Cell motility

Bare bones of the cytoskeleton

Laura M. Machesky and John A. Cooper

Movement is a fundamental feature of living organisms and, on a molecular level, the mechanism of cell movement is highly complex. So it comes as an exciting surprise that Carlier and colleagues (page 613 of this issue[1]) have dissected out a handful of proteins essential for reconstituting the motility of a bacterial propulsion system. The system concerned normally involves bacterial subversion of a host cell's own cytoskeletal machinery.

Cells adopt specialized shapes, change their shape and move around in response to various cues. In multicellular organisms, cell motility is essential for normal development and differentiation, as well as the response to disease. Cell shape and movement largely depend on actin, energy derived from the nucleotide ATP, and a host of other proteins associated with actin. Actin subunits spontaneously polymerize into filaments, which provide structural support and regulate the viscoelasticity and porosity of the cytoplasm; and polymerization of actin filaments is the driving force for extension of cell processes such as lamellipodia and filopodia. Adhesion and contraction also contribute to cell motility.

The pathogenic bacterium *Listeria monocytogenes* is often used for studying actin-based motility[2]. Bacteria move within the cytoplasm of host cells by recruiting cytoskeletal proteins to their surface. This results in actin polymerization at one pole of the bacterium, driving it forward. The set of components recruited by *Listeria* is presumed to include proteins involved in normal cell motility, so understanding how bacteria move should provide insights into how cells move. So far, analysis of the system has consisted largely of identifying components necessary for motility by depleting and inhibiting proteins in cells and cytoplasmic extracts.

Carlier and colleagues[1], however, have stripped the system down to determine its minimal requirements, and report the first reconstitution of bacterial motility from pure components. In addition to actin, only three other components are needed — the Arp2/3 complex, actin-depolymerizing factor (ADF, also called cofilin) and capping protein, all of which are well-known actin-binding proteins.

The bare bones of actin-based motility are now revealed, as depicted in Fig. 1. The Arp2/3 complex, composed of seven polypeptides including the actin-related proteins Arp2 and Arp3 (refs 3,4) nucleates actin polymerization[5,6]. The complex binds the sides of actin filaments and nucleates the polymerization of new filaments, creating a branching network[6]. The pointed (slow-growing) ends of the new filaments are bound to the Arp2/3 complex, and the barbed (fast-growing) ends are free. Actin subunits add to the free barbed ends, causing the filaments to grow. Over time, capping protein binds to the barbed ends, stopping their growth.

The need for capping protein seems paradoxical because it is expected to inhibit, not stimulate, actin polymerization. The paradox can be explained by a model[7] in which the barbed ends of older filaments are capped. In consequence actin subunits add only to newly created free barbed ends, and polymerization is 'funnelled' to the new ends. In other words, capping protein restricts actin assembly to the zone nearest the bacterium.

The role of ADF is rather complicated and controversial. This protein stimulates the loss of actin subunits from pointed ends, which increases the pool of actin subunits that can be added to free barbed ends. It may also accelerate the rate of addition of subunits to free barbed ends[8] (Fig. 1). The reason why increased filament turnover should be necessary for movement, instead of just stimulating it, is unclear.

For Arp2/3, capping protein and ADF, the dose–response curve for bacterial speed is bell-shaped (See Fig. 1b of the paper on page 614) — at low concentrations, speed increases with the concentration of the component; at high concentrations, speed decreases. High ADF may decrease speed by fragmenting filaments[9] or inhibiting ATP–ADP exchange on monomers[10]. Excess capping protein may cap new barbed ends too quickly, and excess Arp2/3 may aggregate actin filaments or induce polymerization away from the bacterial surface. Notably, for each of the three components, the optimal concentrations for speed were slightly less than their concentrations in cytoplasm. Perhaps this was because the concentration of actin used in the assay was also less than that found in cytoplasm, due to technical considerations.

Carlier and colleagues[1] also report that three other proteins — profilin, VASP (vasodilator-stimulated phosphoprotein) and α-actinin — are important, but not necessary, for movement. Profilin, which sequesters actin monomers and increases bacterial speed, probably acts like capping protein in restricting (or funnelling) actin polymerization to a limited zone near the bacterium. But its likely mechanism is different from that of capping protein; profilin appears to suppress spontaneous actin nucleation, so restricting nucleation to Arp2/3-induced free barbed ends[11]. Other functions for profilin have been proposed, including increasing the rate of addition of subunits to barbed ends[12]. But that effect required the presence of another protein that

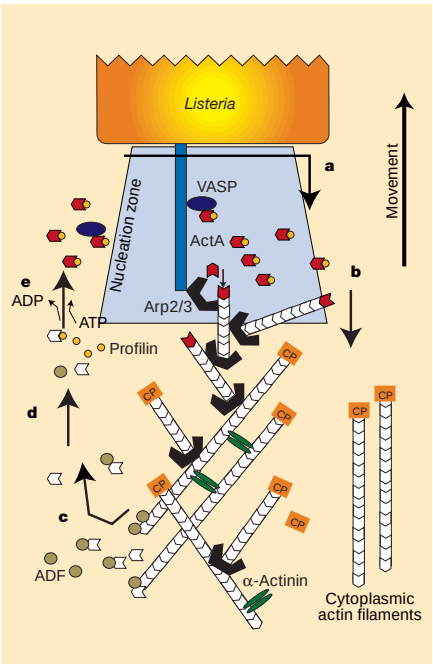


Figure 1 **Propulsion of *Listeria* inside a cell, as reconstituted by Carlier and colleagues[1]. Essential components are the Arp2/3 complex; capping protein (CP); and actin-depolymerizing factor (ADF, also called cofilin). Stimulatory components are vasodilator-stimulated phosphoprotein (VASP); profilin; and α-actinin.** a, **The Arp2/3 complex and VASP bound to *Listeria* ActA promote assembly of actin filament branches. ATP–actin monomers (red chevrons) may be shuttled to the zone of nucleation by profilin bound to VASP/ActA or free in the cytoplasm. VASP may also interact with actin filaments to promote assembly.** b, **The branches eventually become capped at the barbed end by capping protein, and crosslinked by α-actinin, forming a stable scaffold known as the bacterial actin tail. Other actin filaments in the cytoplasm, away from the zone of nucleation, are probably likewise capped.** c, **At the pointed ends of filaments, ADF enhances disassembly of monomers from the filaments.** d, **Profilin will compete with ADF for binding to ADP-bound actin monomers (white chevrons), which will promote the exchange of ADP for ATP to re-charge the monomers for polymerization onto new barbed ends.** e, **Profilin–ATP–actin complexes will then be recycled for use in the nucleation zone. Profilin also prevents spontaneous assembly of ATP-bound actin monomers into nuclei in the cytoplasm outside the nucleation zone.**

sequesters actin monomers, thymosin-β4, which was not included in this study.

Profilin may also bind to VASP, the second stimulatory component examined by Carlier and colleagues. VASP interacts with the bacterial surface protein ActA, and may 'shuttle' profilin–actin subunits to the new barbed ends[13]. However, profilin mutants that do not bind VASP are able to stimulate motility[14]; and VASP without profilin has a stimulatory effect on its own, perhaps by directly interacting with actin filaments[15].

Finally, α-actinin, which crosslinks actin filaments, is known to be necessary for bacterial movement in living cells[16]. Carlier and co-workers found that it did not influence the speed of bacterial movement but did affect the morphology of the tails — without α-actinin, tails were splayed, not compact, because filaments were not crosslinked. This crosslinking function may be essential *in vivo*, where the forces necessary for movement should be greater than those in Carlier and colleagues' purified *in vitro* system.

Other components, not tested here, may also be necessary or stimulatory for bacterial motility in cells. The requirements for motility *in vivo* may be different or more demanding than those in the reconstitution system. For example, the need for profilin may be greater if thymosin-β4 acts to buffer the supply of actin monomers.

As well as providing a wonderfully simple model for actin-based motility, Carlier and colleagues' landmark study provides a new motility assay to analyse components and regulators of the actin cytoskeleton. It complements other methods for measuring actin polymerization and network formation, and greatly increases our ability to analyse actin assembly *in vitro*. ■

Laura M. Machesky is in the Division of Molecular Cell Biology, School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK.
e-mail: L.M.Machesky@bham.ac.uk
John A. Cooper is in the Department of Cell Biology, Washington University, St Louis, Missouri 63110, USA.
e-mail: jcooper@cellbio.wustl.edu

1. Loisel, T. P., Boujemaa, R., Pantaloni, D. & Carlier, M. F. *Nature* **401,** 613–616 (1999).
2. Dramsi, S. & Cossart, P. *Annu. Rev. Cell Dev. Biol.* **14,** 137–166 (1998).
3. Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J. & Pollard, T. D. *J. Cell Biol.* **127,** 107–115 (1994).
4. Welch, M. D., DePace, A. H., Verma, S., Iwamatsu, A. & Mitchison, T. J. *J. Cell Biol.* **138,** 375–384 (1997).
5. Welch, M. D., Rosenblatt, J., Skoble, J., Portnoy, D. A. & Mitchison, T. J. *Science* **281,** 105–108 (1998).
6. Mullins, R. D., Heuser, J. A. & Pollard, T. D. *Proc. Natl Acad. Sci. USA* **95,** 6181–6186 (1998).
7. Carlier, M.-F. & Pantaloni, D. *J. Mol. Biol.* **269,** 459–467 (1997).
8. Carlier, M.-F. *et al. J. Cell Biol.* **136,** 1307–1322 (1997).
9. Maciver, S. K., Pope, B. J., Whytock, S. & Weeds, A. G. *Eur. J. Biochem.* **256,** 388–397 (1998).
10. Blanchoin, L. & Pollard, T. D. *J. Biol. Chem.* **274,** 15538–15546 (1999).
11. Machesky, L. M. *et al. Proc. Natl Acad. Sci. USA* **96,** 3739–3744 (1999).
12. Pantaloni, D. & Carlier, M. F. *Cell* **75,** 1007–1014 (1993).
13. Chakraborty, T. *et al. EMBO J.* **14,** 1314–1321 (1995).
14. Egile, C. *et al. J. Cell Biol.* **146,** 1–14 (1999).
15. Laurent, V. *et al. J. Cell Biol.* **144,** 1245–1258 (1999).
16. Dold, F. G., Sanger, J. M. & Sanger, J. W. *Cell. Motil. Cytoskeleton* **28,** 97–107 (1994).

Plant defence

Long view from a high plateau

Jeff Dangl

Scientific endeavour tends towards a punctuated equilibrium — slow periods during which systems and tools are developed, followed by bursts of new knowledge. For the molecular understanding of plant–pathogen interactions there have been three recent explosions. The first was the isolation of plant disease-resistance (*R*) genes (Fig. 1); second was the ability to isolate mutants in 'model' plants such as *Arabidopsis thaliana* and tomato; and third was the (initially improbable) finding that bacterial pathogens of both plants and animals rely on a conserved delivery system to ferry the effectors of disease into their hosts. Experiments and achievements deriving from these three breakthroughs were reported at two meetings earlier this year*.

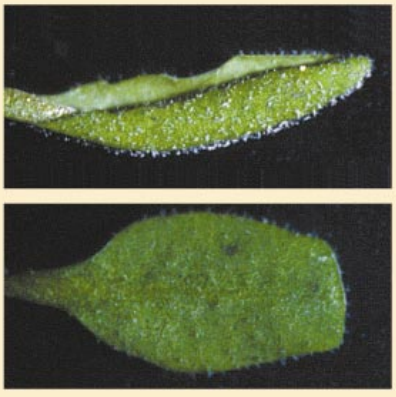
One current debate swirls around how the diversity of the *R* genes evolves and is maintained. This debate reminds me of (and draws from) discussions that followed the isolation of major histocompatibility complex genes in the early 1980s — full of structures and sequence comparisons, with enlightening forays into population genetics and molecular evolution.

Most R proteins contain leucine-rich repeats (LRRs), and there is overwhelming evidence that solvent-exposed surfaces of these repeats are subject to diversifying selection[1,2]. Selection acts on point mutations and on short tracts of DNA exchanged between chromosomes by recombination and, probably, gene conversion. Many *R* genes are found in linked clusters on the chromosomes. At the *R* cluster of each parental chromosome, those *R*-gene sequences (or 'haplotypes') that derived from a common ancestor (orthologues) seem to be more related than those that originated by duplications (paralogues; Richard Michelmore, Univ. California, Davis). If so, *R*-gene divergence is an ancient event[3].

A related and equally compelling argument is that architecture of the *R*-gene cluster determines the recombinational outcome and influences subsequent diversity (Jonathan Jones, Sainsbury Laboratory, Norwich). Two haplotypes at a region known as *RPP5* in *Arabidopsis* are scrambled by rearrangements, so it is impossible to define orthologues here. Recombination between these two haplotypes is suppressed. The extraordinary divergence in the *R*-gene cluster is backlit by considerable homology between the DNA sequences that flank *RPP5*. By contrast, the tomato *Cf-9* and *Cf-4* genes are orthologues embedded in unique, but fairly linear, haplotypes, and evidence for recombination between these two genes can, occasionally, be found.

When combined, these results (Michelmore; Jones) indicate that, at the *R* clusters examined, unequal recombination events can influence the evolution of a particular stretch of DNA, but that they contribute little to its diversification. Evolution of an *R*-gene cluster can also be influenced by chromatin dynamics, a view supported by molecular analyses of the flax *L* and *M* regions. When sequences from 11 of the 13 alleles at *L* were compared, diversity was found to be generated by deletion and expansion of LRRs. This region is also subject to diversifying selection (Peter Dodds, CSIRO, Canberra). So, similar events occur at simple and complex *R* genes.

Diversity aside, what is the function of the R proteins? The simplest idea is that they act as receptors for ligands encoded by the *avr* genes, but this has been difficult to



J. MCDOWELL & D. AVIV

Figure 1 **Downy mildew disease of many plants is caused by obligate oomycete parasites. *Peronospora parasitica* infects one *Arabidopsis thaliana* accession (top), but a second *Arabidopsis* inbred line is resistant (bottom) owing to the action of a single disease-resistance (*R*) gene.**

Attack and Defence: The Thirteenth John Innes Symposium 20–23 July, Norwich, UK; and *Ninth International Congress of Molecular Plant–Microbe Interactions* 25–30 July, Amsterdam, The Netherlands.