including at least one member of both the CHMP2 and CHMP4 protein families, and that different members within each of these two families can function redundantly.

In these screens, we found that two core ESCRT-III subunits, CHMP6 and CHMP3, are dispensable for HIV budding, although their homologs play essential roles in yeast MVB biogenesis. These results indicate that the connections between different ESCRT factors probably differ between yeast and man. For example, the yeast CHMP3 homolog (Vps24p) creates an essential bridge between CHMP2 (Vps2p) and CHMP4 (Snf7p), whereas mammalian CHMP4 proteins interact directly with CHMP2 [41]. This interaction appears to alleviate a strict requirement for CHMP3 in HIV-1 budding (Fig. 1).

It has generally been assumed that the different human ESCRT-III proteins and their isoforms function similarly in MVB vesicle formation to their counterparts in the yeast system [36,44]. However, systematic RNAi depletion experiments of CHMP proteins have not yet been performed, due at least in part to the absence of a facile, sensitive assay for measuring mammalian MVB vesicle formation.

VPS4

VPS4 enzymes belong to the AAA-ATPase (ATPases associated with a variety of cellular activities) protein family. The VPS4 proteins function to disassemble ESCRT-III polymers and thereby recycle them to participate in multiple rounds of membrane fission [1,2]. VPS4 binds LIP5, which serves as an activator of VPS4 assembly and ATPase activities [45,46]. VPS4 enzymes are evolutionally conserved, and there is a single Vps protein in yeast (Vps4p) but two VPS4 proteins in humans (VPS4A and VPS4B). VPS4 enzymes all contain an N-terminal microtubule interacting and transport (MIT) domain connected by a linker to a canonical ATPase cassette [47,48]. The VPS4 MIT domains bind to sequence elements known as MIMs (MIT interacting motifs), located at or near the C-termini of ESCRT-III proteins [49,50]. MIT–MIM interactions link the VPS4 proteins and their ESCRT-III substrates and are required to release ESCRT-III polymers from the membrane.

In HIV-1 budding, depletion of either VPS4A or VPS4B alone has only a modest (~50%) inhibitory effect, whereas co-depletion of both VPS4A and VPS4B dramatically inhibits budding (> 95%) [44,51]. This synergistic inhibition indicates that VPS4A and VPS4B function redundantly in HIV budding (Fig. 1).

Co-depletion of VPS4A and VPS4B also inhibits ligand-dependent EGFR degradation [51], although the effects of depleting each VPS4 isoform individually are not yet known. However, single depletions of either VPS4A or VPS4B have quite a dramatic inhibitory effect on cytokinesis/cell division [42], suggesting that each of the VPS4 isoforms performs a non-redundant function in this pathway (Fig. 1).

Another MIT domain containing AAA-ATPase, Spastin, has been reported to interact with CHMP1 and function in cytokinesis [52,53]. The function of Spastin has been implicated in cytoskeletal rearrangement and dynamics, via microtubule severing. It is not surprising if Spastin may specifically function in cytokinesis, by coordinating both microtubule severing and membrane abscission at the midbody.

Other MIT domain proteins

In addition to VPS4 and Spastin, several MIT-domain-containing proteins have been reported to be involved in the ESCRT pathways. Two ubiquitin hydrolases, UBPY and AMSH, previously identified as binding partners for the STAM-HRS complex [54,55], have recently been shown to possess MIT domains and to use them to interact with specific ESCRT-III proteins [56–58]. Both UBPY and AMSH have deubiquitinating enzyme activities and are thought to modulate the modification levels of ubiquitylated target proteins within ESCRT pathways [59–62].

Another MIT domain protein is calpain-7, one of the cysteine proteases of the calpain superfamily. The MIT domain of calpain-7 associates with CHMP1 and IST1 [63]. However, the role of calpain-7 in cytokinesis is not fully understood. The yeast ortholog of calpain-7, Rim13, regulates proteolytic activation of the transcription factor Rim101, a pH-responsive zinc finger transcription factor [64,65]. Interestingly, several biochemical and genetic experiments have revealed that Rim101 activation is dependent on ESCRT factors [66]. It is therefore conceivable that calpain-7 functions in activation of unknown membrane associated transcription factors that may be activated by the ESCRT pathway in mammalian cells [67].

Conclusion

Comparative studies of ESCRT function during MVB biogenesis, enveloped virus budding and cytokinesis have begun to elucidate the complexity of the ESCRT pathway. However, additional functional studies are required to elucidate the exact roles of different ESCRT factors and their selective requirements for

different biological processes. The ESCRT pathway has been linked to several important classes of human diseases, including tumorigenesis, neurodegeneration and infectious disease, and an improved understanding of the differential requirements for ESCRT factors may therefore assist in the development of therapeutic agents with minimum adverse effects.

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References

1 Henne WM, Buchkovich NJ & Emr SD (2011) The ESCRT pathway. *Dev Cell* **21**, 77–91.
2 Hurley JH & Hanson PI (2010) Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nat Rev Mol Cell Biol* **11**, 556–566.
3 Katzmann DJ, Babst M & Emr SD (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* **106**, 145–155.
4 Morita E & Sundquist WI (2004) Retrovirus budding. *Annu Rev Cell Dev Biol* **20**, 395–425.
5 Saksena S, Wahlman J, Teis D, Johnson AE & Emr SD (2009) Functional reconstitution of ESCRT-III assembly and disassembly. *Cell* **136**, 97–109.
6 Teis D, Saksena S & Emr SD (2008) Ordered assembly of the ESCRT-III complex on endosomes is required to sequester cargo during MVB formation. *Dev Cell* **15**, 578–589.
7 Wollert T & Hurley JH (2010) Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature* **464**, 864–869.
8 Garrus JE, Schwedler von UK, Pornillos OW, Morham SG, Zavitz KH, Wang HE, Wettstein DA, Stray KM, Côté M, Rich RL *et al.* (2001) Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*, **107**, 55–65.
9 Strack B, Calistri A, Craig S, Popova E & Göttlinger HG (2003) AIP1/ALIX is a binding partner for HIV-1 p6 and EIAV p9 functioning in virus budding. *Cell* **114**, 689–699.
10 Bache KG, Brech A, Mehlum A & Stenmark H (2003) Hrs regulates multivesicular body formation via ESCRT recruitment to endosomes. *J Cell Biol* **162**, 435–442.
11 Pornillos O, Higginson DS, Stray KM, Fisher RD, Garrus JE, Payne M, He G-P, Wang HE, Morham SG & Sundquist WI (2003) HIV Gag mimics the Tsg101-recruiting activity of the human Hrs protein. *J Cell Biol* **162**, 425–434.
12 Lu Q, Hope LW, Brasch M, Reinhard C & Cohen SN (2003) TSG101 interaction with HRS mediates endosomal trafficking and receptor down-regulation. *Proc Natl Acad Sci USA* **100**, 7626–7631.
13 Katzmann DJ, Stefan CJ, Babst M & Emr SD (2003) Vps27 recruits ESCRT machinery to endosomes during MVB sorting. *J Cell Biol* **162**, 413–423.
14 Puertollano R & Bonifacino JS (2004) Interactions of GGA3 with the ubiquitin sorting machinery. *Nat Cell Biol* **6**, 244–251.
15 Puertollano R (2005) Interactions of TOM1L1 with the multivesicular body sorting machinery. *J Biol Chem* **280**, 9258–9264.
16 Fabbro M, Zhou B-B, Takahashi M, Sarcevic B, Lal P, Graham ME, Gabrielli BG, Robinson PJ, Nigg EA, Ono Y *et al.* (2005) Cdk1/Erk2- and Plk1-dependent phosphorylation of a centrosome protein, Cep55, is required for its recruitment to midbody and cytokinesis. *Dev Cell* **9**, 477–488.
17 Morita E, Sandrin V, Chung H-Y, Morham SG, Gygi SP, Rodesch CK & Sundquist WI (2007) Human ESCRT and ALIX proteins interact with proteins of the midbody and function in cytokinesis. *EMBO J* **26**, 4215–4227.
18 Lee HH, Elia N, Ghirlando R, Lippincott-Schwartz J & Hurley JH (2008) Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55. *Science*, **322**, 576–580.
19 Morita E, Sandrin V, Alam SL, Eckert DM, Gygi SP & Sundquist WI (2007) Identification of human MVB12 proteins as ESCRT-I subunits that function in HIV budding. *Cell Host Microbe* **2**, 41–53.
20 Kostelansky MS, Schluter C, Tam YYC, Lee S, Ghirlando R, Beach B, Conibear E & Hurley JH (2007) Molecular architecture and functional model of the complete yeast ESCRT-I heterotetramer. *Cell* **129**, 485–498.
21 Carlton JG & Martin-Serrano J (2007) Parallels between cytokinesis and retroviral budding: a role for the ESCRT machinery. *Science* **316**, 1908–1912.
22 Eastman SW, Martin-Serrano J, Chung W, Zang T & Bieniasz PD (2005) Identification of human VPS37C, a component of endosomal sorting complex required for transport-I important for viral budding. *J Biol Chem* **280**, 628–636.
23 Bache KG, Slagsvold T, Cabezas A, Rosendal KR, Raiborg C & Stenmark H (2004) The growth-regulatory protein HCRP1/hVps37A is a subunit of mammalian ESCRT-I and mediates receptor down-regulation. *Mol Biol Cell* **15**, 4337–4346.
24 Konishi H, Tashiro K, Murata Y, Nabeshi H, Yamauchi E & Taniguchi H (2006) CFBP is a novel tyrosine-phosphorylated protein that might function as

a regulator of CIN85/CD2AP. *J Biol Chem* **281**, 28919–28931.
25 Stefani F, Zhang L, Taylor S, Donovan J, Rollinson S, Doyotte A, Brownhill K, Bennion J, Pickering-Brown S & Woodman P (2011) UBAP1 is a component of an endosome-specific ESCRT-I complex that is essential for MVB sorting. *Curr Biol* **21**, 1245–1250.
26 Qian J, Yang J, Zhang X, Zhang B, Wang J, Zhou M, Tang K, Li W, Zeng Z, Zhao X *et al.* (2001) Isolation and characterization of a novel cDNA, UBAP1, derived from the tumor suppressor locus in human chromosome 9p21-22. *J Cancer Res Clin Oncol* **127**, 613–618.
27 Fisher RD, Chung H-Y, Zhai Q, Robinson H, Sundquist WI & Hill CP (2007) Structural and biochemical studies of ALIX/AIP1 and its role in retrovirus budding. *Cell* **128**, 841–852.
28 Katoh K, Suzuki H, Terasawa Y, Mizuno T, Yasuda J, Shibata H & Maki M (2005) The penta-EF-hand protein ALG-2 interacts directly with the ESCRT-I component TSG101, and Ca^{2+}-dependently co-localizes to aberrant endosomes with dominant-negative AAA ATPase SKD1/Vps4B. *Biochem J* **391**, 677–685.
29 Schwedler von UK, Stuchell M, Müller B, Ward DM, Chung H-Y, Morita E, Wang HE, Davis T, He G-P, Cimbora DM *et al.* (2003) The protein network of HIV budding. *Cell* **114**, 701–713.
30 Doyotte A, Mironov A, McKenzie E & Woodman P (2008) The Bro1-related protein HD-PTP/PTPN23 is required for endosomal cargo sorting and multivesicular body morphogenesis. *Proc Natl Acad Sci USA* **105**, 6308–6313.
31 Hierro A, Sun J, Rusnak AS, Kim J, Prag G, Emr SD & Hurley JH (2004) Structure of the ESCRT-II endosomal trafficking complex. *Nature* **431**, 221–225.
32 Teo H, Perisic O, González B & Williams RL (2004) ESCRT-II, an endosome-associated complex required for protein sorting: crystal structure and interactions with ESCRT-III and membranes. *Dev Cell* **7**, 559–569.
33 Babst M, Katzmann DJ, Snyder WB, Wendland B & Emr SD (2002) Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. *Dev Cell* **3**, 283–289.
34 Gill DJ, Teo H, Sun J, Perisic O, Veprintsev DB, Emr SD & Williams RL (2007) Structural insight into the ESCRT-I/-II link and its role in MVB trafficking. *EMBO J* **26**, 600–612.
35 Babst M, Katzmann DJ, Estepa-Sabal EJ, Meerloo T & Emr SD (2002) Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. *Dev Cell* **3**, 271–282.
36 Langelier C, Schwedler von UK, Fisher RD, De Domenico I, White PL, Hill CP, Kaplan J, Ward D & Sundquist WI (2006) Human ESCRT-II complex and its role in human immunodeficiency virus type 1 release. *J Virol* **80**, 9465–9480.
37 Im YJ & Hurley JH (2008) Integrated structural model and membrane targeting mechanism of the human ESCRT-II complex. *Dev Cell* **14**, 902–913.
38 Malerød L, Stuffers S, Brech A & Stenmark H (2007) Vps22/EAP30 in ESCRT-II mediates endosomal sorting of growth factor and chemokine receptors destined for lysosomal degradation. *Traffic* **8**, 1617–1629.
39 Dimaano C, Jones CB, Hanono A, Curtiss M & Babst M (2008) Ist1 regulates Vps4 localization and assembly. *Mol Biol Cell* **19**, 465–474.
40 Rue SM, Mattei S, Saksena S & Emr SD (2008) Novel Ist1-Did2 complex functions at a late step in multivesicular body sorting. *Mol Biol Cell* **19**, 475–484.
41 Morita E, Sandrin V, McCullough J, Katsuyama A, Baci Hamilton I & Sundquist WI (2011) ESCRT-III protein requirements for HIV-1 budding. *Cell Host Microbe* **9**, 235–242.
42 Morita E, Colf LA, Karren MA, Sandrin V, Rodesch CK & Sundquist WI (2010) Human ESCRT-III and VPS4 proteins are required for centrosome and spindle maintenance. *Proc Natl Acad Sci USA* **107**, 12889–12894.
43 Bajorek M, Morita E, Skalicky JJ, Morham SG, Babst M & Sundquist WI (2009) Biochemical analyses of human IST1 and its function in cytokinesis. *Mol Biol Cell* **20**, 1360–1373.
44 Kieffer C, Skalicky JJ, Morita E, De Domenico I, Ward DM, Kaplan J & Sundquist WI (2008) Two distinct modes of ESCRT-III recognition are required for VPS4 functions in lysosomal protein targeting and HIV-1 budding. *Dev Cell* **15**, 62–73.
45 Lottridge JM, Flannery AR, Vincelli JL & Stevens TH (2006) Vta1p and Vps46p regulate the membrane association and ATPase activity of Vps4p at the yeast multivesicular body. *Proc Natl Acad Sci USA* **103**, 6202–6207.
46 Azmi IF, Davies BA, Xiao J, Babst M, Xu Z & Katzmann DJ (2008) ESCRT-III family members stimulate Vps4 ATPase activity directly or via Vta1. *Dev Cell* **14**, 50–61.
47 Scott A, Chung H-Y, Gonciarz-Swiatek M, Hill GC, Whitby FG, Gaspar J, Holton JM, Viswanathan R, Ghaffarian S, Hill CP *et al.* (2005) Structural and mechanistic studies of VPS4 proteins. *EMBO J* **24**, 3658–3669.
48 Scott A, Gaspar J, Stuchell-Brereton MD, Alam SL, Skalicky JJ & Sundquist WI (2005) Structure and ESCRT-III protein interactions of the MIT domain of human VPS4A. *Proc Natl Acad Sci USA* **102**, 13813–13818.
49 Stuchell-Brereton MD, Skalicky JJ, Kieffer C, Karren MA, Ghaffarian S & Sundquist WI (2007) ESCRT-III recognition by VPS4 ATPases. *Nature*, **449**, 740–744.

50 Obita T, Saksena S, Ghazi-Tabatabai S, Gill DJ, Perisic O, Emr SD & Williams RL (2007) Structural basis for selective recognition of ESCRT-III by the AAA ATPase Vps4. *Nature*, **449**, 735–739.
51 Fraile-Ramos A, Pelchen-Matthews A, Risco C, Rejas MT, Emery VC, Hassan-Walker AF, Esteban M & Marsh M (2007) The ESCRT machinery is not required for human cytomegalovirus envelopment. *Cell Microbiol* **9**, 2955–2967.
52 Reid E, Connell J, Edwards TL, Duley S, Brown SE & Sanderson CM (2005) The hereditary spastic paraplegia protein spastin interacts with the ESCRT-III complex-associated endosomal protein CHMP1B. *Hum Mol Genet* **14**, 19–38.
53 Yang D, Rismanchi N, Renvoisé B, Lippincott-Schwartz J, Blackstone C & Hurley JH (2008) Structural basis for midbody targeting of spastin by the ESCRT-III protein CHMP1B. *Nat Struct Mol Biol* **15**, 1278–1286.
54 Kato M, Miyazawa K & Kitamura N (2000) A deubiquitinating enzyme UBPY interacts with the Src homology 3 domain of Hrs-binding protein via a novel binding motif PX(V/I)(D/N)RXXKP. *J Biol Chem* **275**, 37481–37487.
55 Tanaka N, Kaneko K, Asao H, Kasai H, Endo Y, Fujita T, Takeshita T & Sugamura K (1999) Possible involvement of a novel STAM-associated molecule 'AMSH' in intracellular signal transduction mediated by cytokines. *J Biol Chem* **274**, 19129–19135.
56 Row PE, Liu H, Hayes S, Welchman R, Charalabous P, Hofmann K, Clague MJ, Sanderson CM & Urbé S (2007) The MIT domain of UBPY constitutes a CHMP binding and endosomal localization signal required for efficient epidermal growth factor receptor degradation. *J Biol Chem* **282**, 30929–30937.
57 Agromayor M & Martin-Serrano J (2006) Interaction of AMSH with ESCRT-III and deubiquitination of endosomal cargo. *J Biol Chem* **281**, 23083–23091.
58 Solomons J, Sabin C, Poudevigne E, Usami Y, Hulsik DL, Macheboeuf P, Hartlieb B, Göttlinger H & Weissenhorn W (2011) Structural basis for ESCRT-III CHMP3 recruitment of AMSH. *Structure* **19**, 1149–1159.
59 Mizuno E, Kobayashi K, Yamamoto A, Kitamura N & Komada M (2006) A deubiquitinating enzyme UBPY regulates the level of protein ubiquitination on endosomes. *Traffic* **7**, 1017–1031.
60 Mizuno E, Iura T, Mukai A, Yoshimori T, Kitamura N & Komada M (2005) Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. *Mol Biol Cell* **16**, 5163–5174.
61 McCullough J, Row PE, Lorenzo O, Doherty M, Beynon R, Clague MJ & Urbé S (2006) Activation of the endosome-associated ubiquitin isopeptidase AMSH by STAM, a component of the multivesicular body-sorting machinery. *Curr Biol* **16**, 160–165.
62 Row PE, Prior IA, McCullough J, Clague MJ & Urbé S (2006) The ubiquitin isopeptidase UBPY regulates endosomal ubiquitin dynamics and is essential for receptor down-regulation. *J Biol Chem* **281**, 12618–12624.
63 Maemoto Y, Osako Y, Goto E, Nozawa E, Shibata H & Maki M (2011) Calpain-7 binds to CHMP1B at its second {alpha}-helical region and forms a ternary complex with IST1. *J Biochem* **150**, 411–421.
64 Hayashi M, Fukuzawa T, Sorimachi H & Maeda T (2005) Constitutive activation of the pH-responsive Rim101 pathway in yeast mutants defective in late steps of the MVB/ESCRT pathway. *Mol Cell Biol* **25**, 9478–9490.
65 Rothfels K, Tanny JC, Molnar E, Friesen H, Commisso C & Segall J (2005) Components of the ESCRT pathway, DFG16, and YGR122w are required for Rim101 to act as a corepressor with Nrg1 at the negative regulatory element of the DIT1 gene of Saccharomyces cerevisiae. *Mol Cell Biol* **25**, 6772–6788.
66 Maeda T (2012) The signaling mechanism of ambient pH sensing and adaptation in yeast and fungi. *FEBS J* **279**, 1407–1413.
67 Maki M, Maemoto Y, Osako Y & Shibata H (2012) Evolutionary and physical linkage between calpains and penta-EF-hand Ca^{2+}-binding proteins. *FEBS J* **279**, 1414–1421.