When *Shigella* Tells the Cell to Hang On

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OspE, a *Shigella* type III effector binds to integrin-like kinase and enhances cell adhesion to better disseminate and colonize the intestinal epithelium. Because of the existence of OspE orthologues in other enteropathogens such as enteropathogenic *Escherichia coli* or *Salmonella* sp., maintenance of cell adhesion appears as a widespread strategy for bacteria that interact with the intestinal epithelium.

Type III effectors that are injected into host cells have provided exciting and unexpected insights at the molecular and cellular levels in the strategies used by bacterial pathogens to colonize host cell tissues (Coburn et al., 2007). *Shigella*, a Gram-negative enteropathogenic bacterium, invades the colonic mucosa and induces an intense inflammatory reaction leading to tissue destruction and the dysenteric syndrome. Epithelial cells of the colonic mucosa are the primary target of *Shigella*, since following invasion, the bacteria break free in the cell cytosol, replicate intracellularly, and using actin-based motility, can disseminate from cell to cell without extracellular steps (Ray et al., 2009). Critical to its virulence, *Shigella* expresses a type III secretion system (T3SS) to inject type III effectors that divert host cellular processes, driving colonization of the mucosal epithelium and controlling the host inflammatory response (Parrot, 2009). Sequencing of the genome and virulence plasmid from various *Shigella* strains has provided an exhaustive view of type III effectors, with 25–30 identified to date. Roughly, these effectors can be divided in two categories: (i) effectors that are expressed in vitro under normal bacterial culture conditions (early effectors), these include those responsible for bacterial invasion of epithelial cells; and (ii) effectors for which expression is regulated by the T3SS activity (late effectors) and under the control of the transcriptional activator MxiE. The collection of data from various studies supports the emerging picture that early effectors concur to provide the mechanistic means allowing bacterial invasion and multiplication within the tissue, while late effectors regulate the host inflammatory response (Parrot, 2009). Recent works, however, have pointed to another role for the OspE late type III effectors (Miura et al., 2006; Kim et al., 2009).

While in *Shigella flexneri* there are two nearly identical genes encoding OspE1 and OspE2, *Shigella sonnei* expresses only OspE2 because of a frameshift mutation in the OspE1 allele. In earlier works, an *S. sonnei* OspE2 mutant was shown to induce drastic cell rounding during the late stages of bacterial replication and was partially defective for bacterial cell-to-cell spreading (Miura et al., 2006). Interestingly, during bacterial intracellular replication, OspE2 was found to accumulate at focal adhesions (FAs), suggesting that its molecular function was to stabilize cell adhesion to favour bacterial dissemination (Miura et al., 2006). In a recent report, Kim et al. (2009) provide some mechanistic insights into the stabilization of cell adhesion by OspE. Affinity chromatography with recombinant OspE followed by mass spectrometry allowed the identification of integrin-like kinase (ILK) as an OspE-binding partner, an interaction that was confirmed in co-immunoprecipitation experiments of an OspE–ILK complex during bacterial infection of cultured cells (Kim et al., 2009). Despite the fact that since its discovery, ILK has been the subject of intense interest, its precise function remains ill defined, in particular, the role of its kinase activity has been a matter of debate since it shows some divergence in key residues of the catalytic loop and lacks Mg$^{2+}$-chelating residues (Legate et al., 2006). ILK was shown to directly associate with the cytoplasmic tail of $\beta_3$ integrins, but its in vivo recruitment at the levels of FA depends on the FA protein paxillin or kindlins (Legate et al., 2006). ILK is generally believed to function as part of the IPP complex, including PINCH and Parvin implicated in a variety of processes downstream of integrin signalling, including anchoring of the actin cytoskeleton to the membrane or GSK3- and Akt-signalling (Legate et al., 2006). However, there is evidence that ILK could also function on its own (McDonald et al., 2008). OspE could precisely reflect such a peculiar function of ILK since, although OspE binding occurs via the ILK kinase domain, its effect does not seem to implicate the IPP complex formation or GSK3- or Akt-mediated signalling (Kim et al., 2009).

Transfection experiments confirmed that not only OspE accumulates at the levels of FAs, but also that OspE by itself increased the number of FAs. Interestingly, the use of mutant forms of ILK suggests that the ILK kinase domain is required for OspE recruitment at FAs. While OspE does not seem to stimulate assembly of FAs, the ‘nocodazole wash’ test suggests that the increase of FAs observed in OspE transfectants is rather linked to inhibition of FA...
disassembly and that this inhibition also depends on the ILK kinase activity. Of note, however, no obvious changes in ILK kinase activity or ILK-mediated phosphorylation linked to OspE binding could be detected. Thus, it is unclear whether, rather than ILK kinase activity, the ‘kinase domain’ is required to relay OspE-mediated effects through some scaffolding activity that is not found in the K220M kinase dead mutant (Kim et al., 2009). From there, the authors propose a model whereby OspE, secreted through the Shigella T3SS during bacterial intracellular replication, prevents cell detachment by binding to ILK and inhibits FA disassembly. In this model, Shigella would trick the cell where it replicates to prevent cell detachment, which would otherwise limit bacterial dissemination within the colonic epithelium. In these regards, the function of OspE would concur with that of IpaB, the Shigella translocator component, shown by the same group to inhibit cell cycle progression by binding to the APC inhibitor, Mad2L2, in addition to its role in allowing the host cell injection of other Shigella T3SS effectors (Iwai et al., 2007). Both IpaB and OspE would counteract exfoliation, a process that could potentially act as an innate defence mechanism, thereby favouring bacterial multiplication and dissemination within the epithelial tissue. Since OspE orthologues are found in other pathogenic bacteria, including enteropathogenic Escherichia coli, enterohaemorrhagic E. coli and Salmonella, it seems that this strategy could be widely used by pathogens to colonize an epithelium either intra- or extracellularly while bound to the host cell surface (Kim et al., 2009). Along these lines, previous works had shown that Neisseria gonorrhoeae could also fight exfoliation of epithelial cells through a distinct mechanism, by stimulating integrin-mediated cell adhesion through the binding of CEACAM receptors by bacterial Opa proteins (Muenzner et al., 2005). Whether this implicates that exfoliation is a general feature of epithelia that bacterial pathogens infecting epithelial tissue need to systematically deal with is unclear. Although the intestinal epithelium is presumed to be highly dynamic, with constant maturation of intestinal epithelial cells and exfoliation, there has been recent debate over the occurrence of cell detachment in the lumen of the human colonic epithelium or if removal of epithelial cells occurs by alternative processes, such as ingestion by professional phagocytes following colonic epithelial cell apoptosis (Loktionov, 2007). Also, it is likely that the need to fight exfoliation will not be as acute for a pathogen that rapidly crosses an epithelium compared with pathogens that multiply extracellularly while bound to the epithelial cell surface. In the case of Shigella, the issue is even more complex because of the versatility of the Shigella T3SS effectors. For example, the Shigella IpaA effector was shown to lead to FA disassembly and promote cell rounding (Demali et al., 2006). Since the activity of IpaA is presumably opposite to that of OspE, one has to envision coordination between these two T3SS effectors, for example, with IpaA acting during the early invasion steps and OspE stabilizing cell adhesion during later stages of bacterial intracellular replication. Obviously, much is yet to be learned from the sophisticated strategies that bacterial pathogens have developed to colonize their niche. After more than a decade of ‘Cellular Microbiology’, we can only realize that initial breakthroughs were probably the most trivial, since these findings were associated with properties, such as bacterial diversion of cytoskeletal processes by T3SS effectors, for which we had an easy readout. The challenge in future years will lay in our capacity to integrate the molecular functions of these T3SS effectors in a system that recapitulates the essential features of the disease — easier said than done for strict human pathogens.

References