

## Article Addendum

# Cell division and the ESCRT complex

## A surprise from the archaea

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The Archaea constitute the third domain of life, a separate evolutionary lineage together with the Bacteria and the Eukarya.<sup>1</sup> Species belonging to the Archaea contain a surprising mix of bacterial (metabolism, life style, genomic organization) and eukaryotic (replication, transcription, translation) features.<sup>2</sup> The archaeal kingdom comprises two main phyla, the Crenarchaeota and the Euryarchaeota. Regarding the cell division process in archaeal species (reviewed in ref. 3), members of the Euryarchaeota rely on an FtsZ-based cell division mechanism<sup>4</sup> whereas, previously, no division genes had been detected in the crenarchaea. However, we recently reported the discovery of the elusive cell division machinery in crenarchaea from the genus *Sulfolobus*.<sup>5</sup> The minimal machinery consists of three genes, which we designated *cdvA*, B and C (for cell division), organized into an operon that is widely conserved among crenarchaea. The gene products polymerize between segregating nucleoids at the early mitotic stage, forming a complex that remains associated with the leading edge of constriction throughout cytokinesis. Interestingly, CdvB and CdvC were shown to be related to the eukaryotic ESCRT-III protein sorting machinery (reviewed in ref. 6), indicating shared common ancestry and mechanistic similarities to endosomal vesicle formation and viral (HIV) budding in eukaryotes. We also demonstrated that the *cdv* operon is subject to checkpoint-like regulation, and that the genes display a complementary phylogenetic distribution within the Archaea domain relative to FtsZ-dependent division systems.<sup>5</sup> Here, the findings are further explored and discussed, and topics for further investigation are suggested.

### Multiple CdvB Paralogs in Crenarchaeal Genomes

All crenarchaea that harbor the new Cdv machinery contain multiple copies of CdvB-encoding genes, resembling the situation in eukaryotes that contain an even higher number of paralogs.

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The respective roles for each of these CdvB paralogs are currently unclear, and present an interesting challenge for further studies. The evolutionary trajectory that gave rise to the current distribution of CdvB homologs in crenarchaeal species appears to be complex, indicating several (ancient) duplication events and perhaps also cases of horizontal gene transfer (Fig. 1). Irrespective of duplications and transfers, it is plausible that the Cdv-based system was present in the last common ancestor of the Crenarchaeota. Furthermore, the two CdvB clades represented by *S. acidocaldarius* proteins Saci\_0451 and Saci\_1416 appear to share a common origin (Fig. 1), originating from a more recent duplication event. Interestingly, these CdvB homologs have been implicated in formation of membrane vesicles,<sup>7</sup> and might be involved in excretion of enzymes into the environment. These proteins may therefore represent functional analogs of the CHMP proteins, which play important roles in ESCRT-mediated vesicle formation in eukaryotes. Moreover, all CdvB paralogs present in *Sulfolobus solfataricus* are significantly induced upon infection with *Sulfolobus* Turreted Icosahedral Virus (STIV).<sup>8</sup> One of the paralogs, SSO0881, has been found to be consistently present in STIV virion particles,<sup>9</sup> suggesting that this protein, and perhaps the other CdvB paralogs as well, might play a role in viral budding. Another interesting result from the phylogenetic analysis is that the paralogs that belong to the Cdv operon<sup>5</sup> form a monophyletic clade (except for one of the *A. pernix* CdvB proteins), supporting a role in cell division for all proteins within this clade.

The Cdv genes were found to be downregulated upon exposure to UV radiation in two independent studies,<sup>10,11</sup> indicating a checkpoint-like response<sup>5</sup> that enables the cell to repair DNA damage before resumption of cell cycle progression. Interestingly, several of the other CdvB paralogs in *Sulfolobus* are also among the most downregulated genes upon UV exposure (Fig. 1), suggesting that these proteins also may play a role in the division process, further supported by the observation that some of the Cdv paralogs may interact with each other.<sup>12</sup> In addition, the fact that both the UV-repression and STIV-induction (above) appear to affect the same gene set (Fig. 1) provides a striking parallel that may indicate common regulatory features.

The three CdvB copies encoded in each of the genomes of the *Nitrosopumilus maritimus* and *Cenarchaeum symbiosum* form a monophyletic clade, indicating two consecutive duplication events within this lineage. Given that the genomes of these low-temperature crenarchaea also encode an FtsZ homolog, it remains to be shown

whether the Cdv machinery is employed in the process of cell division in these organisms, or whether it may play a role e.g., in vesicle formation (see below).

Altogether, the different CdvB homologs encoded by crenarchaeal genomes appear to be occupied with a wide variety of cellular functions, ranging from cell division to virion release and vesicle formation. Additional experimental work will be necessary to decipher the precise roles of CdvB proteins in each of these processes.

### Lipid Requirements in Vesicle Formation

Lysobisphosphatidic acid has been suggested to induce the formation of multivesicular liposomes in eukaryotes.<sup>13</sup> However, since this compound appears to be absent in yeast,<sup>14</sup> the lipid requirements for vesicle formation are under discussion. Archaeal cell membranes contain a unique combination of the glycerol-1-phosphate stereoisomer ether-linked to various phytanyl lipid derivatives, sometimes even crosslinked into tetraether molecules within a partly monolayer membrane. Archaeal ESCRT-III homologs are, thus, able to function within an entirely different lipid environment as compared to endosomal vesicle formation and virus budding in eukaryotic membranes, which contain fatty acids esterified to glycerol-3-phosphate. This indicates considerable flexibility in the lipid requirements for vesicle formation, at least regarding ESCRT-III mediated processes.

### Archaea as Model Systems for Endosomal Sorting and Viral Budding

The relative simplicity of archaeal species compared to multicellular organisms with many-fold larger gene sets underscores their potential usefulness as model systems for certain aspects of eukaryotic cell biology. Thus, insights into core mechanistic features of ESCRT-III mediated vesicle formation may be gained from an *in vitro* reconstituted *Sulfolobus* system dependent upon significantly fewer components, as compared to the eukaryotic corresponding process. Moreover, archaea that utilize the Cdv machinery may also provide tractable models for central aspects of the budding process of HIV,<sup>15</sup> HBV<sup>16</sup> and other viruses that recruit ESCRT-III functions during release from the host cell. This aspect would become even more relevant if a role for the Cdv proteins, or any of the additional CdvB paralogs, could be demonstrated also in archaeal virus biology, and the induction of the *Sulfolobus solfataricus* *cdv* operon, as well as of the other CdvB paralogs, during infection with STIV,<sup>8</sup> indicates that this may be the case. Thus, further investigation into the budding of STIV and other archaeal viruses could potentially result in mechanistic and regulatory insights of relevance also for eukaryotic virus biology.

### Cell Division in Thermoproteales

An interesting question for future investigation concerns the nature of the unidentified cell division machinery within the Thermoproteales order. Comparative genomics and other bioinformatics approaches may provide initial clues, but experimental evidence will ultimately be needed. The organisms within this order are, however, even less amenable to experimental approaches than *Sulfolobus* species, being strictly anaerobic (except for certain *Pyrobaculum* species) in addition to being hyperthermophiles, often

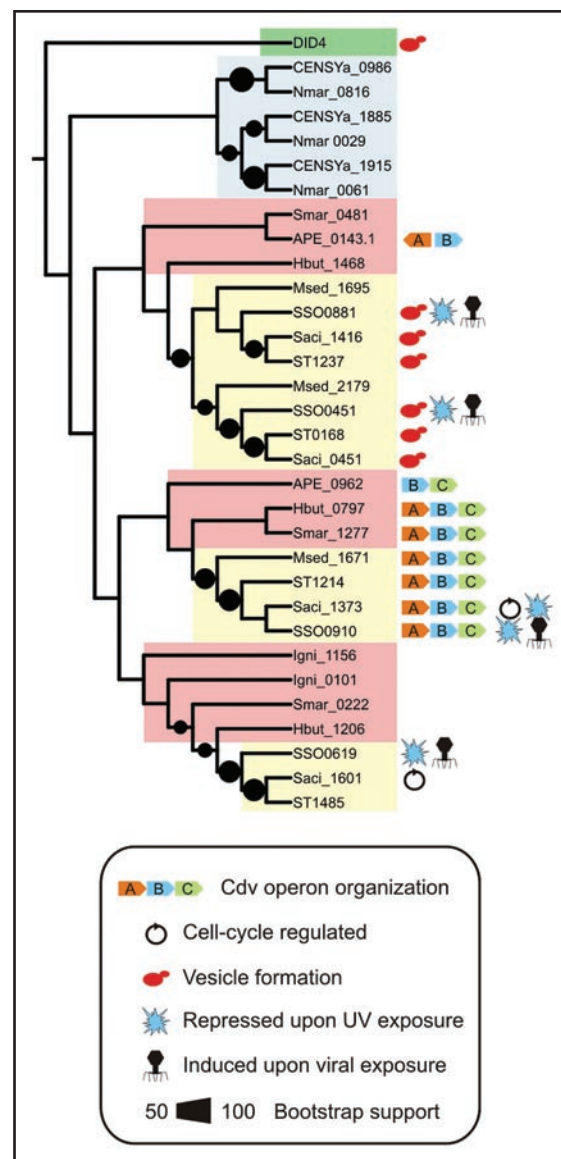


Figure 1. Phylogenetic tree of CdvB paralogs encoded by crenarchaeal genomes. The tree was inferred from the CdvB alignment in reference 5, using RAxML and the WAGPROTGAMMA model for protein evolution.<sup>18</sup> The yeast DID4 protein, which is part of the ESCRT-III machinery, was used to root the tree. Bootstrap support values above 50 are plotted as black circles. The tree is annotated with the following biological features: Cell cycle-specific expression;<sup>19</sup> Induction upon infection by *Sulfolobus* turreted icosahedral virus;<sup>8</sup> Repression upon UV exposure<sup>10,11</sup> and presence in secreted membrane vesicles.<sup>7</sup> Proteins are annotated using their locus tags, which includes the following species abbreviations: APE, *Aeropyrum pernix*; CENSyA, *Cenarchaeum symbiosum*; Hbut: *Hyperthermus butylicus*; Igni: *Ignicoccus hospitalis*; Msed: *Metallosphaera sedula*; Nmar: *Nitrosopumilus maritimus*; Sac: *Sulfolobus acidocaldarius*; Smar: *Staphylothermus marinus* F1; SSO: *Sulfolobus solfataricus* P2; ST: *Sulfolobus tokodaii*. Species belonging to the Sulfolobales, Desulfurococcales and Cenarchaeales orders are shaded yellow, red and blue, respectively. The only eukaryotic sequence (yeast protein DID4) is shaded green.

with even higher growth temperature optima than *Sulfolobus*, and few research groups are able to cultivate and perform well-controlled physiological studies on such species. Furthermore, no efficient genetic systems are available for these organisms. Still, a systematic

search for yet another way to carry out a process as central as cell division is likely to yield novel unexpected physiological and evolutionary insights.

## Cell Division in Low-Temperature Crenarchaea

The fact that the *cdv* and *ftsZ* cell division genes are simultaneously present in two low-temperature crenarchaeal species, *Nitrosopumilus maritimus* and *Cenarchaeum symbiosum*, is intriguing. Do these species combine features of Cdv- and FtsZ-dependent cell division mechanisms into a single process?<sup>17</sup> If so, this opens up a new interesting field for investigation. Alternatively, do new surprises await discovery in these organisms, perhaps in the shape of internal membrane systems, maybe even related to eukaryotic ER, Golgi or lysosomal systems, or concerning processes mediating export of lipid vesicles? Studies of archaea have resulted in a wide range of unexpected findings, often in terms of characteristics related to eukaryotic features, and additional surprises may well await discovery through investigations into the issues outlined above.

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