

Microreview

Yersinia effectors target mammalian signalling pathways

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Summary

Animals have an immune system to fight off challenges from both viruses and bacteria. The first line of defence is innate immunity, which is composed of cells that engulf pathogens as well as cells that release potent signalling molecules to activate an inflammatory response and the adaptive immune system. Pathogenic bacteria have evolved a set of weapons, or effectors, to ensure survival in the host. *Yersinia* spp. use a type III secretion system to translocate these effector proteins, called Yops, into the host. This report outlines how Yops thwart the signalling machinery of the host immune system.

Introduction

Innate immunity and signalling

Innate immunity is the first line of defence against bacterial and viral infection (for a review of innate immunity, see Medzhitov and Janeway, 2000). The cells involved in the immune response use a wide variety of signalling cascades to activate processes such as phagocytosis, cytokine production and release and production of reactive oxygen species. In addition to eliminating pathogens, the innate immune system activates the adaptive immune response through the presentation of foreign peptides to T cells.

Phagocytosis is an essential component of the innate immune system (May and Machesky, 2001). Rearrangement of the actin cytoskeleton during phagocytosis is mediated by the recruitment of proteins that function in actin rearrangement; these include paxillin, p130Cas and focal adhesion kinase (FAK) (Greenberg *et al.*, 1990; 1993; Allen and Aderem, 1996). These proteins may form

focal adhesion complexes within the cell at points of contact with bacteria, although this has not been demonstrated conclusively. The formation of these focal adhesion complexes coupled with signalling events, including the tyrosine phosphorylation of proteins in these complexes, results in rearrangements in the actin cytoskeleton via the activation of the Rho family of GTPases, which includes Rho, Rac and Cdc42 (Aspenstrom, 1999a; Schoenwaelder and Burridge, 1999; Chimini and Chavrier, 2000). Activation of the Rho GTPases induces their interactions with downstream signalling effectors that are involved in actin organization (Aspenstrom, 1999b). These proteins are ideal targets for bacterial virulence factors, as inactivation of these signalling networks would block phagocytosis and allow the survival of the bacteria.

The inflammatory cytokines, including tumour necrosis factor α (TNF α), interleukin-8 (IL-8) and interleukin-1 (IL-1), are involved in activation of the adaptive immune response, macrophage activation and inflammation. Regulation of these cytokines is controlled at the transcriptional level via transcription factors including NF- κ B, the cyclic AMP-responsive element-binding protein (CREB) and AP-1. The NF- κ B transcription factor is held in the cytoplasm through interaction with the protein I κ B (Karin, 1999; Mercurio and Manning, 1999). Translocation of NF- κ B to the nucleus requires the destruction of bound I κ B, which occurs through phosphorylation of I κ B by the I κ B kinase complex (IKK) and subsequent ubiquitination of I κ B (Karin, 1999). CREB and AP-1 transcription factors are activated through phosphorylation via mitogen-activated protein (MAP) kinase signalling cascades (Pearson *et al.*, 2001). One can envisage that inhibition of these signalling cascades, perhaps through effects on these regulatory factors, would be an ideal mechanism for a pathogen to use in order to escape the host inflammatory response.

Yersinia infection

Yersinia pestis causes bubonic plague, whereas *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* both cause gastrointestinal disorders. Enteropathogenic *Yersinia* (*Y. pseudotuberculosis* and *Y. enterocolitica*) enter the lymphoid system of their hosts via passage

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through M cells (microfold cells), which are specialized cells that take up foreign antigens, in intestinal Peyer's patches (Autenrieth and Firsching, 1996). Recognition of *Yersinia* by M cells is mediated via $\beta 1$ integrins on the M-cell plasma membrane and the invasins protein of *Yersinia* (Marra and Isberg, 1997; Clark *et al.*, 1998). Once *Yersinia* has penetrated the M cells, it can localize in the lymphoid tissue of its host.

The recently sequenced genome of *Y. pestis* (Parkhill *et al.*, 2001) suggests that many of the genes that *Yersinia* uses during infection and invasion, including adhesins, secretion systems and insecticidal toxins, have been acquired from other bacteria and viruses. Furthermore, all three pathogenic species of *Yersinia* harbour an extrachromosomal plasmid of 70 kb that is essential for virulence (Portnoy and Martinez, 1985). This plasmid contains the genes of a type III secretion system, a translocation apparatus highly conserved among pathogenic Gram-negative bacteria (for a review, see Cornelis, 1998). This secretion system is responsible for the translocation of the *Yersinia* effectors, termed Yops, into the host cell. Once inside the host cell, Yops carry out disruption of signalling cascades that activate the processes of phagocytosis, cytokine release and respiratory burst. Six Yop effectors have been identified (YopH, YopE, YopJ/P, YpkA/YopO, YopT and YopM). Some of these effectors are known to function in the downregulation of critical signalling cascades of the immune system (Fig. 1). The activities of these effectors and the pathways they target are described below.

YopH

YopH was identified as an essential *Yersinia* virulence

factor that inhibits macrophage phagocytosis (Bolin and Wolf-Watz, 1988; Rosqvist *et al.*, 1988). The 51 kDa YopH protein contains C-terminal sequences similar to the protein tyrosine phosphatase (PTPase) superfamily (Guan and Dixon, 1990). These sequences include residues located within the active-site (D(X)nHC(X)₅R) of the PTPases (Fig. 2A). YopH catalyses phosphate hydrolysis from tyrosine-phosphorylated peptides and proteins (Guan and Dixon, 1990). Bliska *et al.* (1991) demonstrated that bacterially produced YopH can translocate into macrophages. The translocation requires cell contact between the bacteria and the macrophage. Once inside the mammalian cell, the bacterial phosphatase is capable of dephosphorylating host phosphotyrosine-containing proteins, leading to a reduction in invasins-mediated bacterial engulfment. Furthermore, the catalytic activity of YopH is necessary for its role in the virulence of *Yersinia* (Bliska *et al.*, 1991), suggesting that the targets of YopH within the host cell are important in regulating macrophage signalling during the process of phagocytosis during infection. YopH dephosphorylates p130Cas, focal adhesion kinase (FAK), paxillin and the Fyn-binding protein (FBP), thereby inhibiting the formation of focal adhesion complexes (Fig. 1) (Black and Bliska, 1997; Persson *et al.*, 1997; Black *et al.*, 1998; Hamid *et al.*, 1999). YopH bears a similar three-dimensional structure to the mammalian PTPases (Stuckey *et al.*, 1994), suggesting that YopH most probably catalyses tyrosine phosphate hydrolysis via a similar mechanism to the mammalian PTPases.

It is well established that tyrosine phosphorylation plays a role in respiratory burst induction (Yamaguchi *et al.*, 1995). Therefore, it is not surprising that YopH down-

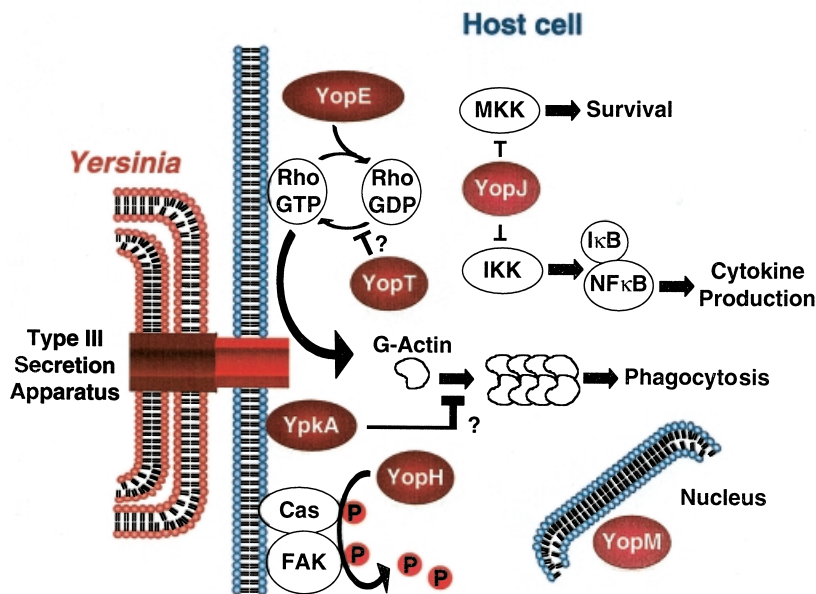


Fig. 1. The *Yersinia* effectors target multiple signalling pathways. The Yop effectors of *Yersinia* are translocated into the host cell via a type III secretion system. Once inside the host cell, each of the effectors targets signalling pathways to ensure survival of the bacteria within its host. YopH, a protein tyrosine phosphatase, dephosphorylates p130Cas, FAK and paxillin, causing disruption of focal adhesion complexes and inhibition of phagocytosis. YopE, a Rho GTPase-activating protein (GAP), inactivates the Rho family of GTPases, resulting in altered actin cytoskeleton. YpkA, a protein serine/threonine kinase, disrupts the actin cytoskeleton. YopJ, a putative cysteine protease, disrupts multiple signalling pathways, resulting in inactivation of MAP kinase and NF- κ B and, ultimately, apoptosis and inhibition of cytokine production. YopT is a protein of unknown function that causes a modification of the Rho family of GTPases, presumably to alter the actin cytoskeleton. YopM, a protein of unknown function, contains leucine-rich repeats, which are presumably used to bind to host proteins.

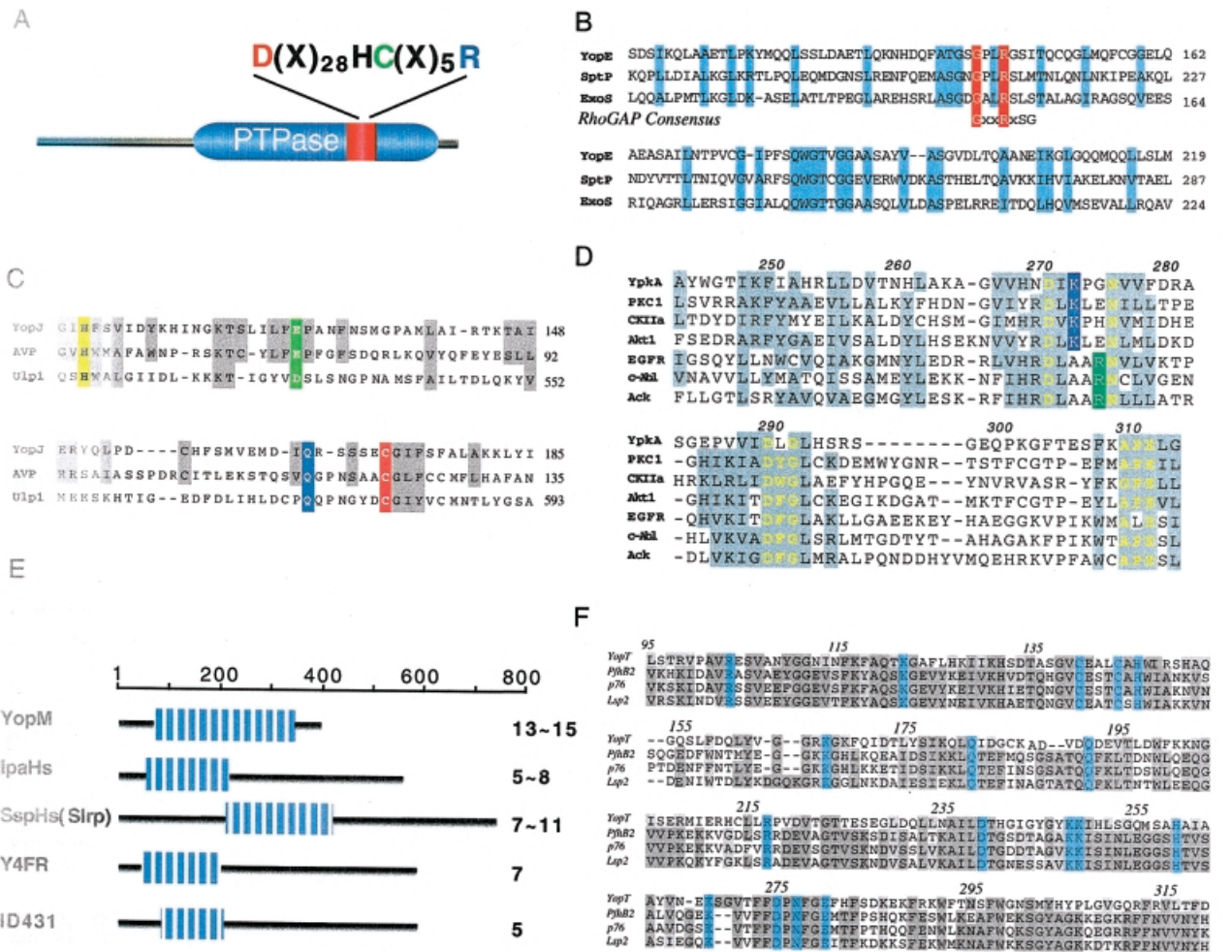


Fig. 2. Sequence alignments and structural diagrams of the Yop effectors and their homologues.
 A. YopH harbours a protein tyrosine phosphatase (PTPase) domain at the carboxy-terminus, which is represented by the presence of the catalytic D(X)₂₈HC(X)₅R motif.
 B. The GAP domains of YopE, SptP and ExoS show significant similarity to each other. However, this domain differs from any of the known eukaryotic GAP domains, except for the presence of the mammalian Rho GAP consensus motif GxxRxSG (in red). The arginine plays a critical catalytic role in GTP hydrolysis activity.
 C. Sequence comparison of YopJ, adenovirus protease (AVP) and Ulp1, a yeast ubiquitin-like protein protease. YopJ shows significant similarity to AVP within the protease domain and shares the catalytic triad including His (in yellow), Asp/Glu (in green) and Cys (in red) for protease activity and an invariant Gln (in blue) located in the oxyanion hole of both AVP and Ulp1.
 D. Sequence alignment of the kinase domain VI, VII and VIII between YpkA and canonical Ser/Thr kinase (PKC1, CKIIa and Akt1) and Tyr kinase (EGFR, c-ABL and Ack). Invariant residues essential for kinase activity, including the catalytic Asp and Asn, Asp-Phe-Gly chelating Mg²⁺ and stabilization loop Ala-Pro-Glu, are highlighted in yellow. The lysine (in blue) within the catalytic loop renders YpkA to be a Ser/Thr kinase. The lysine (blue) or arginine (green) is believed to play a role in stabilization of the transition-state intermediate during catalysis.
 E. Representation of the leucine-rich repeat (LRR)-containing type III secreted virulence factors from *Yersinia* (YopM), *Shigella* (IpaH family), *Salmonella* (SspHs and Slrp) and symbiotic *Rhizobium* (Y4FR) and *Bradyrhizobium* (ID431). LRR is represented by the blue box. The numbers after the diagram indicate the number of LRRs present in each gene or gene family.
 F. YopT exhibits sequence similarity to the C-terminus of surface antigen (p76) from *Haemophilus somnus*, filamentous haemagglutinin-like protein Lsp2 from *Haemophilus ducreyi* and PfhB2 from *Pasteurella multocida*. Several conserved residues that may suggest catalytic activity are highlighted in blue.

regulates signalling cascades required for the activation of Fc-mediated respiratory burst in macrophages (Bliska and Black, 1995) and neutrophils (Ruckdeschel *et al.*, 1996). This inhibition of respiratory burst induction by *Yersinia* requires the tyrosine phosphatase activity of YopH (Bliska and Black, 1995). However, Zymosan-induced oxidative burst inhibition by *Y. enterocolitica* in

murine bone marrow-derived macrophages was not dependent on the catalytic activity of YopH (Green *et al.*, 1995). These results suggest a redundancy in Yop function in the inhibition of zymosan-induced oxidative burst. The proteins that YopH dephosphorylates to inhibit induction of the Fc-mediated respiratory burst have not been identified.

YopH has recently been implicated in inhibiting the adaptive immune response. The phosphatase activity of YopH was shown to be essential for suppressing both T-cell cytokine production and expression of the B-cell co-stimulatory receptor B7.2 (Yao *et al.*, 1999). This downregulation of T-cell and B-cell signalling was most probably caused by dephosphorylation of tyrosine residues on proteins in both the T-cell receptor and the B-cell receptor complexes (Yao *et al.*, 1999). These data suggest that *Yersinia* may function to inhibit both innate and adaptive immunity, although there is no evidence for inhibition of the adaptive immune response by *Yersinia in vivo*.

It should also be noted that other pathogenic bacteria produce a protein tyrosine phosphatase effector. *Salmonella* spp. produce the protein SptP, which contains a YopE-like domain (discussed below) fused to a YopH-like domain (Kaniga *et al.*, 1996). However, the substrates of the phosphatase domain of SptP have not been identified.

YopE

The 23 kDa YopE was one of the earliest identified Yop effectors (Forsberg and Wolf-Watz, 1988) and is an essential virulence determinant. Targeting YopE into both epithelial cells and macrophages leads to a cytotoxic response, in which cells round up and contract away from the extracellular matrix (Rosqvist *et al.*, 1990). Microinjection studies show that this effect results from disruption of the actin microfilament network (Rosqvist *et al.*, 1991a). Mutation of YopE impairs the ability of *Yersinia* to resist phagocytosis (Rosqvist *et al.*, 1990; Ruckdeschel *et al.*, 1996), suggesting that YopE contributes to the antiphagocytic capability of *Yersinia*.

YopE contains a carboxyl-terminal effector domain (Sory *et al.*, 1995; Schesser *et al.*, 1996) that shares a high degree of similarity to the amino-terminal domain of exoenzyme S (ExoS) of *Pseudomonas aeruginosa* and SptP from *Salmonella typhimurium* (Fig. 2B). Fu and Galan (1999) noted that this domain harbours a conserved 'arginine finger' used by mammalian Rho GTPase-activating proteins (GAP) for catalysis (Fig. 2B) and also demonstrated that the amino-terminal half of SptP has GAP activity towards Rac and Cdc42 *in vitro*. Recently, YopE and ExoS were also demonstrated to possess GAP activity towards Rho family GTPases (RhoA, Rac and Cdc42) (Fig. 1) (Goehring *et al.*, 1999; Black and Bliska, 2000; Von Pawel-Rammingen *et al.*, 2000). Activation of Rho GTP-binding proteins is a pivotal signalling event connecting receptor activation to cytoskeletal reorganization during phagocytosis in professional and non-professional phagocytic cells (Chimini and Chavrier, 2000; May and Machesky, 2001). The

mechanism of inhibition of phagocytosis by YopE results from its Rho GAP activity, as substitution of the essential arginine with alanine destroys the ability of YopE to interfere with phagocytosis in cultured HeLa cells (Black and Bliska, 2000). In addition, *Yersinia* strains harbouring only the mutant version (R144A) of YopE are avirulent in a mouse infection model (Black and Bliska, 2000). The crystal structures of the GAP domain of ExoS and SptP (Stebbins and Galan, 2000; Wurtele *et al.*, 2001) reveal that this effector domain mimics the mammalian Rho GAP domain by providing a similar interface to contact Rho GTPases and positioning the catalytic arginine in a position that allows for stabilization of the transitionstate intermediate. This structure bears no similarity to the structure of the Rho GAPs (Stebbins and Galan, 2000), suggesting that the bacterial and mammalian GAP activities arose independently.

Unlike SptP, YopE exhibits no preference towards RhoA, Rac and Cdc42 as its substrate *in vitro* (Black and Bliska, 2000). It will be interesting to unravel which Rho GTPase is preferred under physiological conditions. RhoA and Rac are the two most probable candidates documented in the literature. RhoA is the small GTPase switch controlling stress fibre formation in fibroblasts as well as in macrophages (Hall, 1998). The morphology of actin stress fibre disruption induced by YopE implies that RhoA might be the target of YopE *in vivo* (Rosqvist *et al.*, 1991b). This is substantiated by the observation that overexpression of RhoAV14, an activated form of RhoA, prevents the cytotoxic phenotype induced by YopE in cultured HeLa cells (Black and Bliska, 2000). In addition, overexpression of molecules involved in the yeast Rho1 pathway, the RhoA homologue in yeast, is able to counteract the growth inhibition phenotype in yeast caused by YopE (Von Pawel-Rammingen *et al.*, 2000). However, this hypothesis was challenged by recent morphology studies in cultured endothelial cells (Andor *et al.*, 2001). Membrane ruffling triggered by Cdc42-dependent Rac activation is abrogated by YopE translocation, suggesting that YopE selectively targets Rac (Andor *et al.*, 2001). This result is consistent with the observation that overexpression of Rac1V12, an active form of Rac1, interferes with the antiphagocytic activity of YopE in cultured HeLa cells (Black and Bliska, 2000). Taken together, one can speculate that YopE might target both RhoA and Rac for different purposes. More information about the substrate of YopE *in vivo* awaits further analysis, including the high-resolution crystal structure of YopE and the precise cellular localization of YopE.

Recent studies have demonstrated that selective activation of RhoA, Rac and Cdc42 is required in different phagocytosis processes (Chimini and Chavrier, 2000; May and Machesky, 2001), suggesting that the action of

YopE would allow *Yersinia* to inhibit these multiple processes. Indeed, *Y. pseudotuberculosis* and *Y. enterocolitica* are able to inhibit β 1 integrin-mediated uptake in cultured epithelial cells (Black and Bliska, 2000). *Y. pseudotuberculosis* is capable of inhibiting Fc receptor-mediated phagocytosis in professional phagocytic cells (Fallman *et al.*, 1995). Given that *Yersinia*-infected HeLa cells lose the ability to phagocytose not only *Yersinia* itself, but also other microorganisms (Meccas *et al.*, 1998), *Yersinia* might also antagonize complement receptor (β 2 integrin)-mediated phagocytosis. Studies on the mechanism used by different phagocytosis pathways and identification of physiological targets of YopE will aid in the understanding of not only which GTPases are involved in distinct phagocytosis events, but also how YopE acts to inhibit these processes.

As stated earlier, *Yersinia* encode for a type III secretion apparatus that is essential for the translocation of the Yops into the host cell (Cornelis, 1998). The structure of this apparatus from *Shigella* and *Salmonella* resembles a needle, which probably functions to allow transport across the inner and outer membranes of the bacteria and into the cytoplasm of the host cell (Blocker *et al.*, 1999; Kubori *et al.*, 2000; Tamano *et al.*, 2000). One model suggests that the Yops function in part to prevent a pore from forming in the host cell plasma membrane resulting from insertion of the apparatus. Indeed, mutant strains of *Yersinia* defective for multiple Yop effectors contain a higher activity of pore formation caused by the type III secretion apparatus than *Yersinia* wild-type strains (Hakansson *et al.*, 1996a; Neyt and Cornelis, 1999; Tardy *et al.*, 1999), suggesting that one or more of the Yop effectors prevents pore formation and lysis of the host cell. The GAP activity of YopE is essential in the prevention of pore formation (Viboud and Bliska, 2001), suggesting that pore formation requires activated Rho GTPases and actin polymerization. These data also suggest that the prevention of pore formation by YopE is not simply the occupation of YopE within the pore, but rather involves the downregulation of the Rho GTPases within the host cell.

YopJ

The 32.5 kDa YopJ/YopP was first identified in *Y. pestis* (YopJ) (Straley and Bowmer, 1986) and later in *Y. pseudotuberculosis* and *Y. enterocolitica* (YopP) (Cornelis *et al.*, 1987). YopJ was first shown to be dispensable for the virulence of *Y. pseudotuberculosis* (Galyov *et al.*, 1994). However, it was later demonstrated that the apoptosis induced by YopJ aids in the establishment of a systemic infection *in vivo* (Monack *et al.*, 1998). So far, YopJ is the only Yop effector that has been shown to have an

anti-inflammatory role and to be responsible for the induction of apoptosis in macrophages.

After the transient induction of multiple signalling pathways (MAPK and NF- κ B pathway) by lipopolysaccharide (LPS), *Yersinia* infection results in a severe inhibition of multiple MAPK signalling pathways (Erk, JNK and p38) in macrophages (Ruckdeschel *et al.*, 1997a). Translocation of YopJ through the type III secretion machinery is required for this inhibition (Boland and Cornelis, 1998; Palmer *et al.*, 1998). In addition, YopJ alone is sufficient to cause downregulation of multiple MAPK kinases (Fig. 1) (Palmer *et al.*, 1999). YopJ can also block NF- κ B activation through the inhibition of I κ B degradation and subsequent NF- κ B translocation in both macrophages and epithelial cells (Ruckdeschel *et al.*, 1998; 2001; Schesser *et al.*, 1998). It was difficult to understand how one protein could simultaneously block a multiplicity of signalling pathways that have no apparent common components. Orth *et al.* (1999) made the key observation that YopJ can bind to multiple members of the MAPK kinase superfamily, including MKKs and IKK β , and suppress their activation. The interaction between YopJ and IKK β was also confirmed by co-immunoprecipitation from macrophages (Denecker *et al.*, 2001; Ruckdeschel *et al.*, 2001).

Yersinia infection causes macrophage apoptosis (Ruckdeschel *et al.*, 1997b) as well as the inhibition of cytokine production and an inflammatory response (Nakajima and Brubaker, 1993; Beuscher *et al.*, 1995; Schulte *et al.*, 1996). These processes allow the pathogen to survive and multiply extracellularly in the host lymphoid tissue. Studies on the function of YopJ have offered insights into the mechanism underlying the inhibition of cytokine production. YopJ was shown to be necessary for the inhibition of TNF α and IL-8 production (Boland and Cornelis, 1998; Palmer *et al.*, 1998; Schesser *et al.*, 1998). Inactivation of multiple MAPK pathways and the NF- κ B pathway parallels the decrease in TNF α production (Ruckdeschel *et al.*, 1997a). Considering that the TNF α and IL-8 promoters harbour NF- κ B and AP-1 binding sites (Roebuck, 1999; Liu *et al.*, 2000), and MAPKs and NF- κ B signals converge to the transcription factors AP-1 and NF- κ B, inhibition of cytokine production is probably the result of disruption of MAPK and NF- κ B activation.

YopJ is the only Yop effector required for the induction of apoptosis in murine macrophages (Mills *et al.*, 1997; Monack *et al.*, 1997). However, induction of apoptosis seems to be independent of the role of YopJ in perturbing signalling pathways, as YopJ does not induce apoptosis in fibroblasts even though it disrupts the same signalling pathways within fibroblasts. Recent studies suggest that the apoptotic signalling upstream of Bid

cleavage and cytochrome *c* release, presumably through caspase 8 activation, is responsible for the YopJ-induced apoptosis in macrophages (Denecker *et al.*, 2001). Macrophage apoptosis caused by YopJ most probably results from inactivation of NF- κ B, as inhibition of NF- κ B leads the macrophage to undergo apoptosis (Pagliari *et al.*, 2000).

Recent reports from Orth *et al.* (2000) revealed that YopJ may function as a cysteine protease. The observation arose from the finding that the predicted secondary structure of YopJ was similar to the observed secondary structure determined from the X-ray structure of the adenovirus protease. Furthermore, critical catalytic residues of protease activity were conserved in both YopJ and the adenovirus protease (Fig. 2C). Mutation of the predicted catalytic residues required for cysteine protease activity abolished the ability of YopJ to disrupt MAPK signalling pathways (Orth *et al.*, 2000). This hypothesis was confirmed further by the inability of mutants in the predicted catalytic residues of AvrBst, a predicted YopJ homologue in the plant pathogen *Xanthomonas campestris*, to induce a plant hypersensitive response (Orth *et al.*, 2000). These authors also suggest that YopJ might be a ubiquitin-like protein protease based on the sequence similarity between YopJ and Ulp1 (Fig. 2C), a yeast ubiquitin-like protein protease (Li and Hochstrasser, 1999). These data, taken together with the initial observations that YopJ can block MAPK activation via phosphorylation (Orth *et al.*, 1999), suggest that ubiquitin-like protein modification plays an unidentified regulatory role in MAPK signalling. A growing family of ubiquitin-like proteins has recently been shown to be involved in modulating eukaryotic signalling pathways (reviewed by Yeh *et al.*, 2000). The suggestion that YopJ inhibits signalling by the removal of ubiquitin-like proteins casts new light on deciphering the mechanism of *Yersinia* pathogenesis as well as eukaryotic signalling pathways. Importantly, however, demonstration of *in vitro* protease activity by YopJ and identification of the substrate(s) of YopJ are needed.

YopJ shows similarity to other type III secreted effectors including AvrA from *Salmonella*, AvrRxv, AvrBst, XopJ and AvrXv4 from *X. campestris*, AvrPpiG1 and ORF5 from *Pseudomonas syringae*, ORFB from *Erwinia amylovora* and y4lo from *Rhizobium*. Thus, YopJ may function by a common catalytic mechanism across both animal and plant kingdoms (Orth *et al.*, 2000). This suggests that the signalling pathways disrupted by YopJ family effectors are conserved in both plants and animals. Indeed, both YopJ and AvrBst can induce apoptosis in macrophages and plants respectively (Mills *et al.*, 1997; Monack *et al.*, 1997; Orth *et al.*, 2000). However, it has been demonstrated that AvrA from *Salmonella* does not inhibit the same pathways as YopJ (Schesser *et al.*, 2000), suggesting that some of

the YopJ homologues may function differently mechanistically. However, the similarity among the family does suggest a common catalytic activity.

YpkA

The *Yersinia* protein kinase YpkA was first identified as a secreted protein that is capable of autophosphorylation on serine residues (Galyov *et al.*, 1993). YpkA contains a domain bearing similarity to the eukaryotic serine/threonine protein kinases (Fig. 2D) (Galyov *et al.*, 1993). This study also demonstrated that YpkA is an essential virulence determinant for *Yersinia* infection, as disruption of the YpkA gene in *Yersinia* results in an avirulent strain of bacteria in an animal model. This was the first identification of a bacterial protein kinase playing a direct role in virulence.

Once inside the host cell, YpkA causes a change in morphology distinct from those mediated by YopE, in that the cells containing YpkA round up but do not detach from the extracellular matrix (Hakansson *et al.*, 1996b). Furthermore, YpkA is translocated to the plasma membrane (Fig. 1) (Hakansson *et al.*, 1996b), suggesting that the substrates for YpkA may be localized at the plasma membrane as well. Interestingly, YpkA produced by bacteria exhibits no detectable kinase activity unless a eukaryotic kinase activator is present. Purification of this eukaryotic activator led to its identification as actin (Juris *et al.*, 2000). The C-terminus of YpkA, which is predicted to form an amphipathic helix, is required for YpkA kinase activity and for the interaction between YpkA and actin (Juris *et al.*, 2000). Interaction between YpkA and actin suggested that the morphological changes induced by YpkA might be caused by changes in the actin cytoskeleton. Indeed, YpkA induces disruption of actin stress fibres in HeLa cells in transient transfection experiments (Fig. 1) (Juris *et al.*, 2000). Disruption of the actin cytoskeleton by YpkA might be mediated by its ability to interact with the small GTPase RhoA, which plays an important role in actin dynamics within the cell (Barz *et al.*, 2000; Dukuzumuremyi *et al.*, 2000). Although actin can be phosphorylated *in vitro* (Juris *et al.*, 2000), the *in vivo* substrate is not clear. Phosphorylation of actin by YpkA is not likely to be physiologically significant because of the abundance of actin in a cell. YpkA most probably functions by phosphorylating proteins that are important in the regulation of the actin cytoskeleton within the host cell. The substrates of YpkA probably have an important role in RhoA signalling. Through its ability to disrupt the actin cytoskeleton, YpkA may have an effect on the ability of a macrophage to phagocytose *Yersinia* and to move to areas of infection.

It is intriguing that several pathogenic species of bacteria encode proteins with domains that resemble

eukaryotic-like serine/threonine protein kinase domains (Leonard *et al.*, 1998; Avenue-Gay and Everett, 2000). These proteins control development and stress responses within the bacteria. Only YpkA and a protein kinase from *Pseudomonas aeruginosa* have been shown to be essential virulence determinants (Galyov *et al.*, 1993; Wang *et al.*, 1998). *Yersinia*, and other pathogenic bacteria may use these eukaryotic-like protein kinases to tip the balance of signalling cascades within their hosts by phosphorylating endogenous substrates that regulate immune responses to levels that completely shift the state of those signalling cascades in favour of bacterial survival.

YopM

The *Yersinia* protein YopM is a 41 kDa protein that is a necessary virulence determinant of *Y. pestis* (Leung *et al.*, 1990). YopM is composed almost entirely of sequences resembling leucine-rich repeats (LRRs). The LRR domain is found in a diverse number of proteins, including the RNase inhibitor, the Toll receptors, proteoglycans and platelet glycoproteins (for a review, see Kobe and Deisenhofer, 1994). This domain is believed to be a protein–protein interaction motif functional in a multitude of signalling pathways both within the cell and in the extracellular milieu.

The diversity of signalling pathways governed by LRR-containing proteins has made it difficult to ascertain the exact pathways that YopM may affect. Initial characterization of YopM demonstrated its ability to interact with thrombin and inhibit thrombin-induced platelet aggregation (Reisner and Straley, 1992), suggesting that YopM may actually function in the extracellular environment of the host. However, YopM has also been shown to be translocated into macrophages via the type III secretion apparatus (Boland *et al.*, 1996), making the exact location of YopM during *Yersinia* infection controversial. Recent studies have shown that YopM is not only translocated into HeLa cells during *Yersinia* infection, but also localizes to the nucleus of HeLa cells, trafficking via a vesicle-associated pathway (Skrzypek *et al.*, 1998).

Other LRR-containing proteins have been identified in both pathogenic and symbiotic bacteria. These include the IpaH family from *Shigella flexneri*, SspH 1, SspH 2 and Slrp from *Salmonella typhimurium*, y4fr from *Rhizobium* and ID431 from *Bradyrhizobium* (Fig. 2E). The functions of all these LRR-containing proteins are unknown. These proteins may have been acquired by both pathogenic and symbiotic bacteria to thwart similar pathways within the host. The structure of YopM reveals a parallel β -sheet on its concave face, which is typical of the solved structures of other LRR-containing proteins

(Evdokimov *et al.*, 2001). However, in other LRR-containing proteins, such as the RNase inhibitor (Kobe and Deisenhofer, 1993) and internalin B (Marino *et al.*, 1999), the convex surface is typically helical in structure, whereas on YopM, this face contains an extended structure (Evdokimov *et al.*, 2001). This may be caused by the short length of the LRR of YopM. Furthermore, the YopM structure suggests that it forms a tetramer, which creates a channel with a pore of 35 Å (Evdokimov *et al.*, 2001).

YopT

The function of YopT is largely unknown; however, translocation of YopT into host cells results in cell rounding and disruption of cytoskeleton structure, a cytotoxic response resembling that of YopE (Iriarte and Cornelis, 1998; Zumbihl *et al.*, 1999; Boyd *et al.*, 2000). YopT is secreted in lower amounts than YopE, although both effectors appear to function by altering the host cytoskeleton structures (Iriarte and Cornelis, 1998). Why does *Yersinia* have two cytotoxins (YopT and YopE) to abrogate cytoskeleton structures? The answer to this question is unknown, although there are several possibilities. Unlike YopE mutants, YopT mutants colonize intestinal Peyer's patches as in wild type (Iriarte and Cornelis, 1998), suggesting that YopT and YopE may have different roles in altering cytoskeleton structure during the course of a *Yersinia* infection. Similarly, in *in vitro* experiments, YopE mutant strains of *Y. pseudotuberculosis* exhibit a significant decrease in cytotoxicity (Rosqvist *et al.*, 1991b), whereas YopT mutant strains of *Y. enterocolitica* are as cytotoxic as the wild-type strains (Hartland *et al.*, 1994; Iriarte and Cornelis, 1998). Thus, YopT and YopE may affect different signalling pathways to synergize the cytotoxic effect and secure the survival of the pathogen in lymphoid tissues. Recently, Zumbihl *et al.* (1999) demonstrated that infection of a YopT strain causes an unidentified modification of RhoA (Fig. 1). The GTPase shows an acidic shift in its pI after infection with YopT. Modification of RhoA by YopT may inhibit its ability to alter the cytoskeleton during phagocytosis, allowing for the survival of *Yersinia*.

Sequence comparisons show that YopT is similar to the C-terminus of surface antigen p76 from *Haemophilus somnus* and a small portion of the filamentous haemagglutinin-like protein from *Haemophilus ducreyi* called Lsp1 and Lsp2 as well as PfhB1 and PfhB2 from *Pasteurella multocida* (Fig. 2F). The function of these proteins is unknown. The several conserved residues among these proteins suggest a similar catalytic activity. However, none of the proteins similar to YopT has been shown to modify RhoA. Further studies on the function of the YopT-like domain in these proteins will also provide additional insights into YopT pathogenesis.

Concluding remarks

The *Yersinia* species of bacteria contain six characterized Yop effectors to disrupt vital signalling cascades that are required for innate immunity. Studies on the functions of these effectors have revealed the signalling pathways they target and have also shed light on novel signalling pathways used by the host to induce an immune response. *Yersinia* has probably acquired some (or all) of its effectors via horizontal gene transfer between bacteria and fine tuned these effectors to regulate signalling pathways within its host. Those signalling pathways targeted by the effectors play crucial roles in the elimination of pathogens. Identification of the functions of new effectors, as well as the targets or substrates of these effectors, will aid in the understanding of bacterial pathogenesis as well as innate immunity.

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