Bacterial Serine/Threonine Protein Kinases in Host-Pathogen Interactions*

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In bacterial pathogenesis, monitoring and adapting to the dynamically changing environment in the host and an ability to disrupt host immune responses are critical. The virulence determinants of pathogenic bacteria include the sensor/signaling proteins of the serine/threonine protein kinase (STPK) family that have a dual role of sensing the environment and subverting specific host defense processes. STPKs can sense a wide range of signals and coordinate multiple cellular processes to mount an appropriate response. Here, we review some of the well studied bacterial STPKs that are essential virulence factors and that modify global host responses during infection.

Successful adaptation to a changing environment requires efficient monitoring and a rapid response. The cascade of chemical reactions culminate in gene transcription and a fast metabolic adaptation. These rapid changes are important especially when responses have to be orchestrated from different cellular compartments. Thus, given the importance of signaling in the normal functioning of the host cell, it is not surprising that pathogens exploit host cell signaling networks to optimize their infectious cycles. Signaling systems are commonly involved in regulation of the expression of virulence factors of pathogenic bacteria during disease progression. Previously, the two-component systems were the only tools known for environmental sensing in bacteria (1, 2). In contrast, signaling in eukaryotes is accomplished primarily by a network of protein phosphorylation cascades that require the coordinated action of a number of serine/threonine and tyrosine kinases and their associated phosphatases. These protein kinases transfer a phosphate group from ATP or GTP onto specific serine, threonine, and/or tyrosine residues of a protein substrate. Typically, the phosphorylation functionally activates the substrate to perform either a specific activity or cellular localization and/or transfer the phosphate group to a downstream effector, initiating a cascade of signal-response reactions. The reverse reaction of dephosphorylation restores the activators and effectors to their initial functional state (not phosphorylated), preparing the system for the next signaling event. Thus, kinases and phosphatases function as on/off switches, modulating specific signal transduction pathways (3).

Until recently, it was assumed that signaling in prokaryotic and eukaryotic organisms was mediated by distinct mechanisms. However, recent advances in genetic strategies and genome sequencing have revealed the existence of "eukaryote-like" serine/threonine protein kinases (STPKs) and phosphatases in a number of prokaryotic organisms, including pathogens such as Streptococcus spp. (4–7), Mycobacterium (8–13), Yersinia spp. (14, 15), Listeria monocytogenes (16, 17), Pseudomonas aeruginosa (18), Enterococcus faecalis (19), and Staphylococcus aureus (20–22). In fact, the so-called eukaryote-like Ser/Thr kinases identified in prokaryotes share characteristic signature sequence motifs with the eukaryotic protein kinase superfamily based on sequence homology between their kinase domains (23). These domains are typically organized into 12 subdomains (Hanks domains) that fold in a characteristic two-lobed catalytic core structure, with the catalytic active site lying in a deep cleft formed between the two lobes (23, 24). The kinase catalytic domain can be defined by the presence of specific conserved motifs and nearly invariant residues that are directly or indirectly involved in positioning the phosphate donor ATP molecule and the protein substrate for catalysis. The structural conservation of the catalytic domain between different kinases is remarkable and is maintained across kingdoms.

The discovery of eukaryote-like signaling systems in bacterial pathogens has sparked an interest in understanding their function. This is due partly to the fact that eukaryotic protein kinases are currently the largest group of drug targets, second only to G-protein-coupled receptors (25, 26). A large number of STPK inhibitors have been approved by the Food and Drug Administration for use in humans (26), and ~150 kinase inhibitors are also being tested in clinical trials (27, 28). In addition, STPKs are also being investigated as potential tools in therapeutic strategies (29, 30). Therefore, studies on prokaryotic STPKs in human pathogens have gained interest owing to the prospect of exploiting these signaling components in future anti-infective therapies.

The contribution of STPKs to bacterial growth and pathogenesis is multifaceted, as has been observed for other signaling systems. However, the mechanisms by which these kinases mediate diverse functions in a coordinated fashion remain to be completely understood, particularly their role(s) during host invasion/persistence, as we propose to detail in this minireview. The STPK-directed host-pathogen interactions known so far appear to be of different types: those in which the bacterial STPK phosphorylates a host substrate(s), those in which host defense is disrupted by STPK activity, and those in which the role of STPK is essential but the mechanism of interaction has not yet been clarified.

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2 The abbreviations used are: STPK, serine/threonine protein kinase; T3SS, type III secretion system; GDI, guanine nucleotide dissociation inhibitor; SCV, Salmonella-containing vacuole; IKK, IκB kinase; EPEC, enteropathogenic E. coli; EHEC, enterohemorrhagic E. coli.
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Yersinia YpkA—Bacteria from the genus Yersinia (Y. pestis, Y. pseudotuberculosis, and Y. enterocolitica) secrete the STPK Yersinia protein kinase A (YpkA, also named YopO) into host target cells via a type III secretion system (T3SS) (31). This kinase has been shown to disrupt the actin cytoskeleton and contribute to resisting phagocytosis by macrophages (32, 33). YpkA is a multidomain protein harboring an N-terminal Ser/Thr kinase domain and a C-terminal guanine nucleotide dissociation inhibitor (GDI) domain, followed by an actin-binding domain (ABD) (14, 15, 32, 34). Once secreted into the host, YpkA localizes at the inner surface of the cytoplasmic membrane in eukaryotic cells (15, 34). Its kinase domain seems essential for virulence, as strains harboring an internal deletion in this domain show reduced lethality in infected mice (14, 36). YpkA is necessary for host cell shape and phagocytosis inhibition (36, 37).

The Goq family of heterotrimeric G-proteins is known to activate Ras-mediated pathways and plays a central regulatory role in a number of cellular activities requiring cytoskeletal rearrangements such as phagocytosis and motility (Fig. 1B) (39). Navarro et al. (40) demonstrated that YpkA phosphorylates Goq on Ser-47, a key residue located in the binding loop of the G-protein, inhibiting GTP binding and thereby inhibiting Goq signal transduction (Fig. 1C). Moreover, YpkA has also been shown to interact with other host proteins without phosphorylating them. The YpkA kinase carries a C-terminal Ras GTPase-binding domain that specifically binds and inactivates the small GTPases Ras A and Rac-1, two proteins of the Ras family involved in cytoskeleton integrity (34, 35, 41). YpkA thus mimics a host GDI (35) to “switch off” the Ras A/Rac pathways, causing cytoskeletal disruption and distortion of cell shape. Thus, the kinase- and GTPase-binding domains of YpkA act synergistically to impair specific host cellular functions. These studies highlight the role of YpkA in promoting the immune system failure at various levels.

Staphylococcus Stk1—S. aureus is considered mostly as an extracellular pathogen, but it can invade a variety of mammalian non-professional phagocytes such as epithelial cells (42) and keratinocytes (43) and survive phagocytosis by professional phagocytes such as neutrophils (44) and macrophages (45). S. aureus displays various protective and offensive responses that facilitate its persistence in the host (46, 47). Interestingly, Stk1 (also named PknB) has been shown to be important for infection in mice in an abscess model (48) as well as in a cutaneous model (49), and it is also required for antibiotic resistance (49). Stk1 was thought to be strictly membrane-associated until Miller et al. (50) demonstrated that the full-length protein could be detected in the extracellular medium, although the mechanism remains unknown. In this elegant study, the authors used a peptide microarray loaded with human peptides and identified 68 potential host-phosphorylated substrates...
Bacterial STPKs That Disrupt Host NF-κB Pathways

In a host cell, the transcription factor NF-κB protein complex is critically important in triggering an immune/inflammatory response. In the absence of cognate stimuli, NF-κB is prevented from translocating to the nucleus by inhibitors of the IkB family. In response to stimuli such as a bacterial infection, NF-κB is translocated to the nucleus, where it transcriptionally induces specific genes involved in a variety of processes aimed at eliminating the pathogen. In contrast, certain pathogenic bacteria present mechanisms to counter these attacks, some of which involve bacterial STPKs. Structurally, the different members of the NF-κB family are composed of combinations of five subunits, p50, p52, RelA (p65), RelB, and c-Rel, of which p50 and p52 are derived from p105 and p100, respectively. STPKs from different pathogenic bacteria seem to interact with host factors to disrupt the NF-κB signaling pathways and downstream processes as discussed below (Fig. 2).

Legionella LegK1—Legionella pneumophila infects the lung macrophages and causes the so-called Legionnaires’ disease. Once in the phagosome, this pathogen is able to redirect the classical bacterial phagolysosomal elimination to establish a replicative niche within an endoplasmic reticulum-derived compartment, named the Legionella-containing vacuole, and evade host cell defenses (57–59).

Different species of Legionella harbor three to five genes encoding putative STPKs (legk1–legk3 for L. pneumophila Philadelphia-1 (60) and legk1–legk5 for L. pneumophila Lens (61)). LegK1–LegK4 are known to be secreted by a type IV secretion system called Dot/Icm, which is essential for intracellular growth (58, 59). Only LegK1 has been shown to interfere with the NF-κB pathway, acting as an inflammatory activator. Using an NF-κB-specific luciferase reporter system, Ge et al. (62) demonstrated that ectopic expression of LegK1 in HEK-293T cells triggers activation of the NF-κB cascade (Fig. 2, A (left) and B). The same assay conducted with LegK2, LegK3, and a LegK1 kinase-dead mutant (carrying a mutation in the kinase domain) resulted in no activation of the NF-κB system. Therefore, LegK1 seems to be the only STPK able to interfere with the NF-κB pathway, and its kinase activity is required. Moreover, LegK1 activity is specific to NF-κB and does not affect other innate immune signaling pathways such as MAPK and IFN (62).

Moreover, in the same study, the authors used a cell-free reconstitution system to show that LegK1 is able to phosphorylate IkBα, a central regulator of the NF-κB pathway (Fig. 2A), on Ser-32 and Ser-36 in an IkB kinase (IKK)-independent manner. In vitro assays with IkBα and a variety of LegK1 derivatives confirmed phosphorylation of IkBα by LegK1, indicating that the N-terminal “pre-kinase” part is critical for IkBα phosphorylation. The authors also demonstrated that p100, the precursor of the non-canonical NF-κB complex, is processed into p52 when phosphorylated by LegK1 (Fig. 2B). Taken together, these data highlight the function of the LegK1 kinase as a mimic of the host IKKs in the NF-κB response activation. Thus, L. pneumophila LegK1 is an STPK that phosphorylates host substrates (IkBα and p100) and disrupts the NF-κB pathway, thereby modulating host innate defenses and inflammatory responses during infection.

Shigella OspG—Shigella spp. are the agents of shigellosis in humans, a disease that is characterized by the destruction of the colonic epithelium and that is responsible for 1 million deaths per year (65). Shigella spp. use a T3SS to enter epithelial cells, thus triggering apoptosis (64). About 20 proteins have been identified as substrates of the T3SS (65). One of these, OspG, is an STPK known to manipulate the host innate immune system by down-regulating the canonical NF-κB pathway (Fig. 2A right). Using in vivo and in vitro approaches, Kim et al. (66) demonstrated that OspG is an STPK able to bind to ubiquitylated E2 enzymes such as UbcH7 and UbcH5 without phosphorylating them. They showed that this sequestration leads to a decrease in IkBα degradation, thus blocking the activation pathway of NF-κB. The action of OspG seems to be dependent on its kinase activity, as the inactivated kinase mutant does not generate a similar attenuation of NF-κB signaling. In addition,
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Infection assays carried out on the ligated ileal loop in rabbits confirmed that inactivation of ospG in Shigella strains induces a stronger inflammatory response in vivo (66). Moreover, in a recent study, OspG was found to be able to bind ubiquitin and polyubiquitin. This interaction seems to activate OspG kinase activity in vitro and to be required for attenuating the host NF-κB signaling pathway in vivo (67).

Escherichia NleH1 and NleH2—Enteropathogenic Escherichia coli (EPEC) and enterohemorrhagic E. coli (EHEC) are two diarrheagenic strains of E. coli that contribute to the health burden of food-borne disease. EHEC and EPEC are known to express and secrete effectors into intestinal epithelial cells through a T3SS (68). NleH1 and NleH2 have been biochemically characterized as STPKs and secreted in HeLa cells through a T3SS. These STPKs are able to interact with RPS3, a host protein known to bind the p65 subunit of NF-κB and regulate its affinity for its target genes (Fig. 2C) (69, 70). The interaction of RPS3 with NleH1, but not NleH2, reduces the nuclear abundance of RPS3, causing inhibition of the NF-κB-dependent transcriptional activity. The NF-κB activity seems also to be decreased by NleH2 when IKKβ is overexpressed (71, 72). In vivo tests showed that the EHEC ΔnleH1 strain, but not the ΔnleH2 strain, is hypervirulent in a gnotobiotic piglet infection model, indicating that NleH1 and NleH2 differentially regulate the inflammatory response of the host (70). In a mouse intestine model, EPEC NleH1 and NleH2 seemed to increase colonization and decrease inflammation (71). Phosphorylation of RPS3 on Ser-209 by IKKβ enhances its association with importin-α, thus mediating RPS3 entry into the karyopherin pathway for nuclear translocation (73). The interaction of RPS3 with NleH1 leads to the inhibition of its phosphorylation by IKKβ. Recently, a high-throughput screen to identify a host cell substrate of NleH1 yielded the CRKL (v-Crk sarcoma virus CT10 oncogene-like) protein (74). According to the proposed model, CRKL interacts dually with NleH1 and IKKβ. Interaction of a kinase-active form of NleH1 with CRKL is essential for the ability of NleH1 to inhibit RPS3 phosphorylation by IKKβ.

Bacterial STPKs with Unidentified Host Substrates

In some of the host-pathogen interactions known so far, the kinase activity of the bacterial STPKs is required, but the substrate has not been identified. In addition to E. coli NleH1 and NleH2 described above, this category also includes Legionella LegK2. Similarly, the substrates of most of the mycobacterial STPKs that participate in host-pathogen interactions have not yet been identified.

Legionella LegK2—LegK2 has been identified to be involved in the virulence of L. pneumophila by a combination of in vitro and in vivo approaches (61). A ΔlegK2 mutant showed no defects in in vitro growth but presented less cytotoxicity and delayed intracellular replication in amoebas compared with the wild-type strains. Moreover, vacuoles containing the mutant strain showed less efficient recruitment of endoplasmic reticulum markers. Complementation assays performed with wild-
type and kinase-dead proteins provided evidence that LegK2 kinase activity is required for the normal infectious phenotype of \textit{L. pneumophila} (61). Although no host targets have yet been identified, LegK2 seems to be a crucial virulence determinant involved in the establishment of the replicative niche in the macrophage.

\textit{Mycobacterium tuberculosis} STPKs—\textit{M. tuberculosis} is the causative agent of tuberculosis. It is capable of infection and long-term survival in host macrophages. The bacterium possesses several virulence factors that are expressed at different steps of infection all the way to establishing a latent infection and an eventual resuscitation from dormancy. Genome sequence analyses revealed 11 STPKs (8), four of which have been demonstrated to be involved in virulence in \textit{vivo}: PknH, PknI, PknK, and PknG. Although these STPKs are important virulence factors, their host cell interactors have not yet been identified, except for that of PknG. Studies with genetic mutants of the above STPKs have revealed their roles in establishing an infection. In a mice model, the \textit{pknH} mutant was found to survive and replicate to a higher bacillary load in mouse organs compared with its parental strain (75). Similarly, a \textit{pknI}-null mutant showed increased intracellular growth inside THP-1 macrophage cells and hypervirulence in immunodeficient mice (76). More recently, Malhotra et al. (77) showed that a \textit{pknK} deletion results in increased resistance of the mutant to acidic pH, hypoxia, and oxidative and stationary phase stress \textit{in vitro} and increased survival during persistent infection in mice. Moreover, assays performed on host immune effectors suggested an immunomodulatory function of PknK during acute infection in mice (77).

PknG is a soluble STPK expressed by pathogenic or attenuated mycobacteria such as \textit{M. tuberculosis} and \textit{Mycobacterium bovis} bacillus Calmette-Guérin, but not by \textit{Mycobacterium smegmatis}, a non-pathogenic species. PknG is known to play a role in persistence inside macrophages, presumably by inhibiting the critical step of phagosome-lysosome fusion, as shown for \textit{M. bovis} and \textit{M. smegmatis} in cultured macrophages (78, 79) and in a mouse model (80). Interestingly, the basis for the PknG-mediated enhanced survival in macrophages appears to be its interaction with, but not phosphorylation of, PKCo, an STPK from the host cell that is known to regulate phagolysosome formation (81). Other studies have also highlighted the role of PknG in interfering and disturbing host defense pathways (82–85). Recently, in \textit{Mycobacterium marinum}, SecA2 was identified as the secretion system that likely introduces PknG into the host cell (86).

Conclusions

Thus, the emerging theme from the above examples is that not only are the STPKs in pathogenic bacteria essential for regulating important bacterial processes, but some are secreted such that they can interact with host substrates also, subverting essential host functions such as immune responses and cell shape and integrity. It is not yet clear whether these STPK interactions all involve phosphorylation of a host substrate. In some cases, the phosphorylation of a host substrate has been demonstrated, whereas in others, the STPK kinase activity is seen to be essential, but a phosphorylated substrate has not been identified. The biochemical mechanisms of these pathogen-directed targeted perturbations in the host cell signaling network are being actively investigated. Thus, bacterial STPKs are proving to be molecular switches that play key roles in host-pathogen interactions.

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