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Modulation of Rho GTPases by type III secretion system translocated effectors of *Yersinia*

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Abstract Pathogenic species of the bacterial genus *Yersinia* subdue the immune system to proliferate and spread within the host organism. For this purpose yersiniae employ a type III secretion apparatus which governs injection of six effector proteins (*Yersinia* outer proteins; Yops) into host cells. Yops control various regulatory and signalling proteins in a unique and highly specific manner. YopE, YopT, and YpkA/YopO modulate the activity of Rho GTP-binding proteins, whereas YopH dephosphorylates phospho-tyrosine residues in focal adhesion proteins. Furthermore, YopP/YopJ and YopM affect cell survival/apoptosis and cell proliferation, respectively. In this review the focus will be on the biochemistry and cellular effects of YopT, YopE, YopO/YpkA, and YopH.

Introduction

There are three human pathogenic *Yersinia* species: (1) *Y. pestis* is the causative agent of plague, (2) *Y. pseudotuberculosis*, and (3) *Y. enterocolitica* cause acute or chronic gastroenteric infections. In contrast to other enteropathogenic bacteria such as salmonellae and shigellae, which can replicate intracellularly, yersiniae proliferate extracellularly. The latter also have a preference for lymphatic tissues. To resist the attack of the immune system, pathogenic *Yersinia* spp. are endowed with a 70-kb virulence plasmid (called pYV) which carries genes encoding (1) a protein type III secretion system (TTSS), (2) a set of six effector proteins (*Yersinia* outer proteins, Yops), (3) regulators for gene expression and Yop-secretion/translocation, and (4) a *Yersinia* adhesin (YadA), which among other functions can mediate *Yersinia* attachment to host cells (Cornelis et al. 1998; Hoiczyk et al. 2000). *Y. enterocolitica* and *Y. pseudotuberculosis* also express a chromosomally encoded outer membrane protein—Invasin—that binds with high affinity to $\beta 1$ integrin receptors (Isberg and Leong 1990). In the initial phase of an oral infection invasin triggers internalization of yersiniae into M-cells of the intestinal mucosa and thereby allows transversal of

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the intestinal mucosa. Details about type III secretion, the pYV plasmid, as well as the structure and function of the *Yersinia* adhesins have been reviewed elsewhere (Cornelis et al. 1998; El Tahir and Skurnik 2001; Hueck 1998; Isberg et al. 2000).

Upon contact with target cells, yersiniae employ their TTSS to translocate the six effector proteins named YopH, YopO/YpkA, YopP/YopJ, YopE, YopM, and YopT into the cell. Yops interfere with a variety of cell functions including cytoskeletal regulation, cytokine production, and control of apoptosis to suppress the immune response to infection (Aepfelbacher and Heesemann 2001; Bliska 2000; Cornelis and Wolf Watz 1997; Juris et al. 2002). Controlling the actin cytoskeleton seems to be the major function of at least four Yops, namely YopT, YopE, YopO/YpkA, and YopH. To do so YopT, YopE, and YopO/YpkA modulate the function of Rho GTPases whereas YopH dephosphorylates tyrosine residues of focal adhesion proteins. Noteworthy, *Y. pseudotuberculosis* also produces a homolog of the *E. coli* exotoxin cytotoxic-necrotizing-factor (CNF), which was named CNF-Y (Hoffmann et al. 2004; Lockman et al. 2002). CNF-Y specifically activates RhoA by deamidation of Gln-63, in contrast to CNF from *E. coli* which modifies RhoA, Rac, and Cdc42 (Hoffmann et al. 2004).

Rho GTP-binding proteins constitute ideal targets for pathogens because they are master regulators of the actin cytoskeleton, control cell cycle, intracellular vesicle transport, and gene transcription (Symons and Settleman 2000; van Aelst and D'Souza-Schorey 1997). Most Rho-family proteins are molecular switches which are "on" when bound to GTP and "off" when bound to GDP. In the GTP-bound state Rho proteins stimulate a variety of effector proteins, which include protein kinases, lipid kinases, and multidomain scaffolds (Bishop and Hall 2000). The GDP/GTP cycling of Rho GTPases is regulated by (1) their intrinsic GTPase activity; (2) GTPase activating proteins (GAPs), which greatly increase the intrinsic GTPase activity; and (3) guanine nucleotide exchange factors (GEFs), which promote the exchange of GTP for bound GDP. Furthermore, by binding to guanine nucleotide dissociation inhibitors (GDIs) the hydrophobic isoprenoid moiety at the C-terminus of Rho GTPases is neutralized and this is involved in cytosol/membrane cycling. GDIs also "freeze" Rho GTPases in their actual guanine nucleotide bound state (GDP or GTP) and block their interaction with regulators and effectors (Fig. 1; Olofsson 1999).

An emerging theme is that Yops modulate and exploit the signal transduction pathways which are stimulated initially in host cells after infection with yersiniae (Ruckdeschel et al. 2001; Ruckdeschel 2002). Rho GTPases can get activated through different *Yersinia* factors. Invasin-triggered uptake has been shown to involve Rac, either individually or in cooperation with Rho and Cdc42Hs, dependent on the cell type (Alrutz et al. 2001; McGee et al. 2001; Wiedemann et al. 2001). With the help of cofactors such as phosphatidylinositolphosphates Cdc42Hs and Rac activate N-WASp and WAVE, respectively, which greatly enhance the actin polymerizing activity of Arp2/3 complex (Higgs and Pollard, 1999). Assembly of an actin-rich phagocytic cup by these mechanisms then mediates bacterial uptake. Besides by surface adhesins, Rho and Rac could be activated by lipolysaccharide (LPS) of yersiniae acting via Toll-like receptors (Arbibe et al. 2000). In this review we will focus on the biochemistry and cell biology of the Rho GTPase modulating Yops YopT, YopO/YpkA, YopE, and the focal adhesion regulator YopH. Important features of YopM and YopP will also be mentioned; a more detailed description of their function has been published elsewhere (Juris et al. 2002; Ruckdeschel 2002). Figure 1 de-

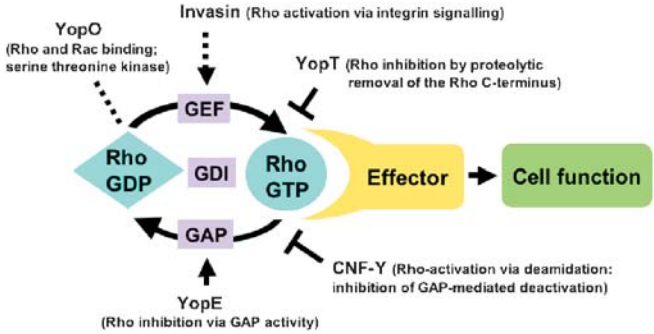

Fig. 1 Interference of *Yersinia* virulence factors with the Rho activation/deactivation cycle. The major biochemical mechanisms by which the respective factors modulate Rho GTPases are in *brackets*. *CNF-Y*, cytotoxic necrotizing factor of yersiniae; *GAP*, GTPase activating protein; *GEF*, guanine nucleotide exchange factor; *GDI*, guanine nucleotide dissociation inhibitor; *RhoGDP*, inactive, GDP-bound Rho GTPase; *RhoGTP*, active, GTP-bound Rho GTPase; *Invasin*, *Yersinia* adhesin binding to β1-integrins.

picts a scheme of the multiple "mechanisms" whereby *Yersinia* virulence factors interfere with the Rho activation/deactivation cycle.

YopT: a cysteine protease removing the isoprenoid group of Rho GTPases

YopT is a 35-kDa protein (322 amino acids) that belongs to the CA clan of cysteine proteases. It is expressed by *Y. enterocolitica*, *Y. pestis*, and only some *Y. pseudotuberculosis* strains (Iriarte and Cornelis 1998; Shao et al. 2002; Zumbihl et al. 1999). In addition to the three *Yersinia yopT* sequences, 16 homologous open reading frames derived from animal pathogens such as *Haemophilus ducreyi*, *E. coli* O157:H7, or *Pasteurella multocida,* plant pathogens such as *Pseudomonas syringae pv. phaseolicola* and entomopathogenic *Photorhabdus luminescens* have been extracted from databases. Despite great sequence diversity, all YopT family members possess conserved C/H/D amino acid residues which appear to be essential for protein activity. The cDNA-inferred YopT family proteins can be divided into two groups. The first group, which includes YopT, contains proteins of 30–40 kDa; the second group contains proteins of >300 kDa which harbor additional functional domains (Shao et al. 2002).

Cellular overexpression and in vitro studies showed that YopT proteolytically removes the geranylgeranyl isoprenoid moiety of RhoA, Rac1, and Cdc42 and this activity is dependent on the invariant C/H/D residues C139, H258, and D274. YopT cleaves just before the C-terminal cysteine to which the geranylgeranyl group is attached via a thioether bond (Shao et al. 2002, 2003). Catalytically inactive YopTC139S can still bind to RhoA and thus can be used for pull-down experiments (Aepfelbacher et al. 2003; Shao et al. 2002; Sorg et al. 2003). Although YopT requires an isoprenoid group for binding and activity, it does not distinguish between geranylgeranylated or farnesylated RhoA. YopT also seems to act equally well on GDP- and GTP-bound RhoA. In the test tube and in cells overexpressing the reaction components, YopT works best on RhoA, is less active on Rac and Cdc42, and does not cleave H-Ras. When the 5 basic amino acid residues (3 x lysine and 2 x arginine) at the C-terminus of RhoA were mutated to glutamine residues, the resulting

RhoA mutant became insensitive to YopT cleavage. Furthermore, an intracellularly expressed GFP fusion construct containing the last 13 amino acids of RhoA (including the 5 basic residues), but not a construct containing the last 4 amino acids of RhoA, was cleaved by YopT, although both GFP constructs became isoprenylated (Shao et al. 2002, 2003). Together these data suggest that YopT recognizes an isoprenoid group in combination with a stretch of basic amino acid at the C-terminus of Rho GTPases. The structural requirements of YopT for binding and cleavage of RhoA were also tested. Whereas deletion of 8 amino acids from the C-terminus abrogated YopT activity, deletion of 74 amino acids from the N-terminus had no effect. When more than 100 amino acids were deleted from the N-terminus of YopT its activity was abolished. In comparison, binding to RhoA was not greatly affected by deleting 14 amino acids from the C-terminus, whereas the N-terminal 74 amino acid deletion mutant displayed considerably reduced RhoA binding. These findings suggest that catalytic activity is mainly located at the C-terminus, whereas Rho GTPase binding also involves the very N-terminal amino acids of YopT (Sorg et al. 2003).

Considering that yersiniae inject only minute amounts of Yops into target cells and intracellular Yop activity is temporally and spatially controlled, cellular infection models provided specific informations as to the physiological functions of Yops. In fact, destruction of actin stress fibers in *Y. enterocolitica*-infected cells was the first activity pointing to the function of YopT (Iriarte and Cornelis 1998). The basis for this YopT effect was subsequently shown to be modification and inactivation of the Rho GTPase RhoA (Zumbihl et al. 1999). Thereafter, the biochemical activity of YopT was identified (Shao et al. 2002, 2003).

RhoA modification by removal of the isoprenoid group has a variety of consequences in *Yersinia*-infected cells. RhoA is released from the plasma membrane and from its cytoplasmic binding partner guanine nucleotide dissociation inhbitor-1 (GDI-1) and accumulates as a monomeric protein in the cytoplasm. Notably, neither Rac1 nor Cdc42 are removed from cell membranes or GDI-1, suggesting that YopT does not work on these proteins in infected cells (Aepfelbacher et al. 2003; Zumbihl et al. 1999). As part of a systematic approach, the role of YopT in preventing opsonized and unopsonized phagocytosis of *Yersinia enterocolitica* by human neutrophils and mouse macrophages was investigated also. Mutant bacteria lacking YopT were phagocytosed in significantly higher amounts than wild-type bacteria both under opsonizing and nonopsonizing conditions. *Yersinia* mutants translocating only YopT were not resistant to phagocytosis by neutrophils or macrophages (Grosdent et al. 2002). However, in primary macrophages YopT overexpressing mutants disrupted actin rich phagocytic cups induced by *Yersinia* invasin as well as podosomal adhesion structures required for chemotaxis (Aepfelbacher et al. 2003). Hence, YopT alone and in combination with partner Yops can disrupt immune cell function thereby promoting *Yersinia* infection. At least in some cell types RhoA seems to be the major target of YopT, whereas Rac and Cdc42 are not affected. However, at present it cannot be excluded that YopT has additional substrates that may be within the large RhoGTPase family or even unrelated to it.

In infected cells YopT is located in membranes, whereas the majority of its target protein RhoA is complexed to GDI in the cytosol. Furthermore YopT can modify RhoA complexed to GDI-1 in vitro only when additional factors such as the membrane lipid phosphatidylinositolbisphosphate (PIP2) are present or when RhoA is artificially loaded with GTP-γS (Aepfelbacher et al. 2003). Thus, YopT likely requires additional signalling molecules (such as PIP2 production and GEFs) to modify RhoA in cells. Taken all these data

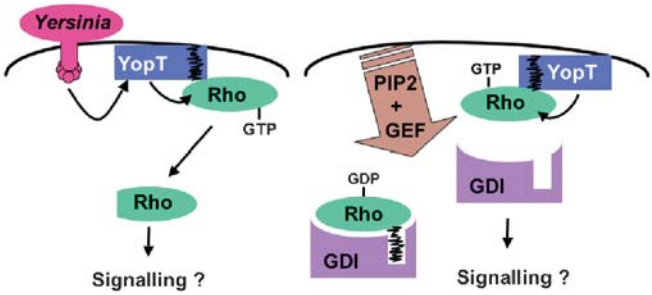

Fig. 2 How YopT may function after translocation into cells. The effects of YopT in cells infected with yersiniae is depicted taking into account temporal and spatial considerations (*left*). Upon injection by the *Yersinia* TTS YopT locates to the plasma membrane where it binds RhoA via its isoprenoid membrane anchor. Through proteolytic removal of the isoprenoid group RhoA is released into the cytosol (*right*). Through action of PIP2 and/or guanine nucleotide exchange factors (*GEFs*) RhoA is released from its cytosolic complex with GDI and then cleaved by YopT. It is unclear what signalling activities the cleaved RhoA and the free GDI in the cytosol have. For details see the section entitled "YopT: A cysteine protease removing the isoprenoid group of Rho GTPases" and reference Aepfelbacher et al. 2003.

together, a model of YopT function within *Yersinia*-infected cells is proposed which includes temporal and spatial considerations: YopT translocated into target cells by the *Yersinia* TTS binds to the plasma membrane where it "meets" RhoA and cleaves off its isoprenoid membrane anchor. The truncated RhoA is released from the membrane and trapped in the cytosol. By a mechanism likely involving membrane lipids and exchange factors, the remaining isoprenylated RhoA is released from GDI and then translocates either directly to YopT or to cell membranes where it is cleaved by YopT. This cycle proceeds until all of the RhoA is modified (Fig. 2).

Within the last years much has been learned about the biochemistry and cell biology of YopT. An exact understanding of the structural basis of YopT cleavage of Rho GTPases will require crystallographic data. The crystal structure of the remote YopT-homolog AvrPphB has been solved and revealed similarities with papain-like cysteine proteases. Consistent with the differing substrate specificities of YopT and AvrPphB, the residues corresponding to the substrate binding sites are highly divergent among the YopT family proteins (Zhu et al. 2004).

YopE: GAP activity for rapid and specific downregulation of Rho GTPases

YopE is a 25-kDa protein (219 amino acids) which, like the homologous domains within exoenzyme S from *Pseudomonas aeruginosa* and SptP from *Salmonella* Typhimurium, works as GAP for Rho-family proteins (Andor et al. 2001; Black and Bliska 2000; Fu and Galan 1999; Goehring et al. 1999; Pawel-Rammingen et al. 2000). The Rho GAP domain of YopE ranges from amino acids 96 to 219. Residues 54–75 have been shown to be both necessary and sufficient for targeting of YopE to an unidentified perinuclear compartment (Krall et al. 2004). The bacterial RhoGAPs, like all mammalian Rho GAPs, possess an arginine finger motif known to be essential for activity (Scheffzek et al. 1998; Wurtele et al. 2001). Mutation of the arginine finger in YopE (R144A) abolished most of the YopE activities in cells and mouse infection models (Aili et al. 2003; Pawel-Rammingen et al.

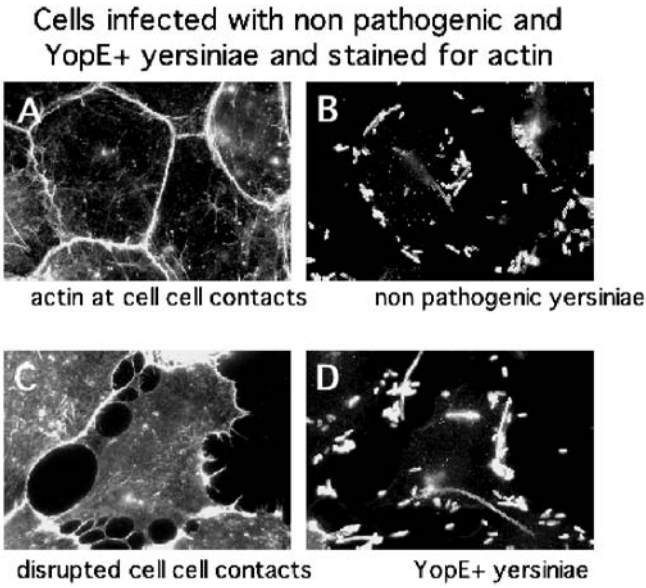

Fig. 3 Disruption of cell–cell contacts by yersiniae expressing YopE. Confluent human endothelial cells were infected with nonpathogenic yersiniae or yersiniae expressing YopE (*YopE+*). Thereafter the actin cytoskeleton of the cells (**A**, **C**) and the bacteria (**B**, **D**) were stained and recorded in separate channels. **A** In confluent cells infected with the nonpathogenic yersiniae, actin is exclusively located at cell-cell contacts. **B** Bacteria attached to the cell in **A** are shown. **C** *Yersinia* YopE+ disrupts actin at cell-cell contacts and changes cell shape. **D** Bacteria attached to the cell in **C** are shown.

2000; Black and Bliska 2000; Andor et al. 2001). Five different triple alanine substitution mutants within amino acids 166–208 abrogated in vitro GAP activity but not cellular cytotoxicity of YopE. It was therefore suggested that YopE may have additional targets/functions within cells which are dependent on the arginine 144 residue but not on GAP activity (Aili et al. 2003). In vitro YopE and ExoS work on Rho, Rac, and Cdc42 whereas SptP acts on Rac and Cdc42 (Black and Bliska 2000; Fu and Galan 1999; Goehring et al. 1999; Pawel-Rammingen et al. 2000). Introduction of YopE into transformed cell lines by *Yersinia* infection or microinjection was found to disrupt actin filaments (Black and Bliska, 2000; Pawel-Rammingen et al. 2000; Rosqvist et al. 1991). In primary endothelial cells, a *Y. enterocolitica* strain that translocates YopE but none of the other effector Yops did not affect direct Rho activation by thrombin, direct Rac activation by sphingosine-1-phosphate, or direct Cdc42 activation by bradykinin, as tested by normal formation of actin stress fibers, membrane ruffles, or filopodia respectively. However, a basal Rac activity required for maintaining cell-cell contacts, as well as Rac activation by Cdc42 resulting in ruffling, was blocked by YopE (Fig. 3; Andor et al. 2001). This finding demonstrates that YopE can modulate Rho GTPase-dependent signal pathways with a remarkable specificity in primary target cells of yersiniae. It also confirms earlier findings showing that the substrate specificity of GAPs may differ in vitro and inside cells (Moon and Zheng 2003; Ridley et al. 1993). The molecular basis for the intracellular specificity of YopE is not known, but YopE may act in a compartmentalized fashion, i.e., may not get access to the membrane compartments in which Cdc42 and Rho are localized. The specific perinuclear membrane localization of YopE supports this notion (Krall et al. 2004). It has not been

tested, however, to what extent the compartments containing the RhoGTPases and YopE overlap.

YopE contributes to the antiphagocytic activity of *Y. enterocolitica* and *Y. pseudotuberculosis* in cooperation with fellow Yops. It blocks phagocytosis of opsonized or unopsonized yersiniae by macrophages, neutrophils, and epithelial cells (Grosdent et al. 2002; Fällman et al. 1995; Ruckdeschel et al. 1996). Furthermore, *Y. pseudotuberculosis* mutants deleted in YopE are clearly attenuated in general virulence/lethality (Rosqvist et al. 1998). Interestingly, in a mouse infection model a YopE-deficient *Y. pseudotuberculosis* strain showed only minor defects in colonization of and persistence in intestinal and lymphoid tissues. However, a YopH/E double mutant was essentially avirulent whereas a YopH mutant wasn't. Furthermore, the YopE-deficient *Y. pseudotuberculosis* strain was outcompeted by *Y. pseudotuberculosis* wild type in coinfection models (Logsdon and Mecsas 2003).

Together these findings suggest that YopE has both individual and redundant functions and synergizes with other Yops during the complex infection cycle of yersiniae. Likely there are additional, hitherto unknown functions of YopE. First, YopE without in vitro GAP activity still has cytotoxic effects (Aili et al 2003). Second, specific intracellular membrane localization of YopE may indicate effects on vesicle transport (Krall et al 2004). Third, although the GAP function individually shuts down activity of Rho GTP-binding proteins, the coordinated activities of GEFs and GAPs can promote some cell functions by enhancing the on-off cycling of RhoGTPases (Symons and Settleman 2000). In addition, some GAPs also work as effector proteins by binding preferentially to the GTP-bound proteins (Moon and Zheng 2003). Thus, introduction of bacterial GAPs into cells may in cooperation with other cellular factors also result in a gain of function.

YopO/YpkA: a serine threonine kinase activated by G-actin and binding to Rho GTPases

YopO from *Y. enterocolitica* (called YpkA in *Y. pseudotuberculosis*) is a 80-kDa (729 amino acids) multidomain protein for which different activities have been demonstrated: (a) serine/threonine kinase activity causing autophosphorylation and phosphorylation of artificial basic substrates, (b) actin binding which results in kinase activation, and (c) binding to the Rho GTPases RhoA and Rac. The protein organization of YopO reflects these distinct activities. An N-terminal secretion/translocation region (aa 1–77) is followed by the predicted catalytic domain (aa 150–400). Mutation of a critical lysine residue in YopO (K269A) or aspartic acid residue in YpkA (D270A), located within the putative catalytic domain, abolished kinase activity (Cornelis et al. 1998; Dukuzumuremyi et al. 2000; Galyov et al. 1993; Juris et al. 2000). The C-terminal half of YopO contains four regions (within aa 436–710) with homology to Rho binding domains (RBDs). In yeast two hybrid assays a construct (residues 442–732) consisting of the RBDs of YpkA was able to bind to RhoA, whereas deletion of residues 543–640 (which include three of the RBDs) from YpkA abolished RhoA binding. The very C-terminal stretch of 21 amino acids (aa 709–729) displays some homology to the actin bundling protein coronin (Dukuzumuremyi et al. 2000; Juris et al. 2000). Removal of these 21 C-terminal amino acids abolished actin binding and actin induced autophosphorylation (Juris et al. 2000). The protein organization of YopO is depicted in Fig. 4.

Purified actin was found to bind to and activate YopO, resulting in autophosphorylation and phosphorylation of artificial substrates (Juris et al. 2000). There may be additional YopO/YpkA activators, because in fetal calf serum the highest YpkA stimulatory activity

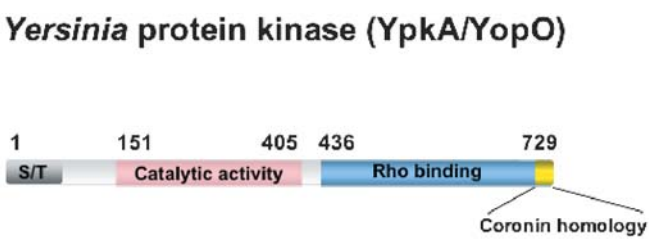

Fig. 4 Domain organization of YpkA/YopO. This figure is based on and summarizes the work of Barz et al. 2000; Cornelis et al. 1998; Dukuzumuremyi et al. 2000; Galyov et al. 1993; and Juris et al. 2000. *S/T*, secretion and translocation domain required by the type III secretion system; *Rho binding*, a region containing four amino acid stretches with homology to Rho binding domains; *coronin homology*, a stretch of 20 amino acids showing homology to the actin bundling protein coronin from *D. discoideum*. The amino acid *numbers* correspond to the sequence of the YopO protein.

was found between 100- to 300-kDa proteins where no actin was present (Dukuzumuremyi et al. 2000).

By yeast two hybrid assays and in immunoprecipitation experiments YpkA was shown to associate with Rho and Rac but not Cdc42Hs (Barz et al. 2000; Dukuzumuremyi et al. 2000). Rho and Rac binding were independent of the nucleotide bound state (GDP or GTP) of the GTPases, although in one report the GDP-bound form of RhoA bound three times more efficiently to YpkA than the GTP-bound form (Dukuzumuremyi et al. 2000). Moreover, YpkA did not alter guanine nucleotide exchange factor-stimulated GDP-release on RhoA in vitro. Although the in vitro data provide no basis for this so far, YpkA was able to reduce the level of GTP-bound RhoA in *Y. pseudotuberculosis*-infected HeLa cells (Dukuzumuremyi et al. 2000). In experiments aimed to relate the phosphorylating and Rho/Rac binding activities of YpkA, it was observed that (a) neither the GDP- nor the GTP-bound forms of Rho or Rac affect autophosphorylation, (b) autophosphorylated YpkA does not show altered binding to the GDP- or GTP-bound forms of Rho or Rac, and (c) in cells the kinase dead D270A mutant of YpkA reduces the levels of GTP-bound Rho as effectively as wild-type YpkA (Barz et al. 2000; Dukuzumuremyi et al. 2000).

HeLa cells infected with a *Y. pseudotuberculosis* mutant overexpressing YpkA responded by cell contraction and formation of pronounced retraction fibers whereby focal adhesions remained intact. In these experiments YpkA was localized at the plasma membrane, a property that was later shown to reside in the N-terminal half of the protein (Dukuzumuremyi et al. 2000; Hakansson et al. 1996). Divergent results where reported concerning the effects of kinase inactive YpkA/YopO mutants on the actin cytoskeleton. In one study, actin filament disruption by YpkAD270A was normal (Dukuzumuremyi et al. 2000), whereas in another study YopOK269A showed considerably attenuated actin filament destroying ability (Juris et al. 2000). In cooperation with YopT and YopE, YpkA contributes to the antiphagocytic activity of yersiniae towards neutrophils and macrophages (Grosdent et al 2002). It also synergizes with other Yops to confer upon yersiniae the ability to colonize and persist in different tissues (Logsdon and Mecsas 2003).

Important information concerning the biology and molecular functions of YopO/YpkA are missing to date. Its physiological substrates in cells are unknown and which of its different biochemical activities are responsible for the cellular and in vivo effects is not clear. Whether these activities work independently or synergistically with each other is worth investigation as well.

YopH: a tyrosine phosphatase working on focal adhesion proteins

YopH is a highly active phosphotyrosine phosphatase of 50 kDa (468 amino acids) with multiple substrates, many of which are components of focal adhesions (Cornelis 2002; Juris et al. 2002). The catalytic domain of YopH is situated in the C-terminal half of the protein (from amino acids 206–408). Mutation of a cysteine residue implicated in phosphate hydrolysis abolishes catalytic activity and leads to a substrate trapping protein (YopHC403S or YopHC403A) which localizes to focal adhesion complexes (Black and Bliska 1997; Persson et al. 1997). The N-terminal 129 amino acids of YopH both harbor the secretion/translocation domain and are crucial for substrate recognition (Black et al. 1998). Four single amino acid mutations (Q11R, V31G, A33D, and N34D) in YopH were identified that interfered with binding to tyrosine-phosphorylated $p130^{Cas}$ but not with YopH translocation into cells. One of these mutants (V31G) was also unable to localize to focal adhesions and dephosphorylate target proteins (Montagna et al. 2001). In addition, deletion of residues 223–226 blocks YopH targeting to focal adhesions and at the same time impairs antiphagocytic activity of *Yersinia* towards macrophages and mouse virulence (Persson et al. 1999).

YopH dephosphorylates $p130^{Cas}$, focal adhesion kinase (Fak), paxillin, Fyn-binding protein (FyB), and the scaffolding protein SKAP-HOM in different cell types such as neutrophils, macrophages, and epithelial cells (Black and Bliska 1997; Persson et al. 1997; Hamid et al. 1999). These YopH substrates regulate the interaction between the actin cytoskeleton and extracellular matrix-binding integrins in focal adhesions/complexes or similar structures (Brakebusch and Fässler 2003; Giancotti 2000). Cytoskeletal uptake structures formed upon interaction of *Yersinia* YadA or invasin with cellular integrins are thought to resemble focal adhesions, and their disruption by YopH may thus explain its antiphagocytic activity.

Previous studies using *Y. enterocolitica* or *Y. pseudotuberculosis* mutants suggested that YopH is responsible for up to 50% of the antiphagocytic activity of yersiniae towards neutrophils and J774 macrophages (Fällman et al. 1995; Ruckdeschel et al. 1996). Further studies showed that translocation of wild-type YopH but not of the catalytically inactive YopHC403S into HeLa cells or J774 macrophages prevented invasin/β1 integrin-mediated uptake of yersiniae (Persson et al. 1997, 1999). YopH has a variety of additional activities in *Yersinia*-infected cells, including suppression of the oxidative burst in macrophages, reduction of Ca^{++} signalling in neutrophils, inhibition of T- and B-lymphocyte activation, and blockage of monocyte chemoattractant protein 1 production by macrophages (Cornelis 2002; Alonso et al. 2004). Whether these functions are due to dephosphorylation of the known YopH substrates or involve unknown substrates is not clear at the moment. Nonetheless, YopH is among the most effective Yops with regard to mouse virulence and antiphagocytic activity.

YopM and YopP/YopJ: effectors modulating cell growth and survival

YopM is an approximately 40-kDa protein the exact size of which depends on the *Yersinia* strain from which it is isolated (Cornelis 2002). Size variation is due to varying numbers (13–20) of a 19-residue leucine-rich-repeat motif (LRR). LRRs which have been implicated in protein-protein interactions make up most of the YopM protein. YopM coimmuno-

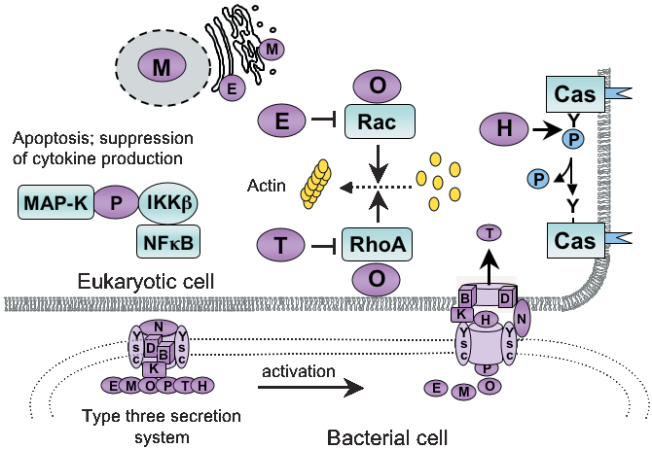

Fig. 5 *Yersinia* type III secretion system mediated injection of Yops into cells and Yop functions. Upon contact of yersiniae with target cells the type III secretion system is activated and a translocation needle is formed that spans the two bacterial membranes and the membrane of the eukaryotic host cell. Through this needle the effector Yops (*single letters in purple circles*) are translocated into the cell where they locate to distinct compartments such as plasma membrane (*YopO*) or Golgi apparatus (*YopE* and *YopM*). The proteins targeted by the Yops are depicted within *blue objects*. *MAP-K*, MAP kinase; *NFκB*, nuclear factor κB; *IKKβ*, inhibitory κB kinase *β*; *Ysc*, yop secretion proteins; *Cas*, p130 Crk-associated substrate; *LcrV*, V-antigen; *P*, phosphate group. For more detailed information, see specific sections of this article.

precipitates from cell lysates with two kinases, protein kinase C-like 2 (PRK2) and ribosomal S6 protein kinase 1 (RSK1). RSK1 is directly activated by YopM and this is required for stimulation of PRK2 (McDonald et al. 2003). The known cellular targets of YopM go well with its ability to affect the expression of genes involved in cell growth and cell cycle (Sauvonnet et al. 2002). It is also noteworthy that YopM is transported to the nucleus in a membrane-vesicle mediated pathway (Skrzypek et al. 1998). It is unclear, however, how this transport event relates to the role of YopM in cell growth or its association with the targets. Although YopM has no obvious antiphagocytic function, it is clearly required for mouse virulence of yersiniae (Leung et al. 1990).

YopP/YopJ (*Y. pseudotuberculosis*) has limited homology to the ubiquitin-like protease Ulp1 of yeast, which cleaves a small ubiquitin related modifier (SUMO1). YopP modulates SUMO activity and concentration in cells overexpressing these reaction components. Moreover, mutation of a critical cysteine or histadine residue within the putative catalytic region prevented some YopP/YopJ functions (Orth et al 1999). YopP/YopJ also associates with members of the MAPK kinase (MKK) superfamily, which represent upstream MAPK activators. Furthermore, it binds and inhibits the IκB kinase-*β* (IKK-*β*), which is the major NF-κB-activating kinase (Orth et al. 1999). Most likely through these properties YopP/YopJ disrupts NF-κB and MAPK signaling pathways, which leads to blockage of the release of TNF*α* by macrophages and of IL-8 by epithelial cells (Ruckdeschel 2002).

Interestingly, by downregulation of NF-κB signaling YopP/YopJ triggers apoptosis in infected macrophages (Ruckdeschel 2002). By enhancing the synthesis of antiapoptotic proteins, NF-κB is thought to protect cells that encounter bacteria or LPS (Karin and Lin 2002). Thus it has been proposed that LPS-responsive signaling by *Yersinia* infection co-

operates with the NF-κB-inhibitory action of YopP/YopJ to mediate apoptosis (Ruckdeschel et al. 2001).

Together, these findings emphasize the variety of strategies pathogenic *Yersinia* spp. have developed to undermine host defense mechanisms. Through the activity of different Yops, yersiniae disrupt the physiological sequence of immune functions that work on distinct cellular and molecular levels (Fig. 5). Initial effects are characterized by blockage of phagocytosis, oxidative burst, and cytokine production, and subsequent responses involve an effect on cell growth/proliferation and/or the induction of an intrinsic macrophage cell death program.

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