ENTH domain alone, hinting that the phenomenon is strictly induced by binding to PIP₂. But the ANTH domain does not cause membrane deformation.

So the authors propose that the burying of PIP₂ deep within epsin's ENTH domain encourages deformation of a membrane bilayer (Fig. 1). They suggest that this burial allows the ENTH domain to insert its N-terminal α -helix into the outer leaflet of the bilayer, pushing the lipid head groups apart. This would provide sufficient mechanical force to bend a flat membrane into an emerging bud. As further work showed that epsin binds to the AP2 adaptor and triggers clathrin-coat assembly, and that the combination of epsin and clathrin induces invagination of lipid monolayers, the authors propose a crucial role for epsin in vesicle budding. According to their model, epsin links its membrane-bending effect to cargo recruitment (through AP2, which binds the tails of transmembrane cargo receptors) and to coat assembly.

Epsin is not the only protein that can induce membrane curvature. At least three other proteins that are involved in clathrin-coated vesicle formation can trigger membrane tubulation independently *in vitro*. These proteins are dynamin, amphiphysin and endophilin^{8–10}. Why are so many actors playing what appears to be the same part?

It seems likely that these proteins have subtly different roles *in vivo*, which are difficult to detect in *in vitro* tests. For instance, for a long time clathrin was envisaged as being sufficient to drive membrane budding, given its *in vitro* ability to self-assemble spontaneously into a polyhedral structure resembling the one surrounding a coated vesicle. Nowadays, that view is being revised, on the basis of experiments and theoretical calculations¹¹, and it seems clear that several proteins must work together to bend a biological membrane. A challenge now is to determine whether these proteins induce membrane curvature *in vivo* as well as *in vitro* by binding to phospholipids.

It will also be important to find out when the proteins work — some might function during the initiation of budding, some later, and some at multiple stages. Although *in vitro* data show that all these proteins can initiate deformation from a flat membrane, suggesting that they take part in early events, this may result only from artificial capping of too many of the target lipids, because artificial templates are more enriched in these lipids than are biological membranes. This might be reinforced if the proteins insert significantly into the outer leaflet of a bilayer, inducing membrane bending by increasing the surface of the leaflet¹².

Of all the proteins that initiate membrane curvature *in vitro*, epsin might be the best candidate for this effect *in vivo*, given that its bending action may go hand in hand with

cargo recruitment — a process that begins when the membrane starts to curve. Nevertheless, it is plausible that some of these proteins induce membrane curvature in several different steps of budding. *In vivo* evidence indicates that endophilin might do so¹². Such could be the case for epsin, too. As well as working in the earliest steps of membrane curvature, epsin might also play a later part, through its binding to Eps15 — a protein that has been detected at the rim of budding coated vesicles¹³.

Understanding all the molecular events that govern the making of a vesicle will require the development of ever more sophisticated approaches, using *in vitro* systems combined with morphological analysis at the ultrastructural level, as exemplified by Ford and colleagues' work¹. This will be complemented by the use of biophysical techniques, some based on high-resolution

imaging, that allow us to study the process in atomic detail¹⁴.

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Virology

Ins and outs

Viruses have a nasty habit of usurping the normal biological processes of their hosts. Take, for instance, adenovirus, which causes mild respiratory infections in humans. This virus takes advantage of a protein that is expressed naturally by the sheets of epithelial cells lining our lungs. It uses this protein, CAR, to latch onto the cells before entering them and multiplying.

Contrary to expectations, the CAR protein is found, not on the exposed surfaces of the cells, but rather on their sides, where one cell abuts on another. It seems that temporary breaks in the epithelium allow a few viruses to detect CAR and enter cells. But Michael J. Welsh and colleagues suspected that adenovirus's finely honed ability to bind this protein must also serve another purpose. As they describe in *Cell* (**110**, 789–799; 2002), they've now discovered what that purpose is.

Welsh and colleagues used a previously developed *in vitro* model of lung epithelial sheets that is similar to the human airway. They infected these sheets with adenovirus, and found that progeny viruses were released at the sides and base (the basolateral surface)

of a cell, appearing only later on the exposed (apical) surface. The apical surface remained undamaged, suggesting that the viruses travelled up between the cells to escape. Supporting this idea, electron microscopy showed that there were unusual spaces between the cells.

The 'proper' function of the CAR protein is to allow epithelial cells to stick to one another and form a continuous sheet. It was known that the adenovirus fibre protein binds to CAR, allowing the virus to enter cells. Welsh and colleagues wondered whether this fibre protein could also cause the breaks in epithelial sheets that are seen after the production of progeny virus. When adenovirus multiplies in epithelial cells, it produces far more fibre protein than can be incorporated into new viral particles. The authors found that when they added fibre protein to the epithelial sheets, it disrupted cell-to-cell contacts, as seen in these before (top) and after (bottom) pictures.

So they propose the following sequence of events. Adenovirus enters cells through temporary natural breaks in the epithelial sheet, which allow it

to use its fibre protein to bind to CAR. It multiplies in the cells, and progeny are released to the basolateral surface, along with surplus fibre protein. That protein again binds to CAR and prevents it from interacting with other cells in the sheet, thereby making large holes through which the virus can escape into the lungs. Neatly, then, the virus uses the same tools for both entry and exit.

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