COMMENTARY

Coated vesicles and protein sorting

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Coated vesicles have been found in virtually every type of eukaryotic cell. They can be recognized in the electron microscope by the characteristic structure of their coat, which forms a lattice of hexagons and pentagons on the cytoplasmic side of the vesicle. The lattice is made out of a protein called clathrin, and it is attached to the vesicle membrane through interactions with two other types of proteins: a family of proteins with M_r values of around 100000, and a 50000 M_r protein (reviewed by Pearse & Crowther, 1987).

The first function to be attributed to coated vesicles was endocytosis of yolk protein in vitellogenic oocytes (Roth & Porter, 1964). It has since been shown that coated vesicles are involved in the endocytosis of a number of other extracellular proteins bound to plasma membrane receptors, including, in mammalian cells, low density lipoproteins, transferrin and growth factors (reviewed by Goldstein *et al.* 1985). Such proteins are highly concentrated in coated pits on the cell surface, while other membrane proteins are efficiently excluded (Bretscher *et al.* 1980), allowing the cell to take up only those molecules that it needs. Once the coated pit has pinched off into the cytoplasm to form a coated vesicle, the coat is rapidly lost, and the vesicle then goes on to fuse with another organelle, the endosome, where further sorting and processing occurs. The coat proteins apparently recycle back to the plasma membrane to begin another round of receptor-mediated endocytosis.

Coated pits and vesicles are not only associated with the plasma membrane. They are found inside the cell as well, particularly in the region of the Golgi apparatus. Although definitive labelling studies still need to be done, existing evidence suggests that the Golgi-associated coated pits serve to concentrate newly synthesized proteins and package them into vesicles, which can then be shunted to other organelles, such as lysosomes and secretory granules (Brown & Farquhar, 1984).

The key issue here is how the cell knows which proteins to put into coated vesicles and which ones to leave out. A simple model would be that the transmembrane proteins (e.g. receptors) that collect in coated pits and vesicles all share a common feature on their cytoplasmic domains that interacts with one of the coat proteins. Although no obvious sequence homologies have been found in proteins for which the primary structure is known, genetic engineering experiments do indeed suggest that the information that directs certain membrane proteins into coated pits resides in the region that protrudes on the cytoplasmic side (Goldstein *et al.* 1985). The most likely candidates for the coat proteins that they might interact with are the 100K and 50K proteins (K = $10^3 M_r$), since these are the closest to the membrane vesicle. Direct binding has in fact been demonstrated between these proteins and the mannose-6-phosphate receptor (Pearse, 1985).

Coated vesicles associated with the plasma membrane and those associated with the Golgi apparatus carry out different functions and are filled with different contents. We might therefore expect the coats on these coated vesicles to be different as well. This appears to be the case. Two different polyclonal antisera that were raised against the 100K proteins preferentially labelled different parts of the cell: one gave stronger plasma membrane staining, while the other gave stronger Golgi staining (Robinson & Pearse, 1986). In addition, two monoclonal antibodies, both of which recognize one of the major 100K species but not the other, exclusively label the plasma membrane (Robinson, 1987). The picture that is emerging, then, is that it is the 100K proteins that give coated vesicles their ability to discriminate between different membrane proteins, while clathrin appears to play a more mechanical or structural role.

Many questions still remain to be answered. For instance, how is the coat lost from the vesicle once it has been pinched off? An ATPase has been purified from a number of different organisms that has the capacity to uncoat coated vesicles *in vitro* and may play a similar role *in vivo* (Schlossman *et al.* 1984). It is not clear, however, how such a molecule could be

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prevented from uncoating coated pits as well as coated vesicles. Another important question is in how many other pathways are coated vesicles involved? For instance, do they participate in traffic from the endoplasmic reticulum (ER) to the Golgi apparatus and in traffic through the Golgi apparatus? In the case of yeast, at least, the answer seems to be no. The clathrin heavy chain gene has recently been cloned from yeast, and a clathrin mutant was then artificially created by replacing the endogenous gene with one that had been disrupted by a large deletion. Such cells grew very slowly and a number of their functions were perturbed, yet protein secretion proceeded at a nearly normal rate (Payne & Schekman, 1985). This result is not inconsistent with ultrastructural observations on mammalian cells: the only part of the secretory pathway where clathrin-coated vesicles have been definitively identified is the very *trans*-most cisterna of the Golgi apparatus (Griffiths *et al.* 1985). Interestingly, it is at this site that sorting decisions are thought to be made (e.g. whether proteins should go to the plasma membrane or be diverted to secretory granules or lysosomes), while traffic from the ER through the Golgi apparatus to the plasma membrane appears to be constitutive.

Forming a coated vesicle is only the first step in this sorting process. The vesicle then has to get to the right organelle and, once there, to recognize it and fuse with it. Although even less is known about these other steps than about the initial formation of a coated vesicle, progress is being made. Promising leads include the discovery of molecules that move vesicles along microtubules (Vale *et al.* 1985) and the establishment of cell-free systems for studying membrane fusion events (Fries & Rothman, 1980; Davey *et al.* 1985). Such approaches, along with further work on coated vesicles, should eventually help to explain how the cell is able to target different proteins to different organelles.

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