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SPOTLIGHT

# Moving simply: *Naegleria* crawls and feeds using an ancient Arp2/3-dependent mechanism

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**Arp2/3-nucleated actin filaments drive crawling motility and phagocytosis in animal cells and slime molds. In this issue, Velle and Fritz-Laylin (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202007158) now show that *Naegleria gruberi*, belonging to a lineage that diverged from opisthokonts around a billion years ago, uses similar mechanisms to crawl and phagocytose bacteria.**

The cytoskeleton, a dynamic polymer meshwork, plays a critical role in the control of cell shape, polarity, division, motility, and phagocytosis in eukaryotic cells (1). It is hardly a surprise that for decades, the protein constituents of the cytoskeleton—in particular the polymer-forming actin and tubulin protein families—were believed to be a "hallmark" of eukaryotic cellular organization, a key defining feature that sets eukaryotes apart from the bacterial and archaeal domains of life. We now know that the actin and tubulin superfamilies are in fact ubiquitous (2) across cellular life on the planet, and eukaryotes likely inherited bona fide actin and tubulin (3), along with a small number of their regulators (4), from their archaeal ancestors around two billion years ago.

However, a dramatic increase in complexity accompanied the transition from prokaryotic to eukaryotic cellular organization. Cellular processes that rely on the cytoskeleton, such as motility and phagocytosis, are achieved through the combined action of hundreds of interacting network components (6) as they self-organize and respond to external cues, and they cannot be explained simply by the acquisition of genes encoding key regulators (5,6).

Given this degree of complexity, how should one study the origin and core organizing principles of cytoskeletal function? Perhaps the only way is by actually assessing cellular phenotypes in a range of experimental models, looking beyond animals and fungi. *Naegleria* is an excellent candidate as it occupies a key position in the eukaryotic tree and has few if any cytoplasmic microtubules in interphase, existing in this state as a rapidly crawling, phagocytosing amoeboid cell. The *Naegleria gruberi* genome (7) was found to encode dozens of actin genes and the full complement of actin regulators—but how does it use them?

In a paper published in this issue of *JCB*, Velle and Fritz-Laylin set out to experimentally probe the *Naegleria* interphase actin cytoskeleton (8). They identify actin-dependent lamellipodia-like ruffles using super-resolution and electron microscopy that are almost entirely abolished by treatment with the Arp2/3 inhibitor CK666. CK666-treated cells also migrated slower and phagocytosed bacteria less effectively than the controls. These results highlight a conserved role for Arp2/3-dependent actin polymerization in *Naegleria* crawling motility and phagocytic activity, a phenotype shared with diverse amoeboid species spanning at least a billion years of evolution, including animals and amoebozoa (Fig. 1).

However, these experiments also serve to underscore the complexity and adaptability of dynamic actin networks and the difficulties of studying them using small molecule inhibitors. Across their experiments, Arp2/3 inhibition using CK666 only partially phenocopied the complete disruption of actin polymerization using Latrunculin B. CK666-treated cells were still able to migrate, albeit slower, and their ability to phagocytose bacteria was only partially compromised.

Are formins then somehow compensating for the absence of branched actin? Based on the use of a small molecule inhibitor, not entirely; cells treated with SMIFH2 exhibited strong defects in directional persistence during migration, but generated lamellipodia and phagocytosed GFP-labeled bacteria just fine. Treatment with both SMIFH2 and CK666 produced no synergistic effects. Moreover, Arp2/3-depleted cells produced long, filopodia-like structures, not seen in unperturbed cells, that were unaffected by the addition of SMIFH2. This implies that either these drugs do not function as expected in *Naegleria*, or that these actin structures are not nucleated by formins either. What is responsible for generating and sustaining these filopodia? There are many possibilities. Arp2/3 inhibition in other systems can drive actin networks to compensate in similar ways (9); other nucleators (e.g., Spire) could come into play; the cells might respond by blocking any further

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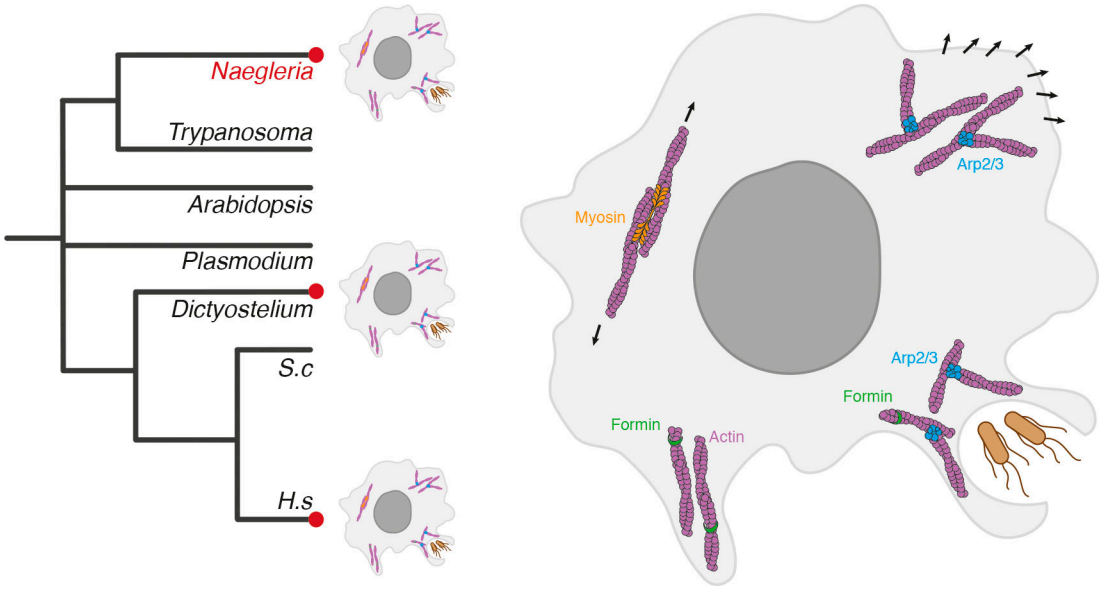


Figure 1. **Representative eukaryotes spanning an estimated one billion years of evolutionary time (H.s, *Homo sapiens*; S.c, *Saccharomyces cerevisiae*).** Red dots and accompanying cell schematics indicate evidence for crawling motility and phagocytosis regulated by Arp2/3-dependent actin networks in amoeboid cell types. On the right, a schematic of a crawling and phagocytosing amoeboid cell highlighting branched, bundled, and contractile filaments.

turnover of filaments (10); and "debranching" of preexisting Arp2/3-nucleated actin networks and bundling can also drive the formation of actin spikes (11).

In a sign that the *Naegleria* actin cytoskeleton probably has other surprises in store, the authors also identify a dense meshwork of (Arp2/3-dependent) actin punctae, and a set of rather mysterious hollow spheres 3–6 µm in diameter. (These latter actin-decorated structures were found in around 30% of the cells.) These observations and the other key findings outlined in this work clearly illustrate the dynamic complexity of the *Naegleria* actin cytoskeleton. In doing so, the paper provides compelling evidence that mechanisms of Arp2/3-dependent motility and phagocytosis employed by amoeboid cells across the eukaryotic tree are truly ancient—at least a billion years old.

Velle and Fritz-Laylin also lay the groundwork for a wave of *Naegleria* cell biology studies in the years to come. For example, it would be great to know how the actin cytoskeletal organization is rewired in preparation for cell division. This work helps make the case for better genetics to rigorously test the functions of individual actins, Arp2/3 homologues, formins, and other actin regulators. Since the *Naegleria fowleri* genome encodes almost exactly the same complement of actins and actin regulators as its free-living cousin, it will also be interesting to examine how its closely related actin cytoskeleton adapts to its pathogenic, "brain-eating" lifestyle (12).

Finally, looking across the tree at other eukaryotes, like *Plasmodium* (13) and *Giardia* (14), it is clear that cells devoid of Arp2/3 can also migrate in a manner that depends on actin, myosin, and formins. It is remarkable that eukaryotic cells have evolved to use the same cytomotive filament, actin, to move in such profoundly different ways.

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