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Review

Conserved structures of ESCRT-III superfamily members across domains of life

Lukas Schlösser,[1] Carsten Sachse,[2,3,4,@] Harry H. Low,[5,@] and Dirk Schneider[1,6,*,@]

Structural and evolutionary studies of cyanobacterial phage shock protein A (PspA) and inner membrane-associated protein of 30 kDa (IM30) have revealed that these proteins belong to the endosomal sorting complex required for transport-III (ESCRT-III) superfamily, which is conserved across all three domains of life. PspA and IM30 share secondary and tertiary structures with eukaryotic ESCRT-III proteins, whilst also oligomerizing via conserved interactions. Here, we examine the structures of bacterial ESCRT-III-like proteins and compare the monomeric and oligomerized forms with their eukaryotic counterparts. We discuss conserved interactions used for self-assembly and highlight key hinge regions that mediate oligomer ultrastructure versatility. Finally, we address the differences in nomenclature assigned to equivalent structural motifs in both the bacterial and eukaryotic fields and suggest a common nomenclature applicable across the ESCRT-III superfamily.

Highlights

Endosomal sorting complex required for transport-III (ESCRT-III) complexes are involved in membrane remodeling.

Recently, ESCRT-III homologs were identified in bacteria and the structures of three bacterial representatives were solved.

A set of minimal structural features is common to all pro- and eukaryotic ESCRT-IIIs.

Conserved interactions stabilize bacterial and eukaryotic oligomeric ESCRT-III assemblies.

Annotation and numbering of ESCRT-III helices has not been consistent in the past; a common nomenclature applicable across the ESCRT-III superfamily is suggested.

Membrane remodeling mediated by ESCRT-III proteins

Membrane remodeling is a critical task by which a eukaryotic cell maintains cellular compartmentalization by membrane-enclosed organelles and membrane remodeling proteins mediate membrane fusion and fission processes as well as membrane repair [1–3]. The protein machinery that is crucially involved in fundamental cellular processes, such as the formation of multivesicular bodies (MVBs), cytokinesis, endosome fission, and lysosome repair in eukaryotes, is termed the 'endosomal sorting complex required for transport' (ESCRT) [4]. In eukaryotes, the ESCRT machinery consists of five multiprotein complexes: ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III, and Vps4, whereby membrane deformation and the final vesicle scission is directly mediated by the ESCRT-III complexes.

Recently, two bacterial proteins were identified as being homologous to eukaryotic ESCRT-IIIs and involvement of these superfamily members in remodeling bacterial membranes is likely. Here, we discuss conservation of ESCRT-III proteins and compare their monomeric and oligomeric structures. Based on this comparison, we propose a common nomenclature for the ESCRT-III superfamily of proteins.

ESCRT-III proteins are conserved in pro- and eukaryotes

Inside-out membrane remodeling induced by the ESCRT core component ESCRT-III buds the membrane away from the cytosol, resulting in, for example, the appearance of MVBs [5]. Like other membrane-deforming systems such as BAR-domain [6] or dynamin-like proteins [7], it is believed that ESCRT-III proteins need to oligomerize to mediate membrane budding. Dynamic assembly of ESCRT-III filaments composed of several different subunits has been suggested to mediate inside-out membrane fission reactions, presumably by promoting negative curvature of the membrane [8,9] while being compatible with the topology away from the cytosol.

[1]Department of Chemistry, Biochemistry, Johannes Gutenberg University Mainz, Germany
[2]Ernst-Ruska Centre for Microscopy and Spectroscopy with Electrons, ER-C-3/Structural Biology, Forschungszentrum Jülich, 52425 Jülich, Germany
[3]Institute for Biological Information Processing/IBI-6 Cellular Structural Biology, Jülich, Germany
[4]Department of Biology, Heinrich Heine University, Universitätsstr. 1, 40225 Düsseldorf, Germany
[5]Department of Infectious Disease, Imperial College, London, UK
[6]Institute of Molecular Physiology, Johannes Gutenberg University Mainz, Mainz, Germany

*Correspondence:
Dirk.Schneider@uni-mainz.de
(D. Schneider).
@Twitter: @DSchneiderLab (D. Schneider), @SachseGroup (C. Sachse), and @thelowlab (H.H. Low).

Yet, experimentally it has been shown that ESCRT-III filaments can also spontaneously stabilize membrane tubes (i.e., membranes with positive curvature in an outside-in geometry) [10–13]. In fact, the tubulated membranes share similarities with membrane tubules formed by dynamins or dynamin-like proteins [7]. Owing to the high number of ESCRT-III isoforms in eukaryotes, homo- as well as hetero-oligomeric complexes have been identified and studied in recent years. Individual proteins form a variety of large macromolecular assemblies *in vitro*, including sheets, rings, filaments, tubules, domes, and spirals, some of which have also been identified in the archaeon *Sulfolobus acidocaldarius* [14–23]. However, the exact mechanism by which these assemblies mediate membrane remodeling is still being unraveled.

In a recent bioinformatic analysis, the bacterial proteins PspA and the IM30 (also known as the vesicle-inducing protein in plastids 1, Vipp1) were identified as eukaryotic ESCRT-III homologs [24]. This observation was supported by secondary structure predictions, as well as tertiary structure coevolutionary analysis. PspA and Vipp1/IM30 proteins are widely distributed across the bacterial kingdom with multiple copies often observed within genomes [24]. Out of a genomic dataset representing 99 bacterial phyla, 45% of the genomes encode at least one *pspA* gene, with up to eight *pspA* genes identified in the genome of *Aneurinibacillus tyrosinisolvens* [25] isolated from methane-rich seafloor sediments. The phylum cyanobacteria is typically rich in *pspA* and *vipp1/im30* genes, likely due to the requirement of maintaining their extended membrane network [24,26]. In addition, PspA is often positioned in the bacterial genome linked with conserved and diverse protein networks [25], indicating lineage-specific functional tuning [27,28]. In contrast to *pspA*, the *im30* gene is essential in cyanobacteria, most likely due to its crucial involvement in thylakoid-specific functions [27,29–34]. Intriguingly, PspA is prevalent in the archaea Methanosarcina, Haloarchaea, and, to a lesser extent, the TACK archaea [24]. However, these PspA forms, the functions of which are unknown, were acquired from bacteria via horizontal gene transfer rather than a common ancestor. In a phylogenetic analysis, the bacterial and archaeal/eukaryotic clades of ESRCT-III are separated by a long branch. On the archaeal/eukaryotic side, an early branch led to the two distinct classes of ESRCT-III proteins with eukaryotic Vps2/Vps24/Vps46 or Vps20/Vps32/Vps60 clustered together [24]. Homologs of these ESCRT-III proteins were previously shown to be present in the Asgard archaea, which represent some of the closest relatives to early eukaryotes [35,36]. Like eukaryotic ESCRT-III superfamily members, bacterial ESCRT-III-like proteins form large oligomeric super-complexes that bind and remodel membranes [24,27,37–43]. Thus, bacterial and eukaryotic ESCRT-III superfamily members not only share a common evolutionary heritage, likely dating to the last universal common ancestor of cells, but also appear to overlap in their functional activities.

In 2021, three cryo-electron microscopy structures of cyanobacterial proteins belonging to the PspA/IM30 protein family were determined independently [24,30,40]. The structures revealed that PspA and IM30 adopt a canonical ESCRT-III-like fold; thereby it has been confirmed that ESCRT-III proteins are indeed conserved in all three domains of life, including bacteria [44]. Although the amino acid (aa) sequence is not highly conserved between pro- and eukaryotic members of the ESCRT-III superfamily [24], the known structures show conserved secondary and tertiary structures of subunits within oligomeric assemblies. Yet, in the case of eukaryotic ESCRT-III monomers, and even more for the bacterial forms, the individual α-helical segments have not always been identified, annotated, and numbered consistently within and between different groups. Therefore, a unifying nomenclature is now needed.

The monomer structure of eukaryotic ESCRT-III proteins

Eukaryotic ESCRT-III proteins form hetero-oligomeric filaments of multiple subunits that appear to be metastable and dynamic. Depending on the species, more than four core ESCRT-III

subunits, in addition to isoforms, have been identified [45], which assemble into (active) hetero-oligomers [18]. The ESCRT-III subunits share a common core structure of five α-helices (Figure 1) and the stoichiometry of functional ESCRT-III proteins has been proposed [46]. However, given the structural and functional diversity in eukaryotes, it is likely that multiple stoichiometric ESCRT-III assemblies exist and thereby confer a specific function to the filamentous oligomer.

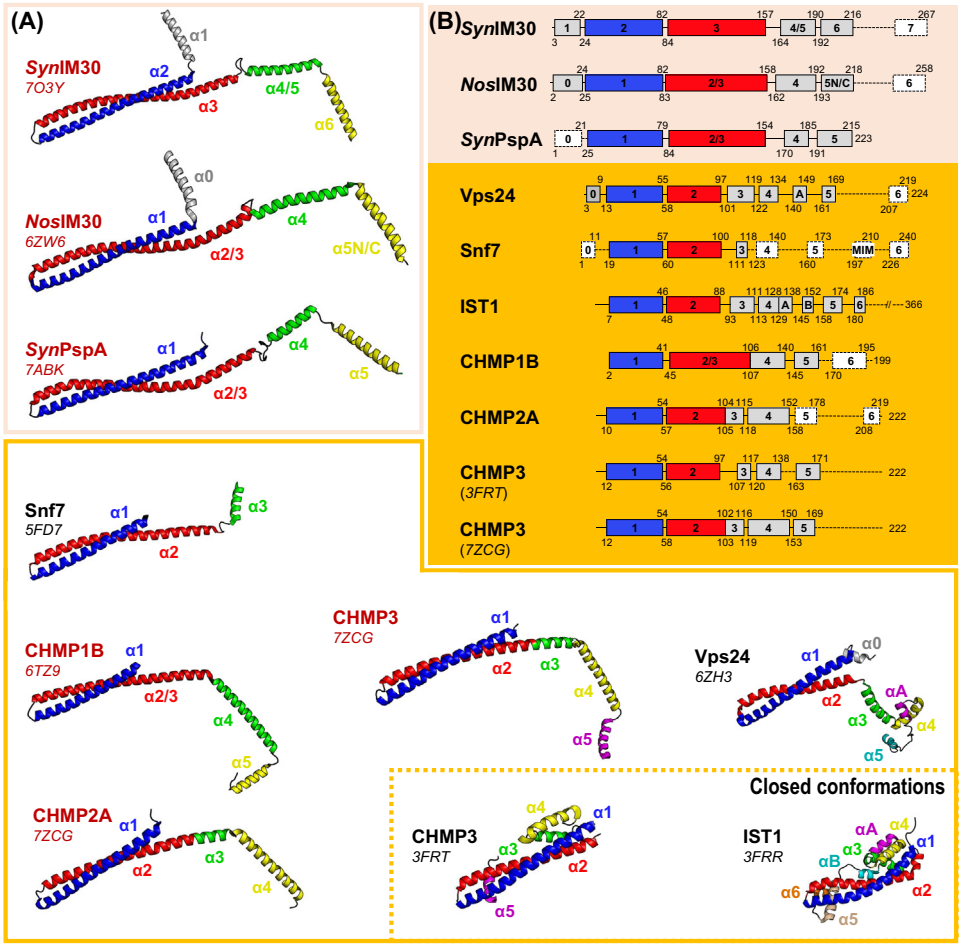

Figure 1. Monomer structure of pro- and eukaryotic endosomal sorting complex required for transport-III (ESCRT-III) superfamily members. (A) The structures of monomeric PspA/IM30 proteins of the cyanobacterium *Synechocystis* sp. PCC 6803 (*Syn*IM30, *Syn*PspA) or *Nostoc punctiforme* (*Nos*IM30) are shown together with eukaryotic ESCRT-III subunits. When the monomer structures have been extracted from oligomeric assemblies (compare Figure 3), the protein identifiers are given in red. The Protein Data Bank (PDB) codes are given below the protein identifiers. α-Helices are numbered as in the original publications and are thus not necessarily conclusive (Box 1). Eukaryotic ESCRT-III monomers with an allegedly closed conformation are especially highlighted. (B) Secondary structure α-helix assignment in pro- and eukaryotic ESCRT-III superfamily members. The two helices forming the core helical hairpin are colored in blue and red, respectively. All other α-helices that were observed in the resolved structures are in grey; predicted α-helices are in white. α-Helices are numbered as in the original publications. The starts and ends of the individual α-helices are given. Known bacterial ESCRT-III-like proteins have an N-terminally extended helix α5 that is absent in the eukaryotic systems [24]. Abbreviations: IM30, inner membrane-associated protein of 30 kDa; MIM, microtubule-interacting and trafficking (MIT)-interacting motif; PspA, phage shock protein A.

Over the past two decades, several structures of eukaryotic ESCRT-III proteins have been solved. In the X-ray crystal structure of a CHMP3 fragment (aa 9–183), five distinct α-helical segments were identified [47]. Similarly, also in the crystal structure of the *Saccharomyces cerevisiae* ESCRT-III protein IST1 N-terminal fragment (IST1: aa 1–193), five α-helices were detected [48]. Based on functional truncation experiments and the available structural data [49], it was suggested early on that the ESCRT-III monomers can adopt two conformations: In a closed conformation, the C terminus is thought to stabilize an autoinhibited, monomeric state via formation of intramolecular helix–helix contacts. Specifically, helix α5 has been observed to fold back against the helix α1/α2 helical hairpin core domain [20]. This conformation is represented, for example, by the IST1 (PDB ID: 3FRR) or the CHMP3 (PDB ID: 3FRT) structures (Figure 1).

During transition to the open conformation, helices α3 and α4 undergo significant structural rearrangements. Thus far, solely for human CHMP3, both conformations have been structurally determined [20,50]. In the open conformation, helix α4 of the ESCRT-III proteins CHMP1B (PDB ID: 6TZ9), CHMP2A (PDB ID: 7ZCG), and CHMP3 (PDB ID: 7ZCG) is stretched compared with the closed conformation represented by the IST1 and CHMP3 structures (Figure 1) and helix α3 forms a continuous extension of helix α2 (of the coiled-coil) so that helices α2 and α3 are not separated by a linker anymore. Due to the observation that helix α3 can be an independent α-helix, in most cases this region is also numbered independently in the open ESCRT-III conformation (i.e., where helix α3 is an extension of helix α2) [10,19,51,52]. Alternatively, this open extended conformation for helix α2 and helix α3 has also been numbered just as 'helix α2' [53] (Figure 1 and Boxes 1 and 2).

The N-terminal segment (aa 1–11) of the yeast ESCRT-III protein Snf7, which has not been resolved forming an α-helix in the previous structures, appears to be involved in ESCRT-III membrane anchoring, as has also been observed for human CHMP2A, CHMP2B, and CHMP3 [50,54] and, in fact, this region has been suggested to form a membrane-inserting amphipathic α-helix (i.e., helix α0, at least in case of Snf7) [55] (Boxes 1 and 2). Furthermore, some ESCRT-III proteins possess additional helices at the C terminus, which, however, appear not to be highly conserved as they host interaction motifs of downstream binding partners, such as the MIM motif bridging ESCRT-III to Vps4 [10,51,56].

In summary, eukaryotic ESCRT-III proteins share a structural core of five α-helical regions, some of which are not always independent α-helices (Box 1) and in some cases additional helices can be found at the N and/or C terminus (Figure 1).

Box 1. Inconsistent annotation and numbering of ESCRT-III helices

Despite the similarities in structure and function, in recent years helix annotation and numbering has not been consistent in the case of eukaryotic ESCRT-IIIs (compare Figure 1 in main text). The recent inclusion of PspA/IM30s as members of a common ESCRT-III superfamily requires a reconsideration of helix numbering. Especially in two cases, the annotations need to be unified:

1. While in eukaryotic ESCRT-IIIs, only in some cases does an additional helix at the N terminus appear to exist, which has been annotated as helix α0. This helix is conserved in prokaryotic PspA/IM30s, where it has typically been annotated as 'helix α1'. Thus, the core of all ESCRT-III superfamily members, the helical hairpin, is 'helix α1 and helix α2' in eukaryotic ESCRT-IIIs and 'helix α2 and helix α3' in prokaryotic counterparts.
2. In several structures of eukaryotic ESCRT-III monomers in the open conformation, where hairpin helix α3 is an extension of helix α2, the numbering of helices has not been consistent. Similarly, also in case of the bacterial counterparts, numbering has not been consistent and sometimes the extended hairpin helix is numbered as a single helix, which even resulted in numbering of the following, shorter helix as helix α4 and helix α5 (instead of just helix α4), whereas in other cases the extended hairpin helix is annotated as the two helical fragments helix α3 and helix α4 (compare Figure 1 in main text).

Box 2. Unifying helix numbering convention in the ESCRT-III superfamily

We now suggest transferring the annotation and nomenclature established for most eukaryotic ESCRT-IIIs to the bacterial proteins and numbering the conserved helices as helices α0–α5, as well as annotating the elongated, extended hairpin helix starting with helix α2 as helices α2 and α3.

1. While it is not necessarily self-explanatory to start the numbering of helices with 'helix α0', not all eukaryotic ESCRT-III proteins appear to have this extra helix at the N terminus (see Figure 1 in main text). Thus, in a cross-kingdom unifying nomenclature, one would either start with 'helix α0' in case of the bacterial and some eukaryotic ESCRT-IIIs, or with 'helix α2' in the case of eukaryotic ESCRT-IIIs that do not have the N-terminal helix. For the sake of unifying the nomenclature, we here suggest starting numbering with the optional 'helix α0'. This also implies that the hinge connecting helices α0 and α1 should be numbered 'Hinge 0' (see Figure 2 in main text), as this ensures that ESCRT-IIIs that lack helix α0 also start with 'Hinge 1'.
2. As outlined in the text, in some eukaryotic ESCRT-III subunit structures, the monomers have a so-called closed conformation, where part of the extended helix α2 (i.e., helix α3) is an extra helix that folds back to contact the hairpin motif formed by helices α1 and α2 (see Figure 1 in main text). While this region is clearly a continuous extension of helix α2 in other structures (see Figure 1 in main text), it definitely has a propensity to form a separated independent helix. Therefore, we suggest maintaining the established ESCRT-III nomenclature, assigning helices α2 and α3 separately, and to transfer this to bacterial PspA/IM30s. While this nomenclature does not properly reflect the organization of the helices resolved in the released structures [24,30,40], helices α2 and α3 are clearly separated by a flexible hinge region (see Figure 2 in main text). Moreover, assigning helix α2 and helix α3 separately unifies the annotations used within the entire ESCRT-III superfamily and at least considers the results of earlier predictions, which have indicated that this region has a propensity to form an independent helix also in prokaryotes, just as in the eukaryotic counterparts.

   The *Syn*PspA and *Nos*IM30 structures (see Figure 1 in main text) have already been annotated following the now proposed numbering.

The structure of bacterial PspA/IM30 monomers

Initial predictions had indicated the presence of four α-helices in the case of *Escherichia coli* PspA, which became a paradigm for PspA-like structures. The proposed four helices comprise most of the protein and have a high propensity to form coiled-coil structures [57,58]. Later on, the initial predictions were refined and for *E. coli* PspA six helices were proposed [59,60]. Additionally, the truncated *E. coli* structure of the conserved hairpin motif formed from helices α1 and α2, common to all PspA/IM30 proteins, was determined [59]. The recently solved full-length structures of two IM30 and one PspA [24,30,40] demonstrated that the structures of these bacterial proteins are similar, with an N-terminal core structure of approximately 220 aa consisting of five α-helices, termed the so-called 'PspA-domain' (Figure 1). Depending on the helical assignment and numbering, these five α-helices have been termed helices α0–5 or α1–6 (Figure 1 and Boxes 1 and 2). A major difference between PspA and IM30 is the presence of an additional C-terminal α-helix in IM30 that is connected to the PspA domain via an extended linker region [37,60–64]. Noteworthy, this extra helix has not been resolved in the recently published structures of IM30 oligomers [24,30]. In contrast to eukaryotic ESCRT-IIIs, prokaryotic proteins appear not to contain any additional domains or helices at the C terminus besides the IM30-specific helix α6 [25].

While in early predictions six and seven helices had been proposed for PspA and IM30 proteins, respectively, the recently solved structures revealed an IM30 monomer [24,30,40] where helix α2 and α3 (Box 2) form a single, continuous helix, as observed for eukaryotic ESCRT-IIIs in the open conformation (Figure 1). Yet, the IM30 monomer structure has a conformational flexibility provided by four hinge regions in the monomer, as illustrated in Figure 2 for a cyanobacterial IM30. In fact, the Hinge 1 region separates helix α2 from helix α3 and the angle between helix α2 and helix α3 increases with increasing sizes of IM30 rings [24,30]. This hinge is also found when comparing eukaryotic ESCRT-IIIs (e.g., CHMP1B and IST1) [52] and it allows eukaryotic ESCRT-IIIs to switch between an open and closed conformation, as observed for CHMP3 (Figure 1). Both PspA and IM30 monomers assemble to form large homo-oligomeric super-complexes, with ring as well as rod structures observed for both. While IM30 of (at least) cyanobacteria, *Arabidopsis thaliana*,

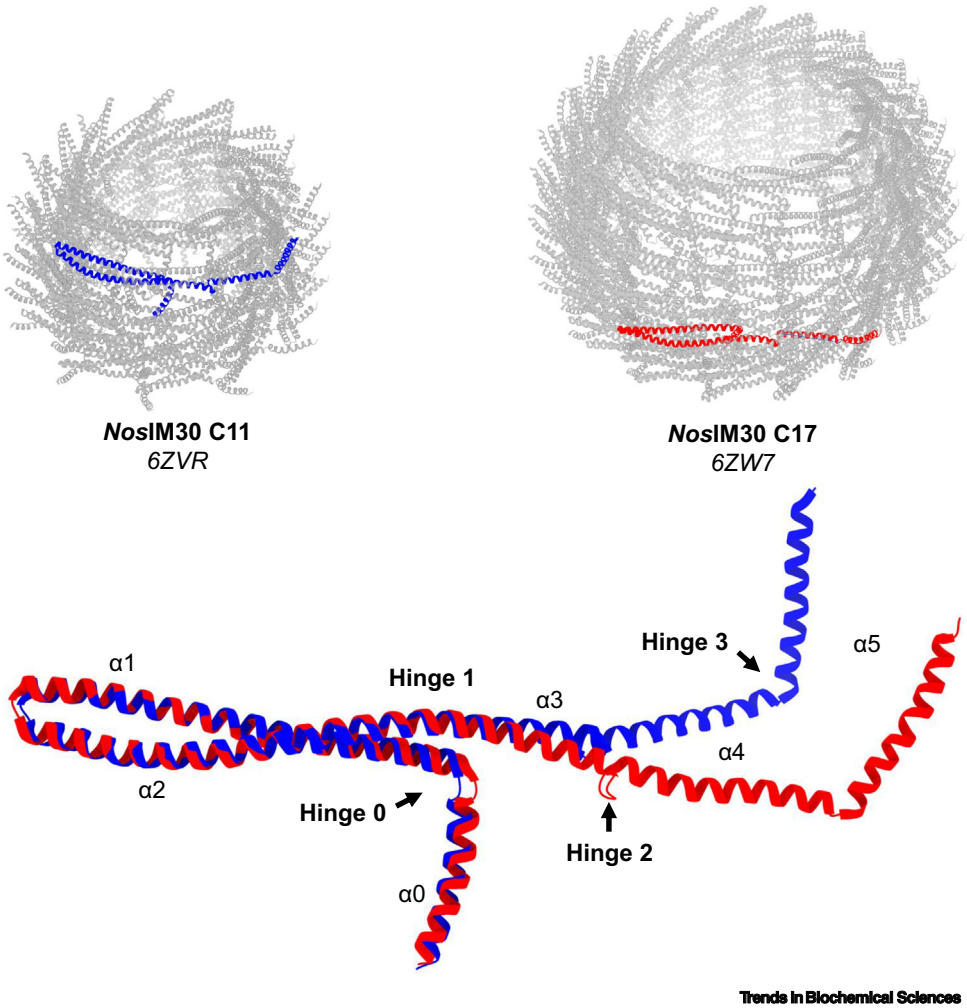

Figure 2. Flexible hinge regions in phage shock protein A (PspA)/inner membrane-associated protein of 30 kDa (IM30) proteins. Superimposition of *Nos*IM30 monomers from layer 2 (from top) of a C11 symmetric ring with the narrowest diameter (blue, PDB: 6ZVR) and the fifth layer of a C17 symmetric ring with the largest diameter (red, PDB: 6ZW7). Structures were aligned onto the helix α1/α2 hairpin. The exact position of Hinge 1 that separates helices α2 and α3 is ill defined. Note that the exact length and starting/ending of helices α4 and α5 vary depending on the ring size and the monomer position in a ring. See also Box 2.

and *Triticum urartu* appear to form ring structures [24,30,61,64–66], as does the *E. coli* PspA [67], IM30 of *Chlamydomonas reinhardtii* [38] and *Nostoc punctiforme* [24] form extended tubular rod structures, as does a cyanobacterial PspA [40] (Figure 3). Experimentally resolved structures of pro- and eukaryotic ESCRT-III proteins (*Syn*IM30, *Nos*IM30, *Syn*PspA, IST1/CHMP1B, CHMP2A/CHMP3) reveal assemblies of tubular structures with outer diameters between 180 and 500 Å, including a solvent-accessible lumen, except for Vps24 filaments [19].

Eukaryotic versus bacterial ESCRT-III oligomers

As discussed earlier, pro- and eukaryotic ESCRT-III proteins share common secondary, tertiary, as well as conserved elements in quaternary structures. Furthermore, ESCRT-III superfamily members appear to display a structural diversity, as they were found capable of forming structurally distinct oligomers, such as helical rods, rings, spirals, filaments, and cones [10,19,52,68,69].

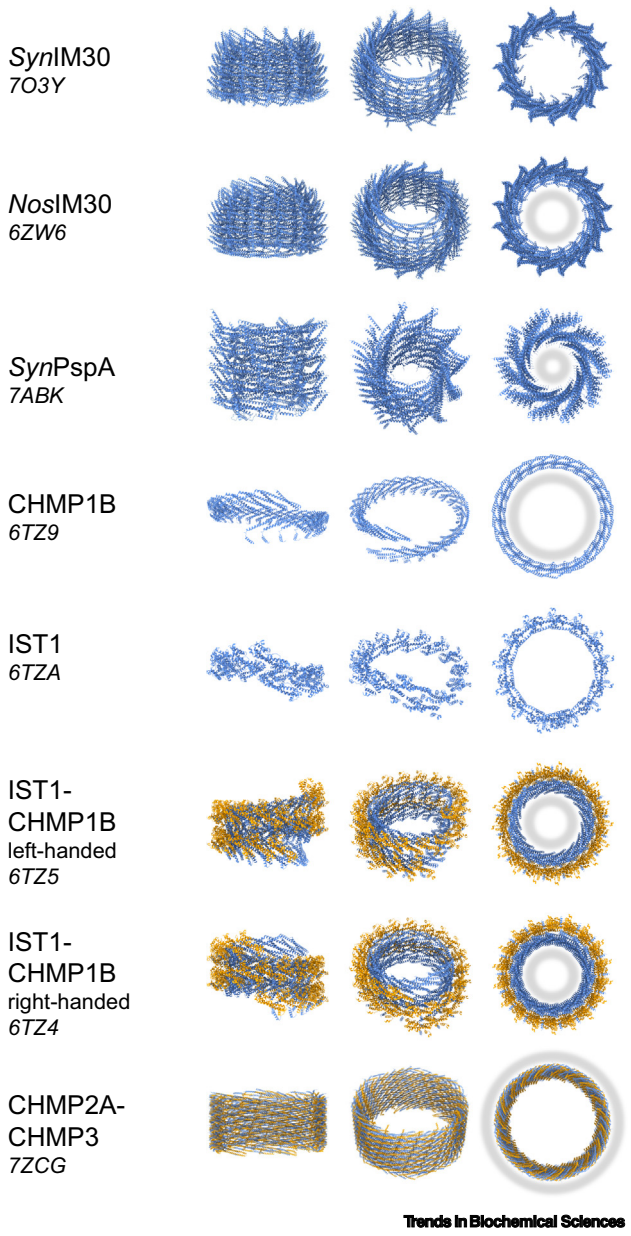

Figure 3. Assembly structures of pro- and eukaryotic endosomal sorting complex required for transport-III (ESCRT-III) super-complexes. Available oligomeric structures of proteins shown in Figure 1 are displayed. For IST1 and CHMP1B the homo-oligomeric rings are shown as well as hetero-oligomeric rings in a left- and right-handed conformation. In the IST1-CHMP1B structures, the IST1 subunits are displayed in orange and CHMP1B in blue. In CHMP2A-CHMP3 the CHMP2A subunit is shown in blue and CHMP3 in orange. When resolved together with the assembly structure, localization of membrane tubes relative to the ESCRT-III oligomers is indicated (grey). Note that CHMP2a/CHMP3 assemblies bind inside membrane tubes, whereas the bacterial ESCRT-IIIs and CHMP1B bind on the outside of membrane tubes.

Similarly, pro- as well as eukaryotic ESCRT-IIIs were observed to form multiple helical rod or ring structures with varying diameters, resulting in structural plasticity [10,19,24,30, 38,40,50–52,61,66].

While the physiological function of this structural plasticity is currently not clear, in the case of eukaryotic ESCRT-III, it has been suggested that sequential disassembly of ESCRT-III filaments is crucial for membrane scission [52]. In case of human IST1-CHMP1B oligomers, the two subunits adopt different structures and, when viewed in cross-section, occupy the outer and inner shell, respectively. While the IST1 subunits of the outer shell are in a closed conformation, in the inner

shell the CHMP1B subunits are in an open, almost entirely α-helical conformation (Figures 1 and 3). Yet, when these IST1-CHMP1B oligomers disassemble, also the monomeric CHMP1B subunits appear to adopt a closed conformation [10]. In contrast, the human CHMP2A-CHMP3 oligomer forms helical super-structures assembled from CHMP2A/CHMP3 heterodimers, where the respective monomers are in the open conformation and the filament forms via oligomerization of this repeating unit [50]. Noteworthy, while the basic structure of the open ESCRT-III conformation is conserved between pro- and eukaryotic ESCRT-IIIs (Figure 1), the position of individual helices, as well as monomer packing, can substantially differ, dictating the final structure of the filaments. In case of the CHMP2A-CHMP3 oligomer, monomer interactions result in a structure with a membrane-interacting outer surface [50], whereas in all other oligomers the membrane-interacting helix α0 lines the inside of the oligomeric assemblies (Figure 3).

For the prokaryotic ESCRT-III superfamily members, a 'closed' conformation of the monomers has not been observed thus far; rather, the monomeric IM30 proteins appear to be largely structurally disordered when not part of an oligomeric assembly [70]. Furthermore, while eukaryotic ESCRT-III oligomer formation involves the assembly of different ESCRT-III subunits, bacterial counterparts have thus far only been shown to form homo-oligomeric complexes and, thus, may represent more ancient ESCRT-III oligomers. However, many bacteria have multiple *pspA* and/or *im30* genes and, therefore, may have the potential to form hetero-oligomeric filaments, as well.

Interactions stabilizing pro- and eukaryotic ESCRT-III oligomers

Pro- and eukaryotic ESCRT-III monomers have been found to assemble into closed ring structures of rotational symmetry that stack to form large barrels, or long chains of helical symmetry that coil and make up rods and filaments. Formation and stabilization of oligomeric ESCRT-III assemblies is mediated by defined interactions between multiple protomers of one layer or helical turn, as well as between different layers (in case of IM30) or helical turns (in case of PspA and eukaryotic ESCRT-IIIs) (Figure 3).

Within an IM30 layer or the PspA filament forming the rod structures, subunit *n* contacts the neighboring subunits n^{-1}, n^{+1} plus n^{+3} (Figure 4, center). In tubular ESCRT-III assemblies with an inner lumen, except for filamentous Vps24 [19], parallel stacking of helix α1–α3 hairpins appears to be an evolutionary conserved and a general mode of ESCRT-III oligomerization [10,50], which is observed in bacterial PspA and IM30 [24,30,40] as well as in eukaryotic ESCRT-III filaments [10,19,50–52]. In fact, a helix α1–α3 fragment of the *Drosophila melanogaster* ESCRT-III protein *Shrub* forms large filaments already in solution, indicating that hairpin stacking is crucial for oligomerization, yet not for ring or rod formation, of ESCRT-IIIs [53].

However, hairpin stacking alone likely is not generally sufficient for oligomerization of pro- and eukaryotic ESCRT-IIIs. While the currently solved structures of ESCRT-III oligomers are unique in several aspects, contacts between helix α5 of one protomer and the helix α1/α2 hairpin of a neighboring protomer appear to be highly conserved and, for example, in human ESCRT-III filaments containing CHMP1B, CHMP2A, or CHMP3, helix α5 packs against the helix α1/α2 hairpin of subunit n^{+4} [10,50,52]. In the cyanobacterial PspA/IM30 proteins, the C-terminal region of helix α5 (helix α5C) of subunit *n* packs approximately perpendicularly against the helix α1/α2 hairpin tip of subunit n^{+3} (Figure 4, upper right) and, when helix α5 of PspA or IM30 proteins was removed, the shortened monomer did not form oligomeric assemblies anymore in solution [24,59,62,63,71]. Replacement of the PspA/IM30 proteins' highly conserved helix α1 residues at the tip of the helix α1/α2 helical hairpin affects the stability of PspA oligomers, as these residues are involved in interactions with helix α5 [40]. Moreover, helix α1 tip mutations even abolished

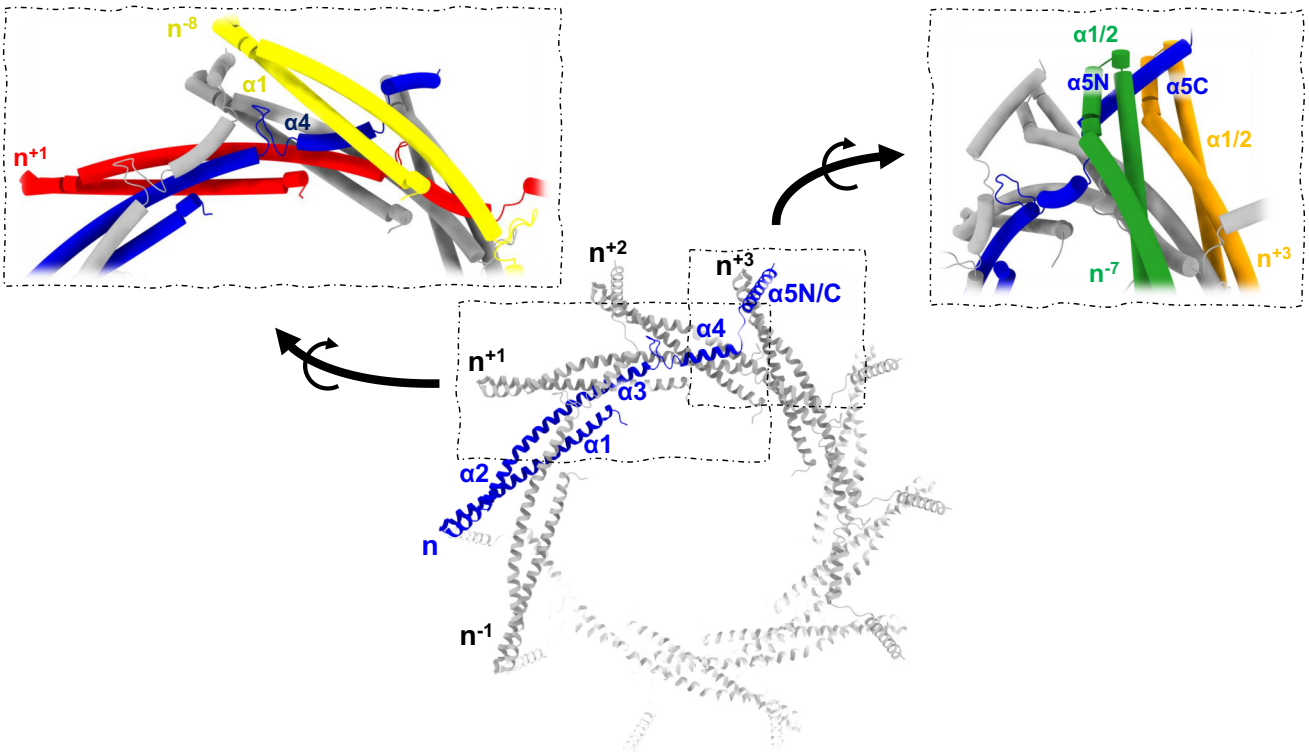

Figure 4. Interactions stabilizing oligomeric endosomal sorting complex required for transport-III (ESCRT-III) assemblies. Shown is one turn of the *Syn*PspA helical filament (center, PDB: 7ABK). Subunit *n* (blue) contacts the neighboring subunits n^{-1}, n^{+1} plus n^{+3}. The helix α1/α2 hairpins stack and the interface formed by helices α1–α3 of subunit *n* interact with the helix α1/α2 hairpin of the subunit n^{-1} and n^{+1}. Upper right: helix α5 of subunit *n* (blue) packs approximately perpendicularly against the helix α1/α2 hairpin tip of subunit n^{+3} (orange). In phage shock protein A (PspA)/inner membrane-associated protein of 30 kDa (IM30) oligomers, helix α5 is extended when compared with eukaryotic ESCRT-IIIs and the extra N-terminal part additionally interacts with the tip of a helix α1/α2 hairpin from an IM30 subunit of the layer above (green, n^{-7}). Upper left: helix α4 of protomer *n* (blue) and helix α2 of a protomer in a following layer (yellow, n^{-8}) interact and mediate stacking of individual IM30 layers. Note that the highlighted interactions repeat between every pair of neighboring subunits and the ones displayed here represent a selected subset for clarity of illustration.

formation of high-molecular mass IM30 oligomers [66]. Similarly, when conserved helix α1 residues of the human ESCRT-III proteins CHMP2A and CHMP3 were mutated, the monomers did not form oligomeric assemblies anymore [50]. Together, these findings highlight a particular role of the hairpin α1/α2-to-α5 interaction in oligomerization of pro- as well as eukaryotic ESCRT-IIIs.

In the bacterial PspA/IM30 oligomers, the N-terminal part of helix α5 that is absent in eukaryotic ESCRT-IIIs [24] additionally interacts with the tip of a helix α1/α2 hairpin from an IM30 subunit of the neighboring layer (Figure 4, upper right). Furthermore, interactions between helix α4 of one protomer and helix α1 of a protomer in a following layer appear to significantly stabilize the IM30 ring structure via mediating stacking of individual IM30 layers (Figure 4, upper left). In fact, helix α4 residues involved in this interface are conserved in PspA/IM30 proteins [40] and mutation of these residues abolished formation of IM30 oligomers [70,72]. A contact of helix α4 of one protomer with the helix α1/α2 hairpin of a neighboring protomer has also been observed within the human CHMP2A/CHMP3 hetero assembly [50].

Furthermore, helix α0 and the helix α1 N terminus of stacked layers interact and stabilize the oligomeric assembly of IM30. The N-terminally located helices α0 face the lumen of IM30 rings where they stack axially, orienting the hydrophobic side of this amphipathic helix towards the

ring lumen and thereby forming a large hydrophobic surface inside IM30 rings that is involved in membrane interaction [24,30]. While helices α0 clearly stack and establish contacts to the helix α1/α2 hairpin, mutation of individual helix α0 residues [30], as well as removal of the entire helix α0, does not abolish oligomerization of IM30 [62,63,71] or human CHMP2A and CHMP3 [18]. Thus, the α0–α1/α2 contact is not *per se* crucial for oligomerization of ESCRT-III members, in line with the observation that not all eukaryotic ESCRT-IIIs contain a helix α0. Yet, helix α0 interactions with the helix α1/α2 hairpin mediate tilting of each helix α0 via Hinge 0 (Figure 2 and Box 2), defining the curvature of the ring lumen and thus the exact geometry of PspA/IM30 oligomers and, upon removal of helix α0, IM30 forms rods rather than rings [71]. Finally, interactions between Hinge 2 (Figure 2) and helix α1 of two stacked layers may further mediate and stabilize stacking of individual IM30 layers [24].

Together, while multiple contacts stabilize bacterial ESCRT-III proteins, stacking of helix α1/α2 hairpins as well as interactions of a helix α1/α2 hairpin with helices α4 and α5, respectively, appear to be evolutionary conserved within the ESCRT-III superfamily. Nevertheless, the observed ESCRT-III quaternary structures differ significantly as the conserved interhelix contacts can originate from different protomers.

Concluding remarks

The recent discovery that bacterial proteins of the PspA/IM30 family adopt an ESCRT-III-like structure confirmed that members of an ancient ESCRT-III superfamily are present in all three domains of life. Despite low sequence conservation [24], the available structures of pro- and eukaryotic ESCRT-III superfamily members show conserved secondary structures when the subunits are part of an oligomeric assembly. Yet, in the case of eukaryotic ESCRT-III subunits, and even more for the bacterial forms, the individual α-helical segments have not always been identified, annotated, and numbered consistently within and between different groups (Box 1). Thus, we here propose a unifying nomenclature (Box 2). The tertiary structure of the subunits, as well as intra-subunit stabilizing contacts, are highly conserved in the superfamily, as are the inter-subunit interactions resulting in the formation of higher-ordered oligomeric ring or rod structures.

Recently, minimal features common to eukaryotic ESCRT-IIIs have been identified and were proposed to be required for their function: (i) spiral formation, (ii) lateral association, and (iii) binding to an AAA+ ATPase [73]. However, (i) the bacterial proteins have not yet been shown to form spirals; and (ii) for bacterial IM30s, an interacting AAA^+ ATPase has not been identified, although an inherent ATP and GTP hydrolysis activity has been observed in IM30 [30,74,75].

Based on the here discussed structures of pro- and eukaryotic ESCRT-III superfamily members, we have identified a set of minimal structural features common to all pro- and eukaryotic ESCRT-IIIs:

1. ESCRT-III superfamily members share five core α-helices (α1–α5).
2. The helical α1/α2 hairpin forms the structural core of ESCRT-III superfamily members.
3. ESCRT-IIIs assemble into oligomeric structures, rings, or rods, originating from filaments, and these structures are typically flexible, resulting in the formation of diverse assemblies composed of a single or diverse protomers.
4. Stacking of the helix α1/α2 hairpin is crucial for ESCRT-III oligomerization and filament formation, finally resulting in rings, when filaments close, or rods when oligomers form helical assemblies.
5. Interactions between helices α4 and α5 of one subunit and the helix α1/α2 hairpin of another subunit are crucial for the stability of ESCRT-III oligomers.
6. ESCRT-III oligomers interact with and remodel membranes.

Outstanding questions

What is the exact physiological function of bacterial ESCRT-III proteins?

How far do the *in vivo* activities of bacterial and eukaryotic ESCRT-III overlap? Can the bacterial ESCRT-III really serve as minimal model systems, also explaining the mechanisms and functions of ESCRT-III complexes in eukaryotes?

For some eukaryotic ESCRT-III monomers, a closed conformation has been observed *in vitro*. Bacterial ESCRT-IIIs appear to partly unfold upon ring disassembly. How relevant is the closed ESCRT-III conformation *in vivo* in bacteria and/or eukaryotes?

Heteromeric ESCRT-III assemblies are critical for the function in eukaryotes. Will hetero-ESCRT assemblies in bacteria be relevant and how could they affect the membrane remodeling?

In eukaryotes, ESCRT-0 to ESCRT-II proteins are involved in the recruitment of ESCRT-III machinery. How are bacterial ESCRT-III proteins targeted to the relevant membrane site and what is the trigger signal of assembly?

While AAA-ATPases are believed to be crucially involved in regulation of ESCRT-III complexes at membranes, these are not identified in all systems yet. How is the structure of ESCRT-III oligomers regulated without AAA-ATPases? All ESCRT-III superfamily members appear to be involved in membrane remodeling upon membrane binding. What is the structure of membrane-bound ESCRT-III? Which parts of the ESCRT-III core structure are involved in and necessary for membrane binding and remodeling?

For eukaryotic ESCRT-IIIs, membrane deformation and inwards as well as outward vesicle budding has been proposed. Are bacterial ESCRT-III superfamily members also involved in outward vesicle budding (i.e., the formation of extracellular vesicles)? And how do the proteins mediate this process?

While the structural features common to ESCRT-III oligomers are now well defined, the structure of membrane-bound prokaryotic ESCRT-III superfamily members still is enigmatic and formation of spirals, as formed by some eukaryotic ESCRT-IIIs, has not been observed yet. More structural and functional data are required to unravel the mechanism of ESCRT-III-mediated membrane remodeling in detail and to link the different ESCRT-III structures to function (see Outstanding questions). Furthermore, understanding the physiological importance of open and closed ESCRT-III monomer conformations and their extended intermediates in the fully assembled and active forms requires further structural investigation. Here, the prokaryotic superfamily members can serve as manageable minimal model systems, as these assemble from a single protomer type. While the exact physiological function of bacterial PspA/IM30 proteins is still unclear, results obtained with eukaryotic systems in the past may help to better understand the physiological function of ESCRT-IIIs in bacterial systems.

Author contributions

All authors wrote the manuscript.

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Declaration of interests

The authors declare no competing interests.

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