A Synergistic Effect of the Multiple Domains of *Yersinia* Protein Kinase A

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Writer's Comment: Prose writing in past English classes was always a difficult, painful endeavor. Much to my delight, however, Henderson's Writing in the Sciences class was geared for writing styles such as mine, straightforward, practical, and undecorated. When the review paper was assigned, I immediately knew it would be on the topic of my ongoing research—plague pathology. I had no idea it would reach a larger audience, so my goals were much more personal; I simply wanted to integrate current research articles into a persuasive argument about an ongoing debate, and to have a piece that would give proof of my ability to establish myself in a field. In retrospect I feel I have stated some of the most important points raised in my paper in plain English, and hope the importance of YpkA will reach a wider audience.

—Matthew Wright

Instructor's Comment: Mr. Matthew Wright was a student in my UWP 104E Science Writing class in winter quarter 2010. One of several major assignments in 104E is a scientific review paper. In this assignment, I challenge my students to write a review that presents a current research topic, using pertinent results and conclusions published in a variety of peer-reviewed papers, and synthesizing these conclusions into a fresh perspective original to the reviewer him or herself. This is what makes a competent and rigorous review paper stand out. Mr. Wright's "A Synergistic Effect of the Multiple Domains of Yersinia *protein kinase A" most certainly has fulfilled this promise, for me, for Matt's target audience, and, of course, for* Prized Writing*'s editorial jury. This extensively researched paper presents a rigorous melding of current research in microbiology, biochemistry, pathology, and genetics, which combine to yield a promising new understanding, and potential for control, of a virulent protein affiliated with the plague and bioterrorism.*

—Brad Henderson, University Writing Program

Introduction

The bacterial genus *Yersinia* is composed of 11 species, three of which are pathogenic to humans. *Y. pestis*, the causative agent of plague, is especially important as it is responsible for an estimated 200 million deaths over three pandemics (1st century; 8th–14th century; 19th–21st century) and has recently emerged in India[1]. In addition, *Y. pestis* is reemerging as a worldwide threat in bioterrorism and has been described as one of the most important bacterial warfare agents[2].

Pathogenic *Yersinia* harbor a 70 kb extra chromosomal virulence plasmid, where *Yersinia* protein kinase A (YpkA/YopO) is generated in addition to several other bacterial effector proteins (Yops). A type three secretion system (TTSS) is also encoded in this pathogenicity island, allowing the bacteria to pierce the target eukaryotic cell and translocate these proteins[3].

YpkA has been extensively studied since its first description in 1993, when it was quickly recognized for its requirement in virulence determination and its extensive homology to eukaryotic Ser/Thr kinases[4]. YpkA is an 82 kDa protein with four domains that have consistently been shown to have non-redundant properties and to target specific components in cells (Figure 1). An N-terminal secretion and translocation domain (S/T) is responsible for transport to the plasma membrane[5]. The rho-binding domain (RBD) inhibits downstream signaling of the small G proteins Rac and Rho[6,7], while the kinase domain inactivates the alpha subunit of the heterotrimeric G protein, Gαq[6]. An actin binding domain (ABD) on the C-terminus of YpkA interacts with host globular actin, activating the kinase domain[8].

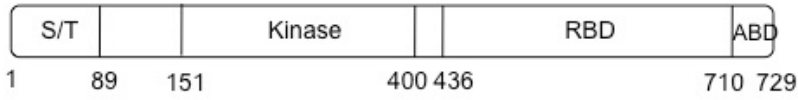

Figure 1: Current model of YpkA.
S/T: Secretion/translocation domain; RBD: Rho Binding Domain; ABD: Actin Binding Domain.

In assays of YpkA and variants thereof, epithelial and leukocyte cell lines are often used. The implications of the choice of cell line and the corresponding domain of study are a matter of interest in this paper and will be discussed throughout. Popular epithelial cell lines include those

derived from human intestines (Henle407) and cervical tissue (HeLa). Lines originating from leukocytes are commonly adherent mouse macrophages (RAW264.7) and non-adherent T-cells (Jurkat). Interestingly, kidney cells are frequently used and include those of the African Green Monkey Kidney cells (COS7) and Human Embryonic Kidney Cells (HEK293A).

YpkA uses its domains in a synergistic way unmatched by its individual parts, while having portions of unresolved function that can be defined and characterized. In addition, the cell lines used to study YpkA are an important influence on results and are frequently overlooked.

Discussion

Contributions of Kinase and Rho Binding Domains

Both the kinase domain and the Rho Binding domain have been shown to independently disrupt host cell machinery. The exact effect on the host cell remains unclear, however, as knockouts continue to show unexpected residual effects, leading to inconclusive roles for each domain in overall virulence. Several studies show mixed phenotypes where certain variables point to key differences—downstream effects which contribute to virulent phenotypes and cell types used in transfection assays—being notable and discussed here.

The kinase domain was demonstrated by Navarro et al. to use Gαq as its target and disrupt downstream signaling by phosphorylating serine 47[6]. This family of G proteins is important for a number of reasons, as it stimulates phospholipase C- β and has a number of cellular effects, ranging from interactions with regulatory, effector, and scaffolding proteins[10] to platelet activation[9]. The latter is of special interest to plague investigators as it accounts for bleeding associated with the disease. Offermann notes in his study of Gαq-deficient mice that increased bleeding times occurred and there was a lower chance of induced blood clots[9].

This warrants the question of what cell types should be used for investigation of the kinase domain; the effect of uncontrolled bleeding is a well known symptom of plague and in many kinase studies, epithelial tissue (including fibroblasts here) is logically used. These experiments may be investigating effects that are beyond the survival stage in real-life infection. One could reason, however, that these results may be used towards remedial medicine when a clearer picture of *Yersinia* infection has been made or when the importance of other downstream effects are

apparent. Kidney cell lines have been frequently used in YpkA research to also give arguably indirect results. When researchers test for basic cell response or obvious morphological differences, kidney cells represent a widespread and reproducible line; it is difficult to affirm, however, that they are a cell type relevant to actual infection.

What about leukocytes as the consensus cell type for *Yersinia* assays? Although food-borne illness caused by *Y. Enterolitica* and *Y. Pseudotuberculosis* is perpetuated through epithelial tissue in the gut, the gut-associated lymphoid tissue is in close association and will ultimately take the brunt of the attack. Additionally, treatable plague (bubonic and septicemic) initially targets the lymphoid or circulatory tissue, making this a strong candidate for primary use.

The RBD is a 274 amino acid stretch at the C-terminal end of YpkA, responsible for keeping cellular G proteins inactive by mimicking Guanine nucleotide-dissociation inhibitor molecules and by directly interacting with Rac1 and RhoA[5,11]. In fact, "rounding up" is frequently the description used in morphological effects of the RBD as cells lose their actin stress fibers[8,12]. How this relates to the disease itself is indirect and may be cell- or tissue-specific, raising the question of how these morphologies relate to the progression of disease. It is clear that the actin cytoskeleton plays a crucial part in host defense as in phagocytosis; however, the details of YpkA inactivation of macrophages beyond simple paralysis remain to be elucidated.

The rationale for choice of cell line for RBD studies is similar to the kinase domain, with minor adjustments. Since this portion of YpkA interacts directly with Rac1 and RhoA, we have a better idea of its possible downstream consequences since it is not at the top of a signaling cascade. Because of this, discrete targets are also better known, which happen to be regulation of the cytoskeleton, among other activities. This again could place leukocytes as a leading investigational tool because of the importance of actin utilization in phagocytosis, and the logical conclusion that the interaction between macrophages and *Yersinia* is of most importance. These details are corroborated by a study by Marketon that explicitly states that "plague bacteria target immune cells"[13].

In recent years, a surge of research has disputed the kinase domain and the RBD as the leading factors in virulence[5,6,7]. Both domains target proteins vital in cellular communication, yet the perpetual theme of functional redundancy remains. Perhaps the pairing of these two

enzymatic functions enable YpkA to remain successful in a variety of cell types, hence the numerous reports and conditions seen in YpkA cell assays. However, for these two domains to truly become independently appreciated and fully characterized, a standard for cell type used in these assays will most likely need to be used.

The sec/tran domain assists the Rho Binding Domain

Groves et al. recently demonstrated a striking synergy between the as of yet uncorrelated or at least unappreciated RBD and S/T domain[14]. The S/T domain escorts YpkA to phagocytic-opsonin receptors, those responsible for destroying bacteria covered in antibodies. It is here that the RBD fulfills its purpose of RhoA/Rac-1 binding and inhibits phagocytic signaling. Experiments showed this to affect a major challenge to the host defense during *Yersinia* infection—anti-phagocytic action. Groves reports up to 60% inhibition of internalization; practically speaking, if this is true of live infections, the immune response is functioning at just 40% of its potential. This anti-phagocytic ability of *Yersinia* is a hallmark of infection, and many previous studies of the RBD have not dealt with this important influence.

In regards to cell assays of the RBD, the choice between leukocytes and any other cell type is important as Fcχ-R expression is limited to a subset of leukocytes. Continued use of non-leukocyte cell lines by transfecting immune-related genes of interest may not accommodate unknown or indirect genes necessary to assay function. Research aimed at systemic infection of plague may fare better by focusing on relevant cells, leukocytes. Another interesting discovery by Groves was the necessity of both Rac and Cdc42 to be inactivated before this phagocytic-sabotage could take place[14]. Previously, only Rac and Rho proteins have been described as affected by YpkA, thus opening another possible target for YpkA. To date, however, no data have shown YpkA to directly interact with Cdc42, resulting in lack of understanding of its role.

The current YpkA model has 100 undefined amino acids

In the last few years, there have been many exciting discoveries that fill the "empty space" of YpkA, including an additional ABD[15], autoactivation sites[15], and an apoptosis inducing domain[16]. These are not placed on the current model of YpkA because they remain unresolved both functionally and spatially.

Arguably the most exciting of these is the apoptosis domain, which actually overlaps with the kinase domain. Surprisingly elusive until its characterization by Park et al. in 2007, it was uncertain whether the observed apoptotic effects were attributed to YpkA, another *Yersinia* effector (YopJ), or both[17]. Since then it has become clear that YpkA has taken on the more active role of apoptosis induction through the intrinsic pathway of mitochondrial depolarization[16], with the other member YopJ taking a complementary role, recalling the theme of synergy in an intermolecular sense. Monack's multicopy plasmid[17] used in the study may also account for the effects not seen by Park, as the excess YopJ may have over-sensitized the MAPK pathway, as has been previously described as [YopJ] mode of action[16]. This discovery has enabled more possibilities for the study of YpkA; further insight into its exact domain boundaries and crucial residues, its relation to kinase activity, if any, and a molecular intracellular target are all relevant research interests.

Due to the large degree of overlap with the kinase domain, a catalytic residue on this domain is doubtful. A simple interaction or sequestering event seems more likely to be inducing apoptosis, possibly related to the role of structure in the kinase domain. While Park showed specific and firm evidence of apoptosis[16], this has been rarely documented in studies of YpkA, including the author's own, hinting at unknown factors occurring in infection. A study by Anderson et al. on pneumonic plague pathology in rats, however, did find a high percentage of apoptosis. At only 48 hours after respiratory infection, they found sections of lung with "severe cell death and destruction of alveoli," a large portion of which consisted of macrophages[18]. Although this study used live cultures of *Y. pestis*, rather than just YpkA, the fact that Park found almost half of his leukocyte transfections dead through apoptosis in just 24 hours leaves a reasonable assumption that YpkA may have the leading effect of virulence[16]. In combination with Anderson's study, apoptosis in leukocytes is a disastrous event, in addition to a grand finale to *Yersinia*'s orchestrated infection.

The concept of YpkA-activation has been known for about a decade; it was shown that actin binding is a crucial step for activation of the kinase domain[8]. Autoactivation is a relatively new concept, though, as Trasak et al. determined a second actin binding site (ABSII) on YpkA (amino acids 400–441) as well as two residues that were intermediate to full activation—serines 90 and 95[15]. These amino acids and domain are

required to become phosphorylated before the kinase domain becomes functional, yet knockout studies of the two residues leave residual activity, indicating other required sites.

This information has two important implications. First, some structural insight is gained with the knowledge that these sites must cooperatively interact. Although the domains reside relatively far from one another, the molecule must fold so that the two actin binding domains, as well as the critical serines (90/95), are in proximity. Second, this sequence of events presents amino acids 89–150 as some sort of regulator of the kinase domain, as well as a possible target for YpkA inactivation. It will be exciting to follow the development of this process.

Concluding thoughts

With so many new discoveries taking place, the development of a new model of YpkA is the logical next step. Soon the "empty space" of approximately 100 amino acids will be well-understood enough to resolve YpkA into exact domains, functions, and interactions. Figure 2 is a glimpse of what this model may resemble, although the region between S/T and kinase activity is still an area of ongoing research. Will this span of 60 amino acids be granted more importance than it is currently ascribed? More than likely, the answer is yes. Equally promising, as more is found out about YpkA, it will be appreciated how all domains work together to exert an effect on host cells that is greater than that achieved by a single domain.

Figure 2: Proposed updated model of YpkA.
AP: Autophosphorylation and activation domain (89–150); ABDII: Actin Binding Domain Two (400–440); ABD and ABDII contact actin prior to phosphorylation of serine 90 and 95, which activates the kinase domain. The apoptosis domain (roughly 133–262) induces apoptosis via the intrinsic pathway.

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