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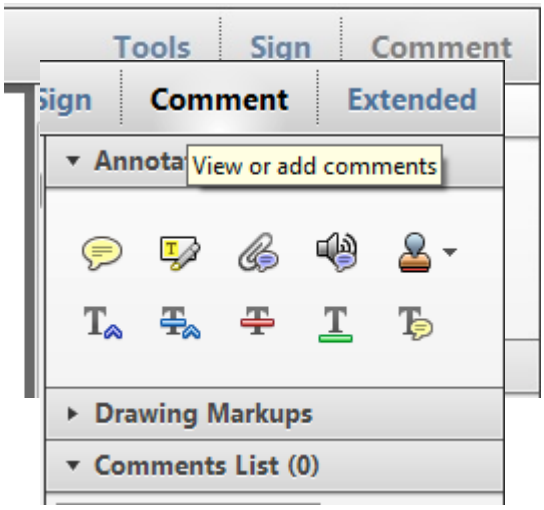

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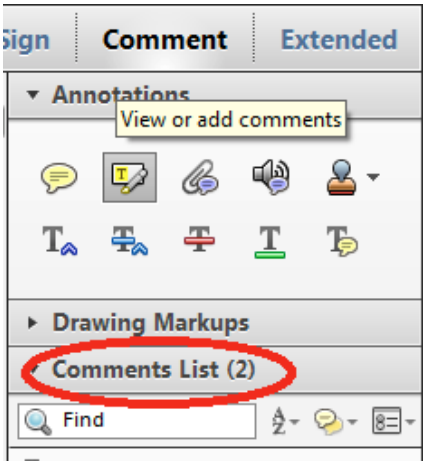

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The Structures of COPI-Coated Vesicles Reveal Alternate Coatomer Conformations and Interactions

Marco Faini,[1] Simone Prinz,[1] Rainer Beck,[2] Martin Schorb,[1] James D. Riches,[1]* Kirsten Bacia,[3] Britta Brügger,[2] Felix T. Wieland,[2]† John A. G. Briggs[1,4]†

Transport between compartments of eukaryotic cells is mediated by coated vesicles. The archetypal protein coats COPI, COPII, and clathrin are conserved from yeast to human. Structural studies of COPII and clathrin coats assembled in vitro without membranes suggest that coat components assemble regular cages with the same set of interactions between components. Detailed three-dimensional structures of coated membrane vesicles have not been obtained. Here, we solved the structures of individual COPI-coated membrane vesicles by cryo–electron tomography and subtomogram averaging of in vitro reconstituted budding reactions. The coat protein complex, coatomer, was observed to adopt alternative conformations to change the number of other coatomers with which it interacts and to form vesicles with variable sizes and shapes. This represents a fundamentally different basis for vesicle coat assembly.

Cellular transport vesicles are formed by conserved protein coats (*1*–*3*). Detailed structural information about vesicle coats assembled on a membrane bilayer has remained elusive. The clearest insights into the architecture of vesicle coats have been obtained by applying electron microscopy (EM) to coat protein complex COPII and clathrin protein cages, assembled in vitro from outer coat protein components in the absence of membranes (*1*, *4*, *5*). The cages have point group symmetries and discrete size distributions (*6*), whereas in vivo formed clathrin-coated vesicles are surrounded by cages that can deviate from point group symmetry (*7*). In both cases, each cage subunit makes the same set of interactions with the same number of partners. The clathrin cage vertex consists of three-fold triskelions, whose arms intertwine to form two-fold symmetrical edges with some flexibility; in COPII, four rod-like edges of the cage converge at each two-fold vertex (fig. S1, A and B). F1

In the COPI system, which transports cargo within the Golgi and from the Golgi to the endoplasmic reticulum (ER) (*8*), the membrane-binding inner coat components are recruited to the membrane en bloc with the outer coat components as a single heptameric complex, called coatomer (*9*). Cytosolic coatomer is a highly flexible complex, precluding high-resolution structural study (*10*). To obtain structural information on the COPI coat, we studied COPI-coated membrane vesicles.

COPI-coated vesicles were formed by reconstituting budding in vitro with giant unilamellar vesicles (GUVs) as donor membranes together with recombinantly expressed heptameric coatomer complex (*11*), the small guanosine triphosphatase Arf1, a guanine nucleotide–exchange factor, and guanosine 5′-*O*-(3′-thiotriphosphate). The budding reaction was plunge-frozen without further purification and analyzed by cryo–electron tomography. Large numbers of coated vesicles were formed, ranging in shape from spherical to slightly elliptical (Fig. 1, A and B). Vesicle membranes were covered with a fuzzy coat ~14 nm thick. The mean vesicle diameter at the membrane was 45 nm ± 6 nm (n = 244) (Fig. 1C), comparable with observations carried out in vivo (*12*) and in vitro (*13*, *14*). In control incubations in the presence of guanosine diphosphate, no coated vesicles were produced.

The variable size and shape of the vesicles precluded averaging of whole vesicles to obtain a high-resolution three-dimensional (3D) structure. We thus applied subtomogram averaging to analyze the repetitive unit of the coat. Subvolumes were extracted at vesicle surfaces and were iteratively aligned and averaged in an unbiased, reference-free manner (*15*–*17*). This procedure was performed on two independent budding reactions, converging to the same structure: a three-fold symmetrical arrangement of leaf-shaped densities surrounding a central platform (Fig. 2A and fig. S2, A to C). Three leaves form a triad, which is ~32 nm across. Subtomogram averaging of further data sets yielded a 3D structure of the triad at 26 Å resolution (Fig. 2, B to D; fig. S3; and movie S1). The triad contacts the membrane below each leaf (Fig. 2C, black arrowhead) and below the central platform (Fig. 2C, white arrowhead). F2

The size of one leaf matched the expected mass of one coatomer-Arf1 complex (*17*), whereas its shape was comparable to that of the cytosolic yeast coatomer complex (*10*) (fig. S4). The crystal structure of the adaptor protein 2 (AP2) trunk domain (*18*), which is homologous to the βδ/γζ-COP sub-complex (*19*), did not fit as a rigid body into the leaf, which indicated conformational differences between crystallized AP2 and the assembled form of βδ/γζ-COP (*17*) (fig. S5, A and B). Upon automated docking of the triskelion-like crystal structure of an αβ′-COP fragment (*20*) into the triad, parts of αβ′-COP protruded from the density (*17*) (fig. S5, C and D). This suggested that, within the assembled coat, either the triskelion does not represent the structural form of αβ′-COP or that the triskelion arms are differently oriented. Upon automated docking of a single copy of αβ′-COP, the two highest-scoring solutions were found within the leaf of the triad such that the α-COP α-solenoid of one solution superimposes on the homologous α-solenoid in β′-COP from the other solution, and vice versa, consistent with the described pseudo–two-fold interaction between α- and β′-COP (*17*, *20*) (fig. S5, E and F).

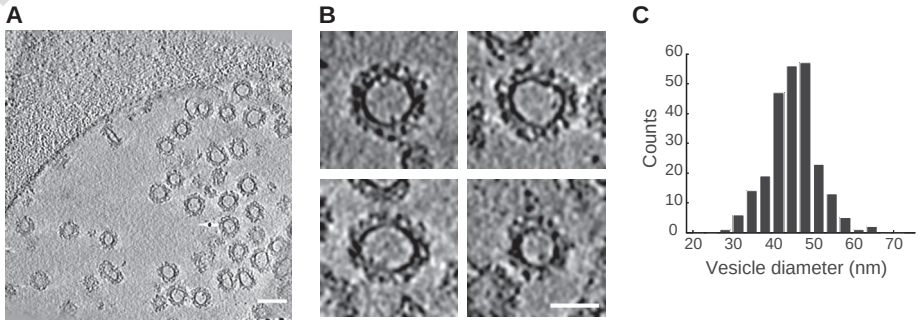

Fig. 1. COPI-coated vesicle budding reconstituted from giant unilamellar vesicles. (**A**) Section through a cryo–electron tomogram of COPI-coated vesicles from an in vitro budding reaction. Scale bar, 100 nm. (**B**) Central sections of vesicles showing variable size and ellipticity, and a fuzzy ~14-nm-thick coat. Scale bar, 50 nm. (A) and (B) were Gaussian filtered to ease visualization. (**C**) Average vesicle diameter measured at the membrane. Distribution mean is 45 nm (n = 244).

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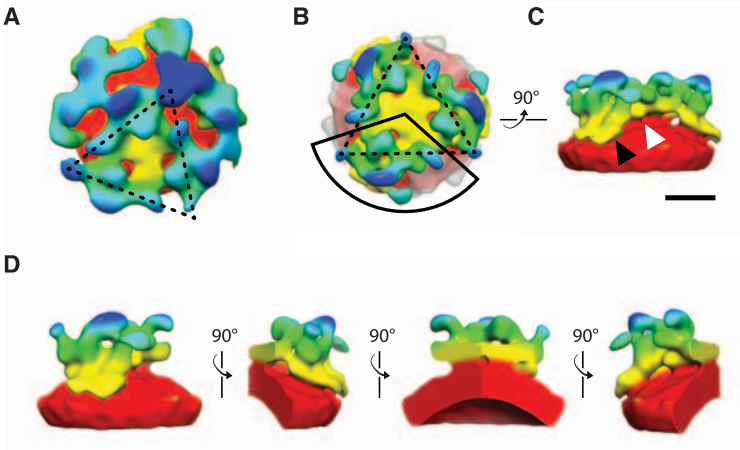

Fig. 2. Structure of a COPI triad. (**A**) Isosurface representation of a reference-free reconstruction of a COPI-coated vesicle subvolume (fig. S2). Coat density is colored from yellow to blue according to radial distance from the membrane (red). A triad consists of three leaf-like coatomer densities, arranged in a pseudo–three-fold fashion (dotted triangle) around a central platform (yellow). (**B**) Top view, 26 Å resolution isosurface representation of a triad after three-fold symmetrization. Dotted triangle highlights three-fold symmetry. Black outline indicates one leaf. Transparent surfaces indicate density from adjacent triads. (**C**) Side view of the triad, showing contacts with the membrane below the platform (white arrowhead), and below the leaf (black arrowhead). Scale bar, 10 nm. (**D**) Side views of the leaf outlined in (B).

To generate "lattice maps" showing the arrangement of triads on individual coated vesicles, we placed triangles at the positions and rotational orientations to which triad subtomograms aligned (*15*, *17*) (Fig. 3, A-D). This showed that the corners and edges of triads are arranged around local three-fold symmetry axes in a triangular lattice (Fig. 3, A and B). All vesicles also had local positions at which the corners of two, instead of three, triads met, either singly (Fig. 3C) or paired (Fig. 3D). Because a triangular lattice is geometrically flat, such two-corner positions are required for assembly of a curved lattice. A relative increase in number of three-corner over two-corner positions, or of paired two-corner over single two-corner positions, increases the diameter of the resulting shell in any curved triangular lattice. The relative numbers of triad patterns found on vesicles of different sizes were consistent with these geometrical principles. For example, two-corner positions were more prevalent relatively to three-corner positions in smaller vesicles with higher membrane curvature (fig. S6A).

The distribution of triads over the vesicle surface did not consistently conform to any point group symmetry. Vesicle coats also contained gaps in the triangular lattice (Fig. 3E). In some cases, the triad arrangement around such gaps precluded the addition of further triads, making it unlikely that gaps resulted from disassembly, prematurely arrested assembly, or unidentified triads; instead, the arrangement suggested that gaps formed during assembly and budding. Electron density seen at most gaps indicated that they contained disordered protein density. Lattice maps of COPI-coated buds that had not completed scission were incomplete at the bud neck (Fig. 3F), which suggested that the gap represents a "budding scar" and that formation of a complete, closed protein coat is not required to mediate vesicle formation in the in vitro budding reaction. Quantitative fluorescence microscopy shows that, under certain conditions, a partially complete clathrin coat can also internalize vesicles from the plasma membrane (*21*, *22*).

To derive structural information about the interactions between triads, we calculated the positional and rotational coordinates of all positions where the corners of three triads met (Fig. 3A), positions where the edges of three triads met (Fig. 3B), single two-corner positions (Fig. 3C), and paired two-corner positions (Fig. 3D). Structures derived by averaging subtomograms extracted at these positions (Fig. 3, G to N, and fig. S6B), revealed that the central platform of the triad and the cores of the leaves were consistent in all structures, whereas the parts of leaves forming corners and edges of the triad adopted different conformations (Fig. 3, K to O). Where three triad corners met, leaves formed a raised three-fold connection (Fig. 3K, arrow). At single and paired two-corner positions, the corner of the triad adopted a different conformation (Fig. 3, M and N, arrows). The connections between triad edges can link three (Fig. 3L, arrowhead) or two triads (Fig. 3, M and N, arrowheads). Hence, dependent on its position within the lattice, coatomer can form homotrimeric or dimeric interactions. This variable valency of interaction is achieved through substantial conformational variability (Fig. 3O).

Structures of triads and linkages were placed at the positions and orientations where they were found in each vesicle and were merged to create continuous density models of individual vesicles (Fig. 4 and movie S2). These models suggest that the COPI coat contains only small apertures (Fig. 4 and fig. S1). Sufficient membrane access for fusion with a target membrane could only be achieved after coat disassembly or through budding scars. In contrast, clathrin and COPII cages form lattices with larger apertures (fig. S1).

In existing models for clathrin and COPII vesicle coats, multiple identical subunits each make the same set of interactions with the same number of neighbors (*1*). Structural flexibility allows formation of vesicles from different total numbers of subunits. Based on these principles, both clathrin-like (*20*) and COPII-like (*23*) models have been proposed for the assembled COPI coat. We found instead that assembled coatomer can adopt different conformations to interact with different numbers of neighbors. By regulating the relative frequencies of different triad patterns in the COPI coat during assembly, for example, by stabilizing particular coatomer conformations, the cell would have a mechanism to adapt vesicle size and shape to cargoes of different sizes.

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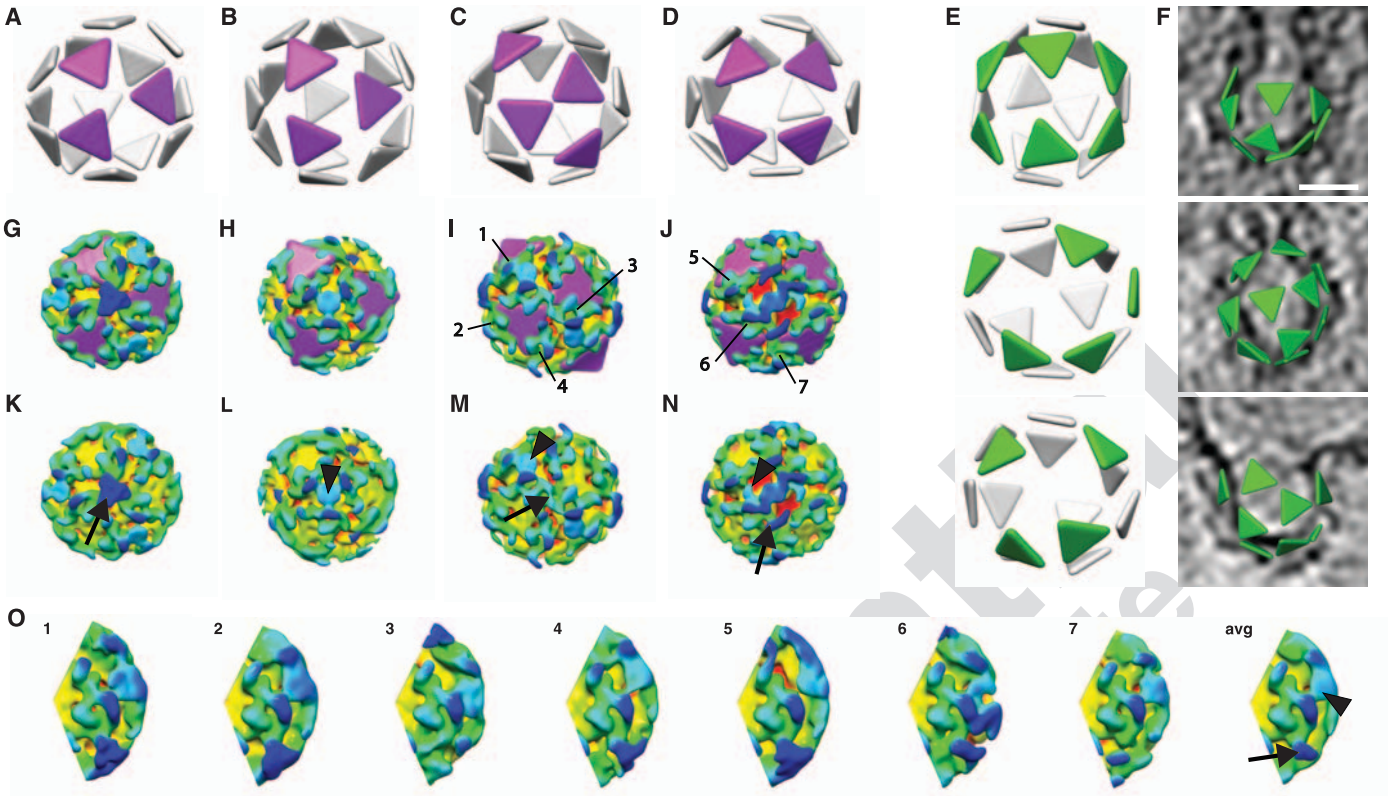

Fig. 3. Arrangement of triads in the vesicle coat and structures of triad patterns. (**A** to **D**) Lattice maps where each triangle represents the position and orientation of a triad identified during subtomogram averaging. Purple triangles indicate characteristic triad arrangements: (A) where the corners of three triads met, (B) where the edges of three triads met, (C) a single two-corner position, and (D) a paired two-corner position. (**E**) Three coated vesicle lattice maps with the budding scar oriented toward the reader. The triad arrangement around the gap (green) precludes closing the gap by placing further triads into the lattice. (**F**) Lattice maps of COPI vesicle buds, superimposed on tomogram slices to show the shape of the membrane. The only gap found is at the bud neck (oriented toward the top). Scale bar, 30 nm. (**G** to **J**) Isosurface representations of structures corresponding to characteristic triad patterns, superimposed on the purple triangles. Numbers in (I) and (J) indicate the seven unique leaf conformations relative to the two-corner positions. (**K** to **N**) As in (G) to (J) without superimposed triangles. Arrows and arrowheads indicate triad corners and edges, respectively, where different conformations are seen in different linkages. (**O**) Isosurface representations of each of the different leaf conformations, these are averages of all leaves found at equivalent positions indicated in (I) and (J). Leaf number 2 is the conformation at the three-corner position. The leaf marked "avg" is the weighted average of leaves 1 to 7. Densities are cut as in the black sector of Fig. 2B. The inner region of the leaves, facing the platform (left side), is constant, whereas the connections with other triads (arrow and arrowhead in avg) show significant structural variability. Densities are colored as in Fig. 2.

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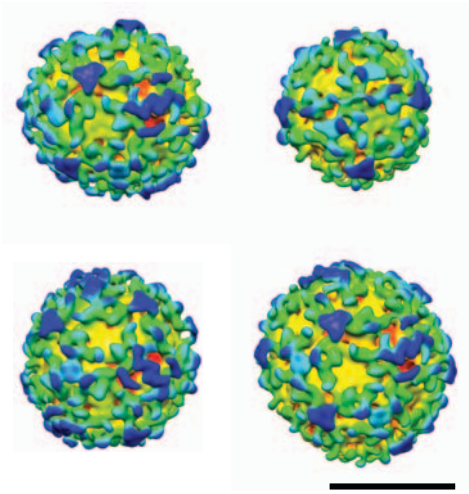

Fig. 4. The structures of COPI-coated vesicles. Isosurface representations of four COPI-coated vesicles produced by positioning reconstructions of triads and triad patterns (Figs. 2 and 3, K to N) at the positions and orientations in space at which they were identified during subtomogram averaging. Densities are colored as in Fig. 2. Scale bar, 50 nm.

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