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When membranes need an ESCRT: endosomal sorting and membrane remodelling in health and disease

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Summary

Originally discovered as regulators of cargo sorting during endosomal trafficking, ESCRT (endosomal sorting complexes required for transport) proteins are emerging as flexible machines that shape the behaviour of membranes throughout the cell. Deregulation of ESCRT activity is observed in neuro-degenerative diseases, virus infection and cancer. However, the mechanisms of pathogenesis involving ESCRTs have not yet fully come into focus. Here, we review the current knowledge of ESCRT function in health and disease and provide educated guesses for future research and focused therapeutic intervention.

Key words: *ESCRT; endosomal sorting; MVB biogenesis; membrane remodelling; signal transduction; nuclear envelope; neurodegeneration; viral infection; cancer*

Introduction

Compartmentalisation is one of the defining features of eukaryotic cells. It is thought to have propelled life towards multi-cellularity and emergence of a nervous system [1, 2]. The plasma membrane and the endo-membranes of a compartmentalised cell constitute the infrastructure for most cellular logistics, which involves incessant trafficking of countless cargoes and associated macromolecules, ultimately shaping the identity and fate of a cell, as well as its relationship with neighbours. In this review, we focus on functions of the ESCRT (endosomal sorting complexes required for transport) machinery, which is emerging as a central regulator of membrane remodelling during trafficking and non-trafficking events (fig. 1).

How ESCRTs work during endosomal sorting

The ESCRT machinery was first identified in yeast by means of genetic isolation of mutants that cause defective protein sorting to the vacuole, the functional equivalent of the lysosome [3, 4]. These mutants, termed "class E-*vps* mutants", possessed enlarged prevacuolar endosome-like compartments containing un-degraded proteins [5]. Most of the class E-*vps* genes were later found to act in succession to concentrate trafficking cargoes and include them in forming late endosomes (also termed multivesicular bodies or MVBs) that eventually fuse with lysosomes for degradation [6]. We now know from a large body of mechanistic studies in yeast and other model organisms that the ESCRT machinery that regulates endosomal sorting is organised into five distinct protein complexes: ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III and the Vps4 AAA-ATPase complex (Vps: vacuolar protein sorting-associated protein; AAA ATPases: ATPases Associated with diverse cellular Activities) (see table 1 for subunit compositions). During sorting, these complexes are recruited from the cytoplasm sequentially by interaction of specific subunits with the endosomal membrane. Ubiquitination of cargoes provides the key signal for initial cargo binding by ESCRT-0 (reviewed in [7]). Indeed, the ESCRT-0 subunits Hrs and Stam, as well as ESCRT-I Vps23/TSG101 and ESCRT-II Vps36,

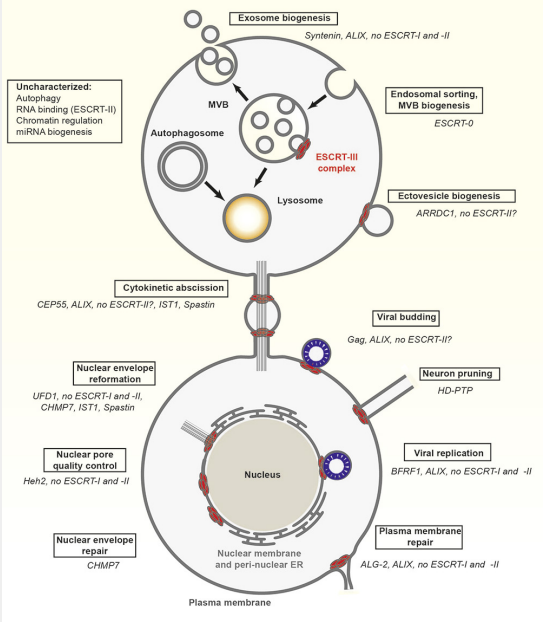

Figure 1

Cell biological functions of ESCRTs (endosomal sorting complexes required for transport).
Overview of ESCRT functions throughout the cell. Factors that are specific for each ESCRT-dependent process (boxed) are listed in italics.

contain ubiquitin-binding domains that interact with ubiquitinated cargoes. ESCRT-0 is also recruited by interaction between the FYVE domain of Hrs and phosphatidylinositol 3-phosphate (PI3P), which is enriched at the endosomal membrane. ESCRT-0 is thought to concentrate ubiquitinated cargoes by organising flat coats of clathrin on the endosomal membrane [8, 9]. ESCRT-0 also summons ESCRT-I, which retains the cargoes by ubiquitin binding and hands them to the ESCRT–II complex. The ESCRT-II complex provides a scaffold for the formation of the ESCRT-III complex, the business end of the ESCRT machinery. The Vps32/Snf7/Chmp4 subunit of ESCRT-III forms multimeric filaments organised in spirals that bend the endosomal membrane away from the cytoplasm to form invaginated buds. Thus, the combined activity of ESCRTs allows sorted cargoes to be corralled and trapped in nascent intraluminal vesicles (ILVs) of the MVBs, which eventually pinch off into the endosomal lumen. The deubiquitinating enzyme Doa4 is recruited by ESCRT-III to remove ubiquitin from cargoes that are included into ILVs. Finally, the Vps4 ATPase complex binds and fully unfolds the ESCRT-III complex in an ATP-dependent manner and favours pinching off the ILV neck, the final step of MVB biogenesis [10–18]. The structure of most ESCRT components has been determined, and detailed extensive knowledge of the ESCRT mechanism of action in endosomal sorting and MVB biogenesis is available (reviewed in [19, 20])

ESCRTs activity during trafficking processes distinct from endosomal sorting

ESCRT-I, -II and -III and the Vps4 complexes are conserved across the eukaryotic lineage [21]. In contrast, ESCRT-0 is present only in a subset of eukaryotes. This indicated early on that they are specialised to couple the core membrane-remodelling activity of ESCRT-III and Vps4 with cargo sorting. Indeed, evidence indicates that additional complexes, such as those containing the protein Tom1 (target of Myb protein 1), might control initial concentration of ubiquitinated cargoes in endosomes [22]. Consistent with the accessory role of ESCRT-0, a large body of studies in the last 25 years revealed that the function of the ESCRTs at membranes is not limited to endosomal sorting and MVB biogenesis. In fact, early work indicated that a number of viruses can recruit ESCRT-III and Vps4 to bud from the plasma membrane [23, 24], leading to subsequent realisation that budding of the plasma membrane operated by ESCRTs occurs also in uninfected cells to form ectovesicles. MVBs can also fuse with the plasma membrane to release ILVs, in this case referred to as exosomes. As in endosomal sorting, deployment of ESCRT-III and Vps4 in the formation of exosomes and ectovesicles (together referred to as exovesicles) appears to depend either on ESCRT-I and -II, or on Alix, and to require adapters different from ESCRT-0. These data indicated that MVB and exovesicle biogenesis can profoundly differ and that multiple pathways of exovesicle formation are likely to exist [25–30].

MVBs also act as main stations for autophagic trafficking [31, 32]. Evidences from *Caenorhabditis elegans*, *Drosophila* and mammalian cells in culture revealed that ESCRTs are required for both microautophagy and macroautophagy [33–36]. During macroautophagy, autophagosomes that are formed *de novo* to clear long-lived proteins, cytoplasmic aggregates or damaged organelles fuse with MVBs and lysosomes to form amphisomes and autolysosomes, respectively, whose content is progressively degraded. Although it recently emerged that ESCRT activity is coordinated with macroautophagic response to starvation [37, 38], how ESCRT regulate autophagy mechanistically is currently unclear.

In summary, the membrane trafficking functions regulated by ESCRTs are crucial for lysosome-mediated cargo degradation, for release of exovesicles and, perhaps indirectly, for autophagy.

The ESCRTs system beyond trafficking

The first evidence of ESCRT functions that are independent of membrane trafficking indicated that ESCRT-III and Vps4 act at the plasma membrane to sever microtubules and release the midbody during cytokinesis. In this case, the recruitment is operated by the midbody protein Cep55

Table 1: Composition of the ESCRT (endosomal sorting complexes required for transport) complexes in endosomal sorting.

Complex	Function	Evolutionary origin	Yeast	*Drosophila*	Human
ESCRT-0	Cargo recognition	Opisthokonta	Vps27	Hrs	HRS
			Hse1	Stam	STAM1, STAM2
ESCRT-I	Upstream adapter	Eukaryotes	Vps23	Tsg101	TSG101
			Vps37		VPS37A, B, C, D
			Mvb12	Mvb12	MVB12A, MVB12B
			Vps28	Vps28	VPS28
ESCRT-II	Bridging adapter		Vps36	Vps36	EAP45
			Vs22(Snf8)	Vps22	EAP30
			Vps25	Vps25	EAP20
ESCRT-III	Membrane remodelling/ filament	Archaea	Vps2	Vps2	CHMP2A, B
			Vps24	Vps24	CHMP3
			Vps32 (Snf7)	Vps32 (Shrub)	CHMP4A, B, C
			Vps20	Vps20	VPS20/CHMP6
			Vps46 (Did2)	Chmp1	CHMP1A, B
			Vps60 (Chm5)	Chmp5	CHMP5
Vps4 –Vta1	Membrane remodelling/ ATPase		Vps4	Vps4	VPS4A, B (SKD1, 2)
			Vta1	CG7967	VTA1 (LIP5)

and ESCRT-III directly recruiting the microtubule-severing protein spastin. This activity is present also in Archaea and plants, suggesting that it is the most ancient evolutionarily [39–41]. Very recent studies showed similar recruitment of spastin during nuclear envelope reformation at the end of mitosis, albeit with a different recruitment system [42, 43]. However, the roles of ESCRTs at the nuclear membrane began to emerge with the recognition that ESCRTs are required for budding of the Epstein-Barr virus through the nuclear membrane [44]. More recently, it was found that the ESCRT machinery also restores membrane integrity after nuclear pore and nuclear envelope damage [43, 45, 46]. These membrane-repair functions of the ESCRT machinery are also observed at the plasma membrane [47] and a likely developmental counterpart of such activity has been observed in neuron remodelling. Indeed, ESCRTs have been shown to be required for the membrane scission that occurs in neuron pruning [48]. ESCRT-dependent neuronal remodelling events were described previously in *Drosophila* development, but had been attributed to endolysosomal trafficking of neuronal receptors [49–51].

Other, less understood, ESCRT functions include control of centrosome number during mitosis [52–55], transcriptional gene regulation [56–61], RNA transport [62], and microRNA biogenesis [63, 64]. Although the mechanistic details of these processes are unclear, it is reasonable to think that they might be linked to bending of membranes away from the cytoplasm, which to date represents the shared topological feature of well-characterised processes operated by ESCRT. In extreme synthesis, the ESCRT machinery is modular and invariantly leads to deployment of ESCRT-III and Vps4, a multipurpose membrane-remodelling complex.

ESCRTs, signalling and tissue architecture

Because a number of signalling proteins are transmembrane or membrane associated, endocytosis and trafficking to lysosomes are crucial to regulation of signal transduction (reviewed in [65]). Indeed, studies in cells of multicellular organisms that followed the initial discovery of ESCRT in yeast revealed that endosomal sorting complexes are essential to downregulate signalling, among which is that stimulated by epidermal growth factor (EGF) [66–74]. Subsequently, *Drosophila* mosaic animals also indicated that ESCRT function is required to regulate Notch signalling, which regulates multiple cell fate decisions. The canonical pathway is activated by binding of ligands to the transmembrane Notch receptor, triggering cleavage and translocation of an activated fragment, Notch intracellular domain, to the nucleus to de-repress transcription of target genes (reviewed in [75]). *Drosophila* organs developing in absence of ESCRT-I, -II or -III activity display increased, and for the most part ligand-independent, Notch signalling activity owing to accumulation on the limiting membrane of endosomes of Notch receptors that fail to be included into MVBs [76–80]. Despite endosomal Notch accumulation, ESCRT-0 mutant organs of mosaic animals do not show ectopic Notch signalling activity [67, 68, 81], highlighting differences in regulation of Notch signalling regulation by endosomal sorting, when compared with EGFR signalling.

In ESCRT-mutant *Drosophila* tissues, cell polarisation is lost, probably because a number of polarity determinants require endosomal trafficking to be maintained at correct levels to polarise membranes and cell-cell junctions [82–86]. Apoptotic response is enhanced as well. However, it is not clear whether this is an indirect consequence of the signalling and polarity defects [79, 87, 88]. In epithelial organs of *Drosophila* lacking ESCRT-I, -II or -III components, ectopic activation of signalling and altered cell polarity contribute to formation of tumour-like tissue that is highly over-proliferative, especially when apoptosis is inhibited. These traits led to the proposal that ESCRT genes act as tumour suppressors in metazoans (reviewed in [89]). *Drosophila* ESCRT-0 genes, however, do not behave as tumour suppressors, perhaps reflecting the distinct evolutionary origin of the complex [81].

Similarly to that of *Drosophila*, analysis of mouse ESCRT knock-outs revealed a requirement for cell survival, proliferation and signalling regulation leading to lethality early in embryogenesis [90–93]. Interestingly, a mouse hypomorph mutant of Vps25, encoding an ESCRT-II component, allows development to occur and reveals a specific requirement for ESCRTs in downregulating Sonic Hedgehog and fibroblast growth factor (FGF) signalling during limb development [94].

Several other signalling pathways have been shown to be deregulated when ESCRT function is impaired in multiple model systems. These include JNK (Jun amino-terminal kinases), JAK/STAT (Janus kinase / signal transducer and activator of transcription), Hedgehog, Wnt, FGF, Toll, nuclear factor kappa-beta (NFκβ) and transforming growth factor-beta (TGF-β) signalling [68, 76, 88, 93–101].

Overall, development and cell biology studies in multicellular organisms have taught us that ESCRTs have essential and pleiotropic functions that deeply impact tissue formation and homeostasis.

ESCRTs and infection

As introduced above, a number of pathogenic viruses including human immunodeficiency virus-1 (HIV-1), hepatitis C virus and Ebola virus hijack ESCRTs for their maturation and eventual budding to release infectious particles from infected cells (table 2). Indeed, plenty of data indicate that viral proteins, such as the Gag protein of HIV-1, recruit TSG101 and Alix, which in turn recruit ESCRT-III and Vps4 proteins, to the neck of the viral particle assembling at the plasma membrane [23, 102–108]. In the absence of TSG101 and ALIX, hepatitis C virus, herpes simplex virus type 1 (HSV-1), and to some extent, HIV-1 are still able to recruit ESCRT-III [102, 109, 110], suggesting that additional proteins mediate these interactions. Alternatively, viral proteins may be able to recruit downstream ESCRT components; for instance, the matrix protein VP40 of Ebola virus, in addition to recruiting TSG101, also directly recruits Vps4, with some other ESCRT proteins, to the site of budding [111].

In addition, a number of reports suggest that several viruses incorporate their proteins, messenger RNAs or microRNAs

into exovesicles of their hosts to promote their spread, to modulate immunity, or to manipulate the microenvironment [112–119]. ESCRT activity is also required for entry of rotaviruses and human papilloma virus, as these are taken up by endocytosis, sorted into ILVs and eventually released in the cytoplasm [120–126]. Finally, a role for ESCRT-II in the replication of HIV-1 has also been reported. Depletion of ESCRT-II subunits in HIV-1-infected human HeLa cells affected the cytoplasmic trafficking of HIV-1 genomic RNA and reduced the expression of the HIV Gag protein [127, 128]. Similar results were reported for the hepatitis B virus [129]. Whether the function of ESCRT-II in this particular aspect of the viral life-cycle corresponds to that in transport of endogenous mRNA in *Drosophila* [62] is currently unclear.

Several non-viral pathogens also exploit the function of ESCRTs in infecting their hosts. Genome-wide screens in *Drosophila* S2 and murine macrophage cells have found that ESCRT components restrict the growth of mycobacteria by impairing phagosome maturation, raising the possibility that mycobacteria may disrupt host ESCRT function for their growth. Indeed, a protein secreted by *Mycobacterium tuberculosis* binds to Hrs to hinder sorting towards the lysosome for degradation [130–132]. A subunit of the lethal anthrax toxin secreted by *Bacillus anthracis* is packaged into ILVs of infected cells, both for a longer half-life and for exosomal secretion [133]. Finally, *Candida albicans*, an opportunistic fungal pathogen that colonises mucosal surfaces, requires ESCRT activity for pathogenesis and colonisation. In contrast to viruses and other pathogens that hijack the host ESCRT machinery, *C. albicans* uses its own ESCRT complex to adapt to the neutral–alkaline pH of the host environment [134–139].

In summary, viruses and other pathogens clearly exploit a wide range of the diverse cell biological functions of ESCRTs, offering multiple points of entry for future innovative therapies.

ESCRTs and cancer

Misexpression of ESCRT subunits has been associated with several types of human cancer. However, the role of ESCRT in tumorigenesis remains highly controversial. One of the most studied ESCRTs in this regard is the ESCRT-I gene TSG101, which was initially isolated in a search for novel tumour suppressor genes (TSG101: tumour susceptibility gene 101). Inactivation of TSG101 in NIH3T3 cells gave rise to metastatic tumours when xenografted in nude mice [140]. Consistent with this, TSG101 expression is significantly downregulated in cervical carcinomas [141]. Despite this, the role of TSG101 as a tumour suppressor has been debated, because it was later found that conditional knock-out of Tsg101 in mouse mammary epithelia did not promote tumour formation but arrested cell growth [142, 143]. Although TSG101 expression seems tightly regulated by an active mechanism [144], a study evaluating the effect of TSG101 overexpression indicated that tumour maintenance and progression rather than ini-

Table 2: Involvement of ESCRT (endosomal sorting complexes required for transport) components in disease.

ESCRT complex	Subunit	Disease/dysfunction	References
Infections			
ESCRT-0	Hrs	Exosomal secretion of hepatitis C virus *Mycobacterium tuberculosis*resistance to degradation	[115] [132]
ESCRT-I/III/VPS4	TSG101, CHMPs, VPS4	Budding of viruses including HIV-1, Ebola.	Reviewed in [251]
ESCRT-II	EAP20, EAP45	Replication of HIV-1	[127, 128]
Cancer			
ESCRT-0	Hrs	Tumorigenesis and metastasis of HeLa cells	[166]
ESCRT-I	TSG101	Cervical cancer	[141]
		Breast cancer	[145, 150]
		Lung cancer	[146]
		Gallbladder adenocarcinoma	[148]
		Ovarian carcinoma	[149, 150, 152]
	VPS37A/HCRP1	Hepatocellular carcinoma	[153, 154]
		Breast cancer	[155]
		Ovarian cancer	[156]
		Renal cell carcinoma	[157]
ESCRT-III	CHMP1A	Renal cell carcinoma	[158]
		Pancreatic carcinoma	[159, 160]
	CHMP4B	Hepatocellular carcinoma	[161]
	CHMP4C	Lung cancer	[162]
VPS4	VPS4A	Hepatocellular carcinoma	[163]
		Ovarian carcinoma	[165]
Neurodegeneration			
ESCRT-I	VPS37A	Hereditary spastic paraplegia	[182]
ESCRT-III	CHMP2B	Frontotemporal dementia	[167, 168]
		Amyotrophic lateral sclerosis	[176, 178]
		Alzheimer's disease, dementia with Lewy bodies	[186, 188–190]
Other diseases			
ESCRT-III	CHMP4B	Progressive childhood posterior subcapsular cataracts	[194]
VPS4	VPS4	Crohn's disease	[196]

tiation might benefit from higher levels of TSG101 [145]. Despite this, the gene has been found to be significantly overexpressed also in lung cancer [146], gallbladder adenocarcinoma [147], papillary thyroid tumours [148] and ovarian carcinomas [149]. These tumours might be addicted to high levels of TSG101, as its depletion was shown to reduce tumour growth, to slow tumour migration, to halt cell cycle progression and to trigger apoptosis of cancer cells [150–152]. TSG101 appears also to be a prognostic marker in some cancers because its high expression correlates with poor prognosis, decreased survival, high tumour stage, and increased metastasis and invasion [147, 152].

Besides TSG101, another ESCRT-I gene, VPS37A, was identified because of its down-regulation in hepatocellular carcinomas and named human hepatocellular protein 1 (HCRP1) accordingly [72, 153]. Reduced VPS37A/HCRP1 expression strongly correlates with depth of tumour invasion, lower survival and higher rate of disease recurrence not only in hepatocellular carcinoma, but also in breast cancer, renal cell carcinoma, and oral and oropharyngeal cancers [153–157]. Most of the effect of VPS37A loss has been attributed to reduced EGF receptor degradation [72, 156], activation of downstream MAPK/ERK signalling and increased matrix metalloproteinase-2 (MMP2) expression: the loss of VPS37A has been suggested to increase tumour proliferation and invasion, and in ovarian cancer patients to lower response to cetuximab treatment [153, 156].

Several subunits of the human ESCRT-III and Vps4 complexes have been also linked to tumour development. CHMP1A appears significantly downregulated in renal cell carcinomas [158] and pancreatic tumours [159, 160], in which it has been suggested to function as a tumour suppressor. Accordingly, non-tumorigenic human embryonic kidney cells acquire the ability to form xenograft tumours when CHMP1A is depleted [160]. CHMP1A overexpression inhibits the proliferation of renal [158] and pancreatic tumour cells [160]. CHMP1A appears to inhibit tumour growth in the pancreas by regulating the activation of ataxia telangiectasia mutated (ATM) kinase and phosphorylation of p53 [159, 160]. Recent reports have identified a strong upregulation (and correlation with poor prognosis) of CHMP4B in hepatocellular carcinoma, and have suggested that CHMP4B and CHMP4C might be required to sustain proliferation and resistance to anticancer treatment in human hepatocellular and lung cancer cell lines, respectively [161, 162]. In a study aimed at characterising microRNAs in exosomes of hepatocellular carcinoma cells, it was found that modulation of Vps4A changed exosome content and activity. Vps4A was also found to act as a tumour suppressor, by repressing the PI3K/Akt pathway [163]. Other studies suggested that VPS4A and exosomes could influence resistance to cancer drugs like cisplatin and doxorubicin by modulating their efflux [164, 165].

Finally, expression of the ESCRT-0 component HRS is significantly increased in human tumour tissues derived from the stomach, colon, liver and cervix and from melanomas — suggesting the existence of a tumour-enhancing function for HRS. Depletion of HRS reduced the tumorigenicity and metastatic ability of HeLa cells and upregulated the protein level of adherens junction component E-cadherin [166]. Since HRS functions in the endolysosomal trafficking and degradation of E-cadherin [83, 166], it has been proposed that, in these tumours, the cargo sorting function of HRS is hijacked to downregulate E-cadherin and promote metastasis.

Overall, the involvement of ESCRT in tumorigenesis is multifaceted and likely to be dependent on the tumour context, reflecting the complexity of the phenotypes observed in ESCRT mutant organs of *Drosophila*.

ESCRTs and neurodegeneration

ESCRT loss is observed frequently in many neuropathologies. Among the best characterised are the form of autosomal dominant frontotemporal dementia (FTD) caused by mutations in CHMP2B, a subunit of ESCRT-III [167, 168]. The mutations lead to loss of the protein C terminus, which controls autoinhibition and interaction with Vps4 [169–173]. Accordingly, enlarged dysmorphic late endosomes have been found in cells of FTD patients [167, 174]. Similar endosomal phenotypes are observed when mutant CHMP2B is overexpressed in human cells [168]. It has been proposed that mutant CHMP2B impairs endosome-to-lysosome fusion by blocking the endosomal recruitment of the GTPase Rab7, by inhibiting ESCRT-III dissociation from endosomes, or by preventing the disassembly of the ESCRT-III complex. Defective autophagy is another mechanism by which CHMP2B mutations might cause FTD. Such a scenario is suggested by the presence of ubiquitin inclusions positive for the autophagy marker p62, which are often observed upon failure of autophagic clearance [173, 175–177]. Overall, the endolysosomal and autophagy defects are thought to lead to accumulation of protein aggregates, inducing neuronal degeneration, which is a hallmark of the disease. CHMP2B mutations have also been identified in amyotrophic lateral sclerosis patients [176, 178] suggesting that defective ESCRT activity may contribute also to the pathogenesis of amyotrophic lateral sclerosis.

Mutations in the microtubule-severing protein spastin, which has been found to be associated with the ESCRT-III complex during cytokinesis and nuclear membrane reformation, cause hereditary spastic paraplegia (HPS) [179]. Spastin function in HPS has been linked to shaping of the endoplasmic reticulum [180] and, recently, to formation of lipid droplets [181]. This indicates that either ESCRT-independent functions of spastin are affected in HPS or that ESCRTs and spastin might cooperate in membrane and microtubule remodelling at the endoplasmic reticulum or in lipid droplets. Underscoring this interesting possibility, mutations in VPS37 (ESCRT-I) have also been identified in HPS patients [182].

Although no mutations have been isolated so far, ESCRT-III function has also been reported to be important for aspects of Alzheimer's disease and of Lewy body dementia (DLB, an umbrella term for two related diagnoses, Parkinson's disease dementia and dementia with Lewy bodies). Lewy bodies are abnormal aggregates containing damaged alpha-synuclein (α-SYN) and other proteins, and α-SYN aggregation is a trait associated with the progression of Parkinson's disease and DLB [183, 184]. A feature of

Alzheimer's disease and of DLB is the prion-like cell-to-cell spreading of α-SYN aggregates leading to rapid disease progression [185]. According to recent studies, α-SYN aggregates are taken up by clathrin-mediated endocytosis, undergo ESCRT-mediated trafficking through MVBs, and are degraded in lysosomes [186–188]. Rapid clearance of α-SYN aggregates and amelioration of the neurodegenerative pathology was observed upon CHMP2B overexpression [186, 189]; on the other hand, depletion of CHMP2B mediated by small interfering RNAs (siRNA) increased the exocytosis and intercellular transmission of α-SYN aggregates [186]. In addition, α-SYN aggregates colocalised with Vps4 [190], and inhibition of Vps4 function using a dominant-negative construct blocked lysosome-mediated degradation and increased extracellular secretion of α-SYN, possibly by means of exosomes [187].

The formation of amyloid-beta aggregates in Alzheimer's disease also appears to involve regulation by ESCRT proteins. In fact, it has been recently shown that amyloid-beta and amyloid protein precursor are sorted into the intraluminal vesicles of MVBs. Depletion of Hrs and Tsg101 increases the intracellular accumulation of amyloid-beta by simultaneously inhibiting lysosomal delivery of amyloid precursor protein and reduced amyloid-beta secretion through an as yet unknown mechanism [191].

Finally, early work showed that fluorescently-tagged polyglutamine aggregates of mutant huntingtin protein required the function of the ESCRT-III protein CHMP3/Vps24 for autophagic clearance [192, 193]. However, no follow-up has further detailed alterations of ESCRT activity in Huntington's disease.

Overall these studies clearly suggest that defects in endosomal sorting, autophagy, exosome release and spastin-dependent membrane remodelling contribute to key aspects of the pathology of a broad range of neurodegenerative diseases and that future modulation of ESCRT activity could provide a major therapeutic benefit.

Other diseases linked to ESCRT function

Mutations in the ESCRT-III subunit CHMP4B have been identified in progressive childhood posterior subcapsular cataracts linked to chromosome 20q [194]. According to Sagona and colleagues, CHMP4B may protect the lens from developing cataract by mediating the autophagolysosomal degradation of micronuclei during lens differentiation, or by ensuring efficient cytokinesis [195]. Intestinal epithelial cells of patients with Crohn's Disease, an inflammatory bowel disease, possess significantly upregulated Vps4B expression. This upregulation facilitates apoptosis of intestinal epithelial cells by activating the MAPK signalling pathway [196].

Future challenges and paths to therapy

In recent years, we have witnessed a dramatic expansion in our knowledge of ESCRT activities. In fact, the current landscape of ESCRT-dependent processes covers a large palette of cellular events involving membrane remodelling, well beyond endosomal sorting. More are likely to surface in the next few years. Some of these are likely to explain the currently unclear involvement of ESCRTs in centrosome, chromatin and RNA regulation. However, it is already clear from the wealth of ESCRT functions, that in the future it will be critical to understand more about the factors and the modifications that regulate ESCRT activity in each different process. In this regard, we know that:

1. ESCRT targeting factors greatly differ. ESCRT-0 is used only for endosomal sorting, Gag for HIV-1 budding, CEP55 is specific for cytokinesis, syntenin and ARRDC1 for exo-vesicle secretion, BFRF1, Heh2 and UFD1 for ESCRT activities at the nuclear envelope and ALG-2 for plasma membrane wound repair.
2. ESCRT-III and Vps4 can use ALIX as an alternative upstream ESCRT in HIV-1 release, cytokinesis, exosome formation, plasma membrane repair and Epstein-Barr virus budding from the nuclear envelope.
3. ESCRT-II appears dispensable as a bridging ESCRT during exosome biogenesis, plasma membrane repair and functions at the nuclear envelope.
4. Special ESCRT-III subunits, such as IST1, that assist cytokinesis and nuclear envelope reformation, or CHMP7 (also involved in nuclear envelope reformation) are often used.
5. A number of other components have been identified as accessory ESCRT-III subunits or regulators of the enzymatic activity of VPS4 with unclear specificity. They include Vta1/LIP5, Vps60/CHMP5, Did2/CHMP1 (reviewed in [197]).

Overall, these differences provide us with an initial glimpse of the functional diversity of ESCRT operations that will allow therapeutic targeting of distinct ESCRT processes in the future.

Our knowledge of the ESCRT modifications that might contribute to specificity and to functional modulation is unfortunately less developed. However, pioneering work has been done on post-translational modification of ESCRTs in the context of endosomal sorting. For instance, it has been shown early on that Hrs becomes rapidly phosphorylated in response to growth factors like HGF (hepatocyte growth factor), EGF and platelet-derived growth factor (PDGF) [198, 199] and subsequently ubiquitinated by the E3 ligase Cbl. Hrs modifications disrupt the interaction of its ubiquitin identification motif (UIM) domain with ubiquitinated (Ub-) cargoes and relocate Hrs to the cytosol to facilitate transfer of Ub-cargoes to downstream Ub-binding ESCRT proteins. Also, relocation of ubiquitinated Hrs from endosomal membranes permits replacement by non-ubiquitinated Hrs to sustain endosomal sorting [200–204]. At the level of ESCRT-I, mahogunin-1 monoubiquitinates TSG101 to favour endolysosomal cargo degradation [205–207], while TAL (Tsg101 associated ligase) specifically polyubiquitinates and aims Tsg101 towards degradation, thus inhibiting endosomal trafficking [208, 209]. Since evidence points to oncogenic alteration of signalling in tumours, and to toxic build-up of cargoes in neurodegeneration, such modifications provide potential targets for therapy.

The exact set of cargoes that can initiate modifications and which other cargo-specific factors can directly regulate

ESCRT activity remains unresolved. Interesting in this context is the case of members of the Lgd/CC2D1 protein family (Lgd in *Drosophila*, CC2D1A and CC2D1B in mammals). They interact with CHMP4/Vps32 and appear required for the function of the CHMP4/Vps32 subunit of ESCRT-III complex. However, at least in *Drosophila*, Lgd regulation of ESCRT-III function appears highly specific to a limited subset of cargoes, as it leads only to alteration of Notch and BMP signalling [210–217]. Such example reminds us also of how little is still our understanding of how major signalling pathways are regulated by the ESCRTs. A case in point is that of Notch signalling, which is perturbed in a wide variety of cancers [218]. In *Drosophila*, Notch peculiarly appears to require fusion of MVBs to lysosomes to be ectopically activated by ESCRT impairment [219]. It is not yet clear whether such a form of regulation applies also to mammalian or cancer cells. However, pharmacological inhibition of endosomal acidification appears to reverse excessive Notch signalling also in mammalian cells [220], highlighting how complex and cargo-specific ESCRT regulation of signalling could be in health as well as disease.

In neuropathologies in which ESCRT mutations clearly lead to cargo accumulation, modifications and factors that might generally upregulate cellular clearance might have therapeutic benefits. A favourable strategy to achieve such a goal might be to activate the transcription factor EB (TFEB), a key regulator of lysosome biogenesis. TFEB activity drives autophagosome-lysosome fusion [221]. Phosphorylation of TFEB by the mechanistic target of rapamycin (mTOR) retains TFEB in the cytosol and prevents its translocation to the nucleus as part of the genetic circuitry that controls amino acid metabolism. Pharmacological inhibition of mTOR (e.g., by rapamycin, Torin1 or 2-hydroxypropyl-β-cyclodextrin) causes activation of TFEB [222–225] and together with TFEB overexpression has shown promise in models of neurodegenerative diseases characterised by autophagic accumulation of toxic protein [226–228]. TFEB overexpression appears to alter also Notch-related development signalling events in *Drosophila* [229], suggesting that the strategy might also beneficially modulate signalling originating from late endosomes.

A more complex example of ESCRT modification is that of Myopic (Mop, HD-PTP in mammals), a member of the protein tyrosine phosphatase (PTP) family that co-localises with ESCRT-0 on endosomes and promotes receptor trafficking towards the lysosome [96, 230–234]. Since the phosphatase activity of Mop is not important for this function and its human orthologue HD-PTP does not possess phosphatase activity [235, 236], exactly how Mop regulates the activity of ESCRT-0 is not well understood. It has been proposed that the PTP domain of HD-PTP/Mop might act as a phospho-tyrosine binding module preventing dephosphorylation or influencing the localisation of ESCRT-0 subunits [236]. Interestingly, HD-PTP activity is also required in lieu of ESCRT-II at the plasma membrane for neuron pruning [48], suggesting that interaction between ESCRTs and Mop might occur at multiple levels of membrane remodelling.

Modifications that specifically affect virus budding have also emerged recently. The primary defence against invading pathogens, including viruses, is the production of type-I interferon (IFN); pathogenic viruses have, however, evolved mechanisms to circumvent this control [237]. IFN upregulates the production of IFN-stimulated genes (ISGs). One of these ISGs is the ubiquitin-like ISG15, which has broad-spectrum antiviral activities [238–240]. Overexpression of ISG15 in HIV-1-infected human cells inhibited the replication of HIV-1 and disrupted the interaction of HIV-1 Gag protein with TSG101 by preventing Gag ubiquitination. [241]. This Gag-TSG101 interaction is crucial for efficient budding of HIV-1. Another mechanism of inhibition of HIV-1 budding by ISG15 is the disruption of VPS4 interaction with LIP5/Vta1 by ISGylating CHMP5 (ISGylation is a ubiquitin-like modification). In the absence of LIP5/Vta1, VPS4 fails to oligomerise and is retained in the cytoplasm. This results in failure to disassemble the ESCRT-III complex required for multiple rounds of scission, thus blocking virus budding [242–245]. The effect of ISG15 on host endosomal sorting has not been fully studied, so it is difficult to predict the level of cytotoxicity associated with this therapy. Gag-TSG101 interaction is also specifically inhibited by overexpression of the N-terminal Gag-binding domain of TSG101 (TSG-5'). Because host endosomal sorting remains relatively intact after TSG101-5' overexpression, some authors proposed the development of TSG101-5' derivatives or similarly-acting Gag-TSG101 inhibitors as specific and potent antiviral therapies [246–248]. Inhibiting the interaction of Ebola virus VP40 protein with ESCRT subunits has also been proposed as a therapeutic strategy [249]. Based on all these observations, small molecules have been, and are still being, developed to disrupt the interaction of viral proteins with host ESCRT or ESCRT-associated proteins [250]. A greater understanding of the cofactors that mediate ESCRT-II function in HIV-1 replication will also aid in the development of appropriate inhibitors at this step. Finally, understanding if these therapeutic strategies and the underlying mechanisms of actions are applicable to other types of ESCRT-mediated viral infections will be of great benefit to public health.

In conclusion, the recent explosion of studies documenting the new functions of ESCRT in membrane remodelling and the increasing evidence of deregulation of ESCRT in a wide range of pathologies demand that we step up our effort towards a deeper and process-specific understanding of ESCRT function, both in physiology and in disease. Such understanding will be invaluable for future preventive medicine and disease treatment.

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Figures (large format)

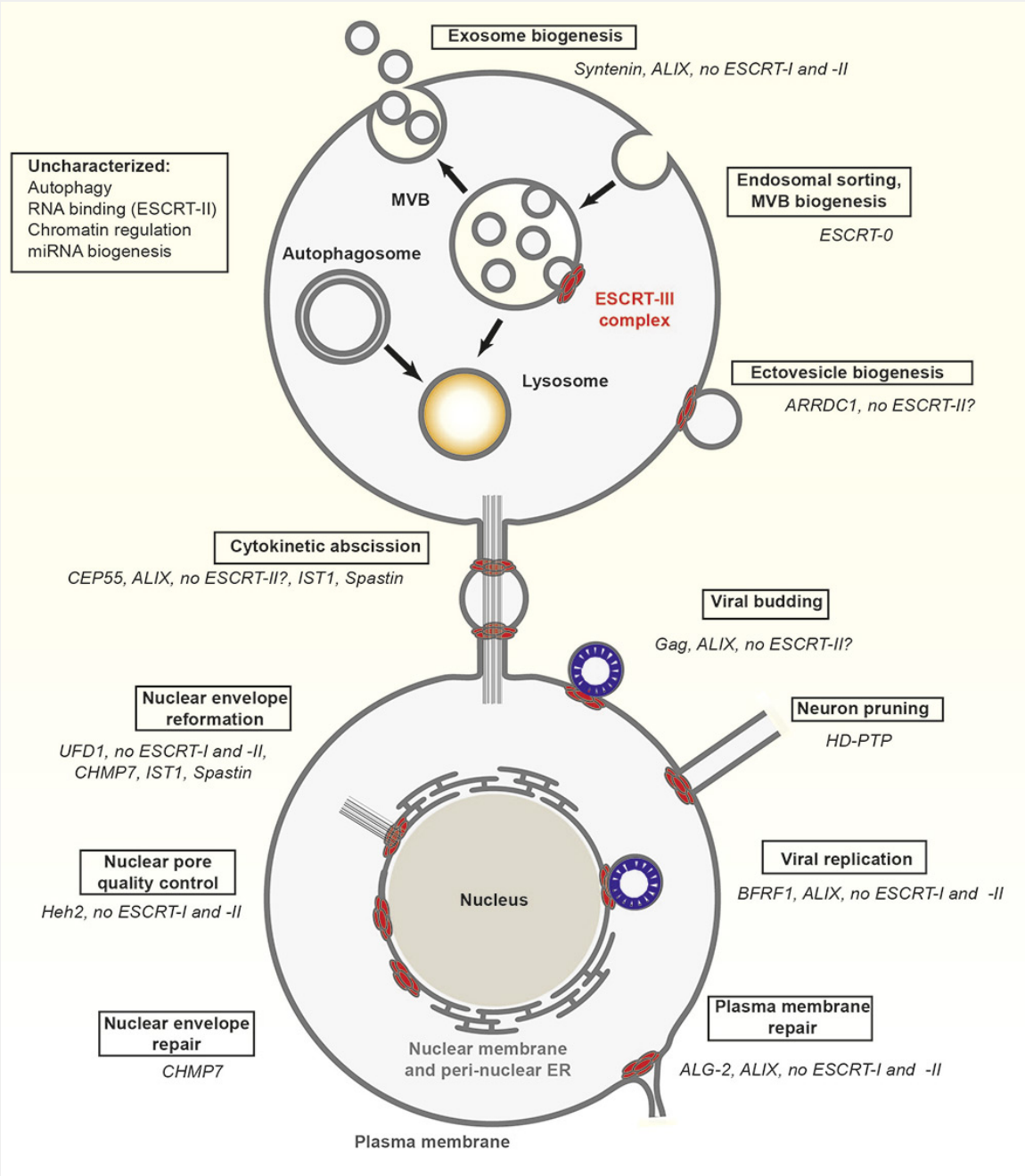

Figure 1

Cell biological functions of ESCRTs (endosomal sorting complexes required for transport).
Overview of ESCRT functions throughout the cell. Factors that are specific for each ESCRT-dependent process (boxed) are listed in italics.