Putting Enterohemorrhagic *E. coli* on a Pedestal

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Many pathogenic bacteria exploit host cytoskeletal pathways to promote infection. In this issue of *Cell Host & Microbe*, Weiss et al. (2009) identify the host factor IRSp53 as the missing link that connects two intracellular bacterial proteins, thereby completing an actin cytoskeletal signaling pathway critical to enterohemorrhagic *Escherichia coli* pathogenesis.

Enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) colonize the human intestinal mucosa, causing diarrhea, which can be severe and, in the case of EHEC, can be accompanied by life-threatening complications. The most common clinical isolate and most extensively studied EHEC is serotype O157:H7. Like several enteric bacterial pathogens, EHEC and EPEC have evolved intricate mechanisms to exploit host cytoskeletal signaling pathways in a manner that enhances infection. In the case of EHEC and EPEC, modulation of the host cytoskeleton enables the organisms to form distinctive "attaching and effacing" (A/E) lesions on infected cells. A/E lesions are characterized by the effacement of the microvilli, intimate attachment of the bacteria to the host cell membrane, and actin filament-filled pseudopods, termed actin pedestals, beneath the sites of bacterial attachment.

A key feature of EHEC and EPEC pathogenesis is the ability of the bacterium to adhere tightly to epithelial cells. Tight association of the bacterium to the host cell surface is mediated by a high avidity interaction between the proteins intimin, presented on the bacterial surface, and Tir (translocated intimin receptor), in the host plasma membrane. Tir proteins are bacterial-encoded and are translocated into the host cell, whereupon they insert into the plasma membrane and serve as a receptor for intimin. Translocation of bacterial proteins occurs via a type III secretion system encoded by the locus of enterocyte effacement (LEE) present in both EHEC and EPEC. Tir adopts a hairpin loop topology, in which the N and C termini lie within the host cytoplasm, and the loop region is exposed on the cell surface, where it interacts with intimin (Kenny et al., 1997). Self-association of intimin molecules leads to clustering of Tir, which triggers downstream signaling events that lead to the formation of actin-rich pedestals directly beneath the bacterial attachment sites, mimicking cellular receptor signaling events that are also triggered by clustering of receptors. Actin pedestal formation requires Tir-dependent activation of the nucleation promoting factor N-WASP (Figure 1). Once activated, N-WASP triggers Arp2/3-mediated actin polymerization. The major pathways of Tir-mediated activation of N-WASP are distinct for EHEC O157:H7 and the most extensively studied EPEC strains. In EPEC, intimin-induced clustering of Tir triggers phosphorylation of Tir tyrosine 474, which lies within its C-terminal domain, by host cell tyrosine kinases. Phosphorylation of tyrosine 474 creates a binding site for the host adaptor protein Nck, which in turn binds to and activates N-WASP (Gruenheid et al., 2001). Actin pedestal formation by EHEC O157:H7 is independent of Tir tyrosine phosphorylation and Nck, but requires an additional bacterial factor, which was independently identified by two labs and named $EspF_U$ (*E. coli* secreted protein F-like from prophage U) or TccP (Tir cytoskeleton coupling protein) (Campellone et al., 2004; Garmendia et al., 2004). $EspF_U$ from different isolates contain two to six almost identical proline-rich repeats that directly interact with the autoinhibitory motif in N-WASP, leading to activation of N-WASP (Cheng et al., 2008; Sallee et al., 2008). Of note, recent surveys of non-O157:H7 EHEC and a variety of EPEC strains reveal heterogeneity in terms of which of these two mechanisms of N-WASP activation are likely utilized during infection (Frankel and Phillips, 2008).

Although Tir does not directly interact with $EspF_U$, translocation of EHEC Tir and $EspF_U$ in the absence of other bacterial effectors is sufficient to trigger actin pedestal formation, indicating that the interaction between Tir and $EspF_U$ is mediated by a host protein. Until now, the identity of this linker protein had remained a mystery. In this issue, Weiss et al. (2009) identify this missing link as the host protein IRSp53 (insulin receptor tyrosine kinase substrate p53). Using a biochemical approach, they demonstrate that IRSp53 interacts with both $EspF_U$ and the C terminus of EHEC Tir. IRSp53 localizes to sites of bacterial attachment in cells lacking either N-WASP or $EspF_U$, and N-WASP and $EspF_U$ recruitment to these sites, as well as actin pedestal formation, is dependent on IRSp53.

IRSp53 is a host adaptor protein that regulates signaling between the protrusive leading edge of cells and the underlying actin cytoskeleton. It is a representative member of a family of proteins whose members contain IMD (IRSp53 and missing-in-metastasis homology domain), important for membrane-binding and -deforming activities associated with filopodia formation. Other functional protein-protein interaction domains within IRSp53 include a partial CRIB domain; an SH3 domain that is involved in interactions with proline-rich sequences in proteins that regulate the cellular actin cytoskeleton, including WAVE2 (WASP-family verprolin-homologous protein 2), Mena/VASP (vasodilator-stimulated phosphoprotein), Eps8, and mDia; and a WH2 domain that binds monomeric actin. Via its partial CRIB and IMD domains, respectively, IRSp53 interacts with activated Cdc42 and Rac, small GTPases that control signaling pathways leading to actin rearrangements. This long list of interactions implicates IRSp53 as a scaffolding

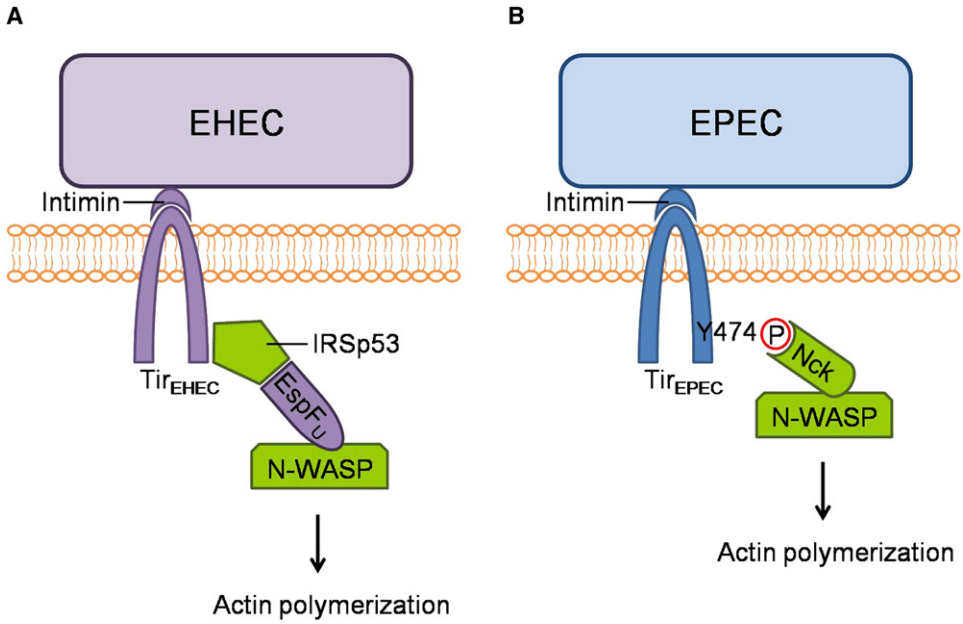

Figure 1. Mechanism of Actin Polymerization upon Attachment of EHEC or EPEC to the Host Cell Surface
(A and B) Adherence of EHEC and EPEC to the host cell is mediated by interactions between the bacterial-encoded receptor Tir, which is translocated into the host cell via the type III secretion machinery, and intimin, which is anchored on the bacterial surface. Upon attachment, EHEC and EPEC induce localized actin rearrangements via two distinct mechanisms. EHEC Tir induces actin polymerization by recruiting the host factor IRSp53, which in turn recruits the secreted bacterial protein EspF$_U$; EspF$_U$ interacts directly with N-WASP, the activation of which leads to actin polymerization (A). Upon receptor binding and clustering, EPEC Tir is phosphorylated on residue Y474 by host cell kinases, thereby creating a binding site for the host adaptor protein Nck; Nck recruits and activates N-WASP, leading to actin polymerization (B).

protein that assembles protein complexes at specific membrane-associated sites; how these interactions are coordinated in physiological conditions is still unclear.

Localization of IRSp53 beneath EHEC is dependent on the presence of its IMD domain, which specifically interacts with 16-amino acid peptides within the C terminus of EHEC and EPEC Tir, known to be critical for Tir function and shown in the present work to be required for IRSp53 recruitment. Although EPEC Tir binds IRSp53, pedestal formation by EPEC is independent of IRSp53. As suggested by the authors, IRSp53 may serve as a scaffold that amplifies EspF$_U$/N-WASP-mediated signaling events. Alternatively, its ability to interact with each of many regulators of the actin cytoskeleton may indirectly enhance pedestal formation. Although IRSp53 binds directly to N-WASP (Lim et al., 2008), EHEC requires EspF$_U$ to activate efficient actin pedestal formation. Perhaps EHEC originally relied solely on IRSp53 to induce minor actin rearrangements and subsequently acquired EspF$_U$, which enhanced the efficiency of pedestal formation and led to an evolutionary advantage. Consistent with this possibility, EspF$_U$ is encoded on a prophage, whereas other type III translocated effector proteins are encoded within the LEE.

Concurrent with the work by Weiss et al. (2009), a second group has independently identified the IRSp53 family member IRTKS (insulin receptor tyrosine kinase substrate) as a critical link between EHEC Tir and EspF$_U$ (Vingadassalom et al., 2009). Both groups show that IRTKS, like IRSp53, localizes to EHEC pedestals and binds Tir and EspF$_U$. In addition, both laboratories demonstrate that the proline-rich repeats in EspF$_U$ that have been shown previously to participate in binding N-WASP (Campellone et al., 2004) are also the target of IRSp53 and IRTKS. However, Weiss et al. (2009) demonstrate that the EspF$_U$ peptide recognized by the SH3 domain of IRSp53 lies within the C-terminal portion of each proline-rich repeat, whereas that which binds N-WASP is known to lie within an N-terminal α-helical portion of each proline-rich repeat (Cheng et al., 2008; Sallee et al., 2008). IRTKS, N-WASP, and EspF$_U$ form a ternary complex (Vingadassalom et al., 2009), indicating that the EspF$_U$ proline-rich repeats can simultaneously bind to N-WASP and IRTKS (or presumably IRSp53). Thus, while some details remain to be clarified, the findings from both laboratories are consistent with the proposed role for one or more member(s) of the IRSp53 family in recruiting a complex of EspF$_U$ and N-WASP to sites of bacterial attachment.

IRSp53 and IRTKS may have some capacity for redundant function in pedestal formation, with the roles of the individual proteins determined by their differential expression in different cell types. Indeed, a notable difference between the experimental setups of the two groups is that Weiss et al. (2009) predominantly infected fibroblasts, whereas Vingadassalom et al. (2009) infected HeLa cells, which may express IRSp53 at lower levels than IRTKS. Together, Weiss et al. (2009) and Vingadassalom et al. (2009) solve one of the mysteries behind EHEC pedestal formation by identifying IRSp53 and the family member IRTKS as host factors that link EHEC Tir signaling to downstream actin polymerization events. The use of

a host factor to link two bacterial proteins is a striking example of bacterial intimacy with the host.

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This Bud's for Vpu

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Successful viruses must overcome the body's immune defenses. In this issue of *Cell Host & Microbe*, Goffinet et al. (2009) provide evidence that the host protein CD317, the target of the HIV Vpu protein, is part of an ancient innate immune response directed against budding viruses.

The human immunodeficiency virus (HIV) is a lentivirus that expresses a small complement of so-called accessory proteins. These proteins—Vif, Vpr, Vpu, and Nef—have been termed "accessory" because they are not required for the nuts and bolts of virus particle construction but are needed for a productive infection in the host. Thus, these proteins are good candidates for factors that are necessary to overcome host defenses.

Indeed, examination of how accessory proteins function has revealed some of the immune defenses the virus must combat to establish a persistent infection. Intriguingly, the accessory proteins all function to degrade host proteins that would otherwise serve protective functions. The Nef protein targets and degrades the major histocompatibility class I molecules HLA-A and -B in lysosomal compartments to evade recognition by cytotoxic T lymphocytes (Roeth et al., 2004). The Vif protein targets and destroys members of the APOBEC family of proteins that disrupt viral genome synthesis by cytidine deamination and subsequent viral genome degradation (reviewed in Malim and Emerman, 2008). Vpr remains somewhat mysterious, but it's known to associate with degradative machinery that targets an as-yet-uncharacterized factor, possibly the cellular protein, UNG, that plays a role in the DNA-damage response (Schrofelbauer et al., 2007). Vpu degrades the viral receptor, CD4, to prevent superinfection of already-infected cells and to decrease detrimental envelope-CD4 interactions within the infected cell (Figure 1). Additionally, in this issue of *Cell Host & Microbe,* Goffinet and colleagues provide evidence that Vpu degrades CD317 (also known as tetherin/BST-2/HM1.24) to reverse the antiviral effects of this protein (Goffinet et al., 2009). The nature of the inhibitory effect of CD317 on viral infection remains unclear, but it targets a very late stage of viral budding and leads to tethering of viral particles to the infected cell surface. Interestingly, the particles can be released by protease digestion indicating that budding particles are linked to the cell surface via a protein tether (Figure 1) (Neil et al., 2007).

CD317 was previously identified as a target of both the Kaposi's sarcoma herpes virus (KSHV) K5 protein and the HIV Vpu protein (Bartee et al., 2006). Targeting of this protein by two unrelated viruses strongly suggested an important but nonspecific antiviral effect. Subsequent studies revealed that CD317 is an interferon alpha-inducible factor that inhibited HIV budding, unless Vpu was expressed (Neil et al., 2008). Vpu was found to alter CD317 surface expression and intracellular localization in some studies (Van Damme et al., 2008), but not in others (Neil et al., 2008), and this effect of Vpu appears to vary with the cell type used (Miyagi et al., 2009). Because Goffinet et al. (2009) demonstrate that the ratio of Vpu to CD317 is important for activity, different results may stem from differences in expression level of these two factors. Alternatively, or in addition, the relative expression of other Vpu targets may impact the observed phenotype.

In this issue of *Cell Host & Microbe*, Goffinet and colleagues demonstrate that human Vpu markedly decreases the half-life of human CD317 in 293 cells expressing ectopic, HA-tagged CD317 and inhibitors of the proteasome reverse that degradation. Notably, these inhibitors also