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Evolution of the cytoskeleton: Emerging clues from the diversification and specialisation of archaeal cytoskeletal proteins

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Recent research in archaeal cell biology has revealed a remarkable diversity of cytoskeletal proteins related to those found in bacteria and eukaryotes, such as the tubulin, actin, and ESCRT protein superfamilies, and archaea-specific proteins that self-assemble and have been implicated in cytoskeletal roles. Here, we outline an emerging view that the archaeal cytoskeleton has several conceptual ties to the sophisticated eukaryotic cytoskeleton. We highlight that duplication and specialisation of protein function is common among archaeal cytoskeletal systems, and that some paralogues show coordinated, opposing functions in the regulation of cell morphogenesis and structural homeostasis. Furthermore, the presence of homologues of eukaryotic cytoskeletal regulators in Asgard archaea, the closest known relatives of eukaryotes, underscores further linkages between eukaryotic and increasingly sophisticated archaeal cytoskeletal systems.

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Introduction

The eukaryotic cytoskeleton is a vastly complex network of cytoplasmic protein fibres fundamental to the intracellular organisation, structure and development of cells. It is constituted by a myriad of cytoskeletal proteins and their binding partners involving sophisticated mechanisms that enable extensive adaptability and multifunctionality in different cell types and conditions. Homologous cytoskeletal systems in bacteria (reviewed in Ref. [1]), despite their overall complexity, are comparatively simpler and are generally thought of as having a single purpose each (Figure 1). This begs the following question: how did the highly sophisticated mechanisms and multifunctionality within the eukaryotic cytoskeleton evolve (Figure 2)?

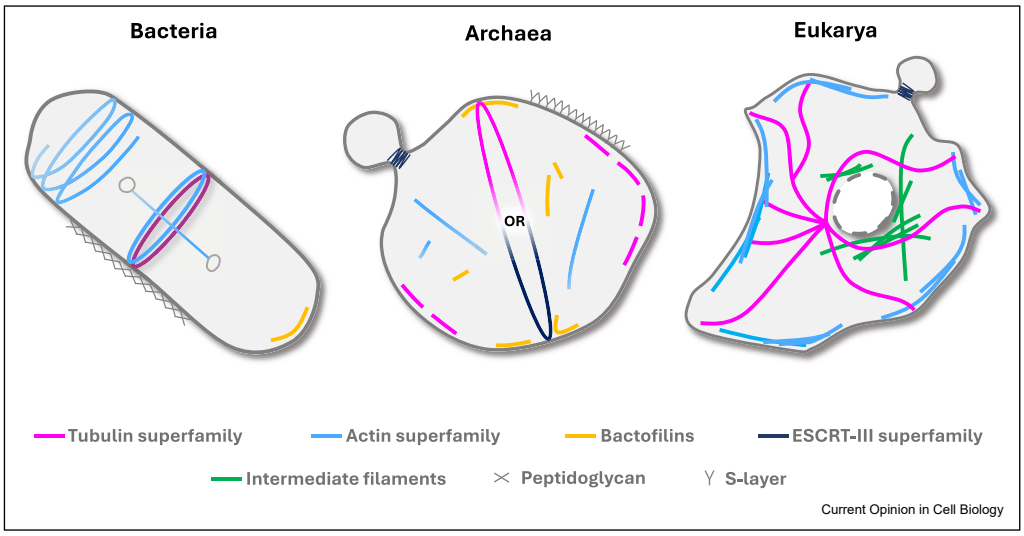
As the closest prokaryotic relatives of eukaryotes, the archaea and their cytoskeletal systems may hold important clues. In this review, we discuss selected cytoskeletal proteins in three of the major groups of archaea, the Euryarchaeota, Crenarchaeota, and Asgard archaea. We focus on *Haloferax volcanii*, *Sulfolobus acidocaldarius*, and *Candidatus* Lokiarchaeum ossiferum, respectively, as diverse model or representative species across the archaeal domain (Figure 3). We also summarise their interesting biological roles and highlight the commonalities among archaeal, bacterial, and eukaryotic cytoskeletal protein homologues (Figure 1) that can provide insights into the early evolution of cells (Figure 2).

The Asgard archaea, as the most recently discovered major archaeal group and most closely related one to eukaryotes, are of particular interest to cell evolution. They have a remarkable abundance of predicted cytoskeletal and intracellular trafficking proteins previously thought to be a hallmark of eukaryotes [2,3]. This, together with the parallel improvements in the tractability of cellular research on archaea generally, has generated renewed interest in their potential to provide clues about the evolutionary history of cytoskeletal systems and the structural transitions that cells underwent during the monumental shifts in complexity accompanying eukaryogenesis around two billion years ago.

Archaeal cell division systems

Cell division is a fundamental process required for the persistence, expansion, and evolution of life, and could be thought of as the primary function of cells, linking all modern-day organisms to the first cells on earth. Cytoskeletal proteins—which assemble into polymers with structural roles in cells—are central to the mechanisms

Figure 1



Generalised cytoskeletal systems and their basic roles in Bacteria, Archaea, and Eukarya. Homologous protein filaments are indicated by colours. Their subcellular positions represent their main roles in the primary cytoskeletal functions of cell division (midcell rings), morphology (envelope-associated filaments/helices), or intracellular segregation/organisation systems (cytoplasmic filaments). Archaeal division rings are based on either FtsZ or ESCRT-III cytoskeletal systems in different phyla. Typical characteristics of the cell envelope are also shown (e.g. peptidoglycan and S-layer), although these are not mutually exclusive and substantial diversity exists within each domain. Many of the Eukarya have an actin–myosin-based division ring and final abscission utilizes ESCRT-III proteins (not shown). Bacteria and archaea cells are of similar size, whereas eukaryotic cells typically have several orders of magnitude greater volumes.

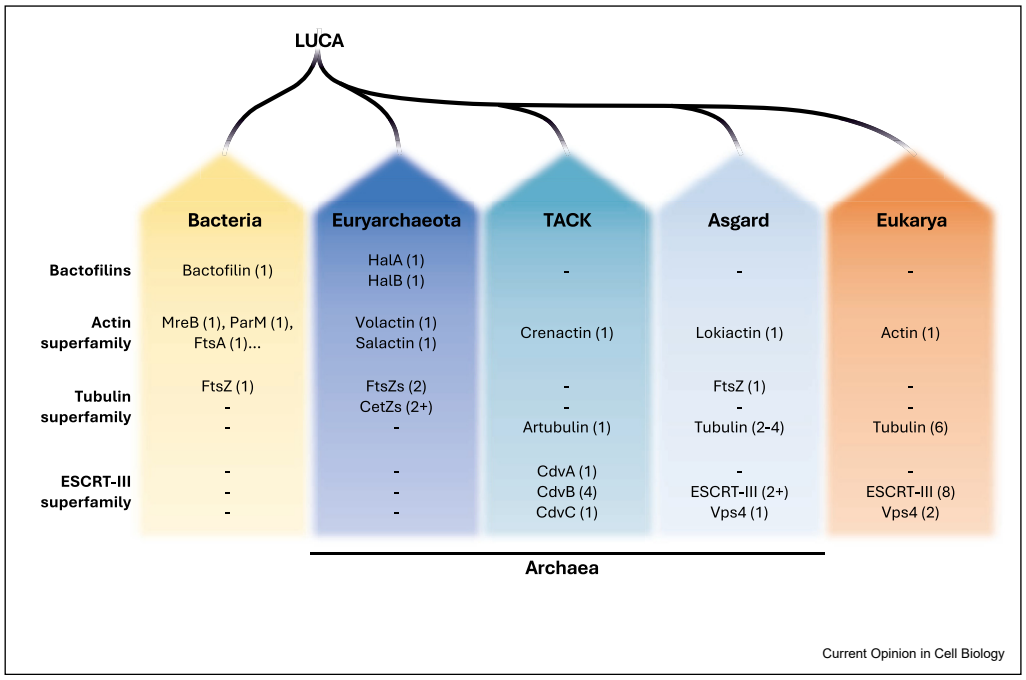
and control of cell division in all cells investigated to date. Archaea generally have either one of the two primary systems used for cell division: (1) the FtsZ-based system, generally homologous in bacteria, and (2) the ESCRT-III based system, which is homologous in eukaryotes, where it plays roles in cell division and other intracellular membrane remodelling systems. Euryarchaeota, which include halophilic and methanogenic archaea, use the FtsZ-based division mechanisms, whereas Crenarchaeota use the ESCRT-III–based system. Asgard archaea harbor inconsistent distributions of both homologous systems, and although these two division systems are typically present mutually exclusively, some Asgard archaea have both FtsZ and ESCRT-III homologues. The potential roles of ESCRT-III and FtsZ in Asgard archaea are unknown [4–6].

ESCRT-III–based division systems

Eukaryotic ESCRT-III (Endosomal Sorting Complex Required for Transport-III) complexes mediate a range of membrane remodelling processes including abscission during cell division (cytokinesis), intracellular membrane trafficking, and the release of extracellular vesicles and viral budding. These functions rely on the polymerisation of ESCRT-III regulated by Vps4, the ATPase responsible for turnover and depolymerisation of ESCRT-III (reviewed in Refs. [7,8]). In archaea, ESCRT-III homologues are distributed throughout broad groups of microorganisms including the TACK superphylum and Asgard archaea [4,9], which are the major archaeal groups thought to be most closely related to eukaryotes.

Archaeal ESCRT-III proteins were first identified and are most well-studied in the model archaeon *Sulfolobus acidocaldarius* [10,11], where they represent a comparatively simplified version of the eukaryotic ESCRT-III-based membrane remodelling complexes [12,13]. Also known as the Cdv system, in *S. acidocaldarius* it consists of CdvA, which has no homologue in eukaryotes, multiple CdvB paralogues (homologous to eukaryotic ESCRT-III), and CdvC (homologous to Vps4). CdvA acts as a membrane association or anchor protein and interacts with CdvB [14]. Together, they are the first to arrive at the division site [15] and form a non-contractile ring required for the recruitment of CdvB1 and CdvB2 [16,17]. CdvC follows and functions similarly to eukaryotic Vps4 [18] by removing CdvB [19], which is targeted for degradation by the proteasome. The remaining CdvB1/B2 ring drives constriction and membrane abscission [16,20]. Interestingly, a fourth CdvB paralogue, CdvB3, is not heavily involved in cell division but has roles in vesicle formation [20,21]. The diversification of roles among the CdvB paralogues, and their incorporation and degradation at specific stages of division allows for precise regulation. This apparent trend towards multiplication and functional specialisation within an archaeal cytoskeletal system is reminiscent of the highly sophisticated roles and multiple isoforms of cytoskeletal proteins like ESCRT-III in eukaryotes.

Figure 2



The main groups of cytoskeletal proteins identified in archaea. The main superfamilies are grouped in rows which list proteins discussed in this review that are generally found in bacteria, eukaryotes, and three main groups of archaea. The nomenclatures and number each protein type typically found in these taxonomic groups are indicated in parentheses, although do not necessarily represent any one model species. The main taxonomic groups of archaea discussed in this review are organised in columns, according to their phylogeny in relation to Bacteria, and Eukarya, stemming from the last universal common ancestor (LUCA); Asgard archaea are most closely related to Eukarya and Euryarchaea are most distantly related. TACK represents a superphylum that includes Crenarchaeota. Artubulins are a small and divergent group of tubulins found in specific Thaumarchaeota.

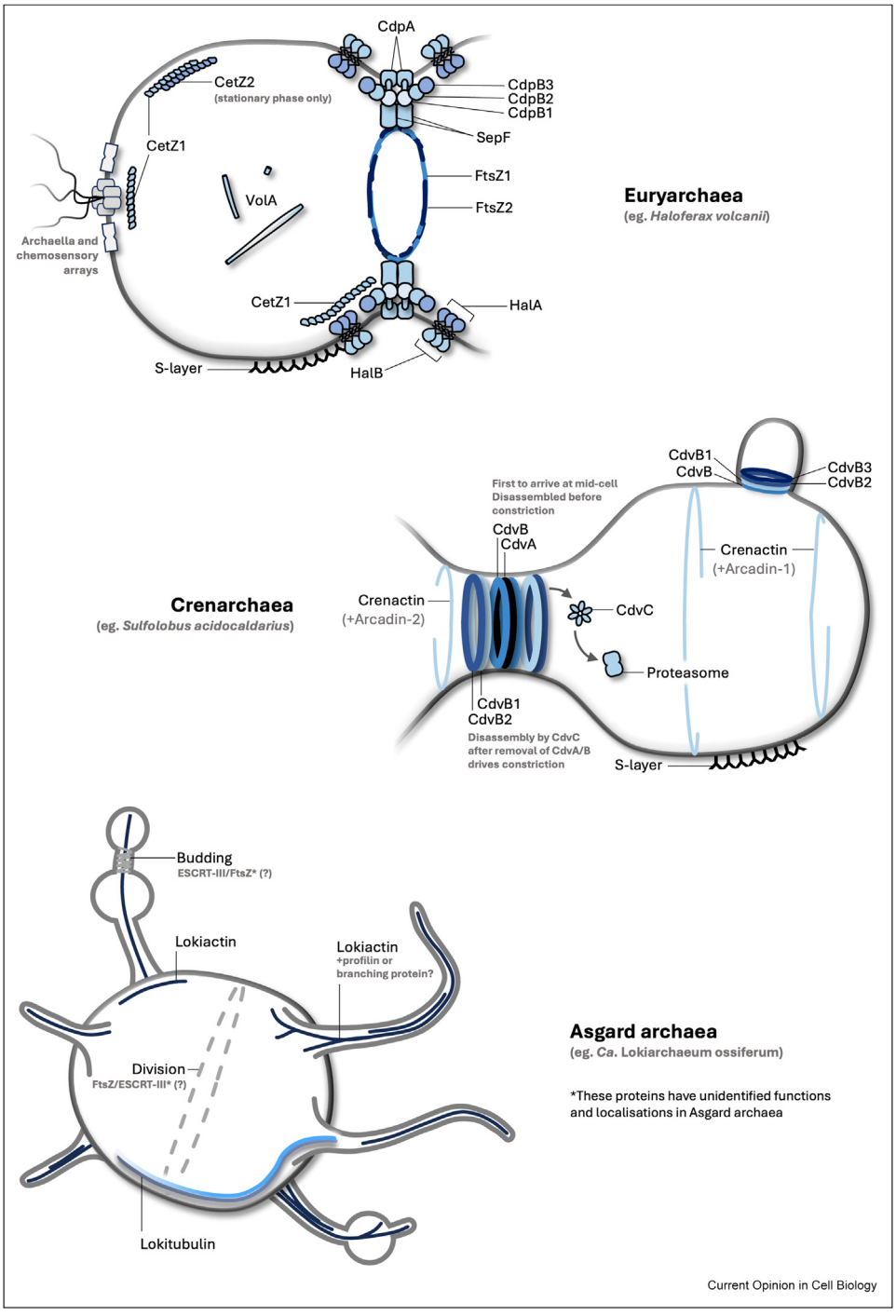
Archaeal FtsZ-based division systems

FtsZ is the prokaryotic homologue of tubulin that is widespread in bacteria and many archaea. FtsZ constitutes one of the three broadest groupings of tubulin superfamily proteins, which also includes tubulins mostly found in eukaryotes and the archaea-specific CetZ proteins involved in cell morphogenesis [22]. FtsZ is a cytoskeletal protein and a major component of the cell division machinery in prokaryotes [23,24]. It is well-characterised in bacteria where it contributes to cell division by forming the Z-ring at the site of division, acting as a recruitment hub for other division proteins, and helping coordinate membrane constriction and cell wall ingrowth to drive cytokinesis [24–28]. In contrast to the single FtsZ in bacteria, archaea typically harbor two FtsZ paralogues [4,22,23], which likely emerged from an early gene duplication [4,22,29,30].

The dual FtsZ system has been studied in the model archaeon *Haloferax volcanii*, where the two FtsZs show specialised functions in division. The primary function of FtsZ1 is to recruit division proteins, including FtsZ2, to form the midcell division ring, whereas FtsZ2 is important for the envelope constriction process through an as-yet unknown mechanism [4,23]. While most archaea such as *H. volcanii* have a lipid membrane and a proteinaceous flexible surface layer (S-layer) shell, certain groups of methanogenic archaea have a type of peptidoglycan (pseudomurein) outer wall, similar to bacterial cell walls. Interestingly, these methanogens, such as the model *Methanobrevibacter smithii* [31], specifically do not possess FtsZ2 and rely on a single FtsZ1 for division [4], suggesting their primary mechanism of division constriction may be the inward-directed biosynthesis of pseudomurein, effectively pushing the lipid membrane inward from the outside as proposed in bacteria [32,33]. In archaea with a monolayer of S-layer proteins, this mechanism appears unlikely, so it has been hypothesised that FtsZ2 primarily pulls the membrane and S-layer inwards from the cytoplasmic side [4].

Recent research has identified several other cell division constituents in haloarchaea, some of which are homologous to the bacterial division proteins, whereas others appear unique to archaea. For example, SepF is thought to act as a membrane anchor for FtsZ, equivalent to its role in bacteria [31,34]. In contrast, CdpA is a transmembrane protein unique to haloarchaea and is thought to also have an FtsZ-anchoring function [35]. CdpB1, CdpB2, and CdpB3 are also highly conserved in haloarchaea, and are paralogous proteins with similar but most likely distinct roles in cell division. CdpB1 and

Figure 3



Cytoskeletal protein functions in eminent cell biological model organisms of Euryarchaeota (*H. volcanii*), Crenarchaeota (*S. acidocaldarius*), and Asgard archaea (*Ca.* L. ossiferum). In *H. volcanii* (upper), CetZ proteins have multiple functions in cell morphogenesis, and potentially at midcell, and are involved in the correct assembly of the motility machinery at the cell poles. Volactin plays a role in maintaining disc-shaped cell morphology, and the halofilins HalA and HalB are involved in cell structural integrity and homeostasis. In cell division, FtsZ1 and FtsZ2 are involved in establishment and constriction of the midcell ring, and cytoskeletal-like proteins CdpB1-3 have been implicated in bracing the membrane anchor proteins CdpA and SepF to organise the division ring. In *S. acidocaldarius* (middle), crenactin forms double-helical filaments that are hypothesised to be involved in maintaining cell shape. The cell division system has multiple ESCRT-III homologs (CdvB/1/2/3) that have different roles in division and are tightly regulated to function at different stages, including through CdvC and proteasome-mediated degradation of CdvB, which triggers the onset of division constriction. Both organisms have a cytoplasmic membrane and protein surface layer as their main envelope structural constituents. *Ca.* L. ossiferum (bottom) is shown as a key representative within the Asgard archaea as it is the most well-studied in regard to cytoskeletal proteins. Both Lokiactin and Lokitubulin are implicated in the control of cell morphogenesis, although their specific biological roles are still unclear. It currently is unknown what proteins contribute to budding and cell division in *Ca.* L. ossiferum; however, ESCRT-III and FtsZ homologues are present and could be involved in these processes.

CdpB2 directly interact with SepF, whereas CdpB3 appears to only interact with CdpB2 but has an unclear role [36,37]. Some of these proteins are predicted to assemble into an 'anchor complex' which organises FtsZ1 and FtsZ2 filaments at midcell for division [35]. The CdpB paralogues appear to represent further examples of multiplication and likely specialisation of cytoskeletal-like proteins in archaea [37]. Furthermore, the conserved archaea-specific proteins in our current model of the haloarchaea division machinery represent an interesting divergence between FtsZ-based division systems in bacteria and archaea. Future comparisons of these systems are anticipated to help identify common and functionally important mechanisms of division amongst diverse microbes.

A patchwork of ESCRT-III and FtsZ in Asgard archaea—the bridge to eukaryotic division?

Asgard lineages encode both FtsZ and ESCRT-III homologues [3,4,29], which is unusual given that these two modes of division are typically mutually exclusive. The ESCRT-III protein sequences group separately from other TACK archaeal ESCRT-III proteins. In the current absence of tractable model systems for Asgard archaea, research has focussed on the activity of purified recombinant proteins. Two phylogenetically and functionally distinct ESCRT-III proteins from Heimdallarchaeota were capable of an ordered series of interactions that resulted in membrane deformation, which was suggested as a simplified precursor of the complex multi-part ESCRT-III system in eukaryotes [9]. Furthermore, a recent study found the Vps4 component of eukaryotic ESCRTs to be almost monophyletic with its Asgard homologues [5]. The authors suggested that vertical inheritance from ancient Asgard-like ancestors, combined with modification of other genes such as the Cdc48 ATPase, came together to form the modern multi-part ESCRT system during eukaryogenesis.

Although FtsZ1, FtsZ2, and SepF are found in Asgard archaea, the FtsZs appear inconsistently present amongst the limited Asgard genomes available [4,5]. FtsZ is present in Lokiarchaeota, Thorarchaeota, and Odinarchaeota, although not in Heimdallarchaeota [38]. This patchy co-occurrence of ESCRT-III and FtsZ-based systems in Asgard archaea may reflect evolutionary transitions in division mechanisms, although it is still unclear which system, neither or both, is used in cell division in the different groups of Asgard archaea. A recent study reported that two paralogous FtsZs from Odinarchaeota formed distinct filament assemblies *in vitro* [39]. While OdinFtsZ1 directly bound lipid membranes, OdinFtsZ2 was recruited to liposomes only in the presence of the Odinarchaeota SepF homologue. This is reminiscent of the distinct and cooperative roles of FtsZ1 and FtsZ2 in *H. volcanii* [4]. It has been hypothesised that Asgard archaea are more likely to rely on FtsZ for division, because ESCRT-III proteins are capable of functions unrelated to division such as vesicle sorting, whereas FtsZs are typically not involved in functions outside of division [5,40]. However, as seen in eukaryotes, ESCRT-III complexes can have multiple roles in different subcellular membrane remodelling processes including vesicle budding and abscission at the final stage of division. Given the remarkable vesiculated, tubulated, and multi-lobed cells of Asgard archaea [41,42], some of which appear to represent unusual forms of division, we speculate that both ESCRT-III and FtsZ proteins are utilised for various steps in the membrane scission and budding events that appear to be common in Asgard archaea—FtsZ might act first in envelope constriction and then ESCRT-III in abscission events.

Future work investigating the distribution, diversity, and functions of cytoskeletal proteins in Asgard archaea is expected to create an integral link between archaeal and eukaryotic cytoskeletons and may be the key to deciphering how the highly sophisticated cytoskeletal systems evolved in eukaryotes [43].

Cell structure and morphogenesis cytoskeletal proteins

Recent research has identified duplication and specialisation of function amongst cytoskeletal proteins that control cell morphogenesis in addition to those that coordinate cell division discussed above. Cytoskeletal systems for the control of cell shape and structure in archaea include actin-like, tubulin-like (CetZ), and bactofilin homologues in Haloarchaea, crenactin in the Crenarchaeota, and eukaryotic-like actin and tubulins in Asgard archaea.

Bactofilins

Bactofilins are a diverse family of self-assembling cytoskeletal proteins that play roles in cell morphology and division in bacteria [44,45]. Bactofilin homologues have recently been identified in archaea and eukaryotes [46]. Bactofilin is also encoded by Asgard archaea and in a minority of eukaryotes, although its presence in eukaryotes may have been due to horizontal gene transfer [46]. In haloarchaea, two distinct subfamilies of bactofilins (referred to as halofilins), HalA and HalB, likely evolved from a duplication and divergence of bactofilin [47]. In *H. volcanii*, HalA prevents the formation of positive membrane curvatures, thereby stabilising the cell's envelope structure, whereas HalB promotes S-layer remodelling at regions of negative curvatures. Together, these complementary actions of HalA and HalB, on opposite sides of the cell membrane, are important for Z-ring condensation and cell morphogenesis, such as the transition from disc- to rod-shaped cells which occurs during the early to mid-exponential phase of the growth cycle. This apparent duplication

and specialisation of halofilins evokes parallels to the duplication and regulated counteractivity of the multiple CdvB division proteins in crenarchaea cell division and the behaviour of CetZ paralogues in cell shape regulation in haloarchaea (described below).

CetZs—multiple homologues with multiple functions

The CetZs are archaea-specific tubulin superfamily proteins [6,22,29,30] found in Euryarchaea, and are the most abundant in Haloarchaea, which typically have at least two CetZ paralogues but can have up to eight [22,29,30]. Haloarchaea almost always possess both CetZ1 and CetZ2 homologues, which form two phylogenetically distinct subfamilies of co-conserved CetZs [30], and have specialised and opposing functions for the regulation of morphological changes [48].

In *H. volcanii*, which has six CetZ paralogues, CetZ1 is required for the morphogenesis of rod-shaped cells during early log growth [22,49—51], and during the development of rod-shaped motile cells [22,49,52]. CetZ1 is also directed to rod cell poles by Min system positioning proteins [53] and facilitates the assembly and positioning of the archaellum (archaeal flagellum) and chemosensory arrays at the poles [52]. During active growth, CetZ2 has no effect on cell morphogenesis or motility [22,52], but as cells enter the stationary phase and shift from rod- to plate (or discoid)-shaped cells, CetZ2 is upregulated and contributes to maintenance of plate shape by antagonising the rod development function of CetZ1 [48]. The opposing functional interplay between CetZ1 and CetZ2 for the regulation of cell morphogenesis is thus reminiscent of that between HalA and HalB, or CdvB and CdvB1/2 described above.

Two phylogenetically distinct and co-conserved subfamilies of CetZs are also present in Archaeoglobales and Methanosarcinales [30]. This may indicate that functional CetZ pairs are important throughout Euryarchaea for coordination of cell shape changes or other cytoskeletal functions. In contrast, Thermococci CetZs exist as a sole homologue [22,29,30]. Although there has been no experimental investigation into their function in Thermococci, it will be informative in the future to investigate whether a single CetZ homologue necessitates multifunctionality or accessory proteins instead of possessing multiple more specialised CetZ homologues.

Actin: duplicated and specialised versus conserved and multifunctional

In eukaryotes, actin is highly conserved but multifunctional, a trait which is largely due to the extensive array of its accessory proteins and binding partners (reviewed in Ref. [54]), which allow actin to assemble diverse architectures and participate in many fundamental cellular processes, such as cell migration, generating intracellular polarity, endocytosis, phagocytosis, and cytokinesis (reviewed in Refs. [55—59]). In contrast, bacterial homologues of actin and other actin-like proteins, such as MreB, FtsA, and ParM, are thought to be monofunctional, contributing to rod shape [60—62], division complex assembly [63,64], and plasmid segregation [65,66], respectively. A recent review [43] suggested that the multifunctional actin cytoskeleton of eukaryotes evolved from monofunctional actin-like proteins in bacteria and suggested that this complexity and multifunctionality likely evolved in archaea.

Actin homologues are diverse and patchily distributed across archaeal taxa [2,67]. In Euryarchaea, two actin homologues from *Halobacterium salinarium* and *H. volcanii* were recently identified, salactin (SalA) and volactin (VolA), respectively. SalA was found to contribute to chromosome segregation [67], while VolA is required for disc-shape formation and maintenance [68]. Both proteins show substantial dynamic cytoplasmic filaments, strongly suggesting they play cytoskeletal roles.

In contrast to the apparent mono-functionality of actins in Euryarchaea, actin homologues from the TACK superphylum, referred to as crenactins, are suggested to be somewhat multifunctional as they are thought to contribute to both cell division and rod development [69]. Several binding partners of crenactin, arcadins, have also been identified and are likely to facilitate crenactin in these differing functions [69,70], evoking parallels to the multiplicity of binding partners of eukaryotic actin which allow for extensive multifunctionality of the eukaryotic actin cytoskeleton [43].

Actin homologues are encoded by many Asgard lineages (reviewed in Ref. [71]) and come packaged with homologues of regulatory proteins like gelsolin [72] and profilin [73,74], some of which have been shown to associate with eukaryotic actin [72,73]. This echoes the multifunctional binding partners of crenactin and those of eukaryotic actin. In *Ca*. L. ossiferum, lokiactin filaments localise in various orientations throughout its characteristic tubular cell extensions, and are structurally akin to eukaryotic actin and crenactin filaments [42]. Similar actin filament-like structures were also very recently observed in *Hodarchaeota*, which has a similar cellular morphology to *Ca*. L. ossiferum [75]. Lokiactin homologues clustered variously with actins from other Asgard archaea in phylogenetic trees, which is likely reflective of a series of gene duplications and losses in the evolution of actin [42]. The presence of actin and its eukaryotic-like regulatory proteins in Asgard archaea may represent a key evolutionary transition from multiple diverse unifunctional actin homologues in bacteria to a multifunctional

highly regulated actin in Asgard archaea and eukaryotes [43].

Tubulin in Asgard archaea

Unlike actin, the distribution of tubulin homologues in Asgard archaea is inconsistent, with homologues only identified in some Asgard genomes. The single Odinarchaetoa homologue (Odintubulin) was not found to assemble into microtubules [76]. Heterodimeric and microtubule forming tubulins have historically been considered a structural hallmark of Eukarya, and how they evolved from ancestral FtsZ/CetZ is unclear. Very recently; however, multiple tubulins were characterised in *Ca*. L. ossiferum [77]. These Asgard tubulins, AtubA, AtubB, and AtubB2, showed structural resemblance to eukaryotic α- and β-tubulins, and formed similar heterodimers, protofilaments, and mini-microtubules *in vitro*. Filaments consistent with these were also observed *in vivo* using cryo-electron tomography and immunofluorescence microscopy [77]. Their basic similarity to eukaryotic microtubules suggest that AtubA/B reflect an early stage of microtubule evolution, and could represent a key transition between the homo-polymerising archaeal/bacterial tubulins and hetero-polymerising eukaryotic tubulins, suggesting that microtubules may have a pre-eukaryotic origin.

Duplication of cytoskeletal proteins occurred multiple times in archaea

The archaeal cytoskeletal proteins discussed here spotlight several examples where gene duplication and sequence divergence appear to have accompanied functional specialisation. This is especially evident amongst archaeal tubulin superfamily proteins that originated from FtsZ in the last universal common ancestor (LUCA) and were eventually elaborated to establish the multiple specialised tubulins and their isoforms in eukaryotes. In archaea, several duplications of tubulin superfamily proteins have occurred [6], resulting not only in two distinct FtsZ homologues, but also in the conception of CetZs, which in themselves have undergone extensive duplication. Duplications of ESCRT-IIIs [5,9] and bactofilins [47] are also evident, demonstrating that duplication of cytoskeletal proteins has occurred multiple times in archaea.

Actin stands out as an exception to this trend, where multiple specialised actin homologues with single functions in bacteria are lost in place of conserved and multifunctional actin in crenarchaea and eukaryotes. Akin to this, the single conserved CetZ homologue in Thermococci may represent an additional example of an evolutionary shift toward a single multifunctional cytoskeletal homologue. However functional studies of these CetZs are yet to be carried out, so it is not yet clear whether these CetZs exhibit multifunctionality. Taken together, the evolutionary history of archaeal cytoskeletal proteins discussed here point toward an interesting evolutionary trade-off between duplicated cytoskeleton proteins with specialised functions and a conserved cytoskeleton with multiple functions.

Concluding remarks: the yin and yang of archaeal cytoskeletal systems

We have outlined several examples of paralogous cytoskeletal proteins which have opposing functions that are coordinated to achieve a single main function. In Euryarchaeota, cell division is driven by the coordination of FtsZ1 and FtsZ2, which stabilise and drive Z-ring constriction, respectively, while the distinct and specialised functions of HalA and HalB ensure proper membrane remodelling and Z-ring condensation. Similarly, opposing functions of CetZ1 and CetZ2 control cell morphogenesis throughout the growth cycle. However, this trend is not only limited to Euryarchaeota as coordination among several functionally distinct CdvB paralogues for the regulation and control cytokinesis is also observed in Crenarchaea. The specialised and opposing functions of these proteins represent remarkable examples of protein duplication and functional specialisation within multiple classes of cytoskeletal proteins in archaea. In each case, the different yet complementary roles of these cytoskeletal proteins achieve a single harmonious outcome that contributes to a fundamental cellular process.

Our understanding of the functions and mechanisms of the archaeal cytoskeleton is limited compared with our relatively vast knowledge of bacterial and eukaryotic cytoskeletal systems. Studying archaeal cytoskeletal proteins will be key in exploring the diversity of archaeal cell functions, and provide key insights into the divergence and conservation of cytoskeletal systems across all life, while promoting further development of archaeal species as model organisms for the study and applications of cell biology. As an evolutionary stepping stone between Bacteria and Eukarya, archaeal cells will also provide important insights into the early evolutionary history of life and its main structural transitions.

Further research into the emergence of complexity, diversity, and multifunctionality of ESCRT-III and tubulin superfamily proteins in archaea is anticipated to provide important insights into the evolution of the highly sophisticated and specialised functions of their eukaryotic counterparts. Similarly, the apparent shift toward multifunctionality of actin in archaea may provide the evolutionary context to the extensive multiple functions and conservation of eukaryotic actin. Together, these archaeal cytoskeletal proteins help to bridge the vast evolutionary landscape between bacteria and eukaryotes, aiding our understanding of evolution and eukaryogenesis. As the study of Asgard archaea or other organisms perhaps yet to

be discovered that further bridge the evolutionary void between prokaryotes and eukaryotes become more feasible, we expect further multiplicity of cytoskeletal function will be discovered and related to increasingly sophisticated cell structures and functions. Piecing these together may allow the eventual creation of a map of the evolution of the modern eukaryotic cell.

Author contributions

HJB – Conceptualisation, Investigation, Writing – original draft, Writing – review and editing; VDS, LB – Investigation, Writing – original draft; IGD – Conceptualisation, Writing – original draft, Writing – review and editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest.

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Data availability

No data was used for the research described in the article.

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* of special interest
** of outstanding interest

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