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COMMENTARY

That's the Way You Do It

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ABSTRACT About one-third of the proteins encoded by the bacterial genomes that have been sequenced to date are proteins of "unknown function." Studies aimed at defining the biological functions of these proteins represent an important frontier in prokaryotic biology. The study presented by J. Herrou et al. (J Bacteriol 201:e00134-19, 2019) in this issue of the *Journal of Bacteriology* provides an excellent example of how to pursue such studies and define a new virulence determinant for an important zoonotic pathogen.

KEYWORDS *Brucella*, DUF protein, domain of unknown function protein, protein of unknown function

We have seen great advances in the experimental approaches that we can use to study prokaryotic biology over the past few decades. Our capacity to determine the complete DNA sequences of bacterial genomes, for instance, is incredibly powerful. The first complete bacterial genome sequence, that of *Haemophilus influenzae* Rd, was published in 1995 (1), and there are currently 13,466 complete bacterial genome sequences available in GenBank. These genome sequences tell us a great deal about bacterial evolution and the metabolic and physiological diversity of bacteria, and they help us better understand how some bacteria have become efficient pathogens. Genome sequence data obtained from uncultivated bacteria provide us with previously unavailable information about microbially diverse populations in many ecological niches and valuable insight into how bacteria interact with each other and their surrounding environments. But one of the fascinating things that we have come to realize from mining these data is that we still have no idea what the functions are for up to one-third of the proteins encoded by prokaryotic genomes (2). Many of these proteins are annotated as proteins having "domains of unknown function" (DUF) (3).

Determining the biological functions of these so-called DUF proteins is one of the new and exciting frontiers of our field. Herrou et al., in this issue of the *Journal of Bacteriology* (4), provide an excellent example of how both old and new experimental approaches can be effectively used to carry out this type of analysis. *Brucella* spp. are Gram-negative bacteria that cause abortion and infertility in their natural mammalian hosts, as well as a chronic debilitating zoonotic disease in humans (5, 6). Like other bacteria, the 775 *Brucella* genome sequences that have been completed contain many genes predicted to encode proteins of unknown function. One of these, a protein belonging to the DUF1849 family, is of particular interest because (i) it appears to only be present in *Brucella* species and other closely related alphaproteobacteria and (ii) Herrou et al. (4) have shown that this protein is an essential virulence determinant for *B. abortus* 2308 in the mouse model of chronic infection.

The DUF1849 protein in question is encoded by the *bab1_1186* gene in the *B. abortus* 2308 genome. Herrou et al. used bioinformatics to determine that this protein is likely located in the periplasm, and phenotypic analysis of a *bab1_1186* deletion mutant suggests that the corresponding gene product plays a role in protecting *Brucella* cells from cell envelope stress (4). Based on these observations, Herrou et al. gave this protein the designation EipB (envelope integrity protein B). They then used

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wild-type and mutated *eipB-phoA* gene fusions to confirm the periplasmic location of EipB in *Brucella*, and they used cryo-electron microscopy to show that loss of this protein did not have a readily observable effect on cell morphology. X-ray crystallography was employed to determine the structure of EipB and gain insight into its function, and site-directed mutagenesis was used to determine whether the highly conserved cysteine residues in the DUF1849 family of proteins are required for EipB function. Finally, Herrou et al. employed a powerful transposon-based synthetic lethality screen to identify another periplasmic protein of unknown function, TtpA, which apparently works in concert with EipB to maintain envelope integrity in *Brucella*, and these researchers used genetic and biochemical approaches to further examine the interactions between these proteins.

In the end, Herrou et al. did not determine the precise function of EipB (4), but they did define important biochemical and biophysical properties of this protein that can be used to design more focused studies to examine its function more closely. Maybe more importantly, the thorough, logical, and meticulous experimental approach this group used to investigate the biological function of EipB serves as a useful "instruction manual" that can be used to examine the biological functions of other bacterial proteins of unknown function. From the perspective of *Brucella* biology and pathogenesis, these studies have also identified a new virulence determinant. This is an important contribution because the brucellae do not employ many of the virulence strategies used by other mammalian pathogens to produce disease in their hosts (7, 8). Consequently, I think that the phrase "that's the way you do it" from the song entitled "Money for Nothing" by Dire Straits perfectly describes the analysis of EipB function by Herrou et al. (4).

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