Shaping development with ESCRTs

Tor Erik Rusten, Thomas Vaccari and Harald Stenmark

Originally identified for their involvement in endosomal sorting and multivesicular endosome (MVE) biogenesis, components of the endosomal sorting complex required for transport (ESCRT) are now known to control additional cellular functions such as receptor signalling, cytokinesis, autophagy, polarity, migration, miRNA activity and mRNA transport. The diverse cell biological functions of ESCRT proteins are translated into a pleiotropic set of developmental trajectories that reflect the wide repertoire of these evolutionarily conserved proteins.

The ESCRT machinery was identified by a combination of yeast genetics and biochemistry for its crucial role in sorting ubiquitinated membrane proteins into the lumen of the lysosome-like vacuole[1–3]. A decade after its initial discovery, this machinery is receiving more attention than ever. There are three main reasons for its popularity: first, parts of the ESCRT machinery are well-conserved among eukaryotic lineages and have ancestral forms in Archaea[4]; second, certain components are involved in cellular functions in addition to protein sorting; third, the ESCRT machinery is important for development and tissue homeostasis, as highlighted by the multiple phenotypes and corresponding diseases associated with loss of ESCRT function. Here, we review the various cellular processes in which ESCRTs participate and discuss the consequences of these engagements in developmental processes.

Composition of the ESCRT machinery

Because the composition of the ESCRT machinery has been described extensively in recent reviews[5–9], it will only be summarized briefly here. The ESCRT machinery consists of four biochemically distinct protein complexes, termed ESCRT-0, -I, -II and -III, according to their chronological involvement in sorting cargo into MVEs (Fig. 1). ESCRT-0, -I and -II contain ubiquitin-binding subunits and function in the recognition and sorting of ubiquitinated cargoes. In addition, ESCRT-I and -II cooperate to form invaginations of the endosomal membrane (Fig. 1)[10]. ESCRT-III recruits deubiquitinating enzymes that ensure conjugated ubiquitin molecules are released before cargo sequestration into MVEs, thereby preventing degradation of ubiquitin[11]. The key function of ESCRT-III, however, is to form spiral-shaped oligomers that constrict to sever the neck of the forming intraluminal vesicle (ILV; Fig. 1)[6,12]. Finally, the ATPase VPS4 is recruited to dissociate ESCRT-III oligomers so that the monomeric subunits can be used in several rounds of ILV budding[6,12,13]. It is important to note that the budding reaction catalysed by the ESCRT machinery has reversed topology when compared with most other budding processes in the cell, such as endocytosis and the formation of transport vesicles.

Signal transduction is regulated by ESCRT-mediated endosomal sorting

Signalling proteins represent an important class of molecules sorted by the ESCRT machinery in metazoans. *Caenorhabditis elegans*, *Drosophila melanogaster*, mouse and human cells mutant for ESCRT-0, -I, -II or -III components or VPS4 possess enlarged endosomes with few ILVs, and fail to sort and degrade components of multiple conserved signalling pathways. These include receptor tyrosine kinases such as the epidermal growth factor receptor and poliovirus receptor, transforming growth factor β receptors, G-protein-coupled receptors, Hedgehog receptors, Notch and its ligand Delta, Wnt receptors, integrins, the T-cell receptor and Toll receptors[14–29]. This evidence points to a widespread requirement of ESCRTs in the degradation of signalling receptors across the animal kingdom.

Indeed, mutations in ESCRT result in prominent alterations of signalling. In most instances, receptor accumulation in ESCRT-mutant cells upregulates signalling, probably as a direct consequence of impaired downregulation of active receptors. However, Notch ectopic signalling in ESCRT-I, -II and -III mutant cells has been shown to be mostly ligand-independent[30]. In addition, although excessive receptor tyrosine kinase signalling in *hrs* (a subunit of ESCRT-0) mutant *Drosophila* embryos correlates with multiple developmental aberrations at stages when epidermal growth factor receptor signalling is required, *hrs-stam* mutant *Drosophila* tracheal cells, which fail to undergo correct morphogenesis, display reduced fibroblast growth factor receptor signalling[18]. Given the diverse mechanisms of signalling activation across multiple, even closely related, pathways, a major future challenge will be understanding how the ESCRT machinery interacts with different receptors and associated signalling molecules in different contexts.

Although most data point to a function for ESCRTs in signal attenuation, an unexpected positive role in cell signalling was also recently identified in the Wnt pathway. The ultimate consequence of Wnt signalling is the stabilization of cytosolic β-catenin, culminating in its nuclear accumulation and activation of target-gene transcription. In

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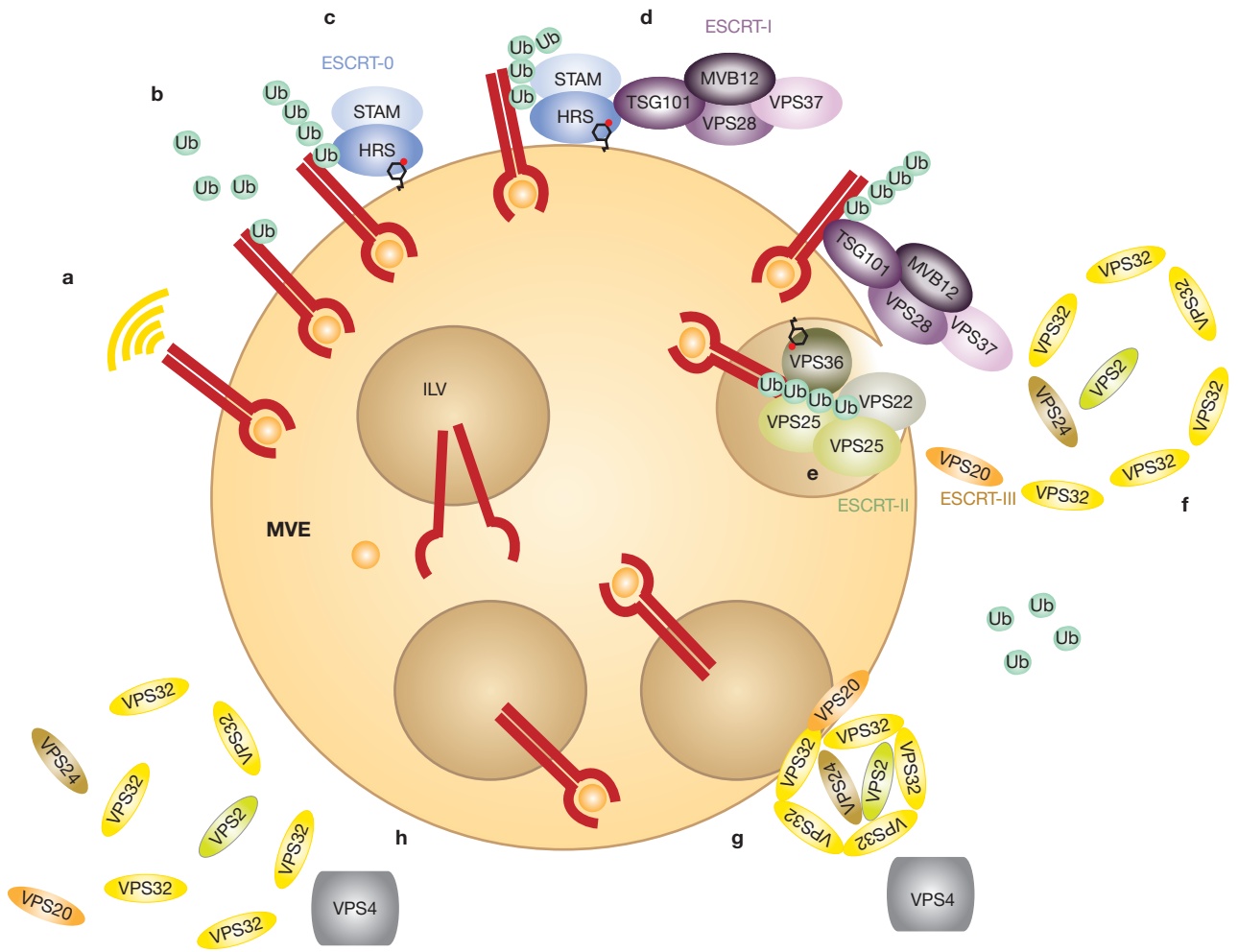

Figure 1 The ESCRT machinery in endosomal sorting and MVE biogenesis. (**a**) Receptors (dark red) in the limiting membrane of the MVE are capable of signalling (yellow signal bars). (**b**) Many receptors are ubiquitinated in response to ligand binding. (**c**) The ubiquitin moieties are recognized by ESCRT-0, which sequesters cargo into specific domains of the limiting membrane. HRS binds to membrane PtdIns(3)P (black hexagon). (**d**) ESCRT-0 also recruits ESCRT-I, presumably in conjunction with the transfer of ubiquitinated cargo. (**e**) Together with ESCRT-II, ESCRT-I mediates invagination of the limiting membrane of the MVE. VPS36 binds to membrane PtdIns(3)P (black hexagon). (**f**) ESCRT-III is recruited by binding to ESCRT-II, and cargo is deubiquitinated by ESCRT-III-associated deubiquitinating enzymes (DUBs). (**g**) Spiral-shaped ESCRT-III filaments assemble around the neck of the forming vesicle to promote its abscission from the limiting membrane, forming an ILV. (**h**) The ATPase VPS4, recruited by ESCRT-III, mediates disassembly of ESCRT-III oligomers so that the subunits are recycled. Note that several parallel nomenclature systems exist for ESCRT subunits[7]. Throughout this manuscript we have used a yeast-centric nomenclature.

unstimulated cells, β-catenin is phosphorylated by glycogen synthase kinase 3 (GSK3), which signals its polyubiquitination and subsequent proteasomal degradation. The mechanism for β-catenin stabilization following Wnt-mediated receptor activation has been elusive, but an attractive possibility arose from the finding that GSK3 sequestered within the ILVs of MVEs in response to Wnt. This event requires HRS and VPS4, which are also required for Wnt-responsive gene activation *in vitro* and Wnt-induced axis duplication in *Xenopus* embryos[31]. How GSK3 becomes sequestered in response to Wnt remains to be established. However, the fact that the Wnt co-receptor Frizzled is ubiquitinated in response to Wnt binding[32], and that several components of the Wnt signalling pathway are sequestered within MVEs[31], argues that GSK3 could follow the ubiquitinated receptor complexes into MVEs through a canonical ESCRT-mediated sorting mechanism. To date, it is not known if this MVE sequestration mechanism is unique to Wnt pathway activation. Intriguingly, however, Toll-mediated innate immunity signalling in *Drosophila* is reported to require ESCRT functions[17], opening the possibility that this unconventional ESCRT-mediated signalling mechanism might apply to multiple pathways.

Developmental consequences of ESCRT-mediated endosomal sorting and signal modulation

The pervasive cellular role of ESCRT in controlling cell–cell signals bears multiple developmental implications, illustrated by the pleiotropic phenotypes of ESCRT-mutant tissue in metazoan animals. An illustrative case is that *Drosophila* epithelial cells lacking ESCRT-I, -II and -III within otherwise wild-type developing imaginal discs over-proliferate, fail to polarize apico-basally, lack terminal differentiation and display increased JNK- and Hippo-dependent apoptosis. Such dramatic changes in cell fate result in the formation of cell masses resembling tumours. The effects of excess proliferation on tumour formation appear to be counteracted by cell death. Indeed, blocking apoptosis in ESCRT-II-mutant cells has

been shown to lead to the formation of even larger tumour masses[21,22]. Conversely, proliferation is bolstered by non-cell-autonomous interactions. In fact, the excess Notch signalling present in *vps25* and *tsg101* (ESCRT-I subunits) mutants correlates with ectopic expression of the soluble Jak/Stat ligand, Unpaired, which exerts its mitogenic effect on the mutant cells themselves as well as on nearby wild-type cells[19–21,30]. Removal of excess endosomal Notch rescues over-proliferation of *vps25* mutant cells, suggesting a causal link between receptor accumulation and excess mitogenic signalling[30]. Whether the effects on apoptosis can also be ascribed to endocytic modulation of JNK and Hippo signal transduction remains to be tested.

Cell migration is directly regulated by ESCRT-mediated endosomal sorting. Indeed, ESCRT controls endosomal sorting and degradation of $\alpha_5\beta_1$-integrin in human fibroblasts, and the localization of cell-matrix attachment kinase Src at focal adhesion points. In cell-based assays, endosomal sorting of integrin complexes is required for proper cell migration[25,33]. In addition, ESCRTs are required in endothelial cells for the degradation of the GTPase RhoB, an endosome-localized regulator of signalling and cell migration[34]. *Drosophila* embryos partially lacking VPS28 function show impairment of morphogenetic processes that require actin cytoskeleton and membrane remodeling[35]. Alteration of the cortical actin cytoskeleton and associated membrane has been observed in other ESCRT-mutant *Drosophila* tissues[36]. The role of ESCRTs in actin and membrane dynamics is still obscure. Although it could be indirectly linked to ESCRT function in cytokinesis, it could also depend on the direct effect of ESCRTs on the modulation of adhesion, which is known to require cytoskeletal and membrane plasticity.

Analyses of mice lacking the ESCRT-0 components HRS and STAM1/2, the ESCRT-I component TSG101 or the ESCRT-III component VPS32 reveal defects in cell survival, proliferation and differentiation, consistent with a role for ESCRT in the trafficking and degradation of signalling components. *Hrs*-knockout embryos display ventral and rostral-to-caudal folding morphogenesis defects, resulting in lethality at embryonic day 11 (ref. 37). This phenotype is associated with a significant block of organ development and apoptosis in the ventral portion of the embryo, and the presence of enlarged early endosomes in multiple embryonic tissues. The latter is a defect observed after loss of HRS expression in many contexts, indicating a correlation between morphogenetic defects and impaired endocytic cargo trafficking. Some embryonic tissues are absent or more affected than others in *hrs*-knockout animals, a phenotype consistent with the impairment of inductive signalling between tissues during development[37]. This might also be the case for embryos lacking the ESCRT-III accessory component VPS60/CHMP5, as they are indistinguishable from *hrs* knockouts. Embryonic cells lacking VPS60 display defects in MVE biogenesis, endosomal transport and cargo degradation, and present persistent ERK and TGFβ signalling. This suggests a causal link between impaired degradation of signalling factors and developmental aberrations[38].

Knocking out the ESCRT-0 component STAM1 in mice causes growth retardation in the third week after birth and degeneration of CA3 pyramidal neurons. Cultured pyramidal neurons derived from *stam1*-knockout mice are vulnerable to cell death induced by excitotoxic amino acids or a nitric oxide donor, consistent with a role for ESCRT-0 in signal attenuation during development[39]. Similarly to *hrs*-knockout mice, *stam1* and *stam2* double-knockout animals also arrest at E11 with ventral folding morphogenesis defects. Thymocytes lacking STAM1 and STAM2 have poor survival rates and their response to proliferative signalling is impaired[40]. These data suggest once again that the multiple alterations of signalling expected from the inability to degrade signalling molecules might underlie the developmental arrest observed in animals with ESCRT genes knocked out.

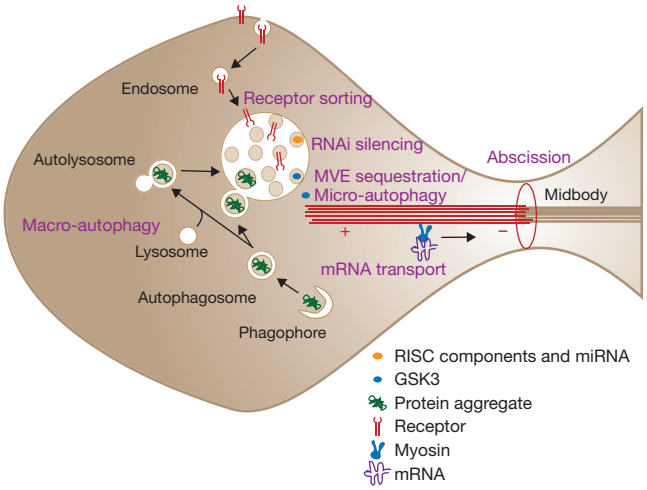

Figure 2 Cell biological processes regulated by ESCRTs. ESCRT components mediate endosomal sorting of ubiquitinated receptors and MVE biogenesis, micro-autophagy, macro-autophagy, cytokinetic abscission, mRNA transport and RNAi-mediated mRNA silencing. In addition (and outside the scope of this review), the ESCRT machinery is also required for budding of HIV-1 and many other enveloped viruses from the plasma membrane[91].

Other developmental processes appear to depend on ESCRT functions, although at present it is less clear if these are controlled through endosomal cargo sorting. For instance, compared with other ESCRT gene knockouts, *tsg101*-knockout embryos die earlier with multiple developmental defects that are already obvious by stage E6. No prominent apoptosis is observed, although *tsg101*-knockout cells display impaired proliferation *ex vivo*. This proliferative block appears to be mediated by p53, as *p53-tsg101* double-knockout embryos survive until stages E8–9. Currently, it is unclear how the loss of cargo sorting might mediate p53 activity[41]. Similarly, SNF7-2/VPS32B-null mice arrest development around stage E8. Consistent with VPS32 controlling dendritic arborization during neuronal morphogenesis in *Drosophila*, cultured mouse cortical neurons depleted of VPS32-2 or expressing a dysfunctional form of the VPS32-2 interactor VPS2B display reduced survival, loss of dendritic integrity and autophagosome accumulation[42,43].

While most of these defects might be explained by sorting-dependent signalling alterations, deconvolution of such complex phenotypes relies on increasing our understanding of how the cellular roles of ESCRTs might impact tissue survival, proliferation, differentiation and morphogenesis *in vivo*.

ESCRTs in establishment and maintenance of cell polarity during development

Cellular polarization is essential for most developmental processes. Studies in *Drosophila* epithelial organs revealed that cell polarity is ESCRT-dependent[19–21,30]. Atypical protein kinase C (aPKC), one of the main regulators of apical polarity, acts genetically upstream of TSG101, and removing aPKC activity in *tsg101*-mutant cells rescues their loss of polarity. In addition *tsg101*-mutant cells accumulate Crumbs, another

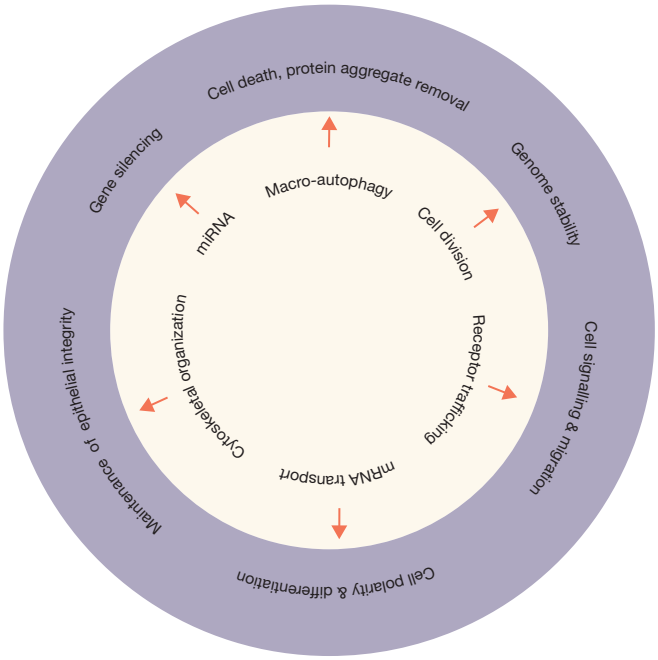

Figure 3 The ESCRT machinery in developmental processes. An illustration of how the various cell biological functions of ESCRT components can be translated into developmental programmes.

important apical polarity determinant, suggesting that stability of apical polarity complexes might depend on endosomal sorting[44]. Given the established role of endocytic trafficking and recycling of E-cadherin in the maintenance of adherence junctions and the fact that *vps25*-mutant cells lose E-cadherin junctions[30], direct involvement of ESCRT in E-cadherin turnover might also underlie polarity defects. Interestingly, a direct role for HRS in the turnover of protocadherins during mouse brain development has been described[45], and TSG101 and HRS are required for degradation of connexins, the main structural proteins of gap junctions[46,47]. In addition, ESCRTs might indirectly modulate polarity by regulating integrin-mediated adhesion signalling and downstream myosin II functions[25]. $\alpha_5\beta_1$-integrin signalling through Src activates myosin light chain kinase, which in turns activates the myosin regulatory light chain, promoting myosin-II-dependent cell polarity and adhesion site turnover[48]. Depletion of ESCRT subunits in fibroblasts causes endosomal accumulation of $\alpha_5\beta_1$-integrin and Src, subsequent failure to activate myosin light chain kinase and the myosin regulatory light chain, as well as impaired polarization and increased cell spreading[49].

ESCRTs and autophagy

The most widely described autophagic pathway, macro-autophagy (Fig. 2), involves sequestration of cytosolic components in a double-membrane vesicle (autophagosome), and subsequent degradation upon fusion with a lysosome to form an autolysosome[50]. Autophagosomes are also capable of fusing with endosomes, underscoring the tightly intertwined relationship between these two vesicular transport pathways[51]. Although ESCRT proteins were not initially isolated in yeast screens for molecules necessary for starvation-induced macro-autophagy, loss of ESCRT proteins has been found to cause accumulation of autophagosomes in *C. elegans*, *Drosophila* and mammals. Moreover, ESCRT proteins were recently shown to be required for a micro-autophagy-like process involving sequestration of cytosolic proteins into the ILVs of MVEs (Fig. 2)[52].

Although the involvement of ESCRTs in micro-autophagy can be readily explained from their function in MVE biogenesis, it is unclear how ESCRTs regulate macro-autophagy[53]. Phenotypes observed after deletion of ESCRT genes in *C. elegans* (*hrs*) and *Drosophila* (*vps28*, *vps25*, *vps32* and *vps4*) suggest that autophagosomal fusion with the endolysosomal system requires the ESCRT machinery[43,54,55]. So far, it is not clear whether induction of macro-autophagy also contributes to these phenotypes, but in *C. elegans* or *Drosophila* mosaic clones, ESCRT inactivation causes the activation of the protein kinase JNK, a potent inducer of macro-autophagy. This suggests that the accumulation of autophagosomes may be due to the combined effects of autophagy induction and a lack of fusion with lysosomes[21,22]. Knockdown of ESCRT-I, -II and -III components in mammalian cells promotes accumulation of autophagosomes and autolysosomes, suggesting that loss of ESCRT function may induce autophagy in addition to inhibiting effective fusion between autophagosomes and lysosomes[43,56,57].

The identification of causative mutations in the ESCRT-III protein VPS2B/CHMP2B in hereditary forms of the neurodegenerative diseases amyotropic lateral sclerosis and frontotemporal dementia has spurred investigations of whether the role of ESCRT 's role in autophagy is linked to the associated protein aggregation and neurodegeneration. Expression of the dominant VPS2B mutation found in ALS causes accumulation of protein aggregates[56]. Moreover, ESCRT knockdown in mammalian cells almost completely abrogates clearance of huntingtin aggregates, and reducing the expression of ESCRT components increases neurotoxicitiy in a *Drosophila* htt model[55,56]. Together, these studies show that ESCRT proteins participate in removing toxic protein aggregates *in vivo* and potentially protect against protein-aggregate diseases.

The degree to which defective autophagy is responsible for the pleiotropic developmental phenotypes caused by ESCRT deficiency is now being explored. As autophagy has been shown to be necessary for developmentally programmed cell death in certain cell types such as *Drosophila* midgut and follicle cells[59,60], it will also be interesting to explore whether some cell death pathways require ESCRT function.

Developmental consequences of ESCRT involvement in cell division

In human cells, ESCRT-I and -III, but not ESCRT-0 or -II, are required for normal completion of cytokinesis[61,62]. ESCRT-I and -III-depleted cells arrest during a late stage of cytokinesis, and a similar phenotype is observed after depletion of Alix, a protein capable of binding both ESCRT-I and ESCRT-III. Recent studies have revealed how Alix and ESCRTs promote completion of cytokinesis. Initiation of ESCRT engagement is defined by the binding of the centrosomal protein CEP55 to the midbody at the onset of cytokinesis[63,64]. CEP55 recruits TSG101 and Alix, and these are followed by ESCRT-III and VPS4[62,65,66]. At a late stage of cytokinesis, a helical 17 nm filament can be observed winding from the midbody to the constriction site where severing of microtubule bundles and membrane scission occurs[67]. Formation of the 17 nm filament depends on VPS32B, and constriction of the helix formed by this filament is thought to generate the force needed for membrane scission. In addition, the tip of the helix defines the site of recruitment of Spastin, a VPS4-related ATPase that mediates microtubule severing[67].

Table 1 ESCRT proteins in development

Cellular function	Developmental consequence of ESCRT inactivation	Species	ESCRT complex investigated	ESCRT component inactivated	Ref
Receptor sorting	***Aberrant cell signalling***				
Notch receptor trafficking	Ligand-independent over-activation of Notch signalling	Dm	I, II, III	Tsg101, Vps25, Vps28, Vps22, Vps32, Vps20,	19, 20, 30, 36
EGFR and FGFR trafficking	Lowered FGFR and EGFR signalling activity	Dm	0	Hrs, Stam	18
EGFR trafficking	EGFR degradation defective, EGFR signal over-activation	Dm	0, I, II, III	Hrs, Vps28, Vps22, Vps25, Vps20, Vps32	14,36
EGFR trafficking	EGFR degradation defective, EGFR signal over-activation	Hs	0, I, II, III	HRS, STAM, TSG101, VPS28, VPS37A, MVB12A, VPS22, VPS20, VPS2B, VPS24, VPS4	14, 23, 42, 80–89
GSK3 sequestration in MVBs	Reduced Wnt signalling	Xl	0, III	Hrs,Vps4	31
Wg (wingless) receptor (Fz, frizzled) trafficking	Increased Wg signalling	Dm	0	Hrs	90
TGFα receptor trafficking	Increased Dpp (TGFα) signalling	Dm	0	Hrs	15
TGFα receptor (TβRII) trafficking	TβRII degradation defective. Increased ERK and SMAD2 signalling	Mm	III	VPS60	38
Thymocyte survival/signaling	Prolonged p38-MAPK and JNK but not ERK and AKT signalling upon T cell receptor stimulation	Mm	0	STAM1/2	40
Macro-autophagy	***Protein aggregate accumulation***				
Autophagosome-lysosome fusion	Protein aggregate accumulation and neuronal cell death	Dm	I, II, III	Vps28, Vps25, Vps32, Vps4	43,55
Autophagosome-lysosome fusion		Ce	0, III	Hrs, Vps32	27
Autophagosome-lysosome fusion	Protein aggregate accumulation and neuronal cell death	Hs	0, I, II, III	TSG101, HRS, VPS22, VPS24, VPS2B, VPS32-2	43, 56, 57
Autophagosome-lysosome fusion	Protein aggregate accumulation	Mm	III	VPS32-2	
Micro-autophagy-like MVE sorting of cytosolic cargo	Unknown	Hs	I, III	TSG101, VPS4	52
Cytoskeletal organization and maintenance of epithelial integrity	***Cytoskeletal dysregulation and loss of epithelial integrity***				
Unknown	Mislocalization of epithelial cell polarity markers, loss of epithelial integrity, actin cytoskeletal defects	Dm	I, II, III	Tsg101, Vps25, Vps28, Vps22, Vps32, Vps20, Vps4	19, 20, 22, 30, 35, 36
Endosomal $\alpha_5\alpha_1$-integrin sorting and degradation	Ectopic Src activation resulting in failure of MLCK and MRLC activation. Defect in actin cytoskeletal dynamics	Hs	0, I, II, III	HRS, TSG101, VPS22, VPS24	49
Cell division (abscission)	***Aneuploidy***				
Necessary for completion of cytokinesis (abscission)	Multinucleate cells	Sa	III	Vps4 (Saci1372)	78
		Hs	I, III (not 0 and II)	TSG101, VPS4	62,65
		At	I	Tsg101(Elch)	70
		Dm	I, II, III	Tsg101, Vps28, Vps2, Vps28, Vps25	36
MVE sorting of miRNA	***Defective RNAi silencing***				
miRNA and RISC loading at MVEs	Impaired RNAi efficiency	Hs	0, II	Hrs, Vps36	71
miRNA and RISC loading at MVEs	Impaired RNAi efficiency	Dm	0, II	Hrs, Vps25	72
mRNA transport	***Dysregulated cell polarity and differentiation***				
Directed mRNA transport	Failure of bicoid mRNA transport and polarization of the egg	Dm	II, not I or III	Vps22, Vps25, Vps36	30,73

Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Ce, *Caenorhabditis elegans*; Mm, *Mus musculus*; Sa-, *Sulfolobus acidocaldarius* (archaebacterium); At, *Arabidopsis thaliana*; Xl, *Xenopus laevis*.

The exact function of VPS4 in cytokinesis remains to be determined, but it is tempting to speculate that it functions in the disassembly of ESCRT-III oligomers, similarly to its proposed function in MVE budding[12]. It is intriguing that cytokinesis does not require the full palette of the ESCRT machinery, as there is no genetic requirement for ESCRT-0 and ESCRT-II. This raises the question of whether other proteins emulate the roles of these ESCRTs during cytokinesis. A key role of ESCRT-0 is initiating ESCRT recruitment to the endosome membrane by binding

phosphatidylinositol-3-phosphate (PtdIns(3)P) in the membrane[68]. As PtdIns(3)P is also required for efficient cytokinesis[69], it is possible that unknown PtdIns(3)P-binding proteins could mediate contacts between the plasma membrane and the 17 nm filament during abscission. In MVE biogenesis, ESCRT-II cooperates with ESCRT-I during membrane invagination and also recruits ESCRT-III (refs 2,10). During cytokinesis there is no need for this kind of membrane invagination, and Alix may replace ESCRT-II in bridging ESCRT-I with ESCRT-III (refs 62,65).

Cultured cells depleted of ESCRT subunits frequently display a multinuclear phenotype[62,65]. This could be attributed partly to cleavage furrow regression as a consequence of cytokinesis arrest; however, ESCRT-III and VPS4 have also recently been found to have an additional role in cell division, specifically centrosome maintenance or proliferation[61]. Depletion of ESCRT-III subunits not only arrests cytokinesis but also results in mono- or multipolar spindles, and defects in chromosome segregation and nuclear morphology. At present, it is unclear how ESCRT-III might control centrosomes, but the fact that all known ESCRT-III subunits are involved points to a functional mechanism that bears resemblance to the function of ESCRT-III in membrane scission[61].

Despite the striking cell division phenotypes observed in cultured ESCRT-depleted cells, there are few *in vivo* phenotypes that have so far been directly attributed to cell division defects in ESCRT-defective multicellular organisms. The clearest example to date is provided by studies of the TSG101 homologue ELCH in *Arabidopsis thaliana*. Interestingly, *elch* mutants show multiple nuclei in various cell types, consistent with a cytokinesis defect, and a subpopulation of trichomes (plant hairs) are binuclear and form double stems[70]. Similarly, the occurrence of multinucleated cells in ESCRT-defective *Drosophila* tissues supports a role for ESCRTs in cell division *in vivo*[36]. Surprisingly, however, the multinucleate *Drosophila* phenotype can be observed even in ESCRT-II defective tissues. As ESCRT-II is dispensable for mammalian cytokinesis[62,65], this suggests that the multinucleate phenotype may not be correlated directly to defective cytokinesis. Given the established importance of ESCRT-I and -III in cell division in cultured human cells, it will now be important to test ESCRT functions in suitable *in vivo* models to establish the importance of this mechanism in the developmental processes of multicellular organisms.

ESCRTs in RNA biology have implications for development

Recent work indicates that ESCRTs are required for post-transcriptional gene silencing. Indeed, in *Drosophila* and human cells, a portion of the miRNA machinery (including components of the RISC complex such as GW182 and AGO1/2, certain miRNA and miRNA-targeted mRNAs) localize to MVEs as part of so-called 'GW-bodies'. Preventing ESCRT function has been shown to reduce RNAi potency[71,72]. The advantage of coupling the RNA silencing machinery to the lysosomal degradation pathway is still unclear but could represent a mechanism for connecting mRNA regulation to receptor signalling and trafficking.

An additional unexpected role of ESCRT proteins in RNA biology was discovered in an unbiased screen for genes affecting the directed transport and retention of *bicoid* mRNA at the anterior pole of the *Drosophila* egg[73]. In this study, a loss-of-function mutant of the ESCRT-II component VPS22 failed to transport and retain *bicoid* mRNA at the anterior pole. Interestingly, all the ESCRT-II subunits, but not ESCRT-0, -I or -III, are necessary for this function. The GLUE domain of VPS36, previously shown to bind endosomal PtdIns(3)P and ubiquitin[74,75], was found to directly bind *bicoid* mRNA, providing a rationale for the specificity of the ESCRT-II complex for this process[73]. So far, *bicoid* mRNA remains the only identified target of ESCRT-II-mediated subcellular mRNA localization, but it will be very interesting to learn whether other RNA molecules (including human mRNAs) depend on ESCRT-II for their localization.

Perspectives

Phylogenetic analyses of ESCRT components show that ESCRT-III and VPS4 are conserved among Archaea, plants and metazoans[76–78]. The involvement of ESCRT proteins in cytokinesis appears to be conserved in plants and mammalian cells[65,70] and is therefore likely to be the most ancient ESCRT function. The diverse functions executed by ESCRT components in eukaryotes (Fig. 3) reflect the evolution of additional complexes that function mostly in concert with ESCRT-III, but which may also have ESCRT-III-independent functions. Given the similar topologies of ILV budding and cytokinetic abscission, the involvement of ESCRTs in these membrane-involution processes can be rationalized. Similarly, the ability of ESCRTs to sort ubiquitinated membrane molecules into MVEs is easy to reconcile with the receptor signalling phenotypes observed in ESCRT-mutant cells and tissues. The most unexpected ESCRT functions to date are those associated with RNA biology — mRNA localization and silencing — and we still need to learn how important these are in various developmental contexts.

For developmental biologists, the broad ESCRT repertoire poses a real challenge to the study of phenotypes associated with loss of ESCRT functions (Table 1). Presumably, most of the observed developmental consequences of ESCRT activity are linked to the canonical function of ESCRT subunits in endosomal receptor sorting. Its impact on receptor signalling — whose mechanistic details are now emerging — might control cell polarity and migration in ways that are currently being clarified. However, the involvement of certain ESCRT components in cell division, autophagy and RNA biology is also very likely to underpin some of the phenotypes observed in ESCRT mutant organisms. For instance, given the widespread role of miRNAs in developmental regulation of gene expression, it is tempting to speculate that a sizable portion of ESCRT ablation effects could be attributed to impaired miRNA function. Finally, considering that most ESCRT components are likely to be constitutively expressed, identification and characterization of developmentally regulated modulators, in addition to the few described[79], will also represent a key aspect in understanding how ESCRTs shape organisms.

COMPETING FINANCIAL INTERESTS
The authors declare that they have no competing financial interests.

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