IN BRIEF

MEMBRANE TRAFFICKING

Insights at the pit

During endocytosis, coated pits are formed by a lattice of clathrin that is linked to the membrane by adaptors. Two imaging studies have shed new light on the initial moments of clathrin-coated vesicle (CCV) formation. Kirchhausen and colleagues used total internal reflection fluorescence (TIRF) microscopy combined with statistical modelling to track events during the first seconds of CCV formation. They used TIRF imaging at a resolution that was sufficient to detect single molecules of clathrin chains or the adaptor protein AP2 fused with EGFP and could demonstrate coincident recruitment of clathrin and AP2 during pit initiation, most commonly with a stoichiometry of one clathrin triskelion and two AP2 complexes. This approach also allowed them to ask which factors are required for initiation of pit formation: they confirmed that the phosphoinositide PtdIns(4,5)P_2 was crucial, whereas the accessory factors FCHO1 (FCH domain only protein 1) and FCHO2 were needed only for continued pit growth.

Briggs and colleagues took a complementary approach of reconstructing the defining steps of endocytosis in budding yeast using correlative fluorescence microscopy and electron tomography. By imaging fluorescently tagged proteins that are recruited during distinct stages of endocytosis, they were able to unambigously identify endocytic events in electron tomograms and to isolate more than 200 endocytic intermediates. This allowed them to reconstruct a virtual 4D 'movie' of the ultrastructural changes that occur during endocytosis and to determine the quantitative changes in membrane shape during this process. They showed that regulators of coat formation are recruited to the membrane while it is still flat and that actin polymerization is important for pit invagination. Moreover, they demonstrated the importance of the amphiphysin component Rvs167, which associates with the parallel membranes of the elongating tubule, for normal vesicle size.

ORIGINAL RESEARCH PAPERS Cocucci, E. *et al.* The first five seconds in the life of a clathrin-coated pit. *Cell* **150**, 495–507 (2012) | Kukulski, W. *et al.* Plasma membrane reshaping during endocytosis is revealed by time-resolved electron tomography. *Cell* **150**, 508–520 (2012)

STEM CELLS

Tracking regenerative behaviour

Rompolas *et al.* describe a new, non-invasive intravital two-photon imaging approach for visualizing cells over long periods of time. The authors used this method to study the physiological regeneration of hair follicles in live mice. They visualized entire hair follicles and monitored skin epithelial stem cells and their progeny through the specific expression of a histone H2B–GFP fusion protein, which provides a strong nuclear signal that can resolve individual cells. Consistent with earlier studies, stem cells were quiescent during the initial stages of hair regeneration, whereas their progeny proliferated more actively. They also observed that follicles could rapidly stretch downwards and that, at advanced growth stages, cells of the lower hair follicle were capable of long-range migration. Thus, this method revealed previously unappreciated dynamic cellular behaviours. Last, laser ablation experiments enabled them to demonstrate that the mesenchyme is required for hair growth initiation, reiterating the importance of epithelial–mesenchymal crosstalk.

ORIGINAL RESEARCH PAPER Rompolas, P. *et al.* Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. *Nature* **487**, 496–499 (2012)