BACTERIAL PHYSIOLOGY

Curl up with *Caulobacter*

Until recently it seemed that bacteria lacked a cytoskeleton and that the cell wall was the main determinant of bacterial shape. However, proteins with remarkable structural similarity to actin — despite very little amino acid sequence homology — were shown to assemble into filaments and help to determine cell shape in *Bacillus subtilis* and *Escherichia coli*. Now, research published in *Cell* has uncovered another bacterial cytoskeletal protein. The characteristic crescent shape of *Caulobacter crescentus* is determined in part by a protein that has features of intermediate filaments (IF), another component of the eukaryotic cytoskeleton.

Ausmees *et al.* used transposon mutagenesis coupled with scrutiny using light microscopy to isolate *C. crescentus* mutants that were rod-shaped. They identified a protein, called crescentin (CreS), that is responsible for the crescent (vibrioid) shape of the cell. To function in determining cell curvature, crescentin forms helical filaments that seem to interact with the cell membrane. Crescentin filaments localize asymmetrically to the concave side of the cell, which contributes to the vibrioid cell shape. IF proteins and crescentin both have extensive coiled-coil motifs arranged in a similar fashion and both can form filaments *in vivo*. Furthermore, since crescentin can form IF-like filaments *in vitro* under conditions similar to those used for filament formation by animal IF proteins, it seems that crescentin is indeed a bacterial IF protein.

It is likely that a combination of actin, IF homologues and peptidoglycan, the major cell-wall component, determine the shape of bacteria. Now that bacterial counterparts of actin, tubulin and IF have been found, it seems that bacteria can be good models for cell-shape determination after all.

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References and links

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WEB SITE

Christine Jacobs-Wagner's laboratory: http://www.biology.yale.edu/facultystaff/jacobs.html

BACTERIAL PATHOGENESIS

Pathogenic *E. coli* — getting intimate

To colonize their hosts successfully, enteropathogenic *Escherichia coli* (EPEC) must attach to and form intimate associations with host intestinal cells, a process that requires rearrangements of the host cytoskeleton at the site of attachment. A recent study by Gad Frankel and colleagues now reveals that the host protein cytokeratin 18 (CK18) is essential for this, the first time that an intermediate filament protein has been implicated in EPEC pathogenesis.

EPEC adhere to host cells through a mechanism that involves a translocated bacterial protein — Tir (translocated intimin receptor) — that is inserted into the host cell plasma membrane and acts as a receptor for the bacterial outer-membrane protein intimin. Translocated Tir binds the host adaptor protein, Nck, which recruits neural Wiskott–Aldrich syndrome protein (N-WASP), the ARP2/3 complex and other actin-binding proteins. The recruitment of these proteins causes rearrangements of the cytoskeleton to form an actin-rich, pedestal-like structure at the site of attachment.

Frankel and colleagues carried out a yeast two-hybrid screen to identify other host proteins that interact with Tir and pulled out CK18. Tir and CK18 were shown to co-immunoprecipitate from cells infected with EPEC, confirming the relevance of the interaction *in vivo*. CK18 was shown to be recruited to sites of EPEC attachment in infected intestinal epithelial cells, where it colocalizes with Tir and ARP3, and this recruitment was found to be dependent on Tir, as it was abolished in cells infected with *tir*$^{-}$ EPEC.

The role of CK18 in EPEC attachment was examined by downregulating its expression in host cells using small interfering RNAs. Cells were then infected with EPEC, and the levels of CK18 expression and the formation of pedestals were analysed. In untransfected cells, with normal levels of CK18, ~63% of adherent bacteria formed pedestals, whereas in cells in which CK18 expression was reduced or abolished, only ~29% did so. In addition, in cells in which CK18 expression was downregulated, but not completely abolished, pedestals were shorter than normal and less ARP3 was associated with these structures.

These results indicate that intermediate filament proteins such as CK18 are required for the formation of the actin-rich pedestal at sites of EPEC attachment, and therefore have an essential role in the pathogenesis of these bacteria. How intermediate filaments modulate actin dynamics is not clear at present, and further studies are needed to elucidate the precise function of CK18 in the modification of the host cytoskeleton by EPEC.

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References and links

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WEB SITE

Gad Frankel's laboratory: http://www.cmmi.ic.ac.uk/gadi.html