Trends in Biochemical Sciences

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Review

Cellular Functions and Molecular Mechanisms of the ESCRT Membrane-Scission Machinery

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The endosomal sorting complex required for transport (ESCRT) machinery is an assembly of protein subcomplexes (ESCRT I-III) that cooperate with the ATPase VPS4 to mediate scission of membrane necks from the inside. The ESCRT machinery has evolved as a multipurpose toolbox for mediating receptor sorting, membrane remodeling, and membrane scission, with ESCRT-III as the major membrane-remodeling component. Cellular membrane scission processes mediated by ESCRT-III include biogenesis of multivesicular endosomes, budding of enveloped viruses, cytokinetic abscission, neuron pruning, plasma membrane wound repair, nuclear pore quality control, nuclear envelope reformation, and nuclear envelope repair. We describe here the involvement of the ESCRT machinery in these processes and review current models for how ESCRT-III-containing multimeric filaments serve to mediate membrane remodeling and scission.

The ESCRT Machinery: Conserved Membrane Scissors

Cellular membranes are highly-dynamic entities that undergo continuous remodeling, fusion, budding, and fission events. The best-characterized type of membrane fission involves budding towards the cytosol, such as endocytosis. This is mediated by cytosolic protein complexes that assemble around the neck of the forming vesicle or tubule. Mechanisms driving membrane budding and fission with the opposite topology, away from the cytosol into the extracellular or luminal space, are less well known. We know that 'inverse' membrane dynamics is mediated by components of a multiprotein machinery known as the ESCRT. First identified for their role in vacuolar protein sorting in budding yeast [1], the ESCRTs constitute an evolutionarily conserved cytosolic membrane machinery that constricts and severs membranes from within the membrane neck; however, the precise mechanism remains elusive. Recent years have seen an explosion in cellular contexts exploiting the ESCRT machinery, currently including the formation of **multivesicular endosomes** (MVEs) (see Glossary) [1], **virus budding** [2], **cytokinetic abscission** [3,4], plasma membrane (PM) repair [5], **neuron pruning** [6], **exovesicle** shedding [7], **nuclear pore complex** (NPC) quality control [8], **nuclear envelope** (NE) reassembly [9,10], NE repair after rupture [11,12] and unconventional protein secretion [13] (Figure 1). Considering the rapid development of the ESCRT field, this list is unlikely to be exhaustive, although it should be noted that the evolutionary conservation of several of the newly identified ESCRT functions remains to be demonstrated.

Trends

The ESCRT machinery is an evolutionarily conserved machinery for scission of membrane necks from their interior.

The ESCRT machinery is a modular system consisting of three subcomplexes named ESCRT-I, -II, and -III. The first two complexes function mainly in protein sorting and in recruitment of ESCRT-III, together with Bro1-domain containing proteins. By contrast, the ESCRT-III complex coordinates the membrane-severing function.

The ESCRT machinery is recruited to sites of action by subfunction-specific targeting modules. These factors include ESCRT-0 (MVE formation), CEP55 (cytokinesis), and Gag (virus budding), that are able to associate with ESCRT components and Bro1-domain proteins.

ESCRT-III subunits assemble into helical filaments that mediate membrane deformation and scission, in cooperation with the ATPase VPS4.

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The ESCRT machinery consists of three biochemically distinct subcomplexes termed ESCRT-I, -II, and -III (Figures 2 and 3). ESCRT-III, together with the AAA ATPase **vacuolar protein-associated sorting 4** (VPS4), harbors the main membrane remodeling and scission function of the ESCRT machinery (Figure 4), whereas the 'earlier' ESCRTs function in a subset of cellular functions and mainly contribute to protein sorting and ESCRT-III recruitment through association with upstream targeting factors (Figure 3). Recruitment of ESCRT-III subunits to their site of action usually involves ESCRT-I and Bro1-domain proteins. These factors present parallel pathways to orchestrate the nucleation and polymerization of cytosolic ESCRT-III monomers into filaments that line the inside of bud necks. Activated ESCRT-III subunits utilize C-terminal motifs to recruit various effector molecules such as VPS4. In a process that crucially relies on VPS4 activity, remodeling of these filaments mediates membrane constriction and scission [14–18]. In this review we discuss the unique and common features of the various ESCRT functions, and focus on the molecular mechanisms that underlie ESCRT-III dynamics in membrane scission.

Diversity of ESCRT Functions in Membrane Scission

ESCRT-Mediated Endosomal Sorting and Intraluminal Vesicle Formation

The endocytic pathway ensures cellular homeostasis and controls cell-to-cell communication in a healthy organism by regulation of nutrient uptake, signaling through growth factors and cytokines, and degradation of transmembrane proteins and misfolded proteins [19]. Upon endocytosis, transmembrane cargo is sorted into **intraluminal vesicles** (ILVs) of endosomes, generating multivesicular endosomes (MVEs). The MVEs then fuse with lysosomes and their content is degraded by lysosomal hydrolases. ESCRT proteins have a dual role in this process, first by mediating the sequestration of cargo at the endosomal membrane, and then by inducing budding and scission of the endosome membrane away from the cytosol to form ILVs loaded with the sequestered cargo. Transmembrane proteins destined for lysosomal degradation are ubiquitinated, and ubiquitin is recognized by ESCRT-0, -I, and -II (Figure 3). The function of ESCRTs in endosomal sorting is briefly summarized in the following paragraph and extensively reviewed in [17,20].

Endosomal ESCRT activity is initiated by binding of the ESCRT-0 protein HRS to the endosomal lipid, phosphatidylinositol 3-phosphate (PI3P) (Figure 2). Together with the other ESCRT-0 subunit STAM and accessory proteins such as Eps15B, HRS binds to ubiquitinated cargo and to the coat protein clathrin, which concentrates ESCRT-0 in endosomal microdomains to facilitate cargo sorting. HRS harbors a PSAP motif that binds to the ESCRT-I subunit TSG101, thereby recruiting the heterotetrameric ESCRT-I complex. ESCRT-I can in turn recruit ESCRT-II, a Y-shaped heterotetramer composed of two EAP20 subunits, one EAP30, and one EAP45 subunit, probably through an interaction between VPS28 and the GLUE domain of EAP45. This GLUE domain also provides another PI3P- and ubiquitin-binding platform. The two EAP20 subunits of ESCRT-II directly interact with CHMP6 molecules [21]. In addition, a direct contact between ESCRT-I and ESCRT-III can occur through interactions between VPS28 and CHMP6 in the respective subcomplexes [22,23]. This generates a nucleation complex that drives the polymerization of ESCRT-III filaments that consist mainly of CHMP4, together with CHMP2 and CHMP3. ESCRT-III subunits interact with the endosomal membrane through clusters of basic residues in their core domain, **myristoylation** (CHMP6), or an N-terminal amphipathic helix (CHMP4) [21,24]. Recent work has indicated that VPS4 function extends beyond ESCRT-III recycling [25] to actively controlling neck constriction and vesicle scission [26].

Parallel to ESCRT-I and ESCRT-II, members of the Bro1-domain protein family contribute to MVE formation by recruiting, activating, or stabilizing ESCRT-III through their Bro1 domains [27–29]. In addition, several Bro1 family members have been shown to bind to ubiquitin and can associate with receptors [30–32]. Whereas ALIX plays a role in stress-induced sorting of

Glossary

Compartment for unconventional protein secretion (CUPS): in yeast, allows the secretion of proteins independently of the endoplasmic reticulum; consists of vesicles and tubules surrounded by a cup-shaped membrane.
Cytokinetic abscission: final step of cell division where the intercellular bridge connecting the two daughter cells is cleaved.
Ectosome: also known as a microvesicle, an extracellular vesicle derived by budding and shedding from the plasma membrane (PM) without endosomal involvement.
Exosome: 30–100 nm small extracellular vesicle; released from cells after fusion of ILV-containing MVEs with the PM.
Exovesicle: extracellular vesicle derived by budding and shedding from the PM with or without endosomal involvement.
Gag: acronym for group-specific antigen; one of three main retroviral proteins in addition to Pol (reverse transcriptase) and Env (envelope protein); Gag is a polyprotein precursor which is cleaved into four separate proteins: capsid protein, matrix protein, nucleocapsid protein, and p6. Together they form the viral core.
Intraluminal vesicle (ILV): a small vesicle inside an endosome; generated by invagination and abscission of a part of the limiting membrane of an endosome.
Late domains: domains consisting of highly conserved motifs in Gag proteins of various viruses which play a crucial role in pinching off virus particles from membranes. The term reflects their function late in the virus budding process.
Multivesicular endosome (MVE): also called the multivesicular body (MVB), the MVE is an endosome where intraluminal vesicles have been formed by invaginating and abscising parts of the endosome membrane, giving the endosome a multivesicular appearance.
Myristoylation: lipid modification of proteins where a myristoyl group, derived from myristic acid, is covalently attached to an N-terminal glycine residue by the enzyme N-myristoyltransferase; myristoylation can anchor a protein to membranes.
Neuron pruning: process by which dendrites and axons from neuronal

unliganded epidermal growth factor receptors (EGFRs) to a subpopulation of MVEs [33], HD-PTP is responsible for efficient degradation of internalized EGFRs after EGF stimulation. HD-PTP makes extensive contacts throughout the ESCRT machinery [32,34], and its binding to STAM and CHMP4B is mutually exclusive. Intriguingly, the switch from ESCRT-0 to ESCRT-III is regulated by recruitment of the de-ubiquitination enzyme UBPY to STAM, followed by deubiquitination of EGFR. Whereas HD-PTP is required for efficient sorting of EGFR into ILVs, it is dispensable for ILV formation [32] and its role in ESCRT-III dynamics remains unknown [35].

Although most transmembrane cargos follow the canonical ESCRT pathway, some cargos use alternative constellations of the ESCRT machinery. For example, virus-induced ubiquitination and degradation of MHC class 1 molecules requires HD-PTP to bridge ESCRT-I and ESCRT-III, whereas ESCRT-II and CHMP6 seem to be dispensable [36,37]. Another example is the growth hormone receptor, which requires TSG101 but not HRS [38]. Some cytosolic proteins can also be internalized into ILVs by a process known as endosomal microautophagy, which requires ESCRT-I, -III, and the molecular chaperone hsc70 [39]. The exact recruitment mechanisms of ESCRTs and cargos in these pathways remain to be elucidated. It also remains to be established how the ESCRTs contribute to bulk degradation of cytoplasm by macroautophagy [40].

ESCRTs in Virus Budding

A large variety of enveloped viruses (summarized in [41]) usurp the ESCRT machinery to bud from the PM of infected cells (Figure 1). This process is best studied for retroviruses and in particular for human immunodeficiency virus (HIV-1). Its structural **Gag** polyprotein localizes to the PM via myristoylation and a $PI(4,5)P_2$ binding site where it assembles into a virus particle. HIV-1 Gag then recruits the cellular ESCRT machinery via its **late domain** to enable scission of the virus particles from the PM (Figure 3). The HIV late domain contains two ESCRT binding motifs: a PTAP-like element that recruits TSG101 [42–44], and a YPXnL motif which interacts with ALIX [45–47]; however, the TSG101-dependent recruitment of ESCRTs seems to predominate in HIV-1 [48]. By contrast, equine infectious anemia virus (EIAV) lacks the TSG101 binding motif and exclusively buds via ALIX recruitment [45–47]. A third late-domain motif is the PPXY-type motif which recruits the ESCRT machinery via Nedd4-like HECT ubiquitin ligases and which is the predominant recruitment motif in murine leukemia virus (MLV) [49]. HIV, EIAV, and MLV budding is strictly dependent on CHMP2A, CHMP4B, and VPS4 [18,48–50], while CHMP1 and CHMP3 seem to be contributing factors [48,51–54]. CHMP6 and ESCRT-II were initially thought not to be required for virus budding [50,53], but more recent evidence supports a contribution of ESCRT-II to HIV-1 assembly [51,55]. Given that HIV-1 has two late domains, the ESCRT-II/CHMP6 recruitment arm may be hard to address by RNAi studies because the ALIX-dependent pathway can compensate for loss of the PTAP-like motif, at least when ALIX is overexpressed [56,57]. In any case it seems unlikely that ESCRT-II, but not the nucleation factor CHMP6, would be involved in virus budding.

ESCRTs in Cytokinesis and the Abscission Checkpoint

During the final steps of cell division, contraction of the actomyosin ring partitions the cytoplasm, resulting in the formation of an intercellular bridge connecting the two future daughter cells. Cytokinesis is completed by cleavage of this thin membrane bridge at the secondary ingression, a constriction zone that forms on either side of the electron-dense structure at the center of the bridge known as the midbody. ESCRT-III proteins are central to this scission process (Figure 1), and depletion or overexpression of dominant-negative alleles of VPS4, ALIX, ESCRT-I, ESCRT-II, or ESCRT-III subunits invariably leads to cytokinesis defects [3,4,29,58–61]. As cytokinesis progresses, the centrosomal protein of 55 kDa (CEP55) localizes to the midbody where it initiates abscission by recruitment of TSG101 and ALIX via recognition of their proline-rich domains [3,4,58,62–64] (Figure 3). In addition to mutual stabilization [3], ALIX and ESCRT-I constitute parallel pathways recruiting ESCRT-III subunits to the midbody [29,61], a process

cells are eliminated, resulting in fewer synaptic connections: a physiologic process thought to increase the efficiency of neuronal transmission; is also required for learning.

Nuclear envelope (NE): also known as the nuclear membrane, nucleolemma, or karyotheca; the NE is double lipid bilayer membrane which surrounds the nucleus in eukaryotic cells.

Nuclear pore complex (NPC): a protein complex forming a channel in the NE that mediates transport between the nucleoplasm and cytoplasm.

Vacuolar protein sorting-associated protein 4 (VPS4): an ATP-dependent enzyme of the AAA + protein family which catalyzes the dissociation of ESCRT proteins from membranes and contributes to the membrane remodeling function of ESCRTs.

Virus budding: enveloped viruses mature by budding at cellular membranes; thereby they acquire a host-derived membrane enriched in viral proteins to form their external envelope.

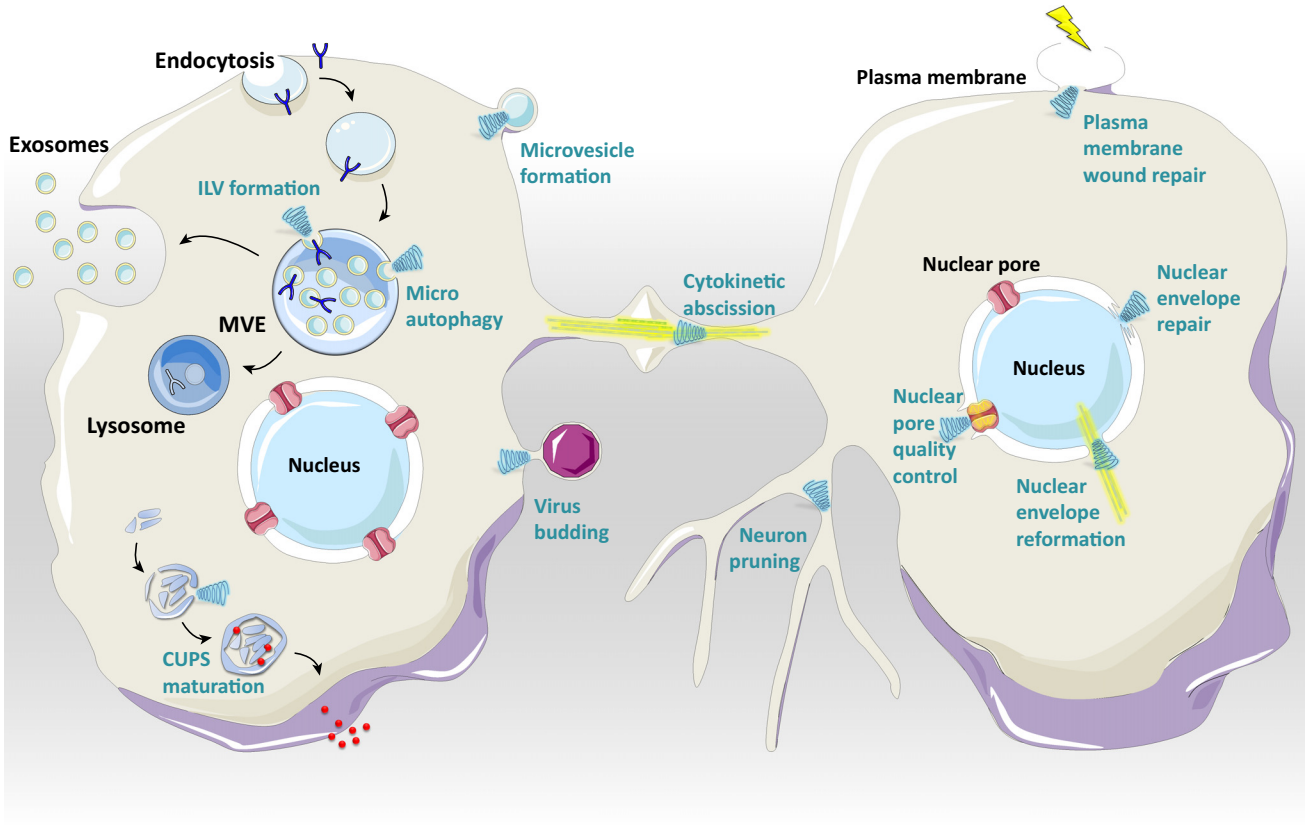

Figure 1. Overview of ESCRT-Dependent Processes. The ESCRT machinery functions in a variety of cellular contexts where it constricts membranes and severs narrow membrane necks from within the cytoplasm. Shown are ESCRT-dependent biological processes as identified to date, superimposed on a mammalian cell. The ESCRT-III machinery is indicated as a blue spiral. Abbreviations: CUPS, compartment for unconventional protein secretion; ESCRT, endosomal sorting complex required for transport; ILV, intraluminal vesicle; MVE, multivesicular endosome.

controlled by phosphorylation-induced conformational changes in ALIX [65]. Subsequently, ESCRT-III-dependent recruitment of the MT-severing AAA ATPase Spastin allows coordination of MT removal from the intercellular bridge with VPS4-controlled membrane scission [60,64,66–68]. It should be noted that ESCRT-III function at the intercellular bridge takes place at larger size dimensions (200–1000 nm) and a longer timescale (around 60 minutes) than other ESCRT functions, indicative of the high versatility of this protein machinery.

Abscission timing is influenced by chromosome segregation defects, intercellular bridge tension, cell adhesion, and NPC integrity [69–74], and is coordinated by multiple kinases. Through a mechanism known as the abscission checkpoint, the Aurora B and ULK3 (Unc-51-like kinase 3) kinases delay the completion of cytokinesis in the presence of chromosome bridges, high tension within the intercellular bridge, or defects in nuclear pore complex assembly [70,72,75–78]. In cells with chromatin permeating the intercellular bridge, persistent Aurora B activity stabilizes the ingressed furrow by phosphorylating the midbody protein MKLP1 [72] and delays abscission by phosphorylating the ESCRT-III subunit CHMP4C [79,80]. Together with the PI3P-binding protein ANCHR, phosphorylated CHMP4C associates with VPS4, which has been proposed to prevent VPS4 activity at the abscission zone [81]. ULK3 similarly phosphorylates CHMP4C as well as IST1 to delay abscission in response to chromosome bridges as well as high intercellular bridge tension [73]. Because phosphorylated IST1 tightly associates with VPS4 [82–85], it could further control

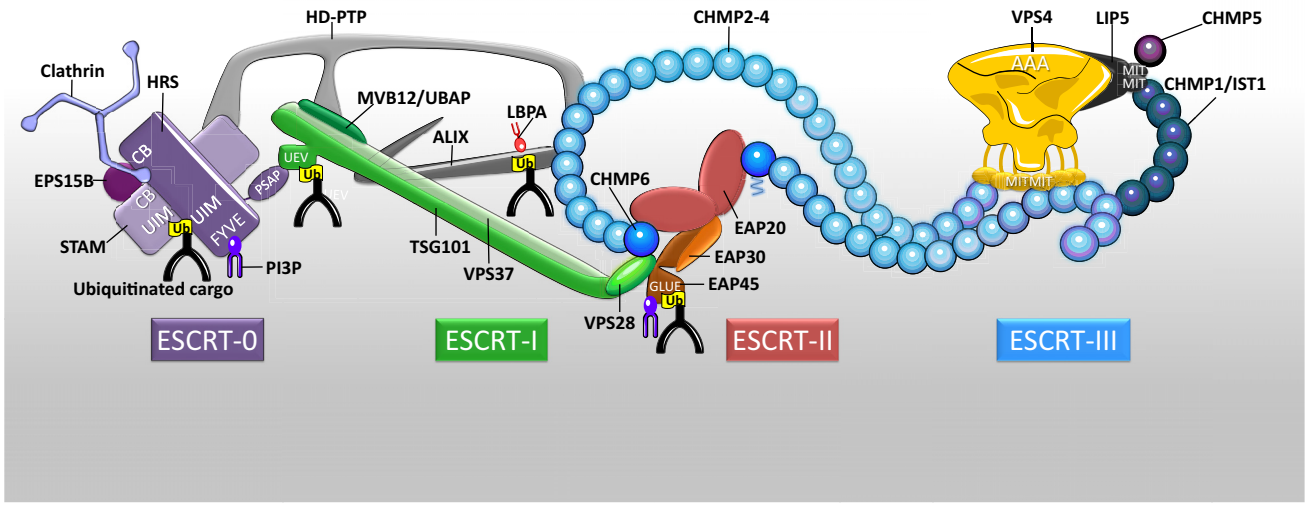

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Figure 2. Composition and Molecular Interactions of the ESCRT Machinery. Illustration of the ESCRT machinery highlighting interactions between different subunits, as well as with ubiquitinated (Ub) cargo, phosphatidylinositol 3-phosphate (PI3P), lyso(*bis*)phosphatidic acid (LBPA), and clathrin, with ESCRT-0 included as a model targeting factor. Note that the constitution and involvement of the complexes varies in different cellular processes. Deubiquitinating enzymes (DUBs) such as AMSH and UBPY, ubiquitin ligases, and the kinases Aurora B and ULK3 are known to interact with several ESCRT subunits. Abbreviations: CB, clathrin-box motif; FYVE, Fab1p/YOTB/Vac1p/EEA1 domain; GLUE, GRAM-like ubiquitin in EAP45 domain; MIT, microtubule interacting and transport domain; Ub, ubiquitin; UEV, ubiquitin-conjugated enzyme E2 variant; UIM, ubiquitin-interacting motif.

VPS4 activity during checkpoint signaling. This network of post-translational modifications may extend to other factors such as ALIX [65]. Whether this control mechanism is restricted to cytokinetic abscission or similarly acts on other ESCRT-III functions remains unknown. In budding yeast, the NoCut checkpoint [75,86] has been shown to control cytokinesis in the presence of chromatin bridges by a mechanism relying on the Aurora B homolog Ipl1p. However, the evolutionary similarities remain limited because ESCRT-III proteins have not been directly implicated in yeast cytokinesis or NoCut signaling.

Cell-to-cell Communication by ESCRT-Dependent Release of Extracellular Vesicles and Proteins

ESCRTs are implicated in two distinct processes that lead to cellular release of extracellular vesicles used for intercellular communication (Figure 1). One of these is the release of **exosomes**, small (30–100 nm) vesicles that are released from cells when ILV-containing MVEs fuse with the PM. The ESCRT machinery contributes to ILV formation and is consequently involved in exosome formation as well as the sorting of cargo into exosomes [87,88]. The other type of extracellular vesicles that are released in an ESCRT-dependent manner are known as microvesicles or **ectosomes**, small vesicles that are released from cells after budding of the PM away from the cytosol. This mechanism shares similarities with the shedding of PM wounds and virus budding.

Interestingly, intercellular signaling by the lipidated morphogen Hedgehog has been proposed to be mediated by both ESCRT-dependent exosomes [89] and ectosomes [90], the latter requiring ALIX, TSG101, EAP30, CHMP1/3/4, and VPS4. It remains to be established whether Hedgehog indeed uses two distinct extracellular carriers or whether the differential results can be explained by differences in assay protocols.

	Species			Interactions		
Targeting	Human	Budding yeast	Features	Proteins	Membrane	Ubiquitin
MVE (ESCRT-0)	HRS	Vps27p	VHS, UIM, GAT, Coiled coil, CB, FYVE, PTAP-like	TSG101, Clathrin	PI3P binding	UIM
	STAM1,2	Hse1p	VHS, UIM, GAT, Coiled coil, CB, SH3	HD-PTP, Clathrin		UIM
Virus budding	GAG		PTAP-like, YPXnL	TSG101, ALIX		
Cytokinesis	CEP55		Coiled coil	TSG101, ALIX		
PM repair	ALG-2		EF hand	ALIX, VPS37, Ca^{2+}		
Microvesicles	ARRDC1		Arrestin, PTAP-like	TSG101, ALIX		
Neuron pruning						
NE reformation						
NE repair						
NPC quality		Heh1,2p	LEM, MCHD	CHMP4	Transmembrane	
Bridging						
ESCRT-I	TSG101	Vps23p	UEV, PRD, Coiled coil	ALIX		UEV
	VPS28	Vps28p	CTD	EAP45, CHMP6		
	VPS37A-D	Vps37p	MOD(r)			
	MVB12A,B	MVB12p	MABP, UMA, UBD			UBD
	UBAP1		SOUBA	HD-PTP		SOUBA
ESCRT-II	EAP20	Vps25p	Winged helix,	CHMP6		
	EAP30	Vps22p	Winged helix			
	EAP45	Vps36p	Winged helix, GLUE	VPS28	PI3P binding	GLUE
Bro1 domain	ALIX	Bro1p	Bro1, Coiled coil, PRD, PTAP-like	CHMP4, TSG101	LBPA binding	Coiled coil
	HD-PTP		Bro1, Coiled coil, PRD, PTAP-like	CHMP4, TSG101, STAM, UBAP1		
	BROX		Bro1	CHMP4, CHMP5	Farnesylation	
		Rim20p	Bro1, Coiled coil	CHMP4		
Filaments						
ESCRT-III	CHMP1A,B	Did2p	MIM1	IST1, CHMP2, VPS4		
	CHMP2A,B	Vps2p	MIM1	CHMP3, VPS4		
	CHMP3	Vps24p	MIM1	CHMP2, 4		
	CHMP4A-C	Snf7p/Vps32p	MIM2, α0, Bro1 domain interacting helix	CHMP3, 6, 7, ALIX, VPS4	α0 insertion	
	CHMP5	Vps60p	MIM1	LIP5		
	CHMP6	Vps20p	MIM2	EAP20, VPS28, CHMP4, VPS4	Myristoylation	
	CHMP7	Chm7p	MIM1, MIM2	CHMP4		
	IST1	Ist1p	MIM1, MIM2	CHMP1, VPS4		
Remodeling						
	VPS4A,B	Vps4p	MIT, AAA+	CHMPs, LIP5, IST1		
	LIP5	Vta1p	MIT, VSL	VPS4, CHMPs, IST1		
	ANCHR		MIM1, FYVE, BBOX	VPS4	PI3P binding	
		Vfa1p	MIM2	VPS4		
	Aurora B	Ipl1p	Ser/Thr kinase	CHMP4		
	ULK3		MIT, Ser/Thr kinase	CHMPs, IST1		
	MITD1		MIT	CHMP1, CHMP2, IST1	PLD	

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Figure 3. Composition, Domains, and Interactions of the ESCRTs. Key components of the ESCRT machinery including nomenclature, domains, and binding features are shown. With exception of ESCRT-III, interactions between components within a complex are not indicated. The remodeling section includes factors that associate with ESCRT-III and affect its function. Aurora B binds to CHMP4 via Borealin. Please refer to the text for references. Abbreviations: α, α-helix; AAA + , ATPases associated with diverse cellular activities; BBOX, B-box-type zinc-finger domain; ALIX, ALG-2-interacting protein X; CB, clathrin-box motif; CHMP, charged multivesicular body protein; CTD, C-terminal domain; FYVE, Fab1p/YOTB/Vac1p/EEA1 domain; GAT, GGA/TOM1 domain; GLUE, GRAM-like ubiquitin in EAP45 domain; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; LBPA, lyso(*bis*)phosphatidic acid; LEM, Lap2/Emerin/MAN1 domain; MABP, MVB12-associated β-prism domain; MCHD, MAN1 C-terminal homology domain; MIM, MIT-interacting motif; MIT, microtubule interacting and transport domain; MOD(r), modifier of rudimentary domain; PI3P, phosphatidylinositol 3-phosphate; PLD, phospholipase D-like; PRD, proline-rich domain; SOUBA, solenoid of overlapping UBAs domain; STAM, signal transducing adaptor molecule; UBD, ubiquitin-binding domain; UEV, ubiquitin-conjugated enzyme E2 variant; UIM, ubiquitin-interacting motif; ULK3, Unc-51-like kinase 3; UMA, UBAP1/MVB12-associated domain; VHS, Vps27/HRS/STAM domain.

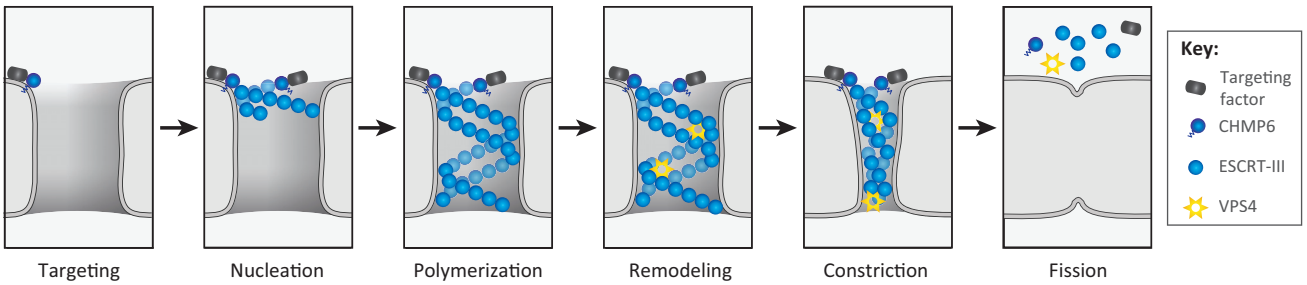

Figure 4. Model of ESCRT-III Function. Simplified model depicting ESCRT-III-mediated membrane neck constriction and fission. CHMP6 is targeted to membrane necks where it nucleates CHMP4 polymerization. Together with CHMP2, CHMP3, CHMP1, and IST1, CHMP4 forms membrane-bound filaments coiling around the invagination. Ultimately, VPS4-dependent ESCRT-III filament remodeling drives membrane constriction and fission. Other models for ESCRT-III mediated abscission are reviewed elsewhere [154–156].

ESCRT-mediated ectovesicle release has also been implicated in arrestin domain-containing protein 1 (ARRDC1)-mediated signaling and T cell receptor signaling. Arrestin proteins associate with G protein-coupled receptors on the PM and regulate their signaling [91]. Arrestin proteins interact directly with TSG101 through a PTAP-like motif, with ALIX through a YPXnL motif, and with several ubiquitin ligases through a PPXY motif [7,92–95]. At the immunological synapse, T cell receptors are secreted in ectovesicles that bud from the center of the synapse by a process that requires TSG101 and VPS4 [96]. This results in transcellular engagement of T cell receptors by neighboring antigen-presenting cells.

In budding yeast, ESCRT-I–III drives the 'unconventional secretion' (meaning that it follows another route than the canonical exocytic pathway) of the protein Acb1p via a double-membrane compartment called the **compartment for unconventional protein secretion** (CUPS) (Figure 1). Even though the biogenesis of this compartment requires ESCRT-III, Vps4p is not involved [13], which is surprising given the conserved role of VPS4 in ESCRT-III-mediated membrane remodeling and scission. The mechanisms of unconventional protein secretion in higher eukaryotes probably share some similarities, and recently ESCRT-0 and ESCRT-I have been implicated in unconventional secretion of interleukin-1β in mammalian macrophages [97].

PM Repair by the ESCRT Machinery

PM injury due to mechanical stress, pathological conditions, or pore-forming toxins generates lesions of different shapes and sizes and causes a massive toxic influx of Ca^{2+}. Consequently, rapid PM resealing occurs through patching, shedding, exocytosis, and endocytosis [98]. ESCRTs have been described to function in the removal of PM lesions after injury [5,99].

ESCRT-III recruitment to the lesion occurs between 30 seconds and 3 minutes after injury, followed by the repair of the wound within minutes after injury. The proposed mechanism, which is consistent with the topology of other known ESCRT-III functions, is that the ESCRT machinery is recruited close to the lesion to mediate shedding of an exovesicle that contains the lesion [5]. How is the ESCRT-III machinery recruited close to PM lesions? Neither CHMP6, nor ESCRT-0 or ESCRT-II, seem to be involved, but instead ALIX is necessary for the recruitment of TSG101 and the ESCRT-III machinery. ALIX directly binds to Ca^{2+} and the lysosomal lipid, lyso(*bis*)phosphatidic acid (LBPA) [100], and ALIX recruitment to the PM also requires the EF-hand Ca^{2+} sensor ALG-2 [99]. Fusion of lysosomes with the PM is another known mechanism to repair PM lesions [98]. This causes insertion of acid sphingomyelinases into the PM and leads to loss of PM tension [98,101]. It is possible that this might be coordinated

with ESCRT-mediated exovesicle budding, and trigger the recruitment of ALIX to the wounded PM, facilitated by Ca^{2+} and LBPA-binding [5].

ESCRTs in Neuron Pruning

During maturation of the developing nervous system of mammals and invertebrates axons and dendrites undergo major morphological changes, involving growth and subsequent elimination of neuronal processes, the latter process called 'neuron pruning'. Recent work has implicated the ESCRT machinery in this process through endolysosomal downregulation of the cell adhesion molecule Neuroglian [102] and the Hedgehog receptor Patched, which negatively control neuron pruning [103]. Such an indirect function of ESCRTs in neuron pruning is supported by its reliance on the endosomal ESCRT-0 complex. Remarkably, an RNAi screen in *Drosophila* neurons has implicated ESCRT-I (TSG101), ESCRT-III (Shrub), VPS4, Mop/HD-PTP, and UBPY directly in dendrite pruning [6]. An MVE-independent role directly in membrane scission is supported by clonal analysis highlighting the independence of ESCRT-0 and ESCRT-II, as well as the localization of ESCRT-III and upstream regulators to sites of pruning. Characterization of the targeting mechanism will be crucial to assess the direct and indirect contributions of ESCRTs to neuron pruning.

ESCRT-III Functions at the NE

With ESCRT functions firmly established at endosomes and the PM, a physiological role for ESCRT-III at the NE has only recently begun to emerge (Figure 1). Using budding yeast as a model system, Vps4p and Snf7p/Vps32p (budding yeast homolog of CHMP4) were shown to be recruited by the inner NE LEM-domain proteins Heh1p and Heh2p to regulate the removal of improperly assembled nuclear pore complexes (NPCs). Although the exact mechanism of action remains to be elucidated, several models have been proposed [8,104,105]. These include ESCRT-III-mediated budding of NPC vesicles into the perinuclear space, analogous to ESCRT-III involvement in nuclear egress of Epstein–Barr virus [106].

ESCRT-III also plays a crucial role in the sealing of membrane annuli during NE reformation upon mitotic exit [9,10]. Failure to seal annuli results in compromised nucleocytoplasmic compartmentalization and accumulation of DNA damage at the nuclear periphery. This function exploits most ESCRT-III subunits, VPS4, and Spastin [10] to sever impeding spindle MTs at sites of NE sealing. However, none of the canonical upstream targeting modules, or bridging or nucleation factors, appear to be involved. Instead, the UFD1 subunit of the p97 AAA ATPase [9] and the poorly characterized ESCRT-III subunit CHMP7 [10] have been put forward as ESCRT-III recruitment factors.

Extending these functions beyond cell division, recent elegant work has shown that ESCRT-III also regulates the repair of interphase NE lesions that arise upon nuclear deformation in migrating cells [11,12]. Importantly, this function similarly relies on CHMP7 because its depletion delays NE resealing. Considering CHMP7 domain architecture and the apparent absence of CHMP6 at the NE, it is tempting to speculate that CHMP7 functions as an NE-specific ESCRT-III nucleator complex. Indeed, recent work has confirmed an essential role for LEM-domain proteins in recruiting CHMP7 to the NE (bioRxiv.org 10.1101/049312, 10.1101/049148, and 10.1101/049221). Although the exact mechanisms remain to be resolved, these observations make a strong case for CHMP7 as an evolutionarily conserved module that targets ESCRT-III to the NE.

Molecular Mechanisms of ESCRT-III Functions

Whereas much is known about the cell biological processes requiring the ESCRT machinery (Figure 1), our mechanistic understanding of how its subunits function in cells is poorly understood and largely relies on *in vitro* studies. The ESCRT-III components are small proteins (~200 aa) sharing a core domain composed of a positively charged four-helical bundle. The two longest

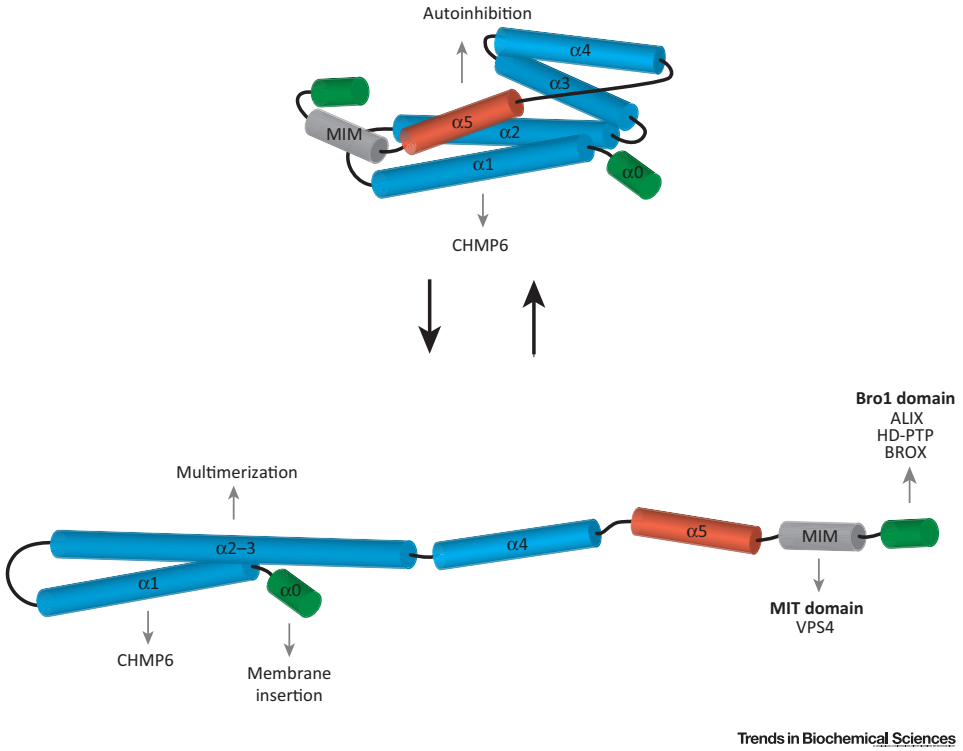

Figure 5. Dynamic CHMP4B Structure. Schematic representation of CHMP4B cycling between an inactive, closed and an activated, open conformation enabling multimerization and interaction with effectors. In crystals helix α4 has been shown to adopt different orientations [28]. Abbreviations: MIM, MIT-interacting motif; MIT, microtubule interacting and transport domain.

helices α1 and α2 are important for membrane binding and dimerization [24,107,108], and form a flexible helical hairpin [109] that packs against the smaller helices α3 and α4 to form a bundled core [24,107,108]. C-terminal to this core domain reside several regulatory elements, including the autoinhibitory α-helix 5 that can fold across the positively charged N-terminal core, thereby inhibiting protein function (Figure 5) [110]. In addition, the C-terminus contains one or two MIM elements [MIT (microtubule interacting and transport)-interacting motif] [111]. These are helical (MIM1) or extended conformation (MIM2) elements that mediate interaction with MIT domain-containing effectors such as VPS4 [112,113], its activator LIP5 [114], MITD1 [115,116], the microtubule-severing enzyme Spastin [67], and the deubiquitinating enzymes UBPY and AMSH [117]. The MIM2-containing CHMP4 isoforms uniquely employ their α-helix 6 to interact with Bro1 protein family members (ALIX, HD-PTP, BROX) [118].

ESCRT-III subunits cycle between an inactive cytoplasmic monomer and an active open state capable of binding to other ESCRT-III subunits and MIT-domain proteins [107,110,111,119–123]. This involves conformational changes including displacement of the inhibitory C-terminus, and extension of the α2 and α3 helices into a single 90 Å helix [28,110,123,124]. These rearrangements expose extensive electrostatic and hydrophobic interaction surfaces important for inter-subunit interaction and association with the lipid bilayer [28,50,123]. In crystal structures of 'open' ESCRT-III subunits, α4 was found in two conformations, either interacting with neighboring subunits within the same protofilament, or interacting in *trans* with neighboring protofilaments [28,123]. Because several ESCRT-III subunits have evolutionarily conserved proline residues in their α3–α4 linker, it will be interesting to see whether this imposes conformational constraints that could translate to functional differences between ESCRT-III subunits during filament maturation [125].

ESCRT-III Targeting and Nucleation

Classically, ESCRT-II binding and membrane association activate the myristoylated ESCRT-III subunit CHMP6, which then initiates ESCRT-III filament assembly by recruiting CHMP4B, the main ESCRT-III component [21,125–130]. CHMP6 is capable of inducing conformational changes within CHMP4B that promote the movement of the inhibitory helix away from the core, thereby exposing and enabling the CHMP4B core regions to interact with other ESCRT-III subunits [125,127]. Both the presence of ESCRT-II and CHMP6, and the negative membrane curvature inside membrane necks, accelerate this nucleation step [131]. Interestingly, CHMP4B can also be recruited via Bro1-domain proteins [29,35,56,57] which constitute a second parallel pathway amending the classical ESCRT-I–ESCRT-II–CHMP6 axis. However, it is not clear whether Bro1-domain proteins directly activate CHMP4B, increase the local concentration of available CHMP4B, or function as filament stabilizers or scaffolding proteins [27,35,132]. As described above, in some ESCRT-mediated processes CHMP4B recruitment relies on CHMP7 [10,12]. Even though the CHMP7 domain architecture resembles that of ESCRT-II–CHMP6 [133], with an ESCRT-II-like winged helix and a CHMP6-like ESCRT-III core domain, it remains to be elucidated whether it is capable of nucleating CHMP4B filaments.

ESCRT-III Filament Assembly and Membrane Contribution

Remarkably, how ESCRT-III filaments contribute to membrane remodeling in cells is still a mystery. All ESCRT-III subunits extensively interact with membranes through their basic clusters on their core domain, and several ESCRT-III subunits contain an additional N-terminal amphipathic helix (α0) that inserts into regions of positive membrane curvature. Whether this helix plays a role in inducing positive curvature in forming membrane necks or contributes to positioning of ESCRT-III filaments during the deformation and scission process remains unknown. However, the fact that membrane anchors that do not impose membrane curvature can rescue Snf7p α0 deletion argues against a prominent role in membrane deformation [24]. Classically, ESCRT-III filaments are thought to line the inside of membrane necks with their membrane interacting surfaces facing outwards, and as such mostly associate with regions of negative membrane curvature. Unexpectedly, recent work has shown that IST1 and CHMP1B can co-assemble into helical structures that coat regions of positive curvature on tubules protruding into the cytoplasm [123]. Further investigations will be essential to understand this remarkable difference in topology between structurally similar ESCRT-III family members.

Activated ESCRT-III assembles into highly-flexible spiral protofilaments, 4 nm in width, that are stabilized through membrane anchorage and subunit interactions within filaments, and lateral interactions between filaments [24,28,108,109,123,125,131,134–139]. Furthermore, activated CHMP4 can recruit downstream ESCRT-III subunits such as CHMP2, CHMP3, CHMP1, and IST1 that have been shown to individually polymerize into filaments *in vitro* [108,123,125,135,137]. Association of CHMP2 and CHMP3 has been shown to alter the architecture of preformed CHMP4 filaments [125,127], but whether they function by capping these filaments to form heterotypic assemblies, or whether they form parallel homotypic assemblies as described for other ESCRT-III proteins [123] is unknown. More recently, *in vitro*, Snf7p filaments assembling on supported lipid membranes were shown to form compressed spiral springs that release their energy to deform membranes. In this setting lateral compression is provided artificially by neighboring filaments, and it will be interesting to see whether such constraints exist in cellular settings.

It should be kept in mind that many ESCRT-III functions do not assemble on flat membranes but instead line pre-existing membrane necks, making it unclear when such spiral spring relaxation comes into play [135,140]. Instead, 17 nm spiral filaments have been identified in the intercellular bridge by electron microscopy [60,141]. If these are indeed ESCRT-III assemblies, the thickness of each filament would require it to be composed of parallel bundles of filaments. Confirmation of

such parallel assemblies would have far-reaching implications for ESCRT function and membrane constriction.

The Role of VPS4 in ESCRT-III-Dependent Processes

ESCRT-III processes crucially rely on the activity of VPS4 that provides the only known ATPase input in the membrane-scission reaction [18,142]. VPS4 rapidly depolymerizes ESCRT-III filaments by unfolding and threading individual subunits through its central pore [143]. This function is essential to recycle inherently stable ESCRT-III filaments and ensure high cytosolic levels of ESCRT-III monomers. Accordingly, depletion or expression of dominant-negative VPS4 rapidly depletes the soluble ESCRT-III pool [53]. Whereas it was originally thought to be solely required for post-scission recycling [25,54,144], recent evidence points towards an important role for VPS4 in ESCRT-III remodeling during the pre-scission stages [26].

VPS4 activation is a complex process involving multimerization into ATP-bound asymmetric hexameric rings [145,146]. Higher-order assemblies have been reported but their physiological relevance remains to be explored [147,148]. In addition to multimerization, VPS4 ATPase activity is controlled by association of cofactors. Association of LIP5 and its cofactor CHMP5 stimulates oligomerization and leads to a strong increase in VPS4 activity [149]. Furthermore, several other ESCRT-III subunits associate with the MIT domains of LIP5 or VPS4 to further stimulate VPS4 activity [84,114,150]. Because hierarchical recruitment of ESCRT-III subunits has been described [10,151], this could couple maturation of ESCRT-III filaments to transitions in VPS4 activity [150]. Resolving whether and how VPS4 activity and function is coordinated with ESCRT-III remodeling represents one of the major questions in the ESCRT field.

Concluding Remarks and Future Perspectives

With the recent realization that ESCRT-mediated membrane deformation and scission are crucial for multiple cellular processes (Figure 1), it is evident that we need to learn more about how the ESCRT machinery functions (see Outstanding Questions). Largely thanks to yeast genetics, we currently have a relatively good inventory of the various ESCRT subunits and accessory proteins (Figure 3), but for most ESCRT-dependent processes we do not have a complete picture of how ESCRT components are recruited or of how they deform and cut membranes. In the cases of MVE sorting/biogenesis and cytokinesis, the key upstream components that recruit and activate ESCRT-III are known, but this is not the case for the more recently discovered ESCRT functions. Moreover, the mechanisms by which ESCRT proteins cause membrane deformation and scission currently remain at the speculative stage. Both ESCRT-I/-II and single-subunit ESCRT-III helices are capable of achieving membrane deformation in model membranes [135,152], but it remains unclear how ESCRT-mediated membrane deformation is achieved *in vivo*. Even more enigmatic is the mechanism of ESCRT-III-mediated membrane scission, for which there are currently no firm experimental data. Recent data strongly imply the ATPase VPS4 in both membrane remodeling and scission, but its actual functional mechanism needs to be clarified. These are challenging issues to address, and will require clever combinations of biophysical, biochemical, and cell biological approaches.

Given the nearly ubiquitous involvement of ESCRTs in membrane dynamics, and the importance of membrane dynamics for human health, one would expect to observe serious pathophysiological consequences of genetic aberrations that affect ESCRT functions. Indeed, ESCRT dysfunctions are associated with both inherited diseases and cancers, and an increasing range of pathogenic viruses and microorganisms are known to co-opt the ESCRT machinery [153]. Thus, even though ESCRT subunits are hardly suited as drug targets by themselves, identifying pathway-specific ESCRT regulators may provide us with novel drugs for cancers and infectious diseases.

Outstanding Questions

How is ubiquitinated cargo transferred between ESCRT-0, -I, and -II?

How are individual ESCRT subunits regulated by post-translational modifications?

How is ESCRT-III recruited to mediate diverse functions such as neuron pruning, PM repair, and NE sealing?

How does the ESCRT machinery sculpt membranes to form membrane invaginations filled with cytosol?

How do ESCRT-III filaments cut membrane tubes to achieve cytokinetic abscission, nuclear envelope sealing, and ILV formation?

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