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Influenza Exits the Cell without an ESCRT

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Many enveloped viruses depend on the membrane remodeling machinery of their host cells to complete their life cycle. In this issue, Rossman et al. (2010) now demonstrate that influenza virus possesses its own device for releasing nascent virus particles from the plasma membrane, the M2 proton-selective ion channel, which can substitute for the host cell's ESCRT pathway.

Many viruses are severely constrained by their small genomes, and thus, viruses have evolved multiple strategies to coerce their host cells to help complete their life cycle. One of the most well studied examples of this strategy is viruses hijacking the eukaryotic ESCRT (endosomal sorting complex required for transport) pathway. Enveloped viruses, such as HIV, Ebola virus, and Rabies virus, have a lipid bilayer or vesicle surrounding their capsid. The ESCRT pathway generates similar types of vesicles while sorting proteins among the plasma membrane, endosomes, and lysosomes (Hurley and Hanson, 2010). Therefore, it intuitively makes sense that many enveloped viruses engage the ESCRT pathway to facilitate budding from the plasma membrane.

However, dependence on the ESCRT pathway is not universal. In particular, the release of influenza virus from infected cells does not require a functional ESCRT system (Chen and Lamb, 2008), raising the possibility that influenza virus exploits a new cellular pathway to escape from cells. Now, in this issue of *Cell*, Rossman et al. (2010) report an alternative explanation; they find that the influenza virus surprisingly relies on its own protein, the M2 proton-selective ion channel, to cleave viral particles from the plasma membrane.

The assembly and release of enveloped viruses from an infected cell involves two major steps. First, a portion of the plasma membrane curves up into a "bud" projecting away from the cytosol (Figure 1). Then the "neck" of the bud "pinches off" to disconnect the vesicle from the membrane, a process known as membrane scission.

The initial hints that this second step requires assistance from the host cell came when researchers identified the L domains of retroviruses (Bieniasz, 2006). These short peptides are required to release assembled virus particles from the cell, and they are known to ultimately engage the ESCRT-III complex, the component of the ESCRT pathway that mediates cleavage of membrane necks during endosomal budding and cytokinesis (Carlton and Martin-Serrano, 2007; Saksena et al., 2007). L domains were later identified in numerous enveloped viruses, and a considerable number of these are now known to require the ESCRT pathway for budding (Chen and Lamb, 2008). Although the influenza virus lacks a conventional L domain, the matrix protein (M1) of influenza binds to a subunit of the ESCRT-I complex, suggesting that this virus may still rely on the ESCRT pathway for budding (Bruce et al., 2009). However, neither M1 nor a functioning ESCRT system is strictly required for the budding of influenza (Bruce et al., 2009; Chen and Lamb, 2008). Thus, the details of how nascent influenza virus particles disconnect from the plasma membrane have been a mystery.

Interestingly, transmembrane proteins, such as hemagglutinin (HA) and neuraminidase (NA) (Figure 1), drive the formation of influenza buds instead of internal proteins, as is the case for other viruses (Chen and Lamb, 2008). One of these transmembrane proteins, the M2 proton-selective ion channel, has attracted considerable attention because it is the target of the antiviral drug amantadine. When influenza enters a cell by endocytosis, M2 triggers uncoating of the virus by acidifying the interior of the virus particle. Thus, amantadine inhibits an early stage of the viral life cycle by blocking M2's channel (Pinto and Lamb, 2006). Recent studies suggest that M2 also plays a critical but less appreciated role later during viral assembly or budding. This second function depends on specific regions in the channel's cytoplasmic tail (Chen et al., 2008), but the exact role that M2 plays in viral morphogenesis has been difficult to pinpoint.

The study by Rossman and colleagues now reveals that purified influenza M2 can alter the curvature of lipid bilayers in vitro. Moreover, in these liposome assays, M2 possesses similar capabilities as the activated ESCRT-III protein complex (Hurley and Hanson, 2010). Like ESCRT-III, M2 induces the inward budding and detachment of small vesicles inside larger vesicles (i.e., giant unilamellar vesicles), an artificial system that mimics virus budding from the plasma membrane. Furthermore, the authors found that an amphipathic helix in M2's cytoplasmic tail is both required and sufficient for the detachment of vesicle buds in this in vitro model system. Importantly, mutating the hydrophobic face of this helix significantly reduces viral release in vivo, indicating that this 17 amino acid helix is crucial for the release of influenza virus in vivo. Together, these results suggest that M2 serves as a substitute for ESCRT complexes during

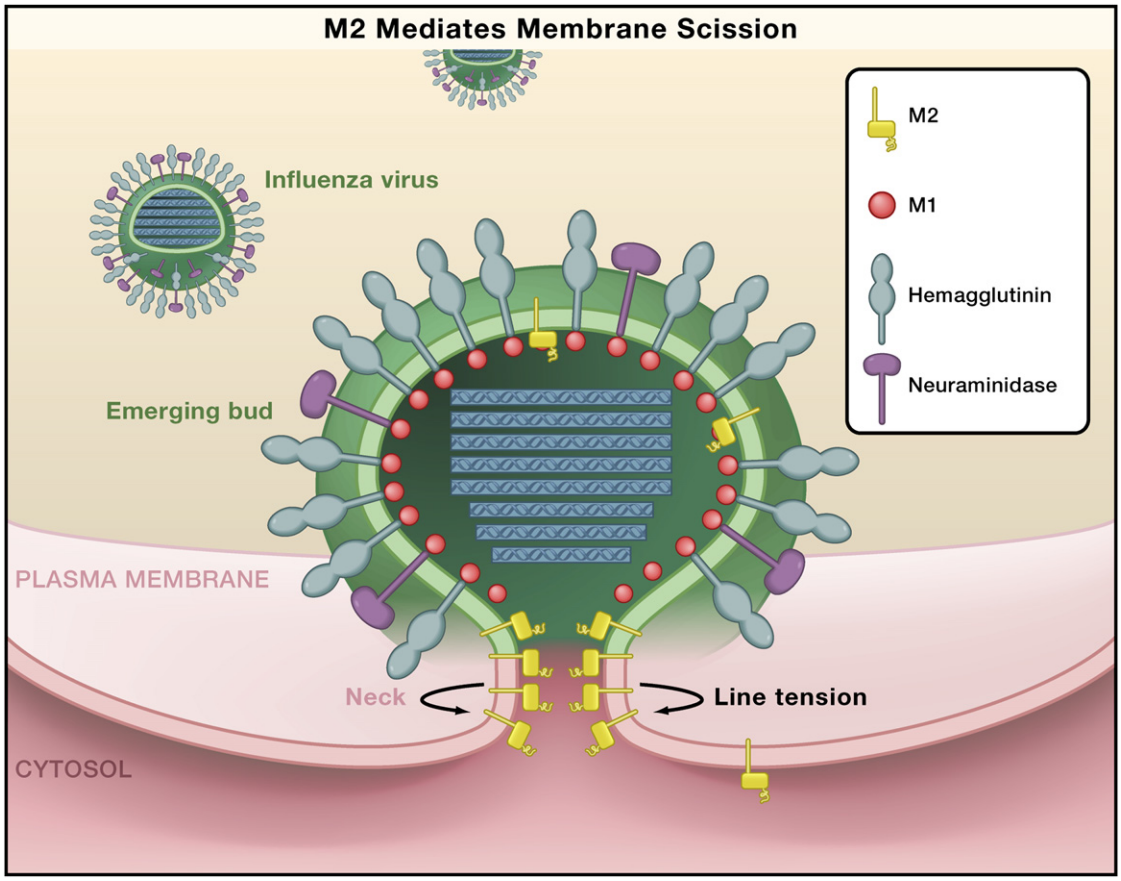

Figure 1. Model of Influenza Virus Budding

The formation of spherical or tubular buds at the plasma membrane is driven by the viral transmembrane proteins hemagglutinin (HA) and neuraminidase (NA). The matrix protein M1 is brought into the virus particle by contacts with HA and NA. In turn, M1 recruits into the emerging bud the viral ribonucleoprotein structures (blue bars) and small quantities of the transmembrane protein M2 (which is a homotetramer but is depicted as a monomer for simplicity). Because HA and NA concentrate in raft-like lipid microdomains, the membrane covering the emerging bud (green) has a higher cholesterol content than the bulk plasma membrane (red). This phase separation results in a line tension that tends to promote bud neck constriction to minimize the strain at the phase boundary. M2 accumulates at the phase boundary, and its amphipathic helix (yellow square) promotes membrane fission by modulating this line tension (Rossman et al., 2010).

influenza virus budding. Furthermore, these results raise the possibility that other enveloped viruses that appear to be independent of the ESCRT system may also encode functional equivalents to M2.

It seems remarkable that a small portion of a single viral protein can replace the function of a cellular machine as complex as the ESCRT pathway, which includes more than 20 proteins in humans. During ESCRT-mediated sorting of cargo into vesicles that bud into endosomes, the upstream components of the pathway, the ESCRT-I and the ESCRT–II complexes, initially form the vesicle buds. The downstream component, ESCRT-III, can also induce buds, but at physiological concentrations it merely mediates membrane scission (Hurley and Hanson, 2010). Rossman and colleagues find that influenza virus lacking M2 can form buds, but the buds fail to detach and instead exhibit a ''beads-on-a-string'' morphology at the plasma membrane. Thus, despite the capacity of the M2 protein to produce buds in vitro, this transmembrane protein probably functions primarily as a membrane scission factor in vivo, similar to ESCRT-III.

The results presented by Rossman and colleagues raise many new questions about the assembly and budding of influenza virus. One important future goal will be to determine how the amphipathic helix of M2 bends membranes and carries out scission. Some cellular trafficking proteins also possess amphipathic helices that induce curvature into membranes. However, these proteins usually insert like wedges into the cytosolic leaflet of the plasma membrane, bending the membrane toward the cytosol (McMahon and Gallop, 2005). In contrast, M2 curves the membrane away from the cytosol, and thus, it is not clear how its amphipathic helix can induce budding in this opposite direction.

Another surprising result from the study by Rossman and colleagues is that M2 facilitates budding only from membranes containing relatively low concentrations of cholesterol. At first, this seems counterintuitive because influenza virus is believed to bud from cholesterol-rich domains of the plasma membrane (Figure 1). The authors speculate that this restriction in M2 activity delays membrane scission until the virus particle is fully assembled. In this intriguing model,

the amphipathic helix of M2 would first contact a cholesterol-rich environment; then as the virus completes assembly, M2 would eventually contact surrounding membrane regions with less cholesterol. At this point, M2 could promote scission by modifying the line tension between the bud and the bulk plasma membrane (Figure 1). This mechanism, however, relies on forces generated at the boundary of two lipid domains. This is perhaps not the complete story because M2 can drive vesicle budding and detachment even from simple model membranes that lack lipids commonly involved in establishing such membrane domains.

Clearly, the study by Rossman and colleagues marks only the beginning of a fascinating story that will shed new light on a fundamental but still poorly understood stage in the life cycle of enveloped viruses. In addition, their study identifies yet another essential function of the M2 channel. It will be interesting to see if M2's role in budding can be exploited as a drug target to treat influenza.

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PARP around the Clock

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Cells possess internal ~24 hr or circadian clocks that synchronize physiological processes with daily cycles of light and nutrient availability. In this issue, Asher et al. (2010) find that PARP-1 (poly(ADP-ribose) polymerase 1) modifies components of the clock machinery in response to feeding, providing a mechanism for how metabolic rhythms coordinate with circadian rhythms.

Many organisms synchronize their behavior, physiology, and metabolism with the 24 hr rotation of the Earth. In mammals, a master pacemaker is located in the hypothalamic region called the suprachiasmatic nucleus (SCN), which contains a cluster of ~10,000 neurons. Each SCN neuron expresses a transcriptional feedback loop that self-generates an oscillation with a period of ~24 hr (Green et al., 2008). Cells in peripheral tissues, such as the liver, contain similar cell-autonomous clocks, and the SCN synchronizes the oscillation of their internal clocks to coordinate rhythms throughout the body. However, in both the SCN and peripheral tissues, the molecular clocks must integrate extracellular cues to maintain synchrony with the environment. Light is the dominant cue for the central clock in the SCN, but for peripheral tissues, metabolic cycles, such as feeding and fasting, can also regulate the internal clocks.

The link between circadian and metabolic rhythms is an area of intense study because disrupting the synchrony is thought to contribute to the etiology of disorders such as diabetes, obesity, and cardiovascular disease (Green et al., 2008). Nonetheless, how circadian and metabolic systems interact remains largely undefined; in particular, how feeding directly modulates the molecular oscillators in peripheral tissues has been a mystery. In this issue of *Cell*, Asher et al. (2010) find that the activity of poly (ADP-ribose) polymerase 1 (PARP-1) in the liver of mice oscillates in synchrony with the feeding-fasting cycle, providing a new link between metabolism and circadian rhythms.

PARP-1 is a highly conserved nuclear protein that adds chains of ADP-ribose molecules to proteins in a process called ADP-ribosylation (or poly ADP-ribosylation). PARP-1 uses nicotinamide adenine dinucleotide (NAD^+) to synthesize these polymers, which can include up to 200 ADP-ribose units. Like other post-translational modifications, ADP-ribosylation alters protein function; chains of ADP-ribose are negatively charged, and thus their addition is believed to disrupt electrostatic interactions, such as those involved in DNA binding. The major substrate of PARP-1 is itself, and