Nature Reviews Microbiology | AOP, published online 12 October 2010; doi:10.1038/nrmicro2458

VIROLOGY

Severing the bud

Although many viruses use the host endosomal complex required for transport (ESCRT) to mediate budding and scission, influenza is thought to use an ESCRT-independent mechanism that was not clear. Lamb and colleagues now reveal that influenza budding and scission depends on the ion channel protein matrix 2 (M2).

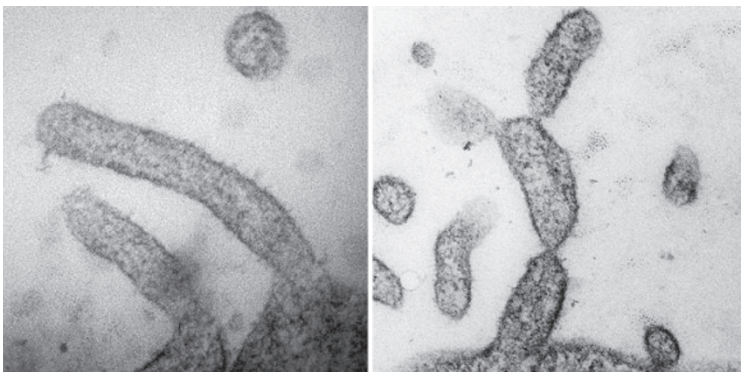
M2 is part of the viral envelope, which also contains the viral glycoproteins haemagglutinin and neuraminidase; these glycoproteins associate with M1, which recruits M2, thereby incorporating it in the virion. M2 is known to be involved in the viral life cycle through its ion channel activities, and recent studies indicate that it might also affect viral budding. Using an *in vitro* reconstitution system, the authors found that M2 affects membrane curvature in a cholesterol-dependent manner and can induce vesicle formation in giant unilamellar vesicles (GUVs) in the presence of low or intermediate (17 mol %) cholesterol levels through its amphipathic helix. Reconstitution of GUVs with this amphipathic helix alone could also induce vesicle formation in the presence of 17 mol % cholesterol (which is comparable to the cholesterol concentration in areas of the plasma membrane), confirming the role of the M2 amphipathic helix in budding and scission.

Because GUVs have only one lipid phase (that is, lipids are spread homogeneously), they do not accurately mimic the plasma membrane, so the authors examined whether M2 can also induce budding in phase-separated GUVs. At low cholesterol levels, the M2 amphipathic helix bound the lipid-disordered phase of the GUV, clustering at the phase boundary, and induced excision of the lipid-ordered phase, initiating budding. Similar observations were made using full-length M2 in plasma membrane spheres, indicating that M2 induces budding by modifying the line tension between lipid phases.

So what is the role of M2 in viral budding *in vivo*? Electron microscopy showed that, in cells infected with wild-type virus, M2 localizes at the base of budding virions, where scission occurs. Interestingly, influenza with a mutated M2 amphipathic helix could bud but showed impaired scission, resulting in a string of attached viral particles. This mutated M2 localized at the constrictions between incompletely budded virions, suggesting that M2 is not required for budding but is necessary for scission.

On the basis of their findings and previous work, the authors propose a model for M2-mediated influenza budding. They suggest that budding is initiated by haemagglutinin, which clusters at lipid rafts and associates with M1. In turn, M1 recruits M2, which, in the cholesterol-rich environment of the rafts, stabilizes the site of budding until other proteins are recruited. When the pool of haemagglutinin is depleted by the assembling virions, M2 can move to the lipid phase boundary (that is, between the virion and the plasma membrane), where cholesterol levels are low, and mediate scission through its amphipathic helix.

Rachel David



Budding of wild-type (left) and amphipathic helix-mutant (right) influenza; the mutant virus fails to pinch off and has a 'beads on a string' morphology. Image courtesy of J. Rossman, Northwestern University, Evanston, Illinois, USA.

ORIGINAL RESEARCH PAPER Rossman, J. S., Jing, X., Leser, G. P. & Lamb, R. A. Influenza virus M2 protein mediates ESCRT-independent membrane scission. *Cell* **142**, 902–913 (2010)