Trends in Microbiology

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Review

ESCRTs – a multi-purpose membrane remodeling device encoded in all life forms

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The ESCRT (endosomal sorting complexes required for transport) membrane remodeling complex, found across all life forms, exhibits a versatility that transcends evolutionary boundaries. From orchestrating the constriction of micron-wide tubes in cell division to facilitating the budding of 50 nm vesicles in receptor degradation, ESCRTs perform diverse functions in animal cells. However, the basis of this functional diversity remains enigmatic. While extensively studied in eukaryotes, the role of ESCRTs in prokaryotes is only beginning to emerge. This review synthesizes data on ESCRT systems across the tree of life, focusing on microorganisms and drawing parallels to their functions in human cells. This comparative approach highlights the remarkable plasticity of the ESCRT system across functional, structural, and genomic levels in both prokaryotes and eukaryotes. This integrated knowledge supports a model in which the ESCRT system evolved as a multipurpose membrane remodeling tool, adaptable to specific functions within and across organisms. Our review not only underscores the significance of ESCRTs in microorganisms but also paves the way for exciting avenues of research into the intricacies of cellular membrane dynamics, offering valuable insights into the evolution of cellular complexity across diverse organisms and ecosystems.

Highlights

Encoded by all domains of life, the ESCRT system manifests as one of the most fundamental machineries for shaping cellular membranes.

ESCRTs demonstrate multilayered plasticity, evident in variable protein cohorts, domain compositions, and structural organizations.

The ESCRT system presents a complex evolutionary trajectory, with different organisms encoding different protein sets and employing the ESCRT system for different cellular functions.

Investigating the cellular function of ESCRTs in eukaryotic microorganisms encoding simplified ESCRT systems can shed light on the evolutionary trajectory of the system.

Revealing the functional landscape of ESCRTs across evolution, particularly in Asgard archaea, may shed light on the membrane remodeling processes that took place during the emergence of eukaryotic cells.

The Eukaryotic ESCRT blueprint

The endosomal sorting complexes required for transport (ESCRT) system was first identified in the early 2000s in yeast as part of the vacuole protein sorting (Vps) machinery that is responsible for sorting cargo into multivesicular bodies (MVBs) during receptor degradation [1]. Over the years, this protein-sorting machinery was recognized as one of the most basic cellular machineries for driving membrane remodeling in eukaryotic cells [2–4]. Operating on almost all eukaryotic cellular membrane types, and mediating both basic and complex membrane-based cellular processes, ESCRTs constitute an all-purpose, robust membrane-remodeling machinery in eukaryotic cells. Notably, while the cellular functions of ESCRTs were mostly studied in animal cells, ESCRT homologs were recently found to be encoded in all domains of life. The realization that the ESCRT membrane remodeling complex – originally found to be part of the eukaryotic endo-membrane system – also functions in prokaryotic cells lacking internal membranes, calls for careful examination of the various functions of this cellular machinery across evolution.

The eukaryotic ESCRT system drives a variety of cellular processes that rely on membrane remodeling, including vesicular transport, cell division, neuronal development, and the surveillance of cellular membranes (Figure 1A). In vesicular transport, ubiquitinated receptors that have been endocytosed from the plasma membrane are recognized by the ESCRT system and internalized into the lumen of endosomes, forming intraluminal vesicles (ILVs). The resulting MVBs are then fused with the lysosome, for protein degradation, or fuse back with the plasma membrane, releasing exosomes. ESCRTs were further shown to mediate the release of extracellular vesicles

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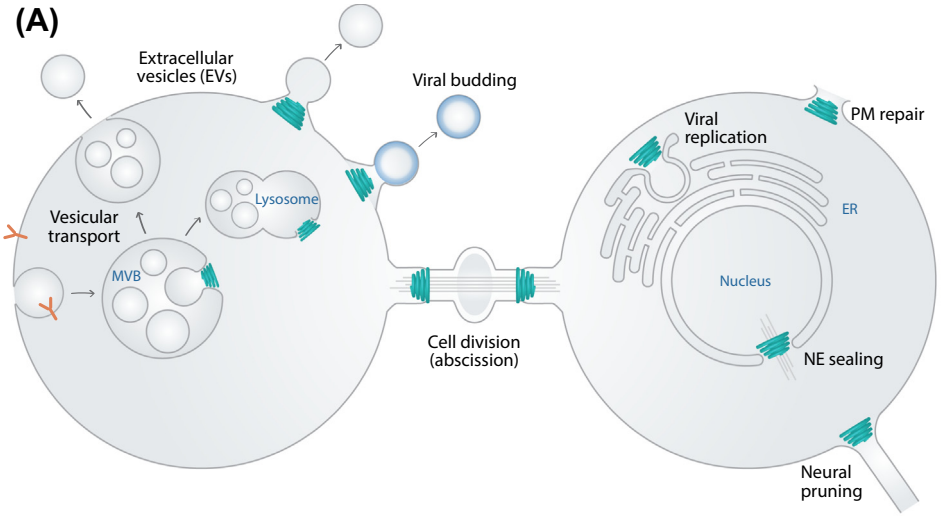

(B)

	Vesicular transport (ubiquitinated cargo)	EVs	Cell division	Membrane integrity	Viral budding and replication
Human	✓	✓	✓	✓	✓
Yeast	✓			✓	✓*
Amoeba				✓	
Red algae			✓		
Eukaryotic microorganisms	✓	✓			
Archaea		✓*	✓		✓
Bacteria				✓	

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Figure 1. Cellular functions of the endosomal sorting complexes required for transport (ESCRT) system across the tree of life. (A) Key processes in animal cells that are driven by the ESCRT machinery. Ubiquitinated receptors, orange; ESCRT complex, turquoise. Reviewed in [2–4]. (B) A table summarizing the conservation of different ESCRT-mediated cellular functions in different life forms with respect to cellular functions of ESCRTs described in animal cells. Detailed description of the role of ESCRTs in these organisms is provided in the main text. Empty boxes indicate the absence of references showing the involvement of ESCRTs in the indicated cellular process. Note that, in these cases, we cannot exclude the possibility that ESCRTs are involved in the specified processes. Asterisks refer to roles that have been suggested but are yet to be established. Note that the illustrations of cellular processes in the top panel refer to the processes described in animal cells and may not reflect on the exact role of ESCRTs in mediating these processes in microorganisms or on the membrane binding topology of ESCRTs in these systems, which have yet to be fully resolved. Abbreviations: NE, nuclear envelope; PM, plasma membrane.

(EVs) via fusion of MVBs with the plasma membrane or by direct release of microvesicles from the plasma membrane. Notably, EVs have been recognized, in recent years, as mediators of cell–cell communication, in health and disease, by carrying cytosolic proteins and genetic material and

transporting it to neighboring cells. In cell division, ESCRTs drive severing of the ~1 μm wide membrane tube, called the intercellular bridge, which connects cells at the end of mitosis. Over the past decade this process, called abscission, which terminates the division process, was found to be well orchestrated and highly regulated. Importantly, the role of ESCRTs in cell division has been found to be associated with critical cell fate decisions, including developmental programming, cellular differentiation, pluripotency, and senescence [5–7]. ESCRTs also drive the cutting of tubes in neuronal development where they execute the release of axonal or dendritic membrane protrusions, during neuronal pruning [8]. One of the emerging roles of ESCRTs is in surveillance of cellular membranes by stitching membrane holes [3]. Plasma membrane ruptures, caused by bacteria or toxins, are sealed by ESCRTs. Triggered by calcium influx, ESCRTs are recruited to the damaged site and close it, presumably by inducing membrane budding. ESCRTs were additionally reported to resist different types of programmed cell death responses (apoptosis, necroptosis, pyroptosis, and ferroptosis) by sealing disintegrated membranes, thereby preventing premature cell death [9]. For instance, in the gasdermin-mediated inflammatory response, ESCRTs are recruited to gasdermin-induced membrane pores and close them, restricting proinflammatory cytokine release and promoting cell survival. Damaged lysosomal membranes induced by pathogens trying to invade the cell's cytosol, or under stress conditions, is also sealed by ESCRTs [10,11]. Finally, ESCRTs have a crucial role in maintaining nuclear membrane integrity. During interphase, ESCRTs contribute to the integrity of the nuclear membrane by closing small ruptures caused during the migration of cells through confined spaces in both normal and cancer cells [12,13]. Loss of nuclear membrane integrity perturbs proper nuclear–cytosol compartmentalization and is associated with the uncontrolled exchange of nucleo-cytoplasmic content, which is crucial for proper cellular function and viability, highlighting yet another vital cellular function of ESCRTs. The role of ESCRTs in the nuclear membrane goes beyond membrane repair as ESCRTs are involved in the reassembly of the nuclear membrane post-mitosis and participate in the removal of defective nuclear pore complexes (NPCs) from the nuclear membrane [14]. These essential ESCRT-dependent nuclear functions suggest an intimate link between the nucleus and ESCRT machinery, implying a coevolutionary relationship critical for cellular homeostasis.

In summary, ESCRTs execute three main tasks: releasing vesicles – sealing membrane holes, and cutting membrane tubes – on different cellular membranes, including the plasma membrane, the nuclear membrane, and the lysosomal membrane [3,15]. Functioning in such a large cellular landscape, ESCRTs were shown to be involved in numerous fundamental cellular processes, including, cell division, signal transduction, cellular communication, membrane surveillance, cellular quality control, cellular differentiation, and neuronal development [2–4]. Therefore, ESCRTs constitute a versatile membrane remodeling machine that can modulate its function according to cellular context and has a critical role in cellular homeostasis. Interestingly, viruses also exploit the eukaryotic ESCRT machinery for budding outside of host cells and for the establishment of a viral replication compartment within the cell's endomembrane system (Figure 1A) [4,16–18]. The ability of viruses to utilize the host ESCRT complex for multiple functions stresses the functional plasticity of the ESCRT system and points to the integral function of these proteins in remodeling both the plasma- and the endo-membrane systems. Notably, as increased membrane complexity and the formation of membrane-bound organelles is a hallmark of eukaryotic cells, understanding the basis for the functional plasticity of the ESCRT complex, and unraveling which of its cellular functions are conserved through evolution, may shed light on the cellular processes that took place during the emergence of eukaryotic cells.

Meet the players

The eukaryotic ESCRT machinery is a multi-protein complex composed of several subfamilies (ESCRT 0–III and the AAA ATPase vacuolar protein sorting-associated protein 4 (Vps4) [19]

(Figure 2). All proteins of the system are cytosolic by nature and are recruited to the designated membrane in response to a specific targeting signal, which differs depending on the cellular process. According to the canonical model, the early ESCRT components (ESCRT 0, I, II, Alix) are the first to arrive at the membrane and stabilize/induce initial membrane curvatures. Membrane-bound early ESCRTs then facilitate membrane recruitment and polymerization of late ESCRT proteins (ESCRT-III). The AAA ATPase, Vps4, induces remodeling and disassembly of the ESCRT-III polymer, ultimately leading to membrane scission (Figure 2) [19]. Membrane recruitment of early ESCRTs is mediated, in some cellular contexts, by binding to ubiquitinated proteins (via the so-called UEV domain), suggesting a functional linkage between these two conserved eukaryotic cellular systems. Notably, while early ESCRT proteins are involved in almost all ESCRT-driven cellular processes, in nuclear ESCRT functions, LEM2 protein directly recruits the late ESCRT-III/

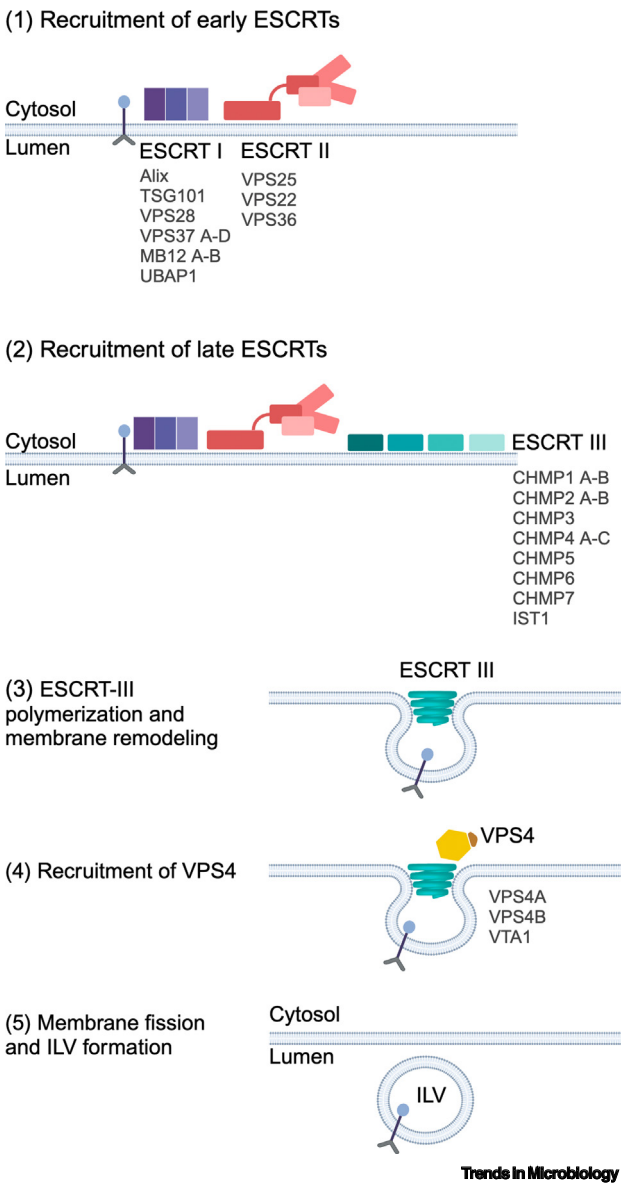


Figure 2. Endosomal sorting complexes required for transport (ESCRT)-mediated membrane remodeling in human cells. The conventional model for the formation of ESCRT-driven intraluminal vesicles (ILVs) in human cells: (1) Ubiquitinated receptors that were removed from the plasma membrane via endocytosis, recruit the early ESCRT proteins ESCRT-0 (not shown) and ESCRT-I (purple) to the outer membrane of the endosome, which in turn facilitates the recruitment of ESCRT-II (magenta). (2) Membrane-associated ESCRT-II then recruits ESCRT-III components (turquoise) to the endosome membrane. (3) Membrane bound ESCRT-III proteins polymerize on the membrane giving rise to internalized membranes that carry the ubiquitinated receptor. (4) Recruitment of the Vps4 complex facilitates fission of the internalized membrane, resulting in ILV formation in the lumen of the endosome and disassembly of ESCRT-III polymer (5). Each ESCRT subfamily consists of several proteins. Proteins constituting each subfamily in human cells are listed (irrespective of their involvement in ILV formation).

Vps4 complex, bypassing the early ESCRT complexes, suggesting a distinct evolutionary trajectory for nuclear ESCRT functions [3,20].

Each ESCRT subcomplex consists of several proteins, with the largest cohort found in the ESCRT-III subfamily, which consists of 12 different ESCRT-III proteins (named charged multivesicular body protein (CHMPs) in human cells [19] (Figure 2). While the reason for the complexity in the ESCRT system is not fully understood, this feature appears to be preserved in most eukaryotes (Figure 3A). Some ESCRT-IIIs have been found to have specific functions (e.g., nuclear functions: CHMP7), while functional differences between other ESCRT-IIIs were not clearly defined, suggesting redundancy. Interestingly, ESCRT systems with a reduced number of proteins are found in some eukaryotes [21] (Figure 3). In the majority of cases, the reduction is manifested by a smaller number of proteins found in each subfamily, suggesting that ESCRTs can function in cells with a reduced set of ESCRT-III components, further supporting redundancy in the ESCRT system of animal cells. While representatives of the ESCRT-III and Vps4 subfamilies were found in all organisms, ESCRT-I and/or ESCRT-II were missing in a few, suggesting that the ESCRT-III and Vps4 subfamilies represent the core of the machinery, as inferred from *in vitro* reconstitution studies [22–24]. These reduced modules offer an exciting model system for tracing the variety of functions carried by the ESCRT system through evolution.

How it works

How the ESCRT machinery drives membrane remodeling is not fully understood, and most of the knowledge obtained so far relies on biochemical, structural, and *in vitro* reconstitution studies of the late ESCRT-III/Vps4 complex. *In vitro*, ESCRT-III proteins were shown to polymerize into helical homo- and hetero-filaments on membranes [23,25–28]. These data were confirmed by the visualization of ESCRT-based rings and spiral organizations at the intercellular tube of mammalian cells that is severed by ESCRTs during cell division [29,30]. While the composition of cellular ESCRT-III filaments is unknown, several ESCRT-III proteins were shown to comprise the ability to self-assemble (e.g., CHMP4B, CHMP1B, CHMP5, CHMP3, and IST1), while others were found to rely on additional ESCRT-III partners for polymerization [25–27,31–36]. Constriction of the membrane is thought to be mediated by remodeling of ESCRT-III polymers that are bound to the inner side of the membrane, driven by Vps4 (Figure 2). Vps4 was found to induce depolymerization of the ESCRT-III complex – but also to facilitate the exchange of ESCRT-III proteins within the polymer both *in vitro* and in cells, thereby contributing to membrane remodeling [25,30,37]. Final fission is proposed to be driven by a dome-like structure formed at the tip of the CHMP2A–CHMP3 polymer, shown *in vitro*, which brings the two membranes in close enough proximity to facilitate fission [38]. Interestingly, different combinations of ESCRT-III proteins self-assemble into helical filaments comprising different biophysical characteristics [23,26,27,39,40]. Based on these findings and computational modeling, it has been proposed that ESCRT-mediated membrane constriction is a stepwise process that relies on the exchange of ESCRT-III monomeric subunits within the polymer, conferring different biophysical properties of the ESCRT polymer [25,41]. While these models have yet to be confirmed in eukaryotic cells, the ability of the ESCRT-III complex to assemble into highly dynamic polymers comprising different compositions, shapes, and biophysical characteristics emphasizes the enormous plasticity of this unique protein complex.

Functions of the eukaryotic ESCRT system in microbial life

There is great benefit in studying the ESCRT machineries encoded in eukaryotic microorganisms in order to track their evolutionary trajectories to higher eukaryotes. Based on sequence homology of *Homo sapiens* and representative eukaryotes along the tree of life, some eukaryotes encode reduced ESCRT systems. The most reduced modules were found in unicellular eukaryotes such

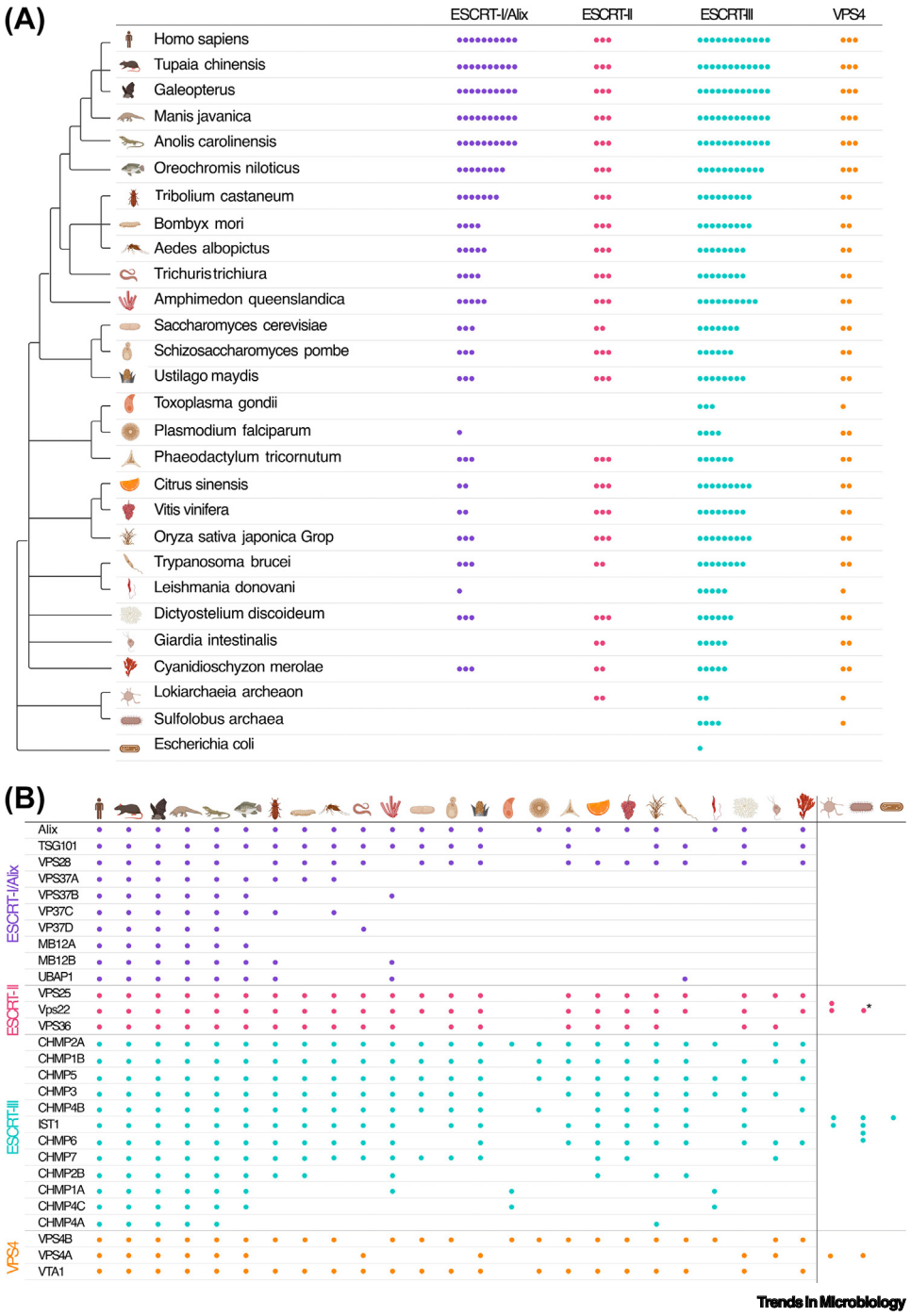


Figure 3. Abundance of endosomal sorting complexes required for transport (ESCRT) proteins across the tree of life. ESCRT homologs in selected taxa along the tree of life were identified by BLAST analysis against individual human ESCRT proteins. Whenever possible, data were verified by comparison to previously reported bioinformatics and experimental results [21,46,48]. In a few cases, ESCRT homologs were not identified by our BLAST analysis but were confirmed experimentally. In these cases, ESCRT homologs were added manually: *Trypanosoma brucei*, Vps28; *Giardia intestinalis*, Vps36, CHMP7 [46]; *Cyanidioschyzon merolae*, Alix, CHMP4B, CHMP6 [54]; *Plasmodium falciparum*, Alix,

(Figure legend continued at the bottom of the next page.)

as *Plasmodium falciparum* and other protozoa, with *Toxoplasma gondii* encoding only three ESCRT-III proteins and a Vps4 [21] (Figure 3A).

Sequence-based comparison of the human ESCRT proteins to ESCRT components encoded in different eukaryotes along the phylogenetic tree suggests that the most conserved late ESCRT proteins are CHMP2A, CHMP1B, CHMP5, and CHMP3, and Vps4 and its cofactor VTA1 (Vesicle Trafficking 1) (Figure 3B). These proteins can be traced back to unicellular eukaryotes encoding partial ESCRT systems. Interestingly, while CHMP2A and CHMP3 were traditionally considered essential eukaryotic ESCRT-III proteins, CHMP1B, CHMP5, and VTA1 were regarded as associated non-essential ESCRT-III components. CHMP4B, which was considered the core ESCRT-III component in animal cells [19], was missing in some unicellular eukaryotes with partial ESCRT systems. The realization that so-called ESCRT-III-associated proteins are conserved in the eukaryotic branch calls for reinvestigation of these proteins. Interestingly, these relatively understudied ESCRT-IIIs appear to carry out unique functions. For example, both CHMP5 and CHMP1B self-assemble into helical filaments, *in vitro*. Only recently, CHMP5 was shown to assemble *in vitro* into homo- and hetero-filaments, incorporating additional ESCRT-III components, but was not able to integrate into CHMP4 polymers, suggesting an alternative/parallel pathway to the well-studied CHMP4 system [32]. CHMP1B, also self-assembled into homo- and co-filaments (with IST1), but induced membrane remodeling in an initially unexpected reverse topology compared to traditional ESCRTs, that is, binding the membrane from the outside rather than the inside [33,34]. Furthermore, CHMP1B was reported to bind DNA and associate with chromatin upon overexpression in cells [34,42,43]. Finally, VTA1, which was traditionally considered a cofactor of Vps4 (as it increases its ATPase activity), was recently found to be involved in regulating mammalian cell division, suggesting additional functions beyond promoting the ATPase activity of Vps4 [44,45]. This comparative, evolutionary-based viewpoint calls for further investigation of these so-called associated ESCRT proteins in order to understand the basic principles of the ESCRT system. Furthermore, they stress the need to study the cellular function of ESCRTs in eukaryotic microorganisms that encode for partial, reduced ESCRT systems.

Unfortunately, limited experimental knowledge has been obtained for ESCRTs in eukaryotic microorganisms. Nevertheless, initial studies already provide valuable insights into the composition and function of ESCRTs in unicellular organisms. Among trypanosomes, a role for ESCRTs in the internalization of ubiquitinated cargo was described for *Trypanosoma brucei*, which encodes ESCRT-I, ESCRT-II, and eight ESCRT-III proteins (Figure 3). In this system, the early ESCRT proteins TSG101 and Vps28 colocalized at endosomal compartments, and the knock-down of Tsg101 led to reduced degradation of the ubiquitinated protein ISG65 [21]. In protozoa *Giardia Intestinalis*, which does not encode for ESCRT-I proteins and encodes for five ESCRT-III proteins (Figure 3), ESCRT were found to localize to the vacuole and to interact with the endomembrane system [21,46]. Introducing a Vps4 mutant to these cells led to reduced ILV formation and exosome release [47]. In this study, the nuclear ESCRT-III, CHMP7, was found to be highly enriched at the endoplasmic reticulum (ER), which is continuous with the nuclear membrane,

CHMP5, CHMP4B [48]. In all cases, accession numbers were verified and checked for no overlap with other homologs. For prokaryotes (Lokiarchaeota, strain GC14_75; *Sulfolobus acidocaldarius*; *Escherichia coli*) data on ESCRT homologs were obtained based on previous publications [66,90,114]. (A) Total number of ESCRT proteins encoded for each ESCRT subfamily in selected taxa. The phylogenetic tree was generated using the web server PhyloT v2, based on taxonomy data from the NCBI and GTDB databases. Note the reduction in number of ESCRT components in each ESCRT subfamily in unicellular organisms compared to multicellular eukaryotes. (B) Homologs for individual ESCRT proteins in the tree of life. ESCRT homologs in prokaryotes do not refer to individual ESCRT proteins but rather to the subfamily. Protein names are as follows: Loki, ESCRT-II: Vps25-like and Vps22/36-like, ESCRT-III CHMP1-3, CHMP4-7; *Sulfolobus* ESCRT II, CdvA (*based on bioinformatic analysis presented in [65]), ESCRT III CdvB 0-3, *Escherichia coli* ESCRT-III PspA.

substantiating a conserved role for CHMP7 in the nuclear membrane. Finally, in *P. falciparum* (encoding only one early ESCRT protein, and four ESCRT-III proteins) a role for ESCRTs in EV production was proposed [21]. The ESCRT proteins Bro1, CHMP4, and CHMP5 were found to interact with one another, localize in vesicular structures, and their depletion led to reduced EV production [48]. Notably, many protozoa were shown to release EVs upon host–parasite and parasite–parasite interactions [49]. While not all parasitic EVs are ESCRT dependent, early ESCRT components were often found inside these EVs, suggesting a prominent role for ESCRTs in EV production. However, as the experimental data on unicellular eukaryotes is mainly derived from parasites, one cannot exclude the possibility that the observed function reflects the parasitic nature of these eukaryotes rather than the functional evolutionary origin of ESCRTs.

A study of the amoeba *Dictyostelium discoideum* shows that this phagocytic species employs the ESCRT machinery to combat bacterial infection by closing holes that pathogenic bacteria generated in the vacuole in order to exit and infect the host cell [50]. In this species, which encodes a reduced set of all ESCRT subcomplexes (Figure 3), ESCRTs were shown to repair damaged plasma membranes, endomembranes, and lysosomal membranes, albeit in a calcium-independent manner, indicating a role for ESCRTs in membrane integrity [50,51] (Figure 1). Both early (TSG101) and late (a CHMP4 homolog and Vps4) ESCRT proteins were shown to participate in these processes. A role for CHMP7 and an IST1 homolog in resealing the nuclear envelope of post-mitotic cells has also been demonstrated in *D. discoideum* [52,53]. Notably, as *D. discoideum* is considered a model organism for understanding the evolution of multicellularity, these findings highlight that key membrane repair ESCRT functions were already present during this critical evolutionary transition.

Despite the established role for ESCRTs in cell division in higher eukaryotes, the only eukaryotic microorganism in which ESCRTs were reported to function in cell division is the red alga *Cyanidioschyzon merolae,* which encodes for representatives of all ESCRT subcomplexes (Figures 1 and 3). In this system, ESCRT localization and function during cell division was strikingly similar to that described for ESCRTs in animal cells. The ESCRT proteins Alix, CHMP 1,2,4,5,6, and Vps4 localized to the tube connecting the two daughter cells forming the typical ring localization pattern. Moreover, a Vps4 mutant disrupted severing of the tube, leaving the two daughter cells connected to one another via elongated membrane tubes [54]. Interestingly, this alga does not encode a contractile actomyosin system, suggesting a complementation of function between these two systems in cell division. Notably, while most cellular functions described for ESCRTs over the years in human cells were validated in budding yeast, in which they were originally identified, so far a role for ESCRTs in yeast cell division could not be identified [2,19] (Figure 1). In fission yeast, which undergoes symmetric division, a role for ESCRTs in cell division was suggested, but in an indirect manner [55,56]. Therefore, although one of the signature functions of the ESCRT machinery in multicellular organisms is in cell division, the experimental knowledge obtained so far raises the possibility that this function may be less common in eukaryotic microorganisms, and that other ESCRT-functions may be more prominent in unicellular life forms.

The many functions documented for ESCRTs in the eukaryotic microorganisms described earlier, stress the notion that the ESCRT system is highly adaptive and can embrace different functions depending on the cellular context and perhaps as a result of different evolutionarily selective pressures. In most eukaryotic microorganisms ESCRTs were found to carry endomembrane functions, including ubiquitinated cargo internalization to MVBs and EV release that involves early ESCRTs. Data from *C. merolae* is consistent with the conserved ability of the complex to mediate cell division, while data from *D. discoideum* supports conservation of membrane repair functions, from the emergence of multicellularity. Further, more systematic studies of these

model microorganisms and establishment of new eukaryotic model organisms that broadly represent the tree of life are crucial for resolving the functional evolution of ESCRTs in eukaryotes.

ESCRTs in prokaryotes

Meet the archaeal players

ESCRT homologs (the Cdv system) were originally identified, over a decade ago, in the archaeal species *Sulfolobus acidocaldarius,* a member of the TACK superphylum (comprising Thaumarchaeota – now Nitrososphaerota, Aigarchaeota, Crenarchaeota, and Korarchaeota), and have been implicated in cell division [57–59]. Phylogenetic analysis revealed that most archaeal phyla that encode the Cdv system do not encode the bacterial FtsZ cell-division machinery and, therefore, ESCRTs were proposed to constitute an alternative cell-division machinery in archaea [59]. The Cdv system comprises the ESCRT-III homologs, CdvB, CdvB1, CdvB2, and CdvB3, and the Vps4 homolog, CdvC [60,61]. Early ESCRTs were not found in the Cdv machinery. Instead, an additional protein, called CdvA, was shown to mediate membrane recruitment of the machinery. Interestingly, CdvA was found to bind DNA, but the physiological role of this property remained elusive [62]. The function of the Cdv system in *S. acidocaldarius* cell division is by far the most characterized ESCRT function in prokaryotes. Using a series of advanced microscopy techniques it has been shown that, during the division, CdvB proteins orderly assemble into ring structures at the cell equator, highly resembling the organization of eukaryotic ESCRT-IIIs in cell division [63,64]. Based on this high spatiotemporal similarity, it was suggested that the function of ESCRT in cell division is conserved through evolution and is mediated by the assembly and remodeling of dynamic ESCRT-III helical filaments [61,63]. However, one cannot exclude the possibility that, as in the red alga, *C. merolae*, which lacks the contractile actomyosin machinery, the Cdv system in *S. acidocaldarius* is specialized in cell division due to the loss of the FtsZ machinery and the consequential need to complement its function [65].

Additional ESCRT-related cellular roles, beyond cell division, have also been described for the Cdv system in *S. acidocaldarius*. The Cdv system was found to function in EV release [66–69]. Investigation of the contribution of different CdvB proteins to cell division and vesicle release, using deletion mutants, revealed that CdvB1 and CdvB2 are more crucial for cell division (with CdvB2 exhibiting the most severe phenotype), while CdvB3 is required mainly for vesicle release [66]. Nevertheless, all three ESCRT-III homologs localized to vesicular buds in cells overexpressing CdvB3 – suggesting that they all participate in the process [66]. Cdv proteins were also shown to participate in the replication and release of archaeal viruses. Upon STIV (*Sulfolobus* turreted icosahedral virus) infection, interactions between viral proteins and the Cdv machinery have been reported; Vps4 was shown to localize at viral exit structures, and over-expression of a Vps4 mutant inhibited viral replication [67,70].

Therefore, the archaeal Cdv system constitutes a reduced ESCRT system, which appears to recapitulate, at least to some extent, the functional plasticity observed for ESCRTs in eukaryotes. Perhaps the most striking manifestation of this plasticity was revealed in a study that investigated the phenotypes of *S. acidocaldarius* that were infected with the STSV2 (*Sulfolobus tengchongensis* spindle-shaped viruses 2) virus. Upon viral infection, expression of ESCRT genes was reduced and cell division was inhibited, giving rise to the formation of giant cells [71]. Cell division in the giant cells then switched from symmetric to asymmetric, where new cells comprising the size of the original cells budded out of the giant cells. Remarkably, ESCRT-III homologs localized at the neck of the budding cells, forming collar-like structures, strongly suggesting their involvement in this aberrant, viral-induced cell division. These results demonstrate an extreme flexibility for the archaeal ESCRT system that can switch from ESCRT-induced symmetric division to ESCRT-induced asymmetric division comprising different

topologies and scales. Importantly, the ability of ESCRTs to quickly adapt to different cell sizes, shown here, could potentially provide the ESCRT system with the versatility needed to function across the cell size changes that occurred during evolution.

Systematic analysis of the distribution of Cdv proteins in different archaeal strains shows that in some taxonomic orders (e.g., Nitrosopumiales, Thermococcales, Methanococcales) both the Cdv and FtsZ machineries are encoded, suggesting alternative functions for either the Cdv or FtsZ systems beyond cell division in these cells [65,72]. Moreover, the number of Cdv components varies between strains [65]. It therefore appears that the reduced ESCRT system encoded in archaea is flexible in terms of the number of ESCRT-III homologous genes and can execute multiple functions, including vesicle release, cell division, and viral response. The latter strongly suggests that plasticity is a core feature of the ESCRT machinery, and that the system may not have been evolved to execute a specific cellular function but rather as a multi-purpose membrane remodeling tool. Exploring the cellular functions of Cdv systems in archaeal cells that encode FtsZ proteins, and in strains encoding different numbers of Cdv genes, will shed light on the plethora of processes driven by ESCRTs in the archaeal domain.

On the bacterial front

Two distant ESCRT-III homologs, PspA and Vipp1, were recently identified in bacteria. Notably, both proteins were known for many years, but their affiliation with ESCRTs only recently became apparent by employing structural and advanced bioinformatic approaches [73,74]. Notably, PspA and Vipp1 participate in distinct cellular processes, and both exhibit self-assembly properties [75]. Homologs of early ESCRTs and Vps4 have yet to be identified in these systems. That said, both PspA and Vipp1 were shown to bind and hydrolyze ATP and GTP, suggesting that the ATP hydrolysis, provided by Vps4 in other ESCRT systems, is contributed, at least to some extent, by the ESCRT-III proteins themselves [73,75–78]. Therefore, the bacterial ESCRT-like system appears to be significantly reduced and distant from all other ESCRT systems identified in the tree of life. Nevertheless, structure–function analysis of these distant homologs can potentially provide insights into the most basic features of the ESCRT system.

PspA (Phage Shock Protein A) is part of the protein stress response complex in prokaryotes (PspA–PspG). In *Escherichia coli*, components of the system were shown to be acutely expressed during phage infection and upon other stress inducers such as high temperature and osmotic shock [79]. While the Psp system is comprised of many components, PspA and PspC are the most conserved. PspA is the most abundant and in some strains encoding several copies of the protein. PspA is also found in archaea but at much lower frequencies. Collectively, these data suggest that PspA has a conserved function beyond its canonical function as part of the Psp complex [80]. While the cellular function of PspA is not fully understood, experimental data suggest that it can counteract cellular stress by polymerizing on the bacterial membrane and surveilling its integrity. In *Bacillus subtilis*, the PspA homolog (LiaH) was found to strongly interact with the membrane anchor protein LiaI and with proteins of the flotillin family, which are involved in membrane microdomain organization, suggesting a role for PspA in membrane organization [80]. *In vitro*, PspA assembled into helical rods that structurally resemble those of human ESCRT-III CHMP1B and was able to bind and invaginate membranes through the interior of the protein tube, supporting functional homology to eukaryotic ESCRTs and membrane remodeling properties for this protein complex [74].

The Vipp1 protein (also called IM30) is involved in the biogenesis and homeostasis of the thylakoid membrane array, where the photosynthetic machinery is assembled, in both oxygenic photosynthetic bacteria, the cyanobacteria, and their eukaryotic organelle descendants, the chloroplasts.

How Vipp1 operates on the thylakoid membrane is unclear. Reduced expression of Vipp1 in model photosynthetic prokaryotic and eukaryotic cells led to impaired thylakoid membrane formation and reduced photosynthetic activity. In the plant model organism, *Arabidopsis thaliana*, and the alga *Chlamydomonas reinhardtii*, reduced Vipp1 expression also led to swelling of the thylakoid membrane in response to strong illumination, suggesting that it is also involved in the stability and maintenance of these crucial membrane arrays [81,82]. Fluorescently tagged Vipp1 expressed in these cells accumulated in small foci and larger filament-like structures [82–84]. In living cyanobacteria, GFP-tagged Vipp1 was found homogeneously distributed in the cytosol as well as in foci, which were located at the periphery of the cell and colocalized with a thylakoid marker [85,86]. Notably, exposure to high light intensity led to dramatic enrichment of VIPP1 in foci, suggesting its acute recruitment to the thylakoid. Cryo-electron tomography in *Chlamydomonas* suggests that the Vipp1 foci represent clusters of Vipp1 that are wrapped around membrane tubules within the chloroplast [73]. This *in vivo* characterization was in agreement with *in vitro* data showing that Vipp1 assembles into helical rods that can invaginate and remodel liposomes [87]. Collectively, these data suggest that Vipp1 constitutes a thylakoid-specific ESCRT-III machinery, involved in both the biogenesis and the surveillance of these highly specialized membrane arrays, which must be resilient to enable efficient photosynthesis under variable environmental conditions, including exposure to intense illumination.

In summary, both PspA and Vipp1 assemble into helical tubes that bind membranes from the interior of the protein tube and have ATP/GTP binding and hydrolysis properties [88]. Additionally, both proteins are associated with the surveillance of membranes in response to stress. That said, each protein appears to function in fundamentally different cellular processes. PspA is part of the bacterial stress response and is recruited to the bacterial membrane to seal holes, suggesting a conserved role for ESCRTs in membrane sealing. Conversely, Vipp1 is encoded in both prokaryotes and in the chloroplast of eukaryotes that carry photosynthetic capabilities and appears to have highly specialized thylakoid membrane functions involving both its biogenesis and maintenance, much like the function described for CHMP7 in the eukaryotic nucleus.

At the prokaryote–eukaryote crossroad: the Asgard superphylum

Prokaryotic ESCRT homologs have garnered increased attention since the discovery of Asgard archaea via advanced metagenome sequencing and assembling methods. The Asgard superphylum encodes eukaryotic signature proteins (ESPs) and is currently regarded as the prokaryotic phylum from which eukaryotic cells emerged. Bioinformatic approaches revealed that genes which encode proteins of the ESCRT system are among the most conserved ESP genes identified in Asgard archaea [89,90]. Notably, the ESCRT system encoded in Asgard archaea includes members of all ESCRT subfamilies (i.e., ESCRT I, II, III, and Vps4) and is the only prokaryotic ESCRT system found to encode early ESCRT proteins, namely ESCRT-I and ESCRT-II (Figure 3A). Additionally, the sequences of ESCRT-III and Vps4 homologs encoded in Asgard archaea are significantly more similar to their eukaryotic homologs than to their archaeal homologs (Cdv) [65,89]. The ESCRT-III sub-complex in Asgard archaea is reduced compared to the eukaryotic system, with some species encoding only two ESCRT-III proteins. Phylogenetic analysis of Asgard and eukaryotic ESCRT-III proteins reveals that they cluster into two groups: one that includes CHMPs 1–3 and another that includes CHMPs 4–7 (Vps2/Vps24/Vps46 and Vps20/Vps32/Vps60, respectively) [89]. At least one gene of each group is encoded in all currently available Asgard metagenomes. AI-based structure predictions of Asgard ESCRT-III homologs suggest that these proteins also adopt the two different folds described for eukaryotic ESCRT-III proteins, with one protein adopting an extended conformation (CHMP4–7) and the other a packed conformation (CHMP1–3) [42]. Therefore, the Asgard ESCRT system constitutes a reduced, prokaryotic ESCRT system that is the closest to the eukaryotic ESCRT system.

Resolving the cellular function and mode-of-operation of Asgard ESCRTs is drawing much attention for two main reasons. First, based on evolutionary theories, the Asgard ESCRT system is at the junction between prokaryotes and eukaryotes and hence may shed light on the evolutionary trajectory of the ESCRT machinery. Second, and perhaps considerably more significant, is that the ESCRT membrane remodeling complex could have contributed to the membrane-compartmentalization processes, which took place in the ancient world, and facilitated the emergence of eukaryotic cells. In this respect, morphological studies of the two Lokiarchaeota species cultured so far show that they comprise an extended membrane network with membrane protrusions extending several folds beyond the size of the archaeal cell body [91,92] (Box 1). Moreover, membrane vesicles were often visualized touching the cell body, supporting the existence of active membrane budding in Lokiarchaeota. Unfortunately, in spite of great interest, functional information on ESCRTs from Asgard archaeal cells is currently missing. This is mainly because the Asgard species cultured so far could not be isolated into pure cultures and were found to be anaerobic and slow-growing, challenging routine laboratory work. Moreover, molecular tools are currently unavailable for these species [91,92]. Hence, the information obtained so far on the Asgard ESCRT system is derived almost exclusively from genomic data and a few *in vitro* studies that we now discuss further.

Remarkably, Asgard archaea also encode proteins of the ubiquitin system that are linked to the ESCRT system in eukaryotes. Moreover, early Asgard ESCRT proteins contain the conserved eukaryotic UEV domain, which mediates their binding to ubiquitinated receptors [93,94]. The Asgard UEV domain was able to bind both Asgard and human ubiquitin, with Asgard UEV binding exhibiting lower affinity for human ubiquitin, supporting coevolution of ubiquitin and early ESCRTs [19]. Finally, Thorarchaeota genomes that encode a partial early ESCRT complex lacking the UEV domain, do not encode proteins of the ubiquitin system [95]. The linkage between the ubiquitin and ESCRT systems, observed in Asgard archaea, suggests that the role of the ESCRT system in vesicle transport, specifically in ubiquitinated cargo sorting, originated in Asgard archaea. If this were the case, then ESCRTs are expected to carry a different cellular function in Thorarchaeota. Interestingly, a recent study suggested that Thorarchaeota encode specific ESCRT proteins, which are missing in most other Asgard phyla, that is, Alix (Bro1), the ESCRT-III protein IST1, and the Vps4 co-factor VTA1 [95]. Hodarchaeota, which is proposed in this study to be the closest Asgard to eukaryotes, was suggested to encode the ubiquitin system, early ESCRTs, and the less abundant ESCRT genes found in Thor. It is, therefore, tempting to speculate that multiple ESCRT functionalities found in different Asgard species converged in the Hodarchaeota, allowing for the emergence of complex membrane functions that ultimately contributed to the establishment of compartmentalized cells. Exploring the cellular functions of ESCRT systems expressed in different Asgard archaea phyla, and understanding their contribution to membrane remodeling may, therefore, shed light on eukaryogenesis.

Recent, studies have investigated the ESCRT-III subfamily in Lokiarchaeota and Heimdallarchaeota, *in vitro*. Through these studies it was found that CHMP 4–7 self-assemble into both homo- and heterohelical filaments [96,97]. CHMP 1–3 proteins did not self-assemble on their own but were shown to bind CHMP 4–7 and to affect the morphology of the ESCRT-III filament [42,96,97]. Whether and how CHMP 1–3 integrate into Asgard ESCRT-III helical filaments and their contribution to ESCRT-III function remain undefined. Asgard Vps4 was found to be able to interact with both Asgard and human ESCRT-III proteins inside a human cell host and to partially rescue the phenotype of a vps4 null yeast mutant [42,98]. Finally, Lokiarchaeota ESCRT-III helical tube assemblies invaginated and deformed vesicles composed of negatively charged phospholipids [97], suggesting a functional similarity among bacterial, Asgard, and eukaryotic CHMP1B ESCRT-III homologs. With respect to the membrane, it should be noted that, based on data from the isolated from

Box 1. The Asgard archaeal cell

Very little is known about the cell biology of Asgard archaea. Recent cultivation efforts have yielded two Lokiarchaeota species, and the development of specific Lokiarchaeota and Heimdallarchaeota labeling probes has enabled the morphological characterization of cells from these taxa in their natural environment. The first cultivated Lokiarchaeota *Candidatus* Prometheoarchaeum syntrophicum strain, MK-D1, was isolated from deep-sea sediments (2.5 km depth) in the Nankai Trough, Japan [91]. This species was found to be anaerobic, dependent on co-symbiosis with *Methanogenium*, and extremely slow growing (doubling time of 14–25 days). Morphologically, MK-D1 cells displayed a relatively small (average diameter of 550 nm) rounded body with extensive membrane protrusions. Membrane blebs were frequently observed near or attached to the cells, but no indication for potential intracellular organelle-like structures could be visualized. Genomic and transcriptomic analyses confirmed the presence of ESP genes and revealed that MK-D1 encodes three potential cell division machineries: actin, FtsZ, and ESCRT complexes. Lipid composition analysis indicated an archaeal-like membrane structure with isoprenoids (C -phytane and C -biphytane) and genes responsible for archaeal ether-type lipid synthesis (but not for bacterial/eukaryotic ester-type lipid synthesis) were identified. The second cultivated Asgard archaea, Lokiarchaeota *Candidatus* Lokiarchaeum ossiferum strain, B35, was isolated from shallow water canal of the Mediterranean Sea at 13–16 cm sediment depth [92]. This species is also anaerobic and forms symbioses with *Methanogenium* but also with three additional species. Lokiarchaeota B35 has a shorter doubling time (7–14 days) and can grow to higher densities than MK-D1, presenting exciting opportunities for future cell biology studies. Morphologically, Lokiarchaeota B35 is similar to MK-D1 with membrane protrusions sometimes extending several folds longer than the cell body (Figure I). Actin-based filaments were observed both within the cell body and protrusions, suggesting a cytoskeleton-like function for actin in these cells. Here too, the three potential cell division machineries were found to be encoded and no sign of organelle-like structures could be detected. Notably, the two Lokiarchaeia strains cultured so far belong to the same class within the phylum Lokiarchaeota, emphasizing the need to culture additional strains both within and beyond this phylum. Finally, the morphology of Lokiarchaeota and Heimdallarchaeota from their natural environment in Arhus Bay was analyzed using fluorescence microscopy [120]. In this study, cell size was estimated at 1–2 µm, and clear spatial separation between DNA and riboplasm was observed. While internal membrane staining was not demonstrated, outer cell surface staining with Wheat germ agglutinin (WGA) revealed extracellular structures extending significantly from the DNA, and riboplasm staining in both Lokiarchaeota and Heimdallarchaeota cells. While much remains to be explored in the cell biology of Asgard archaea, these pioneering studies provide a glimpse into this unique superphylum. More importantly, they open new opportunities for future proteomic studies, immunofluorescence, and for the development of tools for gene manipulation and live-imaging applications, which may potentially shed light on the conditions that permitted the emergence of complex membrane systems in the ancient world.

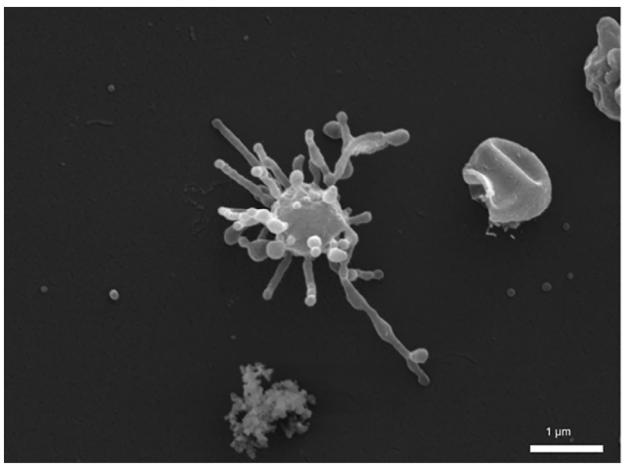


Figure I. A representative scanning electron micrograph (SEM) image of isolated Lokiarchaeota *Candidatus* Lokiarchaeum ossiferum. It was taken by Thiago Rodrigues-Oliveira from the Schleper laboratory (contributed through personal communication).

Lokiarchaeota strain *MK-D1*, Asgard archaea have the typical archaeal isoprenoid signature lipids that are fundamentally different from the phospholipid-based membranes found in bacteria and eukaryotes [91]. Notably, while the experimental data obtained using phospholipid-based vesicles may not reflect the physiological function of the ESCRT complex within the Asgard host, they point to the potential ability of Asgard ESCRTs to remodel both archaeal- and bacterial/eukaryotic-like membranes. The latter may explain the ability of the complex to maintain functionality

during the evolutionary transition from archaeal to eukaryotic membranes. In summary, although these initial findings do not provide comprehensive information on the physiological role of Asgard ESCRTs, they clearly point to an evolutionary and functional linkage between the Asgard and eukaryotic ESCRT systems.

The cellular functions of Asgard ESCRTs may also be inferred based on the extensive functional knowledge of ESCRTs in other systems. First, Asgard archaea are the closest to the TACK archaea superphylum, in which ESCRTs were shown to mediate cell division and vesicle release. A role in vesicle release may be supported by the visualization of shedding vesicles in the two cultures of Lokiarchaeota [91,92] (Box 1). A role for ESCRT in Asgard cell division was considered unlikely based on the presence of the bacterial FtsZ cell division machinery in Asgard genomes. Second, based on the function of the bacterial ESCRT homologs, PspA and Vipp1, a role in membrane integrity was also suggested. Finally, the linkage between the ubiquitin system and the ESCRT system in Asgard archaea, which was also verified experimentally, suggests the establishment of ESCRT function in vesicular trafficking in the Asgard archaea [94]. This ESCRT function is of particular interest during eukaryogenesis as it is one of the most fundamental processes for the function of compartmentalized cells. Importantly, these speculations should be taken with great caution as they rely on very little, if any, experimental data, and on genomic information which may or may not reflect the actual protein expression in these cells. Isolating additional Asgard strains, developing molecular tools for gene manipulation, and providing transcriptomic and proteomic datasets from cultured Asgards will no doubt shed light on the function and diversity of the ESCRT complex in this unique prokaryotic species.

Toward an inclusive perspective of the ESCRT system

The realization that the ESCRT machinery is encoded in almost all living systems known to date provides a unique opportunity for tracing the evolutionary trajectory of this fundamental cellular complex. Over the past two years comparative sequence- and structure-based bioinformatic analysis of ESCRT proteins was performed and several high-resolution structures of ESCRT-III filaments were resolved illuminating new aspects on the basic properties of the machinery, which together may eventually advance our understanding of the functional origin of the machinery.

From the bioinformatic bench

Recent bioinformatic analysis revealed that while the overall sequence similarity between full-length ESCRT proteins is insufficient to identify early ESCRT proteins in prokaryotes outside the Asgard archaeal clade, specific domains appear to be conserved and can be traced through all domains of life [65,72,99]. These studies revealed that multiple domains recur in ESCRT systems across phyla, but are combined in various configurations, resulting ESCRT machineries with distinct domain compositions in different organisms. This mix-and-match feature observed for the ESCRT system, which further highlights the plasticity of this system, may potentially reflect on the different functions documented for ESCRTs in different organisms. Hence, characterizing the potential functions of these domains and tracing their appearance across evolution may contribute to functional understanding of the ESCRT system.

The typical set of ESCRT machinery components identified through recent studies consists of at least four domains: Snf7, AAA ATPase domain, Winged Helix (WH) domain, and Steadiness Box (SB) [65,72,99] (Figure 4). The Snf7 domain is the core domain of ESCRT-III proteins, consisting of five alpha helices and capable of polymerization (see later). The AAA ATPase domain provides the ATP hydrolysis function of Vps4 proteins. While these two domains are well established and are directly linked to specific proteins of the ESCRT system, the two additional domains are much more flexible in the proteins they are part of, and their function is less established. The WH domain

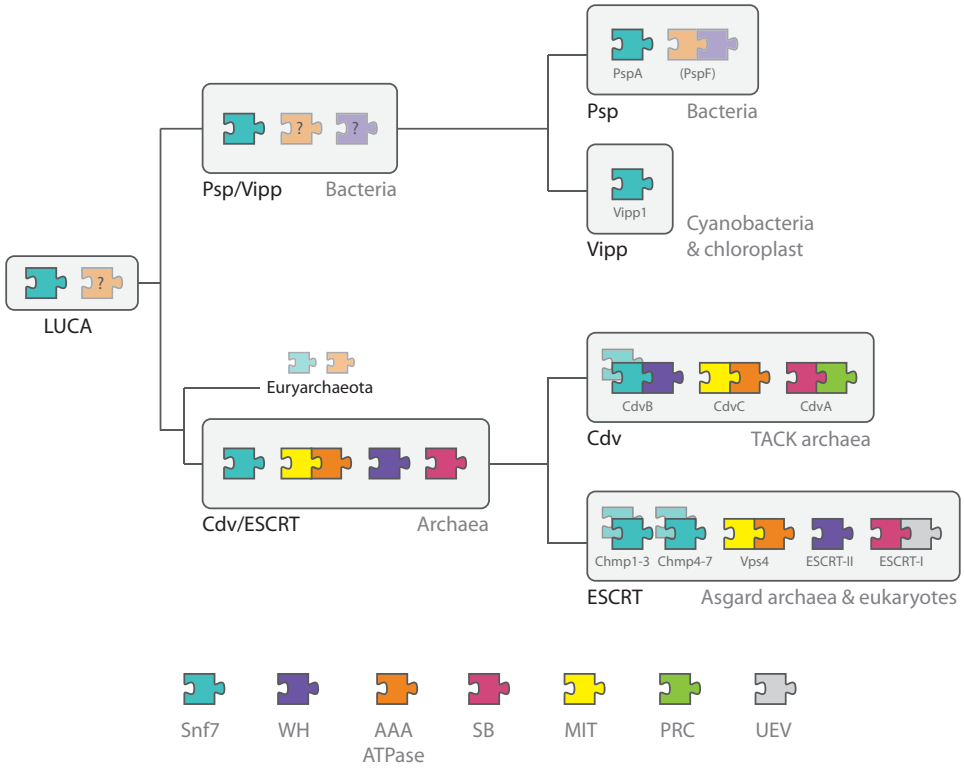


Figure 4. A suggested, bioinformatic-driven model for the evolution of the endosomal sorting complexes required for transport (ESCRT) system. Domains are displayed as puzzle pieces, stitched together to different multi-domain proteins. Opaque domains indicate that they occur in some organisms of this clade, but are not typical. Opaque with a question mark indicates that the presence of these domains is disputable. Domain abbreviations: Snf7, stereotypical ESCRT-III fold; WH, Winged Helix; SB, Steadiness Box; MIT, Microtubule Interacting and Trafficking domain; PRC, Photosynthetic Reaction Center barrel; UEV, Ubiquitin E2 Variant domain.

is a widespread structural motif that is typically associated with DNA recognition [100], but seems to mediate protein–protein interactions in most ESCRT machineries [101,102]. In Eukaryotes and Asgard archaea, this domain is part of the early ESCRT protein ESCRT-II, and is responsible for recruiting downstream ESCRT-III proteins [101,102]. In TACK archaea, however, the WH domain is part of the CdvB protein (ESCRT-III) and mediates interaction with the upstream CdvA protein [58]. Finally, in some bacteria, a reduced WH domain is fused to an AAA ATPase domain of PspF, and mediates DNA binding [99,103]. Interestingly, PspF interacts with the bacterial ESCRT-III homolog PspA in *E.coli*, suggesting that PspF is part of the bacterial ESCRT machinery [103]. However, as PspA is not widely associated with PspF in bacterial genomes, it is not considered part of the typical bacterial ESCRT system [80]. The SB is a recently identified structural domain [65] which is part of the early ESCRT protein ESCRT-I in Eukaryotes and Asgard archaea, and is fused to the UEV domain, linking it to ESCRT/ubiquitin functions [65]. In TACK archaea, the SB domain is integrated into CdvA that recruits ESCRT-IIIs. Interestingly, SB is fused in CdvA to a PRC barrel domain that was recently shown to be an essential domain in archaeal FtsZ-based cell division [104,105]. It is, therefore, tempting to speculate that the PRC barrel domain links the ESCRT system to cell division much like the UEV domain links ESCRT systems to the ubiquitin protein degradation machinery. Finally, an additional domain, conserved in archaea and eukaryotes, is the Microtubule-Interacting and Trafficking (MIT) domain that is fused to the AAA ATPase domain and mediates the interaction between the ESCRT-III and Vps4 proteins [106,107].

Therefore, the MIT domain is most likely an innovation of the last common ancestor of TACK and Asgard archaea.

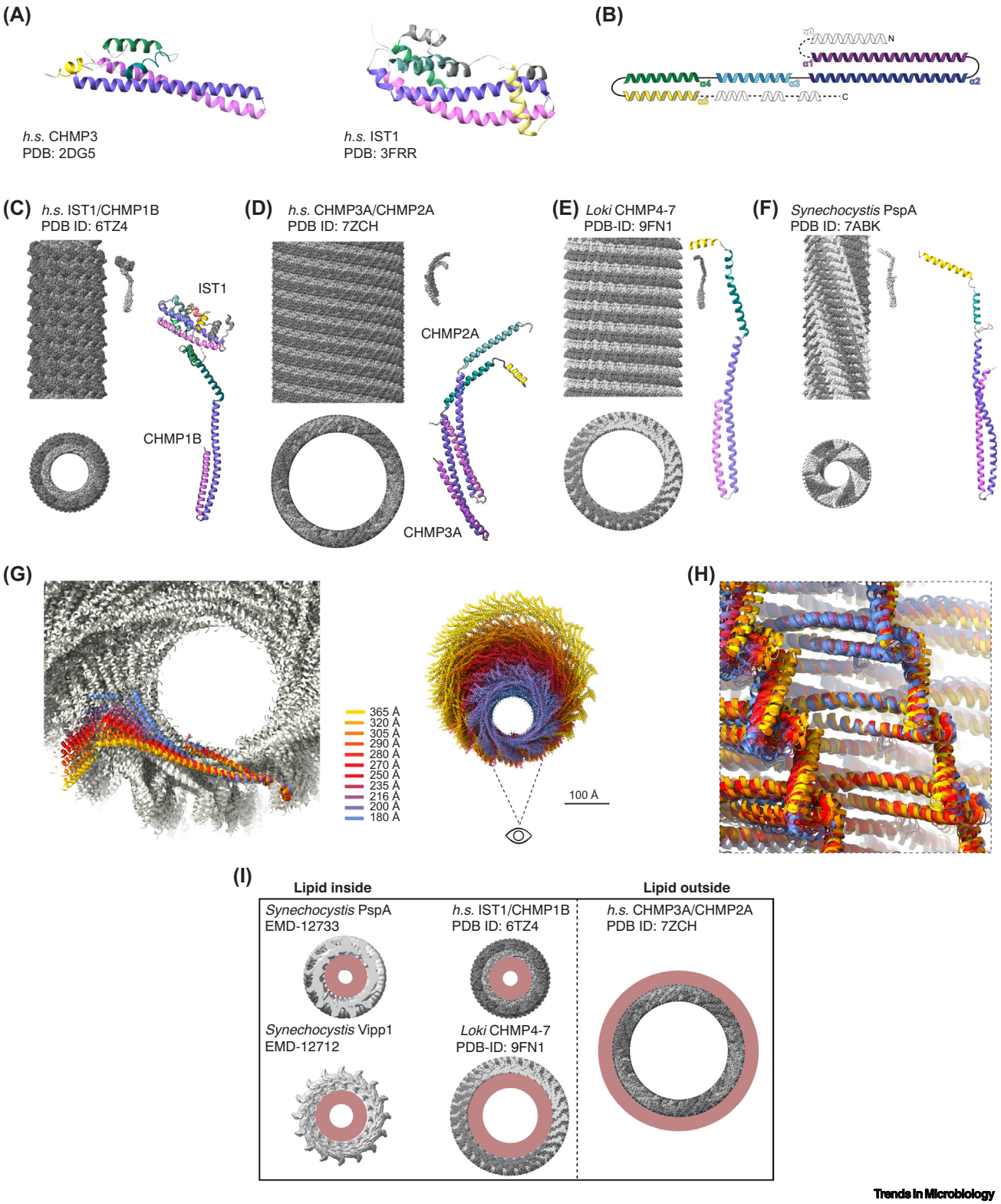
Another conserved feature obtained for ESCRT proteins in all domains of life is gene duplication [65,72,99]. In most eukaryotic and archaeal ESCRT systems multiple ESCRT-III homologs appear to result from gene duplication events that occurred after the phyla had diverged [65,72]. Hence, it was suggested that the last common ancestor of archaea and eukaryotes likely possessed only a single ESCRT-III gene, and later duplications that occurred independently in different species gave rise to the observed diversity of ESCRT-III proteins [65,72]. Notably, the location of ESCRT sequences in the genome, and the number of copies per genome, vary significantly between species across all domains of life, further stressing the dynamic nature of ESCRTs in evolution [65]. Combining these bioinformatic findings suggests an evolutionarily conserved core module of ESCRT machineries in eukaryotes and archaea: the module consists of at least two proteins, Vps4 (comprising a MIT domain and a AAA ATPase domain) and ESCRT-III (comprising an Snf7 domain). This core module is then recruited by upstream proteins, via interactions mediated by the WH and SB domains, presumably to carry out different functions, which are dictated by the arrangement of these domains and their association to additional domains. Multiple duplications of ESCRT-III occurred later in evolution, providing additional functionalities. In bacteria, current knowledge is insufficient to propose an evolutionary trajectory. Yet, the abundance of the PspA gene, its propensity for gene duplication, and its structural homology to ESCRT-IIIs support a shared evolutionary origin for these systems. Together with phylogenetic reconstruction [90,101], these data suggest that all ESCRT machineries evolved from an ancestral ESCRT-III protein, which was already present in the Last Universal Common Ancestor (LUCA) (Figure 4). Whether this system was linked to an AAA ATPase, as in eukaryotes and archaea, or whether it was capable of ATP hydrolysis by itself, as demonstrated for bacterial ESCRTs [88], is yet to be resolved.

Learning from high-resolution structures

Since its discovery, the ESCRT field has been driven by structural data, guiding our understanding of its mode of operation [19]. Consistently, several high-resolution cryo-EM structures of ESCRT-III complexes from both eukaryotes and prokaryotes have been resolved in recent years. Remarkably, the determined assembly structures exhibit large differences, albeit with core secondary structures shared between the observed structures. The current data have not revealed a single evolutionarily conserved tertiary structure for the ESCRT-III complexes; rather, it illuminated a basic conserved fold with several levels of flexibility and plasticity (Figure 5). We now review the current structural knowledge obtained for ESCRT-III polymers from high-resolution cryo-EM.

Early experimentally resolved structures revealed that ESCRT-III monomeric subunits share a common core domain of five α-helixes (α1–5) [108,109] (Figure 5A,B). Some members of the ESCRT-III family, that is, CHMP2A, CHMP2B, CHMP4, and CHMP3 homologs (Snf7 and Vps24, respectively), consist of an additional helix at the N terminus termed α0 that was associated with membrane binding. The C-terminal helix was shown to be important for controlling polymerization [26,110,111]. Based on the obtained structures, ESCRT-III subunits were suggested to coexist in two conformations: a compact conformation with the helixes packed in a globular manner (known as the closed conformation) and an extended conformation in which the helixes are extended, forming an elongated structure (known as the open conformation) [39,112].

The organization of eukaryotic ESCRT-III subunits in the polymer was visualized in the three available cryo-EM structures of eukaryotic ESCRT-III proteins – human CHMP1B/IST1 [39], yeast



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CHMP3 (Vps24) [31], and human CHMP2A/CHMP3 [110] – revealing a complex behavior for the eukaryotic ESCRT-III system. The human IST1/CHMP1B proteins organize in the presence of lipid membranes into a helical tube, that is, a hollow 220 Å wide cylinder, with CHMP1B constituting the inner shell, adopting an extended conformation, and IST1 constituting the outer shell, adopting a packed conformation (Figure 5C). CHMP1B alone – in the absence of IST1 – self-polymerizes into similar structures, forming a wider tube diameter [33]. In both cases, the lipid membrane was observed in the inner lumen of the cylinder. The human CHMP2A/CHMP3 polymer also formed a hollow cylinder in the presence of liposomes, but with both monomers adopting an extended conformation and organized in tandem, making up a single-shell 440 Å wide hollow cylinder (Figure 5D). In this case, the membrane was found at the outside of the lipid tube. In contrast, the yeast CHMP3 homolog (Vps24) polymerized in the absence of membranes and did not form a tubular structure but rather a 160 Å wide twisted double-helical filament with a pitch of 280 Å. The observed Vps24 conformation adopted an intermediate between a compact and an extended fold. Therefore, eukaryotic ESCRT-III proteins can polymerize to form distinct helical polymeric structures. Notably, all the available structures consist of the basic ESCRT-III fold with a minimum of four α-helices, while the relative arrangement of the α-helices changed in each of the structures, and as a result, so did their higher-order assembly architectures.

With the evolutionary discovery of bacterial proteins PspA and Vipp1 being members of the ESCRT-III family [73,74,113], a plenitude of new structural information became available. Recent studies on Asgard archaeal proteins of Lokiarchaeota and Heimdallarchaeota , showed the formation of helical tubes as well as helical filaments based on extended ESCRT-III fold widening the evolutionary distribution of the ESCRT proteins to the archaeal kingdom [96,97] (Figure 5E). PspA from *Synechocystis*, and Vipp1 from *Synechocystis* and *N. punctiforme*, adopted an extended conformation [73,74,113]. In the absence of lipids, PspA was found organized in tubes of helical symmetry (Figure 5F), while Vipp1 formed dome-shaped ring structures of rotational cyclical symmetry. As Vipp1 was found in tapered dome-shaped ring structures, the conformations of each layer varied slightly in the relative orientations of the helices in a total of three hinge loops [73]. PspA revealed a continuous conformational plasticity resulting in a distribution of different diameter tubes from 180 to 365 Å, which could be experimentally shifted upon the addition of nucleotides [114] (Figure 5G,H). The different diameter structures were each made of different helical architectures mediated by variable hydrophobic interactions of α1/α2 hairpin stacking while maintaining the canonical α1/α2 hairpin to helix α5 motif. Notably, removal of hinge 2 connecting helices α3 and α4 resulted in a restrained diameter population from 180 to 250 Å showing the required hinges for the structural plasticity. In these plasticity-restrained mutants, PspA also showed decreased membrane remodeling capabilities [114]. In support of the observed structural plasticity links, recent studies of reconstituted Vipp1 structures alone and with lipid membranes showed a large diversity of resolved cryo-EM structures from rings, stacked rings, helical tubes to carpet-like assemblies together with lipid membranes [115,116]. Whenever PspA and Vipp1 were co-incubated with lipids, lipid membrane could be found in the lumen of the assembled ESCRT-III structures [74,87,113,115].

Figure 5. Structural plasticity of ESCRT-III members in eukaryotes and prokaryotes. (A) Two X-ray crystal structures of *Homo sapiens* (*h.s.*) CHMP3 (PDB ID: 2DG5) and IST1 (PDB ID: 3FRR) shown in ribbon presentation. Color code: α1, orchid; α2, blue; α3, cyan; α4, green; α5, yellow. (B) Topology plot of ESCRT-III α-helical secondary structures with corresponding color code. (C–F) Side and top view of helical tube in gray (left). Surface presentation of IST1/CHMP1B dimer at same scale to the left (center) and ribbon presentation of IST1/CHMP1B dimer with corresponding helix color code (right) for (C) *h.s.* IST1/CHMP1B, (D) *h.s.* CHMP3A/CHMP2A, (E) Lokiarchaeota CHMP4–7 and (F) *Synechocystis* PspA drawn to scale. (G) Left. Top view of structural superposition of 11 PspA helical tubes with a diameter range between 180 and 365 Å in white. In foreground, in color from blue (180 Å), red (270 Å) to yellow (365 Å) 11 superimposed PspA monomer structures of the corresponding diameters with α1/α2 hairpin used for structural alignment. Right. Zoomed-out top view of fully colored 11 helical tubes from blue (180 Å), red (270 Å) to yellow (365 Å). (H) Side view of (G) revealing the close superposition of the neighboring protomers. (I) Summary of determined helical tube structures with lipid binding observed inside the lumen PspA, Vipp1, IST1/CHMP1B, CHMP4-7 (left) and outside the lumen CHMP3A/CHMP2A (right). The lipid bilayer is colored red.

The demonstrated structural plasticity of prokaryotic ESCRT-III homologs is equally applicable across human ESCRT isoforms considering the different conformations observed for IST1/CHMP1B and CHMP2A/CHMP3. Whether the plasticity exists within the same structure as in PspA and Vipp1 or amongst other isoforms of eukaryotic ESCRT-III remains to be established. Comparative analysis of ESCRT-III structures from bacteria, archaea, and eukarya reveals an exceptional structural plasticity with the basic ESCRT-III fold conserved, including the packing motif of helix α5 against the helical α1/α2 hairpin of a neighboring protomer. A direct consequence of this structural plasticity is that small-scale conformational changes give rise to different assembly architectures.

In principle, most of the eukaryotic ESCRT-driven membrane processes are thought to require negatively curved membranes and lipid binding outside the protein tube. A comparison of the observed bacterial, archaeal and eukaryotic ESCRT-III structures with respect to the lipid binding mode (Figure 5I) provide the following insight: human IST1/CHMP1B and CHPM4/CHMP2, yeast Snf7/Vps24/Vps2, bacterial PspA and Vipp1, and Lokiarchaeota CHMP1-3/CHMP4-7 can bind lipid membrane vesicles inside the protein tube [33,39,73,74,97,113–115,117,118], while only the human CHMP2A/CHMP3 tube revealed lipid membrane covering the outside of the protein tube [110]. Thus, the majority of observations, in particular for bacterial and archaeal ESCRT-IIIs, showed inside lipid binding, suggesting that the evolutionarily conserved topology is binding the membrane from the inside of the tube, and binding to the outside may have emerged later in evolution. The inside binding mode provides a shielded environment for stabilizing highly positively curved membrane tubules, which may also represent an important intermediate of an inside-out remodeling activity [74]. A possible explanation for the CHMP2A/CHMP3 observations is that, in the course of evolution, binding modes switched between inside- and outside-binding topologies due to a functional plasticity, allowing the establishment of both modes in human cells, for example, IST1/CHMP1B and CHMP2A/CHMP3 polymers. Given the diversity of different ESCRT functions found in eukaryotes, the observed structural plasticity may also give rise to the functional plasticity of different lipid binding modes, thus enabling both inside-out as well as outside-in fission activities. Further intermediate structures of different isoforms involved in different cellular functions in complex with lipids and budding assays are needed to understand the exact sequence of action of ESCRT fusion and fission.

Concluding remarks

The information presented in the preceding text underscores the exceptional plasticity of the ESCRT system at multiple levels, while maintaining its essential core elements. At the protein level, each ESCRT subfamily comprises multiple proteins, with the number and identity of these proteins varying between organisms, resulting in numerous combinations (Figure 3). At the sequence level, ESCRT systems appear to have conserved functional domains that can be combined in a mix-and-match fashion, potentially increasing their functional diversity (Figure 4). Finally, at the structural level, eukaryotic ESCRT-III proteins can polymerize in different combinations resulting in distinct structures with unique biophysical properties within a single cell. Moreover, individual prokaryotic ESCRT-III proteins can adopt structures with varying dimensions while preserving the core interactions between the monomers (Figure 5). This multi-layer plasticity ultimately translates to the diverse cellular functions described for the ESCRT system (Figure 1). As a result, the ESCRT system presents a complex evolutionary landscape, with different organisms encoding different sets of proteins and employing the ESCRT system for different functions. Taken together, the comparative analysis suggests a scenario in which the ESCRT machinery evolved as a versatile membrane-shaping toolkit, adaptable for specific roles. Tuning can be manifested by domain composition, number, and identity of expressed genes, as well as in protein compositions and structural modulations of the ESCRT-III polymer. Understanding the basis

Outstanding questions

How is the multi-layered plasticity of ESCRTs tuned to drive specific function?

What roles do ESCRT systems employ in the prokaryotic world, and particularly in Asgard archaea?

How does the structural diversity observed for ESCRT-III proteins translate to membrane remodeling in different cellular contexts?

What is the evolutionary trajectory of the ESCRT system, particularly in relation to its cellular functions?

What was the involvement of ESCRTs in the establishment of the first eukaryotic cell?

for the versatility of the ESCRT system may be further used for constructing tunable devices for executing essential membrane remodeling functions such as membrane repair, vesicle release, and cell division for applications in biotechnology and synthetic cell engineering [119].

The comparative overview presented here highlighted new conserved features for the ESCRT system. While CHMP4B was traditionally regarded as the core ESCRT-III component, CHMP1B appears to exhibit greater similarity to prokaryotic ESCRT-III proteins. Interestingly, both CHMP1B and prokaryotic ESCRTs were shown to bind nucleic acids, suggesting a new unexplored venue for the ESCRT system [34,42,43,62,73,74]. Finally, while CHMP1B was originally considered an atypical ESCRT-III because of its unexpected membrane binding topology, recent structural data from Asgard and bacterial ESCRT homologs infer similar membrane binding topology to that of CHMP1B [74,97], stressing the formulation of alternative mechanistic models for ESCRT function.

While the cellular functions of ESCRTs in animal cells have been established, functional knowledge from eukaryotic microorganisms and prokaryotic cells is partial. The four main functions reported for ESCRTs in animal cells – that is, vesicle release, cell division, membrane integrity, and viral replication and release – are also present in prokaryotes (Figure 1), yet no single function can be traced throughout evolution. Notably, the data obtained so far are too little and often biased toward specific functions, and hence may not represent the actual functional repertoire of ESCRTs in these microorganisms. Systematic functional studies of ESCRTs in eukaryotic microorganisms with reduced yet distinct ESCRT complexes are crucial for mapping the functional trajectory of ESCRTs throughout evolution. Further functional and structural studies of ESCRTs encoded in the archaeal domain – using phyla that exhibit diverse combination of ESCRTs, but with a much smaller number of components [42,65,95] – may pave the way for unveiling core properties of this complex protein machinery. Finally, the extraordinary plasticity of the ESCRT system may further infer its ability to deform different types of lipid membranes. Such plasticity may have been crucial during the establishment of the first eukaryotic cells, which may have been composed of both archaeal and bacterial membranes. Future studies deciphering the membrane-shaping properties of ESCRTs using archaeal-like model membranes and elucidating the cellular function of ESCRTs in Asgard archaea may shed light on the potential role of ESCRTs in eukaryogenesis (see Outstanding questions).

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Declaration of interests

The authors declare no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 4.0 for language editing. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

1. Katzmann, D.J. *et al.* (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* 106, 145–155
2. Hurley, J.H. (2015) ESCRTs are everywhere. *EMBO J.* 34, 2398–2407
3. Olmos, Y. (2022) The ESCRT machinery: remodeling, repairing, and sealing membranes. *Membranes (Basel)* 12, 633
4. Gatta, A.T. and Carlton, J.G. (2019) The ESCRT-machinery: closing holes and expanding roles. *Curr. Opin. Cell Biol.* 59, 121–132

5. Adar-Levor, S. *et al.* (2021) Cytokinetic abscission is part of the midblastula transition in early zebrafish embryogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2021210118
6. Chaigne, A. *et al.* (2020) Abscission couples cell division to embryonic stem cell fate. *Dev. Cell* 55, 195–208 e195
7. Gulluni, F. *et al.* (2021) PI(3,4)P2-mediated cytokinetic abscission prevents early senescence and cataract formation. *Science* 374, eabk0410
8. Loncle, N. *et al.* (2015) An ESCRT module is required for neuron pruning. *Sci. Rep.* 5, 8461
9. Yang, Y. *et al.* (2022) The endosomal sorting complex required for transport repairs the membrane to delay cell death. *Front. Oncol.* 12, 1007446
10. Radulovic, M. *et al.* (2018) ESCRT-mediated lysosome repair precedes lysophagy and promotes cell survival. *EMBO J.* 37, e99753
11. Skowyra, M.L. *et al.* (2018) Triggered recruitment of ESCRT machinery promotes endolysosomal repair. *Science* 360, eaar5078
12. Denais, C.M. *et al.* (2016) Nuclear envelope rupture and repair during cancer cell migration. *Science* 352, 353–358
13. Raab, M. *et al.* (2016) ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death. *Science* 352, 359–362
14. Vietri, M. *et al.* (2015) Spastin and ESCRT-III coordinate mitotic spindle disassembly and nuclear envelope sealing. *Nature* 522, 231–235
15. Zhen, Y. *et al.* (2021) Sealing holes in cellular membranes. *EMBO J.* 40, e106922
16. Martin-Serrano, J. *et al.* (2001) HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat. Med.* 7, 1313–1319
17. Garrus, J.E. *et al.* (2001) Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107, 55–65
18. Diaz, A. *et al.* (2015) Host ESCRT proteins are required for bromovirus RNA replication compartment assembly and function. *PLoS Pathog.* 11, e1004742
19. McCullough, J. *et al.* (2013) Membrane fission reactions of the mammalian ESCRT pathway. *Annu. Rev. Biochem.* 82, 663–692
20. Olmos, Y. *et al.* (2015) ESCRT-III controls nuclear envelope reformation. *Nature* 522, 236–239
21. Leung, K.F. *et al.* (2008) Evolution of the multivesicular body ESCRT machinery; retention across the eukaryotic lineage. *Traffic* 9, 1698–1716
22. Saksena, S. *et al.* (2009) Functional reconstitution of ESCRT-III assembly and disassembly. *Cell* 136, 97–109
23. Chiaruttini, N. *et al.* (2015) Relaxation of loaded ESCRT-III spiral springs drives membrane deformation. *Cell* 163, 866–879
24. Schoneberg, J. *et al.* (2018) ATP-dependent force generation and membrane scission by ESCRT-III and Vps4. *Science* 362, 1423–1428
25. Pfitzner, A.K. *et al.* (2020) An ESCRT-III polymerization sequence drives membrane deformation and fission. *Cell* 182, 1140–1155 e1118
26. Lata, S. *et al.* (2008) Helical structures of ESCRT-III are disassembled by VPS4. *Science* 321, 1354–1357
27. Henne, W.M. *et al.* (2012) The endosomal sorting complex ESCRT-II mediates the assembly and architecture of ESCRT-III helices. *Cell* 151, 356–371
28. Ghazi-Tabatabai, S. *et al.* (2008) Structure and disassembly of filaments formed by the ESCRT-III subunit Vps24. *Structure* 16, 1345–1356
29. Goliand, I. *et al.* (2018) Resolving ESCRT-III spirals at the intercellular bridge of dividing cells using 3D STORM. *Cell Rep.* 24, 1756–1764
30. Mierzwa, B.E. *et al.* (2017) Dynamic subunit turnover in ESCRT-III assemblies is regulated by Vps4 to mediate membrane remodelling during cytokinesis. *Nat. Cell Biol.* 19, 787–798
31. Huber, S.T. *et al.* (2020) Structure and assembly of ESCRT-III helical Vps24 filaments. *Sci. Adv.* 6, eaba4897
32. Pfitzner, A.K. *et al.* (2023) Vps60 initiates alternative ESCRT-III filaments. *J. Cell Biol.* 222, e202206028
33. Nguyen, H.C. *et al.* (2020) Membrane constriction and thinning by sequential ESCRT-III polymerization. *Nat. Struct. Mol. Biol.* 27, 392–399
34. Talledge, N. *et al.* (2018) The ESCRT-III proteins IST1 and CHMP1B assemble around nucleic acids. *bioRxiv*, https://doi.org/10.1101/386532
35. Pires, R. *et al.* (2009) A crescent-shaped ALIX dimer targets ESCRT-III CHMP4 filaments. *Structure* 17, 843–856
36. Effantin, G. *et al.* (2013) ESCRT-III CHMP2A and CHMP3 form variable helical polymers *in vitro* and act synergistically during HIV-1 budding. *Cell. Microbiol.* 15, 213–226
37. Maity, S. *et al.* (2019) VPS4 triggers constriction and cleavage of ESCRT-III helical filaments. *Sci. Adv.* 5, eaau7198
38. Fabrikant, G. *et al.* (2009) Computational model of membrane fission catalyzed by ESCRT-III. *PLoS Comput. Biol.* 5, e1000575
39. McCullough, J. *et al.* (2015) Structure and membrane remodeling activity of ESCRT-III helical polymers. *Science* 350, 1548–1551
40. Shen, Q.T. *et al.* (2014) Structural analysis and modeling reveals new mechanisms governing ESCRT-III spiral filament assembly. *J. Cell Biol.* 206, 763–777
41. Jiang, X. *et al.* (2022) Modelling membrane reshaping by staged polymerization of ESCRT-III filaments. *PLoS Comput. Biol.* 18, e1010586
42. Nachmias, D. *et al.* (2023) Asgard ESCRT-III and VPS4 reveal conserved chromatin binding properties of the ESCRT machinery. *ISME J.* 17, 117–129
43. Stauffer, D.R. *et al.* (2001) CHMP1 is a novel nuclear matrix protein affecting chromatin structure and cell-cycle progression. *J. Cell Sci.* 114, 2383–2393
44. Azmi, I.F. *et al.* (2008) ESCRT-III family members stimulate Vps4 ATPase activity directly or via Vta1. *Dev. Cell* 14, 50–61
45. Dvilansky, I. *et al.* (2024) The human AAA-ATPase VPS4A isoform and its co-factor VTA1 have a unique function in regulating mammalian cytokinesis abscission. *PLoS Biol.* 22, e3002327
46. Pipaliya, S.V. *et al.* (2021) Unexpected organellar locations of ESCRT machinery in Giardia intestinalis and complex evolutionary dynamics spanning the transition to parasitism in the lineage Fornicata. *BMC Biol.* 19, 167
47. Moyano, S. *et al.* (2019) Exosome biogenesis in the protozoa parasite *Giardia lamblia*: a model of reduced interorganellar crosstalk. *Cells* 8, 1600
48. Avalos-Padilla, Y. *et al.* (2021) The ESCRT-III machinery participates in the production of extracellular vesicles and protein export during *Plasmodium falciparum* infection. *PLoS Pathog.* 17, e1009455
49. Cruz Camacho, A. *et al.* (2023) Biogenesis of extracellular vesicles in protozoan parasites: the ESCRT complex in the trafficking fast lane? *PLoS Pathog.* 19, e1011140
50. Lopez-Jimenez, A.T. *et al.* (2018) The ESCRT and autophagy machineries cooperate to repair ESX-1-dependent damage at the *Mycobacterium*-containing vacuole but have opposite impact on containing the infection. *PLoS Pathog.* 14, e1007501
51. Talukder, M.S.U. *et al.* (2020) Ca(2+)-calmodulin dependent wound repair in *Dictyostelium* cell membrane. *Cells* 9, 1058
52. Mitic, K. *et al.* (2023) Temporal changes in nuclear envelope permeability during semi-closed mitosis in *Dictyostelium* amoebae. *Cells* 12, 1380
53. Schweigel, U. *et al.* (2022) *Dictyostelium* spastin is involved in nuclear envelope dynamics during semi-closed mitosis. *Nucleus* 13, 144–154
54. Yagisawa, F. *et al.* (2020) ESCRT machinery mediates cytokinetic abscission in the unicellular red alga *Cyanidioschyzon merolae*. *Front. Cell Dev. Biol.* 8, 169
55. Chew, T.G. *et al.* (2020) Inhibition of cell membrane ingression at the division site by cell walls in fission yeast. *Mol. Biol. Cell* 31, 2306–2314
56. Bhutta, M.S. *et al.* (2014) A complex network of interactions between mitotic kinases, phosphatases and ESCRT proteins regulates septation and membrane trafficking in *S. pombe*. *PLoS One* 9, e111789
57. Samson, R.Y. *et al.* (2008) A role for the ESCRT system in cell division in archaea. *Science* 322, 1710–1713
58. Samson, R.Y. *et al.* (2011) Molecular and structural basis of ESCRT-III recruitment to membranes during archaeal cell division. *Mol. Cell* 41, 186–196
59. Lindas, A.C. *et al.* (2008) A unique cell division machinery in the Archaea. *Proc. Natl. Acad. Sci. U. S. A.* 105, 18942–18946

60. Caspi, Y. and Dekker, C. (2018) Dividing the archaeal way: the ancient Cdv cell-division machinery. *Front. Microbiol.* 9, 174
61. Blanch Jover, A. and Dekker, C. (2023) The archaeal Cdv cell division system. *Trends Microbiol.* 31, 601–615
62. Moriscot, C. *et al.* (2011) Crenarchaeal CdvA forms double-helical filaments containing DNA and interacts with ESCRT-III-like CdvB. *PLoS One* 6, e21921
63. Carlton, J.G. and Baum, B. (2023) Roles of ESCRT-III polymers in cell division across the tree of life. *Curr. Opin. Cell Biol.* 85, 102274
64. Tarrason Risa, G. *et al.* (2020) The proteasome controls ESCRT-III-mediated cell division in an archaeon. *Science* 369, eaaz2532
65. Makarova, K.S. *et al.* (2024) Diversity, origin, and evolution of the ESCRT systems. *mBio* 15, e0033524
66. Liu, J. *et al.* (2017) Functional assignment of multiple ESCRT-III homologs in cell division and budding in *Sulfolobus islandicus*. *Mol. Microbiol.* 105, 540–553
67. Ettema, T.J. and Bernander, R. (2009) Cell division and the ESCRT complex: a surprise from the archaea. *Commun. Integr. Biol.* 2, 86–88
68. Liu, J. *et al.* (2021) Archaeal extracellular vesicles are produced in an ESCRT-dependent manner and promote gene transfer and nutrient cycling in extreme environments. *ISME J.* 15, 2892–2905
69. Ellen, A.F. *et al.* (2009) Proteomic analysis of secreted membrane vesicles of archaeal *Sulfolobus* species reveals the presence of endosome sorting complex components. *Extremophiles* 13, 67–79
70. Snyder, J.C. *et al.* (2013) Functional interplay between a virus and the ESCRT machinery in archaea. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10783–10787
71. Liu, J. *et al.* (2021) Virus-induced cell gigantism and asymmetric cell division in archaea. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2022578118
72. Frohn, B.P. *et al.* (2022) Tracing back variations in archaeal ESCRT-based cell division to protein domain architectures. *PLoS One* 17, e0266395
73. Gupta, T.K. *et al.* (2021) Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. *Cell* 184, 3643–3659 e3623
74. Junglas, B. *et al.* (2021) PspA adopts an ESCRT-III-like fold and remodels bacterial membranes. *Cell* 184, 3674–3688 e3618
75. Deo, R.A. and Prinz, W.A. (2021) ESCRTs got your Bac! *Cell* 184, 3591–3592
76. Junglas, B. *et al.* (2020) GTP hydrolysis by *Synechocystis* IM30 does not decisively affect its membrane remodeling activity. *Sci. Rep.* 10, 9793
77. Siebenaller, C. *et al.* (2021) Binding and/or hydrolysis of purine-based nucleotides is not required for IM30 ring formation. *FEBS Lett.* 595, 1876–1885
78. Ohnishi, N. *et al.* (2018) VIPP1 involved in chloroplast membrane integrity has GTPase activity *in vitro*. *Plant Physiol.* 177, 328–338
79. Brissette, J.L. *et al.* (1990) Phage shock protein, a stress protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 87, 862–866
80. Popp, P.F. *et al.* (2022) Phyletic distribution and diversification of the phage shock protein stress response system in bacteria and archaea. *mSystems* 7, e0134821
81. Nordhues, A. *et al.* (2012) Evidence for a role of VIPP1 in the structural organization of the photosynthetic apparatus in *Chlamydomonas*. *Plant Cell* 24, 637–659
82. Zhang, L. *et al.* (2012) Essential role of VIPP1 in chloroplast envelope maintenance in *Arabidopsis*. *Plant Cell* 24, 3695–3707
83. Aseeva, E. *et al.* (2004) Complex formation of Vipp1 depends on its alpha-helical PspA-like domain. *J. Biol. Chem.* 279, 35535–35541
84. Zhang, L. *et al.* (2016) VIPP1 Has a disordered c-terminal tail necessary for protecting photosynthetic membranes against stress. *Plant Physiol.* 171, 1983–1995
85. Bryan, S.J. *et al.* (2014) Localisation and interactions of the Vipp1 protein in cyanobacteria. *Mol. Microbiol.* 94, 1179–1195
86. Gutu, A. *et al.* (2018) Dynamical localization of a thylakoid membrane binding protein is required for acquisition of photosynthetic competency. *Mol. Microbiol.* 108, 16–31
87. Theis, J. *et al.* (2019) VIPP1 rods engulf membranes containing phosphatidylinositol phosphates. *Sci. Rep.* 9, 8725
88. Schlosser, L. *et al.* (2023) Conserved structures of ESCRT-III superfamily members across domains of life. *Trends Biochem. Sci.* 48, 993–1004
89. Spang, A. *et al.* (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 521, 173–179
90. Zaremba-Niedzwiedzka, K. *et al.* (2017) Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 541, 353–358
91. Imachi, H. *et al.* (2020) Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature* 577, 519–525
92. Rodrigues-Oliveira, T. *et al.* (2023) Actin cytoskeleton and complex cell architecture in an Asgard archaeon. *Nature* 613, 332–339
93. Lu, Z. *et al.* (2024) Origin of eukaryotic-like Vps23 shapes an ancient functional interplay between ESCRT and ubiquitin system in Asgard archaea. *Cell Rep.* 43, 113781
94. Hatano, T. *et al.* (2022) Asgard archaea shed light on the evolutionary origins of the eukaryotic ubiquitin-ESCRT machinery. *Nat. Commun.* 13, 3398
95. Eme, L. *et al.* (2023) Inference and reconstruction of the heimdallarchaeial ancestry of eukaryotes. *Nature* 618, 992–999
96. Souza, D.P. *et al.* (2024) Evolutionarily conserved principles of ESCRT-III-mediated membrane remodelling revealed by a two-subunit Asgard archaeal system. *bioRxiv*, https://doi.org/10.1101/2024.07.01.601590
97. Melnikov, N. *et al.* (2025) The Asgard archaeal ESCRT-III system forms helical filaments and remodels eukaryotic-like membranes. *EMBO J.* 44, 665–681
98. Lu, Z. *et al.* (2020) Coevolution of Eukaryote-like Vps4 and ESCRT-III subunits in the Asgard archaea. *mBio* 11, e00417-20
99. Ravi, J. *et al.* (2024) The phage shock protein (PSP) envelope stress response: discovery of novel partners and evolutionary history. *mSystems* 9, e0084723
100. Gajiwala, K.S. and Burley, S.K. (2000) Winged helix proteins. *Curr. Opin. Struct. Biol.* 10, 110–116
101. Im, Y.J. *et al.* (2009) Structure and function of the ESCRT-II-III interface in multivesicular body biogenesis. *Dev. Cell* 17, 234–243
102. Teo, H. *et al.* (2004) ESCRT-II, an endosome-associated complex required for protein sorting: crystal structure and interactions with ESCRT-III and membranes. *Dev. Cell* 7, 559–569
103. Elderkin, S. *et al.* (2002) Mechanism of action of the Escherichia coli phage shock protein PspA in repression of the AAA family transcription factor PspF. *J. Mol. Biol.* 320, 23–37
104. Zhao, S. *et al.* (2023) Widespread PRC barrel proteins play critical roles in archaeal cell division. *bioRxiv*, https://doi.org/10.1101/2023.03.28.534520
105. Nussbaum, P. *et al.* (2024) Proteins containing photosynthetic reaction centre domains modulate FtsZ-based archaeal cell division. *Nat. Microbiol.* 9, 698–711
106. Obita, T. *et al.* (2007) Structural basis for selective recognition of ESCRT-III by the AAA ATPase Vps4. *Nature* 449, 735–739
107. Stuchell-Brereton, M.D. *et al.* (2007) ESCRT-III recognition by VPS4 ATPases. *Nature* 449, 740–744
108. Bajorek, M. *et al.* (2009) Structural basis for ESCRT-III protein autoinhibition. *Nat. Struct. Mol. Biol.* 16, 754–762
109. Muziol, T. *et al.* (2006) Structural basis for budding by the ESCRT-III factor CHMP3. *Dev. Cell* 10, 821–830
110. Azad, K. *et al.* (2023) Structural basis of CHMP2A-CHMP3 ESCRT-III polymer assembly and membrane cleavage. *Nat. Struct. Mol. Biol.* 30, 81–90
111. Bodon, G. *et al.* (2011) Charged multivesicular body protein 2B (CHMP2B) of the endosomal sorting complex required for transport-III (ESCRT-III) polymerizes into helical structures deforming the plasma membrane. *J. Biol. Chem.* 286, 40276–40286
112. Tang, S. *et al.* (2015) Structural basis for activation, assembly and membrane binding of ESCRT-III Snf7 filaments. *Elife* 4, e12548
113. Liu, J. *et al.* (2021) Bacterial Vipp1 and PspA are members of the ancient ESCRT-III membrane-remodeling superfamily. *Cell* 184, 3660–3673 e3618

114. Junglas, B. *et al.* (2025) Structural plasticity of bacterial ESCRT-III protein PspA in higher-order assemblies. *Nat. Struct. Mol. Biol.* 32, 23–34
115. Junglas, B. *et al.* (2024) Structural basis for Vipp1 membrane binding: from loose coats and carpets to ring and rod assemblies. *Nat. Struct. Mol. Biol.*, Published online ahead of print https://doi.org/10.1038/s41594-024-01399-z
116. Naskar, S. *et al.* (2024) Mechanism for Vipp1 spiral formation, ring biogenesis, and membrane repair. *Nat. Struct. Mol. Biol.*, Published online ahead of print https://doi.org/10.1038/s41594-024-01401-8
117. Bertin, A. *et al.* (2020) Human ESCRT-III polymers assemble on positively curved membranes and induce helical membrane tube formation. *Nat. Commun.* 11, 2663
118. Moser von Filseck, J. *et al.* (2020) Anisotropic ESCRT-III architecture governs helical membrane tube formation. *Nat. Commun.* 11, 1516
119. Kretschmer, S. *et al.* (2019) Synthetic cell division via membrane-transforming molecular assemblies. *BMC Biol.* 17, 43
120. Avci, B. *et al.* (2022) Spatial separation of ribosomes and DNA in Asgard archaeal cells. *ISME J.* 16, 606–610