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Review

The archaeal Cdv cell division system

Alberto Blanch Jover[1] and Cees Dekker[1,*]

The Cdv system is the protein machinery that performs cell division and other membrane-deforming processes in a subset of archaea. Evolutionarily, the system is closely related to the eukaryotic ESCRT machinery, with which it shares many structural and functional similarities. Since its first description 15 years ago, the understanding of the Cdv system progressed rather slowly, but recent discoveries sparked renewed interest and insights. The emerging physical picture appears to be that CdvA acts as a membrane anchor, CdvB as a scaffold that localizes division to the mid-cell position, CdvB1 and CvdB2 as the actual constriction machinery, and CdvC as the ATPase that detaches Cdv proteins from the membrane. This paper provides a comprehensive overview of the research done on Cdv and explains how this relatively understudied machinery acts to perform its cell-division function. Understanding of the Cdv system helps to better grasp the biophysics and evolution of archaea, and furthermore provides new opportunities for the bottom-up building of a divisome for synthetic cells.

Highlights

The Cdv system is a protein machinery responsible for cell division in members of the TACK superphylum of archaea.

The Cdv system presents many structural similarities to the eukaryotic ESCRT machinery, and it is presumed to act in a similar manner.

The Cdv system has been found to be implied in a wide range of processes in the cells, such as division, vesicle budding, and virus release.

The role of the CdvB paralogs is key in the division process, as became clear through recent developments in high-temperature live-cell imaging and super-resolution microscopy.

In vitro experiments have shed light on the mutual interactions between CdvA, CdvC, and the various CdvB paralogs.

Introduction

Cell division is a key process in the cell cycle of any living organism. A dedicated set of proteins and complex cellular signaling processes are needed to ensure a faithful splitting of a mother cell into two daughter cells. Eukaryotes make use of the endosomal sorting complex required for transport (ESCRT) for their final stage of cell division, a complex machinery with a large set of proteins that are capable of severing membrane necks from the inside of the cell membrane [1–3]. In virtually all bacteria, the process of cell division is driven by the tubulin-like protein FtsZ [4–6] which guides the cell-wall-synthesis machinery in building an inwards ingression of the cell wall and membrane until the cell splits into two.

Archaea, despite being prokaryotes like bacteria, present a broad heterogeneity in their cell-division machineries. Most archaeal phyla present different tubulin-family proteins that govern shape control [7], including FtsZ homologs that are responsible for cell division [8]. The archaeal FtsZ presents several differences from the bacterial one, as many archaea use two different FtsZ proteins that play different roles during the division process [9]. Unlike in bacteria, where FtsZ has been studied very extensively, the information on how FtsZ acts in archaea is limited, but it appears that the SepF protein plays a major role in cell division by linking one the FtsZ variants to the membrane [10–12]. The current review instead focuses on another interesting membrane-remodeling machinery, that is, the Cdv proteins (Figure 1A) that presents itself in some members of the TACK and Asgard superphyla of archaea. It is worth noting that there are some archaea that lack both the Cdv and the FtsZ machinery [13], and it has been proposed that they might use an actin homologous cytoskeleton to perform their cell division [14]. Conversely, some archaea contain both the Cdv and FtsZ machineries [15]. However, little is known about their function in these rare cases, and generally the different phyla of archaea tend to be distinguished as having an FtsZ- or Cdv-based cell-division system.

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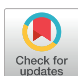

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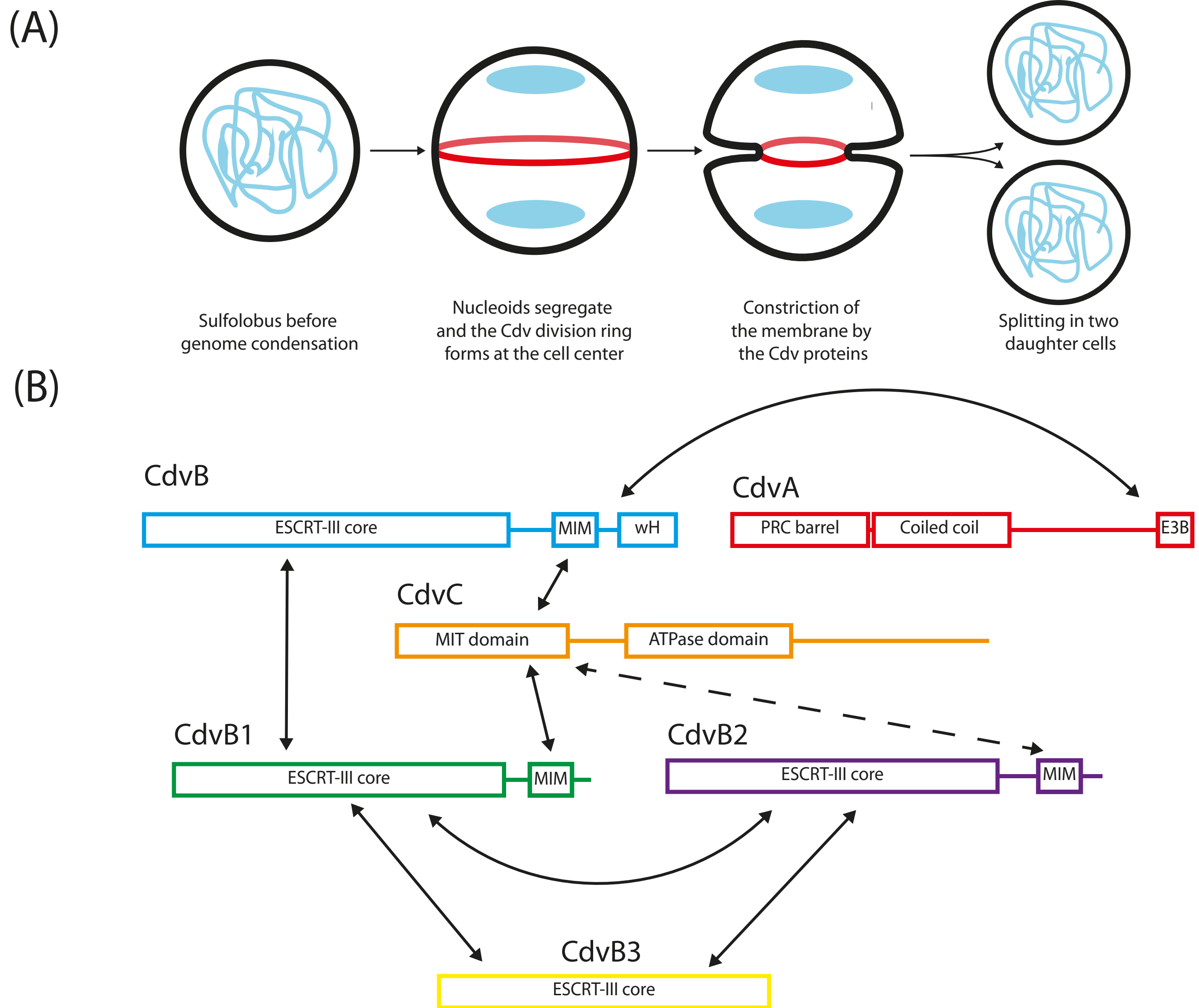

Figure 1. Overview of the Cdv system. (A) Schematic of the cell cycle of the archaeon model organism *Sulfolobus acidocaldarius*. (B) Schematic representation of the protein domains of Cdv proteins from Sulfolobales and the interactions between them. Arrows show domains that interact with each other; dashed arrows show speculative interactions that have not yet been experimentally shown. Abbreviations: ESCRT, endosomal sorting complex required for transport; MIM, MIT-interacting moiety; MIT, microtubule-interacting and transport domain; PRC, photosynthetic reaction center; wH, winged helix.

The Cdv system is a protein machinery responsible for cell division and vesicle deformation in members of the TACK (Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota) superphylum of archaea, and it is also present in the Asgard archaea [16,17]. Various protein families of these archaea are homologous to those found in eukaryotes, such as DNA-processing machineries, ribosomal proteins, ubiquitination systems, and cell-division proteins [18]. These similarities underlie the widely accepted idea of a common evolutionary ancestor between eukaryotes and archaea, and the notion that these two domains of life relate more closely to each other than to bacteria [19]. One of the archaeal protein machineries that exhibit such a homology to eukaryotes is presented in the Cdv system.

A growing interest in the mechanistic understanding of the Cdv proteins in recent years has led to a larger body of published research. In this brief review, we summarize the most relevant research that has been done on the Cdv system since its first description almost 15 years ago [16,20]. We describe the Cdv system, its evolutionary relationship to the eukaryotic ESCRT system, and its functional role in cell division and vesicle formation. We focus on describing the reported experimental findings – mostly from the Sulfolobales – and the picture that they portray of the Cdv system (Figure 1A). While most knowledge has been obtained from genetic and cell-biology studies, more recently, modeling and *in vitro* studies began to disentangle the biophysical mechanism of the various components of the Cdv system. We finish the paper with an outlook that sketches a path forward, as well as discusses the potential use of the Cdv system for the bottom-up building of a divisome for synthetic cells.

The Cdv system in the Sulfolobales

The Cdv system was first described in *Sulfolobus acidocaldarius* [16,20], an extremophile archaeon that inhabits volcanic areas with temperatures of about 80°C and pH as low as 2–3 [21]. Since its discovery, the Cdv system has generally been described as consisting of three classes of proteins: CdvA, CdvB (and multiple paralogs), and CdvC (Table 1). CdvA is an exclusively archaeal protein that has no homologs in eukaryotes. By contrast, all the CdvB paralogs display homology to the eukaryotic ESCRT-III proteins, while CdvC is an AAA ATPase that is homologous to the eukaryotic Vps4 [22].

S. acidocaldarius emerged as a model organism for studying the Cdv system because it was the first to be described [16,20] and because well-established protocols exist for its growth and manipulation [23]. The core proteins of the Cdv system of the Sulfolobales, CdvA, CdvB, and CdvC, are encoded in an operon that is essential for the viability of the organisms [24], while the other CdvB paralogs are found in different parts of the genome [16]. CdvA is a purely archaeal protein that can interact with CdvB [25] through its ESCRT-III-binding region (E3B) (Figure 1B). This E3B region of CdvA interacts with the winged Helix (wH) region present in CdvB [26], which is the only

Table 1. Membrane-deforming Cdv proteins in the Sulfolobales archaea, and their ESCRT eukaryotic homologs

Archaea	Eukarya
CdvA Protein found only in archaea that acts as a membrane anchor, recruiting CdvB to the center of the dividing cell through its E3B domain.	No homologous protein
CdvB Main protein that interacts with CdvA through its wH domain, and which forms a noncontractile ring at the middle of the cell where it recruits the CdvB1 and CdvB2 paralogs involved in membrane constriction.	ESCRT-III Main protein filament that forms a spiral around the division point to promote deformation of the membrane and final scission.
CdvB1 and CdvB2 These CdvB paralogs are recruited to the membrane after the formation of the CdvB ring, and are responsible for membrane constriction.	
CdvB3 CdvB paralog involved in the process of vesicle formation but not in the cell division	
CdvC ATPase responsible for depolymerizing the CdvB rings at the membrane to generate turnover and facilitate constriction.	Vps4 ATPase responsible for depolymerizing ESCRT-III filaments, generating a turnover that facilitates membrane constriction and scission.

CdvB paralog that contains such a region. Since CdvA interacts only with CdvB, and CdvA interacts with the cell membrane (while CdvB does not), it was suggested that the role of CdvA is the recruitment of CdvB to the membrane [25]. Yeast 2 hybrid assays have shown that CdvB is also capable of interacting with CdvB1, while CdvB1 furthermore interacts with CdvB2 and CdvB3 [20]).

In eukaryotes, ESCRT-III proteins are recruited to the membrane through a series of interactions with the ESCRT-0, ESCRT-I, and ESCRT-II complexes of the system [27]. At the membrane, they are structured into filamentous polymers that tend to form spirals [28,29] that deform the membrane through a coordinated depolymerization and reshaping of the filaments into a tighter spiral [30] – a process that ultimately leads to membrane abscission. Similarly, CdvB paralogs of archaea have recently been suggested to deform the cell membrane and perform scission of the cell [31]. Clear images of micron-sized CdvB rings at the division site have been obtained, although their nanoscopic structure has so far remained unclear.

CdvC is the only active Cdv protein with a described ATPase domain [26,32]. The role of CdvC appears to be providing energy to the system to depolymerize the ESCRT-III filaments at the membrane, remodel their structure, and thus deform and cut the membrane, analogously to the eukaryotic machinery [33]. Specifically, by hydrolyzing ATP, CdvC may detach monomers of the membrane-bound CdvB filaments into solution, allowing for a remodeling of the CdvB structures at the membrane that leads to a narrower neck structure until scission occurs [33,34]. Such a scenario is also suggested by the presence of a Microtubule Interacting and Transport (MIT) domain in CdvC that is capable of interacting with the MIT-Interacting Moiety (MIM) (Figure 1B) present in the CdvB proteins [35]. This interaction is well known in eukaryotes, where Vps4 (CdvC homolog) interacts with the ESCRT-III proteins in the same way [35], reinforcing the idea of a similar mechanism of action in the two systems.

The Cdv system in other archaea

After the discovery of the Cdv system in *S. acidocaldarius,* which belongs to the Crenarchaeota phylum, other Cdv systems were found in different archaea, although presenting differences in their composition. Some members of the Euryarchaeota phylum present genes coding for CdvB and CdvC [36], but not much is known about their functional roles. Interestingly, members of the Thaumarchaeota phylum, which are members of the TACK superphylum like Crenarchaea [37], contain genes encoding for both the Cdv machinery and a tubulin homolog similar to FtsZ [5,38]. The thaumarchaeon *Nitrosopumilus maritimus* was found to use the Cdv system for cell division [39]. However, while the thaumarchaeal FtsZ is a member of the tubulin family it is actually distant from the FtsZ clade of proteins and it does not possess the catalytically active domain common in the FtsZs that drives cell division [40]. It can therefore not perform many of the classically associated functions of FtsZ that require the hydrolysis of GTP, and its function in the Thaumarchaeota remains unknown.

More recently, interest emerged in the Cdv proteins from the Asgard archaea. This newly described superphylum of archaea is evolutionarily closest to eukaryotes [41], and their CdvB proteins are the closest to the eukaryotic ESCRT-III as can be seen in the evolutionary tree in Figure 2. This evolutionary tree that we built for CdvB proteins displays interesting relations between the different archaea and the connection of Asgard archaea to Eukarya. This is in line with recent comparative phylogenetic studies which suggest that part of the complexity and characteristics that are commonly associated with eukaryotes started appearing in the Asgard archaea before the formation of eukaryotes [18]. More specifically, it has been suggested that eukaryotes arose from the formation of a symbiont between a Loki archaeon (part of the superphylum

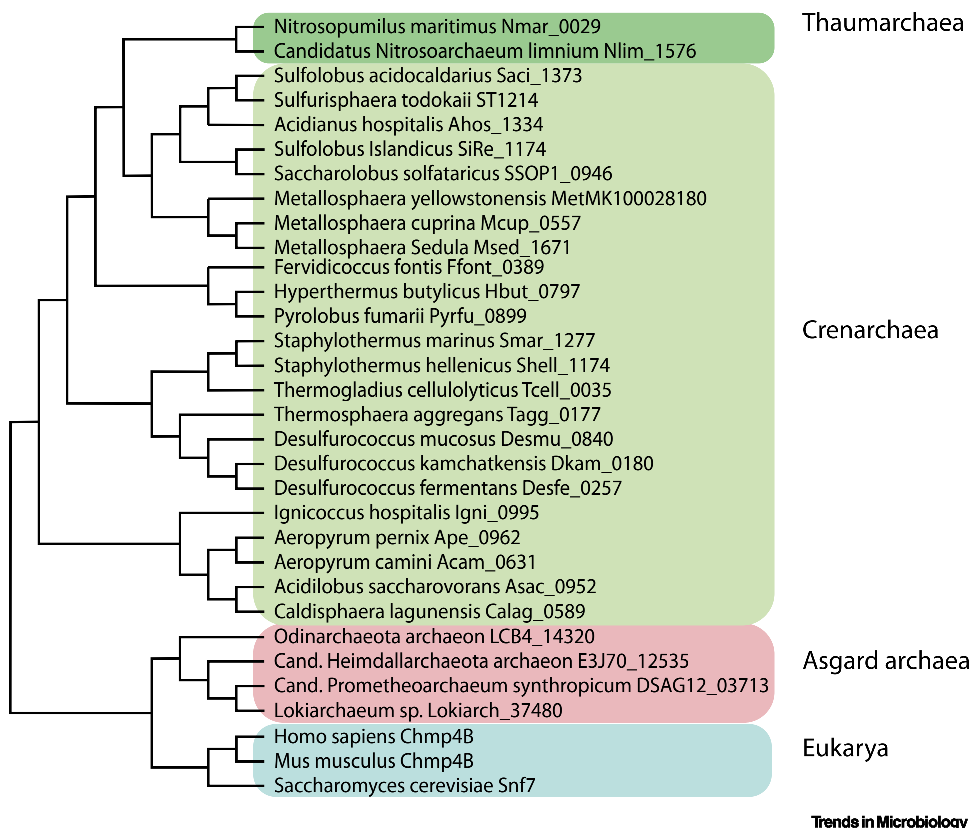

Figure 2. Phylogenetic tree of the evolutionary relationship of the CdvB (ESCRT-III) proteins in different organisms. This tree shows how CdvB from the Asgard archaea is closer to the ESCRT-III of eukaryotes than the Crenarchaeal CdvB, suggesting that the divide between the Asgard and the TACK archaea happened already before the appearance of eukaryotes. Abbreviation: ESCRT, endosomal sorting complex required for transport.

Asgard) and a bacterium, which slowly integrated their genes and functions with each other until forming the first eukaryote [42]. While the Asgards possess Cdv proteins, they also present two FtsZ homologs, like most of the Euryarchaeota [12], which raises questions about the roles of each of these systems in these organisms. The Cdv proteins in Asgards have been found to be much more similar to the eukaryotic ESCRT machinery than the TACK archaea. Remarkably, they present homologs of the ESCRT-I and ESCRT-II machineries, and these interact with the ubiquitylation machinery of the organism, just as in eukaryotes [43]. The Asgards lack the CdvA protein, while CdvB has an ANCHR motif similar to that of the eukaryotic ESCRT-III, which could allow it to directly bind the membrane [44]. There are also some significant structural differences of the CdvB paralogs, which indicates that the protein interactions, and by extension the mechanism of their action, will also be different in the different phyla [44]. This leads to the speculation that, while the Asgard Cdv machinery likely is involved in some membrane-deforming processes in the cell, the process of cell division may be carried out by the FtsZ homologs, just as in the Euryarchaeota [9]. So far, however, there is no experimental proof for this, mostly due to difficulties in culturing and growing Asgard archaea in the laboratory [45].

Cdv is often portrayed as a simpler and more antique version of the ESCRT system [20], and the parallelism between the two domains of life was used for many years to hypothesize how the

archaeal machinery would work, given the extensive literature on the ESCRT and the limited knowledge and understanding of the Cdv proteins. The fact that components of the Cdv machinery like CdvB/ESCRT-III and CdvC/Vps4 are found in all Cdv/ESCRT-bearing organisms, but ESCRT-I, ESCRT-II, and the ubiquitination system are present in only Asgard and Eukaryotes, has led to exciting speculation about their evolutionary relationship. It is, for example, conceivable that Asgard archaea evolved this more complex machinery for endo/exocytosis before eukaryogenesis occurred, and that it was this very same trait that allowed for the successful formation of the internal membrane compartments which allowed for eukaryotes to form [43].

Recently, it was found that the bacterial proteins Vipp1 and PspA belong to the same superfamily as the ESCRT proteins. Both proteins are involved in processes of membrane remodeling: PspA is involved in the Psp stress response in bacteria [46], and Vipp1 takes part on the biogenesis and repair of thylakoids in cyanobacteria and chloroplasts [47]. They present a similar protein sequence, they can bind membranes, they arrange themselves in the same helical tube structure as ESCRT proteins, and they are even able to deform lipid vesicles *in vitro* [48–50]. While the exact mechanics of their functioning is of yet unclear, this finding presents the first evidence that proteins belonging to the ESCRT-III superfamily are present across all domains of life, including eubacteria, raising interesting questions about their evolutionary relationship.

The role of Cdv proteins in cell division in the Sulfolobales

Immunostaining images of dividing *S. acidocaldarius* cells showed a band of CdvA and CdvB forming at the center of the cell, between the two segregated nucleoids, with a band of CdvC proteins observed at the same location. These were some of the first clear indications that the Cdv operon proteins were directly involved in the process of cell division. Cdv protein concentrations also vary during the cell cycle of the organism, with expression of the genes encoded on the Cdv operon occurring before onset of cell division [16]. Gene inactivation experiments in *S. acidocaldarius* showed that removal of the individual CdvB paralogs greatly affected cell growth and generated aberrant cells [51]. This provided the first indications that the CdvB paralogs were involved in the process of cell division as well, although they appeared to not be strictly essential since cells could still survive without them.

Recent super-resolution imaging techniques provided further detail (Figure 3A): at an early stage of cell division, first a CdvB ring forms at the middle of the cell [31]. Then, CdvB1 and CdvB2 are recruited at the same ring location. Subsequently, the proteasome digests the initial ring of CdvB, leaving just a CdvB1 and CdvB2 ring (Figure 3A). This second ring appears to be responsible for the constriction process of membrane ingression and fission. These data showed that the CdvB paralogs play a key role in the cell division process.

The development of high-temperature (75°C) live-cell imaging microscopy of these thermophilic archaea allowed further investigate of the role of these CdvB paralogs in archaeal division [52]. Mutants of *S. acidocaldarius* that lacked CdvB1 presented abnormal division (Figure 3B) in which some cells would suddenly arrest their division process. Mutants of *Sulfolobus islandicus* lacking CdvB1 presented a pearl-collar appearance in which chains of cells were kept attached to each other, while a genome-number analysis showed many cells containing multiple genomes [53]. These data suggest that CdvB1 is implicated in the process of faithful completion of the fission process. Conversely, live imaging of *S. acidocaldarius* cells lacking CdvB2 showed that the division plane in cytokinesis was not located at the middle of the cell, yielding asymmetric division with differently sized daughter cells (Figure 3B). This indicates that the role of CdvB2 is to keep the constricting ring at the correct mid-cell position, and hence ensure symmetric fission.

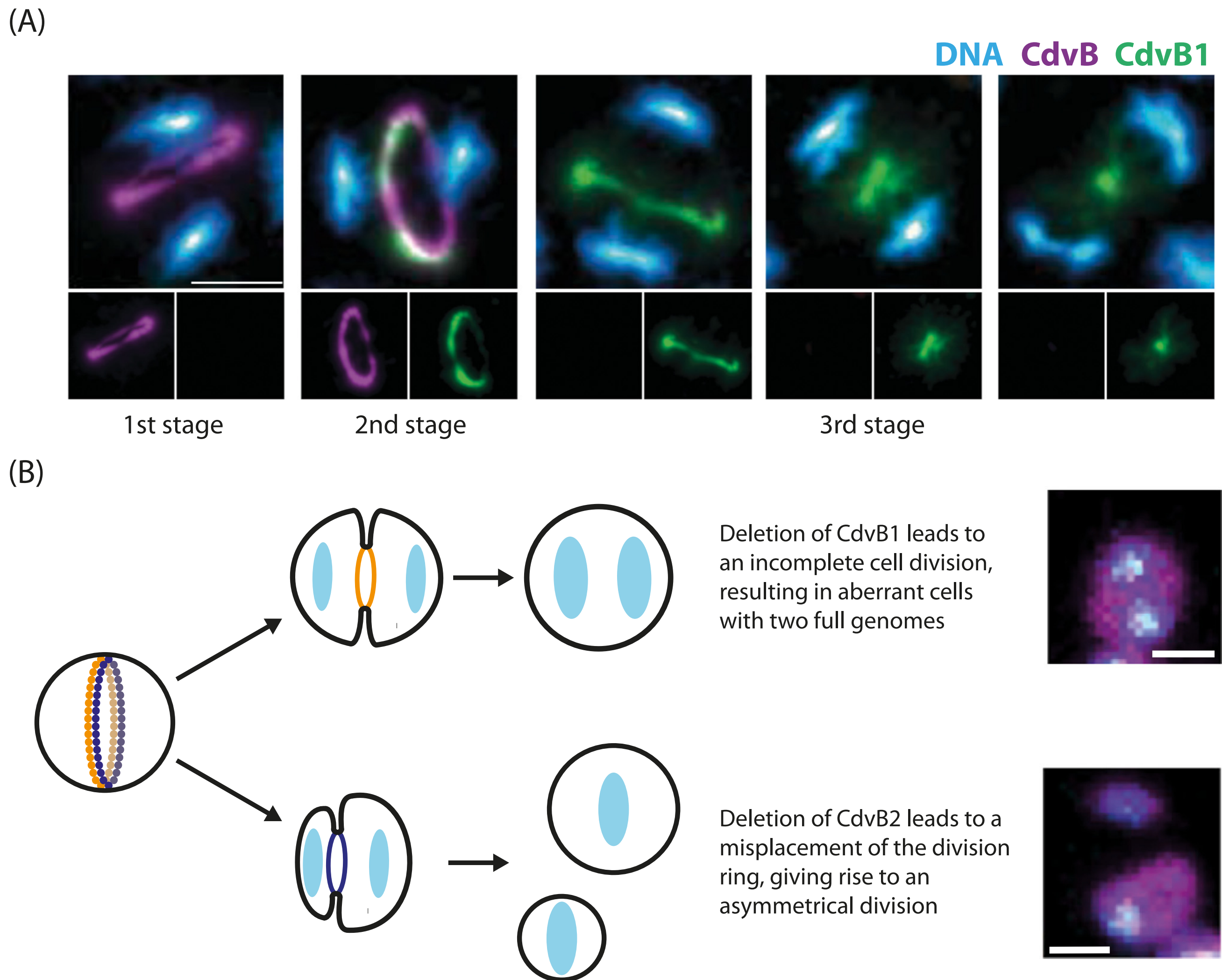

Figure 3. The Cdv system in *Sulfolobus* cells during cell division. (A) High-resolution immunostaining images of *S. acidocaldarius* during cell division, showing (1st stage, left image) how a first ring of CdvB (purple) is formed at the division site between the two replicated genomes (blue), whereupon CdvB1 (green) is recruited, CdvB is removed (2nd stage), and finally only CdvB1 remains which is observed to constrict the cell membrane (3rd stage). Scale bar 0.5 μm. (B) Schematic representation of the effects of the deletion of CdvB1 (purple) and CdvB2 (orange). Top right image shows a CdvB1-lacking cell of *S. acidocaldarius* where scission of the membrane does not occur even after a long time. Bottom right image shows a CdvB2-lacking cell of *S. acidocaldarius* where the two daughter cells have very different sizes right after division. Navy blue: CdvB1. Orange: CdvB2. Scale bars are 1 μm. Panel C is reproduced from [31] with permission; images in B are adapted from [52] with permission.

In contrast to CdvB1 and CdvB2, very little is known about the role of CdvB3 in cell division. In *S. acidocaldarius*, CdvB3 did not form any kind of ring-like structure at the cell center during constriction [51]. Mutants without CdvB3 were still able to survive, although giving rise to fewer and much larger cells. In addition, deletion of the protein led to an aberrant localization of CdvA throughout the cell, and prevented CdvB from forming a defined structure [51]. It thus seems that CdvB3 does not participate directly in the constriction and scission process, but instead it may be involved in the correct positioning of the CdvA/CdvB ring.

In this process of membrane constriction, CdvC seems to play the same role as its eukaryotic homolog, that is, to depolymerize the division rings formed by the CdvC/ESCRT-III proteins to allow

for a correct cell division [54]. Very recent studies of the action of CdvC inside cells indicated that a coordinated step-wise depolymerization of the CdvB paralogs from the membrane is needed for successful cell division, which is coordinated by CdvC.

While all of the different CdvB paralogs are very similar in sequence and structure, they have distinct roles in the process of membrane deformation and cell division. All of these paralogs very likely come from one original gene that started duplicating in the genome, whereupon the newly formed paralogs started specializing in diverging roles of the division process, providing robustness and reliability to the process. It will be interesting to further examine whether the appearance of the CdvB paralogs in archaea happened before eukaryogenesis or whether perhaps this was a parallel convergent evolutionary process in the development of the eukaryotic ESCRT machinery.

While the above data describe the most well-studied Cdv proteins from members of the Sulfolobales, it is worth noting that a large variability in sequence and functional regions exists across the different archaeal species that have the Cdv machinery [44]. For example, many species of Asgard archaea lack some or all of the CdvB paralogs, as well as present different functional domains in the commonly shared proteins. This likely indicates an as yet undiscovered set of protein interactions and mechanisms for the different Cdv machineries.

There also remains a question as to how the process of cell division is coordinated within the cell cycle of the organisms. In eukaryotes, the cell cycle is regulated through the action of cyclin-dependent kinases [55], but no clear proof of such a system has been found in the TACK phylum of archaea. The crenarchaea present a differentiated cell cycle, with growth and chromosome segregation phases that are well differentiated from the cell-division phase [56]. Expression of the genes of the Cdv system is enhanced right before cell division [57]. Microscope images of *S. acidocaldarius* and *N. maritimus* showed how the formation of Cdv rings at the mid cell was coordinated with the segregation of the chromosome prior to cell division [16,39]. There are certain eukaryotic-like kinases present in members of the crenarchaea that seem to regulate processes in the organism (e.g., cell motility) through protein phosphorylation [58,59]. Although some of these kinases present cyclical transcription patterns [57], their implication in the regulation of the cell cycle is not clear. It seems more likely that the regulation of cell division in the cell cycle comes from a regulation in the protein expression. Some indications for this came from recent studies that showed that the transcription factor aCcr1 was indispensable for cell viability of *S. islandicus*, as it downregulates the gene expression of CdvA as well as other proteins and kinases [60]. A peak of aCcr1 gene expression was observed before that of CdvA, leading the authors to suggest that this may constitute a regulatory cycle in which expression of aCcr1 ensures that not too much CdvA goes to the membrane and cell division can occur correctly. Some authors have speculated about a double role for CdvA, implicating it in both cell division and chromosome organization since it also has DNA-binding abilities [26]. There is, however, no direct proof of that so far. Several key questions about the regulation of cell division remain unanswered – such as the sequence of events on Cdv-driven constriction, what drives the placement of the Cdv division ring at the center of the cell, and whether there is any relationship between the Cdv machinery and the deformation of the external S-layer of the archaeal cells. Answers to all of these questions will be important to get the full picture of how these proteins are integrated into the complex cell cycle of the Sulfolobales.

The role of Cdv proteins in vesicle formation

In eukaryotes, the ESCRT complex is involved in many different cellular processes that concern membrane deformation [1]. It was in fact first described and characterized for its main role in

the formation of vesicles in the multivesicular body pathway [61], and its involvement in other membrane-deforming processes was subsequently revealed, for example in cytokinesis, nuclear envelope reformation, and viral budding [62–64]. Basically, the ESCRT system is involved in all processes that require the deformation and pinching of small membrane necks in a reverse topology in cells. Interestingly, such a diversity of functions appears to be conserved in archaea as the Cdv system is involved not only in cell division but also in the formation of membrane vesicles [65].

The release of extravesicular bodies is a widespread phenomenon amongst the *Sulfolobus* genus [65]. These extracellular vesicles (EVs) have the same lipid composition as the archaeal membrane lipids and are covered by an S-layer, showing that they are an outward protrusion of the membrane of the cells. The roles of these EVs are diverse, although its particular purpose for the Sulfolobales is unclear. EVs from *S. islandicus* were found to contain plasmid DNA, suggesting that they can be used for transfer of genetic material [66]. These EVs in *S. islandicus* have furthermore been associated with the release of 'sulfolobicins', protein toxins that inhibit the growth of other Sulfolobales [67]. Mass-spectrometry analysis of EVs showed that they contain several Cdv proteins, suggesting that the Cdv system is involved in the formation of these EVs [65,66]. When knocking down any of the different CdvB paralogs or CdvC using CRSPR [66], cells produced much lower amounts of EVs. Deletion of CdvA, however, largely left the production of vesicles unaffected, indicating that CdvA is dispensable for the process of EV formation. The ability of the Sulfolobales to create this type of EV, using the same proteins, suggests that this is a trait that precedes the formation of eukaryotes, and that therefore the eukaryotic ESCRT machinery already arose with this multiplicity of functions.

Viruses also make use of the ESCRT system, as newly formed virus particles inside infected cells escape the cell encapsulated inside EVs [68]. A similar process was found in *Sulfolobus*, where archaeal viruses induced overexpression or repression of the expression of the Cdv proteins [69,70]. In *Saccharolobus solfataricus*, cells infected with the STIV archaeal virus were observed to overexpress the Cdv proteins [70], and Cdv proteins of their host organisms were involved during the infection cycle [70]. Virus-infected cells have also been useful to determine the role of different Cdv proteins in vesicle budding. The fact that CdvB3 appears not to be involved in the constriction during cell division in *S. islandicus* raised the idea that it might be involved in the vesicle-budding process. Infection of *S. islandicus* cells with the archaeal virus STSV2 promoted the formation of buds coming out of the cells (Figure 4A). For mutants lacking CdvB3, however, these buds did not appear. In a more recent study of the STSV2 virus in *S. islandicus* [71], all Cdv genes were downregulated upon viral infection, and the cells suffered from gigantism, reaching a size up to 20 times larger than normal. It was found that spirals of CdvB1 were formed inside the newly formed buds (Figure 4B) as opposed to the normally occurring mid-cell ring of healthy cells. This shows not only the versatility of the Cdv system in performing different membrane deforming processes including vesicle budding (Figure 4C) but also how different proteins of the system participate in some functions and not in others.

In vitro studies of the *Sulfolobus* Cdv system

Studying the higher-order structures that these ESCRT proteins form *in vitro* provided valuable information about how they may work inside cells. Most eukaryotic ESCRT-III proteins present a duality between a soluble cytosolic state and a membrane-bound polymer form [72]. Interestingly, many of these proteins stay monomeric when purified in their full-length version but polymerize into filaments when removing the last 40 amino acids of their sequence [73,74]. The human ESCRT-III proteins CHMP2A and CHMP3 form helical straight tubes in solution that can be disassembled by the ATPase Vps4 [75]. When incubated with lipid membranes, they polymerize forming straight tubes and cones around lipid tubes [30]. Yeast ESCRT-III was

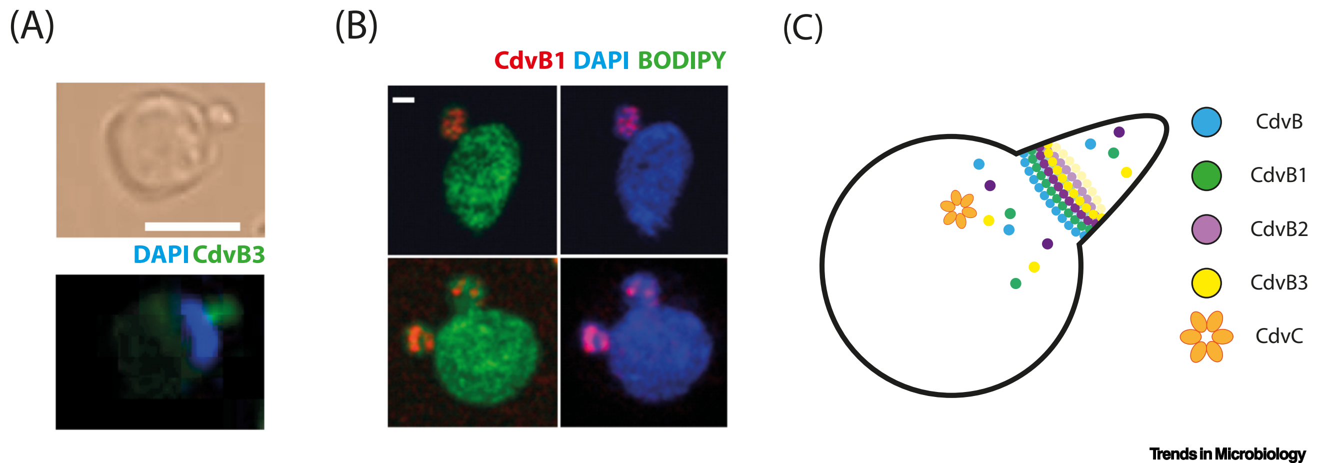

Figure 4. Cdv-mediated vesicle budding for viral release. (A) Immunostaining of *Sulfolobus islandicus* cells infected with the STSV2 virus showing CdvB3 at the neck of the budding vesicle. Top and bottom images are phase contrast and fluorescence, respectively. Scale bar 2 μm. (B) Two examples (top and bottom row) of immunostained STSV2-infected cells of *Sulfolobus islandicus*. Unlike healthy cells that would present CdvB1 at the center of the cell where the membrane shrinks, these infected cells present CdvB1 at the neck of a bud that is performing an asymmetric division. DAPI and BODIPY are DNA stains. Scale bar 1 μm. (C) Schematic of the vesicle-budding function of the Cdv system. Panel A is adapted from [53] with permission. Panel B is adapted from [71] with permission.

shown to form 2D spirals on the surface of lipid bilayers [28], while Vps4 dynamically modified these structures [76]. Furthermore, both human and yeast proteins were shown to assemble into filament bundles along membrane tubes [77,78]. ESCRT-III has been reported to form spirals that deform the membranes and bend them into domes or tubes [30], acting as springs that can exert forces [28].

Similar membrane remodeling has not yet been reported for archaeal protein complexes, but first studies have been conducted to assess the interactions of the proteins with membranes. CdvA was found to be capable of binding the polar lipid fraction E (PLFE) in *Sulfolobus acidocaldarius* [25], whereas CdvB was not. A later study [79] reported that the truncated CdvA(69-238) was unable to polymerize into filaments on its own. However, when the protein was incubated with liposomes of PLFE, CdvA(69-238) assembled into filaments that wrapped around liposomes. These images suggest that CdvA may arrange itself at the center of the cell in a spiral shape that serves as a template that accommodates other divisome components.

CdvA full-length from *Metallosphaera sedula* was found to polymerize *in vitro,* forming double-helical filaments [26]. Much like the human ESCRT-III proteins that do not polymerize *in vitro* when they are full length, full-length CdvB from *M. sedula* did not polymerize [26]. However, upon removal of the C-terminus domain of its sequence, it polymerized into elongated filaments. By contrast, CdvB1 was found to self-assemble into filaments already in its full-length form [80]. These filaments of CdvB1 were shown to be depolymerized by the action of CdvC [80] in the same way that the polymers of ESCRT-III proteins in eukaryotes are depolymerized by Vps4 [75]. This was the first evidence of a depolymerization of a CdvB paralog polymer by the action of CdvC, and it supports the idea that CdvC is responsible *in vivo* for generating a turnover of monomers at the division ring, supporting its sequential assembly and disassembly. In the same study [80], it was reported that the filaments that CdvB1 form *in vitro* cannot bind the lipid membrane, but that monomeric CdvB1 proteins can bind. This indicates that the membrane-binding patch of the protein is not accessible any more upon polymerization, and hence that, *in vivo,* polymerization of the protein likely occurs directly at the membrane. Getting high-

resolution images of the arrangement of these Cdv proteins on lipid membranes, similar to those for the ESCRT proteins, will help us to understand the structure of these proteins and how they facilitate membrane deformation.

The AAA ATPase CdvC presents many similarities with the eukaryotic Vps4. Both CdvC and Vps4 are composed of a MIT region, which is used to interact with the MIM domain of the ESCRT-III proteins, followed by an ATPase region, and a final C-terminal helix (Figure 1B) [26,81]. The monomeric version of the proteins is inactive, whereas the proteins become catalytically active upon oligomerization into hexamers [81]. The hexamers formed by CdvC and Vps4 are actually not closed rings but instead form an open and flexible structure that can wrap itself around the ESCRT-III filaments through their MIM-MIT interactions [82]. The hydrolysis of ATP generates conformational changes in the structure of this hexamer [32], which makes it probably 'walk' along the ESCRT-III filament as it depolymerizes it [34]. Additionally, in yeast, the Vps4 could be deleted and substituted by an Asgard CdvC, and the yeast cells were still viable, further showing the close evolutionary proximity between the Asgard and the eukaryotes [83].

Outlook: towards a physical mechanism for the archaeal Cdv divisome

Based on the current knowledge of the Cdv proteins of the Sulfolobales, one can attempt to sketch a picture that best summarizes how the Cdv system acts during the process of cell division (Figure 5A) [52]. Initial formation of a noncontractile ring involves CdvA and CdvB (Figure 5AI). This ring is located between two segregated chromosomes and is anchored to the membrane by CdvA, which recruits CdvB to the membrane at the mid-cell position. Subsequently, CdvB recruits CdvB1 and CdvB2 (Figure 5AII), thus forming an extended ring at the center of the dividing cell [31,52]. After recruitment of CdvB1 and CdvB2, CdvB is removed from the membrane by CdvC, digested by the proteasome (Figure 5AIII). After removal of the initial CdvB ring, only a CdvB1/CdvB2 contractile ring is left (Figure 5AIV), and these two proteins appear to be responsible for the constriction and scission of the cell [31,52] (Figure 5AV). While we know that CdvB is removed and digested, we are still lacking insight on the fate of CdvA in this process. It seems unlikely that it will remain attached to the contractile ring since there are no interaction domains between CdvA and CdvB1/CdvB2, but it is currently unclear whether and how it is removed.

Once the CdvB1/CdvB2 constricting ring is formed, it needs to shrink and go from the low-curvature state in the fully spherical cell to a high-curvature state, while remodeling the membrane all the way down until scission of the cell. Coarse-grained Molecular Dynamics (MD) simulations have shown that CdvB1/CdvB2 copolymers formed at the center of the cell can deform the membrane by merely transforming from a low-curvature to a high-curvature conformation [31]. This implies that deforming the membrane, almost until the point of division, can be realized by shape changes of polymers without changing their length. However, these polymers would not be able to perform the final scission, as towards the end of the membrane-deformation process, steric hindrance at the neck of the dividing cell impedes the division into two daughter cells. Hence, the cell-division process requires the controlled removal of proteins from the ring. Given the recent studies showing the role of CdvC in controlling the division ring disassembly [54], and because it has been shown that CdvC can depolymerize CdvB1 filaments and detach them from lipid membranes [80], it seems reasonable to hypothesize that CdvC is responsible for this turnover generation at the constricting neck, thus finalizing the process of cell division. It will be interesting to examine how this removal of different CdvB paralogs by CdvC is timed in the cell, and how the

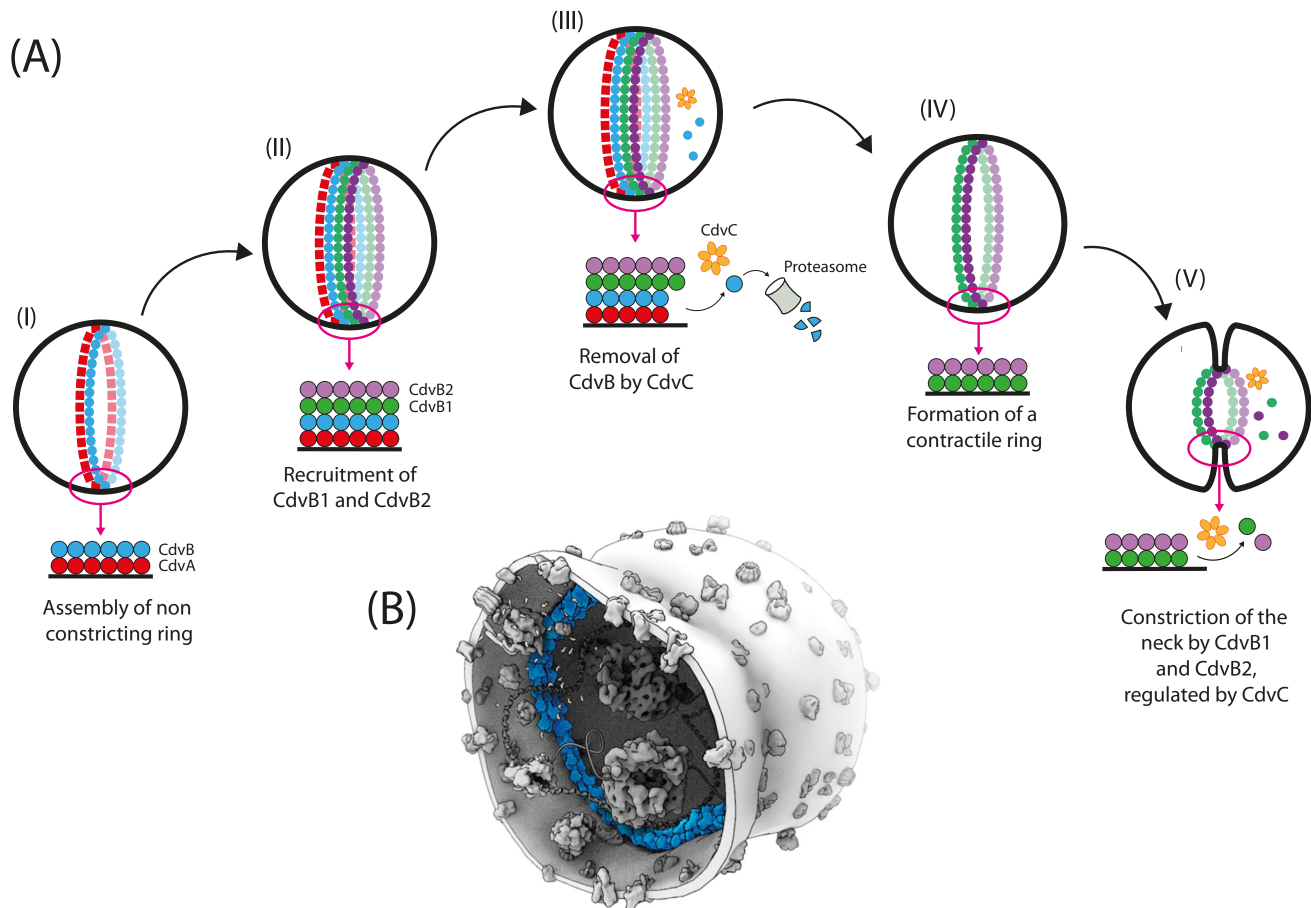

Figure 5. Schematic representation of Cdv-mediated cell division and its potential for synthetic cells. (A) I. Recruitment of CdvA and CdvB at the center of the cell in between the two segregated nucleoids. II. The CdvA/CdvB ring recruits both CdvB1 and CdvB2. III. The initial noncontractile ring is removed from the membrane and CdvB is digested by the proteasome. IV. Only the contractile ring formed by CdvB1 and CdvB2 is left at the division site. V. Constriction of the cell mediated by CdvB1/CdvB2, where CdvC removes the proteins from the membrane. (B) Cartoon of a putative 'synthetic cell', a liposome that is filled with purified proteins that sustain metabolism, a synthetic genome, a divisome, etc. The blue polymer represents a Cdv filament that potentially could serve as a divisome machinery for synthetic cells.

same CdvC protein regulates the stepwise removal of different components of the constriction ring.

In recent MD simulations [84], the CdvB1/CdvB2 copolymers were modeled and tested for different conditions of disassembly. A surprising suggestion from this work was that the Cdv polymers may not, like eukaryotic ESCRT, constrict the membrane with a single helix that spirals all around the membrane. Instead, filaments initially form a ring spanning the full cell circumference, which breaks up into a series of small and highly curved CdvB1/CdvB2 filaments, called hemihelices, that jointly constitute the division ring. These hemihelices effectively reduce the ring circumference, and a controlled unwinding of ring proteins then deforms more and more membrane until a narrow neck is left. At this stage, the hemihelices disappear and the membrane neck may spontaneously break, thus realizing abscission. This model proposes a new mechanism for the Cdv divisome, and it will be interesting to see if future experimental work will be able to verify it.

Concluding remarks

While a certain sequence of events has emerged (Figure 5A), many mechanistic questions are still wide open, for example, on the mechanism of divisome positioning, the regulation of the CdvB versus CdvB1/CvdB2 ring formation, the distinct mechanistic roles of CdvB1 and CdvB2, the mechanism of constriction, the final stage of abscission, etc (see Outstanding questions). The recently reported advances in high-temperature live cell imaging [52,85] open a new window to further cell-biology studies. Beyond that, it will be of interest to image the structural arrangements of the proteins *in vitro* and particularly to observe the induced effects of the protein interactions with lipid membranes. And once an understanding of the Cdv divisome is obtained, new questions will follow, such as its link to other cellular functionalities, specifically chromosome segregation.

Moving forward, a major aim is obtaining a more complete picture of the Cdv functionality across the different species that have this machinery. The work of Frohn *et al*. [44] is a good example of computational work that portrays possible differences across the different species. However, as they point out, experimental work is needed to confirm their hypothesis, especially establishing new model organisms in the Asgard phylum. A first successful laboratory culturing of a member of the Asgard archaea *Candidatus Prometheoarchaeum synthrophicum* [45] was recently reported. Although its slow growth rate and the lack of genetic tools still pose challenges, this may open up studies of the Cdv system in this phylum of organisms.

Finally, the Cdv system is of interest in an entirely different context, viz., for the bottom-up building of a divisome for synthetic cells (Figure 5B). In the past decade, various efforts have been started to assemble *in vitro* protein modules for the various cellular functionalities, with the aim of eventually reconstituting a minimal cell from components [86–88]. The goal of such research is to disentangle the complexity of the cell by separately reconstituting the different cellular functionalities and subsequently putting such modules together to hopefully build a cell from the bottom up. Obviously, such a synthetic cell will also need a division machinery. Efforts so far have largely concentrated on using the bacterial FtsZ [89], but success with this approach has been only modest, since, unlike optimistic early ideas [90], FtsZ does not appear to be able to apply much of a constriction force on liposomes [91,92]. Instead, it appears that FtsZ acts more like a central organizing protein that attracts and coordinates cell-wall-generating proteins that establish the constriction [93], which however make the system complex and less attractive as a minimal divisome for synthetic cells. One could envision to instead use the eukaryotic ESCRT system, but its complexity is staggering as well. Here is where the archaeal Cdv system may come in useful as this is at least somewhat simpler than its eukaryotic counterpart. The Cdv machinery may provide an elegant solution for a synthetic cell-division mechanism as it does not require as many components as the eukaryotic ESCRT (6 proteins in the Sulfolobales versus ~25 in humans [1]).

Indeed, we find it useful to study the Cdv system more closely with this aim in mind. Reconstituting the system *in vitro* not only may take us one step closer to the development of synthetic cells but will also provide valuable information about what minimal sets of the Cdv proteins will be sufficient for function. The idea to use Cdv as a divisome for synthetic cells was first mentioned a decade ago [94] but an understanding of the system was still very basic back then. New developments in recent years, especially new insights of the important role of the CdvB paralogs in the Sulfolobales, now provide a much better starting ground to build this minimal divisome, which opens up an exciting path forward.

Outstanding questions

General questions:

- How does the unique Cdv system mechanistically accomplish cell division in archaea?
- How does the Cdv division ring get placed at the center of the cell?
- Are there differences in the Cdv machineries across different archaeal species?
- Do the Cdv proteins from Asgard organisms present larger structural and mechanistic similarities to the eukaryotic ESCRT?
- Do the Cdv proteins act in the same way during vesicle formation and cell division?

Specific mechanistic questions:

- What is the role of CdvA in the process, and how is it removed from the membrane?
- What structures do the CdvB1 and CdvB2 form during the division process to allow them to perform membrane deformation and scission?
- Does CdvC drive the depolymerization of the various mid-cell rings during cell division?
- Do the Cdv proteins form higher-order structures similar to the ESCRT machinery of eukaryotes?

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Methods

For the phylogenetic tree assembly, the sequences of the CdvB homologs found in the eggNOG [95] database were used (entry COG5491), additionally adding sequences of the thaumarchaean CdvB proteins, the eukaryotic ESCRT-III, and the Asgard CdvB described in [44]. The tree was built using the MEGA 11: Molecular Evolutionary Genetics Analysis version 11 [96]. All the protein FASTA sequences were aligned using MUSCLE alignment, and a Neighbor Joining Tree was generated using Bootsrap method (1000 bootstrap replications) and Poisson substitution model.

Declaration of interests

No interests are declared.

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