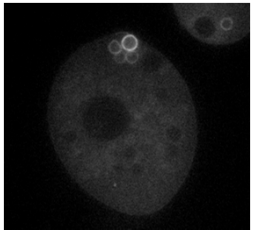
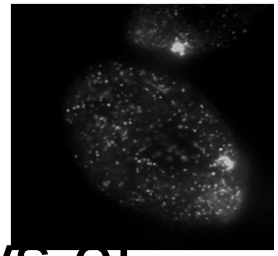
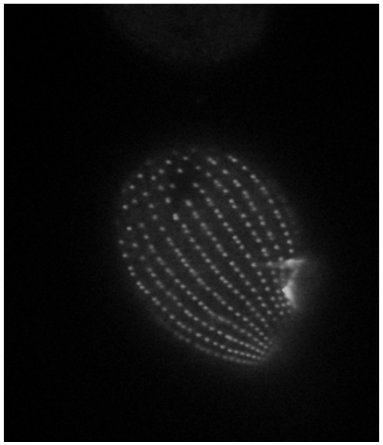




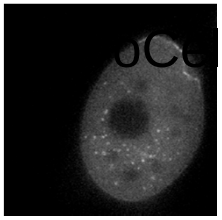
Conservation and innovation in pathways of membrane traffic in *Tetrahymena*



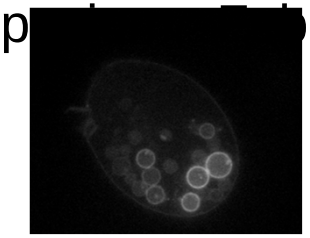
Aaron Turkewitz

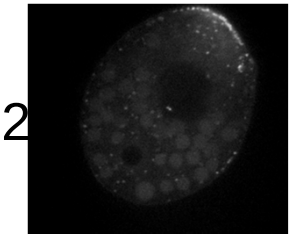
Department of Molecular Genetics and Cell Biology

The University of Chicago



oCell Symp ruary 2012





The complex network of membrane traffic: when and how did it arise?

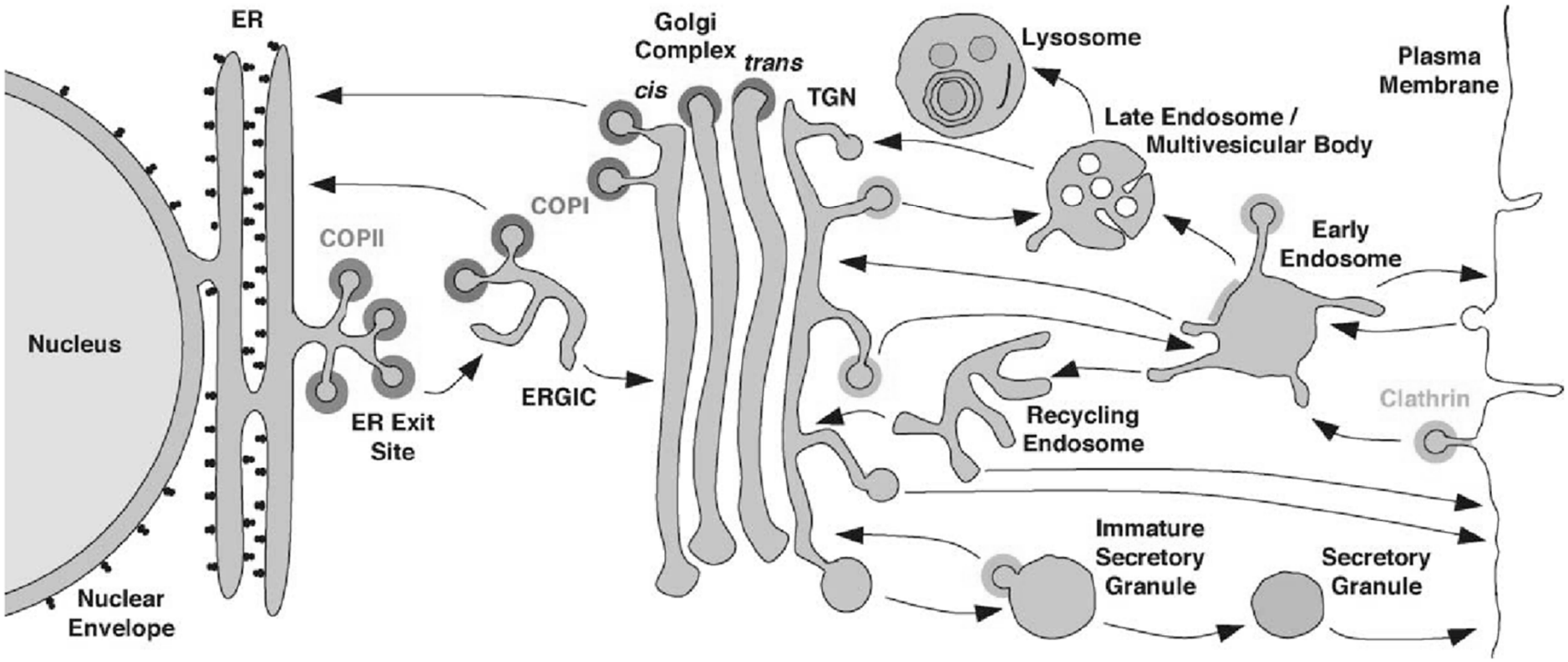
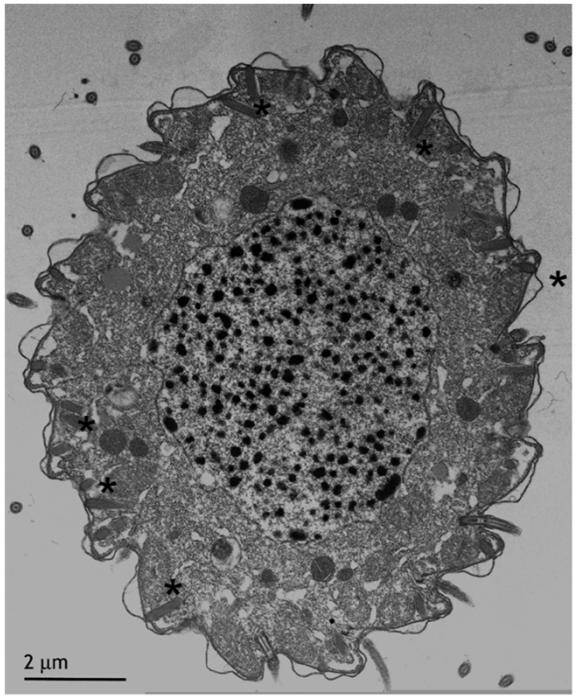
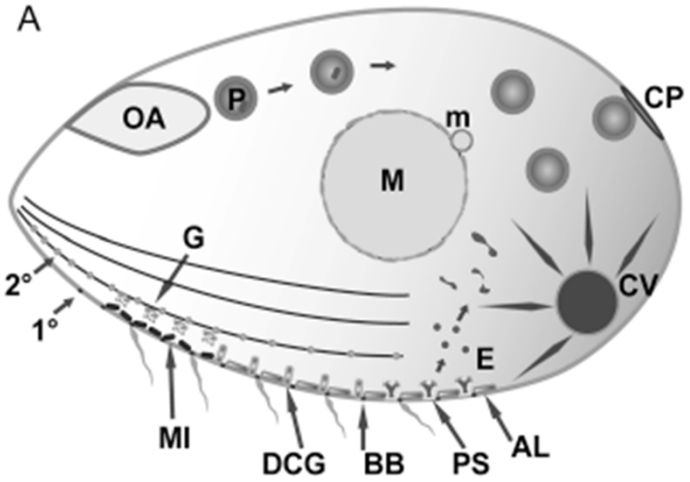


Figure 1. Intracellular Transport Pathways

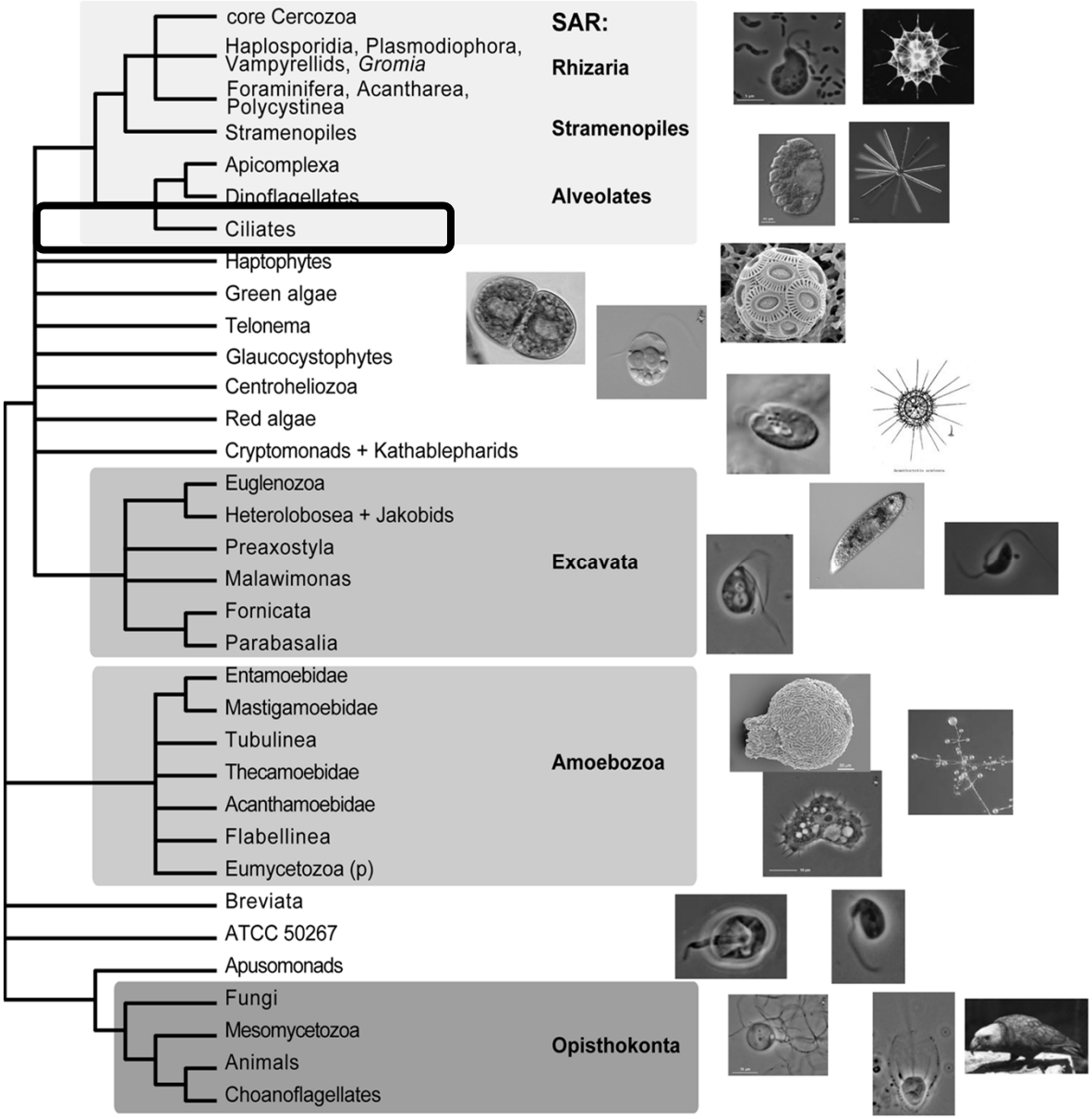
Bonifacino & Glick 2004

Tetrahymena thermophila





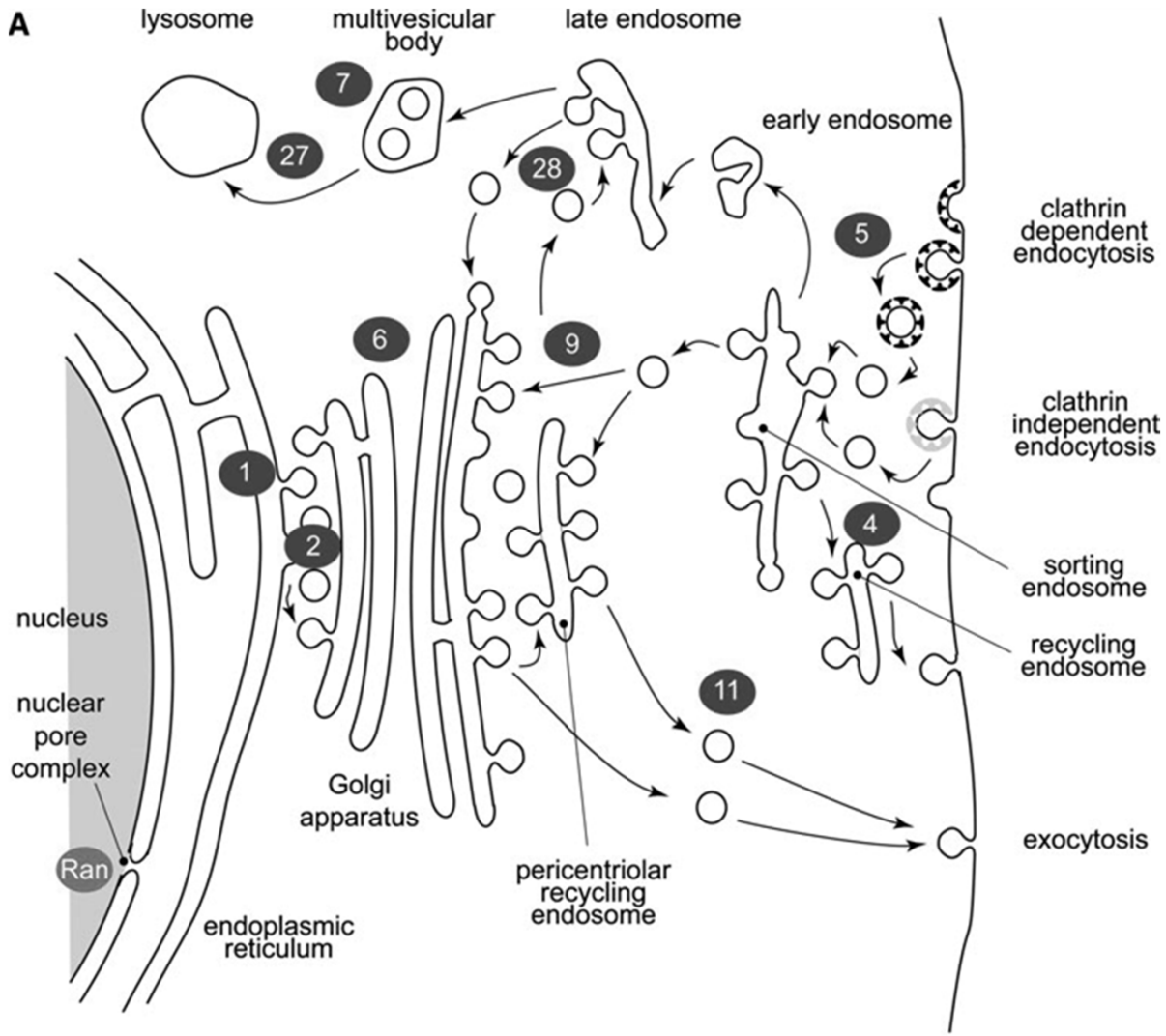
Ciliates are distantly related from most organisms in which membrane traffic is being studied.



Parfrey L W et al. Syst Biol 2010;59:518-533

Rab GTPases can be used to map the network of membrane trafficking and its evolution.

• Each Rab is associated with one or a small number of trafficking steps, for which it acts as a core determinant.



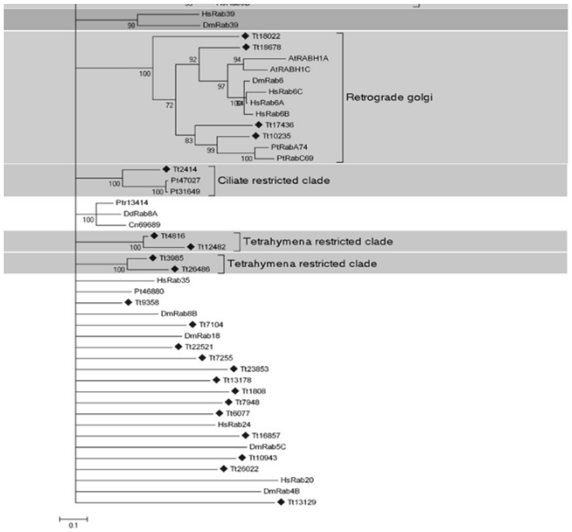
From Brighouse et al, 2010

Tetrahymena and humans encode the same number of Rabs

Organism	Predicted Rabs
Homo sapiens	63
Arabidopsis thaliana	57
Drosophila melanogaster	33
Caenorhabditis elegans	29
Saccharomyces cerevisiae	12
Schizosaccharomyces pombe	8
Trypanosoma brucei	16
Plasmodium falciparum	11
Dictyostelium discoideum	54
Entamoeba histolytica	91
Trichomonas vaginalis	65
Tetrahymena thermophila	*63*

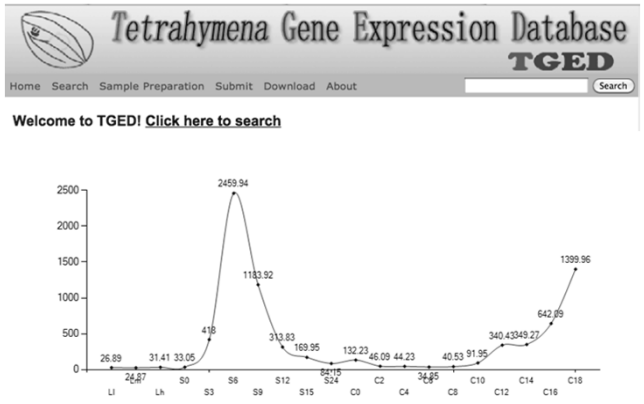
Three-part analysis of the Rab family

Phylogenetic



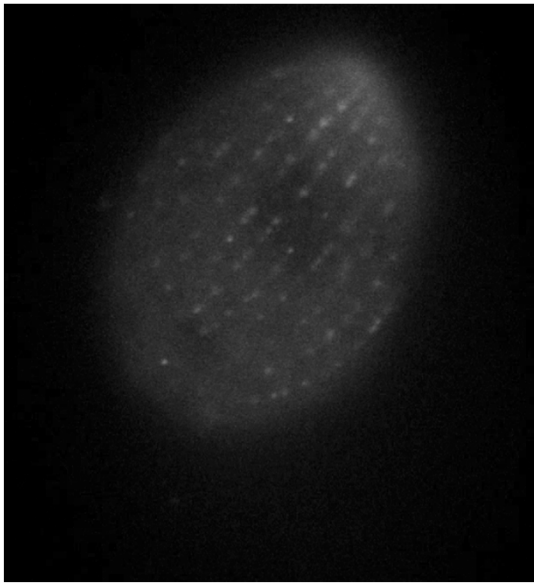
Expression

http://tged.ihb.ac.cn

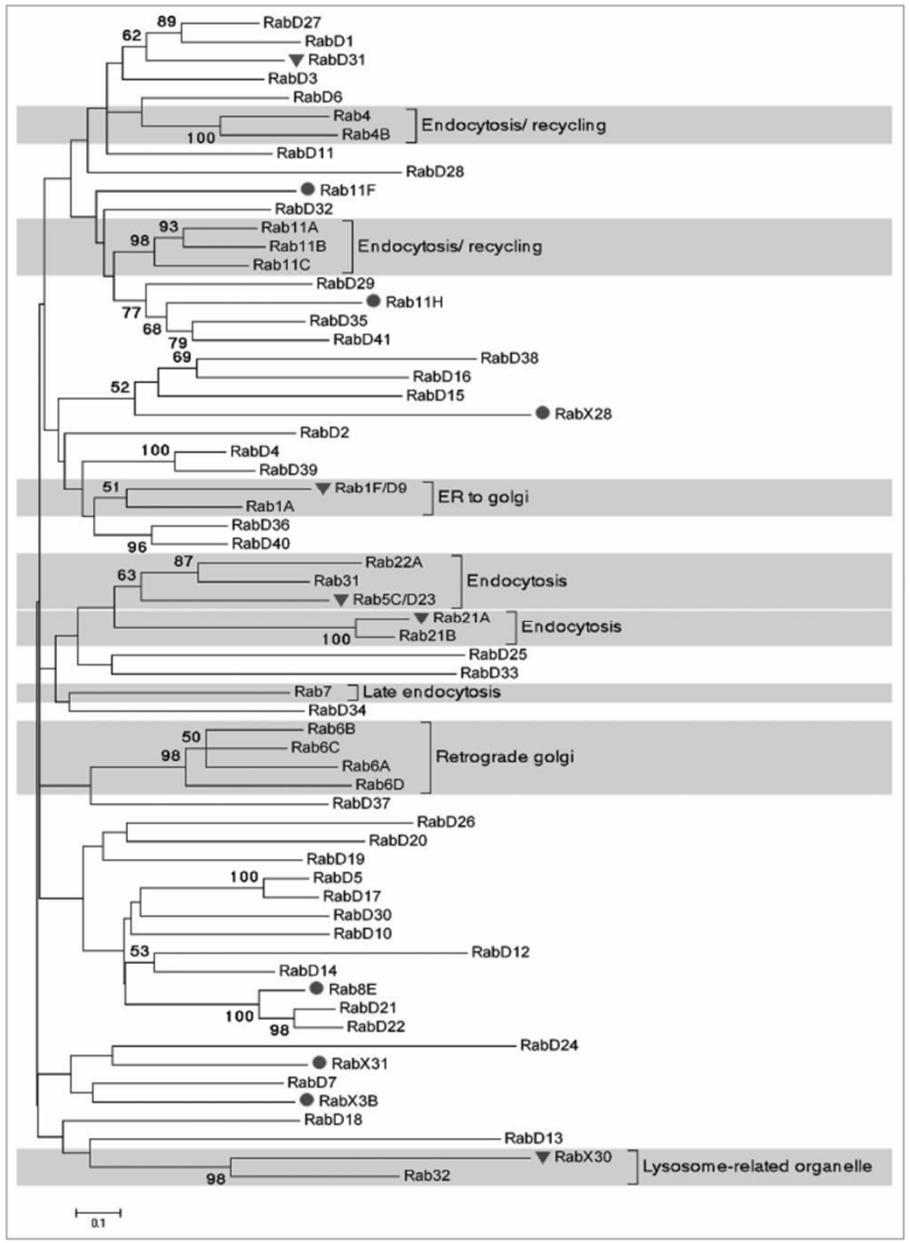


Miao et al 2009

Localization



Phylogenetic analysis of Rabs



Turkewitz & Bright 2011

27/63 Tetrahymena Rabs fall into conserved clades:
Rab 1: 3 paralogs
Rab 2: 4 paralogs
Rab 4: 2 paralogs
Rab 5: 1 gene
Rab 6 : 4 paralogs
Rab 7: 1 gene
Rab 8: 3 paralogs
Rab 11: 6 paralogs
Rab 21: 3 paralogs

36/63 Tetrahymena Rabs appear unconserved.

How much redundancy is there among the 63 Tetrahymena Rabs?

1. No recent chromosome or whole genome duplications.
2. Very few of the Rabs are closely related to one another in sequence.
3. Even sequence-related Rabs can show different localization.

Tetrahymena Gene Expression Database

TGED

Home Search Sample Preparation Submit Download About

Search

Welcome to TGED! Click here to search

TGED is a web-accessible database of information about the *Tetrahymena thermophila* genome-wide gene expression. Currently, TGED provides microarray data of *T. thermophila* during growth, starvation and conjugation completed by the Gorovsky lab at the University of Rochester, Rochester, NY, USA and the Miao Lab at the Institute of Hydrobiolgy, Chinese Academy of Sciences, Wuhan, CHINA.

TGED is intended to be a resource for all members of the scientific research community interested in *Tetrahymena* and other ciliates. As we develop TGED, we would greatly appreciate input from the community so that we can better tailor it to meet your needs. Please feel free to send comments, suggestions, or questions to TGED at tged@ihb.ac.cn

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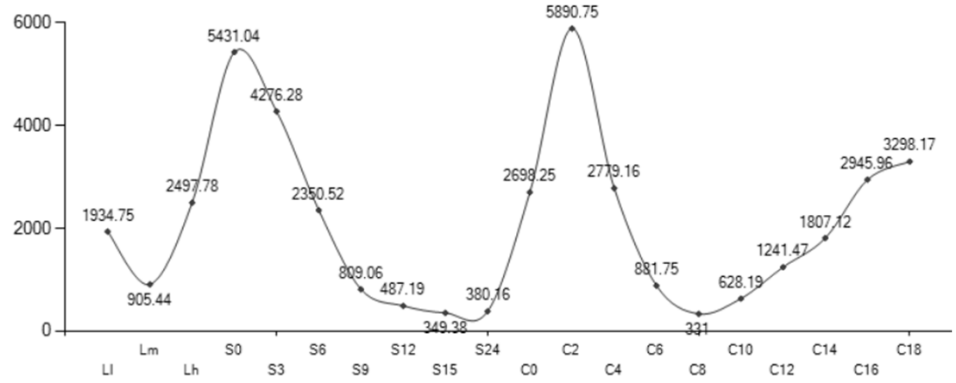
You are quering for TTHERM_00052190

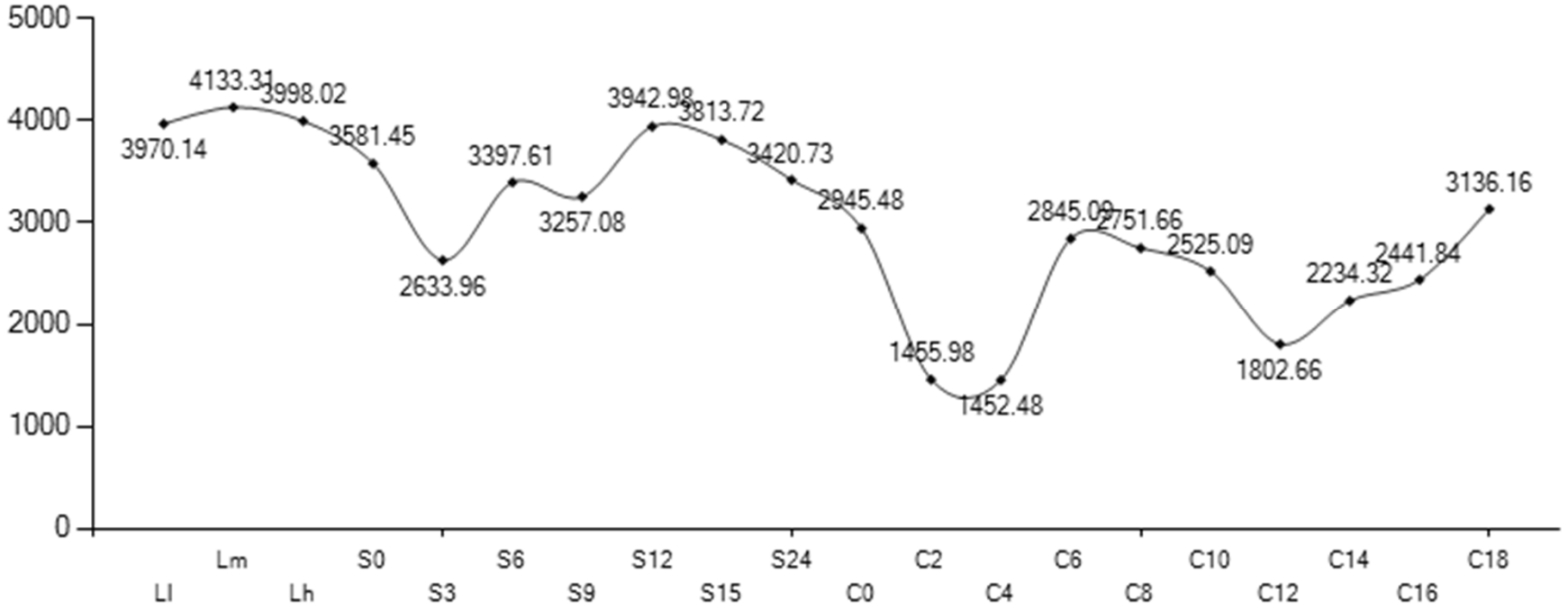
SUMMARY

Gene ID	TTHERM_00052190
Sequence ID	TETRA00S0010250
Gene Type	protein-coding
RNA Name	Eukaryotic aspartyl protease family protein
Sequence	full length cDNA Protein Sequence Probes

Expression Profile

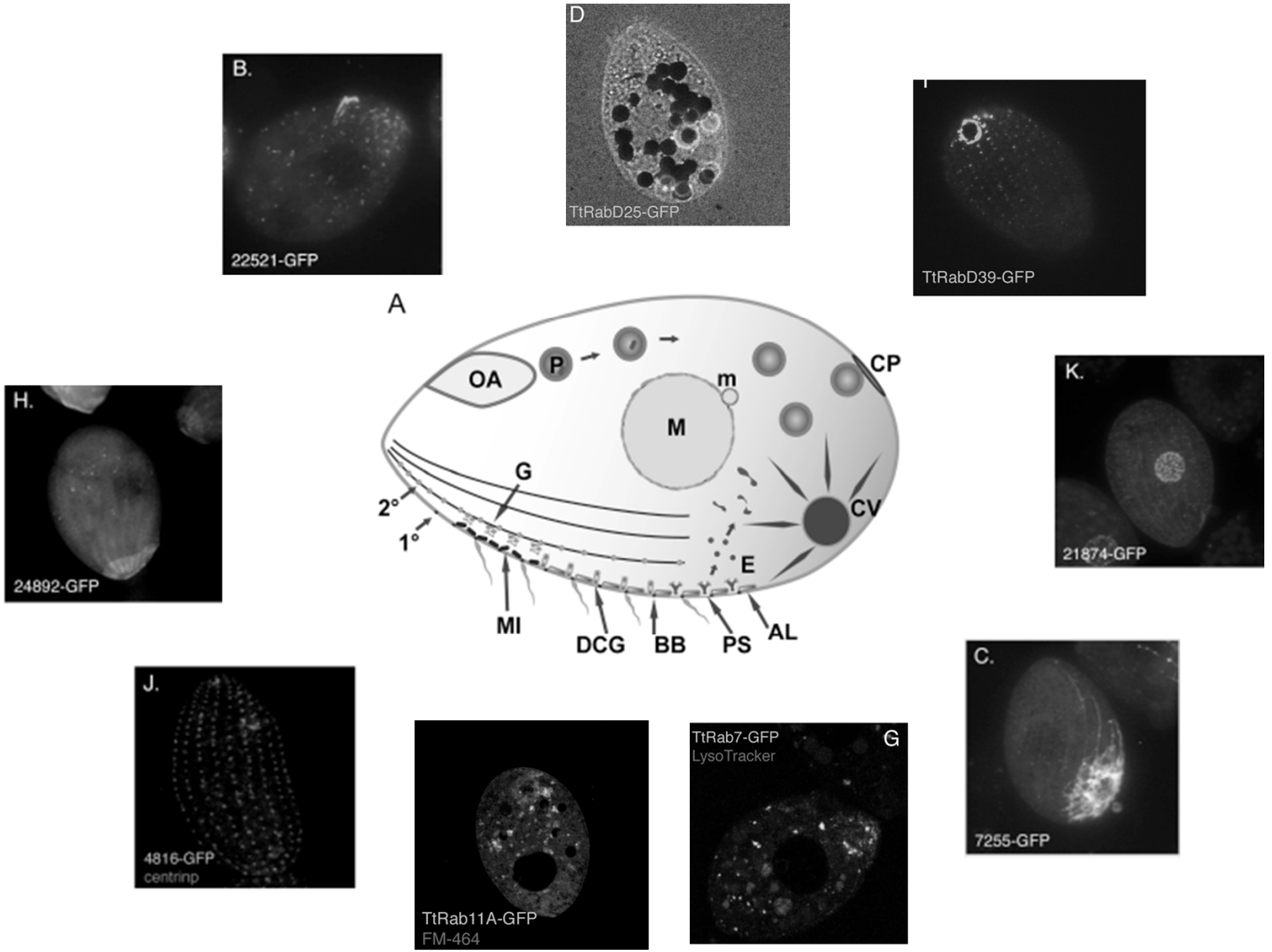


Expression of a “typical” Rab

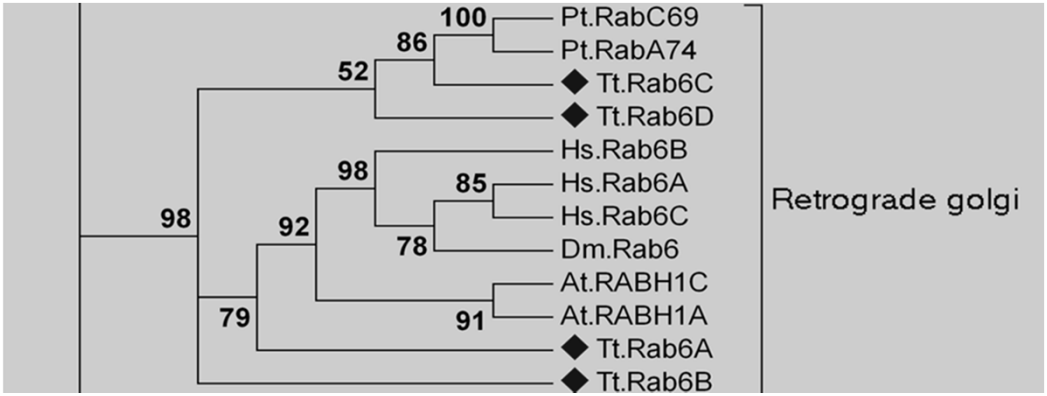


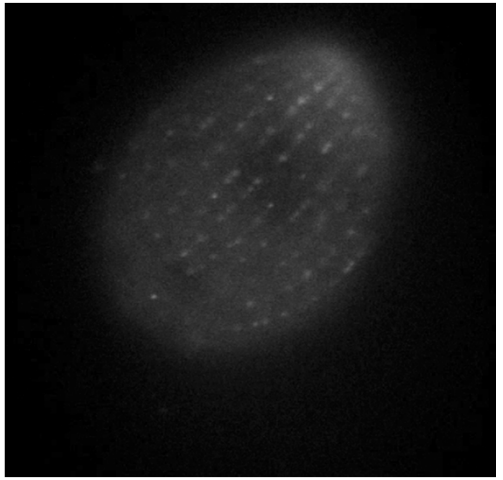
>90% of Rabs expressed in growing cultures; >86% at highest level of gene expression in Tetrahymena

Interpreting Rab localization in Tetrahymena

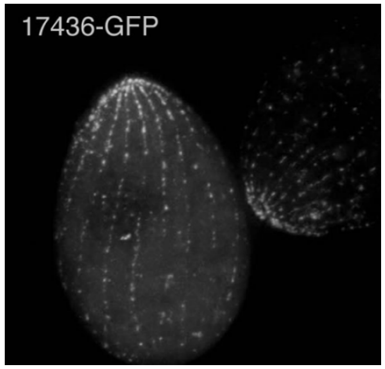


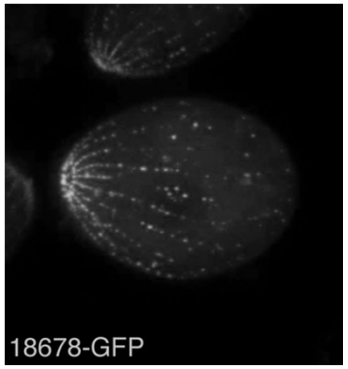
The *Tetrahymena* rab6 paralogs all localize to the Golgi.

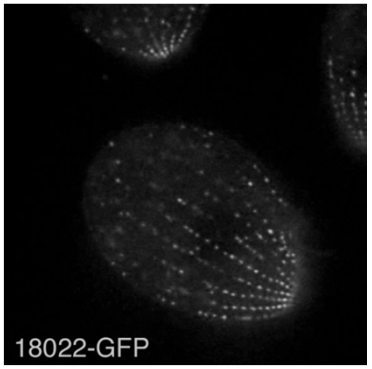




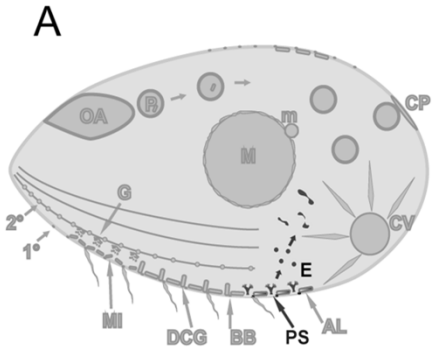
10235 stack

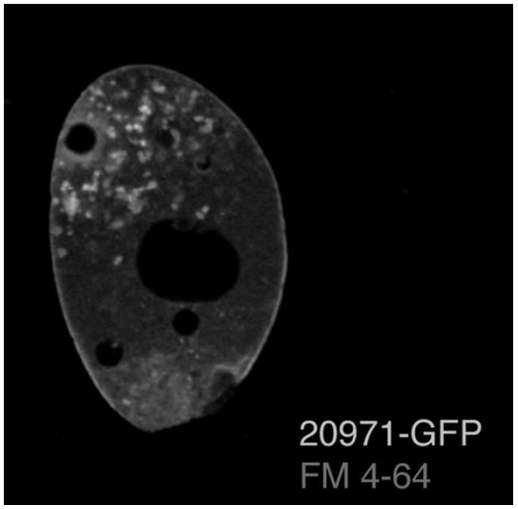


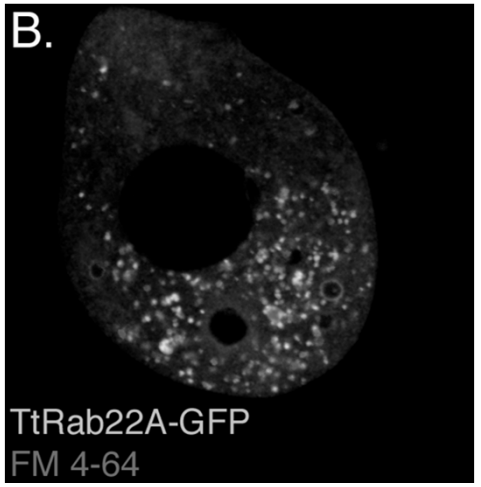


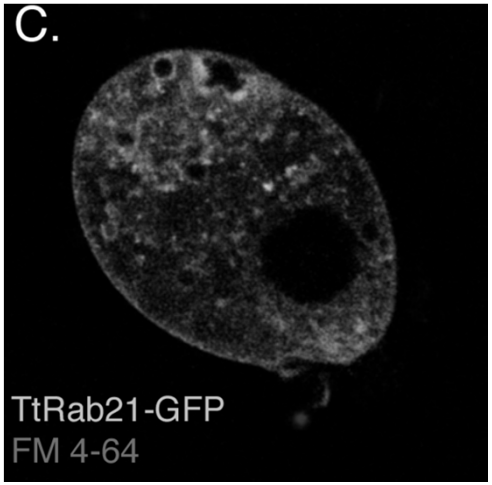


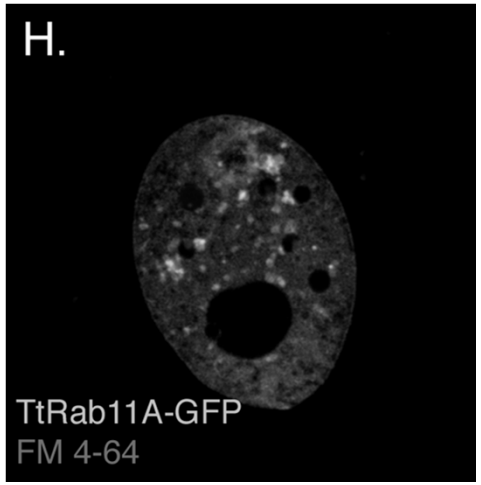
Most Rabs in conserved endocytosis subfamilies are associated with FM4-64-positive endosomes.





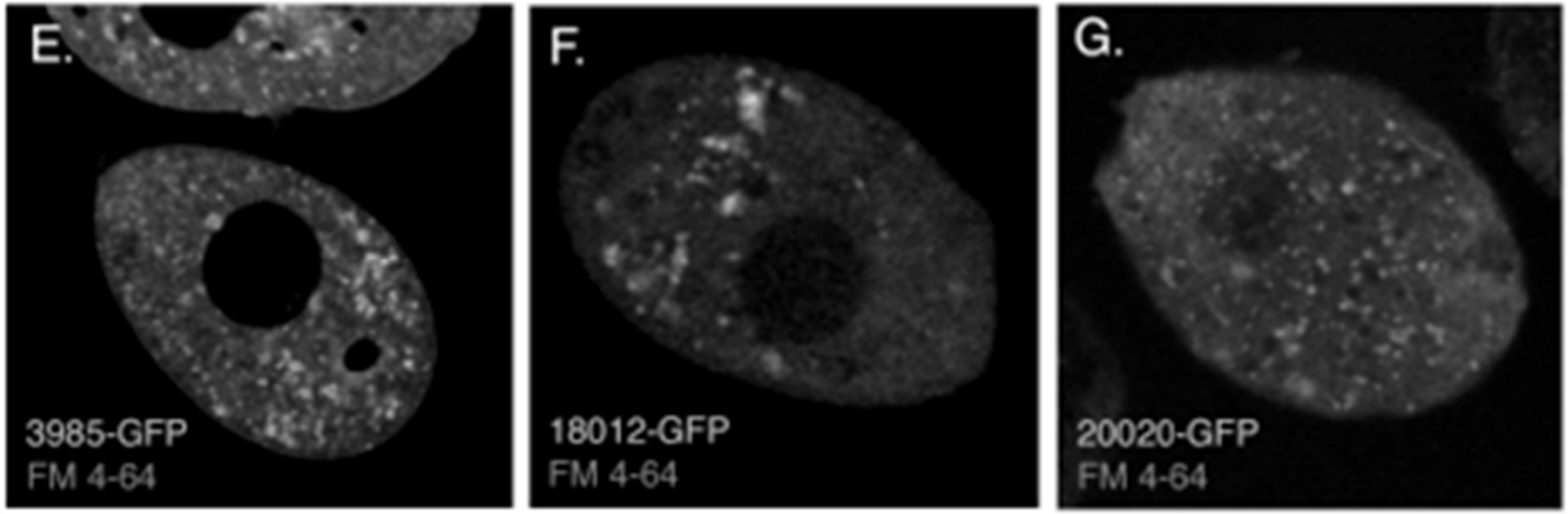




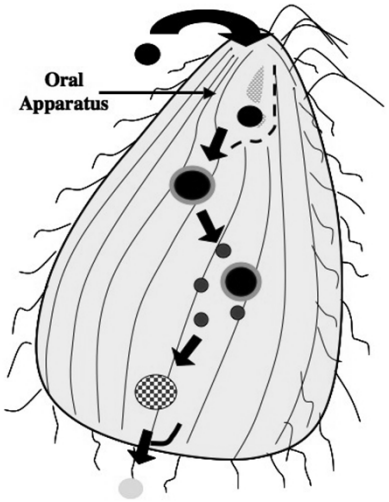


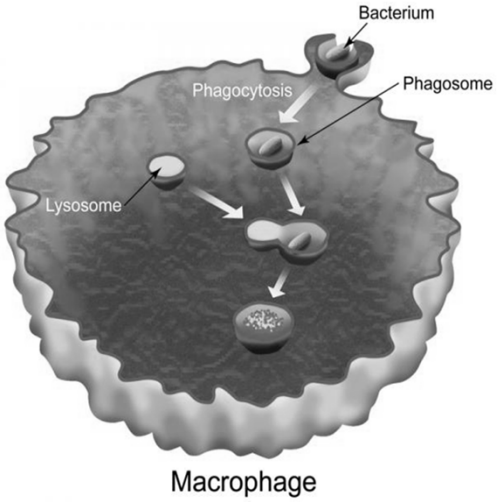
Overall, most (but not all) Rabs in conserved subfamilies were localized consistent with their phylogenetic assignments.

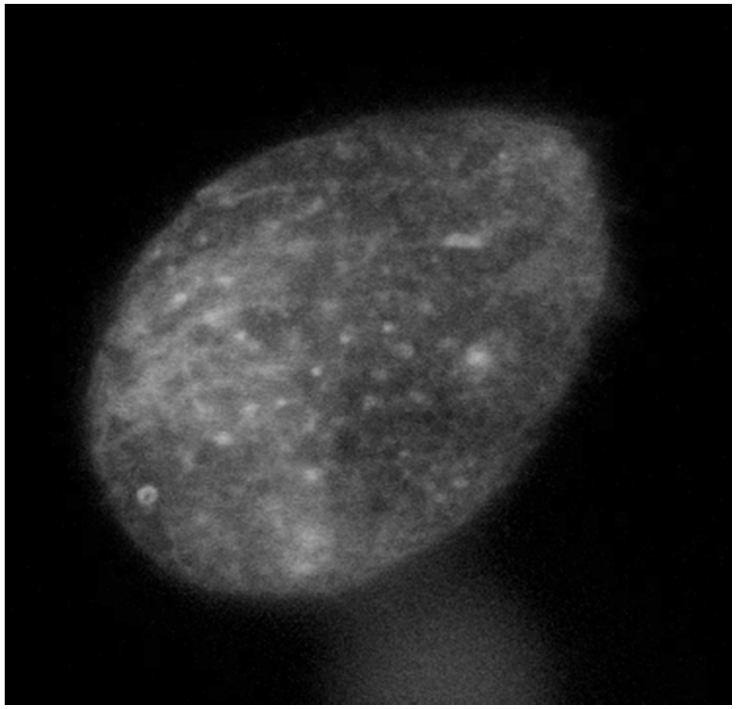
FM4-64 positive endosomes are also labeled by three unconserved Rabs.

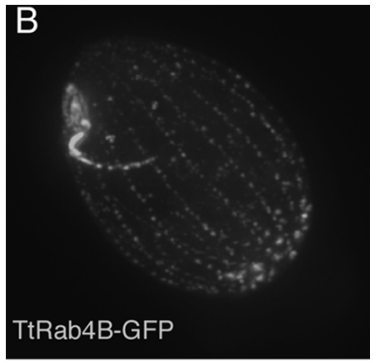


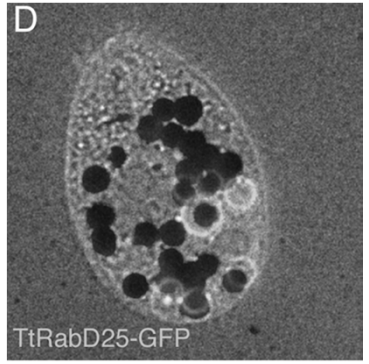
Phagocytosis-associated Rabs.



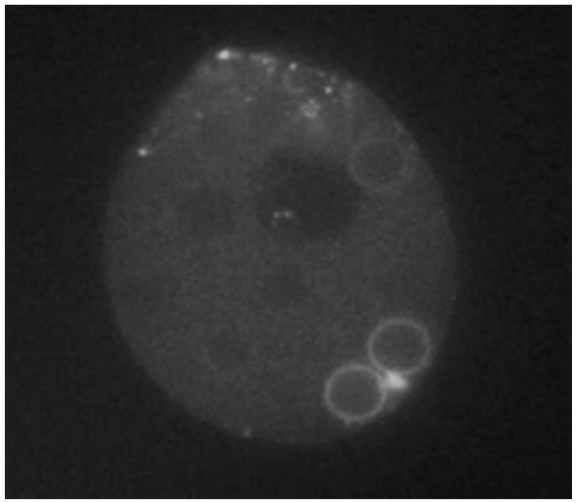




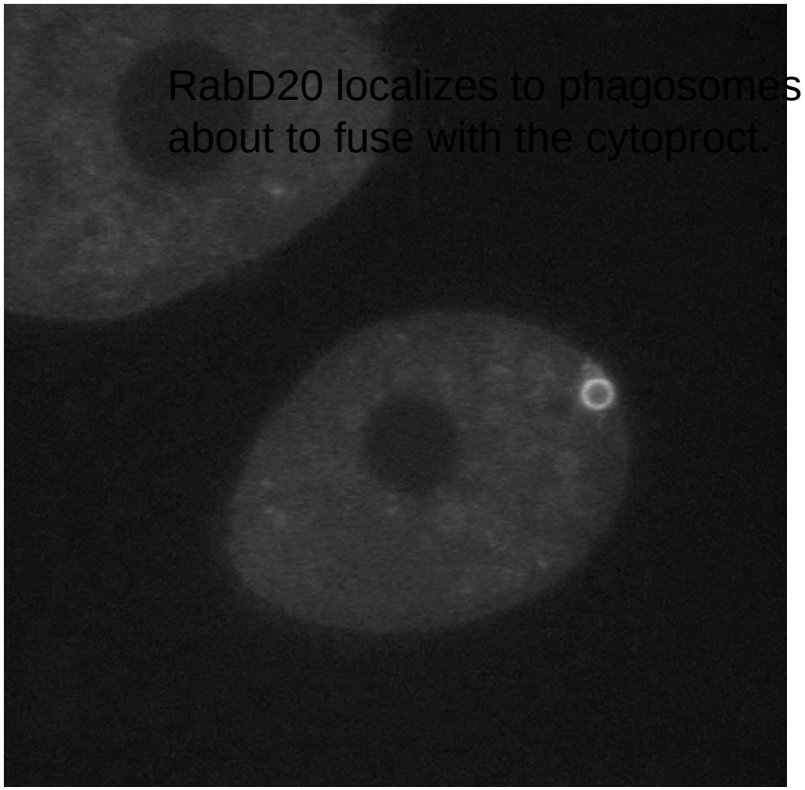




RabD30 is associated with phagosomes en route to the cytoproct zone.



GFP-RabD30



GFP-RabD20

Part I Conclusions

1. GTP-labeled Rabs provide a new set of markers for structures involved in membrane traffic in Tetrahymena.
2. The number of simultanously-expressed Rabs in Tetrahymena suggests that ciliates have evolved pathways of membrane traffic roughly as complex as those in human cells.
3. The putative origins of specific pathways in Tetrahymena could be examined by assessing the functions, inferred via localization, of specific conserved and divergent Rabs.
4. The Golgi appears to be associated only with conserved Rabs predicted to function in that compartment.
5. Endocytosis is associated with a conserved core of Rabs, but a group of non-conserved Rabs has also been recruited to this pathway.
6. Both Tetrahymena and humans have a very large set of phagosomal Rabs, but most of these are unrelated.

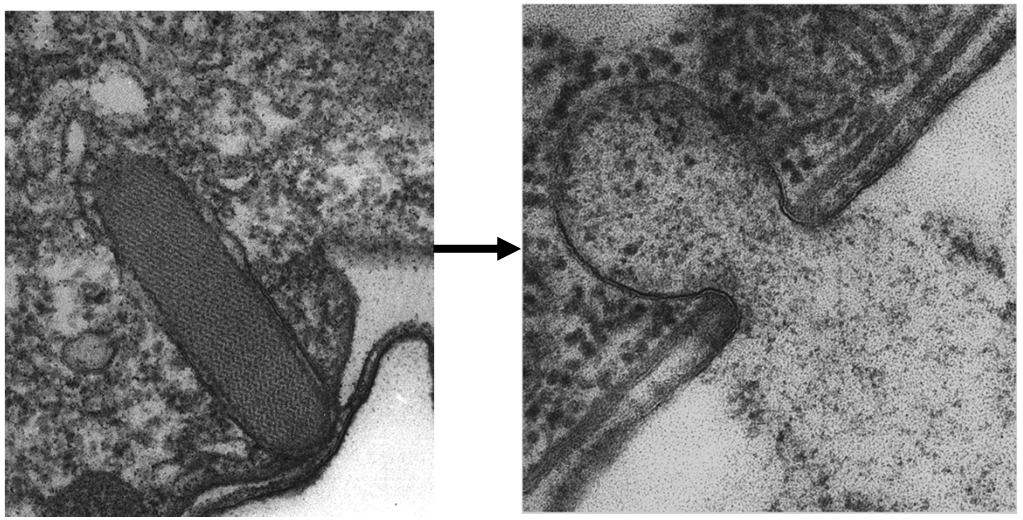
Overall: we hypothesize that lineage-restricted innovation has played a significant role in the evolution of membrane traffic, and such innovations can sometimes converge to produce very similar structures or pathways.

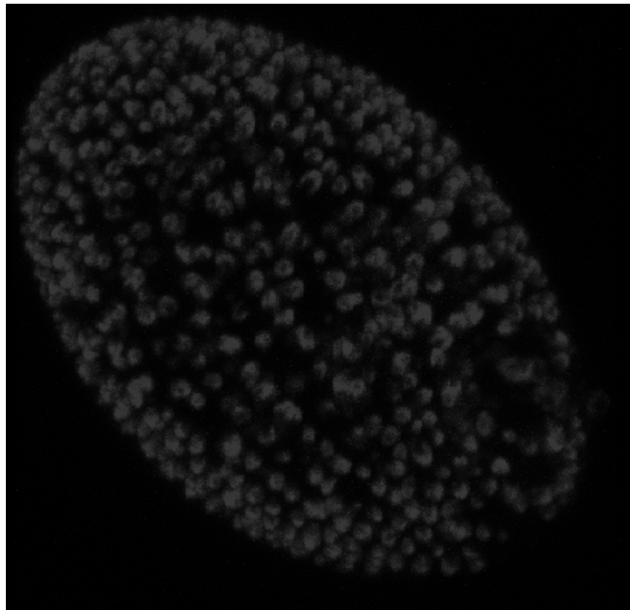
Analysis of a putatively novel pathway of membrane traffic

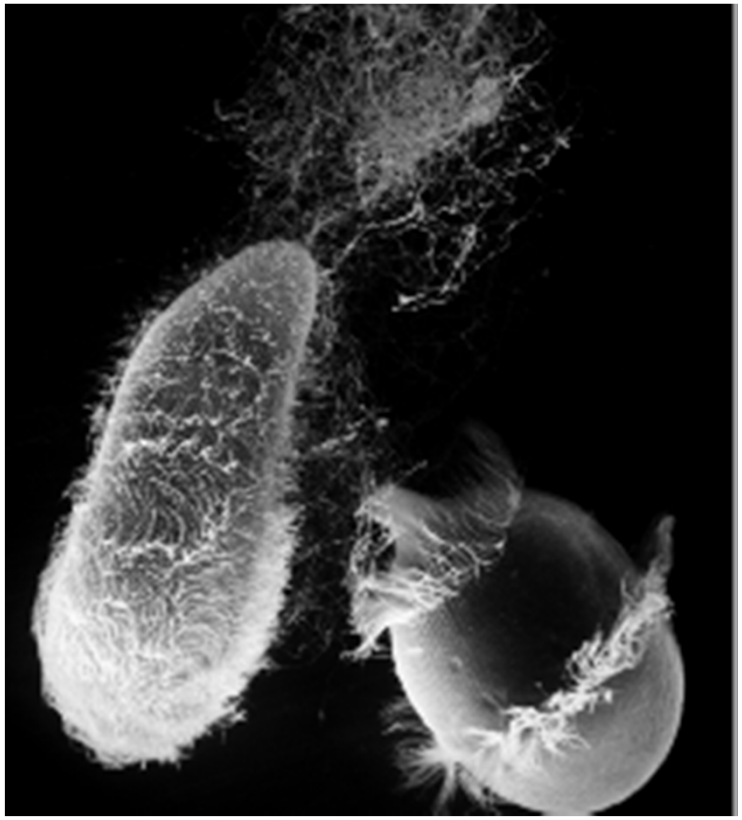
1. Where did it originate?

2. What were the key innovations?

Regulated exocytosis from dense core vesicles (mucocysts) in Tetrahymena







G. Antipa

Dense-core granules facilitate stimulus-dependent secretion of selected cargo.

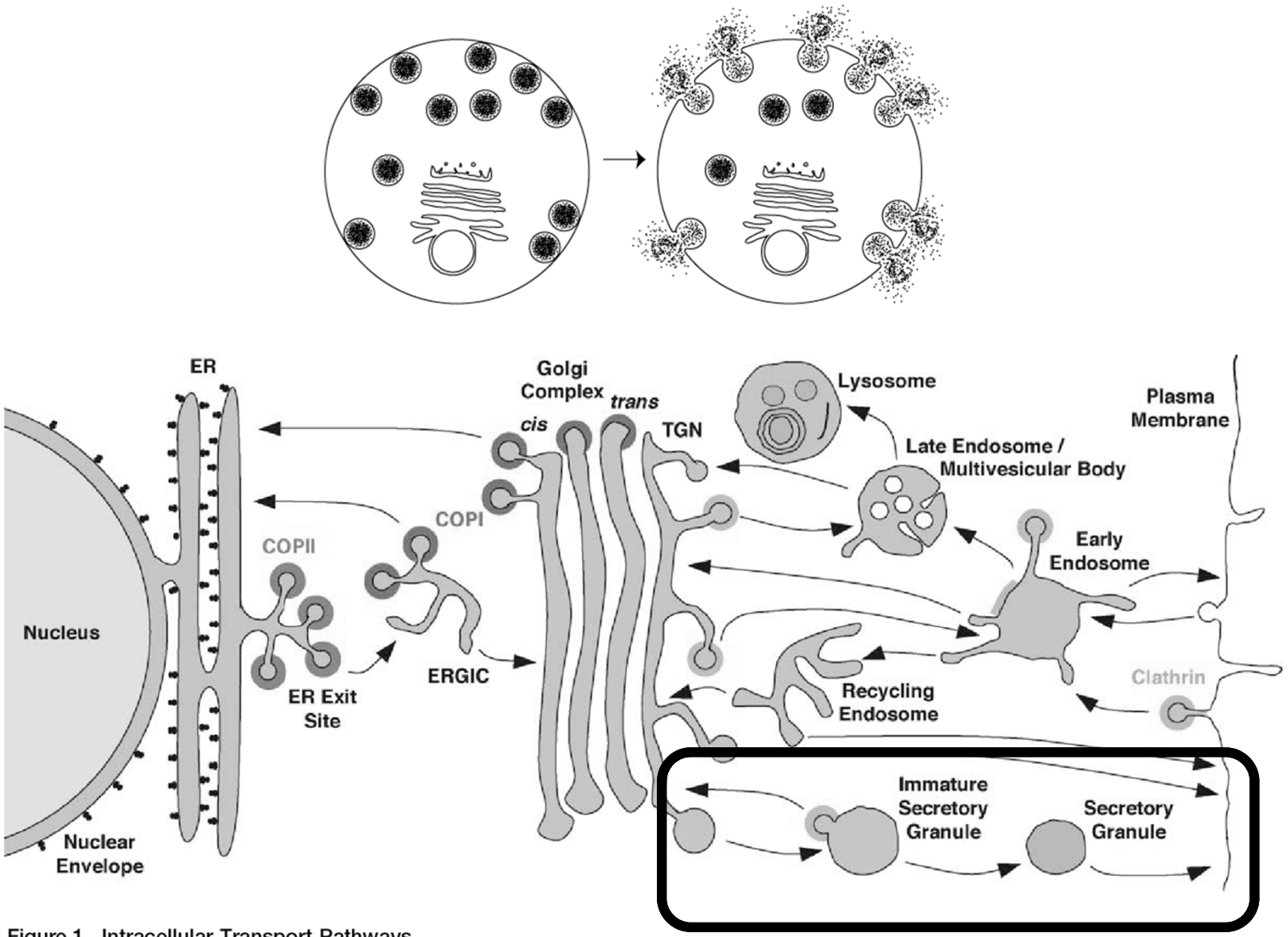
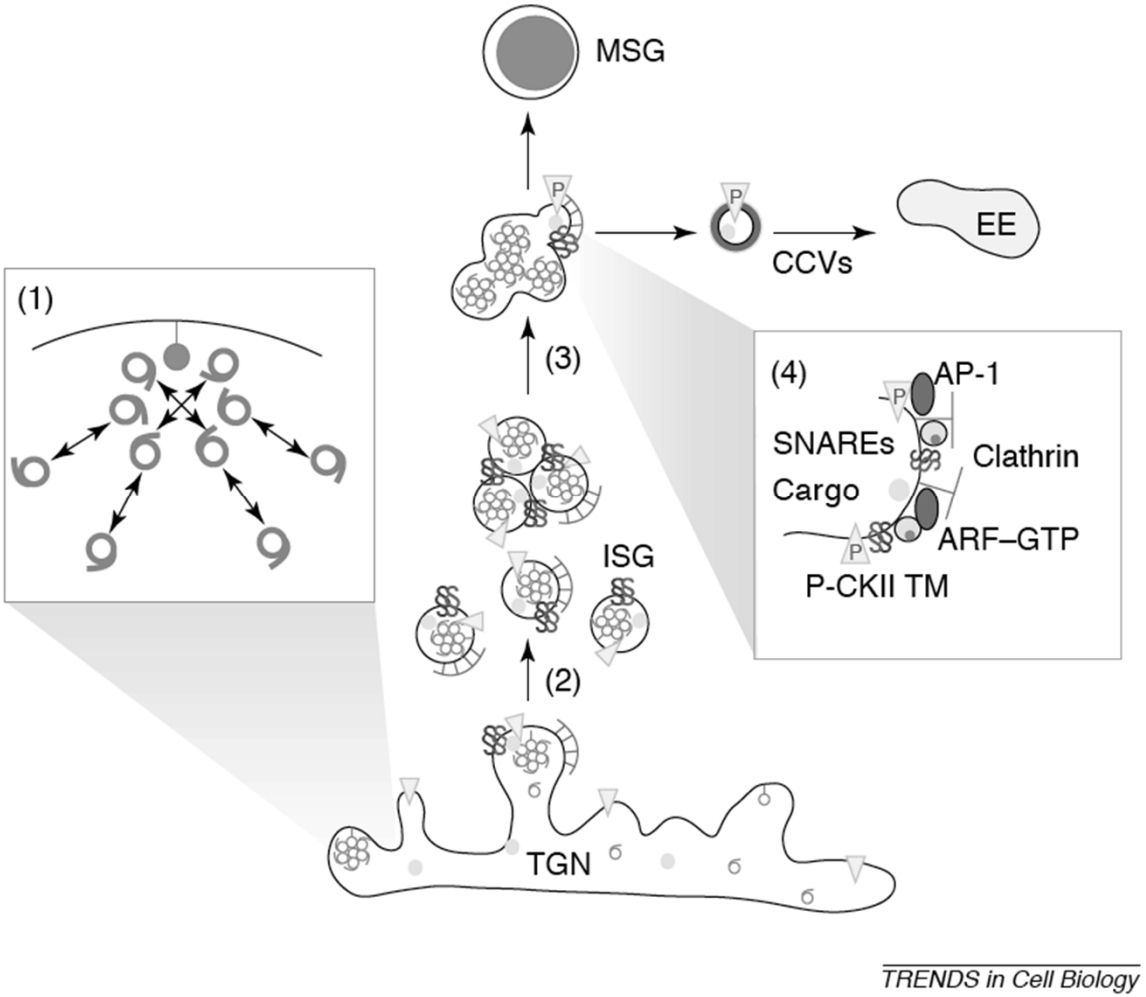
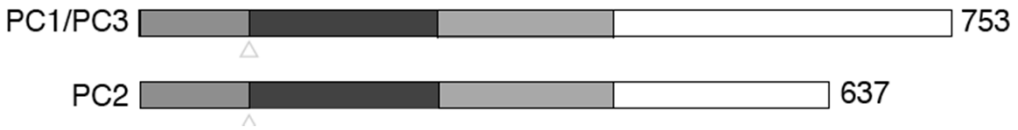


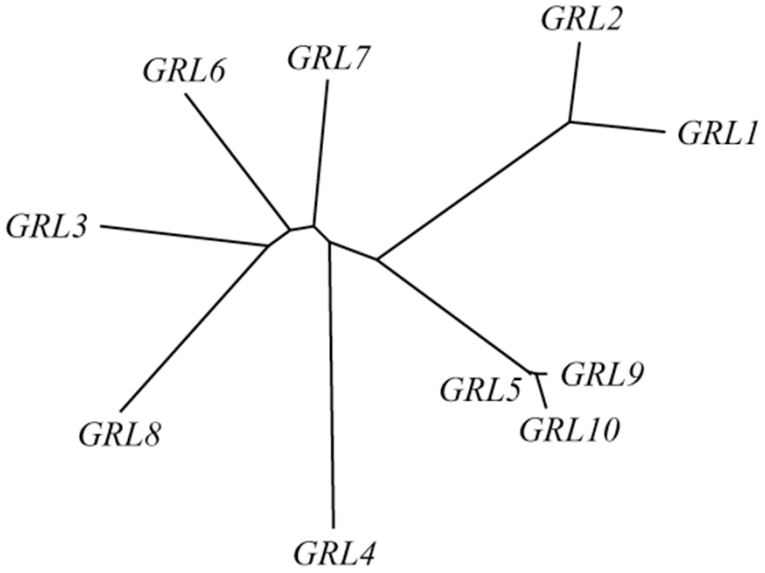
Figure 1. Intracellular Transport Pathways

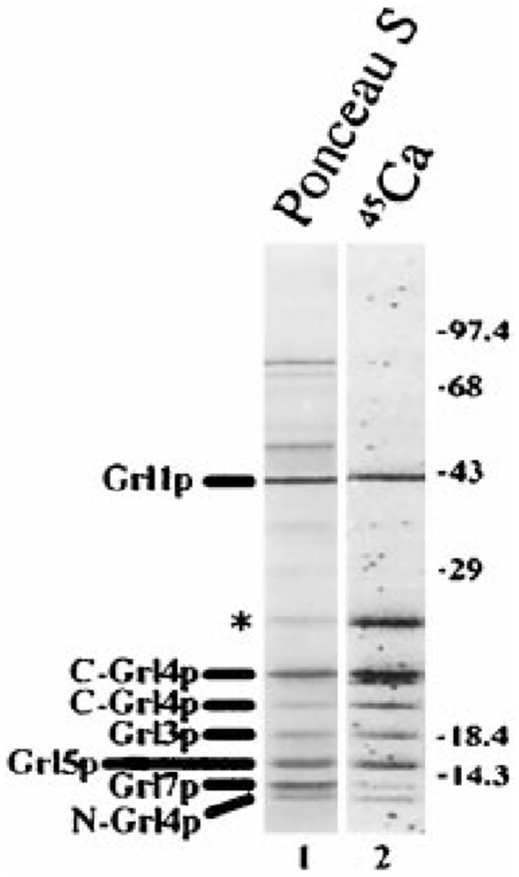




Tooze et al 2001 TICB

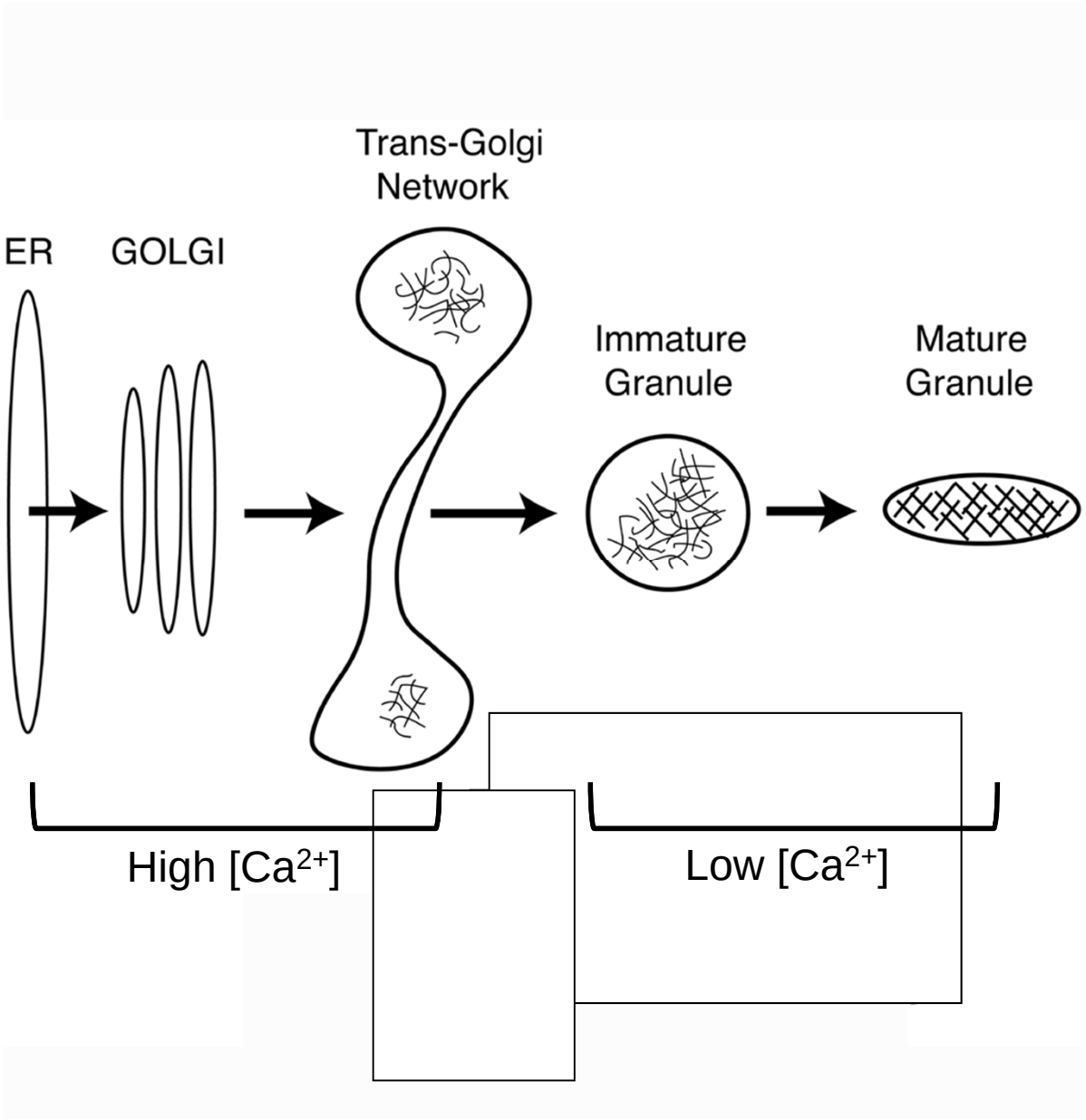
The most abundant mucocyst components, called GRLs, are low affinity calcium-binding proteins.



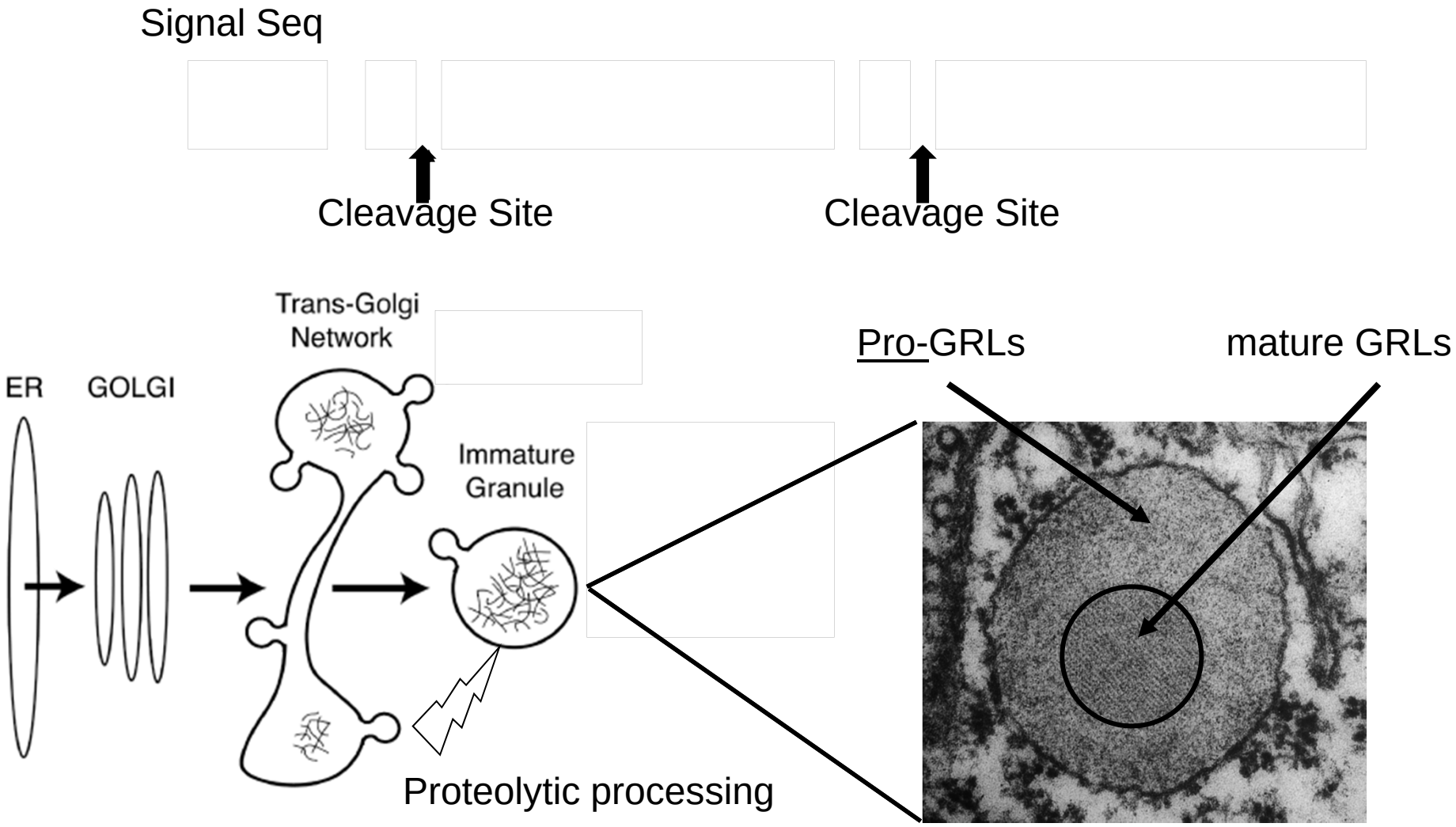


Verbsky and Turkewitz 1998

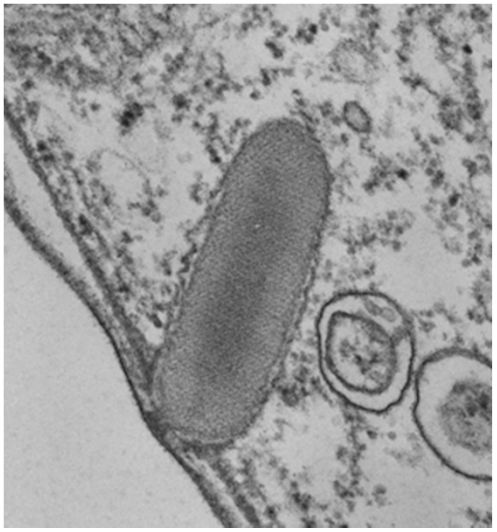
Mature ciliate granules have low lumenal [Ca^{2+}]

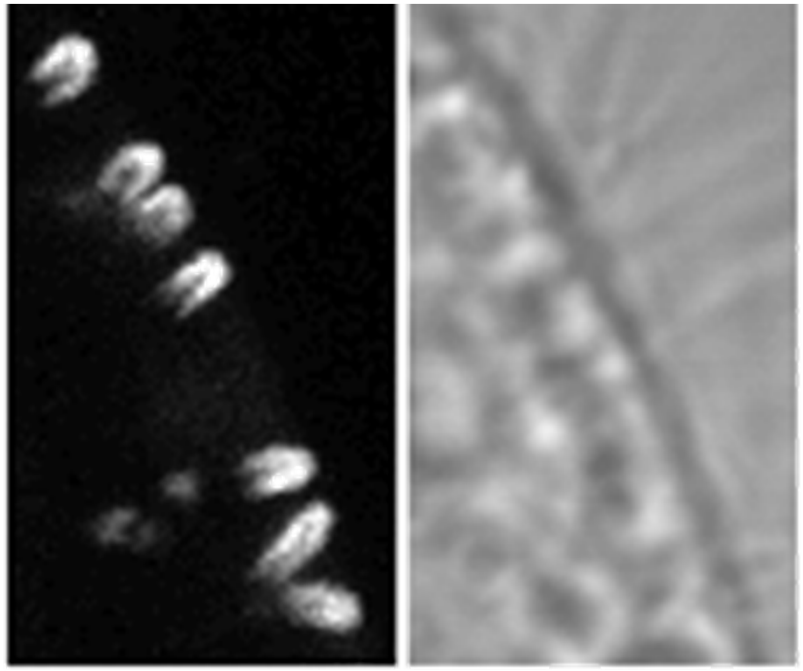


GRL proteins are synthesized as pro-proteins
and proteolytically processed in a post-Golgi compartment.



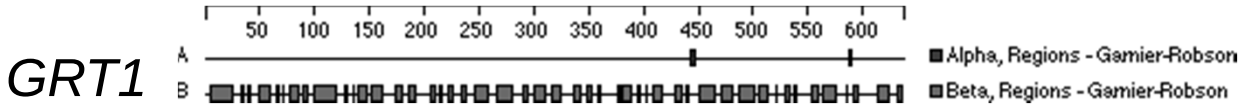
Two gene families encode *Tetrahymena* granule contents

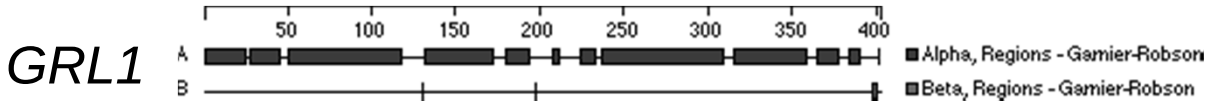




Immunolocalization of Grt1p

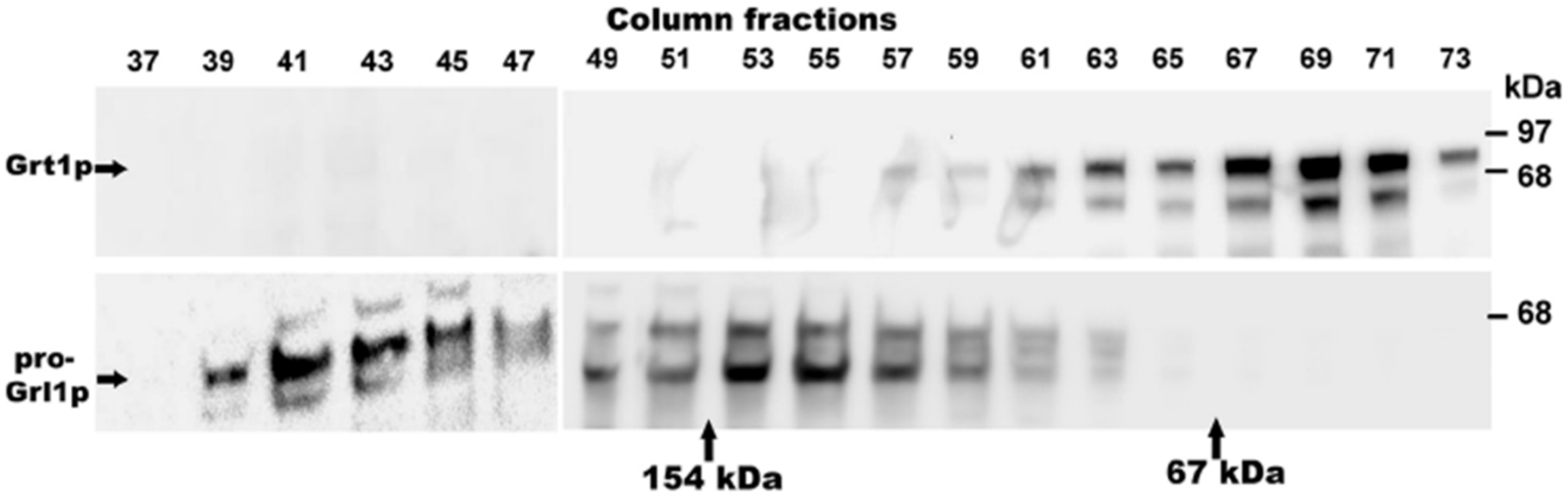
The GRT family: defined by a C-terminal β/γ crystallin domain



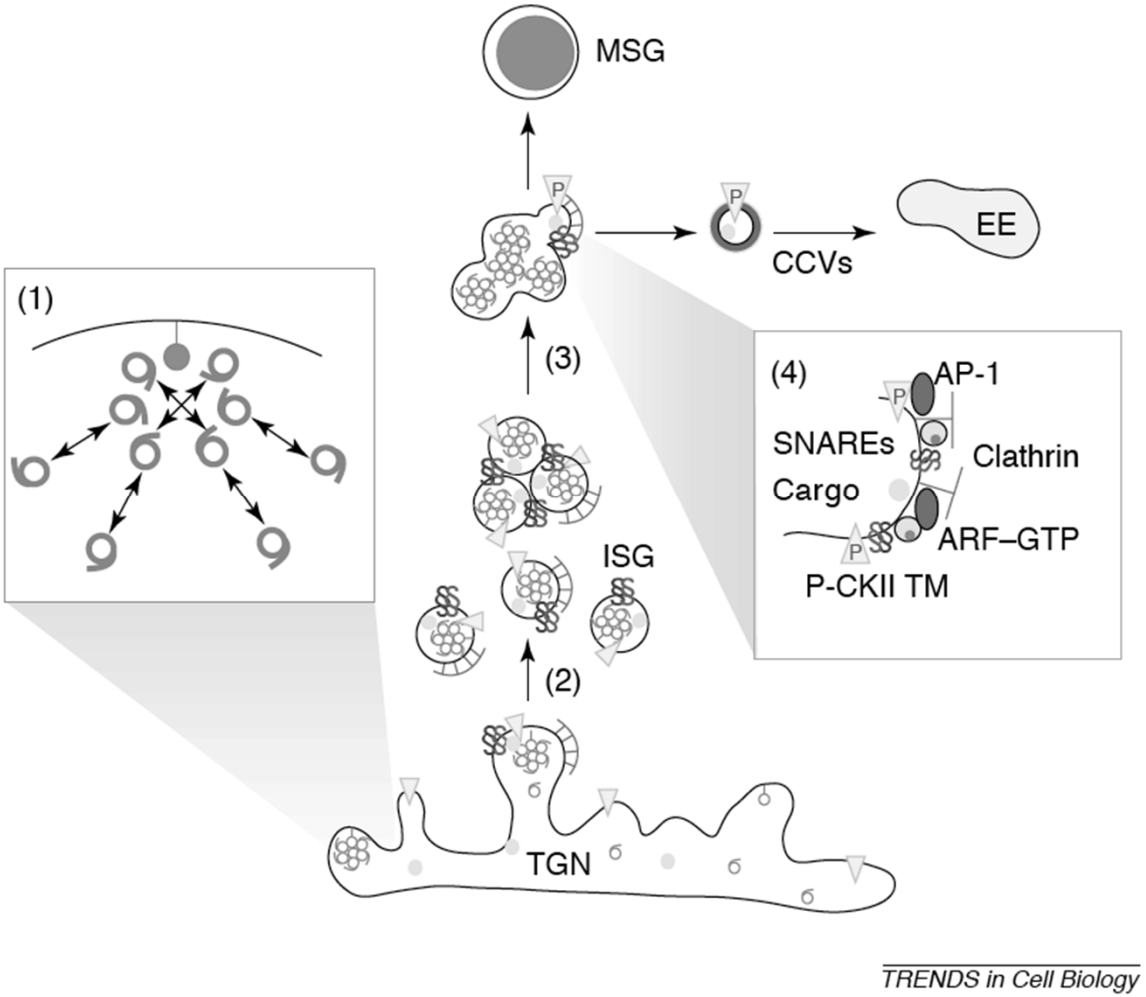


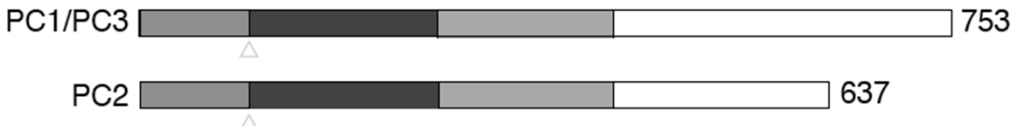
GRT proteins are not processed and do not bind calcium.

Pro-Grl proteins form large aggregates in the secretory pathway, but Grt proteins appear to be monomeric and do not associate with the Grl aggregates



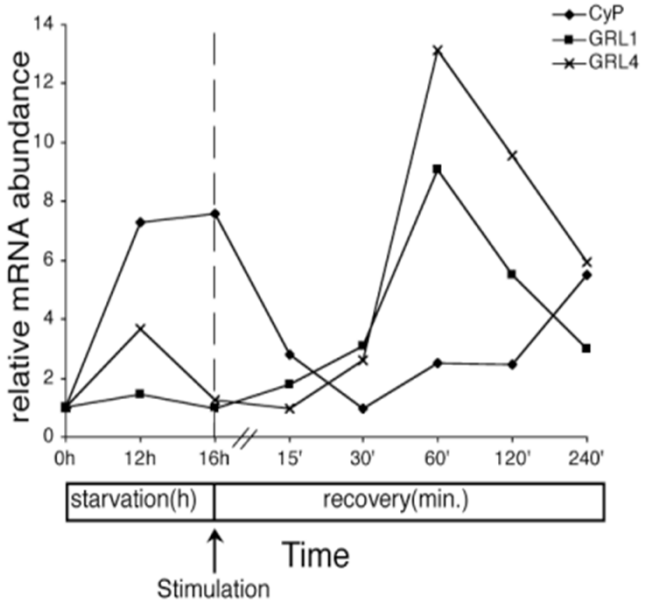
Rahaman et al 2009



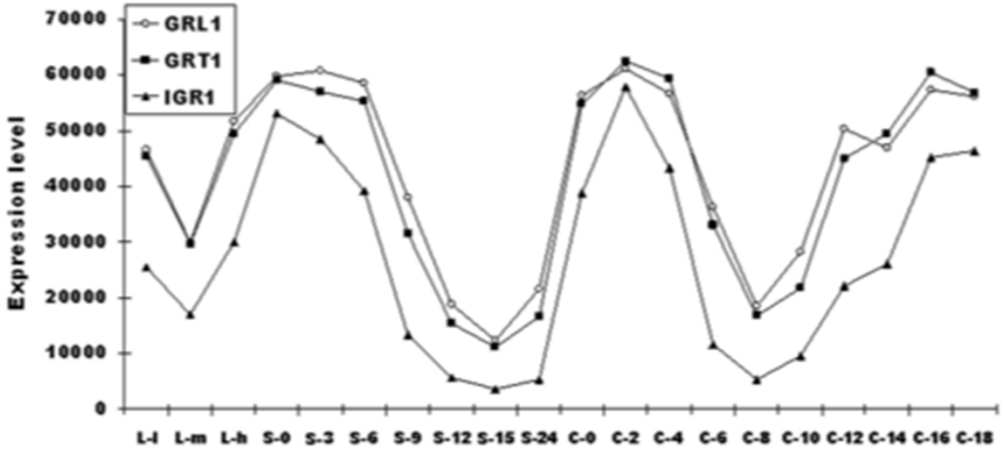


Tooze et al 2001 TICB

All known granule-associated proteins share expression profiles

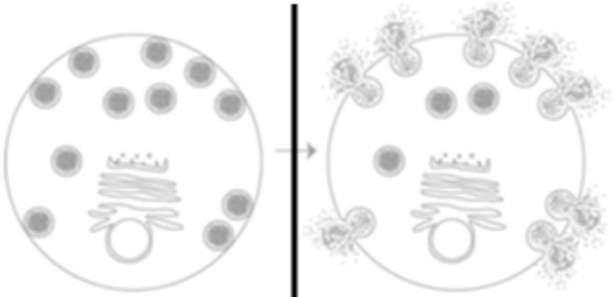


Haddad & Turkewitz 1997



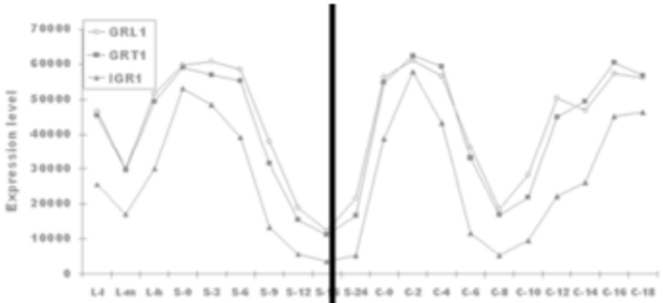
Rahaman et al 2009

Tetrahymena Genome: *~21,100 genes*



Filter 1:
Up-regulated at least 2-fold post global synchronous exocytosis

~1,500



Filter 2:
Co-regulated with DCG proteins

~300

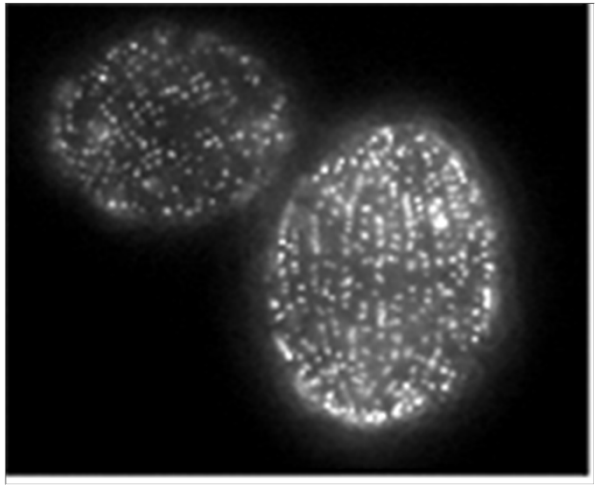
Filter 3:
Conserved across many eukaryotic lineages

14 Candidate Genes

AP-3 adaptin
Ap-3 medium subunit
SEC14
Vps9
Beta-arrestin
GRIP domain protein
V-Type ATPase
Sortilin #1
Sortilin #2
tSNARE
Synaptobrevin
DRP7
Cathepsin
Carboxypeptidase

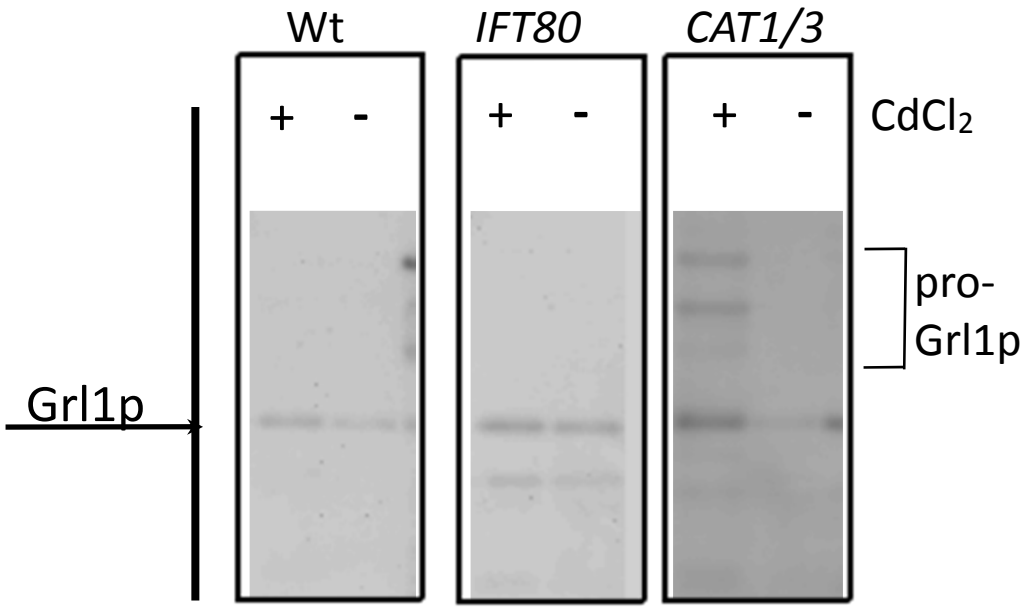
Fold-induction during regranulation	Fold-induction in exo-mutant (neg. control)	Statistical significance	Gene identity
11.1	0.7	0.0008	AP-3 adaptin large subunit
30.0	1.2	0.001	AP-3 medium subunit
6.2	0.4	0.0003	SEC14
11.2	1.1	0.0007	Vps9
7.3	0.6	0.0006	beta-arrestin-related
16.1	0.7	0.0006	GRIP domain protein
7.2	0.4	0.004	V-type ATPase
7.8	1.0	0.0007	Vps10/sortilin (#1)
7.5	1.2	0.0005	Vps10/sortilin (#2)
16.7	1.0	0.002	tSNARE
12.5	1.1	0.001	synaptobrevin
4.1	1.0	0.002	Dynamin-related protein (DRP7)
4.5	0.4	0.0006	cathepsin
5.8	0.7	0.0004	carboxypeptidase

Co-regulation identifies proteases that are targeted to mucocysts



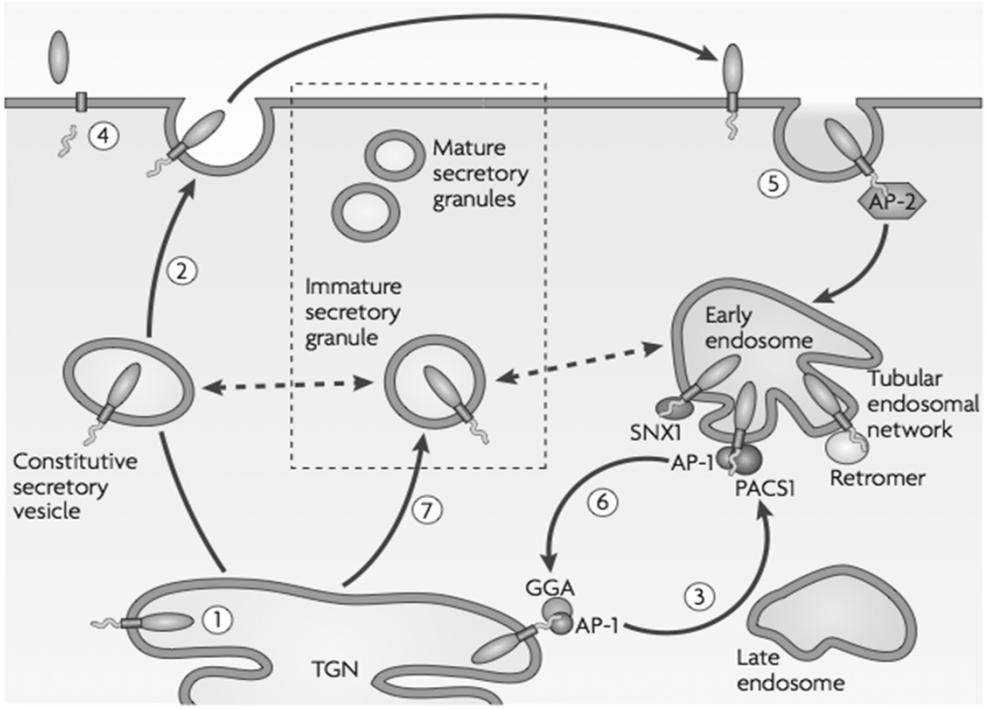
P. Romei

Simultaneous RNAi knockdown of 2 cathepsins results in unregulated secretion of unprocessed precursors



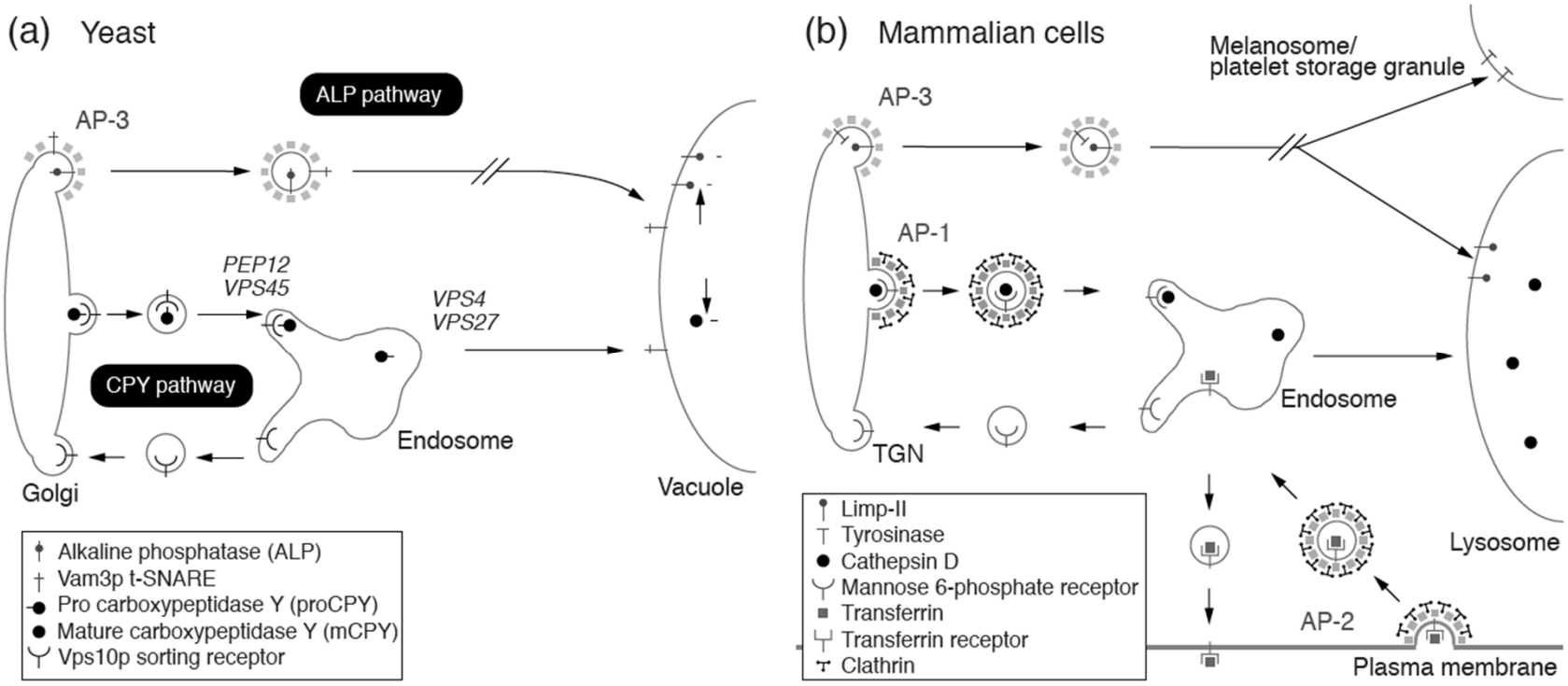
Western blot with anti-Grl1p serum, of cell culture supernatants

Putative roles of sortilins/Vps10 in dense core granule formation

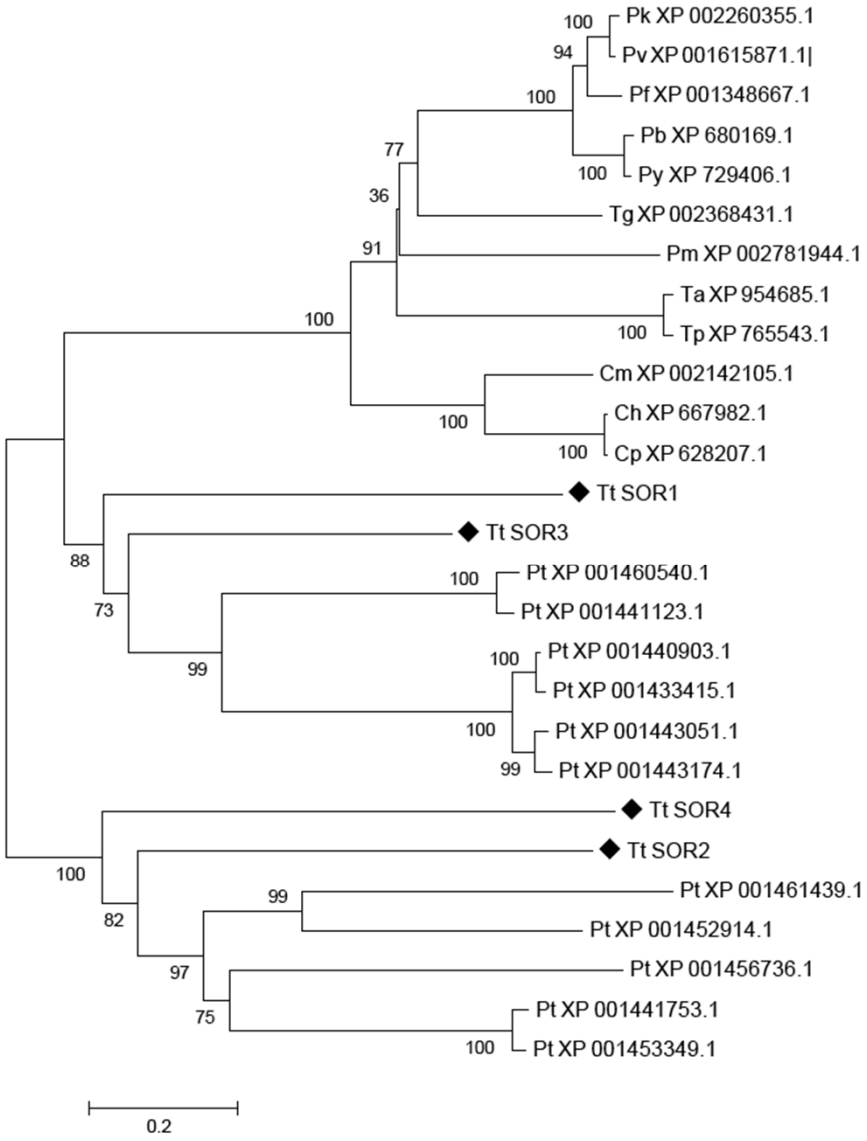


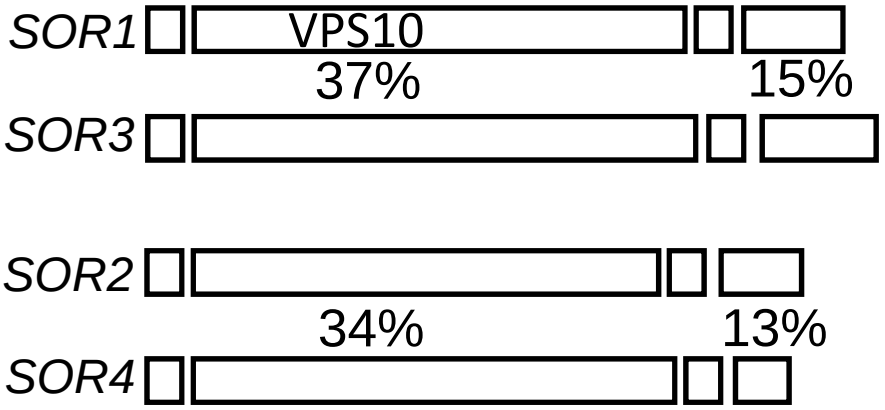
Willnow et al. 2008

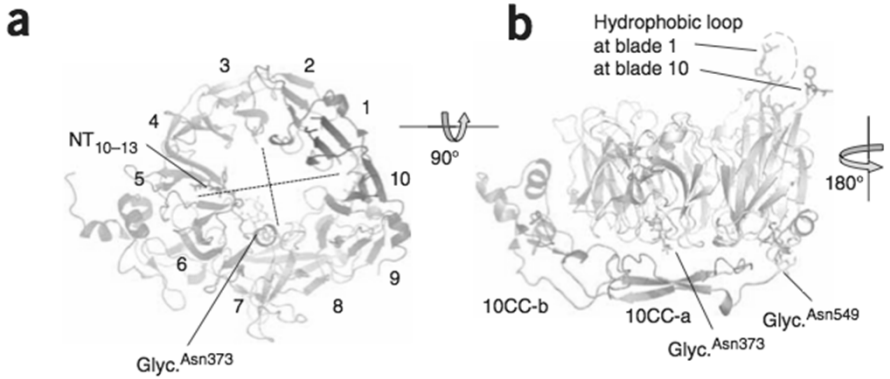
Role of sortilins/Vps10 in lysosome-related organelle (LRO) formation



Tetrahymena encodes four sortilin/Vps10 genes

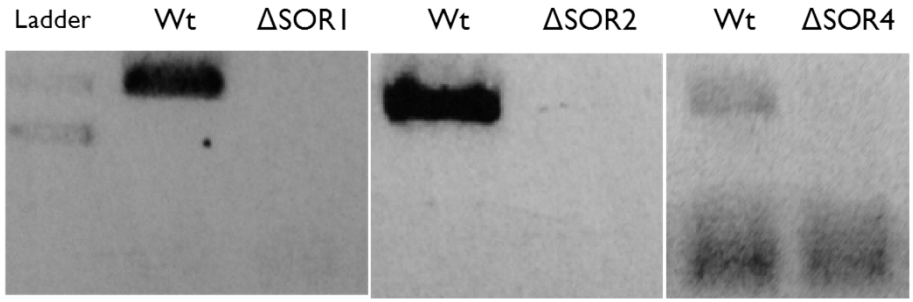




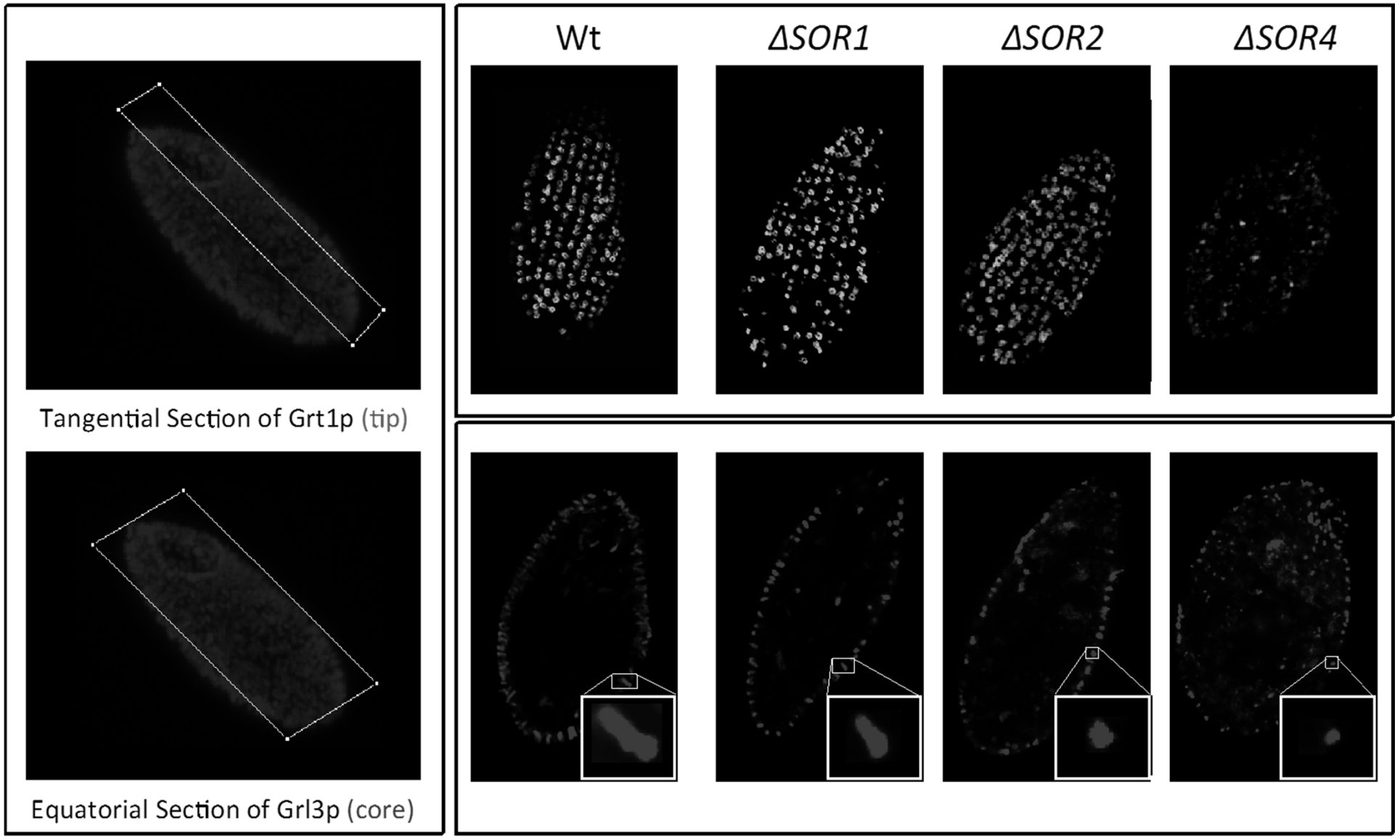


Quistgaard et al. 2009

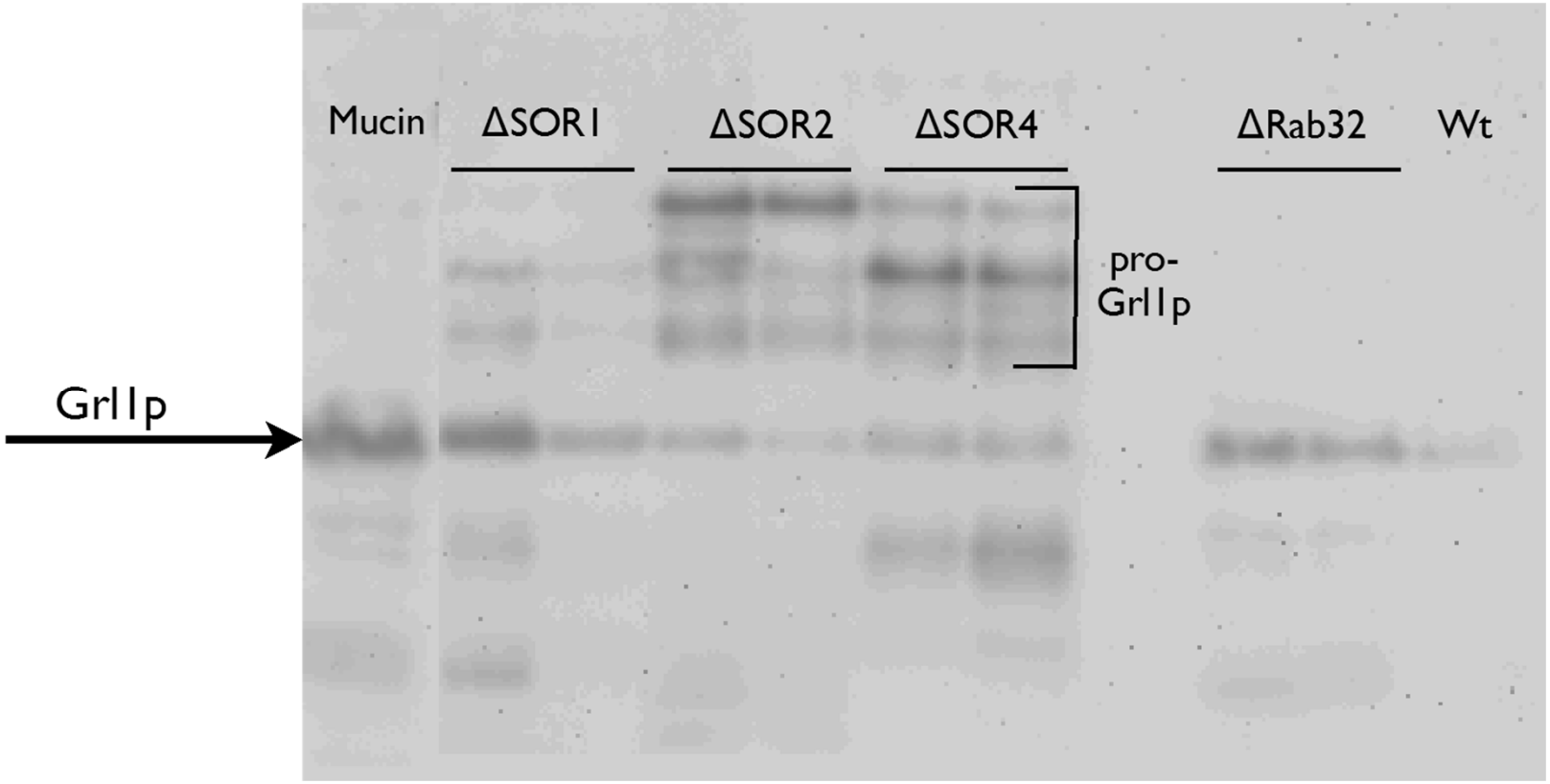
Gene knockout of *SOR1*, *SOR2* and *SOR4*



Sortilin knockout lines make aberrant granules



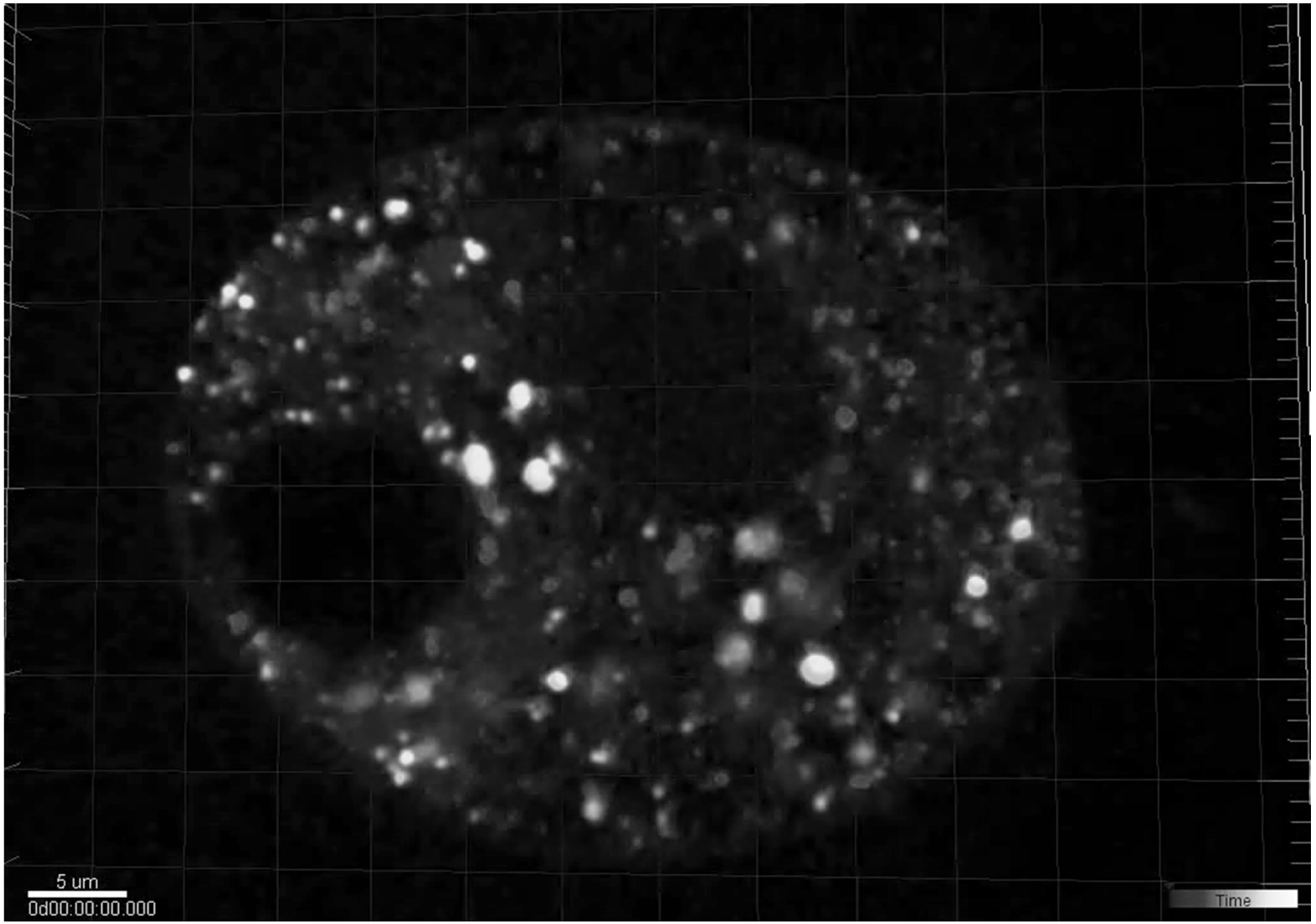
Sortilin knockout lines release proGrl intermediates in an unregulated fashion



Western blot with anti-Grl1p serum, of cell culture supernatants

Hypothesis: sortilins may be required to deliver the processing enzymes during secretory granule maturation

Sor2p-GFP (green) and LysoTracker (red) in an immobilized *Tetrahymena* cell



Enriched summary of part 2

- Mucocysts and mucocyst biogenesis have many morphological and functional similarities with dense core granules and granulogenesis in animals, but ciliates lack Rabs in the conserved regulated exocytosis subgroup. Similarly, the mucocyst-associated SNAREs are lineage-restricted (not discussed, from Paramecium)

- Biochemical and genetic approaches in *Tetrahymena* (and *Paramecium*) identified key components of the mucocysts in these organisms, which generally had no identifiable homologs in animals. This includes proteins required for mucocyst docking (not discussed, from *Paramecium*).

- Expression profiling revealed likely candidates for the mucocyst processing enzymes, and these are not closely related to the functionally-homologous enzymes in animal cell granules.

- Preliminary analysis suggests that Vps10/sortilin receptors play a key role in mucocyst biogenesis, perhaps in an AP-3-associated pathway. These results suggest that regulated exocytosis in ciliates arose from a lysosome-related organelle origin.

An imagined evolution of a regulated secretory vesicle in *Tetrahymena*

	conserved	**lineage-restricted**
AP-3/sortilin-dependent traffic from the transGolgi to a post-Golgi, vacuolar ATPase-positive compartment	yes	
Sortilin-independent (aggregative) sorting (GRLs)	Yes (mech)	Yes (novel proteins)
GRT proteins to form a tip structure	Yes (mech)	Yes (novel proteins)
Novel Rab and SNAREs, or relaxed constraints		Yes (?)
Processing enzymes		Yes
Docking/fusion proteins (ND proteins)		Yes
Ca2+ ⇩ H+ ⇩ Na+ ⇧		

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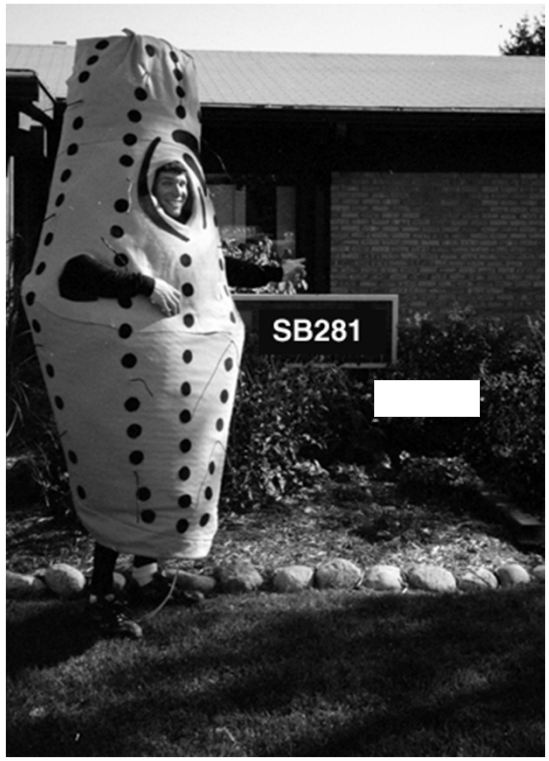
Sharon Melia

Alejandro Nusblat



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