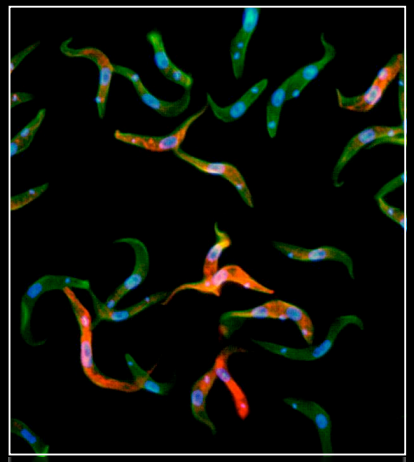
Evolution of eukaryotic trafficking systems



Mark Field

KITP Evo Cell March 1, 2010

UNIVERSITY OF CAMBRIDGE

Deep time and origins of the endomembrane system

The Golgi complex - Sculpting I

Rab proteins and interaction networks - Sculpting II

Evolution of the nucleocytoplasmic transport system

Protocoatomer; putting it all together

Deep time and origins of the endomembrane system

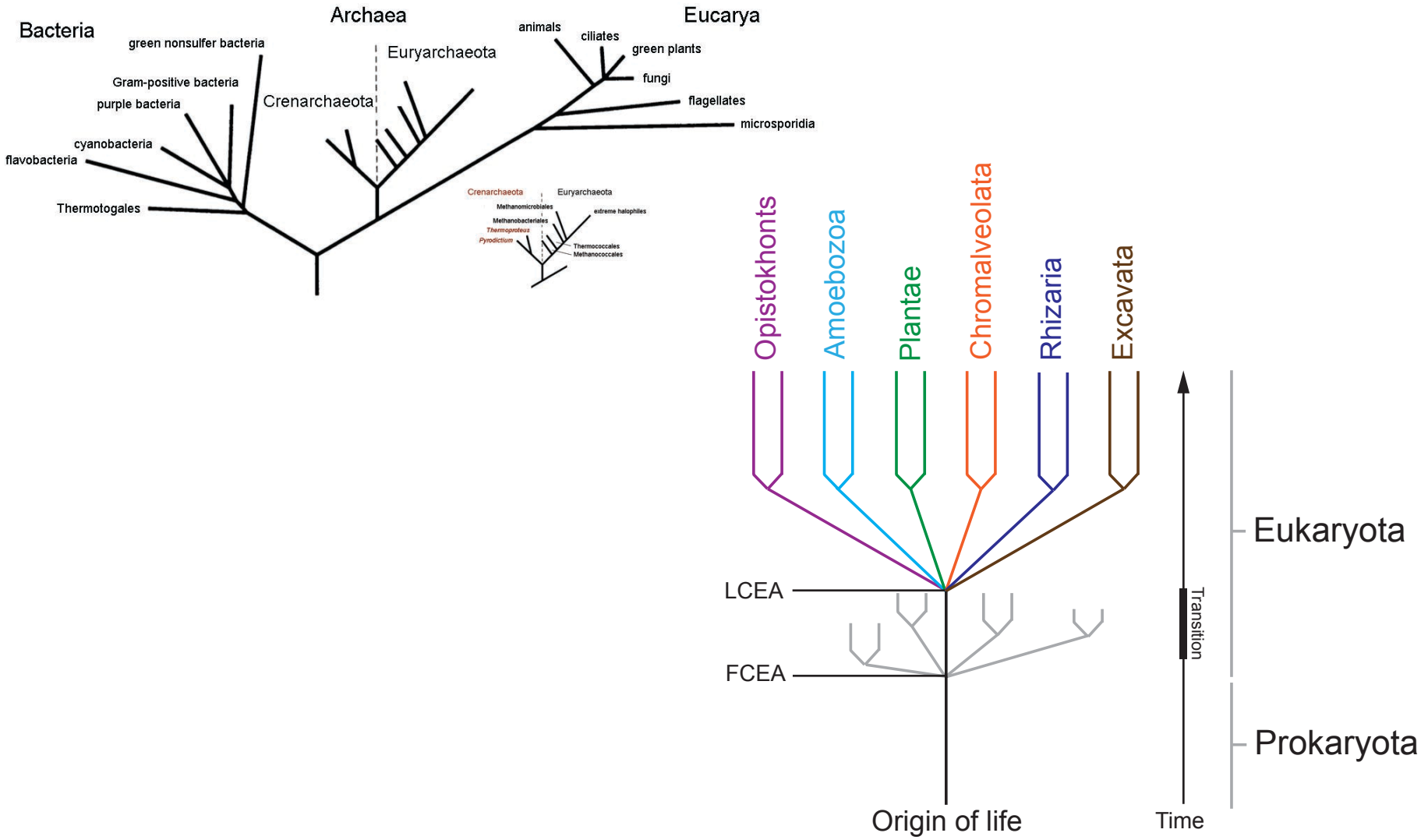
The Golgi complex - Sculpting I

Rab proteins and interaction networks - Sculpting II

Evolution of the nucleocytoplasmic transport system

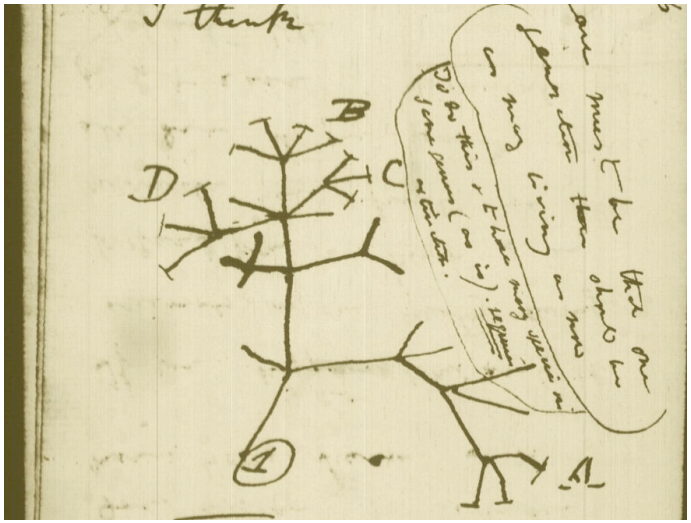
Protocoatomer; putting it all together

The major domains of life

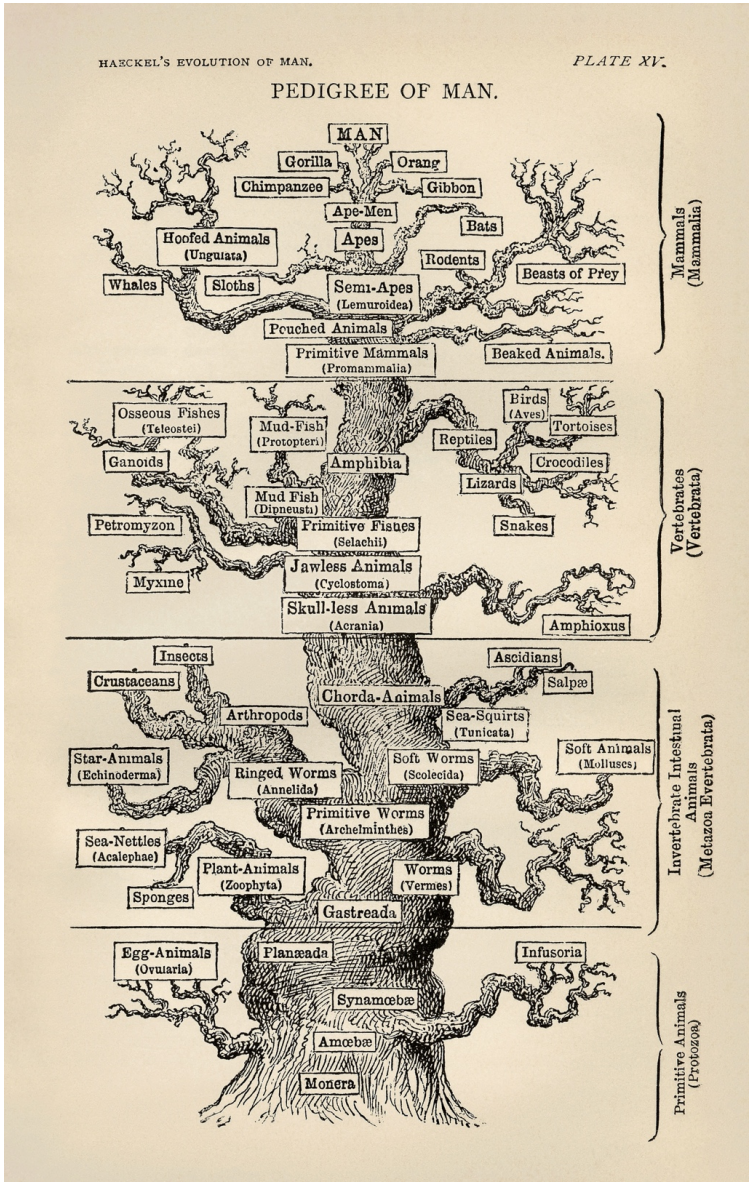


Adapted from Woese *et al.,* 1990, Adl *et al.,* 2005, Field and Dacks 2009

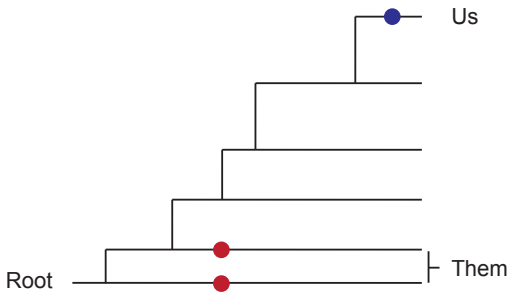
Evolution of supremacy (modern and primitive)



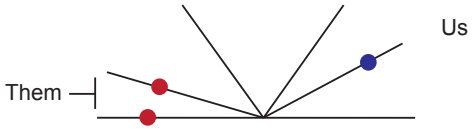
Notebook (1837)
(no timebase)



Darwin 1837, Haeckel 1879



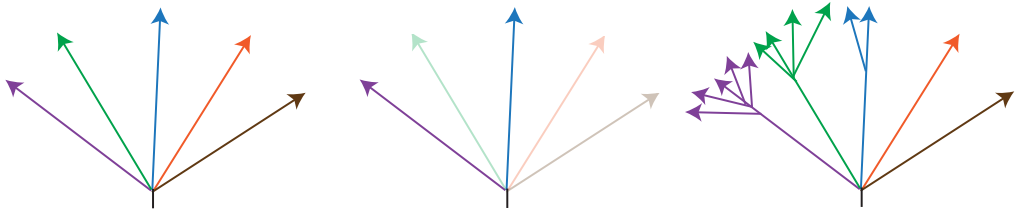
Elitist



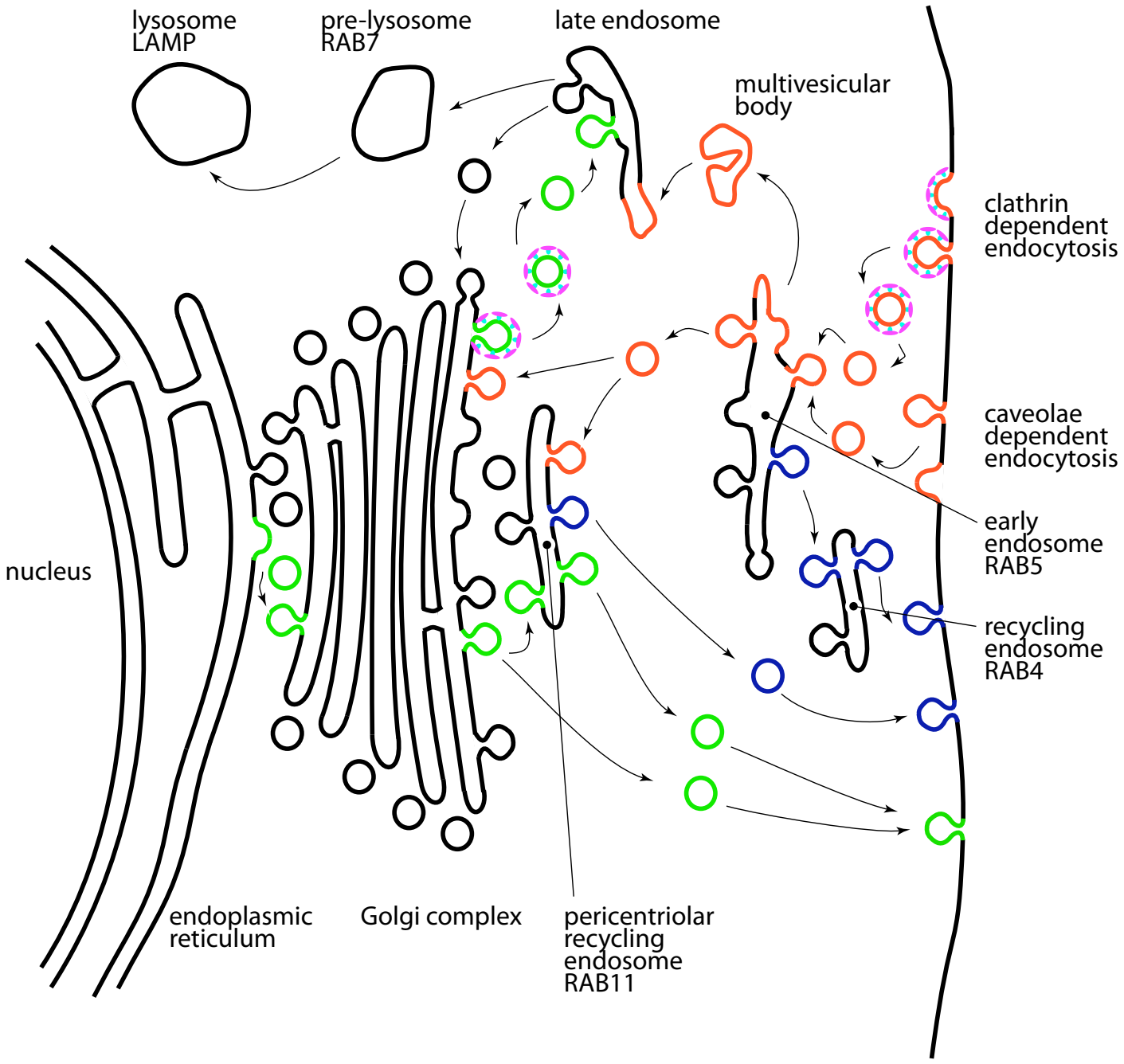
Egalitarian

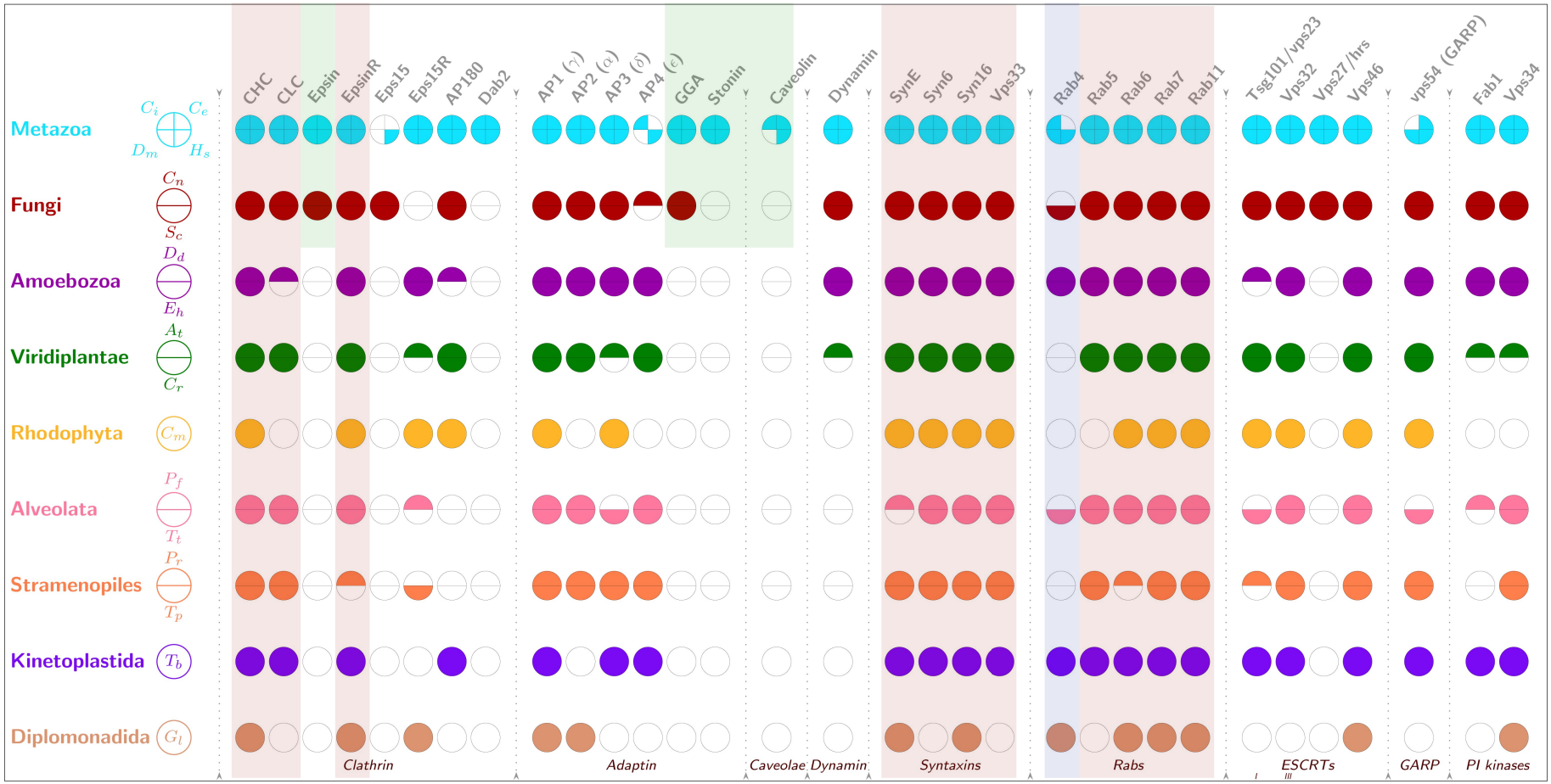
Observations:	● Gene unique to this lineage ● Gene absent from this lineage, present in all others
Inferences:	● Recent adaptation ● Absence equals primitive state prior to evolution of trait Phylogeny has a predicted root ● Lineage-specific adaptation ● Secondary loss Phylogeny is unrooted

Supergroup:
Opisthokonta
Amoebozoa
Plantae
Chromalveolata
Excavata



Evolutionary pattern:	Universal: ancient	Secondary losses: ancient	Expansive: new functions
Rab protein examples:	6, 7, 8, 21, 28	4	1, 5, 11

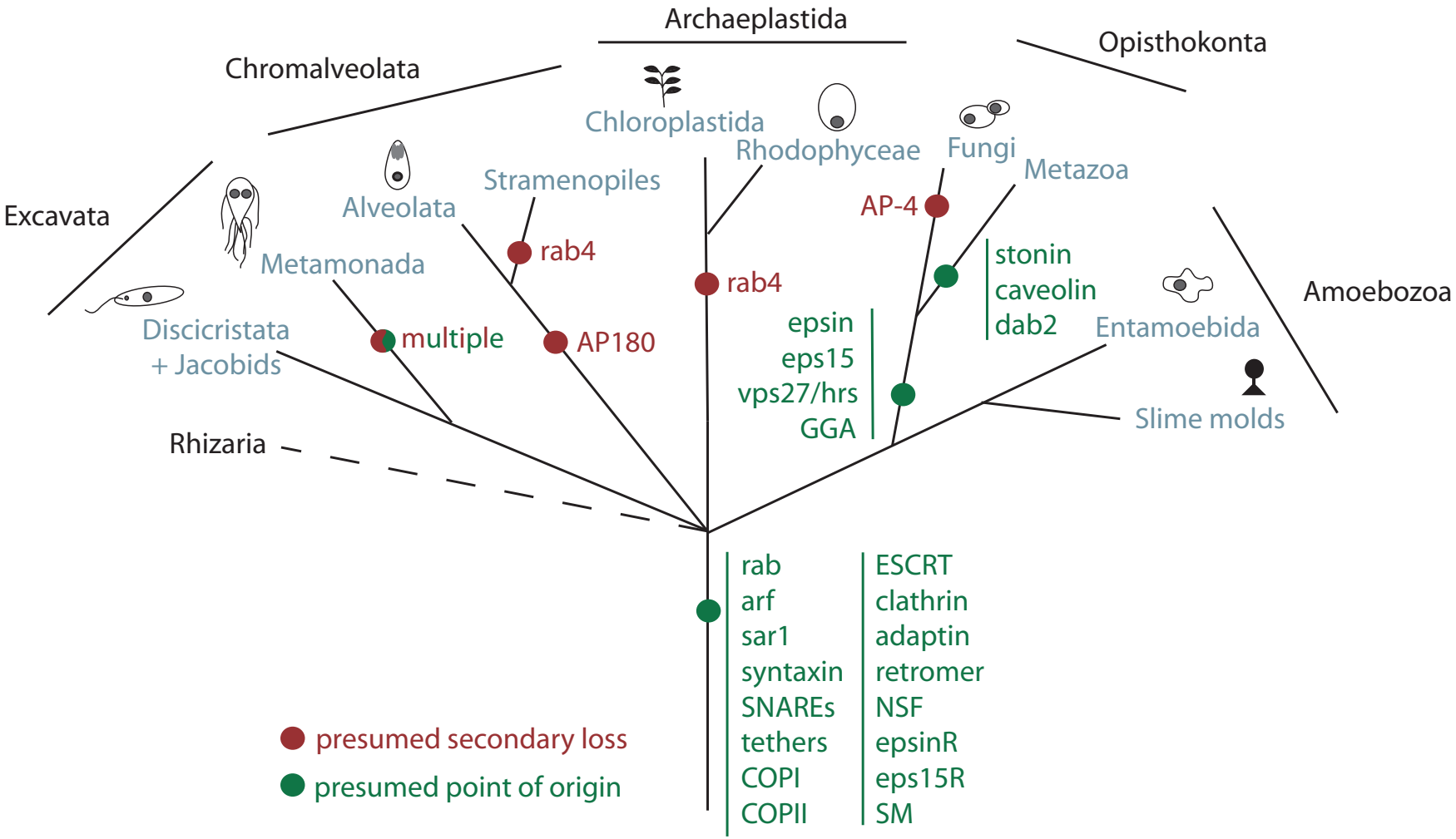

lysosome
LAMP
pre-lysosome
RAB7
late endosome
multivesicular
body
clathrin
dependent
endocytosis
caveolae
dependent
endocytosis
early
endosome
RAB5
recycling
endosome
RAB4
nucleus
endoplasmic
reticulum
Golgi complex
pericentriolar
recycling
endosome
RAB11

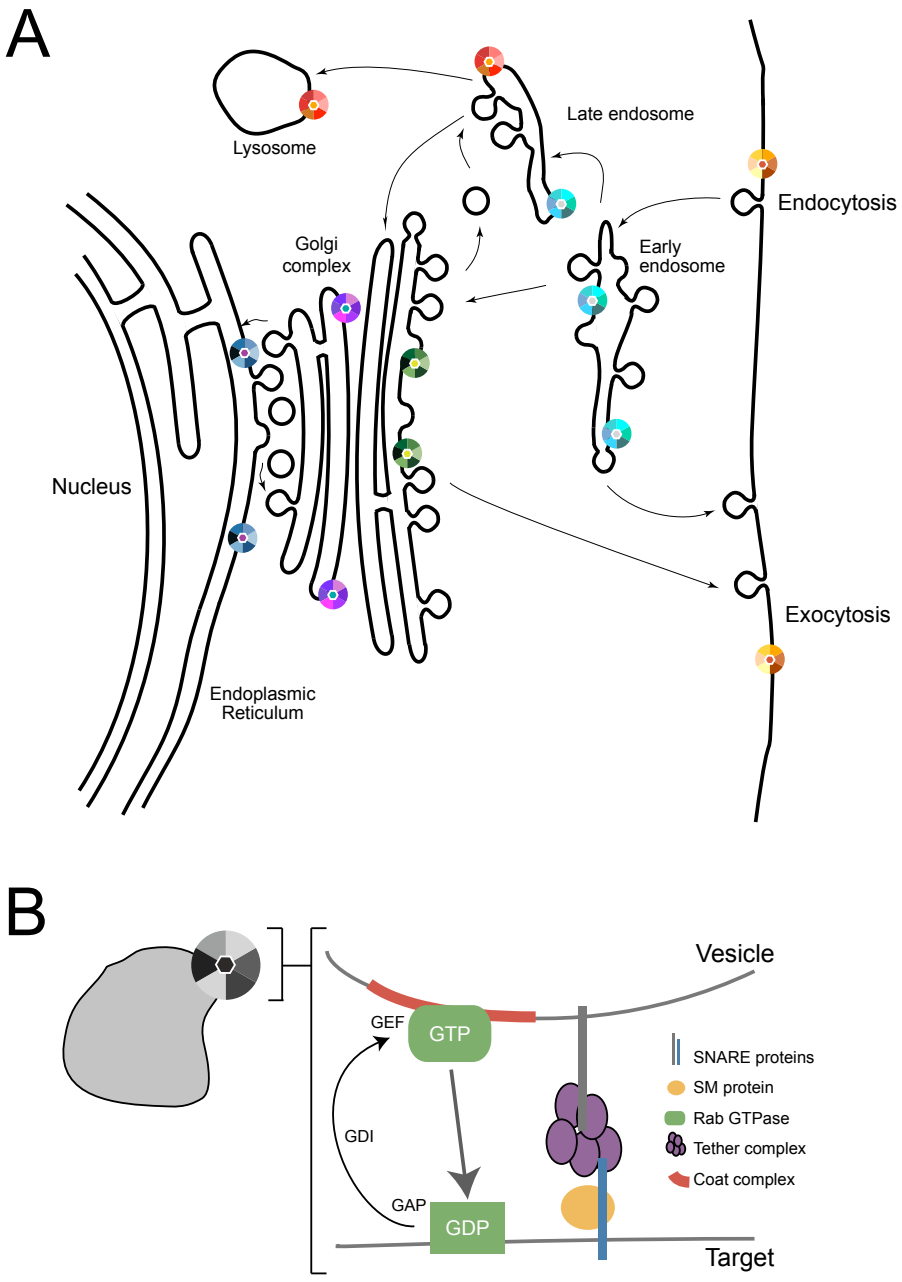


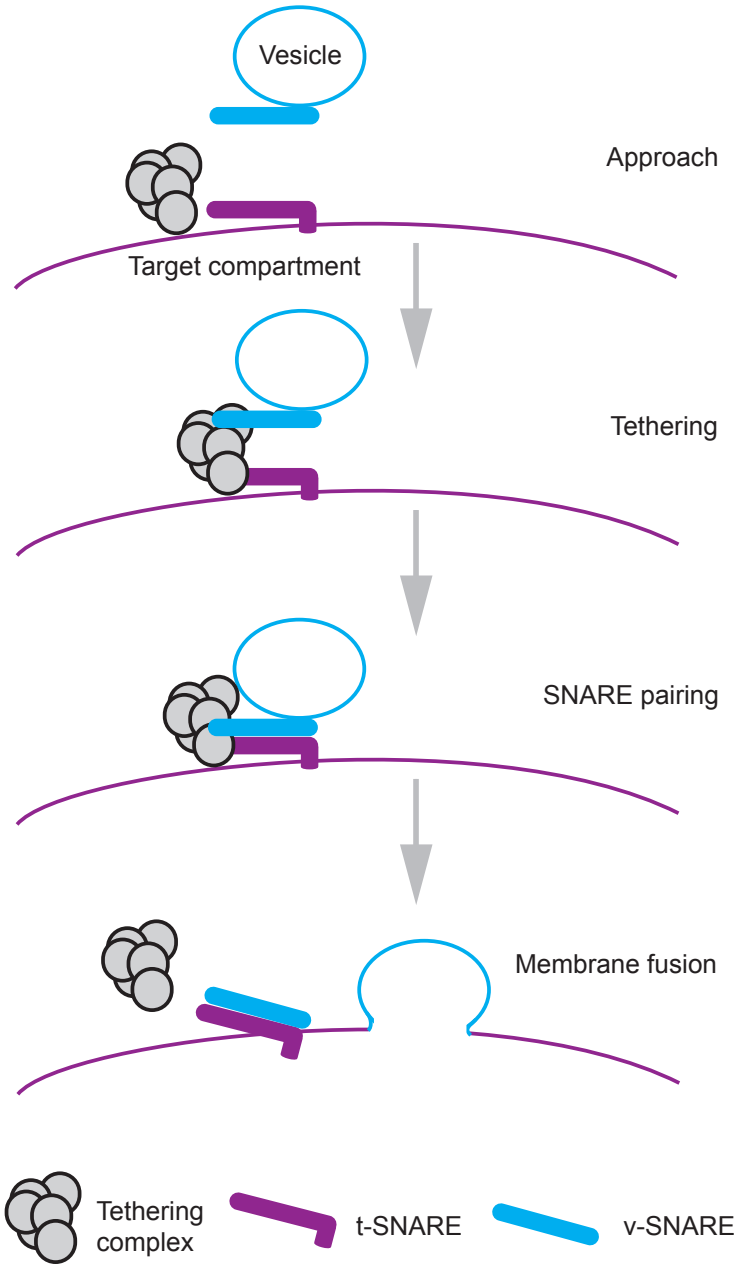
Universal - ancient

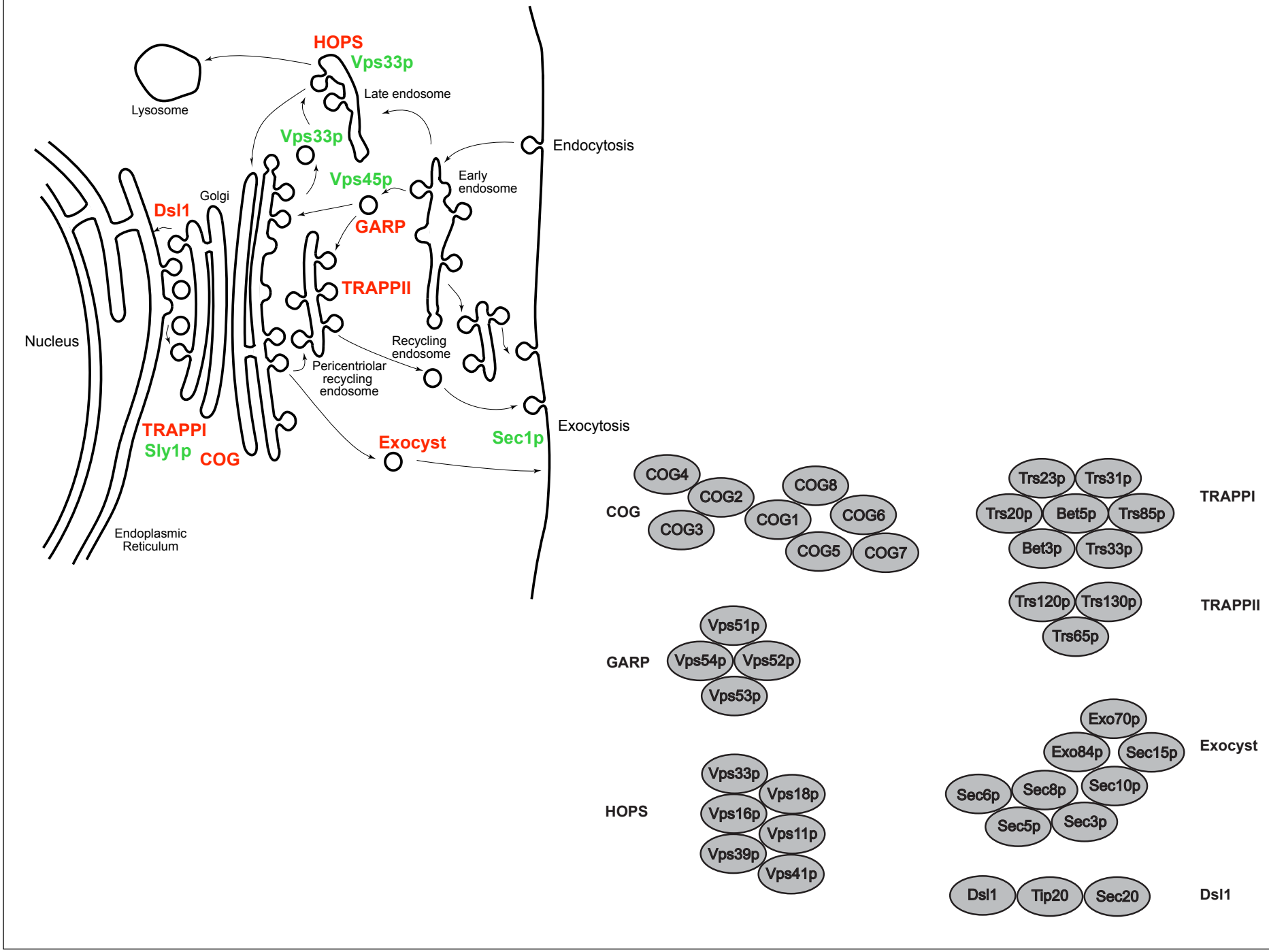
Supergroup restricted - lineage specific innovation

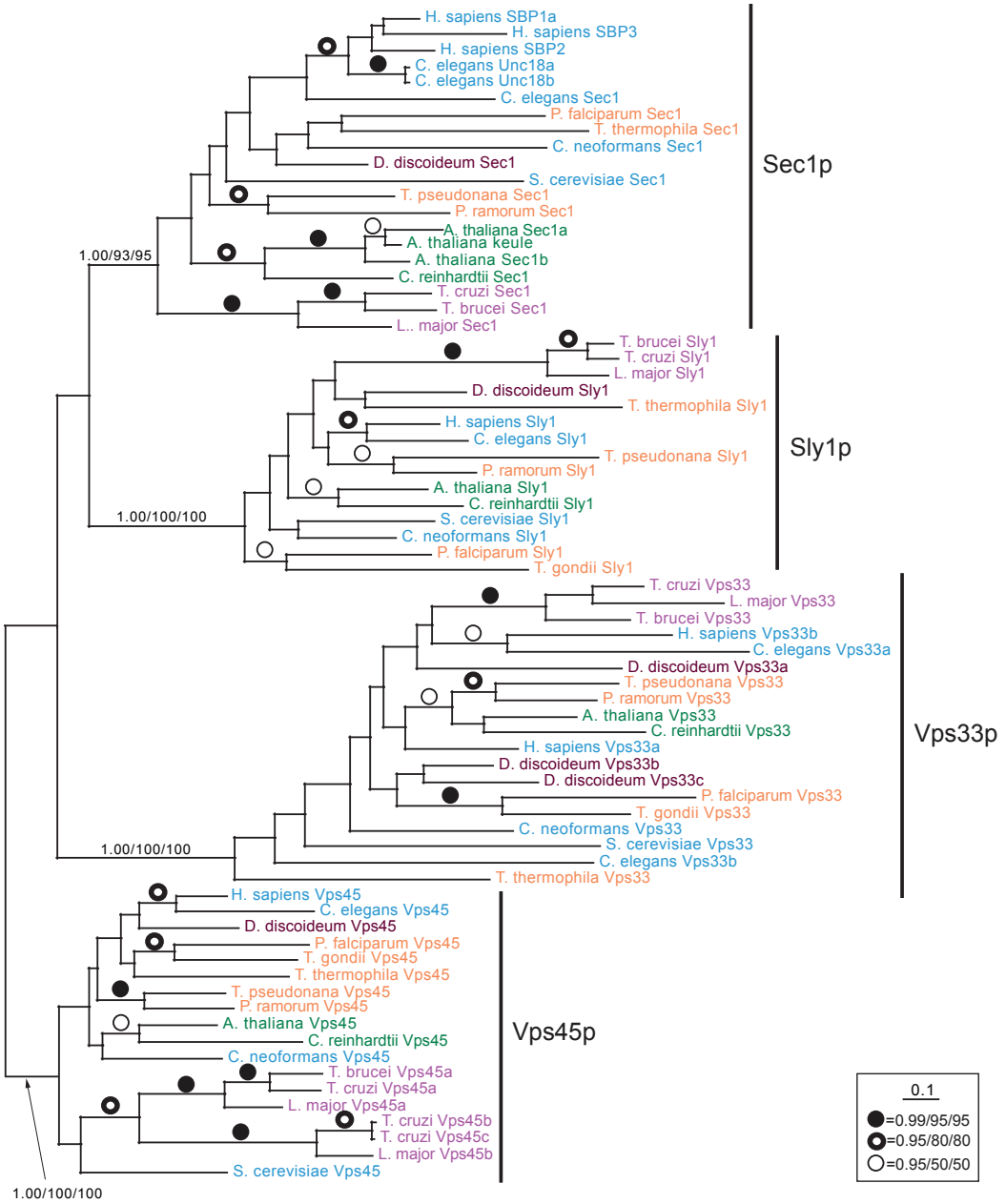
Complex - possible scultping *via* secondary losses

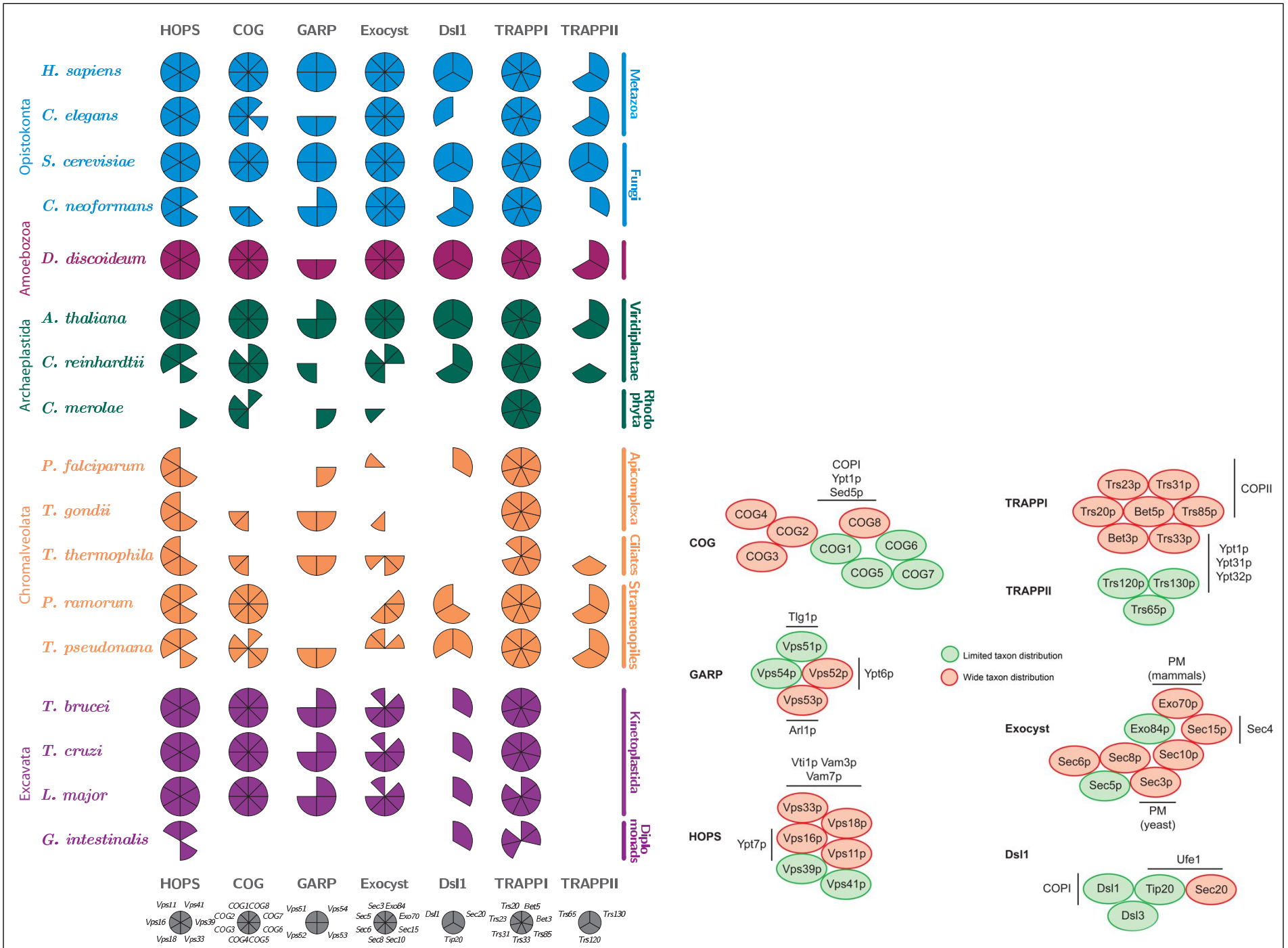

Archaeplastida
Chromalveolata
Opisthokonta
Chloroplastida
Rhodophyceae
Fungi
Metazoa
Stramenopiles
Alveolata
Excavata
AP-4
rab4
stonin
caveolin
dab2
Metamonada
rab4
Amoebozoa
Entamoebida
epsin
eps15
vps27/hrs
GGA
Discicristata
+ Jacobids
multiple
AP180
Slime molds
Rhizaria
rab
arf
sar1
syntaxin
SNAREs
tethers
COPI
COPII
ESCRT
clathrin
adaptin
retromer
NSF
epsinR
eps15R
SM
presumed secondary loss
presumed point of origin

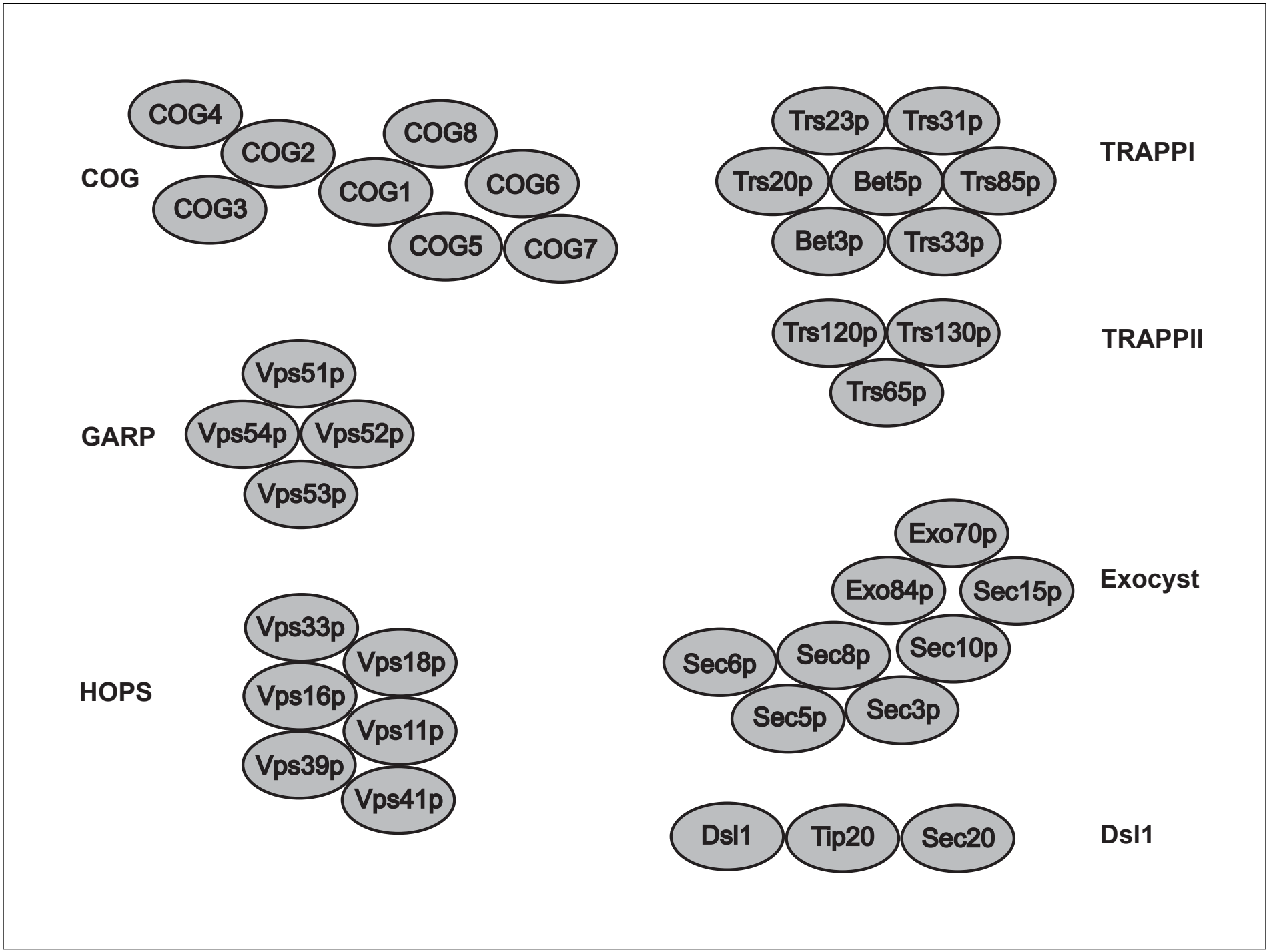

A
Lysosome
Late endosome
Endocytosis
Golgi
complex
Early
endosome
Nucleus
Exocytosis
Endoplasmic
Reticulum
B
Vesicle
GEF
GTP
SNARE proteins
SM protein
Rab GTPase
Tether complex
Coat complex
GDI
GAP
GDP
Target


Vesicle
Approach
Target compartment
Tethering
SNARE pairing
Membrane fusion
Tethering complex
t-SNARE
v-SNARE

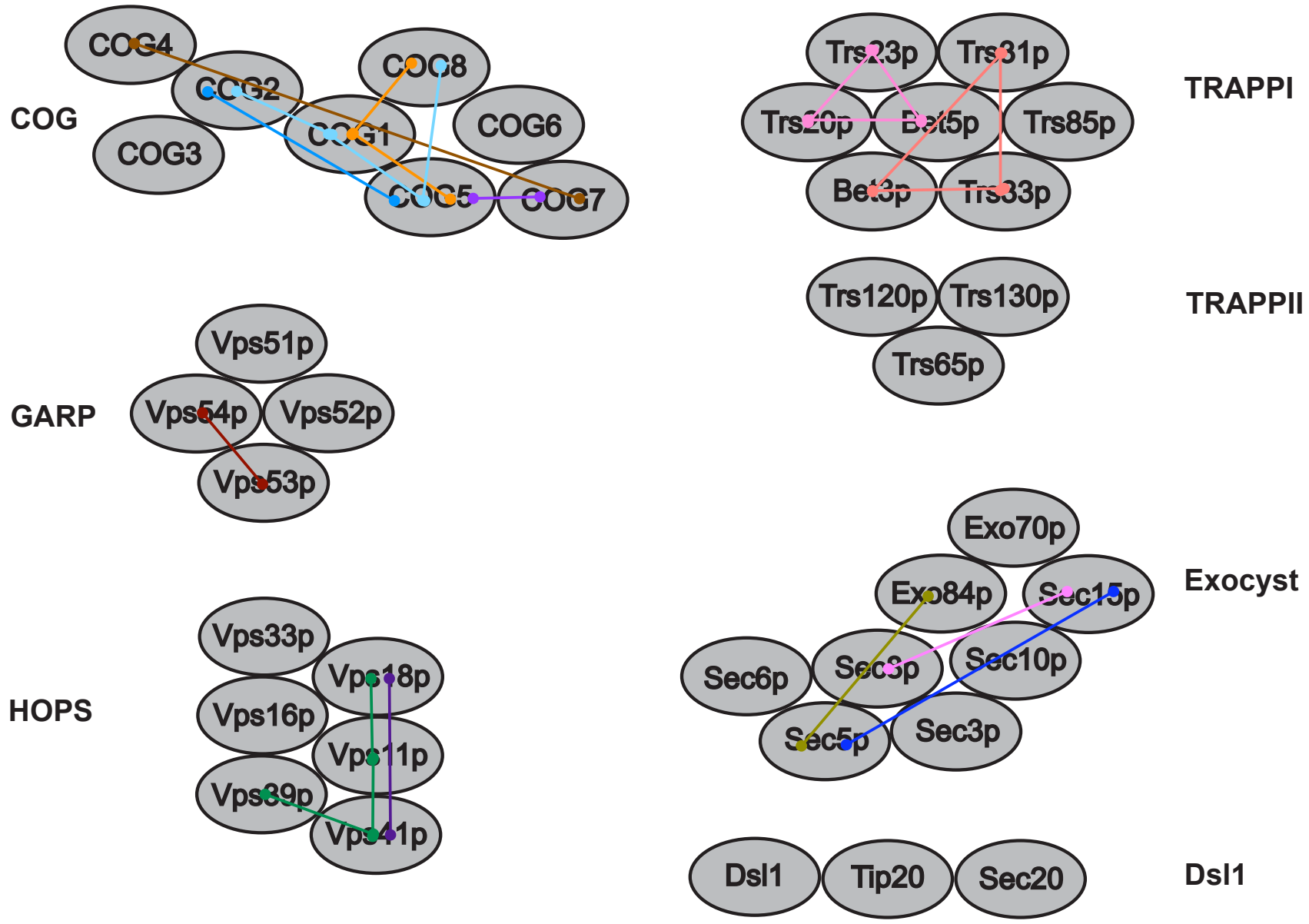

HOPS
Vps33p
Late endosome
Lysosome
Vps33p
Endocytosis
Vps45p
Early
endosome
Golgi
Dsl1
GARP
TRAPPII
Nucleus
Recycling
endosome
Pericentriolar
recycling
endosome
TRAPPI
Sly1p
COG
Exocyst
Sec1p
Exocytosis
Endoplasmic
Reticulum
COG
COG4
COG2
COG3
COG8
COG1
COG6
COG5
COG7
GARP
Vps51p
Vps54p
Vps52p
Vps53p
HOPS
Vps33p
Vps18p
Vps16p
Vps11p
Vps39p
Vps41p
TRAPPI
Trs23p
Trs31p
Trs20p
Bet5p
Trs85p
Bet3p
Trs33p
TRAPPII
Trs120p
Trs130p
Trs65p
Exocyst
Exo70p
Exo84p
Sec15p
Sec6p
Sec8p
Sec10p
Sec5p
Sec3p
Dsl1
Dsl1
Tip20
Sec20

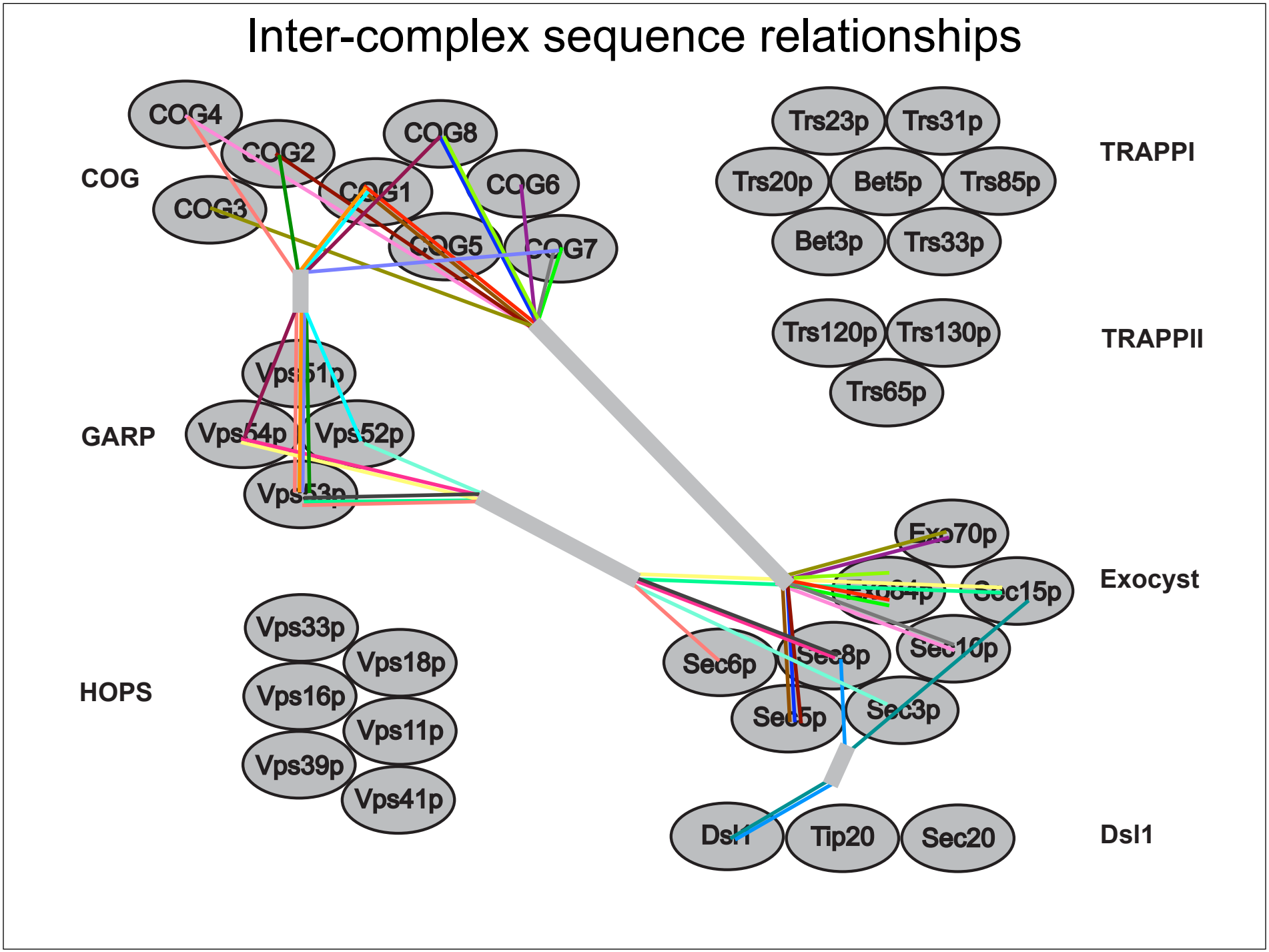

H. sapiens SBP1a
H. sapiens SBP3
H. sapiens SBP2
C. elegans Unc18a
C. elegans Unc18b
C. elegans Sec1
P. falciparum Sec1
T. thermophila Sec1
C. neoformans Sec1
D. discoideum Sec1
S. cerevisiae Sec1
T. pseudonana Sec1
P. ramorum Sec1
A. thaliana Sec1a
A. thaliana keule
A. thaliana Sec1b
C. reinhardtii Sec1
T. cruzi Sec1
T. brucei Sec1
L.. major Sec1
Sec1p
1.00/93/95
T. brucei Sly1
T. cruzi Sly1
L. major Sly1
D. discoideum Sly1
T. thermophila Sly1
H. sapiens Sly1
C. elegans Sly1
T. pseudonana Sly1
P. ramorum Sly1
A. thaliana Sly1
C. reinhardtii Sly1
S. cerevisiae Sly1
C. neoformans Sly1
P. falciparum Sly1
T. gondii Sly1
Sly1p
1.00/100/100
T. cruzi Vps33
L. major Vps33
T. brucei Vps33
H. sapiens Vps33b
C. elegans Vps33a
D. discoideum Vps33a
T. pseudonana Vps33
P. ramorum Vps33
A. thaliana Vps33
C. reinhardtii Vps33
H. sapiens Vps33a
D. discoideum Vps33b
D. discoideum Vps33c
P. falciparum Vps33
T. gondii Vps33
C. neoformans Vps33
S. cerevisiae Vps33
C. elegans Vps33b
T. thermophila Vps33
Vps33p
1.00/100/100
H. sapiens Vps45
C. elegans Vps45
D. discoideum Vps45
P. falciparum Vps45
T. gondii Vps45
T. thermophila Vps45
T. pseudonana Vps45
P. ramorum Vps45
A. thaliana Vps45
C. reinhardtii Vps45
C. neoformans Vps45
T. brucei Vps45a
T. cruzi Vps45a
L. major Vps45a
T. cruzi Vps45b
T. cruzi Vps45c
L. major Vps45b
S. cerevisiae Vps45
Vps45p
1.00/100/100
0.1
=0.99/95/95
=0.95/80/80
=0.95/50/50


HOPS
COG
GARP
Exocyst
Dsl1
TRAPPI
TRAPPII
Opistokonta
Amoebozoa
Archaeplastida
Chromalveolata
Excavata
H. sapiens
C. elegans
S. cerevisiae
C. neoformans
D. discoideum
A. thaliana
C. reinhardtii
C. merolae
P. falciparum
T. gondii
T. thermophila
P. ramorum
T. pseudonana
T. brucei
T. cruzi
L. major
G. intestinalis
Metazoa
Fungi
Viridiplantae
Rhodophyta
Apicomplexa
Ciliates
Stramenopiles
Kinetoplastida
Diplomonads
Vps11 Vps41 Vps16 Vps39 Vps18 Vps33
COG1 COG8 COG2 COG7 COG3 COG6 COG4 COG5
Vps51 Vps54 Vps52 Vps53
Sec3 Exo84 Sec5 Exo70 Sec6 Sec15 Sec8 Sec10
Dsl1 Sec20 Tip20
Trs20 Bet5 Trs23 Bet3 Trs31 Trs33 Trs85
Trs65 Trs130 Trs120
COPI
Ypt1p
Sed5p
COG
COG4
COG2
COG8
COG1
COG6
COG3
COG5
COG7
Tlg1p
GARP
Vps51p
Vps54p
Vps52p
Ypt6p
Vps53p
Arl1p
Vti1p Vam3p
Vam7p
HOPS
Vps33p
Vps18p
Ypt7p
Vps16p
Vps11p
Vps39p
Vps41p
Limited taxon distribution
Wide taxon distribution
TRAPPI
Trs23p
Trs31p
COPII
Trs20p
Bet5p
Trs85p
Bet3p
Trs33p
Ypt1p
Ypt31p
Ypt32p
TRAPPII
Trs120p
Trs130p
Trs65p
PM
(mammals)
Exocyst
Exo70p
Exo84p
Sec15p
Sec4
Sec6p
Sec8p
Sec10p
Sec5p
Sec3p
PM
(yeast)
Dsl1
Ufe1
COPI
Dsl1
Tip20
Sec20
Dsl3

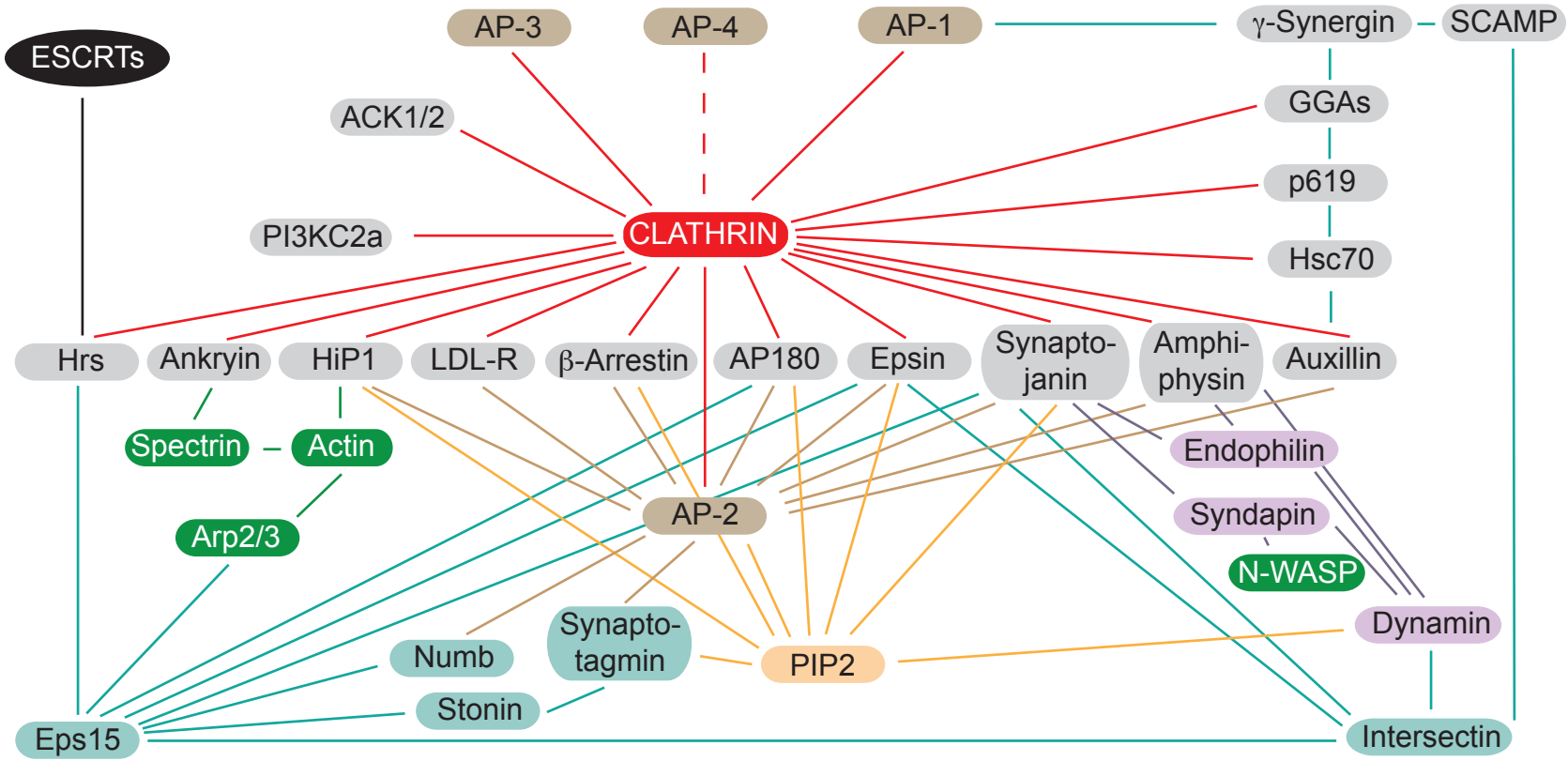

COG
COG4
COG2
COG3
COG8
COG1
COG6
COG5
COG7
TRAPPI
Trs23p
Trs31p
Trs20p
Bet5p
Trs85p
Bet3p
Trs33p
TRAPPII
Trs120p
Trs130p
Trs65p
GARP
Vps51p
Vps54p
Vps52p
Vps53p
Exocyst
Exo70p
Exo84p
Sec15p
Sec6p
Sec8p
Sec10p
Sec5p
Sec3p
HOPS
Vps33p
Vps18p
Vps16p
Vps11p
Vps39p
Vps41p
Dsl1
Dsl1
Tip20
Sec20

Intra-complex sequence relationships



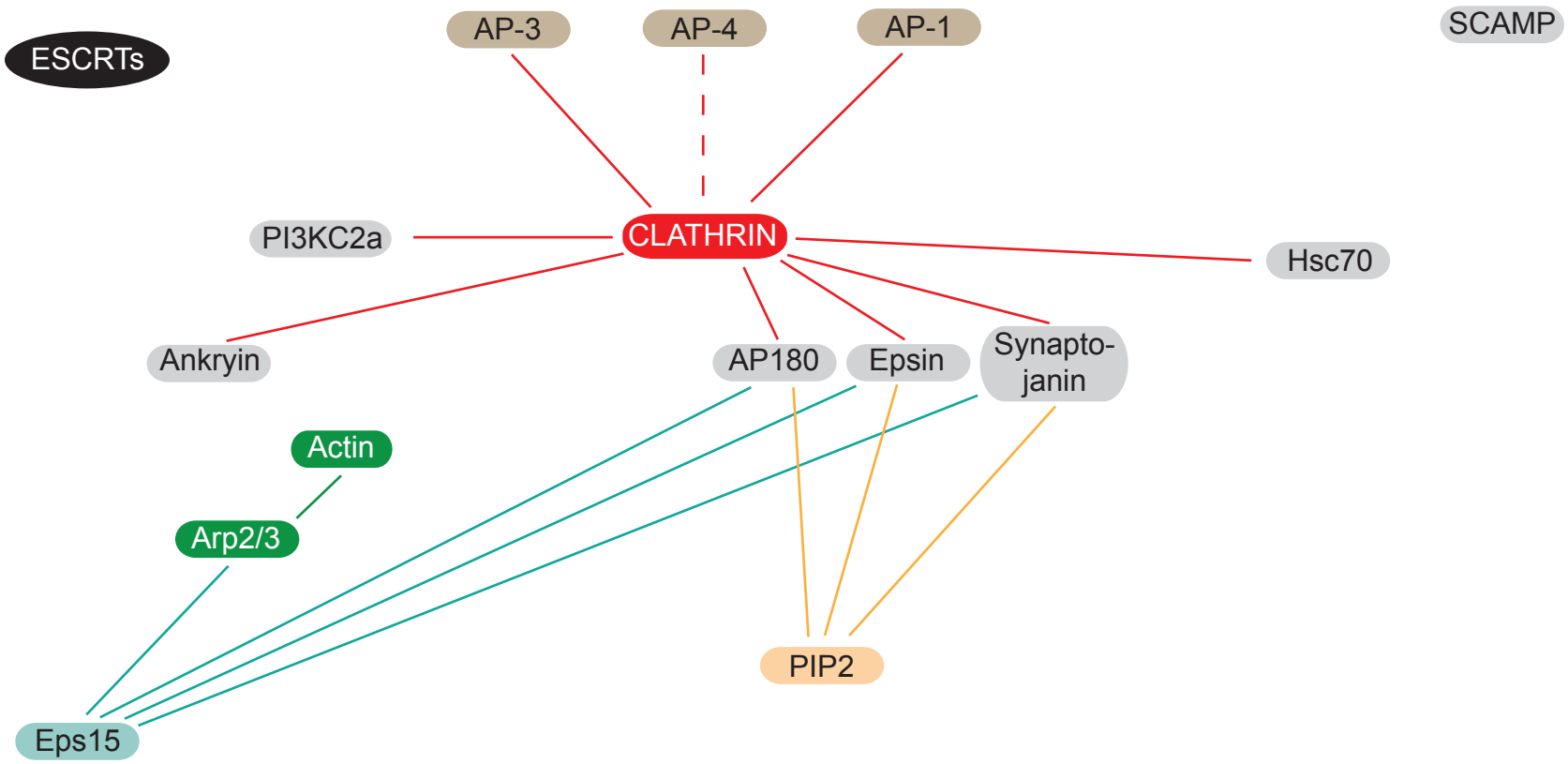

Inter-complex sequence relationships
COG
COG4
COG2
COG8
COG3
COG1
COG6
COG5
COG7
TRAPPI
Trs23p
Trs31p
Trs20p
Bet5p
Trs85p
Bet3p
Trs33p
TRAPPII
Trs120p
Trs130p
Trs65p
GARP
Vps51p
Vps54p
Vps52p
Vps53p
Exocyst
Exo70p
Sec15p
Sec6p
Sec8p
Sec10p
Sec5p
Sec3p
HOPS
Vps33p
Vps18p
Vps16p
Vps11p
Vps39p
Vps41p
Dsl1
Tip20
Sec20
Dsl1

The clathrin interactome



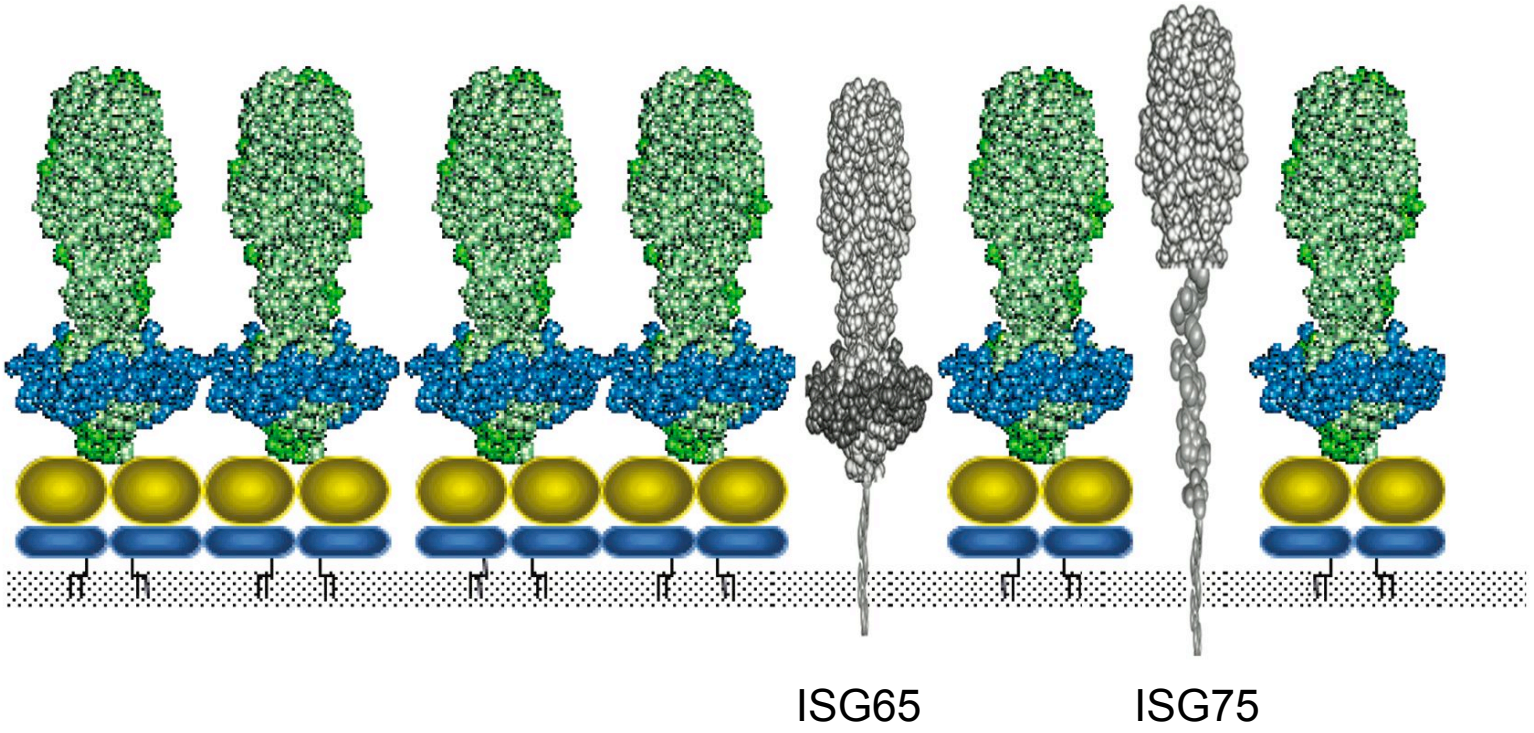
After Lafer (2002)

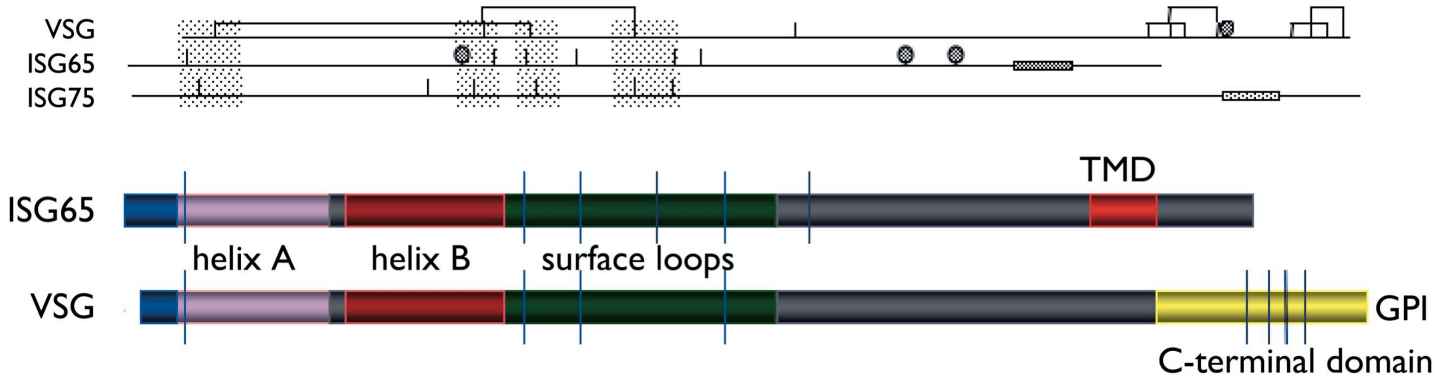
The clathrin interactome



After Lafer (2002)

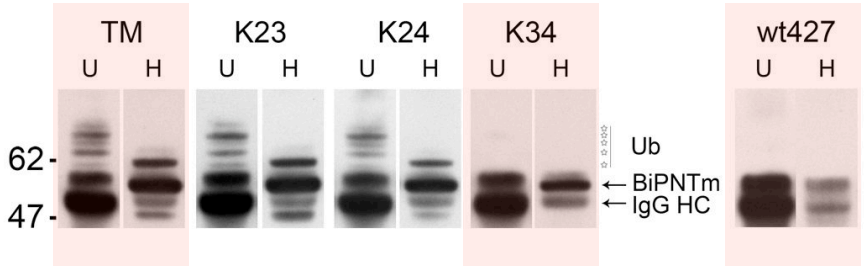
The cell surface of an excavate (*Trypanosoma brucei*)



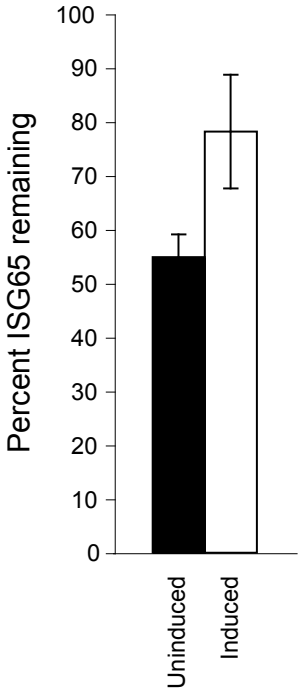


Carrington

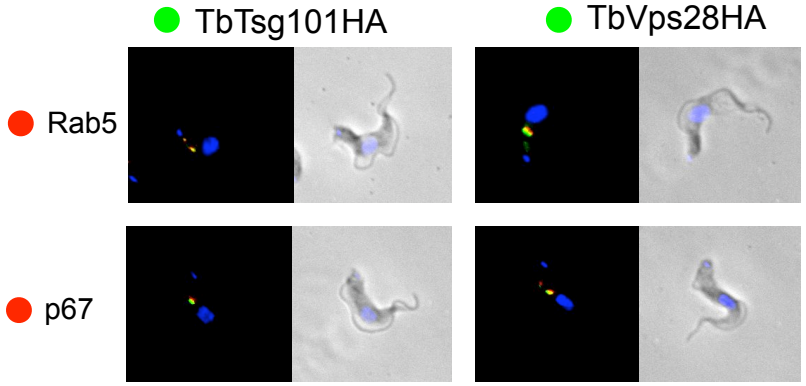
Trans-membrane domain proteins are ubiquitylated



TbTsg101 RNAi



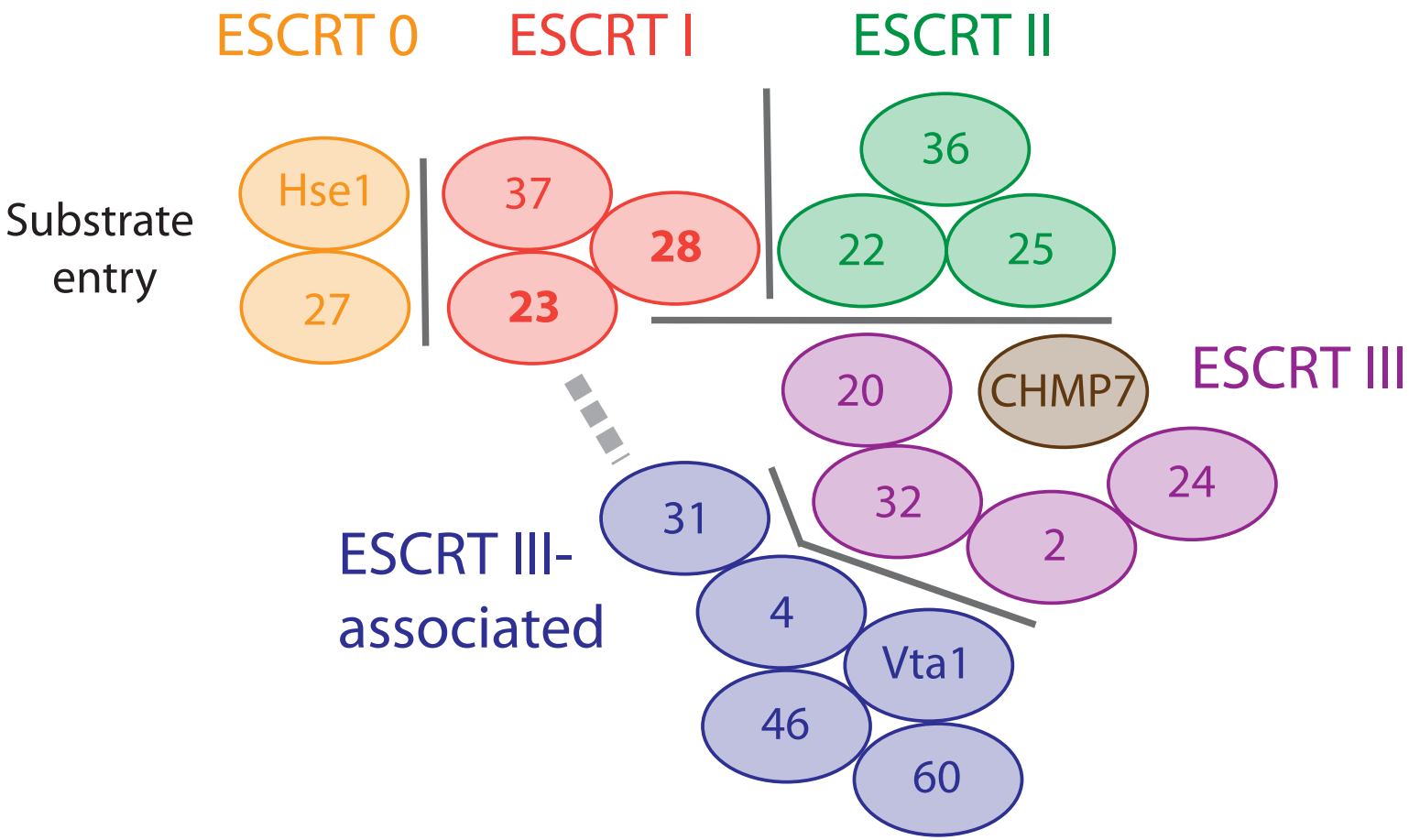
Trypanosome ESCRT I complex factors localize to the endosomal system...



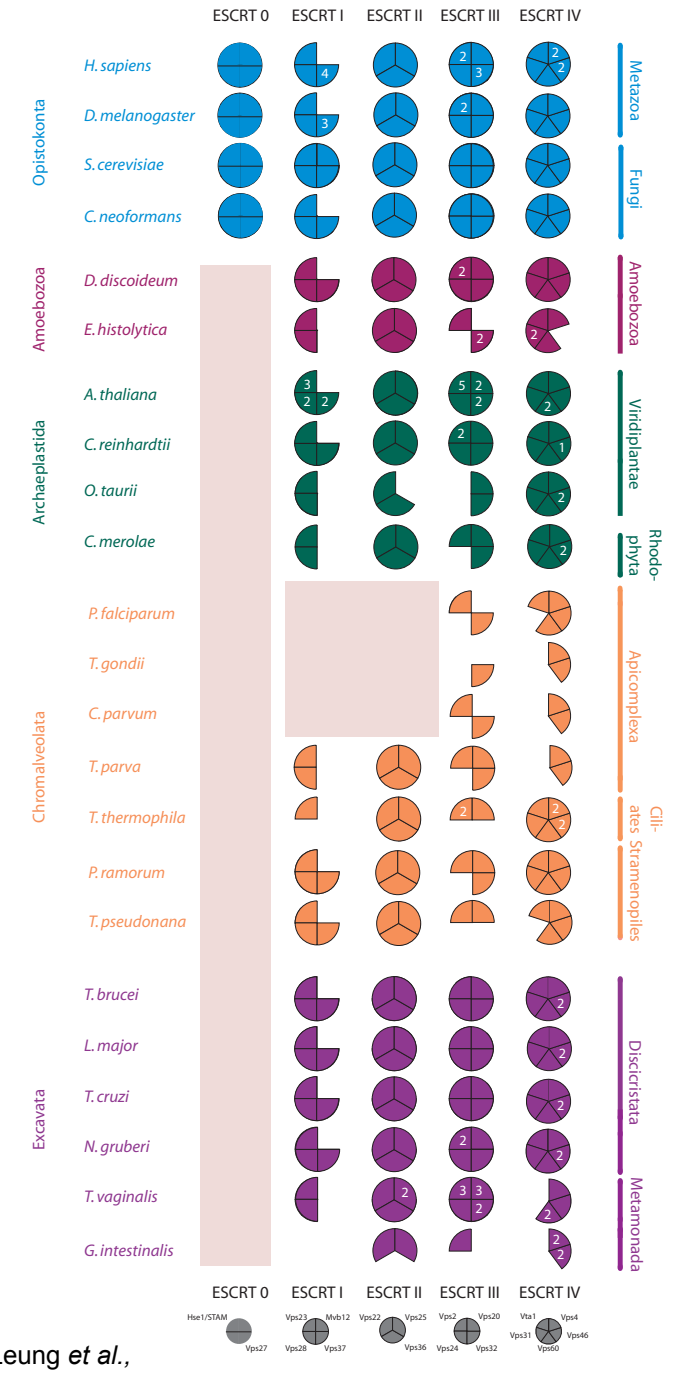
... and are required for turnover

Leung *et al.*, Chung *et al.*,

Composition of ESCRT complexes



Leung *et al.*,

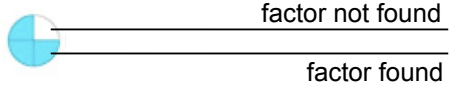


Leung *et al.*,

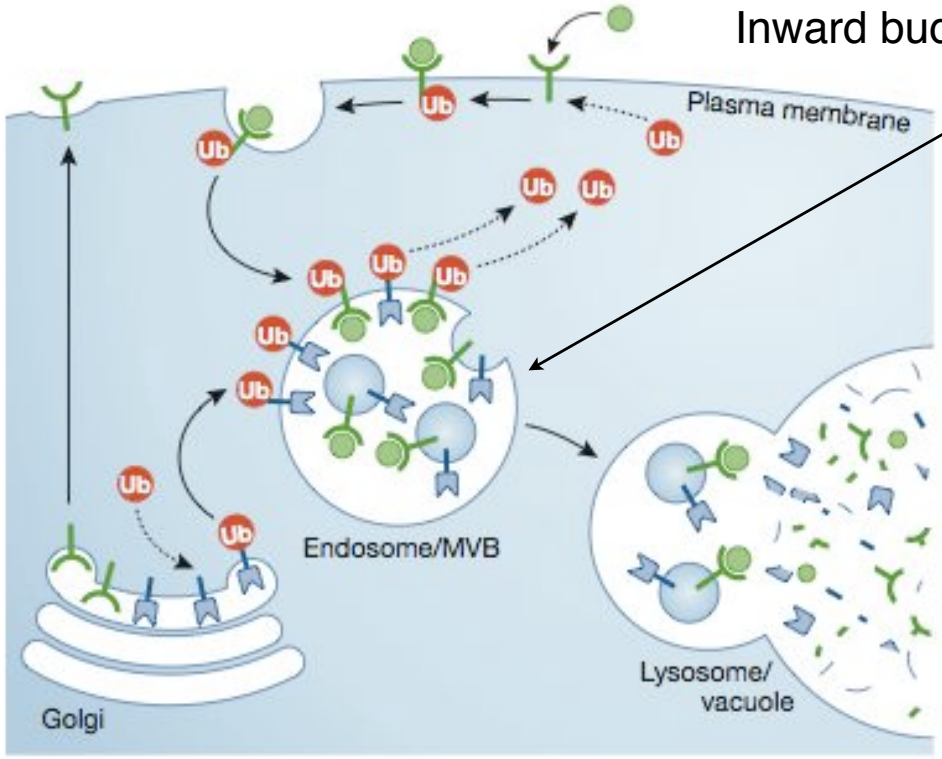
Remarkable level of conservation

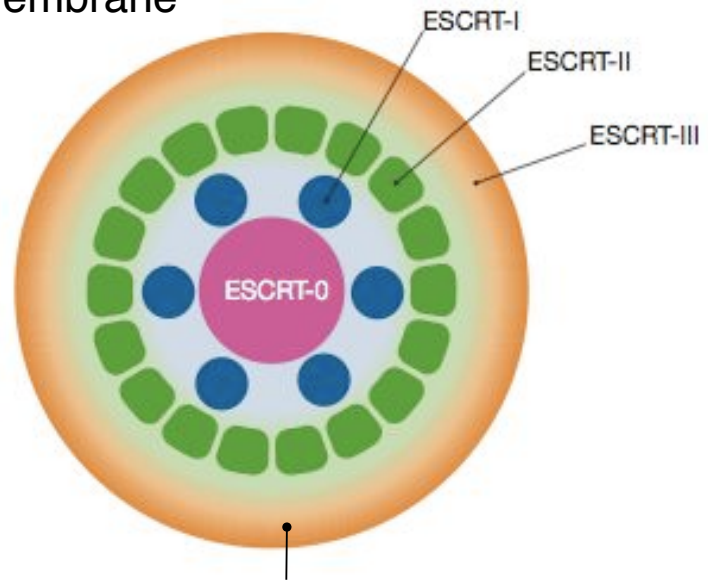
Ancient

ESCRT 0; Opisthokont specialization
ESCRT I and II: Secondary loss

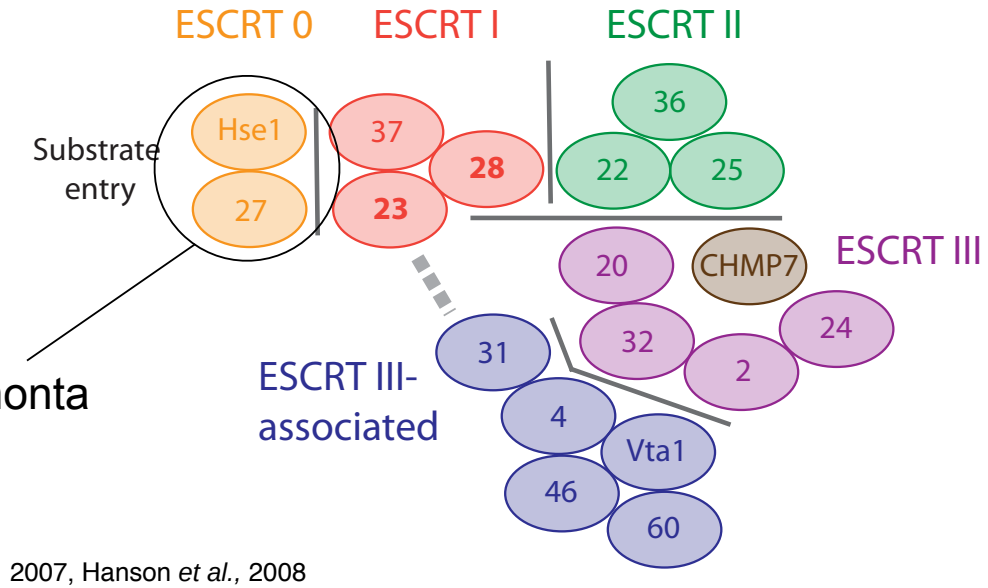


Inward budding of membrane

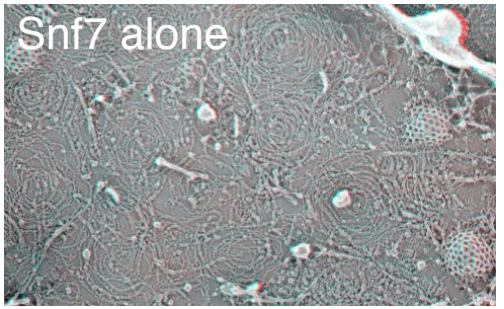


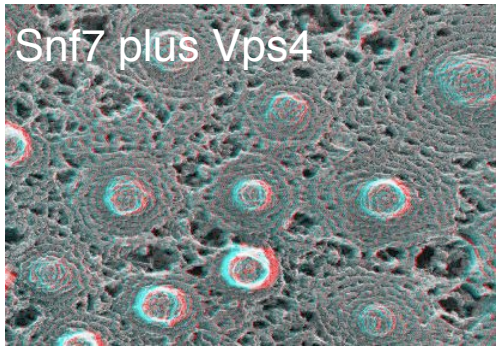


Membrane deformation and ATPase

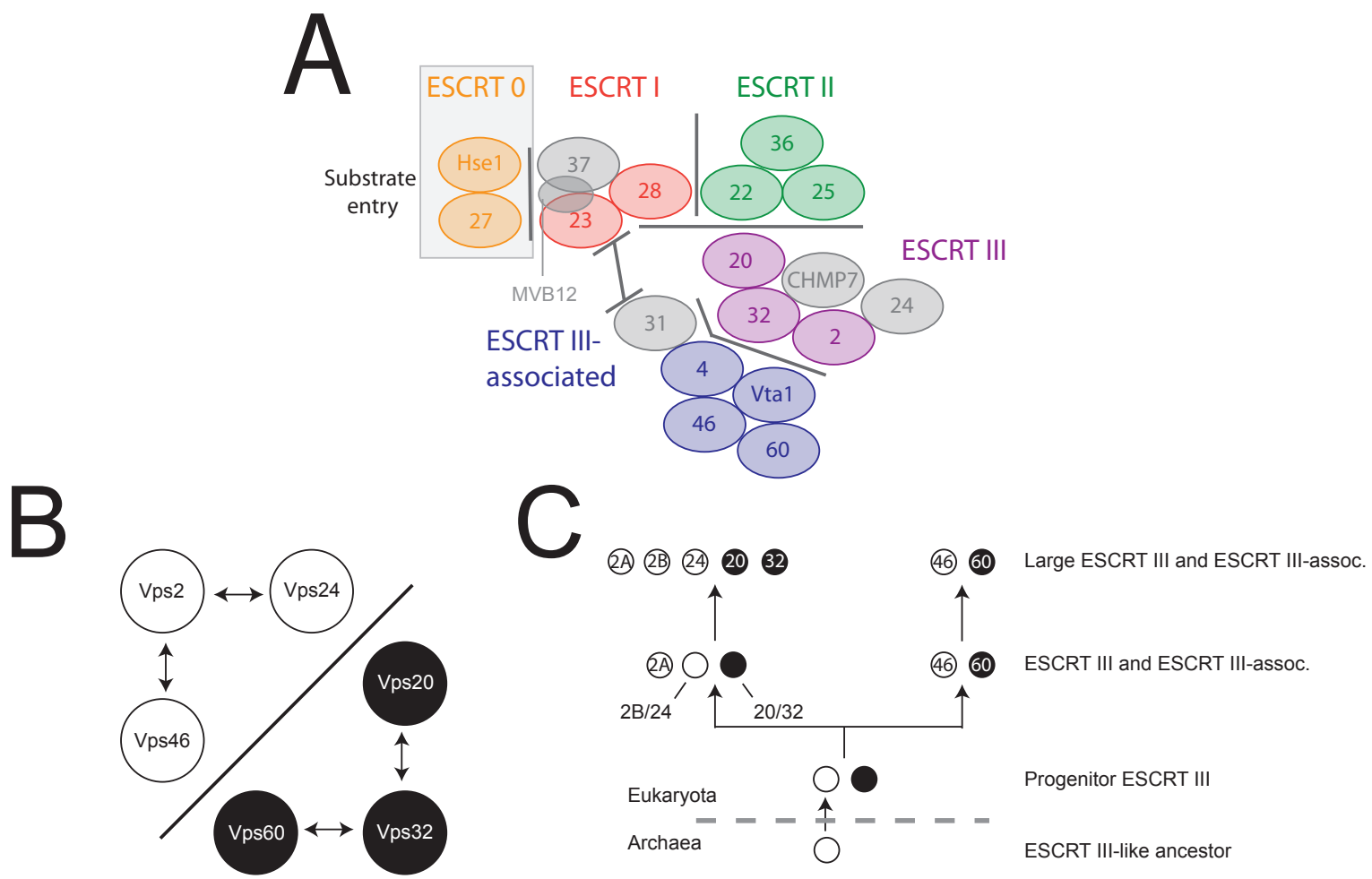


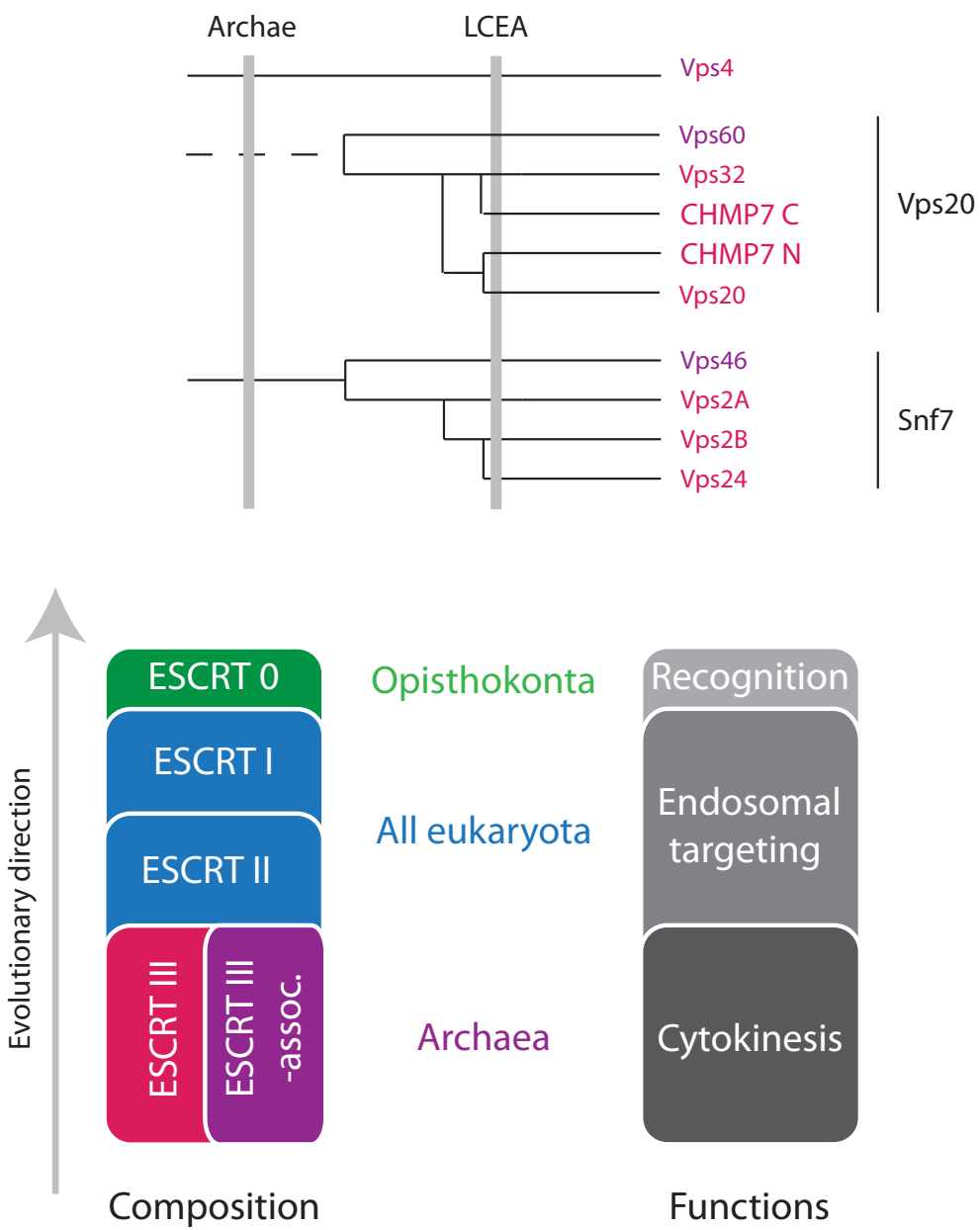
Opistokhonta
specific





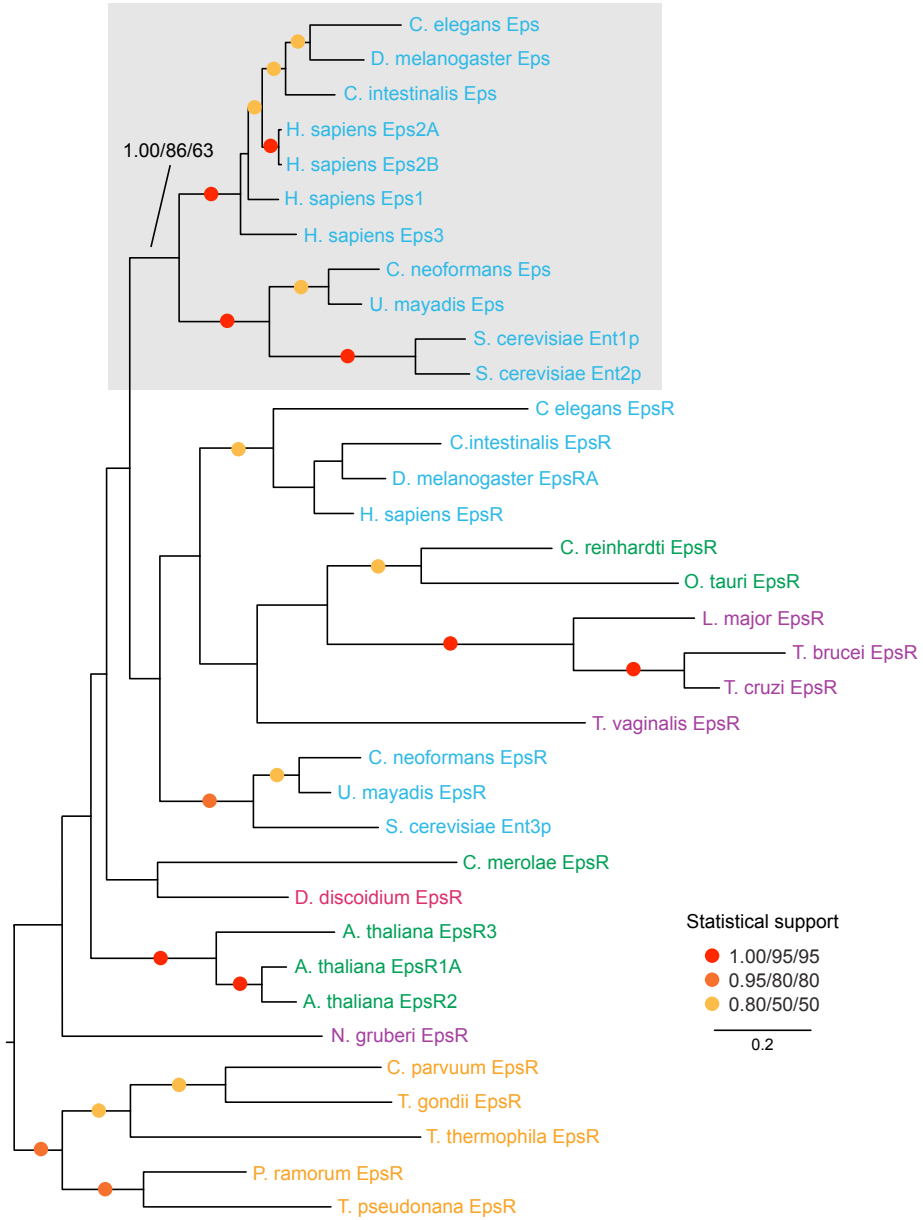
Nickerson *et al.*, 2007, Hanson *et al.*, 2008


A
ESCRT 0
ESCRT I
ESCRT II
Substrate entry
Hse1
27
37
23
28
36
22
25
MVB12
ESCRT III
20
CHMP7
24
31
32
2
ESCRT III-associated
4
Vta1
46
60
B
Vps2
Vps24
Vps46
Vps20
Vps60
Vps32
C
2A 2B 24 20 32
46 60
Large ESCRT III and ESCRT III-assoc.
2A
46 60
ESCRT III and ESCRT III-assoc.
2B/24
20/32
Progenitor ESCRT III
Eukaryota
Archaea
ESCRT III-like ancestor



Field and Dacks

Acquisition of the UIM by the epsin family is lineage-specific

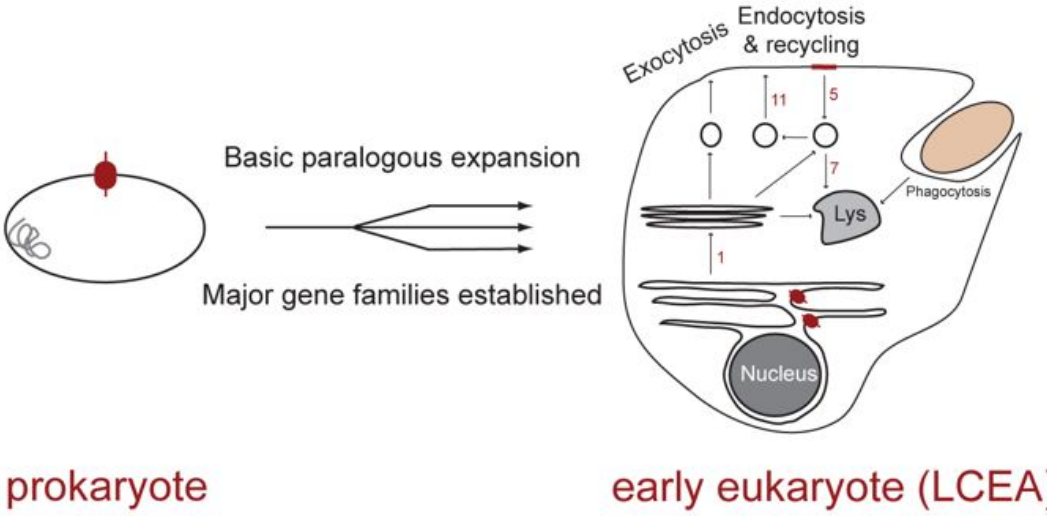


EpsinR

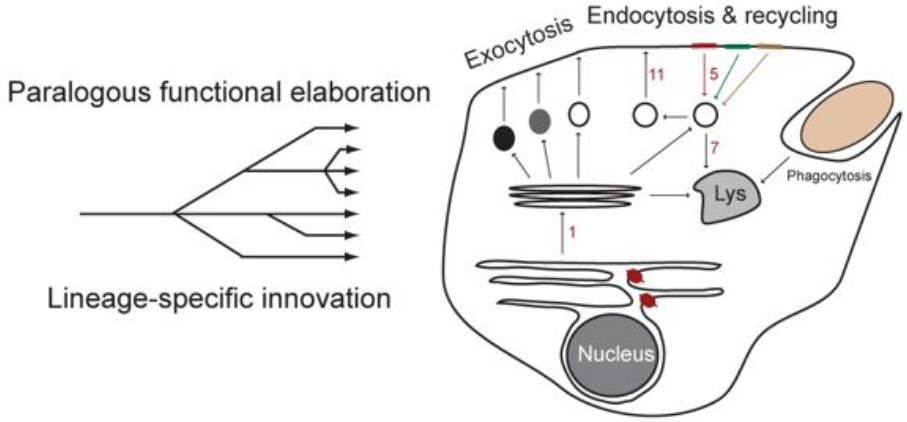
Epsin

Gabernet-Castello, Dacks and Field 2008

Evolution of the endomembrane system

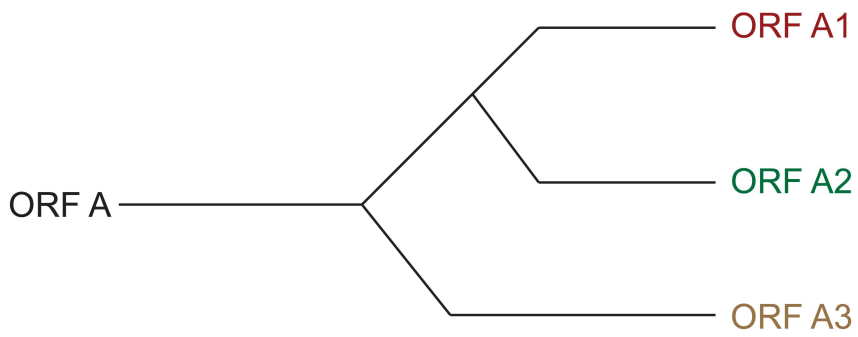


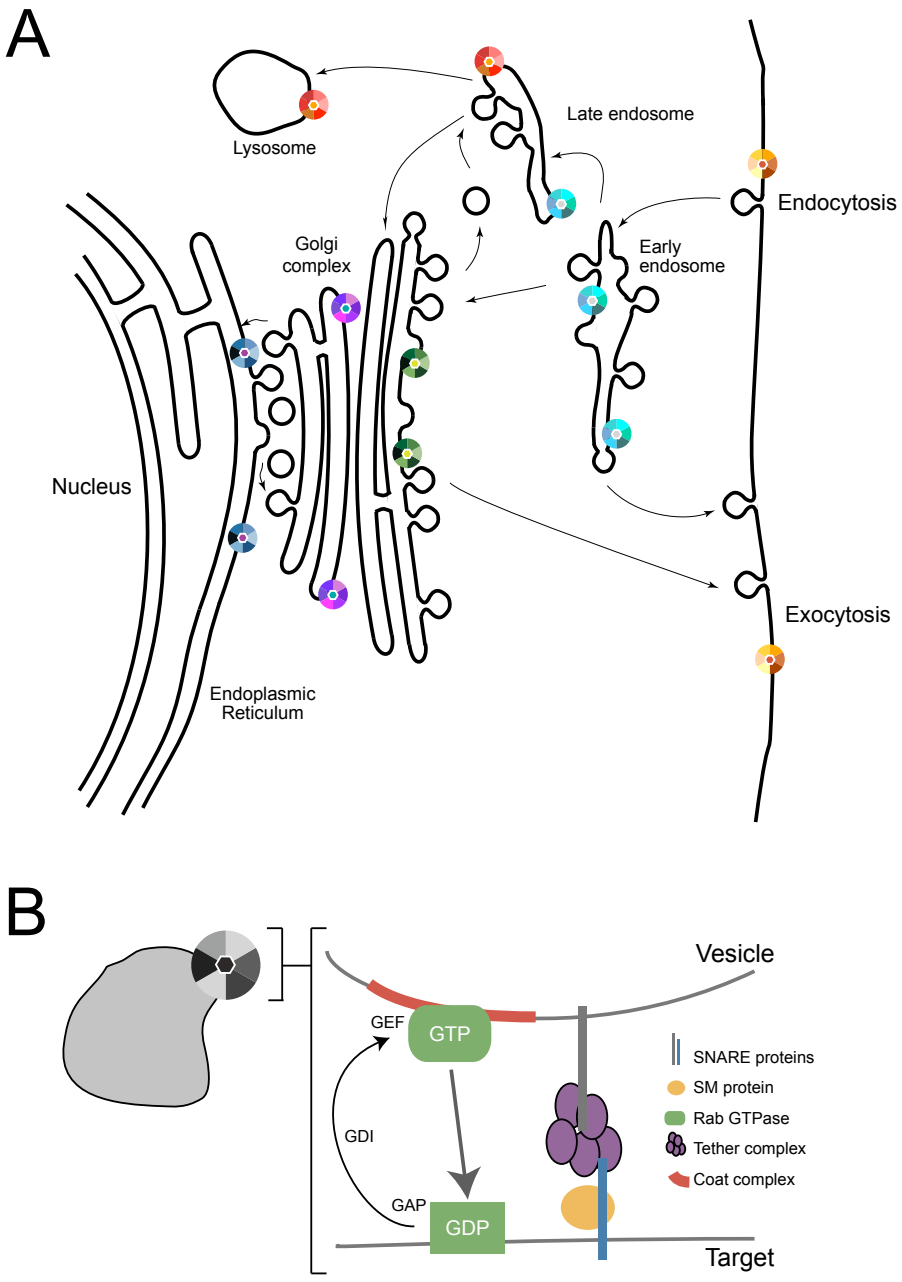
translocon
phagocytic particle
Golgi complex
endocytic pathways
diverse exocytic vesicles
endoplasmic reticulum
prokaryotic DNA
lysosome/vacuole

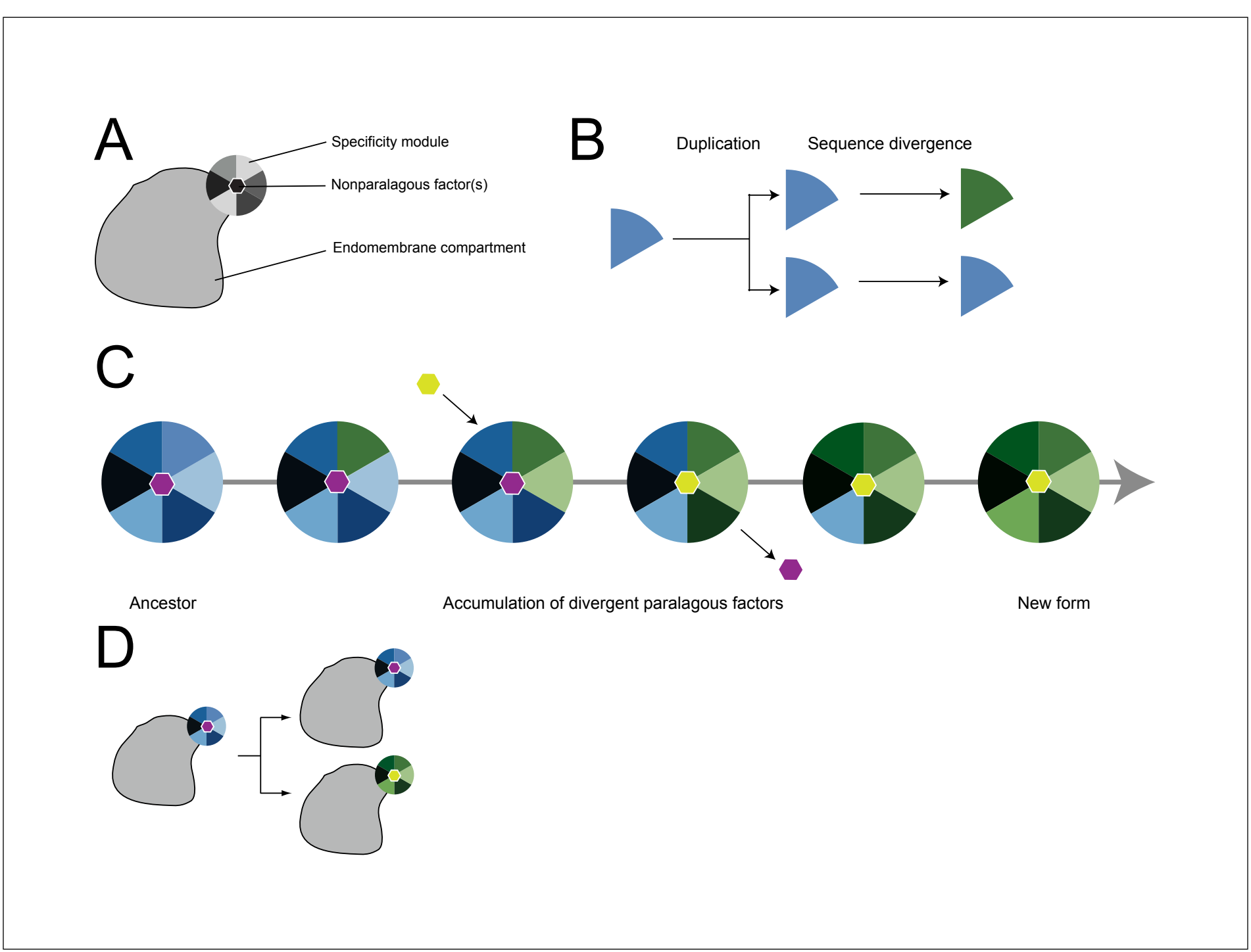


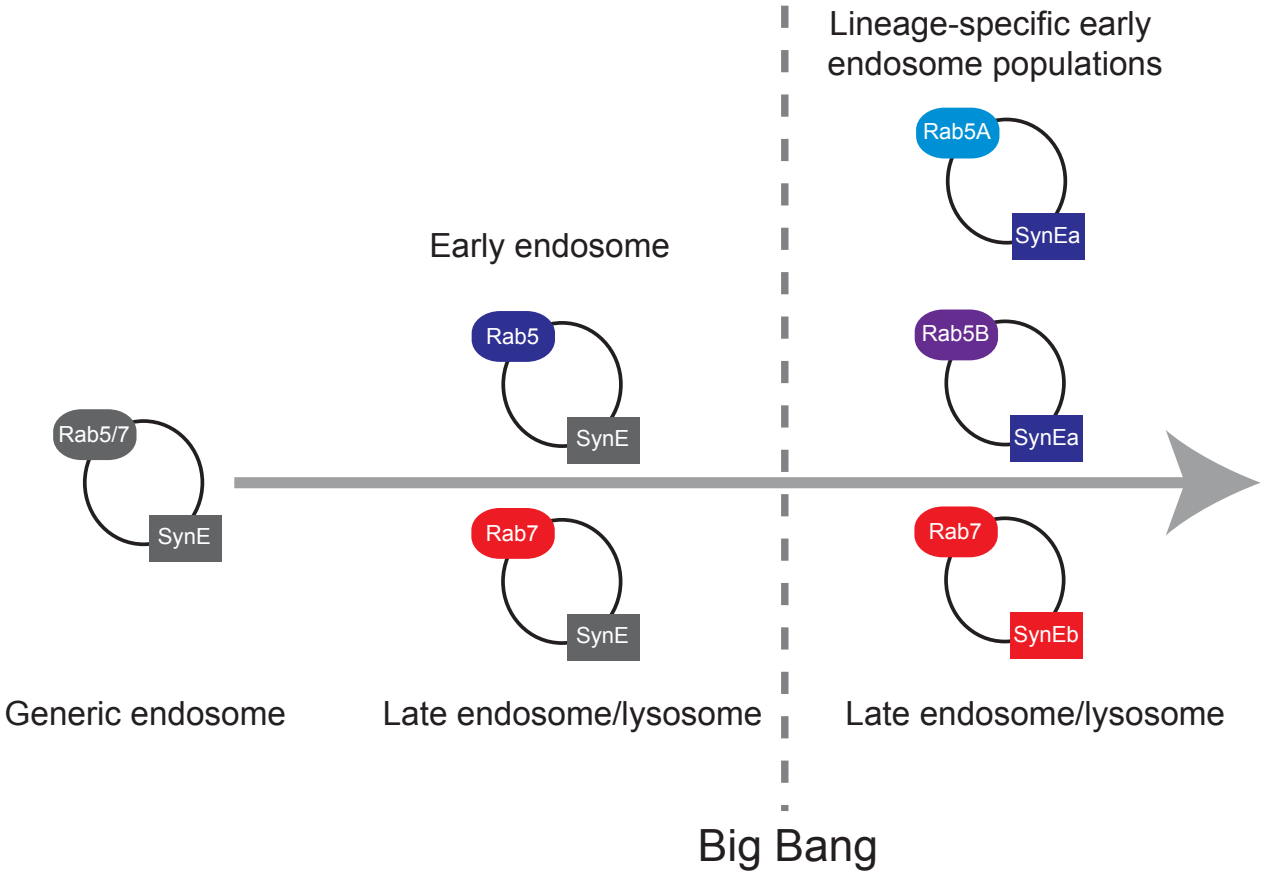
- multiple modes of endocytosis
- multiple modes of exocytosis
- tissue-specific pathways
- lineage-specific pathways
- secondary losses

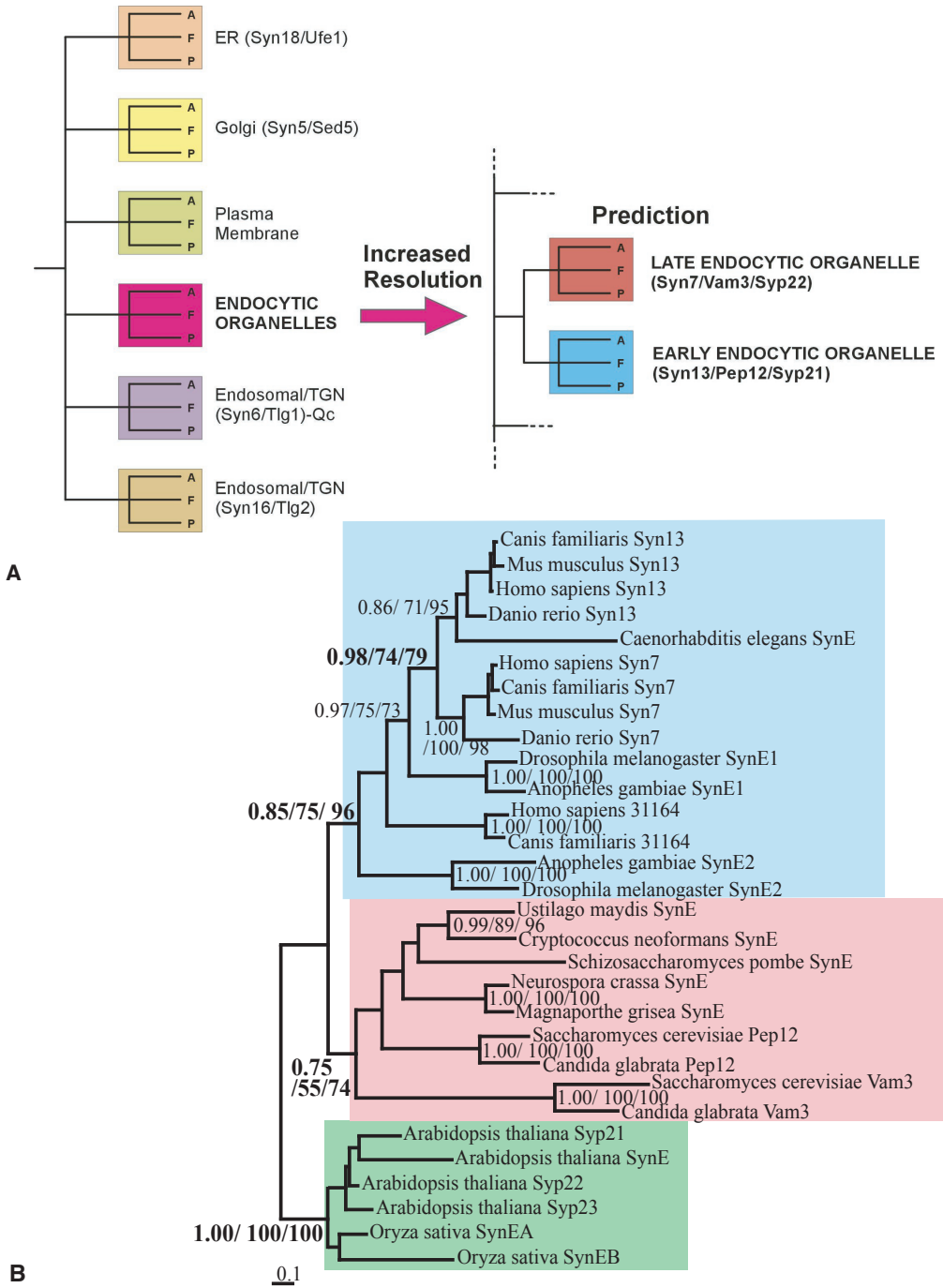
Dacks and Field 2007

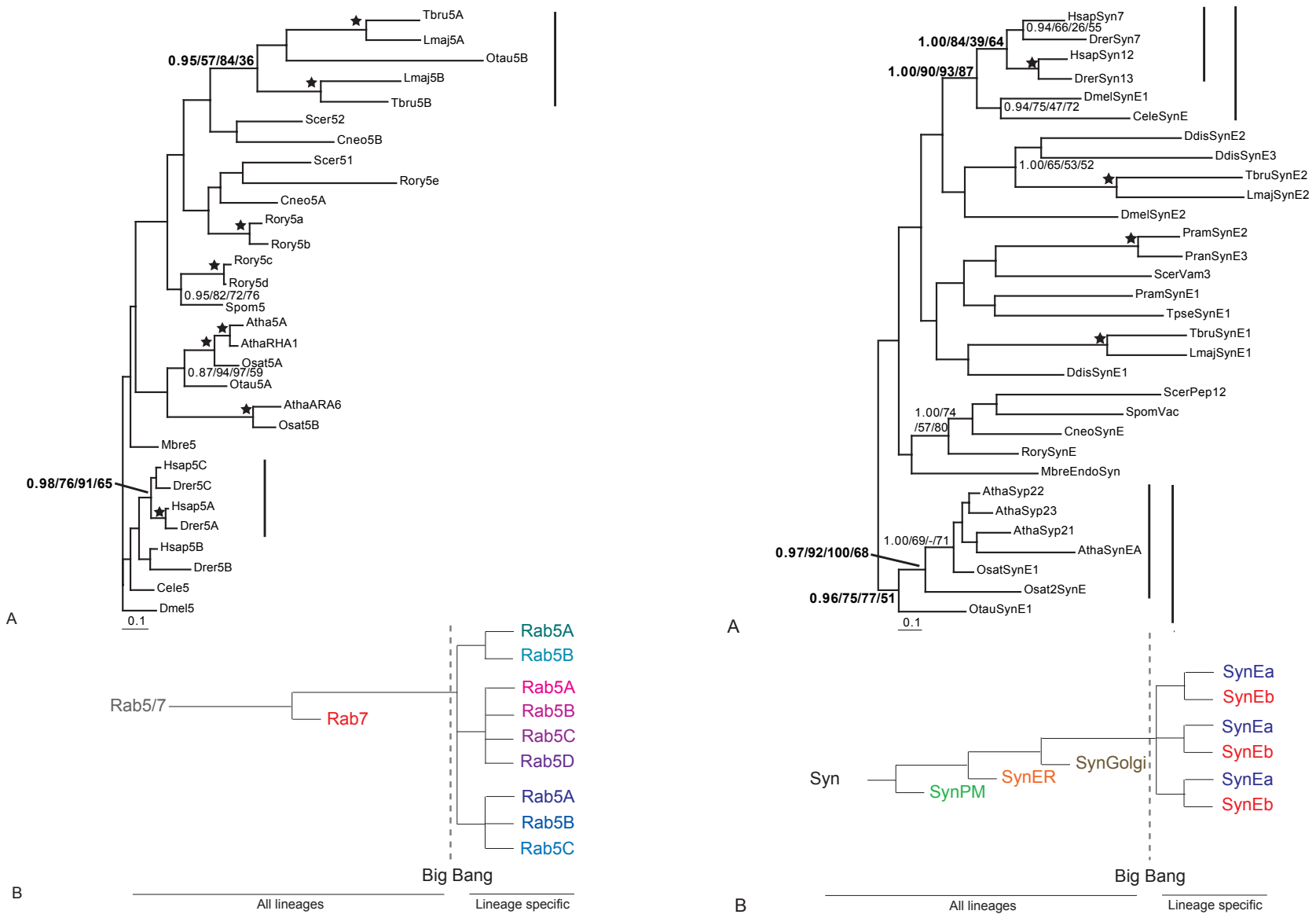

ORF A1
ORF A2
ORF A
ORF A3


A
Lysosome
Late endosome
Endocytosis
Golgi
complex
Early
endosome
Nucleus
Exocytosis
Endoplasmic
Reticulum
B
Vesicle
GEF
GTP
SNARE proteins
SM protein
Rab GTPase
Tether complex
Coat complex
GDI
GAP
GDP
Target

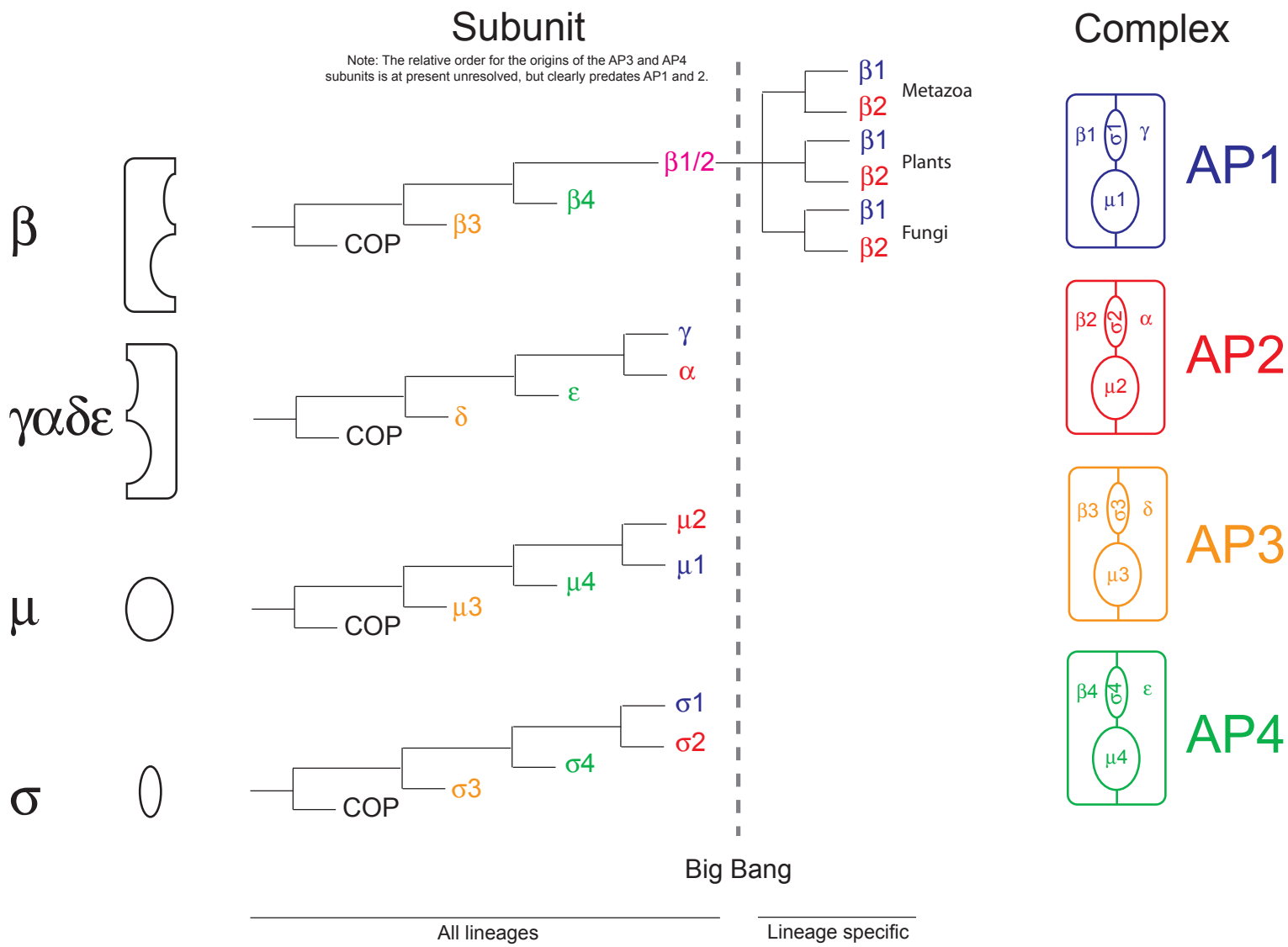

A
Specificity module
Nonparalagous factor(s)
Endomembrane compartment
B
Duplication
Sequence divergence
C
Ancestor
Accumulation of divergent paralagous factors
New form
D


Lineage-specific early
endosome populations
Rab5A
SynEa
Early endosome
Rab5
SynE
Rab5B
SynEa
Rab5/7
SynE
Rab7
SynE
Rab7
SynEb
Generic endosome
Late endosome/lysosome
Late endosome/lysosome
Big Bang

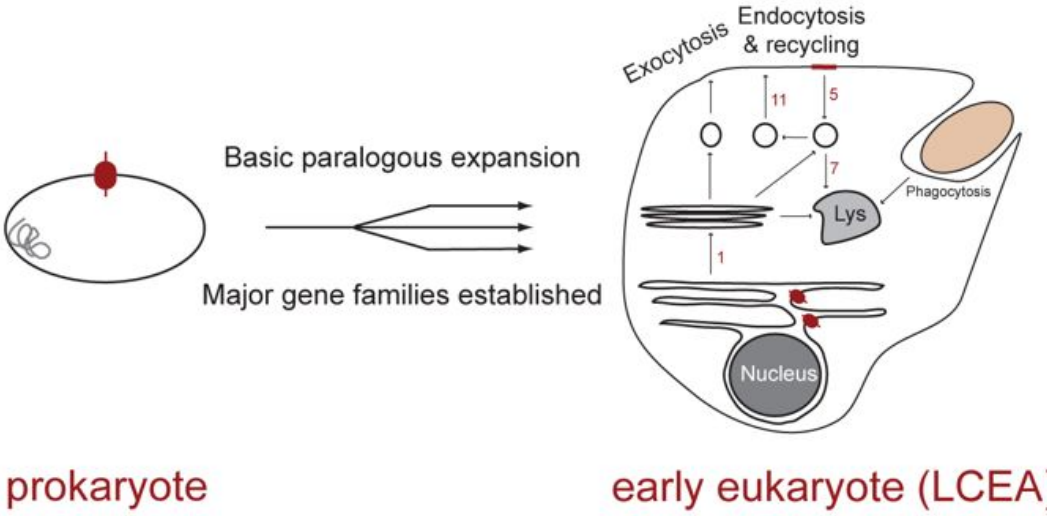

A
F
P
ER (Syn18/Ufe1)
Golgi (Syn5/Sed5)
Plasma Membrane
ENDOCYTIC ORGANELLES
Endosomal/TGN (Syn6/Tlg1)-Qc
Endosomal/TGN (Syn16/Tlg2)
Increased Resolution
Prediction
LATE ENDOCYTIC ORGANELLE (Syn7/Vam3/Syp22)
EARLY ENDOCYTIC ORGANELLE (Syn13/Pep12/Syp21)
A
Canis familiaris Syn13
Mus musculus Syn13
Homo sapiens Syn13
Danio rerio Syn13
Caenorhabditis elegans SynE
Homo sapiens Syn7
Canis familiaris Syn7
Mus musculus Syn7
Danio rerio Syn7
Drosophila melanogaster SynE1
Anopheles gambiae SynE1
Homo sapiens 31164
Canis familiaris 31164
Anopheles gambiae SynE2
Drosophila melanogaster SynE2
Ustilago maydis SynE
Cryptococcus neoformans SynE
Schizosaccharomyces pombe SynE
Neurospora crassa SynE
Magnaporthe grisea SynE
Saccharomyces cerevisiae Pep12
Candida glabrata Pep12
Saccharomyces cerevisiae Vam3
Candida glabrata Vam3
Arabidopsis thaliana Syp21
Arabidopsis thaliana SynE
Arabidopsis thaliana Syp22
Arabidopsis thaliana Syp23
Oryza sativa SynEA
Oryza sativa SynEB
0.86/ 71/95
0.98/74/79
0.97/75/73
1.00 /100/ 98
1.00/ 100/100
0.85/75/ 96
1.00/ 100/100
1.00/ 100/100
0.99/89/ 96
1.00/ 100/100
1.00/ 100/100
1.00/ 100/100
0.75 /55/74
1.00/ 100/100
1.00/ 100/100
0.1
B



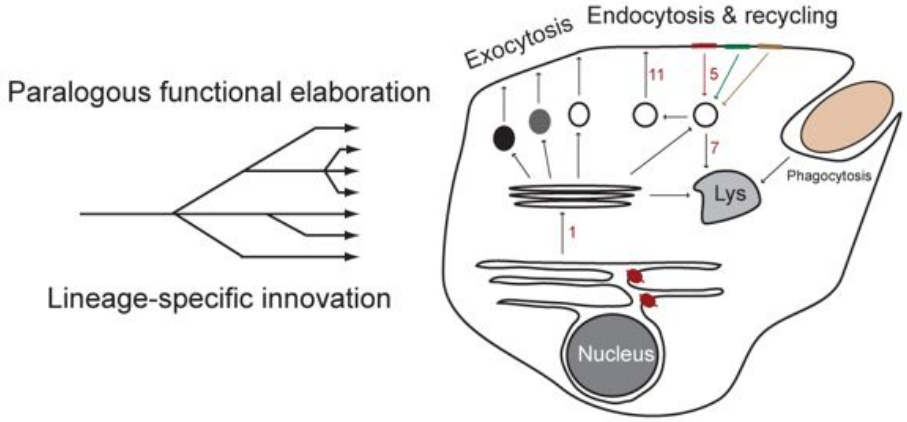
Dacks, Poon, Field and Cavalier-Smith Fig 2


Subunit
Note: The relative order for the origins of the AP3 and AP4 subunits is at present unresolved, but clearly predates AP1 and 2.
β
γαδε
μ
σ
β1/2
β4
β3
COP
γ
α
ε
δ
COP
μ2
μ1
μ4
μ3
COP
σ1
σ2
σ4
σ3
COP
β1
β2
Metazoa
β1
β2
Plants
β1
β2
Fungi
Big Bang
All lineages
Lineage specific
Complex
β1
σ1
γ
μ1
AP1
β2
σ2
α
μ2
AP2
β3
σ3
δ
μ3
AP3
β4
σ4
ε
μ4
AP4

Evolution of the endomembrane system



translocon
phagocytic particle
Golgi complex
endocytic pathways
diverse exocytic vesicles
endoplasmic reticulum
prokaryotic DNA
lysosome/vacuole



- multiple modes of endocytosis
- multiple modes of exocytosis
- tissue-specific pathways
- lineage-specific pathways
- secondary losses

Dacks and Field 2007

Deep time and origins of the endomembrane system

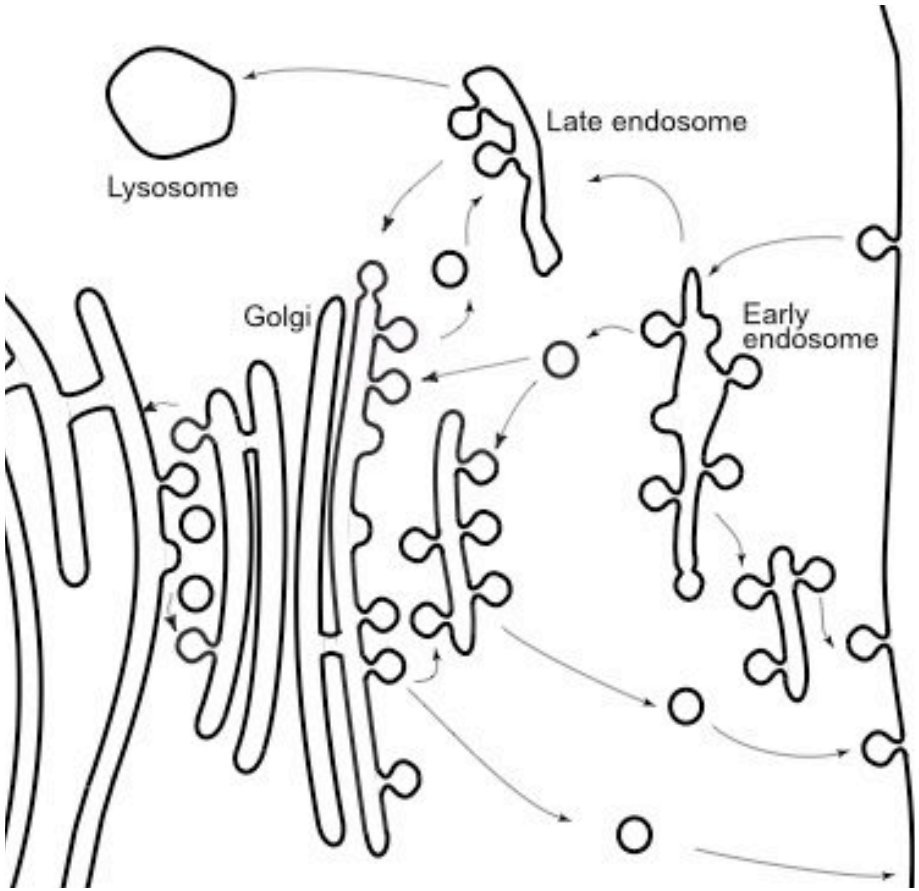
The Golgi complex - Sculpting I

Rab proteins and interaction networks - Sculpting II

Evolution of the nucleocytoplasmic transport system

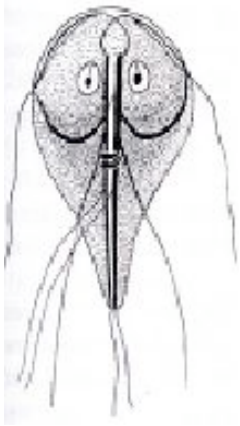
Protocoatomer; putting it all together

The Golgi complex



- Processing
- Modification
- Signaling scaffold
- Sorting & targeting

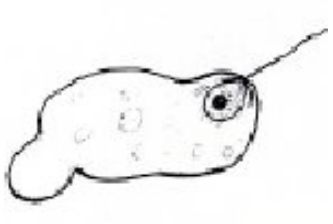
Lineages where an observable Golgi complex is absent



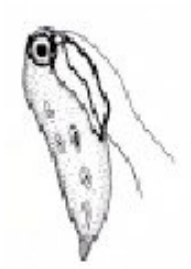
Giardia

Entamoeba

Microsporidia

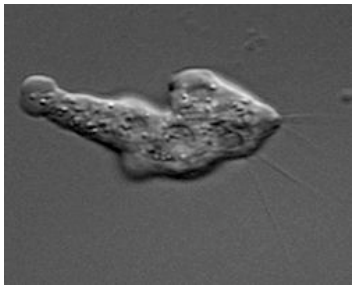


Pelobiont



Retortamonad

Oxymonad



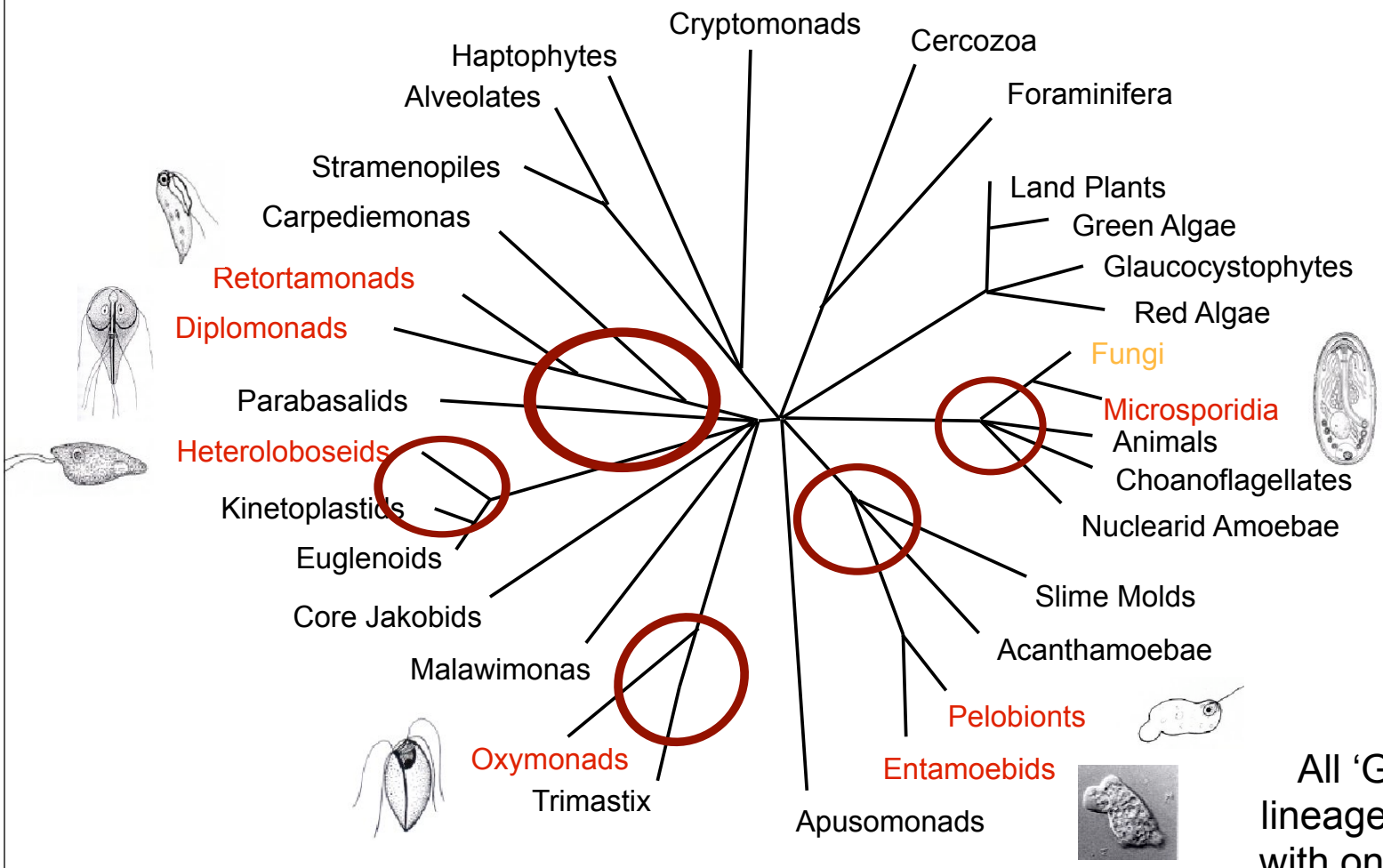
Naegleria

Lee et al, 2002, http://genome.jgi-psf.org/Naegr1/Naegr1.home.html

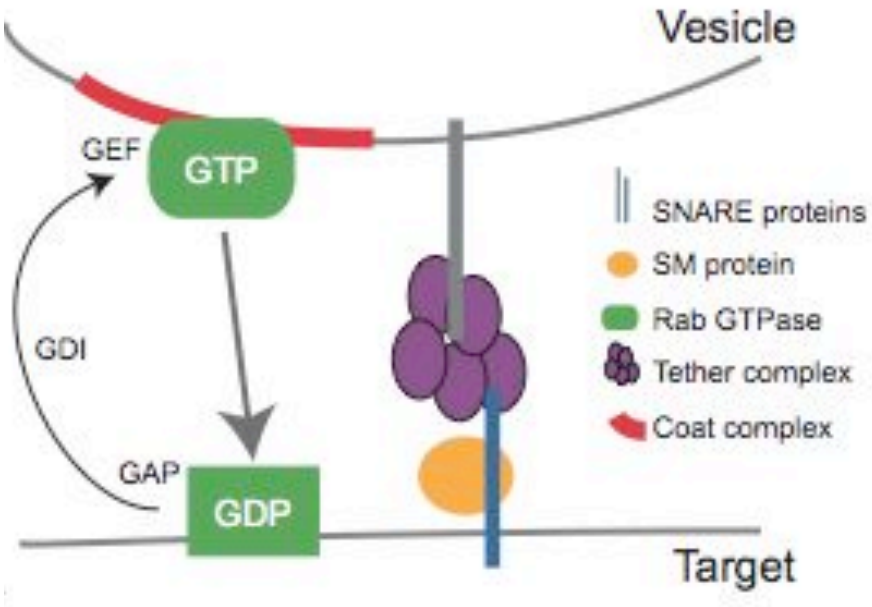
Primitive or derived absence?

- For each lineage
  - Lack of Golgi stacks due to primitive absence or to loss (of recognisable morphology?)
- Overall
  - How many times, and in which lineages, was Golgi stacking lost?
  - What was the structure of the Golgi body in the eukaryotic ancestor?

Phylogenetic evidence



All ‘Golgi-lacking’ lineages are affiliated with ones possessing stacked Golgi complexes



- Vesicle formation
  - COPII, COPI, retromer
  - Adaptins
  - Sar, Arf, Rab
- Vesicle fusion
  - SNAREs, RabS
  - syntaxin-binding (SM)

Genomic Golgi complex signal

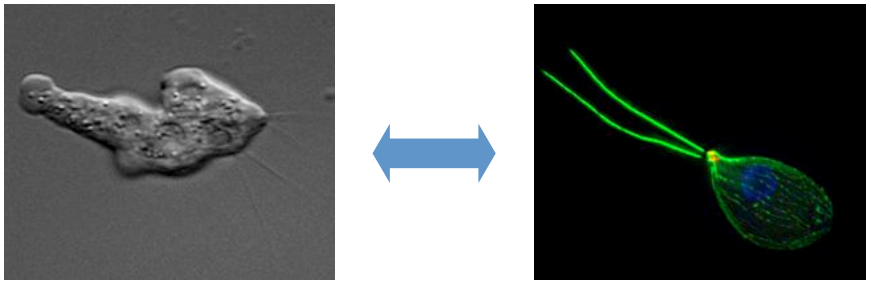
- Gene products functioning exclusively or mostly at the Golgi complex:

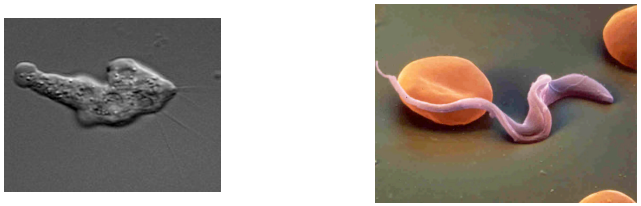
- Coats and adaptors: COPI, Adaptins (AP) 1, 3, 4, retromer
- SNAREs: Syn5/Sed5, Syn16/TLG2, Syn6/TLG1, Gos1, Sec22
- SM proteins: Sly1, Vps45
- Matrix proteins: GRASP, p115

- Far from exhaustive, could also include tethers, rabs, etc...

Naegleria

- Common soil amoeboflagellate
- *N. fowleri* causative agent of primary amoebic meningo-encephalitis
- Related to kinetoplastids, distant from cell biological models
- No recognisable Golgi bodies





Heterolobosea Kinetoplastida

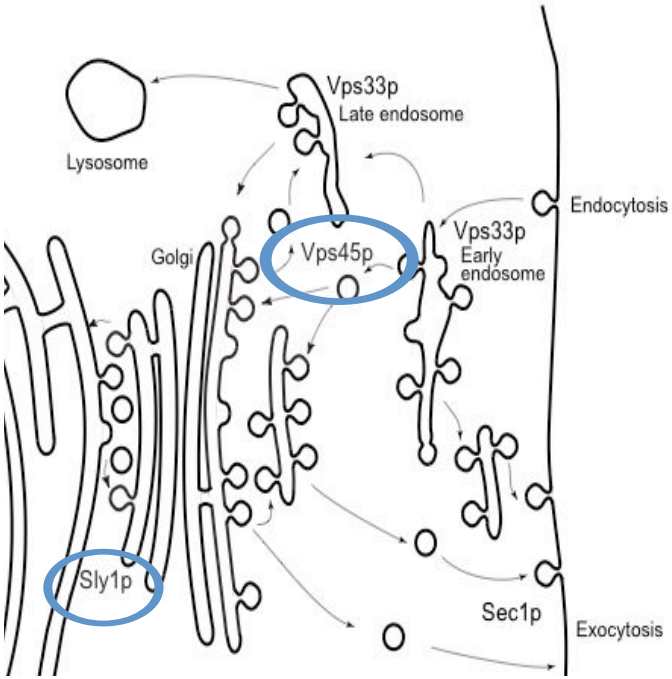
Discicristata

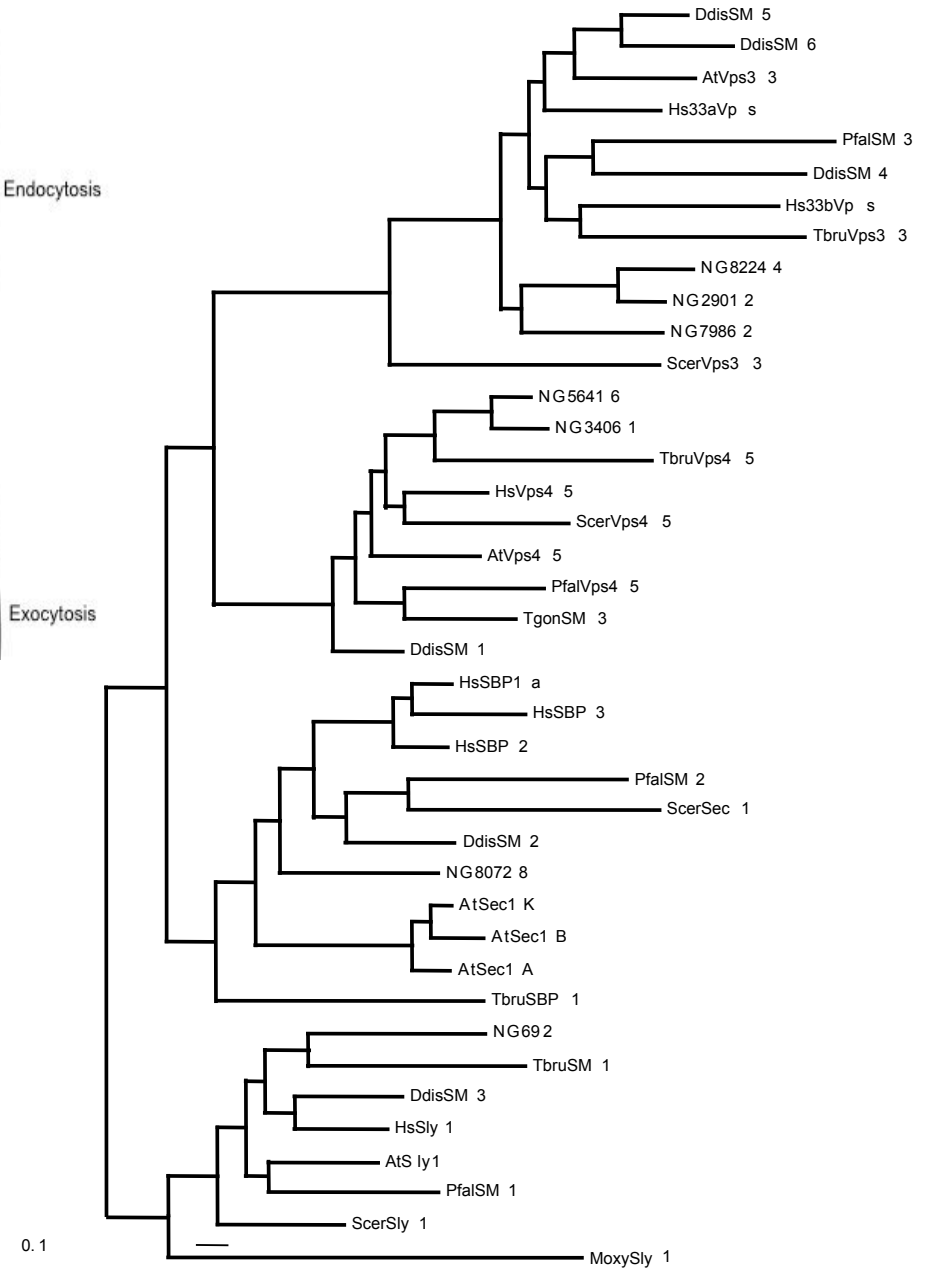
Golgi-specific genes in Golgi-lacking taxa

Higher taxon	Organism	Assignment	Top BLAST hit	Evalue
Entamoebids	*E. histolytica*	Vps 35	Vps35 *A. thaliana*	2E-47
Entamoebids	*E. histolytica*	Vps 35	Vps35 *H. sapiens*	2E-06
Entamoebids	*E. histolytica*	Vps 26	PepA *D. discoideum*	1E-69
Diplomonad	*G. intestinalis*	Vps 26	Vps-like *A. thaliana*	1E-23
Diplomonad	*S. barkhanus*	Vps 26	Vps26 *D. melanogaster*	1E-09
Pelobionts	*M. balamuthi*	Vps 26	PepA *D. discoideum*	5E-18
Diplomonad	*G. intestinalis*	Vps 35	Vps35 *M. musculus*	2E-16
Diplomonad	*S. barkhanus*	b-COP	b-COP *S. cerevisiae*	4E-13
Diplomonad	*G. intestinalis*	Syn16	U00064_6 *C. elegans*	1E-11
Entamoebids	*E. histolytica*	Syn5	SD07852p *D. melanogaster*	2E-16
Pelobionts	*M. balamuthi*	AP3 sigma	AP3 *M. musculus*	3E-53
Heterolobosea	*N. gruberi*	b'-COP	b'-COP- *S.cerevisiae*	8E-13

Coatomer, retromer, adaptins, syntaxins

SM proteins; Golgi and non-Golgi sub-families





Vps33
(1.00/100)

Vps45
(1.00/100)

Sec1
(0.99/90)

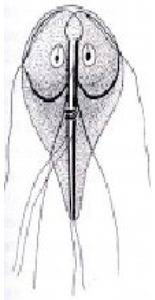
Sly1
(1.00/95)

Genomic evidence for a Golgi complex in *N. gruberi*

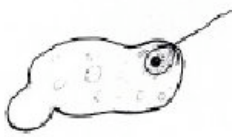
- By both pairwise homology and phylogeny

- Genes involved in receipt of material at the Golgi body, i.e. *cis* and *trans*
- Genes involved in trafficking from the Golgi body (*cis*, intra-Golgi and *trans*)
- Genes involved in Golgi morphology and replication
- Together implies the presence of a Golgi-derived organelle in *Naegleria,* but with cryptic morphology

- Lack of Golgi stacks due to primitive absence or secondary loss?
- Most parsimonious explanation is secondary loss.


diplomonad


entamoebid


pelobiont

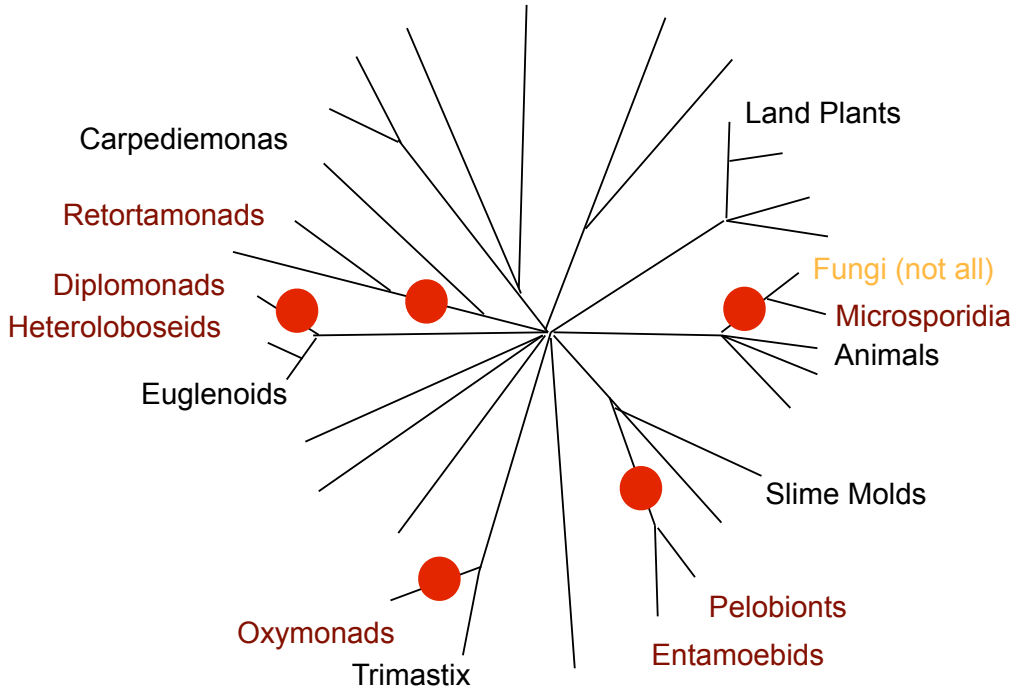

microsporidia


heteroloboseid


oxymonads

- How many times, and from which lineages, was Golgi stacking lost?

At least five times



Fungi, amoebae, three examples in excavates

Note: this is the most parsimonious interpretation - there are likely many more

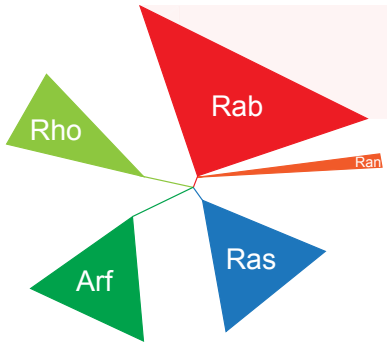
Deep time and origins of the endomembrane system

The Golgi complex - sculpting I

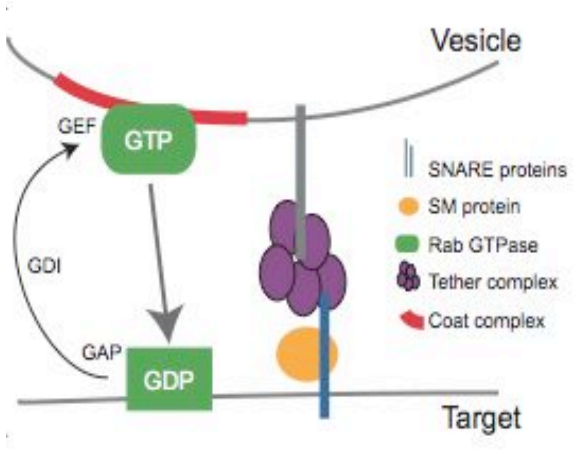
Rab proteins and interaction networks - Sculpting II

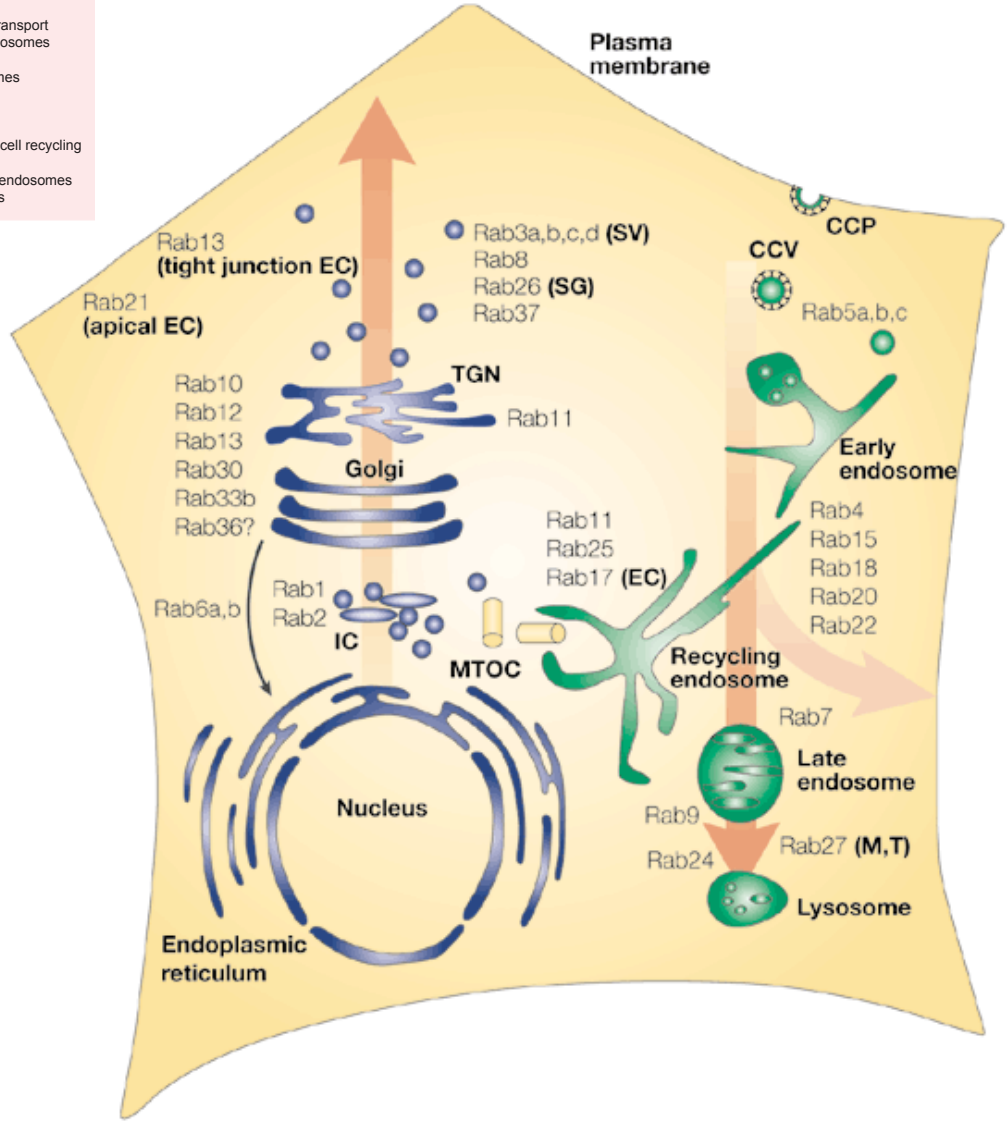
Evolution of the nucleocytoplasmic transport system

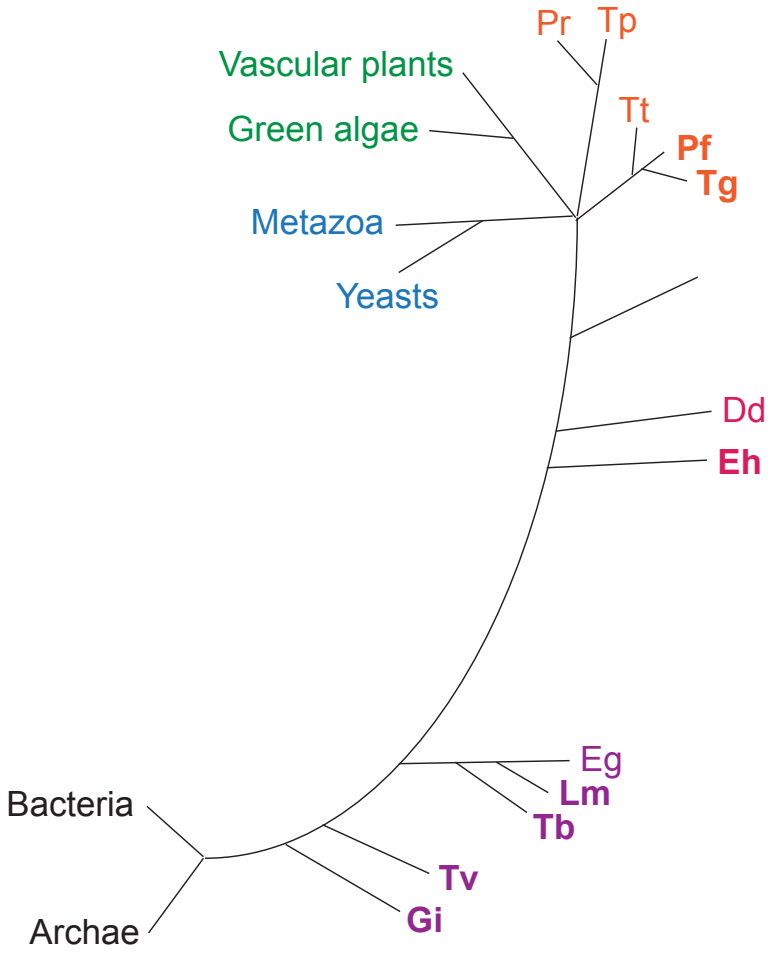
Protocoatomer; putting it all together



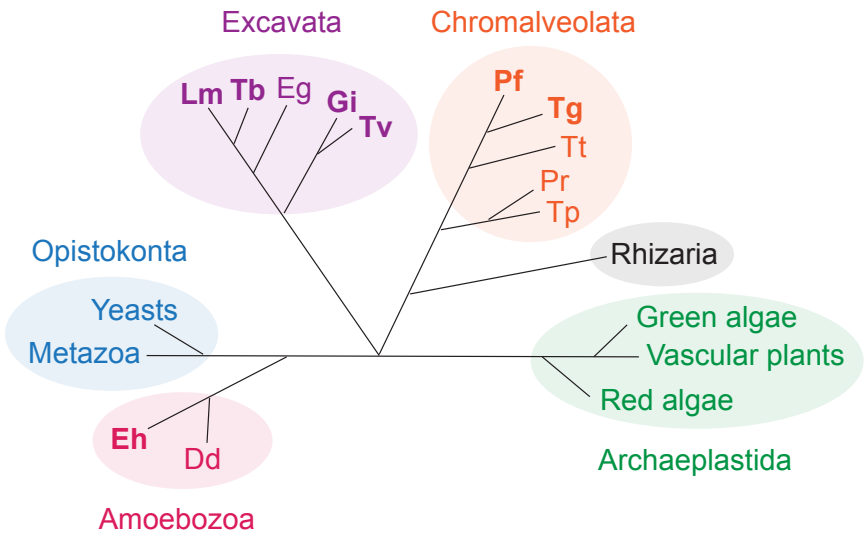
Group	Rabs included	Location/function
I	23	Endosomes, cilia
II	29, 32, 38, 7L1	Post-Golgi, melanosomes
III	L2, L3, L5	
IV	Ran	Nucleocytoplasmic transport
V	7, 9	Late endosomes, lysosomes
VI	28, L4	Endosomes
VII	34, 36	Cell surface, lysosomes
VIII	6, 41	Golgi
IX	5, 17, 20, 21, 22, 24	Endosomes
X	18	Lipid droplets, ER
XI	2, 4, 11, 14, 25, 39, 42	ER, Golgi, polarized cell recycling
XII	19, 30, 33, 43	Golgi
XIII	1, 3, 8, 10, 12, 13, 15, 40	ER, Golgi, recycling endosomes
XIV	26, 27, 37, 44, Rasef	Regulated exocytosis

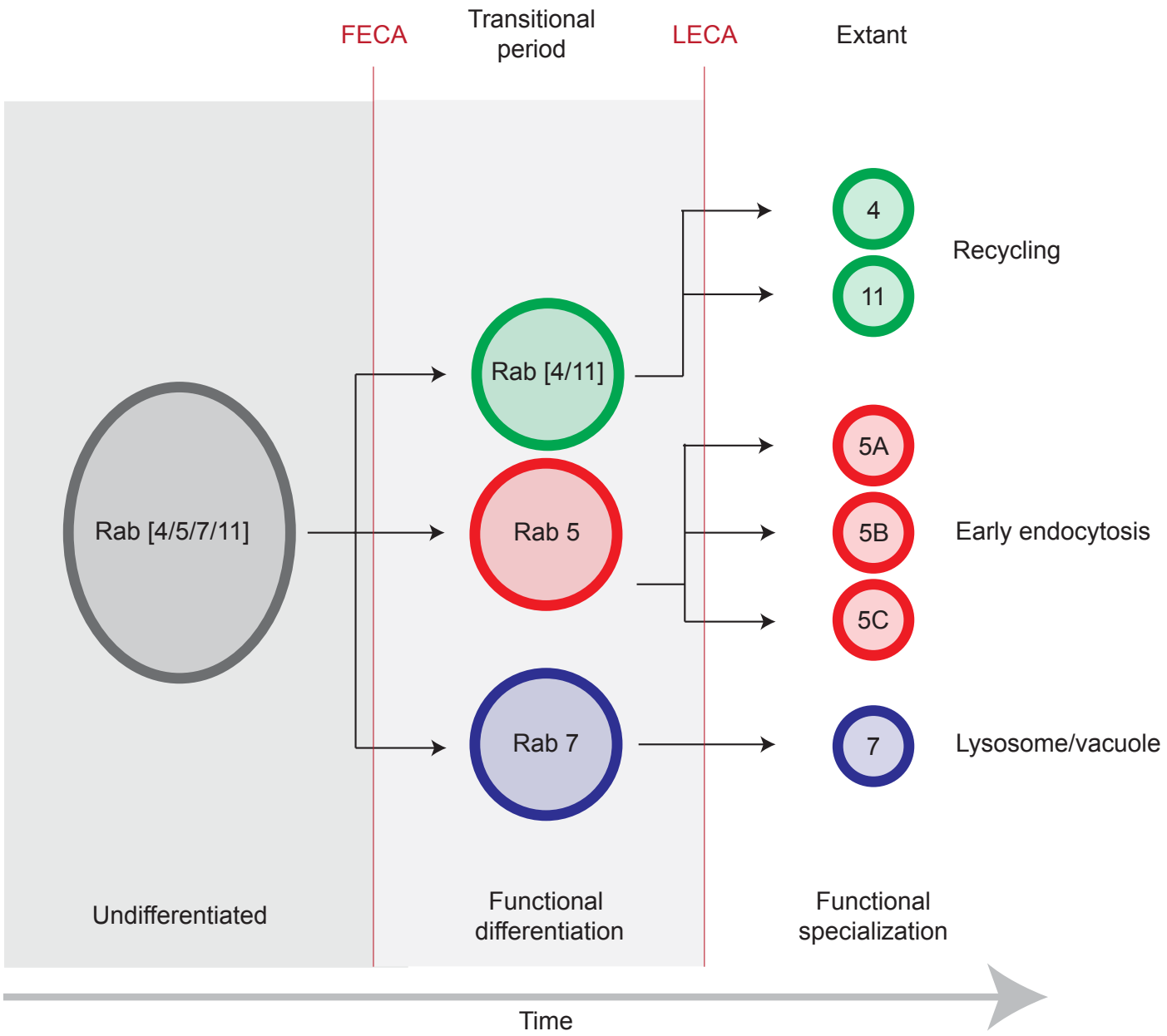


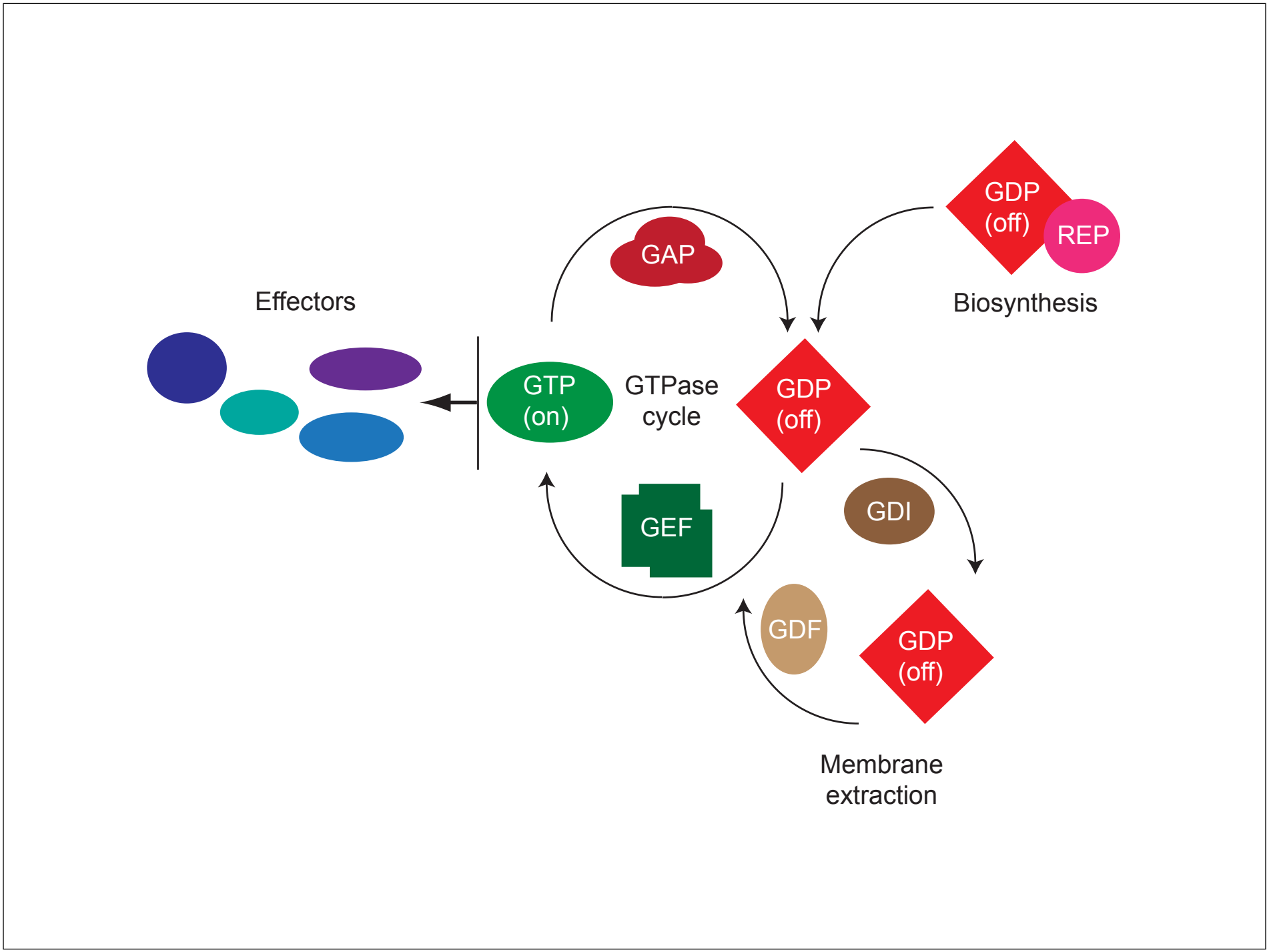


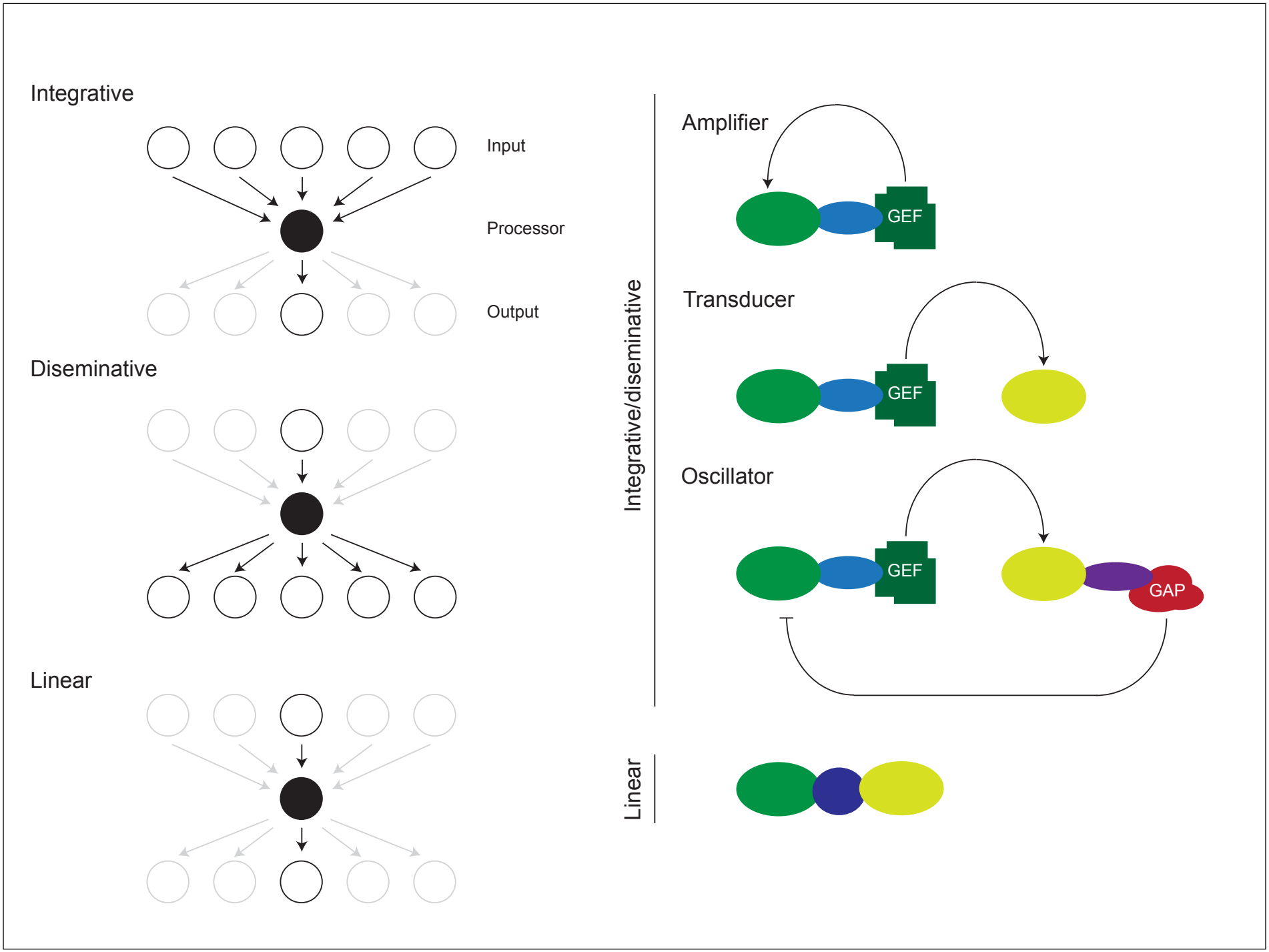


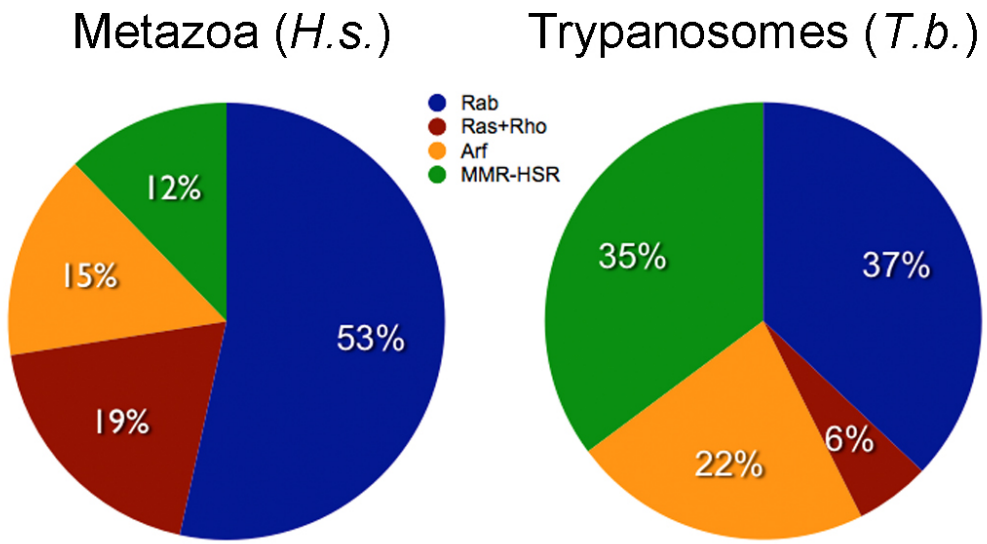
Data paucity and under-sampling
Divergence - inaccurate assignments
Long branch attraction
Ignorance of rate variation

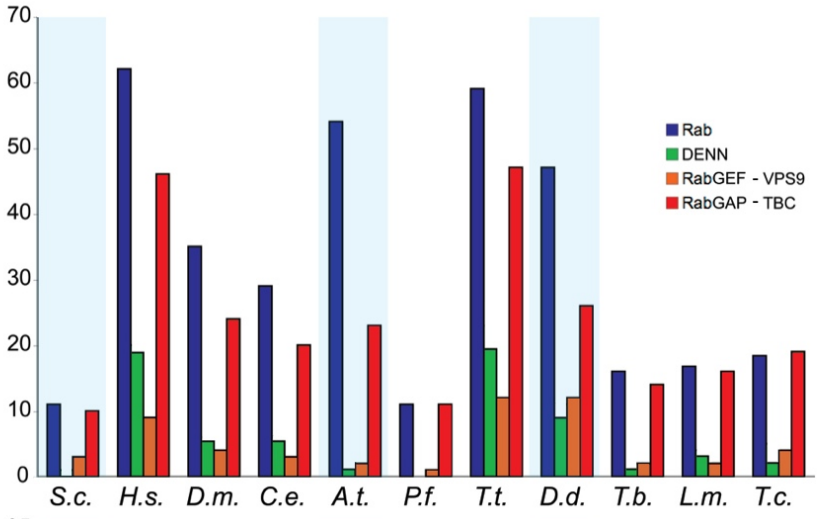


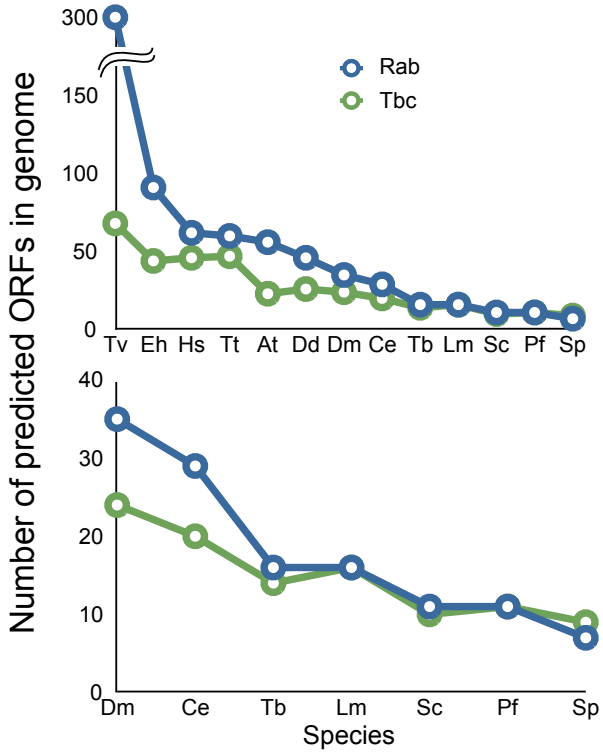

FECA
Transitional period
LECA
Extant
Rab [4/5/7/11]
Rab [4/11]
Rab 5
Rab 7
4
11
5A
5B
5C
7
Recycling
Early endocytosis
Lysosome/vacuole
Undifferentiated
Functional differentiation
Functional specialization
Time

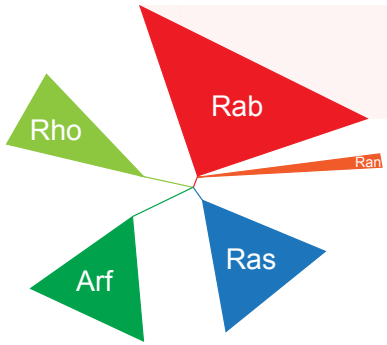

GDP
(off)
REP
GAP
Biosynthesis
Effectors
GTP
(on)
GTPase
cycle
GDP
(off)
GDI
GEF
GDF
GDP
(off)
Membrane
extraction


Integrative
Input
Processor
Output
Diseminative
Linear
Integrative/diseminative
Amplifier
GEF
Transducer
GEF
Oscillator
GEF
GAP
Linear

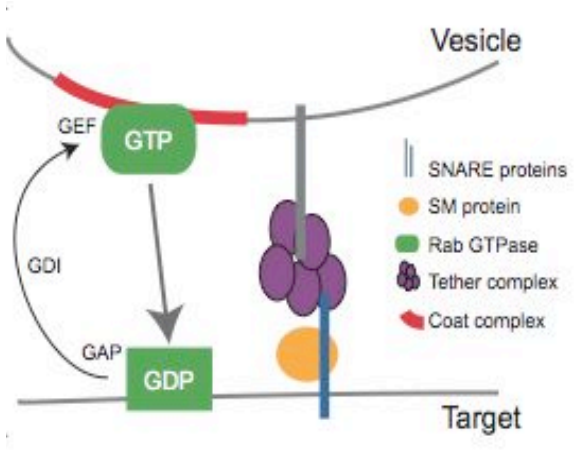

Metazoa (*H.s.*)
Trypanosomes (*T.b.*)
Rab
Ras+Rho
Arf
MMR-HSR
12%
15%
53%
19%
35%
37%
6%
22%

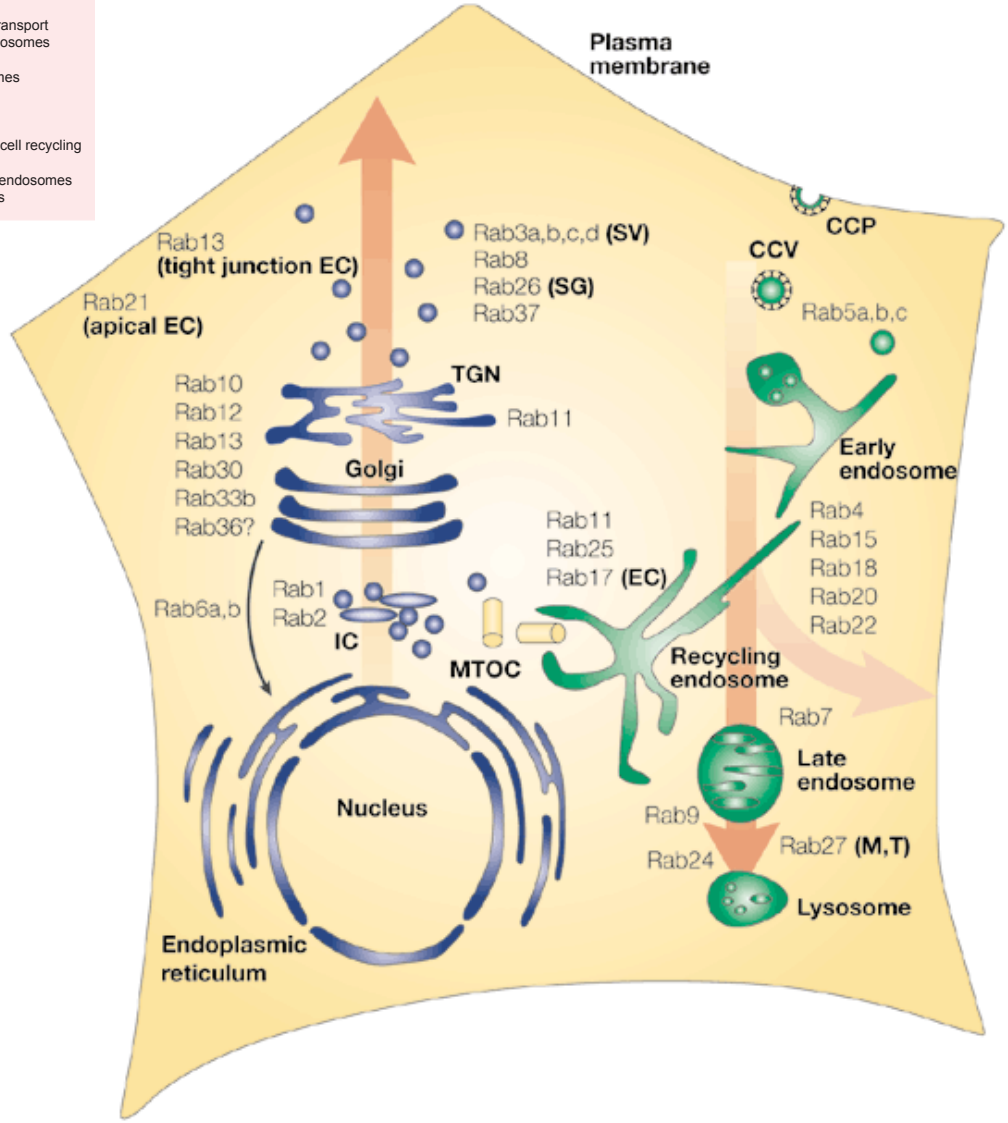

70
60
50
40
30
20
10
0
Rab
DENN
RabGEF - VPS9
RabGAP - TBC
S.c.
H.s.
D.m.
C.e.
A.t.
P.f.
T.t.
D.d.
T.b.
L.m.
T.c.

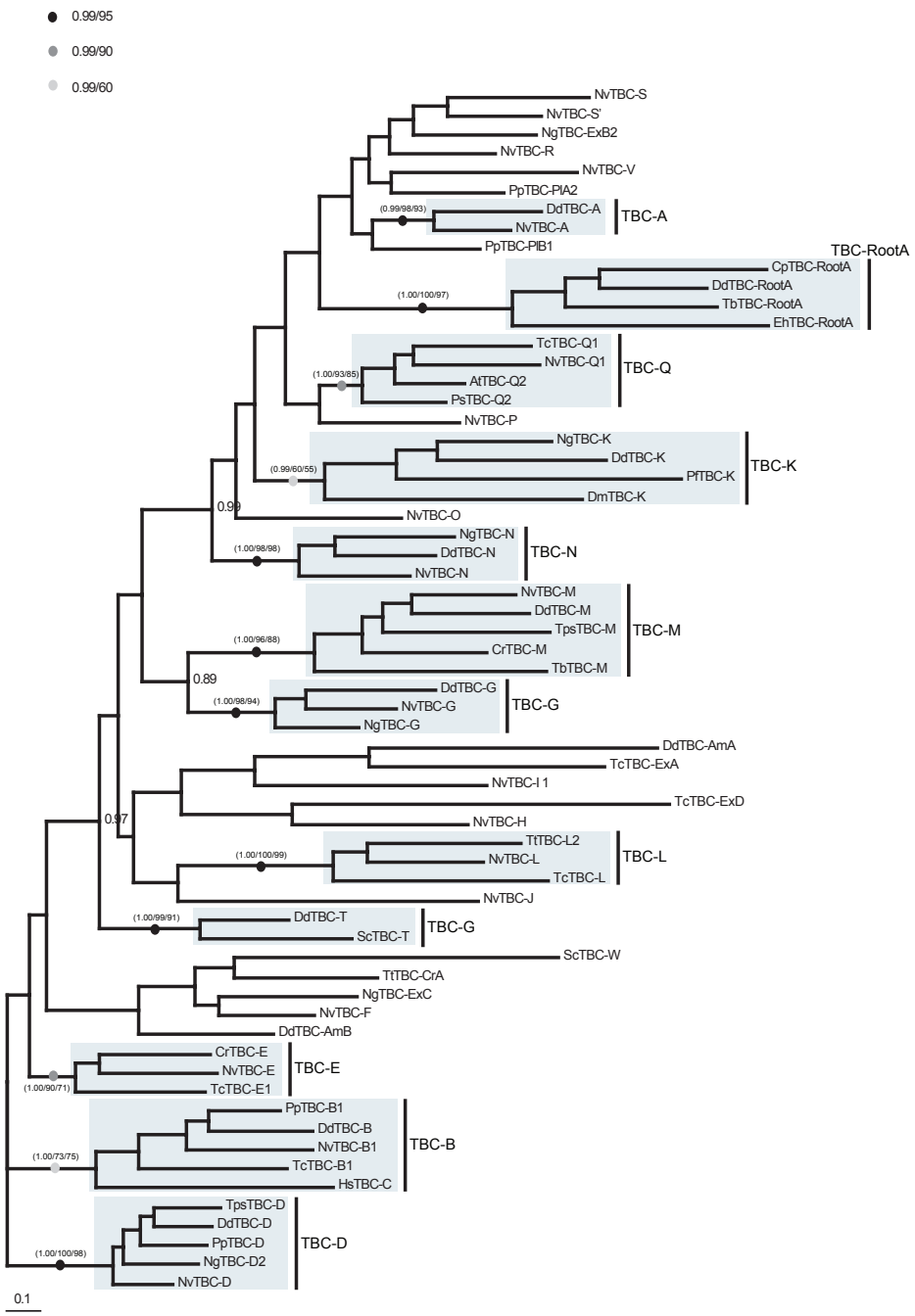

Number of predicted ORFs in genome
300
150
100
50
0
Rab
Tbc
Tv Eh Hs Tt At Dd Dm Ce Tb Lm Sc Pf Sp
40
30
20
10
0
Dm Ce Tb Lm Sc Pf Sp
Species

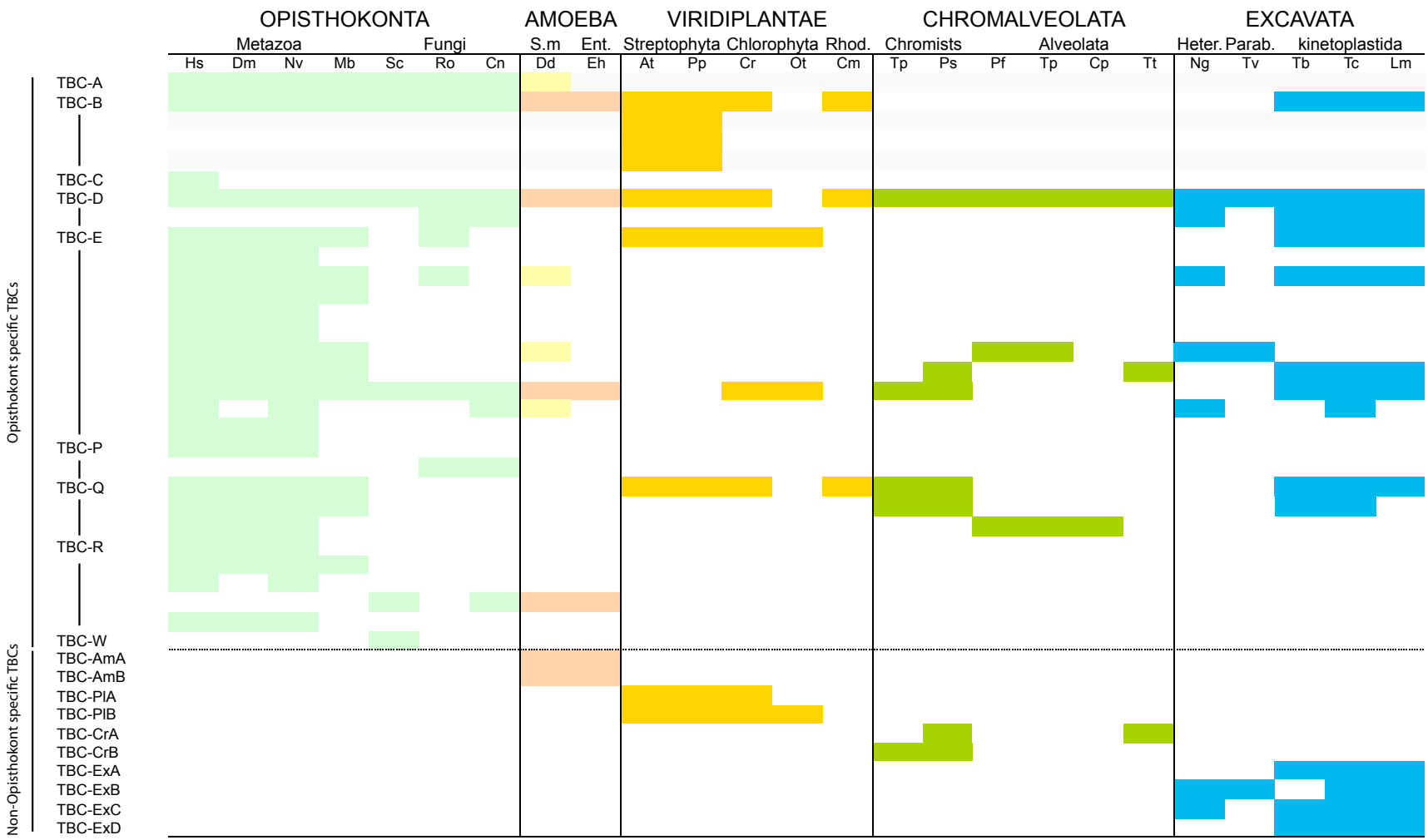


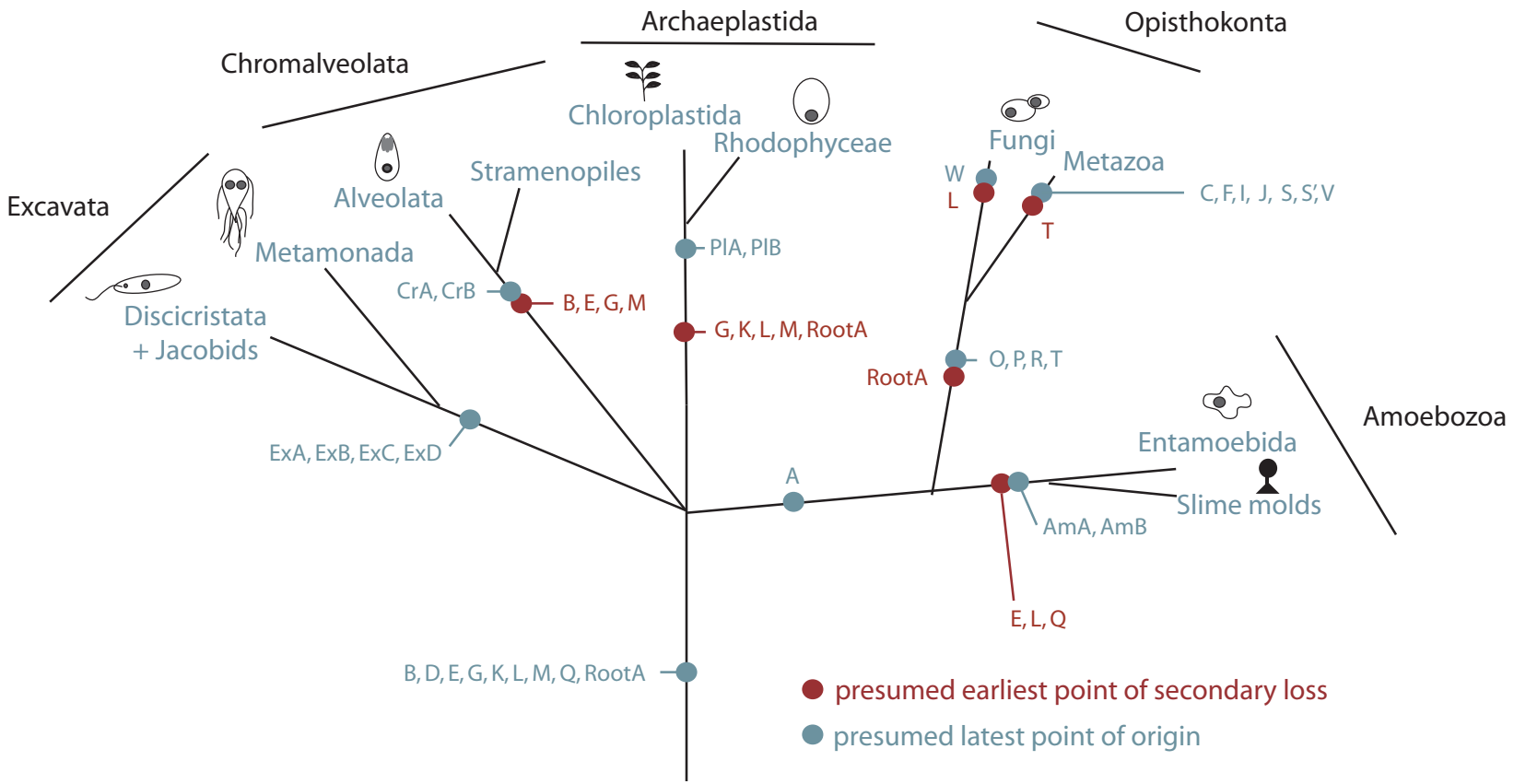
Group	Rabs included	Location/function
I	23	Endosomes, cilia
II	29, 32, 38, 7L1	Post-Golgi, melanosomes
III	L2, L3, L5	
IV	Ran	Nucleocytoplasmic transport
V	7, 9	Late endosomes, lysosomes
VI	28, L4	Endosomes
VII	34, 36	Cell surface, lysosomes
VIII	6, 41	Golgi
IX	5, 17, 20, 21, 22, 24	Endosomes
X	18	Lipid droplets, ER
XI	2, 4, 11, 14, 25, 39, 42	ER, Golgi, polarized cell recycling
XII	19, 30, 33, 43	Golgi
XIII	1, 3, 8, 10, 12, 13, 15, 40	ER, Golgi, recycling endosomes
XIV	26, 27, 37, 44, Rasef	Regulated exocytosis

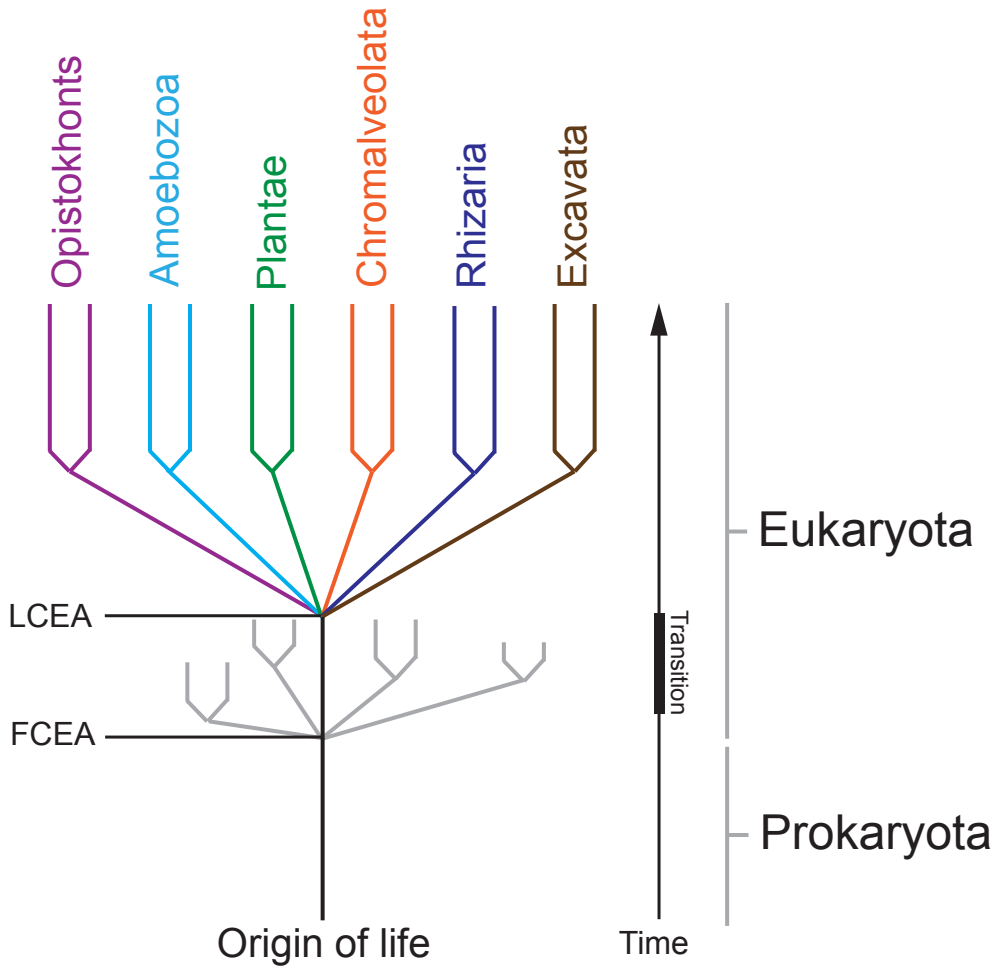





0.99/95
0.99/90
0.99/60
NvTBC-S
NvTBC-S'
NgTBC-ExB2
NvTBC-R
NvTBC-V
PpTBC-PlA2
(0.99/98/93)
DdTBC-A
NvTBC-A
TBC-A
PpTBC-PlB1
TBC-RootA
CpTBC-RootA
DdTBC-RootA
(1.00/100/97)
TbTBC-RootA
EhTBC-RootA
TcTBC-Q1
NvTBC-Q1
(1.00/93/85)
TBC-Q
AtTBC-Q2
PsTBC-Q2
NvTBC-P
NgTBC-K
DdTBC-K
(0.99/60/55)
PfTBC-K
TBC-K
DmTBC-K
0.99
NvTBC-O
NgTBC-N
(1.00/98/98)
DdTBC-N
TBC-N
NvTBC-N
NvTBC-M
DdTBC-M
TpsTBC-M
TBC-M
(1.00/96/88)
CrTBC-M
TbTBC-M
0.89
DdTBC-G
(1.00/98/94)
NvTBC-G
TBC-G
NgTBC-G
DdTBC-AmA
TcTBC-ExA
NvTBC-I 1
TcTBC-ExD
0.97
NvTBC-H
TtTBC-L2
(1.00/100/99)
NvTBC-L
TBC-L
TcTBC-L
NvTBC-J
(1.00/99/91)
DdTBC-T
TBC-G
ScTBC-T
ScTBC-W
TtTBC-CrA
NgTBC-ExC
NvTBC-F
DdTBC-AmB
CrTBC-E
NvTBC-E
TBC-E
(1.00/90/71)
TcTBC-E1
PpTBC-B1
DdTBC-B
NvTBC-B1
TBC-B
(1.00/73/75)
TcTBC-B1
HsTBC-C
TpsTBC-D
DdTBC-D
PpTBC-D
TBC-D
(1.00/100/98)
NgTBC-D2
NvTBC-D
0.1


OPISTHOKONTA
AMOEBA
VIRIDIPLANTAE
CHROMALVEOLATA
EXCAVATA
Metazoa
Fungi
S.m
Ent.
Streptophyta
Chlorophyta
Rhod.
Chromists
Alveolata
Heter.
Parab.
kinetoplastida
Hs
Dm
Nv
Mb
Sc
Ro
Cn
Dd
Eh
At
Pp
Cr
Ot
Cm
Tp
Ps
Pf
Tp
Cp
Tt
Ng
Tv
Tb
Tc
Lm
Opisthokont specific TBCs
Non-Opisthokont specific TBCs
TBC-A
TBC-B
TBC-C
TBC-D
TBC-E
TBC-P
TBC-Q
TBC-R
TBC-W
TBC-AmA
TBC-AmB
TBC-PlA
TBC-PlB
TBC-CrA
TBC-CrB
TBC-ExA
TBC-ExB
TBC-ExC
TBC-ExD


Archaeplastida
Opisthokonta
Chromalveolata
Chloroplastida
Rhodophyceae
Fungi
Metazoa
W
L
C, F, I, J, S, S', V
T
Excavata
Alveolata
Stramenopiles
PlA, PlB
Metamonada
CrA, CrB
B, E, G, M
G, K, L, M, RootA
Discicristata
+ Jacobids
RootA
O, P, R, T
Amoebozoa
Entamoebida
ExA, ExB, ExC, ExD
A
Slime molds
AmA, AmB
E, L, Q
B, D, E, G, K, L, M, Q, RootA
presumed earliest point of secondary loss
presumed latest point of origin


Opistokhonts
Amoebozoa
Plantae
Chromalveolata
Rhizaria
Excavata
LCEA
FCEA
Origin of life
Transition
Time
Eukaryota
Prokaryota

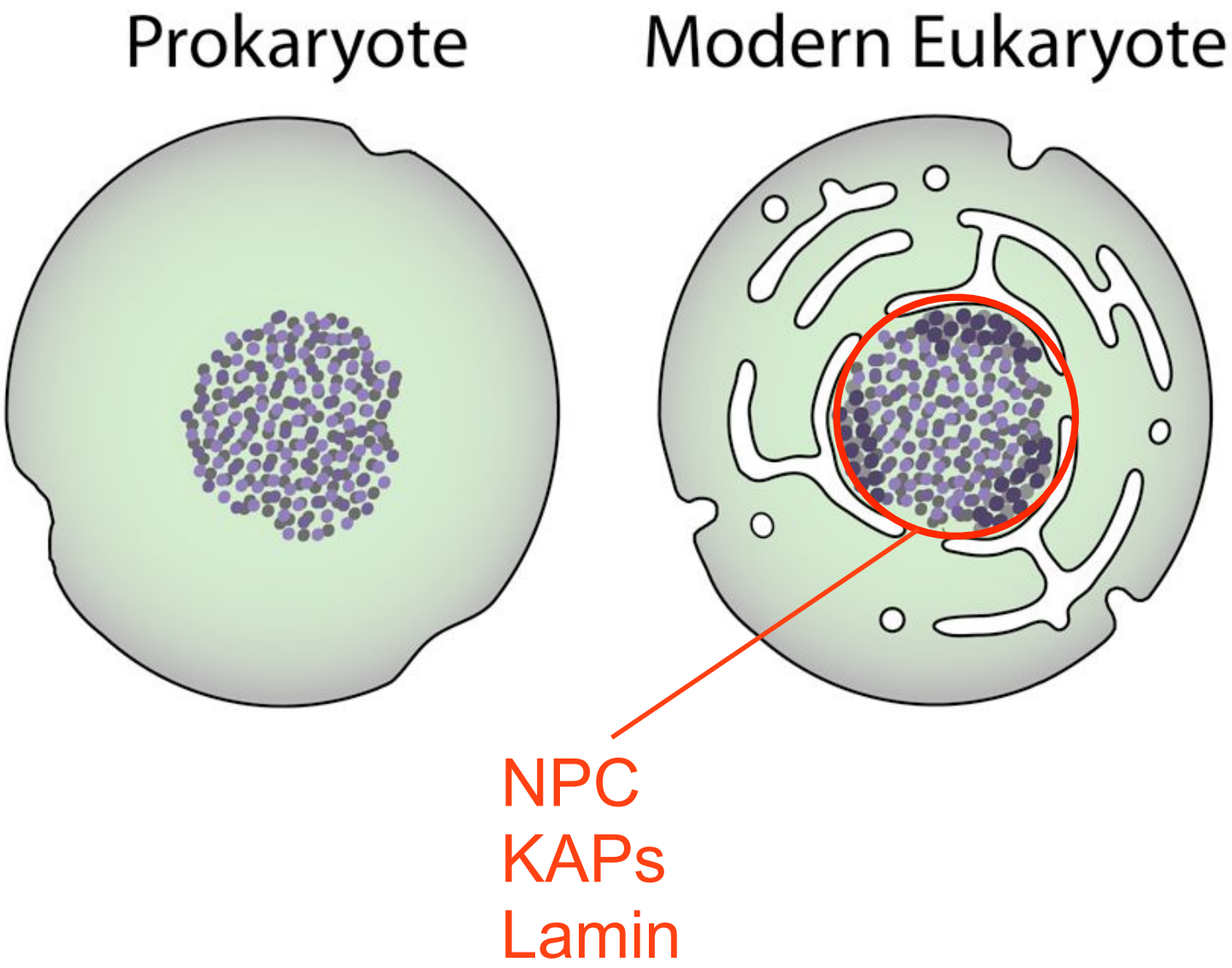
Deep time and origins of the endomembrane system

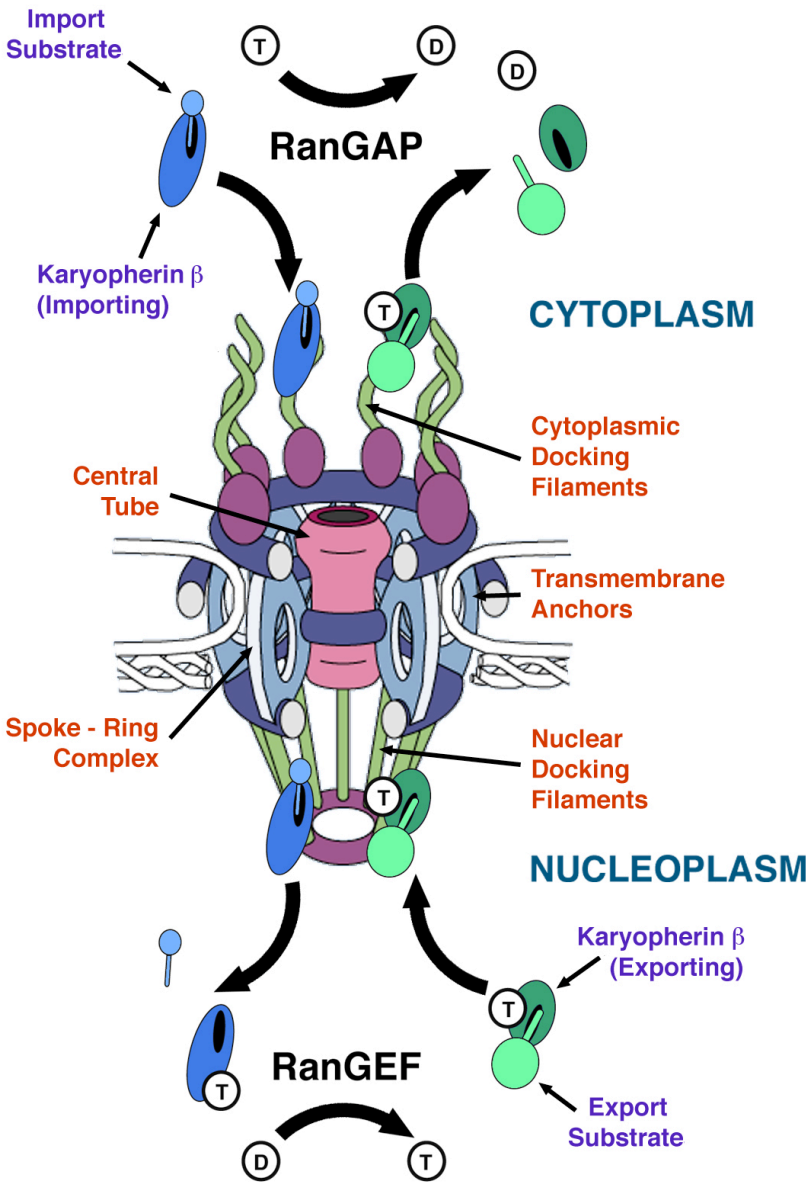
The Golgi complex - Sculpting I

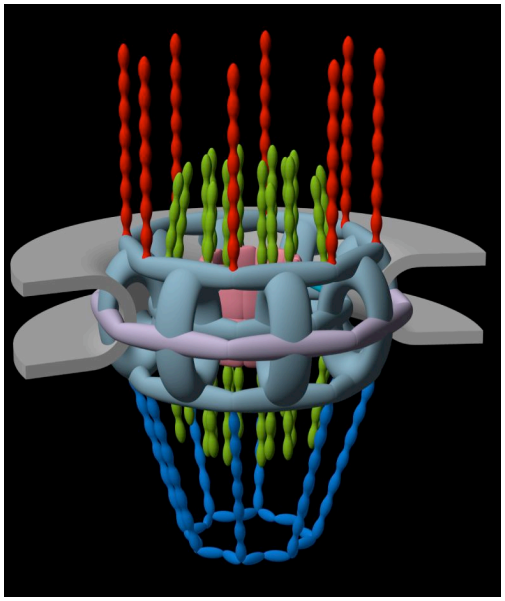
Rab proteins and interaction networks - Sculpting II

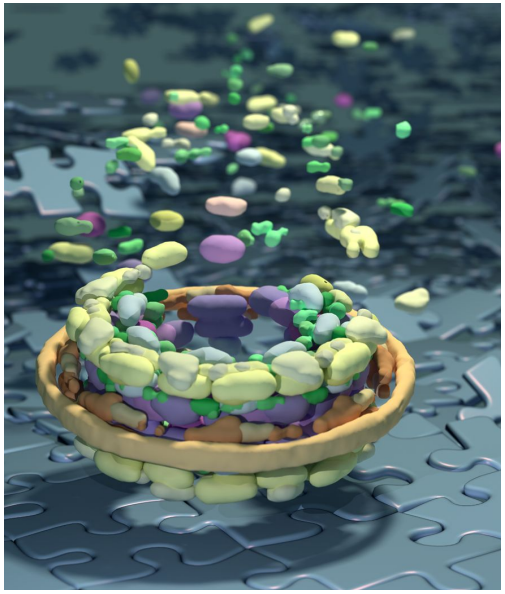
Evolution of the nucleocytoplasmic transport system

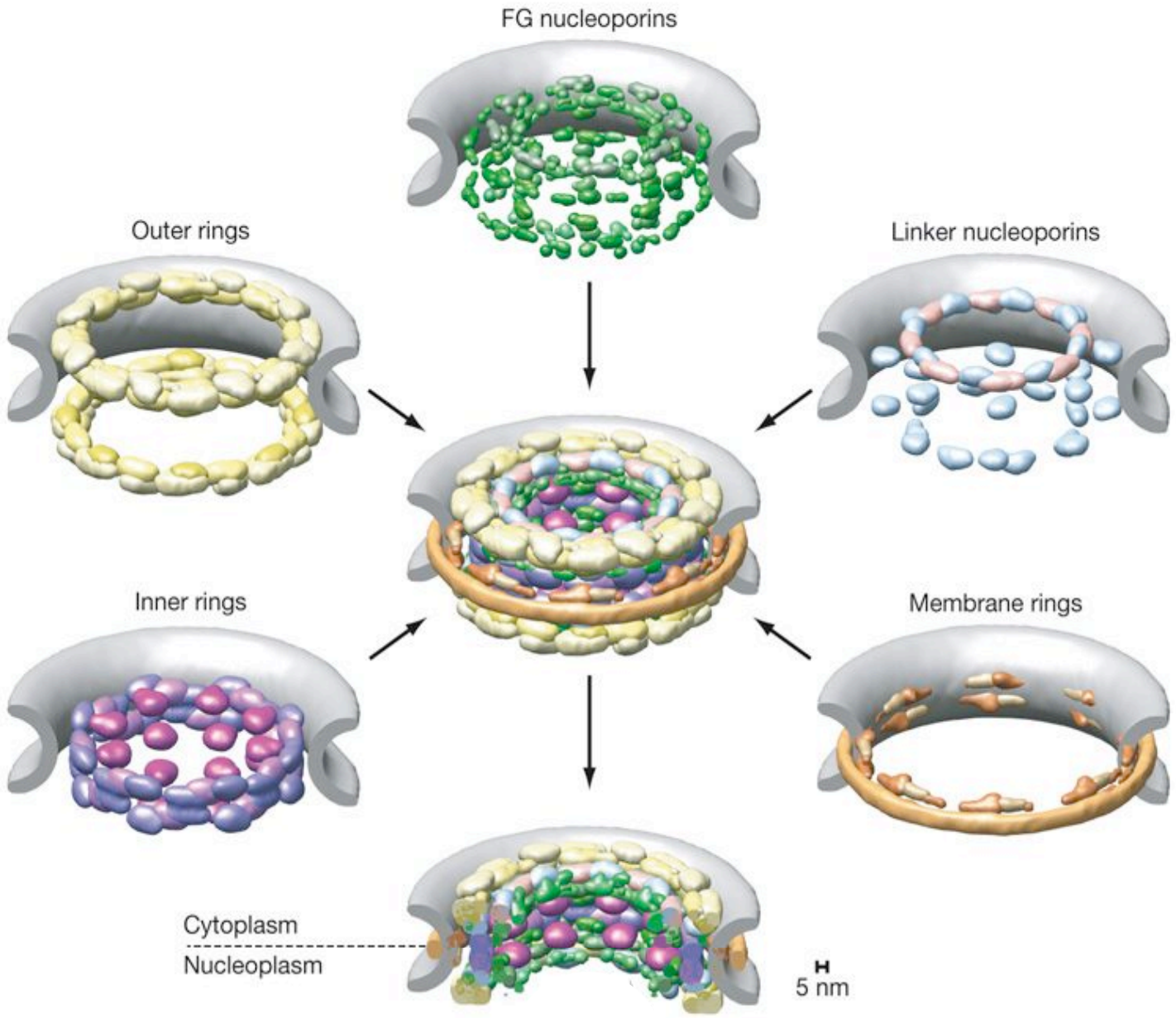
Protocoatomer; putting it all together


Prokaryote
Modern Eukaryote
NPC
KAPs
Lamin


Import Substrate
T
D
D
RanGAP
Karyopherin β (Importing)
CYTOPLASM
T
Cytoplasmic Docking Filaments
Central Tube
Transmembrane Anchors
Spoke - Ring Complex
Nuclear Docking Filaments
T
NUCLEOPLASM
Karyopherin β (Exporting)
T
RanGEF
T
Export Substrate
D
T

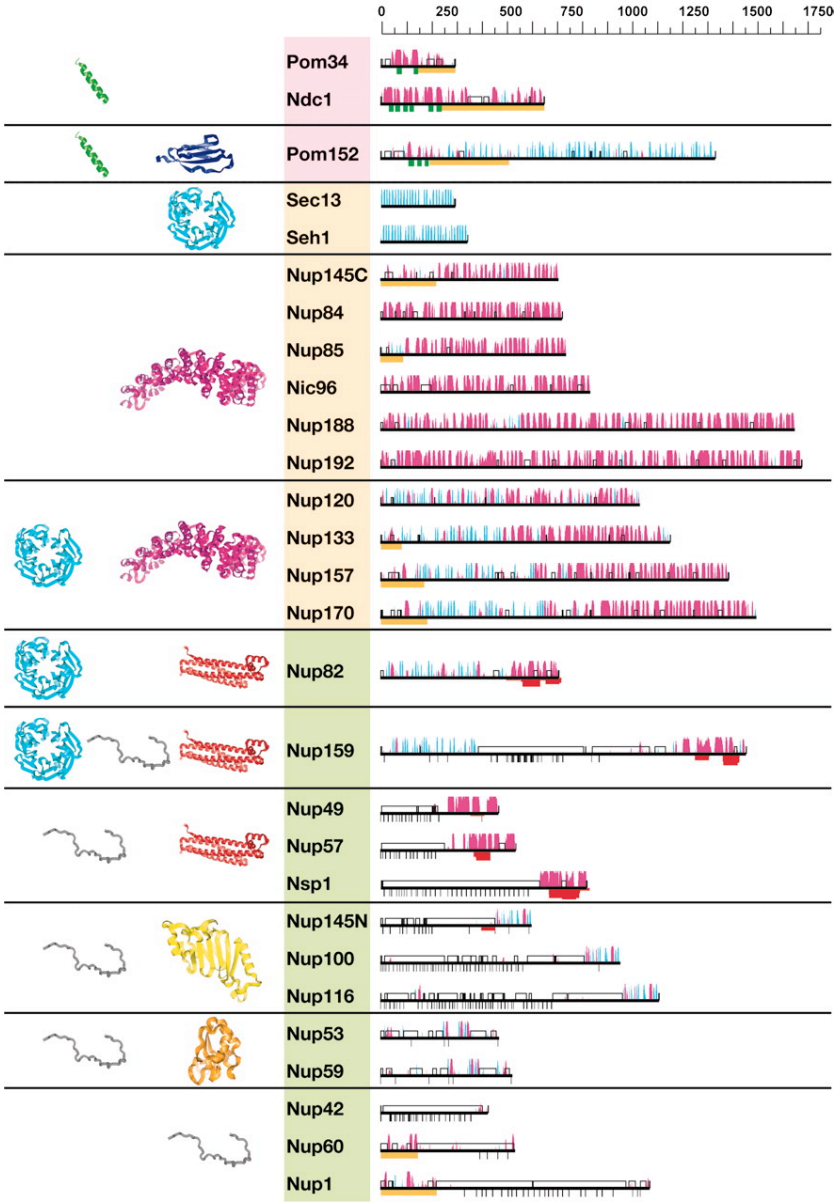






Alber et al. 2007

Opistokhont nucleoporins



Classes of nucleoporins

Trans-membrane

Scaffold

FG repeat

Only 3 major fold types

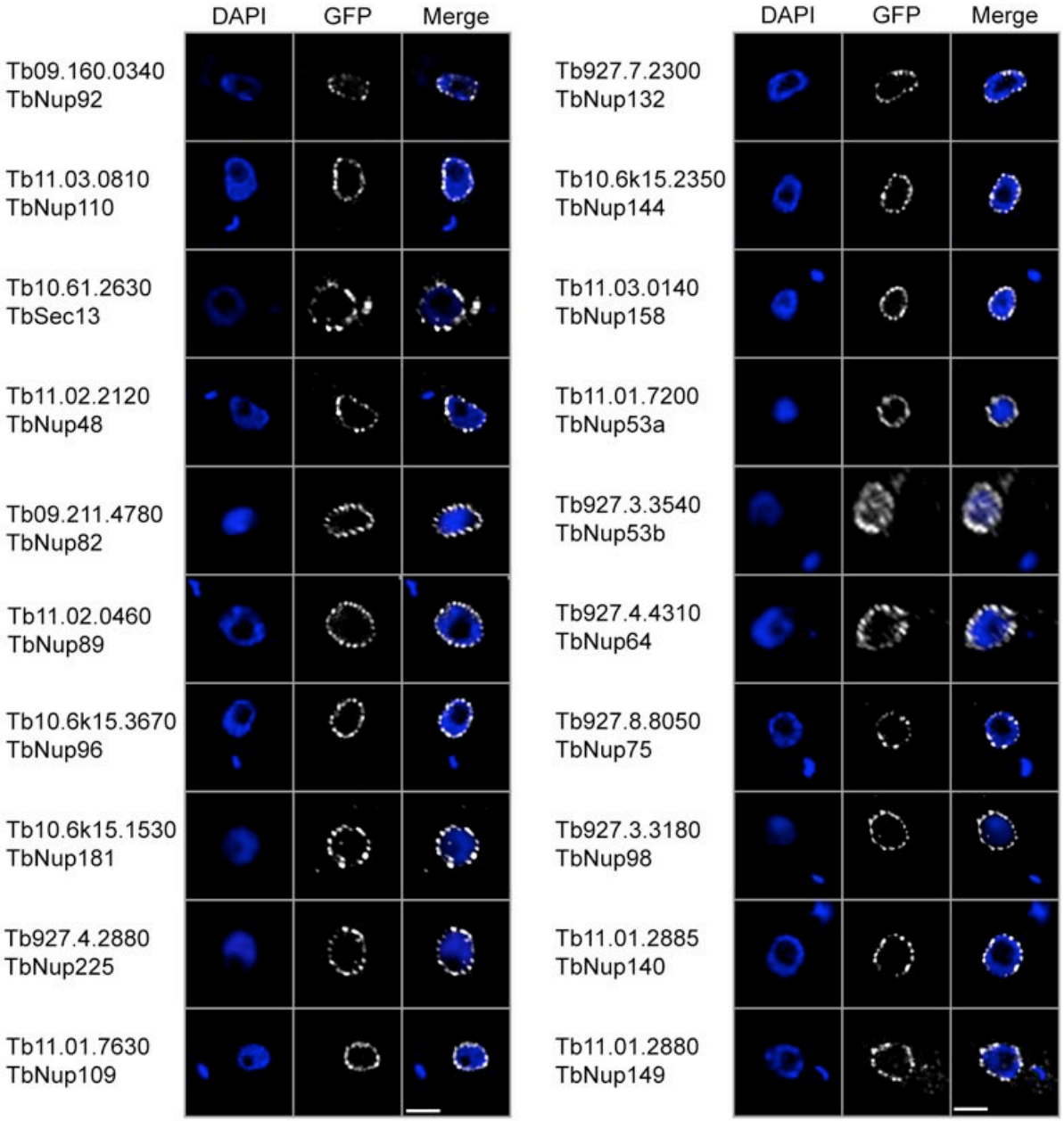
β-propellers

α-solenoid

Disordered FG-repeat

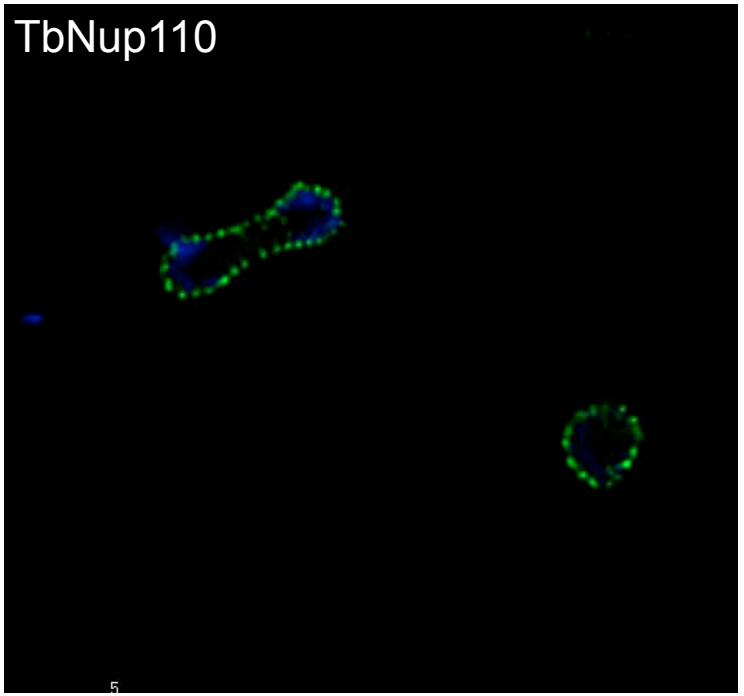
Devos, et. al. 2006

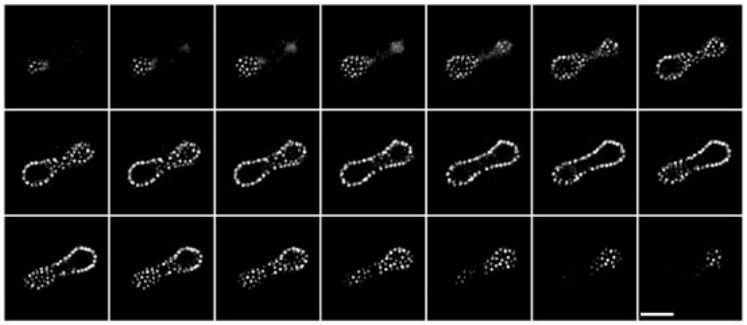
Validation by genomic tagging and localization



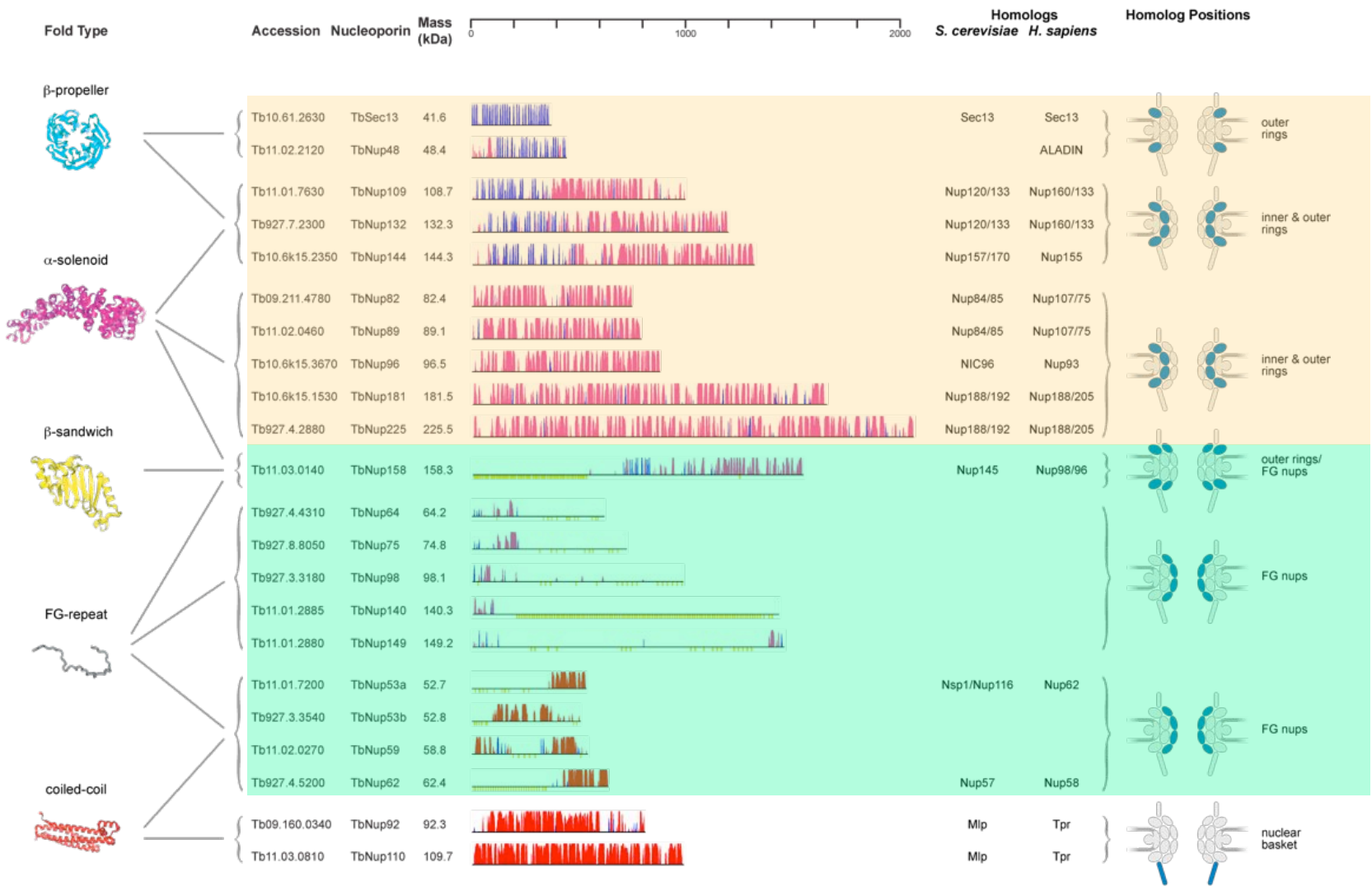
deGrasse and others

TbNup92 has cell-cycle dependent localization

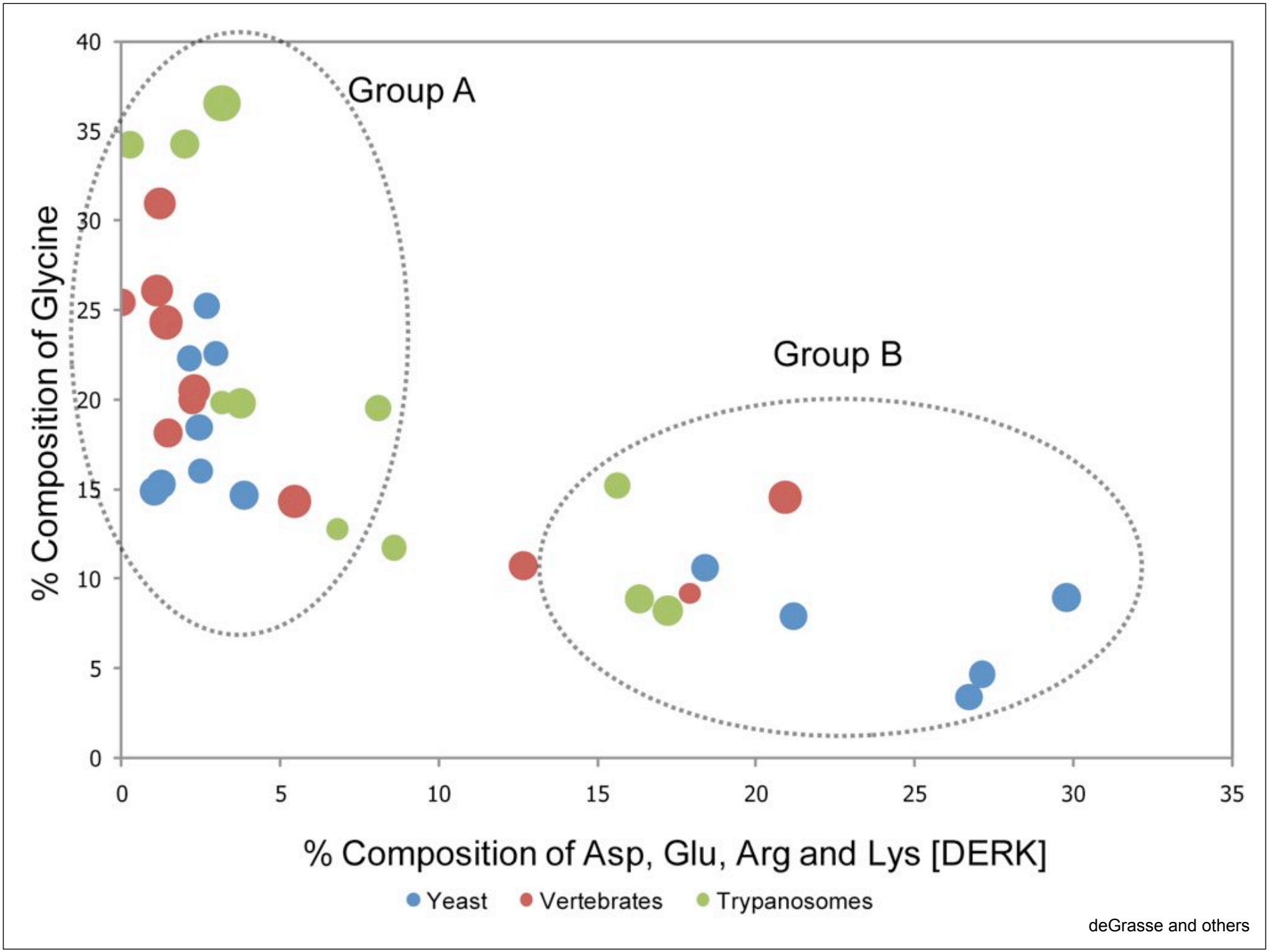


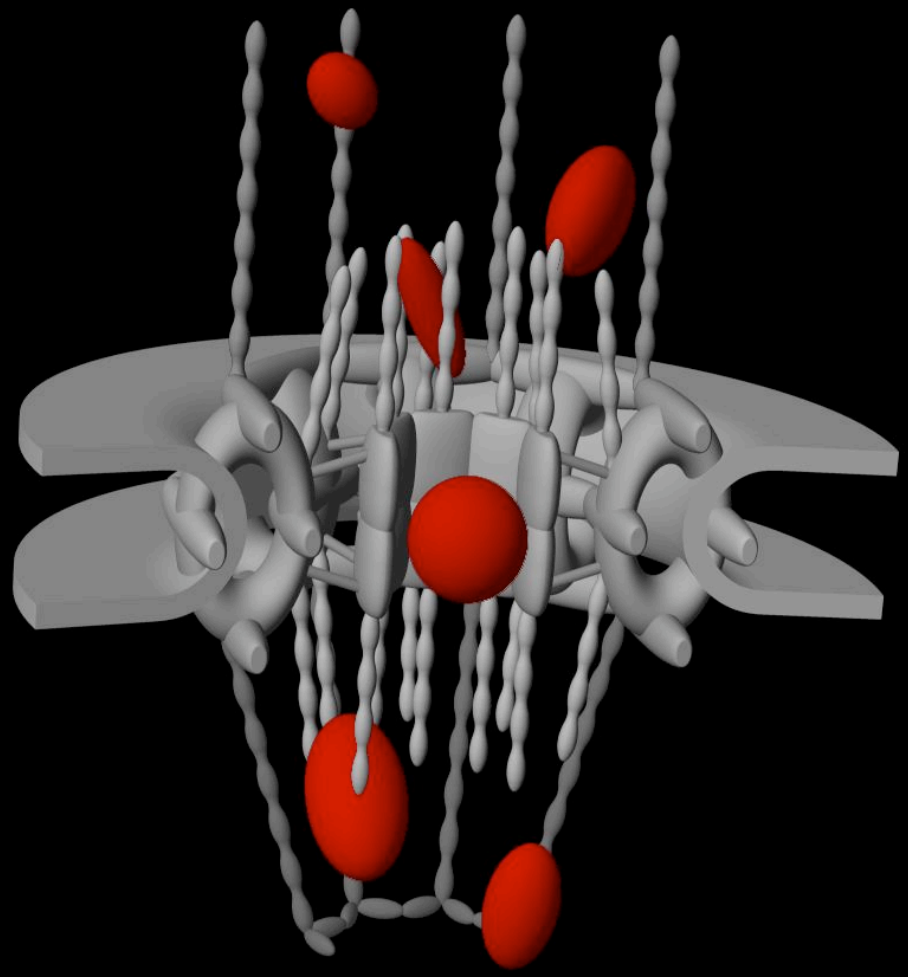


deGrasse and others



deGrasse and others

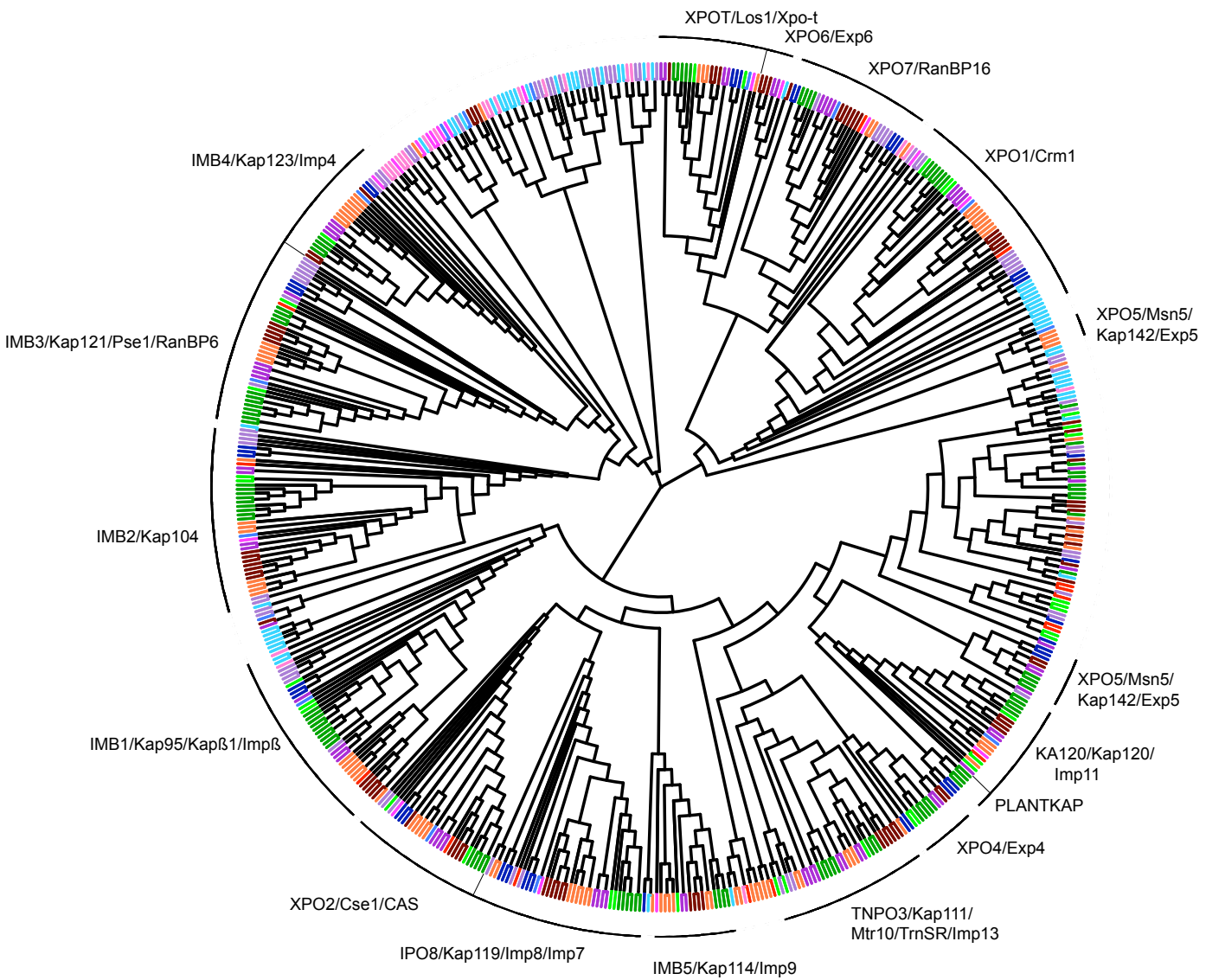

Group A
Group B
% Composition of Glycine
% Composition of Asp, Glu, Arg and Lys [DERK]
0
5
10
15
20
25
30
35
40
Yeast
Vertebrates
Trypanosomes
deGrasse and others



MOBILE PHASE

Rout

The Karyopherins are conserved and ancient



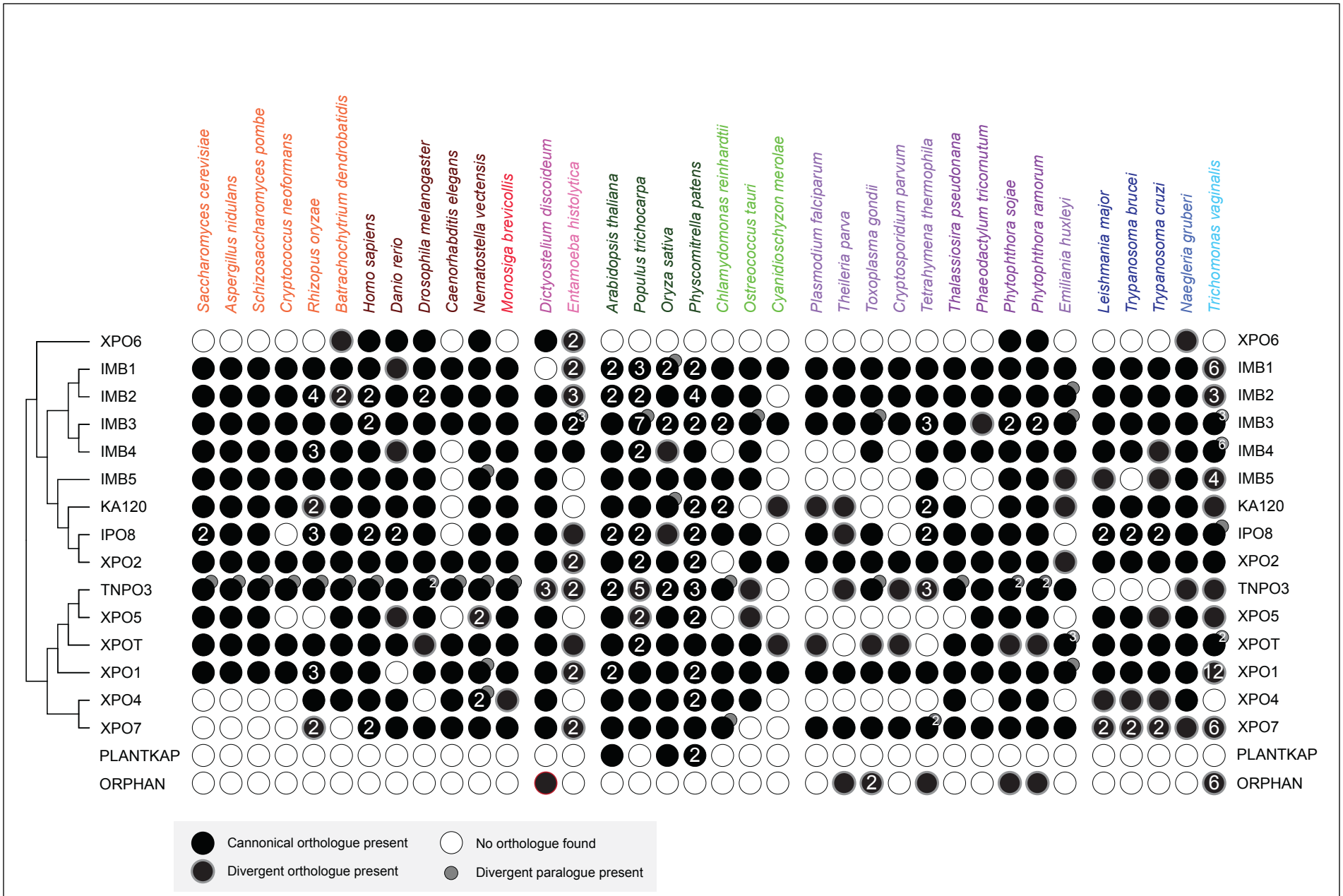
Opisthokonta
- *Saccharomyces cerevisiae*
- *Aspergillus nidulans*
- *Schizosaccharomyces pombe*
- *Cryptococcus neoformans*
- *Rhizopus oryzae*
- *Batrachochytrium dendrobatidis*
- *Homo sapiens*
- *Danio rerio*
- *Drosophila melanogaster*
- *Caenorhabditis elegans*
- *Nematostella vectensis*
- *Monosiga brevicollis*

Amoebozoa
- *Dictyostelium discoideum*
- *Entamoeba histolytica*

Archaeplastida
- *Arabidopsis thaliana*
- *Populus trichocarpa*
- *Oryza sativa*
- *Physcomitrella patens*
- *Chlamydomonas reinhardtii*
- *Ostreococcus tauri*
- *Cyanidioschyzon merolae*

Chromalveolata
- *Plasmodium falciparum*
- *Theileria parva*
- *Toxoplasma gondii*
- *Cryptosporidium parvum*
- *Tetrahymena thermophila*
- *Thalassiosira pseudonana*
- *Phaeodactylum tricornutum*
- *Phytophthora sojae*
- *Phytophthora ramorum*
- *Emiliania huxleyi*

Excavata
- *Leishmania major*
- *Trypanosoma brucei*
- *Trypanosoma cruzi*
- *Naegleria gruberi*
- *Trichomonas vaginalis*


Saccharomyces cerevisiae
Aspergillus nidulans
Schizosaccharomyces pombe
Cryptococcus neoformans
Rhizopus oryzae
Batrachochytrium dendrobatidis
Homo sapiens
Danio rerio
Drosophila melanogaster
Caenorhabditis elegans
Nematostella vectensis
Monosiga brevicollis
Dictyostelium discoideum
Entamoeba histolytica
Arabidopsis thaliana
Populus trichocarpa
Oryza sativa
Physcomitrella patens
Chlamydomonas reinhardtii
Ostreococcus tauri
Cyanidioschyzon merolae
Plasmodium falciparum
Theileria parva
Toxoplasma gondii
Cryptosporidium parvum
Tetrahymena thermophila
Thalassiosira pseudonana
Phaeodactylum tricornutum
Phytophthora sojae
Phytophthora ramorum
Emiliania huxleyi
Leishmania major
Trypanosoma brucei
Trypanosoma cruzi
Naegleria gruberi
Trichomonas vaginalis
XPO6
IMB1
IMB2
IMB3
IMB4
IMB5
KA120
IPO8
XPO2
TNPO3
XPO5
XPOT
XPO1
XPO4
XPO7
PLANTKAP
ORPHAN
Cannonical orthologue present
Divergent orthologue present
No orthologue found
Divergent paralogue present

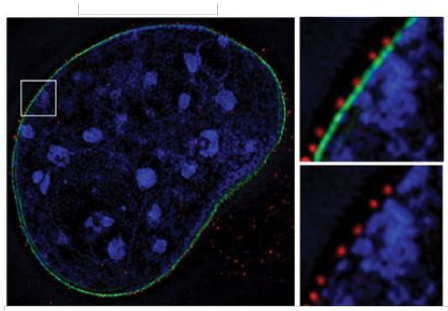
Is there a lamina in excavates?

In higher eukaryotes:

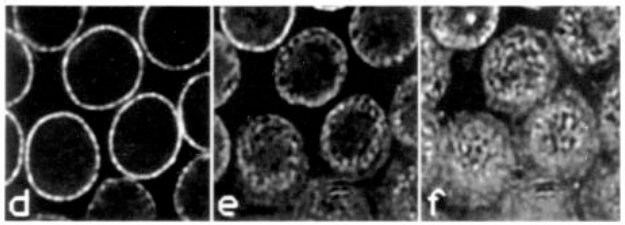
Composed of lamins and lamin-associated proteins

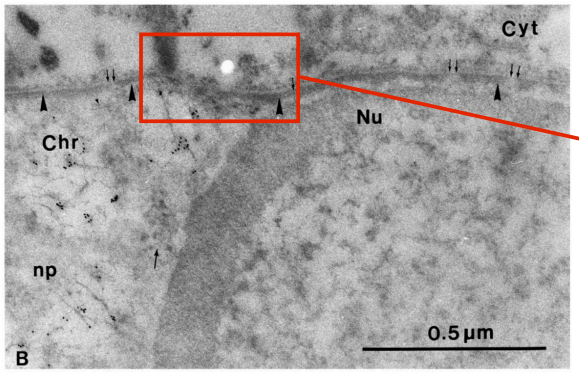
Provide mechanical support for the nucleus

Regulate DNA replication, cell division and heterochromatin organization

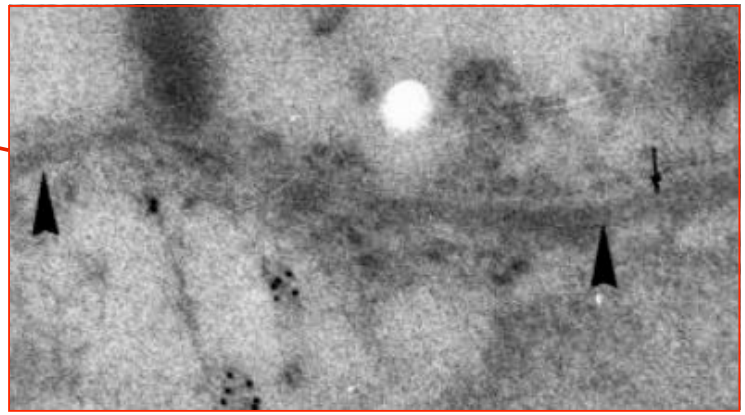


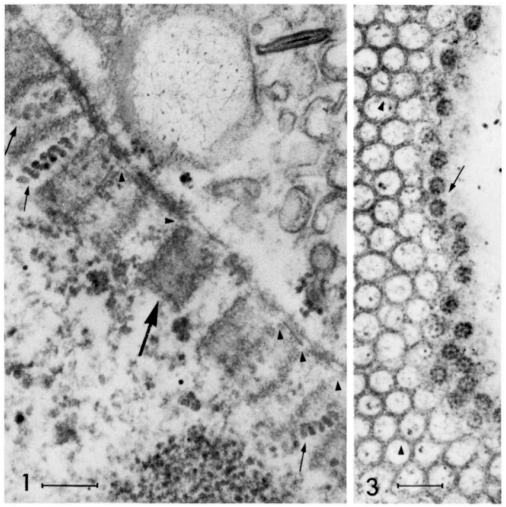
Interphase *Drosophila* nuclei





A. proteus





G. splendens

Lamin orthologues restricted to metazoa

Paddy *et al.,* 1990, Schermelleh *et al.,* 2008, Leeson and Bhatnagar 1975, Minguez 1994

Lamin functions/properties

Maintain structural integrity.

Role in NPC and chromosomal positioning.

Role in gene expression and heterochromatin definition.

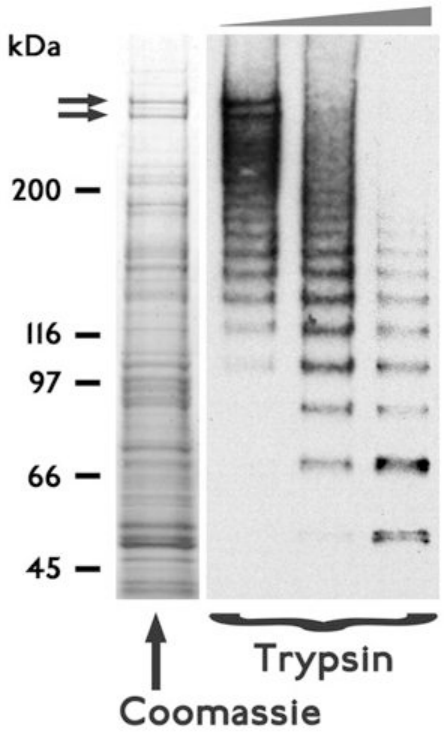
Stable and immobile.

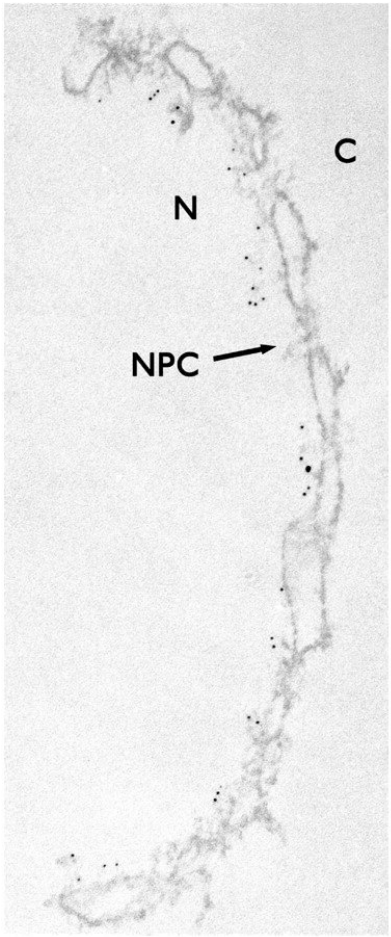
Form filaments.

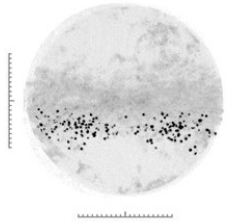
NUP-1

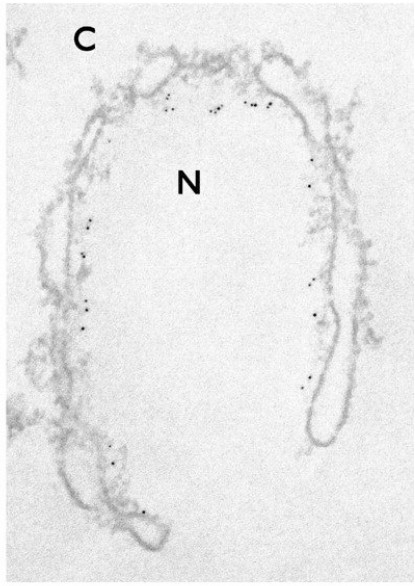
TbNUP-1 contig
CALVSDVAVRNADTDLGTQLASALVALERLAEEREAALEKATEMEERVSTLEKELRTAHSTTKKMSAERELHVTKLTQLEETVSRL
ESYGTTPEQTVAAFTTELQHTQQRLREAEEEIIQLTNKLNAAGVRVRTSQSDKDGNARAALVSDVAVRNADTDLGTQLASALVALE
RLAEEREAALEKATEMEERVSTLEEELRTAHSTTKKMSAERELHVTKLTQLEETVSRLESYGTTPEQTVAAFTTELQHTQQRLREA
EEEIIQLTNK

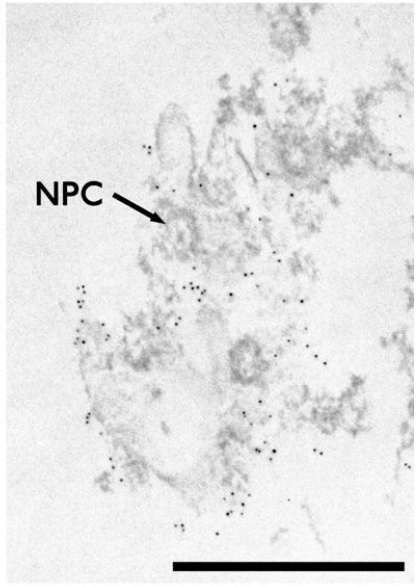
144 repeat	1	E L H V T K L T Q L E E T V S R L E S Y G T T P E Q T V A A F T T E L Q H T Q Q R L R E A E E E I
Partial repeats	1	E L H V T K L T Q L E E T V S R L E S Y G T T P E Q T V A A F T T E L Q H T Q Q R L R E A E E E I
144 repeat	61	V R V R T S Q S D K D G N A R A A L V S D V A V R N A D T D L G T Q L A S A L V A L E R L A E E R
Partial repeats	58	C A L V S D V A V R N A D T D L G T Q L A S A L V A L E R L A E E R
144 repeat	121	E R V S T L E E L R T A H S T T K K M S A E R
Partial repeats	109	E R V S T L E K E L R T A H S T T K K M S A E R





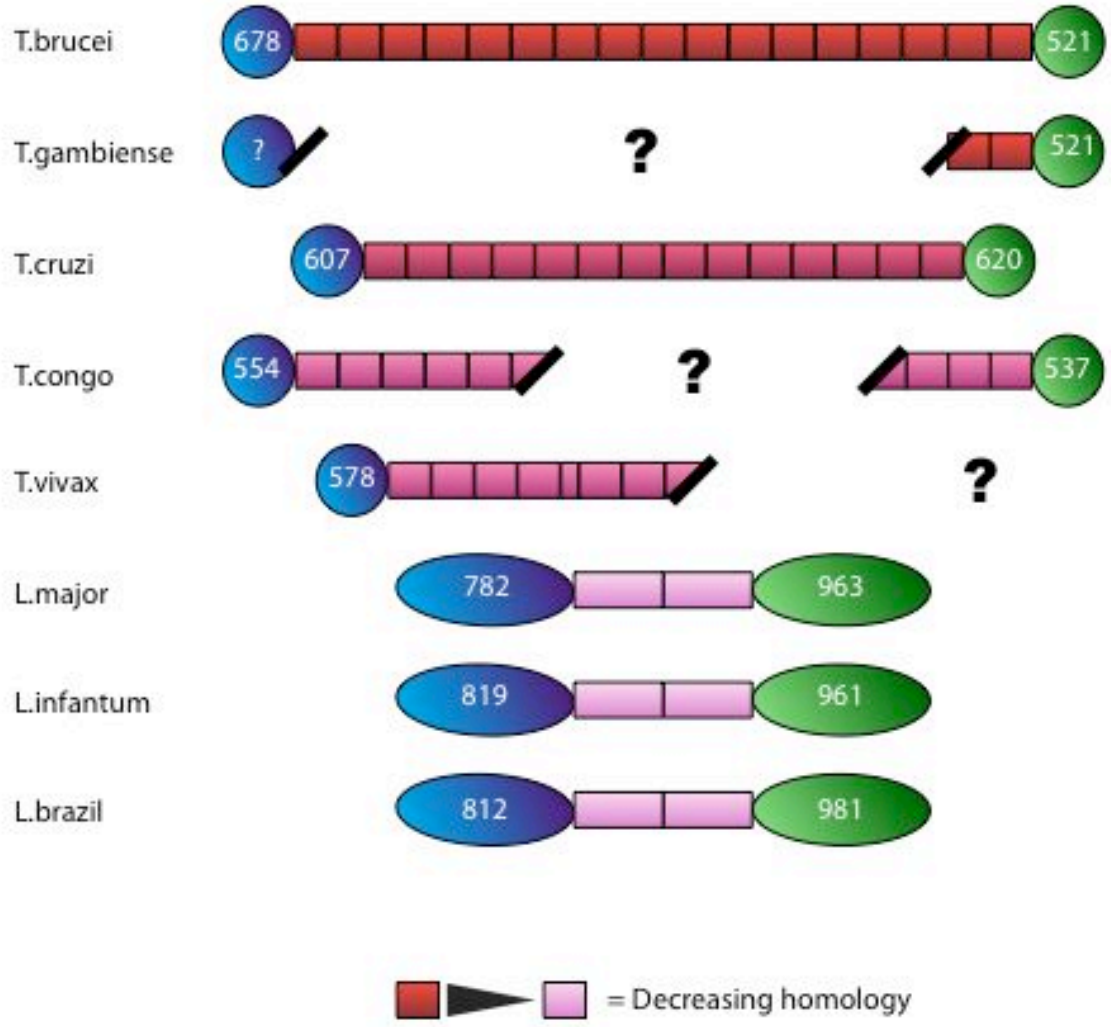






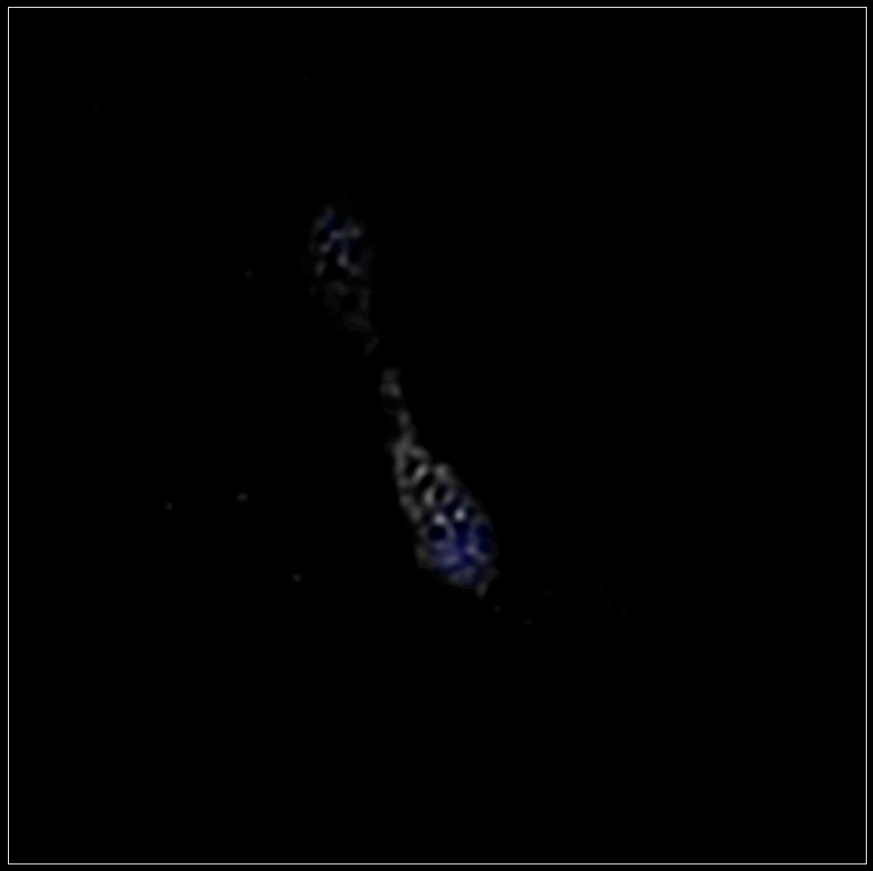
Rout and Field

NUP-1 is conserved in trypanosomes



	No. Repeats	Length of Repeat (bp)
T.brucei	17	144
T.gambiense	≥ 2	144
T.cruzi	14	150
T.congo	≥ 6	147
T.vivax	≥ 7.5	157
L.major	2	256
L.infantum	2	256
L.brazil	2	256

NUP-1 forms a basket

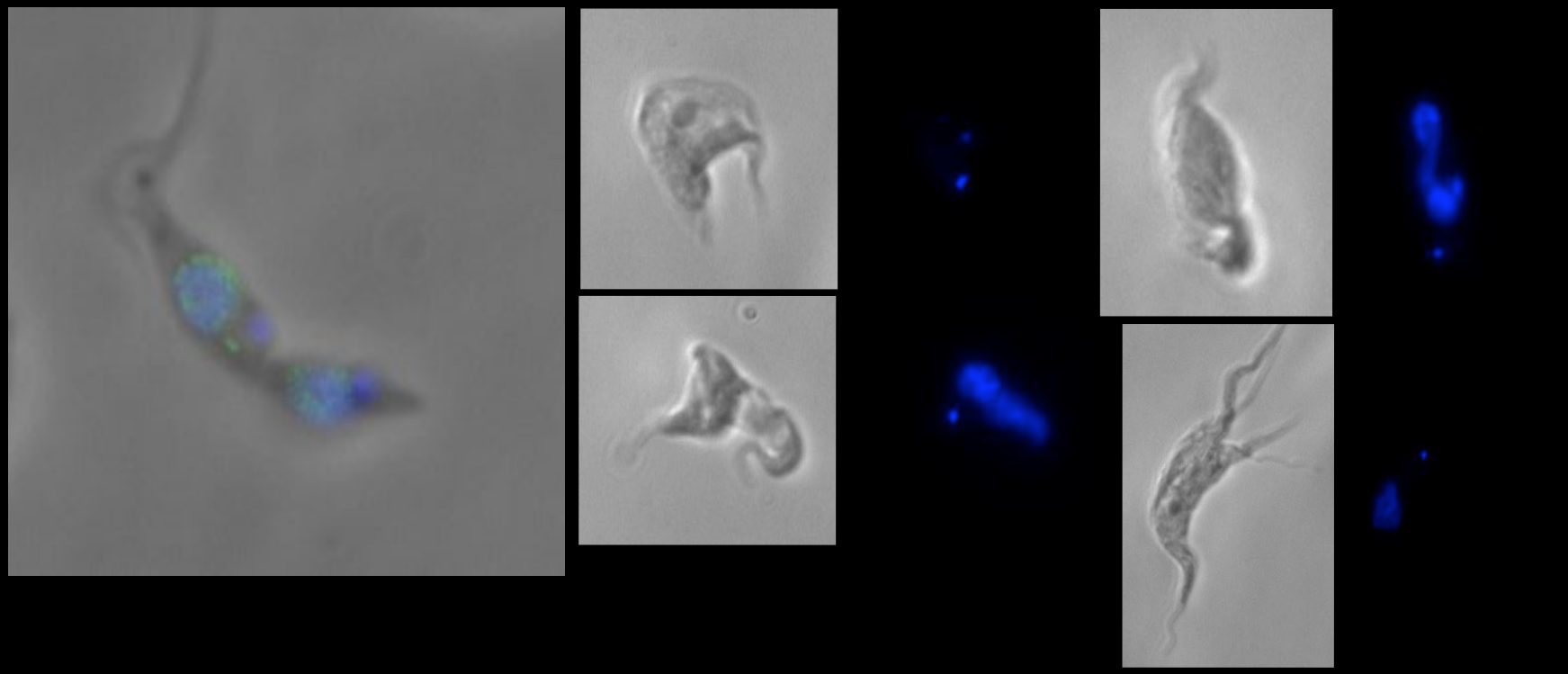


duBois, Field, Rout

NUP-1 is required for nuclear integrity

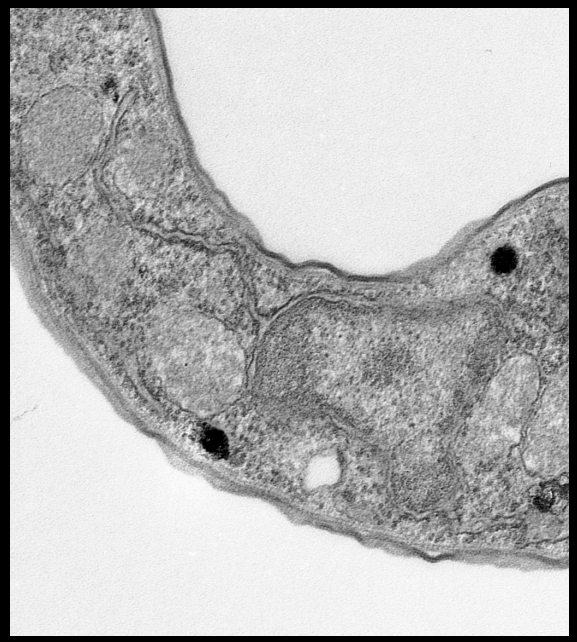
Control

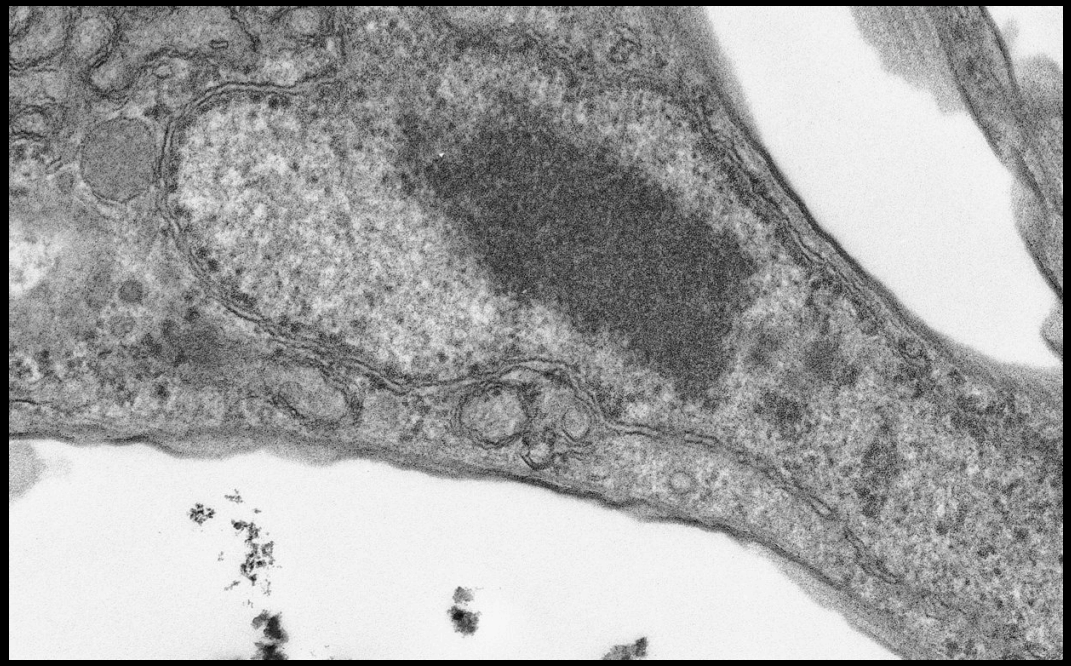
RNAi

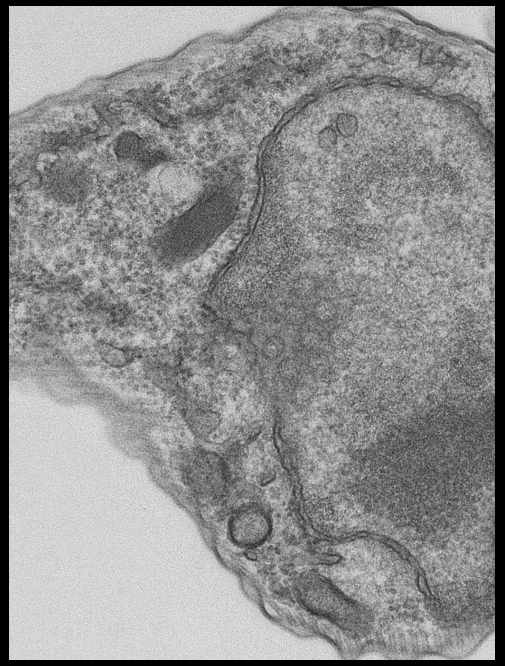


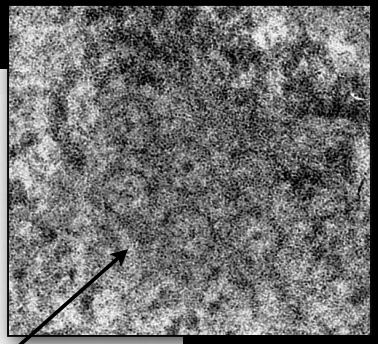
GFP DAPI

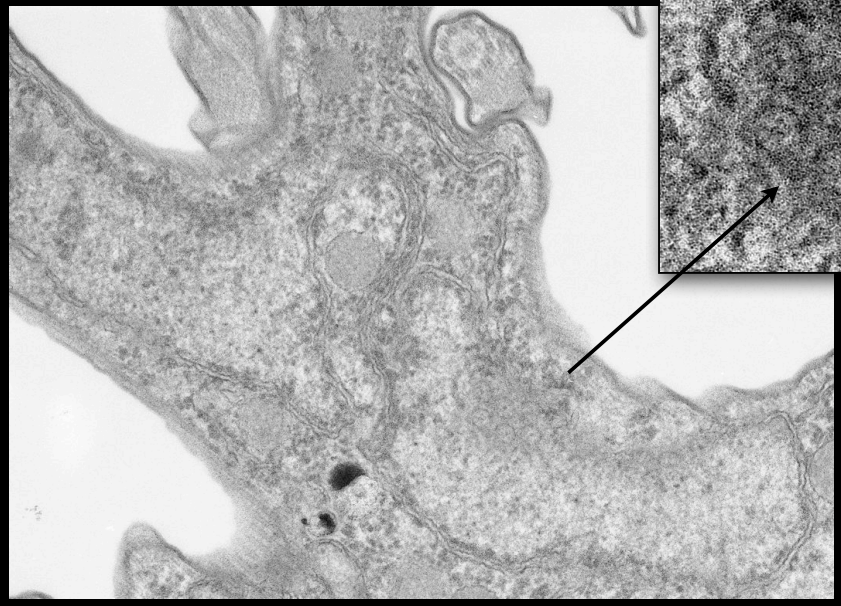
duBois











Lamin functions/properties

Maintain structural integrity.

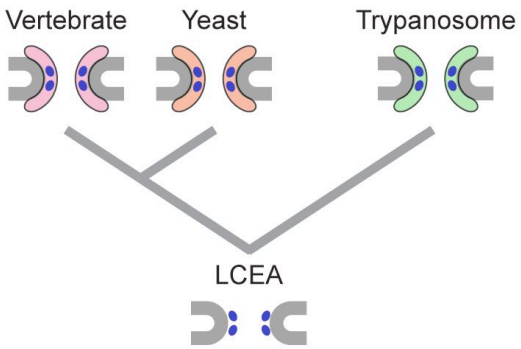
Role in NPC and chromosomal positioning.

Role in gene expression and heterochromatin definition.

Stable and immobile.

Form filaments.

A model for NPC evolution



deGrasse and others

Deep time and origins of the endomembrane system

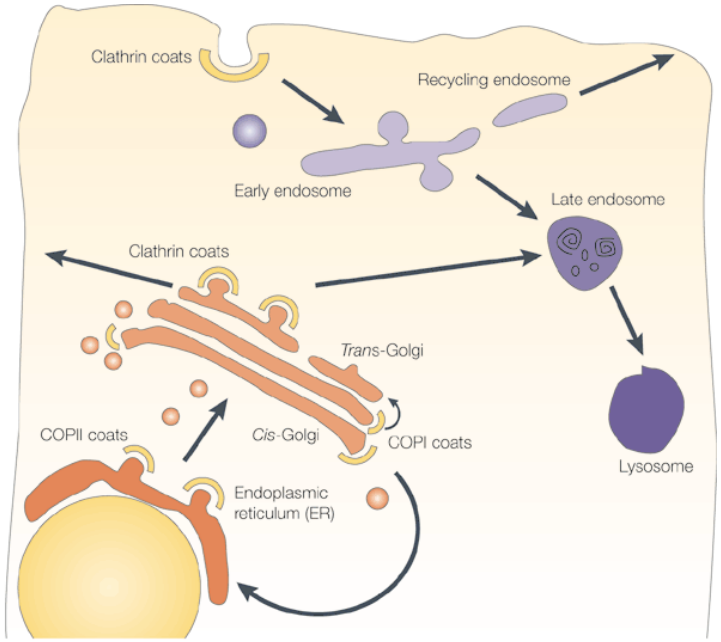
The Golgi complex - Sculpting I

Rab proteins and interaction networks - Sculpting II

Evolution of the nucleocytoplasmic transport system

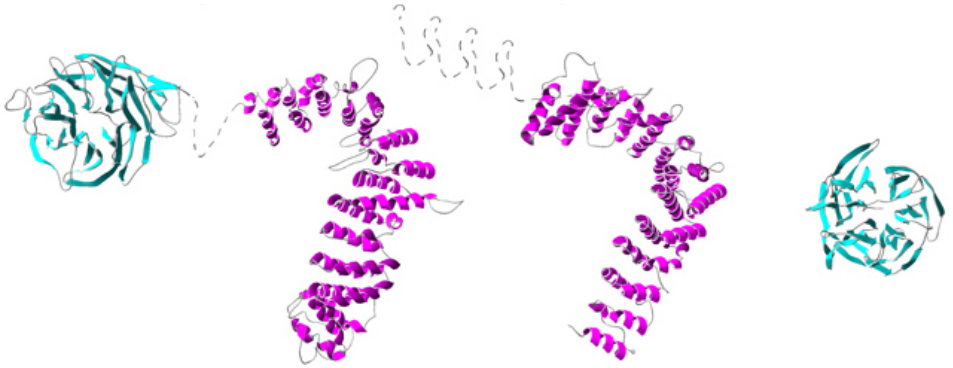
Protocoatomer; putting it all together

Core scaffold: NPC structure and origin



Clathrin and adaptin homologues found in different coated vesicles

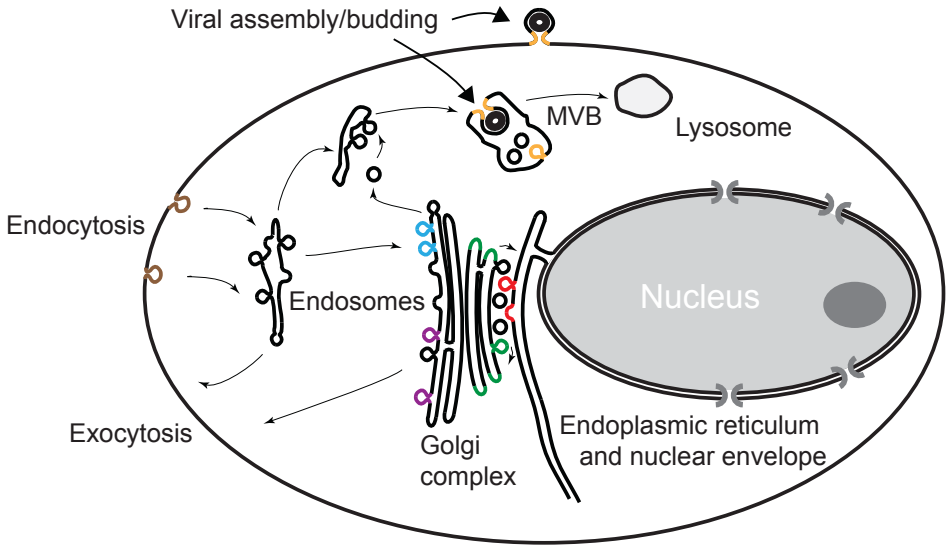
Nup120 Nup84 Sec13



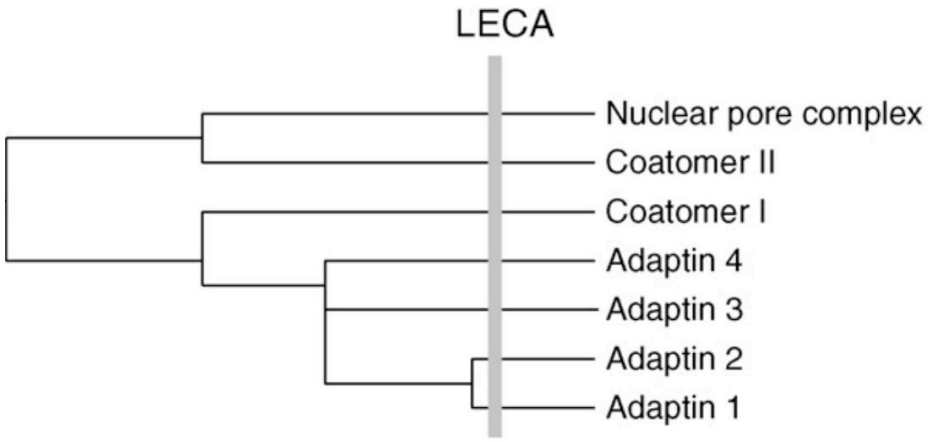
Clathrin-like	Adaptin-like	Sec13-like
Nup133	Nup192	Seh1
Nup120	Nup188	Sec13
Nup170	Nup145C	
Nup157	Nup85	
	Nup84	

Devos *et al.*,

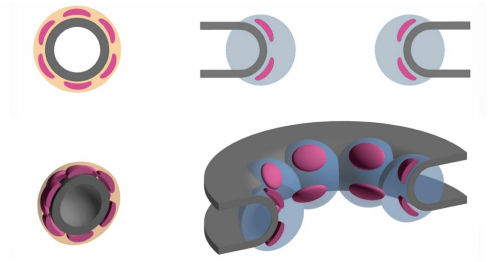
Evolution of vesicle coats and NPCs



Complex	Color	Topology
Nuclear pore complex		+
Coatomer II		+
Coatomer I		+
Adaptin 4		+
Adaptin 3		+
Adaptin 2		+
Adaptin 1		+
ESCRT complex		-



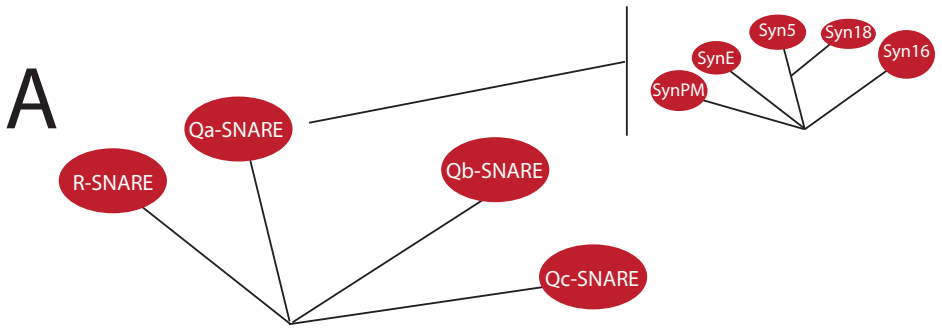
Coated vesicles and NPCs originated in a common precursor



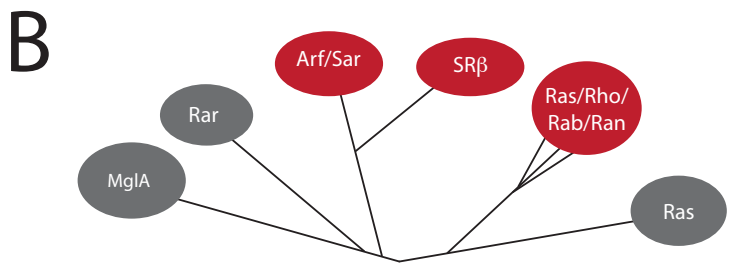
Coating vesicles

Coating pore membranes

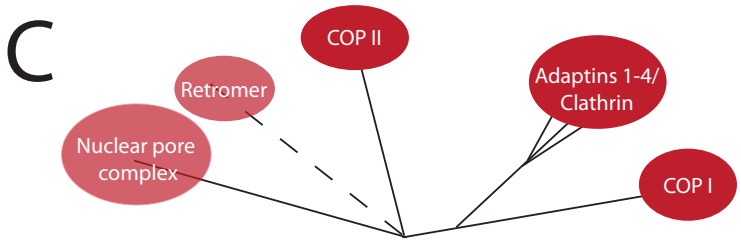
Devos *et al.*, 2004, 2006, Dokudovskaya *et al.*, 2006, Field and Dacks 2009)



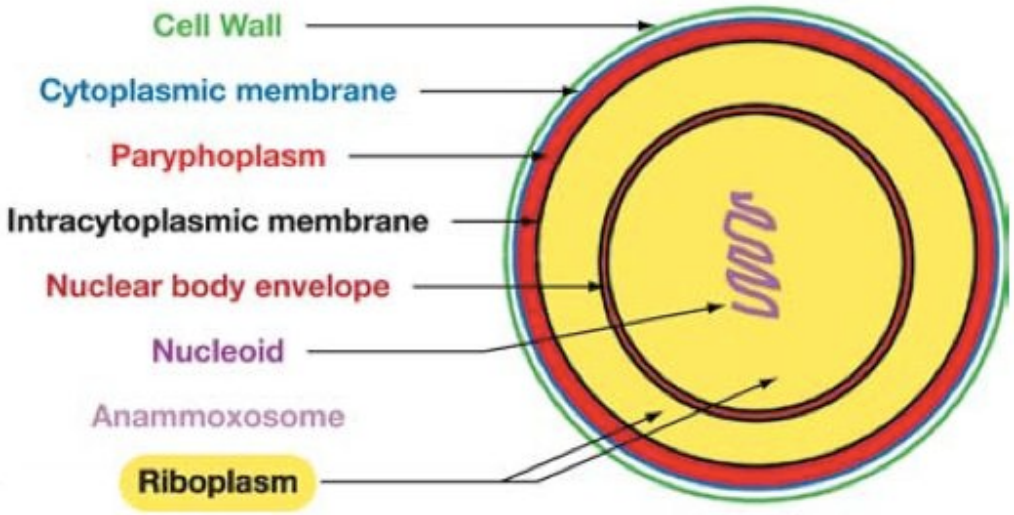
SNARE proteins

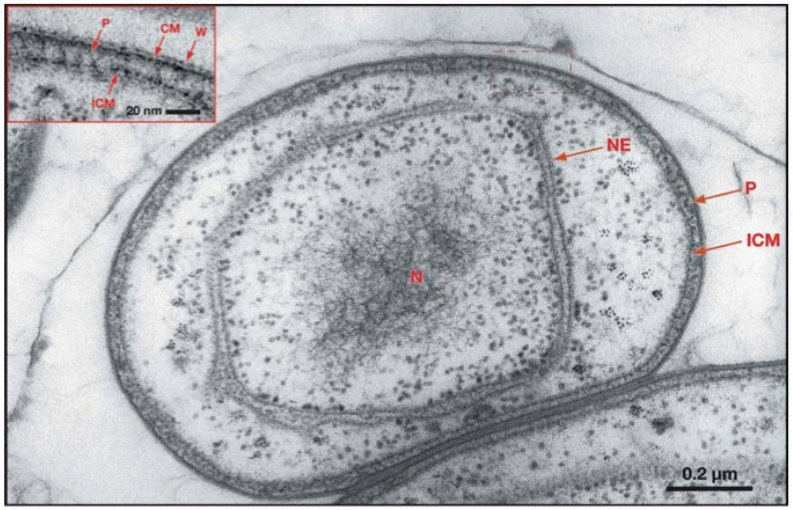


Small GTPases

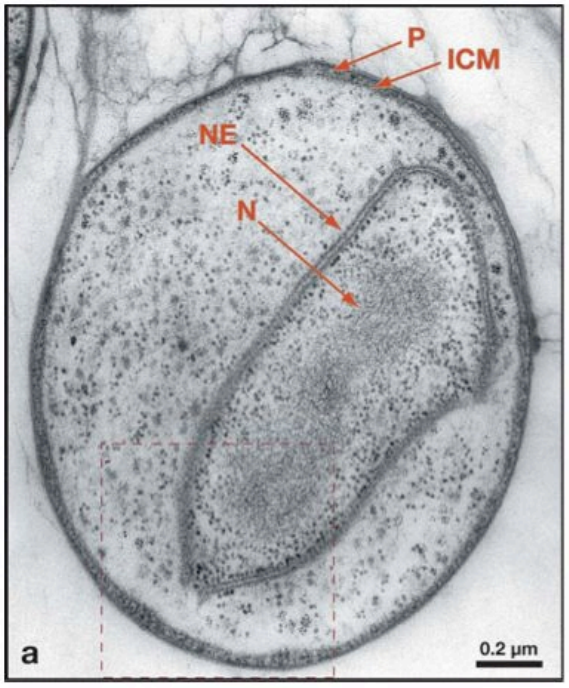


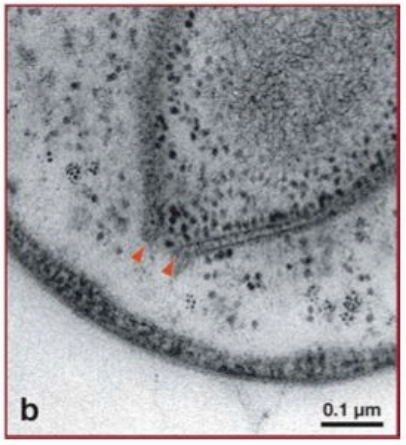
Proto-coatomer

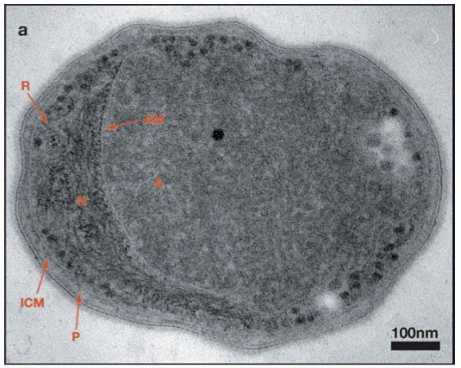




Gemmata







Fuerst 2005

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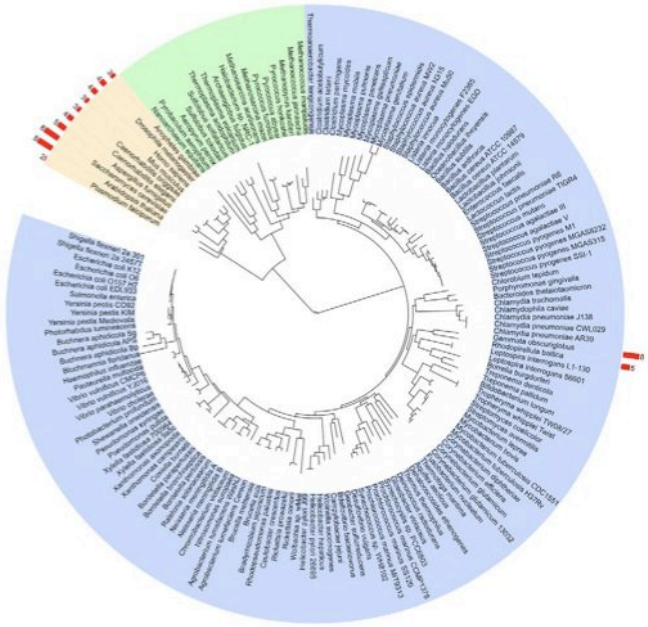
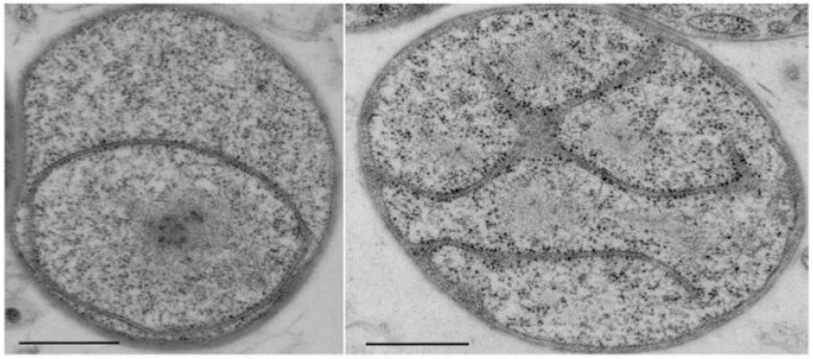
PLoS BIOLOGY

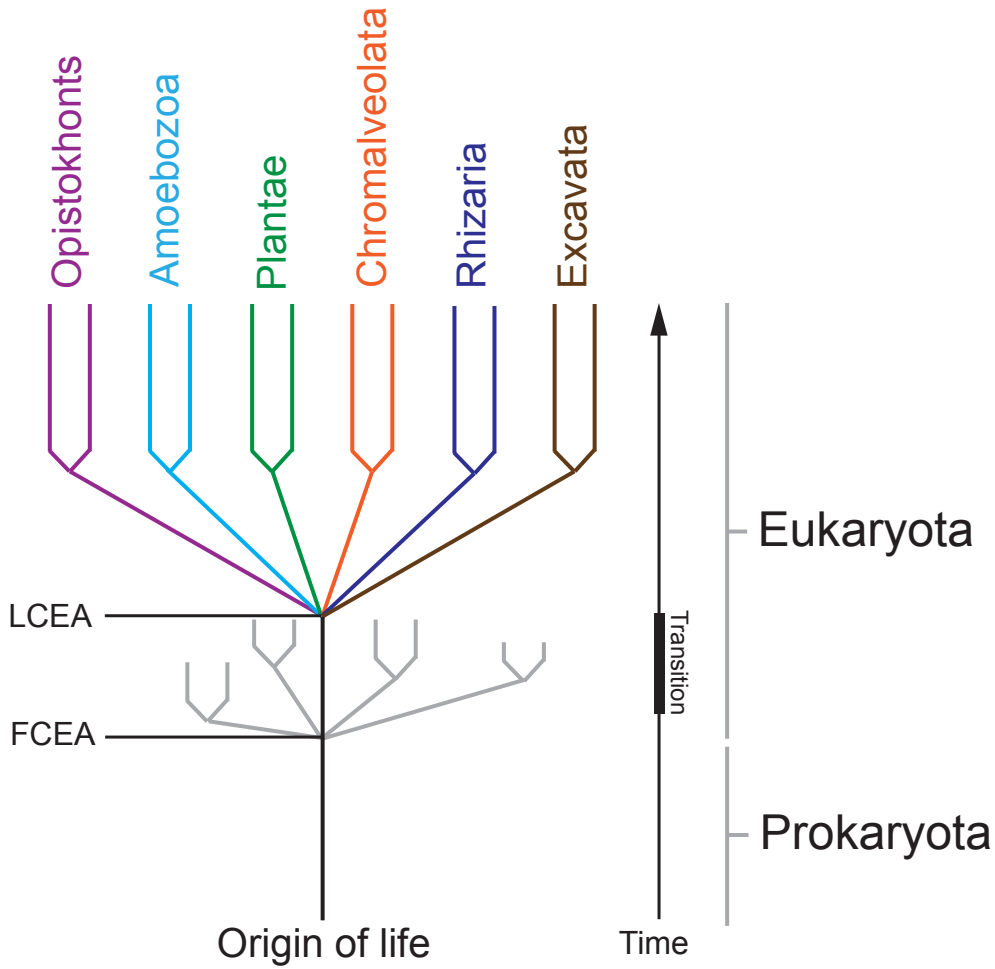
The Compartmentalized Bacteria of the Planctomycetes-Verrucomicrobia-Chlamydiae Superphylum Have Membrane Coat-Like Proteins

Rachel Santarella-Mellwig[1], Josef Franke[2], Andreas Jaedicke[1], Matyas Gorjanacz[1], Ulrike Bauer[1], Aidan Budd[1], Iain W. Mattaj[1], Damien P. Devos[1]*

1 European Molecular Biology Laboratory, Heidelberg, Germany, **2** Laboratory of Cellular and Structural Biology, The Rockefeller University, New York, New York, United States of America






Opistokhonts
Amoebozoa
Plantae
Chromalveolata
Rhizaria
Excavata
LCEA
FCEA
Origin of life
Transition
Time
Eukaryota
Prokaryota

Deep time and origins of the endomembrane system

The Golgi complex - Sculpting I

Rab proteins and interaction networks - Sculpting II

Evolution of the nucleocytoplasmic transport system

Protocoatomer; putting it all together

Today's scientists have substituted mathematics for experiments, and they wander off through equation after equation, and eventually build a structure which has no relation to reality.

Nikola Tesla, *Modern Mechanics and Inventions, July, 1934*

We must continue to gather insightful functional data

Acknowledgments

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The Leverhulme Trust
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