A new gateway into cells

Cells use endocytosis to internalize cargo molecules from the cell surface. During clathrin-dependent endocytosis, cargo is packaged into vesicles with the aid of a clathrin coat. Several pathways that do not use clathrin have been identified, but their mechanistic details remain elusive. Two studies have determined the molecular mechanisms that underlie a specific clathrin-independent pathway for the internalization of activated surface receptors and bacterial toxins.

Boucrot *et al.* used pull-down experiments to identify binding partners of endophilin — a BAR domain-containing protein that deforms membranes — and found that it associates with various G protein-coupled receptors (GPCRs) at the plasma membrane. The authors observed that GPCRs were rapidly internalized following ligand stimulation, and that this was accompanied by the formation of endophilin-positive tubular-vesicular structures migrating from the plasma membrane to the perinuclear area. Cells depleted of endophilin showed decreased internalization of the GPCR β 1-adrenergic receptor and tubule formation, and increased

surface accumulation of the receptor and increased downstream signalling. By contrast, depletion of clathrin had no effect, suggesting that cargo uptake involves a clathrin-independent pathway, which they named fast endophilin-mediated endocytosis (FEME).

Furthermore, Boucrot *et al.* showed that FEME required dynamin and PI3K signalling and that it occurs predominantly at the leading edge of migrating cells. Moreover, they were able to show that for the uptake of activated cargo receptors, FEME carrier formation involves the recruitment of endophilin to the leading edge by membrane-localized phosphatidylinositol-3,4-bisphosphate and its binding protein lamellipodin.

In a second study, Renard *et al.* found that endophilin co-localizes to early uptake structures that are induced at the plasma membrane by the glycosphingolipid receptor-binding parts of the bacterial Shiga (STxB) and cholera toxins. By contrast, clathrin or the clathrin adaptor AP2 did not notably overlap with internalization sites, which suggests that STxB is endocytosed by a clathrin-independent mechanism.

Furthermore, the authors showed that cells expressing exogenous endophilin had shorter STxB-containing tubules than wild-type cells, which is indicative of a role of endophilin in scission of toxin-induced plasma membrane invaginations. To directly test the potential function of endophilin in tubule scission, the authors used liposome-based systems. They observed that endophilin forms a scaffold on STxB-induced membrane tubules, which then sensitizes these tubules to pulling-force-mediated scission, such as that driven by molecular motors. These findings enabled the authors to propose a novel scission modality. Dynamin and actin were also found to contribute to scission of the same tubules to which endophilin is localized, suggesting that a cocktail of molecular factors mediates the detachment of these endocytic invaginations from the plasma membrane.

In summary, these two studies uncover the molecular mechanism underlying a clathrin-independent endocytic pathway that mediates the uptake of activated receptors to control cell signalling and is subverted by bacterial toxins.

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“a specific clathrin-independent pathway for the internalization of activated surface receptors and bacterial toxins”

ORIGINAL RESEARCH PAPERS Boucrot, E. *et al.* Endophilin marks and controls a clathrin-independent endocytic pathway. *Nature* <http://dx.doi.org/10.1038/nature14067> (2014) | Renard, H.-F. *et al.* Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. *Nature* <http://dx.doi.org/10.1038/nature14064> (2014)