



Targeting one of its own: expanding roles of substrates of the *Legionella pneumophila* Dot/Icm type IV secretion system

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Facing the many host defense mechanisms at different stages of the infection, a pathogen needs to employ corresponding arsenals either to hijack host cellular processes for its own use or to thwart the attacks from the host. For numerous bacterial pathogens, one of the effective weapons is effector protein. Type IV protein secretion systems are associated with the virulence of many important pathogens such as *Helicobacter pylori*, *Bartonella pertussis*, *Brucella* spp., *Coxiella burnetii*, and *Legionella pneumophila* (Backert and Meyer, 2006). Although the structures of these transporters appear similar, their protein substrates differ drastically not only in functions but also in abundance. For example, despite extensive efforts, CagA is the only effector identified for the Cag system of *H. pylori* (Hatakeyama, 2008). On the other hand, there are at least 274 experimentally confirmed protein substrates of the *L. pneumophila* Dot/Icm system (Zhu et al., 2011). This count is close to 10% of the predicted genes in the *L. pneumophila* genome (Cazalet et al., 2004; Chien et al., 2004; Schroeder et al., 2010), making the Dot/Icm system arguably the most prolific protein transporter in term of the number of translocated substrates. Similar to effectors of other types of secretion systems, Dot/Icm substrates function to target various host cellular processes, such as vesicle trafficking, cell death, ubiquitination, lipid metabolism, and innate immunity, thus allowing the biogenesis of an intracellular niche permissive for bacterial replication (Isberg et al., 2009; Hubber and Roy, 2010).

To cope with the dynamic response from the host, bacterial pathogens can employ several strategies to achieve temporal regulation of the activity of their virulence factors. The most commonly used mechanism is to regulate expression of virulence factors at the transcriptional level in response to environmental cues present during different phases of infection. For example, many *Legionella* effector genes are induced

when bacteria enter the post-exponential phase and are ready to infect (Bruggemann et al., 2006). The second is to control the translocation efficiency of effectors at post-translational level, which is exemplified by small RNA-mediated regulation of effector transfer in *Salmonella typhimurium* (Padalon-Brauch et al., 2008). The third is to code for effectors capable of causing opposite cell biological effects to neutralize or reverse the effects caused by other effectors. In *S. typhimurium*, bacterial entry is induced by SopE, a guanine nucleotide exchange factor (GEF) for the Rho family of small GTPases, an activity that is antagonized by the GTPase activation protein (GAP) SopE. In this scenario, temporal control is achieved by the inherent differences in the sensitivity of these two proteins to host proteasome degradation (Kubori and Galan, 2003). Similarly, *L. pneumophila* reverses the SidM/DrrA-mediated activation of the small GTPase Rab1 by the GAP protein LepB (Ingmundson et al., 2007). However, the mechanism underlying the temporal control remains elusive. Third, the pathogens can inhibit pathways triggered by themselves. For example, as a result of apoptosis induced by *L. pneumophila* challenge, infected cells contains active caspases 3 and 7, but the activity of these enzymes presumably can be inhibited by IAPs induced by the bacterium itself (Abu-Zant et al., 2005, 2007; Losick and Isberg, 2006; Nogueira et al., 2009). Unfortunately, the bacterial proteins involved in such manipulation remain unknown as does the mechanism underlying the temporal regulation.

Almost all characterized virulence factors exert their effects by targeting one or more host molecules. The discovery by Kubori et al. (2010) extended the roles of bacterial effectors into a completely new domain: direct regulation of the function of other effectors, in this particular case, by targeting a different effector protein for proteasome degradation. LubX is a U-box-type E3

ubiquitin ligase that was previously found to target the host kinase Clk1 (Kubori et al., 2008). In this new study, the authors reported that LubX polyubiquitinates the effector SidH and targets it for degradation (Kubori et al., 2010). Moreover, their data showed that in a fruit fly infection model, deletion of *lubX* caused a hyper-lethality phenotype and reduced bacterial replication. Thus, this discovery established a new mechanism used by a bacterial pathogen to temporally control the activity of its virulence factors. Their results also substantiated some earlier observations on *L. pneumophila* infection. First, they showed that LubX is expressed and translocated by the Dot/Icm system only until after the initial phase of infection has been established, peaking at 10 h post infection (Kubori et al., 2008, 2010), which is in line with the observation that the Dot/Icm system is active for at least 8 h after bacterial internalization (Liu et al., 2008). Second, targeting of SidH did not occur in the initial phase of infection, implying that SidH is important in the early phase of infection and becomes unnecessary or even detrimental to further development of the bacterial phagosome as infection proceeds. Such prediction is consistent with the potential role of SidH in inhibition of host cell death (Laguna et al., 2006), because continued arrest of apoptosis presumably is counterproductive when the infection comes to a close.

This study also provided an explanation to one of the perplexing questions in the study of *L. pneumophila* pathogenesis: Why does this bacterium code for so many effectors? At least two models can be used to explain this phenomenon. The first is that there is a tremendous functional redundancy among effectors targeting a particular host cellular process. This model is supported by the fact that mutations eliminating one single effector gene rarely caused defects in intracellular bacterial growth (Ensminger and Isberg, 2009). Second, these effectors

may be necessary for successful colonization of the taxonomically diverse protozoan hosts encountered by *L. pneumophila* in the environment. The study by Nagai and colleagues added a novel dimension of targets regulated by *L. pneumophila* virulence factors.

Given the complexity of the interactions between *L. pneumophila* and its hosts, it will not be surprising to identify more metaeffectors with modes of action different from that of LubX in future study. It is possible that inhibition of effector activity by a second effector can be achieved by post-translational modifications. An effector can directly reverse the modification made by another effector on its target molecule. For example, such regulation conceivably can be achieved by a pair of effectors with kinase and phosphatase activity, respectively. Alternatively, one effector can directly modify another effector at post-translational level to activate or inactivate its function. As novel post-translational modifications such as AMPylation (Roy and Mukherjee, 2009; Muller et al., 2010) and farnesylation (Ivanov et al., 2010; Price et al., 2010) continued to be identified in *Legionella* effectors, it will be interesting to know whether these modifications are subjected to effector-mediated regulation. Although the existence of these regulatory mechanisms in the control of *L. pneumophila* effector activity is uncertain, it is certain that more excitement will be generated in our continuing study of these several hundreds interesting proteins.

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