

Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode

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Accepted 17 July 2007

Journal of Cell Science 120, 2977-2985 Published by The Company of Biologists 2007
doi:10.1242/jcs.013250

Summary

The emergence of an endomembrane system was a crucial stage in the prokaryote-to-eukaryote evolutionary transition. Recent genomic and molecular evolutionary analyses have provided insight into how this critical system arrived at its modern configuration. The apparent relative absence of prokaryotic antecedents for the endomembrane machinery contrasts with the situation for mitochondria, plastids and the nucleus. Overall, the evidence suggests an autogenous origin for the eukaryotic membrane-trafficking machinery. The emerging picture is that early eukaryotic ancestors had a complex endomembrane system, which implies that this cellular system evolved relatively rapidly after the proto-eukaryote diverged away from the other prokaryotic lines. Many of the components of the

trafficking system are the result of gene duplications that have produced proteins that have similar functions but differ in their subcellular location. A proto-eukaryote possessing a very simple trafficking system could thus have evolved to near modern complexity in the last common eukaryotic ancestor (LCEA) via paralogous gene family expansion of the proteins encoding organelle identity. The descendants of this common ancestor have undergone further modification of the trafficking machinery; unicellular simplicity and multicellular complexity are the prevailing trend, but there are some remarkable counter-examples.

Key words: Eukaryote, Endomembrane, Evolution

Introduction

Eukaryotic cells differ fundamentally from their prokaryotic counter-parts by their possession of internal, membrane-bound, compartments. The origins and mechanisms by which these organelles arose are hotly debated. Much has been published about the evolution of mitochondria and plastids (for reviews, see Barbrook et al., 2006; Embley and Martin, 2006); the origin of the nucleus has received significant but less attention (Lopez-Garcia and Moreira, 2006; Martin, 2005). In the evolutionary transition in which a lineage of prokaryote-like organisms (the proto-eukaryote) diverged from Bacteria and Archaea and eventually gave rise to eukaryotes, the emergence of these organelles would have been important landmarks. No less critical, however, would have been the appearance of cellular machinery for internalization and digestion of extracellular material, targeted intracellular transport, surface remodelling and secretion. These functions are only possible in eukaryotic cells because of the presence of the membrane-trafficking system. Some obvious advantages conferred by such a system include improved heterotrophy (Cavalier-Smith, 1987b; Cavalier-Smith, 2002), the potential for increased cell volume and greater control over surface composition and complexity.

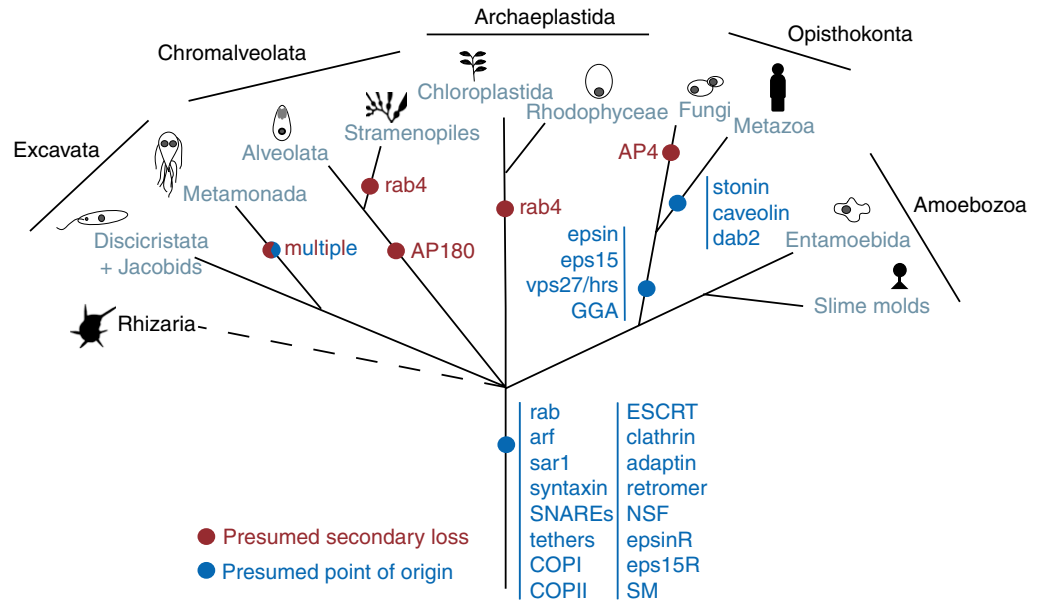
The origins and evolution of the membrane-trafficking system have not received as much attention as those of many other organelles. Proposed paradigms for the evolution of the trafficking system include both endosymbiotic (Gupta and Golding, 1996; Martin and Muller, 1998; Moreira and Lopez-Garcia, 1998) and autogenous origins (Cavalier-Smith, 1975; Cavalier-Smith, 1987b). However, until recently (Cavalier-

Smith, 2002; de Duve, 2007; Jekely, 2003; Mironov et al., 2006), these models were incorporated within larger theories of eukaryogenesis and included relatively few specific molecular details or predictions.

In the past 15 years, studies of yeast (*Saccharomyces cerevisiae*) and metazoan cells have revealed a common core of protein factors involved in transport carrier formation, compartment specificity and membrane fusion (Bonifacino and Glick, 2004). Small GTPases, adaptor proteins and coat protein complexes assemble on membranes to produce cargo carriers (vesicular, tubular or otherwise) that export material from a particular organelle for delivery to another compartment in a highly specific manner (McMahon and Mills, 2004; Robinson, 2004). Protein factors including SNAREs, tethering complexes, syntaxin-binding proteins [also known as Sec1/Munc18 (SM) proteins], and Rab GTPases ensure specificity and fusion of these carriers with the correct target membrane (Jahn et al., 2003). Many of these protein families can be divided into subfamilies in which each paralogue performs a function similar to that of the other members but at a specific organellar location or in a distinct transport pathway (Bonifacino and Glick, 2004). The genes encoding these membrane-trafficking proteins are, therefore, amenable to molecular evolutionary analyses of the type that has yielded information on the emergence and history of the other eukaryotic organelles.

A combination of single-gene, multi-gene and morphological data (Keeling et al., 2005; Simpson and Roger, 2004) has allowed the definition of six eukaryotic 'super groups' (Fig. 1), of which metazoa and fungi populate only one

Fig. 1. Evolutionary origin and loss of endomembrane components across the major eukaryotic lineages. Current understanding of the relationships between major eukaryotic lineages suggests a rapid radiation early in evolution that gave rise to the six major groups. The stem of the tree represents the transition between prokaryotes and eukaryotes, and is a period of radical innovation. Red dots indicate presumed secondary loss of factors, because multiple sampled lineages above the internode lack the relevant gene. Blue dots indicate that most taxa above the symbol possess a given gene or gene complement, suggesting that the system/complex/factor arose at this point. The split dot represents the apparent secondary reduction of multiple trafficking components in the metamonad *Giardia* contrasted with the expansion of trafficking machinery in its sister lineage *Trichomonas*. Significantly, many of the major components are universal, indicating that the basic mechanisms for vesicle specificity and fusion arose very early, together with establishment of major landmarks of the endomembrane system (i.e. clathrin-dependent endocytosis, exocytosis and recycling pathways). In addition, there is evidence for the acquisition of lineage-specific components (e.g. caveolin by metazoa) and multiple secondary losses (e.g. the Rab4 recycling pathway). Sampling bias in choice of experimental taxa means that novel factors in most lineages have not been identified. Rhizaria are shown as a dotted line because, at present, there are no genome sequences for this group. Figure redrawn and modified from Field et al. (Field et al., 2006), with permission from Landes Bioscience and Springer Science + Business Media.



(Adl et al., 2005). Other super groups include the Archaeplastida, the Amoebozoa, the Chromalveolata, the Rhizaria, and the Excavata (Adl et al., 2005)¹. Whole genome sequences are available for representatives from five of the six groups, Rhizaria being the only exception (Bhattacharya and Katz, 2005). This broad sampling enables evolutionary reconstruction of the membrane-trafficking gene complement that would have been present in the common ancestor of the five groups, a reasonable proxy for the last common eukaryotic ancestor (LCEA). Genomes from a vast array of prokaryotic organisms (both bacterial and archaeal) also provide important clues to the origins of the system.

We do not attempt here to argue for one specific hypothesis regarding the origin and evolution of the membrane-trafficking system over any other, nor to summarize the various competing theories (Cavalier-Smith, 2002; de Duve, 2007; Gupta and Golding, 1996; Jekely, 2003; Lopez-Garcia and Moreira, 2006; Martin, 2005; Mironov et al., 2006). Instead, we discuss several trends apparent from recent comparative genomic, phylogenetic and cell biological studies relevant to the evolution of membrane trafficking. These trends directly address the processes by which the endomembrane system is

likely to have originated and increased in complexity. They also shed light on the timing of its emergence and its subsequent evolutionary plasticity.

The paucity of prokaryotic homologues

We can be confident of the prokaryotic origins of mitochondria and plastids from endosymbiotic bacteria, given the numerous directly homologous genes² and structures in alpha-proteobacteria and cyanobacteria, respectively (for reviews, see Keeling, 2004; van der Giezen et al., 2005, and references therein). The machinery for DNA replication, transcription,

¹The Opisthokonta are composed of the animals, fungi and their close unicellular relatives. The Archaeplastida include the higher plants, the red and green algae. The Amoebozoa encompasses the slime moulds, the causative agent of amoebic dysentery (*Entamoeba*), and various additional amoebae. The Chromalveolata include kelp, diatoms, ciliates and the causative agents of malaria (*Plasmodium*) and red tide (dinoflagellates). Rhizaria include cercozoans and the important paleontological group of Foraminifera. Finally, the Excavata includes the causative agents of Giardiasis (*Giardia*) and African Sleeping Sickness (*Trypanosoma brucei*) and holds many of the lineages formerly considered to represent early-evolving eukaryotes. For a full, and more formal, discussion of these groups and the evidence for and against their validity see elsewhere (Adl et al., 2005; Keeling et al., 2005; Parfrey et al., 2006).

²In this article we use the traditional, evolutionary biological, definition of homology: that two homologous objects share common ancestry, i.e. were once the same object in the common ancestor of the organisms that are being considered (Reeck et al., 1987). This definition can be applied to appendages (e.g. mammalian arms and fins), organelles, proteins, genes, amino acids or even nucleotides. In the case of genes and proteins, the argument for homology is usually based on analyses using homology searching algorithms such as BLAST (Altschul et al., 1997). These programs return a statistical value for the homology assumption (an E-value) and some numerical comparison (percentage) of how similar is the sequence of initial interest to the sequences that are returned by the program as candidate homologues. This has given rise to the first common mis-use of the term homology, 'percent homology'. The percentage that is returned by homology-searching programs is either the amount that is exactly the same between the two sequences (identity) or the amount of amino acids that share comparable chemical properties (similarity). This percentage is used to make a judgement of the probability that the two sequences are homologous, but because two things either did or did not share a common ancestor, the term percent homology is non-sensical. Because the two objects being considered were once the same, it may also be assumed that they now share the same role or function in the organisms in which they currently reside. This assumption has given rise to another use of the term homology to mean 'the same function' or alternately use of the term functional homology for two things that may or may not be homologous but do perform the same role. Two proteins that perform the same role based on the fact that they are homologous can be termed functionally homologous. However, two proteins that perform the same role but arrived at this state through convergent evolution might be better termed 'functionally analogous' to avoid confusion. When homology is unclear, functionally analogous is the safer term.

translation and key metabolic pathways are shared between the three domains of life (Bacteria, Archaea and Eukaryota) and therefore must have been possessed in some form by the proto-eukaryote (Ranea et al., 2006). However, the mixed ancestry of these cellular systems, some derived from bacterial progenitors and others derived from archaeal progenitors, leaves the origin of the nucleus currently as an open and highly contentious question (Lopez-Garcia and Moreira, 2006; Martin, 2005). This question is directly related to the evolution of membrane trafficking, however, because of the continuity of the nuclear envelope and the ER. In contrast to components of mitochondria, plastids and much of the nuclear machinery, clear prokaryotic homologues for molecules/organelles of the membrane trafficking system are relatively rare.

A few examples of structures analogous to eukaryotic membrane-trafficking compartments do exist in prokaryotes. Bacteria in the remarkable phylum *Planctomycetes* possess membrane-bound, or partially enclosed, nucleoids, which are reminiscent of the nuclear envelope (Fuerst, 2005). In the archaeon *Ignioccus islandicus*, cytoplasmic vesicles 30–90 nm in diameter and tubules up to 300 nm long have been observed by electron microscopy (Rachel et al., 2002). In *Escherichia coli*, depletion of the signal recognition particle (SRP) component Ffh, or the translocon factor SecE, produces internal stacks of plasma membrane that morphologically resemble ER and contain FtsY-ribosome complexes (Herskovits et al., 2002). However, there is currently little evidence that these structures are directly homologous to the eukaryotic structures that they resemble or that the taxa possessing these structures are direct ancestors of modern eukaryotes (Glockner et al., 2003). This will become increasingly testable, however, as more genome sequences of planctomycetes and of *Ignioccus* become available. Importantly, the presence of these structures within prokaryotes illustrates that the capacity to construct membranous compartments exists in prokaryotes (Herskovits et al., 2002), via processes such as change of localization of one protein or depletion of another, and lends credibility to models favouring the origins of membrane trafficking via spontaneous formation of such structures in the proto-eukaryote, without needing to invoke undefined stochastic events.

Although there are not many clear molecular homologues of membrane-trafficking components in prokaryotes, there are some candidates. For example, Vps29, a central piece of the retromer vesicle coat (Seaman, 2005), has clear prokaryotic homologues in diverse bacterial and archaeal taxa, as demonstrated by recent structural studies (Collins et al., 2005; Wang et al., 2005) and by homology searches of the non-redundant protein database at NCBI (data not shown). Moreover, recent reports have shown that Vps29 has phosphoesterase activity in common with its prokaryotic homologues (Damen et al., 2006). Similarly, a bacterial dynamin that can deform lipid bilayers has been described and provides a direct prokaryotic counterpart for this important component of the endocytic machinery (Low and Lowe, 2006). However, dynamin is less consistently involved in endocytosis (Chanez et al., 2006; Elde et al., 2005; Morgan et al., 2004) than it is in mitochondrial division in many eukaryotic taxa, which implies a secondary and convergent co-opting of dynamin into the endocytic system in different descendents of

the LCEA (Elde et al., 2005). It is possible that the origin of eukaryotic dynamin was associated with the initial mitochondrial endosymbiosis and an initial non-endocytic function; however, there is insufficient evidence at present to tell.

Finally, several components of the prokaryotic SRP/SecY translocation system are retained within the co-translational ER translocational system of eukaryotes (Gribaldo and Cammarano, 1998; Rapoport et al., 1996). This implies not only homology of the core factors of these two systems but also that the ER is topologically derived from the prokaryotic plasma membrane, a point nearly universally included in theories of the origins of the eukaryotic trafficking system. More generally, β -propeller and α -solenoid domains, as well as small GTPases, are all crucial building blocks of the eukaryotic membrane-trafficking machinery: proteins containing these domains are present in both bacteria and archaea (Devos et al., 2004; Pandit and Srinivasan, 2003).

Nevertheless, the number of unequivocal prokaryotic homologues for structures or proteins involved in membrane trafficking is limited. The data are most consistent with an autogenous, rather than a directly endosymbiotic, origin for the eukaryotic trafficking system. The organelles involved also lack additional traits characteristic of endosymbiotically derived structures such as associated genomes and the presence of closely appressed double membranes.

The membrane-trafficking system probably arose autogenously, exploiting pre-existing building blocks of domains and motifs together with a limited repertoire of proteins that were present in the proto-eukaryote or derived early by gene transfer. Since much of the elaboration of the system must have occurred in the progenitors of eukaryotes themselves, in order to understand the origins and elaboration of the trafficking system that produced these complex modern configurations, we may have to focus on the evolutionary history of the components within eukaryotes.

A rapid eukaryotic origin

Related to the basic question of how the eukaryotic membrane-trafficking system originated is how rapidly the system arose. More specifically, did the system evolve rapidly upon separation of the proto-eukaryotic line from the remaining prokaryotes, or did it arise slowly, over the long course of eukaryotic history?³ Each of these alternatives leads to different predictions. If the endomembrane system arose rapidly, then the ancestor of extant eukaryotes is predicted to have possessed an elaborate membrane-trafficking system, potentially approaching the complexity seen in many living

³One potential complication is that it is unclear how much time elapsed between the point of separation of the proto-eukaryotic line from other prokaryotes, and the point when the various extant eukaryotic lineages diverged (i.e. the LCEA). Evidence suggests that life was present on Earth 3.5×10^9 years ago (Schopf, 2006). This life appears to be prokaryotic and provides an earliest limit (Schopf, 2006). The earliest proposed date for the origin of eukaryotes is 2.7×10^9 years ago, based on the contentious argument that evidence of steranes (steroid-like lipids) in the geological record at that point is evidence for the existence of eukaryotes (Brocks et al., 1999). More robust fossil evidence demonstrates a eukaryotic presence as early as 1.8×10^9 years ago (Javaux et al., 2004), with red algal fossils dated back 1.2×10^9 years that are morphologically indistinguishable from the modern genera of *Bangia* (Butterfield, 2000). Similarly, molecular dating studies have placed the divergence of the photosynthetic eukaryotes from the Opisthokonts at around 1.6×10^9 years. This implies that divergence of the LCEA into its descendent lineages had already occurred by around 1.5×10^9 years ago. In reality, the question is whether the membrane-trafficking system evolved slowly over the entire course of eukaryotic history ($\sim 1.5 \times 10^9$ years plus the unknown period of time between the proto-eukaryote and the LCEA) or relatively more rapidly before the LCEA arose.

eukaryotes. By contrast, if the system arose gradually, eukaryotes lacking certain organelles or pathways proposed to be essential for basic cellular trafficking may exist, since such lineages would have speciated away from other eukaryotes prior to the origin of that particular cellular feature. This latter concept was formalized in the mid-1980s in the Archezoan Hypothesis, primarily to explain the evolution of mitochondria (Cavalier-Smith, 1983; Cavalier-Smith, 1987a). Although the Archezoan Hypothesis was supported initially by phylogenetic analyses of SSU rDNA and protein coding genes, it can now be confidently rejected on the basis of diverse and robust lines of evidence, including phylogenetic, molecular evolutionary and cell biological data (reviewed in de Duve, 2007; Embley and Martin, 2006). Evidence indicates that the LCEA possessed a mitochondrion and was probably a complex eukaryotic cell in many additional respects (Dacks and Doolittle, 2001; Embley and Martin, 2006; Roger, 1999; Vanacova et al., 2005). The membrane-trafficking system is no exception.

All studied eukaryotic cells contain ER, endocytic organelles and a plasma membrane (Lee et al., 2002; Roger, 1999). The complexity of endocytic systems varies considerably between lineages, but almost all possess digestive and recycling machinery where this has been examined (i.e. lysosomes and endosomes). A few eukaryotic lineages lack visible Golgi stacks (Cavalier-Smith, 1987a; Patterson, 1999) and were proposed to have diverged from other eukaryotes prior to the origin of the Golgi complex (Cavalier-Smith, 1987b). However, it is now clear, given the placement of these taxa in the eukaryotic tree (Keeling et al., 2005), the presence of genes encoding Golgi-specific components in their genomes (Dacks et al., 2003) and the available cell biological data (Ghosh et al., 1999; Lujan et al., 1995; Marti et al., 2003; Takvorian and Cali, 1994), that a Golgi organelle is, in fact, present in some form in these organisms. Therefore, the LCEA almost certainly possessed what most cell biologists would consider to be the basic complement of membrane-trafficking organelles (i.e. ER, stacked Golgi, endosomes and lysosomes).

At the molecular level, the situation is similar (Fig. 1). Comparative genomic studies demonstrate that the ancestor of eukaryotes would have possessed all of the major protein families that, on the basis of studies in yeast, metazoans and higher plants, are defined as critical for transport carrier formation and fusion (Jahn and Sudhof, 1999; Springer et al., 1999). These include components of the three major coats (COPI, COPII and clathrin), the four SNARE families, GTPase-activating proteins (GAPs), guanine nucleotide exchange factors (GEFs), and NSF ATPases (Dacks and Doolittle, 2001; Dacks and Field, 2004; Hartman and Fedorov, 2002; Sanderfoot, 2007; Yoshizawa et al., 2006). Comparative genomics has similarly confirmed the presence, in a wide variety of eukaryotes, of a complex endocytic system, including the ESCRT complexes (Field et al., 2006), GTPases (Jekely, 2003), and homologues of the retromer complex including Vps35, Vps29 and Vps26 (Dacks et al., 2003; Damen et al., 2006; Nakada-Tsukui et al., 2005), which implies that all of these components must also have been established at a very early stage.

Functional characterization in the amoebae (Bogdanovic et al., 2002; Nakada-Tsukui et al., 2005), chromalveolates such as *Toxoplasma*, *Plasmodium* and the ciliates (Ngo et al., 2003;

Schilde et al., 2006; Struck et al., 2005), excavates such as kinetoplastids (Besteiro et al., 2006; Morgan et al., 2002) and *Giardia* (Lujan and Touz, 2003) and plants further support the idea that the LCEA had a complex membrane trafficking system. These studies have several implications for our understanding of the biology of membrane trafficking. It is crucial to confirm in silico assignments of potential functional conservation, especially in cases where sequence similarity can be extremely poor, as is the case when comparing many diverse taxa with the major model organisms. In addition, the in vivo characterization of vesicle coats, SNAREs, Rabs, ATPases and additional components in such evolutionarily diverse organisms begins to allow us to reconstruct, not only gene complements in early eukaryotes but also ancient trafficking pathways. Finally, the issue of asymmetry (i.e. the under-sampling of potentially unique trafficking features in the majority of taxa) remains to be addressed. The importance of taxon-specific mechanisms can ultimately only be approached through direct experimental analysis and is required before a truly broad and generalizable model for eukaryotic intracellular trafficking can be obtained.

Increasing trafficking complexity by paralogous expansion

More detailed comparative genomics and phylogenetics have reinforced the picture of a complex early eukaryotic ancestor and suggest a mechanism by which this complexity could have arisen. Much of the basic trafficking machinery, down to the level of the subfamily, had already been established via gene duplication prior to the LCEA (Fig. 2A). Small GTPases of the Sar, Arf, Ras, Ran and Rab families are found in diverse eukaryotes and thus in their common ancestor (Jekely, 2003). More specifically, analysis of the Rabs indicate that, at least, the Rab1, Rab2, Rab4, Rab5, Rab6, Rab7, Rab8 and Rab11 subfamilies were present in the ancestor of eukaryotes (Field et al., 2006; Jekely, 2003; Langford et al., 2002). Syntaxin subfamilies syn5, syn18, syn16/TLG2, the anterograde endocytic syntaxins (vam3, pep12, syntaxin 7 and syntaxin 12) and the plasma-membrane-associated syntaxins (sso1, sso2, syntaxins 1-4, and syntaxin 11) all pre-date the LCEA (Dacks and Doolittle, 2002; Dacks and Doolittle, 2004). The syntaxin-binding-protein families Vps33, Vps45, Sly1 and Sec1 strongly resolve into paralogue-specific clades, each containing representative eukaryotes from five eukaryotic super groups (Koumandou et al., 2007). These four important proteins were therefore probably present in the eukaryotic ancestor (Arac et al., 2005). Finally, the various vesicle coats, COPI, COPII, clathrin and adaptins, appear to have arisen via ancient gene duplications (Devos et al., 2004). Comparative genomic (Dacks and Field, 2004) and phylogenetic analyses (Elde et al., 2005; Schledzewski et al., 1999; Singh and Gupta, 2004) reveal that all three coat systems are widely distributed among eukaryotes, which implies their establishment in early eukaryotes. These data all reinforce the complexity of membrane-trafficking in the LCEA and, moreover, suggest a possible mechanism by which the complex membrane-trafficking system of organelles and components may have emerged.

Since the various subfamilies of many important membrane-trafficking components are homologous, and each corresponds to a distinct step or organelle in transport, the gene duplications

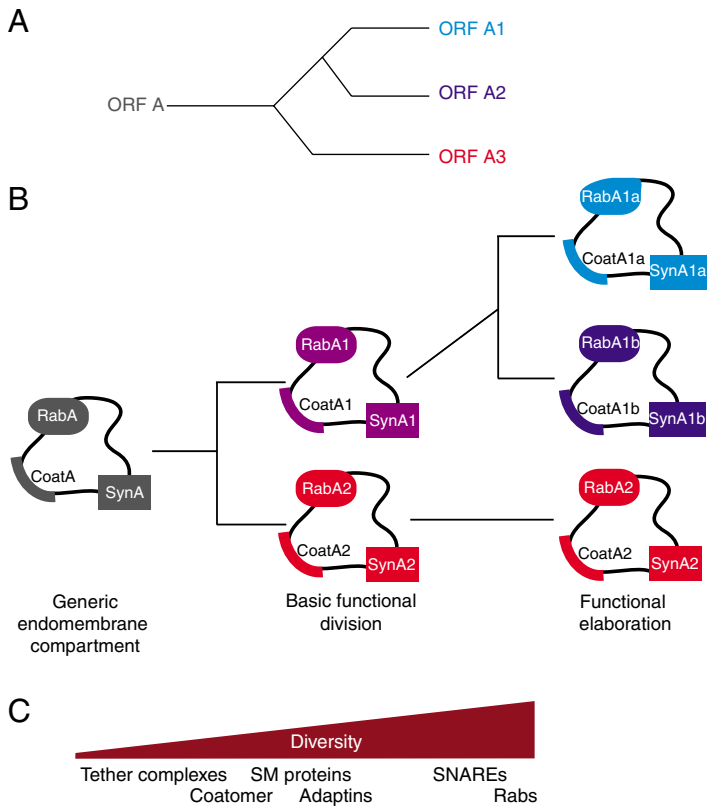


Fig. 2. Acquisition of organelle complexity and function by paralogous gene family expansion. (A) Gene duplication events lead to more than one copy of an open reading frame A (ORF A). This gene redundancy allows each member of the new family to accumulate sequence variation and, hence, potentially new functions. A clear example of this type of event would be the ancestor of the coatomer and adaptin complex subunits. (B) Model for compartment number expansion by gene duplication of protein factors implicated in vesicle identity, specificity and fusion. The progenitor compartment contains, at least, a member of the Rab family, a syntaxin and a coat system (grey). Duplications of the genes encoding these factors facilitate differentiation of function (red, purple), yielding discrete organelles. This process continues in some cases, so that the A1 compartment further is differentiated to A1a and A1b (light and dark blue, respectively), whereas A2 is not elaborated. This may yield a new organelle or differentiation of an organelle into sub-domains or pathways in the case of the coatomers and adaptins (which yielded the *cis*-Golgi and *trans*-Golgi network). This general process could eventually have given rise to the organellar complement and machinery reconstructed as present in the LCEA. (C) Paralogous expansion appears variable across the eukaryotes and across machinery; all of the gene families shown were probably present in the LCEA but exhibit different levels of expansion in modern eukaryotes. Specifically there is little evidence for multiple genes encoding tether complex factors in most genomes, while at the other extreme there are many Rab and SNARE genes. Intermediate situations are found for the coatomer, adaptins and syntaxin-binding (SM) proteins; in some lineages either entire complexes or specific subunit families are expanded.

(Fig. 2A) giving rise to those components may have been concurrent with, or causal to, the origins of the respective organelles (Fig. 2B). In such a mechanism, the observed complexity in eukaryotic membrane trafficking would have been created by several series of gene duplications originating from a basic single organelle and rising in organellar

complexity as the machinery duplicated and diverged. Consider the Golgi as an example: the LCEA probably possessed a stacked Golgi consisting of *cis*- and *trans*-Golgi elements and various post-TGN transport pathways. Since the coats servicing these compartments, the F-COP coatomer subcomplex and the adaptins, respectively, are the product of a set of gene duplications (Schledzewski et al., 1999), the *cis*- and *trans*-Golgi presumably arose from an original undifferentiated Golgi (Fig. 2B). This may have been concurrent with the duplications that produced the coatomer F-COP and the proto-adaptin complexes. Further gene duplications in the adaptin families yielded adaptin complexes AP1-AP4, generating the multiple post-Golgi secretory pathways seen in eukaryotes today.

The idea that paralogous expansion produces organellar complexity (Cavalier-Smith, 2002) has been adopted by various authors, each suggesting their molecule of interest, whether vesicle coats (Cavalier-Smith, 2002), syntaxins (Dacks and Doolittle, 2002), the SNAREs in general (Dacks, 2007; Sanderfoot, 2007; Yoshizawa et al., 2006), GTPases (Jekely, 2003), or SM proteins (Arac et al., 2005) as the key piece in the evolutionary differentiation of the different organelles. Given the role that the various pieces of machinery play determining organelle identity and fusion specificity, and the similarity of their pattern of diversification, they are all likely to have contributed to the development of the membrane-trafficking system (Koumandou et al., 2007).

Interestingly, the degree of expansion of each of these trafficking protein families varies in modern organisms. There are a considerable families of Rabs and SNAREs in most eukaryotes, many family members being the product of recent gene duplications in a specific organismal lineage (Fig. 2C). By contrast, the tether complexes show limited evidence of paralogous gene expansion (Koumandou et al., 2007). Between these two extremes are several families where some expansion has occurred but not to the extent of that displayed by the Rabs or SNAREs. For example, in the adaptin system, multicellular eukaryotes possess tissue-specific isoforms (Boehm and Bonifacino, 2001).

Not all components of the system are consistent with the mechanism described above. The seven different, multi-subunit, tethering complexes were probably already present in the LCEA (Koumandou et al., 2007). However, the evidence for common ancestry of the various complexes is equivocal at best and some of the tethering complexes clearly have independent origins (Koumandou et al., 2007). Thus, although much of the membrane-trafficking system complexity appears to involve paralogous gene expansion, some components have also been added separately.

Loose correlation between membrane-trafficking complexity and multicellularity

Although the LCEA clearly possessed a complex membrane-trafficking system, significant evolutionary change has certainly occurred in its various descendants. The over-riding trend is for unicellular eukaryotes to have maintained the basic

membrane-trafficking machinery, one or a few members of each protein subfamily being present, and for elaboration of the trafficking machinery to have occurred within the multicellular taxa that have been examined. It is worth bearing in mind that the number of fully sequenced eukaryotic genomes is still relatively small, and so it will be important to see how this trend stands up, as more genomes and a broader diversity of genomes become available. Nonetheless, this trend has been described in surveys of multiple components (Bock et al., 2001) and studies of specific components such as the adaptins (Boehm and Bonifacino, 2001), SNAREs (Yoshizawa et al., 2006) and Rabs (Armstrong, 2000; Quevillon et al., 2003). The most convincing example is the discovery that the plasma-membrane-associated Qa and R-SNAREs are more numerous in the genomes of multicellular taxa than in their close unicellular relatives (Dacks and Doolittle, 2002; Sanderfoot, 2007; Yoshizawa et al., 2006). Further description of this phenomenon in additional multicellular lineages (such as brown algae and multicellular fungi), and including other components of the trafficking system, will be important if we are to understand both the evolution of membrane trafficking and the multiple independent evolutionary origins of multicellularity.

The over-riding trend of unicellular simplicity versus multicellular expansion is not universal. The genome of *Entamoeba histolytica* encodes nearly 100 Rab proteins, a repertoire greater than that of mammals (Saito-Nakano et al.,

2005). Genome sequencing of *Tetrahymena thermophila* and *Paramecium tetraurelia* indicate that these ciliates also possess an expanded array of membrane-trafficking genes compared with other unicellular taxa (Aury et al., 2006; Eisen et al., 2006). Finally, the genome of the excavate *Trichomonas vaginalis* encodes a massively expanded array of endocytic components (Carlton et al., 2007). The number of syntaxins, SM proteins, and vesicle coats that are specifically within the endocytosis-associated subfamilies in *Trichomonas* is notably larger than the number of the same components associated with secretion (Carlton et al., 2007). *Trichomonas* also possesses more members of some trafficking protein families than do either humans or *Arabidopsis thaliana*; it has three times the adaptin complement of the multicellular taxa and nearly 300 Rabs (Carlton et al., 2007). What drove selective expansion of endocytic versus secretory machinery in *Trichomonas* is unclear. It could be correlated with the different possible substrates that are endocytosed by the parasite and, hence, a need for a larger number of distinct cargo adaptors. Alternately, since introns are rare in *Trichomonas* (Carlton et al., 2007; Vanacova et al., 2005), diversity that in other organisms is generated by alternative splicing may, in *T. vaginalis*, be generated at the genome level. Whatever the reason, this should be a fruitful area to pursue, given the importance of uptake of material from the host environment in the pathogenic mechanism of this parasite (Rendon-Maldonado et al., 1998).

Degeneration and component loss have also been prevalent

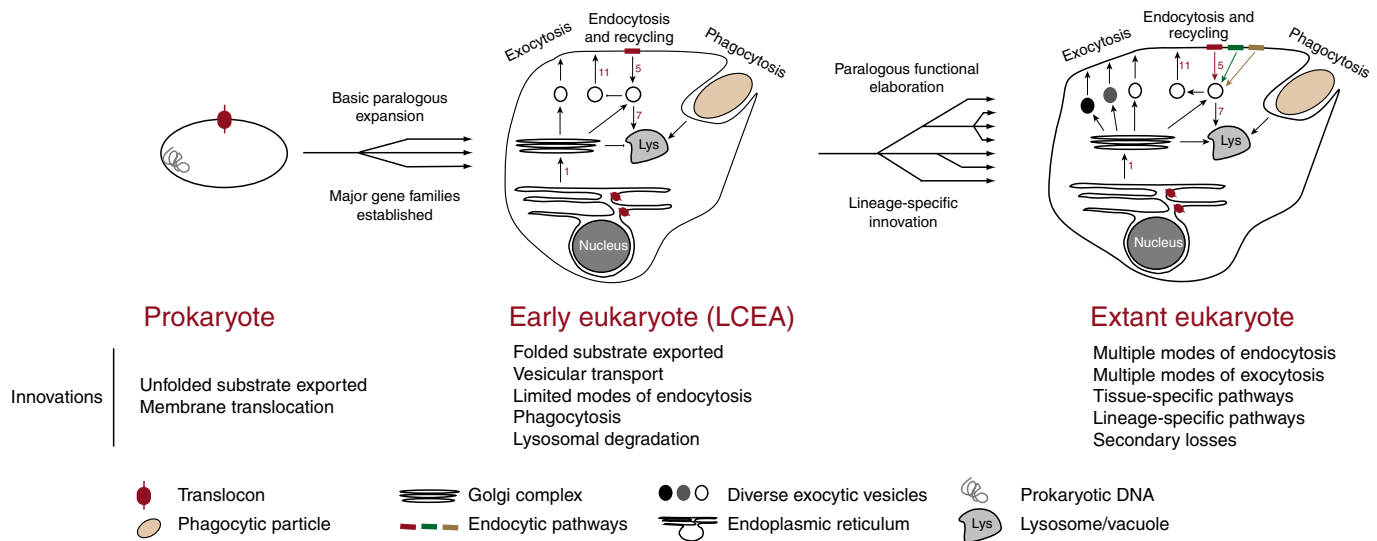


Fig. 3. Major transitions in evolution of the endomembrane system. In prokaryotes, secretion is a comparatively simple matter of translocation of polypeptides across the plasma membrane. Although there are several distinct mechanisms for achieving this, all appear to require an unfolded substrate for translocation. The type I, SRP/SecY-mediated, pathway is homologous to the co-translational ER import pathways of eukaryotes. In the hypothesized LCEA, comparative genomic evidence suggests that the major structures and pathways constituting the endomembrane system were already present, including the ER, Golgi complex and the main features of the endocytosis and recycling systems. The paralogous relationship of the families of SNAREs, Rabs, SM proteins and GTPases, as well as the homology of many coat components to each other and also to components of the nuclear pore complex, provide a potential mechanism for how this system arose. Compartmentalization and gene family expansion led to establishment of multiple protein systems capable of deforming membranes (i.e. transport steps). Elaboration of this basic pattern has been a major driving force for subsequent diversification of the endomembrane system, giving rise to the array of systems present in extant taxa. Subsequent evolution yielded multiple modes of endocytosis, specialized exocytic pathways and increased complexity of post-Golgi pathways. The red ovals indicate a trans-membrane translocation system. Lys, lysosome or vacuole; LCEA, last common eukaryotic ancestor. Small numbers in red indicate an associated pathway-specific Rab protein. The grey structure in the prokaryote indicates the non-compartmentalized genome.

during evolution of the membrane-trafficking system (Fig. 1). Specifically, the stacked Golgi has been lost on at least four separate occasions (Dacks et al., 2003), and sequencing of protist parasite genomes has revealed simplified membrane-trafficking systems in several cases. *Giardia* has undergone remarkable modification of its Golgi complex (Lujan et al., 1995; Marti et al., 2003) and endosomal system (Lanfredi-Rangel et al., 1998) and is missing various key components of the trafficking machinery (Marti et al., 2003). A divergent fungal lineage, the Microsporidia, have also stream-lined their membrane-trafficking-machinery: they have a minimized complement of Rabs, SNAREs and vesicle coats, as well as a complete absence of clathrin genes (Mironov et al., 2006). *Trypanosoma brucei* has lost the AP2 complex while the related kinetoplastid *Leishmania* has dispensed with AP4, as have fungi and invertebrates (Boehm and Bonifacino, 2001; Denny et al., 2005). Many of the key components of the endocytic system, including Rab4, and AP3 and AP4, have probably been lost on multiple occasions (Field et al., 2006). Clearly, as the descendants of the LCEA colonised new environments, their membrane-trafficking systems changed and adapted to suit their ecological niches.

Conclusions

Comparative genomic and molecular evolutionary studies have begun delving into the history of the membrane-trafficking system and four major trends emerge: the paucity of prokaryotic membrane-trafficking homologues, complexity of the trafficking machinery in early eukaryotes, the evolution of complexity by paralogous expansion of trafficking protein families and the correlation of extensive expansion of the trafficking machinery with multicellularity (Fig. 3). These trends provide insight into the history of specific components. They also address the rate at which the system evolved and the nature of the evolutionary processes involved (Fig. 3). However, these patterns are derived from studies of a small fraction of membrane-trafficking gene products and fall well short of a full eukaryotic sampling. Counter-examples have also been documented and hence, at best, these trends provide null hypotheses for testing by new molecular evolutionary results.

Although much has been gained by attempts to reconstruct the LCEA and the evolutionary events that followed, one potentially fruitful but challenging task will be reconstructing events after the divergence of the proto-eukaryote but before the LCEA. Central to this task will be resolving the relationships between paralogues in membrane-trafficking protein families and subfamilies that will reveal the order of component and even organellar origins. Such resolution is elusive but possible (Dacks, 2007; Langford et al., 2002). In particular, the relationships between the SNARE families (Fasshauer et al., 1998; Weimbs et al., 1998), the various small GTPases (Takai et al., 2001), the common vesicle coats and the nuclear pore complex (Devos et al., 2004), and possibly as far afield as the intraflagellar transport system (Jekely and Arendt, 2006), all represent important areas for future investigation. Deep taxonomic sampling and rigorous phylogenetic methods are crucial, because without them results can be misleading, as demonstrated in numerous studies (e.g. Philippe et al., 2000).

The accuracy and speed of molecular evolutionary tools continue to increase. Molecular cell biological studies of

membrane trafficking are providing novel targets for molecular evolutionary analysis at a comforting (alarming!) rate. By incorporating the results from work reviewed here and from studies that are likely to be forthcoming, we can move ever closer to a detailed and reliable reconstruction of the origins, early evolution, diversity and functions of the membrane-trafficking system.

This work was supported by a Travelling Research Fellowship from the Canadian Institutes of Health Research and the Wellcome Trust (to J.B.D.) and Wellcome Trust Program and Project grants (to M.C.F.). We would like to thank Ka-Fai Leung, Lesley Dacks and Giselle Walker (all at the University of Cambridge) for comments on the manuscript.

References

- Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S. et al. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryot. Microbiol.* **52**, 399-451.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389-3402.
- Arac, D., Dulubova, I., Pei, J., Hurryeva, I., Grishin, N. V. and Rizo, J. (2005). Three-dimensional structure of the rSly1 N-terminal domain reveals a conformational change induced by binding to syntaxin 5. *J. Mol. Biol.* **346**, 589-601.
- Armstrong, J. (2000). Membrane traffic between genomes. *Genome Biol.* **1**, reviews104.
- Aury, J. M., Jaillon, O., Duret, L., Noel, B., Jubin, C., Porcel, B. M., Segurens, B., Daubin, V., Anthouard, V., Aiach, N. et al. (2006). Global trends of whole-genome duplications revealed by the ciliate *Paramecium tetraurelia*. *Nature* **444**, 171-178.
- Barbrook, A. C., Howe, C. J. and Purton, S. (2006). Why are plastid genomes retained in non-photosynthetic organisms? *Trends Plant Sci.* **11**, 101-108.
- Besteiro, S., Coombs, G. H. and Mottram, J. C. (2006). The SNARE protein family of *Leishmania major*. *BMC Genomics* **7**, 250.
- Bhattacharya, D. and Katz, L. A. (2005). Frontiers in genomics: insights into protist evolutionary biology, University of Iowa, May 19-21, 2004. *J. Eukaryot. Microbiol.* **52**, 170-172.
- Bock, J. B., Matern, H. T., Peden, A. A. and Scheller, R. H. (2001). A genomic perspective on membrane compartment organization. *Nature* **409**, 839-841.
- Boehm, M. and Bonifacino, J. S. (2001). Adaptins: the final recount. *Mol. Biol. Cell* **12**, 2907-2920.
- Bogdanovic, A., Bennett, N., Kieffer, S., Louwagie, M., Morio, T., Garin, J., Satre, M. and Bruckert, F. (2002). Syntaxin 7, syntaxin 8, Vti1 and VAMP7 (vesicle-associated membrane protein 7) form an active SNARE complex for early macropinosyncytic compartment fusion in *Dictyostelium discoideum*. *Biochem. J.* **368**, 29-39.
- Bonifacino, J. S. and Glick, B. S. (2004). The mechanisms of vesicle budding and fusion. *Cell* **116**, 153-166.
- Brooks, J. J., Logan, G. A., Buick, R. and Summons, R. E. (1999). Archean molecular fossils and the early rise of eukaryotes. *Science* **285**, 1033-1036.
- Butterfield, N. J. (2000). *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiology* **26**, 386-404.
- Carlton, J. M., Hirt, R. P., Silva, J. C., Delcher, A. L., Schatz, M., Zhao, Q., Wortman, J. R., Bidwell, S. L., Alsmark, U. C., Besteiro, S. et al. (2007). Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. *Science* **315**, 207-212.
- Cavalier-Smith, T. (1975). The origin of nuclei and of eukaryotic cells. *Nature* **256**, 463-468.
- Cavalier-Smith, T. (1983). A 6-kingdom classification and a unified phylogeny. In *Endocytobiology II* (ed. H. E. A. Schenk and W. Schwemmler), pp. 1027-1034. Berlin: Walter de Gruyter.
- Cavalier-Smith, T. (1987a). Eukaryotes with no mitochondria. *Nature* **326**, 332-333.
- Cavalier-Smith, T. (1987b). The origin of eukaryote and archaeobacterial cells. *Ann. N. Y. Acad. Sci.* **503**, 17-54.
- Cavalier-Smith, T. (2002). The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int. J. Syst. Evol. Microbiol.* **52**, 297-354.
- Chanez, A. L., Hehl, A. B., Engstler, M. and Schneider, A. (2006). Ablation of the single dynamin of *T. brucei* blocks mitochondrial fission and endocytosis and leads to a precise cytokinesis arrest. *J. Cell Sci.* **119**, 2968-2974.
- Collins, B. M., Skinner, C. F., Watson, P. J., Seaman, M. N. and Owen, D. J. (2005). Vps29 has a phosphoesterase fold that acts as a protein interaction scaffold for retromer assembly. *Nat. Struct. Mol. Biol.* **12**, 594-602.
- Dacks, J. B. (2007). Evolution of the SNARE protein superfamily. In *SNARE Proteins* (ed. D. Banfield and F. Fillippini). Austin: Landes Bioscience (in press).
- Dacks, J. B. and Doolittle, W. F. (2001). Reconstructing/deconstructing the earliest eukaryotes: how comparative genomics can help. *Cell* **107**, 419-425.

- Dacks, J. B. and Doolittle, W. F.** (2002). Novel syntaxin gene sequences from *Giardia*, *Trypanosoma* and algae: implications for the ancient evolution of the eukaryotic endomembrane system. *J. Cell Sci.* **115**, 1635-1642.
- Dacks, J. B. and Doolittle, W. F.** (2004). Molecular and phylogenetic characterization of syntaxin genes from parasitic protozoa. *Mol. Biochem. Parasitol.* **136**, 123-136.
- Dacks, J. B. and Field, M. C.** (2004). Eukaryotic cell evolution from a genomic perspective: the endomembrane system. In *Organelles, Genomes and Eukaryote Phylogeny: An Evolutionary Synthesis in the Age of Genomics* (ed. R. P. Hirt and D. S. Horner), pp. 309-334. London: CRC Press.
- Dacks, J. B., Davis, L. A., Sjogren, A. M., Andersson, J. O., Roger, A. J. and Doolittle, W. F.** (2003). Evidence for Golgi bodies in proposed 'Golgi-lacking' lineages. *Proc. Biol. Sci.* **270** Suppl. 2, S168-S171.
- Damen, E., Krieger, E., Nielsen, J. E., Eygensteyn, J. and van Leeuwen, J. E.** (2006). The human Vps29 retromer component is a metallo-phosphoesterase for a cation-independent mannose 6-phosphate receptor substrate peptide. *Biochem. J.* **398**, 399-409.
- de Duve, C.** (2007). The origin of eukaryotes: a reappraisal. *Nat. Rev. Genet.* **8**, 395-403.
- Denny, P. W., Morgan, G. W., Field, M. C. and Smith, D. F.** (2005). *Leishmania major*: clathrin and adaptin complexes of an intra-cellular parasite. *Exp. Parasitol.* **109**, 33-37.
- Devos, D., Dokudovskaya, S., Alber, F., Williams, R., Chait, B. T., Sali, A. and Rout, M. P.** (2004). Components of coated vesicles and nuclear pore complexes share a common molecular architecture. *PLoS Biol.* **2**, e380.
- Eisen, J. A., Coyne, R. S., Wu, M., Wu, D., Thiagarajan, M., Wortman, J. R., Badger, J. H., Ren, Q., Amedeo, P., Jones, K. M. et al.** (2006). Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. *PLoS Biol.* **4**, e286.
- Eide, N. C., Morgan, G., Winey, M., Sperling, L. and Turkewitz, A. P.** (2005). Elucidation of clathrin-mediated endocytosis in *Tetrahymena* reveals an evolutionarily convergent recruitment of dynamin. *PLoS Genet.* **1**, e52.
- Embley, T. M. and Martin, W.** (2006). Eukaryotic evolution, changes and challenges. *Nature* **440**, 623-630.
- Fasshauer, D., Sutton, R. B., Brunger, A. T. and Jahn, R.** (1998). Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. *Proc. Natl. Acad. Sci. USA* **95**, 15781-15786.
- Field, M. C., Gabernet-Castello, C. and Dacks, J. B.** (2006). Reconstructing the evolution of the endocytic system: insights from genomics and molecular cell biology. In *Evolution of the Eukaryotic Endomembrane System and Cytoskeleton*. Vol. 1 (ed. G. Jekely), pp. 84-96. Austin, TX: Landes Bioscience Publishing.
- Fuerst, J. A.** (2005). Intracellular compartmentation in planctomycetes. *Annu. Rev. Microbiol.* **59**, 299-328.
- Ghosh, S. K., Field, J., Frisardi, M., Rosenthal, B., Mai, Z., Rogers, R. and Samuelson, J.** (1999). Chitinase secretion by encysting *Entamoeba invadens* and transfected *Entamoeba histolytica* trophozoites: localization of secretory vesicles, endoplasmic reticulum, and Golgi apparatus. *Infect. Immun.* **67**, 3073-3081.
- Glockner, F. O., Kube, M., Bauer, M., Teeling, H., Lombardot, T., Ludwig, W., Gade, D., Beck, A., Borzym, K., Heitmann, K. et al.** (2003). Complete genome sequence of the marine planctomycete *Pirellula* sp. strain 1. *Proc. Natl. Acad. Sci. USA* **100**, 8298-8303.
- Gribaldo, S. and Cammarano, P.** (1998). The root of the universal tree of life inferred from anciently duplicated genes encoding components of the protein-targeting machinery. *J. Mol. Evol.* **47**, 508-516.
- Gupta, R. S. and Golding, G. B.** (1996). The origin of the eukaryotic cell. *Trends Biochem. Sci.* **21**, 166-171.
- Hartman, H. and Fedorov, A.** (2002). The origin of the eukaryotic cell: a genomic investigation. *Proc. Natl. Acad. Sci. USA* **99**, 1420-1425.
- Herskovits, A. A., Shimoni, E., Minsky, A. and Bibi, E.** (2002). Accumulation of endoplasmic membranes and novel membrane-bound ribosome-signal recognition particle receptor complexes in *Escherichia coli*. *J. Cell Biol.* **159**, 403-410.
- Jahn, R. and Sudhof, T. C.** (1999). Membrane fusion and exocytosis. *Annu. Rev. Biochem.* **68**, 863-911.
- Jahn, R., Lang, T. and Sudhof, T. C.** (2003). Membrane fusion. *Cell* **112**, 519-533.
- Javaux, E. J., Knoll, A. H. and Walter, M. R.** (2004). TEM evidence for eukaryotic diversity in mid-Proterozoic oceans. *Geobiology* **2**, 121-132.
- Jekely, G.** (2003). Small GTPases and the evolution of the eukaryotic cell. *BioEssays* **25**, 1129-1138.
- Jekely, G. and Arendt, D.** (2006). Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. *BioEssays* **28**, 191-198.
- Keeling, P. J.** (2004). Diversity and evolutionary history of plastids and their hosts. *Am. J. Bot.* **91**, 1481-1493.
- Keeling, P. J., Burger, G., Durnford, D. G., Lang, B. F., Lee, R. W., Pearlman, R. E., Roger, A. J. and Gray, M. W.** (2005). The tree of eukaryotes. *Trends Ecol. Evol.* **20**, 670-676.
- Koumandou, V. L., Dacks, J. B., Coulson, R. M. and Field, M. C.** (2007). Control systems for membrane fusion in the ancestral eukaryote; evolution of tethering complexes and SM proteins. *BMC Evol. Biol.* **7**, 29.
- Lanfredi-Rangel, A., Attias, M., de Carvalho, T. M., Kattenbach, W. M. and De Souza, W.** (1998). The peripheral vesicles of trophozoites of the primitive protozoan *Giardia lamblia* may correspond to early and late endosomes and to lysosomes. *J. Struct. Cell Biol.* **123**, 225-235.
- Langford, T. D., Silberman, J. D., Weiland, M. E., Svard, S. G., McCaffery, J. M., Sogin, M. L. and Gillin, F. D.** (2002). *Giardia lamblia*: identification and characterization of Rab and GDI proteins in a genome survey of the ER to Golgi endomembrane system. *Exp. Parasitol.* **101**, 13-24.
- Lee, J. J., Leedale, G. F. and Bradbury, P.** (2002). *The Illustrated Guide to the Protozoa*. Lawrence, KS: Society of Protozoologists.
- Lopez-Garcia, P. and Moreira, D.** (2006). Selective forces for the origin of the eukaryotic nucleus. *BioEssays* **28**, 525-533.
- Low, H. H. and Lowe, J.** (2006). A bacterial dynamin-like protein. *Nature* **444**, 766-769.
- Lujan, H. D. and Touz, M. C.** (2003). Protein trafficking in *Giardia lamblia*. *Cell. Microbiol.* **5**, 427-434.
- Lujan, H. D., Marotta, A., Mowatt, M. R., Sciaky, N., Lippincott-Schwartz, J. and Nash, T. E.** (1995). Developmental induction of Golgi structure and function in the primitive eukaryote *Giardia lamblia*. *J. Biol. Chem.* **270**, 4612-4618.
- Marti, M., Regos, A., Li, Y., Schraner, E. M., Wild, P., Muller, N., Knopf, L. G. and Hehl, A. B.** (2003). An ancestral secretory apparatus in the protozoan parasite *Giardia intestinalis*. *J. Biol. Chem.* **278**, 24837-24848.
- Martin, W.** (2005). Archaeobacteria (Archaea) and the origin of the eukaryotic nucleus. *Curr. Opin. Microbiol.* **8**, 630-637.
- Martin, W. and Muller, M.** (1998). The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37-41.
- McMahon, H. T. and Mills, I. G.** (2004). COP and clathrin-coated vesicle budding: different pathways, common approaches. *Curr. Opin. Cell Biol.* **16**, 379-391.
- Mironov, A. A., Banin, V. V., Sesorova, I. S., Dolgikh, V. V., Luini, A. and Beznoussenko, G. V.** (2006). Evolution of the endoplasmic reticulum and the Golgi complex. In *Origins and Evolution of Eukaryotic Endomembranes and Cytoskeleton*. Vol. 1 (ed. G. Jekely), pp. 61-72. Austin, TX: Landes Bioscience.
- Moreira, D. and Lopez-Garcia, P.** (1998). Symbiosis between methanogenic archaea and delta-proteobacteria as the origin of eukaryotes: the syntrophic hypothesis. *J. Mol. Evol.* **47**, 517-530.
- Morgan, G. W., Hall, B. S., Denny, P. W., Field, M. C. and Carrington, M.** (2002). The endocytic apparatus of the kinetoplastida. Part II: machinery and components of the system. *Trends Parasitol.* **18**, 540-546.
- Morgan, G. W., Goulding, D. and Field, M. C.** (2004). The single dynamin-like protein of *Trypanosoma brucei* regulates mitochondrial division and is not required for endocytosis. *J. Biol. Chem.* **279**, 10692-10701.
- Nakada-Tsukui, K., Saito-Nakano, Y., Ali, V. and Nozaki, T.** (2005). A retromerlike complex is a novel Rab7 effector that is involved in the transport of the virulence factor cysteine protease in the enteric protozoan parasite *Entamoeba histolytica*. *Mol. Biol. Cell* **16**, 5294-5303.
- Ngo, H. M., Yang, M., Paprotka, K., Pypaert, M., Hoppe, H. and Joiner, K. A.** (2003). AP-1 in *Toxoplasma gondii* mediates biogenesis of the rhoptry secretory organelle from a post-Golgi compartment. *J. Biol. Chem.* **278**, 5343-5352.
- Pandit, S. B. and Srinivasan, N.** (2003). Survey for g-proteins in the prokaryotic genomes: prediction of functional roles based on classification. *Proteins* **52**, 585-597.
- Parfrey, L. W., Barbero, E., Lasser, E., Dunthorn, M., Bhattacharya, D., Patterson, D. J. and Katz, L. A.** (2006). Evaluating support for the current classification of eukaryotic diversity. *PLoS Genet.* **2**, e220.
- Patterson, D. J.** (1999). The diversity of eukaryotes. *Am. Nat.* **154**, S96-S124.
- Philippe, H., Lopez, P., Brinkmann, H., Budin, K., Germot, A., Laurent, J., Moreira, D., Muller, M. and Le Guyader, H.** (2000). Early-branching or fast-evolving eukaryotes? An answer based on slowly evolving positions. *Proc. R. Soc. Lond. B Biol. Sci.* **267**, 1213-1221.
- Quevillon, E., Spielmann, T., Brahimi, K., Chattopadhyay, D., Yeramian, E. and Langsley, G.** (2003). The *Plasmodium falciparum* family of Rab GTPases. *Gene* **306**, 13-25.
- Rachel, R., Wyszchony, I., Riehl, S. and Huber, H.** (2002). The ultrastructure of *Ignicoccus*: evidence for a novel outer membrane and for intracellular vesicle budding in an archeon. *Archaea* **1**, 9-18.
- Ranea, J. A., Sillero, A., Thornton, J. M. and Orenco, C. A.** (2006). Protein superfamily evolution and the last universal common ancestor (LUCA). *J. Mol. Evol.* **63**, 513-525.
- Rapoport, T. A., Jungnickel, B. and Kutay, U.** (1996). Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. *Annu. Rev. Biochem.* **65**, 271-303.
- Reeck, G. R., de Haen, C., Teller, D. C., Doolittle, R. F., Fitch, W. M., Dickerson, R. E., Chambon, P., McLachlan, A. D., Margoliash, E., Jukes, T. H. et al.** (1987). "Homology" in proteins and nucleic acids: a terminology muddle and a way out of it. *Cell* **50**, 667.
- Rendon-Maldonado, J. G., Espinosa-Cantellano, M., Gonzalez-Robles, A. and Martinez-Palomo, A.** (1998). *Trichomonas vaginalis*: in vitro phagocytosis of lactobacilli, vaginal epithelial cells, leukocytes, and erythrocytes. *Exp. Parasitol.* **89**, 241-250.
- Robinson, M. S.** (2004). Adaptable adaptors for coated vesicles. *Trends Cell Biol.* **14**, 167-174.
- Roger, A. J.** (1999). Reconstructing early events in eukaryotic evolution. *Am. Nat.* **154**, S146-S163.
- Saito-Nakano, Y., Loftus, B. J., Hall, N. and Nozaki, T.** (2005). The diversity of rab GTPases in *Entamoeba histolytica*. *Exp. Parasitol.* **110**, 244-252.
- Sanderfoot, A.** (2007). Increases in the number of SNARE genes parallels the rise of multicellularity among the green plants. *Plant Physiol.* **144**, 6-17.
- Schilde, C., Wassmer, T., Mansfeld, J., Plattner, H. and Kissmehi, R.** (2006). A multigene family encoding R-SNAREs in the ciliate *Paramecium tetraurelia*. *Traffic* **7**, 440-455.
- Schledzewski, K., Brinkmann, H. and Mendel, R. R.** (1999). Phylogenetic analysis of components of the eukaryotic vesicle transport system reveals a common origin of adaptor protein complexes 1, 2, and 3 and the F subcomplex of the coatomer COPI. *J. Mol. Evol.* **48**, 770-778.

- Schopf, J. W.** (2006). Fossil evidence of Archaean life. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **361**, 869-885.
- Seaman, M. N.** (2005). Recycle your receptors with retromer. *Trends Cell Biol.* **15**, 68-75.
- Simpson, A. G. and Roger, A. J.** (2004). The real 'kingdoms' of eukaryotes. *Curr. Biol.* **14**, R693-R696.
- Singh, B. and Gupta, R. S.** (2004). Genomic organization and linkage via a bidirectional promoter of the AP-3 (adaptor protein-3) mu3A and AK (adenosine kinase) genes: deletion mutants of AK in Chinese hamster cells extend into the AP-3 mu3A gene. *Biochem. J.* **378**, 519-528.
- Springer, S., Spang, A. and Schekman, R.** (1999). A primer on vesicle budding. *Cell* **97**, 145-148.
- Struck, N. S., de Souza Dias, S., Langer, C., Marti, M., Pearce, J. A., Cowman, A. F. and Gilberger, T. W.** (2005). Re-defining the Golgi complex in *Plasmodium falciparum* using the novel Golgi marker PfGRASP. *J. Cell Sci.* **118**, 5603-5613.
- Takai, Y., Sasaki, T. and Matozaki, T.** (2001). Small GTP-binding proteins. *Physiol. Rev.* **81**, 153-208.
- Takvorian, P. M. and Cali, A.** (1994). Enzyme histochemical identification of the Golgi apparatus in the microsporidian, *Glugea stephani*. *J. Eukaryot. Microbiol.* **41**, 63S-64S.
- van der Giezen, M., Tovar, J. and Clark, C. G.** (2005). Mitochondrion-derived organelles in protists and fungi. *Int. Rev. Cytol.* **244**, 175-225.
- Vanacova, S., Yan, W., Carlton, J. M. and Johnson, P. J.** (2005). Spliceosomal introns in the deep-branching eukaryote *Trichomonas vaginalis*. *Proc. Natl. Acad. Sci. USA* **102**, 4430-4435.
- Wang, D., Guo, M., Liang, Z., Fan, J., Zhu, Z., Zang, J., Zhu, Z., Li, X., Teng, M., Niu, L. et al.** (2005). Crystal structure of human vacuolar protein sorting protein 29 reveals a phosphodiesterase/nuclease-like fold and two protein-protein interaction sites. *J. Biol. Chem.* **280**, 22962-22967.
- Weimbs, T., Mostov, K., Low, S. H. and Hofmann, K.** (1998). A model for structural similarity between different SNARE complexes based on sequence relationships. *Trends Cell Biol.* **8**, 260-262.
- Yoshizawa, A. C., Kawashima, S., Okuda, S., Fujita, M., Itoh, M., Moriya, Y., Hattori, M. and Kanehisa, M.** (2006). Extracting sequence motifs and the phylogenetic features of SNARE-dependent membrane traffic. *Traffic* **7**, 1104-1118.