Title:

Host Cell Contribution to Type III Secretion in Yersinia pseudotuberculosis

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Abstract:

Type III secretion (T3S) is a highly conserved effector delivery mechanism in many different Gram-negative bacteria including Yersinia pseudotuberculosis. Previous studies of Yersinia spp. type III secretion have focused on characterization of 6 Yop (Yersinia outer proteins) effectors and the components of the translocation apparatus. However, the role of the host cell in Yop effector translocation, cellular trafficking and subsequent localization with target is unknown. To investigate the contribution of the host cell to T3S, we have designed a pooled RNAi screen to discover host genes required for the cytotoxic effects associated with the Yersinia translocated substrate YopE, a GTPase activating protein that inactivates the small Rho GTPases. Fluorescence Resonance Energy Transfer (FRET) of a Rho GTPase Biosensor can measure the efficiency of RhoA inactivation by YopE in RNAi depleted cells challenged with Y. pseudotuberculosis. RNAi knockdown of host cell genes critical for YopE intoxication should block YopE inactivation of the Rho GTPases. Therefore, cells maintaining an active Rho GTPase after incubation with YopE can be positively selected by fluorescence activated cell sorting (FACS) in order to identify the candidate genes disrupting YopE cytotoxicity. Flow cytometry experiments confirm that the Rho Biosensor accurately measures the activation state of RhoA as shown by analyzing genetic point mutations or following incubation with a Y. pseudotuberculosis strain secreting just YopE. As a proof of principal, we demonstrate that FRET positive cells can be enriched from a mixed population of cells. Candidate genes are identified as shRNAs enriched in the FRET positive cells versus input cells as determined by Illumina sequencing. Overall, we have performed a unique screen to elucidate the role of host cell factors in T3S of Y. pseudotuberculosis. The results obtained from this screen should provide a breadth of new information pertaining to T3S in Yersinia spp., which can also be extrapolated to T3S systems in many other bacteria.