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### Lessons from the deep

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# Lessons from the deep: mechanisms behind diversification of eukaryotic protein complexes

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## ABSTRACT

Genetic variation is the major mechanism behind adaptation and evolutionary change. As most proteins operate through interactions with other proteins, changes in protein complex composition and subunit sequence provide potentially new functions. Comparative genomics can reveal expansions, losses and sequence divergence within protein-coding genes, but *in silico* analysis cannot detect subunit substitutions or replacements of entire protein complexes. Insights into these fundamental evolutionary processes require broad and extensive comparative analyses, from both *in silico* and experimental evidence. Here, we combine data from both approaches and consider the gamut of possible protein complex compositional changes that arise during evolution, citing examples of complete conservation to partial and total replacement by functional analogues. We focus in part on complexes in trypanosomes as they represent one of the better studied non-animal/non-fungal lineages, but extend insights across the eukaryotes by extensive comparative genomic analysis. We argue that gene loss plays an important role in diversification of protein complexes and hence enhancement of eukaryotic diversity.

*Key words:* molecular evolution, evolutionary mechanisms, gene replacement, constructive neutral evolution, protein complexes, evolutionary divergence.

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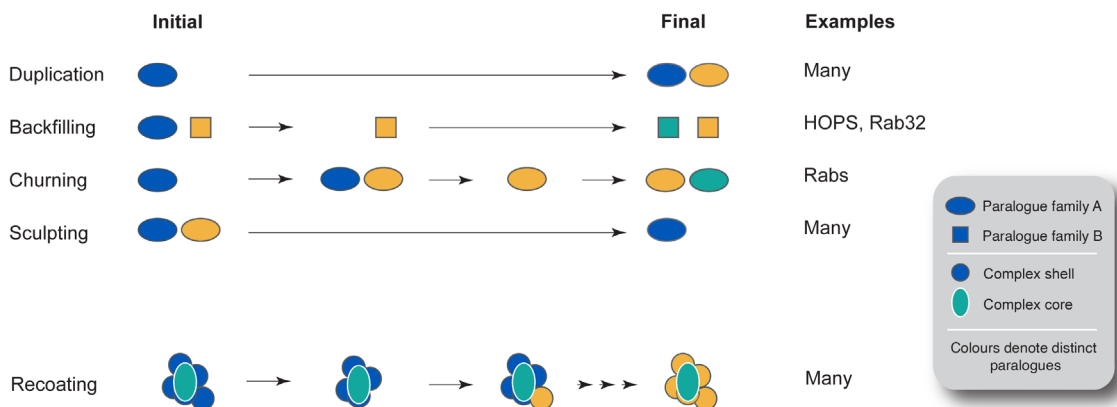
## I. INTRODUCTION

Eukaryogenesis, the transition from prokaryotic to eukaryotic cells, encompassed multiple innovations, with emergence of complex intracellular compartmentalisation being one of the most dramatic. Intracellular organelles are either of endogenous origin, such as the nucleus, flagellum and endomembrane system, or result from endosymbiotic events, exemplified by the mitochondrion and plastids. For endogenous origin compartments, the genes required to support these subcellular structures are derived from ancestral archaeal genes, but are now frequently expanded into large paralogous families (Dacks & Field, 2018; Field & Rout, 2019). Further, a considerable contribution emanated from endosymbiont genomes to support the mitochondrion and plastids (Gray, 2012). Regardless of their ultimate origins, it is generally accepted that the major eukaryotic compartments with the exception of plastids emerged in the period between the first and the last eukaryotic common ancestor (FECA and LECA, respectively) (Martin, Garg & Zimorski, 2015; Speijer, 2020).

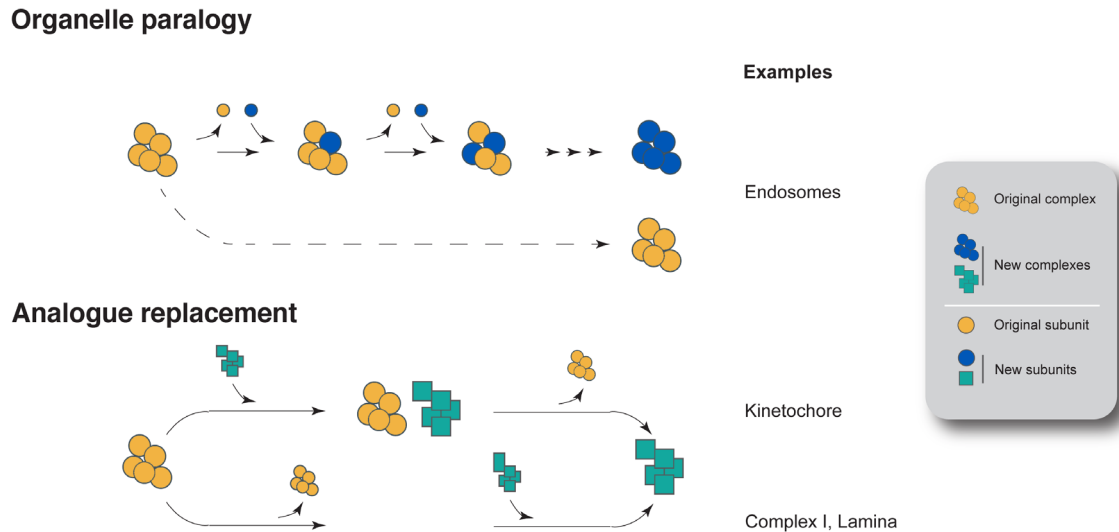
We, and others, have reported that the LECA was likely considerably more complex in terms of the numbers of organelles, protein-coding capacity and size of many paralogous families than many extant lineages (Koumandou *et al.*, 2013; O'Malley, Wideman & Ruiz-Trillo, 2016; Wideman & Muñoz-Gómez, 2016). Considering the dynamic nature of eukaryotic genomes post-LECA, this

indicates that gene loss has played a significant role in the evolution of many eukaryotic lineages (Koumandou *et al.*, 2013). A number of mechanisms for diversification within paralogous families and protein complexes have been described (Dacks & Field, 2018): (i) gene duplication; (ii) replacement upon gene loss by recruitment of an unrelated/analogous protein either allowing for, or compensating for, loss of the original protein (backfilling); (iii) expansion and contraction of paralogue families (churning); and (iv) loss of a gene (sculpting) (Fig. 1). Each of these processes, as well as other possibilities not considered here, can lead to changes in the composition of protein complexes and, in some cases, the evolution of novel complexes (Fig. 2).

The LECA existed at least  $10^9$  years BCE (Eme *et al.*, 2014) and, assuming a 90-min generation time (typical for *Saccharomyces cerevisiae*), more than  $10^{12}$  generations might have passed since, allowing a huge variety of forms and lifestyles to arise. Among extant eukaryotes, microbial lineages contained within the supergroups TSAR (Telonemia, Stramenopila, Alveolata, and Rhizaria), Metamonada and Discoba dominate the global biomass and are highly genetically diverse (Katz, 2012). Evolutionary innovations in microorganisms are likely facilitated by both short generation times and population bottlenecks. The latter increases the frequency of fixation of alleles (Wolf & Koonin, 2013), and is a common occurrence in parasitic protists and other potentially isolated populations. Notably, the composition of many protein complexes in protists can approach or even



**Fig. 1.** Selected modes of protein complex evolution. Evolution of protein complexes *via* paralogous gene duplication, backfilling, churning, sculpting or recoating. In duplication, a simple event creating a second paralogue allows the evolution of a new function. In backfilling, a lost component is substituted by the expansion of the remaining subunits. For churning, which is a special case of duplication, continual generation of new paralogues coupled with losses serves to diversify a gene family without an apparent change in overall number of paralogues. Sculpting represents a case of simple gene loss. Lastly, recoating describes the process whereby a complex protein core, that is surrounded by an additional protein shell of interactors, sees replacement of the shell but retention of the core subunits. Colours represent different paralogues. HOPS, homotypic fusion and vacuole protein sorting complex; Rabs, Ras-related in brain proteins.



**Fig. 2.** Organelle paralogy and replacement in the evolution of complexes. Top: original organelle paralogy model. Duplication of one paralogue encoding a protein complex subunit allows one of the copies to gain new functions (blue circle), evolving interactions with the original subunits (yellow circles), as well as with new proteins (additional blue circles), these possibly facilitated by mutations and subsequent changes in binding specificity. Importantly, the original complex is retained. Bottom: analogue replacement, a more severe form of complex evolution. In the upper row, exemplified by the evolution of an apparently novel kinetochore in kinetoplastids, the entire complex (or the vast majority) is replaced. We speculate that this took place *via* a neofunctionalisation mechanism, as kinetochore functionality is essential and therefore loss is unlikely to occur. In the lower row, there are clear cases of loss of a system, and its replacement. Respiratory complex I loss is known as are examples of organisms that lack an apparent lamina, *albeit* with some functionality subtended by non-conventional means. See text for details.

exceed that of multicellular organisms in terms of distinct gene products, demonstrating that protists are, at the cellular level, highly sophisticated and complex organisms (Gray *et al.*, 2020). Although large protein complexes are intuitively assumed to have the capacity for more sophisticated functionality (regulation, flexibility, etc.), this may not always be the case. As postulated by Constructive Neutral Evolution (CNE) theory, complexity may increase in the absence of positive selection, hence bringing no selective advantage, at least initially (Stoltzfus, 1999; Lukeš *et al.*, 2011). CNE seems to explain some of the diversity of mitochondrial respiratory complexes and protein import machineries. Although additional evidence is needed, the available data indicate that, in these cases, an increase in compositional complexity *per se* does not provide new functions or increased fitness (Muñoz-Gómez *et al.*, 2021). This view is supported by the varying composition of protein complexes from the diverse eukaryotic lineages considered below.

A growing interest in divergent lineages, due to recognition of their importance in multiple contexts, such as ecology and human health, and an increase in genome sequencing, has led to direct experimental characterisation of protein complexes from a taxonomically broad range of organisms. Here, we consider experimental and genomics evidence from organisms across the eukaryotic tree and assess the origins and elaboration of complexes supporting organelle biogenesis and function. We focus on key complexes of the endomembrane system, nucleus, and mitochondrion from multiple organisms, but in particular *Trypanosoma brucei*, a

member of the Kinetoplastea (phylum Euglenozoa). Despite being a parasitic organism with somewhat reduced complexity, trypanosomes remain one of the most intensively studied unicellular organisms outside of the opisthokonts. Importantly, this includes direct experimental evidence for a wealth of protein complexes and extends to localisation of the vast majority of proteins encoded by the genome (Tinti & Ferguson, 2022; Billington *et al.*, 2023). We compare and discuss some well-studied protein complexes in eukaryotes based on the available literature, and for the less-investigated examples we performed additional comparative genomic analyses using a representative (i.e. covering a large swathe of total eukaryotic diversity) set of 23 eukaryotic genomes/transcriptomes. We suggest that many complexes retain a conserved core with, unexpectedly, widely varying peripheral components. In extreme cases, entire complexes are replaced. We also found indications that gene loss has facilitated these substitutions.

## II. DIVERSITY WITHIN THE ENDOMEMBRANE SYSTEM

The endomembrane system encompasses the majority of eukaryotic organelles, including transport systems for protein targeting, flagellum construction, nuclear transport and other features. Proteins forming coats, tethers, specificity modules (i.e. providing address codes for identification of

specific compartments) and factors mediating formation and stability of these organelles almost exclusively belong to large paralogous families, with many of the organelle-specific paralogues having already evolved prior to the LECA (reviewed in Dacks & Field, 2018). The most prominent and best-supported proposal for how the organelles of the endomembrane system evolved (Dacks & Field, 2007) suggests that organelle-specificity proteins, which participate in transport vesicle formation, compartment specificity and membrane fusion, generally expanded by gene duplication and co-evolution of interacting proteins to allow organelle differentiation, with new compartments generated iteratively (Fig. 2 top panel). A special example of this recognises the architecture and homology between  $\alpha$ -solenoid/ $\beta$ -propeller fold-containing proteins that form vesicle coats, nuclear pore complexes (NPCs) and intraflagellar transport (IFT) complexes as evidence for common descent (Rout & Field, 2017). Comparative genomics established that a fully fledged organelle and protein complement was already present in the LECA (Dacks & Field, 2018), highlighting that gene expansion is not the sole process shaping endomembrane systems, and that loss clearly contributes towards specialisation. These paralogous families exhibit conservation ranging from ubiquitous and/or near universal to infrequently retained yet present in a wide range of taxa, indicating a presence in the LECA and underscoring the high frequency of secondary loss.

One comprehensively studied family is heterotetrameric adaptor complex-containing coats. The best-known examples are those containing adaptins and membrane-deformation proteins such as clathrin, but the family also includes coat protein complex I (COPI) and TSET (Dacks & Robinson, 2017). While COPI mediates transport within the Golgi complex, the remaining complexes function in post-Golgi transport and/or endocytic pathways (Dacks & Robinson, 2017). The distribution of this family illustrates the full span of evolutionary patterns. The adaptor protein 1 (AP-1) complex and the associated clathrin coat, as well as COPI, appear essentially universal. AP-2, involved in endocytosis from the cell surface, is also very well retained, with the striking exception being loss in African trypanosomes (Manna, Kelly & Field, 2013). The intestinal parasite *Giardia lamblia*, despite being highly reduced, retains AP-1, AP-2, and COPI (Touz *et al.*, 2012), but other complexes seem more dispensable. The AP-3 complex, which operates between the trans-Golgi network to, from and between various types of endosomes (Simpson *et al.*, 1997; Theos *et al.*, 2005), has been lost at least four times, and the AP-4 complex lost multiple times (Dacks & Robinson, 2017). The AP-5 complex has been lost even more frequently (Ebenezer *et al.*, 2019). Finally, TSET, which, similarly to AP-2, may function in an endocytic pathway from the cell surface, is present in diverse eukaryotes (Hirst *et al.*, 2014). However, it also has been lost frequently (Lee *et al.*, 2015; Richardson & Dacks, 2022), and, strikingly, is absent from animal and fungal model systems (Hirst *et al.*, 2014). This particular taxonomic distribution of being widespread in

eukaryotes but not in well-studied model systems is increasingly apparent and has relevance to creating truly generalised cell biological models (More *et al.*, 2020). There are also cases where model organisms possess expanded AP complex complements, best characterised in metazoans where tissue- or cargo-specific subunit expression occurs (Boehm & Bonifacino, 2001). However, expanded complements are present in *Trichomonas vaginalis* (Carlton *et al.*, 2007) and embryophyte plants, and appear to be the result of recent convergent duplications (Larson, Dacks & Barlow, 2019). Moreover, there are notable instances of new cargo adaptors working as monomers such as the Golgi-localised  $\gamma$  ear-containing Arf-binding (GGA) proteins (Hirst, Lindsay & Robinson, 2001), which are homologous to the adaptin  $\gamma$  interaction domain and can represent functions of single subunits or partial losses. For the TSET complex, the remaining medium subunit serves as a novel clathrin adaptor (Umasankar *et al.*, 2014; Zaccai *et al.*, 2022). Overall, the observed pattern of strict conservation of some complexes and loss of others is interpreted as sculpting (Elias *et al.*, 2012), i.e. cutting away proteins and pathways during cellular system evolution. However, sculpting is not the sole process involved in the evolutionary dynamics of the endomembrane system.

Backfilling, the occurrence of equivalent function being undertaken by non-homologous components, can be seen in the machinery mediating interactions between conserved components. Of several reported modes, the major endocytic pathway is supported by the protocoatmer family member clathrin, which is also involved in additional transport and sorting pathways (Robinson, 2015). The clathrin heavy chain is retained in the vast majority of eukaryotes, where it performs a well-observed role, serving as one of the several interaction hubs operating within the endosomal system (Schmid & McMahon, 2007). By contrast, the regulatory light chain is less frequently detected and at least in the diplomonad flagellates (including *G. lamblia*), appears to have been functionally replaced by an analogous protein (Santos *et al.*, 2022). Furthermore, only few of the conventional clathrin protein partners, i.e. those identified in animals and fungi, are present in trypanosomatids, and comparisons across eukaryotic lineages reveal that many are indeed specific to animals and fungi, or even just to metazoans (Field, Gabernet-Castello & Dacks, 2007). An exception is the retention by trypanosomatids of epsin N-terminal homology (ENTH) and AP180 N-terminal homology (ANTH) domain containing proteins, which possess a phosphoinositide-binding domain at the N-terminus and clathrin-interaction boxes within a largely disordered C-terminal domain (Gabernet-Castello, Dacks & Field, 2009; Manna *et al.*, 2015). However, trypanosomatids do possess a significant cohort of distinct clathrin interactors, many of which have been identified directly from affinity isolation studies and validated in both African and American trypanosomes (Adung'a, Gadelha & Field, 2013; Manna *et al.*, 2015; Kalb *et al.*, 2016). A minority function in association with clathrin and share architectural similarities with the ANTH-domain proteins (Manna *et al.*, 2015), but without obvious sequence homology. The majority, however, are clearly even more

highly divergent and not shared beyond these flagellates (Adung'a *et al.*, 2013; Manna *et al.*, 2015). This suggests that the trypanosome lineage has most likely lost many canonical genes but that this reduced repertoire has been 'backfilled' by the recruitment/emergence of new factors.

Further examples of backfilling have been identified in the trypanosome endocytic system, particularly in case of Ras-related in brain (Rab) proteins. Rabs are identity markers coordinating vesicle fusion through interactions with tethering complexes, and facilitate transport through cytoskeletal elements (Stenmark, 2009; Kjos *et al.*, 2018). Rab11 controls a major recycling pathway returning molecules to the cell surface after internalisation, a role conserved in trypanosomatids (Jeffries, Morgan & Field, 2001; Umaer, Bush & Bangs, 2018). In metazoans, Rab11 functions are mediated in part *via* coiled-coil Rab11-family interacting proteins (FIPs) and additional interactions with Sec15, a subunit of the exocyst, a heterooctameric complex responsible for late stages of exocytosis (Synek, Sekereš & Zárský, 2014). While FIPs are Metazoa-specific, the Sec15–Rab11 interaction is maintained in *T. brucei*, but with two novel proteins supporting the interactions likely similar to those provided by FIPs. One, Rab11-binding protein of 74 kDa (RBP74), interacts with both Rab11 and Rab5, shares a coiled-coil architecture with FIPs and maintains a role in coordinating endocytosis and recycling, despite lacking sequence similarity to FIPs. The second is Exo99, a novel ninth subunit of 99 kDa within the trypanosomatid exocyst. The eight canonical exocyst subunits share similar overall architecture (Mei *et al.*, 2018), suggesting an ancestral complex possessing one or few subunits. While there are many examples of canonical exocyst subunit expansion or loss (Zárský *et al.*, 2020), Exo99 has no similarity to the canonical subunits, and is predicted to be architecturally related to  $\alpha$ -solenoid/ $\beta$ -propeller fold-containing proteins. Exo99 is essential for complex functionality (Boehm *et al.*, 2017), and present throughout the trypanosomatids (Boehm & Field, 2019) suggesting recruitment of a novel protein to build a new version of the exocyst.

The final process under consideration is churning, specifically the birth and death of proteins of the same paralogous family, rather than replacement by non-paralogous proteins. Good examples are once again Rab GTPases. The Rab complement of extant eukaryotes ranges widely, from only five in *Neophytopia* (formerly *Pyropia*) *yezoensis*, to 12 in yeasts, over 60 in Metazoa and several hundred in *Entamoeba* and *Trichomonas* (Diekmann *et al.*, 2011; Elias *et al.*, 2012; Klöpper *et al.*, 2012; Petřelková & Eliáš, 2014). Reconstructions of the LECA identify between 19 and 24 ancient Rab subfamilies, depending on how subfamilies are categorised (Diekmann *et al.*, 2011; Elias *et al.*, 2012; Klöpper *et al.*, 2012). Clearly loss sculpted this protein family, but examples of churning can be discerned. The first is metazoan Rab32, which plays roles within the endocytic system, but also in several endoplasmic reticulum (ER) functions (Wasmeier *et al.*, 2006; Ortiz-Sandoval *et al.*, 2014). Rab32A and Rab32B are ancient Rab32 paralogues, with Rab32B lost from plants, fungi and the base of the vertebrate lineage (Elias *et al.*, 2012; Ortiz-Sandoval *et al.*, 2014). However, Rab32A has been duplicated at least twice,

to give rise to Rab29 (at the base of the holozoan radiation, i.e. the group encompassing animals and their single-celled relatives) and Rab38 in vertebrates. Rab29 and Rab38 retain similar functions with Rab32, and provide clear examples of loss followed by re-expansion within a protein family (Wasmeier *et al.*, 2006; Ortiz-Sandoval *et al.*, 2014; Wang *et al.*, 2014). More striking examples of changes to Rab complement come from land plants. Here, ancestral losses in the Archaeplastida supergroup removed over seven ancestral Rab subfamilies, with further loss of another five later in evolution in the lineage leading to land plants (Petřelková & Eliáš, 2014). As a consequence, the ancestral multi-cellular plant, i.e. embryophyte, possessed a reduced Rab complement, specifically lacking Rabs associated with flagellar function (Rab28, Rab12, IFT27) and endocytosis/phagocytosis (Rab4, 20, 22, 32A/B, 34) (Rutherford & Moore, 2002; Petřelková & Eliáš, 2014). Reduction in the endocytic Rab complement has been compensated by backfilling from Rab5 and Rab11, which act at early and recycling endosomes, respectively (Stenmark, 2009), as there is evidence for the emergence of a new Rab5 paralogue (RabF1/ARA6) in the Chloroplastida stem lineage, and expansion of Rab11 paralogues in Embryophyta (Petřelková & Eliáš, 2014).

An example that is intermediate between backfilling and churning is provided by the class C core vacuole/endosome tethering (CORVET) and homotypic fusion and vacuole protein sorting (HOPS) complexes within ciliates. This multi-subunit tethering assembly functions in the process of vesicle fusion, interacting with Rab5 and Rab7 during early endosome maturation into late endosomes. These complexes are formed of four core subunits [vacuolar protein sorting-associated protein (Vps) 11, 16, 18, and 33], with CORVET-(Vps3 and 8) or HOPS-(Vps39 and 41) specific subunits (Balderhaar & Ungermann, 2013; Solinger & Spang, 2013). The CORVET/HOPS complexes are an ancient eukaryotic feature (Field *et al.*, 2007; Klinger, Klute & Dacks, 2013), but their specific subunits are missing from some key lineages, including apicomplexans and ciliates (Woo *et al.*, 2015). Vps8 subunits expanded at the base of the oligohymenophorean ciliate class or potentially earlier, and HOPS subunits were subsequently lost, with the Vps8 paralogues replacing HOPS subunit function (Sparvoli *et al.*, 2018, 2020). As the Vps8 subunit is distantly related to the lost Vps39 and Vps41 subunits, this represents churning, but since it is not an expansion of the same paralogue, it is also an example of backfilling. Additional losses of multi-subunit tethering complexes in ciliates have also been suggested, raising the possibility of additional non-homologous subunit replacement (Richardson & Dacks, 2022).

### III. REPLACEMENTS AND LOSSES IN THE NUCLEUS

The evolutionary processes described above have also contributed to shaping other organelles, including the nucleus. Despite conserved and central roles of the organelle, nuclear composition varies considerably across eukaryotes. Although

the morphology of the nuclear envelope (NE) is remarkably conserved, the origins of many protein components indicate compositional diversity. The NE is punctuated by NPCs and also other structures such as the mitotic spindle and spindle pole body (SPB). In many lineages a proteinaceous lamina is present on the NE inner face (Gruenbaum *et al.*, 2003). Mitotic division is supported by a tubulin spindle in most eukaryotes. We highlight several examples of protein complexes to illustrate the range of divergence for nuclear structures.

Nuclear division occurs *via* a multitude of distinct mechanisms. Association of specific microtubule organising centres (MTOCs), such as the SPB or centrosomes, with anchoring of the spindle at the NE is neither universal, mechanistically conserved, nor even always the same in different developmental stages within the same organism (Devos, Gräf & Field, 2014; Bouhlel *et al.*, 2015; Ito & Bettencourt-Dias, 2018). Spindle-organising MTOCs are structurally conserved, with a 9 + 0 tubulin architecture in common with the 9 + 2 arrangement of axonemes, attesting to shared origin. However, SPBs in fungi and centrosomes in animal cells also exhibit clear divergence, offering a strong example of sculpting. Beyond the tubulin core, both SPBs and centrioles contain an additional subcomplex, the pericentriolar matrix (PCM). Multiple proteins are shared between the SPB and the centrosome PCM, but several subunits are unique to centrosomes or SPBs. Elegant reconstruction of PCM evolution suggests a pattern of secondary losses from a more complete ancestral form (Ito & Bettencourt-Dias, 2018) and is supported by the presence of centrosome-specific components in the PCM of basal fungi. Hence, some SPB PCM subunits have been acquired post-loss of centrosome-specific proteins during fungal evolution.

Eukaryotic genomes are packaged as chromatin, but remarkably, even this fundamental aspect of nuclear biology exhibits diversity. In most lineages, including animals, plants, fungi and almost all protists, histones comprise the chromatin protein core. Five core histones are recognised, with H2A, H2B, H3, and H4 forming the nucleosome and the multifunctional ‘linker’ histone H1 completing assembly and stabilising higher-order chromatin structures. Several divergent histones are also present and employed to mark a DNA strand for specific functions, including initiation/termination of transcription and binding of kinetochores during mitosis. These major isoforms are ancient, present in the LECA and, together with some of the variant histones, quite probably universal (Stevens *et al.*, 2020; Ding *et al.*, 2021; Osakabe & Molaro, 2023), *albeit* with the likelihood of some churning taking place such that some histone variants have been generated post-LECA and paralogues of some LECA histones lost. In the absence of detailed reconstruction of histone evolution, however, the mechanism and modes of diversification of this central eukaryotic gene family remain to be fully addressed.

In dinoflagellates (Alveolata), a significant alteration has occurred, exemplifying both replacement and extreme divergence. In this lineage histones are hyperdivergent, with extended N- and C-termini, and are supported by additional

core chromatin proteins (Gornik *et al.*, 2019). Dinoflagellates have very high DNA content (>30-fold that of Metazoa), which likely necessitates unusual packaging mechanisms. Core histones are at very low abundance and contain variant canonical residues compared to those of animals (Marinov & Lynch, 2016; Riaz & Sui, 2018). Mutation patterns of canonical histone modification sites suggest that dinoflagellate histones retain canonical functions in transcription (Gornik *et al.*, 2019), but two additional protein families accompany dinoflagellate histones: histone-like basic proteins (HLPs) and dinoflagellate/viral nucleoprotein (DVNP). Both likely originated from lateral gene transfer (LGT) events: the former from bacterial histone-like proteins (Wong *et al.*, 2003) and the latter from viruses (Gornik *et al.*, 2012). Interestingly, HLPs are represented by two distinct subfamilies derived from different bacterial progenitors (Janoušková *et al.*, 2017a), leading to a model whereby DVNP proteins were acquired early, which facilitated increased genome compaction, with HLPs acquired later from at least two LGT events. While LGT is an unusual mechanism for supply of new proteins in eukaryotes, this is an excellent example of a case of such provision, most likely facilitating functional divergence of otherwise highly conserved proteins.

The nuclear lamina provides structural support and organisational functions to the nucleus. There are multiple molecular forms, but the system in animal cells likely represents the LECA state (Koreny & Field, 2016). In Metazoa and many other taxa, the lamina is comprised of lamins that assemble into higher-order filaments. Viridiplantae and Euglenozoa lack detectable lamin orthologues, and lamina functions are performed by distinct protein systems, the nuclear matrix constituent proteins (NMCPs) in plants and nuclear pore proteins (NUPs) 1 and 2 in glycomonads (the subphylum encompassing kinetoplastids and diplomonids) (see online Supporting Information, Table S1; Koreny & Field, 2016; Groves *et al.*, 2020; Butenko *et al.*, 2021). NUP1 and 2 are repetitive, coiled-coil proteins forming networks, with both the N- and C-termini of NUP-1 acting as interaction hubs, an assembly distinct from metazoan lamins (DuBois *et al.*, 2012; Maishman *et al.*, 2016; Padilla-Meija *et al.*, 2021). NUP1 and 2 position NPCs and silence subtelomeric genes while providing structural support, similar to the animal lamina (DuBois *et al.*, 2012). However, since NUP1/2 are unrelated to lamins, this indicates a striking replacement of lamins during the emergence of glycomonads, or even earlier, as conventional lamins were not identified in *Euglena gracilis* (Koreny & Field, 2016). Similarly, the plant NMCP proteins also possess considerable coiled-coil regions and multiple NMCP paralogues that expanded from an ancestral gene on several occasions (Ciska & Moreno Diaz de la Espina, 2013). Since metazoan-related lamins are widespread amongst different eukaryotic lineages (Table S1), the most parsimonious model is backfilling, with loss followed by replacement, although the alternative, where NUP1 and 2 or NMCP evolved alongside lamins before their loss is also possible.

Monocentric kinetochores recognise centromeric chromosomal regions, linking chromosomes to the spindle during cell

division. Inner and outer kinetochore subcomplexes are recognised, the inner interacting with centromeric DNA, usually *via* recognition of a variant histone H3, with the outer complex recruiting spindle microtubules. In nearly all taxa, kinetochores comprise many distinct proteins (Table S2), probably arising by duplication of genes in a stepwise manner (Musacchio & Desai, 2017; Field, 2019; Tromer *et al.*, 2019). The trypanosomatid kinetochore stands as the sole known exception (Akiyoshi & Gull, 2014). While also complex and apparently functioning analogously to canonical kinetochores, there is only weak evidence for conservation of any component (Table S2; Akiyoshi & Gull, 2014; D'Archivio & Wickstead, 2017; Ebenezer *et al.*, 2019; Butenko *et al.*, 2020). The trypanosome lamina and kinetochore proteins interact and probably function together during chromosomal segregation (D'Archivio & Wickstead, 2017; Padilla-Mejia *et al.*, 2021). Recent evidence of homology between some trypanosomatid kinetochore proteins and components of the eukaryotic synaptonemal complex suggests that trypanosomatid kinetochores may have evolved by repurposing meiotic components already present in the LECA, and supporting a fully unique origin (Tromer *et al.*, 2021). Again, the most parsimonious model is backfilling, with loss followed by replacement, especially as potential ancestral kinetoplastid kinetochore components were already present in the LECA, although alternative pathways cannot be excluded.

The NPC is responsible for transport across the NE and many additional functions. It is comprised of several subcomplexes that together ensure selective transfer of cargo molecules. Morphologically the NPC is highly conserved, exhibiting eight-fold symmetry and comprising concentric proteinaceous rings surrounding a central transport channel. Fibrous extensions are present at both nuclear and cytoplasmic NPC faces, with roles in regulating transport and messenger RNA (mRNA) maturation. Although much of the transport mechanism and core NPC structure is conserved across eukaryotes (Field & Rout, 2019), salient differences are present. Many of these are only apparent using high-resolution morphological analysis (Makarov, Padilla-Mejia & Field, 2021). There is one example, however, where detailed information is available for NPC remodelling. In trypanosomes, the NPC has lost components of the RNA quality control system, which actively monitor the integrity and functionality of RNA molecules and are normally located at the NPC cytoplasmic face (Obado, Field & Rout, 2017). This surveillance system ensures that defective mRNAs are not translated and, hence, that *cis*-splicing is correct. As trypanosomal splicing is restricted to 5' untranslated region (UTR) *trans*-splicing, with *cis*-splicing almost absent, this verification step seems to have become superfluous. Retention of this NPC subcomplex in euglenids and diplomonads, both of which do exhibit extensive *cis*-splicing, supports this interpretation of a clear example of secondary loss in trypanosomatids (Butenko *et al.*, 2021). Additional changes in the trypanosome NPC are associated with losses and possible replacements, including proteins of the nuclear basket, where the canonical subunits are replaced by a trypanosome-

specific analogue (Holden *et al.*, 2014). Overall, we suggest that the NPC represents an example where multiple evolutionary mechanisms likely contributed, but that loss of subunits is clear and this may well have led to additional innovation. In these examples, the NPC has most probably been backfilled with the acquisition of novel components.

#### IV. EXTREME DIVERGENCE OF MITOCHONDRIAL PROTEIN COMPLEXES

Mitochondria, the most prominent organelle of endosymbiotic origin (Martin & Mentel, 2010), contribute many essential functions. They are highly specialised in morphology and composition and show extensive diversity (Pánek *et al.*, 2020). In trypanosomes and related flagellates, mitochondria harbour a structurally unusual genome (Jensen & Englund, 2012), the transcripts of which undergo extensive editing and processing (Aphasizheva *et al.*, 2020). Some anaerobic protists are completely devoid of mitochondrial DNA and instead of canonical mitochondria retain mitochondrion-related organelles (MROs). These MROs are not involved in oxidative phosphorylation, but perform other functions (Leger *et al.*, 2019); MROs have been excluded from our comparative analysis as they represent a special case of gene loss.

##### (1) Mitochondrial import

In the course of endosymbiotic integration, most mitochondrial genes were either lost or relocated to the nuclear genome (Roger, Muñoz-Gómez & Kamikawa, 2017). Consequently, nuclear-encoded proteins are conveyed into the organelle *via* dedicated import systems (Wiedemann & Pfanner, 2017). There are at least five major pathways for protein import to mitochondria, which require distinct machineries, including TOM and TIM (translocase complex of the outer and inner membranes, respectively), SAM (sorting and assembly machinery), and MIM (mitochondrial import machinery) (Wiedemann & Pfanner, 2017). For the purposes of this review, we will focus on the two former complexes that mediate import of presequence-containing (TOM and TIM23 complexes) and polytopic hydrophobic carrier (TOM and TIM22) proteins, respectively.

The TOM complex is composed of a pore-forming, bacteria-derived, Tom40, associated receptors (Tom20, 22 and 70) and various chaperones (Bausewein *et al.*, 2020; Wang *et al.*, 2020). As the central pore, Tom40 is retained in essentially all eukaryotes [Fig. S1 (see Fig. S0 for information on how to read the dartboard charts); Table S3]. The Tom22 receptor, playing an important role in oligomerisation of the TOM complex, is also nearly ubiquitous. However, Tom70 and Tom 20 appear to have been lost on multiple occasions, which presumably reflects a non-essential role in pore formation (Fig. S1; Table S3). Sculpting of the TOM complex by the loss of receptors in some lineages might be at least partly explained by their overlapping substrate



specificities and the ability to compensate reciprocally for each other's absence (Yamano *et al.*, 2008; Backes *et al.*, 2018). TOM complex sculpting also involves so-called small Toms (Tom5–7), which do not directly contribute to the protein translocation function (Wiedemann & Pfanner, 2017), with Tom5 and 6 likely present in the LECA but retained only in opisthokonts, and chloroplastids, while Tom6 is also preserved in some discobans (Fig. S1; Table S3). A clear case of a subunit replacement by its functional analogue is that of the ancestral Tom70-like receptor by ATOM69 (atypical outer membrane translocase 69) and OM64 (outer mitochondrial membrane protein of 64 kDa) in trypanosomes and plants, respectively (Schneider, 2020). The trypanosomatid complex shares only Tom40/ATOM40 and Tom22/ATOM14 with other eukaryotic lineages, albeit most of the additional subunits are recognised as functional analogues of the canonical TOM subunits (Fig. S1; Table S3), a further example of re-coating a conserved core with a divergent shell of interacting proteins (Fig. 1; Mani, Meisinger & Schneider, 2016).

Upon interaction with the TOM complex, presequence-containing proteins destined for the mitochondrial matrix and metabolite carriers of the inner membrane (IM) are directed to their locations by the TIM23 complex and the TIM22 carrier-insertase, respectively (Wiedemann & Pfanner, 2017). The core components of both complexes (pore-forming Tim22 and Tim23; Tim17 closely associated with Tim23) are present in all eukaryotes possessing aerobic mitochondria. However, trypanosomes have retained only a single TIM17/22/23 family transporter (TbTim17), most closely related to TIM22 (Fig. S2; Table S4; Žárský & Doležal, 2016). Unique absence of Tim23 and a canonical eukaryotic Tim17 in trypanosomatids is, most likely, another example of a derived 'simplification', involving (secondary) gene loss. The most plausible scenario is that an initially neutral recruitment of *T. brucei* specific presequence translocase-associated motor subunit 27 (TbPam27) and two rhomboid proteins (TimRhom I and II) gave TbTim17 the ability to translocate presequence-containing substrates, thus making the function of the ancestral TIM23 complex redundant (Schneider, 2018). The losses of core subunits are only described in trypanosomatids and a few other groups, but mainly MROs in those latter instances (Pyrihová *et al.*, 2018). However, losses of chaperones Tim8–10 and 13 are more common (Fig. S2; Table S4), and especially evident in the case of Tim8 and 13, forming a subcomplex, at least in yeast, and responsible for relocation of only a narrow group of precursor proteins. This may explain rather frequent loss of one or both paralogues (Fig. S2; Table S4).

In addition to highlighting gene losses, variations in TIM complex composition illustrate the existence of moonlighting proteins. Examples include the Sdh3 subunit of succinate dehydrogenase in *S. cerevisiae* (Gebert *et al.*, 2011), NADH dehydrogenase (respiratory complex I) subunits B14.7-like and Tim 23-2 in *A. thaliana* (Wang *et al.*, 2012), acyl glycerol kinase in Metazoa (Valpadashi *et al.*, 2021), and acyl-CoA dehydrogenase (ACAD) in *T. brucei*, which in addition to their

more widely known functions are components of TIM translocase in these organisms (Singha *et al.*, 2012). However, it is unclear whether the recruitment of these proteins to the TIM complex in certain lineages created functional redundancies, possibly allowing subsequent losses of some of the original subunits, or whether these proteins substituted for some ancestral subunits, already lost, instead.

## (2) Mitochondrial contact site and cristae organising system (MICOS)

As the name implies, the primary function of the MICOS is in organising cristae and anchoring them to the mitochondrial IM (Wollweber, von der Malsburg & van der Laan, 2017). In addition, MICOS interacts with the Sam50 component of SAM and TOM complex components to create contact sites between the IM and OM. In opisthokonts, MICOS is composed of two dynamic subcomplexes, organised around broadly conserved core proteins Mic10 and Mic60, respectively (Zerbes *et al.*, 2012; Barbot *et al.*, 2015). Mic60 contains a mitofilin domain originating from ancestral bacteria (Muñoz-Gómez *et al.*, 2015), and together with Mic10 and Mic19 traces back to the LECA (Fig. S3; Table S5). Mic12 is also proposed to have been present in the LECA (Huynen *et al.*, 2016).

The trypanosomatid MICOS is also bipartite, although unlike opisthokont MICOS, is divided into integral and peripheral subcomplexes (Eichenberger *et al.*, 2019). Altogether, trypanosomes possess eight MICOS subunits, with most being lineage specific, indicating a near-complete replacement, albeit with a shared core of Mic60 and Mic10 (Huynen *et al.*, 2016; Kaurov *et al.*, 2018). TbMic60 and its orthologue in *E. gracilis* lack the mitofilin domain (Kaurov *et al.*, 2018; Hammond *et al.*, 2020), and the absence of the domain is suggested to be compensated by mitofilin domain-containing TbMic34, a core component of the peripheral subcomplex (Kaurov *et al.*, 2018; Eichenberger *et al.*, 2019). TbMic60 has a domain architecture resembling that of the N-terminus of the canonical Mic60, including a mitochondrial presequence, *trans*-membrane and coiled-coil domain (Kaurov *et al.*, 2018). It seems that in Euglenozoa the ancestral Mic60 gene split into two parts, one encoding the core Mic60 region (=TbMic60) and the other encoding the mitofilin domain (TbMic34) (Fig. S3).

The euglenozoan MICOS also possesses a number of lineage-specific components, among which is a novel thioredoxin-like subunit, Mic20 (Fig. S3; Table S5), which performs a still unassigned role in the stabilisation of inter-membrane space proteins and which may well represent a novel subunit and function (Kaurov *et al.*, 2018, 2022). Whether major deviations from opisthokont counterparts, in both composition and structure of MICOS, similar to those observed in trypanosomes, occur in other lineages, is not known. Furthermore, the basic mechanism for cristae formation that originated from the ancestral bacteria was complexified by duplications, including Mic19 and 25 in opisthokonts and Mic10-1/2 in trypanosomes and was later sculpted by gene loss and backfilled with novel subunits.

### (3) Oxidative phosphorylation system

Oxidative phosphorylation (OxPhos) is mediated through respiratory complexes I–IV and ATP synthase, all located in the IM. A number of core subunits of alphaproteobacterial origin are conserved, surrounded by subunits of eukaryotic origin, only some of which were already present in the LECA (Timmis *et al.*, 2004; Roger *et al.*, 2017).

Complex I, NADH:ubiquinone oxidoreductase, is the major entry for electrons into the respiratory chain (Brandt, 2006). In yeast, some plants, and myzozoans, there is a complete loss of complex I combined with alternative electron entry points (De Vries & Marres, 1987; Gardner *et al.*, 2002; Petersen *et al.*, 2015). By contrast, opisthokonts, euglenozoans and *Tetrahymena thermophila* possess extremely large complexes of ~40–60 subunits (Huynen & Elurbe, 2022).

Eukaryotes share a highly conserved set of 17 alphaproteobacterial subunits, which encompasses all redox centres and hence the catalytic core of the complex (Yip *et al.*, 2011). Additionally, the LECA had incorporated a large number of accessory subunits, almost tripling subunit composition during early eukaryotic evolution (Cardol, 2011). While the losses of core subunits in eukaryotes are extremely rare, some accessory subunits are lost more frequently, with the notable example of a  $\gamma$ -carbonic anhydrase absent in opisthokonts (Gawryluk & Gray, 2010). Gains of lineage/species-specific accessory subunits are also very common. The acquisition of additional subunits occurred from various sources (Yip *et al.*, 2011; Elurbe & Huynen, 2016), and most are products of gene duplications and/or recruitment from other mitochondrial protein families, suggesting paralogue expansion (Szklarczyk & Huynen, 2009; Elurbe & Huynen, 2016).

In *T. brucei* at least seven accessory subunits are encoded by what seem to be duplications of genes encoding other accessory subunits, either species/lineage-specific, or more universal (Table S6; Duarte & Tomás, 2014). In some lineages novel accessory subunits even seem to form new functional modules (e.g. an extra matrix ‘FAS domain’ in *T. brucei*) (Duarte & Tomás, 2014). It was suggested that in the euglenozoan *Paradiplonema* (formerly *Diplonema*) *papillatum*, novel accessory subunits might have replaced some of the absent accessory proteins which are otherwise nearly universally distributed (Valach *et al.*, 2018). Overall, our comparative analysis again confirms that 43 subunits were already part of complex I in the LECA (Fig. S4; Table S6).

Succinate:ubiquinone oxidoreductase (complex II) in opisthokonts consists of four proteobacteria-derived subunits, with succinate dehydrogenase (SDH) subunit A (SDHA) and SDHB forming a soluble catalytic domain anchored in the IM by the hydrophobic anchor composed of SDHC and SDHD (Moosavi *et al.*, 2019). While the catalytic subunits are universal (Fig. S5; Table S7; Huang & Millar, 2013), SDHC and SDHD appear to have been lost several times, for example in alveolates (Fig. S5; Evers *et al.*, 2021). It was assumed that the accessory lineage-specific subunits may have replaced the ancestral membrane-anchoring ones in some groups (Maclean *et al.*, 2022).

However, the most recent experimental data hint that the genomes of these organisms encode extremely divergent integral membrane subunits (Mühleip *et al.*, 2023), and the observed absences might be attributed to the limitations of bioinformatic tools. The genes encoding the subunits of proteobacterial origin underwent duplication in some lineages (e.g. in chloroplastids, some amoebozoans and stramenopiles) (Table S7). In Euglenozoa the *sdhb* gene has been split into two separately transcribed and translated parts. The separate protein products correspond to the N and C termini of a typical SDHB protein (Morales *et al.*, 2009; Perez *et al.*, 2014). In discobans, chloroplastids and alveolates, whenever complex II was experimentally characterised, the classical opisthokont four-subunit composition turned out to be augmented by a variable number of additional subunits (Fig. S5; Table S7; Morales *et al.*, 2009; Huang *et al.*, 2019b; Evers *et al.*, 2021). Of these, at least the homologues of plant SDH5 and SDH6 are readily identifiable outside the lineage and were likely present in the LECA (Huang *et al.*, 2019b; Gray *et al.*, 2020). This would imply that the LECA possessed a more complex succinate:ubiquinone oxidoreductase than model opisthokont (yeast and mammalian) systems, and that the observed bacteria-like composition of complex II in that group is the result of ancient sculpting events.

Complex III, ubiquinol:cytochrome *c* oxidoreductase, forms homodimers (Berry *et al.*, 2000). The catalytic engine incorporating cytochromes *b* and *c1*, and Rieske Fe-S protein is of proteobacterial origin and is conserved across eukaryotes (Fig. S6; Table S8; Smith, Fox & Winge, 2012). The accessory subunits (COR1 and 2, QCR7–10) involved in the stabilisation of complex III and the formation of III + IV supercomplexes (Maclean *et al.*, 2022), can also be traced back to the LECA, with QCR8 and 9 either lost or diverged beyond recognition in several lineages (Fig. S6; Table S8). It is noteworthy that complex III of apicomplexans, euglenozoans, and a chloroplastid (*Chlamydomonas reinhardtii*) also acquired a small, extra cohort of species- or lineage-specific subunits (Fig. S6; Table S8; Perez *et al.*, 2014; Salinas *et al.*, 2014; Evers *et al.*, 2021). Some of the lineage-specific subunits, such as QCRTB1 and QCRTB2 1 in Euglenozoa (Perez *et al.*, 2014), again are the result of duplicating a gene encoding one of the LECA-derived complex III subunits (COR1/QCR1) in the ancestor of the group.

Complex IV, also known as cytochrome *c* oxidase, which couples oxygen reduction to proton translocation across the IM, is surprising with regard to the amount of variation in subunits when comparing different protists to the well-studied yeast and mammalian models (Ziková *et al.*, 2008; Maclean *et al.*, 2022; Zhou *et al.*, 2022). Indeed, euglenozoans, alveolates and plants, in addition to the universally conserved catalytic core (subunits COX1–3) of proteobacterial origin and several supernumerary subunits demonstrating a patchier distribution among eukaryotes, have acquired multiple lineage/species-specific complex IV components (Fig. S7; Table S9). These subunits are predicted to be involved in the assembly and regulation of the complex, in

supercomplex formation, and in maintenance of its structural integrity and/or unusual species-specific secondary functions (Perez *et al.*, 2014; Evers *et al.*, 2021; Zhou *et al.*, 2022). Along with the accretion of novel subunits and the apparent losses of others in certain groups, gene duplication played a profound role in the evolution of complex IV (e.g. COX7A and B in mammals, COX6C in *T. thermophila*), including the duplications of genes encoding subunits of other respiratory complexes [e.g. the COX4/COX5b subunit is a duplication product of the NADH:ubiquinone oxidoreductase subunit S6 (NDUFS6) complex I subunit gene] (Elurbe & Huynen, 2016). Overall, complex IV in many eukaryotes is often somewhat expanded compared to its opisthokont counterpart and was mainly shaped, along with a few cases of losses and/or extreme divergence, by gene duplication of ancestral subunits and accretion of dozens of species/lineage-specific subunits.

Complex V, or F<sub>O</sub>F<sub>1</sub>-ATP synthase consists of the F<sub>O</sub> moiety embedded in the IM, and the soluble F<sub>1</sub> moiety extending into the matrix (Walker, 2013). The F<sub>1</sub> subcomplex incorporating the catalytic head subunits  $\alpha$  and  $\beta$ , as well as the  $\gamma$ ,  $\delta$ , and  $\epsilon$  subunits forming the central stalk, is nearly universally conserved across eukaryotes. The very rare absences are probably the result of either incomplete data or limitation of bioinformatic searches (Fig. S8; Table S10). The only known conspicuous addition to the F<sub>1</sub> subcomplex occurred in Euglenozoa, where the newly acquired subunit p18 is associated with the F<sub>1</sub> head. This Euglenozoa-specific component does not appear to contribute directly to the catalytic function of the enzyme but is essential for its integrity (Gahura *et al.*, 2018). All other eukaryotic F<sub>1</sub> subunits are conserved in Euglenozoa, which makes the incorporation of this novel subunit rather challenging to explain unless a neutral process is assumed, ending with this extra, chaperonin-like subunit, compensating for progressive loss of stabilising protein–protein interactions between the F<sub>1</sub> subunits.

The composition of the F<sub>O</sub> subcomplex, except for the conserved subunits a and c which form the H<sup>+</sup> channel, is more variable with several losses being evident (Fig. S8; Table S10). These losses include peripheral stalk subunits b and d in *Naegleria gruberi*, ATP8 in *Capsaspora owczarzaki*, Rhodophyta, and *Emiliania huxleyi*, and subunit h in several lineages. The peripheral stalk (stator) subunits putatively involved in ATP synthase dimerization (f, e, g, i/j, and k) demonstrate an even more patchy distribution (Fig. S8; Table S10). Experimental evidence indicates the presence of numerous species/lineage-specific F<sub>O</sub> subunits in alveolates, euglenozoans, chlorophytes and amoebozoans (Vázquez-Acevedo *et al.*, 2006; Balabaskaran Nina *et al.*, 2010; Perez *et al.*, 2014). Some of these subunits, however, were recently found to be divergent homologues of the (nearly) universal subunits (Sinha & Wideman, 2023). The F<sub>O</sub> subcomplex has undergone multiple losses along with gains of species/lineage-specific subunits, hinting that backfilling might be a dominant mode of ATP synthase evolution. However, the results of functional studies in line with this assumption are currently not available, and it is not

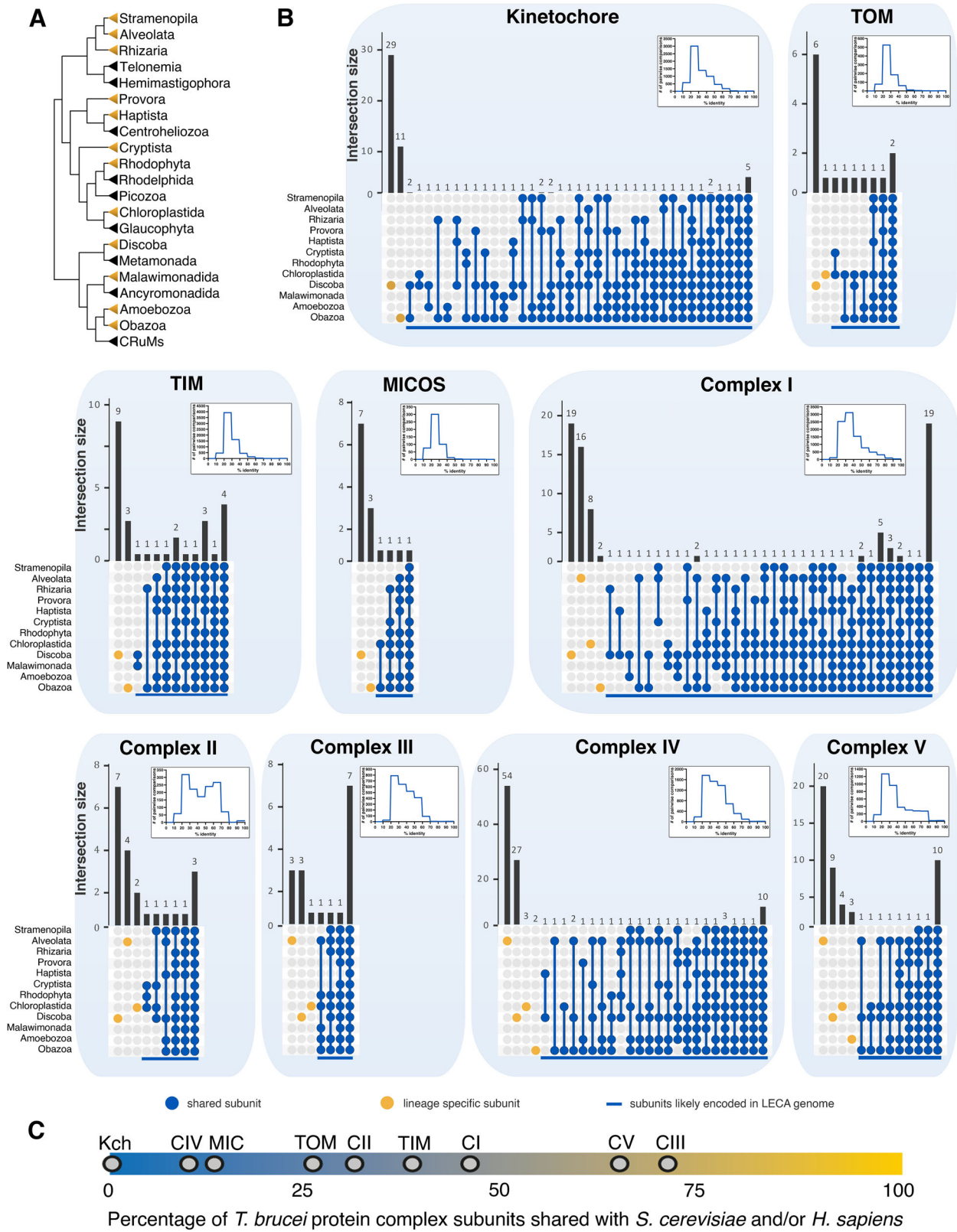
feasible to draw such conclusions based on comparative genomics data alone. Another view suggests that most if not all species/lineage-specific subunits are accretions that do not replace missing subunits. Overall, experimental and comparative genomics analyses performed by us and others (Sinha & Wideman, 2023) suggest that the LECA complex V possessed 17 subunits (identified by experimental studies in at least two eukaryotic supergroups), which represent functional homologues across eukaryotes. There is one additional protein (ATPTG9) which was likely encoded in the LECA genome but this might not represent a *bona fide* complex V subunit (Fig. S8; Table S10).

#### (4) Mitochondrial secretion system

Recently, a surprising new addition to the set of mitochondrial protein complexes was found. Multiple core homologues of a bacterial secretion system (type 2; T2SS) used to secrete proteins across the outer membrane of certain Gram-negative bacteria are detected in the genomes of heteroloboseans, jakobids, malawimonads and hemimastigotes, while being absent from model opisthokonts (Horváthová *et al.*, 2021). Some of these proteins are present in mitochondria, and from their experimental behaviour infer the presence of a T2SS-derived system in mitochondria (miT2SS). The phylogenetic evidence and distribution patterns of key T2SS subunits across eukaryotes strongly indicate that a miT2SS pathway was present in the LECA (Horváthová *et al.*, 2021). Direct experimental evidence is lacking but phylogenetic profiling identifies over 20 protein families (seven containing family members linked by, for instance, sequence features or functional data), with indications of peroxisomes as secretion targets. This adds to the ever-increasing list of peroxisome–mitochondria connections (Schrader & Yoon, 2007). More important for our present discussion is that the preliminary genomic patterns of retention, loss, replacement and addition of putative miT2SS subunits mirrors the patterns we found for the other eukaryotic complexes. Details can be found in Horváthová *et al.* (2021).

## V. THE DEEP: PATTERNS OF VARIATION ACROSS EUKARYOTES

Extensive *in silico* analysis of selected complexes was undertaken, specifically including taxonomically relevant organisms (Fig. 3A) with high-quality genome assemblies to broaden understanding of complex evolution (Table S11; Fig. S9; Appendix S1) and revealed that the LECA genome encoded somewhere from four (for MICOS) to several dozen (for complexes I and IV) subunits for a respective protein complex (Fig. 3B). Moreover, for almost every lineage of extant eukaryotes for which experimental data exist, multiple species- or lineage-specific subunits were identified (Tables S2–S10; Figs S1–S8). The number of species/lineage-specific subunits is comparable to, or even exceeds, the



(Figure 3 legend continues on next page.)

number of ancestral subunits predicted in the LECA, including the MICOS complex in *T. brucei* and respiratory complexes I and IV in alveolates and euglenozoans.

In some cases, species/lineage-specific proteins form a completely novel functionally analogous complex replacing the ancestral eukaryotic machinery, with the kinetoplastid kinetochore (Fig. 3C) and plant and kinetoplastid lamins being prominent examples, whilst elsewhere species/lineage-specific subunits form a divergent shell around conserved cores of ancestral subunits, for example, in TOM and most respiratory complexes. Functions of these supernumerary subunits include assembly and stabilisation, replacing the functions of lost ancestral subunits and facilitating the formation of supercomplexes (Huynen & Elurbe, 2022; Maclean *et al.*, 2022).

Although complexification is a prevalent trend for the experimentally characterised set of complexes discussed herein, secondary simplification as the result of the loss of ancestral subunits is also observed (e.g. the loss of presumably ancestral  $\gamma$ -carbonic anhydrase, and SDH5 and SDH6 subunits in case of the respiratory complexes I and II respectively in opisthokonts). Such divergent and relatively well-studied organisms as *T. brucei* are valuable indicators of complex diversity, as experimental data are only very rarely available for species outside model opisthokonts and plants, which collectively represent only two eukaryotic supergroups. Combining experimental and *in silico* data (Fig. 3C) indicates that *T. brucei* shares somewhere between zero and 70% of complex subunits with opisthokonts. Extreme divergence of protein complexes is manifested not only in highly variable subunit composition, but also in low sequence similarity among the respective subunits (Fig. 3B).

## VI. DISCUSSION

Multiple reconstructions demonstrate that the LECA was a highly complex organism and in possession of a larger intracellular compartment cohort than many extant organisms (Koumandou *et al.*, 2013; O'Malley *et al.*, 2016; Wideman & Muñoz-Gómez, 2016). Such a high level of complexity is further demonstrated by the combined experimental and *in silico*

evidence discussed here. Many modes for evolution of gene families and protein complexes have been proposed, including duplication, churning, backfilling and sculpting (Fig. 1; Elias *et al.*, 2012). While sculpting is simply carving away of genes encoding protein subunits, churning is exemplified by rapid birth and death of new paralogues. Backfilling is a two-step process by which a subunit gene is lost, but retention of functional integrity, and even functional diversification, is facilitated through expansion of other genes within the pathway or complex (Gray *et al.*, 2010; Padilla-Meija *et al.*, 2021). We initially defined backfilling by considering paralogue expansion only, as exemplified by the HOPS/CORVET and Rab32 examples here, but we found broader cases, where non-paralogous genes can act, as in the case of TOM and MICOS complexes. Further, each mechanism can operate in combination, such that sculpting followed by backfilling is in essence a special example of churning. While all of these events seem widely attested to shape evolution of eukaryotic protein complexes, we suggest that gene loss is a major initiator, as loss can generate space for a new gene (Dacks & Field, 2018). Importantly, the processes described provide an opportunity for selection to operate, but the initial loss itself should be considered as essentially neutral.

We provide examples in support of these multiple scenarios from simple loss to replacement of entire structures by apparently unrelated proteins. The presence of large paralogous families populating much of the endomembrane system provides a flexible means by which cells can evolve, as new paralogues can be accommodated within existing machinery. However, even within the less closely related gene cohorts of mitochondrial complexes, the presence of multiple paralogues is noteworthy. Furthermore, the ability of these paralogous families to recruit and adapt a shell of less-retained proteins to augment function is broadly similar for many of the complexes considered here. *In silico* evidence highlights the patchy retention of many subunits surrounding a conserved core, emphasising the frequency and hence importance of such events.

Overall, this suggests a potent role for CNE. While gene loss is in itself a comparatively minor evolutionary event and facilitated by an environment where the lost gene product presumably has little or no impact on fitness, additional factors including population size and the stability or dynamic

(Figure legend continued from previous page.)

**Fig. 3.** Relative conservation of selected protein complexes across eukaryotes. (A) A cladogram depicting the phylogenetic relationships among major eukaryotic supergroups. Taxa for which a comparative analysis was conducted in this study are marked in yellow. Based on Tice *et al.* (2021). CRuMs, clade consisting of Collodictyonidae, Rigifilida and *Mantamonas*. (B) Retention of individual subunits among eukaryotic supergroups is indicated by the number of lineage-specific and shared subunits in UpSetR plots (Conway *et al.*, 2017). A subunit was considered present in a supergroup when it was identified in at least one species from this group. Retention patterns for the subunits shared by two or more supergroups, and thus likely encoded in the genome of the last eukaryotic common ancestor (LECA), are shown in blue; lineage-specific subunits are shown in yellow. See Tables S2–S10 for the species where a particular complex was experimentally characterised. Pairwise amino acid identities were calculated using the *EMBOSS* module in Biopython with the Needleman–Wunsch algorithm (Cock *et al.*, 2009). Histograms depict distributions of pairwise per cent identities for shared subunits. (C) Distribution of *Trypanosoma brucei* (Euglenozoa) protein complexes with respect to the relative number of subunits shared with *Saccharomyces cerevisiae* and/or *Homo sapiens* (Opisthokonta). Positions of individual complexes on this scale are marked: Kch, kinetochore; MIC, mitochondrial contact site and cristae organising system (MICOS); CI–V, respiratory complex I–V. The total number of complex subunits in *T. brucei* is set at 100%.

nature of the environment are of importance for fixing a new allele, or the irreversible loss of a gene. We suggest that this combination of factors provides a mechanism for the evolution of novelty. Thus, paradoxically, gene loss is a major evolutionary force driving divergence of the highly elaborate eukaryotic protein complexes.

## VII. CONCLUSIONS

- (1) We present multiple scenarios for evolution of protein complexes, with most incorporating a gene loss event.
- (2) In many complexes a core of subunits coordinates an interacting shell of lineage-specific components.
- (3) There are examples of entire complexes, considered to be indispensable, being replaced by functional analogues.
- (4) We propose that gene loss is a major evolutionary force for divergence by providing opportunity for novel subunit recruitment.

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## IX. REFERENCES

References identified with an asterisk (\*) are cited only within the Supporting Information.

- \*ACESTOR, N., ZÍKOVÁ, A., DALLEY, R. A., ANUPAMA, A., PANIGRAHI, A. K. & STUART, K. D. (2011). *Trypanosoma brucei* mitochondrial respiratome: composition and organization in procyclic form. *Molecular & Cellular Proteomics* **10**, M110.006908.
- ADUNG'A, V. O., GADELHA, C. & FIELD, M. C. (2013). Proteomic analysis of clathrin interactions in trypanosomes reveals dynamic evolution of endocytosis. *Traffic* **14**, 440–457.
- AKIYOSHI, B. & GULL, K. (2014). Discovery of unconventional kinetochores in kinetoplastids. *Cell* **156**, 1247–1258.
- APHASIZHEVA, I., ALFONZO, J., CARNES, J., CESTARI, I., CRUZ-REYES, J., GÖRINGER, H. U., HAJDUK, S., LUKEŠ, J., MADISON-ANTENUCCI, S., MASLOV, D. A., McDERMOTT, S. M., OCHSENREITER, T., READ, L. K., SALAVATI, R., SCHNAUFER, A., *ET AL.* (2020). Lexis and grammar of mitochondrial RNA processing in trypanosomes. *Trends in Parasitology* **36**, 337–355.
- \*ATTEIA, A., ADRAIT, A., BRUGIÈRE, S., TARDIF, M., VAN LIS, R., DEUSCH, O., DAGAN, T., KUHN, L., GONTERO, B., MARTIN, W., GARIN, J., JOYARD, J. & ROLLAND, N. (2009). A proteomic survey of *Chlamydomonas reinhardtii* mitochondria sheds new light on the metabolic plasticity of the organelle and on the nature of the alpha-proteobacterial mitochondrial ancestor. *Molecular Biology and Evolution* **26**, 1533–1548.
- BACKES, S., HESS, S., BOOS, F., WOELLHAF, M. W., GÖDEL, S., JUNG, M., MÜHLHAUS, T. & HERRMANN, J. M. (2018). Tom70 enhances mitochondrial

- preprotein import efficiency by binding to internal targeting sequences. *Journal of Cell Biology* **217**, 1369–1382.
- BALABASKARAN NINA, P., DUDKINA, N. V., KANE, L. A., VAN EYK, J. E., BOEKEMA, E. J., MATHER, M. W. & VAIDYA, A. B. (2010). Highly divergent mitochondrial ATP synthase complexes in *Tetrahymena thermophila*. *PLoS Biology* **8**, e1000418.
- BALDERHAAR, H. J. K. & UNGERMANN, C. (2013). CORVET and HOPS tethering complexes – coordinators of endosome and lysosome fusion. *Journal of Cell Science* **126**, 1307–1316.
- \*BALSA, E., MARCO, R., PERALES-CLEMENTE, E., SZKLARCZYK, R., CALVO, E., LANDÁZURI, M. O. & ENRÍQUEZ, J. A. (2012). NDUFA4 is a subunit of complex IV of the mammalian electron transport chain. *Cell Metabolism* **16**, 378–386.
- BARBOT, M., JANS, D. C., SCHULZ, C., DENKERT, N., KROPPEN, B., HOPPERT, M., JAKOBS, S. & MEINECKE, M. (2015). Mic10 oligomerizes to bend mitochondrial inner membranes at cristae junctions. *Cell Metabolism* **21**, 756–763.
- \*BARLOW, L. D., Maciejowski, W., More, K., Terry, K., Vargová, R., Záhonová, K. & Dacks, J. B. (2023). Comparative genomics for evolutionary cell biology using AMOEBAE: Understanding the Golgi and beyond. *Methods in Molecular Biology* **2557**, 431–452.
- BAUSEWEIN, T., NAVEED, H., LIANG, J. & NUSSBERGER, S. (2020). The structure of the TOM core complex in the mitochondrial outer membrane. *Biological Chemistry* **401**, 687–697.
- BERRY, E. A., GUERGOVA-KURAS, M., HUANG, L. S. & CROFTS, A. R. (2000). Structure and function of cytochrome *bc* complexes. *Annual Review of Biochemistry* **69**, 1005–1075.
- \*BIGGINS, S. (2013). The composition, functions, and regulation of the budding yeast kinetochore. *Genetics* **194**, 817–846.
- BILLINGTON, K., HALLIDAY, C., MADDEN, R., DYER, P., BARKER, A. R., MOREIRA-LEITE, F. F., CARRINGTON, M., VAUGHAN, S., HERTZ-FOWLER, C., DEAN, S., SUNTER, J. D., WHEELER, R. J. & GULL, K. (2023). Genome-wide subcellular protein map for the flagellate parasite *Trypanosoma brucei*. *Nature Microbiology* **8**, 533–547.
- BOEHM, M. & BONIFACINO, J. S. (2001). Adaptins: the final recount. *Molecular Biology of the Cell* **12**, 2907–2920.
- BOEHM, C. & FIELD, M. C. (2019). Evolution of late steps in exocytosis: conservation and specialization of the exocyst complex. *Wellcome Open Research* **4**, 112.
- BOEHM, C. M., OBADO, S., GADELHA, C., KAUPISCH, A., MANNA, P. T., GOULD, G. W., MUNSON, M., CHAIT, B. T., ROUT, M. P. & FIELD, M. C. (2017). The trypanosome exocyst: a conserved structure revealing a new role in endocytosis. *PLoS Pathogens* **13**, e1006063.
- BOUHLEL, I. B., OHTA, M., MAYEUX, A., BORDES, N., DINGLI, F., BOULANGER, J., VELVE CASQUILLAS, G., LOEW, D., TRAN, P. T., SATO, M. & PAOLETTI, A. (2015). Cell cycle control of spindle pole body duplication and splitting by Sfi1 and Cdc31 in fission yeast. *Journal of Cell Science* **128**, 1481–1493.
- BRANDT, U. (2006). Energy converting NADH:quinone oxidoreductase (complex I). *Annual Review of Biochemistry* **75**, 69–92.
- \*BRUSINI, L., D'ARCHIVIO, S., McDONALD, J. & WICKSTEAD, B. (2021). Trypanosome KKIPI1 dynamically links the inner kinetochore to a kinetoplastid outer kinetochore complex. *Frontiers in Cellular and Infection Microbiology* **23**, 641174.
- BUTENKO, A., HAMMOND, M., FIELD, M. C., GINGER, M. L., YURCHENKO, V. & LUKEŠ, J. (2021). Reductionist pathways for parasitism in euglenozoans? Expanded datasets provide new insights. *Trends in Parasitology* **37**, 100–116.
- BUTENKO, A., OPPERDOES, F. R., FLEGONTOVA, O., HORÁK, A., HAMPL, V., KEELING, P., GAWRYLUK, R. M. R., TIKHONENKOV, D., FLEGONTOV, P. & LUKEŠ, J. (2020). Evolution of metabolic capabilities and molecular features of diplomonads, kinetoplastids, and euglenids. *BMC Biology* **18**, 23.
- \*CAMACHO, C., COULOURIS, G., AVAGYAN, V., MA, N., PAPADOPOULOS, J., BEALER, K. & MADDEN, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421.
- CARDOL, P. (2011). Mitochondrial NADH:ubiquinone oxidoreductase (complex I) in eukaryotes: a highly conserved subunit composition highlighted by mining of protein databases. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1807**, 1390–1397.
- \*CARDOL, P., FIGUEROA, F., REMACLE, C., FRANZÉN, L.-G. & GONZÁLEZ-HALPHEN, D. (2009). Oxidative phosphorylation: building blocks and related components. In *The Chlamydomonas Sourcebook*, Second Edition (eds E. H. HARRIS, D. B. STERN and G. B. WITMAN), pp. 469–502. Academic Press, Oxford, UK.
- \*CARDOL, P., VANROBAEYS, F., DEVREESE, B., VAN BEUMEN, J., MATAGNE, R. F. & REMACLE, C. (2004). Higher plant-like subunit composition of mitochondrial complex I from *Chlamydomonas reinhardtii*: 31 conserved components among eukaryotes. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1658**, 212–224.
- 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., SICHERITZ-PONTEN, T., NOEL, C. J., DACKS, J. B., FOSTER, P. G., SIMILLION, C., *ET AL.* (2007). Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. *Science* **315**, 207–212.
- \*CHACINSKA, A., KOEHLER, C. M., MILENKOVIC, D., LITHGOW, T. & PFANNER, N. (2009). Importing mitochondrial proteins: machineries and mechanisms. *Cell* **138**, 628–644.

- CISKA, M., DIAZ, M. & DE LA ESPINA, S. (2013). NMCP/LINC proteins: putative Lamin analogs in plants? *Plant Signaling & Behavior* **8**, e26669.
- COCK, P. J., ANTAO, T., CHANG, J. T., CHAPMAN, B. A., COX, C. J., DALKE, A., FRIEDBERG, I., HAMELRYCK, T., KAUFF, F., WILCZYNSKI, B. & DE HOON, M. J. L. (2009). Biopython: freely available python tools for computational molecular biology and bioinformatics. *Bioinformatics* **25**, 1422–1423.
- CONWAY, J. R., LEX, A. & GEHLENBORG, N. (2017). UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* **18**, 2938–2940.
- DACKS, J. B. & FIELD, M. C. (2007). Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. *Journal of Cell Science* **120**, 2977–2985.
- DACKS, J. B. & FIELD, M. C. (2018). Evolutionary origins and specialisations of membrane transport. *Current Opinion in Cell Biology* **53**, 70–76.
- DACKS, J. B. & ROBINSON, M. S. (2017). Outerwear through the ages: evolutionary cell biology of vesicle coats. *Current Opinion in Cell Biology* **47**, 108–116.
- D'ARCHIVIO, S. & WICKSTEAD, B. (2017). Trypanosome outer kinetochore proteins suggest conservation of chromosome segregation machinery across eukaryotes. *The Journal of Cell Biology* **216**, 379–391.
- \*DAS, J., MILLER, S. T. & STERN, D. L. (2004). Comparison of diverse protein sequences of the nuclear-encoded subunits of cytochrome *C* oxidase suggests conservation of structure underlies evolving functional sites. *Molecular Biology and Evolution* **21**, 1572–1582.
- DE VRIES, S. & MARRES, C. A. M. (1987). The mitochondrial respiratory chain of yeast. Structure and biosynthesis and the role in cellular metabolism. *Biochimica et Biophysica Acta (BBA) – Reviews on Bioenergetics* **895**, 205–239.
- DEVOS, D. P., GRÄF, R. & FIELD, M. C. (2014). Evolution of the nucleus. *Current Opinion in Cell Biology* **28**, 8–15.
- DIEKMANN, Y., SEIXAS, E., GOUW, M., TAVARES-CADETE, F., SEABRA, M. C. & PEREIRA-LEAL, J. B. (2011). Thousands of Rab GTPases for the cell biologist. *PLoS Computational Biology* **7**, e1002217.
- DING, D., NGUYEN, T. T., PANG, M. Y. H. & ISHIBASHI, T. (2021). Primate-specific histone variants. *Genome* **64**, 337–346.
- DUARTE, M. & TOMÁS, A. M. (2014). The mitochondrial complex I of trypanosomatids – an overview of current knowledge. *Journal of Bioenergetics and Biomembranes* **46**, 299–311.
- DUBOIS, K. N., ALSFORD, S., HOLDEN, J. M., BUISSON, J., SWIDERSKI, M., BART, J. M., RATUSHNY, A. V., WAN, Y., BASTIN, P., BARRY, J. D., NAVARRO, M., HORN, D., AITCHISON, J. D., ROUT, M. P. & FIELD, M. C. (2012). NUP-1 is a large coiled-coil nucleoskeletal protein in trypanosomes with Lamin-like functions. *PLoS Biology* **10**, e1001287.
- EBENEZER, T. E., ZOLTNER, M., BURRELL, A., NENAROKOVA, A., NOVÁK VANCLOVÁ, A., PRASAD, B., SOUKAL, P., SANTANA-MOLINA, C., O'NEILL, E., NANKISSOR, N. N., VADAKEDATH, N., DAIKER, V., OBADO, S., SILVA-PEREIRA, S., JACKSON, A. P., ET AL. (2019). Transcriptome, proteome and draft genome of *Englena gracilis*. *BMC Biology* **17**, 11.
- EICHENBERGER, C., OELJELKAUS, S., BRUGGESSER, J., MANI, J., HAENNI, B., KAUROV, I., NIEMANN, M., ZUBER, B., LUKEŠ, J., HASHIMI, H., WARSCHIED, B., SCHIMANSKI, B. & SCHNEIDER, A. (2019). The highly diverged trypanosomal MICOS complex is organized in a nonessential integral membrane and an essential peripheral module. *Molecular Microbiology* **112**, 1731–1743.
- ELIAS, M., BRIGHOUSE, A., GABERNET-CASTELLO, C., FIELD, M. C. & DACKS, J. B. (2012). Sculpting the endomembrane system in deep time: high resolution phylogenetics of Rab GTPases. *Journal of Cell Science* **125**, 2500–2508.
- ELURBE, D. M. & HUYNEN, M. A. (2016). The origin of the supernumerary subunits and assembly factors of complex I: a treasure trove of pathway evolution. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1857**, 971–979.
- EME, L., SHARPE, S. C., BROWN, M. W. & ROGER, A. J. (2014). On the age of eukaryotes: evaluating evidence from fossils and molecular clocks. *Cold Spring Harbor Perspectives in Biology* **6**, a016139.
- \*EUBEL, H., JÄNSCH, L. & BRAUN, H. P. (2003). New insights into the respiratory chain of plant mitochondria. Supercomplexes and a unique composition of complex II. *Plant Physiology* **133**, 274–286.
- EVERS, F., CABRERA-OREFICE, A., ELURBE, D. M., KEA-TE LINDERT, M., BOLTRYK, S. D., VOSS, T. S., HUYNEN, M. A., BRANDT, U. & KOIJ, T. W. A. (2021). Composition and stage dynamics of mitochondrial complexes in *Plasmodium falciparum*. *Nature Communications* **12**, 3820.
- \*FERNÁNDEZ-VIZARRA, E. & ZEVIANI, M. (2015). Nuclear gene mutations as the cause of mitochondrial complex III deficiency. *Frontiers in Genetics* **6**, 134.
- \*FETTERMAN, J. L., LIU, C., MITCHELL, G. F., VASAN, R. S., BENJAMIN, E. J., VITA, J. A., HAMBURG, N. M. & LEVY, D. (2018). Relations of mitochondrial genetic variants to measures of vascular function. *Mitochondrion* **40**, 51–57.
- FIELD, M. C., GABERNET-CASTELLO, C. & DACKS, J. B. (2007). Reconstructing the evolution of the endocytic system: insights from genomics and molecular cell biology. *Advances in Experimental Medicine and Biology* **607**, 84–96.
- FIELD, M. C. & ROUT, M. P. (2019). Pore timing: the evolutionary origins of the nucleus and nuclear pore complex. *F1000Research* **8**, F1000 Faculty Rev-369.
- FIELD, M. C. (2019). The kinetochore and the origin of eukaryotic chromosome segregation. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 12596–12598.
- \*FLYGAARD, R. K., MÜHLEIP, A., TOBIASSON, V. & AMUNTS, A. (2020). Type III ATP synthase is a symmetry-deviated dimer that induces membrane curvature through tetramerization. *Nature Communications* **11**, 5342.
- \*FUKASAWA, Y., ODA, T., TOMII, K. & IMAI, K. (2017). Origin and evolutionary alteration of the mitochondrial import system in eukaryotic lineages. *Molecular Biology and Evolution* **34**, 1574–1586.
- \*GABALDÓN, T., RAINEY, D. & HUYNEN, M. A. (2005). Tracing the evolution of a large protein complex in the eukaryotes, NADH:ubiquinone oxidoreductase (complex I). *Journal of Molecular Biology* **348**, 857–870.
- GABERNET-CASTELLO, C., DACKS, J. B. & FIELD, M. C. (2009). The single ENTH-domain protein of trypanosomes; endocytic functions and evolutionary relationship with epsin. *Traffic* **10**, 894–911.
- \*GAHURA, O., MÜHLEIP, A., HIERRO-YAP, C., PANICUCCI, B., JAIN, M., HOLLAUS, D., SLAPNÍČKOVÁ, M., ZÍKOVÁ, A. & AMUNTS, A. (2022). An ancestral interaction module promotes oligomerization in divergent mitochondrial ATP synthases. *Nature Communications* **13**, 5989.
- GAHURA, O., ŠUBRTOVÁ, K., VÁCHOVÁ, H., PANICUCCI, B., FEARNLEY, I. M., HARBOUR, M. E., WALKER, J. E. & ZÍKOVÁ, A. (2018). The F1-ATPase from *Trypanosoma brucei* is elaborated by three copies of an additional p18-subunit. *The FEBS Journal* **285**, 614–628.
- GARDNER, M. J., HALL, N., FUNG, E., WHITE, O., BERRIMAN, M., HYMAN, R. W., CARLTON, J. M., PAIN, A., NELSON, K. E., BOWMAN, S., PAULSEN, I. T., JAMES, K., EISEN, J. A., RUTHERFORD, K., SALZBERG, S. L., ET AL. (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511.
- \*GAWRYLUK, R. M., CHISHOLM, K. A., PINTO, D. M. & GRAY, M. W. (2012). Composition of the mitochondrial electron transport chain in *Acanthamoeba castellanii*: structural and evolutionary insights. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **11**, 2027–2037.
- \*GAWRYLUK, R. M., CHISHOLM, K. A., PINTO, D. M. & GRAY, M. W. (2014). Compositional complexity of the mitochondrial proteome of a unicellular eukaryote (*Acanthamoeba castellanii*, supergroup Amoebozoa) rivals that of animals, fungi, and plants. *Journal of Proteomics* **109**, 400–416.
- GAWRYLUK, R. M. & GRAY, M. W. (2010). Evidence for an early evolutionary emergence of  $\beta$ -type carbonic anhydrases as components of mitochondrial respiratory complex I. *BMC Evolutionary Biology* **10**, 176.
- \*GAWRYLUK, R. M. R. & STAIRS, C. W. (2021). Diversity of electron transport chains in anaerobic protists. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1862**, 148334.
- GEBERT, N., GEBERT, M., OELJELKAUS, S., VON DER MALSBERG, K., STROUD, D. A., KULAWIAK, B., WIRTH, C., ZAHEDI, R. P., DOLEZAL, P., WIESE, S., SIMON, O., SCHULZE-SPECKING, A., TRUSCOTT, K. N., SICKMANN, A., REHLING, P., ET AL. (2011). Dual function of Sdh3 in the respiratory chain and TIM22 protein translocase of the mitochondrial inner membrane. *Molecular Cell* **44**, 811–818.
- \*GENTLE, I. E., PERRY, A. J., ALCOCK, F. H., LIKIĆ, V. A., DOLEZAL, P., NG, E. T., PURCELL, A. W., MCCONNVILLE, M., NADERER, T., CHANEZ, A. L., CHARRIÈRE, F., ASCHINGER, C., SCHNEIDER, A., TOKATLIDIS, K. & LITHGOW, T. (2007). Conserved motifs reveal details of ancestry and structure in the small TIM chaperones of the mitochondrial intermembrane space. *Molecular Biology and Evolution* **24**, 1149–1160.
- GORNIK, S. G., FORD, K. L., MULHERN, T. D., BACIC, A., MCFADDEN, G. I. & WALLER, R. F. (2012). Loss of nucleosomal DNA condensation coincides with appearance of a novel nuclear protein in dinoflagellates. *Current Biology* **22**, 2303–2312.
- GORNIK, S. G., HU, I., LASSADI, I. & WALLER, R. F. (2019). The biochemistry and evolution of the dinoflagellate nucleus. *Microorganisms* **7**, 245.
- GRAY, M. W. (2012). Mitochondrial evolution. *Cold Spring Harbor Perspectives in Biology* **4**, a011403.
- GRAY, M. W., BURGER, G., DERELLE, R., KLIMEŠ, V., LEGER, M. M., SARRASIN, M., VLČEK, Č., ROGER, A. J., ELIŠ, M. & LANG, B. F. (2020). The draft genome sequence and predicted mitochondrial proteome of *Andalucia godoyi*, a protist with the most gene-rich and bacteria-like mitochondrial genome. *BMC Biology* **18**, 22.
- GRAY, M. W., LUKEŠ, J., ARCHIBALD, J. M., KEELING, P. J. & DOOLITTLE, W. F. (2010). Cell biology. Irremediable complexity? *Science* **330**, 920–921.
- GROVES, N. R., BIEL, A., MOSER, M., MENDES, T., AMSTUTZ, K. & MEIER, I. (2020). Recent advances in understanding the biological roles of the plant nuclear envelope. *Nucleus* **11**, 330–346.
- GRUENBAUM, Y., GOLDMAN, R. D., MEYUHAS, R., MILLS, E., MARGALIT, A., FRIDKIN, A., DAYANI, Y., PROKOCIMER, M. & ENOSH, A. (2003). The nuclear lamina and its functions in the nucleus. *International Review of Cytology* **226**, 1–62.
- HAMMOND, M. J., NENAROKOVA, A., BUTENKO, A., ZOLTNER, M., DOBÁKOVÁ, E. L., FIELD, M. C. & LUKEŠ, J. (2020). A uniquely complex mitochondrial proteome from *Englena gracilis*. *Molecular Biology and Evolution* **37**, 2173–2191.
- \*HARSMAN, A. & SCHNEIDER, A. (2017). Mitochondrial protein import in trypanosomes: expect the unexpected. *Traffic* **18**, 96–109.

- \*HARTLEY, A. M., LUKOYANOVA, N., ZHANG, Y., CABRERA-OREFICE, A., ARNOLD, S., MEUNIER, B., PINOTIS, N. & MARÉCHAL, A. (2019). Structure of yeast cytochrome *c* oxidase in a supercomplex with cytochrome *bc1*. *Nature Structural & Molecular Biology* **26**, 78–83.
- \*HE, J., FORD, H. C., CARROLL, J., DOUGLAS, C., GONZALES, E., DING, S., FEARNLEY, I. M. & WALKER, J. E. (2018). Assembly of the membrane domain of ATP synthase in human mitochondria. *Proceedings of the National Academy of Sciences of the United States of America* **115**, 2988–2993.
- \*HEAZLEWOOD, J. L., HOWELL, K. A. & MILLAR, A. H. (2003). Mitochondrial complex I from *Arabidopsis* and rice: orthologs of mammalian and fungal components coupled with plant-specific subunits. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1604**, 159–169.
- \*HERMAN, E. K., GRENINGER, A., VAN DER GIEZEN, M., GINGER, M. L., RAMIREZ-MACIAS, I., MILLER, H. C., MORGAN, M. J., TSAOUSIS, A. D., VELLE, K., VARGOVÁ, R., ZÁHONOVÁ, K., NAJLE, S. R., MACINTYRE, G., MULLER, N., WITTEW, M., *ET AL.* (2021). Genomics and transcriptomics yields a system-level view of the biology of the pathogen *Naegleria fowleri*. *BMC Biology* **19**, 142.
- HIRST, J., LINDSAY, M. R. & ROBINSON, M. S. (2001). GGAs: roles of the different domains and comparison with AP-1 and clathrin. *Molecular Biology of the Cell* **12**, 3573–3588.
- HIRST, J., SCHLACHT, A., NORCOTT, J. P., TRAYNOR, D., BLOOMFIELD, G., ANTROBUS, R., KAY, R. R., DACKS, J. B. & ROBINSON, M. S. (2014). Characterization of TSET, an ancient and widespread membrane trafficking complex. *eLife* **3**, e02866.
- HOLDEN, J. M., KORENY, L., OBADO, S., RATUSHNY, A. V., CHEN, W. M., CHIANG, J. H., KELLY, S., CHAIT, B. T., AITCHISON, J. D., ROUT, M. P. & FIELD, M. C. (2014). Nuclear pore complex evolution: a trypanosome Mlp analogue functions in chromosomal segregation but lacks transcriptional barrier activity. *Molecular Biology of the Cell* **25**, 1421–1436.
- HORVÁTHOVÁ, L., ŽÁRSKÝ, V., PÁNEK, T., DERELLE, R., PYRIH, J., MOTYČKOVÁ, A., KLÁPŠTOVÁ, V., VINOPALOVÁ, M., MARKOVÁ, L., VOLEMAN, L., KLIMĚŠ, V., PETRŮ, M., VAITOVÁ, Z., ČEPIČKA, I., HRYZÁKOVÁ, K., *ET AL.* (2021). Analysis of diverse eukaryotes suggests the existence of an ancestral mitochondrial apparatus derived from the bacterial type II secretion system. *Nature Communications* **12**, 2947.
- \*HU, B., WANG, N., BI, X., KARASLAN, E. S., WEBER, A.-L., ZHU, W., BERENDZEN, K. W. & LIU, C. (2019). Plant lamin-like proteins mediate chromatin tethering at the nuclear periphery. *Genome Biology* **20**, 87.
- HUANG, S. & MILLAR, A. H. (2013). Succinate dehydrogenase: the complex roles of a simple enzyme. *Current Opinion in Plant Biology* **16**, 344–349.
- \*HUANG, R., DING, J., GAO, K., CRUZ DE CARVALHO, M. H., TIRICHINE, L., BOWLER, C. & LIN, X. (2019a). A potential role for epigenetic processes in the acclimation response to elevated pCO<sub>2</sub> in the model diatom *Phaeodactylum tricornutum*. *Frontiers in Microbiology* **14**, 3342.
- HUANG, S., BRAUN, H. P., GAWRYLUK, R. M. R. & MILLAR, A. H. (2019b). Mitochondrial complex II of plants: subunit composition, assembly, and function in respiration and signaling. *The Plant Journal* **98**, 405–417.
- HUYNEN, M. A. & ELURBE, D. M. (2022). Mitochondrial complex complexification. *Science* **376**, 794–795.
- HUYNEN, M. A., MÜHLMEISTER, M., GOTTHARDT, K., GUERRERO-CASTILLO, S. & BRANDT, U. (2016). Evolution and structural organization of the mitochondrial contact site (MICOS) complex and the mitochondrial intermembrane space bridging (MIB) complex. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research* **1863**, 91–101.
- ITO, D. & BETTENCOURT-DIAS, M. (2018). Centrosome remodelling in evolution. *Cell* **7**, 71.
- \*IWAMOTO, M., HIRAOKA, Y. & HARAGUCHI, T. (2016). Uniquely designed nuclear structures of lower eukaryotes. *Current Opinion in Cell Biology* **40**, 66–73.
- JANOŠKOVIC, J., GAVELIS, G. S., BURKI, F., DINH, D., BACHVAROFF, T. R., GORNIK, S. G., BRIGHT, K. J., IMANIAN, B., STROM, S. L., DELWICHE, C. F., WALLER, R. F., FENSOME, R. A., LEANDER, B. S., ROHWER, F. L. & SALLDARRIAGA, J. F. (2017a). Major transitions in dinoflagellate evolution unveiled by phylotranscriptomics. *Proceedings of the National Academy of Sciences of the United States of America* **114**, E171–E180.
- \*JANOŠKOVIC, J., TIKHONENKOV, D. V., BURKI, F., HOWE, A. T., ROHWER, F. L., MYLNIKOV, A. P. & KEELING, P. J. (2017b). A new lineage of eukaryotes illuminates early mitochondrial genome reduction. *Current Biology* **27**, 3717–3724.e5.
- JEFFRIES, T. R., MORGAN, G. W. & FIELD, M. C. (2001). A developmentally regulated rab11 homologue in *Trypanosoma brucei* is involved in recycling processes. *Journal of Cell Science* **114**, 2617–2626.
- JENSEN, R. E. & ENGLUND, P. T. (2012). Network news: the replication of kinetoplast DNA. *Annual Review of Microbiology* **66**, 473–491.
- KALB, L. C., FREDERICO, Y. C. A., BOEHM, C., MOREIRA, C. M. D. N., SOARES, M. J. & FIELD, M. C. (2016). Conservation and divergence within the clathrin interactome of *Trypanosoma cruzi*. *Scientific Reports* **6**, 31212.
- KATZ, L. A. (2012). Origin and diversification of eukaryotes. *Annual Review of Microbiology* **66**, 411–427.
- KAUROV, I., HELLER, J., DEISENHAMMER, S., POTĚŠIL, D., ZDRÁHAL, Z. & HASHIMI, H. (2022). The essential cysteines in the CIPC motif of the thioredoxin-like *Trypanosoma brucei* MICOS subunit TbMic20 do not form an intramolecular disulfide bridge in vivo. *Molecular and Biochemical Parasitology* **248**, 111463.
- KAUROV, I., VANCOVÁ, M., SCHIMANSKI, B., CADENA, L. R., HELLER, J., BÍLÝ, T., POTĚŠIL, D., EICHENBERGER, C., BRUCE, H., OELJEKLAUS, S., WARSCHIED, B., ZDRÁHAL, Z., SCHNEIDER, A., LUKEŠ, J. & HASHIMI, H. (2018). The diverged trypanosome MICOS complex as a hub for mitochondrial cristae shaping and protein import. *Current Biology* **28**, 3393–3407.e5.
- KJOS, I., VESTRE, K., GUADAGNO, N. A., BORG DISTEFANO, M. & PROGIDA, C. (2018). Rab and Arf proteins at the crossroad between membrane transport and cytoskeleton dynamics. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research* **1865**, 1397–1409.
- KLINGER, C. M., KLUTE, M. J. & DACKS, J. B. (2013). Comparative genomic analysis of multi-subunit tethering complexes demonstrates an ancient pan-eukaryotic complement and sculpting in Apicomplexa. *PLoS One* **8**, e76278.
- KLÖPPER, T. H., KIENLE, N., FASSHAUER, D. & MUNRO, S. (2012). Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis. *BMC Biology* **10**, 71.
- \*KOLLMAR, M. (2015). Polyphyly of nuclear lamin genes indicates an early eukaryotic origin of the metazoan-type intermediate filament proteins. *Scientific Reports* **5**, 10652.
- \*KÖNIG, A. C., HARTL, M., BOERSEMA, P. J., MANN, M. & FINKEMEIER, I. (2014). The mitochondrial lysine acetylome of *Arabidopsis*. *Mitochondrion* **19**, 252–260.
- KORENY, L. & FIELD, M. C. (2016). Ancient eukaryotic origin and evolutionary plasticity of nuclear lamina. *Genome Biology and Evolution* **8**, 2663–2671.
- \*KÖSEM, S., ÖRTEN, Z., HO, T. H., TROMMLER, G., KOONCE, M. P., SAMEREIER, M. & MÜLLER-TAUBENBERGER, A. (2013). A non-mitotic CENP-E homolog in *Dictyostelium discoideum* with slow motor activity. *Biochemical and Biophysical Research Communications* **431**, 490–495.
- KOUMANDOU, V. L., WICKSTEAD, B., GINGER, M. L., VAN DER GIEZEN, M., DACKS, J. B. & FIELD, M. C. (2013). Molecular paleontology and complexity in the last eukaryotic common ancestor. *Critical Reviews in Biochemistry and Molecular Biology* **48**, 373–396.
- LARSON, R. T., DACKS, J. B. & BARLOW, L. D. (2019). Recent gene duplications dominate evolutionary dynamics of adaptor protein complex subunits in embryophytes. *Traffic* **20**, 961–973.
- LEE, L. J., KLUTE, M. J., HERMAN, E. K., READ, B. & DACKS, J. B. (2015). Losses, expansions, and novel subunit discovery of adaptor protein complexes in haptophyte algae. *Protist* **166**, 585–597.
- \*LERMONTOVA, I., SANDMANN, M. & DEMIDOV, D. (2014). Centromeres and kinetochores of Brassicaceae. *Chromosome Research* **22**, 135–152.
- \*LERMONTOVA, I., SANDMANN, M., MASCHER, M., SCHMITT, A. C. & CHABOUTÉ, M. E. (2015). Centromeric chromatin and its dynamics in plants. *The Plant Journal* **83**, 4–17.
- LEGER, M. M., KOLÍSKO, M., STAIRS, C. W. & SIMPSON, A. G. B. (2019). Mitochondrion-related organelles in free-living protists. In *Hydrogenosomes and Mitosomes: Mitochondria of Anaerobic Eukaryotes. Microbiology Monographs* (Volume 9, ed. J. TACHEZY), pp. 287–308. Springer, Cham.
- \*LI, L., NELSON, C. J., CARRIE, C., GAWRYLUK, R. M., SOLHEIM, C., GRAY, M. W., WHELAN, J. & MILLAR, A. H. (2013). Subcomplexes of ancestral respiratory complex I subunits rapidly turn over in vivo as productive assembly intermediates in *Arabidopsis*. *The Journal of Biological Chemistry* **288**, 5707–5717.
- \*LKIĆ, V. A., PERRY, A., HULETT, J., DERBY, M., TRAVEN, A., WALLER, R. F., KEELING, P. J., KOEHLER, C. M., CURRAN, S. P., GOOLEY, P. R. & LITHGOW, T. (2005). Patterns that define the four domains conserved in known and novel isoforms of the protein import receptor Tom20. *Journal of Molecular Biology* **347**, 81–93.
- \*LIU, R., VAISHNAV, R. A., ROBERTS, A. M. & FRIEDLAND, R. P. (2013). Humans have antibodies against a plant virus: evidence from tobacco mosaic virus. *PLoS One* **8**, e60621.
- LUKEŠ, J., ARCHIBALD, J. M., KEELING, P. J., DOOLITTLE, W. F. & GRAY, M. W. (2011). How a neutral evolutionary ratchet can build cellular complexity. *IUBMB Life* **63**, 528–537.
- \*MAČASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M. C., MULHERN, T. D. & LITHGOW, T. (2004). Tom22, an 8-kDa trans-site receptor in plants and protozoans, is a conserved feature of the TOM complex that appeared early in the evolution of eukaryotes. *Molecular Biology and Evolution* **21**, 1557–1564.
- MACLEAN, A. E., HAYWARD, J. A., HUET, D., VAN DOOREN, G. G. & SHEINER, L. (2022). The mystery of massive mitochondrial complexes: the apicomplexan respiratory chain. *Trends in Parasitology* **38**, 1041–1052.
- MAISHMAN, L., OBADO, S. O., ALSFORD, S., BART, J. M., CHEN, W. M., RATUSHNY, A. V., NAVARRO, M., HORN, D., AITCHISON, J. D., CHAIT, B. T., ROUT, M. P. & FIELD, M. C. (2016). Co-dependence between trypanosome nuclear lamina components in nuclear stability and control of gene expression. *Nucleic Acids Research* **44**, 10554–10570.



- MAKAROV, A. A., PADILLA-MEJIA, N. E. & FIELD, M. C. (2021). Evolution and diversification of the nuclear pore complex. *Biochemical Society Transactions* **49**, 1601–1619.
- \*MALDONADO, M., PADAVANNIL, A., ZHOU, L., GUO, F. & LETTIS, J. A. (2020). Atomic structure of a mitochondrial complex I intermediate from vascular plants. *eLife* **25**, e56664.
- MANI, J., MEISINGER, C. & SCHNEIDER, A. (2016). Peeping at TOMs-diverse entry gates to mitochondria provide insights into the evolution of eukaryotes. *Molecular Biology and Evolution* **33**, 337–351.
- MANNA, P., KELLY, S. & FIELD, M. C. (2013). Adaptin evolution in kinetoplastids and emergence of the variant surface glycoprotein coat in African trypanosomatids. *Molecular Phylogenetics and Evolution* **67**, 123–128.
- MANNA, P. T., GADELHA, C., PUTTICK, A. E. & FIELD, M. C. (2015). ENTH and ANTH domain proteins participate in AP2-independent clathrin-mediated endocytosis. *Journal of Cell Science* **128**, 2130–2142.
- MANNI, M., BERKELEY, M. R., SEPPEY, M., SIMÃO, F. A. & ZDOBNOV, E. M. (2021). BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Molecular Biology and Evolution* **38**, 4647–4654.
- MARINOV, G. K. & LYNCH, M. (2016). Diversity and divergence of dinoflagellate histone proteins. *G3 Genes | Genomes | Genetics* **6**, 397–422.
- MARTIN, W. F., GARG, S. & ZIMORSKI, V. (2015). Endosymbiotic theories for eukaryote origin. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **370**, 20140330.
- MARTIN, W. & MENDEL, M. (2010). The origin of mitochondria. *Nature Education* **3**, 58.
- \*MARUYAMA, S., KUROIWA, H., MIYAGISHIMA, S. Y., TANAKA, K. & KUROIWA, T. (2007). Centromere dynamics in the primitive red alga *Cyanidioschyzon merolae*. *The Plant Journal* **49**, 1122–1129.
- \*MAZUR, M., WOJCIECHOWSKA, D., SITKIEWICZ, E., MALINOWSKA, A., ŚWIDERSKA, B., KMITA, H. & WOJTKOWSKA, M. (2021). Mitochondrial processes during early development of *Dictyostelium discoideum*: from bioenergetic to proteomic studies. *Genes* **12**, 638.
- \*MAZUR, M., WOJTKOWSKA, M., SKALSKI, M., SŁOCIŃSKA, M. & KMITA, H. (2019). The TOB/SAM complex composition in mitochondria of *Dictyostelium discoideum* during progression from unicellularity to multicellularity. *Acta Biochimica Polonica* **66**, 551–557.
- MEI, K., LI, Y., WANG, S., SHAO, G., WANG, J., DING, Y., LUO, G., YUE, P., LIU, J. J., WANG, X., DONG, M. Q., WANG, H. W. & GUO, W. (2018). Cryo-EM structure of the exocyst complex. *Nature Structural & Molecular Biology* **25**, 139–146.
- \*MERALDI, P., MCAINSH, A. D., RHEINBAY, E. & SORGER, P. K. (2006). Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. *Genome Biology* **7**, R23.
- \*MEYER, E. H., TAYLOR, N. L. & MILLAR, A. H. (2008). Resolving and identifying protein components of plant mitochondrial respiratory complexes using three dimensions of gel electrophoresis. *The Journal of Proteome Research* **7**, 786–794.
- \*MICHAUD, M., GROS, V., TARDIF, M., BRUGIÈRE, S., FERRO, M., PRINZ, W. A., TOULMAY, A., MATHUR, J., WOZNY, M., FALCONET, D., MARÉCHAL, E., BLOCK, M. A. & JOUHET, J. (2016). AtMic60 is involved in plant mitochondrial lipid trafficking and is part of a large complex. *Current Biology* **26**, 627–639.
- \*MILLAR, A. H., EUBEL, H., JÄNSCH, L., KRUFFT, V., HEAZLEWOOD, J. L. & BRAUN, H. P. (2004). Mitochondrial cytochrome *c* oxidase and succinate dehydrogenase complexes contain plant specific subunits. *Plant Molecular Biology* **56**, 77–90.
- \*MISUMI, O., MATSUZAKI, M., NOZAKI, H., MIYAGISHIMA, S. Y., MORI, T., NISHIDA, K., YAGISAWA, F., YOSHIDA, Y., KUROIWA, H. & KUROIWA, T. (2005). *Cyanidioschyzon merolae* genome. A tool for facilitating comparable studies on organelle biogenesis in photosynthetic eukaryotes. *Plant Physiology* **137**, 567–585.
- MOOSAVI, B., BERRY, E. A., ZHU, X. L., YANG, W. C. & YANG, G. F. (2019). The assembly of succinate dehydrogenase: a key enzyme in bioenergetics. *Cellular and Molecular Life Sciences* **76**, 4023–4042.
- MORALES, J., MOGI, T., MINEKI, S., TAKASHIMA, E., MINEKI, R., HIRAWAKE, H., SAKAMOTO, K., OMURA, S. & KITA, K. (2009). Novel mitochondrial complex II isolated from *Trypanosoma cruzi* is composed of 12 peptides including a heterodimeric Ip subunit. *The Journal of Biological Chemistry* **284**, 7255–7263.
- MORE, K., KLINGER, C. M., BARLOW, L. D. & DACKS, J. B. (2020). Evolution and natural history of membrane trafficking in eukaryotes. *Current Biology* **30**, R553–R564.
- \*MORIYAMA, T., SAKURAI, K., SEKINE, K. & SATO, N. (2014). Subcellular distribution of central carbohydrate metabolism pathways in the red alga *Cyanidioschyzon merolae*. *Planta* **240**, 585–598.
- MÜHLEIP, A., FLYGAARD, R. K., BARADARAN, R., HAAPANEN, O., GRUHL, T., TOBIASSON, V., MARÉCHAL, A., SHARMA, V. & AMUNTS, A. (2023). Structural basis of mitochondrial membrane bending by the I-II-III<sub>2</sub>-IV<sub>2</sub> supercomplex. *Nature* **615**, 934–938.
- MUÑOZ-GÓMEZ, S. A., BILOLIKAR, G., WIDEMAN, J. G. & GEILER-SAMEROTTE, K. (2021). Constructive neutral evolution 20 years later. *Journal of Molecular Evolution* **89**, 172–182.
- MUÑOZ-GÓMEZ, S. A., SLAMOVITS, C. H., DACKS, J. B., BAIER, K. A., SPENCER, K. D. & WIDEMAN, J. G. (2015). Ancient homology of the mitochondrial contact site and cristae organizing system points to an endosymbiotic origin of mitochondrial cristae. *Current Biology* **25**, 1489–1495.
- \*MUÑOZ-GÓMEZ, S. A., SNYDER, S. N., MONTOYA, S. J. & WIDEMAN, J. G. (2020). Independent accretion of TIM22 complex subunits in the animal and fungal lineages. *PLoS ONE* **15**, 1060.
- \*MURCHA, M. W., ELHAFEZ, D., LISTER, R., TONTI-FILIPPINI, J., BAUMGARTNER, M., PHILIPPAR, K., CARRIE, C., MOKRANJAC, D., SOLL, J. & WHELAN, J. (2007). Characterization of the preprotein and amino acid transporter gene family in *Arabidopsis*. *Plant Physiology* **143**, 199–212.
- \*MURCHA, M. W., LISTER, R., HO, A. Y. & WHELAN, J. (2003). Identification, expression, and import of components 17 and 23 of the inner mitochondrial membrane translocase from *Arabidopsis*. *Plant Physiology* **131**, 1737–1747.
- \*MURCHA, M. W., NARSAI, R., DEVENISH, J., KUBISZEWSKI-JAKUBIAK, S. & WHELAN, J. (2015). MPIC: a mitochondrial protein import components database for plant and non-plant species. *Plant and Cell Physiology* **56**, e10.
- MUSACCHIO, A. & DESAI, A. (2017). A molecular view of kinetochore assembly and function. *Biology (Basel)* **6**, 5.
- \*NERUSHEVA, O. O., LUDZIA, P. & AKIYOSHI, B. (2019). Identification of four unconventional kinetoplastid kinetochore proteins KKT22–25 in *Trypanosoma brucei*. *Open Biology* **9**, 190236.
- OBADO, S. O., FIELD, M. C. & ROUT, M. P. (2017). Comparative interactomics provides evidence for functional specialization of the nuclear pore complex. *Nucleus* **8**, 340–352.
- O'MALLEY, M. A., WIDEMAN, J. G. & RUIZ-TRILLO, I. (2016). Losing complexity: the role of simplification in macroevolution. *Trends in Ecology & Evolution* **31**, 608–621.
- ORTIZ-SANDOVAL, C. G., HUGHES, S. C., DACKS, J. B. & SIMMEN, T. (2014). Interaction with the effector dynamin-related protein 1 (Drp1) is an ancient function of Rab32 subfamily proteins. *Cellular Logistics* **4**, e986399.
- OSAKABE, A. & MOLARO, A. (2023). Histone renegades: unusual H2A histone variants in plants and animals. *Seminars in Cell & Developmental Biology* **135**, 35–42.
- PADILLA-MEJIA, N. E., MAKAROV, A. A., BARLOW, L. D., BUTTERFIELD, E. R. & FIELD, M. C. (2021). Evolution and diversification of the nuclear envelope. *Nucleus* **12**, 21–41.
- PÁNEK, T., ELIÁŠ, M., VANCOVÁ, M., LUKEŠ, J. & HASHIMI, H. (2020). Returning to the fold for lessons in mitochondrial crista diversity and evolution. *Current Biology* **30**, R575–R588.
- PEREZ, E., LAPAILLE, M., DEGAND, H., CILIBRASI, L., VILLAVICENCIO-QUEIJEIRO, A., MORSOMME, P., GONZÁLEZ-HALPHEN, D., FIELD, M. C., REMACLE, C., BAURAIN, D. & CARDOL, P. (2014). The mitochondrial respiratory chain of the secondary green alga *Euglena gracilis* shares many additional subunits with parasitic Trypanosomatidae. *Mitochondrion* **19**, 338–349.
- PETERSEN, G., CUENCA, A., MÖLLER, I. M. & SEBERG, O. (2015). Massive gene loss in mistletoe (*Viscum*, *Viscaceae*) mitochondria. *Scientific Reports* **5**, 17588.
- PETRŽELKOVÁ, R. & ELIÁŠ, M. (2014). Contrasting patterns in the evolution of the Rab GTPase family in Archaeplastida. *Acta Societatis Botanicorum Poloniae* **83**, 303–315.
- PYRIHOVÁ, E., MOTYČKOVÁ, A., VOLEMAN, L., WANDYSZEWSKA, N., FIŠER, R., SEYDLOVÁ, G., ROGER, A., KOLÍSKO, M. & DOLEŽAL, P. (2018). A single Tim translocase in the mitosomes of *Giardia intestinalis* illustrates convergence of protein import achines in anaerobic eukaryotes. *Genome Biology and Evolution* **10**, 2813–2822.
- \*QI, L., WANG, Q., GUAN, Z., WU, Y., SHEN, C., HONG, S., CAO, J., ZHANG, X., YAN, C. & YIN, P. (2021). Cryo-EM structure of the human mitochondrial translocase TIM22 complex. *Cell Research* **31**, 369–372.
- RIAZ, S. & SUI, Z. (2018). Molecular cloning, transcriptome profiling, and characterization of histone genes in the dinoflagellate *Alexandrium pacificum*. *Journal of Microbiology and Biotechnology* **28**, 1185–1198.
- RICHARDSON, E. & DACKS, J. B. (2022). Distribution of membrane trafficking system components across ciliate diversity highlights heterogenous organelle-associated machinery. *Traffic* **23**, 208–220.
- \*RICHTER, F., DENNERLEIN, S., NIKOLOV, M., JANS, D. C., NAUMENKO, N., AICH, A., MACVICAR, T., LINDEN, A., JAKOBS, S., URLAUB, H., LANGER, T. & REHLING, P. (2019). ROMO1 is a constituent of the human presequence translocase required for YME1L protease import. *Journal of Cell Biology* **218**, 598–614.
- ROBINSON, M. S. (2015). Forty years of clathrin-coated vesicles. *Traffic* **16**, 1210–1238.
- ROGER, A. J., MUÑOZ-GÓMEZ, S. A. & KAMIKAWA, R. (2017). The origin and diversification of mitochondria. *Current Biology* **27**, R1177–R1192.
- \*ROJAS, F., KOZELA, J., BÚA, J., LLORENTE, B., BURCHMORE, R., AUER, M., MOTTRAM, J. C. & TÉLLEZ-IÑÓN, M. T. (2017). The ubiquitin-conjugating enzyme CDC34 is essential for cytokinesis in contrast to putative subunits of a SCF complex in *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases* **11**, e0005626.
- ROUT, M. P. & FIELD, M. C. (2017). The evolution of organellar coat complexes and organisation of the eukaryotic cell. *Annual Review of Biochemistry* **86**, 637–657.
- RUTHERFORD, S. & MOORE, I. (2002). The *Arabidopsis* Rab GTPase family: another enigma variation. *Current Opinion in Plant Biology* **5**, 518–528.

- SALINAS, T., LAROSA, V., CARDOL, P., MARÉCHAL-DROUARD, L. & REMACLE, C. (2014). Respiratory-deficient mutants of the unicellular green alga *Chlamydomonas*: a review. *Biochimie* **100**, 207–218.
- SANTOS, R., ÁSTVALDSSON, Á., PIPALIYA, S. V., ZUMTHOR, J. P., DACKS, J. B., SVÄRD, S., HEHL, A. B. & FASO, C. (2022). Combined nanometric and phylogenetic analysis of unique endocytic compartments in *Giardia lamblia* sheds light on the evolution of endocytosis in Metamonada. *BMC Biology* **20**, 206.
- SCHMID, E. M. & MCMAHON, H. T. (2007). Integrating molecular and network biology to decode endocytosis. *Nature* **448**, 883–888.
- SCHNEIDER, A. (2018). Mitochondrial protein import in trypanosomatids: variations on a theme or fundamentally different? *PLoS Pathogens* **14**, e1007351.
- SCHNEIDER, A. (2020). Evolution of mitochondrial protein import – lessons from trypanosomes. *Biological Chemistry* **401**, 663–676.
- SCHRADER, M. & YOON, Y. (2007). Mitochondria and peroxisomes: are the ‘big brother’ and the ‘little sister’ closer than assumed? *Bioessays* **29**, 1105–1114.
- \*SIMON, D. N. & WILSON, K. L. (2013). Partners and post-translational modifications of nuclear lamins. *Chromosoma* **122**, 13–31.
- SIMPSON, F., PEDEN, A. A., CHRISTOPOULOU, L. & ROBINSON, M. S. (1997). Characterization of the adaptor-related protein complex, AP-3. *Journal of Cell Biology* **137**, 835–845.
- SINGHA, U. K., HAMILTON, V., DUNCAN, M. R., WEEMS, E., TRIPATHI, M. K. & CHAUDHURI, M. (2012). Protein translocase of mitochondrial inner membrane in *Trypanosoma brucei*. *The Journal of Biological Chemistry* **287**, 14480–14493.
- SINHA, S. D. & WIDEMAN, J. G. (2023). The persistent homology of mitochondrial ATP synthases. *iScience* **26**, 106700.
- \*SMITH, D. G., GAWRYLUK, R. M., SPENCER, D. F., PEARLMAN, R. E., SIU, K. W. & GRAY, M. W. (2007). Exploring the mitochondrial proteome of the ciliate protozoan *Tetrahymena thermophila*: direct analysis by tandem mass spectrometry. *Journal of Molecular Biology* **374**, 837–863.
- SMITH, P. M., FOX, J. L. & WINGE, D. R. (2012). Biogenesis of the cytochrome *bc<sub>1</sub>* complex and role of assembly factors. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1817**, 872–882.
- SOLINGER, J. A. & SPANG, A. (2013). Tethering complexes in the endocytic pathway: CORVET and HOPS. *The FEBS Journal* **280**, 2743–2757.
- \*SOTO, A. R., ZHENG, H., SHOEMAKER, D., RODRIGUEZ, J., READ, B. A. & WAHLUND, T. M. (2006). Identification and preliminary characterization of two cDNAs encoding unique carbonic anhydrases from the marine alga *Emiliania huxleyi*. *Applied and Environmental Microbiology* **72**, 5500–5511.
- SPARVOLI, D., RICHARDSON, E., OSAKADA, H., LAN, X., IWAMOTO, M., BOWMAN, G. R., KONTUR, C., BOURLAND, W. A., LYNN, D. H., PRITCHARD, J. K., HARAGUCHI, T., DACKS, J. B. & TURKEWITZ, A. P. (2018). Remodeling the specificity of an endosomal CORVET tether underlies formation of regulated secretory vesicles in the ciliate *Tetrahymena thermophila*. *Current Biology* **28**, 697–710.e13.
- SPARVOLI, D., ZOLTNER, M., CHENG, C. Y., FIELD, M. C. & TURKEWITZ, A. P. (2020). Diversification of CORVET tethers facilitates transport complexity in *Tetrahymena thermophila*. *Journal of Cell Science* **133**, jcs238659.
- SPEIJER, D. (2020). Debating eukaryogenesis-part 1: does eukaryogenesis presuppose symbiosis before uptake? *Bioessays* **42**, e1900157.
- \*STEINEGGER, M., MEIER, M., MIRDITA, M., VÖHRINGER, H., HAUNSBERGER, S. J. & SÖDING, J. (2019). HH-suite3 for fast remote homology detection and deep protein annotation. *BMC Bioinformatics* **20**, 473.
- STENMARK, H. (2009). Rab GTPases as coordinators of vesicle traffic. *Nature Reviews Molecular Cell Biology* **10**, 513–525.
- STEVENS, K. M., SWADLING, J. B., HOCHER, A., BANG, C., GRIBALDO, S., SCHMITZ, R. A. & WARNECKE, T. (2020). Histone variants in archaea and the evolution of combinatorial chromatin complexity. *Proceedings of the National Academy of Sciences of the United States of America* **117**, 33384–33395.
- STOLTZFUS, A. (1999). On the possibility of constructive neutral evolution. *Journal of Molecular Evolution* **49**, 169–181.
- \*SUBRAHMANYAN, N., REMACLE, C. & HAMEL, P. P. (2016). Plant mitochondrial complex I composition and assembly: a review. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1857**, 1001–1014.
- \*SURUJON, D. & RATNER, D. I. (2016). Use of a probabilistic motif search to identify histidine phosphotransfer domain-containing proteins. *PLoS One* **11**, e0146577.
- SYNEK, L., SEKEREŠ, J. & ZÁRSKÝ, V. (2014). The exocyst at the interface between cytoskeleton and membranes in eukaryotic cells. *Frontiers in Plant Science* **4**, 543.
- SZKLARCZYK, R. & HUYNEN, M. A. (2009). Expansion of the human mitochondrial proteome by intra- and inter-compartmental protein duplication. *Genome Biology* **10**, R135.
- THEOS, A. C., TENZA, D., MARTINA, J. A., HURBAIN, I., PEDEN, A. A., SVIDERSKAYA, E. V., STEWART, A., ROBINSON, M. S., BENNETT, D. C., CUTLER, D. F., BONIFACINO, J. S., MARKS, M. S. & RAPOSO, G. (2005). Functions of adaptor protein (AP)-3 and AP-1 in tyrosinase sorting from endosomes to melanosomes. *Molecular Biology of the Cell* **16**, 5356–5372.
- TICE, A. K., ŽIHALA, D., PÁNEK, T., JONES, R. E., SALOMAKI, E. D., NENAROKOV, S., BURKI, F., ELIŠ, M., EME, L., ROGER, A. J., ROKAS, A., SHEN, X. X., STRASSET, J. F. H., KOLÍŠKO, M. & BROWN, M. W. (2021). PhyloFisher: a phylogenomic package for resolving eukaryotic relationships. *PLoS Biology* **19**, e3001365.
- TIMMIS, J. N., AYLIFFE, M. A., HUANG, C. Y. & MARTIN, W. (2004). Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nature Reviews Genetics* **5**, 123–135.
- TINTI, M. & FERGUSON, M. A. J. (2022). Visualisation of proteome-wide ordered protein abundances in *Trypanosoma brucei*. *Wellcome Open Research* **7**, 34.
- TOUZ, M. C., RIVERO, M. R., MIRAS, S. L. & BONIFACINO, J. S. (2012). Lysosomal protein trafficking in *Giardia lamblia*: common and distinct features. *Frontiers in Bioscience-Elite* **4**, 1898–1909.
- \*TRELLE, M. B., SALCEDO-AMAYA, A. M., COHEN, A. M., STUNNENBERG, H. G. & JENSEN, O. N. (2009). Global histone analysis by mass spectrometry reveals a high content of acetylated lysine residues in the malaria parasite *Plasmodium falciparum*. *Journal of Proteome Research* **8**, 3439–3450.
- TROMER, E. C., VAN HOOFF, J. J. E., KOPS, G. J. P. L. & SNEL, B. (2019). Mosaic origin of the eukaryotic kinetochore. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 12873–12882.
- TROMER, E. C., WEMYSS, T. A., WALLER, R. F. & AKIYOSHI, B. (2021). Repurposing of synaptonemal complex proteins for kinetochores in Kinetoplastida. *Open Biology* **11**, 210049.
- UMAER, K., BUSH, P. J. & BANGS, J. D. (2018). Rab11 mediates selective recycling and endocytic trafficking in *Trypanosoma brucei*. *Traffic* **19**, 406–420.
- UMASANKAR, P. K., MA, L., THIEMAN, J. R., JHA, A., DORAY, B., WATKINS, S. C. & TRAUB, L. M. (2014). A clathrin coat assembly role for the mucin protein central linker revealed by TALEN-mediated gene editing. *eLife* **3**, e04137.
- VALACH, M., LÉVEILLÉ-KUNST, A., GRAY, M. W. & BURGER, G. (2018). Respiratory chain complex I of unparallegled divergence in diplomonids. *The Journal of Biological Chemistry* **293**, 16043–16056.
- VALPADASHI, A., CALLEGARI, S., LINDEN, A., NEUMANN, P., FICNER, R., URLAUB, H., DECKERS, M. & REHLING, P. (2021). Defining the architecture of the human TIM22 complex by chemical crosslinking. *FEBS Letters* **595**, 157–168.
- \*VAN DER SLUIS, E. O., BAUERSCHMITT, H., BECKER, T., MIELKE, T., FRAUENFELD, J., BERNINGHAUSEN, O., NEUPERT, W., HERRMANN, J. M. & BECKMANN, R. (2015). Parallel structural evolution of mitochondrial ribosomes and OXPHOS complexes. *Genome Biology and Evolution* **7**, 1253–1251.
- \*VAN DOOREN, G. G., STIMMLER, L. M. & MCFADDEN, G. I. (2006). Metabolic maps and functions of the *Plasmodium* mitochondrion. *FEMS Microbiology Reviews* **30**, 596–630.
- \*VAN HOOFF, J. J., TROMER, E., VAN WIJK, L. M., SNEL, B. & KOPS, G. J. (2017). Evolutionary dynamics of the kinetochore network in eukaryotes as revealed by comparative genomics. *EMBO Reports* **18**, 1559–1571.
- VÁZQUEZ-ACEVEDO, M., CARDOL, P., CANO-ESTRADA, A., LAPAILLE, M., REMACLE, C. & GONZÁLEZ-HALPHEN, D. (2006). The mitochondrial ATP synthase of chlorophycean algae contains eight subunits of unknown origin involved in the formation of an atypical stator-stalk and in the dimerization of the complex. *Journal of Bioenergetics and Biomembranes* **38**, 271–282.
- \*VELOURS, J. & ARSELIN, G. (2000). The *Saccharomyces cerevisiae* ATP synthase. *Journal of Bioenergetics and Biomembranes* **32**, 383–390.
- \*VERMA, G. & SUROLIA, N. (2014). The dimerization domain of *Pf* CENP-C is required for its functions as a centromere protein in human malaria parasite *Plasmodium falciparum*. *Malaria Journal* **13**, 475.
- WALKER, J. E. (2013). The ATP synthase: the understood, the uncertain and the unknown. *Biochemical Society Transactions* **41**, 1–16.
- WANG, S., MA, Z., XU, X., WANG, Z., SUN, L., ZHOU, Y., LIN, X., HONG, W. & WANG, T. (2014). A role of Rab29 in the integrity of the trans-Golgi network and retrograde trafficking of mannose-6-phosphate receptor. *PLoS One* **9**, e96242.
- WANG, W., CHEN, X., ZHANG, L., YI, J., MA, Q., YIN, J., ZHUO, W., GU, J. & YANG, M. (2020). Atomic structure of human TOM core complex. *Cell Discovery* **6**, 67.
- WANG, Y., CARRIE, C., GIRAUD, E., ELHAFEZ, D., NARSAI, R., DUNCAN, O., WHELAN, J. & MURCHA, M. W. (2012). Dual location of the mitochondrial preprotein transporters B14.7 and Tim23-2 in complex I and the TIM17:23 complex in *Arabidopsis* links mitochondrial activity and biogenesis. *The Plant Cell* **24**, 2675–2695.
- WASMEIER, C., ROMAO, M., PLOWRIGHT, L., BENNETT, D. C., RAPOSO, G. & SEABRA, M. C. (2006). Rab38 and Rab32 control post-Golgi trafficking of melanogenic enzymes. *Journal of Cell Biology* **175**, 271–281.
- \*WELCHEN, E. & GONZALEZ, D. H. (2005). Differential expression of the Arabidopsis cytochrome *c* genes *Cyt-c-1* and *Cyt-c-2*. Evidence for the involvement of TCP-domain protein-binding elements in anther- and meristem-specific expression of the *Cyt-c-1* gene. *Plant Physiology* **139**, 88–100.
- \*WHEELER, T. J. & EDDY, S. R. (2013). nhmmer: DNA homology search with profile HMMs. *Bioinformatics* **29**, 2487–2489.
- \*WIDEMAN, J. G., GAWRYLUK, R. M., GRAY, M. W. & DACKS, J. B. (2014). The ancient and widespread nature of the ER-mitochondria encounter structure. *Molecular Biology and Evolution* **30**, 2044–2049.

- WIDEMAN, J. G. & MUÑOZ-GÓMEZ, S. A. (2016). The evolution of ERMIONE in mitochondrial biogenesis and lipid homeostasis: an evolutionary view from comparative cell biology. *Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids* **1861**, 900–912.
- WIEDEMANN, N. & PFANNER, N. (2017). Mitochondrial machineries for protein import and assembly. *Annual Review of Biochemistry* **86**, 685–714.
- \*WOJTKOWSKA, M., BUCZEK, D., STOBINIENIA, O., KARACHITOS, A., ANTONIEWICZ, M., SŁOCINSKA, M., MAKALOWSKI, W. & KMITA, H. (2015). The TOM complex of Amoebozoans: the cases of the amoeba *Acanthamoeba castellanii* and the slime mold *Dictyostelium discoideum*. *Protist* **166**, 349–362.
- \*WOJTKOWSKA, M., BUCZEK, D., SUZUKI, Y., SHABARDINA, V., MAKALOWSKI, W. & KMITA, H. (2017). The emerging picture of the mitochondrial protein import complexes of Amoebozoa supergroup. *BMC Genomics* **18**, 997.
- WOLF, Y. I. & KOONIN, E. V. (2013). Genome reduction as the dominant mode of evolution. *Bioessays* **35**, 829–837.
- WOLLWEBER, F., VON DER MALSBERG, K. & VAN DER LAAN, M. (2017). Mitochondrial contact site and cristae organizing system: a central player in membrane shaping and stalk. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research* **1864**, 1481–1489.
- WONG, J. T. Y., NEW, D. C., WONG, J. C. W. & HUNG, V. K. L. (2003). Histone-like proteins of the dinoflagellate *Cryptophodinium oohii* have homologies to bacterial DNA-binding proteins. *Eukaryotic Cell* **2**, 646–650.
- WOO, Y. H., ANSARI, H., OTTO, T. D., KLINGER, C. M., KOLISKO, M., MICHÁLEK, J., SAXENA, A., SHANMUGAM, D., TAYYROV, A., VELUCHAMY, A., ALI, S., BERNAL, A., DEL CAMPO, J., CIHLÁŘ, J., FLEGONTOV, P., ET AL. (2015). Chromerid genomes reveal the evolutionary path from photosynthetic algae to obligate intracellular parasites. *eLife* **4**, e06974.
- \*YAMADA, M. & GOSHIMA, G. (2017). Mitotic spindle assembly in land plants: molecules and mechanisms. *Biology (Basel)* **6**, 6.
- YAMANO, K., YATSUKAWA, Y., ESAKI, M., HOBBS, A. E., JENSEN, R. E. & ENDO, T. (2008). Tom20 and Tom22 share the common signal recognition pathway in mitochondrial protein import. *Journal of Biological Chemistry* **283**, 3799–3807.
- YIP, C. Y., HARBOUR, M. E., JAYAWARDENA, K., FEARNLEY, I. M. & SAZANOV, L. A. (2011). Evolution of respiratory complex I: "supernumerary" subunits are present in the  $\mathbb{E}$ -proteobacterial enzyme. *Journal of Biological Chemistry* **286**, 5023–5033.
- ZACCAI, N. R., KADLECOVA, Z., DICKSON, V. K., KOROBCHEVSKAYA, K., KAMENICKY, J., KOVTUN, O., UMASANKAR, P. K., WROBEL, A. G., KAUFMAN, J. G. G., GRAY, S. R., QU, K., EVANS, P. R., FRITZSCHE, M., SROUBEK, F., HÖNING, S., ET AL. (2022). FCHO controls AP2's initiating role in endocytosis through a PtdIns(4,5)P<sub>2</sub>-dependent switch. *Science Advances* **8**, eabn2018.
- ŽÁRSKÝ, V. & DOLEŽAL, P. (2016). Evolution of the Tim17 protein family. *Biology Direct* **11**, 54.
- ŽÁRSKÝ, V., SEKEREŠ, J., KUBÁTOVÁ, Z., PEČENKOVÁ, T. & CVRČKOVÁ, F. (2020). Three subfamilies of exocyst EXO70 family subunits in land plants: early divergence and ongoing functional specialization. *Journal of Experimental Botany* **71**, 49–62.
- ZERBES, R. M., BOHNERT, M., STROUD, D. A., VON DER MALSBERG, K., KRAM, A., OEJJEKLAUS, S., WARSCHIED, B., BECKER, T., WIEDEMANN, N., VEENHUIS, M., VAN DER KLEI, I. J., PFANNER, N. & VAN DER LAAN, M. (2012). Role of MINOS in mitochondrial membrane architecture: cristae morphology and outer membrane interactions differentially depend on mitofilin domains. *Journal of Molecular Biology* **14**, 183–191.
- ZHOU, L., MALDONADO, M., PADAVANNIL, A., GUO, F. & LETTS, J. A. (2022). Structures of *Tetrahymena's* respiratory chain reveal the diversity of eukaryotic core metabolism. *Science* **31**, eabn7747.
- ZÍKOVÁ, A., PANIGRAHI, A. K., UBOLDI, A. D., DALLEY, R. A., HANDMAN, E. & STUART, K. (2008). Structural and functional association of *Trypanosoma brucei* MIX protein with cytochrome *c* oxidase complex. *Eukaryotic Cell* **7**, 1994–2003.
- Fig. S1.** Dartboard chart representation of the translocase of the outer mitochondrial membrane (TOM) complex.
- Fig. S2.** Dartboard chart representation of the translocase of the inner mitochondrial membrane (TIM) complex.
- Fig. S3.** Dartboard chart representation of the mitochondrial contact site and cristae organising system (MICOS) complex.
- Fig. S4.** Dartboard chart representation of respiratory chain complex I (NADH:ubiquinone oxidoreductase).
- Fig. S5.** Dartboard chart representation of respiratory chain complex II (succinate: ubiquinone oxidoreductase).
- Fig. S6.** Dartboard chart representation of respiratory chain complex III (ubiquinol:cytochrome *c* oxidoreductase).
- Fig. S7.** Dartboard chart representation of respiratory chain complex IV (cytochrome *c* oxidase).
- Fig. S8.** Dartboard chart representation of complex V (F<sub>0</sub>F<sub>1</sub>-ATP synthase).
- Fig. S9.** Genome/transcriptome completeness for the species in the reference data set estimated using genome/transcriptome-derived proteins as the query and eukaryota\_odb10 as the database in BUSCO v5.4.3 (Manni *et al.*, 2021).
- Table S1.** Constituents of the lamina complex and their amino acid sequences across eukaryotes.
- Table S2.** Constituents of the kinetochore complex and their amino acid sequences across eukaryotes.
- Table S3.** Constituents of the translocase of the outer mitochondrial membrane (TOM) complex and their amino acid sequences across eukaryotes.
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- Table S5.** Constituents of the mitochondrial contact site and cristae organising system (MICOS) complex and their amino acid sequences across eukaryotes.
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- Table S10.** Constituents of respiratory complex V (F<sub>0</sub>F<sub>1</sub>-ATP synthase) and their amino acid sequences across eukaryotes.
- Table S11.** Reference data set used in our study.

## X. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1.** Supplementary materials and methods.

**Fig. S0.** How to read the dartboard charts.

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