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Archaeal actins and the origin of a multi-functional cytoskeleton

Arthur Charles-Orszag,[1] **Natalie A. Petek-Seoane,**[1] **R. Dyche Mullins**[1]

AUTHOR AFFILIATION See affiliation list on p. 8.

ABSTRACT Actin and actin-like proteins form filamentous polymers that carry out important cellular functions in all domains of life. In this review, we sketch a map of the function and regulation of actin-like proteins across bacteria, archaea, and eukarya, marking some of the *terra incognita* that remain in this landscape. We focus particular attention on archaea because mapping the structure and function of cytoskeletal systems across this domain promises to help us understand the evolutionary relationship between the (mostly) mono-functional actin-like filaments found in bacteria and the multi-functional actin cytoskeletons that characterize eukaryotic cells.

KEYWORDS actin cytoskeleton, archaeal cell biology, bacterial cell biology, evolutionary cell biology, eukaryogenesis

COMPARE AND CONTRAST: BACTERIAL AND EUKARYOTIC ACTINS

The identification of MreB as a *bona fide* filament-forming actin homolog in bacteria (1) marked the beginning of an age of discovery during which more than 40 classes of bacterial actin-like proteins (ALPs) were identified (2). Many of these actin-like proteins have been associated with specific biological functions; some have been purified and studied *in vitro* (3–7), and a few have been used to reconstitute complex cellular processes (8–10). Although more work remains to be done, several themes have emerged from these studies. A useful way of organizing these themes is by comparing bacterial and eukaryotic actins along two different axes: conservation/divergence and single/multiple function.

Conservation versus divergence

Borrowing a line from Tolstoy, we could say that: "all eukaryotic actins look alike, but each bacterial actin is unique." By this, we mean that (except for a few outliers) the sequence and structure of eukaryotic actins are highly conserved, while those of bacterial actin-like proteins are remarkably divergent. For example, the amino acid sequences of major non-muscle actin isoforms diverge by less than 7% across all animals, less than 17% from yeast to humans, and less than 12% from the discoba to the opisthokonts (11). In contrast, bacterial actin-like proteins share very little sequence similarity with each other. In a groundbreaking sequence analysis of bacterial actin-like proteins, the Pogliano group (2) settled on an identity cutoff of 20% to define the various subtypes of actin-like proteins. Even by this highly inclusive definition, sequence diversity yielded more than 40 subtypes of actin-like proteins encoded by bacterial genomes and plasmids. Given that each bacterial actin family is held together by as little as 20% sequence identity, the similarity *between* different families can be vanishingly small.

We observe a similar contrast when comparing the architecture of filaments formed by eukaryotic and bacterial actins. All eukaryotic actins studied to date form polymers with the same core architecture, which can be described as a right-handed, two-start helix with a pitch of 72 nm. Eukaryotic actin filaments also exhibit a structural and kinetic polarity, with fast- and slow-growing ends, also known as the "barbed" and "pointed" ends, respectively. In contrast, bacterial actin-like proteins form a variety of

Editor Julie A. Maupin-Furlow, University of Florida Department of Microbiology and Cell Science, Gainesville, Florida, USA

Address correspondence to R. Dyche Mullins, Dyche.Mullins@ucsf.edu, or Arthur Charles-Orszag, arthur.charles-orszag@ucsf.edu.

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two-stranded filaments that vary in pitch, handedness, and polarity. Specifically, some bacterial actin-like proteins, including MamK, form right-handed, two-start helices (12), while others, such as ParM and AlfA, are better described as left-handed helices (13, 14). The pitch of the helices can also vary. AlfA filaments comprise two strands with a pitch of 39.4 nm (14), while the plasmid-encoded protein Alp12 forms a mixed polarity bundle of two filaments (15), each with a pitch of 141 nm, and the anti-parallel strands of FtsA and MreB have essentially infinite pitch (6, 16). The anti-parallel arrangement of polymer strands in MreB and FtsA (17) filaments also demonstrates that even polarity is not universally conserved among bacterial actin-like filaments (16).

Mono- versus multi-functional filaments

In eukaryotes, the actin cytoskeleton carries out many essential cellular functions, all (or almost all) of which involve moving, shaping, or reinforcing membranes (Fig. 1). These functions include pseudopod protrusion, phagocytosis, endocytosis, cell-cell and cell-matrix adhesion, cytokinesis, and endosome processing (18, 19). How can highly conserved actins that create a single, stereotyped polymer carry out so many different functions in the same cell? The answer to this question lies in the large number of actin binding and regulatory proteins expressed in eukaryotic cells. These factors include multiple families of nucleation factors, crosslinkers, monomer-binding proteins, and myosin-family motors. This multiplicity of binding partners places strong constraints on the sequence and structure of eukaryotic actin filaments. A non-conservative mutation that perturbs a binding interface on the surface of the filament, for example, might require compensatory mutations in multiple actin-binding regulators to avoid producing a growth defect. Even a mutation that subtly alters filament architecture could perturb an essential cellular process such as cytokinesis or a complex developmental process such as left-right symmetry breaking in animal embryos (20). Occasionally, an organism can acquire specialized actin isoforms, via gene duplication and subsequent sequence divergence. The presence of these divergent and more specialized actins, however, has not affected the conservation of the most abundant, multi-functional actin isoforms in these species (21).

In contrast to the case of eukaryotes, one is tempted to characterize two decades of research on a variety of bacterial species by saying that each bacterial actin-like filament performs a single cellular function, aided by one (or occasionally two) regulatory factors (23, 24). While this simple formulation might not turn out to be strictly true (especially given the ambiguity of the phrase "cellular function"), bacterial actin-like proteins are certainly more specialized than their eukaryotic counterparts. ParM, AlfA, and Alp7A (2, 25, 26), for example, specialize in segregating plasmid DNA; MreB forms a scaffold specific for cell wall synthesis machinery (27–29) and cell division proteins (30); and MamK's only job appears to be aligning magnetite-containing organelles in magnetotactic bacteria (31) (Fig. 1). By adapting its structure and assembly dynamics to carry out one function has enabled the bacterial actin-like proteins to dispense with many of the accessory factors that regulate eukaryotic actin filaments. The assembly dynamics of ParM, for example, are tuned so that it can create a simple bipolar spindle that segregates plasmid DNA without relying on other proteins to regulate filament nucleation, nucleotide exchange, crosslinking, or filament disassembly (3, 10).

The very low sequence conservation *between* subtypes of bacterial actin-like proteins makes it difficult to trace their evolutionary history and suggests that they may not be a monophyletic protein family. Given their structural diversity, it seems likely that functional filaments evolved multiple times from one or more ancient members of the actin superfamily. On the other hand, the low sequence identity *within* each ALP subtype may be a consequence of their mono-functionality. Having one function and only one or two binding partners reduces the potential cost of mutations in an ALP gene and relieves constraints on genetic drift. In addition, Hyland et al. neatly demonstrated that functional interference between related ALPs in the same cell provides positive selection for sequence divergence (32).

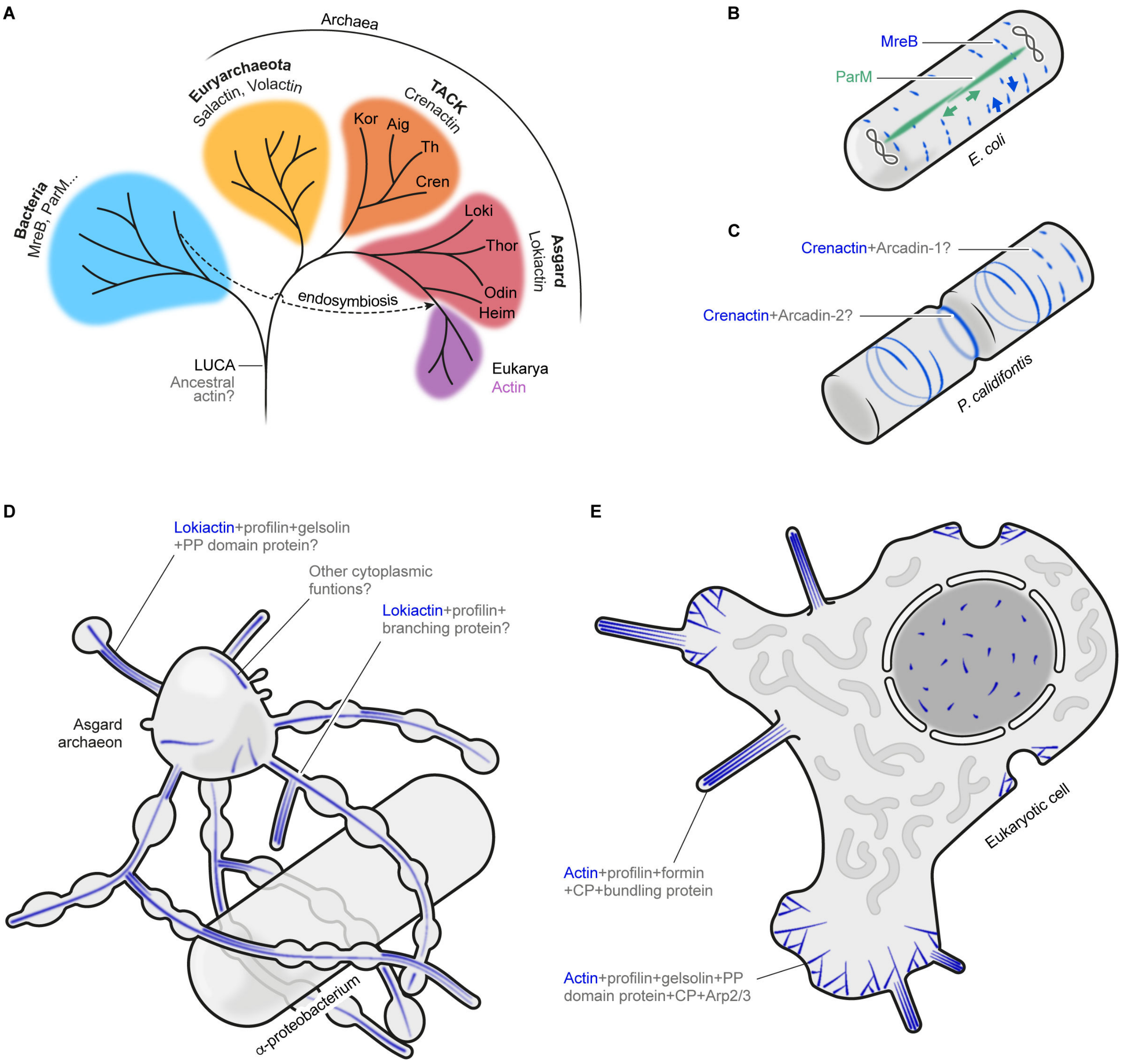


FIG 1 Functions of actin proteins across the phylogenetic tree of life. (A) Actin is ubiquitous across all domains of life (Bacteria, Archaea, and Eukarya) and was likely present in the last universal common ancestor (LUCA). (B) In bacteria such as *Escherichia coli*, actin-like proteins assemble filaments that perform unique cellular functions such as plasmid DNA segregation (ParM) or cell wall synthesis (MreB), with little to no regulatory proteins. (C) Some crenarchaea like *Pyrobaculum calidifontis* encode crenactin, an actin homolog that is thought to assume multiple functions in the cell that may be determined by specific subcellular localizations and a set of crenactin-regulating proteins (arcadins), suggesting that a multi-functional actin cytoskeleton originated in archaea. (D) Asgard archaea, the prokaryotes most closely related to eukaryotes, encode a eukaryote-like actin homolog called lokiactin as well as homologs of eukaryotic actin-regulating proteins profilin and gelsolin, suggesting that Asgard archaea assemble a dynamic, eukaryotic-like, multi-functional actin cytoskeleton. It is unknown whether lokiactin produces force-generating filaments capable of shaping biological membrane, although lokiactin filaments are found in the abundant membrane protrusions produced by *Lokiarchaeum ossiferum* (22), suggesting a key role for the lokiactin cytoskeleton in the acquisition of a bacterial endosymbiont by the archaeal ancestor of eukaryotes. (E) In eukaryotes, actin assembles a unique filament that adopts multiple architectures, and a plethora of cellular functions, dictated by a great number of actin-regulating proteins. Actin polymerization generates forces that are capable of moving membranes (e.g., filopodia and lamellipodia used in cell migration, internalization of endocytic vesicles, etc.). PP: polyproline motif.

Travels among the archaea

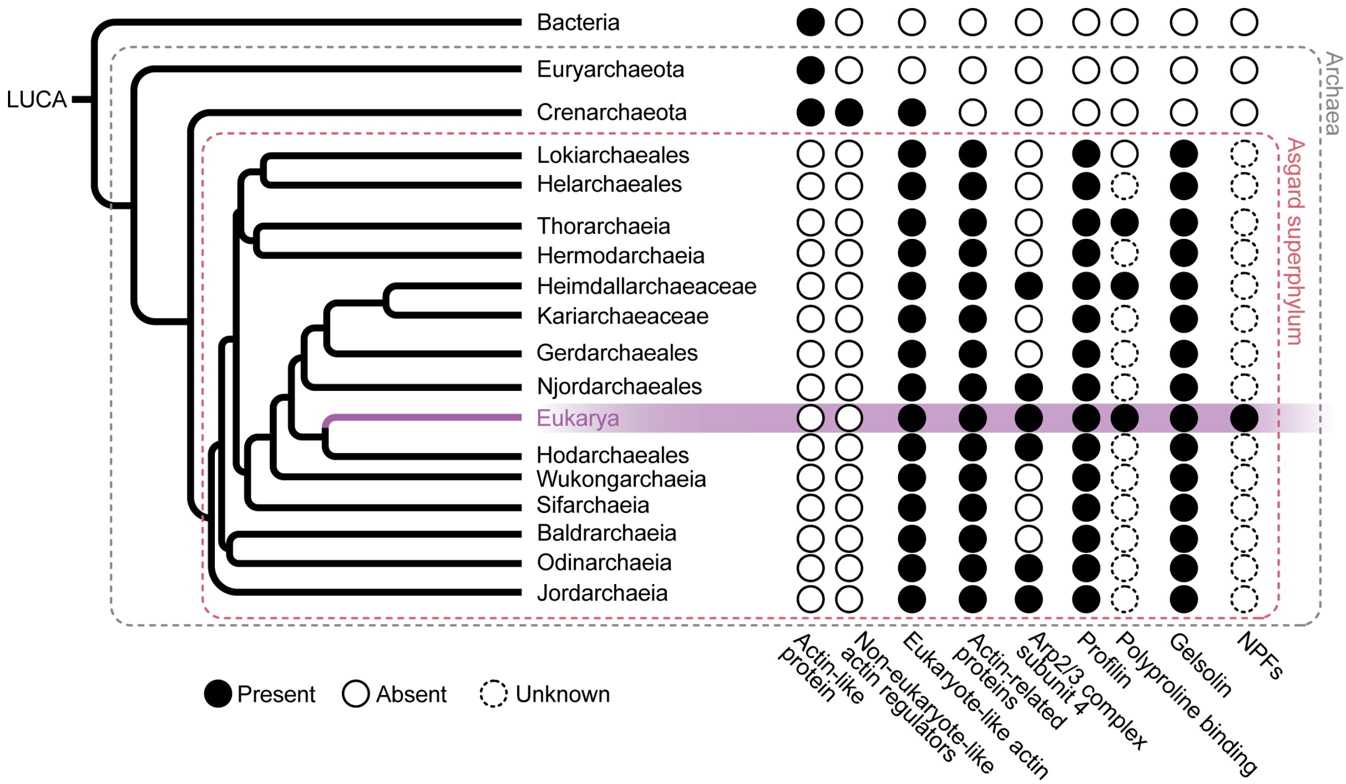
Recent work has begun to reveal the evolutionary landscape that lies between the mono-functional actin-like proteins of bacteria and the multi-functional actin cytoskeleton of eukaryotes. Much of the interesting territory lies within the archaeal domain (Fig. 1). The major archaeal clades span a vast range of diversity, from the Nanoarchaeota and Euryarchaeota, which share many molecular characteristics with bacteria, to the TACK superphylum, which contains organisms with distinctly eukaryotic features, to the more recently discovered Asgard archaea, which contain many eukaryotic signature proteins (ESPs) including relatives of the actin regulators profilin, cofilin, and gelsolin (Fig. 2). In the remainder of this review, we will describe, in turn, archaeal actins found in the Euryarchaeota, TACK, and Asgard lineages. We will briefly discuss how the structure and function of these molecules may relate to those of bacterial and eukaryotic actins, and we will highlight important unanswered questions associated with each molecule.

EURYARCHAEAL ACTINS: A BIT LIKE BACTERIAL ACTINS?

Many archaeal genomes encode actin-like proteins related to those found in bacteria (e.g., MreB and FtsA). It is generally assumed that these archaeal proteins carry out the same cellular functions as their bacterial counterparts, such as cell shape control (MreB) or cell division (FtsA). Genomics, cell biology, and biochemistry have also identified potential euryarchaeal-specific actins, including Ta0583, salactin, and volactin. Although more work will be required to understand the assembly dynamics and cellular functions of these proteins, recent studies suggest that, similar to bacterial actin-like proteins, each is adapted to carry out a single cellular function.

The actin-like protein Ta0583 appears in several species of Thermoplasmatales but its closest relatives are actually the plasmid-encoded ParM proteins found in rod-shaped, enteric bacteria (34). Interestingly, ParM proteins assemble into bipolar, DNA-segregating spindles (10, 13). The atomic structure of Ta0583 supports its relationship to ParM proteins, and its distribution among archaeal genomes suggests that it was acquired by lateral gene transfer (34). If Ta0583 was acquired when a bacterial plasmid made its way into an archaeal cell and integrated into the genome, it is possible that the protein retains its original function and has been harnessed to promote chromosome segregation. The only study of its *in vitro* assembly dynamics (35) found that Ta0583 polymerizes in an ATP-dependent manner and forms filament bundles. Interestingly, this study did not report evidence of dynamic instability in these polymers—a key feature of ParM filaments that enables them to carry out their cellular function. An alternative possibility is that Ta0583 was co-opted for a new function, perhaps regulating cell shape or cell-cell interactions. Thermoplasmatales species lack both a cell wall and an S-layer and display amorphous (amoeboid?) cell shapes. Some Thermoplasmatales species also form protrusions that link cells together into a "network phase" (36), reminiscent of membrane tubules and protrusions observed in Asgard archaea (22, 37). Ta0583 (for which we suggest the new name "thermoplasmactin") is a potentially interesting blank spot on our map and understanding its function will require more biochemical and cell biological studies.

By searching archaeal genomes for sequences related to bacterial MreB, Zheng et al. (38) recently identified an actin-like protein in *Halobacterium salinarum* and related species. The authors christened this protein salactin and demonstrated that its loss leads to a conditional defect in chromosome segregation. In rich media, salalactin knockout cells appear normal. In low phosphate media, which reduce genome copy number, these cells exhibit a significant growth defect, aberrant chromosome localization, frequent chromosome loss, and occasional morphology defects. These observations, together with the close proximity of the *salactin* gene to genes encoding DNA replication and repair factors, led to the proposal that salactin may function as part of a chromosome partitioning system in *H. salinarum* and closely related species. Time-lapse imaging of fluorescent proteins in live *H. salinarum* reveals salactin filaments growing from one cell pole and switching between phases of steady elongation and rapid shortening. These



	Actin-like protein	Non-eukaryote-like actin regulators	Eukaryote-like actin	Actin-related proteins	Arp2/3 complex subunit 4	Profilin	Polyproline binding	Gelsolin	NPFs
Bacteria	●	○	○	○	○	○	○	○	○
Euryarchaeota	●	○	○	○	○	○	○	○	○
Crenarchaeota	●	●	●	○	○	○	○	○	○
Lokiarchaeales	○	○	●	●	○	●	○	●	◌
Helarchaeales	○	○	●	●	○	●	◌	●	◌
Thorarchaeia	○	○	●	●	○	●	●	●	◌
Hermodarchaeia	○	○	●	●	○	●	◌	●	◌
Heimdallarchaeaceae	○	○	●	●	●	●	●	●	◌
Kariarchaeaceae	○	○	●	●	○	●	◌	●	◌
Gerdarchaeales	○	○	●	●	○	●	◌	●	◌
Njordarchaeales	○	○	●	●	●	●	◌	●	◌
Eukarya	○	○	●	●	●	●	●	●	●
Hodarchaeales	○	○	●	●	●	●	◌	●	◌
Wukongarchaeia	○	○	●	●	○	●	◌	●	◌
Sifarchaeia	○	○	●	●	○	●	◌	●	◌
Baldrarchaeia	○	○	●	●	○	●	◌	●	◌
Odinarchaeia	○	○	●	●	●	●	◌	●	◌
Jordarchaeia	○	○	●	●	●	●	◌	●	◌

FIG 2 Distribution of actin, actin-like proteins, and actin-regulating proteins in Archaea. The presence or absence of genes coding for actin, actin-like proteins, and actin-regulating proteins, as described in the literature, was mapped against published Bacteria, Euryarchaeota, Crenarchaeota, and Asgard archaea genomes. The eukaryotic lineage is highlighted in purple. NPFs: nucleation promoting factors. The phylogenomic tree was adapted from reference (33) to highlight Hodarchaeales, a recently proposed Asgard order identified as the closest to eukaryotes.

observations strongly suggest that, like eukaryotic microtubules and bacterial ParM filaments, salactin filaments are dynamically unstable. Additional *in vitro* studies of purified salactin assembly yielded results consistent with dynamic instability. Although it was identified by its similarity to MreB, salactin actually shares more sequence identity with MamK, an actin-like protein found in magnetotactic bacteria.

Schiller et al. (39) recently identified a class of actins in another halophilic Euryarchaeon, *Haloferax volcanii*, which they named volactin. Unlike Zheng et al. who searched genomes for actin-like sequences (38), Schiller et al. performed forward genetic screens for genes that alter or maintain cell shape. Volactin emerged from this screen as a protein required to establish and maintain the disc shape of late log phase cells. In fluorescence images of disc-shaped Haloferax cells, green fluorescent protein (GFP)-volactin fusion proteins appeared as filaments spanning the cytoplasm, with their tips at or near the membrane surface. In time-lapse movies, these filaments elongate and shorten, but their shortening does not appear to be as catastrophic as that of salactin, suggesting that volactin might not exhibit the same sort of dynamic instability and that the two proteins may have significantly different cellular functions and mechanisms of regulation.

TACK ACTINS: EVIDENCE FOR MULTIFUNCTIONALITY?

While chasing the evolutionary origin of eukaryotes, Guy and Ettema (40) identified a deep-branching superphylum of archaea with more eukaryotic features than previously identified clades (Fig. 1A). This superphylum, christened TACK (40, 41), comprises the Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota, and contains at least one unique and widely distributed actin homolog: crenactin (42). The name crenactin reflects its discovery within a subgroup of Crenarchaeota (now Thermoproteota), but related genes appear in Aigarchaeota and Korarchaoeta species. Crenactin is a distant homolog of both eukaryotic actin *and* bacterial MreB, although closer to the latter (with ~20% sequence identity). In addition to sequence similarity, purified crenactin mixed with ATP at physiological salt concentrations forms two-start, helical filaments that are much more similar to eukaryotic actin than MreB (43).

Crenactin is an ATPase with optimal activity at 90°C and, by immunofluorescence, it forms linear, helical structures that run the length of *Pyrobaculum calidifontis* cells (42) (Fig. 1). To date, crenactin orthologs have been found only in rod-shaped TACK archaea, supporting the hypothesis that this ancient actin gene helps control cell shape, similar to MreB in bacteria. However, since most archaea do not assemble a peptidoglycan-based cell wall-like bacteria, it is unclear how crenactin promotes a rod shape in archaea. Interestingly, in a fraction of cells, crenactin was observed to form a ring at mid-cell, suggesting a possible additional function during cell division (Fig. 1). Consistent with this idea, crenactin genes are found only in species that do not encode the ESCRT-like proteins that drive membrane constriction during cytokinesis in many other members of the Thermoproteota and Thaumarchaeota (42). Unlike bacterial actins, crenactin filaments appear to interact with multiple regulatory proteins, called arcadins. Up to four arcadin genes (*rkd-1* to *rkd-4*) are typically found clustered with a crenactin gene. Arcadin-1, arcadin-3, and arcadin-4 form a crenactin-like helical structure in *P. calidifontis* cells, suggesting that all three proteins bind to or co-polymerize with crenactin. The structures arcadin-1 and arcadin-2 did not reveal any homology with known eukaryotic actin-binding proteins, but both were found to bind crenactin *in vitro* (43). In particular, arcadin-2 binds crenactin with nanomolar affinity, and the last 17 residues of arcadin-2 are sufficient to induce depolymerization of crenactin filaments, likely through competition for binding to crenactin's D-loop, similar to some actin monomer-binding proteins in eukaryotes, such as DNAseI or thymosin-β4 (44, 45). Arcadin-1 binds much more weakly than arcadin-2 to crenactin (~15 µM) and does not appear to affect polymerization.

More cell biological studies will be required to determine whether crenactin actually carries out multiple cellular functions. Although genetic tools are not yet available in *P. calidifontis*, recent developments in high-temperature, live-cell imaging (46–48) will likely be key to investigate such dynamic processes *in vivo*.

ASGARD ACTINS: THE CRADLE OF EUKARYOTIC ACTIN REGULATION?

Our understanding of archaea and their role in eukaryogenesis increased substantially with the discovery of the Asgard superphylum (49, 50) (Fig. 1), first identified through metagenome assembly of DNA found in deep marine sediments near Greenland hydrothermal vents named Loki's Castle. The Asgard archaea comprise four separate clades (Lokiarchaeota, Thorarchaeota, Odinarchaeota, and Heimdallarchaeota) that encode a number of “eukaryotic signature proteins” (ESPs), once thought unique to eukaryotes. In addition to ESCRT-like proteins that are also found in the TACK superphylum, Asgard archaea express Ras-like small GTPases, actins similar to eukaryotic actin, and homologs of a number of actin regulatory proteins, including close relatives of profilin and gelsolin (Fig. 2). These discoveries support an engulfment model—similar to the earlier “eocyte” hypothesis (51)—in which eukaryotic cytoplasm was inherited from an archaeon that engulfed a bacterium.

Asgard actins were first found in Lokiarchaea (49) and dubbed lokiactins. Similar lokiactins were later identified in the other Asgard lineages (50), including in Hodarchaeales, a recently proposed Asgard order identified as the closest to eukaryotes (33). Lokiactins share 58–60% similarity with human cytoplasmic β-actin and are more closely related to eukaryotic actin than to crenactin or other archaeal actin-like protein. In an actin family tree, however, they branch between eukaryotic actin-related proteins 2 and 3 (Arp2 and Arp3). The kinship between lokiactins and eukaryotic actins may have been complicated by the intervention of viruses. Da Cunha et al. reported that virally encoded actins—“viractins”—in the *Imitervirales* family may have been acquired from proto-eukaryotes around the time of the last eukaryotic common ancestor. Based on sequence analysis of viractins, these authors raise the possibility that modern eukaryotic actins are the product of one or more rounds of horizontal gene transfer from a proto-eukaryote into a virus and then back again (52).

Regardless of whether viruses played a role in the creation of eukaryotic actin, the presence of profilins and gelsolin domain-containing proteins in Asgard genomes argues strongly that the assembly of lokiactins is regulated by eukaryote-like mechanisms (Fig. 2). Unfortunately, recombinant lokiactin has proved challenging to express and purify in sufficient quantities to investigate its assembly properties. Akıl and Robinson, however, bypassed this roadblock by employing eukaryotic actin to study the regulatory potential of Asgard profilins and gelsolins (53–55). *In vitro*, purified Asgard profilins bind monomers of rabbit skeletal muscle actin and alter their assembly kinetics (53). When expressed in human tissue culture cells, a GFP fused to a Thorarchaeal profilin interacts with cytoplasmic actin (55). Like their eukaryotic cousins, purified Asgard profilins also bind phosphatidylinositol-4,5-bisphosphate (PIP2) lipids. Although PIP2 is not found in archaeal membranes, this result at least raises the possibility that lokiactin assembly is regulated by membranes (53). Similar to the results with Asgard profilins, purified gelsolin orthologs from Thorarchaeota bind rabbit actin filaments *in vitro* with affinities between 7.7 and 135 µM (54). Purified Archaeal gelsolin-like proteins also display some of the *in vitro* activities of their eukaryotic cousins, including nucleation, capping, severing, and bundling of actin filaments and sequestration of actin monomers. Similar to their eukaryotic cousins, these various activities of Thorarchaeal gelsolins are altered—possibly regulated—by calcium. When expressed in human tissue culture cells, Asgard gelsolins fused to GFP co-localize with endogenous actin structures in a calcium-sensitive manner (55). Recently, Survery et al. reported purifying small amounts of a recombinant and polymerization-competent lokiactin from Heimdallarchaeota (56), paving the way for more *in vitro* biochemical studies.

One key question is whether Asgard profilins can deliver actin monomers to cytoskeletal polymerases similar to eukaryotic Ena/VASP proteins. Ena/VASP-family polymerases bind profilin-actin complexes and facilitate their delivery to growing ends of actin filaments, either by forming multi-protein oligomers or dense, membrane-associated clusters (57–59). Profilins (and profilin-actin complexes) bind proline-rich sequences in Ena/VASP proteins, leading eukaryotic actin aficionados to wonder whether Asgard profilins also bind polyproline (PP) motifs, and whether endogenous polyproline-containing proteins regulate archaeal actin assembly. Akıl and Robinson reported that profilins from Lokiarchaeota do not bind proline-rich regions from human VASP, and they failed to detect polyproline-rich proteins in Loki genomes (53). More recent work by Survery et al. hints that profilins from Thorarchaeaota and Heimdallarchaeota—closer relatives to eukaryotes than Lokiarchaeota—can bind both proline-rich motifs and PIP2. Structural data suggest that profilins from other Asgard phyla may also bind proline-containing sequences similar to those found in eukaryotic VASP (56). Survery et al. went on to identify potential polyproline motif-containing protein candidates in both Heimdallarchaeota and Thorarchaeota. Taken at face value, these studies suggest the possibility that the structural and functional diversity of Asgard profilins reflect the evolution of eukaryotic style regulatory mechanisms within the Asgard lineage, close to the point of origin of modern eukaryotes.

Lokiactins may have played a key role in eukaryogenesis. The first isolation and culturing of Lokiarchaeal species, "*Candidatus* Prometheoarchaeum syntrophicum" strain MK-D1 (37) and *Lokiarchaeum ossiferum* (22), revealed cells with small (~500 nm) coccoid bodies and long, thin protrusions, reminiscent of eukaryotic filopodia. Both of these recently isolated Lokiarchaeota live in close association with syntrophic bacterial partners, leading to the hypothesis that filopodial protrusions evolved to promote close cell-cell contact and nutrient exchange. This hypothesis formed the basis of an entangle-engulf-endogenize (E3) model for eukaryogenesis, in which archaeal membrane protrusions encircling a bacterial partner fused at some point to produce an archaeal host cell with a stably engulfed bacterial endosymbiont (37). Where does actin fit into this model? In the case of *Lokiarchaeum ossiferum* (22), cryo-electron tomography revealed that filopodial membrane protrusions are filled with protein polymers likely to be lokiactin filaments (Fig. 1D). Although electron microscopy failed to identify similar actin-like filaments in the filopodia of "*Candidatus* Prometheoarchaeum syntrophicum," this may reflect failure of the fixation procedure to preserve fragile filaments. Additional work on a wider variety of Asgard species will provide a clearer picture of the role of lokiactin in the formation of archaeal filopodia.

In eukaryotes, actin filament assembly can be initiated by formins (60), spire-family proteins (61), or the Arp2/3 complex (Fig. 1E). Asgard genomes sequenced to date contain no obvious formin or spire-like proteins, but they do contain proteins related to actin (Fig. 2). In eukaryotes, filament nucleation by the Arp2/3 complex relies on the close structural similarity between actin and the actin-related proteins Arp2 and Arp3. The *Lokiarchaeum ossiferum* genome encodes four actin homologs. The most abundant homolog, lokiactin, also has the most sequence identity to eukaryotic actins. The three other actin homologs appear to be expressed at lower levels and show more sequence similarity to eukaryotic actin-related proteins than actin itself (22). Could one or more of these lokiactin homologs initiate the formation of new lokiactin filaments in a manner similar to Arp2 and Arp3? This is an especially intriguing possibility given the recent discovery of homologs of Arp2/3 complex subunits (e.g., ARPC4) in genomes of Asgard lineages such as the Hodarchaeales, which are the closest known relatives of eukaryotes (Fig. 2) (33). Intriguingly, many of the filaments observed by cryo-electron microscopy in *Lokiarchaeum ossiferum* cells were arranged in parallel bundles while some appeared to form branching arrays (22). Future *in vitro* biochemical studies will help us understand whether Asgard archaea evolved filament crosslinkers and/or branching nucleation factors.

From this brief survey, we can see that the "multi-functional" nature of the eukaryotic actin cytoskeleton mainly reflects multiple ways in which actin networks can interact with cellular membranes to move, shape, and reinforce them. The functional connection between membranes and actin filaments, as well as some of the most highly conserved actin regulatory proteins, now appear to be innovations of the Asgard archaea. We look forward to the isolation and propagation of stable Lokiarchaeal cultures (22, 37), which will further revolutionize our understanding of both eukaryogenesis and the evolutionary history of the actin cytoskeleton.

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AUTHOR AFFILIATION

[1]Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, California, USA

AUTHOR ORCIDs

Arthur Charles-Orszag http://orcid.org/0000-0002-5185-4124
Natalie A. Petek-Seoane http://orcid.org/0009-0003-8253-729X
R. Dyche Mullins http://orcid.org/0000-0002-0871-5479

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