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A role for convergent evolution in the secretory life of cells

Nels C. Elde[1], Manyuan Long[2] and Aaron P. Turkewitz[3]

[1] Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
[2] Department of Ecology and Evolution, The University of Chicago, Chicago, IL 60637, USA
[3] Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637, USA

The role of convergent evolution in biological adaptation is increasingly appreciated. Many clear examples have been described at the level of individual proteins and for organismal morphology, and convergent mechanisms have even been invoked to account for similar community structures that are shared between ecosystems. At the cellular level, an important area that has received scant attention is the potential influence of convergent evolution on complex subcellular features, such as organelles. Here, we show that existing data strongly argue that convergent evolution underlies the similar properties of specialized secretory vesicles, called dense core granules, in the animal and ciliate lineages. We discuss both the criteria for judging convergent evolution and the contribution that such evolutionary analysis can make to improve our understanding of processes in cell biology. The elucidation of these underlying evolutionary relationships is vital because cellular structures that are assumed to be analogous, owing to shared features, might in fact be governed by different molecular mechanisms.

Convergent evolution: from molecules to ecosystems

The fins of whales and fish both promote efficient swimming, but are not primarily the result of shared inheritance. Instead, natural selection honed features in separate lineages to produce functionally equivalent limbs. Many examples of such convergent evolution of morphological features are known, including wing pattern mimicry in butterflies, bright coloration accompanied by toxicity in poison frogs and skeletal features in isolated populations of stickleback fish [1–3].

Parallel adaptations associated with convergent evolution are also observed at other levels of biological organization. For the cases cited above, morphological traits of individual species are influenced by specific environmental conditions. However, convergent evolution might also be relevant to higher levels of biodiversity. This concept, called community convergence, proposes that similar environments might independently shape phenotypes of groups of species to fill similar niches. For example, the parallel adaptation of certain neotropical lizards to similar microhabitats resulted in similar communities albeit with different species compositions, across the West Indian islands (summarized in Ref. [4]). The existence of convergent evolution is also increasingly recognized at the other extreme of biological organization, where selective pressures acting at the molecular level shape the evolution of macromolecules.

Studies of convergent evolution at the molecular level have primarily focused on the acquisition of shared functions for individual proteins. For example, the stomach enzyme lysozyme independently acquired similar properties in leaf-eating birds, langur monkeys and ruminants, owing to common selective pressures to digest plant matter [5–7]. Another example comes from antifreeze proteins, which have arisen independently in antarctic notothenioid fish and arctic cod to restrict the growth of ice crystals in the circulatory system [8,9]. A case of convergent evolution was also uncovered in the visual pigment proteins of humans and blind cave fish, where identical amino acid substitutions independently gave rise to red pigments from green ones [10]. Convergent evolution also extends to changes in gene expression [11].

These analyses add significantly to our understanding of adaptation by convergent evolution at the level of proteins, whole organisms and even ecological communities. Here, we propose that functional convergence also occurs at the previously unrecognized level of cellular features and complex structures, such as organelles.

This proposal arose from the historical observation, based largely on electron microscopy, that cells from a vast array of existing organisms share similar subcellular features. A prevailing and parsimonious interpretation of these observations is that shared features are exclusively due to descent from a common ancestor already possessing such features. However, given the demonstrations of convergent evolution at various levels of biological organization, an alternative hypothesis is that some shared subcellular features could also be the result of similar selective pressures in different lineages, resulting in fixation of lineage-specific adaptations whose similarities belie their independent origins. This scenario is challenging to imagine for cellular features depending on multiple proteins that must maintain functional interactions while undergoing adaptation in a convergent scenario. Nonetheless, such a case was recently uncovered in the pathway of endocytosis, where it was demonstrated that at least one feature of clathrin-coated pits has been shaped by convergent evolution (Box 1) [12]. Another example was discovered for mitochondrial import [13].

Corresponding author: Turkewitz, A.P. (apturkew@midway.uchicago.edu).
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Box 1. Various forms of evolution in cell biological pathways: the case of clathrin-mediated endocytosis

Proteins involved in endocytosis illustrate the varied evolutionary forces shaping complex cellular pathways (Figure Ia). Clathrin is a cytosolic protein that mediates formation of vesicles at the plasma membrane. Clathrin-mediated endocytosis is broadly conserved in modern eukaryotes and all evidence suggests this is due to inheritance from an early common ancestor, that is, divergent evolution (Figure Ib). Interestingly, recent duplications of clathrin genes during chordate evolution produced isoforms with tissue-specific functions [72]. Gene duplications commonly lead to functional divergence through the 'birth' of new genes, which adapt to perform new or specialized tasks in specific lineages (Figure Ic) [67]. A similar evolutionary history, based on widespread inheritance supplemented by lineage-specific isoforms, can explain the modern distribution of a protein family called 'adaptors', which are involved in selective protein uptake during clathrin-mediated endocytosis (Figure Ib) [73].

Not surprisingly, some proteins involved in endocytosis are found only in restricted lineages, and might represent lineage-specific innovation. Recently, an unexpected example of similar innovations in two parallel lineages revealed that convergent evolution has also shaped the pathway of endocytosis (Figure Ic). This example involved dynamins. Dynamins are self-assembling GTPases that promote membrane scission. In animals, one dynamin isoform functions in formation of endocytic vesicles [74]. A dynamin isoform also has this role in ciliates. However, endocytic isoforms were not inherited from a common ancestor but instead evolved independently. Indeed, dynamins in independent lineages have converged to function in the same pathway through independent duplication events after divergence from a common ancestor (Figure Id).

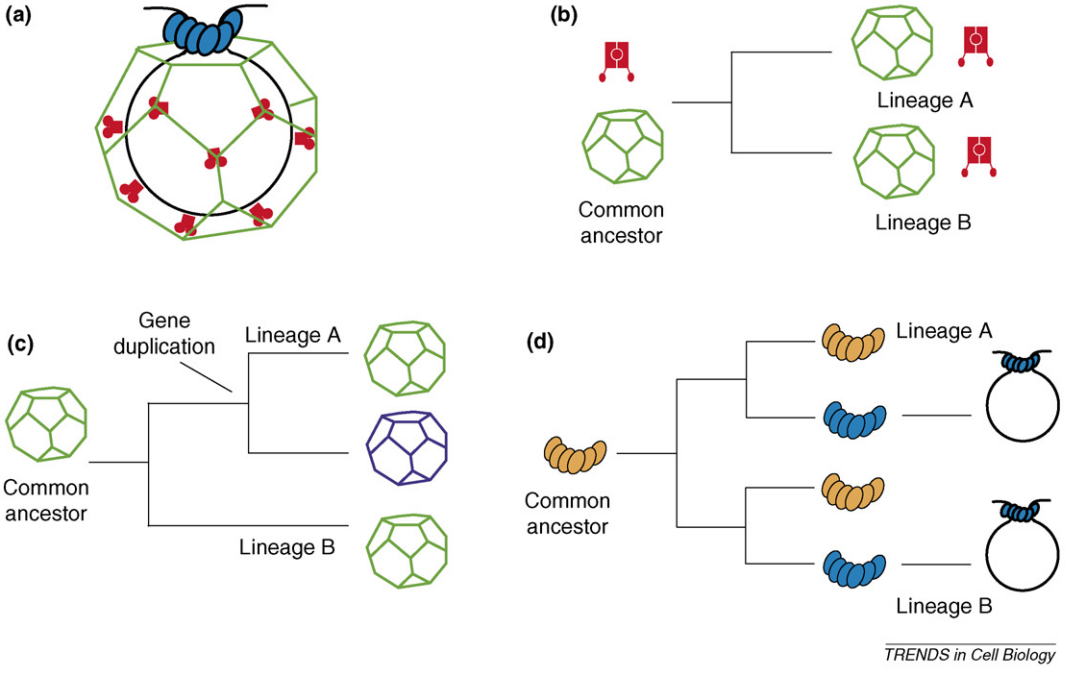

Figure I. Evolution of components involved in clathrin-mediated endocytosis. **(a)** A cartoon highlighting the major components of a clathrin-coated vesicle. Clathrin (green) is assembled around a nascent vesicle formed at the plasma membrane (black). AP-2 (red) acts as an adaptor protein between clathrin and components (e.g. receptors) internalized in vesicles; dynamin (blue) forms an oligomeric ring promoting the scission of membranes and the release of vesicles into the cytoplasm. **(b)** A simple schematic phylogeny of AP-2 and clathrin demonstrating divergent evolution. Primitive forms of AP-2 and clathrin with ancestral roles in endocytosis continue to carry out these functions in diverging lineages. **(c)** A phylogeny showing a gene duplication of clathrin leading to a specialized, lineage-specific role for a new isoform (purple) in lineage A. **(d)** A phylogenetic scheme showing the recruitment of dynamin to the endocytic pathway by convergent evolution. A primitive dynamin (orange) with a role unrelated to endocytosis diverges in separate lineages and, after separate duplication events, independently adopts new and similar roles in endocytosis (blue) for both lineages.

One of the current challenges is to extend these observations from the evolution of an individual component to that of interactions in cellular pathways. Another challenge lies in surveying a broad range of subcellular structures for signs of convergent evolution. Here, we review the evidence demonstrating that at least one class of secretory vesicles, called dense core granules, has been shaped by convergent evolution, and also argue that functional convergence has a more common role in the evolution of cellular structures than is currently recognized.

Dense core granule biology and evolution

Dense core granules (DCGs) are a class of specialized secretory vesicles that release their contents to the cell exterior by fusing with the plasma membrane. The DCG lumen contains a condensed protein core that assembles during DCG formation through self-aggregation of soluble proteins within the *trans*-Golgi network (TGN) of the secretory pathway. Reorganization of the protein core continues during a subsequent remodeling phase called maturation [63]. Because DCG cargo is condensed, these vesicles serve as efficient storage reservoirs compared with other classes of vesicles that transport soluble cargo. A second defining feature of DCGs is that their fusion with the plasma membrane is not spontaneous but instead responsive to specific stimuli. DCGs accumulate within the cell cytoplasm until an extracellular event triggers the fusion of the vesicles and plasma membranes by a process called 'regulated exocytosis' [33]. Although both the condensed core and regulated exocytosis are characteristic of DCGs, neither is unique to them; indeed, some secretory

lysosomes, such as the lytic granules in haemopoietic cells, possess both of these features [14]. DCGs, however, are distinct from lysosomes in fundamental aspects relating to their biosynthetic pathway; for example, the lysosomal protein sorting at the TGN involves adaptors and coat proteins [15,16].

Because DCGs are relatively large and possess dense cores, they are easily detected by conventional electron microscopy. Micrographs of cells from far-flung eukaryotic lineages suggest that membrane-bound carriers with DCG-like features are widely present and serve an equally wide array of functions, as illustrated in Box 2. Although DCGs have been most intensely studied in mammalian cells, even organisms representing the most ancient eukaryotic branches possess DCG-like vesicles. In *Giardia lamblia*, for example, exocytosis of dense vesicles is a crucial step for encystment, which is part of the pathogenic cycle of this organism [17]. The wide distribution of DCG-like vesicles raises an important question: was the pathway of DCG formation already present in an early eukaryotic ancestor, and subsequently inherited in an array of descendents? If this were the case, the absence of DCGs in lineages such as that of fungi could be accounted for by lineage-specific loss. The alternate scenario is that DCG-like vesicles arose independently by convergent evolution in a variety of lineages.

One important consideration, however, is whether the variety of dense-cored structures seen in electron micrographs should actually be considered DCGs. This cannot be determined based solely on morphological criteria because biochemical features defining DCGs cannot be discerned by appearances alone. Therefore, the proper identification of DCGs also requires the analysis of the vesicles' biosynthetic pathway. For example, scale formation in some algae is driven by protein self-assembly in the TGN, but whether subsequent steps resemble DCG biogenesis and exocytosis in animals is unknown [18]. The DCG-like vesicles in some lineages have been intensely studied and, therefore, offer good test cases for our hypothesis of convergent evolution.

Of the DCG-like vesicles in non-animal lineages, the best characterized are those found in ciliates, particularly in *Tetrahymena* and *Paramecium* [19,20]. Granules in *Paramecium*, called trichocysts, are involved in defense against predators [21]. The corresponding *Tetrahymena* secretory granules, whose function is still unknown, are called mucocysts. Both are large vesicles with strong similarities to their animal cell counterparts. First, assembly occurs through a multi-step process that includes granule remodeling ('maturation') following the initial appearance of granules from the TGN [22–24]. Second, maturation within the granule lumen entails the proteolytic processing of proproteins, which underlies a remodeling of the dense core structure in both ciliates and many animal granules [25–30]. Extraneous lumenal proteins that are removed from the granule during the remodeling phase are secreted from both ciliate and animal cells by a stimulus-independent pathway, which suggests that the connections between granule biogenesis and other pathways of membrane traffic are also similar in these two lineages [31,32]. Third, granules in both lineages undergo a classical regulated exocytosis [33]. As in many animal cells, exocytosis-inducing stimuli in ciliates mobilize calcium from intracellular stores; the resulting rise in local cytosolic free calcium then triggers exocytosis [34,35]. Exocytosis in ciliates, as in animals, is mediated by SNAREs (soluble NSF attachment receptors) as revealed by genetic manipulation of the SNARE disassembling factor, NSF [36]. Taken together, these observations support the view that ciliate granules share fundamental hallmarks of animal cell DCGs. Are these similarities due to inheritance of DCGs in ciliates and animals from a common ancestor? Early evidence appeared consistent with this idea, because some lumenal proteins within ciliate granules were reactive with antibodies against mammalian granule proteins [37]. However, the basis for the cross-reactivity was never established, and subsequent molecular analysis, detailed below, indicates that animal and ciliate granules are unlikely to contain any homologous proteins inherited from a common ancestor.

Evidence for convergent evolution of dense core granules

The principal lumenal proteins comprising dense cores have been characterized from *Paramecium* and *Tetrahymena*. The trichocyst matrix proteins (Tmps) of *Paramecium* and granule lattice proteins (Grls) of *Tetrahymena* are clearly products of a single gene family present in ciliates but not in animals [28,29,38,39]. A second family of lumenal granule proteins involved in granule polarization in *Tetrahymena* also has clear homologs in *Paramecium* [40,41]. Similarly, these proteins have no identifiable homologs in animal cell granules, although individual domains are shared with many prokaryotic and eukaryotic proteins. Importantly, the same appears to be true for granule-localized integral membrane proteins required for docking and exocytic fusion [42,43]. Eight such proteins identified in *Paramecium* have clear homologs in *Tetrahymena*. Although several of these proteins have individual domains also found within animal proteins, none of those animal proteins is associated with secretory granules or even with the secretory pathway. These data suggest the independent appearance of secretory granules with distinct molecular compositions in the animal and ciliate lineages, rather than inheritance from a common ancestor.

A hypothesis of independent innovations giving rise to similar features is further bolstered by observations relating to DCG maturation. In animals, the best characterized endoproteases involved in core maturation are the prohormone convertases, which are subtilisin-family proteases that recognize sequence-specific motifs in their targets [44]. Early analysis of the Tmps and Grls in ciliates suggested similar rules for cleavage site recognition [29,38]. However, testing of the processing requirements in *Tetrahymena* provided strong evidence that site recognition is fundamentally different in animals and ciliates [45]. Furthermore, the recently sequenced genomes of *Tetrahymena thermophila* and *Paramecium tetraurelia* have no identifiable homologs of prohormone convertases [46,47].

The most informative genes for analyzing the evolutionary relationships between vesicles found in different lineages belong to groups characterized by compartment-specific family members. The proteins encoded by

Box 2. Diverse cellular roles of dense core granules and similar structures

Many eukaryotes rely on rapid and regulated release of dense core granules (DCGs) under certain conditions for survival. The need for DCGs is reflected by intense selective pressure on regulated secretory pathways to perform properly in critical situations. Molecular studies of distantly related species (Figure I) are beginning to reveal different molecular bases for the shared features and functions of DCGs having important roles in the following processes:

Predation and defense

A variety of ciliates have morphologically complex DCGs that aid in capturing other ciliates as prey. Such DCGs, often called toxicysts, release molecules that subdue other ciliates upon contact [75,76]. Non-'carnivorous' ciliates sometimes deploy DCGs as countermeasures against predators. For example, the release of DCGs from *Paramecium* confers protection from predation [21,77].

Fungi are not recognized for possessing DCGs. However, the predatory fungus *Arthrobotrys oligospora* accumulates an abundance of electron dense bodies resembling DCGs in 'trap' cells, which are involved in capturing and digesting nematode prey [78]. Although not secreted, such microbodies appear to fuse with vacuoles in a regulated manner during feeding.

Pathogenicity and protection

Electron dense granules of *Entamoeba histolytica* secrete collagenases and a variety of other proteases that facilitate invasion [79]. The parasite *Toxoplasma gondii* contains three distinct secretory reservoirs called micronemes, rhoptries and dense granules. These are deployed sequentially during and after invasion of a host cell [80]. Structures called encystation secretory vesicles from the anciently derived eukaryote *Giardia lamblia* contain proteins that are incorporated into the cyst wall [81]. Encystation provides protection from the environment and facilitates wide dissemination of this parasite. The prespore vacuoles of the social amoeba *Dictyostelium discoideum* also confer protection from harsh conditions. At a key point during development, the secretion of spore coat proteins results in spore cells resistant to temperature extremes and drought [82]. In some cases, the relationship between these compartments and *bona fide* dense core granules is not well characterized. However, the secretory organelles of *Toxoplasma gondii* have different biochemical features in comparison to DCGs from ciliates and animals [83,84].

Metazoan specific functions

Metazoans have numerous classes of DCGs. The neuroendocrine system of mammals relies on a variety of DCGs, including neurotransmitter-containing granules of chromaffin cells [85] and pancreatic insulin-storage granules.

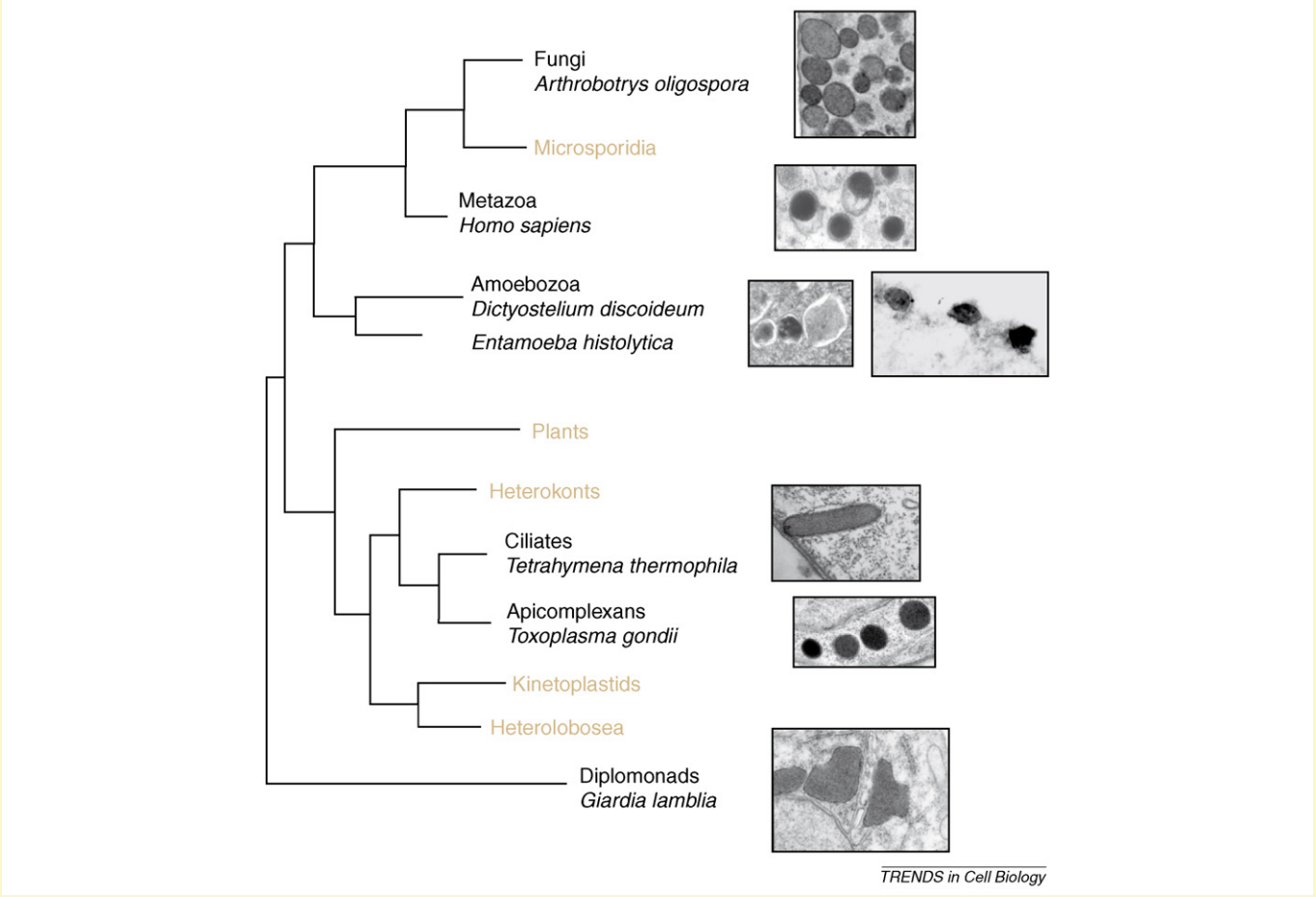

Figure I. Phylogenetic sampling of dense core granules and related vesicles. Electron micrographs showing a variety of dense core granules arranged according to a phylogeny of major eukaryotic groups. Shown from top to bottom are microbodies of the predatory fungus *Arthrobotrys oligospora* (image courtesy of Marten Veenhuis), DCGs of a mammalian chromaffin cell from adrenal medulla tissue (Chad Grabner), and prespore vacuoles of *Dictyostelium discoideum* (Supriya Srinivasan and Steven Alexander; image reprinted with permission from the Journal of Biological Chemistry). Also pictured are electron dense granules of *Entamoeba histolytica* (María de Lourdes Muñoz), DCGs of *Tetrahymena thermophila*, dense granules of *Toxoplasma gondii* (David Sibley and Wandy Beatty), and encystation secretory vesicles of *Giardia lamblia* (Michael McCaffery and Frances Gillin). The phylogeny is based on recently proposed tree constructions of eukaryotes [86,87]. The images of *Tetrahymena* and *Giardia* were adjusted to highlight granule morphology.

these genes are fundamental to the budding and fusion of vesicles involved in membrane traffic. Precise budding and fusion are essential in the secretory pathway; for example, a vesicle carrying newly synthesized lysosomal enzymes from the TGN must be targeted for fusion with the pre-lysosomal compartment rather than with the endoplasmic reticulum. This specificity can be explained by the distribution of crucial proteins that provide molecular addresses. In particular, members within some large protein families are targeted to specific membranes, conferring unique addresses. Two families that are particularly informative for exocytic vesicles are the SNAREs and the Rabs. SNAREs promote membrane fusion [48,49]. Each SNARE is targeted to one, or a small subset, of the cell's organellar membranes [50]. The distribution of SNAREs suggests that gene duplication within this family proceeded hand-in-hand with compartmental differentiation during eukaryotic evolution [51].

The sites of action of many SNAREs have been defined, especially in fungi and animals. One crucial observation is that the SNAREs associated with a specific compartment, across the animal and fungal lineages, are more closely related to one another than they are to those associated with another compartment [49,52]. For example, SNAREs associated with the TGN in *Saccharomyces cerevisiae* are more similar in sequence to TGN-localized SNAREs in *Homo sapiens*, than to SNAREs associated with other compartments in either yeast or humans [52]. This relationship implies that the TGN, and its associated SNAREs, were inherited in animals and fungi from a common ancestor in which this compartment was already present. Whereas all SNAREs are *homologs* of one another, the TGN-localized SNAREs in yeast and humans are further defined as *orthologs* because they exhibit a vertical descent from a common ancestor. Additionally, they maintain the same function. A phylogenetic representation is shown in Figure 1b where, in each case, genes from humans and yeast are found in clades consistent with the species tree, which is indicative of descent from a common ancestor.

Rabs, which are regulatory GTPases, represent a second family of compartment-specific proteins [53–56]. In animals, several Rabs associated with DCGs have been characterized [57,58]. Genome analysis reveals that the number of Rabs encoded in the *Tetrahymena* genome is similar to that of animals Rabs [46]. Such great diversity in the Rab family of a unicellular eukaryote might reflect the cell biological complexity of ciliates [59]. A subset of the ciliate Rabs is associated with granules [60]. An important question relating to the evolution of DCGs is whether granule-associated Rabs in ciliates are orthologous to DCG-associated Rabs in animals or, alternately, if they were independently recruited to a shared role in granule function. Although the granule-associated Rabs in ciliates have not yet been isolated, it is possible to ask whether the *Tetrahymena* genome includes any Rab that is closely related to the DCG-associated Rabs in animals. Importantly, phylogenetic analysis indicates that none of the *Tetrahymena* Rabs can be considered orthologous to the DCG-associated Rabs in animals (see Figure S7 in Ref. [46]). Given this result, we hypothesize that granule-associated Rabs in *Tetrahymena*

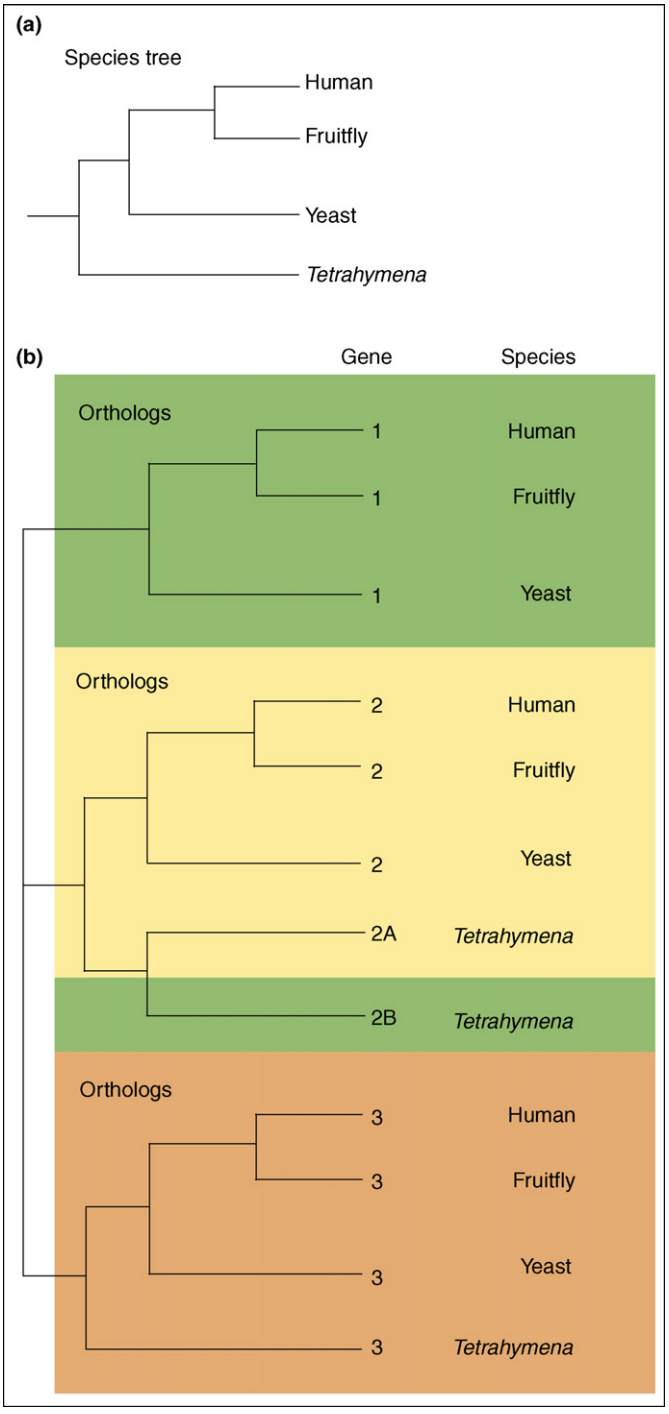

Figure 1. Recognizing patterns of convergent evolution in phylogenetic trees. **(a)** A simple phylogeny showing the evolutionary relationship between several distantly related species. Because of long divergence times, this arrangement generally holds up for comparisons of any gene shared by these species. **(b)** A phylogenetic tree of a hypothetical, three-gene family highlighting orthologous relationships between genes, which in most cases perform the same function for each species (green, orange and yellow boxes). The green boxes are consistent with an example of convergent evolution of gene function. The differences in phylogenetic arrangement in comparison to the species tree are consistent with the independent evolution of an equivalent function as shown in green. Importantly, proper interpretation of such trees depends on the inclusion of complete gene families and assumes that extensive gene loss has not masked the true relationships between orthologous groups of genes. Also, the possibility of accelerated divergence in a lineage-specific fashion needs to be considered when proposing a hypothesis of functional convergence. Adapted from Ref. [71].

will instead be found in a phylogenetic clade restricted to ciliates and perhaps other close relatives. A representation of this arrangement can be found in Figure 1b (green boxes). For simplicity only one *Tetrahymena* gene is shown in green, however, orthologous genes from related species would also be in this region of the tree as opposed to the larger green box (Figure 1b). Such lineage-specific phylogenies support a hypothesis of independent innovation rather than shared inheritance (see Figure 8 in Ref. [12]). Significantly, recent phylogenetic analysis of *Paramecium* SNAREs is also consistent with our hypothesis [61]. For compartments that were likely to be present in an early eukaryotic ancestor, such as the Golgi complex, the associated human and *Paramecium* SNAREs cluster as orthologs. However, there are no *Paramecium* orthologs for human DCG-associated SNAREs. This suggests that the DCG-associated SNAREs in *Paramecium* will be found in ciliate-specific clades. Confirmation of these predictions with functional analysis would provide strong evidence that the DCG-like vesicles in ciliates are indeed related to those in animals by convergent evolution, rather than by inheritance from a common ancestor.

Detecting convergent evolution in cellular pathways

Functional convergence and the acquisition of new genes involved in shaping cell biological pathways might be more widespread than currently appreciated. Identifying cases of convergence at the level of cellular structures will be facilitated by the sequencing of genomes from divergent eukaryotes to generate more informative phylogenetic trees, in addition to functional analyses now possible in many non-model organisms. From the phylogenetic analysis of protein families associated with cellular processes, patterns consistent with an independent acquisition of cellular functions can become apparent, even for comparisons of divergent lineages (Figure 1). In addition to phylogenetic analyses, it is crucial to confirm the shared functions of genes suspected of functional convergence, because lineage-specific expansions of gene families could also reflect the evolution of novel protein functions and, therefore, give a false signature resembling convergence. The case of clathrin-mediated endocytosis described in Box 1 is an example that depended on both phylogenetic and functional analysis, in a highly divergent lineage, to uncover functional convergence [12].

Certain cellular pathways might be more prone to convergent evolution than others. For the pathway of DCG formation, the fundamental requirement might simply be the presence of one or more proteins, targeted to the secretory pathway, which assemble into large oligomers to form protein cores, and perhaps also interact with the membrane [62,63]. This idea is consistent with experiments in which a protein that forms the dense cores in mammalian endothelial DCGs was heterologously expressed in tissue culture cells that do not normally make granules [64,65]. Remarkably, these transfected cells accumulated vesicles with dense cores, suggesting that the core protein induced the formation of its own specialized vesicle. These results implied that the capacity to make granules was inherent in the basic organization of the Golgi complex and TGN, because it could also occur in such non-specialized tissue culture cells. If the formation of granules from the TGN does not require a specialized cytoplasmic machinery and can be driven by individual lumenal proteins, we posit that numerous mutations over evolutionary time could have resulted in the induction of primitive DCGs. Under this scenario, granules arose independently in different lineages as a consequence of lineage-restricted mutations in the secretory proteins themselves. The specific targeting and regulated exocytosis of such granules would have depended on independent radiations and functional divergence within gene families, such as the Rabs and SNAREs. An interesting issue is the extent to which ciliate and animal granules, notwithstanding their putative independent origins, would have been constrained to rely on similar cytosolic machinery for membrane remodeling during maturation.

Why convergent evolution matters

Generally speaking, searching for the conservation of molecular features including genes, their functions and mechanisms has become a major biological pursuit [66]. In particular, most studies in cell biology make inherent assumptions that all conserved factors are inherited by divergent evolution. Such assumptions obscure our understanding of the diversity of mechanisms underlying cellular processes and the flexibility built into these systems. For example, current data on dense core granules do not support the notion that the conserved morphological and biochemical features in this class of vesicles exclusively reflects common ancestry. Instead, both molecular genetic and evolutionary analysis suggest that this pathway might have been acquired independently by several eukaryotic lineages. Our hypothesis might be relevant to the entire set of eukaryotic lineages in which secretory granules, or granule-like structures, have been noted. Further study will help uncover the biological richness and diversity of mechanisms honed by natural selection to produce this important class of cellular compartments.

The traditional emphasis on divergent evolution might be due, at least in part, to a historical focus on a small number of model organisms and a failure to appreciate the rapid and extensive changes that occur in genomes. Because the currently fashionable model organisms are in actual fact relatively closely related to one another (see Figure I in Box 2; note proximity of fungi and metazoans), they represent an incomplete sampling of cellular diversity. Understanding the origins of organelles in more distant lineages, such as those of ciliates, provides a more comprehensive appreciation of evolutionary history. Rather than reflexively invoking shared ancestry, rigorously establishing convergence in a basic cellular process can illustrate the importance of shared selective pressures on the conservation of complex and fundamental phenotypes. Such considerations are also important for studies of closely related species because genes and genomes evolve rapidly, on an evolutionary time scale much shorter than the divergence between ciliates and animals [67]. New genes have arisen in the genomes of humans [68], *Drosophila* [69] and plants [70] within the past few million years. Novel gene interactions are also likely to form on a similar time scale. Taken as a whole, these observations suggest

that genetic systems controlling cellular diversity can evolve rapidly and independently, increasing the probability of generating similar cellular phenotypes in different organisms.

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