

100-kD Coated Vesicle Proteins: Molecular Heterogeneity and Intracellular Distribution Studied with Monoclonal Antibodies

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Abstract. Proteins with molecular weights of around 100,000 (designated 100K) are found in all coated vesicles. Five monoclonal antibodies have been raised against the major 100K proteins of bovine brain coated vesicles, which migrate on SDS gels as three closely spaced bands. One antibody stains the middle band (band B), two stain both upper and lower bands (bands A and C), and two stain the lower band (band C) only. Thus, the polypeptides in bands A and C are related (but not identical), a result confirmed by NH₂-terminal sequencing. Other tissues were found to express proteins corresponding to, and co-migrating with, bands B and C but not band A. Only the two antibodies that recognize both A and C stained fixed and permeabilized tissue culture cells; they both showed a punctate pattern in the plane of the plasma membrane. Double labeling with anti-clathrin antibod-

ies confirmed that the dots correspond to coated pits and vesicles. However, perinuclear staining seen with anti-clathrin, corresponding to Golgi-derived coated vesicles, was conspicuously absent with the two monoclonal antibodies. Affinity-purified polyclonal antisera against the 100K proteins, reported earlier, gave perinuclear as well as punctate staining; these included one antiserum which gave mainly perinuclear staining (Robinson, M. S., and B. M. F. Pearse, 1986, *J. Cell Biol.*, 102:48-54). Thus, different 100K proteins appear to be found in different membrane compartments. Since the 100K proteins are thought to lie between clathrin and the membrane proteins of the vesicle, these results may help to explain how different membrane proteins can be sorted into coated vesicles in different parts of the cell.

CLATHRIN-COATED pits and vesicles play an important part in the sorting of proteins during intracellular membrane traffic. On the plasma membrane, coated pits concentrate selected membrane proteins and cause them to be efficiently internalized during receptor-mediated endocytosis (8) and recycling of secretory granule components (15). Inside the cell, coated vesicles are most abundant in the region of the Golgi apparatus. Here they are thought to be involved in the targeting of proteins to organelles such as lysosomes (3) and secretory granules (14).

Recent studies suggest that a family of proteins, with molecular weights of around 100,000 (designated 100K), may provide the molecular basis for the sorting of proteins by coated vesicles. Both biochemical and structural work indicate that the 100K proteins are positioned between clathrin and the vesicle membrane (23, 24). In addition, Pearse (17) has been able to demonstrate that the mannose-6-phosphate receptor, one of the membrane proteins concentrated in coated pits, binds directly to complexes containing the 100K proteins and a closely associated 50-kD protein. Genetic engineering experiments performed on other such membrane proteins indicate that the cytoplasmic domain contains the information that directs them into coated pits (8, 22). Thus,

these results all suggest the 100K proteins may interact with the cytoplasmic tails of selected transmembrane proteins and link them to clathrin, causing them to be sequestered into coated pits and vesicles for transfer to another organelle.

We have purified the 100K proteins from bovine brain coated vesicles and have shown that they can be divided into two groups, based on their behavior on hydroxylapatite columns (18). The group comprising the bulk of the protein has been designated HA-II (for hydroxylapatite group II). The 100K proteins in this group migrate on SDS gels as three closely spaced bands, but the upper and lower bands can be separated from the middle band by running a second hydroxylapatite column in the presence of SDS. Peptide mapping by limited proteolysis indicates that the upper and lower bands are related to each other, but are markedly different from the middle band (21).

When polyclonal antisera were raised against the HA-II 100K proteins, each antiserum showed a somewhat different labeling pattern on immunoblots. Two of these antisera were able to stain cells and also showed somewhat different labeling patterns by immunofluorescence (21). This result raised the possibility that different 100K proteins might be found in different types of coated vesicles, and thus suggested a means

whereby different membrane proteins could be sorted into coated vesicles in different membrane compartments. However, because we were working with polyclonal antisera, we could not really correlate the patterns seen on blots with the patterns seen in intact cells.

I have now raised and characterized five monoclonal antibodies against the HA-II 100K proteins. These antibodies have been useful biochemical tools for characterizing the proteins further. In addition, two of the antibodies stain tissue culture cells, and provide further support for the idea that different 100K proteins may be found in coated vesicles embarked on different pathways of membrane traffic.

Materials and Methods

Antibody Production

The HA-II 100K proteins were purified and prepared for injection as previously described (21). BALB/c mice were immunized with 50 µg of protein and were boosted after 2 and 8 wk. 1 wk after the final injection, the antisera were screened on immunoblots (21). The mice that were to be used for fusions were then injected intraperitoneally with 50 µg of protein without Freund's adjuvant on the fifth and fourth days before the fusion.

Fusions were carried out using NSO myeloma cells, essentially as described by Galfre and Milstein (6). Cells were plated into four 24-well plates, and the supernatants were assayed when yellow on immunoblots. Positives were then cloned twice before being grown in large flasks for culture supernatants or injected into mice for ascites fluid. Antibody subclasses were typed using a kit provided by Cambridge Bioscience (Cambridge, England).

Immunoblots

Immunoblots were prepared by electrophoresing protein samples on minigels, transferring them onto nitrocellulose, and labeling them with antibodies and radioactive probes as described (21). Antibodies B1-M6 and AC1-M11 were detected with ¹²⁵I-protein A, while the other three antibodies, which do not bind protein A, were detected with affinity-purified ¹²⁵I-rabbit anti-mouse immunoglobulin. Among the protein samples treated in this way were bovine brain 100K proteins that had been separated on hydroxylapatite in the presence of SDS (21), human placental coated vesicles (16), and rat liver coated vesicles (20). Whole tissue homogenates were also blotted; these were prepared by cutting small pieces of fresh tissue, homogenizing the tissue in boiling sample buffer, and sonicating and centrifuging the samples before running them on a gel. In addition, blots were made of column fractions of rat liver coated vesicles chromatographed on hydroxylapatite in the presence of SDS. This was done by incubating the coated vesicles (~1 mg in 0.5 ml) with 1% SDS at 37°C for 15 min, then diluting them to 0.1% SDS and dialyzing them against the column starting buffer. The protein was applied to a 1-ml hydroxylapatite column and eluted with a gradient of 0.2 to 0.5 M phosphate as described (21).

Immunoprecipitation

Purified HA-II 100K protein (~3–4 µg in 5 µl) was made 0.2% in SDS and heated to 37°C for 15 min. This solution was then mixed with 100 µl 10% fixed *Staphylococcus aureus* suspended in PBS containing 0.05% Tween-20, and 3 µl ascites fluid from hybridoma AC1-M11. The tube was rotated for 1 h and then centrifuged, and the pellet was washed twice. The final pellet was made up to the same volume as the first supernatant, and both samples were boiled in sample buffer, subject to electrophoresis, and blotted.

Immunofluorescence

Primary cultures of bovine fibroblasts were generously provided by Dr. Ann Dane (ARC Institute of Animal Physiology, Babraham, England). They were grown on multiwell test slides and prepared for immunofluorescence in several different ways. The fixation that worked best for the monoclonal antibodies was immersion of the slide in -20°C methanol for 5 min, followed by air drying. The anti-clathrin antiserum gave the best staining on cells that had been fixed for 30 min in PBS containing 2% paraformaldehyde and 0.01% glutaraldehyde, blocked by a 15-min incubation in 1 mg/ml so-

Table I. Antibody Characterization

Antibody	Subclass	Specificity	Cell staining
B1-M6	IgG2a	Middle (B)	-
AC1-M11	IgG2a	Upper and lower (A and C)	+
AC2-M15	IgG1	Upper and lower (A and C)	+
C1-M15	IgM	Lower (C)	-
C2-M15	IgM	Lower (C)	-

dium borohydride, and permeabilized for 10 min in 0.1% Triton X-100. For double labeling, cells were fixed for 10 min in 3.7% paraformaldehyde in PBS, followed by 5 min in -20°C methanol and 30 s in -20°C acetone.

Antibody labeling was carried out essentially as described (21). The monoclonal antibodies were used at a dilution of 1:10 for the culture supernatants and 1:100 for the ascites fluid, although staining could still be detected at much greater dilutions. Cells that had been incubated with monoclonal antibodies alone were then incubated with affinity-purified fluorescein rabbit anti-mouse Ig (Miles Laboratories, Inc., Naperville, IL). The mounting medium contained 0.05 µg/ml of the DNA stain 4,6 diamido-2-phenylindole (DAPI)¹ so that nuclei could be visualized with the appropriate filter combination.

Anti-clathrin light chain antibodies were affinity purified from a rabbit antiserum that had been prepared against bovine brain triskelions by Dr. Ernst Ungewickell while he was in this laboratory. For double labeling with wheat germ lectin, the anti-clathrin incubation was followed by incubations in affinity-purified fluorescein goat anti-rabbit (Sigma Chemical Co. Ltd., Dorset, England) and rhodamine wheat germ lectin, as described (21).

Cells that were to be double labeled with anti-clathrin and anti-100K were first incubated with the monoclonal anti-100K, then with the anti-clathrin. This was followed by an incubation with a mixture of fluorescein sheep anti-mouse and rhodamine sheep anti-rabbit (Cappel Laboratories, Cochranville, PA). Both of these secondary antibodies had been affinity purified with the appropriate immunoglobulin, then absorbed with immunoglobulin from the other species to remove any cross-reacting antibodies. In control experiments, one or the other of the two primary antibodies was omitted, which abolished the respective fluorescent staining and showed that the labeling observed was indeed due to the presence of the specific antibody.

NH₂-terminal Sequencing

Pure preparations of the three 100K bands were obtained by hydroxylapatite chromatography in the presence of SDS followed by preparative gel electrophoresis, as previously described (21). The bands were eluted into dialysis bags and extensively dialyzed against 20% ethanol containing 0.2% 2-mercaptoethanol. Samples were then lyophilized, dissolved in 70% formic acid, desalted by gel filtration on Bio-Gel P-6 (Bio-Rad Laboratories, Whatford, England), and lyophilized a second time. About 100 µg of pure protein was recovered from each band. The proteins were then dissolved in 70% formic acid and sequenced on an Applied Biosystems 470A gas phase protein sequencer, using off-line high performance liquid chromatography to identify the phenylthiohydantoin derivatives (5).

Results

Characterization of the Five Antibodies

Five monoclonal antibodies were obtained from mice that had been immunized with total HA-II 100K proteins. Table I lists these antibodies and their specificities. The nomenclature was kept as simple as possible, with the name of each antibody telling its specificity and the mouse from which it was derived. Thus, for instance, AC1-M11 is an antibody against the upper and lower bands (A and C), derived from mouse 11. As can be seen in Fig. 1, one of the antibodies (B1-M6) is against the middle band (B), two (AC1-M11 and

1. Abbreviation used in this paper: DAPI, 4,6 diamido-2-phenylindole.

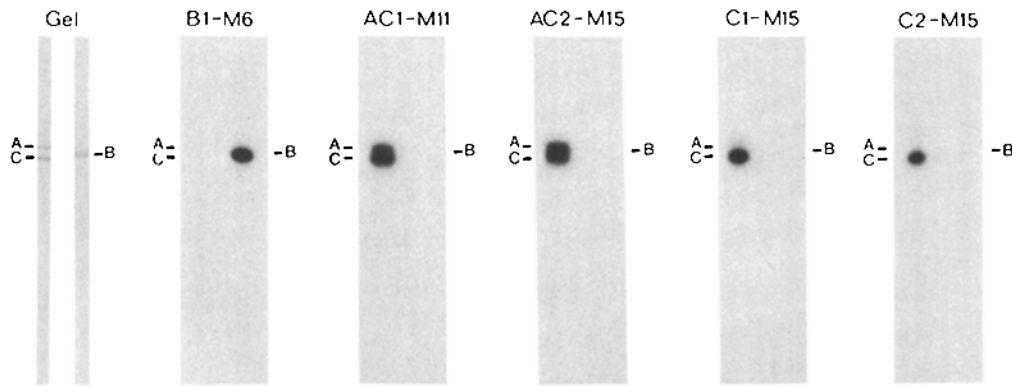


Figure 1. Immunoblots of HA-II 100K proteins from bovine brain coated vesicles. The bands were separated by hydroxylapatite chromatography in the presence of SDS, and fractions containing either A and C (*left lanes*) or B (*right lanes*) were subject to electrophoresis and blotted. The blots were stained with Ponceau S before blocking, and the positions of the bands were marked with pencil so that the autoradiographs and blots could be aligned. One antibody, B1-M6, stains band B; two others, AC1-M11 and AC2-M15, stain both A and C; and the last two, C1-M15 and C2-M15, stain band C only.

AC2-M15) are against both the upper and lower bands (A and C), and two (C1-M15 and C2-M15) are against the lower band (C) only.

NH₂-terminal Sequences of the 100K Proteins

The ability of two of the monoclonal antibodies to recognize both upper and lower bands indicates that the polypeptides in these two bands share common antigenic determinants. To compare the NH₂-terminal sequences of the different 100K proteins, each band was electrophoretically purified and subjected to Edman degradation. The protein or proteins in the middle band, B, could not be sequenced, presumably because of a blocked NH₂ terminus. However, the first 10 amino acids of bands A and C could be identified, and appeared to be identical:

Pro Ala Val Ser Lys Gly Ser Gly Met Gly

It is unlikely that band C is merely a breakdown product

of band A, however. Their peptide maps are related but show several distinct differences (21). Moreover, two of the monoclonal antibodies exclusively recognize band C. Thus, the polypeptides in bands A and C appear to have identical NH₂ termini and are closely related, but nevertheless must vary in other regions of their amino acid sequences.

100 K Proteins in Other Tissues and Species

The antibodies were all tested for their ability to cross-react with the 100K proteins of coated vesicles purified from human placenta and rat liver. Fig. 2 shows that cross-reactivity is generally good: all the antibodies except for C1-M15 reacted strongly with human placenta (Fig. 2 *a*), while all the antibodies except for AC2-M15 reacted strongly with rat liver (Fig. 2 *b*). Immunoblots of human and rat brain homogenates indicate that the relative inability of these antibodies to cross-react is due to the difference in species rather than the difference in tissue (data not shown).

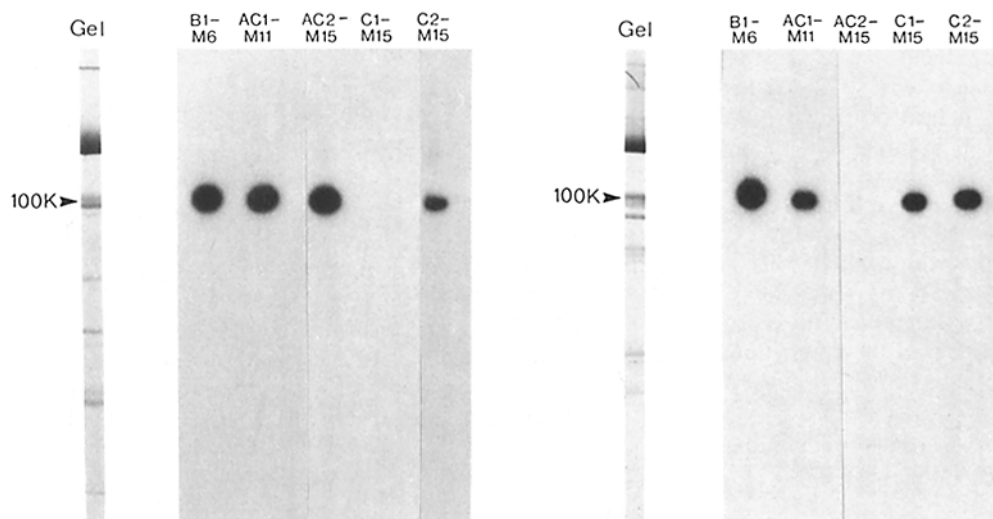


Figure 2. Coated vesicles purified from other sources. Coated vesicles were purified from human placenta (*a*) and rat liver (*b*), subject to electrophoresis, and blotted. In both cases, four out of the five antibodies show strong labeling of the 100K region, but the antibody that does not cross-react is different in the two blots.

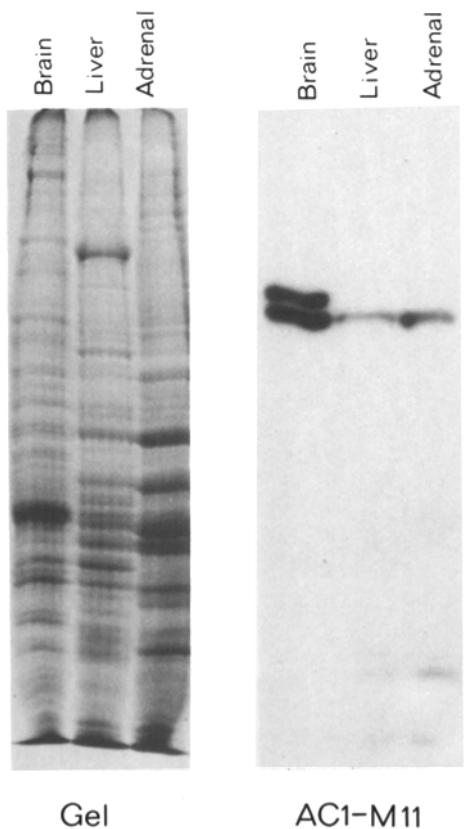


Figure 3. Immunoblots of whole tissue homogenates from three different rat organs, run on a long gel. Antibody AC1-M11 labels a doublet, bands A and C, in the lane of brain protein, but only detects a single band, co-migrating with band C, in the other two lanes, which contain protein from liver and adrenal gland.

Although the 100K proteins of human placenta and rat liver coated vesicles can be labeled with all three of the different types of antibodies (anti-B, anti-A and C, and anti-C), the pattern of bands in the 100K region looks different from that of bovine brain. Two experiments indicate that coated vesicles from these other tissues contain bands corresponding to, and co-migrating with, bands B and C from brain but not band A. First, whole homogenates of a number of different rat organs, three of which are shown in Fig. 3, were subject to electrophoresis on a long gel, blotted, and stained with AC1-M11. Although two bands can be seen in the lane of brain protein, the other lanes only show one band when stained with the antibody, which co-migrates with band C from brain. Second, the 100K proteins of rat liver coated vesicles were separated on hydroxylapatite in the presence of SDS (Fig. 4 a). The rat liver band that co-migrates with band C from brain peaked at around fraction 30, while the band that co-migrates with band B peaked at around fraction 40. A similar elution profile is seen when bovine brain 100K proteins are chromatographed on hydroxylapatite with SDS (21). The identity of the two rat liver bands was confirmed by staining blots of the column fractions with monoclonal antibodies specific for band B (Fig. 4 b) and bands A and C (Fig. 4 c). These results indicate that bands B and C are expressed in all tissues, while band A so far appears to be brain specific.

Immunoprecipitation

Although none of the antibodies were able to precipitate the 100K proteins in their native state, antibody AC1-M11 brought down proteins that had first been denatured in SDS (Fig. 5). When the supernatants and pellets from such an experiment are compared, it can be seen that bands A and C have been selectively precipitated. To test whether the other antibodies bind to the same polypeptides as AC1-M11, supernatants and pellets from an immunoprecipitation experiment were blotted and probed with the five different antibodies (Fig. 5). As expected, B1-M6, which is against band B, almost exclusively labeled the supernatant, while AC1-M11 itself mainly labeled the pellet. All of the other antibodies also mainly labeled the pellet. Thus, antibodies AC2-M15, C1-M15, and C2-M15 all recognize polypeptides that are also recognized by AC1-M11.

Immunofluorescence

Before using the antibodies for immunofluorescence, it was necessary to show that they labeled bands of the correct

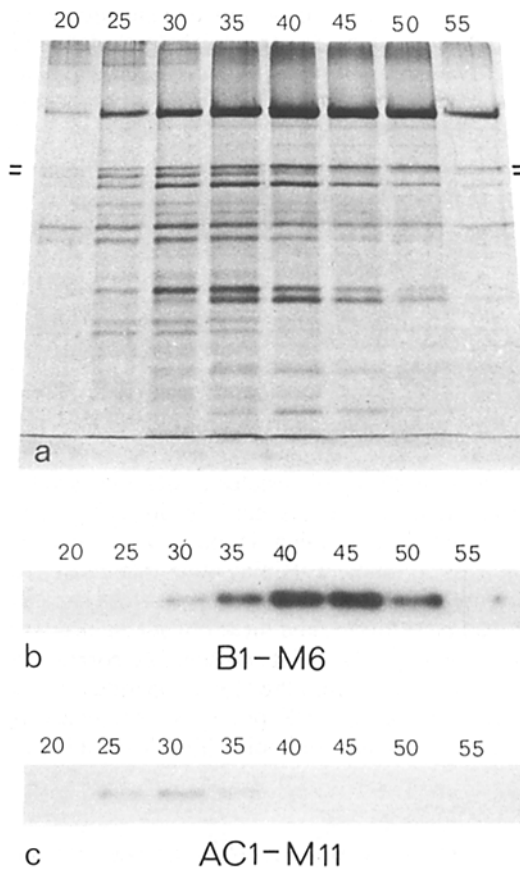


Figure 4. Rat liver coated vesicles separated by hydroxylapatite chromatography in the presence of SDS. (a) Silver-stained gel showing eight of the column fractions. Bands co-migrating with bands B and C of brain are indicated; the band just below is apparently unrelated. The band co-migrating with C elutes before the band co-migrating with B, consistent with the behavior of brain 100K proteins on hydroxylapatite with SDS. (b) Blot of a similar gel stained with B1-M6. Only the 100K region is shown. (c) Blot stained with AC1-M11. The antibody labeling confirms the identity of the two bands.

molecular mass on blots of tissue culture cells. Fig. 6 shows that three of the antibodies, B1-M6, AC1-M11, and AC2-M15, are clearly able to detect a 100K band on blots of bovine fibroblasts. Although B1-M6 works well on blots, it has so far not worked for immunofluorescence, probably because the antigenic site recognized by this antibody is not accessible under any of the fixation conditions that have been tried. However, antibodies AC1-M11 and AC2-M15, both of which label bands A and C on blots of brain 100K proteins, gave good immunofluorescent staining on methanol-fixed cells.

Fig. 7, *a* and *b*, shows bovine fibroblasts stained with the two antibodies, and demonstrates that the two staining patterns are very similar. The cells are covered with fluorescent dots, often in clusters and sometimes linearly arranged. Focusing up and down on the cells indicates that the dots are in the plane of the plasma membrane. However, perinuclear staining in these cells is conspicuously absent: in fact, in some cells it is difficult to determine where the nuclei are located unless the cells are double labeled with a nuclear stain such as DAPI (Fig. 7, *c* and *d*).

When cells are stained with a rabbit antiserum against clathrin light chains (Fig. 8 *a*), the punctate labeling looks very similar to that seen with the two monoclonal antibodies. However, in addition, there is strong labeling of a perinuclear reticulum. Such staining is typically seen with antibodies against clathrin, both polyclonal (1) and monoclonal (4), as well as with polyclonal antibodies against total 100K proteins both in 3T3 cells (21) and in bovine fibroblasts (not shown). Double labeling with wheat germ lectin demonstrates that the perinuclear staining seen with anti-clathrin corresponds to the Golgi apparatus (Fig. 8 *b*).

To confirm that the monoclonal antibodies were labeling a subset of the structures stained with anti-clathrin, cells were double labeled with anti-100K and anti-clathrin. Fig. 9 demonstrates that most of the discrete fluorescent dots in the spread margins of the cells are identical with the two antibodies (*a* and *b*, *c* and *d*). Essentially all the dots stained with anti-100K are also stained with anti-clathrin, providing further evidence that uncoating of vesicles involves removal of both clathrin and 100K and not just clathrin alone (21). However, bright perinuclear staining is only seen with the anti-clathrin. This perinuclear staining often extends in a reticular pattern into the surrounding cytoplasm. Here, individual dots can be resolved, and these are not stained with the anti-100K (*e* and *f*). These dots presumably correspond to coated vesicles budding from the Golgi apparatus, which contain clathrin but not the 100K protein(s) recognized by these particular monoclonal antibodies (AC1-M11 and AC2-M15).

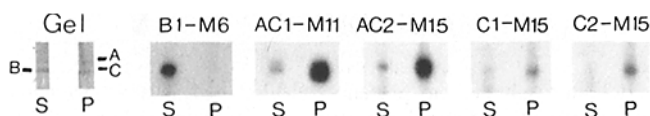


Figure 5. Immunoprecipitation of brain 100K proteins with AC1-M11. The supernatants (*S*) and pellets (*P*) were made up to the same volume. The gel demonstrates that most of the protein in bands A and C has been precipitated, while band B has remained in the supernatant. Blots of the two samples show that antibody B1-M6 labels the supernatant, while the other four antibodies mainly label the pellet.

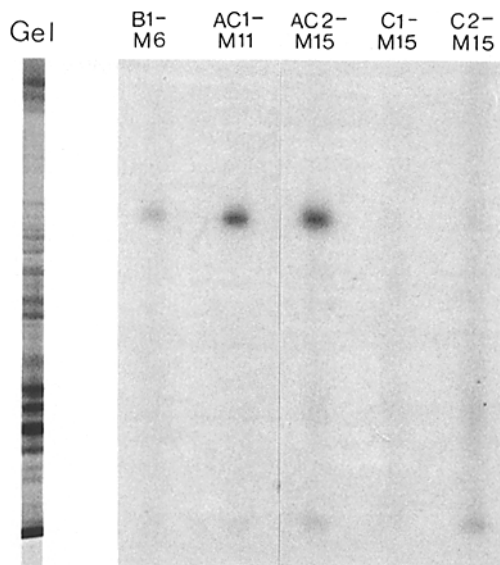


Figure 6. Gel and blots of a total homogenate of bovine fibroblasts. Antibodies B1-M6, AC1-M11, and AC2-M15 are all able to label a band in the 100K region.

Discussion

Monoclonal antibodies provide a means of distinguishing between different members of the 100K family of coated vesicle proteins. The antibodies described in this paper confirm earlier results indicating that there are at least three distinct polypeptides in the HA-II group of 100K proteins, corresponding to the three bands that can be resolved on SDS gels (21). Bands B and C appear to be expressed in all tissues, while band A has so far only been detected in brain. Bands A and C are closely related, even to the extent that they have identical NH₂ termini, but the ability of two of the antibodies to recognize band C but not band A indicates that band C is not simply a breakdown product of band A. None of these results rule out the possibility that there might be more than one polypeptide per band.

One subset of 100K proteins, containing bands A and C, appears by immunofluorescence to exist only in a particular subset of coated vesicles: those associated with the plasma membrane and not those in the Golgi region. This result provides clear evidence for the idea that different 100K proteins are found in different types of coated vesicles. That this might be the case was first suggested when different polyclonal antisera against the 100K proteins showed different amounts of staining of the plasma membrane compared with the Golgi apparatus. By raising monoclonal antibodies, it has been possible here to localize defined 100K species. Antibodies AC1-M11 and AC2-M15 both recognize bands A and C but not B on blots. They do not recognize the same epitope, however: AC1-M11 cross-reacts much more strongly with rat 100K proteins (see Fig. 2), and the two antibodies also label different bands on one-dimensional peptide maps (not shown). When used for immunofluorescence, both of these antibodies give plasma membrane staining but no Golgi staining.

It should be possible to refine the localization of the 100K proteins further. The number of different pathways that make

AC1-M11

AC2-M15

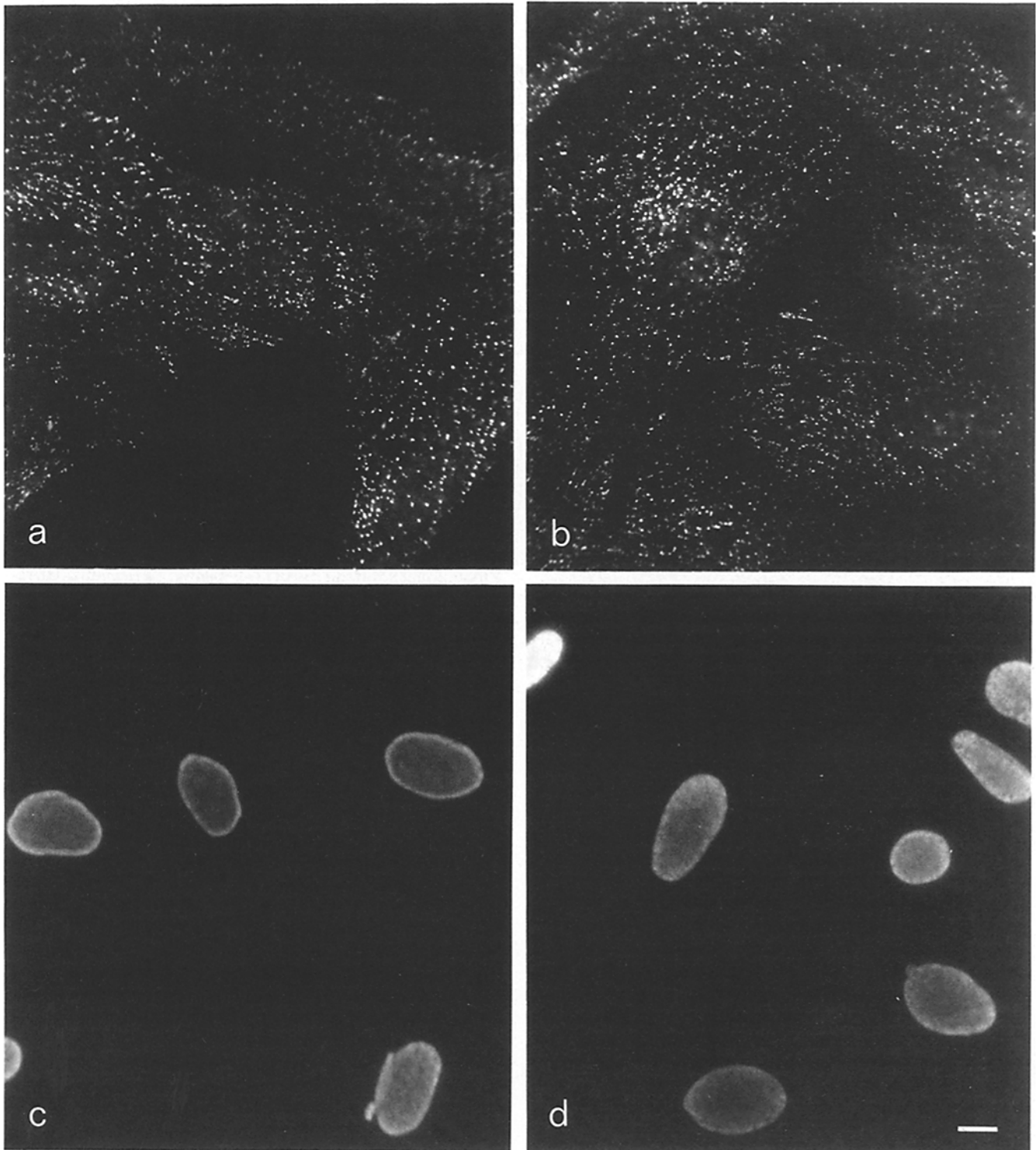


Figure 7. Bovine fibroblasts stained with AC1-M11 (*a*) and AC2-M15 (*b*). The nuclei of the cells in *a* are shown in *c*, and those of the cells in *b* are shown in *d*, labeled with the DNA stain DAPI. Punctate labeling in the plane of the plasma membrane can be seen with both antibodies, but perinuclear labeling is conspicuously absent. Bar, 10 μ m.

use of coated pits and vesicles is not yet known, but in addition to being associated with the plasma membrane and the Golgi apparatus, coated vesicles have also been reported to bud from endosomes (2, 7, 12), and there may be other path-

ways as well. Interestingly, when cells were double labeled with the monoclonal antibodies and anti-clathrin, faint dots could occasionally be seen in the spread margins of the cells that were only stained with anti-clathrin (see Fig. 9, *c* and

Anti-Clathrin

Wheat Germ Lectin

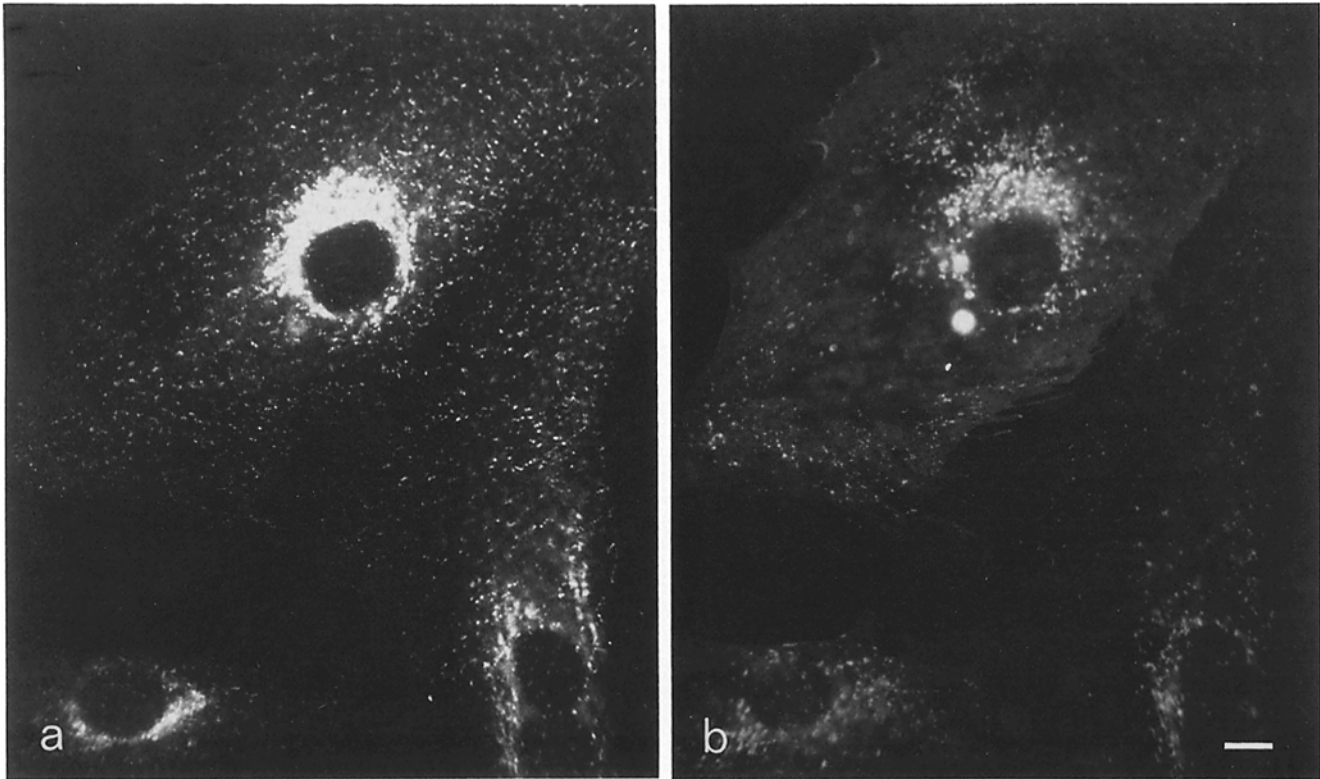


Figure 8. Cells labeled with affinity-purified rabbit anti-clathrin (*a*) and wheat germ lectin (*b*). The punctate labeling looks similar to that shown in Fig. 7, but there is strong perinuclear labeling as well. Wheat germ lectin, which binds to sugars that are added to glycoproteins in the Golgi apparatus and thus can be used as a Golgi marker, gives similar perinuclear staining to that seen with anti-clathrin, confirming that the antibody is labeling Golgi-derived coated pits and vesicles. Bar, 10 μ m.

d). Such dots may correspond to another subset of coated vesicles, such as coated vesicles budding from endosomes. If appropriate fixation conditions can be worked out, immunoelectron microscopy should greatly improve the resolution of the localization of the different antibodies.

Most important, however, will be to raise more monoclonal antibodies against the 100K proteins (HA-I as well as HA-II) and to select for those that stain cells, in order to complete the picture of where all the different 100K proteins are located. By staining cells with polyclonal antisera, we have been able to show that at least one component of the HA-II 100K proteins must be located in Golgi-derived coated vesicles, especially since one such antiserum (antiserum 3) actually gave stronger Golgi staining than plasma membrane staining when compared with anti-clathrin (21). The appearance of antiserum 3 on blots is misleading, however, and points out the problems that arise when trying to correlate blot staining with cell staining using a polyclonal antiserum. Antiserum 3 stained band A particularly strongly; but the two monoclonal antibodies that stain both A and C on blots give no Golgi staining at all. Moreover, when antiserum 3 was used to probe blots of 100K proteins immunoprecipitated with antibody AC1-M11 (see Fig. 5), the heavy labeling of band A was found in the pellet, not in the supernatant (not shown). Thus, it seems likely that a component of antiserum 3 that gives relatively weak staining on blots (possibly antibodies against band B) is responsible for most of the cell staining. In any case, it should be possible to raise mono-

clonal antibodies against the 100K proteins that stain the perinuclear region of the cell, and thus to define which of these proteins are present in coated vesicles that bud from the Golgi apparatus.

Three papers have recently appeared which report attempts to separate different types of coated vesicles biochemically. First, Pfeffer et al. (19) used monoclonal antibodies against synaptic vesicle antigens to affinity purify two subsets of bovine brain coated vesicles and found no obvious differences in their 100K proteins. However, it is possible that such a treatment would select for coated vesicles embarked on more than one pathway, since synaptic vesicle antigens might be expected to be present not only in coated vesicles that recycle at the synapse, but also in coated vesicles budding from the Golgi apparatus containing newly synthesized protein destined for specialized (i.e., regulated) secretory granules (13, 14). More recently, Helmy et al. (10) have used a novel density shift method to separate endocytic and exocytic coated vesicles purified from rat liver and again saw no clear differences in their 100K proteins. However, the two bands in rat liver corresponding to bands B and C in brain migrate very close together and cannot be resolved on heavily loaded gels. Finally, Kedersha et al. (11) have shown that agarose gel electrophoresis results in partial separation of different types of rat liver coated vesicles: different fractions are enriched in different content proteins. Interestingly, their gels, which were lightly loaded and silver stained, do show a slight difference in the 100K region of the various fractions, and one

AC1-M11

Anti-Clathrin

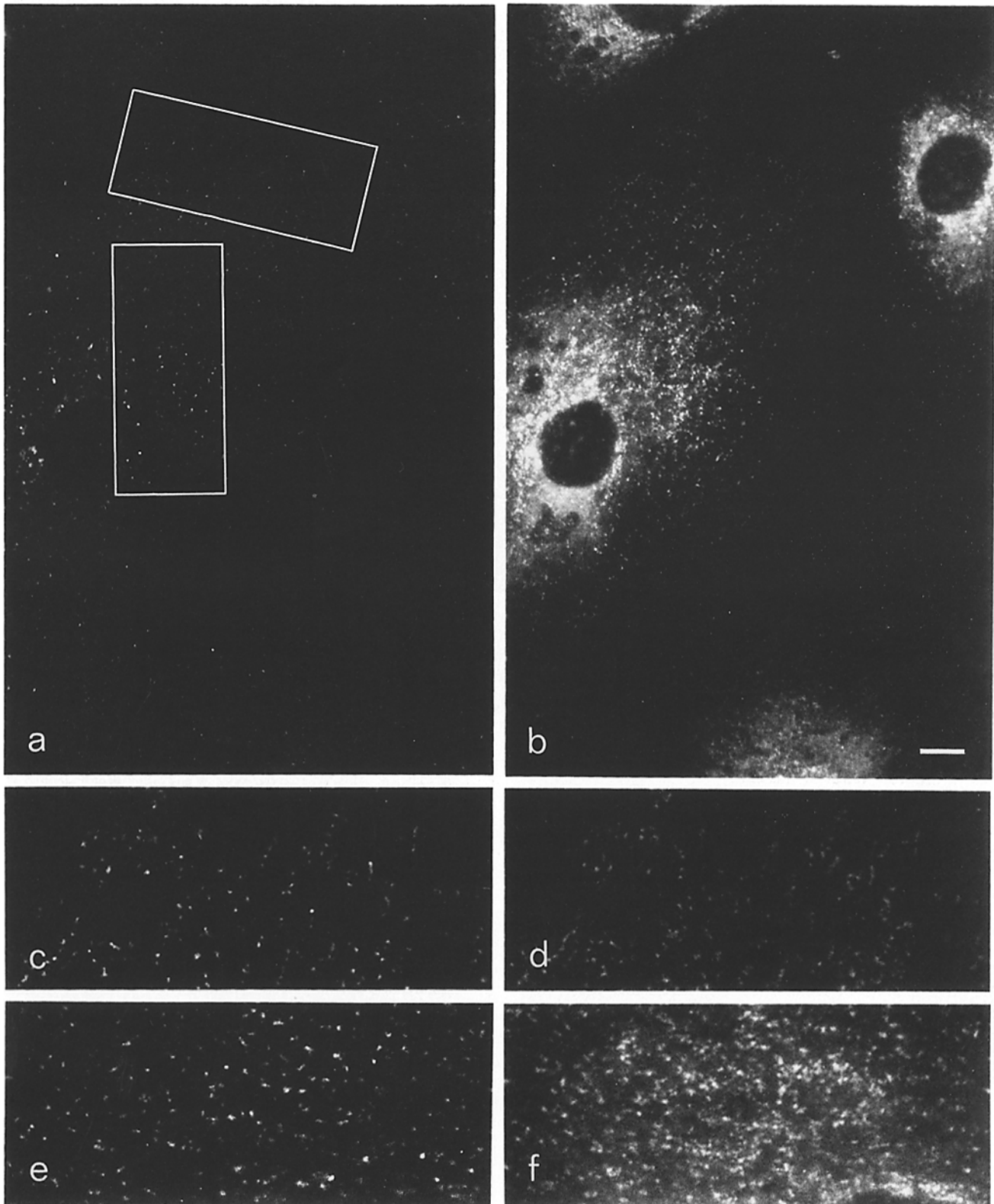


Figure 9. Double labeling with monoclonal antibody AC1-M11 (*a*, *c*, and *e*) and affinity-purified rabbit anti-clathrin (*b*, *d*, and *f*). The two regions indicated in *a* are shown enlarged in *c-f*, both rotated counter-clockwise. (*c* and *d*) In the spread margin of the cell, most of the fluorescent dots are identical with the two antibodies, although some of the fainter dots stained with anti-clathrin are not stained with the monoclonal antibody. (*e* and *f*) In the perinuclear region, many clusters of dots are seen that are only stained with anti-clathrin. These presumably correspond to coated vesicles budding from the Golgi apparatus. An occasional suggestion of perinuclear staining with the monoclonal antibody can be attributed to the shape of the cells: coated pits and vesicles associated with the upper plasma membrane around the nucleus appear diffusely fluorescent when the microscope is focused on the lower cell surface. Bar, 10 μm .

that is consistent with the possibility that band B may be found in Golgi-derived coated vesicles and band C in endocytic ones. The monoclonal antibodies against the 100K proteins could be used in conjunction with any of these methods as an additional tool for defining different subpopulations of coated vesicles. In addition, with the right monoclonal antibodies, it should be possible to affinity purify different types of coated vesicles based on their 100K protein content and then to look for other ways in which they differ from each other.

Coated vesicles are mainly seen budding either from the plasma membrane or from the Golgi apparatus. These two types of coated vesicles have different functions and are filled with different membrane and content proteins. For instance, the envelope glycoprotein of vesicular stomatitis virus, G protein, is concentrated in coated pits and vesicles when it is on the host cell surface (22), but is apparently excluded from coated vesicles when it is in the Golgi apparatus (9). The ability of the cell to select different proteins for inclusion into coated vesicles in different membrane compartments is essential for the correct targeting of proteins from one part of the cell to another. It has recently been suggested that there may be no difference in the coats on these different types of coated vesicles, but only in their membranes and contents (10, 11, 19). However, if the function of the coat is to specify the composition of the vesicle, by binding to selected membrane proteins (which are often receptors for content proteins), then it is difficult to imagine how the cell can form different types of coated vesicles without using different coat proteins. The results presented in this paper show that the 100K coat proteins, which are thought to provide the link between the clathrin outer cage and the vesicle membrane (24), do indeed appear to differ in different types of coated vesicles. Although there are still many questions that remain to be answered, this finding may provide a first step toward understanding how coated vesicles can carry out different functions in different parts of the cell.

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