374 CD
DOI: 10.1017/S1431927606067882
Microsc Microanal 12(Supp 2), 2006

Visualizing the Clathrin and Assembly-Regulating Proteins of Coated Vesicles by Cryo-Electron Tomography

J. Bernard Heymann[*], Dennis C. Winkler[*], Yang-In Yim[**], Evan Eisenberg[**], Lois E. Greene[**], Alasdair C. Steven[*]

[*] Laboratory of Structural Biology, NIAMS, and
[**] Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD 20892

Clathrin provides scaffolding for the formation of invaginations during one type of endocytosis, aiding in the transformation from a flat hexagonal lattice on the plasma membrane into a curved coated pit and finally releasing a coated vesicle (CV). The CV is then rapidly uncoated by the ATPase, Hsc70, and the clathrin triskelions recycled. In a cryo-EM reconstruction of clathrin baskets loaded with Hsc70 at pH 6 where it is unable to uncoat, we detected diffuse densities associated with the spars of the clathrin lattice, representing Hsc70 in its initial binding site prior to effecting the detachment of triskelions (Fig. 1B) [1]. The diffuse nature of these densities indicates partial occupancy, with Hsc70 binding in two or more, mutually occluding locations.

The uncoating occurs rapidly after the formation of a CV, and the lifetime of a CV is only a few seconds [2]. Therefore, small CVs prepared from tissues are thought to mainly arise from coated pits. These CVs are highly polymorphic and, mostly, less regular than the D6 barrels that we used for cryo-EM reconstruction (Fig. 1B) [1]. To better appreciate the interactions of clathrin lattices with enclosed vesicles and the distributions of regulatory and adaptor proteins on individual CVs, we have been performing cryo-electron tomography of CVs isolated from bovine brain (Fig. 1A). They range in size from ~ 660 Å to 1200 Å, with spherical or ovoid shapes. Each CV has an enclosed membrane vesicle with a diameter of 230 Å to 570 Å (purple sphere in Fig. 1C), whose surface is separated from the clathrin legs by 160 Å to 340 Å. The closest approach between the clathrin legs and the membrane is consistent with the clathrin N-terminus contacting the membrane, while greater separation may accommodate other proteins involved in CV function and mediating its interaction with the membrane. Fig. 1C shows a model of the clathrin network built into a CV from tomography. Many of the spars in the clathrin network of the CV are very clear, especially where the spar is oriented along the z-axis (see the white circle in Fig. 1A and the vertical spars in Fig. 1C). Other spars are less clear in the surface rendering at the top and bottom of the particle, but from the slices in the top and bottom rows in Fig. 1A, the lattice is well defined. The lattice has 12 pentagonal faces (following Euler's theorem for polyhedra) and 14 hexagonal faces. We are attempting to reconstruct the known parts of the CV (the clathrin network and the vesicle membrane), with the aim of identifying densities representing other proteins associated with the CV.

[1] Heymann et al. (2005) Visualization of the Binding of Hsc70 ATPase to Clathrin Baskets: Implications for an Uncoating Mechanism. J. Biol. Chem. 280: 7156-7161.
[2] Kaksonen et al. (2005). A modular design for the clathrin- and actin-mediated endocytosis machinery. Cell **123**(2): 305-20.

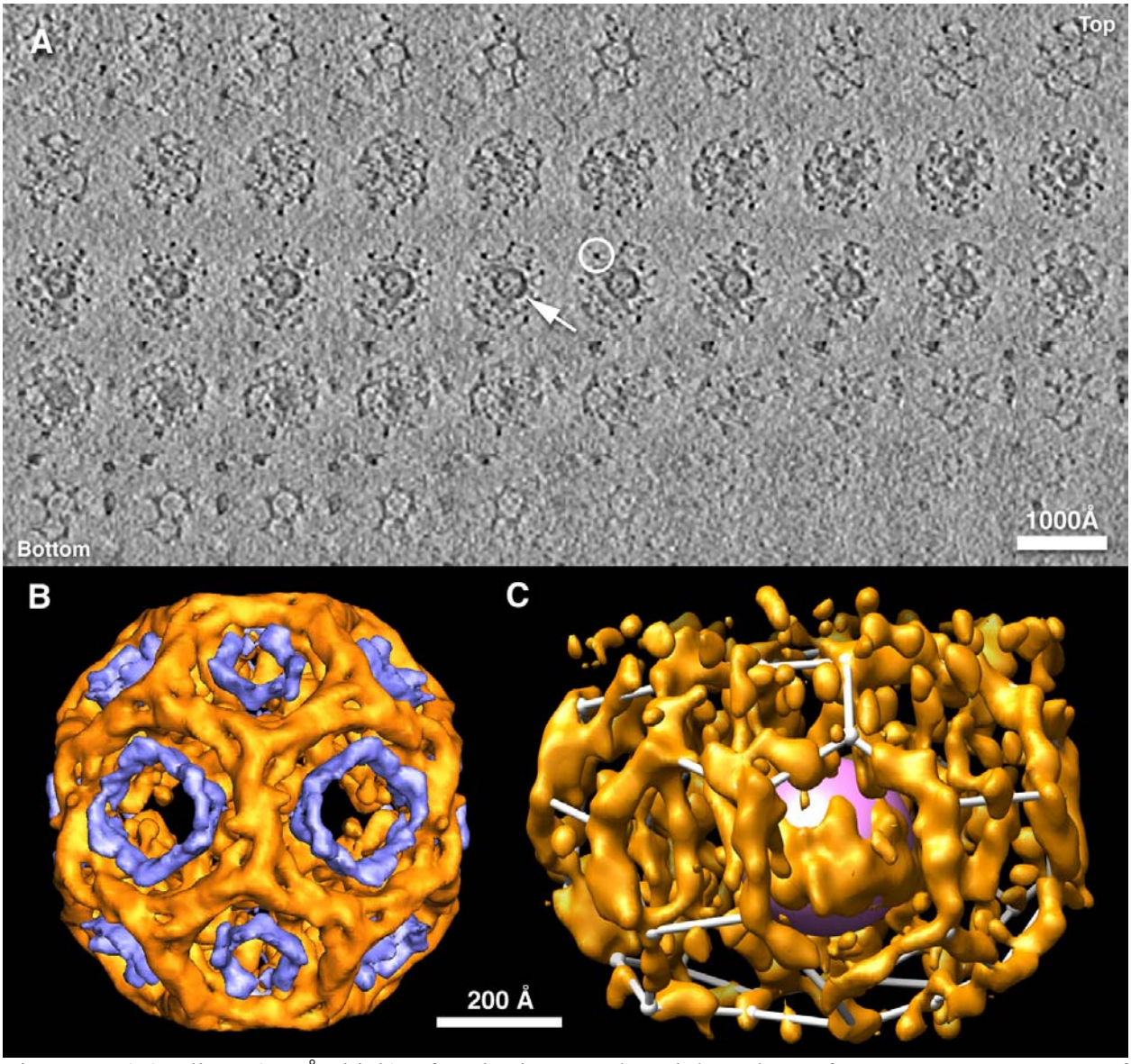

Figure 1: (A) Slices (16 Å thick) of a single coated vesicle volume from a cryo-tomogram of bovine clathrin coated vesicles. The white arrow points to the enclosed vesicle with a diameter of ~330 Å, and the white ring indicates a spar of the clathrin lattice (black spot in the middle) oriented along the view. (B) A clathrin basket in vitro assembled with C58J (required to bind Hsc70) and the uncoating ATPase, Hsc70 (blue densities). (C) A surface rendering of the coated vesicle in (A), with a purple sphere in the center aligned with the inner vesicle, and a network of nodes and links built to follow the clathrin network (white spheres and cylinders). The vesicle is flattened between the two ice surfaces that coincide with the top and bottom surfaces in the picture.

https://doi.org/10.1017/S1431927606067882 Published online by Cambridge University Press