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Multiple functions of YopN in the *Yersinia pseudotuberculosis* type III secretion system

From regulation to *in vivo* infection

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Title
Multiple functions of YopN in the *Yersinia pseudotuberculosis* type III secretion system, from regulation to in vivo infection

Abstract
The type 3 secretion systems (T3SSs) are virulence mechanisms used by various Gram-negative bacteria to overcome the host immunity. They are often target-cell contact induced and activated. Activation results in targeting of virulence effector substrates into host cells. One class of secreted substrates, translocators, are required for the intracellular targeting of the second class, the virulence effectors, into host target cells. T3SSs are mainly regulated at 2 levels; a shift from environmental to host temperature results in low level induction of the system whereas target cell contact further induces and activates the system. In the Yersinia T3SS, YopN, one of the secreted substrates, is involved in the latter level of activation. Under non-inducing conditions, YopN complexes with TyeA, SycN and YscB and this complex suppresses the T3SS via an unknown mechanism. When the system is induced, the complex is believed to dissociate and YopN is secreted resulting in the activation of the system. Earlier studies indicated that YopN is not only secreted but also translocated into target cells in a T3SS dependent manner. TyeA, SycN and YscB bind to the C-terminal and N-terminal YopN respectively but so far the central region (CR) of YopN has not been characterized. In this study we have focused on the function of the YopN central region.
We therefore generated in-frame deletion mutants within the CR of YopN. One of these deletion mutants, aa 76-181, showed decreased early translocation of both YopE and YopH into infected host cells and also failed to efficiently block phagocytosis by macrophages. However, the YopNΔ76-181 protein was expressed at lower levels compared to wt YopN and also showed a slightly deregulated phenotype when expressed from its native promoter and were as a consequence not possible to use in in vivo infection studies.
Therefore, we generated mutants that disrupted a putative coiled coil domain located at the very N-terminal of CR. Similar to YopNΔ76-181, these substitution mutants were affected in the early translocation of effector proteins. Importantly, they were as stable as wt YopN when expressed from the native promoter. One of these mutants was unable to cause systemic infection in mice indicating that YopN indeed also has a direct role in virulence and is required for establishment of systemic infection in vivo.

Keywords
Type III secretion system, *Yersinia,* YopN, virulence, phagocytosis, mouse infection, kinetics

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