Membrane trafficking

Coat control by curvature

Jennifer Lippincott-Schwartz and Wei Liu

The main transport vehicles inside cells are spherical vesicles that form when patches of membrane curve into buds and then pinch off. 'Coat' proteins both control, and are controlled by, this membrane curvature.

Many cells use small, membrane-bounded carriers — vesicles — to release and take up molecules and to move proteins between membrane-clad intracellular compartments. When and where a carrier forms within the cell, and what it contains, depends on a remarkable system of protein-based 'coats'[1,2]. Components of these coats are recruited individually to membranes, where they assemble into a lattice. This allows cargo proteins to be concentrated into the patch defined by the lattice, and the patch to deform into a bud that pinches off as a vesicle. For this system to work, the assembly and disassembly of the coats must be carefully controlled. Coat components can self-associate into lattices, but unless the lattices are kept from disassembling they will never grow large enough to deform the membrane or pinch off vesicles. However, they must also be able to dissociate quickly after a vesicle has pinched off, so that fusion of the vesicle with its target membrane is not inhibited. How do cells control these events?

Bigay *et al.*[3] put forth a promising model on page 563 of this issue. Focusing on the coatomer (COPI)-type coat, which mediates vesicle trafficking between two intracellular compartments — the Golgi apparatus and endoplasmic reticulum — they show that membrane curvature induced by the coat stimulates coat disassembly. The kind of continuous, self-regulating mechanism they propose provides a new framework for understanding the function and dynamics of protein coats. It also suggests parallels with the dynamics of other protein polymers within cells, such as microtubule filaments.

Bigay and colleagues' work builds on the previous finding that assembly of a COPI coat is controlled by the Arf1 protein. This protein's activity is in turn determined by whether it binds the small molecule GDP (guanine diphosphate) or GTP (guanine triphosphate). When it binds GTP, which ousts GDP, Arf1 is 'on': it binds to membranes and recruits coatomer, a heptameric complex that forms in the cytoplasm. Coatomer then further interacts with ArfGAP1 (ref. 4). Together, coatomer, Arf1–GTP and ArfGAP1 constitute the tripartite COPI coat unit[5].

It was known that these coat units can assemble on membranes into a lattice that is stabilized through multiple low-affinity hydrophobic and non-covalent interactions between coatomer complexes. Coat disassembly occurs if ArfGAP hydrolyses the GTP on Arf1 to GDP[6], producing inactive Arf1. But exactly when coat release from membranes occurs relative to GTP hydrolysis, and how these processes are spatially and temporally regulated for efficient vesicle formation and budding, was not known.

To address these questions, Bigay and colleagues[3] made liposomes — artificial, hollow spheres wrapped in a bilayer of lipids — with a lipid composition similar to that of Golgi membranes. They then sequentially added purified Arf1–GTP, coatomer and ArfGAP1. The authors used two approaches to monitor coat dynamics on the liposomes: the first to measure the rate of Arf1–GTP hydrolysis, and the second to determine when the coat dissociates. In previous work[7], the authors had found that ArfGAP1 binds more efficiently to, and so is activated more by, liposomes containing small-head-group, conical lipids (which are loosely packed) than liposomes containing large-head-group, cylindrical lipids (which are more tightly packed). Now they began by testing whether the kinetics of Arf1 inactivation, catalysed by ArfGAP1, was different depending on the radius of the liposome. They reasoned that highly curved membrane surfaces have looser lipid packing than flatter ones, and so might stimulate ArfGAP1 activity irrespective of the lipid composition.

And that's just what they found: Arf1 inactivation occurred much more quickly on small liposomes (with high membrane curvature) than on large ones (with low membrane curvature). Moreover, the stimulation of ArfGAP1 activity was greatest at radii approaching that of a typical coated vesicle, about 35 nm. The results hint that membrane curvature might serve as a sensor for controlling the timing of Arf1–GTP hydrolysis. One way in which this might work is if Arf1GAP's conformation in curved membranes favours its interaction with Arf1–GTP.

Once Arf1–GTP is hydrolysed and released from membranes, the remaining coat components, including coatomer and Arf1GAP, are thought to persist for a time before disassembling[8,9] (but also see ref. 10). So the next question the authors addressed was whether disassembly of these coat

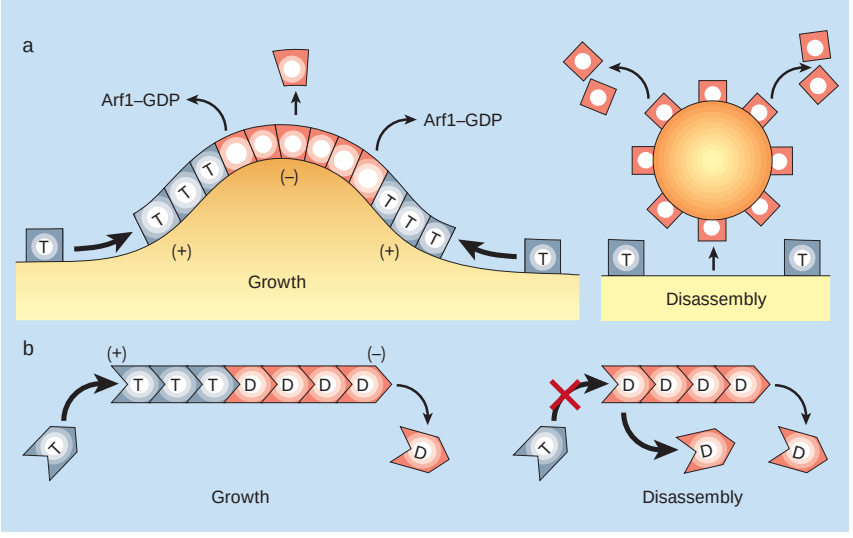

Figure 1 **Similarities in the dynamics of coats and filaments.** a, **Possible dynamics of vesicle coats, incorporating the new findings[3]. The tripartite coat unit (T) comprises Arf1–GTP, coatomer and ArfGAP1. The units first assemble into small aggregates; as these grow, they bend the membrane. This stimulates ArfGAP1, leading to GTP hydrolysis and dissociation of the resulting Arf1–GDP. The lattice (and membrane) thus becomes more bent, inducing the remaining bipartite coat (empty box) to dissociate. Faster addition of tripartite units to the edges (+) relative to bipartite unit loss from the interior (–) causes the coated surface to grow into a bud (left). If the bud dissociates as a vesicle, rapid uncoating occurs (right), because tripartite units are no longer being added.** b, **Microtubule filaments. The β-tubulin subunit of microtubules may be either GTP-bound (T) or GDP-bound (D). Microtubule formation begins through assembly of the T form. Within the polymer, the T form converts to the D form, which ultimately dissociates. The T form is added only at one end (+); in the presence of the T form, the D form dissociates only from the opposite end (–). The filament grows if T-form addition is faster than D-form loss. If addition of the T form is inhibited or slowed, the filament disassembles.**

components was also sensitive to membrane curvature. Remarkably, it was: the rate of dissociation increased by almost two orders of magnitude (from 700 seconds to 11 seconds) as the liposome radius decreased from 140 nm to 35 nm.

These findings can be interpreted in the context of an interesting new model, proposed by Bigay *et al.*, for coat assembly and disassembly (Fig. 1a). In it, coatomer is recruited to membranes by GTP-bound Arf1 and then interacts with ArfGAP1, forming the tripartite coat unit. As long as these units remain associated with flat membranes, no GTP hydrolysis occurs on Arf1. But because coatomer can undergo low-affinity interactions, coat units diffusing in the membrane begin to nucleate into small aggregates. Once the aggregates grow large enough, they begin to bend the membrane by forming basket-like lattices. This stimulates ArfGAP1 activity, causing GTP hydrolysis. The release of Arf1–GDP then changes the conformation of coat components within the lattice, and this tends to force the lattice into an even more curved shape.

Within the lattice, the low abundance of Arf1–GTP and the existence of membrane curvature start to produce coatomer dissociation. A state can then arise in which coat units containing Arf1–GTP are added at the (flatter) lattice rim, and units without Arf1 are released from the (curved) lattice interior. As long as unit addition is faster than unit release, the coat lattice grows and ultimately becomes spherical, forming a coated vesicle. When the vesicle detaches from the membrane, continued dissociation of coat components, in the absence of any component addition, leads to rapid vesicle uncoating.

This model shows remarkable parallels with the dynamics of microtubule filaments[11] (Fig. 1b). Microtubules are composed of α-tubulin and β-tubulin subunits, and, again, the assembly–disassembly cycle is coupled to GTP–GDP exchange, with assembly favoured in the presence of GTP-bound β-tubulin and disassembly favoured in the presence of GDP-bound β-tubulin. GTP-bound subunits are added to only one end of the microtubule, paralleling the entry of coat units in their active (GTP-bound) state only at the edges of the lattice. In both processes, these high-energy units are then transformed to a lower-energy form that dissociates from the structure over time. For microtubules, this can result in the growth, maintenance or dissipation of the filament. For protein coats, it can result in membrane curvature and vesicle generation.

The new model for coat assembly and disassembly is consistent with several observed features of COPI coat dynamics (see, for example, refs 8, 9). It also allows new predictions to be made. Because microtubules can maintain their length for certain periods without significant growth or shrinkage (a process called treadmilling[11]), so might coated buds be capable of stability, neither pinching off nor shrinking back into the membrane. If so, then they could have additional roles, for example in creating membrane tension[12] that could lead to a lateral separation of lipids and proteins into membrane domains distant from the coated bud. Bigay and colleagues' work, and the model it invokes, provides a promising framework for explaining how bud formation is possible through a continuous, self-regulating mechanism. This opens the way for work on a more general model of coat dynamics that incorporates the previous, successful use of concepts developed for other dynamic polymer arrays. ■

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Chemistry

Internal combustion

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It's sometimes difficult to observe combustion *in situ* — inside, say, a porous material or an industrial reactor. But with the help of nuclear magnetic resonance, a new vista has opened up.

It was a burning candle that introduced generations of scientists to the discipline of observation. From the glow of the wick to the hues and flickers of the flame, combustion became the point of entry into the world of experimental science. Combustion continues to be a passion for many, who are drawn to the rich interplay of kinetics, thermodynamics and transport phenomena that describe modern combustion technologies.

Despite such allure, *in situ* experimental observations of combustion remain difficult in environments inaccessible to light. Measurements of reactions within porous media are particularly problematic. For example, many reactors are filled with solid particles to combust fuel catalytically (thereby reducing the combustion temperature and lowering emission of environmentally harmful nitrogen oxides); but the presence of these solids makes experimental access to temperature, pressure and composition inside the reactor very difficult. Looking to the future, nanoscale combustion engines embedded in a silicon chip will not be easily monitored using current combustion diagnostics.

In a contribution to the *Journal of the American Chemical Society*, Anala *et al.*[1] demonstrate the potential of nuclear magnetic resonance (NMR), one of the most effective toolkits in experimental science, to study combustion reactions and transport in opaque media. Magnetic resonance methods, both spectroscopic and imaging, rely on the collective observation of the angular momentum of around 10^{18} nuclei. Such huge numbers of nuclei are needed because the energy differences between the various angular-momentum states of individual nuclei — which are the basis of the measurement — are small compared with the thermal energy available at room temperature. But this requirement hinders the acquisition of quantitative NMR data in gas-phase systems. To counteract this, Anala *et al.* have exploited a clever technique: xenon gas can be prepared so that individual atoms have angular momenta characteristic of extremely low temperatures, and then delivered to the NMR experiment at ambient temperatures (for a review, see ref. 2); using this 'hyperpolarized' xenon overcomes the sensitivity limitations inherent in such measurements.

Anala *et al.*[1] were thus able to detect NMR signals from xenon atoms in a methane flame burning inside a porous material (a zeolite molecular sieve). Their measurements reveal differing zones of temperature and pressure within the flame. For example, from the subtle shifts in the many resonance frequencies of the xenon atoms the authors determined the temperature changes experienced by xenon atoms inside the micropores of the material. They also noted slight changes in pressure experienced by xenon atoms in and above the bed of porous material.

In a second experiment, Anala *et al.* monitored the exchange of xenon atoms between different locations in the reaction region, manipulating and storing the observables associated with nuclear angular momenta using specially crafted radio-frequency pulses[3] and time delays. In this scheme,