

is now abundant evidence in many organisms (including *Drosophila*) that DNA damage repair proteins are normally bound at telomeres. Presumably their repair function is blocked, either by unique telomeric proteins or DNA structure. Indeed, elimination of HP1 does result in very high levels of telomeric fusion, indicating telomere dysfunction. However, mutation of other telomere proteins, such as the repair proteins MRE11, ATM, and Ku subunits also result in telomeric fusions, suggesting that there are many ways to disrupt telomere function (Bi et al., 2004; Oikemus et al., 2004; see Harrington, 2004, for a review). It is not clear why mutation of general repair proteins leads to telomere dysfunction. Perhaps the telomere is trapped as an unprocessed broken end, where repair proteins are bound but cannot convert the DNA into a full-blown repair substrate (Takai et al., 2003).

One could imagine that HP1 forms repressive heterochromatin at telomeres, which blocks the function of repair proteins. However, Perrini's insights on HP1 at telomeres seem to rule out this simple hypothesis. They distinguish two functional interactions at the telomere: HP1 is bound to DNA, which along with the RNAi system is required for H3K9-me marking of telomeric chromatin. Presumably, additional HP1 localization depends on H3K9-me modification. Perrini et al. suggest that DNA-bound HP1 nucleates telomeric heterochromatin by recruiting a methyl-transferase, which then generates a domain of marked chromatin. Interestingly, they also examine an HP1 mis-sense mutation that abolishes binding to H3K9-me. This relieves silencing of telomeric HeT and TART genes but does not cause end-to-end fusions, thus genetically separating heterochromatin and capping. The nature of the block to DNA repair at telomeres remains mysterious. But the general picture that emerges from this and other studies of telomere proteins is that *Drosophila* has a clear separation between its retroposon systems for solving the end-replication problem and its protein complexes for capping. This makes their telomeres simple to dissect, and ongoing genetic screens promise to reveal more.

This is not the first time that a different mode of binding has been uncovered in HP1. The chromodomain of HP1 is the H3K9-me binding domain, but can also bind to nucleosome cores (Nielsen et al., 2001). Previous work

has suggested that HP1 homologs can interact with DNA or RNA through the adjacent hinge region of the protein. Strikingly, HP1 is even recruited to actively transcribed genes in *Drosophila*, apparently by binding RNA, where it promotes transcription (Piacentini et al., 2003)! *Drosophila* telomeres give one example of how HP1 modes of binding may work together. Perhaps its DNA binding activity generally nucleates HP1 domains, while nucleosome core binding presents additional HP1 to histone tails, thereby increasing its affinity for H3K9-me. Indeed, some HP1 is retained even in SuVar3-9 mutants (Schotta et al., 2002), and in RNAi mutants HP1 is widely distributed over chromatin (Pal-Bhadra et al., 2004). Deciphering these different binding modes will be important to understanding this versatile chromatin protein.

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## A New Twist in the Hijacking of the Actin-Nucleating Machinery

Many microbial pathogens have evolved specific adaptations to harness the host cell actin cytoskeleton. The understanding of these mechanisms reveals a striking level of complexity and diversity in the strategies utilized by different pathogens to module actin dynamics.

While *Escherichia coli* K-12 is a ubiquitous and harmless workhorse of every molecular biology laboratory, many of its close relatives are a serious public health problem. For example, enterohemorrhagic *Escherichia coli* (EHEC) is an important human pathogen that causes hemolytic

uremic syndrome, a serious illness that often results from the consumption of tainted food products. Central to the pathogenicity of these bacteria is their ability to modulate the actin cytoskeleton of intestinal epithelial cells leading to the formation of characteristic “pedestals” where the microvilli are disrupted by localized filamentous (F)-actin rearrangements at the place of intimate bacterial attachment (Campellone and Leong, 2003; Celli et al., 2000). Formation of actin pedestal is also induced by other bacterial pathogens including the closely related enteropathogenic *E. coli* (EPEC). To induce the formation of these actin pedestals, both EHEC and EPEC rely on a type III secretion apparatus, which translocates bacterial effector proteins into host cells and is encoded within a pathogenicity island known as the “locus of enterocyte effacement” (LEE) (Campellone and Leong, 2003). Although the components of the type III secretion systems in EHEC and EPEC are highly con-

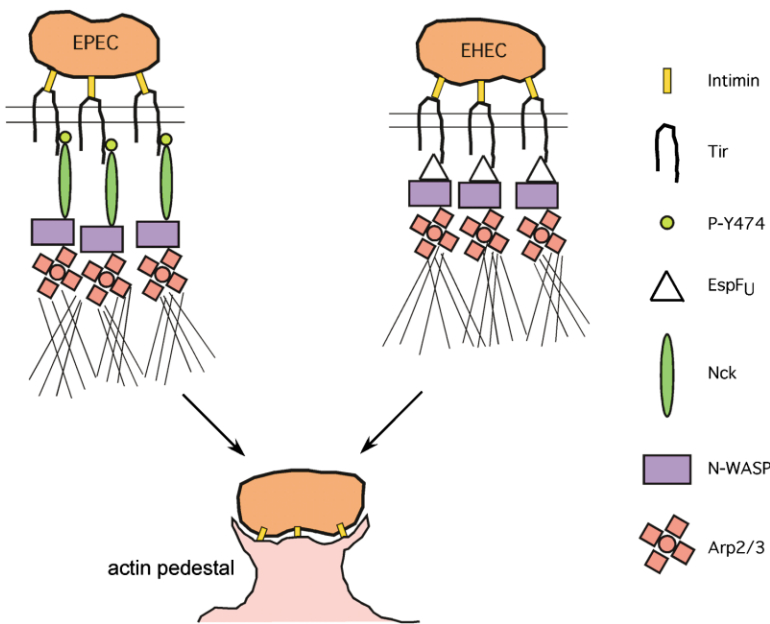


Figure 1. Model for Actin Pedestal Formation Mediated by EHEC or EPEC  
See text for details.

served, the effector proteins that are delivered by these systems are less conserved. This observation suggests that despite the morphological similarities between the actin-rich structures induced by EHEC and EPEC, there are some distinct features in the mechanisms that lead to their formation. Indeed, the available evidence supports this hypothesis. For example, EPEC relies on a single translocated effector protein to induce actin pedestal formation. This effector is known as “translocated intimin receptor” (Tir). Upon its translocation into the host cell membrane, Tir becomes the receptor for Intimin, a bacterial surface adhesin that anchors the bacterium to the plasma membrane. Intimin binding triggers clustering of Tir and its phosphorylation on tyrosine 474 by the tyrosine kinase Fyn (Phillips et al., 2004), thus creating a binding site for the adaptor protein Nck (Gruenheid et al., 2001). This adaptor protein recruits N-WASP, which through its ability to activate the Arp2/3 complex, initiates the actin polymerization events that lead to pedestal formation (Campellone and Leong, 2003; Celli et al., 2000). Therefore, in EPEC, Tir clustering is essential and sufficient for pedestal formation.

EHEC also possesses a LEE-encoded Tir effector that is essential for actin pedestal formation. However, EHEC Tir is unable to induce actin-pedestal formation when expressed in EPEC, indicating that, unlike its EPEC counterpart, EHEC Tir is not sufficient to induce actin nucleation (Campellone and Leong, 2003). In fact, EHEC Tir lacks the corresponding critical Y474 residue and does not recruit Nck. However, EHEC is able to recruit N-WASP, which like in the case of EPEC, is essential for actin nucleation and pedestal formation (Lommel et al., 2004). If Nck is not recruited to the EHEC pedestals, how is N-WASP recruited to initiate actin nucleation after infection with these bacteria? In the current issue of *Developmental Cell*, Campellone et al. report a series of elegant experiments that provide an answer to this question.

Cells infected with an EPEC strain that had been engi-

neered to deliver EHEC Tir to the plasma membrane did not form actin pedestals. However, actin pedestals were induced if cells were co-infected with this engineered EPEC strains and an EHEC strain harboring a functional type III secretion but lacking Tir, which by itself does not induce pedestal formation (DeVinney et al., 2001). These results suggested that an additional EHEC factor(s) delivered via the type III secretion system must act in conjunction with EHEC Tir. The genome sequence of the prototypic EHEC strain revealed the presence of several pathogenicity islands specific for enterohemorrhagic *E. coli* O157:H7 (O-islands) that are absent from the genome of the laboratory *E. coli* K-12 strain. Campellone et al. reasoned that the additional effector(s) acting in concert with EHEC Tir to induce the formation of actin pedestals should be encoded within one of the O-islands and therefore generated EPEC strains carrying specific deletions in these islands. Elimination of only one of the O-islands resulted in the inability of EPEC to induce actin pedestal formation. Further analysis determined that only one ORF within this island, termed EspF<sub>U</sub>, was critical for pedestal formation by an EHEC strain containing an intact LEE. This ORF is capable of encoding a polypeptide with modest but significant sequence similarity to EspF, a protein that is translocated by the LEE-encoded type III secretion system and is required for EPEC's and EHEC's ability to disrupt tight junctions. Expression of EspF<sub>U</sub> in an EPEC strain engineered to encode the EHEC Tir was capable of inducing the formation of actin pedestals, indicating that EspF<sub>U</sub>, in conjunction with the LEE-encoded Tir and type III secretion system, are sufficient to induce actin pedestal formation.

Campellone et al. then conducted a series of experiments that provides the framework for a very plausible model by which EspF<sub>U</sub> exerts its function (Figure 1). The first clue came from the observation that after translocation through the type III secretion system, EspF<sub>U</sub> localizes to actin-pedestals where Tir is also present. This

suggested that EspF<sub>U</sub> exerts its activity directly at that site of actin polymerization and not indirectly by regulating other cellular or bacterial processes. Consistent with this hypothesis, EspF<sub>U</sub> was not required for efficient translocation and localization of EHEC Tir. Unlike EPEC Tir, EHEC Tir by itself was unable to recruit N-WASP and the Arp2/3 complex, requiring the co-translocation of EspF<sub>U</sub>, suggesting a role for EspF<sub>U</sub> as an intermediary in N-WASP recruitment. Additionally, EspF<sub>U</sub> was shown to interact directly with N-WASP, and form a complex with Tir. These results indicate that EHEC has evolved a remarkable mechanism to focalize the actin cytoskeleton to the plasma membrane at the point of bacterial attachment by delivering two proteins, Tir and EspF<sub>U</sub>, which through their concerted action recruit N-WASP and the actin-nucleating machinery.

The ability of EspF<sub>U</sub> to recruit N-WASP is reminiscent of the mechanism of action of another bacterial protein, IcsA, which binds N-WASP to focalize the actin cytoskeleton at one of the bacterial poles to mediate the actin-based motility of *Shigella* spp. within the cytoplasm of infected cells (Goldberg, 2001). Interestingly, both IcsA and EspF<sub>U</sub> bind a region of N-WASP immediately adjacent to the Cdc42 binding site. Since binding of Cdc42 to N-WASP results in a conformational change that leads to its activation (Higgs and Pollard, 2001), it is possible that binding of the bacterial proteins may also lead to similar conformational changes in N-WASP. In this sense, both IcsA and EspF<sub>U</sub> would be intersecting into “hard-wired” mechanisms of activation of the actin-nucleating machinery. However, there are distinct differences between the actin-cytoskeletal rearrangements induced by EspF<sub>U</sub> or IcsA with those induced by the activation of Cdc42. Activation of Rho-family GTPases tends to lead to transient actin cytoskeleton rearrange-

ments. In contrast, the formation of actin pedestals by EHEC or *Shigella*'s intracellular motility requires continuous focalized actin polymerization. Whether recruitment of N-WASP by these bacterial proteins results in its long-lasting activation is unknown. As the details of the molecular and even atomic interfaces between microbial pathogens and their hosts are beginning to be understood, it is becoming even more apparent that the study of host/pathogen interactions can not only reveal potential entries for therapeutic intervention but can also teach us important lessons in basic cell biology.

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