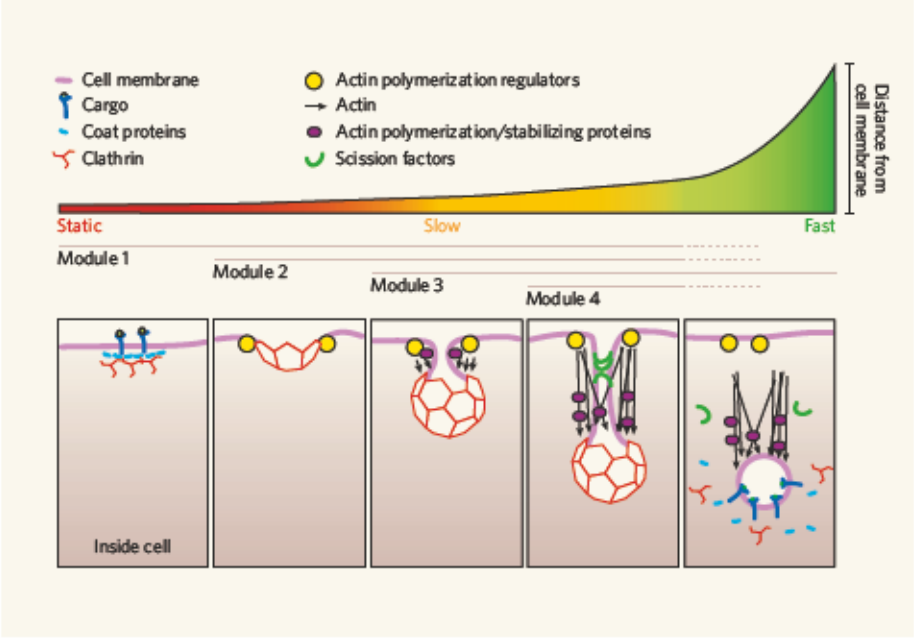

Figure 1 | The dynamics of endocytic vesicle formation. Initially, at the outer membrane of the cell, an immobile coat module assembles from clathrin and other cytoplasmic proteins (module 1). The coat captures cargo through interactions with the intracellular regions of the cargo proteins. Next, actin polymerization regulators (module 2) and actin polymerization/stabilizing modules associate with the coat, leading to the formation of actin filaments that drive slow inward movement of the coat (module 3). Finally, a scission module is recruited to separate the budded vesicle from the membrane (module 4). The freed vesicle moves rapidly into the cell and the coat components dissociate from the vesicle for additional rounds of vesicle formation.

(Fig. 1). Endocytosis begins with assembly at the plasma membrane of a clathrin-coat module containing proteins involved in cargo recruitment and coat formation. In cells lacking clathrin, the other coat components still assemble and their kinetic behaviours are only modestly affected. But in these cells the number of endocytic sites on the plasma membrane is severely reduced, implying that clathrin facilitates the assembly of the endocytic machinery.

The second module associates with the coat module and consists of a group of proteins involved in regulation of actin polymerization. Actin is a small protein that polymerizes into filaments; filament polymerization can generate the force necessary to move proteins and membranes.

Proteins that promote actin filament formation, accelerate filament assembly and stabilize new filaments make up the third module. Once this module assembles, stable actin filaments become apparent and the endocytic patch on the membrane begins to move, probably indicating membrane invagination (but this remains to be demonstrated). Treatment with a drug that abolishes actin polymerization, or genetic deletion of third-module components, trapped the endocytic patches at the plasma membrane, so it seems that actin polymerization is involved in vesicle invagination.

The fourth module appears transiently and contains proteins that, when purified, can constrict spherical membrane structures into tubules. Remarkably, in cells lacking these proteins, the endocytic patch began to jut into the cell but sometimes snapped back to the cell surface. The authors suggest that this behaviour indicates a defect in vesicle release, consistent with membrane-constricting activity of the proteins. Once the vesicle is released, the coat protein module disassembles and the actin network continues to drive the vesicle deeper into the cell.

How well do the events in yeast correspond to those in mammalian cells? Although many components of the endocytic machinery are evolutionarily conserved between the two, there are differences in their requirements for actin and clathrin. In yeast, endocytosis is strictly dependent on actin assembly, but overall can proceed slowly without clathrin[6]. In contrast, clathrin-mediated endocytosis in mammalian cells seems to rely more on clathrin than on actin[4]. The results from yeast indicate that clathrin is an integral component of endocytic coats[2,7], with a significant but non-obligatory role in coat assembly. This confirms a long-standing observation that inactivation of clathrin causes immediate but partial endocytic defects[8]. Recent live-cell imaging of mammalian fibroblast cells revealed that, as in yeast, actin and actin-polymerizing proteins associate with most, if not all, clathrin coats, and promote invagination and vesicle movement[9,10]. However, inhibition of actin polymerization causes only partial defects in endocytosis, indicating that although actin is important it is dispensable for endocytosis in fibroblasts. So, overall, the fundamental features and components of clathrin-mediated endocytosis have been well conserved. The variation between yeast and mammalian cells probably reflects cell-type-specific requirements. For example, the higher internal pressure in yeast might present an energy barrier to vesicle formation that makes actin force-generating mechanisms more significant than in some mammalian cells.

The ability to visualize single vesicles forming in living cells and to perturb the process by gene inactivation provides an unprecedented opportunity to probe the mechanism of endocytosis at a molecular level. Drubin and colleagues have made a good start, but there are still many questions to be answered. How are endocytic sites selected, for example, or are they randomly initiated? What regulatory mechanisms ensure ordered progression from coat assembly to vesicle release and coat disassembly? How is actin polymerization triggered and then harnessed to drive membrane invagination and perhaps scission? And how is endocytosis coordinated with other cellular processes? It is evident that the dance has just begun. ■

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1. Conner, S. D. & Schmid, S. L. *Nature* **422**, 37–44 (2003).
2. Kaksonen, M., Toret, C. P. & Drubin, D. G. *Cell* **123**, 305–320 (2005).
3. Roth, T. F. & Porter, K. R. *J. Cell Biol.* **20**, 313–332 (1964).
4. Perrais, D. & Merrifield, C. J. *Dev. Cell* **9**, 581–592 (2005).
5. Kaksonen, M., Sun, Y. & Drubin, D. G. *Cell* **115**, 475–487 (2003).
6. Baggett, J. J. & Wendland, B. *Traffic* **2**, 297–302 (2001).
7. Newpher, T. M., Smith, R. P., Lemmon, V. & Lemmon, S. K. *Dev. Cell* **9**, 87–98 (2005).
8. Tan, P. K., Davis, N. G., Sprague, G. F. & Payne, G. S. *J. Cell Biol.* **123**, 1707–1716 (1993).
9. Merrifield, C. J., Perrais, D. & Zenisek, D. *Cell* **121**, 593–606 (2005).
10. Yarar, D., Waterman-Storer, C. M. & Schmid, S. L. *Mol. Biol. Cell* **16**, 964–975 (2005).

Correction

In "Chemical biology: Bring them back alive" by Michael Yarus (*Nature* **438**, 40; 2005), the references were jumbled. The reference list should read as follows:

1. Fusz, S., Eisenführ, A., Srivatsan, S. G., Heckel, A. & Famulok, M. *Chem. Biol.* **12**, 941–950 (2005).
2. Yarus, M. *Annu. Rev. Biochem.* **74**, 179–198 (2005).
3. Wilson, D. S. & Szostak, J. W. *Annu. Rev. Biochem.* **68**, 611–647 (1999).
4. Lilley, D. M. J. *Curr. Opin. Struct. Biol.* **15**, 313–323 (2005).
5. Tarasow, T. M., Tarasow, S. L. & Eaton, B. E. *Nature* **389**, 54–57 (1997).
6. Helm, M., Petermeier, M., Ge, B., Fiammengo, R. & Jäschke, A. *J. Am. Chem. Soc.* **127**, 10492–10493 (2005).
7. Yarus, M. & Knight, R. in *The Genetic Code and the Origin of Life* (ed. de Pouplana, L.) 75–91 (Landes Bioscience, Georgetown, Texas, 2004).
8. Lawrence, M. S. & Bartel, D. P. *RNA* **11**, 1173–1180 (2005).
9. Yarus, M. *Cold Spring Harb. Symp. Quant. Biol.* **66**, 207–215 (2001).