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Human ESCRT-II Complex and Its Role in Human Immunodeficiency Virus Type 1 Release†

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The budding of many enveloped RNA viruses, including human immunodeficiency virus type 1 (HIV-1), requires some of the same cellular machinery as vesicle formation at the multivesicular body (MVB). In *Saccharomyces cerevisiae*, the ESCRT-II complex performs a central role in MVB protein sorting and vesicle formation, as it is recruited by the upstream ESCRT-I complex and nucleates assembly of the downstream ESCRT-III complex. Here, we report that the three subunits of human ESCRT-II, EAP20, EAP30, and EAP45, have a number of properties in common with their yeast orthologs. Specifically, EAP45 bound ubiquitin via its N-terminal GRAM-like ubiquitin-binding in EAP45 (GLUE) domain, both EAP45 and EAP30 bound the C-terminal domain of TSG101/ESCRT-I, and EAP20 bound the N-terminal half of CHMP6/ESCRT-III. Consistent with its expected role in MVB vesicle formation, (i) human ESCRT-II localized to endosomal membranes in a VPS4-dependent fashion and (ii) depletion of EAP20/ESCRT-II and CHMP6/ESCRT-III inhibited lysosomal targeting and downregulation of the epidermal growth factor receptor, albeit to a lesser extent than depletion of TSG101/ESCRT-I. Nevertheless, HIV-1 release and infectivity were not reduced by efficient small interfering RNA depletion of EAP20/ESCRT-II or CHMP6/ESCRT-III. These observations indicate that there are probably multiple pathways for protein sorting/MVB vesicle formation in human cells and that HIV-1 does not utilize an ESCRT-II-dependent pathway to leave the cell.

Enveloped RNA viruses like human immunodeficiency virus (HIV) acquire lipid bilayers and exit infected cells by budding through limiting membranes. The process of HIV budding shares a number of similarities with the cellular process of vesicle formation at the multivesicular body (MVB) (reviewed in references 2, 14, 25, 26, 33, 40, and 51). MVB vesicles and HIV virions both bud away from the cytoplasm (58) and share the following mechanistic similarities: (i) a requirement for ubiquitin (Ub) in formation and cargo incorporation (reviewed in references 40 and 81), (ii) recruitment and utilization of the cellular ESCRT-I complex (6, 15, 19, 22, 47, 48, 52, 70, 79), and (iii) a requirement for ESCRT-III, LIP5, and VPS4 ATPase activities (22, 46, 69, 83, 85). In spite of these similarities, however, MVB biogenesis and viral budding ultimately create different vesicles, and it is therefore of interest to determine the precise relationship between the two processes.

Genetic screens in *Saccharomyces cerevisiae* defined the basic machinery of MVB vesicle formation and identified 17 soluble yeast proteins that are essential for MVB biogenesis (the "class E" proteins) (40, 59). Complementary biochemical analyses revealed that MVB vesicle formation proceeds through an ordered pathway in which a series of soluble class E complexes, termed ESCRT-I (39), ESCRT-II (4), and ESCRT-III (3), are sequentially recruited to endosomal membranes, where they function in vesicle formation (Fig. 1A). Although the processes of vesicle formation and cargo incorporation are not yet understood in mechanistic detail, ESCRT-I and -II appear to function primarily as adaptors that recognize protein cargoes and help recruit ESCRT-III, which in turn appears to function more directly in protein sorting and vesicle formation.

As the central complex in the yeast ESCRT pathway, ESCRT-II bridges ESCRT-I and ESCRT-III and also interacts with ubiquitylated cargoes. As shown in Fig. 1B, crystal structures of the yeast ESCRT-II core (30, 74) have revealed that the yeast ESCRT-II complex contains two copies of Vps25p (EAP20) that bind asymmetrically to single copies of Vps22p (EAP30) and Vps36p (EAP45) (human protein names are provided in parentheses for reference). The two Vps25p (EAP20) proteins project away from the Vps22p-Vps36p dimer, so that the overall complex assumes a trilobed "Y" shape. The three ESCRT-II subunits are related structurally, as each contains two copies of the winged-helix (WH) protein motif. Two of the proteins also contain N-terminal extensions: Vps22p (EAP30) has an extended helix (Fig. 1B), and Vps36p (EAP45) contains a linker and an N-terminal GRAM-like ubiquitin-binding in EAP45 (GLUE) domain (not present in the core structure). Structural and biochemical analyses of the Vps36p GLUE domain have shown that it is a split PH domain that binds phosphatidylinositol phosphates, particularly PI(3)P (67, 73). An extended insert within the GLUE domain contains two Npl4 zinc fingers (NZF), which are small globular zinc binding modules that mediate protein-protein interactions (49). The first, NZF-N, binds Vps28p (ESCRT-I) and helps recruit ESCRT-II to the endosomal membrane (73), whereas the second, NZF-C, binds ubiquitin and is required for efficient sorting of ubiquitylated cargoes (1).

The human and yeast ESCRT-II complexes are likely to be related structurally, because mammalian ESCRT-II can be iso-

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† Supplemental material for this article may be found at http://jvi.asm.org.