



Evolutionary origins and specialisation of membrane transport

Joel B Dacks¹ and Mark C Field²

From unicellular protists to the largest megafauna and flora, all eukaryotes depend upon the organelles and processes of the intracellular membrane trafficking system. Well-defined machinery selectively packages and delivers material between endomembrane organelles and imports and exports material from the cell surface. This process underlies intracellular compartmentalization and facilitates myriad processes that define eukaryotic biology. Membrane trafficking is a landmark in the origins of the eukaryotic cell and recent work has begun to unravel how the revolution in cellular structure occurred.

Addresses

¹Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

²School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK

Corresponding author: Field, Mark C (mfield@mac.com)

Current Opinion in Cell Biology 2018, 53:70–76

This review comes from a themed issue on **Membrane trafficking**

Edited by **Anne Spang** and **Jitu Mayor**

<https://doi.org/10.1016/j.ceb.2018.06.001>

0955-0674/© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

The sophisticated last eukaryotic common ancestor

Many studies of membrane trafficking evolution focused on determining the organelles and proteins present in the last eukaryotic common ancestor (LECA), a hypothetical organism living $\sim 10^9$ years ago. As discussed in detail elsewhere [1,2], the numbers of predicted transport pathways and/or components within the LECA likely exceeded many extant organisms and LECA possessed all the canonical endomembrane organelles [1], extending to a *cis*, medial and *trans*-cisternal differentiated Golgi complex [3^{*}]. These inferences also provide insights into the basic mechanisms of vesicle formation and fusion [1].

LECA is deduced to have possessed at least nine distinct vesicle coat complexes (including clathrin/AP1-5, COPI, TSET, COPII, retromer, ESCRT), ARF/ARF-like GTPases and their regulators [1,2]. LECA also possessed complex fusion machinery, including an extensive SNARE complement [4^{**}], multisubunit tethering

complexes [5], Rab GTPases [6–8] and regulatory factors [9]. Thus, LECA was capable of endocytosis, secretion and complex sorting, and while this is perhaps surprising, metabolism, cytoskeleton, mitochondrial functions, nuclear transport and many other cellular systems demonstrate similar predicted complexity.

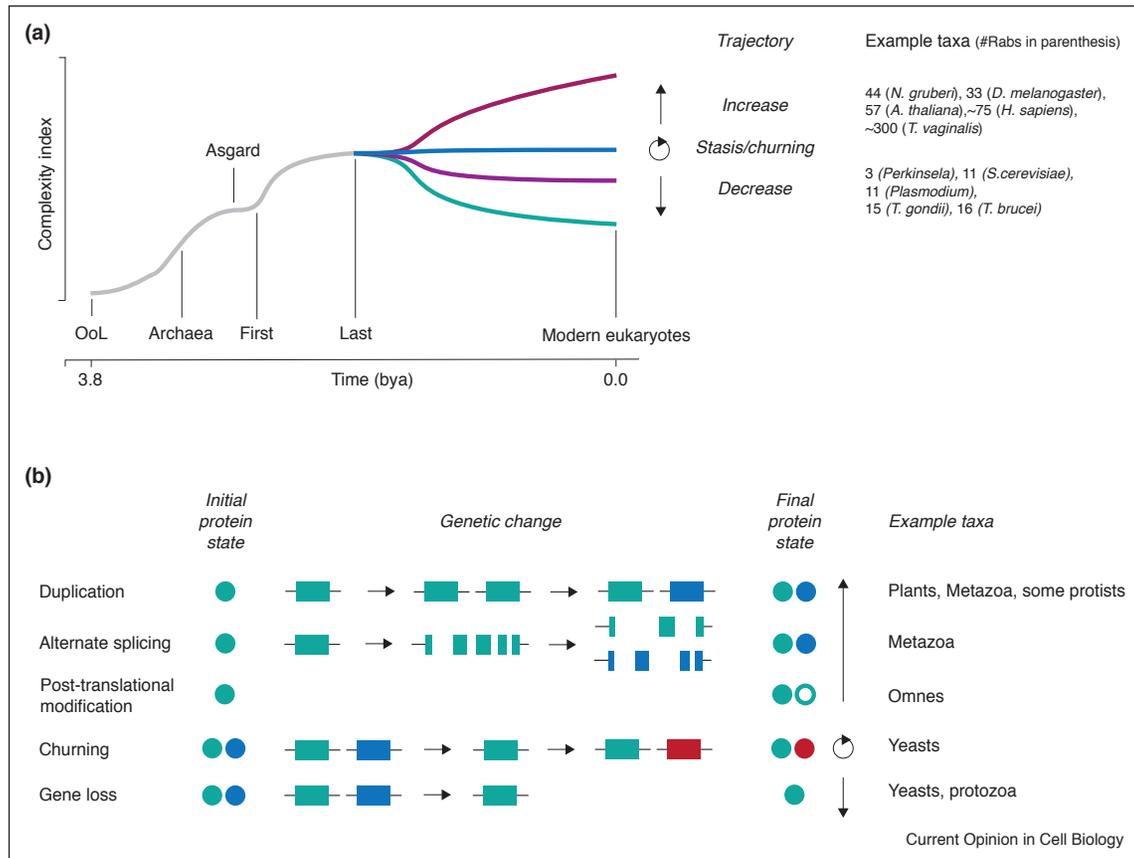
As new components and pathways are discovered within transport and sorting machinery, their relevance to LECA and subsequent evolution can be addressed, for example recent descriptions of vesicle formation machinery such as TSET [10], Tepsin [11,12^{*}], TSSC1 [13^{*}] and novel clathrin adaptors [14^{*}]. As a complex LECA should now be taken as a starting assumption, within trafficking systems and elsewhere, this complexity leads to two questions; what preceded LECA and how has subsequent evolution unfolded?

How did complex membrane trafficking evolve?

The most basic evolutionary question, how did an endomembrane system originate, cannot be resolved by reconstructing LECA, as this represents an already advanced cell. As we assume complexity arose from a simpler state, this implies that transition from the first eukaryotic common ancestor (FECA; the first differentiated lineage from archaea giving rise only to organisms possessing some eukaryotic traits) to LECA required a revolution in cellular mechanisms (Figure 1). Promisingly, details of this revolution are now being discerned [15].

The basic vesicle trafficking machinery involves several protein families, each member of which functions at a specific organelle or transport pathway. Furthermore, organelle and pathway identity arises via combinatorial protein–protein interactions [16]. Different combinations of Rabs, SNAREs and tether complexes interact and substituting one or a few components can alter intracellular localisation. As these families evolved via gene duplication (and subsequent neofunctionalisation (Figure 1)), a mechanism for organelle evolution can be proposed; that organelle complexity arose where a primordial set of vesicle formation and fusion proteins allowed for transport and, through gene duplications and co-evolution of interacting proteins, developed new specificity. One pathway became two, and by simple iteration, many. This mechanism, the ‘organelle paralogy hypothesis’, found experimental support and has been elaborated upon repeatedly since the original proposal [2,9,17^{*},18,19^{*},20,21].

Figure 1



Generation of complexity. **(Panel a)** Timeline for alterations to complexity with cells and genomes across the history of life, with emphasis on the eukaryotic lineage. The ‘Complexity Index’ is an abstract concept to express compartmental sophistication, and is assumed to have increased during the ascent of eukaryotes (gray line), including the contributions from the archaea and bacterial (not shown) donors. Significantly, there are examples of extant taxa where the number of compartments, as predicted by the size of the Rab gene cohort, have increased (red), decreased (purple, teal) or remained approximately constant (blue) from the predicted state in the LECA. OoL; Origin of life. Note that points of complexity increase, as well as the curve topology, are arbitrary and for illustrative purposes; the true topology is unknown. **(Panel b)** Mechanisms for the generation of molecular complexity, based on examples from Rab protein evolution. Proteins are shown as circles, and genes as rectangles. The open circle denotes a modified protein, for example phosphorylated or acetylated.

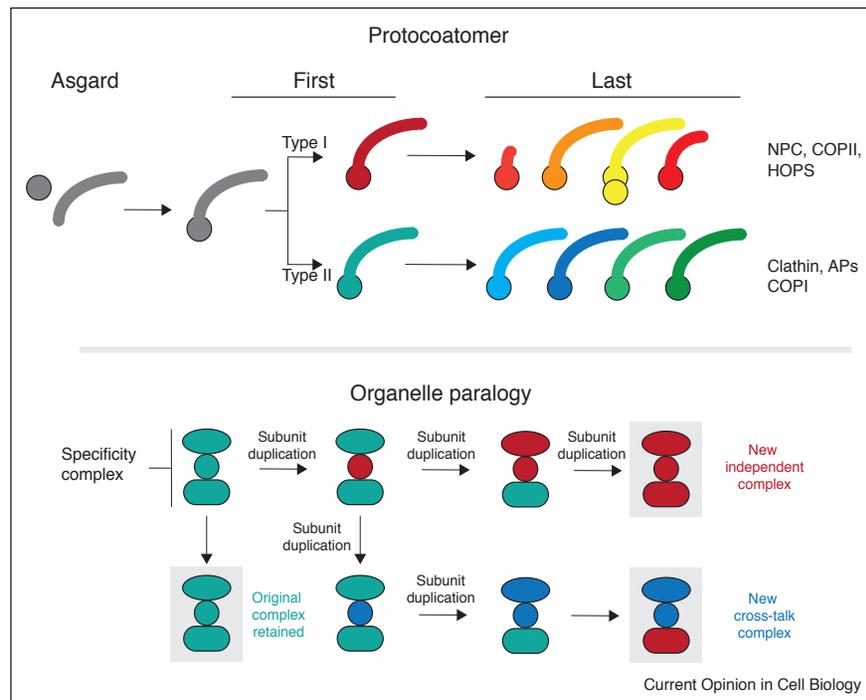
An important corollary to the concept of trafficking complexity emerging via incremental steps based on gene duplications is that, if the order of these steps can be resolved, the order in which the pathways originated would emerge. While significant challenges remain, over two-thirds of predicted LECA Rabs fall into either an endocytic or broadly secretory grouping [7]. Adaptins can be resolved to allow inference of multiple independent origins of pathways for internalization from the cell surface and post-Golgi transport [10]. Similar information for any membrane trafficking protein can, theoretically, determine the order of organelle origins, presently an exciting prospect.

While adaptin complex genes are easily identifiable, a deep connection is also likely present between many additional coat proteins. Homology here is based on

retention of one or more β -propellers followed by an α -solenoid, a ‘protocoatmer’ configuration, and members can be grouped into two subfamilies, with distinct structural features (Figure 2). Importantly this encompasses more complexes than classic coat proteins and includes the nuclear pore and SEA complexes and intraflagellar transport system, uniting the origins of most nonendosymbiotic organelles [22^{**}]. Reconstructing the evolutionary relationships remains a tremendous challenge due to massive sequence divergence and recent attempts have provided only partial solutions (e.g. [23^{*}]). Further, the NPC may contain multiple protocoatmer subfamily architectures, suggesting an origin post-dating establishment of several organelles [24,25,26^{*}].

There are relatively few hypotheses for membrane trafficking’s ultimate origin, and most are part of models

Figure 2



Paradigms for molecular evolution of eukaryotic cellular compartments and function. **(Top)** The protocoatomer hypothesis is predicated on the recognition that components of many membrane coating complexes share a particular architecture, specifically β -propeller plus α -solonoid secondary structural elements. While α and β structural elements are obviously common and present in all genomes, the combination of an N-terminal β -propeller plus α -solonoid configuration appears to be a hallmark of membrane deforming complexes, as well as being a eukaryotic signature. Complexes incorporating these proteins include the classical coats (COPI, COPII, clathrin), as well as the nuclear pore complex (NPC), adaptins and several others. Current evidence supports the following model: $\beta + \alpha$ proteins are encoded on the same cistron in Asgard archaea (but not as a single gene), which are presumed to have become fused at least by the time of the first eukaryotic common ancestor (First). Evidence suggests that there are at least two distinct types of protocoatomer in extant eukaryotes, based on the presence of several distinct accessory domains as well as structural criteria; these are arbitrarily termed type I and type II. It is unclear when these arose, but presumed an early event in transitioning from the first to last (Last) eukaryotic common ancestor (red/teal). Subsequent paralog expansions led to many distinct coats. Type I, which contains the NPC and COPII, appears more structurally diverse than Type II. Significant details for protocoatomer evolution remain to be determined. β -propeller structures are represented by a circle and α -solonoid by a curved bar of varying length. **(Lower)** Expanded version of the organelle paralogy hypothesis. Organelles are defined by the presence of members of paralog families, which include the Rab and SNARE proteins. Here we assume that three proteins (teal) are sufficient to define an organelle. Duplication of the circular factor allows the second copy (red) to neofunctionalise, as the original complex is retained. Initially this factor is able to interact with all of the original complex factors, but mutations will facilitate a change in specificity and the ability to bind a red eclipse and subsequently a red rounded rectangle. These latter factors are also the products of paralog expansion. Further mutation of the red circle (blue) can allow both a similar trajectory as before, as well as the possible sharing of components, again assisted by the paralogous nature of the various components.

explaining the origin of the eukaryotic cell itself [27–31,32^{••}]. The best of these suggest both a coherent model as well as incorporate existing data objectively. As new data arises, for example the demonstration that hybrid archaeal and bacterial lipid membranes are biochemically and biologically viable [33^{••}], some theories need to be modified or discarded in favour of hypotheses better supported by data. Perhaps the most available data at present, and thus best incorporated into models is the phylogenetic affinity and the relevant origins of membrane-trafficking components. For the overwhelming majority of these proteins, origins are of archaeal ancestry, supporting an autogenous evolution rather than endosymbiosis.

This model was provided a major boost with descriptions of the Lokiarchaeota [34] and subsequently a larger clade of related taxa, the Asgardarchaeota [35^{••}]. Asgardarchaeota biology is inferred via metagenomic assemblies from locales as exotic as deep-sea sediments and thermal springs to those as mundane as marine estuaries. Very recent environmental surveys have uncovered additional candidate Asgardarchaea-related sequences suggesting wide prevalence [36]. Phylogenetics indicates that eukaryotes emerged from within the Asgardarchaea, that is, that many Asgardarchaea genes are the most similar of all prokaryote sequences to their eukaryote descendants. These metagenomes encode proteins previously considered eukaryote-specific, including ESCRT subcomplex I

and II components, longin domain-containing proteins, expanded GTPase families similar to Rabs and Arf-like superfamilies, [37,38,39], putative COPII components and possible protocoatmer-related proteins. These features are consistent with the Asgardarchaea as an ancestral source for many membrane-trafficking components [35,37,38]. However, technical and methodological concerns regarding these genomes have been raised which need to be addressed, not least the authenticity of the metagenomic assemblies as derived from a single species (see [40,41,42] for the latest in this debate). Isolation and culturing of an Asgardian remains crucial for evaluating their contribution to eukaryogenesis.

How has the complex membrane trafficking system modified in LECA's descendants?

Understanding the origins of LECA complexity is one part of evolutionary study of membrane-trafficking; the counterpart is defining processes that shaped complexity post-LECA and what diversity has since arisen. Some components, for example, COPI and AP1 complexes, are near ubiquitous [10], suggesting they are both ancient and indispensable. Other components expanded in certain lineages or introduced novel domains [14,43]. Other components still, such as AP5 [44] and DSCR3 [45], are present in organisms spanning eukaryotic diversity, but frequent losses suggest that these ancient complexes are expendable, under some conditions. Several components (e.g. the SNARE NPSN) are lost from animals and fungi, indicating that opisthokonts have lineage-specific gains and losses, just as any other. While parasite genomes tend to be reduced, there are striking examples of gene family expansions in *Entamoeba* [46–48] and *Trichomonas* [48,49].

Inferring biology from genome sequence implies functional homology, that is, that a given gene retains the same function in different lineages. Evidence supports this for many gene products, including Rab5, 7 and 11, AP1 and 2 and ESCRT (reviewed extensively in [37,38]). Examples where functional homology is less apparent include organelles absent from animals or fungi, such as the osmoregulatory contractile vacuoles and modified secretory lysosomes associated with predation or parasitism, for example, mucocysts in ciliates and rhoptries in apicomplexa.

Trypanosomatids: a detailed case study

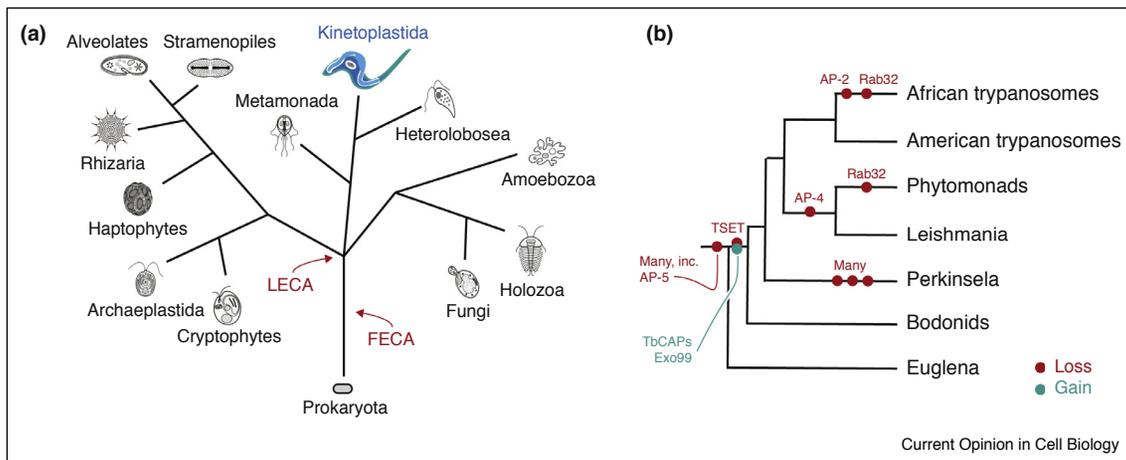
Understanding the extent to which adaptations are associated with smaller scale changes requires fine-scale investigations coupling genomics and cell biology. One well studied group of protists are the Euglenozoa, which are distantly related to animals and fungi (Figure 3). A vast array of lifestyles within the lineage, ranging from the free living and photosynthetic *Euglena* spp., [57] to *Bodo saltans*, [58] a phagophore, and many parasitic forms, including trypanosomes and *Leishmania*,

provides a perfect opportunity for evaluating predictions of post-LECA trafficking trends. High quality genome and experimental resources allow this to be investigated with some rigour.

Trypanosome endocytosis is exclusively clathrin-mediated, with an intriguing amalgam of conserved proteins, for example, epsinR, CALM, AAK and AP-1, losses, for example, Dab2, and lineage-specific innovations conserved throughout kinetoplastids, for example, TbCAPs [14,50,51]. An emerging paradigm is of a conserved core with a secondary 'shell' of lineage-specific proteins, *albeit* frequently retaining common architectural features. For example TbCAP80 and TbCAP141 are both phosphoinositide-binding proteins with an N-terminal lipid-interacting domain and disordered C-terminus, similar to organisation of ANTH and ENTH proteins [14,51]. Similarly, kinetoplastid exocytic pathways are modified, with an additional lineage-specific subunit, Exo99, as a component of an otherwise conventional octameric exocyst Boehm *et al.* [52]. The functional implications of these innovations remain cryptic.

A major overall trend across the lineage is of secondary loss in the Rab and SNARE proteins, *albeit* following a gradual shift in complexity. Kinetoplastids retain essentially all SNARE proteins predicted to be present in LECA, but lack several Rabs, including Rab8, 34 and 50. Whilst the former functions in post-Golgi transport, Rab34 and Rab50 are uncharacterised. Simplification of anterograde pathways is unsurprising as exocytosis in trypanosomes does not appear to be significantly differentiated into multiple pathways. Within the kinetoplastids, *Bodo saltans* has the largest Rab and SNARE gene complement, which undergoes a gradual diminishment as one progresses through the Leishmanias, American trypanosomes (*Trypanosoma cruzi* and relatives) and finally to African trypanosomes (*T. brucei* and relatives) (Figure 3). Significantly, the plant parasitic Phytomonads are also reduced. These alterations of trafficking complexity likely reflect life style; for example *B. saltans* must adapt to rapid environmental and nutrient changes and has a large repertoire of Rab7 and Rab32-related Rabs facilitating autophagic and complex digestive functions, as well potentially as the osmoregulatory contractile vacuole. *Leishmania* and *T. cruzi* invade host cells and retention of a more complex transport system by *T. cruzi*, may reflect this and specifically a need to adapt and exploit autophagic mechanisms if resources are scarce. Both Phytomonads and African trypanosomes are distinguished by remaining extracellular in their respective plant and mammalian hosts; it is probably significant that both lack Rab32, have very few lineage-specific Rab proteins and also, in the case of Phytomonas, have a significant loss of the endocytic Rab21 and 28. Significantly, African trypanosomes lost the AP-2 complex as an adaptation to antigenic variation and a need for extremely

Figure 3



Phylogeny of eukaryotes, emphasising the kinetoplastida. **(Panel a)** The tree is based on most recent views of eukaryotic relationships, and each clade is accompanied by an illustrative diagram for a representative species. Positions of LECA and FECA are indicated in red, and kinetoplastida are highlighted in teal. **(Panel b)** Evolutionary relationships between the organisms discussed in the text, together with indications of where reconstruction suggest various proteins have been lost or gained during evolution.

rapid endocytosis for immune evasion [53]. However, recently we found that *T. cruzi*, which does possess the genes for all AP-2 subunits, apparently does not use this complex for endocytosis [54], indicating that AP-2 independent endocytosis is more widespread than inferred by comparative genomics and perhaps serves to underscore the importance of experimental study. Most recently, the genome sequence for *Perkinsela*, an intracellular kinetoplastid parasite with a greatly reduced genome has been reported; this organism has but three Rab1/Rab2-like proteins [55**], providing a provocative example of the flexibility of the trafficking system and its evolution.

Conclusion

Eukaryotic diversity is immense and has direct bearing upon our health, agriculture and environment. Understanding how such distinctiveness came to be is a major goal of evolutionary cell biology [56]. Genome sequencing, direct experimentation and increased sampling of environments have all revealed pathways that shaped the multiplicity of cellular forms and architectures, with membrane trafficking retaining a position centre stage. With considerable knowledge, we are in the exciting position of beginning to understand the origins of trafficking, and to explore the many facets of these pathways in multiple lineages.

Acknowledgements

Work in the authors' laboratories is supported by the Medical Research Council (MR/N010558/1 and MR/P009018/1 to MCF), the Wellcome Trust (204697/Z/16/ Z to MCF) and an NSERC Discovery grant (RES0021028 to JBD). JBD is the Canada Research Chair (Tier II) in Evolutionary Cell Biology. MCF is a Wellcome Trust Investigator.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- of special interest
- of outstanding interest

1. Koumandou VL, Wickstead B, Ginger ML, van der Giezen M, Dacks JB, Field MC: **Molecular paleontology and complexity in the last eukaryotic common ancestor**. *Crit Rev Biochem Mol Biol* 2013, **48**:373-396.
2. Schlacht A, Herman EK, Klute MJ, Field MC, Dacks JB: **Missing pieces of an ancient puzzle: evolution of the eukaryotic membrane-trafficking system**. *Cold Spring Harb Perspect Biol* 2014, **6**:a016048.
3. Barlow LD, Nývltová E, Aguilar M, Tachezy J, Dacks JB: **A sophisticated, differentiated Golgi in the ancestor of eukaryotes**. *BMC Biol* 2018, **16**:27 <http://dx.doi.org/10.1186/s12915-018-0492-9> Erratum in: *BMC Biol.* 2018 Mar 2018;16(1):35.
This paper provides a bioinformatic reconstruction of the organizational complexity of the Golgi apparatus in the Last Eukaryotic Common Ancestor. Not only does it strongly suggest the presence of a differentiated Golgi, comprising multiple distinct compartments, it also suggests tantalizing implications for the mechanism of Golgi stacking in modern eukaryotes.
4. Venkatesh D, Boehm C, Barlow LD, Nankissoor NN, O'Reilly A, Kelly S, Dacks JB, Field MC: **Evolution of the endomembrane systems of trypanosomatids – conservation and specialisation**. *J Cell Sci* 2017, **130**:1421-1434.
Reconstruction of the evolutionary history of the trafficking machinery in the kinetoplastids, using Rab, Rab GAPs and SNARE genes as predators of compartments and complexity. The study illuminates pathways of sculpting as this group of organisms exploit specific hosts and adapt to differing environments.
5. Koumandou VL, Dacks JB, Coulson RM, Field MC: **Control systems for membrane fusion in the ancestral eukaryote; evolution of tethering complexes and SM proteins**. *BMC Evol Biol* (7):2007:29.
6. Diekmann Y, Seixas E, Gouw M, Tavares-Cadete F, Seabra MC, Pereira-Leal JB: **Thousands of rab GTPases for the cell biologist**. *PLoS Comput Biol* 2011, **7**:e1002217.

7. Elias M, Brighthouse A, Gabernet-Castello C, Field MC, Dacks JB: **Sculpting the endomembrane system in deep time: high resolution phylogenetics of Rab GTPases.** *J Cell Sci* 2012, **125** (Pt 10):2500-2508.
8. Klöpffer TH, Kienle N, Fasshauer D, Munro S: **Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis.** *BMC Biol* 2012, **10**:71.
9. Gabernet-Castello C, O'Reilly AJ, Dacks JB, Field MC: **Evolution of Tre-2/Bub2/Cdc16 (TBC) Rab GTPase-activating proteins.** *Mol Biol Cell* 2013, **24**:1574-1583.
10. Hirst J, Schlacht A, Norcott JP, Traynor D, Bloomfield G, Antrobus R, Kay RR, Dacks JB, Robinson MS: **Characterization of TSET, an ancient and widespread membrane trafficking complex.** *Elife* 2014, **3**:e02866.
11. Mattera R, Guardia CM, Sidhu SS, Bonifacino JS: **Bivalent motif-ear interactions mediate the association of the accessory protein Tepsin with the AP-4 adaptor complex.** *J Biol Chem* 2015, **290**:30736-30749.
12. Frazier MN, Davies AK, Voehler M, Kendall AK, Borner GH, Chazin WJ, Robinson MS, Jackson LP: **Molecular basis for the interaction between AP4 β 4 and its accessory protein, Tepsin.** *Traffic* 2016, **17**:400-415.
- An excellent example of a lineage-specific protein involved in the trafficking pathway.
13. Gershlick DC, Schindler C, Chen Y, Bonifacino JS: **TSSC1 is novel component of the endosomal retrieval machinery.** *Mol Biol Cell* 2016, **27**:2867-2878.
- See annotation to Ref. [12].
14. Manna PT, Obado SO, Boehm C, Gadelha C, Sali A, Chait BT, Rout MP, Field MC: **Lineage-specific proteins essential for endocytosis in trypanosomes.** *J Cell Sci* 2017, **130**:1379-1392.
- Detailed investigation of two lineage-specific proteins in trypanosomes identified by proteomics that share architectural features with conserved endocytic proteins, but not sequence. This suggests possible constraints in sorting receptor/clathrin-associated proteins in terms of the overall structure and functionality.
15. Dacks JB, Robinson M: **Outerwear through the ages: evolutionary cell biology of vesicle coats.** *Curr Opin Cell Biol* 2017, **47**:108-116.
16. Cai H, Reinisch K, Ferro-Novick S: **Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle.** *Dev Cell* 2007, **12**:671-682.
17. Shukla A, Bhattacharyya A, Kuppusamy L, Srivas M, Thattai M: **Discovering vesicle traffic network constraints by model checking.** *PLOS ONE* 2017, **12**:e0180692.
- Are the known components, and theories for compartment identity sufficient to account for eukaryote evolution and diversification? This modelling paper addresses this with a mathematical treatment to describe what constraints are most relevant for understanding the demands of organelles identity.
18. Dacks JB, Field MC: **Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode.** *J Cell Sci* 2007, **120** (Pt 17):2977-2985.
19. Mani S, Thattai M: **Wine glasses and hourglasses: non-adaptive complexity of vesicle traffic in microbial eukaryotes.** *Mol Biochem Parasitol* 2016, **209**:58-63.
- A mathematic treatment that demonstrates that new organelles can arise by neutral evolution - and which is consistent with several experiments studies indicating rapid birth and death of pathways.
20. Dacks JB, Poon PP, Field MC: **Phylogeny of endocytic components yields insight into the process of nonendosymbiotic organelle evolution.** *Proc Natl Acad Sci U S A* 2008, **105**:588-593.
21. Bright LJ, Gout JF, Lynch M: **Early stages of functional diversification in the Rab GTPase gene family revealed by genomic and localization studies in *Paramecium* species.** *Mol Biol Cell* 2017, **28**:1101-1110.
22. Rout MP, Field MC: **The evolution of organellar coat complexes and organization of the eukaryotic cell.** *Annu Rev Biochem* (86):2017:637-657.
- Lays out the current evidence for the protocoatmer hypothesis and implications for the origin s and specialisations of eukaryotes.
23. Promponas VJ, Katsani KR, Blencowe BJ, Ouzounis CA: **Sequence evidence for common ancestry of eukaryotic endomembrane coatomers.** *Sci Rep* 2016, **6**:22311.
- Given the huge divergence in sequences of the protocoatmer family of proteins, conventional phylogenetic methods have failed to reconstruct the full family, with the obvious issue that the order that such complexes arose cannot be predicted. This study utilises a sophisticated set of similarity assessments to arrive at a possible set of relationships.
24. Sampathkumar P, Kim SJ, Upla P, Rice WJ, Phillips J, Timney BL, Pieper U, Bonanno JB, Fernandez-Martinez J, Hakhverdyan Z, Ketaren NE, Matsui T, Weiss TM, Stokes DL, Sauder JM, Burley SK, Sali A, Rout MP, Almo SC: **Structure, dynamics, evolution, and function of a major scaffold component in the nuclear pore complex.** *Structure* 2013, **21**:560-571.
25. Shi Y, Fernandez-Martinez J, Tjioe E, Pellarin R, Kim SJ, Williams R, Schneidman-Duhovny D, Sali A, Rout MP, Chait BT: **Structural characterization by cross-linking reveals the detailed architecture of a coatmer-related heptameric module from the nuclear pore complex.** *Mol Cell Proteomics* 2014, **13**:2927-2943.
26. Kim SJ, Fernandez-Martinez J, Nudelman I, Shi Y, Zhang W, Raveh B, Herricks T, Slaughter BD, Hogan JA, Upla P, Chemmama IE, Pellarin R, Echeverria I, Shivaraju M, Chaudhury AS, Wang J, Williams R, Unruh JR, Greenberg CH, Jacobs EY, Yu Z, de la Cruz MJ, Mironska R, Stokes DL, Aitchison JD, Jarrold MF, Gerton JL, Ludtke SJ, Akey CW, Chait BT, Sali A, Rout MP: **Integrative structure and functional anatomy of a nuclear pore complex.** *Nature* 2018, **555**:475-482.
- Reconstruction of the yeast nuclear pore complex to high resolution. Demonstrates the presence of a high degree of diversity in the arrangement of protocoatmer subunits and the possibility that the nuclear pore is a later assemblage of type 1 and type 2 protocoatmer subunits. This also suggests that the evolution of the nuclear envelope is a possibly later event in eukaryogenesis.
27. Martin WF, Neukirchen S, Zimorski V, Gould SB, Sousa FL: **Energy for two: New archaeal lineages and the origin of mitochondria.** *Bioessays* 2016, **38**:850-856.
28. Gould SB, Garg SG, Martin WF: **Bacterial vesicle secretion and the evolutionary origin of the eukaryotic endomembrane system.** *Trends Microbiol* 2016, **24**:525-534.
29. Baum DA, Baum B: **An inside-out origin for the eukaryotic cell.** *BMC Biol* 2014, **12**:76.
30. Martijn J, Ettema TJ: **From archaeon to eukaryote: the evolutionary dark ages of the eukaryotic cell.** *Biochem Soc Trans* 2013, **41**:451-457.
31. López-García P, Eme L, Moreira D: **Symbiosis in eukaryotic evolution.** *J Theor Biol* 2017, **434**:20-33.
32. Zachar I, Szilágyi A, Számadó S, Szathmáry E: **Farming the mitochondrial ancestor as a model of endosymbiotic establishment by natural selection.** *Proc Natl Acad Sci U S A* 2018. pii:201718707.
- This sophisticated mathematical model addresses the dynamics of establishing a mitochondrial endosymbiont in the proto-eukaryote. Intriguingly, it suggests that a direct benefit from the symbiont is not a necessary condition for establishment but does imply that phagocytotic ability by the host is needed. Regardless of the staying power of the specific model, the paper is notable for relying on robust mathematically based methodology and emphasizing ecological and population-level adaptation in the debate on eukaryogenesis.
33. Caforio A, Siliakus MF, Exterkate M, Jain S, Jumde VR, Andringa RLH, Kengen SWM, Minnaard AJ, Driessen AJM, van der Oost J: **Converting *Escherichia coli* into an archaeobacterium with a hybrid heterochiral membrane.** *Proc Natl Acad Sci U S A* 2018, **115**:3704-3709.
- Experimentally addresses the issue of apparent incompatibility between archaea and eukaryotic/eubacterial lipids by engineering *E. coli* to produce both. This supposed incompatibility has been raised as a major barrier in eukaryogenesis. The data described in this paper indicate that such a hybrid is viable and hence that mixed stereochemistry does not necessarily represent a barrier to evolution.

34. Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, C Schleper, Guy L, Ettema TJ: **Complex archaea that bridge the gap between prokaryotes and eukaryotes.** *Nature* 2015, **521**:173-179.
35. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, ● Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, Stott MB, Nunoura T, Banfield JF, Schramm A, Baker BJ, Spang A, Ettema TJ: **Asgard archaea illuminate the origin of eukaryotic cellular complexity.** *Nature* 2017, **541**:353-358.
- Extended dataset of the Asgard Archaea, and which identifies several more putative representatives of the group, and from a varied set of environments. The new findings suggests that these organisms are quite widespread throughout the biosphere, and that they are diverse, but possess genes that are the closest prokaryotic homologs of eukaryotic genes.
36. Karst SM, Dueholm MS, Mclroy SJ, Kirkegaard RH, Nielsen PH, Albertsen M: **Retrieval of a million high-quality, full-length microbial 16S and 18S rRNA gene sequences without primer bias.** *Nat Biotechnol* 2018, **36**:190-195.
37. Klinger CM, Ramirez-Macias I, Herman EK, Turkewitz AP, Field MC, Dacks JB: **Resolving the homology-function relationship through comparative genomics of membrane-trafficking machinery and parasite cell biology.** *Mol Biochem Parasitol* 2016, **209**:88-103.
38. Klinger CM, Spang A, Dacks JB, Ettema TJ: **Tracing the archaeal origins of eukaryotic membrane-trafficking system building blocks.** *Mol Biol Evol* 2016, **33**:1528-1541.
39. Surkont J, Pereira-Leal JB: **Are There Rab GTPases in archaea?** ● *Mol Biol Evol* 2016, **33**:1833-1842.
- An analysis of the predicted small GTPases present in Lokiarchaea. The study masses important residues and sequence motifs to determine that many are close relatives of Rab proteins, but fall outside of the definition of a Rab in extant organisms.
40. Da Cunha V, Gaia M, Gabelle D, Nasir A, Forreter P: **Lokiarchaea ● are close relatives of Euryarchaeota, not bridging the gap between prokaryotes and eukaryotes.** *PLoS Genet* 2017, **13**: e1006810.
- Detailed challenge to the integrity and position of the Asgard archaea. Provides evidence for issues with assembly, suggesting extensive chimerism, and distinct lineages for many genes. Calls into doubt the entire idea that the Asgard archaea contributed to eukaryogenesis.
41. Spang A, Eme L, Saw JH, Caceres EF, Zaremba-Niedzwiedzka K, Lombard J, Guy L, Ettema TJ: **Asgard archaea are the closest prokaryotic relatives of eukaryotes.** *PLoS Genet* 2018, **14**: e1007080.
42. Da Cunha V, Gaia M, Nasir A, Forreter P: **Asgard archaea do not close the debate about the universal tree of life topology.** *PLOS Genet* 2018, **14**:e1007215.
43. Kontur C, Kumar S, Lan X, Pritchard JK, Turkewitz AP: **Whole ● genome sequencing identifies a novel factor required for secretory granule maturation in *Tetrahymena thermophila*.** *G3 (Bethesda)* 2016, **6**:2505-2516.
- Example of a SAR supergroup-specific trafficking protein, and demonstration of specific evolution within another supergroup.
44. Hirst J, Barlow LD, Francisco GC, Sahlender DA, Seaman MN, Dacks JB, Robinson MS: **The fifth adaptor protein complex.** *PLoS Biol* 2011, **9**:e1001170.
45. Koumandou VL, Klute MJ, Herman EK, Nunez-Miguel R, Dacks JB, Field MC: **Evolutionary reconstruction of the retromer complex and its function in *Trypanosoma brucei*.** *J Cell Sci* 2011, **124**(Pt 9):1496-1509.
46. Nakada-Tsukui K, Saito-Nakano Y, Husain A, Nozaki T: **Conservation and function of Rab small GTPases in *Entamoeba*: annotation of *E. invadens* Rab and its use for the understanding of *Entamoeba* biology.** *Exp Parasitol* 2010, **126**:337-347.
47. Herman E, Siegesmund MA, Bottery MJ, van Aerle R, Shather MM, Caler E, Dacks JB, van der Giezen M: **Membrane trafficking modulation during *Entamoeba* encystation.** *Sci Rep* 2017, **7**:12854.
48. Lal K, Field MC, Carlton JM, Warwicker J, Hirt RP: **Identification of a very large Rab GTPase family in the parasitic protozoan *Trichomonas vaginalis*.** *Mol Biochem Parasitol* 2005, **143**:226-235.
49. Carlton JM, Hirt RP, Silva JC, Delcher AL, Schatz M, Zhao Q, Wortman JR, Bidwell SL, Alsmark UC, Besteiro S, Sicheritz-Ponten T, Noel CJ, Dacks JB, Foster PG, Simillion C, Van de Peer Y, Miranda-Saavedra D, Barton GJ, Westrop GD, Müller S, Dessi D, Fiori PL, Ren Q, Paulsen I, Zhang H, Bastida-Corcuera FD, Simoes-Barbosa A, Brown MT, Hayes RD, Mukherjee M, Okumura CY, Schneider R, Smith AJ, Vanacova S, Villalvazo M, Haas BJ, Perteu M, Feldblyum TV, Utterback TR, Shu CL, Osoegawa K, de Jong PJ, Hrdy I, Horvathova L, Zubacova Z, Dolezal P, Malik SB, Logsdon JM Jr, Henze K, Gupta A, Wang CC, Dunne RL, Upcroft JA, Upcroft P, White O, Salzberg SL, Tang P, Chiu CH, Lee YS, Embley TM, Coombs GH, Mottram JC, Tachezy J, Fraser-Liggett CM, Johnson PJ: **Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*.** *Science* 2007, **315**:207-212.
50. Adung'a VO, Gadelha C, Field MC: **Proteomic analysis of clathrin interactions in trypanosomes reveals dynamic evolution of endocytosis.** *Traffic* 2013, **14**:440-457.
51. Manna PT, Gadelha C, Puttick AE, Field MC: **ENTH and ANTH domain proteins participate in AP2-independent clathrin-mediated endocytosis.** *J Cell Sci* 2015, **128**:2130-2142.
52. Boehm CM, Obado S, Gadelha C, Kaupisch A, Manna PT, ● Gould GW, Munson M, Chait BT, Rout MP, Field MC: **The trypanosome exocyst: a conserved structure revealing a new role in endocytosis.** *PLoS Pathog* 2017, **13**:e1006063.
- Demonstration, using proteomics and reverse genetics, of an additional subunit for the exocyst with highly restricted distribution. Good example of a lineage-specific acquisition of a new component for an otherwise well conserved complex, but retaining clear functional similarity.
53. Manna PT, Kelly S, Field MC: **Adaptin evolution in kinetoplastids and emergence of the variant surface glycoprotein coat in African trypanosomatids.** *Mol Phylogenet Evol* 2013, **67**:123-128.
54. Kalb LC, Frederico YC, Boehm C, Moreira CM, Soares MJ, Field MC: **Conservation and divergence within the clathrin interactome of *Trypanosoma cruzi*.** *Sci Rep.* (6):2016:31212.
55. Tanifuji G, Cenci U, Moog D, Dean S, Nakayama T, David V, Fiala I, ● Curtis BA, Sibbald SJ, Onodera NT, Colp M, Flegontov P, Johnson-MacKinnon J, McPhee M, Inagaki Y, Hashimoto T, Kelly S, Gull K, Luke J, Archibald JM: **Genome sequencing reveals metabolic and cellular interdependence in an amoeba-kinetoplastid symbiosis.** *Sci Rep* 2017, **7**:11688.
- Genome sequence and analysis of Perkinsela, a kinetoplastid endoparasite. The organism is fully dependent on its host. Significantly, the genome is hugely reduced, and lacks a major cohort of trafficking proteins.
56. Dacks JB, Field MC, Buick R, Eme L, Gribaldo S, Roger AJ, Brochier-Armanet C, Devos DP: **The changing view of eukaryogenesis – fossils, cells, lineages and how they all come together.** *J Cell Sci* 2016, **129**:3695-3703.
57. Ebenezer T, Zoltner M, Burrel A, Nenarokova A, Novák Vanclová AMG, Prasad B, Soukal P, Santana-Molina C, O'Neill E, Nankissoor NN, Vadakedath N, Daiker V, Obado S, Jackson AP, Devos D, Luke J, Lebert M, Vaughan S, Hampl V, Carrington M, Ginger ML, Dacks JB, Kelly S, Field MC: **Decoding the evolution, cell biology, significance to kinetoplastid parasitism and biotechnological potential of *Euglena gracilis*.** *bioRxiv* 2018 <http://dx.doi.org/10.1101/228015>.
58. Jackson AP, Otto TD, Aslett M, Armstrong SD, Bringaud F, ● Schlacht A, Hartley C, Sanders M, Wastling JM, Dacks JB, Acosta-Serrano A, Field MC, Ginger ML, Berriman M: **Kinetoplastid phylogenomics reveals the evolutionary innovations associated with the origins of parasitism.** *Curr Biol* 2016, **26**:161-172.
- Detailed consideration of the mechanisms of evolution of parasites, using well defined and understood systems. The major conclusion is that there is no obvious signal, and that changes are gradual, at least in terms of the membrane trafficking system complexity. Perhaps a caution that simple gene complement is insufficient to reconstruct complex evolutionary events.