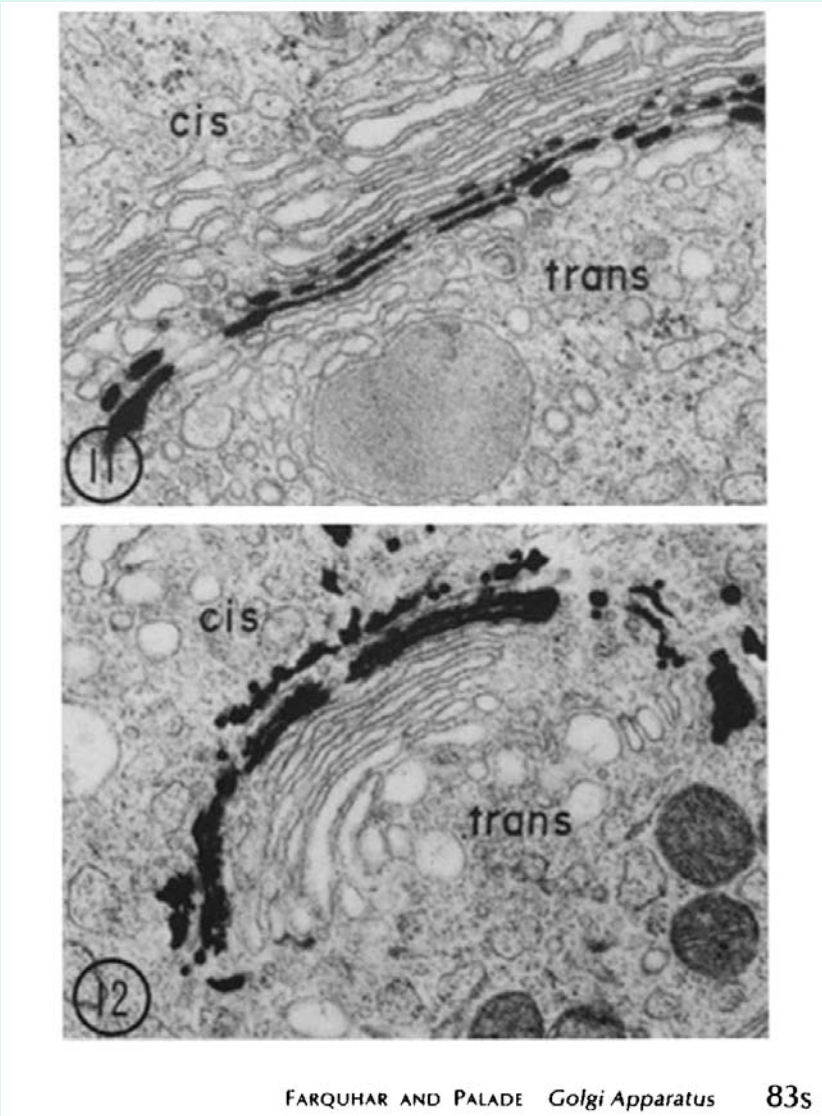


Fig. 1. Golgi complex (G) of a rat epididymal cell. Cross section at the supranuclear region. Large channels through stacks of cisternae illustrated at the arrows (and enlarged in Fig. 3). A mitochondrion (M) and tubules are visible in the channels. ×20,000.

Golgi stacks are polarized

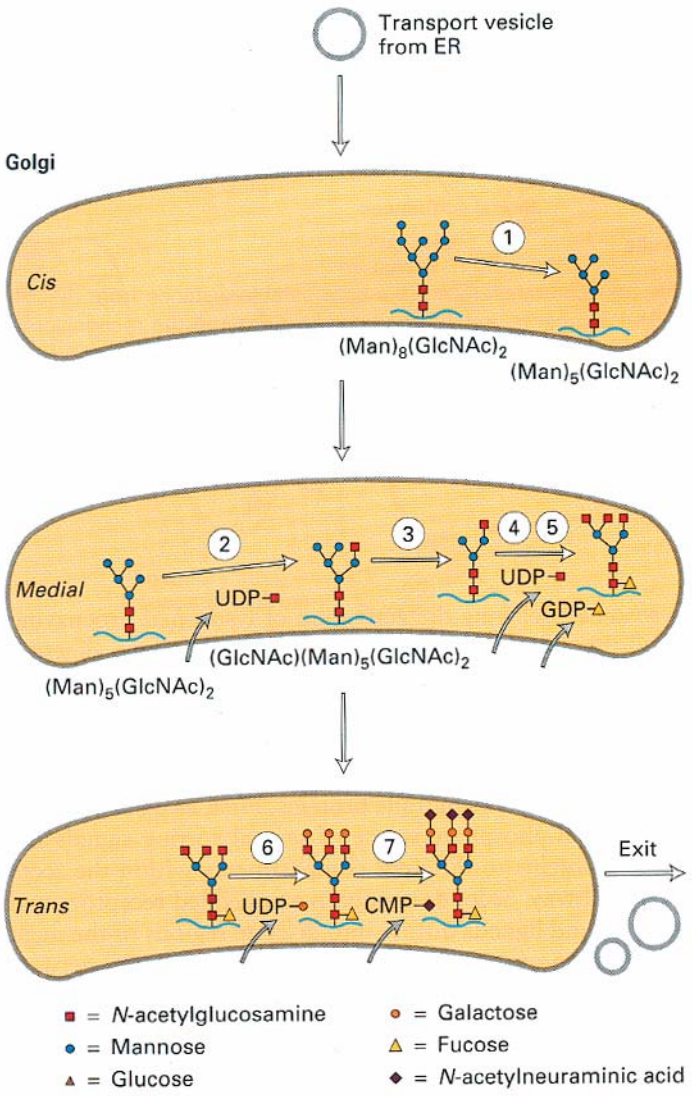


The Golgi has two main functions

(1) It serves as a “carbohydrate factory”.

(2) At the trans-Golgi network (TGN), cargo molecules are sorted into different types of carriers for delivery to their final destinations.

igosaccharides are modified in the Gol



◀ **FIGURE 17-38 Processing of glycoproteins within *cis*-, *medial*-, and *trans*-Golgi cisternae to yield *N*-linked complex oligosaccharides in vertebrate cells.** The enzymes catalyzing each step are localized to the indicated compartments. After removal of three mannose residues in the *cis*-Golgi (step ①), the protein moves by cisternal progression to the *medial*-Golgi. Here, two more mannose residues are removed (step ③), three GlcNAc residues are added (steps ②, ④), and a single fucose is added (step ⑤). Processing is completed in the *trans*-Golgi by addition of three galactose residues (step ⑥) and finally linkage of an N-acetylneuraminic acid residue to each of the galactose residues (step ⑦). Note that added sugars are transferred to the oligosaccharide, one at a time, from sugar nucleotide precursors by specific transferase enzymes, as in the synthesis of *O*-linked oligosaccharides (see Figure 17-32). The sugar nucleotides are imported from the cytosol. [See R. Kornfeld and S. Kornfeld, 1985, *Annu. Rev. Biochem.* **45**:631.]

The Golgi has two main functions

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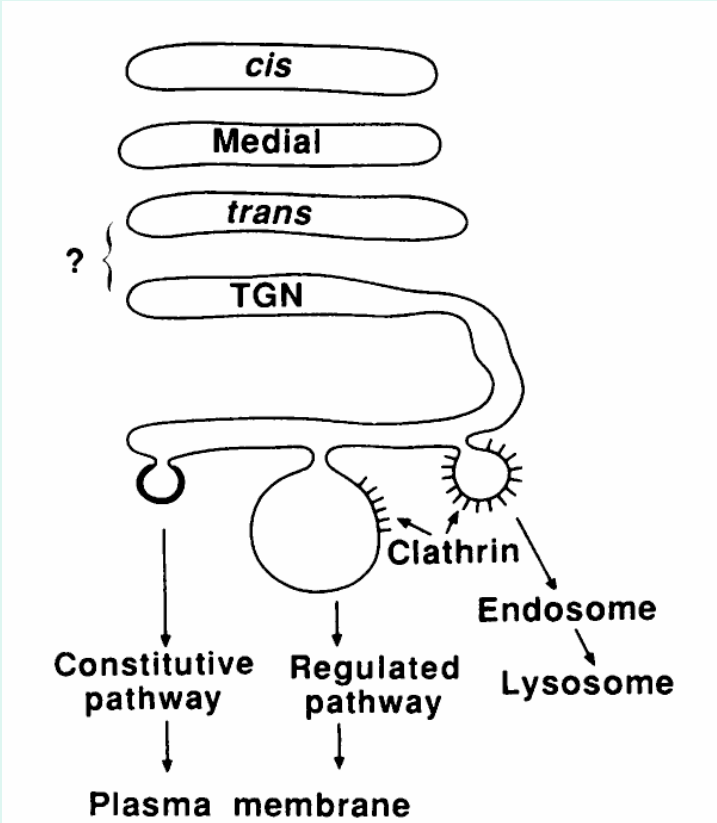
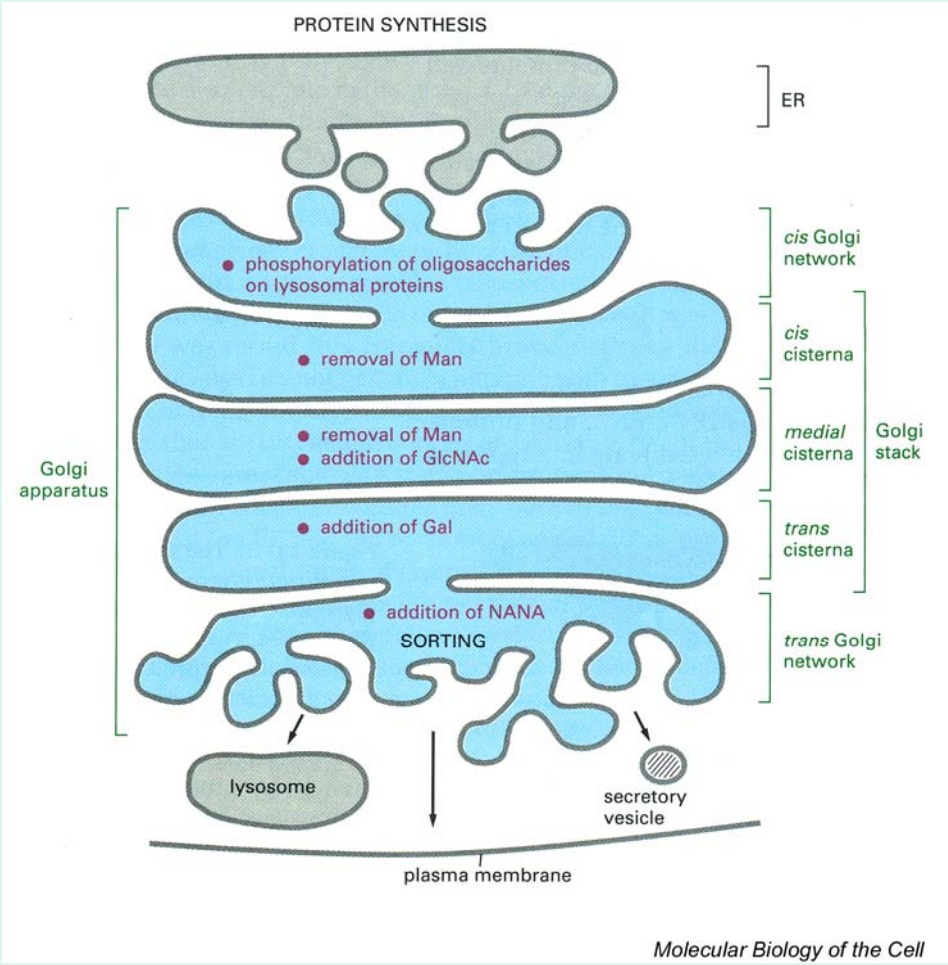
Cargo sorting occurs at the TGN



Science. 1986. 234:438-43.

Some features of the secretory pathway are highly conserved



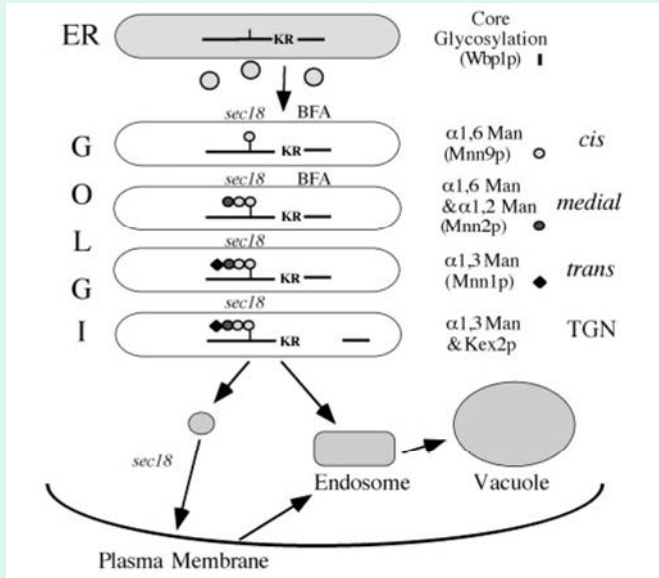
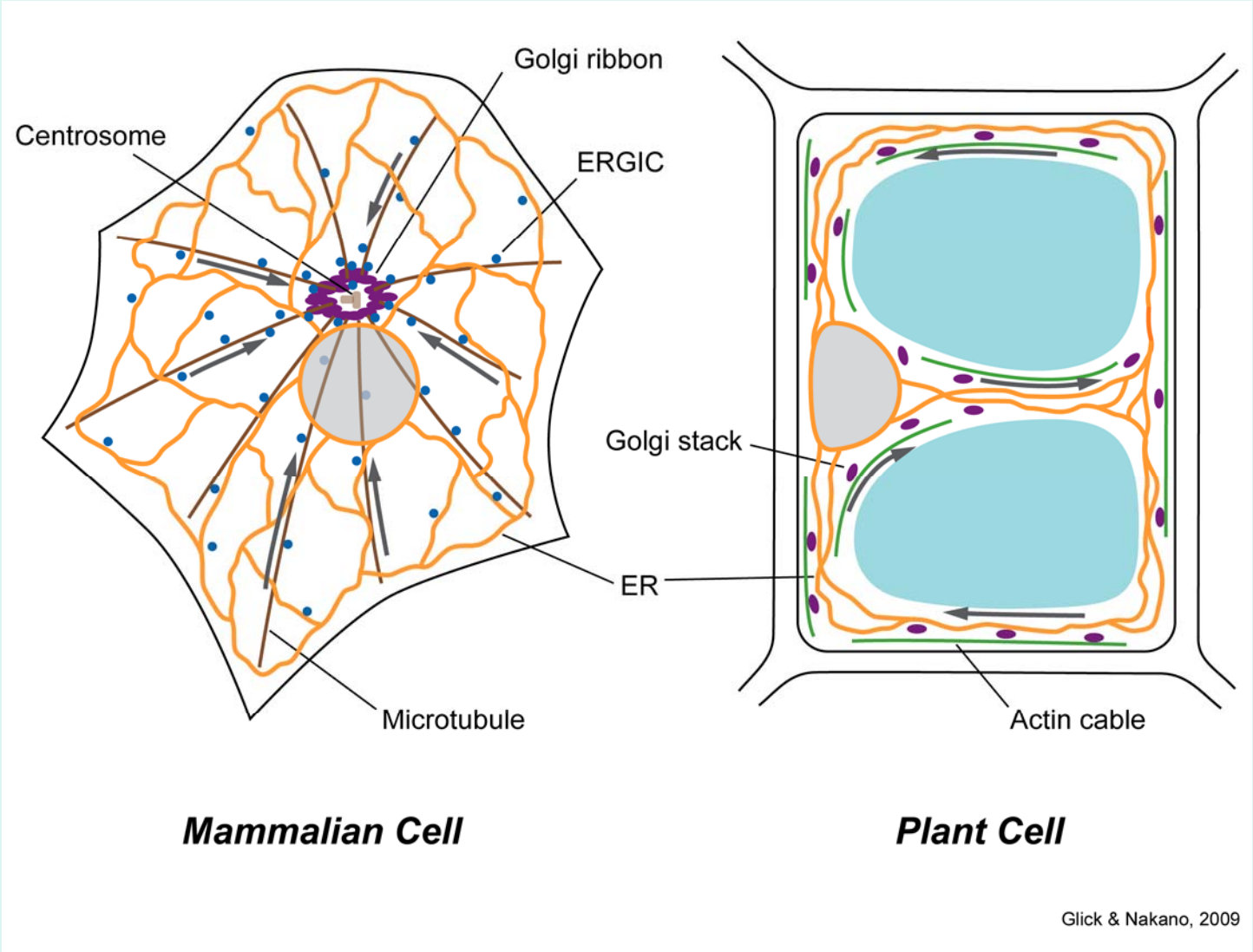


Figure 8. Compartmental organization of the yeast Golgi complex.

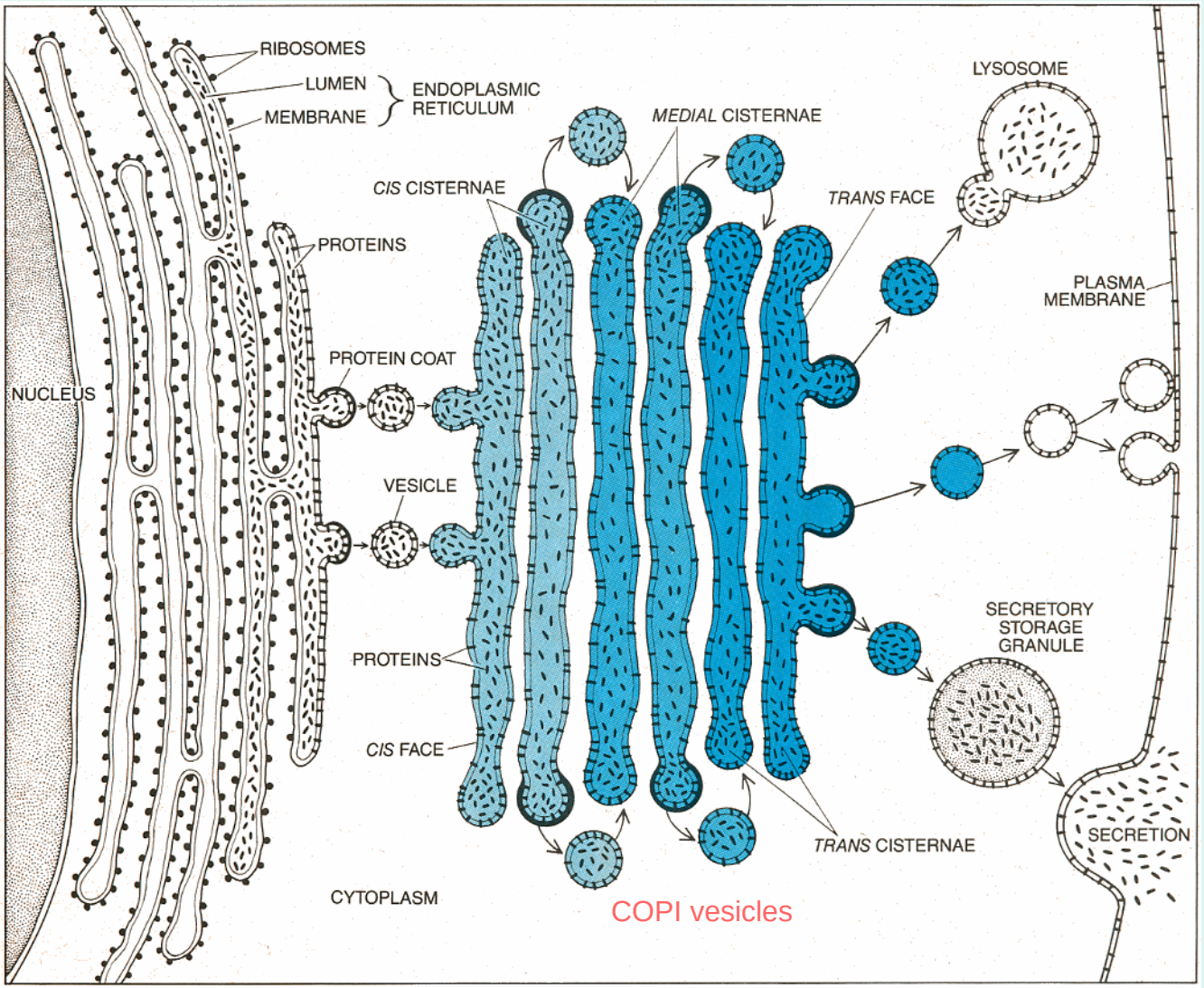
Brigance et al., 2000

Other features are specific to certain cell types



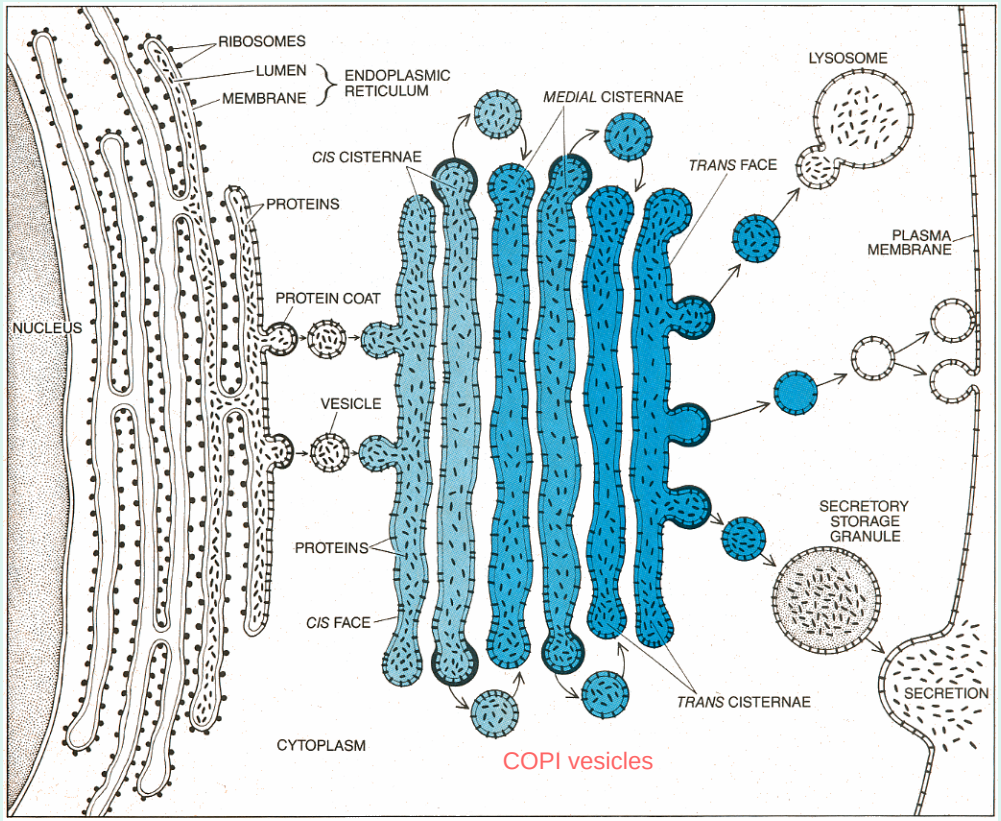
How does secretory cargo traverse the Golgi?

The Stable Compartments Model



Scientific American. 1985. 253:74-89

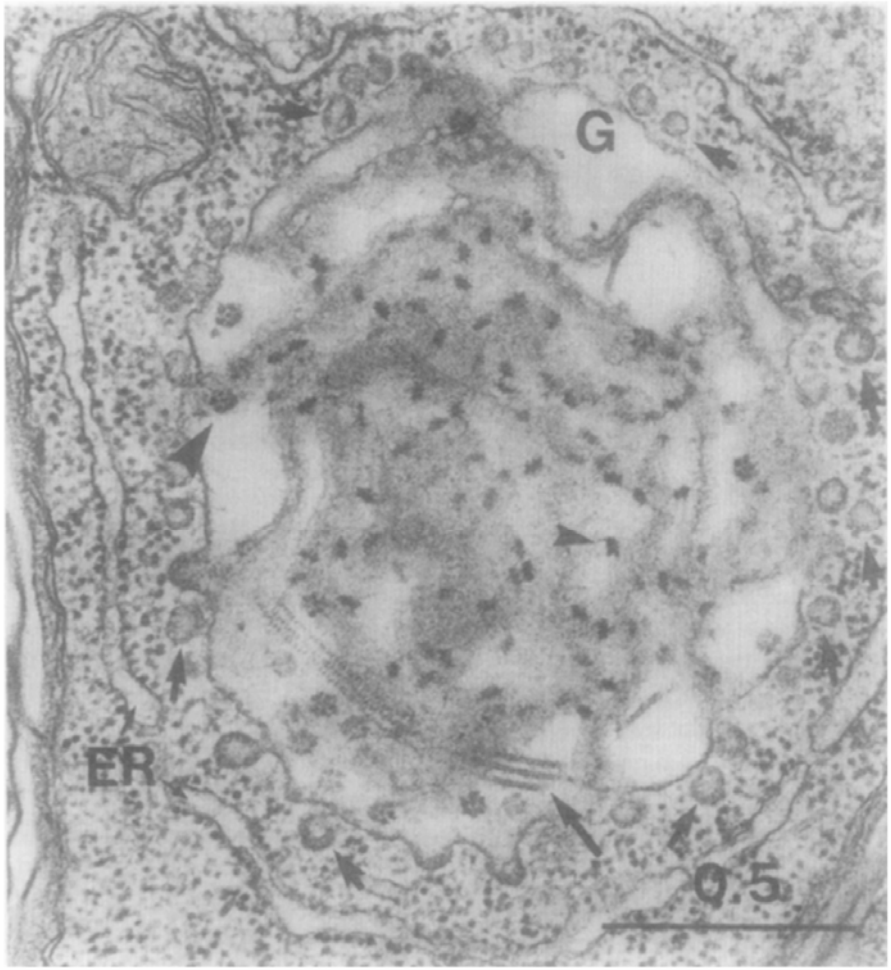
stable compartments model encountered probl



Scientific American. 1985. 253:74-89

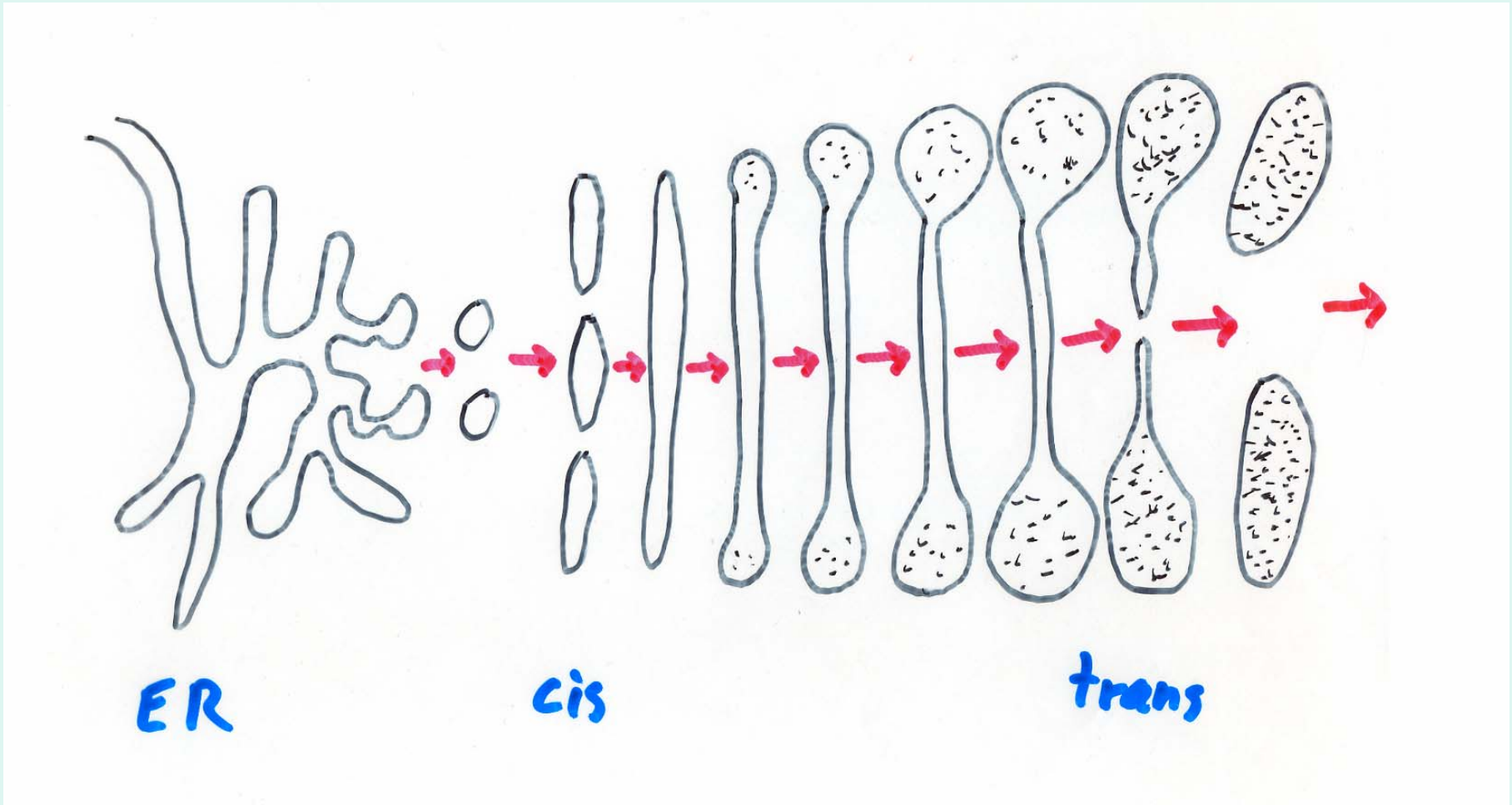
The most serious problem is that big secretory cargoes transit through the Golgi, and many of these cargoes are larger than a COPI vesicle.

Big things move through the Golgi

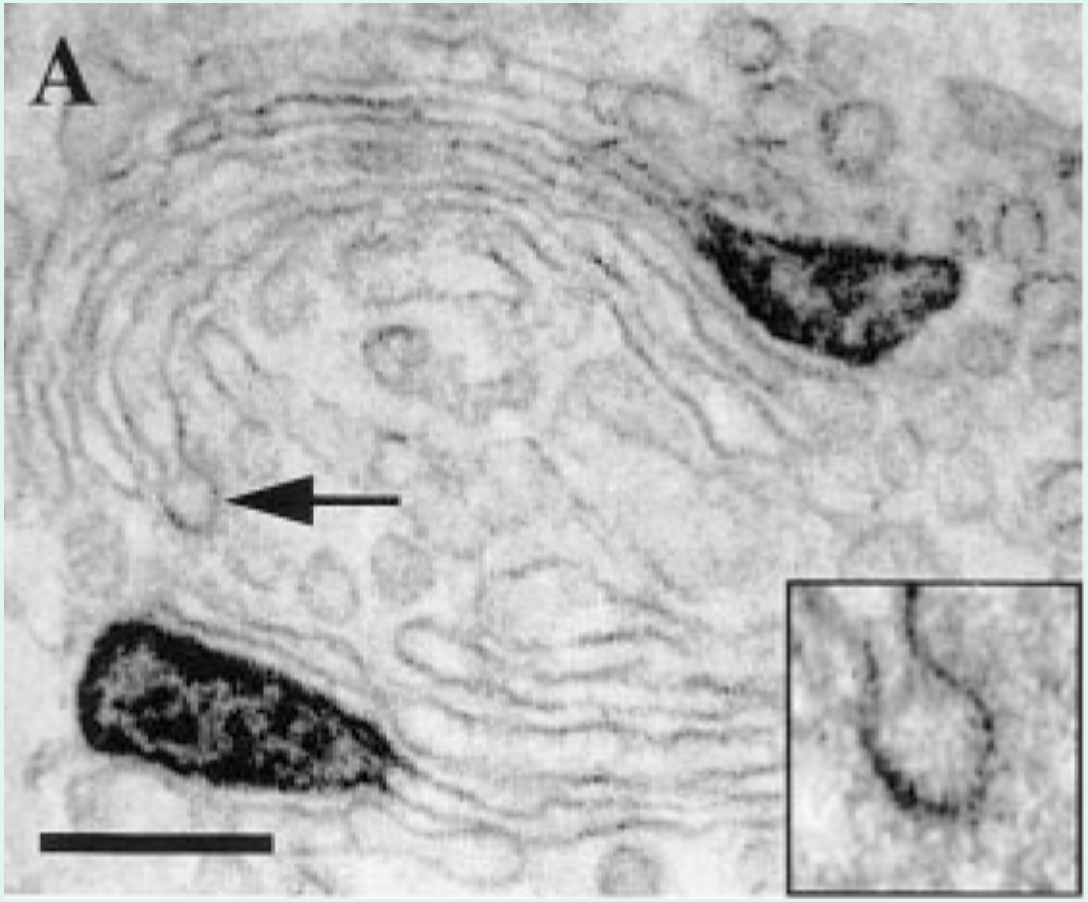


TRENDS IN CELL BIOLOGY VOL. 5 AUGUST 1995

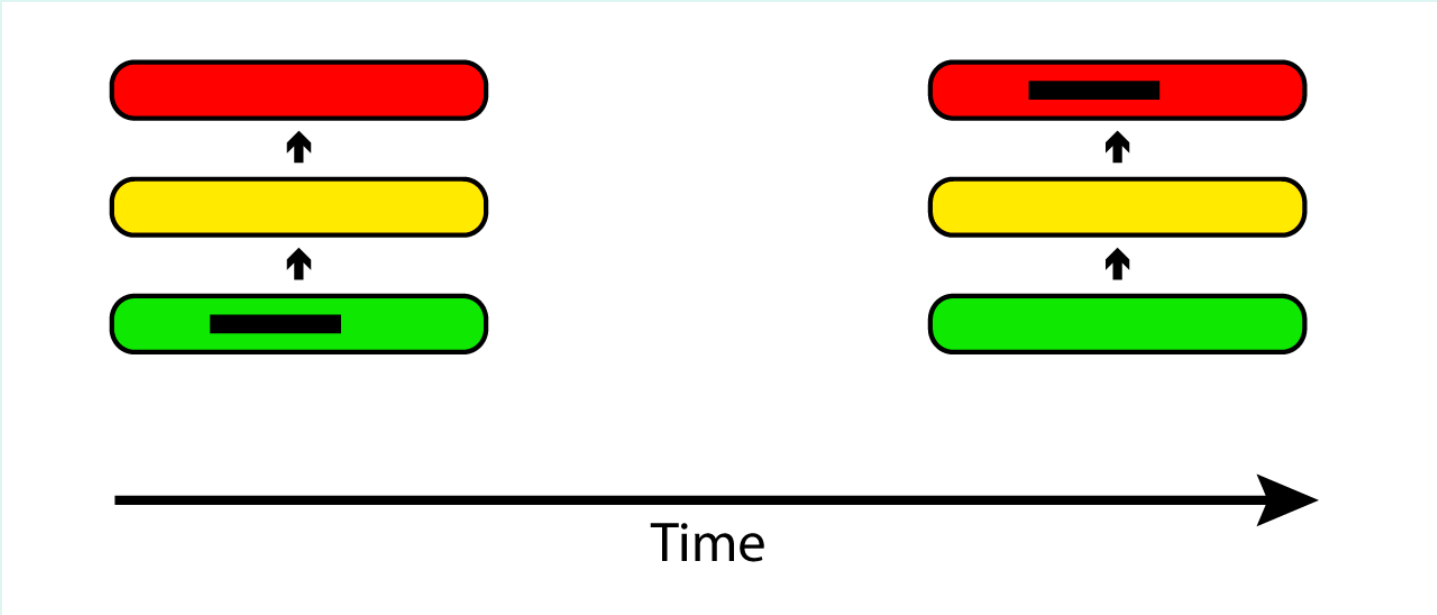
The Cisternal Progression Model



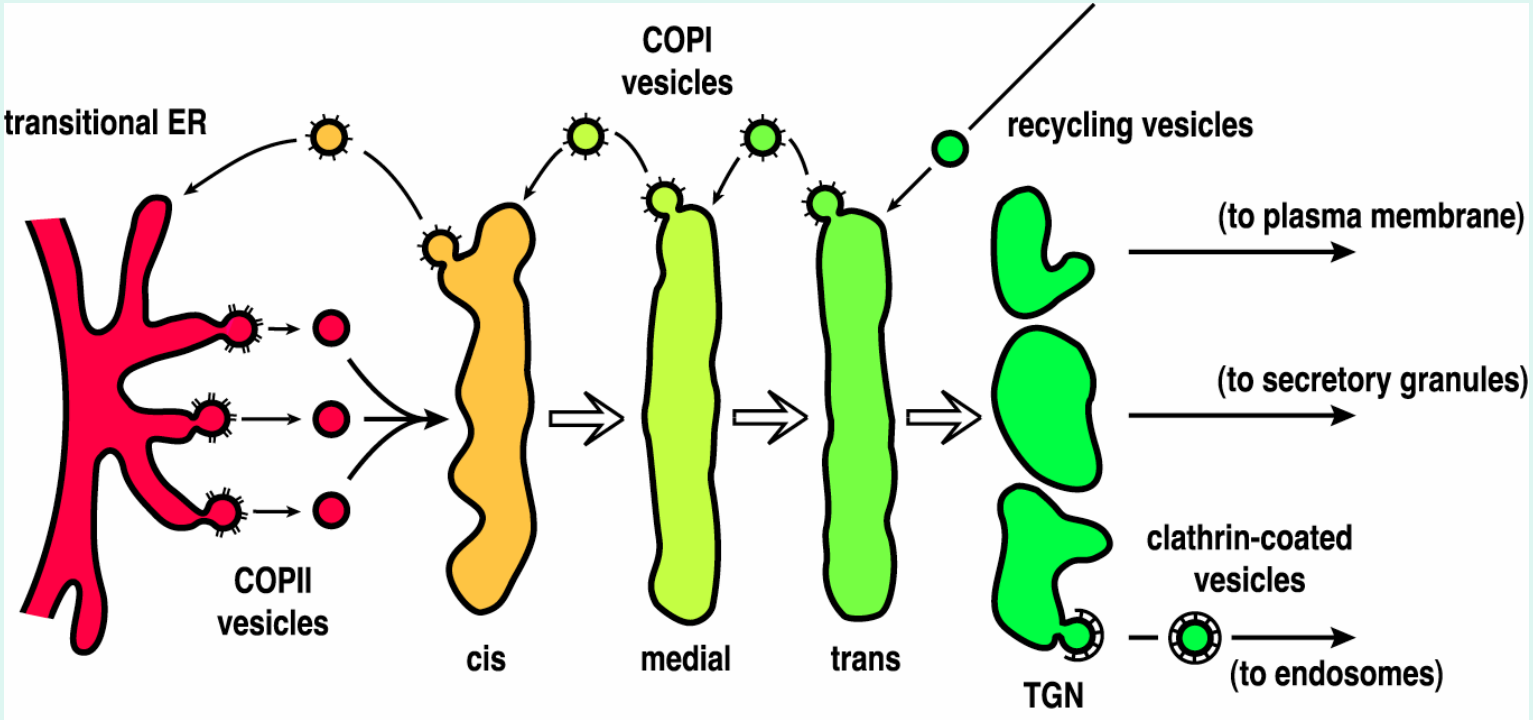
ollagen is transported by cisternal progres



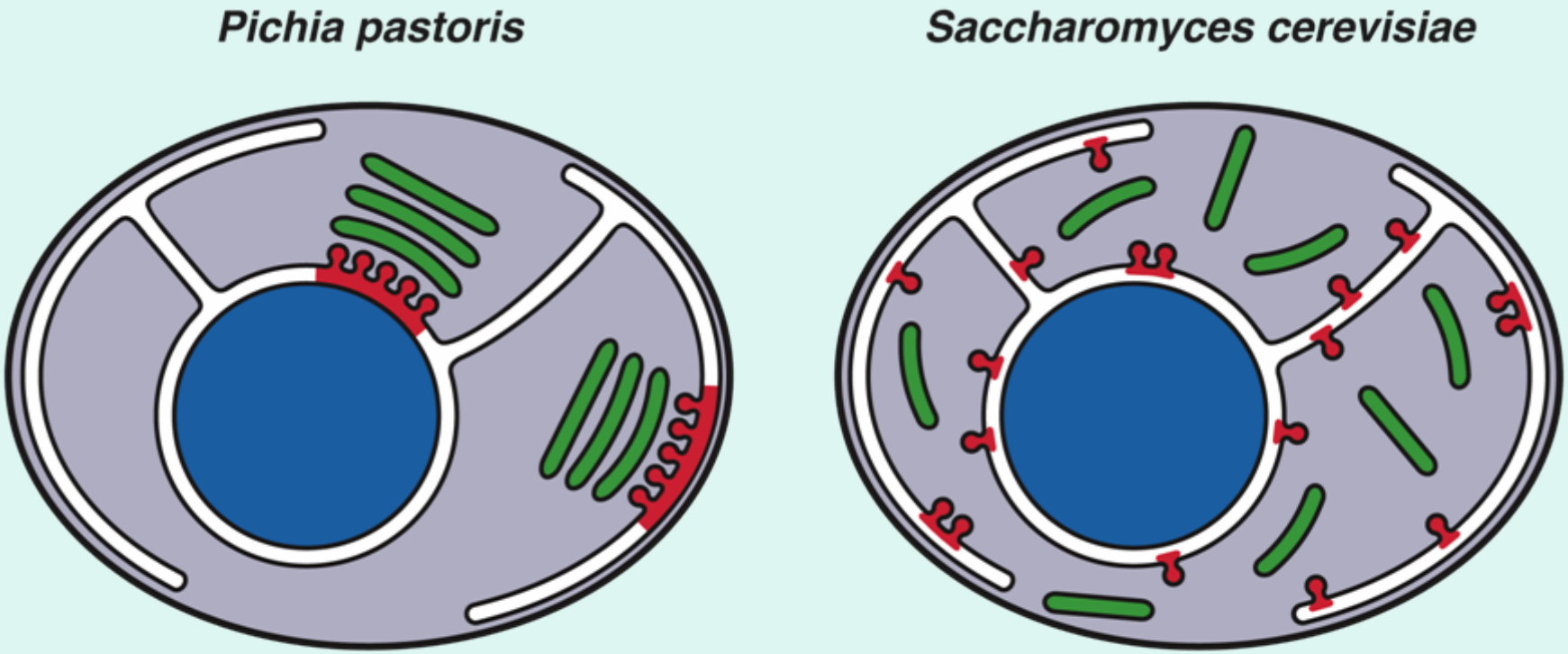
ternal progression implies cisternal maturat



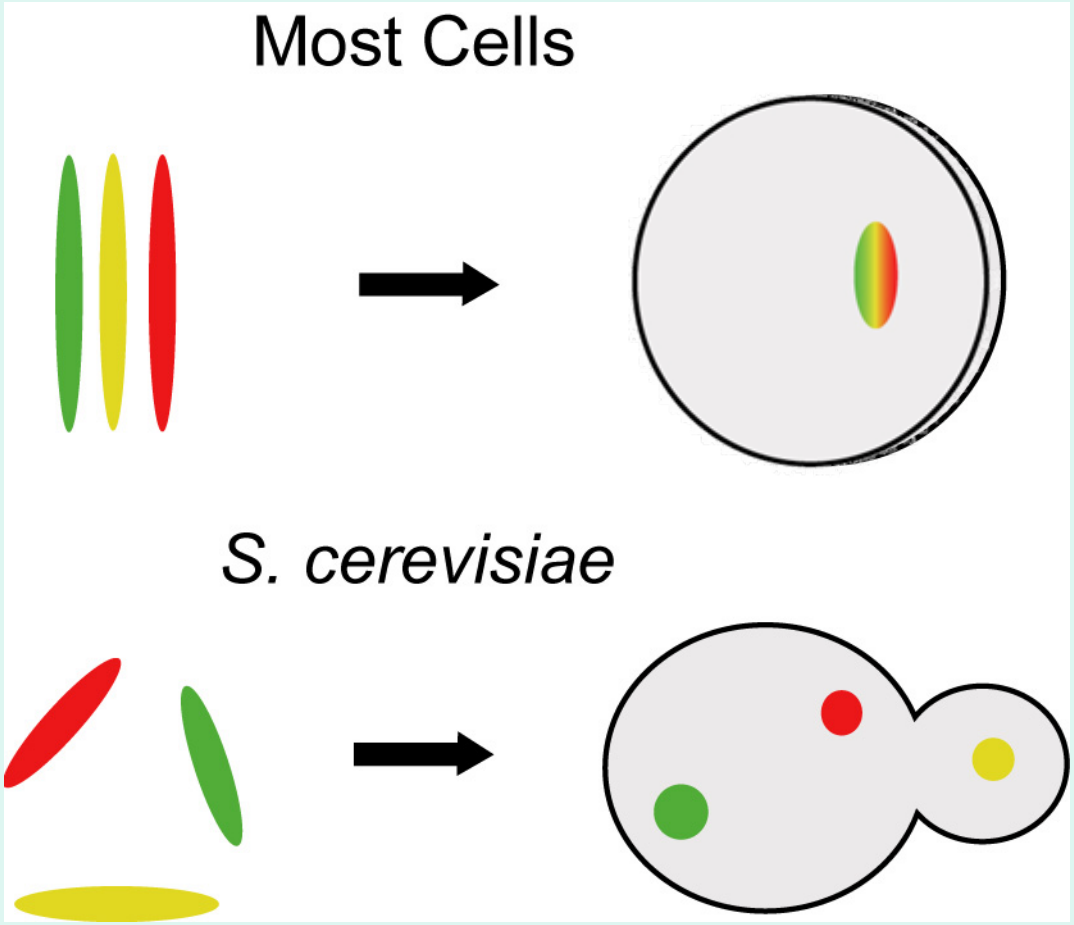
*** The Cisternal Maturation Model ***



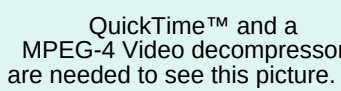
ding yeasts are a good experimental system studying the secretory pathway

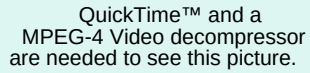


Saccharomyces cerevisiae has a nonstacked Golgi



Late
(trans)

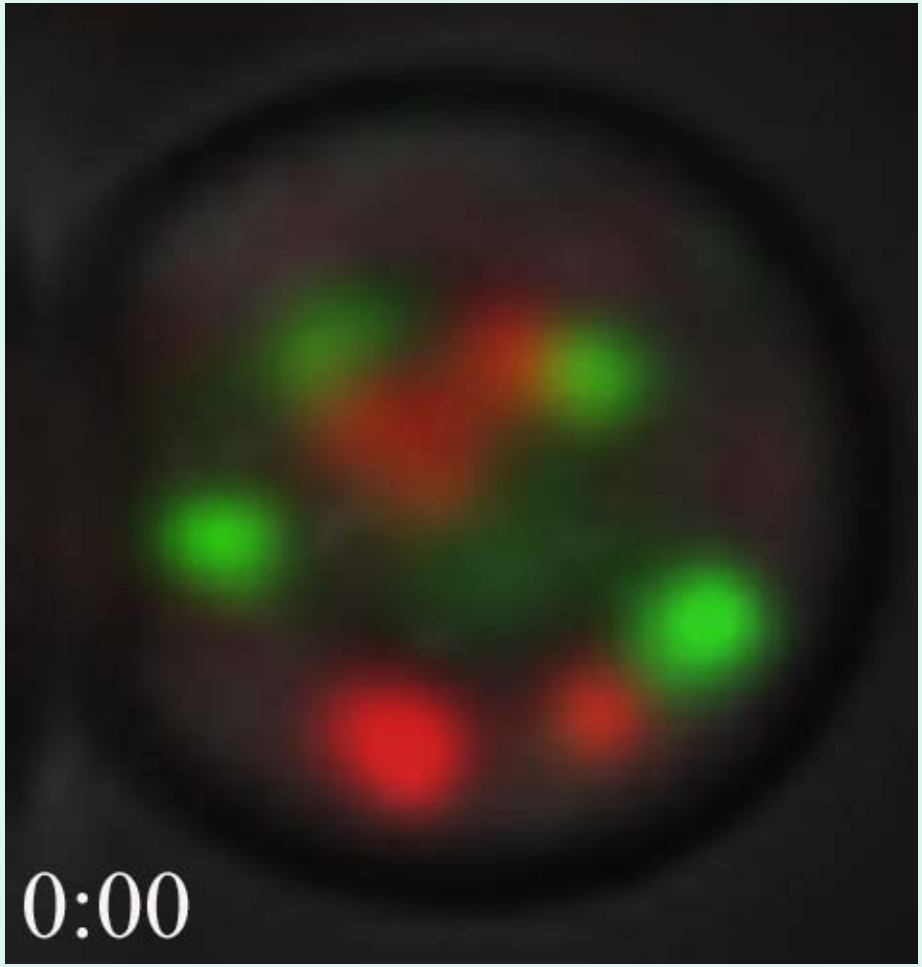




Early
(cis)

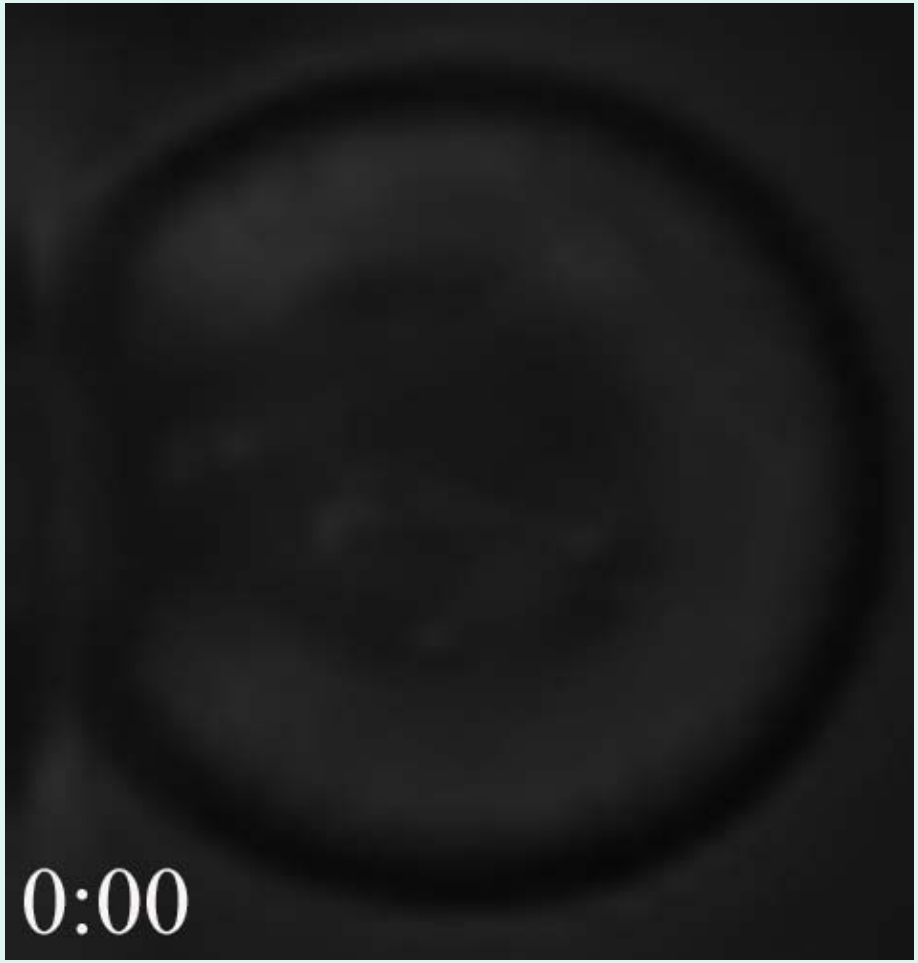
Cisternal Maturation vs. Stable Compartments

Golgi Dynamics in *Saccharomyces*



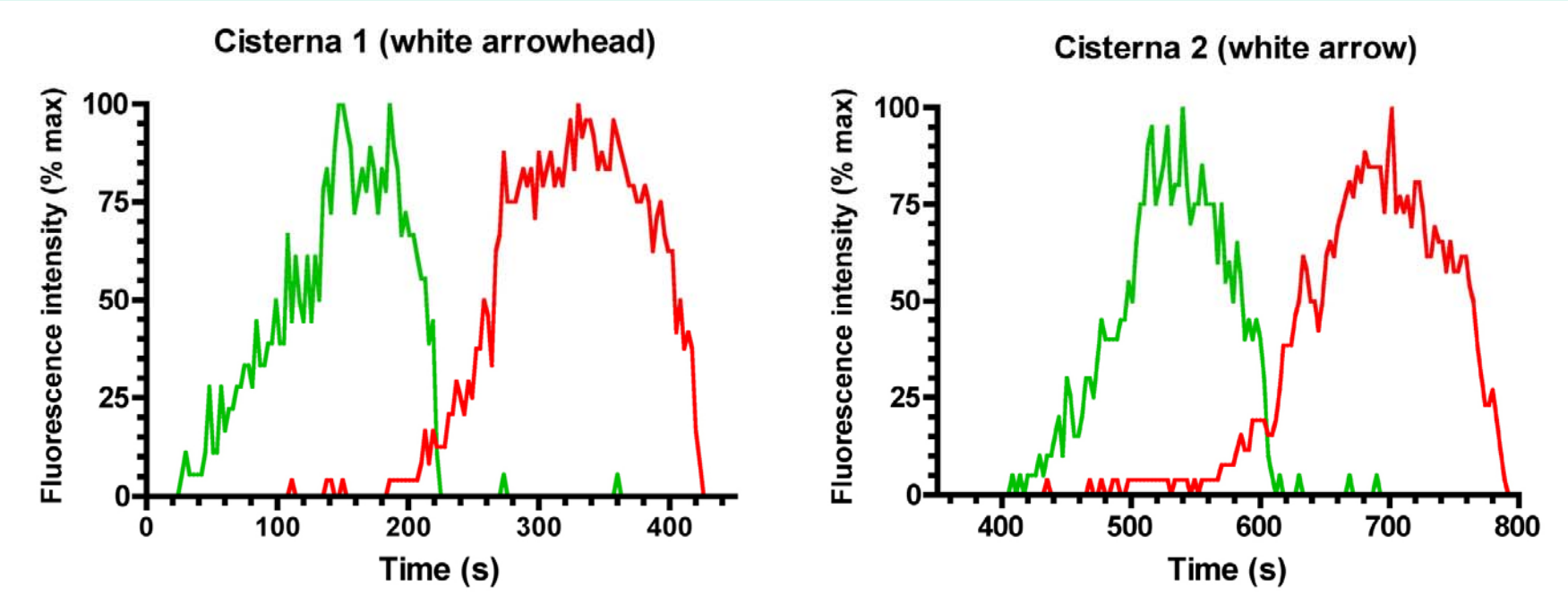
Green: GFP-Vrg4 Red: Sec7-DsRed-Monomer

Golgi Dynamics in *Saccharomyces* (Edit



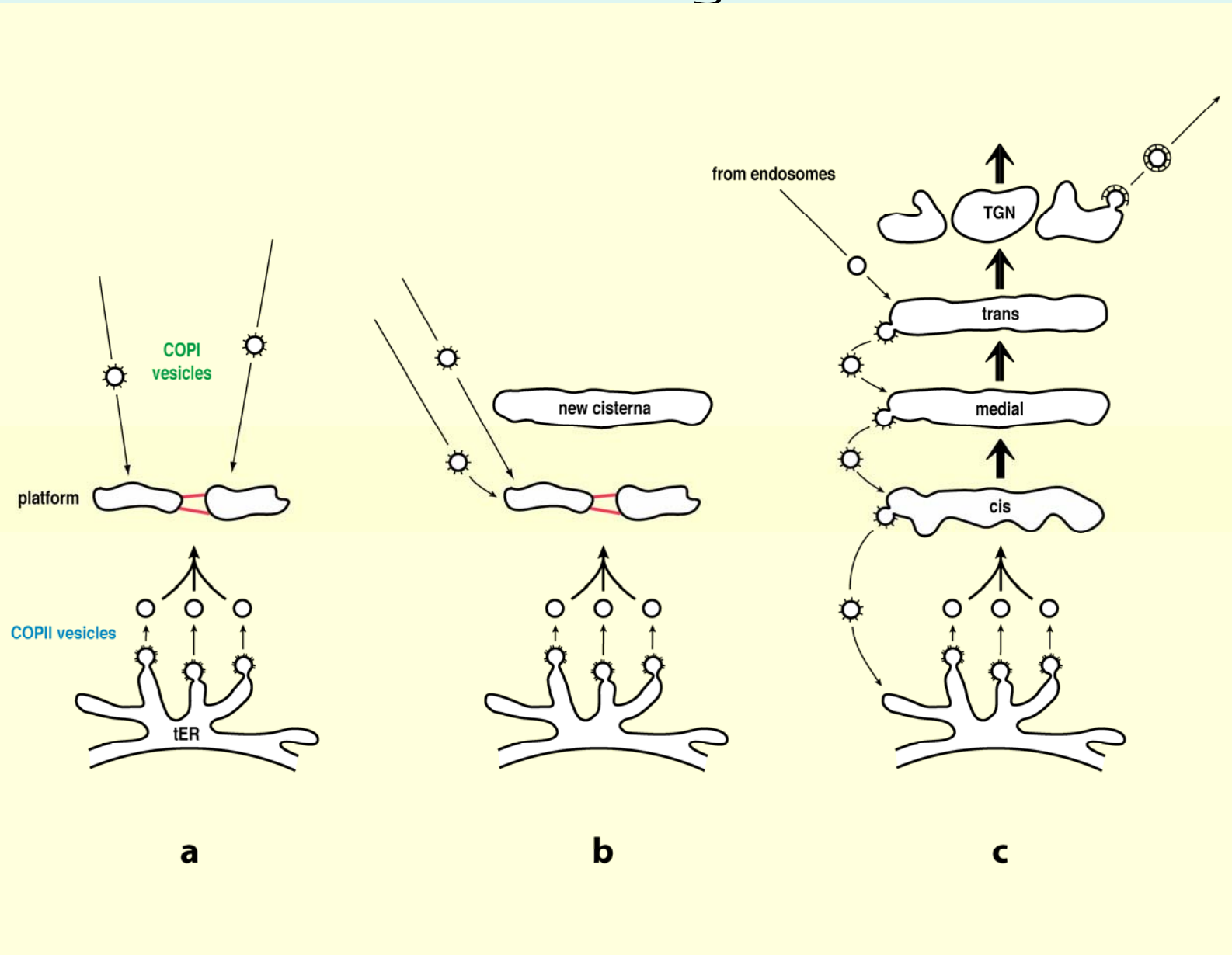
Green: GFP-Vrg4 Red: Sec7-DsRed-Monomer

n *Saccharomyces*, early Golgi cisternae matu
into late Golgi cisternae

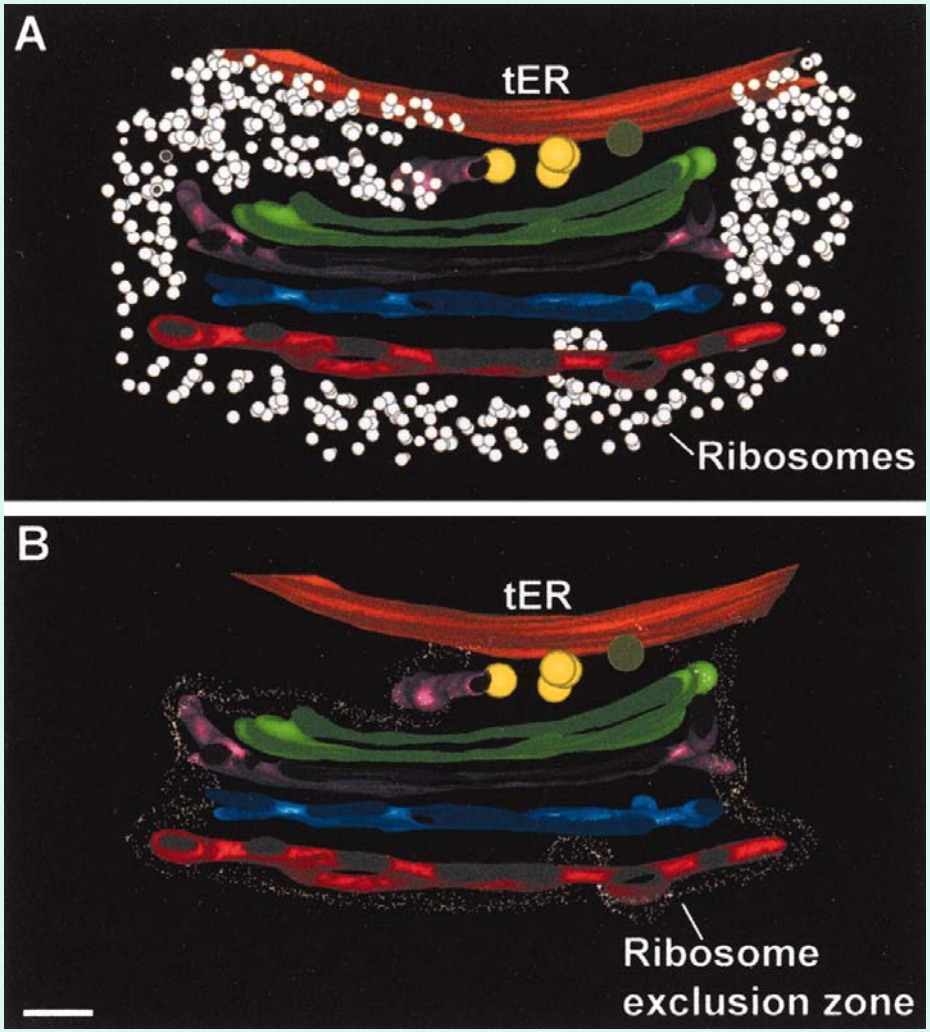


Green: GFP-Vrg4 Red: Sec7-DsRed-Monomer

Is the tER the birthplace of the Golgi?



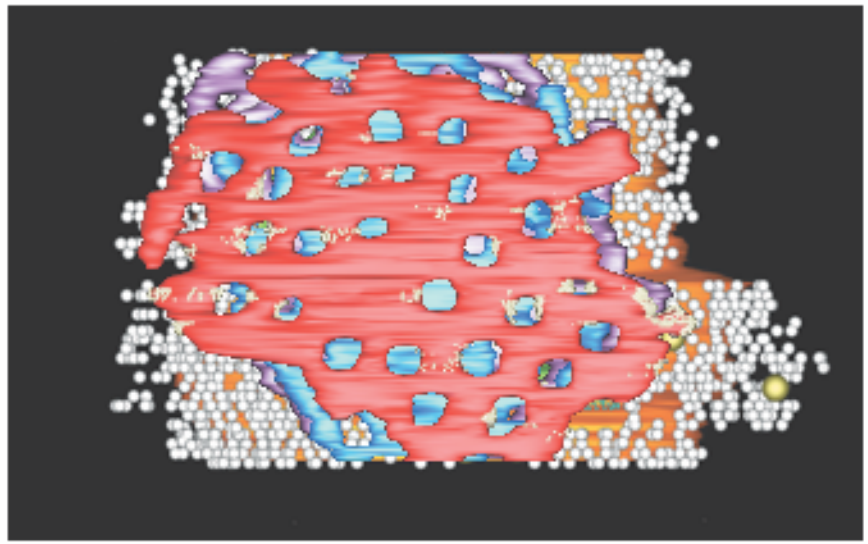
Pichia has beautiful tER and Golgi organization



A tER-Golgi unit in *Pichia*

Staehelin Lab, Boulder, CO

tER-Golgi Units in *Pichia*



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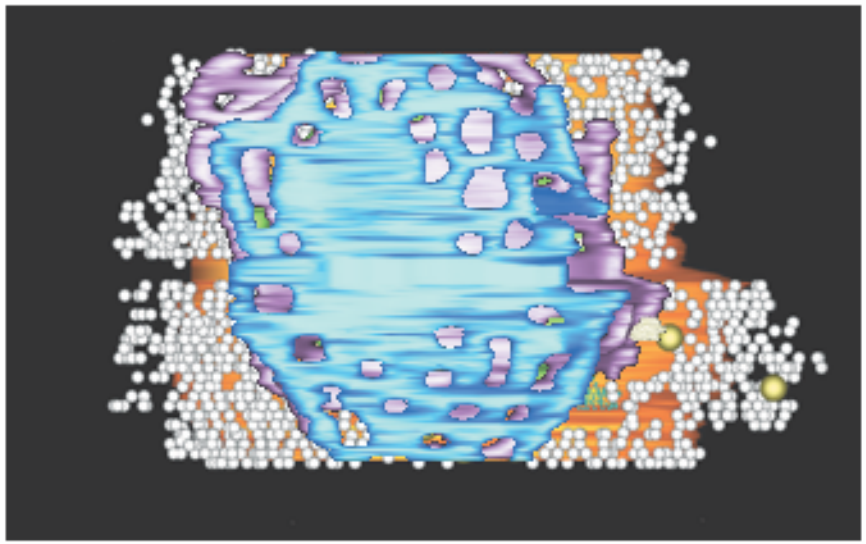
tER-Golgi Units in *Pichia*



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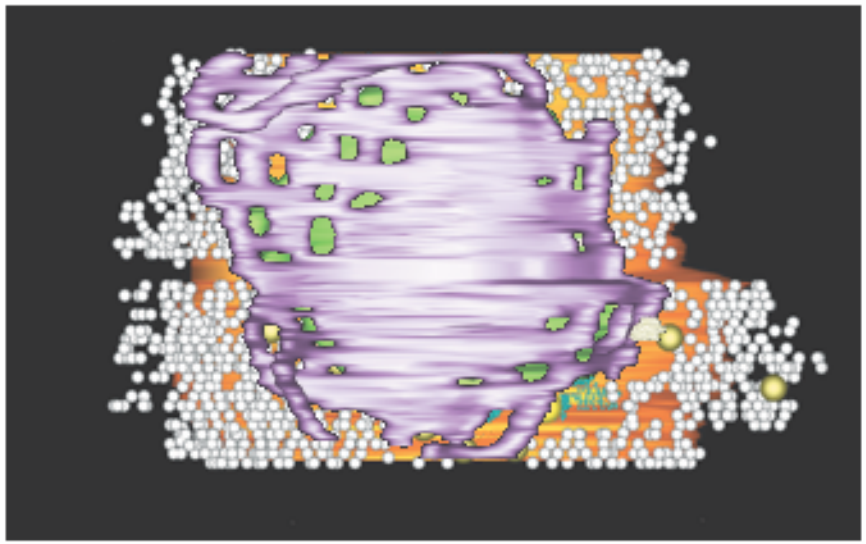
tER-Golgi Units in *Pichia*



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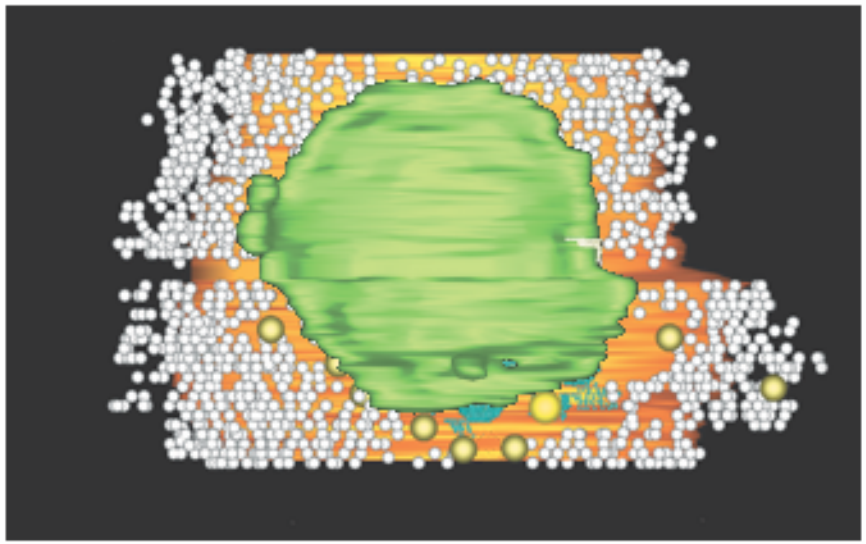
tER-Golgi Units in *Pichia*



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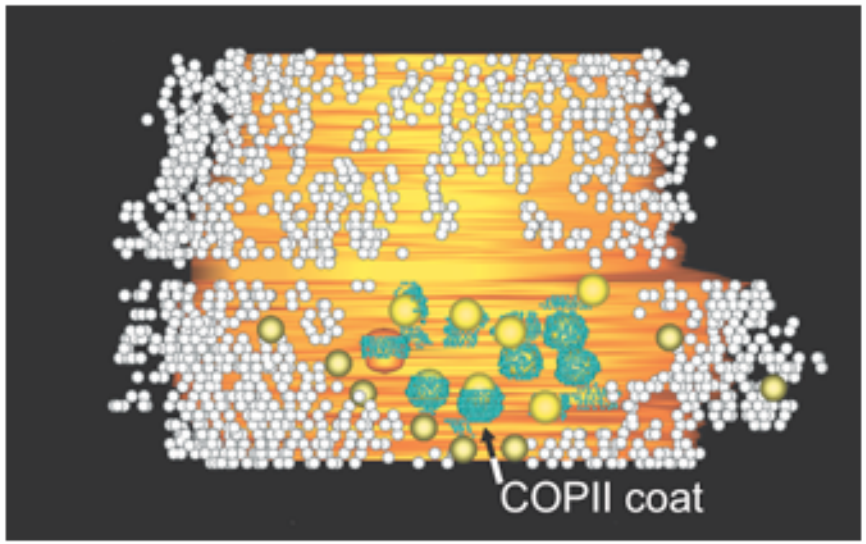
tER-Golgi Units in *Pichia*



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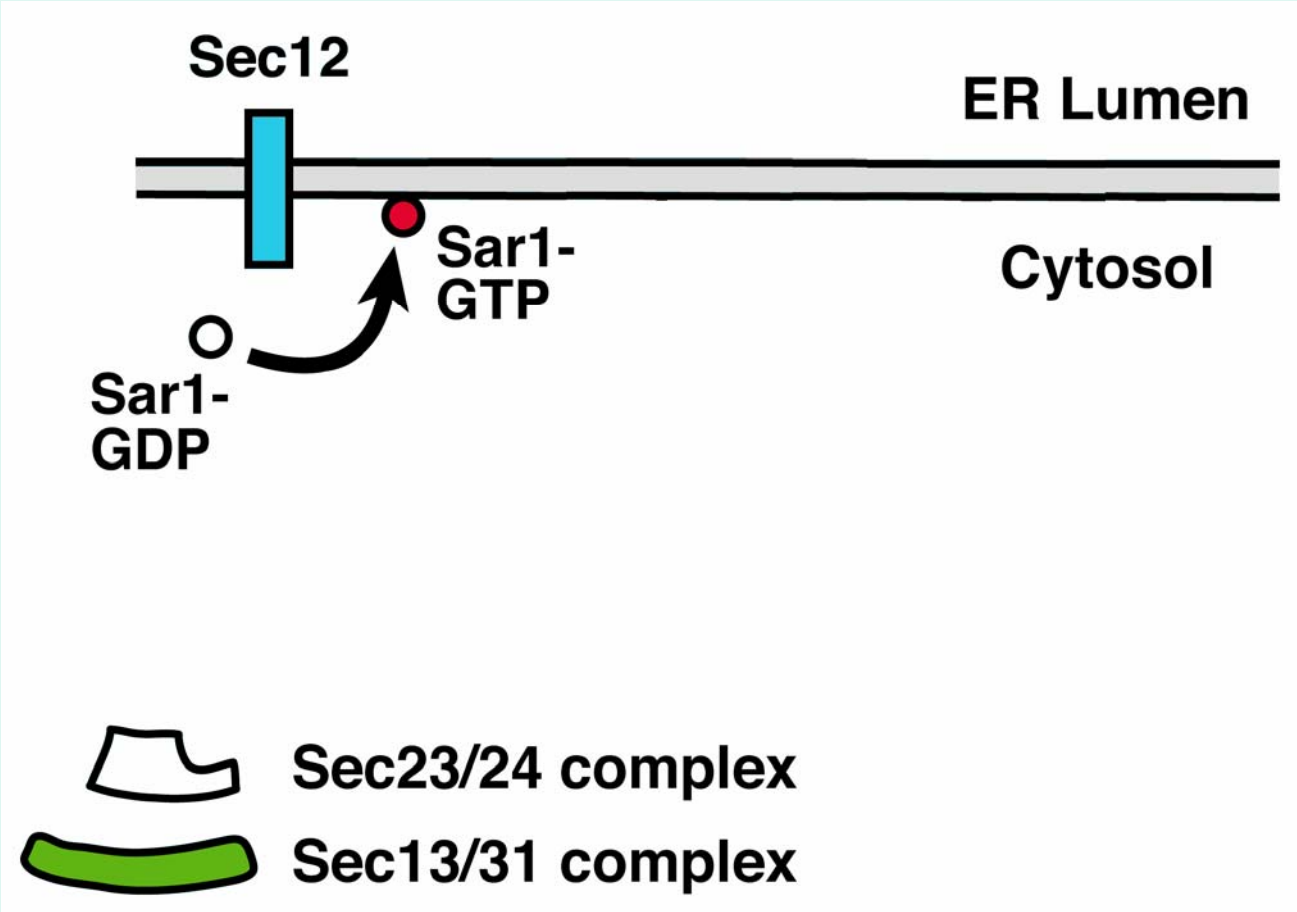
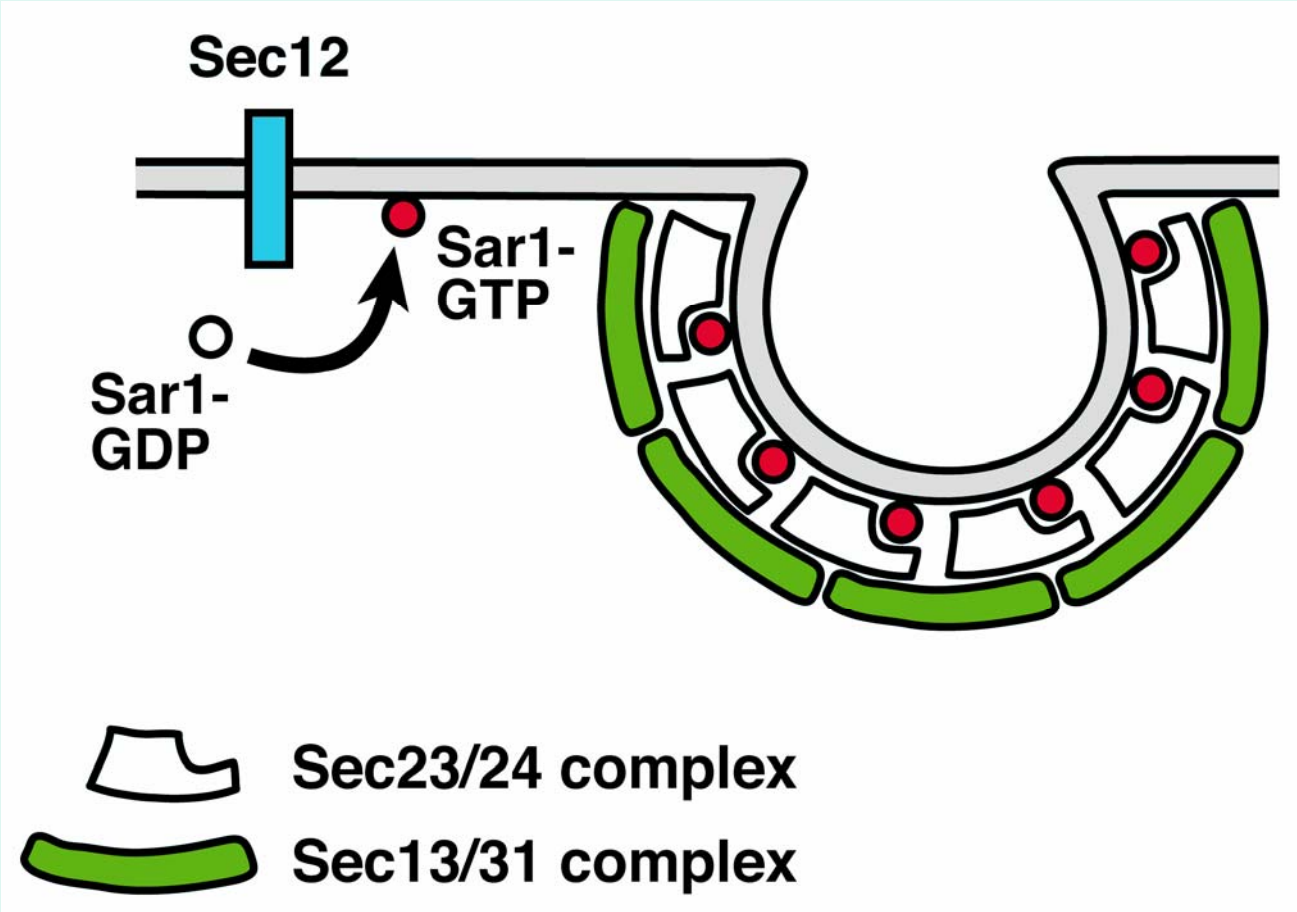
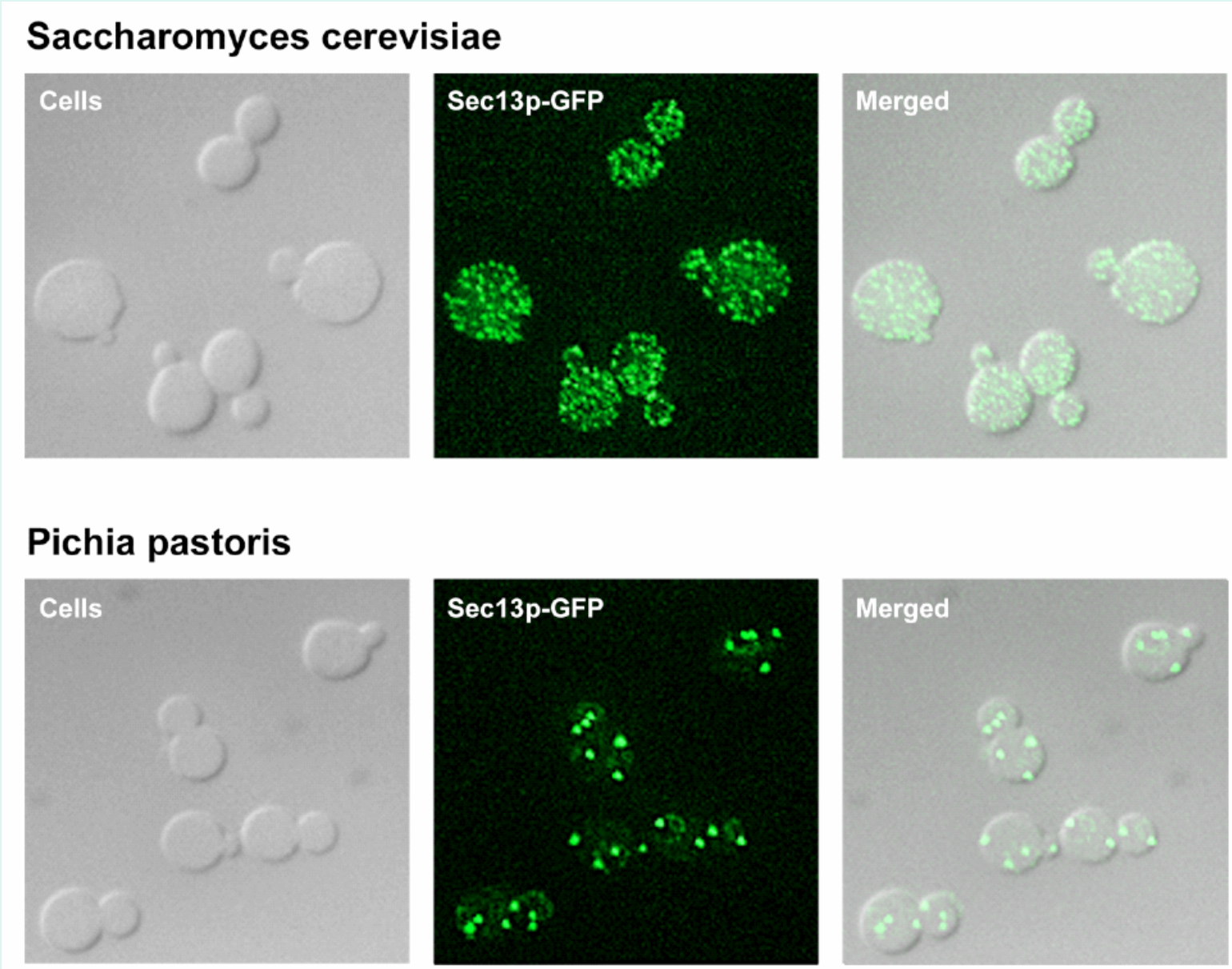
COPII Vesicle Formation



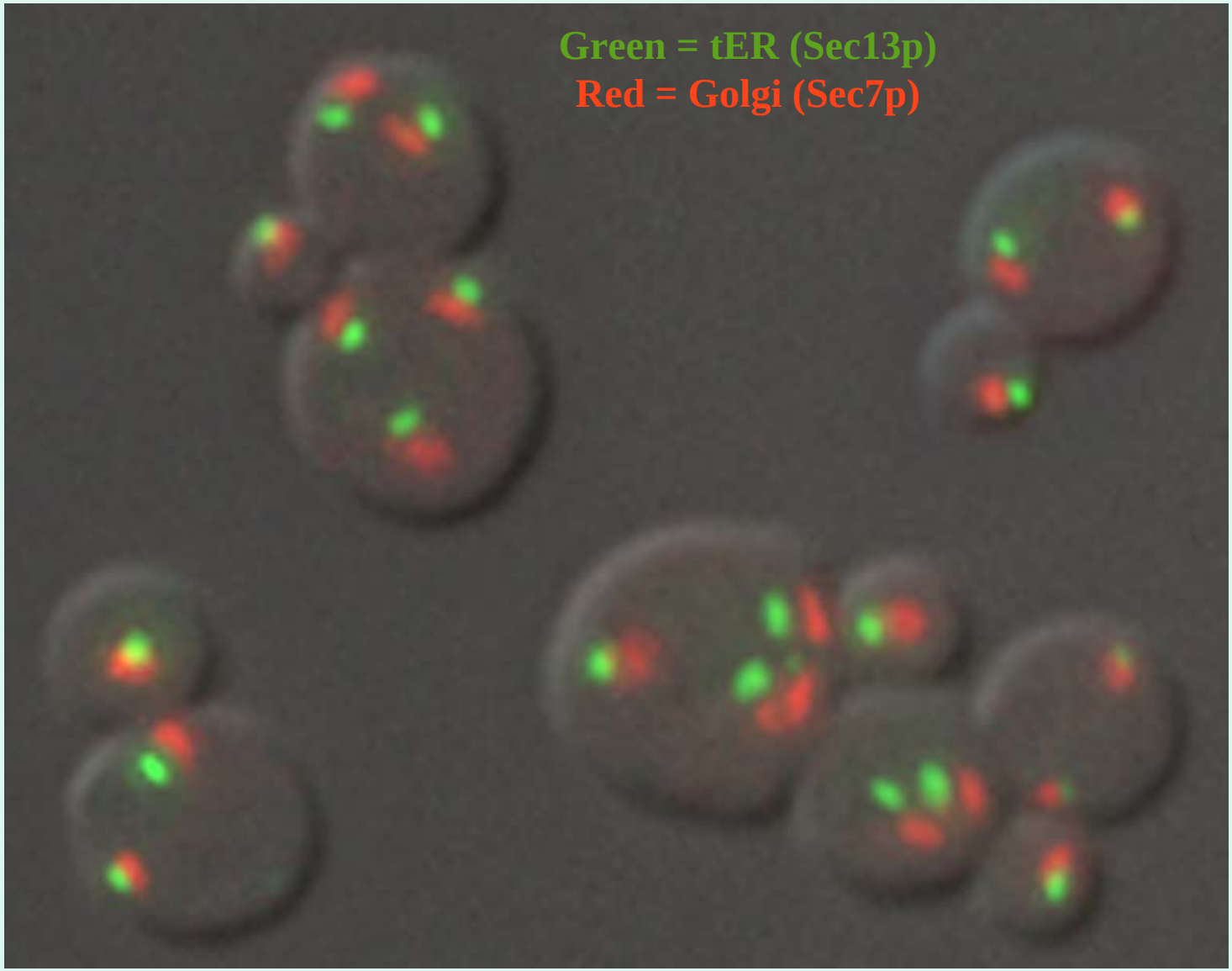
COPII Vesicle Formation



tER Organization in the Two Yeasts



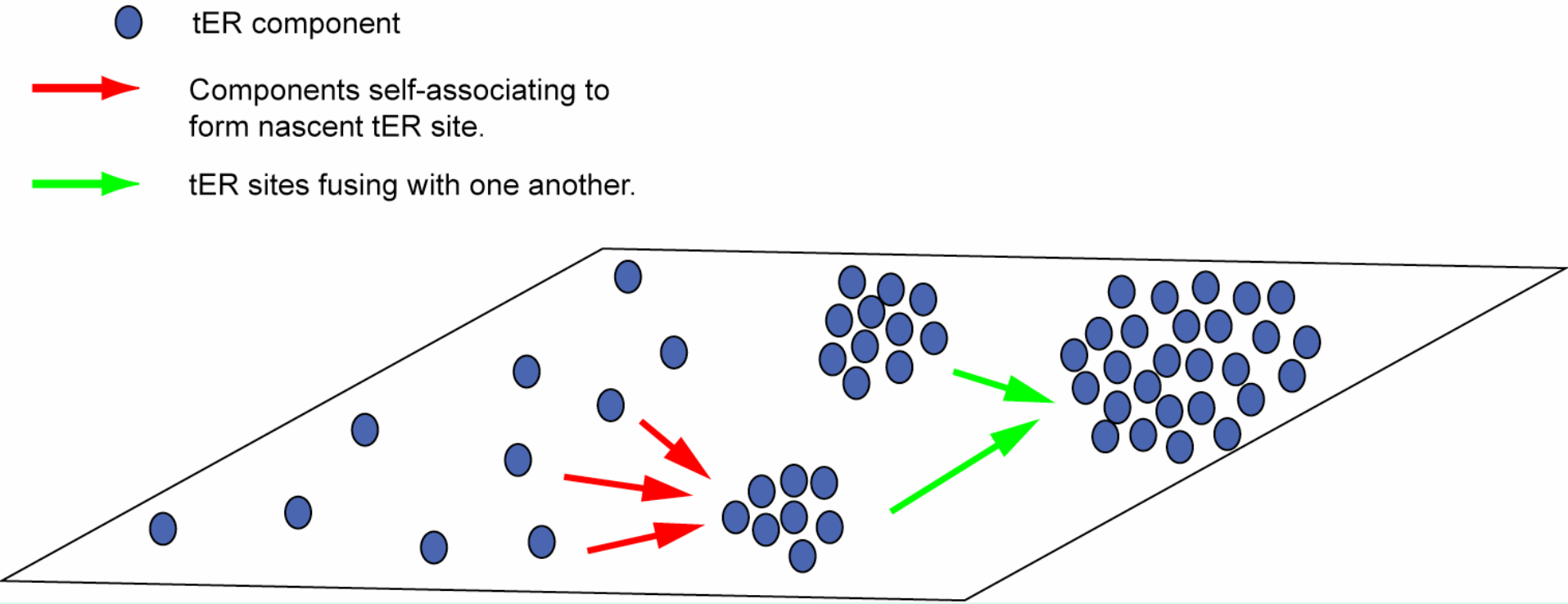
tER and Golgi in *Pichia*



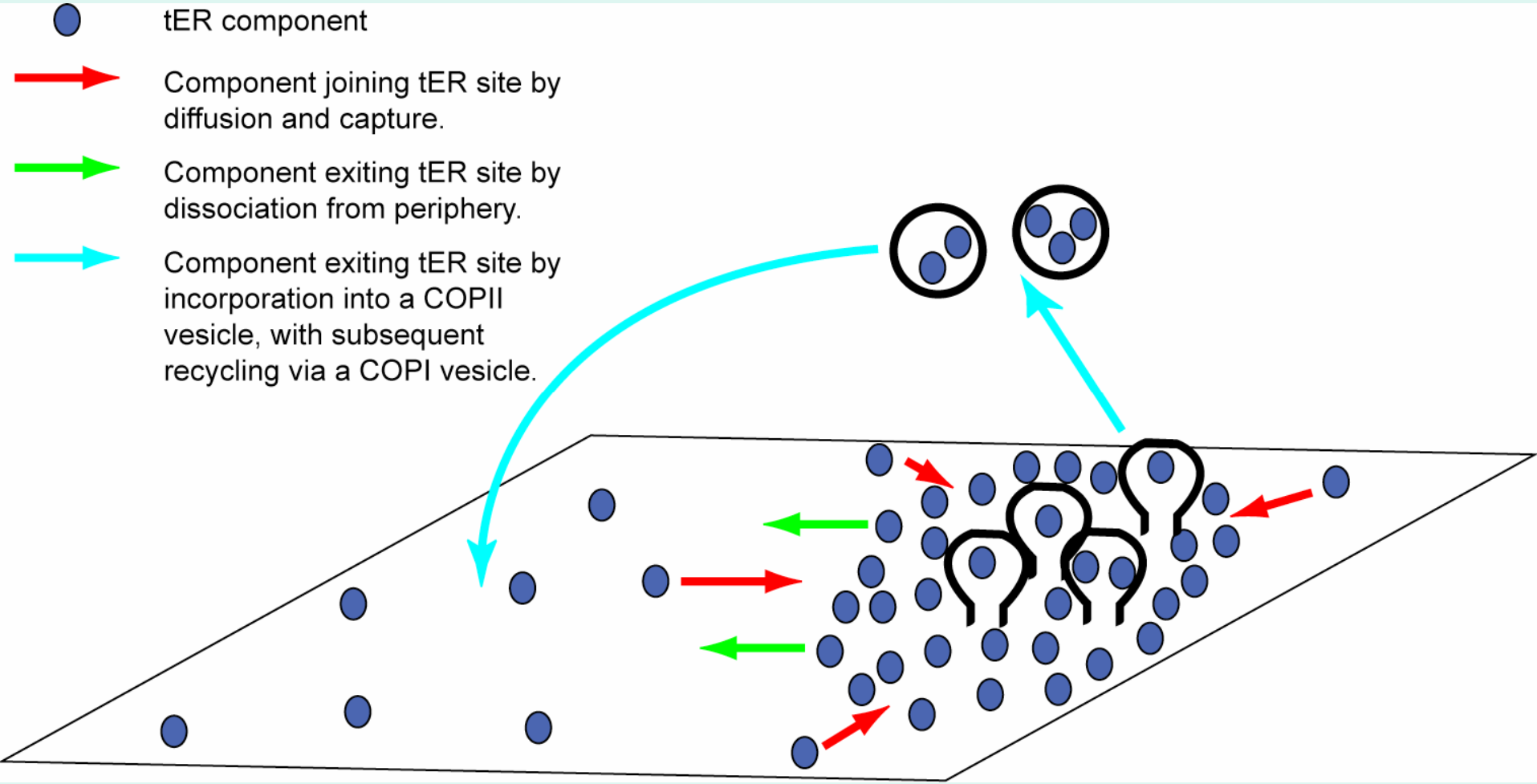
Pichia tER sites form *de novo* and fuse

QuickTime™ and a
H.264 decompressor
are needed to see this picture.

Self-Organization Model for tER Site Formation



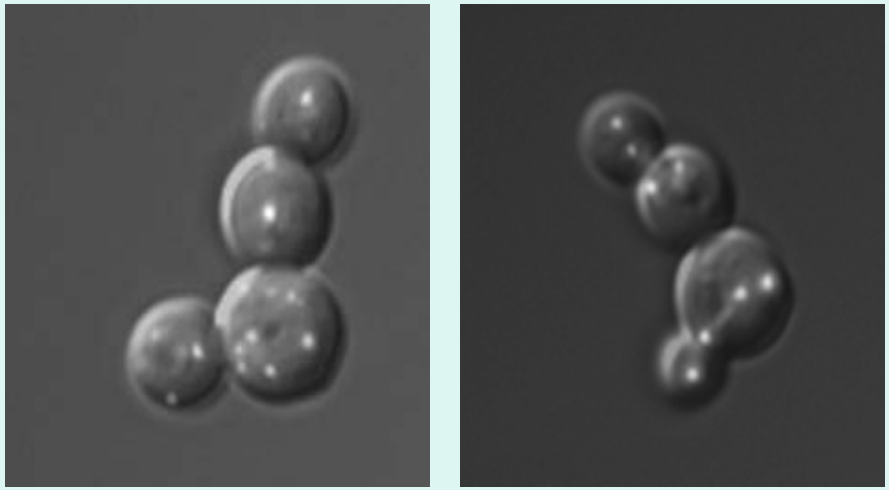
Model for tER Site Dynamics



Pichia tER Organization Mutant

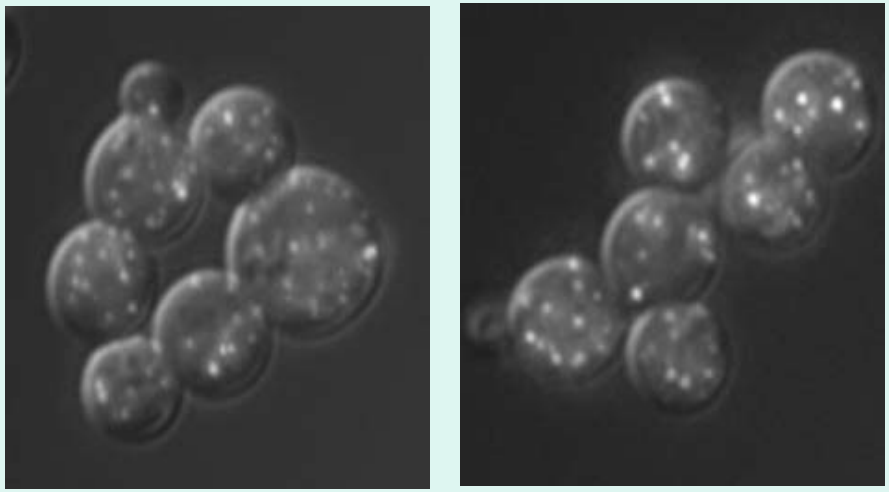
Mean number of spots per cell ± SEM

Wild-Type



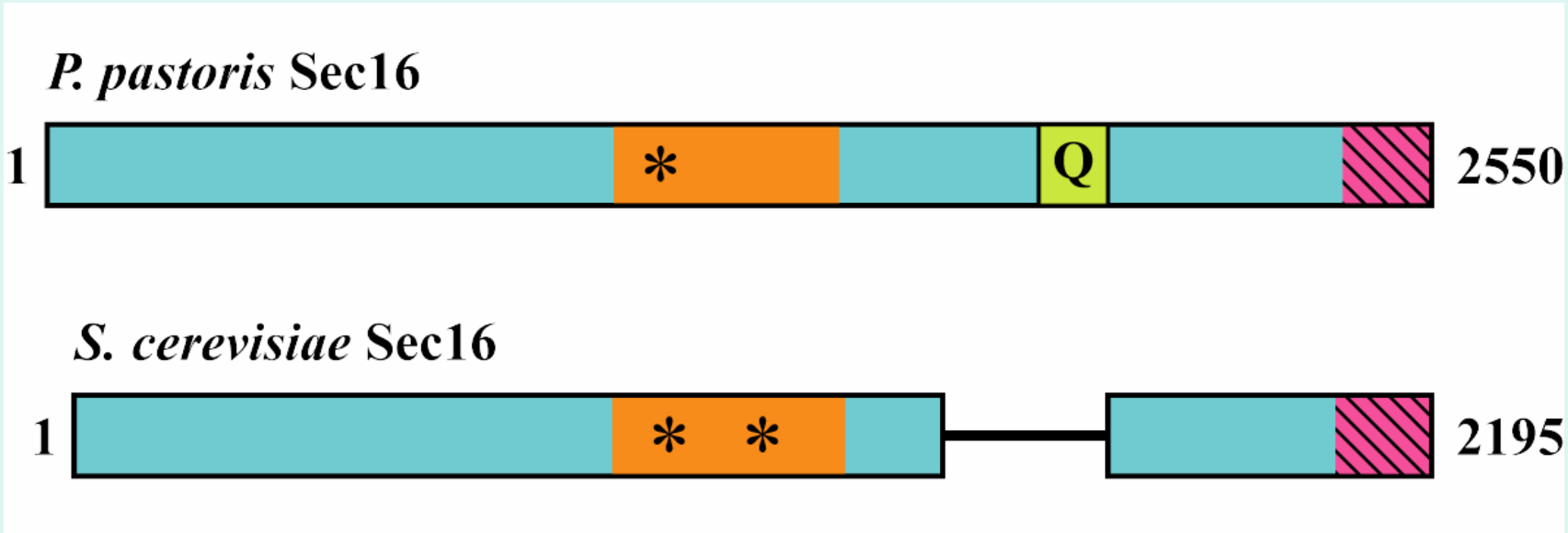
3.5 ± 0.2

Mutant



10.9 ± 0.5

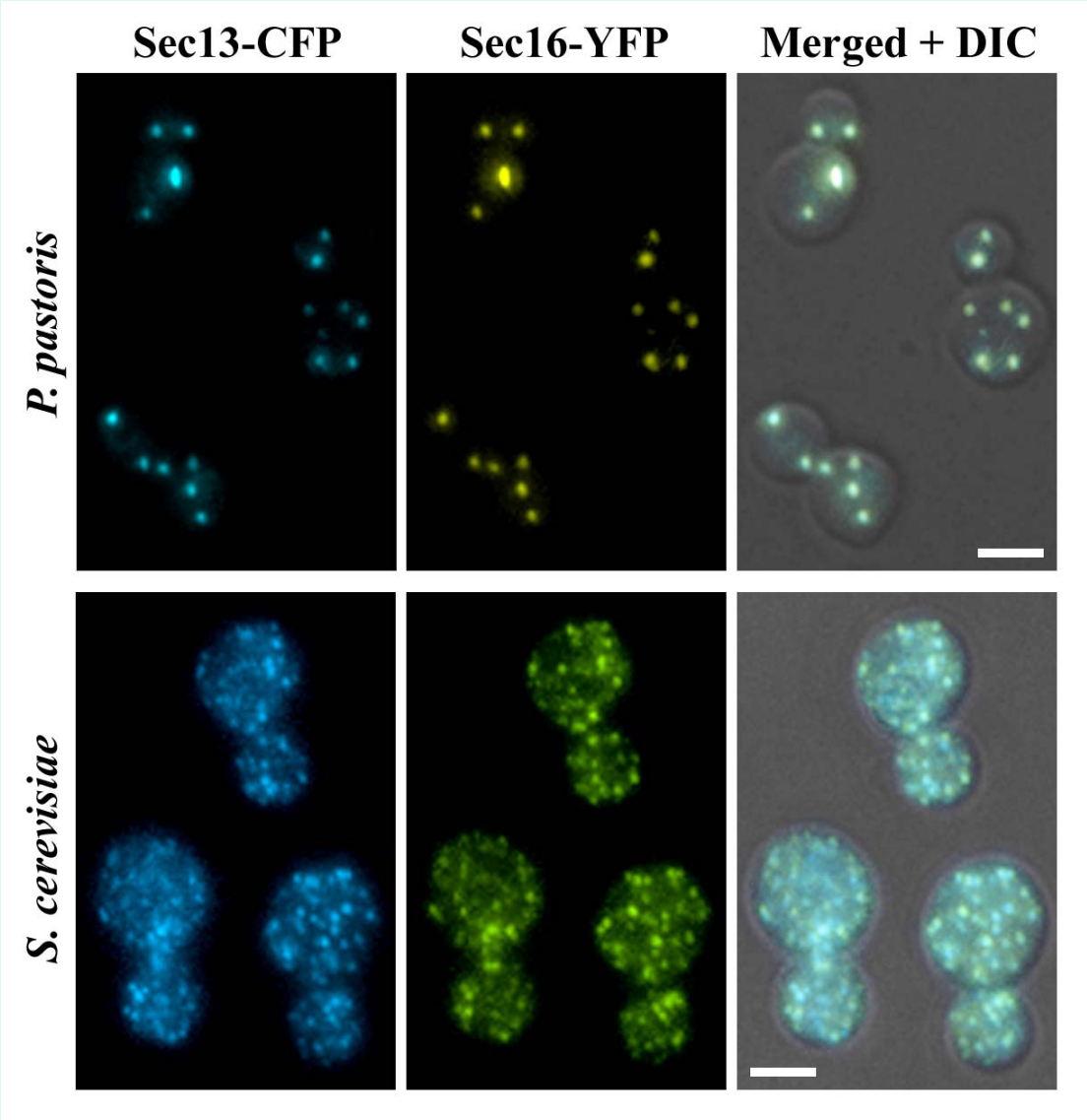
The mutation that alters tER sites is in *SEC16*



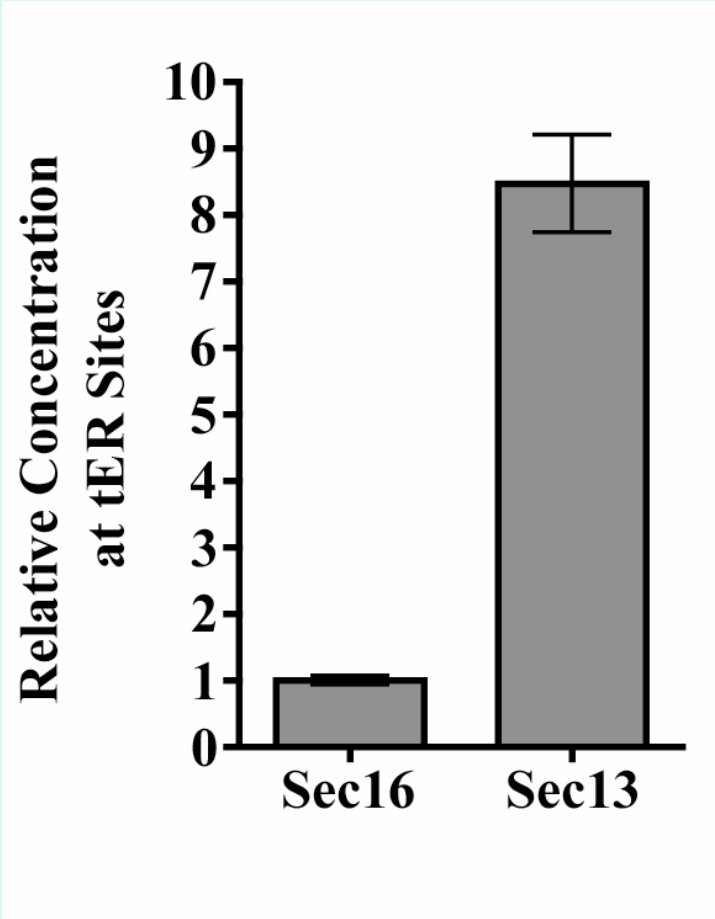
* ts mutations

Sec16 is a large peripheral ER membrane protein that interacts with COPII components. The mutation that yields many small tER sites is a P1092L substitution.

Sec16 colocalizes with COPII coat proteins



Sec16 is much less abundant at tER sites than a COPII coat protein



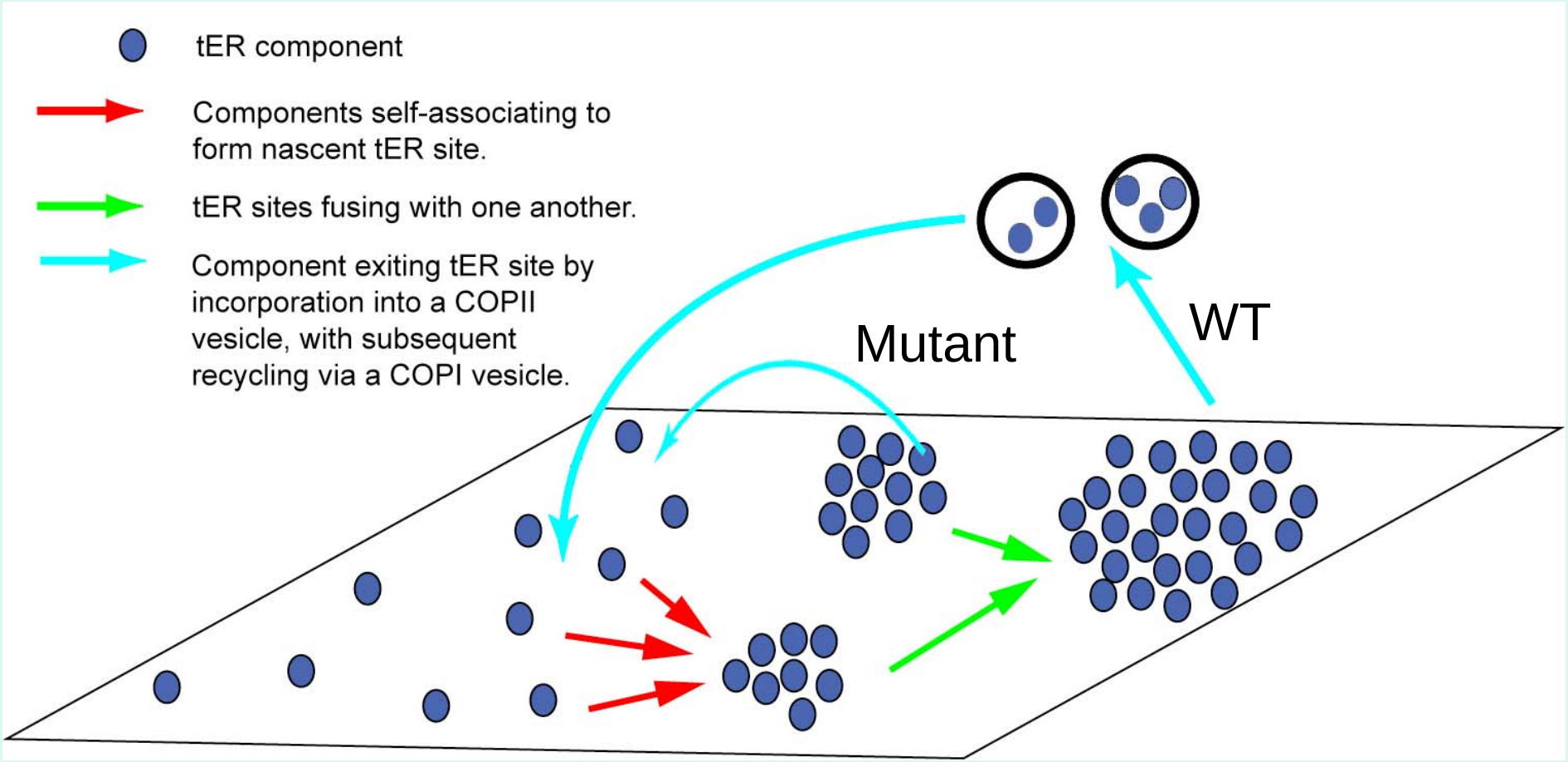
Data Summary

Sec16 is a regulator of COPII vesicle budding. It has both positive and negative regulatory effects.

The P1092L mutation selectively blocks the inhibitory effect. As a result, the mutant Sec16 is hyperactive.

How can we explain why the P1092L mutant cells have more tER sites?

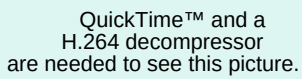
Self-Organization Model for tER Site Formation



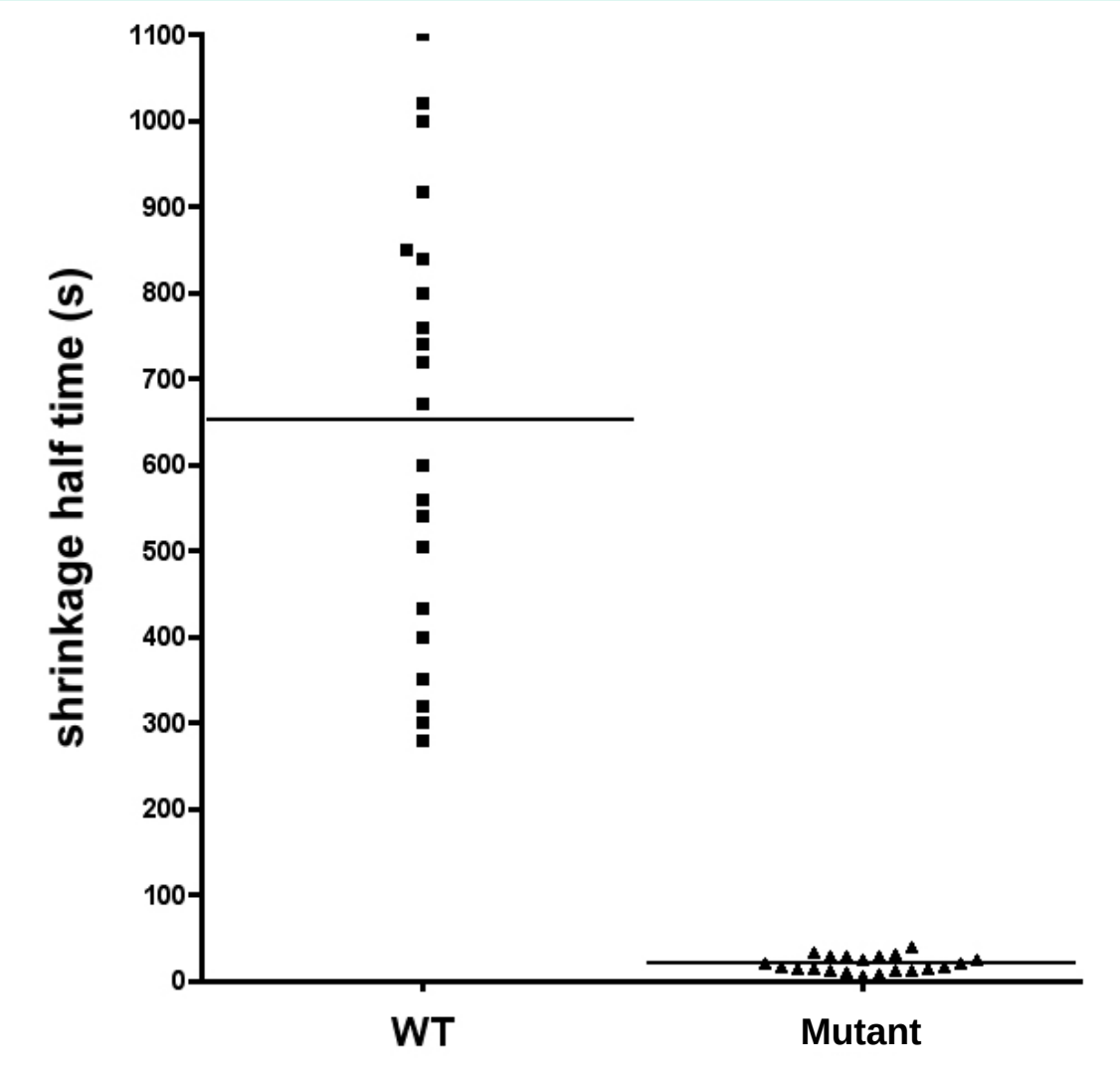
Prediction

tER sites in *sec16-P1092L* cells should shrink faster than in wild-type cells.

tER sites in *sec16-P1092L* cells show greatly accelerated dynamics



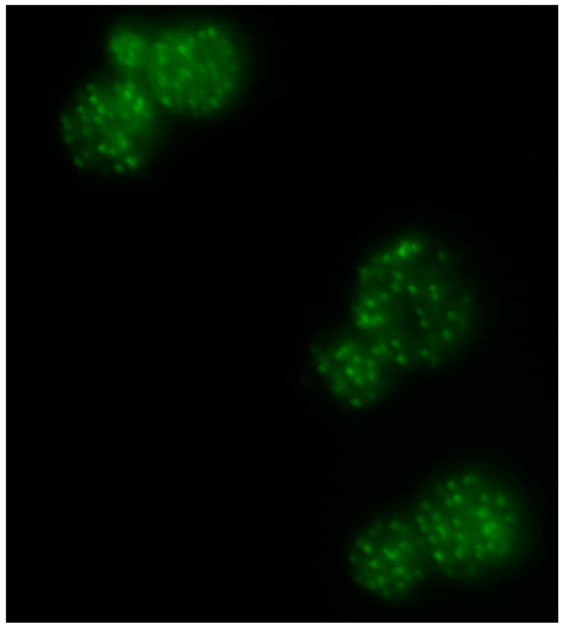
tER sites shrink faster in *sec16-P1092L* cells



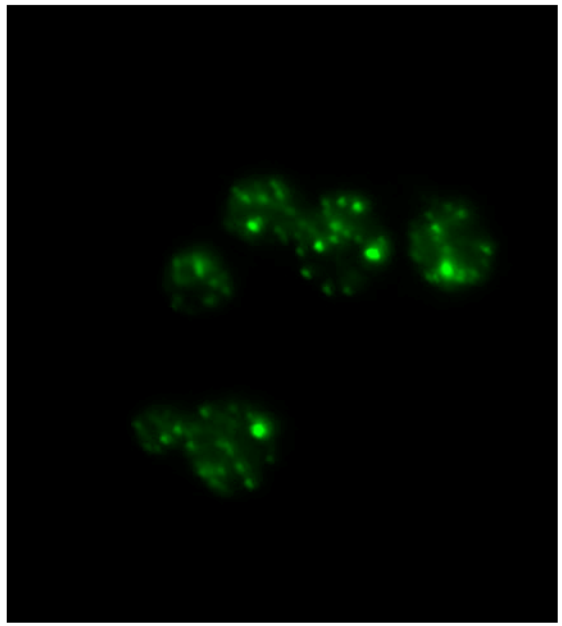
Could *Saccharomyces* be made to resemble *Pichia* simply by slowing ER export?

Blocking ER export in a *Saccharomyces sec12^{ts}* mutant enlarges tER sites

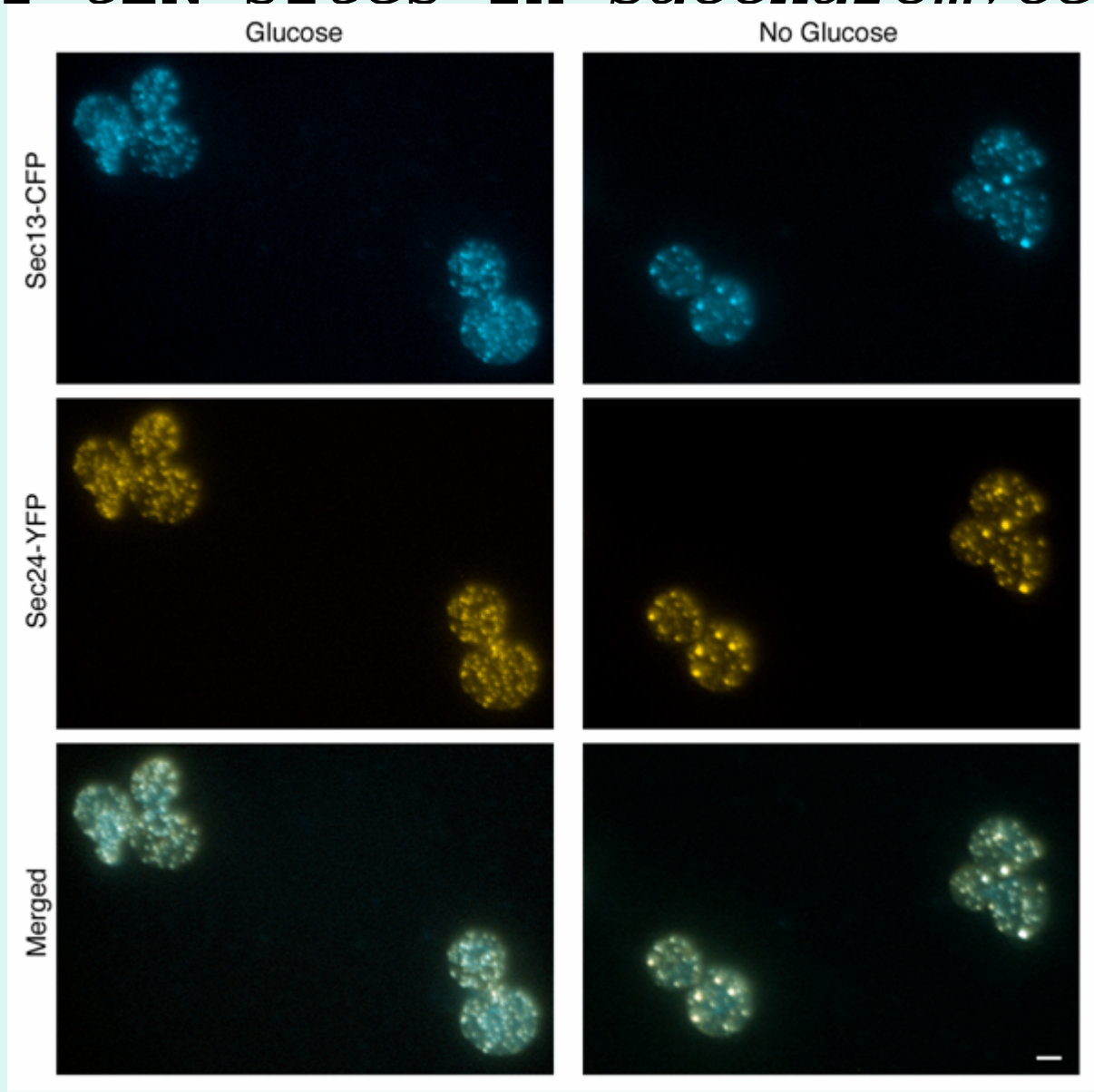
25° C



37° C



Glucose deprivation causes a similar enlargment of tER sites in *Saccharomvces*

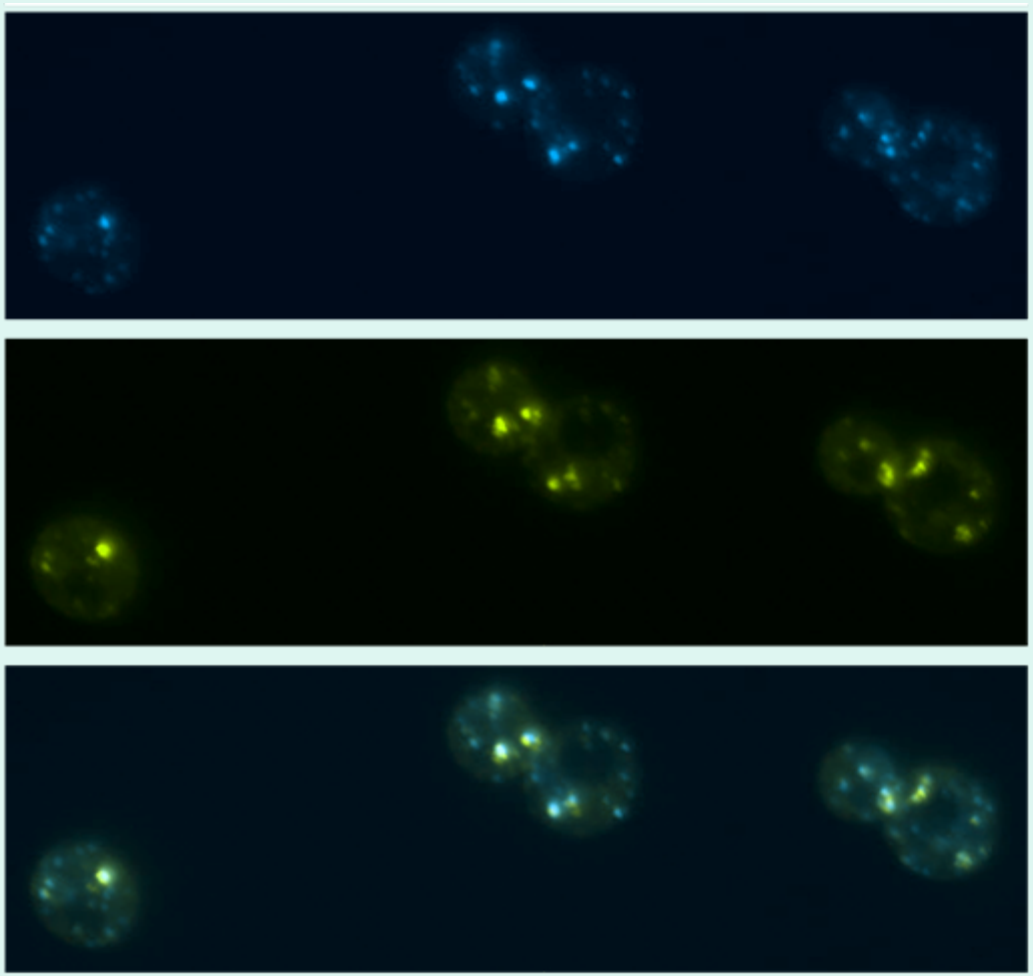


Glucose deprivation reveals a tER-*cis*-Golgi association in *Saccharomyces*

COPII
(Sec13-CFP)

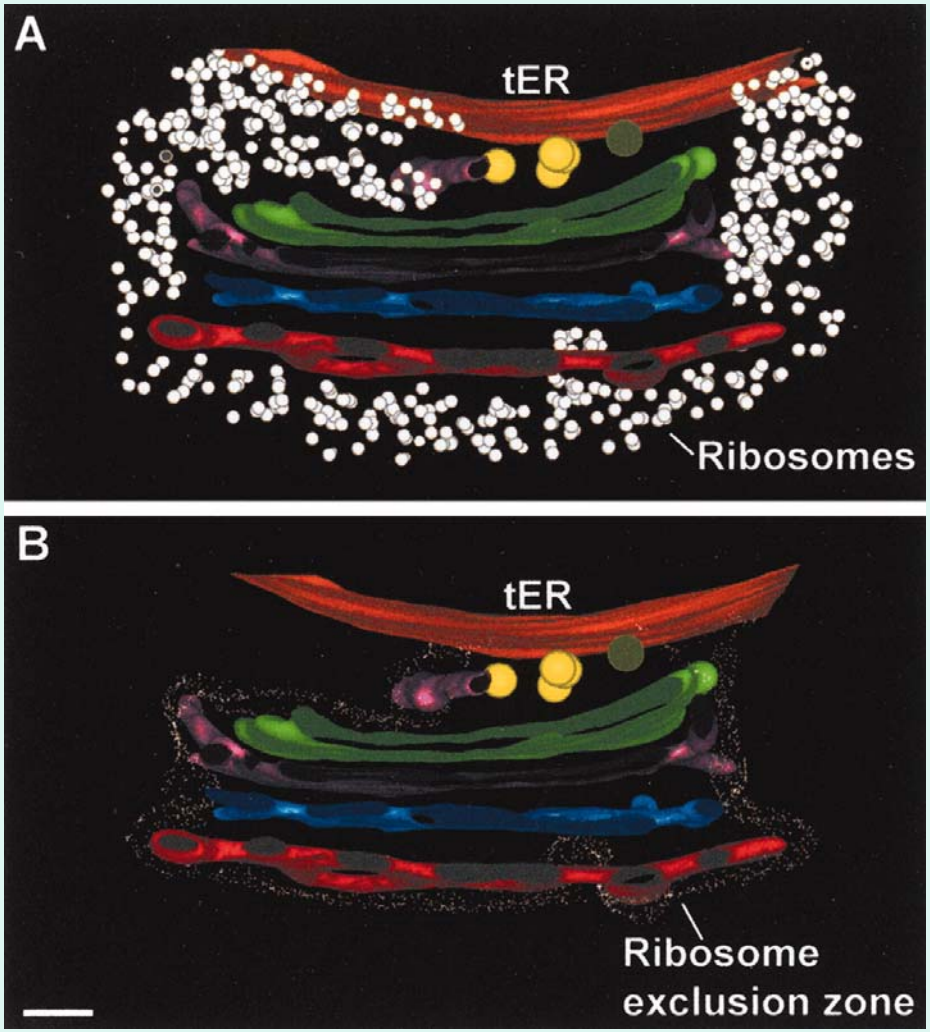
COPI
(Sec21-YFP)

Merged



The *trans*-Golgi is still separate.

Saccharomyces is not so different from *Pichia* after all!



A tER-Golgi unit in *Pichia*

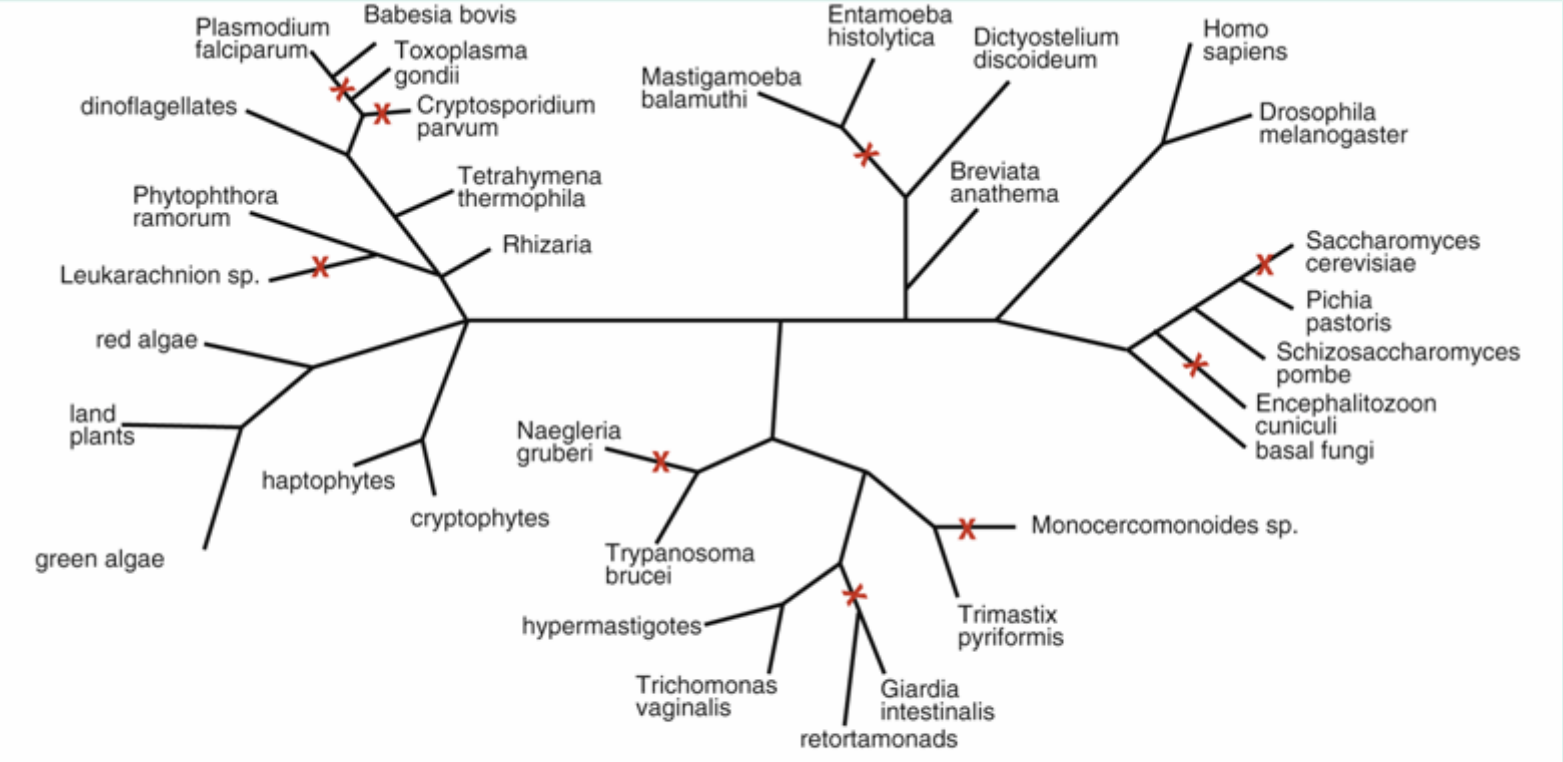
Staehelin Lab, Boulder, CO

Interpretation

A tER-Golgi association may be universal.

Golgi cisternal stacking seems to be “optional”.

Golgi stacking has been lost multiple times



K. Mowbrey, J.B. Dacks / FEBS Letters 583 (2009) 3738–3745

The nonstacked Golgi in *Saccharomyces* is probably a viable system for studying general principles of Golgi dynamics.

“That’s how it works in yeast… but mammalian cells may well be different.”

:-o The yeast experiments were inspired by data from algae and mammalian cells. Indeed, our analysis only made sense in light of those earlier studies.

:-o Basic cell biological mechanisms tend to be conserved. Golgi operation presumably falls into this category.

TGN cisternae "peel off" from the stack

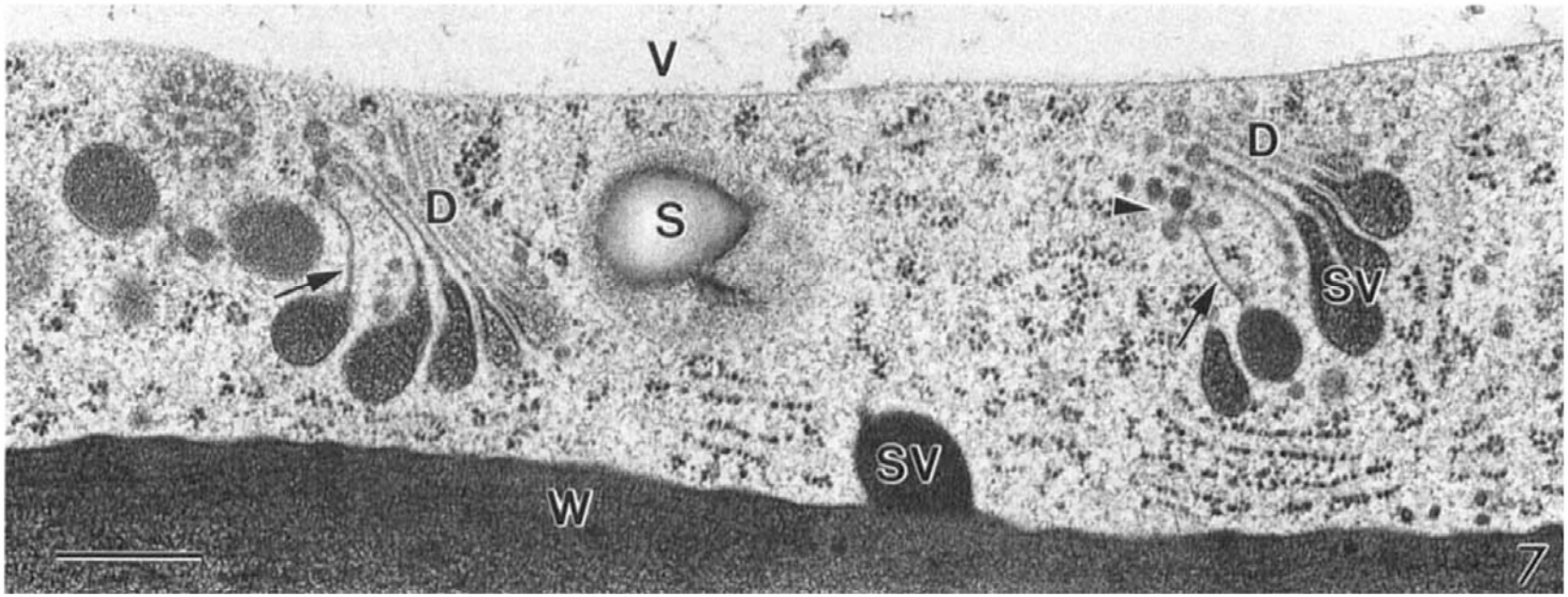


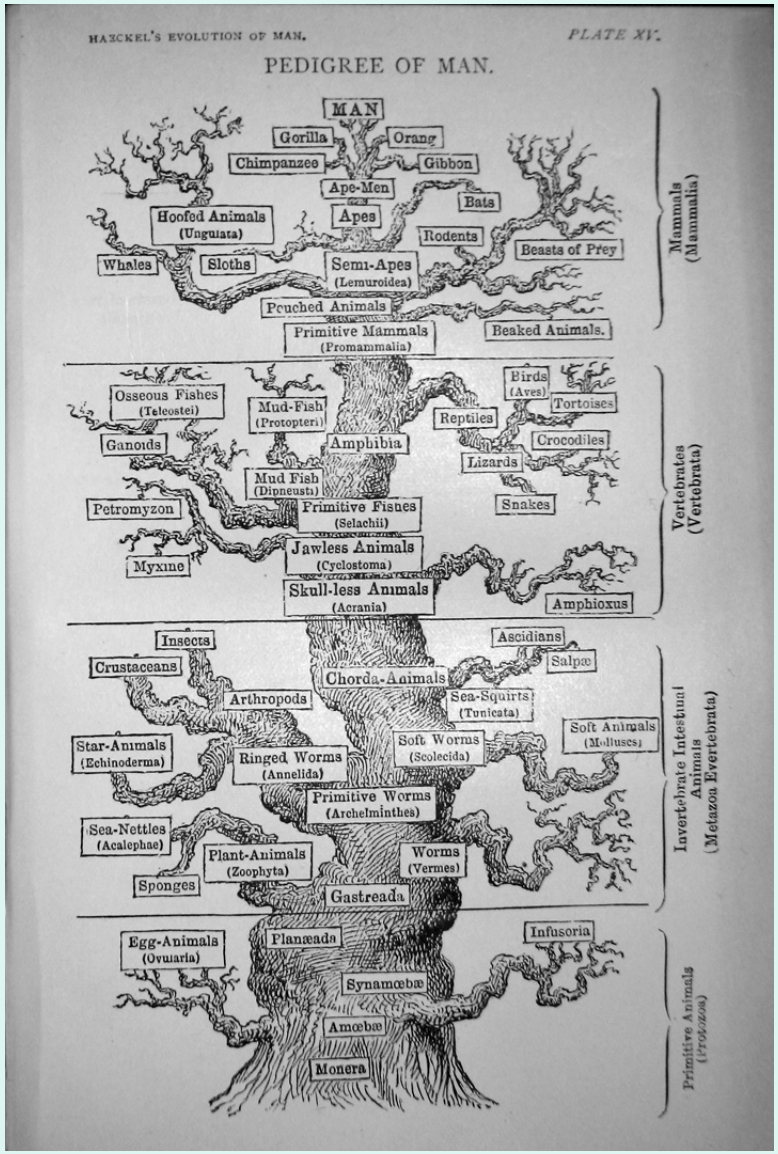
Fig. 7. Maize rootcap cell illustrating dictyosomes (D) and trans cisternae that have separated from the stack (arrows). Note the vesicular buds on one of the separated cisternae (arrowhead). Cell wall (W), vacuole (V), secretory vesicle (SV), starch in amyloplast (S). Bar = 0.5 μm (ca.).

J. Electron Microsc. Tech. 1991. 17:2-14.

We've heard this argument before…

[3] At present the only case in which a cis-trans movement of Golgi cisternae appears to be established is that of scale-producing algae (118). However, this may represent a rare formula connected with the unusual geometry and size of the product: a whole cisterna is needed to accommodate each scale under construction.

asic Golgi mechanisms are probably conserve



CONCLUSIONS

- The tER and Golgi are intimately associated. This relationship is evolutionarily conserved.
- Self-organization models can explain the formation and dynamics of tER and Golgi structures.

structure-function link is still a mys

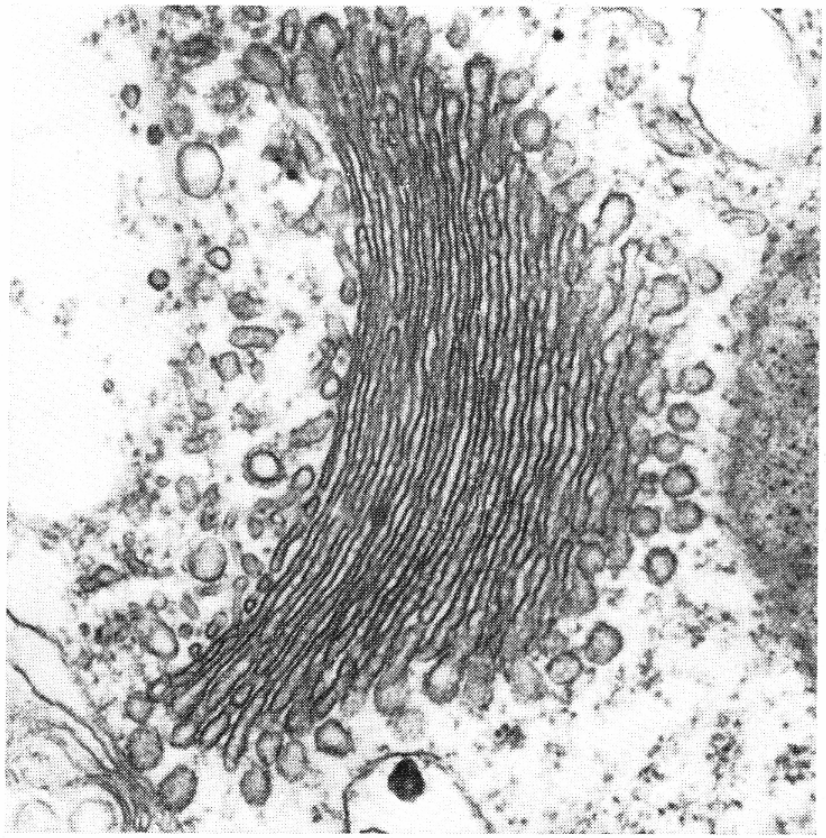
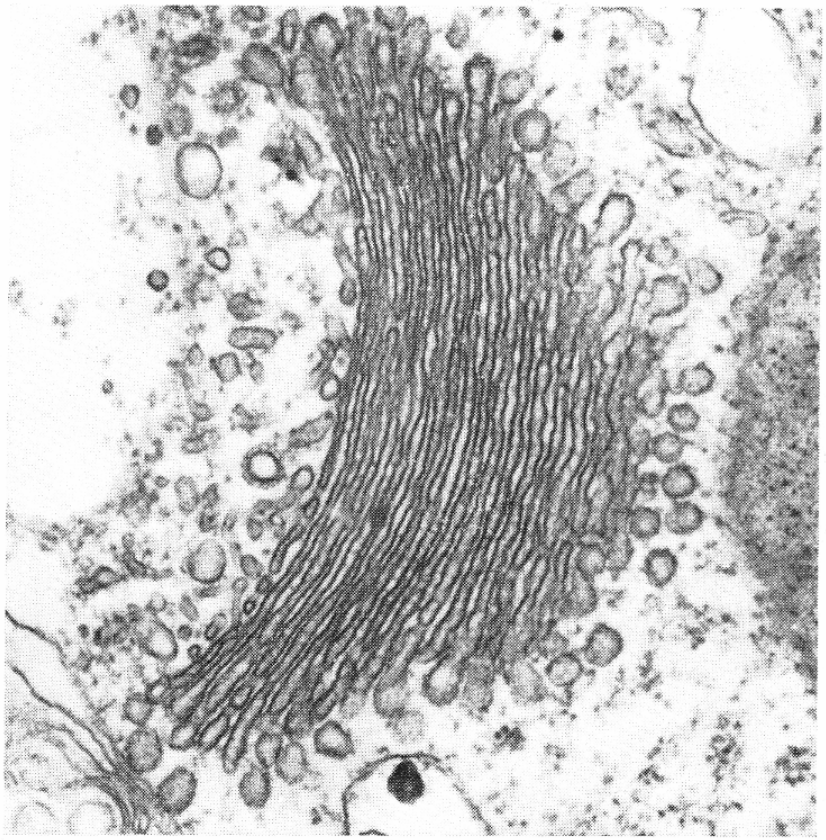


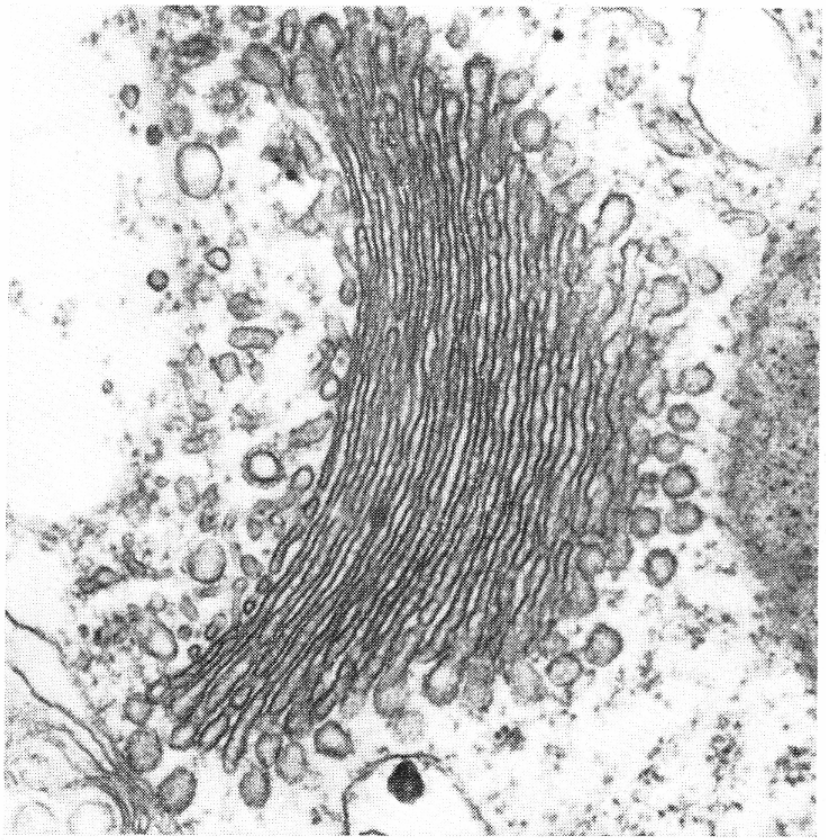
Fig. 12. Golgi apparatus of an individually discrete type from *Euglena* showing a large number of cisternae. From ARNOTT in WHALEY, in: The biological basis of medicine, Vol. **1** (BITTAR, E. E., and N. BITTAR, eds.). London-New York: Academic Press. 1968. ×30,000.

structure-function link is still a mys



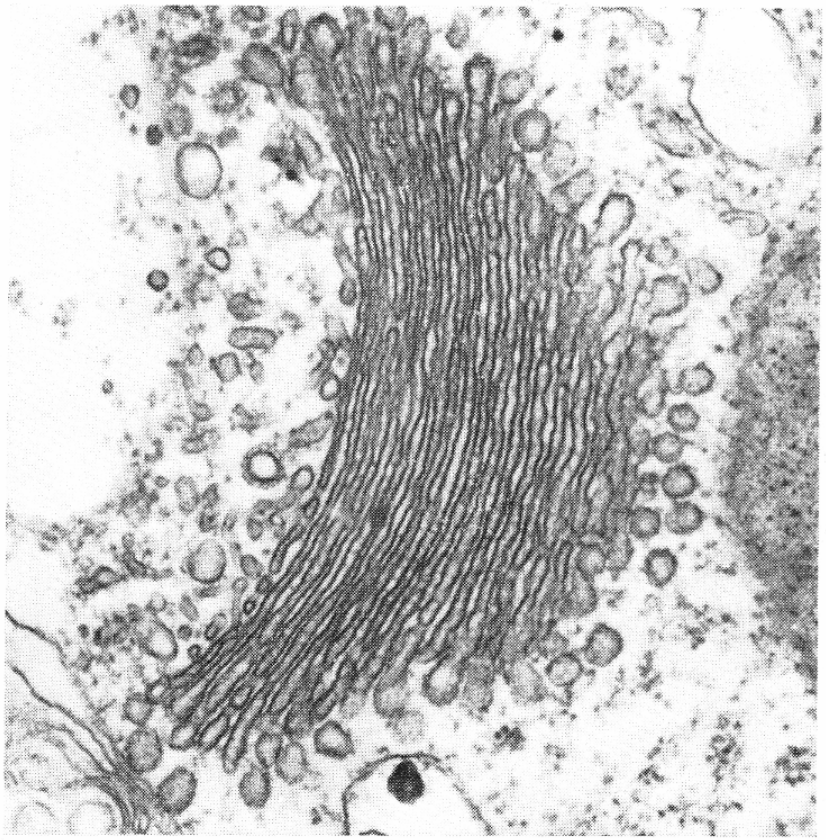
Why is the Golgi divided into cisternae?

structure-function link is still a mys



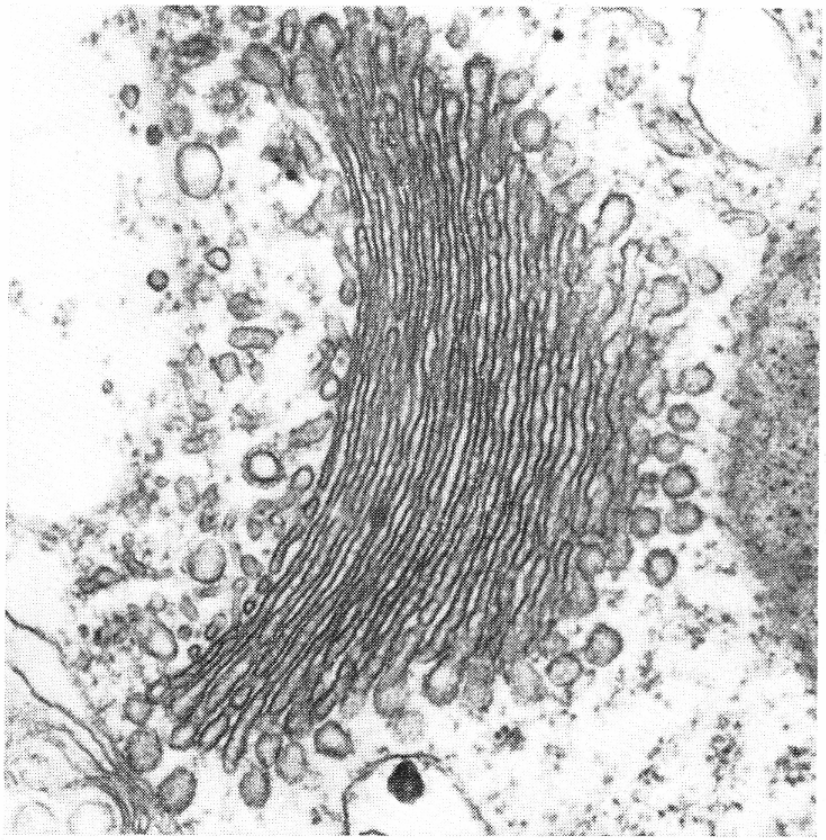
Why do some cells have so many cisternae?

structure-function link is still a mys



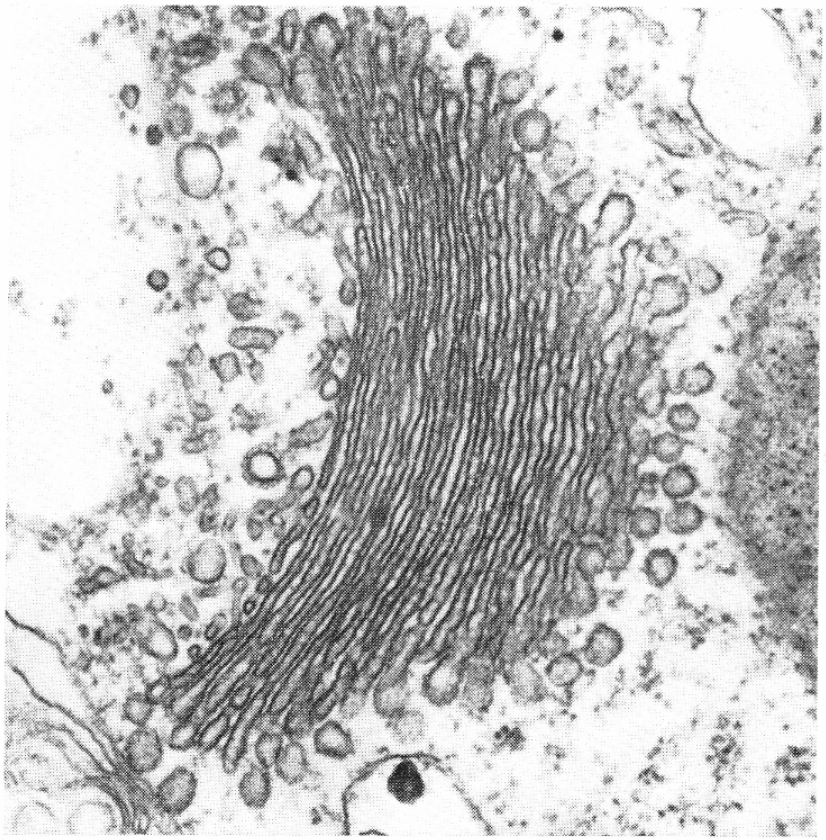
How is cisternal number regulated?

structure-function link is still a mys



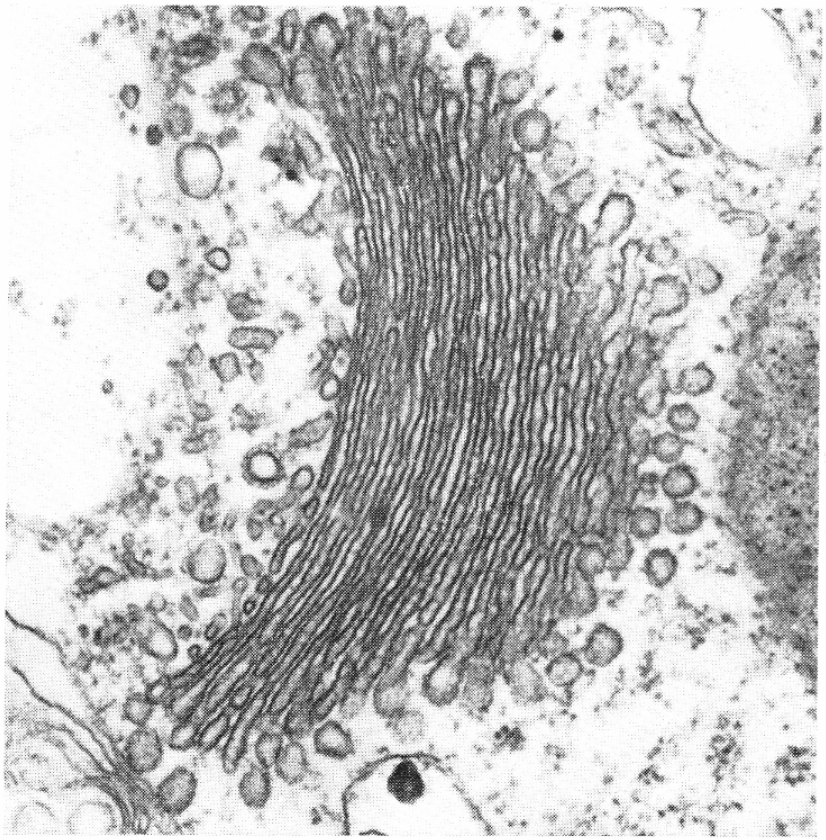
What “glues” the cisternae together?

structure-function link is still a mys



How do TGN cisternae become unglued?

structure-function link is still a mys



What defines Golgi compartmentation?

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