



A fresh look at the type III secretion system: two-step model of effector translocation in pathogenic bacteria

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A commentary on

Translocation of surface-localized effectors in type III secretion

by Akopyan, K., Edgren, T., Wang-Edgren, H., Rosqvist, R., Fahlgren, A., Wolf-Watz, H., and Fallman, M. (2011). *Proc. Natl. Acad. Sci. U.S.A.* 108, 1639–1644.

Acquisition of genetic elements such as virulence plasmids or pathogenicity islands (PI) by horizontal gene transfer can endow pathogenic bacteria with an arsenal of virulence factors that promote bacterial survival and replication within their hosts. Despite the differences in the host organisms and pathology caused by important pathogenic bacteria such as *Escherichia coli*, *Yersinia*, *Salmonella*, and *Shigella*, a common virulence mechanism exists in the form of a needle-like structure that translocates bacterial proteins into host cells to hijack the host machinery and modulate the host immune response (Ghosh, 2004).

Enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) belong to a family of attaching and effacing (A/E) pathogens responsible for diarrheal diseases in humans and animals. The diseases are characterized by the effacement of the intestinal microvilli, bacterial colonization, and attachment on pedestals induced by localized actin polymerization upon contact with enterocytes and disruption of tight junctions (Dean and Kenny, 2009; Croxen and Finlay, 2010). A type III secretion system (T3SS) encoded by the locus of enterocyte effacement (LEE) secretes proteins called effectors to form A/E lesions in the host and subvert various host processes such as disruption of the host cytoskeletal network and modulation of the host innate immune signaling (Sharma et al., 2006; Ruchaud-Sparagano et al., 2007; Khan et al., 2008). *Yersinia* employs the plasmid-encoded Ysc-Yop T3SS to deliver effectors called Yops (*Yersinia* outer proteins) to the host cytosol to paralyze phagocytes

and block bacterial uptake (Cornelis et al., 1989; Rosqvist et al., 1990). This tactic of evading the host immune response ensures an environment conducive for the lifestyle of *Yersinia*. In contrast, *Salmonella* possesses two PI encoding distinct T3SS called SPI-1 and SPI-2. During invasion, the SPI-1 secretion apparatus deploys effectors to the host cell milieu to promote phagocytosis (Galán, 1999) while the SPI-2 T3SS activity creates a niche for replication and survival of *Salmonella* within target cells (Cirillo et al., 1998). The components of the T3SS are generally conserved among Gram-negative bacteria and even heterologous effectors can be secreted in another host bacteria such as the case of the *Yersinia* effector YopE expressed in *Salmonella enterica* serovar Typhimurium (Rosqvist et al., 1995).

The *Yersinia* injectisome consists of a membrane-spanning basal body and a hollow conduit of YscF polymers through which effectors transit for secretion (Hoiczyk and Blobel, 2001). Dedicated T3SS chaperones bind to their cognate effectors and keep them in a locally unfolded, secretion-competent state (Ghosh, 2004). The chaperone-effector interaction is thought to provide specificity for effector docking on the secretion apparatus. The N-terminal domain or the 5' end of most secreted effectors contains the signal sequence for secretion, translocation, and chaperone binding (Sory et al., 1995; Miao and Miller, 2000). However, there is no clear consensus sequence for the signals due to the degeneracy of the sequences at the amino acid or RNA level (Ghosh, 2004). How these signals are recognized by the secretion apparatus is not well understood but differential signal recognition by the chaperones or translocon components is thought to be the basis for the hierarchical secretion of effectors (Lara-Tejero et al., 2011; Osborne and Coombes, 2011). A defined order of secretion of effectors ensures that effector functions are activated in a spatial and temporal manner. A recent study revealed a cytoplasmic complex made up of SpaO/OrgA/

OrgB that functions as a platform for sorting chaperone-effector pairs prior to secretion (Lara-Tejero et al., 2011). Differential binding of the specific chaperones to the complex leads to the sequential loading of substrates. The translocators YopB and YopD are needed to complete the translocation of effectors across the host cell membrane and deletion of these translocators results in the extracellular secretion but not translocation of effectors into the host cytosol (Håkansson et al., 1996; Neyt and Cornelis, 1999). In *Yersinia*, the first ~15 amino acids at the N-terminus is sufficient for secretion but not for translocation of the effectors leading to the conclusion that the presence of YopB/YopD and a distinct translocation signal are required for proper effector translocation (Sory et al., 1995). In the absence of translocators, secretion of effectors can be induced by growing bacteria in media that mimic environmental cues for T3SS activation such as low calcium, phosphate or magnesium (Michiels et al., 1990; Yu et al., 2010). Hence, although secretion and translocation are both necessary for infection, these events have different regulatory and structural requirements.

YopB/D, as well as the related translocators in *E. coli* (EspB/D), *Shigella* (IpaB/C/D), and *Salmonella* (SipB/C/D), contain hydrophobic domains and are proposed to form a pore by inserting into the host cell membrane (Ghosh, 2004). However, direct evidence for effectors being transported through this pore is lacking. In the one-step microinjection model, the effectors are injected directly by the T3SS into the host cytosol. However, one issue with this model lies in the structural and functional relationship of the translocators and the injectisome. It still remains to be elucidated whether the injectisome itself actually pierces the host cell membrane or the translocators act as the terminal connection of the injectisome to the target cells by creating a membrane pore (Hoiczyk and Blobel, 2001; Cornelis, 2002).

A recent paper by Akopyan et al. (2011) aimed to elucidate the translocation mechanism of the T3SS. They demonstrated that *Y. pseudotuberculosis* effectors localized to the bacterial surface are translocated by the T3SS. They first observed that the effectors YopE and YopH and the translocator YopD were evenly distributed on the bacterial surface prior to host cell contact (Schesser et al., 1996; Akopyan et al., 2011). Interestingly, previous studies also found secreted Ipa effectors on the surface of *Shigella* before injection into the host cell (Watarai et al., 1995). The significance of these findings is unclear but it was proposed that the extracellular effectors serve a protective role for the pathogen against the onslaught of the host immune attack (Schesser et al., 1996). Mutational analysis of the N-terminal region of YopE showed that distinct domains are needed for secretion, surface localization, and translocation across the host cell membrane. YopE disrupts the host cytoskeleton to prevent phagocytosis and having a distinct pool of surface-localized YopE could provide an early protection for the bacterium before host cell contact (Schesser et al., 1996).

Akopyan et al. (2011) further investigated the role of surface-localized effectors by demonstrating that bacteria coated with recombinant purified YopH induced YopH-mediated inhibition of early calcium response in neutrophils during infection. These results were dependent on a functional T3SS and the translocators YopB and YopD. To prove that the response was due to the translocation of the surface-localized effector, they coated bacteria with a recombinant YopH-Bla fusion protein and showed β lactamase activity in the host cytosol after infection. Truncations at the N-terminal region of YopH-Bla showed that distinct domains are required for effector secretion and translocation. Coating of bacteria with YopH₁₋₁₇-Bla did not result in the translocation of the mutant effector, substantiating previous studies that showed expression of the first 17 amino acids of YopH is sufficient for T3SS-mediated secretion of the effector but not translocation into the host cytosol (Sory et al., 1995; Akopyan et al., 2011). On the other hand, a distinct signal sequence found between amino acids 18 to 49 was required for effector translocation, as recombinant YopH₁₈₋₄₉-Bla or YopH₁₈₋₉₉-Bla used to coat bacteria were exported into

the host cell. However, these YopH mutants were not secreted when expressed in bacteria and in the absence of surface localization, strongly suggesting that effector secretion, and translocation are two separate events (Boyd et al., 2000).

Based on the findings of this study, the authors proposed a two-step model in which formation of an effector-translocator complex represents the intermediate step between effector secretion by the injectisome and translocation through the pore formed by YopB and YopD. In this model, the N-terminal translocation domain is critical for the formation of this intermediate and could explain why separate N-terminal secretion and translocation domains are needed for efficient delivery of T3SS substrates. However, direct evidence of the intermediate is still lacking and whether the binding of the effector to YopB/D occurs through the translocation domain has yet to be determined. It is hypothesized that YopB/YopD block the membrane pore to prevent host membrane damage after infection (Viboud and Bliska, 2001). It would be interesting to study whether the formation of the effector-translocator intermediate induces conformational changes in YopB/D to enable opening of the pore.

Akopyan et al. (2011) proposed that pre-formed translocation intermediates could be found on the bacterial surface or bacteria-host membrane interface prior to translocation. How does surface-localization relate to the observed hierarchical secretion of effectors? It is possible that some of the surface-localized effectors compete with the pool of T3SS substrates originating directly from the bacterial cytosol for binding with the translocators. The rest of the surface-localized effectors could function in protecting the pathogen. This dual role for T3SS effectors, as well as the presence of an available pool of extra- and intracellular T3SS substrates, might be beneficial for the pathogen as this primes the bacteria during its transit from the extracellular environment to target tissues. Alternatively, the secretion hierarchy could be maintained by secreting early-acting effectors that are then localized to the bacterial surface in preparation for host cell contact. In *Salmonella*, it has been shown that expression of SPI-2 genes occurs before invasion of intestinal epithelium (Brown

et al., 2005). Whether effectors from both T3SS are localized to the surface of the bacteria before invasion is not known. The possibility for surface-localized effectors in *Salmonella* was supported by experiments showing *S. Typhimurium* coated with recombinant YopH also translocated the effector into host cells in a SPI-1 T3SS-specific manner (Akopyan et al., 2011). Thus, the two-step model challenges our view of the translocation mechanism of the T3SS and provides a fresh look at the events occurring at the pathogen-host cell interface. Future work on the identification and translocation of surface-localized effectors in *E. coli*, *Salmonella*, and other pathogenic Gram-negative bacteria will provide needed insight into the two-step translocation mechanism of T3SS. It will particularly be interesting to see whether this mechanism of effector translocation also occurs in alternative host settings, particularly the host-pathogen interactions leading to commensalism of EHEC and EPEC in animals.

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