

Timing and targeting of Type III secretion translocation of virulence effectors in *Yersinia*

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Abstract

The Type III secretion system (T3SS) is an important virulence mechanism that allows pathogenic bacteria to translocate virulence effectors directly into the cytoplasm of eukaryotic host cells to manipulate the host cells in favor of the pathogen. Enteropathogenic *Yersinia pseudotuberculosis* use a T3SS to translocate effectors, Yops, that prevent phagocytosis by immune cells, and is largely dependent on it to establish and sustain an infection in the lymphoid tissues of a mammalian host.

Translocation into a host cell requires specific translocator proteins, and is tightly controlled from both the bacterial and host cell cytoplasm. We aimed to investigate two of the regulatory elements, YopN and LcrV, to gain more insight into the translocation mechanism.

Two separate regulatory complexes regulate expression and secretion of Yops, however, the processes are linked so that expression is induced when secretion is activated. A complex, including YopD, prevents expression of *yops*, while YopN-TyeA and LcrG block secretion. LcrV is required to relieve the secretion block, by sequestering LcrG. We verified that LcrG binds to the C-terminal part of LcrV, which is consistent with what has been shown in *Y. pestis*. In addition to their regulatory roles, both LcrV and YopD are translocators and are assumed to interact at the bacterial surface, where LcrV promotes insertion of YopB and YopD into the host cell membrane. However, here we show that purified YopD failed to interact with LcrV, instead YopD solely interacted with a complex of LcrV-LcrG. This indicates that LcrV and YopD interact in the bacterial cytosol, which may be important for regulation of *yop* expression and secretion.

The established role of YopN is to block secretion prior to host cell contact. We found that deleting the central region (amino acids 76-181) had no effect on the regulatory role of YopN in expression and secretion of Yops. Interestingly, we found that, even though the YopN $_{\Delta 76-181}$ mutant secreted the translocators with similar kinetics as the wild type strain, translocation of the effector YopH, into HeLa cells, was significantly reduced. Consequently, the YopN $_{\Delta 76-181}$ mutant was unable to block phagocytosis, almost to the same level as the $\Delta lcrV$ mutant which is completely unable to translocate YopH. Our results indicate that YopN is involved in the translocation step in addition to its role in regulating secretion.

Further, we show that the amino terminal of LcrV, in the context of translocation, is involved in the early intracellular targeting of YopH in order to block phagocytosis efficiently and sustain an *in vivo* infection. LcrV mutants that failed to efficiently target YopH intracellularly were severely attenuated also for *in vivo* virulence.

All together, we show that LcrV and YopN are involved in more steps in the regulation of translocation, than what was known before. Our studies also highlight that early translocation is essential for *Yersinia* to block phagocytosis, which in the end is essential for *in vivo* virulence.

Keywords

Type III secretion system, virulence, translocation, *Yersinia pseudotuberculosis*, LcrV, YopN, effector targeting, phagocytosis inhibition, YopH, *in vivo* infection

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