EUKARYOTIC TARGETS OF A BACTERIAL PROTEIN KINASE VIRULENCE FACTOR

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INTRODUCTION. Several animal- and plant-interacting Gram-negative bacteria possess the so-called type III secretion system or TTSS, which functions to inject proteins (or ‘effectors’) directly into eukaryotic cells[1]. TTSS effectors play important roles in a variety of host-microbe interactions including *Rhizobium*-mediated root nodulation, plant hypersensitivity reactions, and in animals, modulating immune responses. The pathogenic yersiniae (*Yersinia pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*) inject five TTSS effectors all of which are required for full virulence in the mouse model. As might be expected since they function within host cells, *Yersinia*’s TTSS effectors possess clear eukaryotic-like protein domains or motifs. One of these effectors, the *Yersinia* protein kinase A (YpkA), contains the canonical 11 subdomains (I-XI) characteristic of eukaryotic ser/thr protein kinases with a highly conserved catalytic core sequence. In addition to the amino-terminally located kinase domain, YpkA, also contains in its carboxyl-terminal region sequences that resemble eukaryotic RhoA-binding domains.

RESULTS AND DISCUSSION. We have previously shown by flow cytometry that the expression of TTSS effectors increase in individual bacteria following contact with cultured macrophage-like cells[2]. In a cell culture infection assay, a strain of *Y. pseudotuberculosis* expressing an enzymatic inactive variant of YpkA (YpkA_{D270A}) was greatly attenuated in its ability to proliferate compared to the wild-type strain when incubated with eukaryotic cells. To identify eukaryotic proteins that physically interact with YpkA, we performed yeast two-hybrid screening using YpkA’s kinase domain as bait. In a library prepared from human spleen, we discovered that the calcium- and integrin-binding (CIB) protein interacts with YpkA and have gone on to show that the YpkA-CIB association occurs in an *in vitro* protein interaction assay. We are currently testing whether CIB (a.k.a. ‘calmyrin’), a peripheral membrane protein[3,4] in fact localizes YpkA to the inner face of the plasma membrane following its injection into eukaryotic cells[5]. We have also found that YpkA’s kinase activity was not required in a cell morphology assay in which YpkA induces HeLa cells to undergo retraction. Instead the carboxyl-terminal region of YpkA, which contains sequence similarity to eukaryotic RhoA-binding domains, is required for YpkA-mediated morphological changes of eukaryotic cells[5]. YpkA does indeed interact with RhoA (and Rac but not Cdc42) in protein interaction assays, but interestingly, unlike

eukaryotic RhoA-binding proteins, with which it shares sequence similarity, YpkA binds both the activated GTP-bound form of RhoA as well as inactive GDP-bound RhoA.

Although the significance of CIB and RhoA targeting by YpkA, both in terms of the cell biology and, in a larger sense, pathogenesis, is currently unclear, we believe that characterizing these events will eventually lead us to an understanding of how this virulence factor promotes bacterial proliferation vis-à-vis the eukaryotic host cell.

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