MEMBRANE TRAFFICKING

Roll out the barrel

Clathrin-coated vesicles are vital for intracellular membrane trafficking, and new insights into clathrin coats have now been provided by Grigorieff, Harrison, Kirchhausen, Walz and colleagues in two papers in *Nature*.

Clathrin triskelions — which are composed of three heavy chains that radiate from a central hub — assemble to form clathrin coats or lattices. Each heavy-chain 'leg' contains a proximal segment near the hub, followed by a 'knee', a distal segment, an 'ankle', a 'linker' and a terminal domain. Previous work showed that in an assembled clathrin lattice, a triskelion hub lies at each vertex and the proximal segments extend towards the three neighbouring vertices, projecting slightly inwards. After a curved knee, each distal segment projects towards the next vertex, where three ankles converge beneath the triskelion hub centered there and the terminal domains extend inwards.

In the first study, Fotin *et al.* reconstituted clathrin coats *in vitro* using bovine-brain triskelions and adaptor-protein complexes (adaptors link clathrin to cargo). Using electron cryomicroscopy, they obtained a 7.9-Å-resolution view of a clathrin hexagonal barrel and, by fitting crystal structures and homology models into the electron-microscopy density map, the authors traced most of the 1,675 residues of the heavy chain.

In addition, they defined a helical tripod — formed by C-terminal regions of the heavy chains — that projects inwards from each triskelion hub and interacts, beneath the hub, with ankles from triskelions that are centered two vertices away. At each vertex, this 'ankle brace' might help to hold the 'invariant hub assembly' together — that is, the tripod, the three proximal segments that radiate from the vertex, distal segments from triskelions centered at the nearest-neighbour vertices, and a triangle of ankles at the base of the tripod.

Fotin *et al.* also determined the structure of a mini-coat, which has the same overall organization and triskelion packing as the barrel. Clathrin can form structures of variable curvature and, by comparing the barrel and the mini-coat, the authors showed that variations in curvature are achieved by changing the crossing angle of overlapping proximal and distal segments, rather than by changing the organization at the vertex.

In the second study, Fotin *et al.* used electron cryomicroscopy to determine the structure of *in-vitro*-assembled clathrin coats bound to a C-terminal fragment of auxilin. Auxilin is a co-chaperone that specifically recruits the 70-kDa heat-shock cognate protein (Hsc70), which has a clathrin-uncoating activity. The authors showed that auxilin binds within the clathrin lattice, making contacts with a terminal domain, two ankles and a part of the 'ankle brace' — a region that is crucial for coat stability. Auxilin binding perturbs heavy-chain contacts, so the authors propose that "…auxilin could create a local strain, release the neighbouring C-terminal segment from its interactions with the ankle, and recruit Hsc70 to clamp and sequester the segment thus exposed." In this way, local destabilization could promote uncoating.

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References and links

ORIGINAL RESEARCH PAPERS Fotin, A. *et al.* Molecular model for a complete clathrin lattice from electron cryomicroscopy. *Nature* 24 Oct 2004 (doi:10.1038/nature03079) | Fotin, A. *et al.* Structure of an auxilin-bound clathrin coat and its implications for the mechanism of uncoating. *Nature* 24 Oct 2004 (doi:10.1038/nature03078)

FURTHER READING Kirchhausen, T. Clathrin. *Annu. Rev. Biochem.* **69**, 699–727 (2000)

WEB SITES

Nikolaus Grigorieff's laboratory: http://www.bio.brandeis.edu/faculty01/grigorieff.html

Steve Harrison's laboratory: http://crystal.med.harvard.edu/harrison.html

Tom Kirchhausen's laboratory: http://cbr.med.harvard.edu/investigators/kirchhausen/lab/

Thomas Walz's laboratory: http://cellbio.med.harvard.edu/faculty/walz/