PERSPECTIVES: CELL BIOLOGY

A Lipid Oils the Endocytosis Machine

David J. Gillooly and Harald Stenmark

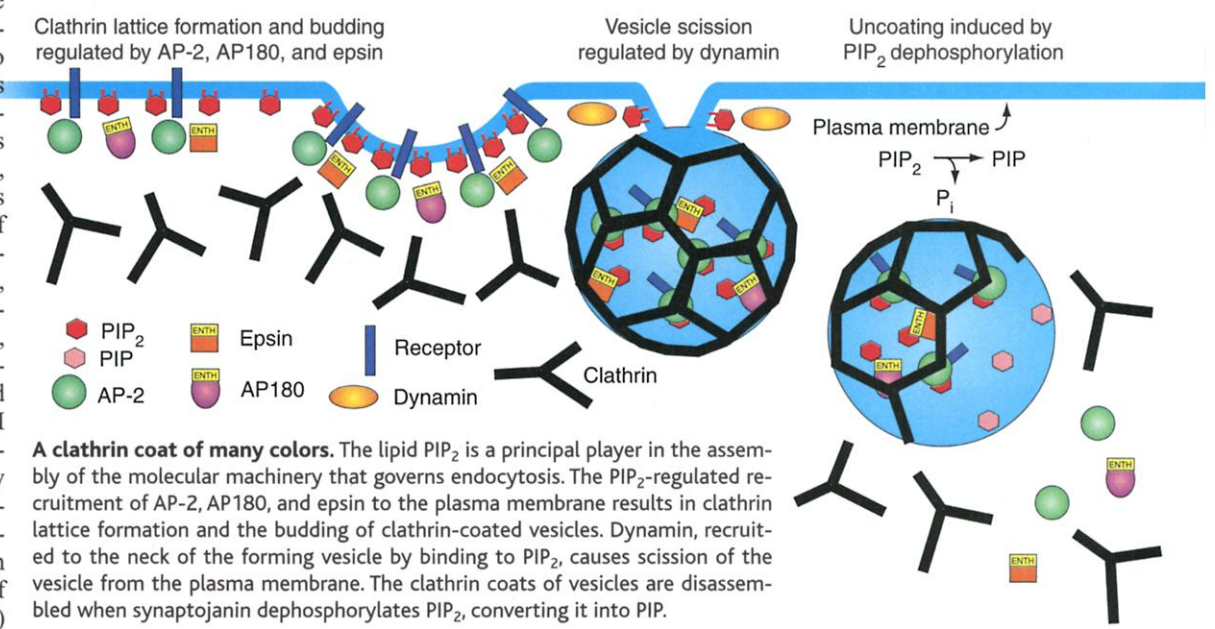
Vital cellular processes such as the acquisition of nutrients, the down-regulation of receptors, and the transmission of nerve impulses all depend on endocytosis, the inward budding of vesicles from the plasma membrane. Given the importance of endocytosis, cell biologists are striving to dissect the molecular machinery that governs this highly regulated vesiculation process. It is known that endocytosis begins with the formation of a lattice, composed of the protein clathrin, at the cytosolic face of the plasma membrane. This lattice recruits receptors and other cargo molecules into clathrin-coated indentations (pits) in the plasma membrane that then bud off as vesicles (see the figure) (*1*, *2*). A number of proteins regulate the assembly of the clathrin lattice and subsequent vesicle budding, including CALM, its brain-specific homolog AP180, and epsin. These three proteins contain a conserved amino-terminal ENTH (epsin N-terminal homology) domain, but exactly how this domain is implicated in endocytosis remains unclear (*3*). Now, on pages 1051 and 1047 of this issue, Ford *et al.* (*4*) and Itoh *et al.* (*5*) report that the ENTH domains of CALM (*4*) and epsin (*5*) bind to the membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP_2). Moreover, binding of PIP_2 by AP180 and epsin is a necessary prerequisite for endocytosis to proceed.

Ford *et al.* (*4*) deduce from the crystal structure of CALM's ENTH domain that it is a superhelix consisting of nine α helices. The seven amino-terminal helices are very similar to those of the epsin ENTH domain (*6*), which has eight α helices in total. The binding site for PIP_2 in the CALM ENTH domain (determined by x-ray diffraction) (*4*) is distinct from that in the epsin ENTH domain (determined by nuclear magnetic resonance) (*5*). The PIP_2 binding site of CALM ENTH consists of a cluster of lysine amino acid residues, which is a conserved feature of a subgroup of ENTH domains. In contrast, the binding site of the epsin ENTH domain consists of a pocket of basic amino acids, a feature that is conserved in all ENTH domains. The superhelical fold of the ENTH domain is shared by several other domains. The VHS domain, found in several proteins implicated in intracellular trafficking (*7*), has a superhelical fold that is very similar to that of the ENTH domain. There is also reasonable structural similarity between the ENTH domain and both the Arm domain of β-catenin and the Heat domain of karyopherin-β (*6*). It will be intriguing to discover if these domains also interact with PIP_2 or other membrane lipids.

That proteins containing ENTH domains bind to PIP_2, a lipid known to be essential for endocytosis (*8*), fits into an emerging picture of PIP_2 as a major recruiter of the endocytic molecular machinery (see the figure). In addition to CALM and epsin, several components of the endocytic machinery have been found to bind to PIP_2. These include AP-2, synaptotagmin, and β-arrestin, which recruit receptors and other transmembrane proteins into clathrin-coated pits (*2*, *9*), and dynamin, which causes the scission of clathrin-coated vesicles from the plasma membrane (*10*). Dynamin may possibly recruit the lipid-modifying enzyme endophilin to help in this scission activity (*11*).

So what is so special about PIP_2? This lipid is formed by phosphorylation of the inositol head group of the membrane phospholipid phosphatidylinositol (PI) through the action of specific enzymes, the PI 4- and 5-kinases. PIP_2 is the most abundant of PI's phosphorylated derivatives and is localized mainly on the cytoplasmic face of the plasma membrane. The abundance and restricted localization of PIP_2 make it an ideal seed for the assembly of clathrin lattices at the plasma membrane.

With many proteins of the endocytosis machinery identified and characterized (*1*), it may soon be feasible to reconstitute endocytosis in vitro using purified components (*12*). Ford *et al.* (*4*) have made important progress in this regard by showing that a clathrin lattice can be formed on a PIP_2-containing lipid monolayer in the presence of AP180. This, together with previous evidence that AP180 strongly stimulates clathrin assembly (*2*), indicates that AP180 is a crucial component in the nucleation and assembly of the clathrin lattice. Another component that stimulates clathrin coat assembly in vitro is the clathrin adaptor protein AP-2. When AP-2, AP180, and clathrin are added to PIP_2-containing monolayers, clathrin-coated pits start to form. Thus, the first steps of endocytosis can now be reconstituted in



A clathrin coat of many colors. The lipid PIP_2 is a principal player in the assembly of the molecular machinery that governs endocytosis. The PIP_2-regulated recruitment of AP-2, AP180, and epsin to the plasma membrane results in clathrin lattice formation and the budding of clathrin-coated vesicles. Dynamin, recruited to the neck of the forming vesicle by binding to PIP_2, causes scission of the vesicle from the plasma membrane. The clathrin coats of vesicles are disassembled when synaptojanin dephosphorylates PIP_2, converting it into PIP.

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vitro from only a few components, although it remains to be established whether these in vitro clathrin-coated pits form with the same kinetics and have the same morphology as those formed in vivo. Exactly how AP180 and AP-2 stimulate clathrin coat assembly and vesicle budding is not known, but their ability to interact both with each other and with clathrin suggests that they may coordinate clathrin coat assembly through dynamic and complex interactions. It is likely that their interaction with PIP_2 tethers the clathrin lattice to the plasma membrane.

Given the importance of AP-2 and AP180 in clathrin coat assembly and vesicle budding in vertebrate cells, it is puzzling that the yeast homologs of AP-2 and AP180 are dispensable for clathrin-coated vesicle formation (*2*). In contrast, epsins are crucial for endocytosis in both yeast and mammalian cells (*2*, *13*). Like AP180, epsin is capable of interacting with both AP-2 and clathrin. In addition, it also interacts with several multidomain proteins, including Eps15, which in turn can interact with other proteins implicated in endocytosis. Itoh *et al.* (*5*) show in cultured mammalian cells that the overexpression of epsin containing a mutation in the ENTH domain, which prevents epsin from binding to PIP_2, blocks clathrin-dependent endocytosis. Moreover, the ENTH domains of the yeast homologs of epsin are essential for endocytosis (*13*). This indicates that binding of epsin to PIP_2 is critical for the formation of clathrin-coated vesicles in vivo, although exactly how epsin is involved remains to be established. It is possible that epsin is part of a complex protein network that can fine tune endocytosis in response to phosphorylation and other protein modifications (*1*).

As PIP_2 is a central player in the assembly of the endocytosis machinery, regulation of its formation and turnover are of great importance for endocytic vesicle trafficking. This is illustrated by the finding that synaptojanin, which dephosphorylates PIP_2, converting it into phosphatidylinositol 4-phosphate (PIP), is involved in the uncoating of clathrin-coated vesicles, a process that is required before they can fuse with other membranes (*12*). Future studies should reveal how the PI 4- and 5-kinases and synaptojanin are regulated, and where exactly PIP_2 is localized in cellular membranes. The focal assembly of clathrin lattices implies that there may be PIP_2-rich patches in the plasma membrane. Recent technological developments such as fluorescence resonance energy transfer microscopy and evanescent wave microscopy promise to reveal whether these patches exist.

References and Notes

1. M. Marsh, H. T. McMahon, *Science* **285**, 215 (1999).
2. T. Kirchhausen, *Annu. Rev. Cell Dev. Biol.* **15**, 705 (1999).
3. B. K. Y. M. Kay, B. Wendland, S. D. Emr, *Protein Sci.* **8**, 435 (1999).
4. M. G. J. Ford *et al.*, *Science* **291**, 1051 (2001).
5. T. Itoh *et al.*, *Science* **291**, 1047 (2001).
6. J. Hyman *et al.*, Brunger, *J. Cell Biol.* **149**, 537 (2000).
7. Y. Mao *et al.*, *Cell* **100**, 447 (2000).
8. M. Jost *et al.*, *Curr. Biol.* **8**, 1399 (1998).
9. I. Gaidarov, J. H. Keen, *J. Cell Biol.* **146**, 755 (1999).
10. S. L. Schmid, M. A. McNiven, P. De Camilli, *Curr. Opin. Cell Biol.* **10**, 504 (1998).
11. N. Ringstad *et al.*, *Neuron* **24**, 143 (1999).
12. O. Cremona *et al.*, *Cell* **99**, 179 (1999).
13. B. Wendland, K. E. Steece, S. D. Emr, *EMBO J.* **18**, 4383 (1999).
14. We thank the Norwegian Cancer Society, the Research Council of Norway, and the Novo-Nordisk Foundation for financial support.

PERSPECTIVES: MATERIALS SCIENCE

The Simplicity of Complexity—Rational Design of Giant Pores

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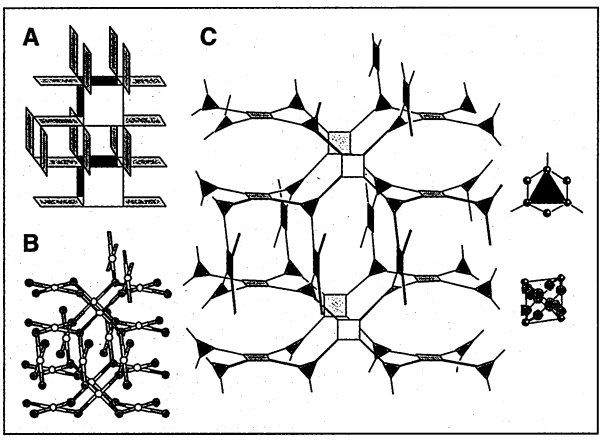
Porous materials with regular, accessible cages and tunnels are increasingly in demand for applications in catalysis, separations, sensors, and electronics. Depending on their structure and pore size, these materials allow only molecules of certain shapes and sizes to enter the pores. Furthermore, giant pores may act as nanoreactors. The confined volume may generate reactions that do not occur in the bulk. In this respect, the larger the pores, the wider the range of reactants that can be combined.

During the last decade, there has been a tremendous increase in the synthesis of new porous solids, both inorganic and organic (*1*, *2*). But researchers aiming to increase pore sizes have encountered two obstacles: a drastic decrease in material stability with pore size and the tendency of many materials to form two interpenetrated identical subnetworks by concatenation. Sometimes one strikes lucky, but usually these factors restrict the size and accessibility of pores, even if the accessibility can be optimized (*3*). Several design strategies have been pursued to circumvent these difficulties. Three approaches currently prevail. Müller *et al.* (*4*) and Khan (*5*) create linkages between isolated very large molecular moieties, such as $Mo_{72}Fe_{30}$ spheres. My group introduced the concept of "scale chemistry" (*6*), in which the size of the "secondary building blocks" (SBUs) in a structure is increased while maintaining the same connectivity between them; the larger the SBU, the larger the pores. Finally, the validity and power of a concept developed by O'Keeffe and Yaghi and co-workers last year (*7*) are demonstrated by Chen *et al.* on page 1021 of this issue (*8*).

The authors define three requirements: The final framework must be as thermally stable as possible, avoid or minimize interpenetration, and preserve the accessibility of the pores. To fulfill these conditions, they first choose a topology, select the tectons that are able to create this topology, and only then select a chemistry to reach the goal.

The originality of the concept is to start from very simple structure types, which are illustrated in every textbook and often correspond to thermodynamically stable structures, to describe them in terms of connected nets (*9*–*11*), and to decorate ("augment") these nets. In Chen *et al.*'s work (*8*), the simple structure is Pt_3O_4, a three-dimensional network of corner-shared square planes (see



How to design a porous solid. Chen *et al.* start from the simple three-dimensional network adopted by Pt_3O_4 (**A**), in which O and Pt atoms are three- and fourfold coordinated, respectively (**B**). They then replace the O atoms by triangles and the Pt atoms by squares (**C**). Any chemical species with the right connectivity (see examples to the right) can take up the places of these units.

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