BACTERIAL PATHOGENICITY

Formin' a comet tail

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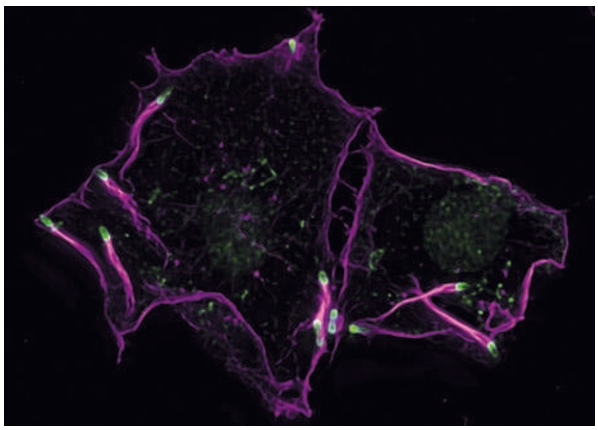
Many intracellular pathogens, such as *Listeria monocytogenes* and vaccinia virus, produce nucleation-promoting factors (NPFs) that co-opt the Arp2/3 complex in the host cell to trigger the formation of branched actin filaments, known as 'comet tails', that propel the pathogens through the cytosol. However, writing in *Nature Cell Biology*, Haglund *et al.* now show that comet tail formation in many *Rickettsia* spp. is independent of both the Arp2/3 complex and the previously identified NPF RickA and instead relies on the formin-like protein surface cell antigen 2 (Sca2) to mediate actin nucleation.

The first indications that *Rickettsia* spp. may use a different mechanism of actin nucleation were the findings that Arp2/3 could not be observed in rickettsial comet tails and that the actin filaments in the tails are unbranched. To identify factors that might have a role in actin-based motility in *Rickettsia* spp., the authors searched for proteins containing Wiskott–Aldrich syndrome protein (WASP) homology 2 (WH2) motifs, which are actin-binding motifs found in NPFs. One such protein, Sca2, which has recently been implicated in host cell invasion, was found to contain a central cluster of three WH2 motifs flanked by two proline-rich domains (PRDs). In *in vitro* actin polymerization assays, Sca2 exhibited a dose-dependent actin nucleation activity that was not enhanced by the addition of RickA. Using truncated versions of Sca2, the authors found that efficient nucleation required the WH2 motifs, the PRDs and also the amino-terminal region of the protein.



A *Rickettsia parkeri*-infected *Drosophila melanogaster* S2R+ cell. Surface cell antigen 2 (Sca2) is immunostained with Sca2-specific antibodies (green), and actin is stained with Alexa Fluor 488 phalloidin (purple). Image courtesy of M. Welch, University of California, Berkeley, USA.

In eukaryotic cells, unbranched actin filaments are assembled by formins, which bind to the barbed end of an actin filament and, in combination with profilin, enable filament elongation. Consistent with formin-like activity, Sca2 alone produced actin filaments *in vitro* with a median length of 0.6 μm, and the addition of profilin increased the median length of the filaments to 5.7 μm. The actin filaments were unbranched, similar to those produced by formins and those observed in the rickettsial comet tail. Furthermore, using total internal fluorescence microscopy, the authors observed that Sca2 modulates the elongation of unbranched filaments by processively riding the elongating filament barbed end, just like a eukaryotic formin.

Using immunofluorescence, the authors detected Sca2 on the bacterial cell surface and saw that it was enriched at the point of contact between the actin tail and the bacterial cell surface but was not present along the length of the tail. Finally, when Sca2-coated polystyrene beads were incubated in *Xenopus laevis* egg extracts, dense arrays of actin formed around the beads, indicating that Sca2 is sufficient to nucleate actin filament formation from cytosolic components in the absence of any other bacterial factors.

So, if *Rickettsia* spp. rely solely on Sca2 to nucleate comet tail formation, what is the role for RickA? The authors propose that RickA functions together with the Arp2/3 complex during host cell invasion but not during actin-based motility.

This is the first study to identify a bacterial protein with formin-like activity and suggests that many *Rickettsia* spp. have two distinct mechanisms for subverting the host cells actin cytoskeleton at different points in the bacterial life cycle.

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ORIGINAL RESEARCH PAPER Haglund, C. M. *et al. Rickettsia* Sca2 is a bacterial formin-like mediator of actin-based motility. *Nature Cell Biol.* **12**, 1057–1063 (2010)
FURTHER READING Ray, K., Marteyn, B., Sansonetti, P. J. & Tang, C. M. Life on the inside: the intracellular lifestyle of cytosolic bacteria. *Nature Rev. Microbiol.* **7**, 333–340 (2009)