nature structural & molecular biology

Lessons from *Listeria*

© 2005 Nature Publishing Group http://www.nature.com/nsmb

We really owe much of our understanding of many important cellular processes to microorganisms. For example, the pioneering studies on bacteriophage lambda gave us the framework for understanding gene regulation across species. Likewise, the mechanism used by enveloped viruses to enter host cells helped explain eukaryotic receptor mediated endocytosis and intracellular transport. And studies on *Listeria* pathogenicity have led the way in understanding actin nucleation in the cell.

Listeria cause a severe form of food poisoning. It is particularly problematic because it grows well in the temperature range used for refrigeration, so it can be transmitted in ready-to-eat foods that have been properly refrigerated. The Center for Disease Control estimates 2,500 human cases and 500 deaths annually in the U.S. are caused by listeriosis (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis_g.htm). Groups particularly at risk are those with compromised immune systems, pregnant mothers and their unborn fetuses.

The bacterium is able to invade a host cell and manipulate cellular processes to move intra- and intercellularly, resulting in the rapid spread of infection. While *Listeria* can move by means of flagella, this is not the primary means of movement at host body temperatures. Instead, the bacteria use host actin filaments to transport themselves through the host cytoplasm. Actin filaments form part of the cytoskeletal framework supporting many different types of cell extensions and cellular actin-based movement is important for neural outgrowth, intracellular vesicle movement, and a variety of other cellular processes.

In the absence of additional factors, actin filaments do not readily polymerize *de novo*. Actin filament assembly starts with the formation of an unstable dimer, followed by the addition of another monomer to form a more stable actin trimer that is the nucleus for filament growth. This nucleation is the rate-limiting step in filament formation. The filament is elongated by the addition of actin monomers to one end. The pointed end, which faces towards the cell body, has a lower affinity for actin monomers and is believed to be where actin disassembly occurs. The barbed end has a high affinity for actin monomers. It faces towards the plasma membrane within the cell and is the site of actin assembly during cell projection.

The *Listeria* cell wall contains a surface protein, ActA, that extends into the host cytosol to direct actin filament assembly. For a long time it was unclear how ActA could manipulate a host's actin network. Then in 1995, it was discovered that ActA initiates actin nucleation on the outer surface of *Listeria*, leading to rapid filament growth which creates an actin scaffold for the bacterium to move along.

Microorganisms frequently usurp host proteins for their own purposes. In an effort to find host proteins that can be hijacked by ActA, cytoskeletal extracts were purified and the fractions containing actin assembly-promoting activity were isolated. A complex containing actin-related proteins Arp2 and Arp3 along with five other proteins was identified as sufficient to promote actin assembly by *Listeria*. The Arp2/3 complex is found in eukaryotes from yeast to humans and near the lamellae in motile cells. In a *Listeria* infected cell, Arp2/3 is localized along the actin scaffold in the wake of the moving bacterium.

It was soon shown that ActA directly recruits Arp2/3 and stimulates it to nucleate filament polymerization. The complex remains attached at a filament's pointed end, and further experiments showed that Arp2/3 can nucleate branches off of an existing filament. But what host protein is responsible for nucleation under normal cell conditions?

A yeast two-hybrid screen for host cell factors that interact with the Arp2/3 complex revealed the SCAR/WASp family of proteins. These proteins have several interesting features: they have a WASP-homology 1 domain that helps localize SCAR/WASp to membranes; they have a proline-rich region that interacts with SH3-containing signaling proteins; and they interact with Arp2/3 through a WA/VCA domain (which has actin binding and cofilin homology subdomains). Members of this family were shown to stimulate Arp2/3 mediated nucleation, suggesting that ActA may be mimicking their activity.

Until very recently, it was unclear what role the WASp family members were playing. It was proposed that Arp2 and Arp3 might initiate nucleation by forming an actin-like dimer, and that WASp proteins might recruit actin monomers to the branch site to form the nucleating trimer. But the crystal structure of Arp2/3 complex didn't support this theory. While EM studies had shown that Arp2/3 forms a horseshoe shaped complex, the crystal structure of inactive Arp2/3 showed that Arp2 and Arp3, located essentially on opposing ends of the horseshoe, were too far apart to form a pseudo-actin dimer. However, cross-linking studies of free and SCAR/WASp-bound Arp2/3 suggested conformational changes in Arp2/3.

Now, an EM study on page 26 by Goode and co-workers shows that the Arp2/3 complex exists in an equilibrium of several conformations ranging from open to closed. Using yeast and bovine Arp2/3 complexes, they show that the open conformation is the inactive state and that all WASp-bound complexes are closed. WASp, along with the filament-binding p35 subunit, stabilizes the closed conformation of the complex, holding the Arp2 and Arp3 subunits together to allow the formation of a pseudo-actin dimer. While closure of the Arp2/3 complex activates it, WASp must still provide an actin monomer to the Arp barbed ends to promote nucleation. How it does this is not yet clear.

The studies on *Listeria* ActA have paved the way to understanding Arp2/3-mediated actin nucleation in eukaryotes. Pathogens are evidently very good at subverting host factors to their own benefit. We should pay closer attention when they do since it almost always teaches us something fundamental about basic processes occurring in our own cells. ■