karyotes than in yeast. One level of increased complexity is suggested by the fact that unique yeast class E proteins often have multiple orthologs in mammalian cells. Examples include the 4 different human homologs of the single yeast Vps37p protein (6, 19, 70), the 10 different human homologs of the six yeast ESCRT-III proteins (51), and the 2 (or more) different human homologs of yeast Vps4p (60). Thus, unique proteins or complexes that are essential for MVB formation in yeast may play redundant or overlapping roles in higher eukaryotes. For example, simultaneous depletion of both VPS37B and VPS37C inhibits PTAP-dependent retrovirus budding to a greater extent than depletion of either single VPS37 paralog alone (48).

A second level of complexity is suggested by the fact that even unique ESCRT-I or ESCRT-II subunits do not appear to be required for metazoan MVB vesicle formation in all contexts. For example, a knockout of the *Drosophila* Vps28/ESCRT-I protein has only modest effects on MVB morphology and no measurable effect on the downregulation of several different cell surface receptors in vivo, even though there is only a single *vps28* gene in *Drosophila* (63). Similarly, agonist-induced lysosomal degradation of the delta opioid receptor is inhibited by dominant-negative VPS4B mutants and by depletion of HRS, but not by depletion of TSG101/ESCRT-I (31). Hence, an intact ESCRT-I complex is apparently not required for downregulation of all cell surface receptors via the MVB pathway (27, 31). Similarly, EAP20/ESCRT-II is not required for MHC-I receptor downregulation induced by Kaposi's sarcoma-associated herpesvirus KK3 (reference 11 and data not shown).

Retroviruses also differ in their requirements for ESCRT-I. HIV-1, which binds directly to TSG101, requires all of the known ESCRT-I components for efficient release (19, 22, 48), whereas equine infectious anemia virus and Moloney murine leukemia virus, which bud primarily via ALIX and the Nedd4 E3 ligases, respectively, exhibit little or no requirement for TSG101/ESCRT-I (22, 48, 62, 64, 72). Nevertheless, all retroviruses are inhibited by dominant-negative ESCRT-III and VPS4 proteins, suggesting a common use of these downstream factors (22, 46, 69, 83). This model is consistent with the idea that ESCRT-I and ESCRT-II may function primarily as adaptor complexes that help recruit cargoes into the pathway, whereas the ESCRT-III/VPS4 machinery functions more directly in vesicle formation. Indeed, the genomes of *Plasmodium falciparum* and *Toxoplasma gondii* reportedly lack ESCRT-I and ESCRT-II entirely but do encode ESCRT-III and VPS4 proteins and require VPS4 to create multivesicular bodies (87). Finally, we note that even the ESCRT-III/VPS4 machinery appears to be dispensable in some cases, as the melanosomal protein Pmel17 is sorted into MVB vesicles via an ESCRT-independent pathway that is also insensitive to inhibition by dominant-negative forms of VPS4 (75). The apparent diversity of MVB sorting pathways raises the intriguing possibility that viral systems may also have evolved to utilize such "noncanonical" MVB pathways to escape the cell (34).

Roles for mammalian ESCRT-I and ESCRT-II in receptor downregulation. Although ESCRT-I and ESCRT-II are not absolutely required for the efficient downregulation of all receptors or for the release of all retroviruses, both complexes clearly play very important roles in downregulating certain cell surface receptors. Indeed, a number of recent studies indicate that both ESCRT-I and ESCRT-II exhibit growth/tumor suppressor activities by virtue of their roles in cell surface receptor downregulation. For example, all three known ESCRT-I components (TSG101, VPS28, and VPS37) have now been implicated in cell growth and tumor suppression. TSG101 was initially identified in a genetic screen for genes with tumor suppressor activity, and depletion of TSG101 induces NIH 3T3 cell overproliferation in culture and metastatic tumor formation in nude mice (44). Similarly, deletion of *erupted*, the *Drosophila* TSG101 ortholog, inhibits Notch receptor degradation and activates the JAK-STAT (and possibly other) signaling pathway and thereby induces tissue overproliferation (50). Likewise, depletion of one of the four mammalian VPS37 proteins (6, 19, 70), VPS37A/HCRP-1, enhances the growth of cultured hepatocellular carcinoma BEL-7404 cells and elevates their invasive ability (86). Interestingly, VPS37A/HCRP-1 is also downregulated in some hepatocellular carcinomas. Finally, we have observed that depletion of VPS28 can relieve contact-inhibited growth in cultured 293T cells (J. Garrus, personal communication). ESCRT-II also appears to play an important role in growth receptor downregulation, as deletion of the *Drosophila vps25* gene leads to tissue overproliferation through aberrant stabilization of the Notch and DPP (and possibly other) receptors (76, 77). Similarly, we observed that depletion of EAP20 (human VPS25) reduces the degradation rates of EGFR (Fig. 9 and 10), ferroportin (De Domenico, et al., unpublished), and presumably other cell surface receptors following ligand stimulation. Thus, just as the proteasome functions to degrade soluble proteins, the mammalian ESCRT pathway, including ESCRT-II, can play a critical role in mediating both homeostatic and regulated lysosomal degradation of integral membrane proteins.

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