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Essay

The evolution of animal cell motility

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Eukaryotic cells use a number of diverse mechanisms to swim through liquid or crawl across solid surfaces. The two most prevalent forms of eukaryotic cell motility are flagellar-dependent swimming and actin-dependent cell migration, both of which are used by animal cells and unicellular eukaryotes alike. Evolutionary cell biologists have used morphological and molecular phenotypes to trace the evolution of flagellar-based swimming. These efforts have resulted in a large body of evidence supporting a single evolutionary origin of the eukaryotic flagellum, an origin that dates back to before the diversification of modern eukaryotes. Actin-dependent crawling, in contrast, involves mutiple distinct molecular mechanisms, the evolution of which is just beginning to be explored.

Cell motility is vital to nearly every aspect of our lives. Before we are born, our fathers' sperm swim in search of an egg. Our embryonic development relies on the concerted movement of sheets of cells that move together, as well as individual cells, like primordial germ cells, that venture out on their own to find their destined developmental niches. Our health depends on the rapid motility of white blood cells that rush to sites of injury and hunt down pathogens. Even our deaths are often a consequence of cell movement, from the invasion of infectious parasites to the migration of metastatic cancer cells.

The cell locomotion that underlies these critical behaviors can be divided into two main categories: flagellar motility and actin-dependent cell migration (Figure 1). Flagellar motility is powered by whip-like organelles called flagella that propel cells through liquid or induce flow across surface-attached cells. Our lungs, for example, rely on the wave-like beating of flagella — in this context called cilia — to clear airways. In contrast, actin-dependent cell migration is driven by a variety of molecular mechanisms, all reliant on the dynamic turnover of actin networks that push and pull cells across or between solid surfaces [1–6].

Flagellar motility and actin-based cell migration are not limited to humans, or even to animals; although the cells of other multicellular organisms like plants and fungi are sessile, many of their unicellular relatives swim and crawl from place to place [7,8]. The pervasiveness of cell motility across the eukaryotic tree raises interesting questions as to how and when these complex cell behaviors evolved. Here I discuss what we know — and, more importantly, what we don't know — about the evolution of eukaryotic cell motility. Although eukaryotes display an impressive diversity of mechanisms used for cell locomotion, here I focus on actin-dependent cell migration and flagellar motility because of their ubiquity across the eukaryotic tree, as well as their importance to human health.

Cell motility is not limited to animals

Many single-celled organisms rely on cell motility for the same basic functions as our own cells, from sexual reproduction to hunting bacteria, as well as for their own unique purposes such as evading predation. Unlike human cells, however, unicellular microbes are not limited to flagellar swimming and actin-based cell migration. Indeed, single-celled organisms move by a wide variety of distinct, and inventive, mechanisms [7]. For example, many bacterial species use propeller-like appendages to swim, while others pull themselves along using molecular machines that function like grappling hooks [9]. Some species of bacteria have even been suggested to move by exuding glycosylated proteins that swell as they absorb water to propel the cell forward like a rocket engine.

There is diversity among eukaryotic microbes, too. Some species of ciliates bundle multiple cilia together and sweep them through water like Viking ships, or use them as legs to crawl along leaf blades, while species of alveolates build molecular tracks

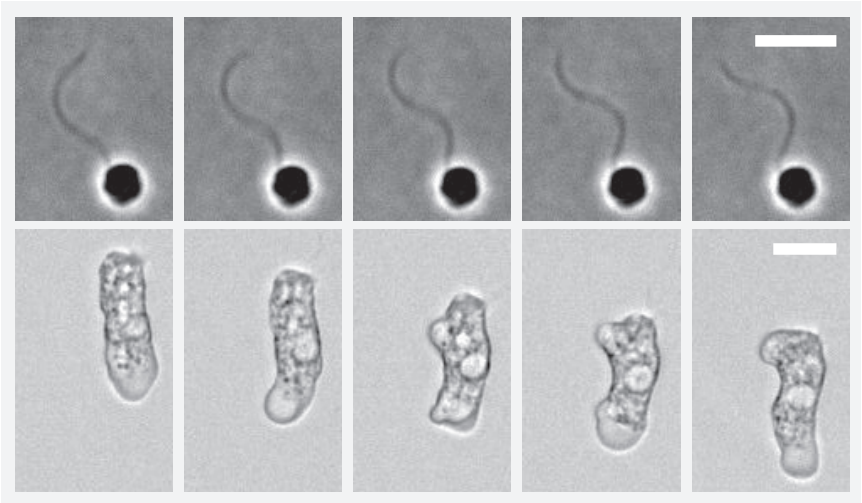

Figure 1. Flagellar-based swimming and actin-dependent crawling are the two predominant forms of eukaryotic cell motility.
(Top) Species from every branch of the eukaryotic tree use flagella to swim through liquids. Some cells, including human sperm and the *Batrachochytrium dendrobatidis* fungal cells shown here, use flagella to push cells. Other cells, like *Chlamydomonas* and *Naegleria* flagellates, use flagella to pull themselves through liquid. Flagellar motility is often very rapid; these cells were imaged once every millisecond. (Bottom) The other predominant form of eukaryotic cell motility is the actin-dependent crawling motility used by cells to crawl across or between solid surfaces. In contrast to the rigid and stable microtubules that are the basis of flagellar motility, crawling motility relies on the dynamic turnover of ephemeral actin polymer networks. Many eukaryotic species take advantage of the ability to crawl on solid surfaces *and* swim through liquids, including this *Naegleria gruberi* cell (here imaged every 2 seconds) that eats and replicates as a crawling amoeba, but under stress can differentiate into a swimming flagellate. Both scale bars represent 5 μm.

and trundle over them like trains in an actin-dependent form of cell motility that is distinct from cell crawling [10]. In fact, cells move using nearly every mechanical system you can imagine, and similar-seeming mechanisms can be found scattered across the tree of life [3,7,11,12].

Included within this remarkable diversity are unicellular organisms that appear to move using mechanisms related to those used by human cells. The most obvious parallels are the sperm-like swimming of flagellated protists and the crawling motility of amoebae that is nearly indistinguishable from the movements of our white blood cells (Figure 1). Such similarities raise a profound question: are these behaviors, central to the function of human and microbial cells alike, evolutionarily related, or are they the result of convergent evolution? It turns out that the answers to these questions are complex and depend upon the form of motility being studied.

The evolution of eukaryotic cell motility in the context of multi-functional cytoskeletons

Cell motility did not evolve in a void, and any serious attempt to trace its evolutionary history must take into account the relevant cellular context. Eukaryotic cell motility is driven by dynamic cytoskeletal polymer systems, particularly microtubules, which form the core of eukaryotic flagella, and actin, which powers crawling motility. These polymers are found in all eukaryotic cells and were undoubtedly present in the last common eukaryotic ancestor alongside key cytoskeletal regulators [13,14]. Actin and microtubule networks also play roles in a variety of other essential cellular activities, from nutrient uptake and intracellular trafficking, to cell polarity and separating chromosomes during mitosis. The ubiquity and diversity of these vital functions mean that actin and microtubule cytoskeletal networks are conserved whether or not cells use them for motility.

Although genes encoding actin and tubulin monomers have long been attributed to the last common eukaryotic ancestor, it was not until relatively recently that distant homologs of these proteins were discovered in bacteria [13]. The relationships between these ‘bacterial actins’ and ‘bacterial tubulins’ and their eukaryotic counterparts are too distant to be detected at the sequence level and were only revealed by comparing their 3D protein structures (reviewed in [13]). The discovery of bacterial actins and tubulins, as well as additional homologs in archaea, implies that the evolution of these polymers predates the origin of eukaryotes. Despite their ancient roots, however, the diversification of tubulin and actin polymer networks remains a hallmark of eukaryotic cell biology.

Eukaryotic cells assemble actin and microtubule polymers into a huge variety of distinct structures that facilitate a wide array of cellular processes. Cell biologists have identified hundreds of cytoskeletal regulators that control specific biochemical functions, including nucleators that initiate the formation of new polymers, proteins that enhance or inhibit polymer elongation, factors that induce polymer severing, bundlers that connect polymers together, and motors that walk along polymers. Compared with actin and tubulin, whose protein sequences are strikingly well conserved across eukaryotes, the evolutionary histories of cytoskeletal regulators are highly variable [13]. Some regulators are only found in individual eukaryotic lineages, while others are broadly distributed. Some regulators share nearly identical protein sequences in distant taxa, while others have diverged greatly. The resulting sequence variability provides useful information with which to trace the evolution of the individual cytoskeletal regulators along with the phenotypes they encode, including cell motility. Because the field has come to a strong consensus about the evolutionary history of flagellar motility, I will begin there.

The evolution of flagellar-based swimming motility

Cells from each of the three domains of life swim using whip-like appendages, all of which have been called flagella. Despite sharing a name, cellular function, and overall form, bacterial, archeal, and eukaryotic flagella represent clear examples of convergent evolution (reviewed in [11]). Bacterial and archeal flagella are both assembled on the outside of the cell from unrelated proteins that are, unfortunately, both called flagellins. Eukaryotic flagella, on the other hand, are assembled beneath the cell membrane from microtubules and hundreds of associated proteins. In addition to being built from unrelated components, bacterial, archaeal, and eukaryotic flagella are also powered by distinct motors. Bacterial and archeal flagella are powered by rotary motors at the base of the flagellum that cause the flagellum to twist. Eukaryotic flagella, in contrast, are powered throughout their length by the movement of dynein motors that cause microtubules to slide past each other and the entire flagellum to bend.

Although distinct from bacterial and archaeal flagella, there is a general consensus that eukaryotic flagella evolved once, before the radiation of extant eukaryotes [11–13]. This means that eukaryotic flagella were present in the ancestor of all living eukaryotes and that the current distribution of flagella among eukaryotic lineages results from loss over evolutionary time. For example, organisms like yeast, *Arabidopsis*, and *Dictyostelium* that do not have flagella evolved from ancestors who did, and they have since lost the genes used specifically for flagella. The idea of a single ancestral origin of eukaryotic flagella is supported by two lines of evidence: morphological similarity and molecular phylogenetic analyses.

For decades, cell biologists have used electron microscopy to observe eukaryotic flagella and basal bodies, the organelles at the base of the flagellum that organize flagellar microtubules. Since the beginning, biologists have noted that cross-sections through flagella and basal bodies are not only staggeringly complex, but also strikingly similar across distant phyla [12]. This conservation of structural complexity was the first line of evidence that eukaryotic flagella were most likely conserved through evolutionary time, i.e. that they are homologous.

The second major line of evidence comes from molecular phylogenetic analysis of flagellar components from species that span the eukaryotic tree. Based on large-scale, multi-gene phylogenetic analysis, eukaryotes can be divided into somewhere between six and ten major groups, each of which contain species with microtubule-based

flagella (Figure 2). Genetic, genomic, and proteomic analyses of flagellar composition across a wide diversity of eukaryotic lineages has made it clear that eukaryotic flagella from all lineages share dozens of proteins (*e.g.* [15–17]). Remarkably, many of these proteins are *only* found in species that have flagella, and appear to be used *only* for flagella [15]. There are two obvious possible hypotheses about the evolutionary history of flagella-specific genes: flagella-specific genes were vertically inherited and conserved only in lineages that have retained flagella; or flagella-specific genes were traded across the eukaryotic tree by horizontal gene transfer. Phylogenetic analysis of flagella-specific proteins results in trees that have similar topologies to the topology of eukaryotic species trees (*e.g.* [18]). The vertical inheritance of flagella-specific genes, coupled with the sheer number of flagella-specific genes absent from lineages that do not build flagella (currently in the hundreds [19]), provide overwhelming support for a single evolutionary origin of eukaryotic flagella.

Because eukaryotic flagella all share a common ancestor, the next obvious question is how and why did the eukaryotic flagellum evolve? Cell biologists have hypothesized that eukaryotic flagella evolved by processes ranging from symbiotic bacterial associations (an idea now believed to be highly unlikely), to a viral origin [20], to gradual accumulation of complexity [12,21,22]. Although they all remain provocative, testing any of these hypotheses appears, as of now, to be an intractable problem. It is impossible to place the origin of flagella within the eukaryotes because all eukaryotes have flagella or evolved from an ancestor that did. Moreover, bacteria and archaea do not have homologs of flagella-specific genes. This leaves us without a useful outgroup to use for determining which evolutionary origin is most likely, a deficiency that may be remedied by the isolation and sequencing of additional microbial lineages from all three domains of life.

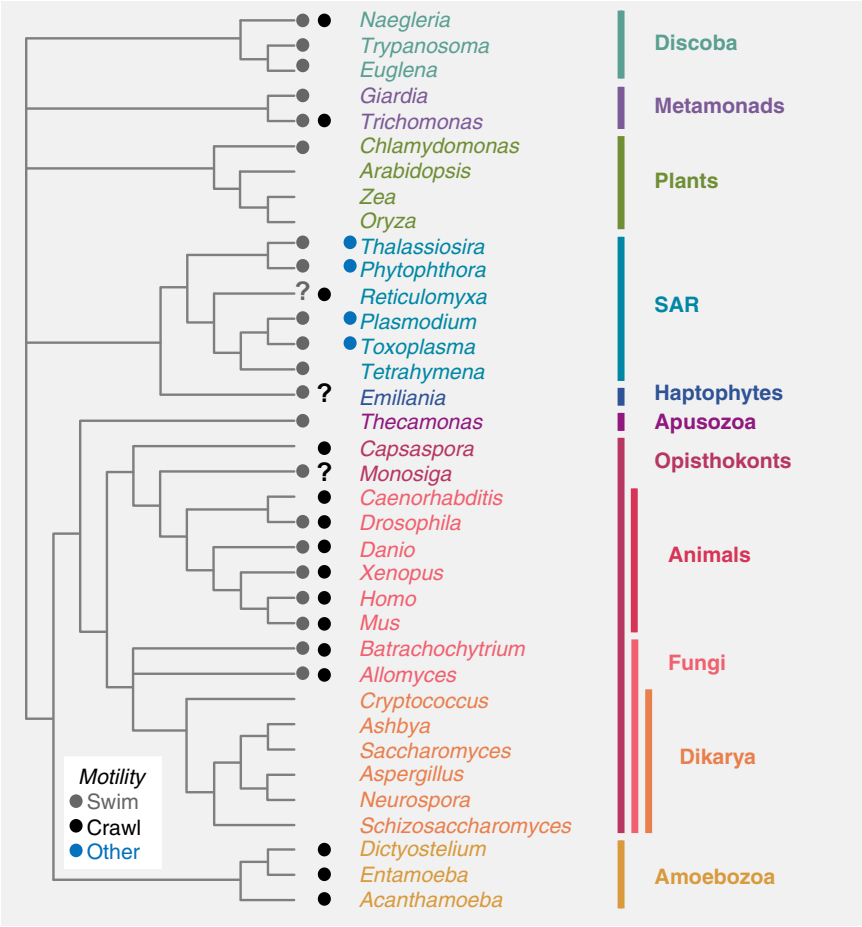

Figure 2. Cells from diverse eukaryotic phyla crawl and swim.
This diagram illustrates the phylogenetic relationships between selected species of animals, plants, fungi, and unicellular eukaryotes, many of which have motile cells. Gray circles indicate species with cells that swim using microtubule-based flagella, while black circles highlight species with at least one form of crawling motility. Blue shows the presence of another form of motility. This list is by no means exhaustive; many other eukaryotes have motile cells, and cell motility can take additional forms. Diagram adapted from [14].

Crawling motility can be driven by diverse molecular mechanisms

We can use the evolution of flagella as an intellectual framework for studying the evolution of crawling motility by determining what, if any, structures are shared between cells that crawl, and ascertaining the evolutionary history of genes used specifically to build and control these structures.

Before that, however, let's explicitly define what we mean by 'crawling motility'. Crawling motility is any form of cell motility that involves the following three activities: a pushing out of the membrane at the front of the cell to provide forward movement; contraction of the rear of the cell to keep the cell from simply flattening out indefinitely; and interaction with one or more surfaces to provide traction, either through molecular adhesions [23], or by pushing against multiple surfaces (e.g. [1,2,24]). Because it is the source of forward movement, crawling motility is often defined by the molecular mechanism used to push out the plasma membrane. This protrusive force can stem from a wide variety of molecular mechanisms, many of which rely on actin polymerization (Figure 3).

Although cells can employ multiple distinct mechanisms to push out their leading edges, the most heavily studied is the actin-dependent mode used by animal cells, *Dictyostelium*, and other microbial eukaryotes. This type of crawling motility is often called 'lamellipodial', or 'mesenchymal' motility after the assembly of flat, sheet-like lamellipodia at the leading edge of mesenchymal animal cells. Lamellipodia are built by the Arp2/3 complex, which

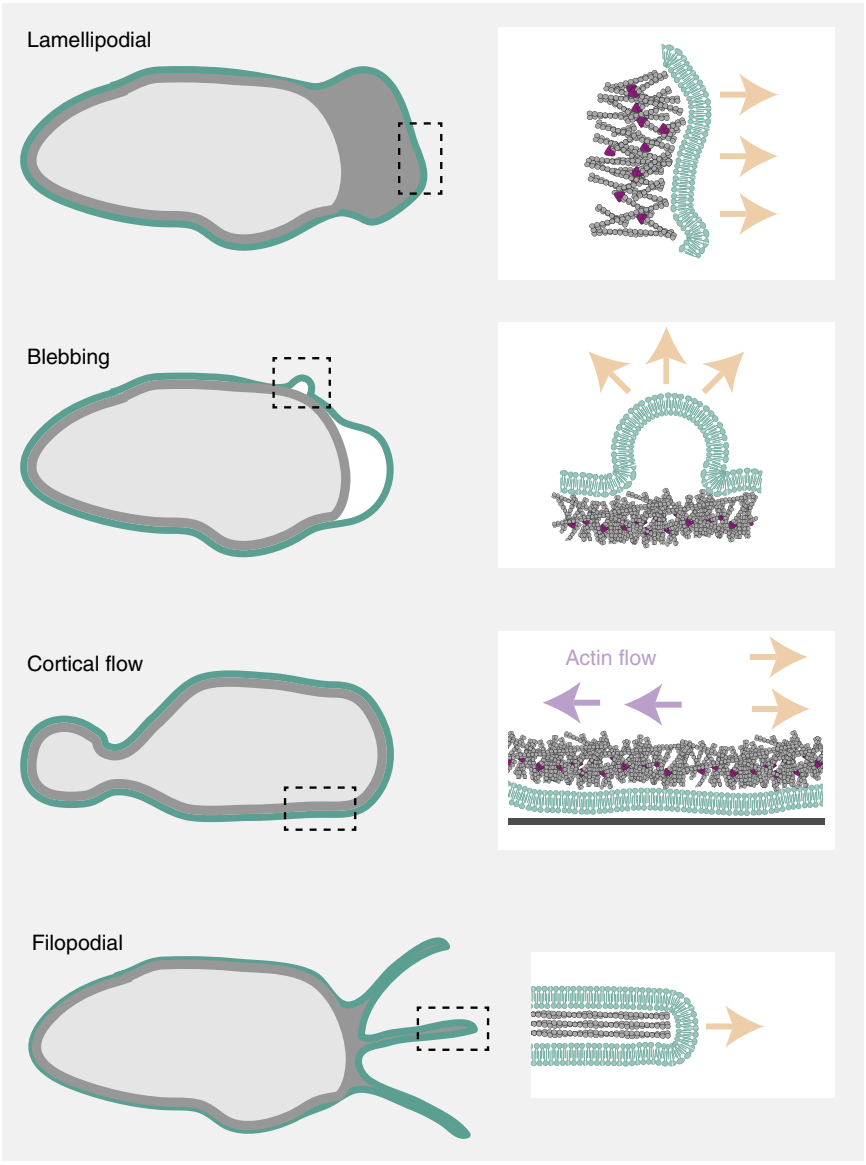

Figure 3. Cells can crawl using several mechanistically distinct modes of actin-dependent cell migration.
Although each molecular mechanism is unique, all modes of actin-dependent cell motility rely on dynamic actin networks to produce net displacement of the plasma membrane (yellow arrows). **Lamellipodial motility** relies on branched actin networks nucleated by the Arp2/3 complex. Elongation of these filaments pushes the front edge of the cell membrane forward. **Blebbing motility** results from dissociation of the plasma membrane from an underlying layer of actin called the 'actin cortex'. Intracellular pressure then pushes out the plasma membrane to form a blister-like 'bleb'. The actin cortex assembles under the bleb and the cycle repeats. **Cortical flow** arises when myosin motors contract actin networks at the rear of the cell, pulling the actin cortex backwards (purple arrows). Similar to how the backwards push on tank treads propel vehicles forward, cortical flow can result in forward movement of the cell. **Filopodial motility** uses actin bundles called filopodia that grow by adding actin monomers to their tips. Some cells build filopodia in conjunction with lamellipodia, while other cells use filopodia alone to move.

builds branched polymer networks by nucleating new actin polymers on the side of pre-existing actin filaments. Growth of this branched actin network pushes out the leading edge of the plasma membrane. Cells without Arp2/3 do not make lamellipodia [25], meaning that Arp2/3 activity is a requirement for this form of cell migration, although cells with mutations in Arp2/3 still move using other mechanisms [25].

Normal cells from diverse species can also push their leading edges forward using Arp2/3-independent mechanisms (Figure 3). These alternative mechanisms often rely on other actin nucleators, particularly the evolutionarily ancient and highly diversified formin family proteins [26] that sometimes cooperate with WH2-domain actin nucleators [27]. Some human cells and single-celled amoebae, for example, can move using cortical actin networks that hug the plasma membrane, either through sequential delamination of the membrane by blister-like blebbing [4,5], or through activating myosin-mediated flow of the actin cortex while pushing against surfaces [1,2]. Cells can also push their membranes forward using long actin bundles called filopodia [8,25]. Adding yet another layer of complexity, many cells mix and match these types of actin-based motility by either adopting multiple migration modes simultaneously or by switching between modes depending on their particular environment and/or cell state [4,5,28].

The evolution of crawling motility

Despite relying on overlapping subsets of actin regulators, each mode of actin-dependent crawling motility relies on molecularly distinct mechanisms. Therefore, to trace the evolution of actin-dependent cell motility, we need to: define distinct modes of cell motility, determine which eukaryotic species use each mode, and identify the actin regulators specific to each mode. Only then can we begin to unravel the evolutionary history of the phenotypes these regulators encode.

When we overlay examples of cells known to use Arp2/3-dependent crawling motility onto the eukaryotic species tree, we see this mode of motility spread across many lineages [3]. This phylogenetic distribution makes it tempting to infer that, like flagellar motility, Arp2/3-dependent crawling motility is ancestral and has been retained and lost in various lineages. But making this assumption without additional evidence would be a mistake because the branched

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actin networks that drive this form of crawling motility are *also* used for other cell functions, particularly endocytosis. It is therefore possible that cells across the eukaryotic tree maintain Arp2/3-dependent branched actin networks to facilitate endocytosis, and have co-opted them for cell migration multiple times throughout eukaryotic history.

To discern between these two scenarios — convergent evolution of Arp2/3-dependent motility or vertical inheritance of an ancestral behavior — we must first identify genes that are *unique* to this form of cell migration and determine their evolutionary history, as we have done for flagellar-specific genes. Along these lines, a study set out to identify genes likely used specifically for Arp2/3-dependent cell migration, by employing phylogenetic profiling to identify genes conserved *only* in the genomes of diverse species that use this mode of cell motility [29]. A follow-up analysis provided the first predictive molecular signature of crawling motility — the evolutionary retention of two Arp2/3 activators, SCAR and WASP [3]. This finding is consistent with a single evolutionary origin of Arp2/3-dependent motility and its vertical inheritance, but does not prove it. Because cell motility relies on the coordinated activity and localization of many dozens of proteins that interact in complex regulatory networks, understanding the evolution of this complex cell behavior will require layering phylogenetic evidence from more than just one or two genes [8].

Other forms of crawling motility are clear examples of convergent evolution. The most obvious example of a distinct molecular mechanism for crawling motility is the amoeboid sperm of *Ascaris suum* nematodes [30]. Nematodes have lost the genes needed for motile flagella, and the sperm of these animals do not swim. Instead, *Ascaris* sperm cells crawl. The crawling of *Ascaris* sperm appears strikingly similar to the motility of fish keratocytes and other cell types that assemble actin polymer networks to push forward the leading edge. Despite the morphological similarity to actin-dependent crawling motility, and the presence of actin in the *Ascaris* genome, *Ascaris* sperm do not use actin polymers for motility. Rather, *Ascaris* sperm push the membrane forward using analogous networks assembled by major sperm protein, an unrelated polymer system that is controlled by kinase-mediated phosphorylation (reviewed in [30]). This makes *Ascaris* sperm motility a clear-cut example of convergent evolution, and highlights the obvious problem with using *Ascaris* sperm cells to infer anything about the evolution of actin-dependent cell motility. For tracing the evolutionary history of cell motility and other complex cell behaviors, morphological similarity is not enough. Although this is conceptually straightforward for *Ascaris* sperm, other types of crawling motility are less obviously distinct. If we want to understand the evolution of these important phenotypes, we must first determine the molecular mechanisms underlying the motility of evolutionarily diverse cell types.

Moving forward

Although we are well on our way towards understanding the evolution of swimming motility, the evolutionary history of crawling motility remains largely unexplored, despite its central role in the lives of humans and eukaryotic microbes alike. This is, in part, due to the diversity of molecular mechanisms that give rise to crawling motility as well as the overlap between actin networks used for cell migration with other cell functions [8,14]. To overcome these barriers, we need to determine which species use which molecular mechanisms and identify the genes that are unique to each mode of motility in a wide diversity of species. With these genes in hand, we can easily trace the evolutionary history of these behaviors using comparative genomics and phylogenetic analysis.

There remains, however, a serious practical hurdle to understanding how and when cell motility evolved: the limited information we have on diverse eukaryotic species. Cell biologists are just beginning to explore the diversity of cell motility among animal cells, and there is much to discover about the motility of other eukaryotes, particularly those that inhabit the large swaths of the eukaryotic tree for which we have no experimentally tractable species to study. The development of inexpensive and rapid genome sequencing, along with easily portable CRISPR/Cas9 gene-editing technology, is quickly opening the door to molecular hypothesis testing in a wide array of species. Reverse genetics, however, is not useful for identifying novel and unexpected genes. For this we need to continue to invest in the development of forward genetics and/or high-throughput biochemical screening methods in species across the eukaryotic tree.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two videos and can be found with this article online at https://doi.org/10.1016/j.cub.2020.03.026.

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Primer

Protocells

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The cell is the basic unit of life as we know it. But are cells truly necessary for life? To probe this question, one may start with NASA's 'working definition' of life that is now widely used: a self-sustaining chemical system capable of Darwinian evolution. The term 'self-sustaining' encompasses many interesting aspects, such as metabolism and environmental driving forces (e.g., diurnal cycling). This aside, an information-bearing molecular system that replicates should meet this definition of life, since errors (mutations) are an inevitable feature of real chemical systems. Artificial molecular replicators have been made from RNA, DNA, peptides and even small molecules, albeit with varying potential for variation and evolution.

Considering life on Earth, it is parsimonious, experimentally expedient and increasingly plausible to consider RNA as the basis for a simple kind of life, given its remarkable capacity to both store information and serve as a biocatalyst. Imagine that writing about a horse would actually create a horse from those letters (Figure 1) — such is the wondrous nature of RNA. The idea that life began with RNA (the 'RNA World') has been extensively debated and reviewed. The great scientific utility of this concept is to provide a concrete, experimentally attainable vision of a primitive life form: a system of RNAs with catalytic activities (ribozymes) that enable sequence replication and any necessary metabolic functions. The complexity needed in such a system depends on the richness of the environment provided. For example, an RNA-dependent RNA polymerase (an RNA replicase) might fulfill NASA's definition of life if the environment could supply nutrients such as nucleotides, primers and Mg^{2+}. More pointedly, a couplet of mutually dependent RNA ligases, each catalyzing formation of the other, undergoes Darwinian evolution as demonstrated by Lincoln and Joyce in 2009. So, why do we need cells?

There are at least two fundamental reasons why cells are important to life. First, they serve as a semi-permeable barrier for nutrients and waste products while keeping the molecular genome and the metabolism of an organism linked (Figure 2). To understand the second reason, consider an RNA replicase whose sequence arises by chance (e.g., by non-enzymatic polymerization) in an environment conducive to self-replication. If the replicase diffuses freely in solution, it encounters other RNAs and copies them, but no other molecules copy the replicase. There may be some non-specific copying of the replicase, but its activity actually creates a selection pressure for good templates, not good catalysts. Such situations result in parasitic sequences overwhelming the selection (Figure 2), and the altruistic replicase disappears.

The first in vitro molecular incarnation of such evolution occurred in Sol Spiegelman's famous Qβ replicase experiment. The Qβ replicase is a protein enzyme (from phage) that replicates the RNA that encodes it. When provided with an in vitro environment enabling replication and translation, truncated replicase mutants that lacked enzymatic activity but served as preferred templates arose and accumulated. Eventually, Spiegelman's 'monsters', unable to replicate themselves, drove the system to a halt. This is indeed Darwinian evolution. But it is not particularly interesting on its own. Biological evolution on Earth has exhibited tremendous creativity and innovation, which can be termed 'open-ended evolution'. A minimal requirement for such evolution is to prevent parasites from crashing the system. There are many mechanisms that select for cooperative traits, and one such trait that is available to even simple molecular systems is compartmentalization. By physically separating different genomes from each other, cells create a new unit of selection. Cells containing parasites