Host factors in virus budding – Insights from yeast

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The budding yeast, *Saccharomyces cerevisiae,* is an excellent model organism for the study of eukaryotic cellular processes such as endocytosis [1]. Like other eukaryotic cells, yeast take up extracellular material by invagination of the plasma membrane to form a vesicle. The internalised material is transported to a membrane-bound compartment, the early endosome. As the early endosome matures, internal vesicles form within the lumen giving it the appearance of a multivesicular body (vesicles enclosed by a membrane). The machinery required for endosome maturation is highly conserved between yeast and mammalian cells. In mammalian cells this machinery is also required for the budding of enveloped viruses. Here we discuss how studies of endosome maturation in *S. cerevisiae* have given valuable insights into the mechanism by which clinically important enveloped viruses, including human immunodeficiency virus (HIV) and hepatitis B virus, are released from mammalian cells.

Endocytosis and endocytic trafficking

The cell membrane is a dynamic structure that separates the interior of the cell from the extracellular milieu. Eukaryotic cells have evolved an endocytic vesicular trafficking system that allows the uptake of extracellular material. During endocytosis (Figure 1), a region of the plasma membrane, including membrane-associated receptors, invaginates to forms a vesicle incorporating both plasma membrane and extracellular material. The vesicle is transported to a membrane-bound compartment, an early endosome, which then matures into a late endosome. During maturation, some membrane proteins are sorted into internal vesicles that form by invagination of the endosome limiting membrane. These internal vesicles give the endosome the appearance of a multivesicular body (MVB) and the process is referred to as MVB sorting.

Mature MVBs fuse with the lysosome (a degradative organelle) allowing degradation of their contents including the internal vesicles. This process is important for down-regulation of signalling receptors and defects in this process are implicated in cancer. In some cells, MVBs fuse with the plasma membrane and their internal vesicles may function in inter-cellular signalling. For example, internal vesicles have been reported to be released by antigen presenting cells [2-4].

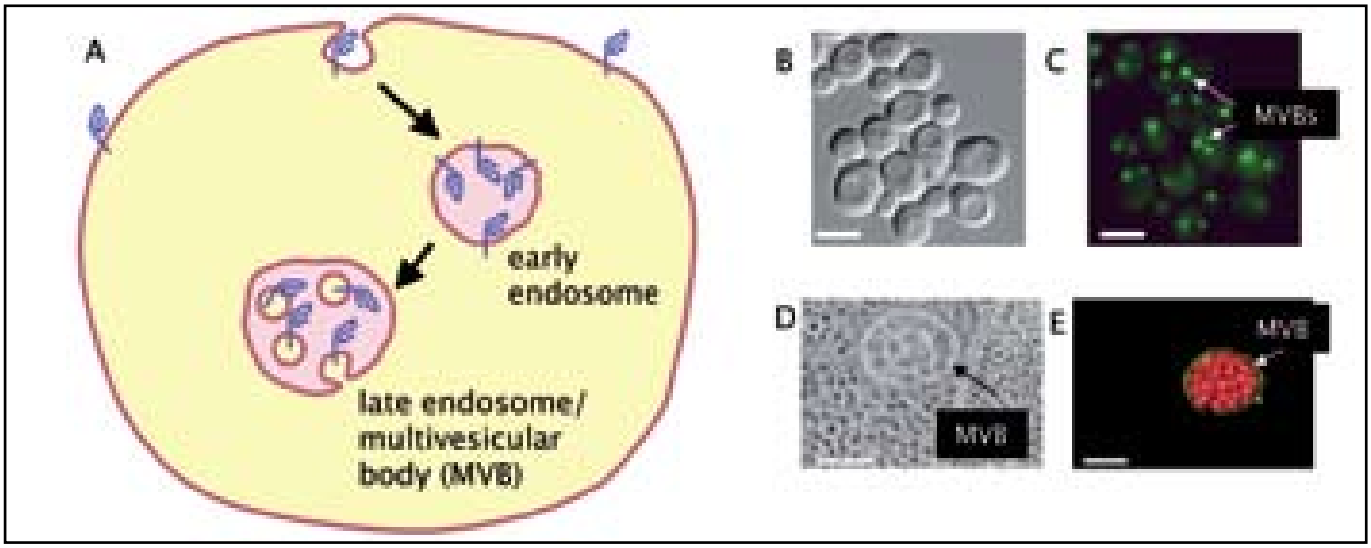

Figure 1. (A) Simplified schematic illustrating transport of endocytosed material to an early endosome and maturation of the early endosome into a multivesicular body (late endosome). Integral membrane proteins (drawn in blue) are sorted into internal vesicles that form by invagination of the endosome limiting membrane. (B & C) Budding yeast cells expressing a GFP-tagged Vps4 construct showing Vps4 association with endosomes. The same field of cells is shown under brightfield with differential interference contrast [B] or fluorescence [C]. Scale bar in B and C is 5 mm. (D) A representative cross-section of an electron tomogram of a single MVB from yeast cells. E Three dimensional reconstruction of a single MVB derived from electron tomograms. Scale bar in D and E is 100 nm. (D & E reproduced from *Journal of Cell Biology*, ©Rockefeller University Press) [14].

Identification and characterisation of the multivesicular body (MVB) sorting machinery in yeast

Identification of the machinery of MVB sorting has been made possible by genetic screens in yeast [5]. Subsequent studies in mammalian cells showed that there are homologues of each of the components of the yeast MVB sorting machinery. Our current understanding of the mechanism of MVB sorting is largely based on studies from yeast.

There are numerous advantages of studying the mechanism of MVB sorting in yeast. First, yeast is easy to genetically manipulate. Viable yeast knockout strains lacking key MVB sorting machinery components are available (where tested, knockout of the mammalian homologues is lethal). Second, the ease of genetic crosses and the availability of a collection of yeast gene knockouts [6] allow the identification of genetic interactions on a genome wide scale. Third, the ability to express tagged forms of a protein under the endogenous promoter avoids potential artefacts caused by overexpression, which is often unavoidable using mammalian expression plasmids. Finally, in yeast a single isoform of each component of the MVB sorting machinery is present compared to mammalian cells, which contain multiple isoforms of several components thus complicating gene knock experiments.

The mechanism of MVB sorting is starting to emerge. There are eighteen components of the MVB sorting machinery in yeast and many of these assemble into three sub-complexes, referred to as endosomal sorting complexes required for transport (ESCRT complexes I-III). During MVB sorting, ESCRT complexes are sequentially recruited to the endosome membrane by integral membrane proteins that are destined for MVB sorting [5].

One component of the MVB sorting machinery that is recruited late in the process is an ATPase known as vacuolar protein sorting 4 (Vps4). Vps4 releases the MVB sorting machinery from the endosome limiting membrane as it buds to form an internal vesicle. Efforts in our lab and many others are directed toward understanding the mechanism by which Vps4 mediates these events. Our recent findings indicate that Vps4 acts directly on multiple components of the MVB sorting machinery to mediate its release from the endosome membrane.

Hijacking of the MVB sorting machinery by enveloped viruses

Interest in understanding the mechanism of MVB sorting has intensified since the discovery that components of the MVB sorting machinery are utilised by enveloped viruses for budding (Table 1) [7]. Both enveloped virus budding and MVB sorting are topologically similar, since both involve budding of a membrane away from the cytoplasm. Short sequences found within viral envelope polypeptides are essential for budding of certain enveloped viruses. These sequences, referred to as late domains, recruit the MVB sorting machinery, in a manner analogous to endogenous membrane proteins destined for MVB sorting [8]. Different viruses use different components of the MVB sorting machinery, but most viruses examined require Vps4 [8-12]. In the absence of Vps4, the virus particles are trapped at the stage of virus budding (Figure 2).

Viruses that use the MVB sorting machinery for budding include HIV, hepatitis B virus and Ebola virus. Some of the diseases in humans caused by these viruses are devastating and incurable (Table 2).

The MVB sorting machinery as a target for antiviral drugs

Current attempts to treat viral infections rely on therapeutic agents directed against viral encoded components. However, due to the rate at which the viruses mutate and develop resistance, there is a growing consensus that therapeutic agents directed against host cell components utilised for virus entry and budding may be worth exploring [13]. Our studies to understand the mechanism of MVB sorting will concomitantly lead to a greater understanding of the mechanism of enveloped virus budding and have the potential to contribute to the development of new antiviral drugs.

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Table 1. Host factors implicated in enveloped virus budding from mammalian cells.

Proteins	Functions in MVB sorting	Viruses dependent of host proteins
Tsg101 (#*S.c.* Vps23)	Yes	human immunodeficiency virus (HIV), human T-cell leukaemia virus (HTLV)
Vps28 (#*S.c.* Vps28)	Yes	HIV
Chmp2 (#*S.c.* Vps2)	Yes	HIV, murine leukemia virus (MLV)
Chmp3 (#*S.c.* Vps24)	Yes	HIV
Chmp4 (#*S.c.* Vps32)	Yes	HIV
ALIX (#*S.c.* Bro1)	Yes	HIV, equine infectious anaemia virus (EIAV)
Chmp5 (#*S.c.* Vps60)	Yes	HIV
mVps4 (#*S.c.* Vps4)	Yes	HIV, MLV, EIAV, hepatitis B virus, ebola virus, parainfluenza virus

#*S.c.* = *S. cerevisiae*

Table 2. Incidence of diseases caused by enveloped viruses that rely on Vps4 for budding

Virus	Host	Incidence
HIV	Human	39.5 million cases worldwide
Hepatitis B virus	Human	350 to 400 million cases worldwide
Ebola virus	Human, Gorilla	a recent outbreak caused 143 infections, mortality rate is 90% estimated to have wiped out one-quarter of the world's gorillas
Parainfluenza virus 5	Dog	ND*
EIAV	Horse, Mule, Donkey	ND*
Murine leukaemia virus	Mouse	ND*

*ND = not determined

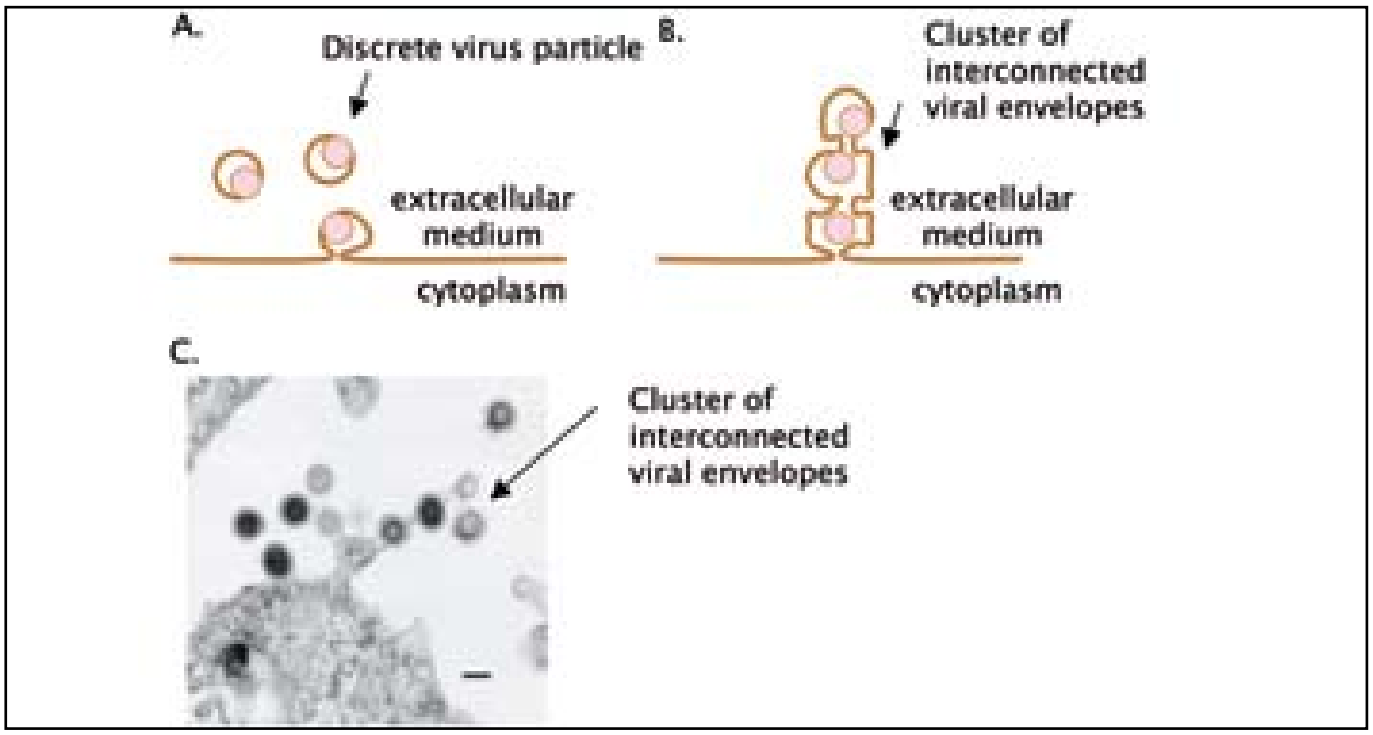

Fig. 2 Arrest of enveloped virus budding upon inhibition of MVB sorting. (A) Schematic representation of enveloped viruses budding from the plasma membrane. (B) Schematic representation of a cluster of interconnected viral envelopes that remain connected to the plasma membrane upon inhibition of a component of the MVB sorting machinery, such as Vps4. (C) A representative thin section electron micrograph showing the accumulation of murine leukaemia virus on the surface of infected cells upon inhibition of Vps4. Scale bar 100 nm. (C Reprinted from Cell 2001;107, with permission from Elsevier ©2001 [8]).

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Note: Following the submission of this article, two recent papers[15,16] have shown that the MVB sorting machinery is also implicated in the release of both Marburg virus and Epstein-Barr virus.