The *Yersinia* Yop Virulon, a Bacterial System to Subvert Cells of the Primary Host Defense*

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**ABSTRACT.** The Yop virulon enables *Yersinia* (*Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*) to survive and multiply in the lymphoid tissues of their host. It is an integrated system allowing extracellular bacteria to communicate with the host cell's cytosol by injection of effector proteins. It is composed of four elements: (1) a contact or type III secretion system called Ysc, devoted to the secretion of Yop proteins; this secretion apparatus, made of some 22 proteins, recognizes the Yops by a short N-terminal signal that is not cleaved off during secretion, (2) a system designed to deliver bacterial proteins into eukaryotic target cells; this system is made of YopB, YopD and LcrV, (3) a control element (YopN) and (4) a set of effector Yop proteins designed to disarm these cells or disrupt their communications (YopE, YopH, YopM, YpkA/YopO, YopP). The whole virulon is encoded by a 70-kb plasmid called pYV. Transcription of the genes is controlled both by temperature and by contact with a eukaryotic cell.

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1 INTRODUCTION: THE *Yersinia* LIFE STYLE

For a long time, the view was held that each invasive pathogenic bacterium has its own life style, and that there is a great diversity of individual bacterial virulence strategies. However, recent data from several laboratories challenge this view and reveal the existence of related major virulence systems in various pathogenic bacteria, including phytopathogens. One of these systems involves the delivery of bacterial proteins inside eukaryotic cells by surface-bound bacteria that are in close contact with the target cell surface. The Yop virulon of the genus *Yersinia* represents the archetype of this growing family of systems.

The genus *Yersinia* includes three species that are pathogenic for rodents and humans: *Y. pestis* is the agent of black death, *Y. pseudotuberculosis* causes adenitis and septicemia and *Y. enterocolitica*, the most prevalent in humans, causes a broad range of gastrointestinal syndromes. In spite of differences in the infection routes, they share a common tropism for lymphoid tissues and a common capacity to resist the nonspecific immune response. Pathological examinations of artificially infected mice show that *Yersinia* forms extracellular microcolonies. In accordance with these *in vivo* observations, *Yersinia* is resistant to phagocytosis *in vitro*, by macrophages and polymorphonuclear leukocytes. Once they are phagocytosed, *Y. pseudotuberculosis* and *Y. enterocolitica* are generally killed.

2 THE CLUE: CALCIUM DEPENDENCE

It has been known since the mid-fifties that *Y. pestis* does not grow at 37 °C in Ca²⁺-deprived media. Since the loss of this unusual property correlates with the loss of virulence, nonvirulent mutants could easily be detected and selected. Both phenotypes are determined by a 70-kb plasmid (pYV) which *in vitro* governs the massive release of a set of about 12 proteins called Yops (for *Yersinia* outer proteins). Genetic analyses revealed that these Yops are essential for virulence but three observations were disturbing: (1) Yops are not produced in the presence of Ca²⁺ at mmol/L concentrations that prevail in the extracellular fluid; (2) *in vitro*, Yops have no obvious toxic activity of their own; and (3) Yops form large and insoluble aggregates in the culture medium.

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3 FROM THE ENIGMA TO A MODEL

Rosqvist et al. (1991) first showed that extracellular adherent Yersinia induce a cytotoxic effect on HeLa cells and that YopE is involved in this action. However, crude preparations containing YopE had no cytotoxic effect unless they were microinjected into HeLa cells, indicating that the target of YopE is intracellular. A yopD mutant was also unable to affect HeLa cells but a preparation of Yops secreted by this mutant was cytotoxic when microinjected into the cytosol of HeLa cells. They concluded from this that YopD plays a role in translocating YopE across the plasma membrane of the target cell to reach the cytosolic compartment. They also showed that intracellular bacteria are not cytotoxic which ruled out that YopE is translocated across the membrane of a phagosome. Evidence for YopD-mediated translocation of YopE was confirmed by two independent approaches. One used immunofluorescence and confocal laser scanning microscopy to show that YopE appeared in the perinuclear region of HeLa cells infected with wild-type Y. pseudotuberculosis. However, when infection was carried out with a yopD mutant, YopE was only found in spots in the vicinity of bacteria adhering to the cell surface (Rosqvist et al. 1994). The other approach was based on a reporter enzyme strategy. The reporter consisted of the calmodulin-activated adenylate cyclase domain (Cya) of the Bordetella pertussis cyclolysin. Since the catalytic domain of cyclolysin is unable to enter eukaryotic cells by itself, accumulation of cAMP reflects Yop internalization. Infection of HeLa cells with Y. enterocolitica producing a hybrid YopE–Cya resulted in a marked increase in cAMP, even when internalization of the bacteria themselves was prevented by cytochalasin D. Infection with a yopBD mutant did not lead to cAMP accumulation, confirming the involvement of YopD and/or YopB in translocation of YopE across eukaryotic membranes (Sory and Cornelis 1994).

A coherent picture emerged from these two approaches and mechanistic insight now takes precedence over phenomenology. According to the model, the Yops form two distinct groups of proteins: some, like YopE, are effectors that are delivered inside eukaryotic cells by extracellular yersinia, while YopB, YopD and possibly other Yops form a delivery apparatus. Both groups of proteins are secreted by the same specialized secretion system. The pYV plasmid thus encodes an integrated anti-host system that we call the Yop virulen. For the rest of this review, we will call "secretion" the crossing of the two bacterial lipid membranes and "translocation" the crossing of the eukaryotic cell plasma membrane. Yops involved in the translocation of effectors will be referred to as "translocators".

4 INTRACELLULAR EFFECTORS

Five Yop effectors have been formally identified: YopE, YopH, YopO/YpkA, YopM and YopP. The 23-kDa YopE causes disruption of the actin microfilament structure of cultured HeLa cells. However, it does not disrupt actin filaments polymerized in vitro, even in the presence of NAD⁺, suggesting that its action is indirect. The target of YopE is still unknown, but it is interesting to note that YopE shares homology with exoenzyme S of Pseudomonas aeruginosa that was recently shown to be secreted by a contact secretion pathway (Yahr et al. 1996) and which elicits the same cytotoxicity as YopE when injected by a recombinant Y. pseudotuberculosis (Frithz-Lindsten et al. 1997). This may indicate that the two proteins have the same target(s). Since ExoS modifies small G-proteins involved in the regulation of the actin network (for a recent review see Goranson and Frank 1996) it is possible that the effect of YopE is also mediated by some modification of small G-proteins.

YopH is a 51-kDa, broad-spectrum, protein tyrosine phosphatase related to eukaryotic PTPases. Though the catalytic domain is only ~20 % identical to human PTP1B, the Yersinia PTPase contains all of the invariant residues present in eukaryotic PTPases and forming the phosphate-binding loop (P-loop) including the nucleophilic Cys⁴⁰³ which forms a phosphocysteine intermediate during catalysis (Stuckey et al. 1994). It acts on macrophage tyrosine-phosphorylated proteins (Andersson et al. 1996), which contributes to the inhibition of bacterial uptake (Andersson et al. 1996) and oxidative burst (Bliska and Black 1995), presumably by dephosphorylating key proteins involved in signal transduction. YopH also obstructs the invasin stimulated uptake of Yersinia by HeLa cells. This uptake is associated with increased tyrosine phosphorylation and recruitment to peripheral complexes of p130Cas and FAK, two proteins that are dephosphorylated by YopH (Persson et al. 1997; Black and Bliska 1997).

YpkA is an 81-kDa serine/threonine kinase that shows noticeable sequence similarity to eukaryotic counterparts (Galyov et al. 1993). It is targeted to the inner surface of the plasma membrane of the eukaryotic cell (Håkansson et al. 1996a). Given the kinase activity of YpkA and its spatial localization it is reasonable to suggest that YpkA also interferes with some signal transduction pathway of the eukaryotic cell.