Lipid Membranes: the Third Fundamental Component of Cells

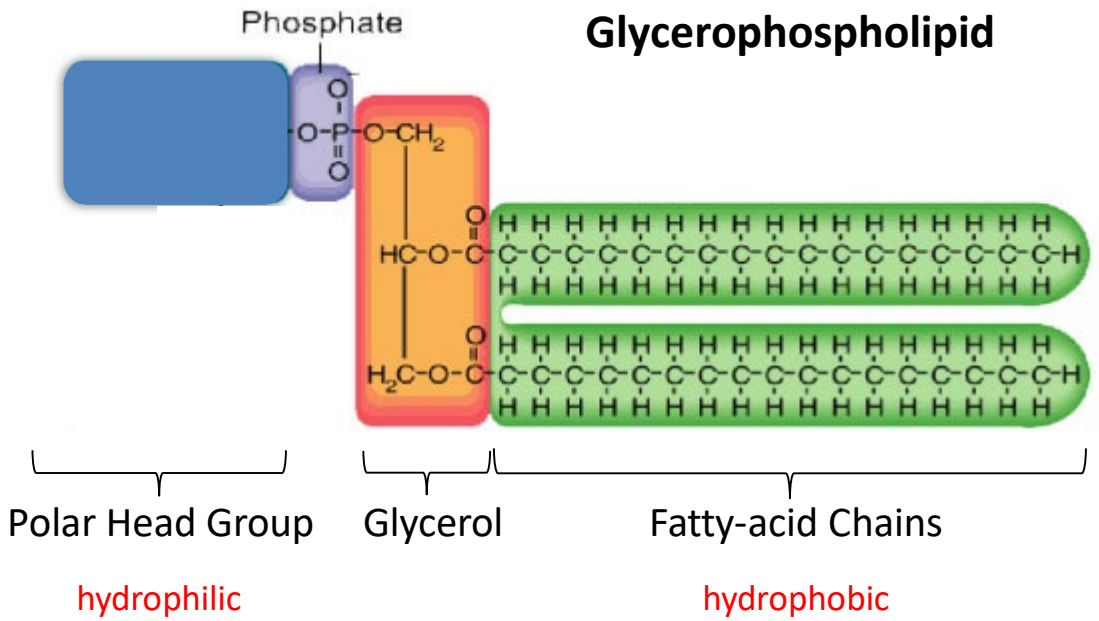
- Provide a barrier to the external environment, and in eukaryotes provide the basis for intracellular substructure.

- Ensure individuality, a critical requirement for heritable evolutionary processes.

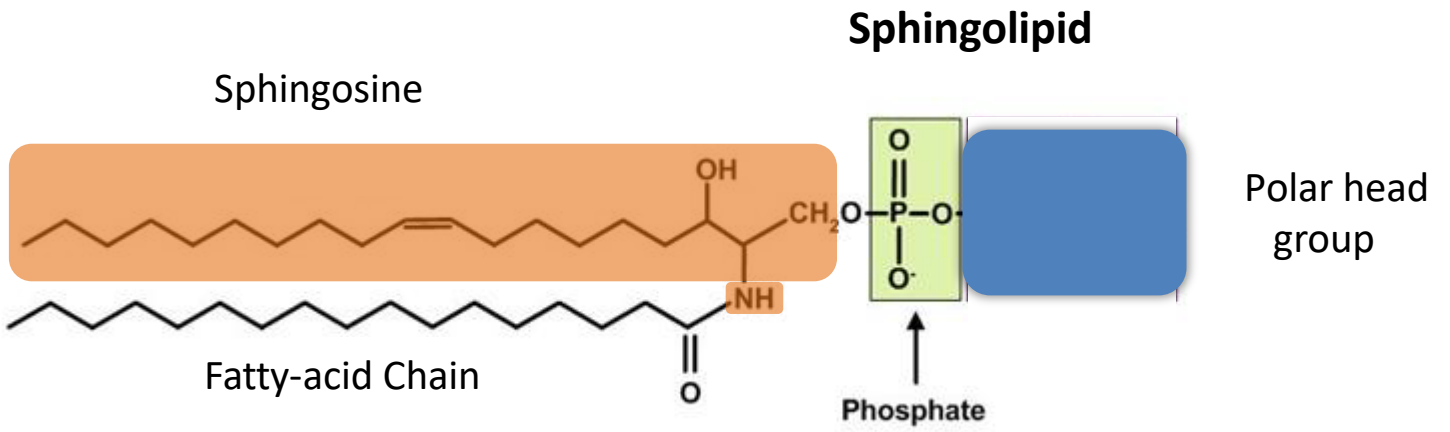
- Provide platforms for the residence of key proteins with diverse functions, e.g., signal-transduction systems (for communication), channels (for import/export), electron transfer chains and ATP synthases (for energy production), nuclear-pore complexes for the guided exit of mRNAs and entry of nuclear proteins (transcription factors, histones).

- Considerable energetic costs of producing lipids.

Basic Architecture of Phospholipid Molecules



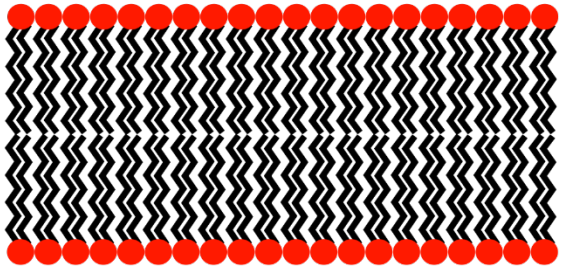
- How did phospholipids come to be deployed in membranes, and does this tell us anything about the origin of life?



The Most Thermodynamically Stable State: Fluid Bilayers of Amphipathic Lipids

hydrophilic polar head

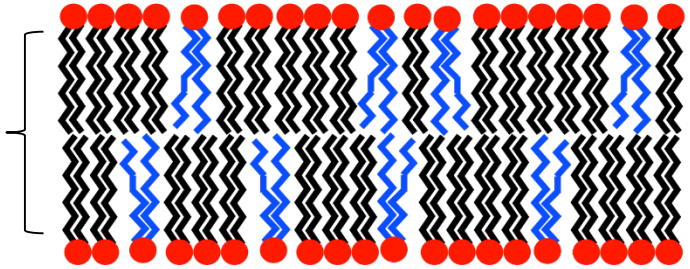
hydrophobic nonpolar tail



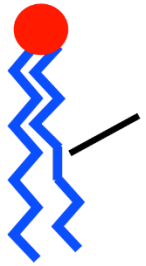
Saturated lipids only

Saturated

tail length defines membrane thickness



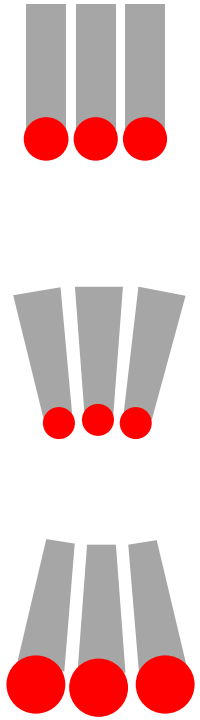
Mixed saturated and unsaturated



Double bond

Monounsaturated

spontaneous membrane bending via tail architecture

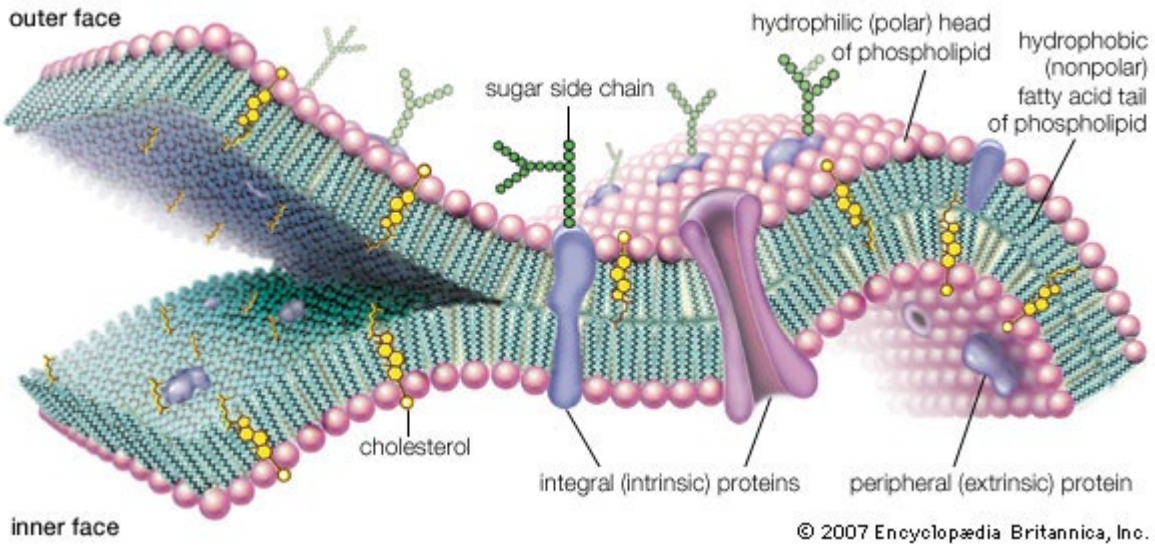


- Average diffusion rate is 3.5 microns/second, so individual molecules can traverse the length of a eukaryotic cell in minute or so.

Enormous Combinatorial Structural Diversity of Lipid Families

- Nature of the head groups:
  - Most common in glycerophospholipids: choline, ethanolamine, serine, glycerol, inositol, and phosphatidyl glycerol.
- Number of carbon atoms in the fatty-acid chain: typically 14 to 22 carbons.
- Number of double C=C bonds in the fatty-acid chain (inserted between two -CH groups, which are referred to as unsaturated): typically 0 to 5.

Today's Membranes Have Many Embellishments



- Phospholipids are the bilayer formers in Bacteria and Eukaryotes, but the Archaea employ ether-lipid based membranes.

J. Mol. Biol. (1983) **166**, 211–217

BARBARA A. LEWIS†
DONALD M. ENGELMAN

Lipid Bilayer Thickness Varies Linearly with Acyl Chain Length in Fluid Phosphatidylcholine Vesicles

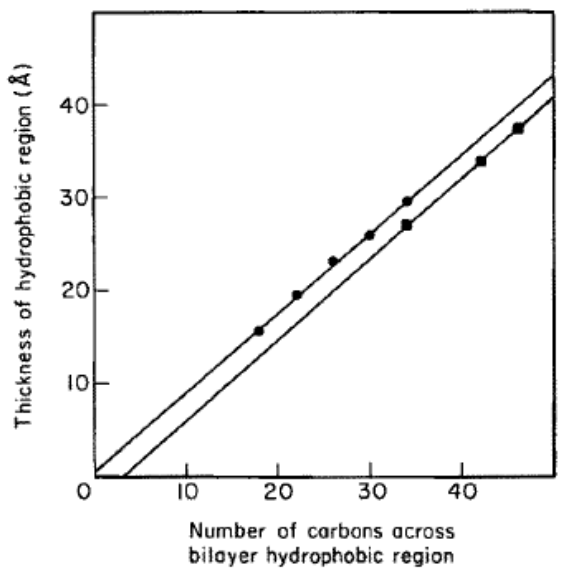


FIG. 3. Plot of the measured thickness of the bilayer hydrocarbon region *versus* the number of carbons across the hydrophobic region. The number of carbons is taken starting at C-2 of each acyl chain. Estimated errors in the thickness measurement correspond to the size of the plotted points. The lines are least-squares fits to the data. Circles, saturated acyl chains; squares, monounsaturated acyl chains.

- Eukaryotes and bacteria share most classes of lipids.

- Most membranes consist of a mixture of types.

Table 8.1. Fractional contributions of lipid molecules to plasma membranes in select species.

Organism	PC	PE	PG	PI	PS	C	LPG	O
Bacteria:								
Bacillus subtilis	0.00	0.24	0.35	0.00	0.00	0.18	0.23	0.00
Caulobacter crescentus	0.00	0.00	0.88	0.00	0.00	0.12	0.00	0.00
Escherichia coli	0.00	0.75	0.19	0.00	0.00	0.06	0.00	0.00
Staphylococcus aureus	0.00	0.00	0.53	0.00	0.00	0.07	0.40	0.00
Zymomonas mobilis	0.13	0.62	0.20	0.00	0.00	0.01	0.00	0.03
Eukaryotes:								
Mus musculus, thymocytes	0.57	0.21	0.00	0.07	0.10	0.00	0.00	0.06
Vigna radiata, seedings	0.47	0.35	0.05	0.05	0.08	0.00	0.00	0.00
Dictyostelium discoideum	0.29	0.55	0.01	0.08	0.02	0.02	0.00	0.03
Dunaliella salina	0.15	0.41	0.15	0.06	0.00	0.00	0.00	0.22
Saccharomyces cerevisiae	0.17	0.18	0.00	0.23	0.21	0.03	0.00	0.19
Schizosaccharomyces pombe	0.42	0.23	0.00	0.25	0.03	0.06	0.00	0.02

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinsoitol; PS, phosphatidylserine; C, cardiolipin; LPG, lysophosphatidylglycerol; O, other.

References: *Mm:* van Blitterswijk et al. (1982); *Vr:* Yoshida and Uemura (1986); *Dd:* Weeks and Herring (1980); *Ds:* Peeler et al. (1989); *Sc:* Zinser et al. (1991); Tuller et al. (1999); Zinser et al. (1991); Blagovic et al. (2005); *Sp:* Koukou et al. (1990); *Bs:* Op den Kamp (1969); Lopez et al. (1998); *Cc:* Contreras et al. (1978); *Ec:* Raetz et al. (1979); Rietveld et al. (1993); *Sa:* Haest et al. (1972); Mishra and Bayer (2013); *Zm:* Carey and Ingram (1983).

Eukaryotic Organelles: membrane-bound intracellular inclusions.

- Mitochondria and plastids are **exogenous** in origin.

- All others, e.g., the endoplasmic reticulum (ER), the Golgi, peroxisomes, and vesicle sorting systems, appear to have originated **endogenously,** by “descent with modification,” and to have been present in LECA.

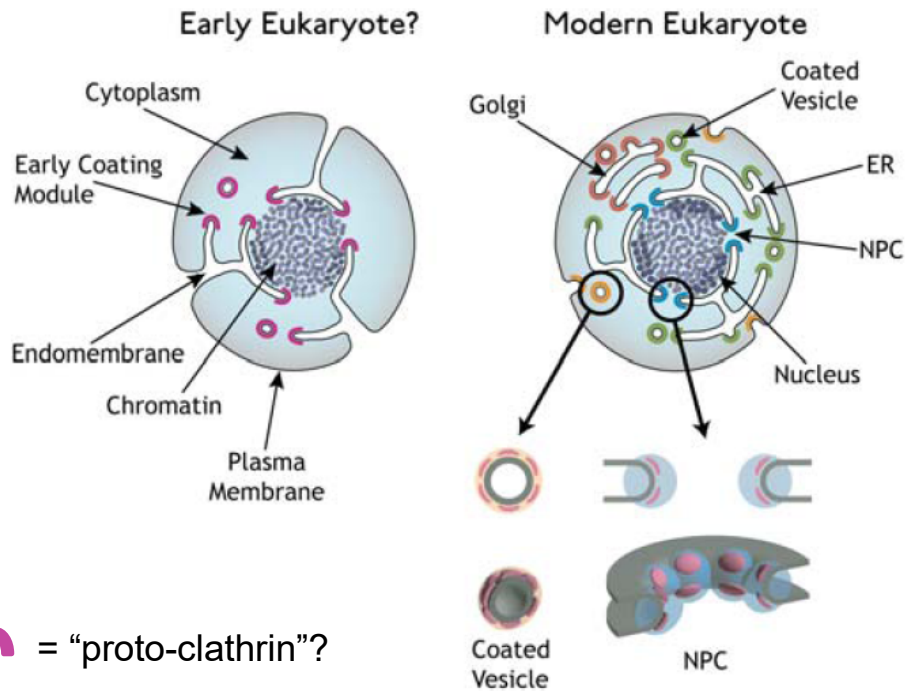
- Approximately 10% of the genes in a eukaryotic genome are devoted to membrane / vesicle trafficking.

- A wide range of features of organelle systems appear to have evolved by repeated rounds of duplication and divergence.

- Subcellular architecture can be viewed as having some advantages, but there are also costs to building and maintaining such structures.

  - Why haven’t many prokaryotes gone down this route, and did the structure of the eukaryotic cell emerge as an adaptation or as a simple consequence of drift-like processes?

The Protocoatamer Hypothesis for the Evolution of Internal Membranes in Eukaryotes



= "proto-clathrin"?

Figure 5. Proposed Model for the Evolution of Coated Vesicles and Nuclear Pore Complexes

Early eukaryotes (left) acquired a membrane-curving protein module (purple) that allowed them to mold their plasma membrane into internal compartments and structures. Modern eukaryotes have diversified this membrane-curving module into many specialized functions (right), such as endocytosis (orange), ER and Golgi transport (green and brown), and NPC formation (blue). This module (pink) has been retained in both NPCs (right bottom) and coated vesicles (left bottom), as it is needed to stabilize curved membranes in both cases.

1) Early acquisition of a membrane-curving protein resulted in the molding of the plasma membrane into internal compartments such as the ER and golgi.

2) Juxtaposition of two complexes led to a proto-nuclear pore.

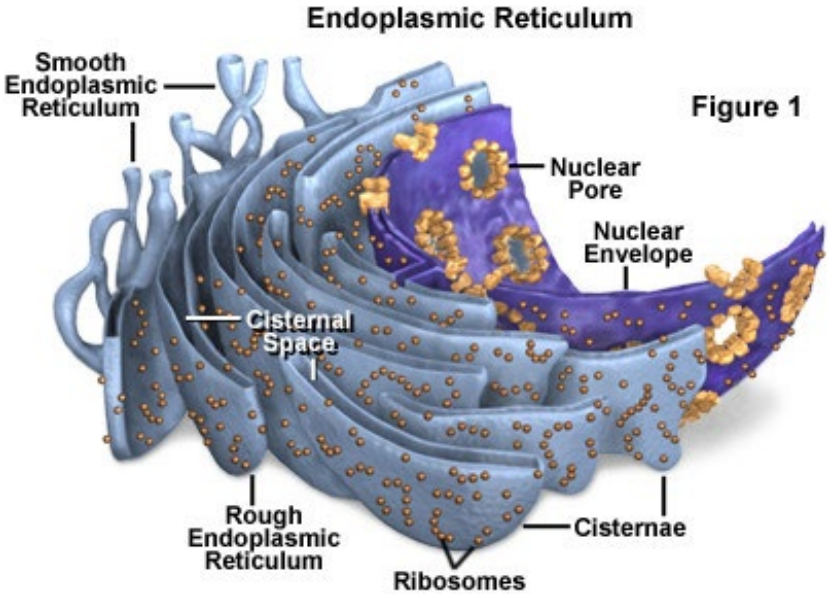
3) Plasma-membrane invaginations eventually became sealed off.

4) Accrual of specialized proteins lead to a gated nuclear pore.

Devos et al. (2004, PLoS Biology)

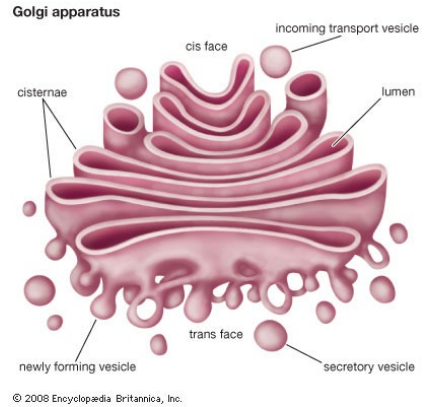
Endoplasmic Reticulum (ER)

- Ribosomes in the cytosol bind to the rough ER when translating a protein that is destined to be secreted into the ER; such peptides have a short leading sequence that is cleaved upon entry to the ER, releasing the ribosome.
- Site of protein folding by chaperones.
- Site of lipid synthesis.
- Continuous with the nuclear envelope.
- All known eukaryotes have an ER, so LECA almost certainly did too.



Stacked Golgi

- Central headquarters for the membrane-trafficking system.
- Receives newly synthesized proteins and lipids from the ER by vesicle transport, and modifies and distributes them through the vesicle system to their cellular destinations.
- Site of post-translational modification of proteins, e.g., glycosylation (sugar-group addition).



- Some eukaryotes lack stacked Golgi, but all appear to be examples of loss, as the genes encoding for coat proteins employed in Golgi-associated vesicles are still present.
- Lineages with no identifiable Golgi may harbor Golgi-like organelles or functions.
- Conclusion: LECA very likely had stacked Golgi.

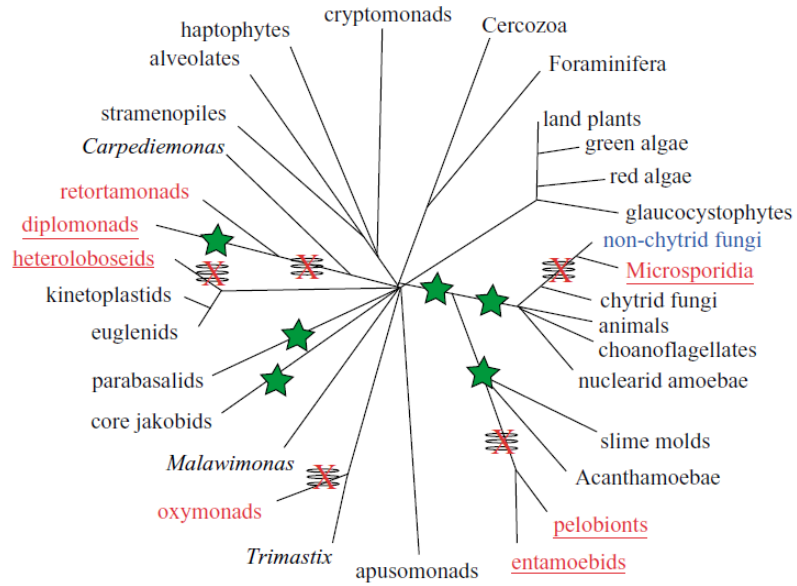
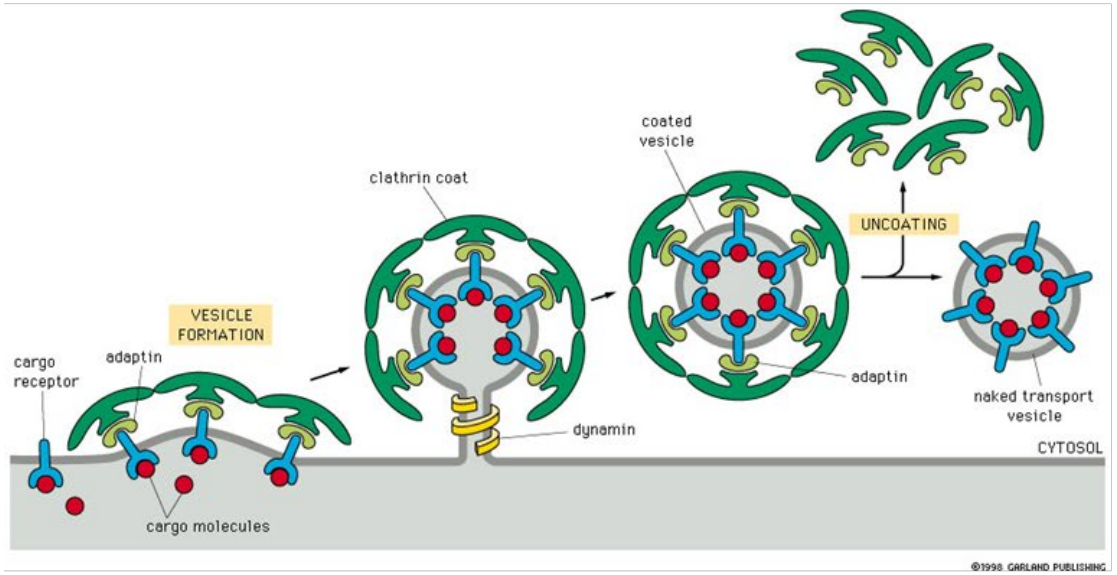


Figure 1. Schematic of proposed eukaryotic relationships. This illustration is based on combined molecular and morphological data reviewed in Dacks & Doolittle (2001), as well as more recent data (Keeling *et al.* 2000; Keeling 2001; Bapteste *et al.* 2002; Simpson *et al.* 2002*a*,*b*). Taxa in red lack Golgi stacks. Underlined red taxa possess molecular biological evidence for a Golgi apparatus. Non-chytrid fungi are shown in blue as most lack Golgi stacks but, based on extensive molecular evidence, are widely accepted to possess the organelle. The cartoon Golgi stacks with a red X represent possible shifts from stacked to a non-canonical morphology. Stars represent potential placements of the root of eukaryotes.

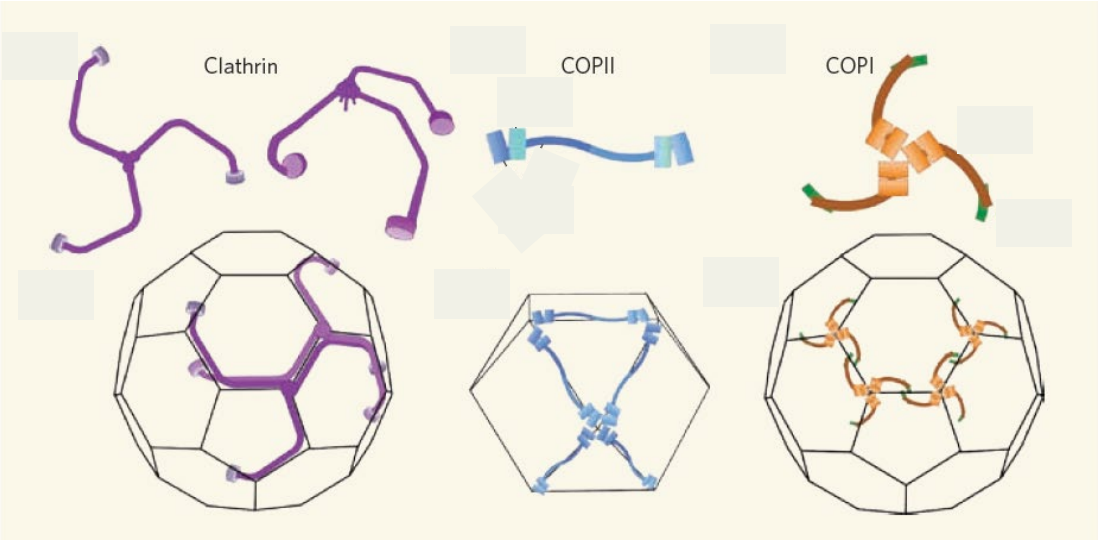
Vesicle Construction and Delivery



- **Cargo capture**: external molecules (red) are initially bound by specific cargo-transport proteins (blue).
- **Vesicle budding and coating**: specific adaptor proteins (light green) bind to the cargo receptors, and in turn recruit vesicle coating proteins (dark green), inducing membrane curvature (dark gray).
- **Vesicle scission**: coat proteins continue to be recruited, and the stem is eventually squeezed off with a concatamer of dynamin molecules (yellow).
- **Vesicle uncoating**: vesicle is free to bind to a recipient membrane.

The Higher-Order Structure of the Protein Coats of Eukaryotic Lipid Vesicles

Edeling et al. (2006); Harrison and Kirchhausen (2010)



Clathrin: cell membrane to endosomes.

COPI (coat protein complex I): between compartments of the Golgi.

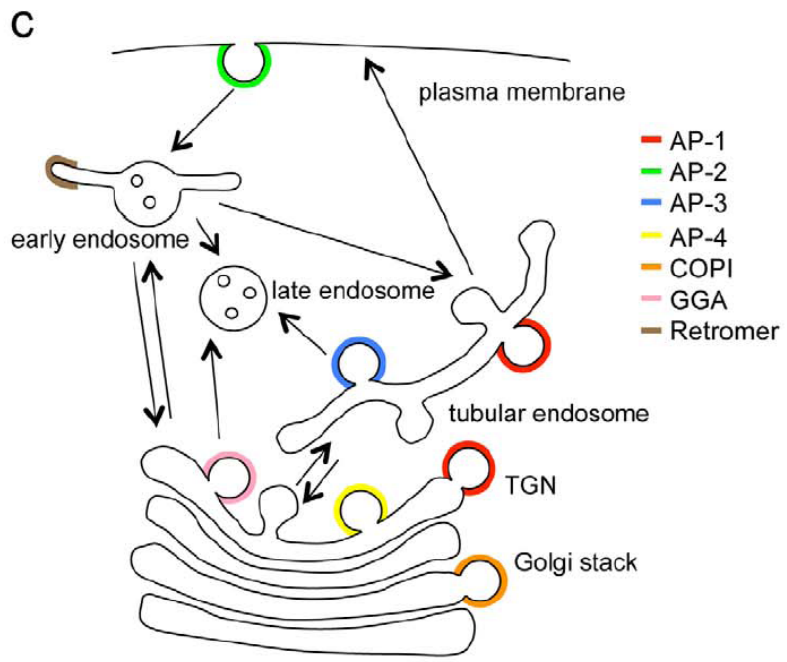
COPII: export of newly synthesized proteins from the ER.

- Subunits of clathrin and COPI coats are homotrimeric, whereas those for COPII vesicles are heterodimeric.
- Subunits organize into lattices with distinct geometric shapes, the dimensions of which are defined by the lengths of the monomeric-subunit domains.
- It remains to be seen whether such structures vary in any meaningful way phylogenetically.

A monomeric subunit of clathrin. Each linear domain of the arm consists of a long series of alpha helices, the numbers of which define the overall dimensions of the lattice.

Deciphering the Order of Evolutionary Events From a pre-LECA Gene Genealogy

- Adaptor proteins are specialized to bind to specific cargo receptors.
- Five adaptor proteins appear to have been present in LECA.



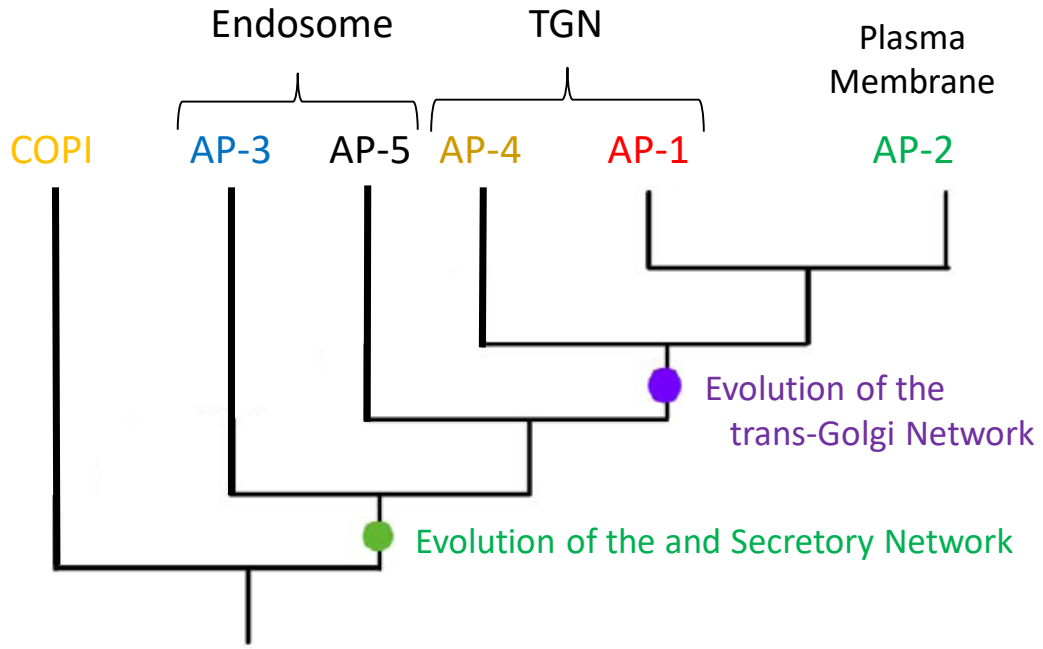
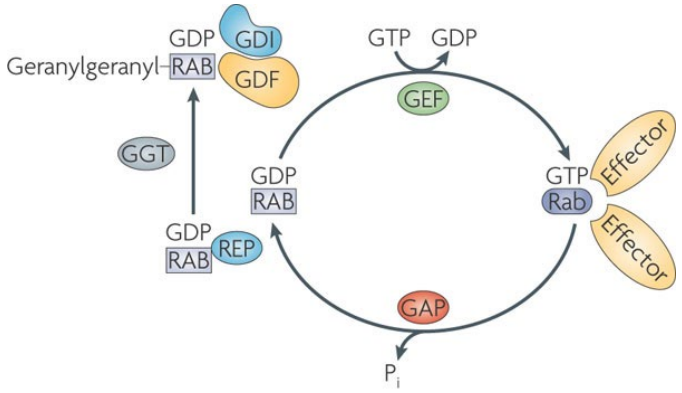


Figure 1. Overview of AP complexes. (a) Structure of an AP complex, showing the positions of the four subunits, and indicating some of the domains on the μ and β subunits. (b) List of subunits in the four AP complexes and F-COPI. (c) Diagram of trafficking pathways and machinery. We have called the AP-1- and AP-3-positive endosomes "tubular endosomes" because this is how they appear by electron microscopy [43,57]; functional names such as recycling or early endosomes are more contentious.
doi:10.1371/journal.pbio.1001170.g001

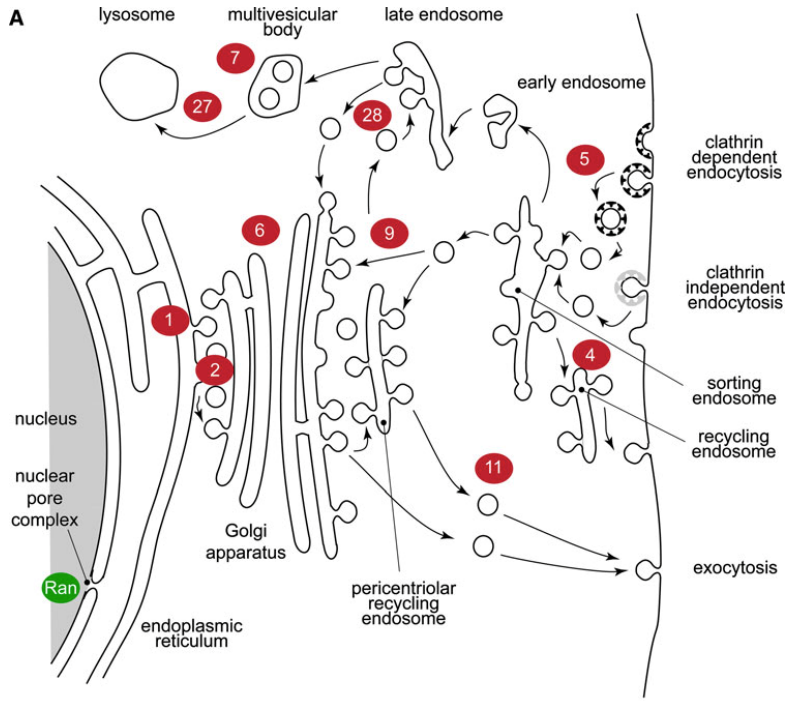
Hirst et al., 2011, PLoS Biology

Edeling et al., 2006, Nature Rev. Mol. Cell Biol.

RAB GTPase Cycle: control where vesicles go.



- GEF = guanine nucleotide exchange factor
- GAP = GTPase-activating protein



Number of RABs per genome:

Yeasts	~10
Arabidopsis	57
Caenorhabditis	29
Homo	60
Entamoeba	~90
Tetrahymena	88
Paramecium	229
Plasmodium	11
Toxoplasma	~20

- RAB proteins are found in large gene families, formed by duplication and diversification in sequence and function.
- Membrane-bound in the GTP state, and soluble in the cytosol in the GDP state.
- Prenylation (post-translational addition of lipid groups to C terminus) assists in binding to membranes.
- Similar numbers of RABs, GEFs, and GAPs within genomes suggests coevolution.

The RAB family had as many as 23 specificity subfamilies in LECA, with distinct lineages then experiencing various loss events

(Elias et al. 2012, J. Cell Science)

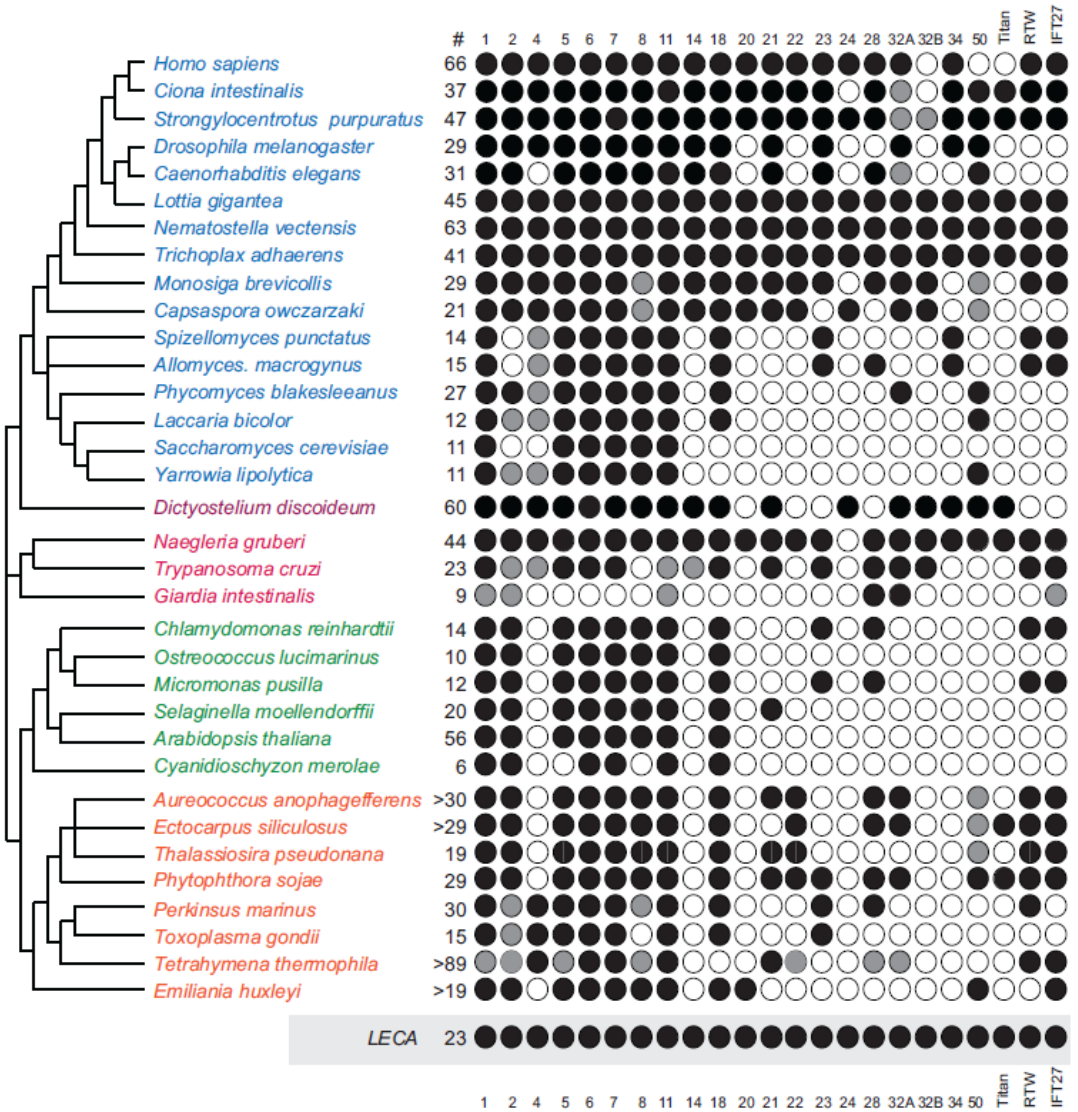


Fig. 4. Rab representation for select eukaryotes. Individual Rab clades, inferred as present in the LECA, are shown as columns. Taxa are shown as rows, with the hypothetical LECA as the lowest row (grey box). A schematic phylogeny for the taxa is drawn on the left and derived from Walker et al. and references therein (Walker et al., 2011). The total number of Rabs found in each genome is also indicated on the right of the taxon labels, and by a hash. Here, and in Fig. 5, black circles indicate at least one member of the clade has been identified with phylogenetic support (>0.80/50/50 MrBayes/PhyML/RaxML) and grey circles indicate naming based on BLAST results. Taxa are colour-coded by supergroup as in Fig. 3.

Does the Broader Phylogeny of RAB-related Proteins Reflect the Timing of Origin of Eukaryotic Endomembranes?

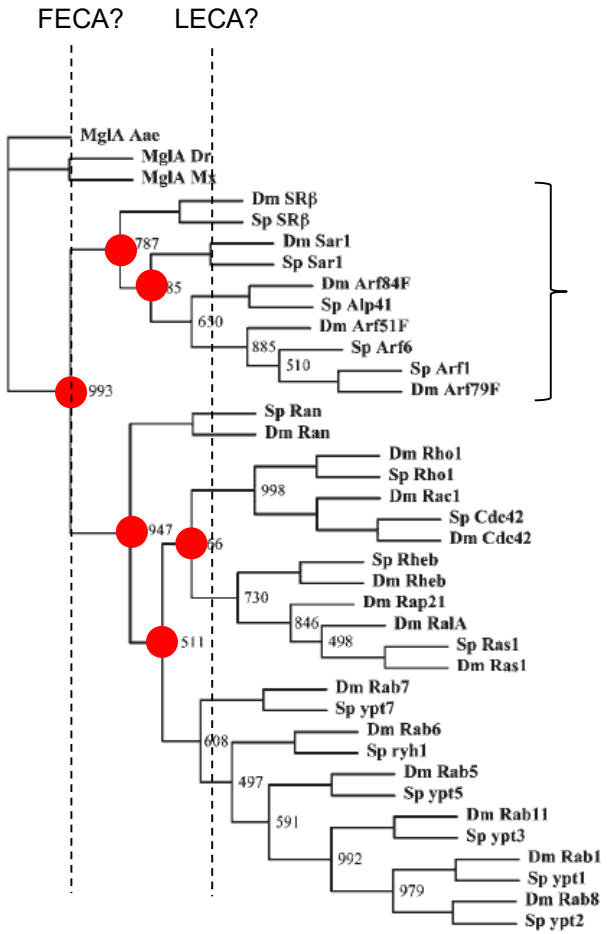


Figure 1. Phylogeny of the Ras superfamily based on animal and fungal sequences. Neighbour-joining tree of selected *D. melanogaster* (Dm) and *S. pombe* (Sp) small GTPases rooted with bacterial MglA-like sequences. Bootstrap values of 1000 pseudo-replicates are shown. Key nodes in the tree are numbered. The tree was generated using the Phylip program package, version 3.6. The multiple alignment used for the construction of the tree was based on a structural alignment of selected GTPase (as found in the FSSP database; www.ebi.ac.uk/dali/fssp/fssp.html). Additional sequences were added using

Prokaryotes – myxobacteria

SRβ - ribosomal docking to the ER

Sar1 - regulates COPII assembly and cargo selection

ARF - regulates COPI coat assembly and transport through Golgi

Association with secretory membranes

Ran - nuclear function and transport across nuclear envelope

Rho - regulation of actin cytoskeleton

Ras – transmission of extracellular signals = “rat sarcoma”

Rab – vesicular identity and regulation of vesicular transport = “RAS-associated binding proteins”

Jekely, 2003, BioEssays

Membrane Trafficking: a multi-layered intracellular communication system.

At least five specificity languages involving signalers and receptors.

Vesicle production:

1) Cargo receptor proteins – recognize cargo.

2) Adaptor proteins – interface between cargo receptor proteins and coat proteins.

Vesicle delivery:

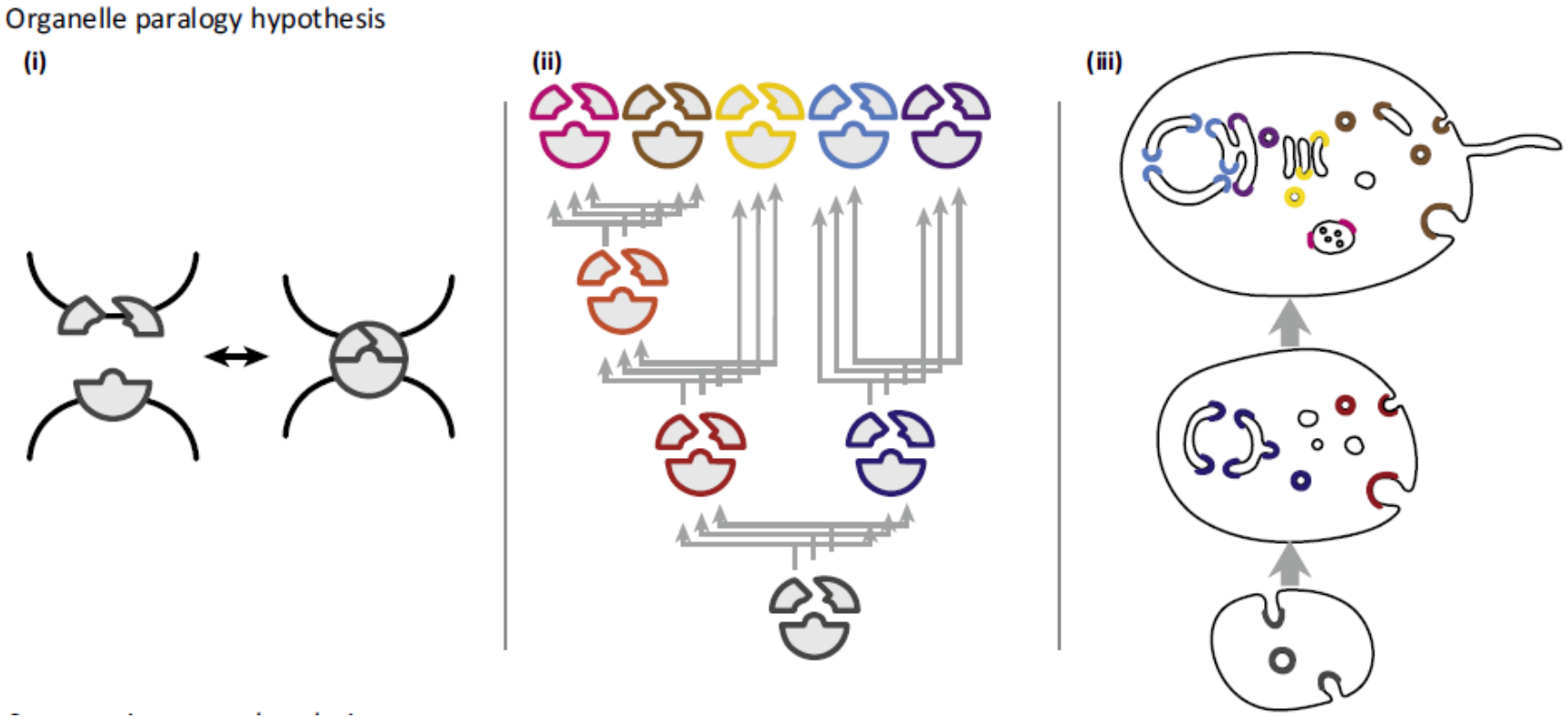
1) RAB proteins – surface markers on vesicles (and/or target membranes) identify their origin and type of cargo;

2) RAB effector proteins – recognize active RAB-GTPs and help tether the vesicle to the membrane.

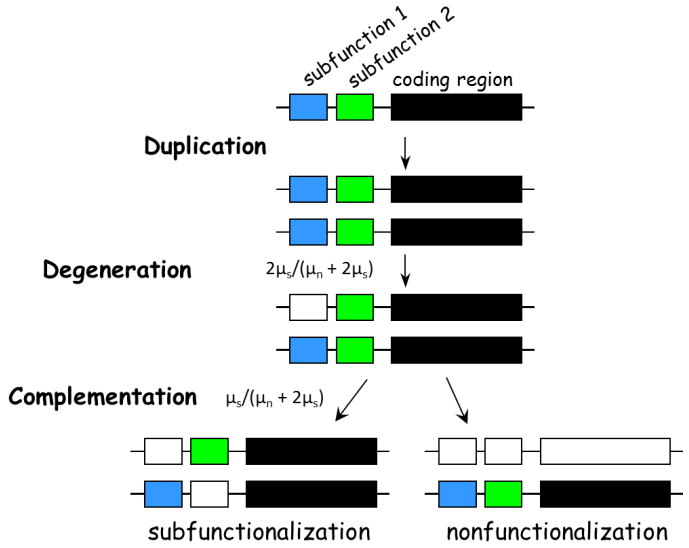
Vesicle fusion:

SNARES (soluble NSF attachment protein receptor) proteins – v (vesicle) and t (target) pairs dock vesicle and catalyze fusion.

The Organelle Paralogy Hypothesis (Dacks and Field 2007; Mast et al. 2014).



Single-gene Duplication:



Probability of Partial Preservation Greatly Exceeds That of Mutual Preservation, at Least Under the Subfunctionalization Hypothesis

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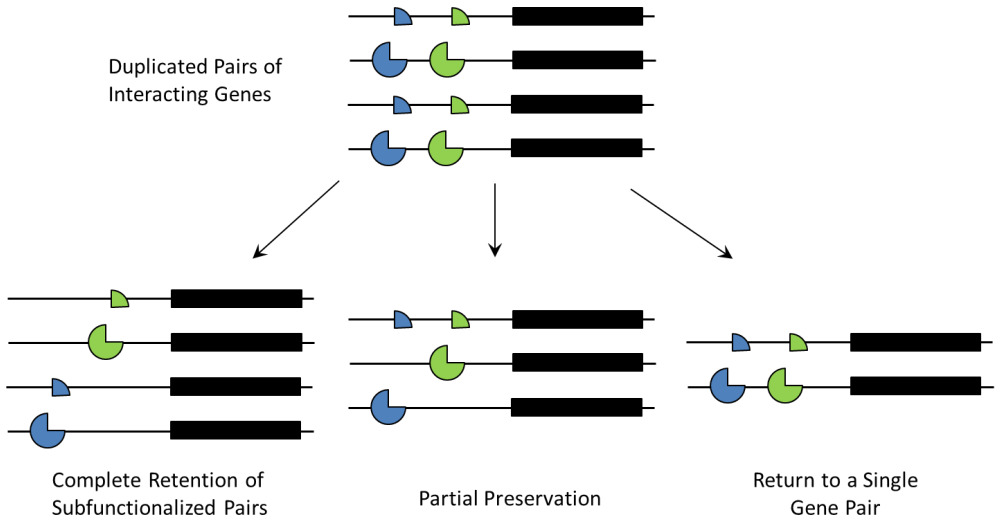
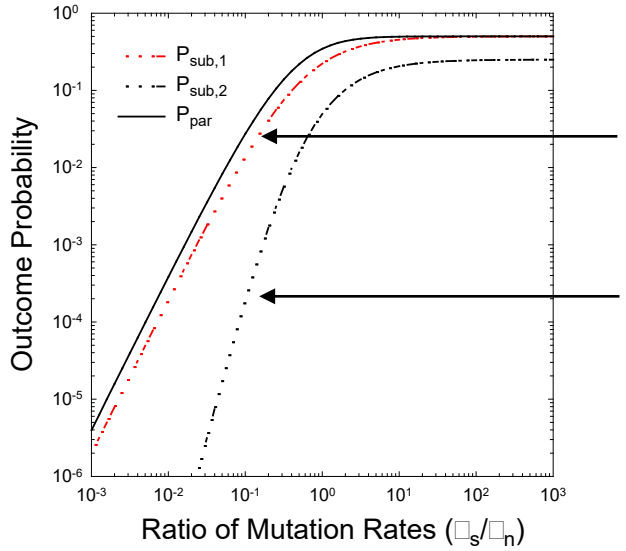
Gene-pair Duplication:





Partial preservation

Complete subfunctionalization

Some Unanswered Questions Regarding Vesicle Transport

- What universal eukaryotic features were present in FECA?
  - Which features arose in the direct line of descent between FECA and LECA?
  - What was the order of events?

- Coevolution of regulatory languages?
  - How do RABs (specifiers of localization addresses) coevolve with their regulatory proteins (GEFs and GAPs)?
  - How do adaptor proteins coevolve with vesicle-type determinants?
  - How do vesicle SNARES coevolve with tethering SNARES?

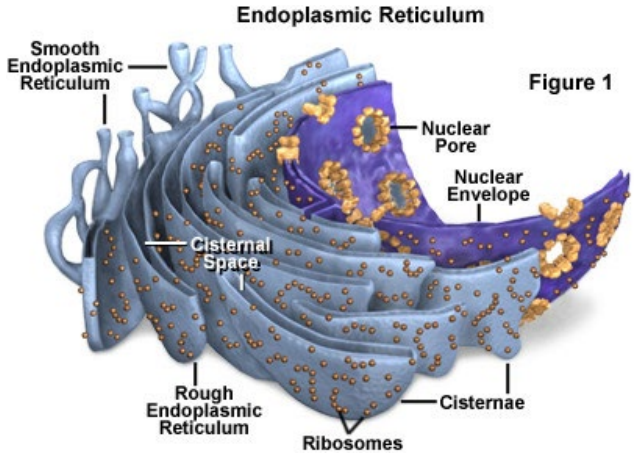
- Did whole-genome duplication allow unfettered subfunctionalization / neofunctionalization of the full repertoire of the membrane-trafficking system?

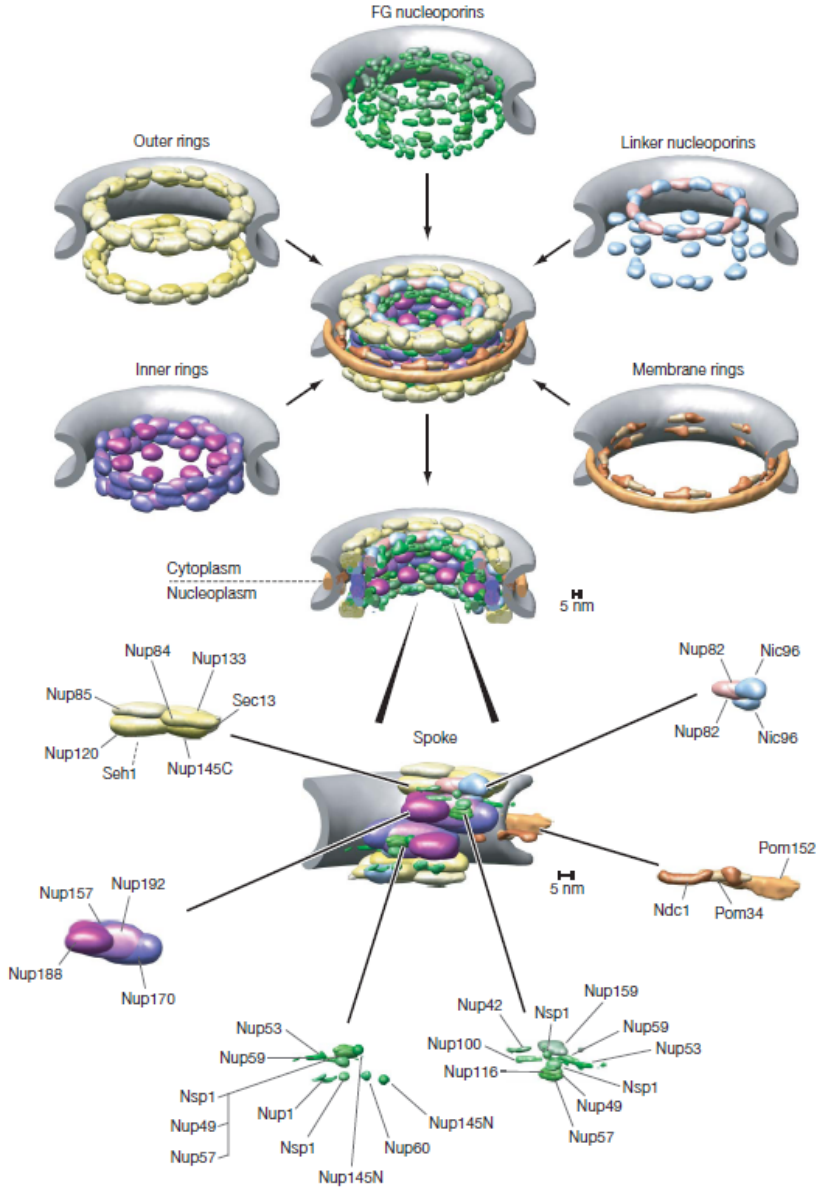
The Nuclear Pore Complex (NPC): the largest protein complex in eukaryotic cells.

- ~30 distinct proteins contribute to an overall structure of ~456 individual molecules.

Evolutionary Roots:

- Relationship to membrane trafficking system.
- Duplication and divergence of parts.





Alber et al. (2007, Nature)

A Hierarchical Structure Involving Repetition of Structural Modules, Arising by Duplication and Divergence

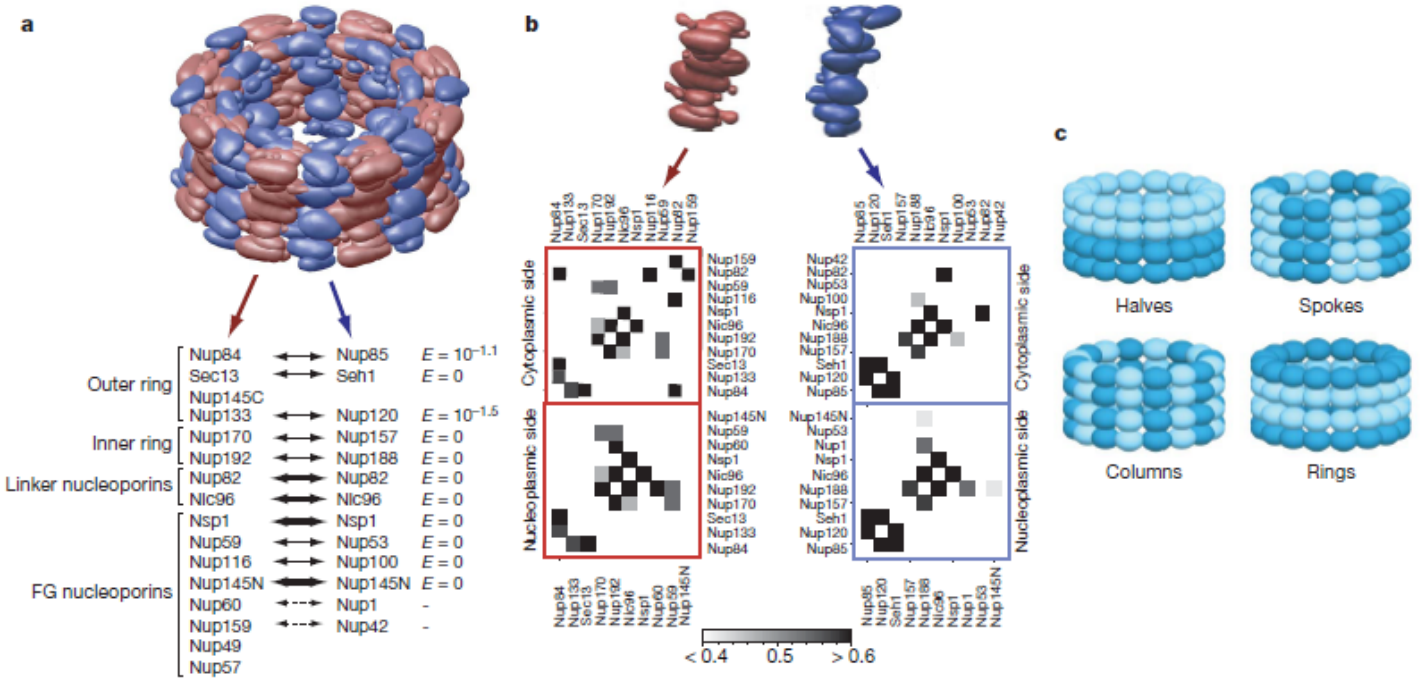


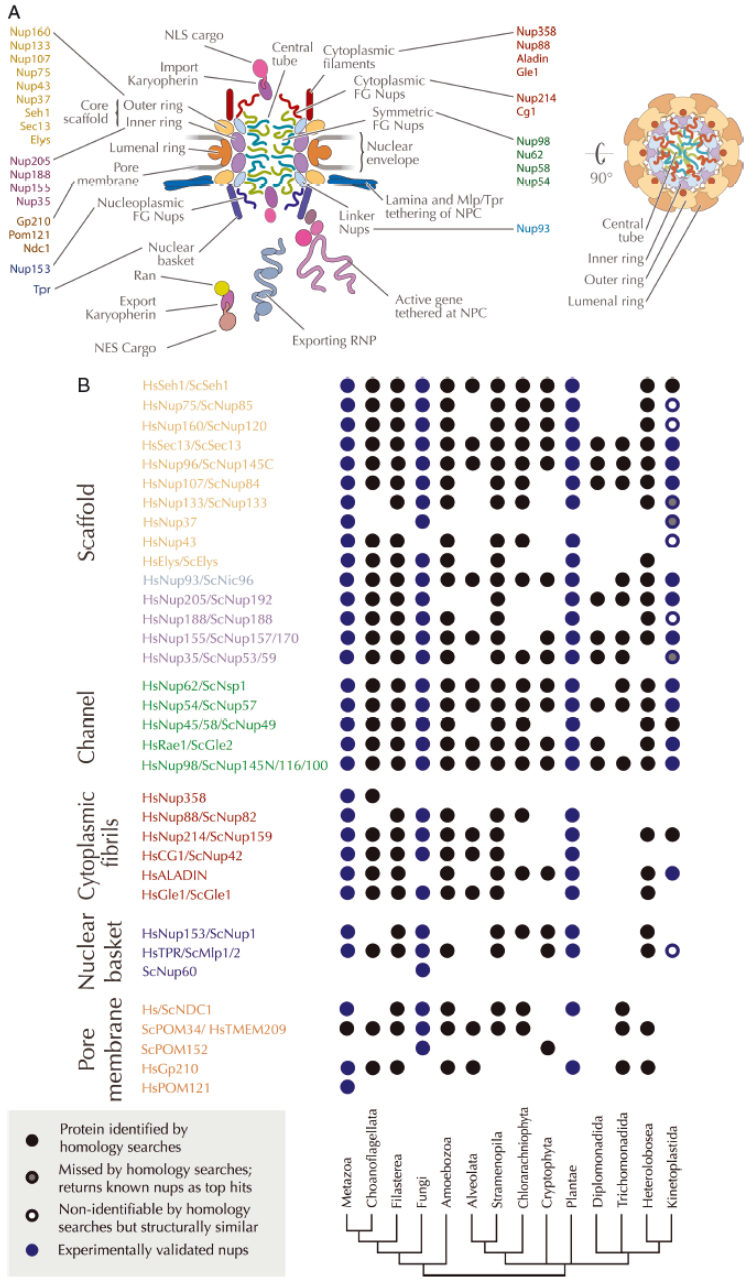
Figure 5 | Modular duplication in the NPC. **a**, The NPC can be divided into two alternating equivalent groups of nucleoporins arranged in parallel columns, as indicated here in red and blue. The nucleoporins in the red column are listed below left, and those in the blue column are listed below right. Almost every nucleoporin in the red column contains a counterpart in the blue column related by approximate position, as well as size and fold arrangement (pairs linked by dashed arrows), strong sequence similarity (those linked by thin arrows), or as duplicate copies with one copy in each column (thick arrows). The membrane rings and FG repeat regions were removed for clarity. Also shown are the *E*-values generated by HMMsearch[57] for the most significant local matches of the corresponding protein pairs; the *E*-value for a sequence match is the expected number of false positives per database search with a score at least as good as this sequence match. **b**, Network of protein contacts in the cytoplasmic (upper row) and nucleoplasmic (lower row) half-spokes for proteins in the red column (left) and blue column (right), showing that the homologous constituents have equivalent neighbours in each column. The networks are shown as instance contact frequency maps[14] (Supplementary Information). Notably, many contacts among proteins in one column are also present between the equivalent proteins in the second column. **c**, A large portion of the NPC can be divided into pairs of structurally similar modules: nuclear and cytoplasmic halves, eight radially disposed spokes, 16 radially disposed columns, and inner and outer rings.

- Origin of the 16-column structure via an ancient duplication event.
- Symmetrical features of the inner and outer rings suggest another duplication.

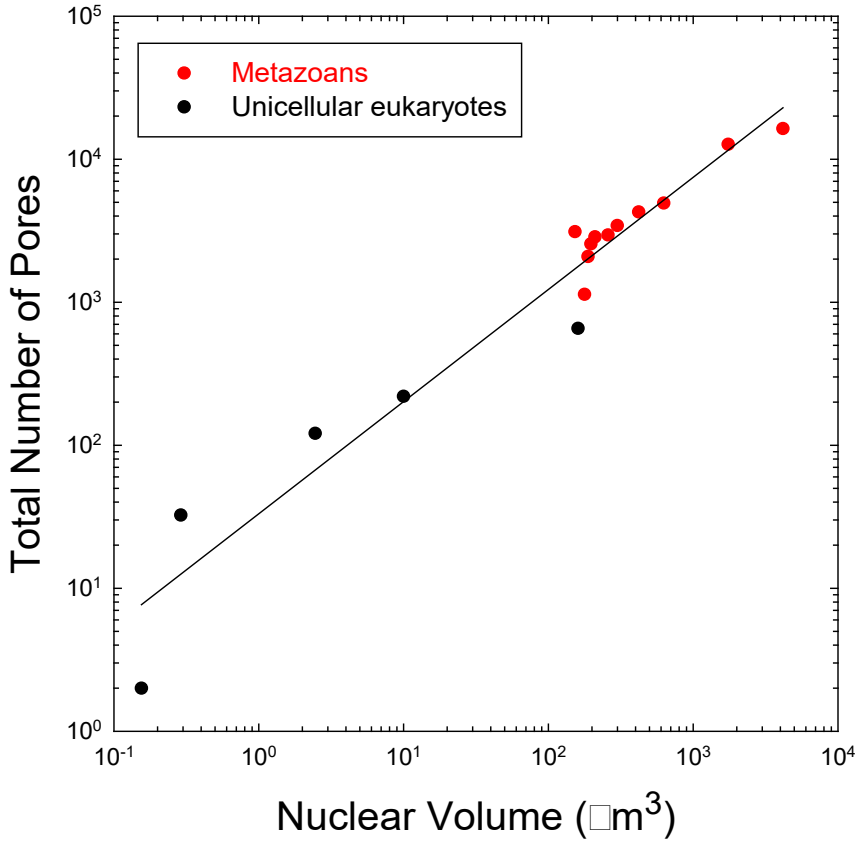
LECA likely had a conventional Nuclear Pore Complex (NPC), although lineage-specific differences appear to exist.

(Neumann et al. 2010; Field et al. 2013,)

- One protein component of the outer ring, Sec13, is a component of both the NPC and the COPII vesicle coat.
- Consistent with the protocoatamer hypothesis.



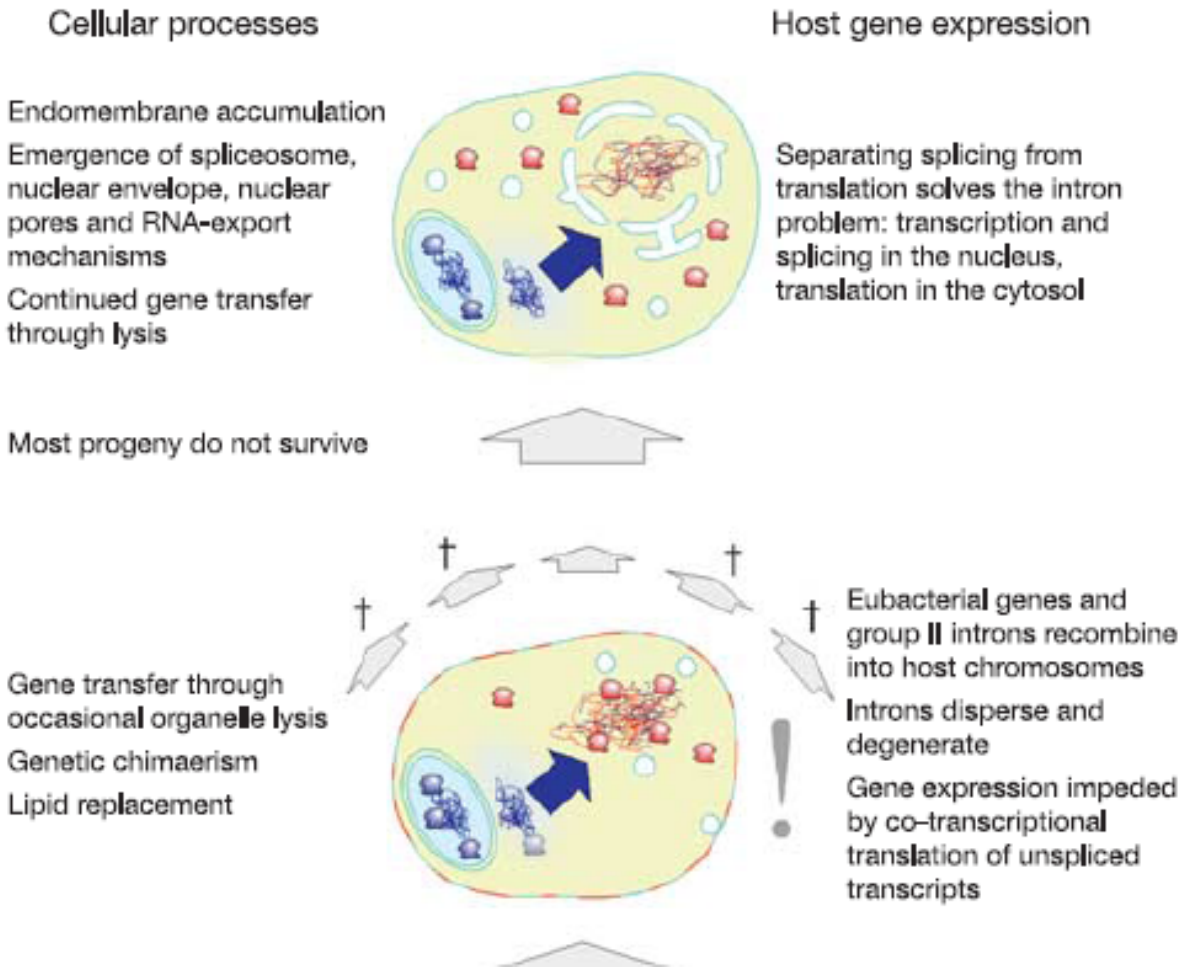
Pore Number Scales with ~(Nuclear Volume)$^{0.78}$; and So Nearly Proportional to Nuclear Envelope Surface Area, ~(SA)$^{0.11}$



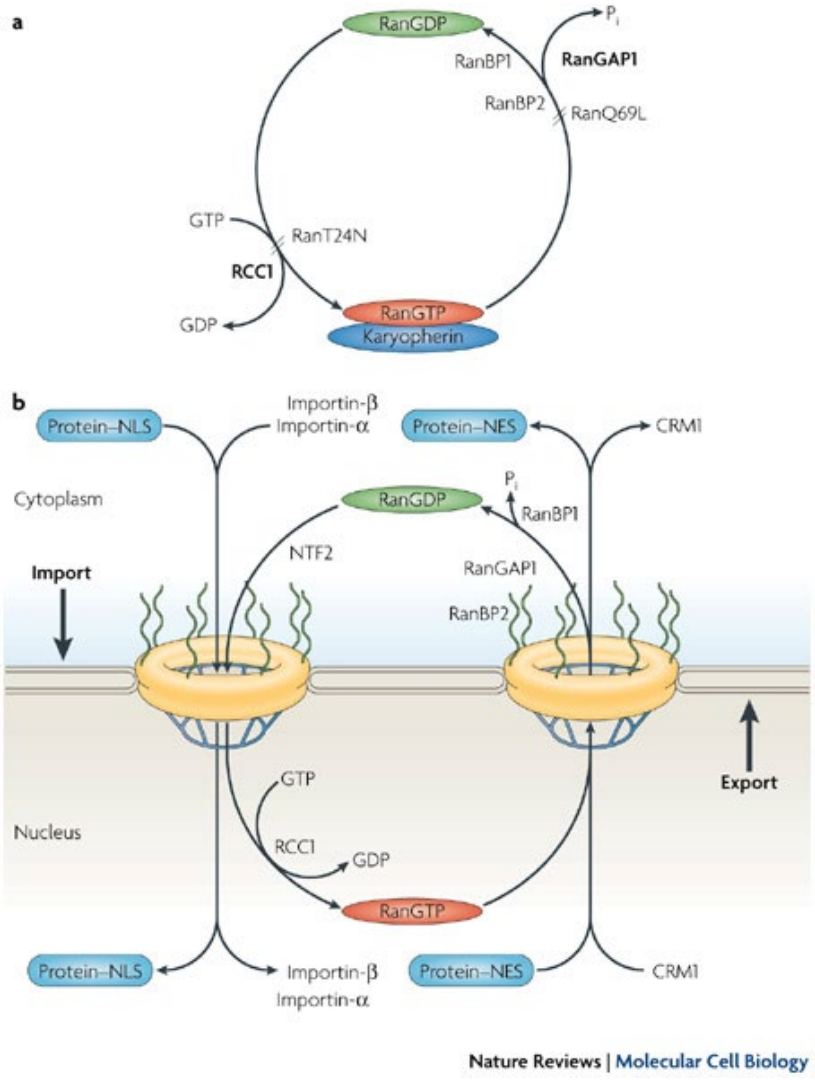
Why Did the Nuclear Envelope Evolve?

- Martin and Koonin (2006) proposed that the origin of introns created problems that forced the origin of the nuclear envelope.

- A more plausible hypothesis is that the prior origin of the nucleus provide a permissive environment for the colonization of introns and transposable elements.



Nuclear Transport: evolutionary consequences of the nuclear-pore complex.



- Energy for import / export is provided by Ran GTPase, which cycles between GTP- and GDP-bound states.
- Imported proteins have nuclear localization signals recognized by a family of karyopherins (importin-α). Importin-β docks the complex to the NPC.
- Cargo is released in the interior upon RanGTP binding to the karyopherin-cargo complex.
- There is also a family of exportins, which recognize cargos having appropriate nuclear export signals – exportins bind with RanGTP prior to export.

Alternative Models for Transport of Molecules Through the Maze of Nups (Grunwald et al. 2011, Nature)

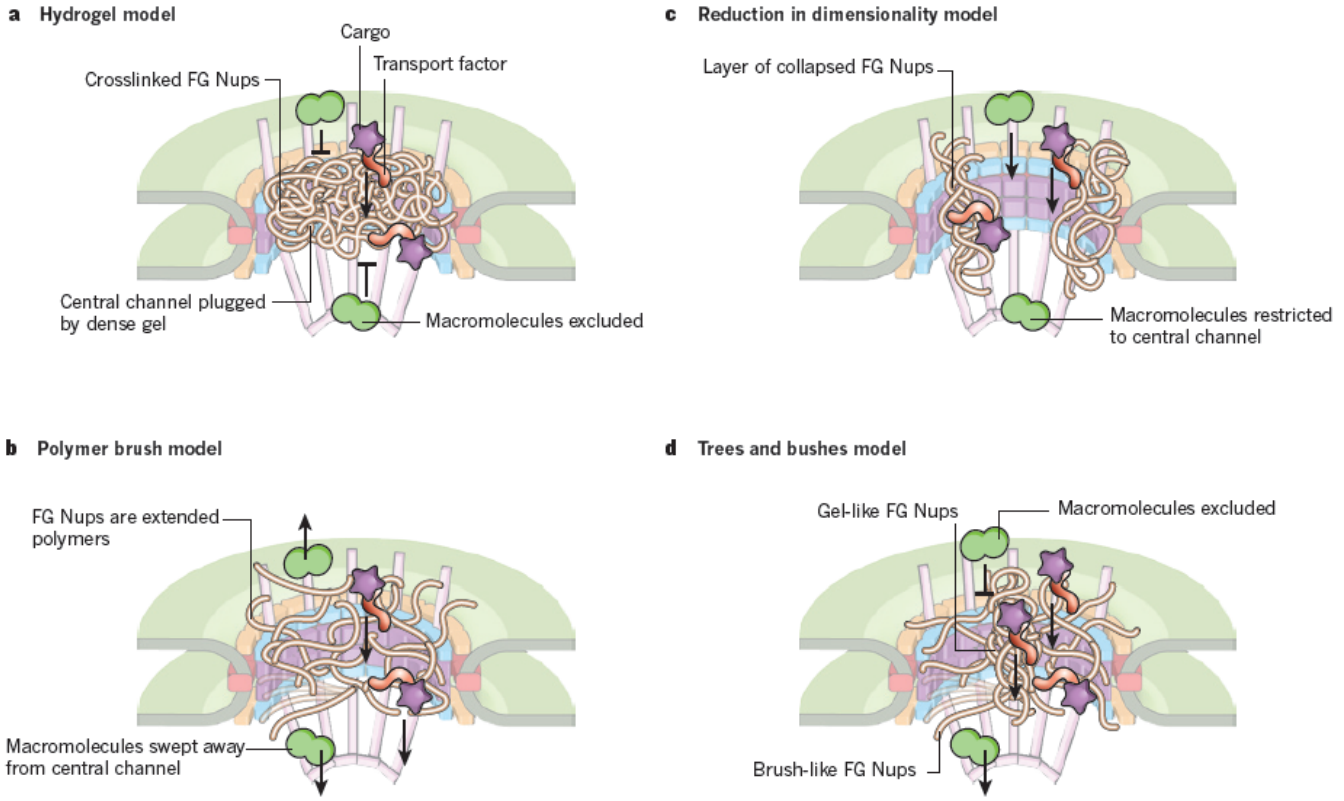


Figure 2 | Modes of transport. Various models for how the FG Nups mediate the selective barrier function of the NPC are shown. The detailed distribution of FG repeat domains is not illustrated here. **a**, FG Nups polymerize into a gel through which transport receptors pass by binding to the FG Nups and dissolving the crosslinks[25]. **b**, The FG repeat filaments diffuse around their tether, and other molecules are excluded from this region. Transport factors pass through by binding to the FG Nups[28,29]. The FG Nups might also act as a molecular brush that collapses once transport receptors have bound other molecules[24]. **c**, FG Nups collapse after binding by transport factors to form a layer along the walls of the channel. This layer is impenetrable to inert molecules but permeable to transport factors[52]. Inert macromolecules are able to pass through the central channel only. **d**, FG Nups form two categories of disordered filaments: collapsed coils, which are gel-like; and extended coils, which are brush-like[31]. Transport factors can pass through both configurations, but macromolecules are excluded. An argument can also be made (not shown) that the central channel *in vivo* will always be permeated with transport receptors, loaded or unloaded with cargo, resulting in a highly crowded environment. This could have a profound influence on the physical state of the FG Nups[33,34].

FG Nups evolve at unusually rapid rates: relaxed selection or diversifying selection resulting from coevolutionary arms races?

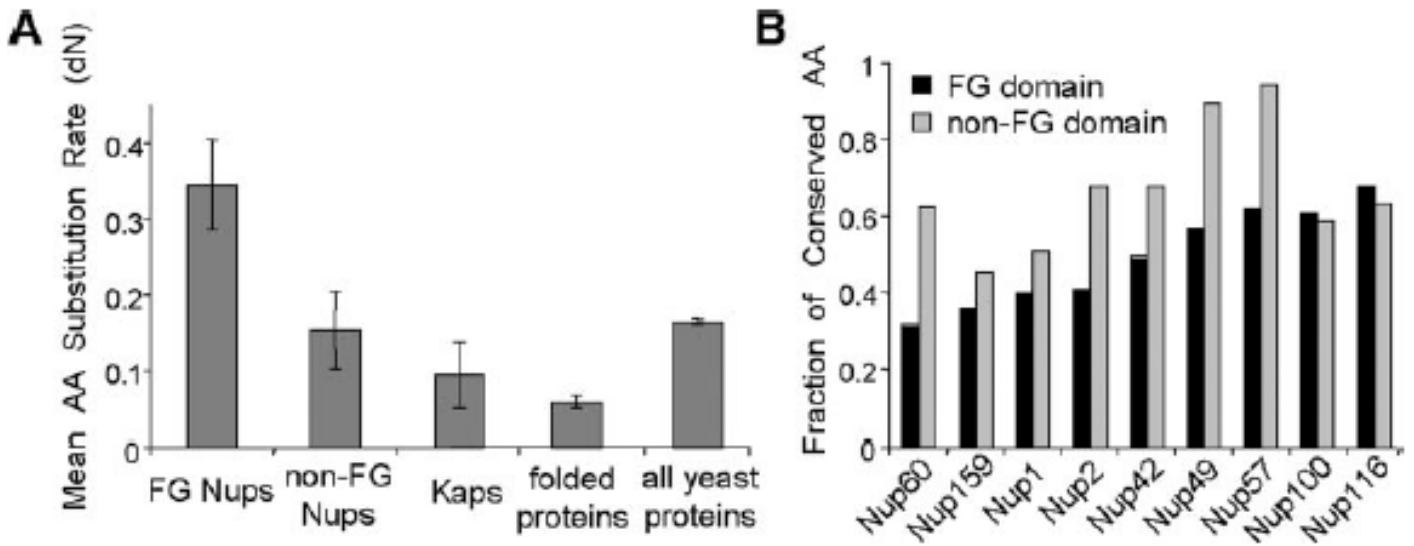


FIG. 1. **The FG Nups in budding yeast have evolved rapidly.** *A*, the mean AA substitution rates for FG Nups, non-FG Nups, Kaps, a set of 105 yeast proteins with highly significant homologs in the Protein Data Bank of NMR and crystal structures, and all yeast proteins were derived from alignments of syntenic orthologs from *S. cerevisiae*, *S. paradoxus*, *S. mikatae*, and *S. bayanus* (analysis of variance, $p = 2.2 \times 10^{-7}$). *Error bars* represent 95% confidence intervals for each mean. *B*, the fraction of conserved AAs in the FG domains and non-FG domains calculated from alignments of the FG Nups of four *Saccharomyces* species.

Denning and Rexach (2007, Mol. Cell. Proteomics)

Exploitation by viruses and mobile elements

- The LTR retrotransposon Tf1 in the yeast *S. pombe* has a requirement for the FXFG nuclear pore factor, Nup124. Three nuclear localization signals in the Gag protein of this mobile element are responsible for this interaction (Dang and Levin 2000; Kim et al. 2005).

- This same Nup is exploited by HIV (Varadarajan et al. 2005; Woodward et al. 2009; Lee et al. 2010); whereas it prevents entry of hepatitis B virus, specifically via the FXFG repeats, apparently by binding with the capsids (Schmitz et al. 2010).

- Many other examples exist for virus/mobile-element exploitation of Nup proteins in various ways:

  1) FIV (Lee et al. 2010);

  2) mengovirus enters mammalian cells by hyperphosphorylation of Nup62 (Bardina et al. 2009), and interactions with this same Nup are essential for mobility of some cardioviruses (Porter and Palmenberg 2009) and pos viruses (Sivan et al. 2013);

  3) poliovirus and rhinovirus employ protease-dependent cleavage of several Nups.

A link between Nup protein evolution and speciation?

- Nup160 and Nup96, have diverged between sister species *Drosophila melanogaster* and *D. simulans* by positive selection, and serve as incompatibility factors.

Table 3. Recurrent adaptive evolution at the hybrid lethality gene *Nup160* in both *D. melanogaster* and *D. simulans*. R, replacement; S, synonymous.

	Polymorphic		Divergent				Fisher's exact
	R	S	R/S	R	S	R/S	*P* value
D. melanogaster–D. simulans pooled	27	154	0.175	58	64	0.906	9.3×10^{-10}
D. melanogaster lineage	10	48	0.208	19	33	0.576	0.0299
D. simulans lineage	11	85	0.129	26	21	1.238	7.1×10^{-8}

- Possible mechanisms driving divergence:

  1) genetic conflict between hosts and pathogens;

  2) genetic conflict over centromeric drive (after nuclear disassembly, some Nup proteins localize to kinetochores during cell division).

Tang and Presgraves (2007, Science)
Presgraves and Stephan (2007, Mol. Biol. Evol.)

Ciliates have dual nuclei: the germline micronucleus and the somatic macronucleus, with distinct Nup features.

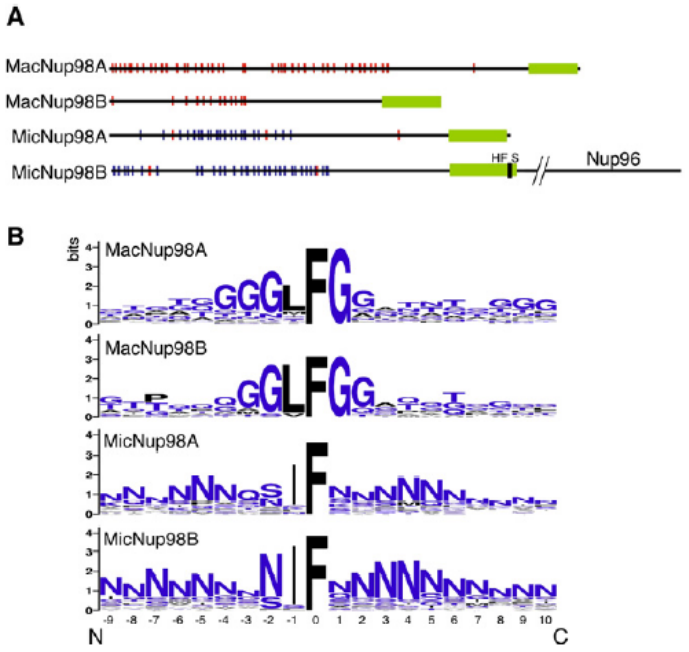
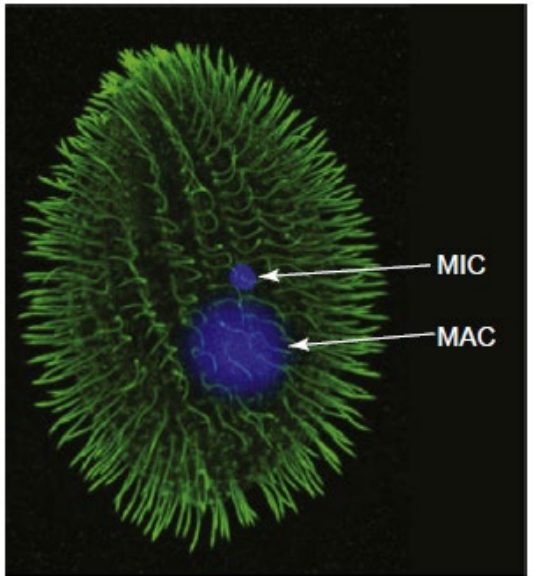


Figure 2. Molecular Characteristics of Four Nup98 Homologs in *T. thermophila*

(A) Domain architecture representing the positions of repeat sequences (red or blue vertical bars) and the conserved "Nucleoporin2" domain (green boxes). Out of the four Nup98 homologs, only MicNup98B features the amino acid sequence HFS (a solid black vertical line) conserved as an auto-cleavage site in the Nucleoporin2 domain; thus, the proform generates two gene products, MicNup98B with HF at the C terminus and Nup96 with S at the N terminus. Red and blue vertical bars represent the positions of FG or Fx (wherein x = N, Q, or S) repeats, respectively.

(B) An amino acid multiple alignment of the repeat regions of *Tetrahymena* Nup98 homologs was performed by WebLogo software (http://weblogo.berkeley.edu/). The y axis represents the relative frequency of each amino acid present in the repeats. GLFG and NIFN are well conserved in MacNup98A/B and MicNup98A/B, respectively. Black and blue letters represent hydrophobic and hydrophilic residues, respectively.

- In T*etrahymena*, the two nuclei share 12 of the identified Nups. However, for one group that occupies the central channel (98a and b), the mac- proteins contain conventional GLFG repeats, while the mic-proteins contain NIFN repeats.
- Swapping of the repeat domains abrogates pore function, suggesting that these Nups serve to prevent misdirection of nucleus-specific protein transport.



Iwamoto et al. (2009, Current Biol.)
Malone et al. (2008, Eukaryotic Cell)