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Clathrin, Cages, and Coated Vesicles

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Transfer of membranes and associated components between cellular compartments involves vesiculation from one membrane bilayer and fusion of the vesicle with another. Coated vesicles are widely involved in the first of these steps. Formation of a lattice-like coat from clathrin and associated proteins accompanies vesiculation. The coat must then be shed for fusion with a target membrane. The cycle of assembly and disassembly occurs on a time scale of a few minutes, as estimated by receptor recycling times in endocytosis or by the rate of passage of newly synthesized proteins through the Golgi apparatus. The structure and biochemistry of coated vesicles suggest some of the mechanisms that regulate this cycle.

The major protein component of coated vesicles is the 180 kd polypeptide first identified by Pearse, who named it clathrin. It appears to be the same size in all vertebrate tissues. Other proteins associated with coated vesicles vary in size with the source. All tissues so far examined contain a pair of polypeptides, migrating with apparent sizes close to 35 kd, that have come to be known as clathrin light chains. Brain coated vesicles, the best studied preparation, show in addition a group of bands in the 100–110 kd range (on SDS–polyacrylamide gel electrophoresis), a band at 55 kd identified as tubulin (probably associated nonspecifically), and several other much fainter components.

Clathrin can be dissociated from its vesicle in various ways, such as incubation with 2 M urea or with Tris at pH 8. The light chains always remain associated with 180 kd heavy chains; depending upon the conditions, other proteins are also solubilized. Clathrin thus prepared is a trimer of three heavy chains and three light chains; its striking shape, first visualized by Ungewickell and Branton (Nature *239,* 420–422, 1981), has suggested the descriptive term "triskelion." Each arm is composed of one heavy chain (HC) and one associated light chain (LC) (Kirchhausen and Harrison, Cell *23,* 755–761, 1981). Calf-brain clathrin has light chains of 36 kd (LC_a) and 33 kd (LC_b) in a 1:2 ratio. Their location on the trimer arm has been visualized by a biotin–avidin–ferritin method (Ungewickell et al., CSHSQB *57,* 723–731, 1982) and by immunoelectron microscopy with monoclonal antibodies to LC_a (Kirchhausen et al., PNAS *80,* 2481–2485, 1983; and see Figure 1). They bind near the apex, probably close enough that antibodies can bridge from one to another on the same trimer. The 1:2 ratio of LC_a:LC_b would be consistent with a uniform 1:2 stoichiometry for all trimers, but in calf-brain clathrin this appears not to be the case. Quantitative immunoprecipitation of clathrin with monoclonal antibodies to LC_a shows that the two species of light chain are randomly distributed among the three binding sites on each trimer (Kirchhausen et al., op. cit.). Light chains may be removed from heavy chain trimers by limited proteolysis with elastase or by treatment with agents such as thiocyanate or urea.

Purified trimers reassemble into coat-like structures, referred to as cages (or baskets), when they are dialyzed into a buffer of about pH 6.2 in the presence of Ca^{2+} or Mg^{2+} (Keen et al., Cell *16,* 303–312, 1979). Removal of the light chains by elastase digestion appears to reduce the yield of properly formed cages—various "monster" assemblies arise instead (Kirchhausen and Harrison, op. cit.). It is not clear, however, whether the light chains have a direct regulatory function in assembly, or whether the heavy chains are simply somewhat unstable in their absence. Proteolytic cleavage of cages with trypsin releases a 50 kd fragment from the distal end of the clathrin trimer (as well as digesting the light chains), but this treatment does not lead to disassembly (Schmid et al., PNAS *79,* 91–95, 1982).

The arrangement of clathrin trimers in cages is suggested by the shape and length of the arms, which can clearly span two sides of the lattice, as shown in Figure 2. Moreover, in all observed cage structures, three edges join at a vertex. By placing a trimer at each such local threefold position, a packing is generated with similar molecular contacts throughout (Kirchhausen and Harrison, op. cit.; Crowther and Pearse, JCB *91,* 790–797, 1982). A single edge is composed of two proximal arm segments and two distal segments (see Figure 2; the packing shown is based on Crowther and Pearse, op. cit.). The proximal segments emanate from trimers centered on adjacent vertices, and they are related by local dyad symmetry of an edge. Light chains could participate in these contacts. The distal segments, likewise twofold related, belong to trimers centered one further vertex away. The appearance of negatively stained cages suggests that distal–distal pairs lie beneath the proximals. Proximal–proximal contacts and distal–distal contacts may be conserved along all edges of the lattice. The various observed designs for cages and coated vesicles nonetheless imply some flexibility in the trimers. That is, the symmetries of the cage structures are such that different trimers must have somewhat different packing geometries. There is an analogous situation in the construction of spherical viruses, where identical subunits are not all in symmetrically related positions. Flexibility in clathrin could be restricted principally to the elbow and to the pucker at the vertex. The range of possible angles need not be very great to account for the range of cage diameters. Extended, flat, hexagonal lattices are, for example, never seen in reassembly experiments, suggesting that the trimer may not ordinarily be capable of flattening at its center.

Electron micrographs of coated vesicles from brain show several structures of which one predominates, a barrel-like lattice of D_6 symmetry with 8 hexagonal and 12 pentagonal facets (Crowther et al., JMB *103,* 785–798, 1976; and see Figure 3). Cages reassembled from purified

calf-brain clathrin have a sharply bimodal size distribution (Nandi et al., Biochem. *19*, 5917–5921, 1980), with some cages of apparently icosahedral symmetry. It is also striking that careful reassembly gives rise to well-formed and accurately closed cages with a very restricted number of distinct designs—many fewer than the number of possible closed shells having 12 pentagons and variable numbers of hexagons. It must therefore be a property of the assembly that once a particular structure is nucleated, subsequent addition of trimers occurs so as to locate the positions of pentagonal and hexagonal facets correctly. This involves the correct choice of "flex angles" for trimers as they join the lattice. Errors would give rise to spirals, bulged lattices, and other irregular forms. An important property of the molecule that can account for accurate propagation is its extended configuration: trimers centered at second- and third-nearest neighbor positions in the cage are in contact, making possible direct conformational switches. Similar direct switches are found in virus assembly. In 180-subunit plant viruses, such as tomato bushy stunt virus, extended arms of a symmetrically related subset of 60 of the subunits make an internal framework to select conformational alternatives. In proheads of bacteriophage P22, a scaffold protein is needed for accurate formation of the 420-subunit shell, and mostly irregular structures appear when it is absent. There is evidence that auxiliary proteins in coated vesicles may similarly regulate assembly and select a particular structure (Irace et al., Biochem. *21*, 5764–5769, 1982).

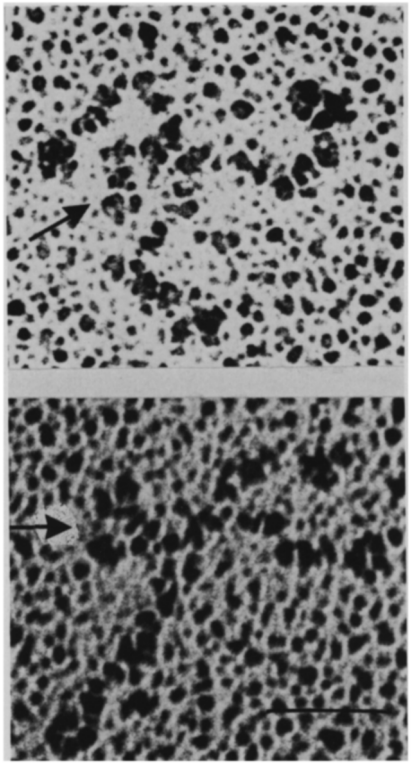

Figure 1. A monoclonal antibody that specifically recognizes a light chain (LC_a) binds close to the center of a clathrin trimer. Bar = 200 Å.

Coat-like cages self-assemble from clathrin trimers. What about coated vesicles themselves? Some striking micrographs of Heuser's (JCB *84*, 560–583, 1980), showing extended hexagonal arrays of clathrin on the inside surface of cell membrane, have given rise to the belief that coated pits and coated vesicles form by rearrangement of such structures. The properties of clathrin assembly in vitro do not support this notion. On the contrary, curvature and closure appear to be built into the geometry and bonding properties of the trimer. The same assembly process that occurs in solution could drive coated vesicle formation, if suitably nucleated on the membrane and coupled to it by repeated contacts. One or more of the "100 kd" group of

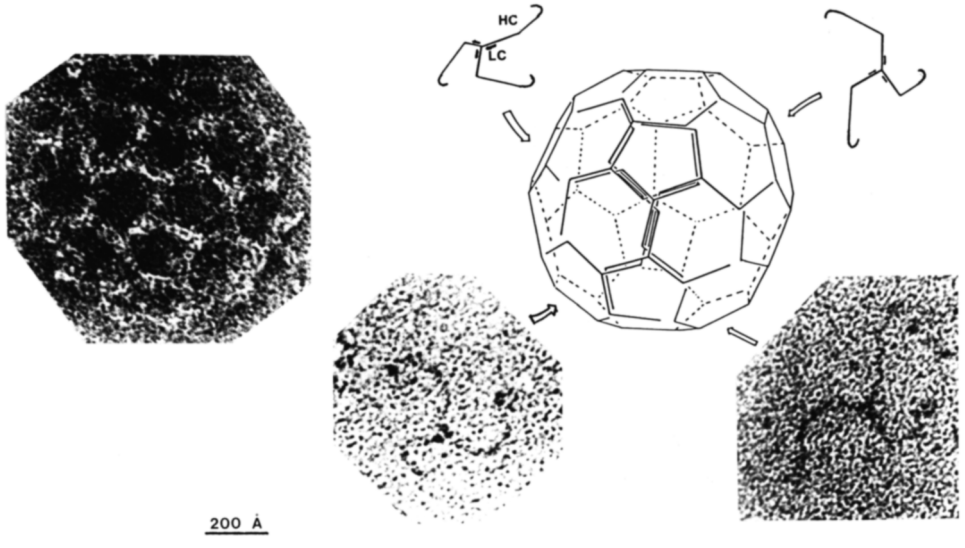

Figure 2. Diagram of clathrin trimers and of their packing arrangement in a cage. Micrographs show negatively stained isolated trimers and a cage similar to the one in the drawing. Bar = 200 Å.

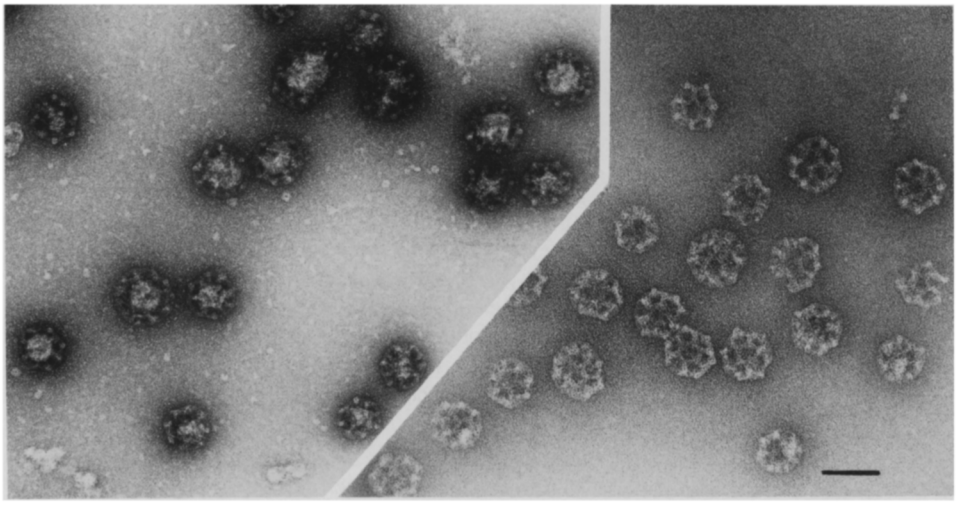

Figure 3. Comparison of a field of coated vesicles isolated from calf brain (left) with cages assembled in vitro from purified clathrin (right). Bar = 1000 Å.

proteins appears to mediate the binding of clathrin to membranes (Ungewickell et al., op. cit.). An analogous process occurs in the budding of enveloped viruses. In vesicular stomatitis virus, for example, the internal tubular structure initiates assembly at the membrane and wraps the bilayer around itself, probably by successive formation of contacts with glycoprotein tails. This sort of process is rapid and efficient. Clathrin cage assembly in solution is likewise very rapid (the time scale is probably seconds, as suggested by results of Crowther and Pearse, op. cit.), comparable to reassembly times for shells of small spherical viruses in appropriate pH-jump experiments). Rearrangement of a flat lattice is by contrast a very inefficient process. To transform a sixfold to a fivefold position, an entire 60° "pie slice" must be removed, and massive disassembly would have to accompany assembly. Extended arrays of clathrin have also been seen in thin sections of cultured HeLa cells (Maupin and Pollard, JCB *96,* 51–62, 1983) and in macrophages ingesting latex spheres (Aggler and Werb, JCB *94,* 1982), but only where the cells are in contact with the plastic substrate or bead. These observations are consistent with the view that such arrays are special configurations for clathrin rather than direct precursors of coated pits or vesicles.

If coated vesicles form directly by assembly from clathrin trimers, then the mechanistic requirements are relatively simple. Specific initiation must occur on the membrane. This might in some cases involve recognition of the location of liganded receptors or other molecules to be engulfed. Recent evidence indicates that endocytotic coated vesicles contain a proton pump (Forgac et al., PNAS *80,* 1300–1303, 1983; Stone et al., JBC *258,* 4059–4062, 1983). Since initiation is the only unique step, the pump ATPase might also be expected to be part of the assembly origin. We have seen that propagation of assembly can proceed from trimers in such a way as to ensure closure. Completion of the coat can probably produce the necessary pinching off of the membrane bilayer, just as in the budding of enveloped viruses, where no particular fusion activity seems to be involved. In this picture of coated vesicle formation, a coated pit is essentially an intermediate stage, rather than an independent structure.

Clathrin function implies cyclic association and dissociation. If intracellular conditions normally favor assembly at appropriate nucleation sites or membranes, then disassembly must be an active process. Patzer et al. (JCB *93,* 230–236, 1982) have provided evidence that disassembly of coated vesicles occurs in the presence of a cytosolic factor and ATP. Enzymatic disassembly must be able to distinguish between completed vesicles, which indeed are rapidly uncoated, and assembling coated vesicles (coated pits). One distinguishing property of complete coated vesicles is a closed interior that can undergo acidification or other ionic changes. Could occurrence of these changes be coupled to a signal on the coated surface of the membrane to enable or trigger the process? Such a mechanism is one way protein interactions at any single location in the coat can be influenced by whether the structure is a vesicle or a pit.

Formation of a coated vesicle corresponds to the assembly of a clathrin cage, directed and localized by interaction with a number of specific proteins. Study of these regulatory interactions is likely to give useful clues to mechanisms for organizing coated vesicle activity.