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The *Yersinia pestis* type III secretion system: expression, assembly and role in the evasion of host defenses

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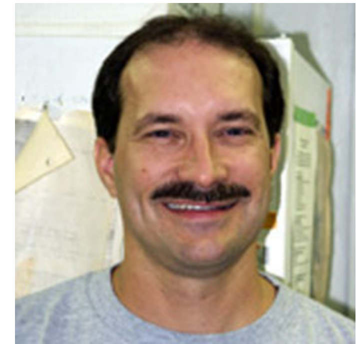

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Abstract *Yersinia pestis*, the etiologic agent of plague, utilizes a type III secretion system (T3SS) to subvert the defenses of its mammalian hosts. T3SSs are complex nanomachines that allow bacterial pathogens to directly inject effector proteins into eukaryotic cells. The *Y. pestis* T3SS is not expressed during transit through the flea vector, but T3SS gene expression is rapidly thermoinduced upon entry into a mammalian host. Assembly of the T3S apparatus is a highly coordinated process that requires the homo- and hetero-oligomerization over 20 *Yersinia* secretion (Ysc) proteins, several assembly intermediates and the T3S process to complete the assembly of the rod and external needle structures. The activation of effector secretion is controlled by the YopN/TyeA/SycN/YscB complex, YscF and LcrG in response to extracellular calcium and/or contact with a eukaryotic cell. Cell contact triggers the T3S process including the secretion and assembly of a pore-forming translocon complex that facilitates the translocation of effector proteins, termed *Yersinia* outer proteins (Yops), across the eukaryotic membrane. Within the host cell, the Yop effector proteins function to inhibit bacterial phagocytosis and to suppress the production of pro-inflammatory cytokines.

Keywords Secretion · Virulence · Apparatus · Effector · Yops

Introduction

Plague, caused by the Gram-negative bacterium *Yersinia pestis*, has devastated the human population throughout recorded history [1]. Improvements in public health measures and medicine have brought this disease under control in most developed countries; however, plague remains a major public health problem in many underdeveloped countries, particularly in Africa [2]. In addition, sporatic cases of human plague still occur in endemic areas of Asia, South America and North America, including the western United States.

In the wild, plague is primarily a disease of rodents and other small mammals and is transmitted between hosts by infected fleas [3]. Human plague occurs in three different forms: bubonic, septicemic and pneumonic. The most common form of plague is bubonic which normally occurs following the bite of an infected flea. Regurgitated bacteria initially disseminate to, and replicate within, draining lymph nodes, leading to the formation of enlarged lymph nodes, termed buboes. Bacteria eventually spread systemically, leading to septicemia (septicemic plague), shock, disseminated intravascular coagulation, peripheral gangrene and often death. If untreated, bacteria can also spread to the lungs, producing a secondary pneumonic plague that can subsequently be transmitted from person to person via infectious droplets. Inhalation of infected droplets can result in primary pneumonic plague, which has an extremely high fatality rate.

The extreme virulence of *Y. pestis* primarily stems from its ability to avoid or thwart the defenses of its host and to eventually overwhelm the host with bacterial growth [4]. To accomplish this task, *Y. pestis* deploys a variety of virulence factors, including the assembly and deployment

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of a protein injection device termed a type III secretion system (T3SS) [5]. T3SSs are complex protein secretion systems that function to transport antihost proteins, termed effectors, across the bacterial inner and outer membranes as well as across a eukaryotic membrane. This review will focus on the expression, assembly and regulation of the *Y. pestis* T3S apparatus (T3SA) as well as the role of the injected effector proteins in the pathogenesis of plague. The genes encoding both the T3SA and the effector proteins, termed *Yersinia* outer proteins (Yops), are encoded on a large virulence plasmid termed pCD1 in *Y. pestis* [6]. Plasmids encoding highly related T3SSs are also found in the other human pathogenic yersiniae (*Y. enterocolitica* and *Y. pseudotuberculosis*). Importantly, the T3S process is critical for the virulence of all three human pathogenic yersiniae [4, 7]. Finally, although the focus of this review is on *Y. pestis*, many of the contributions to our understanding of *Yersinia* T3SS function originate from studies of the T3SSs of other bacterial pathogens, particularly the closely related enteropathogenic yersiniae.

Regulation of T3SS gene expression

Yersinia pestis alternates between fleas (25 °C) and a variety of different mammalian hosts (37 °C) during its lifecycle [3]. Plasmid pCD1, which encodes the T3SS, plays no role in flea colonization, and T3SS genes are not expressed at flea temperature. In contrast, the T3S process plays a critical role during mammalian infections, and T3SS genes are maximally expressed at mammalian body temperature (37 °C). Expression of T3SS genes is strictly dependent upon the AraC-type DNA-binding protein LcrF [8], whose production is tightly thermoregulated at both the level of transcription and translation [9, 10] (Fig. 1). At temperatures below 30 °C, transcription of the *yscW-lcrF* operon is minimal due to the inhibitory action of the *Yersinia* modulator A (YmoA) protein [10, 11]. YmoA is a nucleoid-associated protein that is homologous to the *E. coli* regulator of high hemolysin activity (Hha) [12], interacts directly with the DNA-binding global regulator H-NS [13] and influences DNA supercoiling [14]. Recent studies have definitively demonstrated that YmoA in complex with H-NS directly binds to the 5′-UTR region of *yscW* and downregulates *yscW-lcrF* operon transcription [10]. Importantly, the level of YmoA protein is tightly thermoregulated in *Y. pestis* by the ClpXP and Lon proteases [15]. Thus, YmoA is stable at 25 °C and functions to repress *yscW-lcrF* operon transcription; in contrast, at 37 °C, YmoA is rapidly degraded, leading to the upregulation of *yscW-lcrF* operon transcription.

LcrF expression is also tightly thermoregulated at the level of translation [9, 10]. Indeed, *lcrF* mRNA transcripts

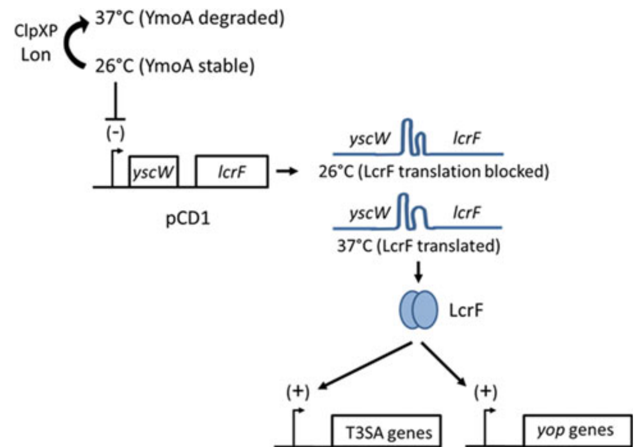

Fig. 1 Thermoregulation of *Y. pestis* T3SS gene expression. Transcription of plasmid pCD1 T3SS genes requires the AraC-type transcriptional activator LcrF. LcrF is highly expressed at 37 °C but not at 26 °C. Transcription of the *yscW-lcrF* operon is repressed at 26 °C by the nucleoid-associated YmoA protein. At 37 °C, YmoA is rapidly degraded by the ClpXP and Lon proteases, leading to the activation of *yscW-lcrF* transcription. LcrF expression is further thermoregulated at the level of translation by a twin hairpin structure that masks the *lcrF* ribosome-binding site at 26 °C. At 37 °C, localized melting of the critical stem–loop structure allows access to the *lcrF* ribosome-binding site. Stable expression of LcrF at 37 °C leads to the activation of T3SA and *yop* gene expression

are translated efficiently at 37 °C but not at temperatures below 30 °C [9]. The temperature-dependent translation of *lcrF* transcripts was originally hypothesized to involve a thermolabile stem–loop structure that functions to conceal the *lcrF* ribosome-binding site at temperatures below 30 °C. Recent studies have confirmed the presence of a cis-acting twin stem–loop RNA structure upstream of the *lcrF* coding sequence that plays a major role in the thermoregulated expression of LcrF [10]. The second hairpin loop of this structure is predicted to contain four uracil residues that directly pair with the *lcrF* ribosome-binding site. Importantly, mutations designed to specifically disrupt this predicted structure alter the thermoregulated expression of LcrF and *Y. pestis* virulence.

The mechanism by which LcrF activates T3SS genes is not fully understood; however, LcrF is homologous to the well-characterized AraC-type DNA-binding protein ExsA, which activates T3SS gene expression in *Pseudomonas aeruginosa* [16]. ExsA is normally monomeric but self-associates to a dimer when bound to DNA [17]. In contrast, LcrF forms a stable dimer in solution and when bound to DNA [18]. Remarkably, the amino acids that compromise the helix-turn-helix (HTH) DNA-binding motifs of ExsA and LcrF are nearly identical, and recent studies indicate that ExsA and LcrF both recognize the ExsA consensus-binding site (AaAAAnwmMyGrCynnnmTGayAk), which is present in *Y. pestis* promoter regions [18]. Thus, LcrF appears to activate T3SS gene expression by binding as a

preformed dimer to consensus-binding sites in promoter regions and recruiting the RNA polymerase complex to these promoters.

Assembly of the T3SA

The *Y. pestis* T3SA is a complex nanomachine that must be assembled in an efficient and highly coordinated manner to ensure rapid availability of the T3S process following entry into a mammalian host. Expression of over 20 *Yersinia* secretion (Ysc) proteins is required to assemble the minimal T3SA, which consists of a membrane-bound base structure and an external needle structure that extends approximately 40 nm from the bacterial surface [4]. The assembly process is coordinated via a complex series of homo- and hetero-oligomeric protein interactions that generate multiple intermediate assembly complexes that are subsequently joined or function as a platform for further assembly.

Studies by Diepold et al. [19] indicate that the YscR, YscS, YscT, YscU and YscV integral inner membrane proteins initially assemble an inner membrane platform. Individual homo-oligomerization of YscR, YscS, YscT or YscU was not detected; however, homo-oligomerization of YscV, which consists of an N-terminal membrane-bound domain (8 predicted transmembrane segments) and a C-terminal soluble cytoplasmic domain [20], was readily detected and likely results in the assembly of a nanomeric ring structure [19, 21]. The assembled YscRSTUV inner membrane platform further recruits the MS ring-forming proteins YscJ and YscD [19, 22]. In separate events, the outer membrane secretin YscC, with assistance from the YscW pilotin, assemble the 12–14 subunit outer membrane ring structure of the T3SA [22, 23]. Joining of the outer membrane YscC subassembly and the inner membrane subassembly is mediated by interactions between the periplasmic–localized regions of the YscD and YscC proteins [24]. Other cytoplasmic/peripheral membrane proteins, including YscK, YscQ, YscL and the YscN ATPase, direct the assembly of a cytoplasmic complex or sorting platform that is believed to be involved in the ordered recognition and processing of T3S substrates [25, 26]. Interaction of the ATPase and sorting complex with the membrane-bound components of the T3SA is likely mediated by interactions between YscN and/or YscQ with the cytoplasmic domains of YscD, YscU and/or YscV [27–29]. Additional essential components of the T3SA whose function and interactions are not fully characterized include YscO, YscX and YscY [30–32].

Completion of base assembly results in a T3SA that lacks both an inner rod and an external needle structure. Final assembly of the T3SA requires the T3S process to complete these structures. Initially, the T3SA recognizes and secretes only early (needle-type) substrates, which include YscI (the rod subunit) [33], YscF (the needle subunit) [34] and YscP (needle length control) [35, 36]. In the absence of YscP, YscF is continuously secreted and needles of variable and often extraordinary length are generated [37]. In contrast, in the presence of YscP, needles of relatively uniform length (approximately 40 nm in *Y. pestis*) are assembled and YscF secretion is terminated following needle assembly. Journet et al. [37] demonstrated that YscP directly determines needle length by acting as a molecular ruler. Furthermore, the C-terminal domain of YscP contains a type III secretion substrate specificity switch (T3S4) domain that functions with YscU to switch the substrate specificity of the T3SA from early (needle type) to late (Yop-type) type III secretion substrates upon the completion of needle assembly [38]. Finally, assembled needles have also been shown to possess a needle tip complex composed of a pentamer of the secreted LcrV protein [39]. The tip complex primarily functions after contact with a eukaryotic cell and is predicted to function as a platform for the assembly of the pore-forming translocon proteins [40].

Regulation of Yop effector protein secretion

The activity of all T3SSs is primarily controlled at the level of secretion. This is unusual as the activity of most protein secretion systems is largely controlled at the level of substrate production. *Y. pestis* T3SS components that play a direct role in regulating the secretion of the Yop effector proteins include YopN, TyeA, SycN, YscB and LcrG [41]. In vitro, the secretion of the Yop effector proteins is blocked in the presence of millimolar levels of calcium and triggered by the removal of calcium [42]. In vivo, levels of calcium are sufficient to prevent Yop secretion until the bacteria contact a eukaryotic cell (Fig 2). *Y. pestis* strains deficient in the expression of YopN, TyeA, SycN, YscB or LcrG constitutively secrete the Yop effector proteins regardless of the presence or absence of calcium [43]. Thus, the YopN, TyeA, SycN, YscB and LcrG proteins function to prevent the secretion of the Yop effector proteins until a T3S activation signal is encountered. Furthermore, as each T3SA is regulated independently, only the T3SAs that are in direct contact with a targeted eukaryotic cell are activated, which avoids the spurious loss of effector proteins to the extracellular environment (Fig. 2).

LcrG is a small cytosolic protein that is required to prevent Yop secretion in the presence of calcium and prior to contact with a eukaryotic cell [44]. The mechanism by which LcrG blocks secretion is unknown; however, LcrG

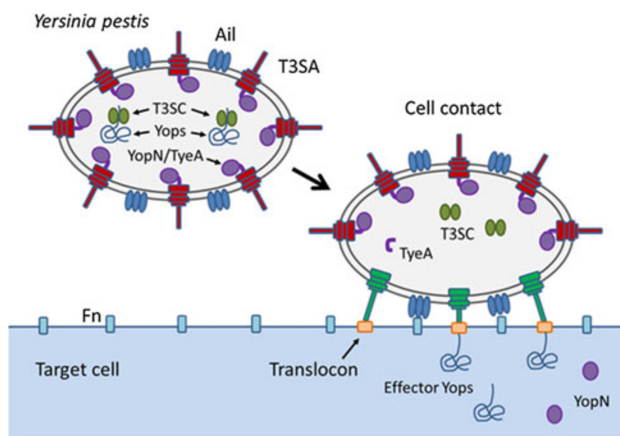

Fig. 2 Contact with a eukaryotic cell triggers Yop secretion and translocation. Upon entry into a mammalian host, T3SS gene expression and T3SA assembly are initiated. Following the completion of external needle assembly, the substrate specificity of the T3SA switches from early (needle-type) substrates to late (Yop-type) substrates. Importantly, cytoplasmic pools of the Yop effectors in complex with their cognate T3SCs are not secreted due to the regulatory action of the YopN/TyeA complex and LcrG. Ail-mediated attachment of *Y. pestis* to a host cell triggers the T3S process, translocon assembly, and secretion and translocation of the Yop effector proteins

likely prevents Yop secretion via direct interaction with the T3SA. LcrG also directly interacts with cytosolic LcrV, an interaction that inhibits LcrG's ability to block secretion [45]. Upon contact with a eukaryotic cell, levels of LcrV dramatically increase relative to the levels of LcrG. The excess LcrV is hypothesized to bind LcrG and relieve the LcrG-dependent block in Yop secretion.

YopN is a secreted protein that also plays a critical role in the regulation of Yop secretion [46]. In the bacterial cytoplasm, YopN directly interacts with the SycN/YscB T3S chaperone (T3SC) and TyeA [47, 48]. The SycN/YscB T3SC binds to the N-terminal chaperone-binding domain (CBD) of YopN and functions to promote YopN secretion and translocation. TyeA, on the other hand, binds to a C-terminal region of YopN and functions to reduce YopN translocation [43, 49]. Alanine scanning mutagenesis of *tyeA* identified two surface-exposed regions that were required for TyeA to regulate Yop secretion [50]. One region mediates the interaction of TyeA with YopN; the second region was hypothesized to mediate the interaction of TyeA with the T3SA. YopN mutants that constitutively block Yop secretion required TyeA to function but no longer required the YopN secretion signal, CBD or the SycN/YscB T3SC, indicating that the C-terminal region of YopN in complex with TyeA functions to block Yop secretion from a cytosolic location [51]. Overall, these studies indicate that the YopN/SycN/YscB/TyeA complex interacts with the T3SA as a T3S substrate via the YopN secretion signal, CBD and SycN/YscB T3SC and as a regulatory protein via the C-terminal region of YopN in complex with TyeA. Triggering of the T3S process is hypothesized to disrupt the interaction of TyeA with the T3SA. Disruption of the TyeA–T3SA interaction allows YopN to be exported, removing the block in Yop secretion.

Current evidence indicates that LcrG and the YopN/TyeA/SycN/YscB complex control Yop secretion from a cytosolic location [51, 52]; however, the signals that regulate the T3S process are extracellular calcium or contact with a eukaryotic cell. This suggests that a signal transduction mechanism is required to control the activity of the cytosolic localized regulatory proteins. Current evidence suggests that the T3SA itself and, in particular, the T3SA needle structure play a direct role in the detection and/or transmission of these extracellular signals [53, 54]. Mutant needle (YscF) proteins were identified that secreted the Yop effector proteins in both the presence and absence of calcium and prior to contact with a eukaryotic cell, indicating that the YscF needle plays a role in the regulation of Yop secretion [54]. Furthermore, a number of the YscF needle point mutants exhibited altered responses to extracellular calcium with different mutants requiring from 1 mM to 7.5 mM extracellular calcium to prevent Yop secretion. These studies indicate that the YscF needle plays a role in the detection and/or transmission of the extracellular calcium and/or cell contact signals. Thus, the YscF needle and possibly other components of the T3SA in conjunction with the LcrG and YopN/TyeA/SycN/YscB complex function as a signal detection and response system that controls the activity of the T3SA in response to specific environmental signals.

Translocation of the Yop effector proteins

In general, the activity of all virulence-associated T3SSs is controlled, in part, by contact with a eukaryotic cell or membrane [55]; however, the mechanisms by which bacteria detect cell contact, activate the T3S process and translocate the effector proteins across a eukaryotic membrane, are poorly understood. The YscF needle, LcrG and YopN/TyeA/SycN/YscB complex play an important role in the cell-contact-mediated activation of the T3S process; however, the assembly of an additional multiprotein complex, termed the translocon, is required to form the pore structure responsible for the transport of the Yop effector proteins across the eukaryotic membrane [56]. *Y. pestis* secretes three proteins (translocators) that function specifically to facilitate the translocation of the Yop effector proteins across the eukaryotic membrane: LcrV [57], YopB [58] and YopD [59]. LcrV is the needle tip protein that assembles into the needle tip complex [40]. The needle tip complex is hypothesized to serve as an assembly platform

for YopB and YopD, the two hydrophobic pore-forming translocators [60]. Attempts to determine the stoichiometry and/or structure of the pore complex have been largely unsuccessful; however, a YopB/YopD pore complex of approximately 500–700-kDa that corresponds to approximately 15–20 YopB/YopD monomers has been isolated from erythrocyte membranes [61].

In vitro, *Y. pestis* is capable of injecting effector Yops into essentially any cell; however, in vivo, *Y. pestis* primarily targets dendritic cells, macrophages and neutrophils [62]. In general, the interaction between a bacterial pathogen and the cells of its host is determined by the expression of bacterial adhesins and the distribution of specific receptors on the different host cell types. In the enteropathogenic yersiniae, two prominent adhesins, YadA and Invasin, mediate bacterial attachment and Yop injection [63]; however, all *Y. pestis* strains lack expression of YadA and invasin [64]. Instead, the Ail outer membrane protein has been shown to function as the primary *Y. pestis* adhesin mediating cell adhesion and Yop injection [65, 66]. The mechanism by which *Y. pestis* specifically targets selected cell types in vivo is not fully understood but likely involves specific bacterial adhesins as well as host factors such as surface receptors and opsonins, such as C3b [63].

The Yop effector proteins

Since the Yop effector proteins are delivered directly into host cells by surface-attached bacteria, their respective contributions to virulence are believed to be due to directly modulating the physiology of the infected cell: This belief is bolstered by the fact that five (of the six) possess eukaryotic-like structural domains and/or biochemical activities. The six Yop (*Yersinia* outer proteins) effector proteins of *Y. pestis* can be roughly divided up into two functional groups: YopE, YopH, YopT and YpkA have been shown to interact with components of the host cell cytoskeleton, whereas YopJ inhibits the activation of host signaling pathways (the function of YopM has remained largely enigmatic).

Yops functionally interacting with the host cell cytoskeleton

In studies performed in the 1980s using tissue culture-based models (hereafter referred to as ‘in vitro’), it was observed that *Yersinia*-infected eukaryotic cells rapidly undergo a catastrophic collapse of their actin-based cytoskeleton. Subsequent genetic analyses eventually led to the discovery of the T3SA-encoding 70-kb extrachromosomal plasmid of *Yersinia* and eventually, in 1994, to the demonstration that at least one of the substrates of this T3SA, YopE, is directly injected from the bacterium into the host cell [67]. The cytoskeletal-disrupting activity of *Yersinia* enhances the virulence of this pathogen by interfering with the structural rearrangements required for the normal phagocytic process to occur in host cells. As it turns out, the cytoskeletal-disrupting activity of *Yersinia* is primarily due to YopE and YopH.

Although the effects on the host cell caused by YopE are dramatic, its precise mechanism remained obscure until it was recognized that it acts as a GTPase-activating protein (GAP) toward the RhoA family of GTPases (RhoA, Rac and Cdc42) [68]. These GTPases function as molecular switches such that when bound by GTP they are ‘on’ and when this GTP is hydrolyzed to GDP they are ‘off’. The relative equilibrium between these two states determines, among many other things, the coordination of actin dynamics that occurs during processes such as phagocytosis. The GAP activity of YopE results in RhoA, Rac and Cdc42 to be primarily in their ‘off’ states, which in turn inhibits actin polymerization, thus accounting for the cytoskeletal collapse observed in *Yersinia*-infected cells. Thus, it is through this means that YopE contributes to the antiphagocytic activity of this pathogen.

YopH also contributes to *Yersinia*’s antiphagocytic activity. Unlike YopE, the biochemical activity of YopH was almost immediately recognized based on its sequence (and structural) similarity to eukaryotic protein tyrosine phosphatases [69]. Using a substrate-trap mutant of YopH, it was demonstrated that following its injection into the host cell, YopH physically interacts with p130Cas and focal adhesion kinase (FAK) [70]. These latter host factors are normally activated by phosphorylation following bacterial adhesion and subsequently promote the assembly of new focal adhesion complexes. Focal adhesion complexes link the cellular cytoskeleton to the plasma membrane during various cellular processes including phagocytosis. What is believed to occur is that YopH, following its injection into the host cell, rapidly dephosphorylates any p130Cas and FAK that had become activated during the initial contact between the host cell and *Yersinia*. There have been other host proteins suggested to be targeted by YopH (i.e., the Fyn-binding protein and SKAP-HOM), but it has been difficult to determine whether these are true ‘targets’ of YopH during an infection or whether these host factors are in fact innocent bystanders that are indiscriminately dephosphorylated by the potent phosphatase activity of YopH.

Unlike YopE and YopH that both alter cell function by mimicking the activities of host regulatory factors, YopT possesses a biochemical activity that irreversibly inactivates host cell proteins. YopT is a protease that specifically cleaves RhoA, Rac and Cdc42 near their carboxyl termini

[71]. These GTPases are normally anchored to plasma membrane via a covalently bound lipid moiety at their carboxyl termini. By cleaving these GTPases just 'upstream' of this post-translational modification, YopT effectively releases the GTPases from the membrane, thereby removing these host factors from their normal cellular location. Whereas YopE and YopH are found in all clinical and environmental isolates of *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*, YopT is absent in several clinical isolates of *Y. pseudotuberculosis* which may indicate that this virulence factor plays a redundant role.

An additional Yop that affects the host cell cytoskeleton (at least under certain experimental conditions) is the *Yersinia* protein kinase A (YpkA). YpkA possesses two easily recognizable domains: residues 136–408 of YpkA are similar to eukaryotic Ser/Thr kinases of the PKA family, whereas the carboxyl–terminal region (residues ≈ 500-700) consists of sequences resembling GTPase-binding modules [72]. These latter sequences mediate the binding of RhoA and Rac (but not Cdc42), and in an infection model in which YpkA is overexpressed in the absence of the other Yops, this binding activity is necessary for YpkA-dependent cytoskeletal disruption of the host cell [73]. Although identifying the eukaryotic 'target' of the kinase activity of YpkA has been elusive, a *Yersinia* strain expressing a kinase-inactive version of YpkA (YpkAD270A) is attenuated in both in vitro and in vivo (mouse) infection models [74]. Although the GTPase-binding domain of YpkA has an extremely high affinity for RhoA and Rac, precisely how this property of YpkA is related to its overall cellular functioning is still unknown. When YpkA is overexpressed in cells either via plasmid-mediated transfection or by using strains engineered to highly express YpkA (and that lack YopE and YopH), YpkA clearly negatively affects the integrity of the actin-based cytoskeleton. However, since in natural *Yersinia* strains, YpkA is expressed at levels that are far below YopE and YopH, it has been so far impossible to determine whether YpkA impacts cytoskeletal dynamics in cells infected with YopE/H-expressing *Yersinia*. It is somewhat surprising that the cellular activity of a virulence factor with two well-defined biochemical activities has remained obscure.

YopJ and host cell signaling

In addition to growth in host tissues due to the antiphagocytic activities of YopE, YopH (and possibly YopT and YpkA), another characteristic of *Yersinia* infections is that this pathogen proliferates in tissues without provoking an inflammatory-like response. Visceral organs such as the liver become heavily colonized by mice infected with *Y. pestis* in stark contrast to mice infected with plasmid-*cured Y. pestis* (i.e., strains lacking the T3SS). In the latter situation, inflammatory cells are rapidly recruited to the site of infection forming protective granulomas which limit both the proliferation and dissemination of the bacterium to other tissue sites. Inflammation occurs in response to heightened cytokine expression at infection sites. Inducible cytokine secretion by infected cells is a result of a multistep process: A danger signal is perceived at the cell surface that activates signaling pathways that culminate in the expression of genes encoding pro-inflammatory cytokines (e.g., TNFα, IL8, etc.). Several different research groups have used a variety of approaches to show that YopJ, following its T3SA-mediated injection into the host cell by surface-bound *Yersinia*, blocks signaling pathways that normally are activated in host cells following bacterial contact. Using various models, several different cellular activities have been attributed to YopJ, including deubiquination of Iκβ and TNF receptor-associated factors (TRAFs) as well as acetylation of MKKs. Whether any of these activities (which are not mutually exclusive) occur when YopJ is introduced into cells via the T3SA following infection is unknown. Therefore, like YpkA, precisely how YopJ exerts its activity during infection is presently unclear.

Linking the activity of a 'cytoskeletal' disruptor with that of a 'signaling' inhibitor

To try to identify eukaryotic factors that were responsive to YpkA, a yeast-based mutagenesis screen was performed. Through such an approach, it was discovered that the eIF2 signaling pathway (a stress-responsive signaling pathway that regulates protein synthesis) is specifically activated by YpkA and that this response reduces the toxicity of YpkA toward the host cell [75]. Entirely unexpectedly, it was found that the cellular activity of YopJ (blocking stress-induced MAP kinase signaling that is distinct from translation-level eIF2 pathway) is also dependent on a properly functioning eIF2 signaling pathway. Although these findings were unexpected and their meaning for infection remains to be determined, it is noteworthy that YpkA and YopJ are encoded on the same transcription unit, which, in bacteria, is usually an indicator that gene products are functionally linked.

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