ENDOCYTOSIS

Gaining independence

The vast majority of our present knowledge about endocytic pathways concerns classic clathrin-dependent endocytosis. Clathrin-independent pathways are poorly understood by comparison, although, in recent years, evidence has accumulated for clathrin-independent pathways that are mediated by caveolae (caveolin-1-containing structures) or lipid rafts. Now, in *The Journal of Cell Biology*, two papers — the first by Parton and colleagues, the second by Helenius and co-workers — provide new insights into endocytic pathways that are independent of both clathrin and caveolae.

In the first paper, Parton and colleagues studied the internalization of the cholera-toxin binding subunit (CTB). It is known that CTB can be endocytosed by clathrin-independent mechanisms, but whether caveolae are involved has remained controversial. The authors first developed an ultrastructural assay that allowed them to identify budded caveolin-1-positive structures for the first time. In wild-type mouse embryonic fibroblasts (MEFs), they found that a small fraction of CTB- and caveolin-1-positive structures budded from the plasma membrane, and that this budding could be stimulated.

However, they also showed that this is not the predominant pathway for CTB uptake in MEFs, as CTB trafficking to the Golgi was found to be identical in wild-type and caveolin-$1^{-/-}$ MEFs. In addition, they showed that blocking clathrin-mediated endocytosis in caveolin-$1^{-/-}$ MEFs only partially inhibited the Golgi accumulation of CTB. It therefore seems that a significant proportion of CTB uptake can occur through a pathway that is independent of caveolae and clathrin, and Parton and colleagues speculate that this might represent a primordial endocytic route.

Through further characterization of this pathway, they found that the internalization was dynamin and Arf6 independent, but relatively cholesterol dependent. They also showed that the endocytic vehicles were uncoated tubular/ring-like structures with diameters of 40–80 nm. These structures were found to contain CTB, glycosylphosphatidylinositol-anchored proteins (GPI-APs) and fluid-phase markers. They are therefore probably GEECs (GPI-AP-enriched early endosomal compartments), structures that have been previously shown to be involved in the CDC42-dependent uptake of GPI-APs and fluid-phase markers.

In the second paper, Helenius and co-workers studied the internalization of simian virus-40 (SV40), which is known to be able to enter cells through a clathrin-independent, caveolae-dependent pathway. However, by using a caveolin-1-deficient cell line and caveolin-$1^{-/-}$ MEFs and by blocking clathrin-mediated endocytosis, they found that SV40 can also enter cells through a clathrin- and caveolae-independent pathway. This pathway was more rapid than caveolae-dependent uptake and, by using various inhibitors, they showed that it was dynamin-2 and Arf6 independent, but cholesterol and tyrosine-kinase dependent.

The authors further characterized this pathway and found that, after binding to the plasma membrane, SV40 rapidly associated with lipid rafts and then remained associated with these rafts. They also showed that SV40 was first internalized into small, tight-fitting vesicles (60 nm in diameter) before being transported to pH-neutral organelles that resembled caveosomes but were devoid of caveolin. From these caveosome-like structures, which they speculate might be where the caveolae-dependent and caveolae-independent pathways intersect, SV40 then moved through microtubule-mediated vesicular transport to the endoplasmic reticulum (ER) — a step that was required for infectivity.

Helenius and co-workers have therefore identified an endocytic pathway that can transport SV40 from the plasma membrane to the ER in a clathrin- and caveolae-independent manner. And, although these two papers have provided new information regarding clathrin- and caveolae-independent endocytic pathways, they also raise the question, just how many clathrin-independent pathways are there?

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References and links

ORIGINAL RESEARCH PAPERS

Kirkham, M. *et al.* Ultrastructural identification of uncoated caveolin-independent early endocytic vehicles. *J. Cell Biol.* **168**, 465–476 (2005) | Damm, E.-M. *et al.* Clathrin- and caveolin-1-independent endocytosis: entry of simian virus 40 into cells devoid of caveolae. *J. Cell Biol.* **168**, 477–488 (2005)

FURTHER READING Parton, R. G. & Richards, A. A. Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. *Traffic* **4**, 724–738 (2003)

WEB SITES

Ari Helenius' laboratory:
http://www.bc.biol.ethz.ch/people/groups/arih/

Robert Parton's laboratory:
http://www.imb.uq.edu.au/index.html?page=11688&pid=12015

WEB WATCH

AD test strikes gold

A nanoparticle test reported in the *Proceedings of the National Academy of Sciences* could herald the advent of a living diagnosis for Alzheimer's disease (AD). The test detects the prevalence of amyloid-β-derived diffusible ligands (ADDLs), and is a million times more sensitive than other approaches.

ADDLs are "…invisible to conventional neuropathology, but their presence or absence may be the real determinants of memory loss," according to William L. Klein, a member of the research team (*Science News Online*, 5 February 2005), and they have been shown to target synapses well before symptoms can be spotted at present. At the moment, a firm diagnosis of AD requires an autopsy, and other forms of diagnosis, such as memory tests and brain scans, fall short of 100% accuracy.

Although ADDLs were suspected to be present in the cerebrospinal fluid, no tests were sensitive enough to detect their presence there. The approach taken by Dimitra Georganopoulou and colleagues used antibodies directed against ADDL. Some of the antibodies were attached to iron particles, and could therefore be extracted using a magnetic field. Other antibodies, which were attached to gold nanoparticles, could also hitch a lift in the extraction process. Along with the gold were 'barcode' DNA strands, which, when released by dehybridization, bound to complementary DNA on a glass plate and were detected using a highly sensitive scanometric method.

Faced with the challenge of trying out the technique on easier-to-obtain blood and urine samples, in which ADDL concentrations are expected to be far lower, the team remains confident. "We're nowhere near the limits of the test's sensitivity." (*NewScientist.com*, 31 January 2005.)

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