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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 (TNFα), 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-κB, the cyclic AMP-responsive element-binding protein (CREB) and AP-1. The NF-κB transcription factor is held in the cytoplasm through interaction with the protein IκB (Karin, 1999; Mercurio and Manning, 1999). Translocation of NF-κB to the nucleus requires the destruction of bound IκB, which occurs through phosphorylation of IκB by the IκB kinase complex (IKK) and subsequent ubiquitination of IκB (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 β1 integrins on the M-cell plasma membrane and the invasin 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 invasin-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-

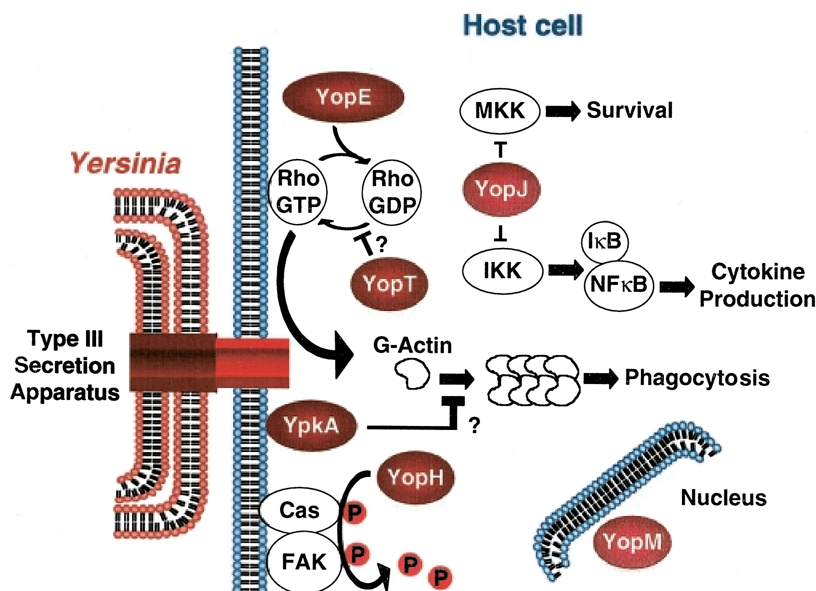

Fig. 1. The *Yersinia* effectors target multiple signalling pathways. The Yop effectors of *Yersinia* are translocated into the host cell via a type III secretion system. Once inside the host cell, each of the effectors targets signalling pathways to ensure survival of the bacteria within its host. YopH, 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-κB 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.

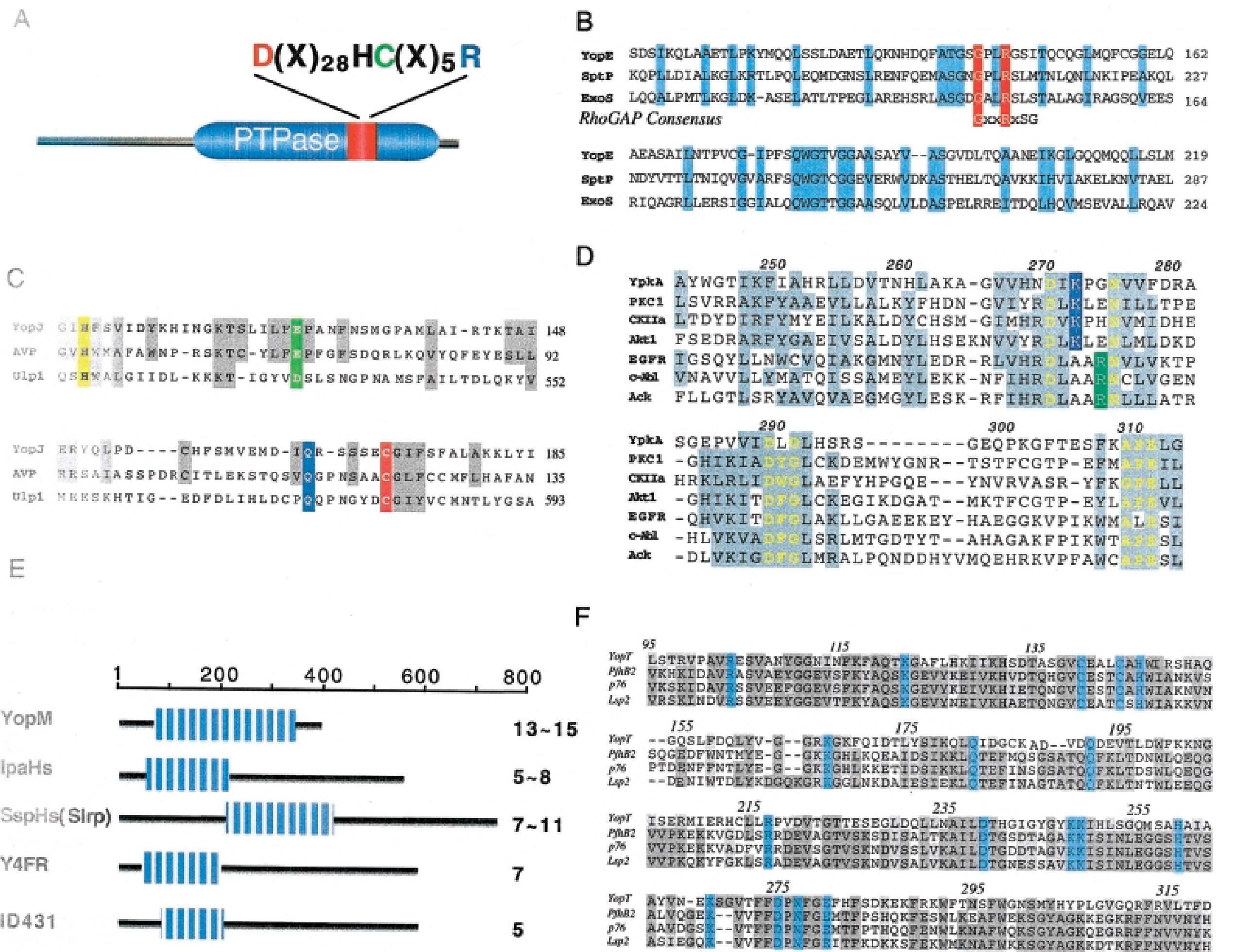

Fig. 2. Sequence alignments and structural diagrams of the Yop effectors and their homologues.

A. YopH harbours a protein tyrosine phosphatase (PTPase) domain at the carboxy-terminus, which is represented by the presence of the catalytic $D(X)_{28}HC(X)_5R$ 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 VII 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 Mg^{2+} 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 β1 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 (Mecsas *et al*., 1998), *Yersinia* might also antagonize complement receptor (β2 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-κB 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-κB activation through the inhibition of IκB degradation and subsequent NF-κB 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 IKKβ, and suppress their activation. The interaction between YopJ and IKKβ 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 TNFα and IL-8 production (Boland and Cornelis, 1998; Palmer *et al*., 1998; Schesser *et al*., 1998). Inactivation of multiple MAPK pathways and the NF-κB pathway parallels the decrease in TNFα production (Ruckdeschel *et al*., 1997a). Considering that the TNFα and IL-8 promoters harbour NF-κB and AP-1 binding sites (Roebuck, 1999; Liu *et al*., 2000), and MAPKs and NF-κB signals converge to the transcription factors AP-1 and NF-κB, inhibition of cytokine production is probably the result of disruption of MAPK and NF-κB 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

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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-κB, as inhibition of NF-κB 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*, AvrPpiG1and 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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References

Allen, L.A., and Aderem, A. (1996) Molecular definition of distinct cytoskeletal structures involved in complement- and Fc receptor-mediated phagocytosis in macrophages. *J Exp Med* **184:** 627–637.

Andor, A., Trulzsch, K., Essler, M., Roggenkamp, A., Wiedemann, A., Heesemann, J., and Aepfelbacher, M. (2001) YopE of *Yersinia*, a GAP for Rho GTPases, selectively modulates Rac-dependent actin structures in endothelial cells. *Cell Microbiol* **3:** 301–310.

Aspenstrom, P. (1999a) The Rho GTPases have multiple effects on the actin cytoskeleton. *Exp Cell Res* **246:** 20–25.

Aspenstrom, P. (1999b) Effectors for the Rho GTPases. *Curr Opin Cell Biol* **11:** 95–102.

Autenrieth, I.B., and Firsching, R. (1996) Penetration of M cells and destruction of Peyer's patches by *Yersinia enterocolitica*: an ultrastructural and histological study. *J Med Microbiol* **44:** 285–294.

Av-Gay, Y., and Everett, M. (2000) The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. *Trends Microbiol* **8:** 238–244.

Barz, C., Abahji, T.N., Trulzsch, K., and Heesemann, J. (2000) The *Yersinia* Ser/Thr protein kinase YpkA/YopO directly interacts with the small GTPases RhoA and Rac-1. *FEBS Lett* **482:** 139–143.

Beuscher, H.U., Rodel, F., Forsberg, A., and Rollinghoff, M. (1995) Bacterial evasion of host immune defense: *Yersinia enterocolitica* encodes a suppressor for tumor necrosis factor alpha expression. *Infect Immun* **63:** 1270–1277.

Black, D.S., and Bliska, J.B. (1997) Identification of p130Cas as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J* **16:** 2730–2744.

Black, D.S., and Bliska, J.B. (2000) The RhoGAP activity of the *Yersinia pseudotuberculosis* cytotoxin YopE is required for antiphagocytic function and virulence. *Mol Microbiol* **37:** 515–527.

Black, D.S., Montagna, L.G., Zitsmann, S., and Bliska, J.B. (1998) Identification of an amino-terminal substrate-binding domain in the *Yersinia* tyrosine phosphatase that is required for efficient recognition of focal adhesion targets. *Mol Microbiol* **29:** 1263–1274.

Bliska, J.B., and Black, D.S. (1995) Inhibition of the Fc receptor-mediated oxidative burst in macrophages by the *Yersinia pseudotuberculosis* tyrosine phosphatase. *Infect Immun* **63:** 681–685.

Bliska, J.B., Guan, K.L., Dixon, J.E., and Falkow, S. (1991) Tyrosine phosphate hydrolysis of host proteins by an essential *Yersinia* virulence determinant. *Proc Natl Acad Sci USA* **88:** 1187–1191.

Blocker, A., Gounon, P., Larquet, E., Niebuhr, K., Cabiaux, V., Parsot, C., and Sansonetti, P. (1999) The tripartite type III secretion of *Shigella flexneri* inserts IpaB and IpaC into host membranes. *J Cell Biol* **147:** 683–693.

Boland, A., and Cornelis, G.R. (1998) Role of YopP in suppression of tumor necrosis factor alpha release by macrophages during *Yersinia* infection. *Infect Immun* **66:** 1878–1884.

Boland, A., Sory, M.P., Iriarte, M., Kerbourch, C., Wattiau, P., and Cornelis, G.R. (1996) Status of YopM and YopN in the *Yersinia* Yop virulon: YopM of *Y. enterocolitica* is internalized inside the cytosol of PU5-1.8 macrophages by the YopB, D, N delivery apparatus. *EMBO J* **15:** 5191–5201.

Bolin, I., and Wolf-Watz, H. (1988) The plasmid-encoded Yop2b protein of *Yersinia pseudotuberculosis* is a virulence determinant regulated by calcium and temperature at the level of transcription. *Mol Microbiol* **2:** 237–245.

Boyd, A.P., Grosdent, N., Totemeyer, S., Geuijen, C., Bleves, S., Iriarte, M., *et al*. (2000) *Yersinia enterocolitica* can deliver Yop proteins into a wide range of cell types: development of a delivery system for heterologous proteins. *Eur J Cell Biol* **79:** 659–671.

Chimini, G., and Chavrier, P. (2000) Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. *Nature Cell Biol* **2:** E191–E196.

Clark, M.A., Hirst, B.H., and Jepson, M.A. (1998) M-cell surface beta1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. *Infect Immun* **66:** 1237–1243.

Cornelis, G.R. (1998) The *Yersinia* Yop virulon, a bacterial system to subvert cells of the primary host defense. *Folia Microbiol (Praha)* **43:** 253–261.

Cornelis, G.R., Vanooteghem, J.C., and Sluiters, C. (1987) Transcription of the *yop* regulon from *Y. enterocolitica* requires trans acting pYV and chromosomal genes. *Microb Pathog* **2:** 367–379.

Denecker, G., Declercq, W., Geuijen, C.A., Boland, A., Benabdillah, R., van Gurp, M., *et al*. (2001) *Yersinia enterocolitica* YopP-induced apoptosis of macrophages involves the apoptotic signaling cascade upstream of bid. *J Biol Chem* **276:** 19706–19714.

Dukuzumuremyi, J.M., Rosqvist, R., Hallberg, B., Akerstrom, B., Wolf-Watz, H., and Schesser, K. (2000) The *Yersinia* protein kinase A is a host factor inducible RhoA/Rac-binding virulence factor. *J Biol Chem* **275:** 35281–35290.

Evdokimov, A.G., Anderson, D.E., Routzahn, K.M., and Waugh,

D.S. (2001) Unusual molecular architecture of the *Yersinia pestis* cytotoxin YopM: a leucine-rich repeat protein with the shortest repeating unit. *J Mol Biol* **312:** 807–821.

Fallman, M., Andersson, K., Hakansson, S., Magnusson, K.E., Stendahl, O., and Wolf-Watz, H. (1995) *Yersinia pseudotuberculosis* inhibits Fc receptor-mediated phagocytosis in J774 cells. *Infect Immun* **63:** 3117–3124.

Forsberg, A., and Wolf-Watz, H. (1988) The virulence protein Yop5 of *Yersinia pseudotuberculosis* is regulated at transcriptional level by plasmid-plB1-encoded *trans*-acting elements controlled by temperature and calcium. *Mol Microbiol* **2:** 121–133.

Fu, Y., and Galan, J.E. (1999) A *salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion (see comments). *Nature* **401:** 293–297.

Galyov, E.E., Hakansson, S., Forsberg, A., and Wolf-Watz, H. (1993) A secreted protein kinase of *Yersinia pseudotuberculosis* is an indispensable virulence determinant. *Nature* **361:** 730–732.

Galyov, E.E., Hakansson, S., and Wolf-Watz, H. (1994) Characterization of the operon encoding the YpkA Ser/Thr protein kinase and the YopJ protein of *Yersinia pseudotuberculosis*. *J Bacteriol* **176:** 4543–4548.

Goehring, U.M., Schmidt, G., Pederson, K.J., Aktories, K., and Barbieri, J.T. (1999) The N-terminal domain of *Pseudomonas aeruginosa* exoenzyme S is a GTPase-activating protein for Rho GTPases. *J Biol Chem* **274:** 36369–36372.

Green, S.P., Hartland, E.L., Robins-Browne, R.M., and Phillips, W.A. (1995) Role of YopH in the suppression of tyrosine phosphorylation and respiratory burst activity in murine macrophages infected with *Yersinia enterocolitica*. *J Leukoc Biol* **57:** 972–977.

Greenberg, S., Burridge, K., and Silverstein, S.C. (1990) Colocalization of F-actin and talin during Fc receptor-mediated phagocytosis in mouse macrophages. *J Exp Med* **172:** 1853–1856.

Greenberg, S., Chang, P., and Silverstein, S.C. (1993) Tyrosine phosphorylation is required for Fc receptor-mediated phagocytosis in mouse macrophages. *J Exp Med* **177:** 529–534.

Guan, K.L., and Dixon, J.E. (1990) Protein tyrosine phosphatase activity of an essential virulence determinant in *Yersinia*. *Science* **249:** 553–556.

Hakansson, S., Schesser, K., Persson, C., Galyov, E.E., Rosqvist, R., Homble, F., and Wolf-Watz, H. (1996a) The YopB protein of *Yersinia pseudotuberculosis* is essential for the translocation of Yop effector proteins across the target cell plasma membrane and displays a contact-dependent membrane disrupting activity. *EMBO J* **15:** 5812–5823.

Hakansson, S., Galyov, E.E., Rosqvist, R., and Wolf-Watz, H. (1996b) The *Yersinia* YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane. *Mol Microbiol* **20:** 593–603.

Hall, A. (1998) Rho GTPases and the actin cytoskeleton. *Science* **279:** 509–514.

Hamid, N., Gustavsson, A., Andersson, K., McGee, K., Persson, C., Rudd, C.E., and Fallman, M. (1999) YopH dephosphorylates Cas and Fyn-binding protein in macrophages. *Microb Pathog* **27:** 231–242.

Hartland, E.L., Green, S.P., Phillips, W.A., and Robins-Browne, R.M. (1994) Essential role of YopD in inhibition of the respiratory burst of macrophages by *Yersinia enterocolitica*. *Infect Immun* **62:** 4445–4453.

Iriarte, M., and Cornelis, G.R. (1998) YopT, a new *Yersinia* Yop effector protein, affects the cytoskeleton of host cells. *Mol Microbiol* **29:** 915–929.

Juris, S.J., Rudolph, A.E., Huddler, D., Orth, K., and Dixon, J.E. (2000) A distinctive role for the *Yersinia* protein kinase: actin binding, kinase activation, and cytoskeleton disruption. *Proc Natl Acad Sci USA* **97:** 9431–9436.

Kaniga, K., Uralil, J., Bliska, J.B., and Galan, J.E. (1996) A secreted protein tyrosine phosphatase with modular effector domains in the bacterial pathogen *Salmonella typhimurium*. *Mol Microbiol* **21:** 633–641.

Karin, M. (1999) The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation. *J Biol Chem* **274:** 27339–27342.

Kobe, B., and Deisenhofer, J. (1993) Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. *Nature* **366:** 751–756.

Kobe, B., and Deisenhofer, J. (1994) The leucine-rich repeat: a versatile binding motif. *Trends Biochem Sci* **19:** 415–421.

Kubori, T., Sukhan, A., Aizawa, S.I., and Galan, J.E. (2000) Molecular characterization and assembly of the needle complex of the *Salmonella typhimurium* type III protein secretion system. *Proc Natl Acad Sci USA* **97:** 10225–10230.

Leonard, C.J., Aravind, L., and Koonin, E.V. (1998) Novel families of putative protein kinases in bacteria and archaea: evolution of the 'eukaryotic' protein kinase superfamily. *Genome Res* **8:** 1038–1047.

Leung, K.Y., Reisner, B.S., and Straley, S.C. (1990) YopM inhibits platelet aggregation and is necessary for virulence of *Yersinia pestis* in mice. *Infect Immun* **58:** 3262–3271.

Li, S.J., and Hochstrasser, M. (1999) A new protease required for cell-cycle progression in yeast. *Nature* **398:** 246–251.

Liu, H., Sidiropoulos, P., Song, G., Pagliari, L.J., Birrer, M.J., Stein, B., *et al*. (2000) TNF-alpha gene expression in macrophages: regulation by NF-kappa B is independent of c-Jun or C/EBP beta. *J Immunol* **164:** 4277–4285.

Marino, M., Braun, L., Cossart, P., and Ghosh, P. (1999) Structure of the lnlB leucine-rich repeats, a domain that triggers host cell invasion by the bacterial pathogen *L. monocytogenes. Mol Cell* **4:** 1063–1072.

Marra, A., and Isberg, R.R. (1997) Invasin-dependent and invasin-independent pathways for translocation of *Yersinia pseudotuberculosis* across the Peyer's patch intestinal epithelium. *Infect Immun* **65:** 3412–3421.

May, R.C., and Machesky, L.M. (2001) Phagocytosis and the actin cytoskeleton. *J Cell Sci* **114:** 1061–1077.

Mecsas, J., Raupach, B., and Falkow, S. (1998) The *Yersinia* Yops inhibit invasion of *Listeria*, *Shigella* and *Edwardsiella* but not *Salmonella* into epithelial cells. *Mol Microbiol* **28:** 1269–1281.

Medzhitov, R., and Janeway, C., Jr (2000) Innate immunity. *N Engl J Med* **343:** 338–344.

Mercurio, F., and Manning, A.M. (1999) Multiple signals converging on NF-kappaB. *Curr Opin Cell Biol* **11:** 226–232.

Mills, S.D., Boland, A., Sory, M.P., van der Smissen, P., Kerbourch, C., Finlay, B.B., and Cornelis, G.R. (1997) *Yersinia enterocolitica* induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. *Proc Natl Acad Sci USA* **94:** 12638–12643.

Monack, D.M., Mecsas, J., Ghori, N., and Falkow, S. (1997) *Yersinia* signals macrophages to undergo apoptosis and YopJ is necessary for this cell death. *Proc Natl Acad Sci USA* **94:** 10385–10390.

Monack, D.M., Mecsas, J., Bouley, D., and Falkow, S. (1998) *Yersinia*-induced apoptosis *in vivo* aids in the establishment of a systemic infection in mice. *J Exp Med* **188:** 2127–2137.

Nakajima, R., and Brubaker, R.R. (1993) Association between virulence of *Yersinia pestis* and suppression of gamma interferon and tumor necrosis factor alpha. *Infect Immun* **61:** 23–31.

Neyt, C., and Cornelis, G.R. (1999) Insertion of a Yop translocation pore into the macrophage plasma membrane by *Yersinia enterocolitica*: requirement for translocators YopB and YopD, but not LcrG. *Mol Microbiol* **33:** 971–981.

Orth, K., Palmer, L.E., Bao, Z.Q., Stewart, S., Rudolph, A.E., Bliska, J.B., and Dixon, J.E. (1999) Inhibition of the mitogen-activated protein kinase kinase superfamily by a *Yersinia* effector. *Science* **285:** 1920–1923.

Orth, K., Xu, Z., Mudgett, M.B., Bao, Z.Q., Palmer, L.E., Bliska, J.B., *et al*. (2000) Disruption of signaling by *Yersinia* effector YopJ, a ubiquitin-like protein protease (see comments). *Science* **290:** 1594–1597.

Pagliari, L.J., Perlman, H., Liu, H., and Pope, R.M. (2000) Macrophages require constitutive NF-kappaB activation to maintain A1 expression and mitochondrial homeostasis. *Mol Cell Biol* **20:** 8855–8865.

Palmer, L.E., Hobbie, S., Galan, J.E., and Bliska, J.B. (1998) YopJ of *Yersinia pseudotuberculosis* is required for the inhibition of macrophage TNF-alpha production and downregulation of the MAP kinases p38 and JNK. *Mol Microbiol* **27:** 953–965.

Palmer, L.E., Pancetti, A.R., Greenberg, S., and Bliska, J.B. (1999) YopJ of *Yersinia* spp. is sufficient to cause downregulation of multiple mitogen-activated protein kinases in eukaryotic cells. *Infect Immun* **67:** 708–716.

Parkhill, J., Wren, B.W., Thomson, N.R., Titball, R.W., Holden, M.T.G., Prentice, M.B., *et al*. (2001) Genome sequence of *Yersinia pestis*, the causative agent of plague. *Nature* **413:** 523–527.

Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.E., Karandikar, M., Berman, K., and Cobb, M.H. (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* **22:** 153–183.

Persson, C., Carballeira, N., Wolf-Watz, H., and Fallman, M. (1997) The PTPase YopH inhibits uptake of *Yersinia*, tyrosine phosphorylation of p130Cas and FAK, and the associated accumulation of these proteins in peripheral focal adhesions. *EMBO J* **16:** 2307–2318.

Portnoy, D.A., and Martinez, R.J. (1985) Role of a plasmid in the pathogenicity of Yersinia species. *Curr Topics Microbiol Immunol* **118:** 29–51.

Reisner, B.S., and Straley, S.C. (1992) *Yersinia pestis* YopM: thrombin binding and overexpression. *Infect Immun* **60:** 5242–5252.

Roebuck, K.A. (1999) Regulation of interleukin-8 gene expression. *J Interferon Cytokine Res* **19:** 429–438.

Rosqvist, R., Bolin, I., and Wolf-Watz, H. (1988) Inhibition of phagocytosis in *Yersinia pseudotuberculosis*: a virulence plasmid-encoded ability involving the Yop2b protein. *Infect Immun* **56:** 2139–2143.

Rosqvist, R., Forsberg, A., Rimpilainen, M., Bergman, T., and Wolf-Watz, H. (1990) The cytotoxic protein YopE of *Yersinia* obstructs the primary host defence. *Mol Microbiol* **4:** 657–667.

Rosqvist, R., Forsberg, A., and Wolf-Watz, H. (1991a) Microinjection of the *Yersinia* YopE cytotoxin in mammalian cells induces actin microfilament disruption. *Biochem Soc Trans* **19:** 1131–1132.

Rosqvist, R., Forsberg, A., and Wolf-Watz, H. (1991b) Intracellular targeting of the *Yersinia* YopE cytotoxin in mammalian cells induces actin microfilament disruption. *Infect Immun* **59:** 4562–4569.

Ruckdeschel, K., Roggenkamp, A., Schubert, S., and Heesemann, J. (1996) Differential contribution of *Yersinia enterocolitica* virulence factors to evasion of microbicidal action of neutrophils. *Infect Immun* **64:** 724–733.

Ruckdeschel, K., Machold, J., Roggenkamp, A., Schubert, S., Pierre, J., Zumbihl, R., *et al*. (1997a) *Yersinia enterocolitica* promotes deactivation of macrophage mitogen-activated protein kinases extracellular signal-regulated kinase-1/2, 38, and c-Jun NH2-terminal kinase. Correlation with its inhibitory effect on tumor necrosis factor-alpha production. *J Biol Chem* **272:** 15920–15927.

Ruckdeschel, K., Roggenkamp, A., Lafont, V., Mangeat, P., Heesemann, J., and Rouot, B. (1997b) Interaction of *Yersinia enterocolitica* with macrophages leads to macro-phage cell death through apoptosis. *Infect Immun* **65:** 4813–4821.

Ruckdeschel, K., Harb, S., Roggenkamp, A., Hornef, M., Zumbihl, R., Kohler, S., *et al*. (1998) *Yersinia enterocolitica* impairs activation of transcription factor NF-kappaB: involvement in the induction of programmed cell death and in the suppression of the macrophage tumor necrosis factor alpha production. *J Exp Med* **187:** 1069–1079.

Ruckdeschel, K., Mannel, O., Richter, K., Jacobi, C.A., Trulzsch, K., Rouot, B., and Heesemann, J. (2001) *Yersinia* outer protein P of *Yersinia enterocolitica* simultaneously blocks the nuclear factor-kappa B pathway and exploits lipopolysaccharide signaling to trigger apoptosis in macrophages. *J Immunol* **166:** 1823–1831.

Schesser, K., Frithz-Lindsten, E., and Wolf-Watz, H. (1996) Delineation and mutational analysis of the *Yersinia pseudotuberculosis* YopE domains which mediate translocation across bacterial and eukaryotic cellular membranes. *J Bacteriol* **178:** 7227–7233.

Schesser, K., Spiik, A.K., Dukuzumuremyi, J.M., Neurath, M.F., Pettersson, S., and Wolf-Watz, H. (1998) The yopJ locus is required for *Yersinia*-mediated inhibition of NF-kappaB activation and cytokine expression: YopJ contains a eukaryotic SH2-like domain that is essential for its repressive activity. *Mol Microbiol* **28:** 1067–1079.

Schesser, K., Dukuzumuremyi, J.M., Cilio, C., Borg, S., Wallis, T.S., Pettersson, S., and Galyov, E.E. (2000) The *Salmonella* YopJ-homologue AvrA does not possess YopJ-like activity. *Microb Pathog* **28:** 59–70.

Schoenwaelder, S.M., and Burridge, K. (1999) Bidirectional signaling between the cytoskeleton and integrins. *Curr Opin Cell Biol* **11:** 274–286.

Schulte, R., Wattiau, P., Hartland, E.L., Robins-Browne, R.M., and Cornelis, G.R. (1996) Differential secretion of interleukin-8 by human epithelial cell lines upon entry of virulent or non-virulent *Yersinia enterocolitica*. *Infect Immun* **64:** 2106–2113.

Skrzypek, E., Cowan, C., and Straley, S.C. (1998) Targeting of the *Yersinia pestis* YopM protein into HeLa cells and intracellular trafficking to the nucleus. *Mol Microbiol* **30:** 1051–1065.

Sory, M.P., Boland, A., Lambermont, I., and Cornelis, G.R. (1995) Identification of the YopE and YopH domains required for secretion and internalization into the cytosol of macrophages, using the cyaA gene fusion approach. *Proc Natl Acad Sci USA* **92:** 11998–12002.

Stebbins, C.E., and Galan, J.E. (2000) Modulation of host signaling by a bacterial mimic: structure of the *Salmonella* effector SptP bound to Rac1. *Mol Cell* **6:** 1449–1460.

Straley, S.C., and Bowmer, W.S. (1986) Virulence genes regu-

lated at the transcriptional level by Ca2+ in *Yersinia pestis* include structural genes for outer membrane proteins. *Infect Immun* **51:** 445–454.

Stuckey, J.A., Schubert, H.L., Fauman, E.B., Zhang, Z.Y., Dixon, J.E., and Saper, M.A. (1994) Crystal structure of *Yersinia* protein tyrosine phosphatase at 2.5 A and the complex with tungstate (see comments). *Nature* **370:** 571–575.

Tamano, K., Aizawa, S., Katayama, E., Nonaka, T., Imajoh-Ohmi, S., Kuwae, A., *et al*. (2000) Supramolecular structure of the *Shigella* type III secretion machinery: the needle part is changeable in length and essential for delivery of effectors. *EMBO J* **19:** 3876–3887.

Tardy, F., Homble, F., Neyt, C., Wattiez, R., Cornelis, G.R., Ruysschaert, J.M., and Cabiaux, V. (1999) *Yersinia enterocolitica* type III secretion-translocation system: channel formation by secreted Yops. *EMBOJ* **18:** 6793–6799.

Viboud, G.I., and Bliska, J.B. (2001) A bacterial type III secretion system inhibits actin polymerization to prevent pore formation in host cell membranes. *EMBO J* **20:** 5373–5382.

Von Pawel-Rammingen, U., Telepnev, M.V., Schmidt, G., Aktories, K., Wolf-Watz, H., and Rosqvist, R. (2000) GAP activity of the *Yersinia* YopE cytotoxin specifically targets the Rho pathway: a mechanism for disruption of actin microfilament structure. *Mol Microbiol* **36:** 737–748.

Wang, J., Li, C., Yang, H., Mushegian, A., and Jin, S. (1998) A novel serine/threonine protein kinase homologue of *Pseudomonas aeruginosa* is specifically inducible within the host infection site and is required for full virulence in neutropenic mice. *J Bacteriol* **180:** 6764–6768.

Wurtele, M., Wolf, E., Pederson, K.J., Buchwald, G., Ahmadian, M.R., Barbieri, J.T., and Wittinghofer, A. (2001) How the *Pseudomonas aeruginosa* ExoS toxin downregulates Rac. *Nature Struct Biol* **8:** 23–26.

Yamaguchi, M., Oishi, H., Araki, S., Saeki, S., Yamane, H., Okamura, N., and Ishibashi, S. (1995) Respiratory burst and tyrosine phosphorylation by vanadate. *Arch Biochem Biophys* **323:** 382–386.

Yao, T., Mecsas, J., Healy, J.I., Falkow, S., and Chien, Y. (1999) Suppression of T and B lymphocyte activation by a *Yersinia pseudotuberculosis* virulence factor, yopH. *J Exp Med* **190:** 1343–1350.

Yeh, E.T., Gong, L., and Kamitani, T. (2000) Ubiquitin-like proteins: new wines in new bottles. *Gene* **248:** 1–14.

Zumbihl, R., Aepfelbacher, M., Andor, A., Jacobi, C.A., Ruckdeschel, K., Rouot, B., and Heesemann, J. (1999) The cytotoxin YopT of *Yersinia enterocolitica* induces modification and cellular redistribution of the small GTP-binding protein RhoA. *J Biol Chem* **274:** 29289–29293.