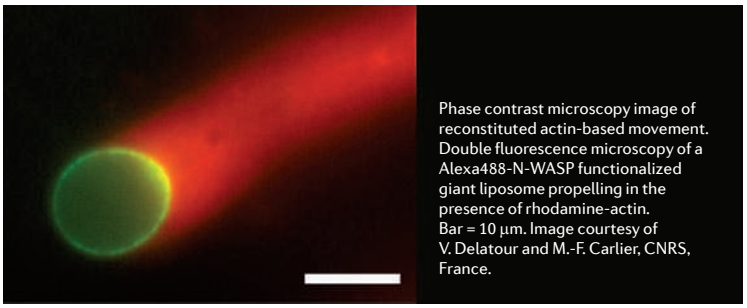

Phase contrast microscopy image of reconstituted actin-based movement. Double fluorescence microscopy of a Alexa488-N-WASP functionalized giant liposome propelling in the presence of rhodamine-actin. Bar = 10 μm. Image courtesy of V. Delatour and M.-F. Carlier, CNRS, France.

MILESTONE 25

Reconstituting motility

Cell shape and motility are fundamentally important during development, for the physiological functions of unicellular and multicellular organisms, and for disease processes. The actin cytoskeleton is not only a major determinant of cell shape and migration, but also participates in many other cellular processes, such as the trafficking of membrane compartments and signalling.

Over the years, many factors have been identified that participate in the dynamic regulation of actin-filament and actin-network formation. A key step forward came with the identification of the actin-related protein-2/3 (Arp2/3) complex, which is a seven-subunit protein complex that was found to trigger the nucleation of new actin filaments (see Milestone 23). It therefore regulates the formation of branching actin networks, which, for example, generate the force for movement at the leading edge of migrating cells. Importantly, Arp2/3 was found to be a target of signalling pathways that regulate cell motility.

The Arp2/3 complex is also required to promote the movement of bacteria, such as *Listeria monocytogenes* or *Shigella*. These microorganisms hijack the cellular actin-polymerization machinery, allowing them to propel themselves forward within infected host cells. In a landmark paper, Marie-France Carlier and colleagues were able to reconstitute the actin-based motility of these bacteria *in vitro*, using purified components, to identify for the first time the minimal requirements for actin-based movement. They found that, in addition to actin, ATP and Arp2/3, sustained bacterial motility in defined solutions required actin-depolymerizing factor (ADF; also known as cofilin) and capping protein.

The actin-stimulated propulsion of bacteria is due to actin polymerization at one end of actin filaments (the so-called barbed end) on the surface of the bacterium, and depolymerization at the other end of actin filaments (the pointed end) in a treadmilling process. Capping protein and ADF support this process. Capping protein binds to barbed ends and thereby prevents actin polymerization at actin filaments that are no longer attached to bacteria, to restrict actin polymerization to where it is needed. ADF increases actin depolymerization at the pointed end, thereby increasing the amount of available (and ATP-bound) monomeric globular-actin needed for actin polymerization elsewhere.

Although not found to be essential, profilin which is involved in treadmilling, and — in the case of *L. monocytogenes* — vasodilator-stimulated phosphoprotein (VASP), were found to enhance bacterial movement further.

These findings provided a key assay and lay the foundations to dissect the biochemical mechanisms that govern actin-based motility, and thereby to understand in molecular detail one of the most important and fundamental cell biological processes.

Barbara Marte, Senior Editor, Nature

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