MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 2011, p. 192–212 Vol. 75, No. 1
1092-2172/11/$12.00 doi:10.1128/MMBR.00042-10

Eukaryote-Like Serine/Threonine Kinases and Phosphatases in Bacteria

Sandro F. F. Pereira, Lindsie Goss, and Jonathan Dworkin*

Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

INTRODUCTION

The survival of an organism relies on its capacity to quickly respond and adapt to a constantly changing environment. Underlying this adaptive potential is the ability of cells to sense and transduce external and internal signals. Protein kinases, together with their cognate phosphatases, play a central role in signal transduction by catalyzing reversible protein phosphorylation. Upon sensing external stimuli, kinases undergo autophosphorylation and proceed to transphosphorylate substrate proteins. Phosphorylation on specific amino acid residues, most commonly serine (Ser), threonine (Thr), tyrosine (Tyr), histidine (His), and aspartate (Asp), can control the activity of target proteins, either directly, for example, by inducing conformational changes in the active site, or indirectly, by regulating protein-protein interactions. While most bacterial kinases have been thought to target only His/Asp residues, increasing attention is being paid to the Ser/Thr kinases and their partner phosphatases. This review focuses primarily on bacterial kinases that show homology in their catalytic domains to eukaryotic Ser/Thr kinases (eSTKs) and, to a lesser extent, on their partner eukaryote-like phosphatases (eSTPs).

Ser/Thr PROTEIN KINASES

Protein phosphorylation on Ser, Thr, and Tyr residues was first described for eukaryotes (36, 90, 94, 176). After these initial studies, the functional relevance of these modifications was soon realized, and it quickly became clear that protein phosphorylation is a key mechanism to regulate protein activity and, consequently, to control cellular functions. However, Ser, Thr, and Tyr phosphorylation was assumed for a long time to occur exclusively in eukaryotes (8). Eukaryotic Ser/Thr and Tyr kinases are grouped together in the eukaryotic protein kinase superfamily based on sequence homology between their kinase domains (63). These domains are typically organized into 12 subdomains 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 (63, 79) (Fig. 1A). The smaller, N-terminal lobe is involved primarily in binding and orienting the phospho-donor ATP molecule, whereas the larger, C-terminal lobe binds the protein substrate and initiates the transfer of the phosphate group. The structural conservation of the catalytic domain between different kinases is remarkable and is maintained across kingdoms (Fig. 1B) (also see "Structural and functional studies of bacterial eSTKs"). While subdomains vary in size, there is little sequence homology between members of the superfamily. However, the kinase catalytic domain can be defined by the presence of specific conserved motifs and 12 nearly invariant residues which are directly or indirectly involved in positioning the phosphate donor ATP molecule and the protein substrate for catalysis (63) (Fig. 1C and D).

Kinases are molecular switches that exist in either an "off," inactive state or an "on," active state (67). The transition to the activated state is tightly controlled by diverse mechanisms, including the binding of allosteric effectors and the subcellular localization. Both the αC helix in the N-terminal lobe and the activation loop in the C-terminal lobe undergo extensive conformational changes essential for this transition (Fig. 1C and D). When the αC helix is directed toward the active site, it allows multiple interactions between the two lobes that are

* Corresponding author. Mailing address: Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, 701 W. 168th St., Room 1218, New York, NY 10032. Phone: (212) 342-3731. Fax: (212) 305-1468. E-mail: jonathan.dworkin@columbia.edu.

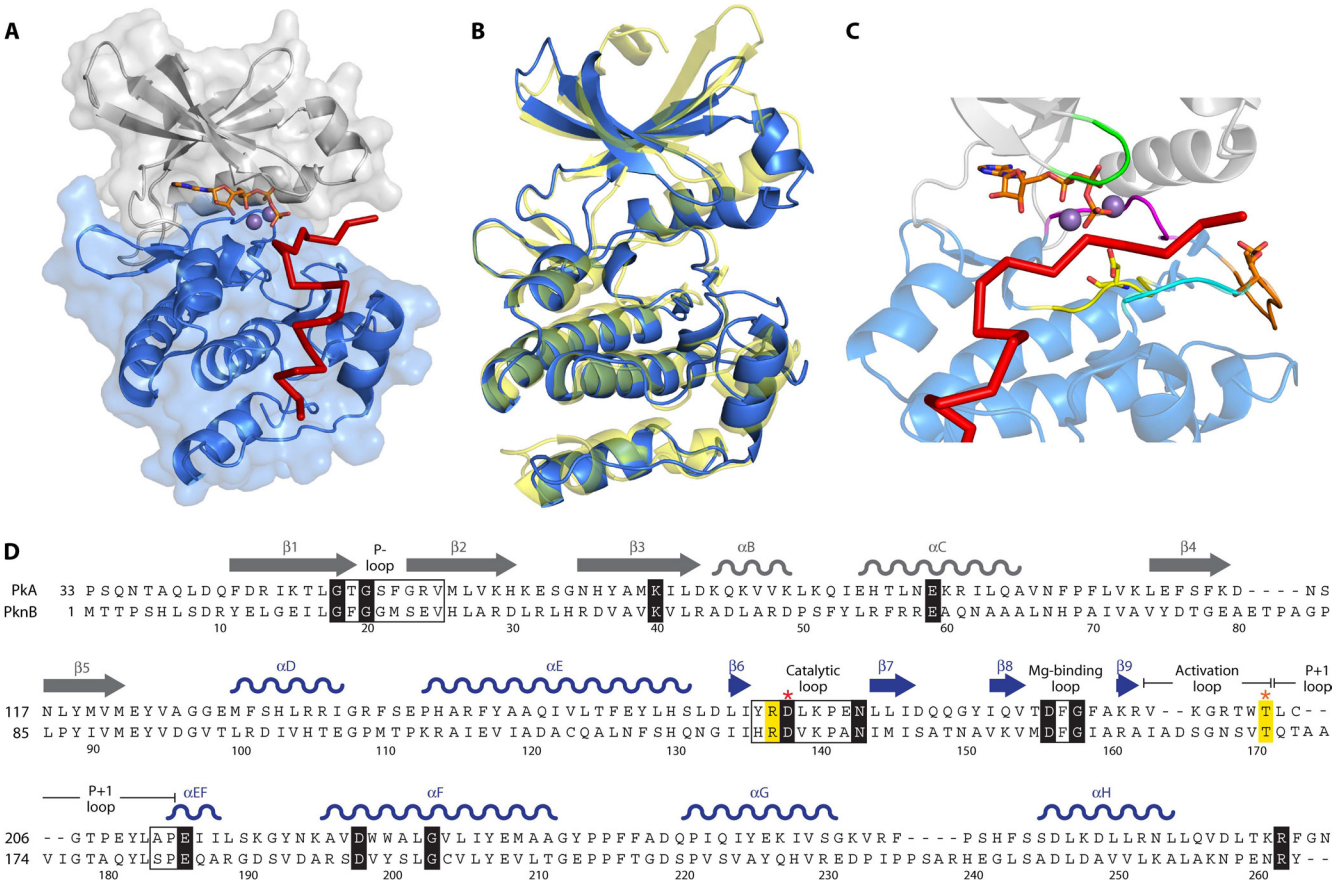

FIG. 1. Structure of the Ser/Thr kinase catalytic domain. (A) Crystal structure of the mouse PKA catalytic domain in complex with an ATP molecule and an inhibitor peptide (Protein Data Bank [PDB] accession number 1ATP). The PKA N-terminal lobe is shown in gray, and the C-terminal lobe is shown in blue. ATP is represented as sticks, with two manganese ions shown as spheres, and the inhibitor peptide is shown as a red line. (B) Superimposition of tertiary structures of PKA and the *M. tuberculosis* eSTK PknB (PDB accession number 1MRU). PKA is shown in blue, and PknB is shown in yellow. (C) The regulatory elements that comprise the catalytic cleft formed between the N- and C-terminal lobes of the Ser/Thr kinase catalytic domain are indicated in the structure of PKA as follows: green, P loop; yellow, catalytic loop with the catalytic Asp residue; magenta, magnesium-binding loop; orange, activation loop with the phosphorylated Thr residue; and cyan, P+1 loop. ATP is represented as sticks, with two manganese ions shown as spheres, and the inhibitor peptide is shown as a red line. (D) Primary sequence alignment between the PKA (residues 33 to 283) and PknB (residues 1 to 266) catalytic domains. The N- and C-terminal lobes of PKA are shown in gray and blue, respectively. Conserved motifs are shown in boxes, and the invariant residues are depicted in black. Other important residues are highlighted and/or shown in bold. Red and orange asterisks indicate the catalytic Asp and phosphorylated Thr residues, respectively.

essential for the kinase activity. The activation loop is part of the most important regulatory element of kinase activity, the so-called activation segment, which is defined as the region between and including the conserved DFG and APE motifs (122) (Fig. 1C and D). This segment includes the magnesium-binding loop, the activation loop, and the P+1 loop. The activation loop is involved in determining substrate specificity and is the most variable region of the activation segment. Many kinases are activated by phosphorylation on at least one Ser/Thr (Tyr for tyrosine kinases) residue in the activation loop, by either autophosphorylation or transphosphorylation by another kinase. This modification promotes several interactions that stabilize the loop in a conformation that allows substrate binding and catalysis (67, 122). These include the critical interaction between the phosphorylated Ser/Thr residue in the activation loop and the conserved arginine that precedes the catalytic aspartate in the catalytic loop of the so-called arginine/aspartate (RD) kinases. The phosphorylated Ser/Thr residue can also interact with the αC helix, bringing the two lobes together in an active conformation. The activation loop is also a site of protein-protein interaction for activity modulators in many kinases. The P+1 loop is a critical point of contact between the kinase and its substrate and is a major determinant of the distinct substrate specificity between Ser/Thr and Tyr kinases. In the Ser/Thr kinases, the loop includes a conserved Ser or Thr residue that interacts with the catalytic loop. Also of relevance is the glycine-rich consensus motif, called the P loop, since it covers both the β- and γ-phosphates and plays an important role in both phosphoryl transfer and ATP/ADP exchange during the catalytic cycle (Fig. 1C and D). All of these conformational changes bring the ATP γ-phosphate, the kinase catalytic Asp residue, and the substrate phospho-acceptor residue together, allowing the transfer of γ-phosphate from ATP to the phospho-acceptor Ser or Thr residue (Tyr in tyrosine kinases) in the substrate protein.

The two main groups of the superfamily, the Ser/Thr kinases

and the Tyr kinases, can be subdivided further into smaller families composed of enzymes that show similar substrate specificities and modes of regulation (63). This review focuses on bacterial kinases with catalytic domains that share structural and functional homology with eukaryotic Ser/Thr kinases. These kinases are referred to herein as eSTKs to distinguish them from other prokaryotic enzymes that can also phosphorylate Ser/Thr residues (see the next section). Although outside the scope of this review, phosphorylation in bacteria also occurs at Tyr residues, and it should be noted that most bacterial tyrosine kinases belong to the BY kinase family and do not resemble eukaryotic enzymes (reviewed in references 57 and 58).

eSTKs in Bacteria

In prokaryotes, protein phosphorylation was assumed for a long time to occur only on histidine and aspartic acid residues involving two-component systems (TCS) (64, 172). The *Escherichia coli* tricarboxylic acid cycle enzyme isocitrate dehydrogenase (IDH) was the first example of Ser/Thr phosphorylation described for bacteria (53). However, IDH was phosphorylated on a Ser by a bifunctional kinase/phosphatase which lacked sequence homology with eSTKs (86). The first characterized eSTK in bacteria was *Myxococcus xanthus* Pkn1 (114). When Pkn1 was overexpressed in *E. coli*, it underwent autophosphorylation on Ser and Thr residues. Although the gene was not essential, a *pkn1* deletion strain showed premature differentiation, indicating that Pkn1 is required for normal *M. xanthus* development. The identification of a transmembrane eSTK, *M. xanthus* Pkn2, suggested that membrane receptor kinases, such as the transforming growth factor beta (TGF-β) receptor kinases that play a central role in eukaryotic cellular regulation, also exist in bacteria (183; see the next section). The weak phenotypes of *pnk1* and *pkn2* mutants suggested the presence of additional kinases with redundant functions. This was confirmed in subsequent studies that revealed a large number of eSTKs interacting in intricate signaling pathways involved in the control of the complex *M. xanthus* life cycle (118–120; also see "Physiological roles and targets of eSTKs in bacteria").

The availability of prokaryotic genomic sequencing data led to the rapid identification of numerous bacterial eSTKs containing characteristic signature sequence motifs (78, 83, 89, 138, 165) (see Ser/Thr Protein Kinases). The number of eSTKs identified in prokaryotes has continued to increase since those initial efforts, and these enzymes are now considered ubiquitous. It has also become clear that prokaryotes show a remarkable degree of diversity in signal transduction pathways (51, 78). A comprehensive and regularly updated list of eSTKs and other prokaryotic signal transduction molecules can be found at http://www.ncbi.nlm.nih.gov/Complete_Genomes/SignalCensus.html (51). Variability exists at the level of the modular organization of prokaryotic eSTKs (83; also see "Structural and functional studies of bacterial eSTKs") as well as in the catalytic kinase core domain itself, as exemplified by the *E. coli* YihE kinase (200). YihE belongs to the category of so-called "atypical" kinases, which share homology with the catalytic core of eSTKs but do not conserve all of the usual kinase motifs (152). It should also be emphasized that not all bacterial Ser/Thr kinases are eSTKs. In addition to the IDH kinase/phosphatase previously mentioned and the HPr kinase/phosphorylase (81), several other non-eSTK Ser/Thr kinases have been described, including *Bacillus subtilis* SpoIIAB, RsbT, and RsbW. SpoIIAB phosphorylates the anti-anti-sigma factor SpoIIAA on a Ser as part of a regulatory circuit that controls the activity of the sigma factor σ^F in sporulation (116). Although SpoIIAB is not an eSTK, its phosphatase partner SpoIIE is a eukaryote-like Ser/Thr phosphatase (40). RsbT and RsbW, which are members of the ATPase/kinase superfamily (41), phosphorylate their substrates on a Ser as part of a complex regulatory network that controls the activity of σ^B, the major sigma factor involved in the stress response (193).

Cross talk between eSTKs and TCS can also occur. The *M. xanthus* transcription factor Mrp is under the control of both the MrpA/MrpB TCS and the eSTKs Pkn8 and Pkn14 (119, 173; also see "Physiological roles and targets of eSTKs in bacteria. (i) Developmental processes"). Similarly, the CovR/CovS TCS and the eSTK Stk1 both regulate expression of the β-hemolysin/cytolysin, which is critical for survival of group B streptococci in the bloodstream and for resistance to oxidative stress (145). The *Mycobacterium tuberculosis* response regulator DosR of the DosS/DosR TCS, which controls the DosR regulon involved in hypoxia- and nitric oxide-induced dormancy, is phosphorylated on Thr198 and Thr205 by PknH, and in conjuction with phosphorylation on Asp54 by DosS, this cooperatively enhances DosR DNA affinity for target genes (25).

Structural and functional studies of bacterial eSTKs. The first bacterial eSTK structure described was that of *M. tuberculosis* PknB (130, 195). The cocrystal of the PknB catalytic domain in complex with an ATP analog revealed that PknB is structurally very similar to the mouse cyclic AMP (cAMP)-dependent protein kinase (PKA) in the activated state (100, 130, 195) (Fig. 1B). The PknB catalytic domain exhibits the typical two-lobed structure, with the nucleotide analog tightly bound in the catalytic cleft between the two lobes and in contact with the regulatory structural elements (see Ser/Thr Protein Kinases and Fig. 1). Both the overall conformation of PknB and the conformation of the majority of the regulatory regions resemble those for the activated state of eukaryotic Ser/Thr kinases (67, 100). Nevertheless, two important exceptions were observed. First, the PknB αC helix is oriented away from the active site, in a position characteristic of the inactive state (67). Despite this orientation, the essential interaction between the conserved Lys40 residue (Lys72 in PKA) in the β3 strand and the conserved Glu59 residue (Glu91 in PKA) in the αC helix is still maintained, as well as the interaction with the α- and β-phosphates of the nucleotide (67) (Fig. 1C). Second, the PknB activation loop was found to be disordered, which is also characteristic of an inactive kinase. However, this might be explained by the phosphorylation state of the activation loop or the absence of the protein substrate, both of which are known to stabilize this region (67, 122).

Overexpression of the PknB kinase domain in *E. coli* resulted in the phosphorylation of four residues in the activation loop (Ser166, Ser169, Thr171, and Thr173) (195). Interestingly, PknB Thr171 corresponds to the phospho-receptor Thr197 residue in the PKA activation loop, which undergoes autophosphorylation during the activation of the kinase (100) (Fig. 1C). In addition, when the sequences of 22 PknB or-

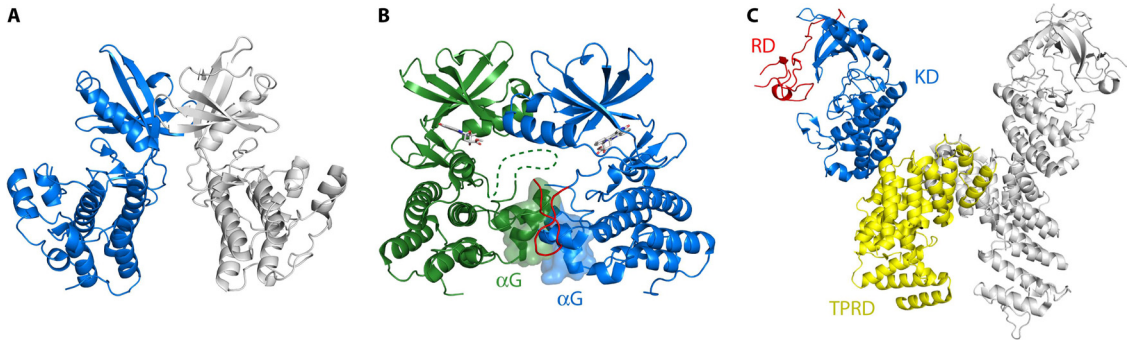

FIG. 2. Dimerization interfaces involved in bacterial eSTK activation. (A) Back-to-back dimer revealed in the crystal structure of the *M. tuberculosis* PknB kinase domain (PDB accession number 1MRU). Two PknB monomers interact through a dimerization interface located in the back sides of the N-terminal lobes (130, 195). (B) Asymmetric front-to-front dimer found in the cocrystal of a mutant PknB kinase domain in complex with an ATP competitive inhibitor (PDB accession number 3F69). This structure resembles an activation complex involving the contact between the αG helixes of two monomers (103). One of the monomers (blue) shows an ordered activation loop (red), characteristic of the active state, whereas the other monomer (green) shows a disordered activation loop (represented by a dashed line). (C) Dimerization of two *M. tuberculosis* PknG kinase monomers. The complete structure of PknG is shown (PDB accession number 2PZI), including the N-terminal rubredoxin domain (RD) and the C-terminal tetratricopeptide repeat domain (TPRD) that surround the kinase catalytic domain (KD). In contrast with the case for PknB, dimerization of PknG occurs through interaction between the TPRDs of two monomers (153).

thologs were mapped on the surface of the PknB structure, extensive conservation was found both in the ATP binding cleft, in residues adjacent to the ATP γ-phosphate binding site, and in the surface analogous to the PKA substrate-binding groove (195). This suggests a common activation mechanism shared by homologous eukaryotic and prokaryotic Ser/Thr kinases.

In both structures, PknB crystallized as a dimer, indicating interactions between the opposite or "back" sides of the N-terminal lobes of two catalytic domains (130, 195) (Fig. 2A). This dimerization interface was conserved among PknB orthologs (195) and was also found in the structure of *M. tuberculosis* PknE, despite the low level of sequence conservation between the two kinases in this region (54). Consistent with a common activation mechanism between eukaryotic and prokaryotic Ser/Thr kinases, the eukaryotic double-stranded RNA-activated protein kinase PKR also crystallized as a back-to-back dimer (30). Moreover, PKR was shown to be activated by a ligand-induced dimerization mechanism (for a review, see reference 28). Experimental data support a similar model of activation for prokaryotic eSTKs (Fig. 3A). The replacement of the *M. tuberculosis* PknD ligand-binding domain (see below) by rapamycin-induced dimerization domains resulted in activation of unphosphorylated kinase (59). The importance of dimerization in kinase activation was further documented by mutagenesis studies in which the replacement of conserved

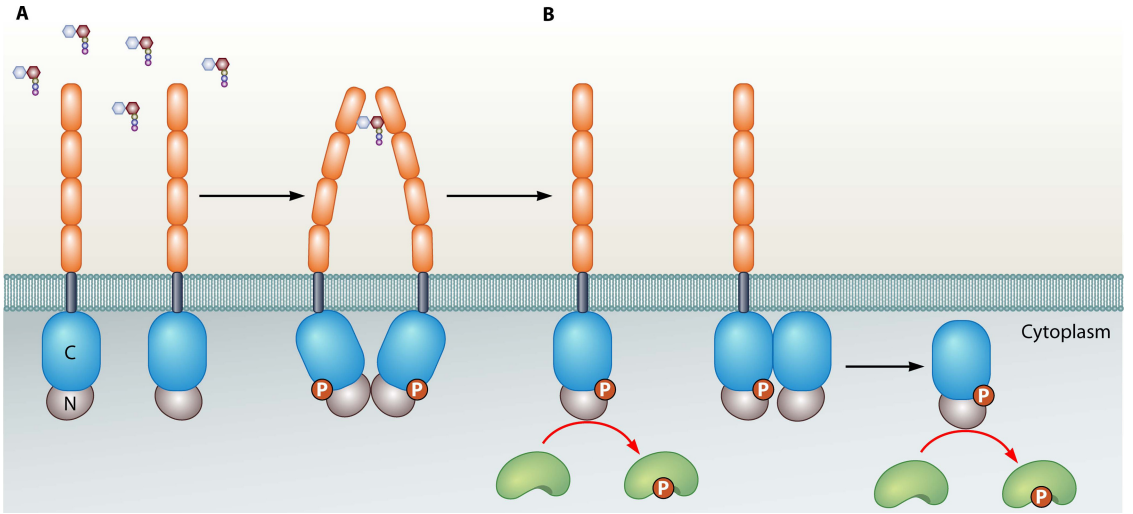

FIG. 3. Activation model for eSTKs. *M. tuberculosis* PknB was chosen for the purpose of illustrating the hypothesized eSTK activation pathways. (A) In the presence of a ligand, two or more PknB monomers bind to a single ligand molecule through their extracellular domains. This brings the intracellular catalytic domains closer, resulting in the formation of a symmetric back-to-back dimer and in the consequent activation of the kinases by autophosphorylation (see text for details). (B) An activated kinase can directly phosphorylate a downstream protein target or activate a soluble kinase through the formation of an asymmetric front-to-front dimer, which will then phosphorylate downstream targets as part of a signaling pathway.

residues in the PknD back-to-back N-terminal lobe dimerization interface reduced autophosphorylation and altered substrate specificity (59). Similar observations were also reported for *Pseudomonas aeruginosa* PpkA (66).

While these observations clearly support a role for dimerization in the activation of eSTKs, the mechanism by which dimerization results in autophosphorylation remains unknown. It is unlikely that it occurs through intermolecular phosphorylation, since in a back-to-back dimer the active sites of the kinase domains are oriented away from each other (Fig. 2A and 3A). In addition, a heterodimer composed of an inactive PknD catalytic site mutant and a wild-type monomer still activated the wild-type catalytic domain (59). These results are consistent with allosteric activation of the kinase upon dimerization, but it is also possible that activation can occur through transphosphorylation by another monomeric or dimeric activated kinase (Fig. 3).

Ligand-promoted dimerization is unlikely to be the only activation mechanism for bacterial eSTKs. First, the phosphorylated PknD kinase domain is fully active even in the absence of dimerization (59). Second, this mechanism does not explain the activation of eSTKs that lack a ligand-binding domain. Consistently, the structure of a PknB mutant kinase cocrystallized with an inhibitor molecule revealed a second dimerization interface in which the two monomers bound to the inhibitor form an asymmetric front-to-front dimer (103) (Fig. 2B). This dimerization interface occurs through interactions between the αG helix and the ordered activation loop of one kinase domain and the αG helix of the second kinase domain. The conformations of the two proteins suggest that one monomer functions as an activator and the other functions as a substrate. In agreement, mutagenesis studies showed that the asymmetric dimer interface mimics a *trans*-autophosphorylation complex (103). This dimerization provides a new mechanism by which an allosterically activated kinase could phosphorylate and thereby activate other kinases that are not associated with a receptor domain (Fig. 3B).

The *M. tuberculosis* soluble kinase PknG has a unique modular organization, with the kinase domain sandwiched between an N-terminal rubredoxin domain and a C-terminal tetratricopeptide repeat domain (TPRD) (153) (Fig. 2C). While PknG crystallizes as a dimer, in contrast with PknB and PknD, dimerization does not occur through the kinase domain but via the TPRD (Fig. 2C). Also, the mechanism of PknG activation seems to differ from that of PknB and most kinases. First, the activation loop of PknG is not phosphorylated, but it is fully ordered and stabilized despite being in an open and extended conformation. Second, the conserved Arg immediately preceding the invariant catalytic Asp residue is absent in the PknG catalytic loop (see above and Ser/Thr Protein Kinases). Activation of PknG is controlled by its rubredoxin domain, which is often found in electron transfer proteins, suggesting that the redox status of the environment may regulate PknG (153).

The catalytic domains of many bacterial eSTKs are associated with an additional domain(s) (83). The diversity in this modular organization is well illustrated in organisms that express a large number of these regulatory proteins, such as in myxobacterial species and *Streptomyces coelicolor* (138, 140). While these extra domains can be enzymatic, they most commonly mediate ligand binding or protein-protein interactions. For example, virtually all Gram-positive bacteria contain at least one transmembrane eSTK composed of a cytoplasmic catalytic kinase domain linked by a transmembrane segment to an extracellular domain consisting of a variable number of PASTA (*p*enicillin-binding protein *a*nd *S*er/*T*hr kinase-*a*ssociated) repeats (194). PASTA repeats were first observed in the crystal structure of *Streptococcus pneumoniae* PBP2X, where two approximately 70-residue-long repeats adopted a unique $\beta_3\alpha$ structural topology (35). However, the PASTA repeats of *M. tuberculosis* PknB and its *Staphylococcus aureus* homolog adopt a linear organization (12, 134) (Fig. 4A). The PBP2X structure includes a molecule of the β-lactam antibiotic cefuroxime interacting with one of the PASTA repeats. The portion of cefuroxime bound to the PASTA repeat is structurally analogous to unlinked peptidoglycan, suggesting that these repeats bind peptidoglycan (194). This hypothesis was confirmed by demonstrating that peptidoglycan bound the PASTA repeats of *B. subtilis* PrkC, providing the first evidence for a ligand of a bacterial receptor eSTK (160; also see "Physiological roles and targets of eSTKs in bacteria. (i) Developmental processes"). This observation, together with the structural studies of PknB proteins from *M. tuberculosis* and *S. aureus*, supports a ligand-induced dimerization model (see above and Fig. 3) in which the activation of these eSTKs results from dimerization of two kinase molecules that bind peptidoglycan through their extracellular PASTA repeats (12, 160). *M. tuberculosis* PknD is also a transmembrane eSTK, but its catalytic domain is linked to an extracellular domain consisting of a rigid β propeller with six blades symmetrically arranged around a central pore (56) (Fig. 4B). While the ligand(s) of PknD remains unknown, this motif is found in eukaryotic proteins with a wide variety of functions, and among bacteria, it appears to be present only in pathogenic mycobacteria.

Physiological roles and targets of eSTKs in bacteria. Open reading frames encoding eSTKs are found in many sequenced microbial genomes (51). However, complete characterization of their essentiality and the identification of their specific substrates have been difficult to achieve, likely resulting from functional redundancy and/or substrate promiscuity. Table 1 lists the identified substrates of eSTKs, but it should be emphasized that the majority of these substrates were identified by phosphoproteomic approaches and/or *in vitro* kinase assays and site-directed mutagenesis and lack *in vivo* confirmation. In addition, only limited information exists concerning the effect of phosphorylation on the activity of substrates.

Eukaryotic Ser/Thr kinases form complex signaling networks that involve phospho-dependent protein-protein interactions mediated by conserved protein modules or domains (137). One example found in some bacterial proteins is the FHA (forkhead-associated) domain, which interacts preferably with phosphorylated Thr residues (62). Proteins containing FHA domains include *M. tuberculosis* GarA, which is involved in glycogen recycling and the tricarboxylic acid cycle (14, 121). GarA is the substrate of several kinases, including PknB and PknG (128, 189; also see "Physiological roles and targets of eSTKs in bacteria. (i) Developmental processes"), and its FHA domain interacts with the phosphorylated activation loop of PknB. This interaction led to a model of substrate recruitment in which the PknB activation loop provides a secondary docking site for FHA-mediated binding (189). Alternatively, this

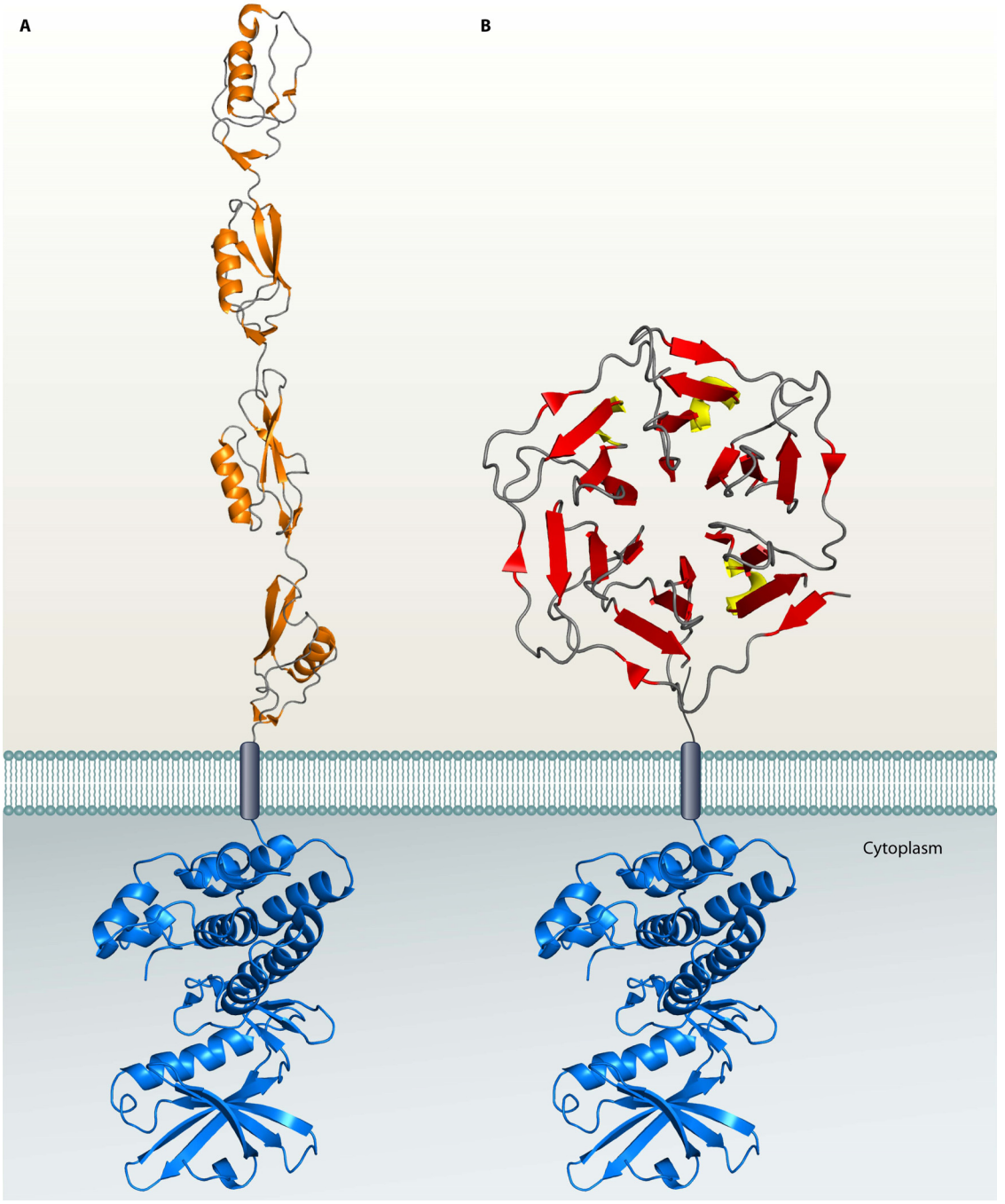

FIG. 4. Structures of *M. tuberculosis* PknB and PknG sensor domains. (A) The extracellular domain of *M. tuberculosis* PknB is composed of four PASTA repeats organized linearly (PDB accession number 2KUI) (12). (B) The highly symmetric six-bladed β propeller formed by the extracellular domain of *M. tuberculosis* PknG kinase (PDB accession number 1RWI) (56). The PknB catalytic domain is shown to represent the intracellular catalytic kinase domain.

interaction may stabilize the PknB activation loop and thereby activate the kinase, reminiscent of the case for many eukaryotic kinases. Regardless of the exact activation mechanism, the PknG/PknB-GarA interaction results in the phosphorylation of GarA at Thr21 (PknG) or Thr22 (PknB and other kinases) (128, 189), and this modification inhibits its function (44, 123).

The *M. tuberculosis* Ser/Thr phosphoproteome was recently analyzed, and the phosphorylation site motif for the *M. tuberculosis* eSTKs was determined (142). The identified motif, Xαααα**T**X(X/V)ϕ(P/R)I, in which T corresponds to the phosphorylated Thr residue, α corresponds to an acidic residue, and ϕ corresponds to a large hydrophobic residue, is shared by 6 of the 11 *M. tuberculosis* kinases (PknA, PknB, PknD, PknE, PknF, and PknH). There are, however, differences in the optimal substrate sequences for each kinase, including the hydrophobic residue at the third position after the phospho-Thr

TABLE 1. eSTKs in bacteria[a]

Species	eSTK	Substrate	Function	Phospho-residue(s)	Methodology	Reference(s)
Bacillus subtilis	PrkC	AlsD	α-Acetolactate decarboxylase; central metabolism	S88	*In vitro* kinase assay, mass spectrometry	1a, 141
		CpgA	GTPase; peptidoglycan deposition	Thr(s)/Ser(s)	*In vitro* kinase assay, site-directed mutagenesis	1
		EF-G	Elongation factor; protein translation	Thr(s)	*In vivo* immunoprecipitation, *in vitro* kinase assay	50, 159, 160
		EF-Tu	Elongation factor; protein translation	T384	*In vitro* kinase assay, site-directed mutagenesis	1
		GlnA	Glutamine synthetase; central metabolism	S207, T26, T147, T286	*In vitro* kinase assay, mass spectrometry	141
		Icd	Central metabolism	T138, T14, T396	*In vitro* kinase assay, mass spectrometry	141
		HPr	Kinase, phosphotransferase system	S12	*In vitro* kinase assay, mass spectrometry	141
		YezB	Stress	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis, 2D electrophoresis	1
		YwjH	Transaldolase; central metabolism	S55, T26, T82, T125, T159, T184	*In vitro* kinase assay, mass spectrometry	141
Chlamydia trachomatis	Pkn1	IncG	Pathogenesis	NA	Bacterial two-hybrid assay, *in vitro* kinase assay	186
	PknD	Pkn1	eSTK	NA	Bacterial two-hybrid assay, immunoprecipitation, *in vitro* kinase assay	186
Corynebacterium glutamicum	PknA	FtsZ	Cell division	T108	*In vitro* kinase assay, 2D electrophoresis, mass spectrometry	157
		MurC	Cell wall synthesis	T51, T120, T133, T16, T362, T365	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	48
		PknG	Soluble eSTK	T451, T787	*In vitro* kinase assay	49
		OdhI	Glutamate catabolism	T15	*In vitro* kinase assay, mass spectrometry	13, 49, 157
	PknB	FtsZ	Cell division	NA	*In vitro* kinase assay	157
		OdhI	Glutamate catabolism	T15	*In vivo* and *in vitro* kinase assays	13, 49, 157
	PknG	OdhI	Glutamate catabolism	T14	*In vivo* 2D electrophoresis, *in vitro* kinase assay, mass spectrometry, site-directed mutagenesis	49, 121, 157
	PknL	FtsZ	Cell division	T63, S353, T388	*In vitro* kinase assay, 2D electrophoresis, mass spectrometry	157
Mycobacterium tuberculosis	PknA	EmbR	Arabinan synthesis, cell wall	NA	*In vitro* kinase assay	162
		FadD	Mycolic acid biosynthesis	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	109
		FabH	Mycolic acid pathway; cell wall biosynthesis	T45	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	187
		FipA	Cell division under oxidative stress	T77	*In vivo* and *in vitro* kinase assays, mass spectrometry	175
		FtsZ	Cell division	T343	*In vitro* kinase assay, mass spectrometry	175, 179
		GlmU	Cell wall synthesis	Thr(s)	*In vitro* kinase assay	135
		GroEL1	Heat shock protein	NA	*In vitro* kinase assays, mass spectrometry	23
		KasA	Mycolic acid biosynthesis	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	109
		KasB	Mycolic acid biosynthesis	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	109
		MabA	Mycolic acid biosynthesis	T21, T114, T191	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	188
		MurD	Cell division	NA	*In vitro* kinase assay	178
		PknB	eSTK	NA	*In vitro* kinase assay	75
		Rv1422	NA	T325	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	75
		Wag31	Cell division	T73	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	75

PknB	EmbR	Arabinan synthesis, cell wall	NA	*In vitro* kinase assay	162
	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	GarA	Glycogen recycling, tricarboxylic acid cycle	T22	Phosphoproteomic kinase assays, site-directed mutagenesis	189
	GlmU	Cell wall synthesis	Thr(s)	*In vitro* kinase assay	135
	GroEL1	Heat shock protein	NA	*In vitro* kinase assays, mass spectrometry	23
	KasA	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	KasB	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	MabA	Mycolic acid biosynthesis	T21, T114, T191	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	188
	PBPA	Cell wall synthesis	T437, T362	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	32
	PknA	eSTK	NA	*In vitro* kinase assay	75
	RshA	SigH anti-sigma factor, oxidative stress	T94	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	136
	Rv0020c	FHA-containing protein	NA	*In vitro* kinase assay	60
	Rv1422	NA	T325	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	75
	Rv1747	Putative ABC transporter	NA	*In vitro* kinase assay	60
	SigH	Alternate sigma factor; oxidative stress	T26, T106	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	136
PknD	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	FabH	Mycolic acid pathway, cell wall biosynthesis	T45	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	187
	GarA	Glycogen recycling, tricarboxylic acid cycle	NA	*In vitro* kinase assays	189
	GroEL1	Heat shock protein	NA	*In vitro* kinase assays, mass spectrometry	23
	KasA	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	KasB	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	MabA	Mycolic acid biosynthesis	T21, T114, T191	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	188
	Mmpl7	Membrane transporter; resistance, nodulation, and cell division family	NA	2D electrophoresis, mass spectrometry	139
	Rv0516c	Anti-anti-sigma factor	T2	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	59a
	Rv1747	Putative ABC transporter	NA	*In vitro* kinase assay	60
PknE	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	FabH	Mycolic acid pathway, cell wall biosynthesis	T45	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	187
	GarA	Glycogen recycling, tricarboxylic acid cycle	NA	*In vitro* kinase assays	189
	GroEL1	Heat shock protein	NA	*In vitro* kinase assays, mass spectrometry	23
	KasA	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	KAsB	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	MabA	Mycolic acid biosynthesis	T21, T114, T191	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	188
	Rv1747	Putative ABC transporter	NA	*In vitro* kinase assay	60
PknF	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	112
	FabH	Mycolic acid synthesis, cell wall	T45	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	187
	GarA	Glycogen recycling, tricarboxylic acid cycle	NA	*In vitro* kinase assays	189

Continued on following page

TABLE 1—*Continued*

Species	eSTK	Substrate	Function	Phospho-residue(s)	Methodology	Reference(s)
		GroEL1	Heat shock protein	T25, T54	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis	23
		KasA	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		KasB	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		Rv0020c	FHA-containing protein	NA	*In vitro* kinase assay	60
		Rv1747	Putative ABC transporter	NA	*In vitro* kinase assay	112
	PknG	GarA	Glycogen recycling, tricarboxylic acid cycle	T21	Kinase assays, mass spectrometry, site-directed mutagenesis, *in vivo* studies	128
	PknH	DacB1	Penicillin-binding protein, cell wall	Thr(s)	*In vitro* kinase assay, site-directed mutagenesis	201
		DosS	Dormancy	T198, T205	*In vitro* kinase assays, mass spectrometry, site-directed mutagenesis	25
		EmbR	Arabinan synthesis, cell wall	Thr(s)	*In vitro* kinase assay	111
		FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		FabH	Mycolic acid pathway, cell wall biosynthesis	T45	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	187
		GroEL1	Heat shock protein	NA	*In vitro* kinase assays, mass spectrometry	23
		KasA	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		KasB	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		Rv0681	TetR class transcription factor	T170	*In vitro* kinase assay, site-directed mutagenesis	201
	PknI	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
	PknJ	Pyruvate kinase A	Glycolysis	S37	*In vitro* kinase assay	6
		EmbR	Arabinan synthesis, cell wall	NA	*In vitro* kinase assay	71
		MmaA4	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	71
		PepE	Dipeptidase	NA	*In vitro* kinase assay	71
	PknK	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		VirS	Transcriptional regulator	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	84
	PknL	FadD	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		GroEL1	Heat shock protein	NA	*In vitro* kinase assays, mass spectrometry	23
		KasA	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		KasB	Mycolic acid biosynthesis	NA	*In vitro* kinase assay	109
		MabA	Mycolic acid biosynthesis	T21, T114, T191	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	188
		Rv2175c	DNA-binding protein, putative cell wall/cell division protein	T9	*In vivo* and *in vitro* kinase assays, mass spectrometry, site-directed mutagenesis, phospho-amino acid analysis, 2D electrophoresis	24, 27
Mycoplasma pneumoniae	PrkC	HMW1-3	Cytadherence	NA	Pro-Q Diamond staining of cell lysates	155
		MPN474	Surface protein	NA	Pro-Q Diamond staining of cell lysates	155
		P1	Adhesin	NA	Pro-Q Diamond staining of cell lysates	155
Myxococcus xanthus	Pkn2	HUα	Histone-like protein	T59	Suppressor screen in *E. coli*, *in vitro* kinase assay, site-directed mutagenesis	182
		β-Lactamase	Ampicillin resistance	Thr(s)	Phospho-amino acid analysis	183
	Pkn4	PFK	Glycolysis	T226	*In vitro* kinase assay, site-directed mutagenesis	117
	Pkn8	Pkn14	Soluble eSTK	Thr(s)	*In vitro* kinase assay	119
	Pkn14	MrpC	Transcription factor; fruiting body development	Thr(s)	*In vitro* kinase assay	119
		PFK	Glycolysis	T226	*In vitro* kinase assay, site-directed mutagenesis, phospho-amino acid analysis	117
Pseudomonas aeruginosa	PpkA	Fha1	Protein secretion, virulence	T362	*In vitro* kinase assay, mass spectrometry, site-directed mutagenesis	66, 113

Staphylococcus aureus	PknB	AFT-2	Human transcription factor	Thr73	Peptide arrays, *in vitro* kinase assay, mass spectrometry	107
		MgrA	Global transcriptional regulator	NA	*In vitro* kinase assay	181
		PurA	Purine biosynthesis	NA	*In vitro* kinase assay	39
		SarA	Transcription regulator; virulence	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	38
		SA0498	Ribosomal protein L7/L12; central metabolism	Thr(s), Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA0545	Phosphate acetyltransferase; central metabolism	Thr(s), Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA0729	Triose isomerase; central metabolism	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA0731	Enolase; central metabolism	Thr(s), Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA0944	Pyruvate dehydrogenase; central metabolism	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA1359	Elongation factor P; central metabolism	Thr(s), Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA1499	Trigger factor; central metabolism	Thr(s), Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA2340	Glyoxalase; central metabolism	Thr(s), Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
		SA2399	Fructose biphosphate aldolase; central metabolism	Thr(s)	*In vitro* kinase assay, phospho-amino acid analysis	96
Streptococcus agalactiae	Stp1	CovR	Response regulator; toxin expression	T65	*In vitro* kinase assay	93, 145
		DivIA	Cell division	NA	*In vivo* phosphopeptide enrichment approach, *in vitro* kinase assay	168
		PpaC	Mn-dependent inorganic pyrophosphatase; virulence	Ser(s)	*In vitro* kinase assay, phospho-amino acid analysis	144
		PurA	Purine biosynthesis	NA	*In vitro* kinase assay	146
Streptococcus pneumoniae	StkP	DivIVA	Cell division	Thr(s)	*In vivo* 2D electrophoresis, mass spectrometry, *in vitro* kinase assay	124
		FtsZ	Cell division	Thr(s)	*In vitro* kinase assay	55
		GlmM	Cell wall	NA	2D electrophoresis, *in vitro* kinase assay	125
		PpaC	Mn-dependent inorganic pyrophosphatase; virulence	Thr(s)	*In vivo* 2D electrophoresis, mass spectrometry, *in vitro* kinase assay	124
		RitR	Transcriptional regulator; iron transport	NA	*In vitro* kinase assay	184
		Spr0334	NA	Thr(s)	*In vivo* 2D electrophoresis, mass spectrometry, *in vitro* kinase assay	124
Streptococcus pyogenes	SP-STK	SP-HLP	Histone-like protein	Thr(s)	*In vitro* kinase assay	72
Streptomyces coelicolor	AfsK	AfsR	Transcriptional factor; secondary metabolism	Ser(s), Thr(s)	*In vitro* kinase assay	102
Yersinia spp.	YpkA	Eukaryotic actin	Cytoskeleton	NA	*In vitro* kinase assay	74

[a] NA, not available.

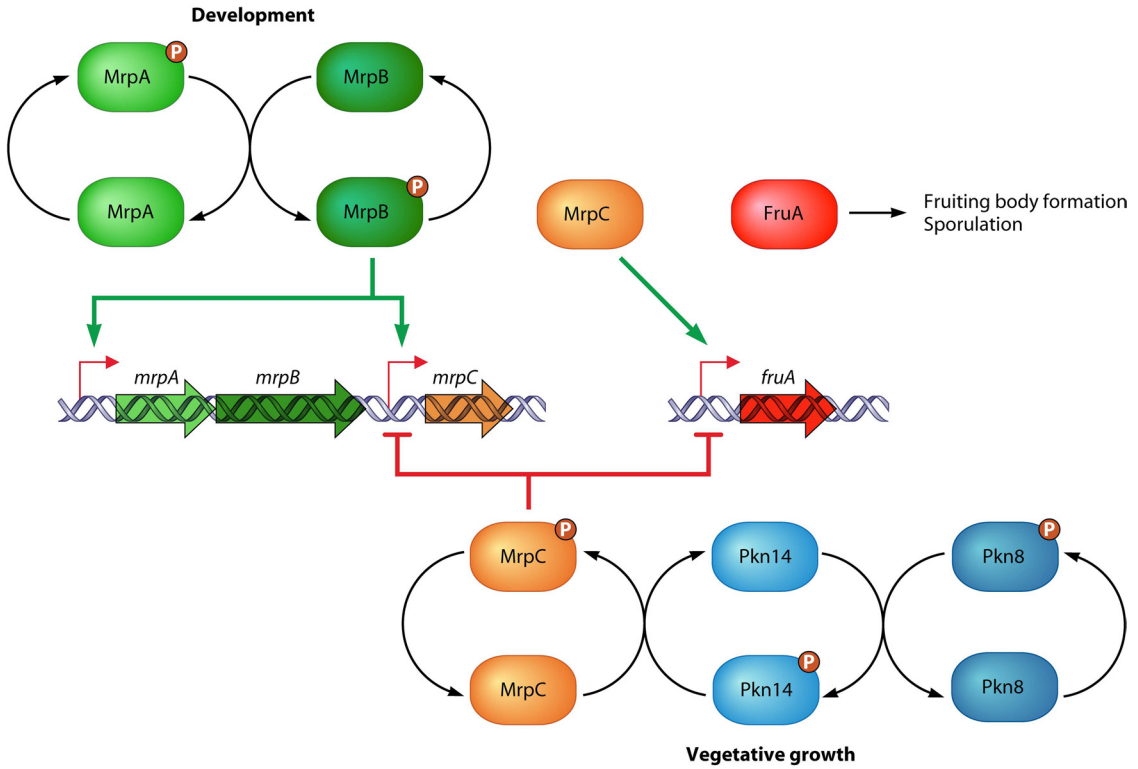

FIG. 5. Regulation of the developmental cycle of *Myxococcus xanthus*. The *M. xanthus* transcriptional activator MrpC controls the expression of *fruA*, which encodes a major regulator of fruiting body formation and sporulation. MrpC is itself under dual regulation of an eSTK signaling cascade and a TCS. During vegetative growth, phosphorylation by the eSTK PknB14/8 signaling cascade decreases MrpC's affinity for the *fruA* promoter and its own promoter. In response to starvation signals, the MrpA/MrpB TCS activates the transcription of *mrpC* as well as itself, leading to the expression of *fruA* and the consequent developmental commitment.

residue, the positions of acidic residues preceding the phospho-Thr residue, and the preference for Pro/Arg at the fourth position after the phospho-Thr residue. The inability of a Ser residue to substitute for a Thr residue as the phospho-acceptor for any kinase demonstrates the specificity of the kinase-substrate interaction. The phosphorylation sites of several substrates of *S. aureus* PknB were also analyzed, and although the most frequently found residues were serine and threonine, tyrosine phosphorylation was also identified (107). In addition, a proline residue was found next to the phosphorylated residue, indicating that PknB is the first identified prokaryotic representative of the proline-directed kinases, a major family of eukaryotic regulators involved in cellular physiology (also see "Physiological roles and targets of eSTKs in bacteria. (iv) Essentiality, virulence, and central metabolism").

In the following sections, we discuss some examples that illustrate the diversity of biological processes and the complexity of signaling pathways in which bacterial eSTKs are involved. We describe the identified substrates and, if known, how phosphorylation affects their activity.

(i) Developmental processes. *M. xanthus* grows unicellularly and forms large swarms of cells in the presence of nutrients. However, cells aggregate upon nutrient depletion and form multicellular fruiting bodies composed of around 100,000 cells. If unfavorable conditions persist, the aggregated cells can form metabolically dormant myxospores until nutrients become available again. The tight regulation of this complex developmental cycle has been reviewed in detail (69). Briefly, FruA is a key transcription factor that regulates the expression of a large number of genes involved in both fruiting body formation and sporulation. Another transcription factor, MrpC, controls *fruA* expression and is itself under a dual regulatory mechanism involving both a TCS and an eSTK. Transcription of *mrpC* is controlled by the MrpA/MrpB TCS, encoded on the same locus. In response to starvation signals, the sensor His kinase MrpA is activated and phosphorylates the response regulator MrpB, which in turn positively regulates *mrpC* expression, leading to aggregation. The eSTK Pkn14 also regulates MrpC by phosphorylating it on a Thr residue(s), reducing its affinity for both its own promoter and the *fruA* promoter (119, 120). The transmembrane eSTK Pkn8 phosphorylates Pkn14, and it has been suggested that Pkn8 and -14 form an eSTK cascade that inhibits the activity of MrpC during vegetative growth (69, 119) (Fig. 5). Consistent with this model, MrpC accumulated in Δ*pkn8* and Δ*pkn14* deletion strains, leading to *fruA* expression during early stationary phase and, consequently, to a faster developmental phenotype (119). Similar observations were reported for other organisms, such as cyanobacteria (197, 198) and streptomycetes (115, 190).

Bacillus subtilis forms heat- and desiccation-resistant spores under conditions of nutrient limitation. These dormant spores germinate when stimulated by various nutrient sources, including specific amino acids. Peptidoglycan fragments released by growing cells can also induce germination, and the transmem-

brane eSTK PrkC is necessary for this response (160). Peptidoglycan fragments bind the PASTA repeats in the extracellular domain of PrkC and lead to kinase activation and subsequent germination. The essential translational GTPase elongation factor EF-G is phosphorylated in this PrkC-dependent germination pathway (160). PrkC-dependent phosphorylation of EF-G on a Thr residue(s) *in vivo* has also been described for vegetative *B. subtilis* cells (45, 50, 91, 98).

In addition to the phylogenetically conserved phosphorylation of EF-G identified in the phosphoproteomes of *Corynebacterium glutamicum* (17), *E. coli* (97), and *Mycoplasma pneumoniae* (156), other proteins involved in translation have been implicated as targets of eSTKs. Ser/Thr phosphorylation of another elongation factor, EF-Tu, has been reported for *E. coli* (158), *Thermus thermophilus* (95), *Streptomyces coelicolor* (65, 106), *B. subtilis* (1, 45), and *S. pneumoniae*. Importantly, EF-Tu phosphorylation inhibited binding to amino-acylated tRNA and to kirromycin, an antibiotic that inhibits the elongation step of translation by preventing the release of EF-Tu–GDP from the ribosome (5). For *Streptomyces collinus*, a ribosome-associated kinase was reported to phosphorylate several ribosomal proteins on Ser and Thr residues, which led to a significant reduction in protein synthesis (104, 105). Finally, Ser/Thr phosphorylation of a number of other ribosomal and ribosome-associated proteins has been observed in phylogenetically diverse bacteria by use of phosphoproteomic methodologies (17, 97, 125, 142, 156, 171, 174).

Regulation of translational initiation and elongation by phosphorylation in response to diverse stimuli is well established for eukaryotes (21). A bacteriophage T7 kinase expressed during *E. coli* infection phosphorylates EF-G and ribosomal protein S6, suggesting that this modification is important for the specific translation of phage mRNAs (148). An appealing but as yet untested hypothesis is that eSTKs direct the protein translation machinery to specific mRNAs that are required in the transition between different growth stages or in response to particular growth conditions.

(ii) Secondary metabolism. *Streptomyces* spp. are Gram-positive filamentous soil bacteria that undergo complex morphological differentiation and synthesize a wide variety of small molecules, including many clinically relevant antibiotics. The production of actinorhodin by *S. coelicolor* is under the control of the regulator AfsR, which itself is a target of the membrane-associated eSTK AfsK (102). AfsK phosphorylates AfsR *in vitro* on a Ser and/or Thr residue(s), and the production of actinorhodin is reduced in a Δ*afsK* strain. Also, the transcription of *afaS*, a gene located immediately downstream of *afsR* that encodes a small protein involved in secondary metabolism, is delayed in a Δ*afsK* mutant. Phosphorylation of AfsK was shown to both increase its affinity for the *afsS* promoter and modulate AfsR ATPase activity, which is essential to activate the expression of *afsS*, resulting in the production of actinorhodin (87). The activity of AfsK itself is under regulation. The protein KbpA interacts with the AfsK kinase domain in its unphosphorylated form and subsequently inhibits AfsK autophosphorylation and, consequently, its activation (185). KbpA is encoded by a gene located upstream of *afsK*, and its transcription increases in response to the production of actinorhodin, suggesting that KbpA controls the production of secondary metabolites by modulating AfsK activity. In addition to AfsK, AfsR is also phosphorylated *in vitro* by at least two other eSTKs, AfsL and PkaG (151).

(iii) Cell division and cell wall synthesis. A large number of eSTK substrates involved in cell division or cell shape have been reported. Here we focus on proteins with either mutant/overexpression phenotypes or direct *in vivo* phosphorylation. Those for which only *in vitro* data support phosphorylation are listed in Table 1. Given the presence of numerous eSTKs in bacteria such as *M. tuberculosis* (110) and the potential for *in vitro* artifacts (101), caution must be used in interpreting *in vitro* phosphorylation data to make definitive assignments of physiologically relevant kinase-substrate interactions. For example, PknG is the primary kinase responsible for phosphorylating *M. tuberculosis* GarA (128); however, PknB, PknD, PknE, and PknF also phosphorylate GarA *in vitro* (189).

Bacterial operons often contain functionally related genes, and there is a well-established relationship between bacterial morphology and the order of genes in a cluster involved in cell division (177). Thus, the presence of eSTK genes such as those for *M. tuberculosis* PknA and PknB in the operon containing the genes encoding penicillin-binding protein A (PBPA) and RodA suggests a role for these eSTKs in cell shape. Both *pknA* and *pknB* are expressed preferentially during exponential growth phase, and their overexpression results in long, broad, and in some cases branched cells, whereas partial depletion results in long cells (75). PknB phosphorylated PBPA on Thr362 and Thr437 when the proteins were coexpressed in *E. coli* (32). Moreover, a PBPA Thr437Ala mutant did not complement a *pbpA* deletion in a heterologous system and led to an increased number of nucleoids per cell and to mislocation of the protein. Another *in vivo* substrate of PknB is Wag31, a homolog of the essential cell shape/cell division protein DivIVA, and overexpression of a mutated form of Wag31 in which the Thr residue identified as the phosphoacceptor was replaced by a Glu residue resulted in morphological defects (75). PknA phosphorylates FtsZ, a homolog of eukaryotic tubulin and a major component of the division septum, on a Thr residue(s) *in vitro* (179). The coexpression of both proteins in *E. coli* resulted in significantly reduced FtsZ GTPase and polymerization activities. The *S. pneumoniae* eSTK StkP colocalized with FtsZ at midcell (55) and interacted with and phosphorylated FtsZ *in vitro*, but this modification was not confirmed *in vivo*. In the actinobacterium *Corynebacterium glutamicum*, overexpression of the PknA and PknB kinases also resulted in growth and morphological defects (49). In addition, PknA inhibits the activity of the muropeptide ligase MurC by phosphorylating it on multiple Thr residues *in vivo* (48). The enzyme GlmM, which catalyzes the interconversion of *N*-acetylglucosamine-6-phosphate into GlcN-1-P, which serves as a precursor for peptidoglycan synthesis, is activated by phosphorylation in *E. coli* (73) and is also a substrate of *S. pneumoniae* StkP (125).

The involvement of eSTKs in cell wall synthesis and division has been reported for other Gram-positive pathogens. For example, a *Staphylococcus aureus* Δ*pknB* strain grew slower and exhibited severe cell division defects, including multiple and incomplete septa, bulging, and irregular cell size (16). Consistent with this phenotype, a Δ*pknB* mutant also revealed a strong regulatory impact on the expression of genes encoding proteins involved in cell wall metabolism and autolysis (39),

and this strain showed increased susceptibility to antimicrobials that target the cell wall (16, 39). The importance of PknB and its cognate phosphatase in the synthesis of peptidoglycan and wall teichoic acid was further supported by the impact of the Δ*pknB* mutation on *S. aureus* metabolism (92). Similar division and growth defects, as well as antibiotic susceptibility phenotypes, were described for *Streptococcus pyogenes*, *Streptococcus agalactiae*, *S. pneumoniae*, and *Streptococcus mutans* (9, 37, 72, 132, 144, 149, 174), as well as *Enterococcus faecalis* (82).

(iv) Essentiality, virulence, and central metabolism. While eSTKs regulate many essential bacterial processes, most eSTKs are not themselves essential, with *M. tuberculosis* PknA and PknB being prominent exceptions (47, 75, 150). The reported essentiality of the *C. glutamicum* PknA and PknB homologs appears to be strain specific (49, 157). While mutations in *B. subtilis* PrkC (50, 159) or in the eSTKs of several streptococcal species (68, 72, 144) revealed that these proteins are not, strictly speaking, essential, they led to significant stationary-phase survival defects. Two plausible explanations for this discrepancy are the existence of one or more kinases with redundant functions and the fact that the *in vitro* conditions in which the majority of the studies were carried out differed considerably from the *in vivo* conditions.

Several eSTKs appear to have important roles in bacterial pathogenesis. An *M. tuberculosis* Δ*pknG* strain exhibited delayed mortality in SCID mice and reduced viability in a BALB/c mouse infection model, with significant reductions in the bacillary loads in the lungs, spleen, and liver (29). Similarily, an *M. tuberculosis* Δ*pknH* mutation increased the bacillary load in a BALB/c mouse infection model (133). PknH phosphorylates the transcription activator EmbR (involved in antibiotic production in *S. coelicolor* [see above]) through interaction with its FHA domain (111; also see "Structural and functional studies of bacterial eSTKs"). Phosphorylation of EmbR activates the expression of the *embCAB* operon, which encodes arabinosyltransferases involved in the metabolism of arabinan, an essential component of the mycobacterial cell wall, and resistance to ethambutol (163). Since EmbC catalyzes the conversion of lipomannan into lipoarabinomannan, a cell wall lipoglycan that prevents the host proinflammatory response (20), PknH may modulate the host response during infection by controlling the ratio of these two cell wall components (163). PknH also phosphorylates DosR on two Thr residues, and DosR together with DosS controls the DosRS regulon responsible for the nitric oxide and hypoxia response, an important evasive response to host macrophages (25).

The *Yersinia pseudotuberculosis* eSTK YpkA is absolutely essential for virulence (52). YpkA is produced in an inactive form that is activated upon secretion into the host cell and interaction with the host actin (74). Activation of YpkA results in disruption of the host actin cytoskeleton, presumably leading to the inhibition of macrophage function during infection. *S. aureus* PknB was also found to be released into the external milieu (107), with the first report of a prokaryotic proline-directed kinase with the potential to phosphorylate host target proteins involved in essential processes such as signal transduction, cell communication, gene regulation, and immune responses (also see "Physiological roles and targets of eSTKs in bacteria" and Table 1). The eSTK PrkC is required for *M. pneumoniae* adhesive growth and cytotoxicity (155) (Table 1). PrkC phosphorylates several cytadherence proteins as well as the major adhesin P1 and the surface protein MPN474, and it was observed that these modifications affect the accumulation of these proteins. Homologs of PrkC have also been implicated in *S. pyogenes* adherence and invasion and in *E. faecalis* persistence (72, 82). The involvement of eSTKs in virulence has also been reported for several other pathogens, such as *Streptococcus pneumoniae*, *E. faecalis*, *Pseudomonas aeruginosa*, and *Salmonella enterica* serovar Typhi (33, 43, 46, 144–146, 191).

M. tuberculosis PknG exemplifies the involvement of eSTKs in central metabolism. A Δ*pknG* mutant showed higher intracellular levels of glutamate, glutamine, and derivatives, suggesting that PknG controls glutamate synthesis in response to nutritional stress signals (29). Consistent with this hypothesis, deletion of the *C. glutamicum pknG* homolog impaired glutamine utilization. OdhI, an FHA-containing protein that inhibits the 2-oxoglutarate dehydrogenase (ODH) complex of the tricarboxylic acid cycle, was inhibited by phosphorylation and identified as an *in vivo* PknG substrate (121). Similarly, *M. tuberculosis* PknG phosphorylates GarA, an OdhI homolog, on Thr21 *in vivo* (128).

Ser/Thr PROTEIN PHOSPHATASES

Reversible phosphorylation occurs in organisms from bacteria to humans (166). In prokaryotes, the need for dedicated phosphatases was not initially appreciated in the context of two-component systems and phosphorelay signal transduction (64), since both phosphohistidine and aspartyl-phosphate residues undergo relatively rapid hydrolysis (167, 172, 196), and therefore a dedicated enzyme to remove the phosphate groups was not thought to be necessary. In contrast, phosphorylated Ser, Thr, and Tyr residues are not as labile, and therefore cognate phosphatases can be necessary in order to quench signaling cascades (3). Homologs of four eukaryotic protein phosphatase superfamilies are present in archaea and bacteria, including conventional and low-molecular-weight protein tyrosine phosphatases, phosphoprotein phosphatases (PPPs), and metal-dependent phosphatases (PPMs) (78, 165). We focus here on the last two classes (for a review of tyrosine phosphatases, see reference 10). Eukaryote-like serine/threonine phosphatases (eSTPs) belong to two structurally distinct phosphatase families: PPPs and PPMs. The PPP family members are formally described as serine/threonine phosphatases but can also dephosphorylate phosphohistidine and phosphotyrosine residues (10). For example, PrpE, a *B. subtilis* PPP family member, exclusively removes phosphate groups from phosphotyrosines *in vitro* (70). Other PPP family members have been annotated for bacteria and archaea, and *in vitro*, most are dual-specificity phosphatases that dephosphorylate tyrosine residues in addition to serine and threonine residues (85, 88, 108, 164, 170).

The serine/threonine Mg^{2+}- or Mn^{2+}-dependent phosphatases of the PPM family share a conserved catalytic domain with eukaryotic PP2C that contains 11 to 13 signature motifs containing eight conserved amino acids (2, 19, 78, 165, 199) (Fig. 6A). Bacterial PPMs can be divided into two subfamilies depending on the presence of motifs 5a and 5b (199). One subfamily, which includes the *B. subtilis* sporulation-specific

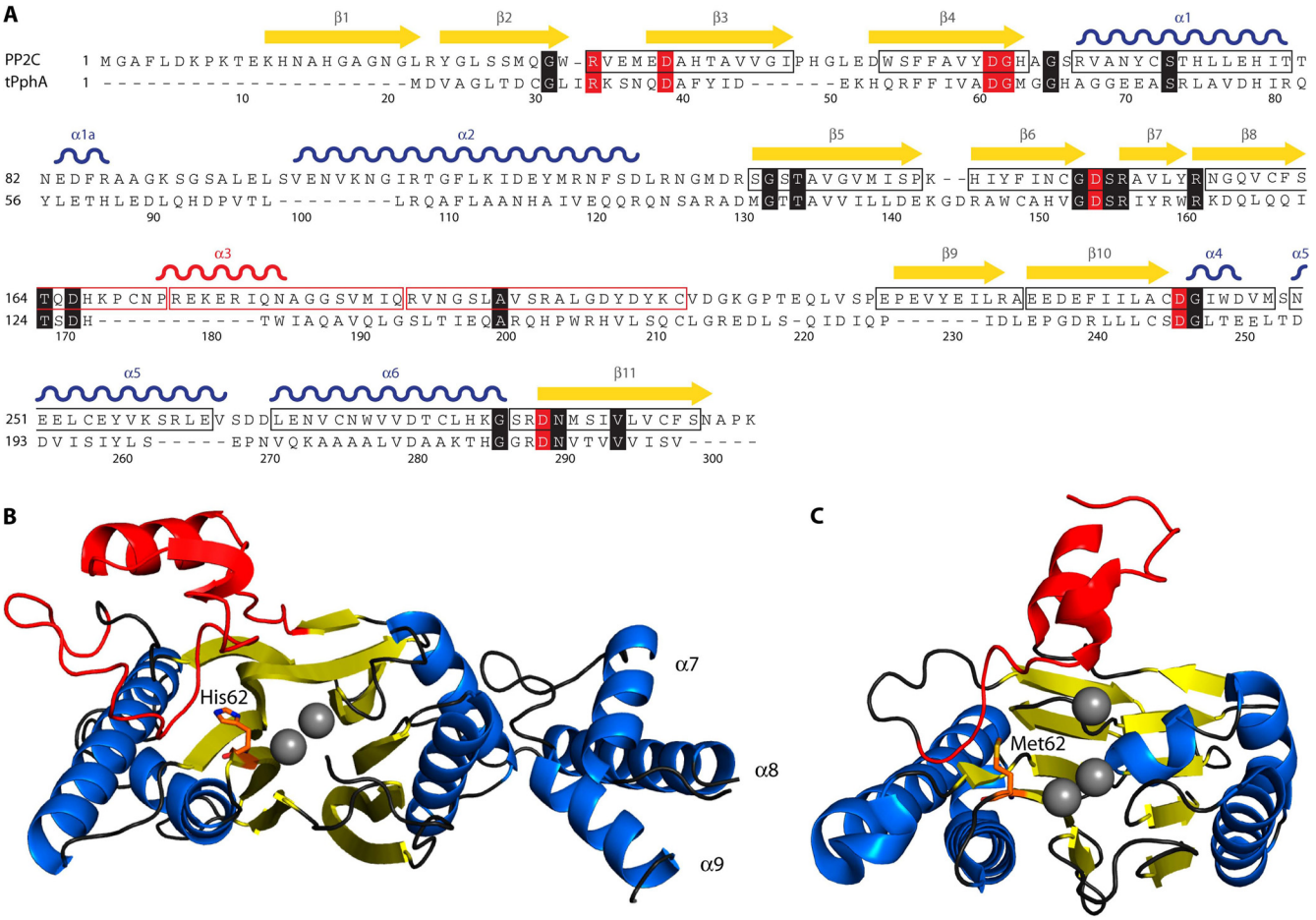

FIG. 6. Ser/Thr phosphatase structures. (A) Primary sequence alignment of human PP2C (residues 1 to 299) and PphA (residues 1 to 241) catalytic domains. Conserved amino acids are indicated in bold, and those that also form part of the metal-binding pocket are shown in red. Motifs 1 to 5, 5a, 5b, and 6 to 11, as defined by Bork et al. (19), are indicated in boxes. (B) Crystal structure of the human PP2C structure (PDB accession number 1A6Q). The β-sandwich is represented in yellow, α-helices are represented in blue, the large irregular loop is shown in red, and two manganese ions are shown in gray. His62 (orange) is predicted to be a general acid. The C-terminal domain (α7, α8, and α9) is characteristic of mammalian PP2C and is absent from prokaryotic family members. (C) Crystal structure of the *Thermosynechococcus elongatus* eSTP PphA (PDB accession number 2J86). The β-sandwich is shown in yellow, the α-helices are shown in blue, the three magnesium ions are shown in gray, and the large irregular loop is shown in red. The flap subdomain (red) appears flexible and is thought to be involved in substrate binding and catalytic activity. Instead of His62, Met62 (orange) occupies the homologous position.

phosphatase SpoIIE (40) and the stress response phosphatases RsbU and RsbX (34, 42, 76, 126, 193), lacks the 5a and 5b catalytic domain motifs (77, 199). This subfamily also includes phosphatases that are cognate to non-eukaryote-like serine/threonine kinases and is not discussed further. Members of the second subfamily (eSTPs) contain all 11 signature motifs and are cognate phosphatases to the eSTKs discussed above. This subfamily is discussed in detail in the next section and is of particular biological importance, as there are no known inhibitors of these proteins.

PPM Family eSTPs

eSTPs have been characterized for both Gram-negative and Gram-positive bacteria (Table 2). While typically there appears to be one cognate phosphatase per kinase and phosphatases are often encoded in the same operon as their partner eSTKs, *M. tuberculosis* has 11 eSTKs and a single eSTP (7, 80). The physiological relevance of this discrepancy has yet to be ascertained, but it suggests that, at least in *M. tuberculosis*, phosphatases are subject to complex regulation.

Structure and function of eSTPs. The conserved catalytic core domain of human PP2C contains a central β-sandwich comprised of two five-stranded antiparallel β sheets, each flanked by a pair of antiparallel α helices (31) (Fig. 6B). A binuclear metal center is located within the channel of the β-sandwich, with the two metal ions located at the base of the cleft and hexa-coordinated with water and amino acids. The mechanism of dephosphorylation is thought to occur by metal-activated water nucleophilic attack of the phosphorus atom. This mechanism is supported by the high level of conservation of active site residues among eukaryotic and bacterial homologs. There are three additional α helices (helices 7 to 9) that associate with the core domain and are also hypothesized to be involved in substrate specificity and/or regulation, but these are absent in bacterial PP2C family members (Fig. 6B).

TABLE 2. eSTPs in bacteria[a]

Species	eSTP	Type	Partner kinase	Substrate	Function	Phospho-residue(s)	Methodology	Reference
Bacillus anthracis	Ba-Stp1	PP2C	Ba-Stk1	Ba-Stk1-P	eSTK	NA	*In vitro* phosphatase assay	161
Bacillus subtilis	PrpC	PP2C	PrkC	EF-G	Translation factor	NA	*In vitro* phosphatase assay	50
				CpG	Small ribosome-associated GTPase	NA	*In vitro* phosphatase assay	1
				EF-Tu	Translation factor	NA	*In vitro* phosphatase assay	1
				HPr kinase	Phosphotransferase system	S46	*In vitro* phosphatase assay	169
				PrkC-P	eSTK	NA	*In vitro* phosphatase assay	129
				YezB	Stress response	NA	*In vitro* phosphatase assay	1
	SpoIIE	PPM		SpoIIAA	Phosphoprotein anti-anti-sigma factor	NA	*In vitro* phosphatase assay	127
	RsbU	PPM	RsbV-P	RsbV-P	Regulation of sigma B	NA	*In vitro* phosphatase assay	193
	RsbX	PPM	RsbS-P	RsbS	Regulation of sigma B	NA	*In vitro* phosphatase assay	193
Listeria monocytogenes	Stp	PPM		EF-Tu	Translation factor	NA	2D phosphoprotein gel electrophoresis, *in vitro* phosphatase assay, decreased kirromycin sensitivity	5
Mycobacterium tuberculosis	PstP	PPM	PknB	PBPA	PBP, cell wall	NA	*In vitro* phosphatase assay	32
				PknB-P	eSTK	T171/T173	Mass spectrometry	18
Mycoplasma pneumoniae	PrpC	PP2C		HPr	Phosphocarrier protein of phosphotransfer system	S46	*In vitro* phosphatase assay	61
Myxococcus xanthus	Pph1	PP2C	Pkn5	Pkn5	Negative effector of development	NA	Yeast two-hybrid interaction	180
Staphylococcus aureus	Stp1	PP2C	Stk1	Stk1-P	eSTK	NA	*In vitro* phosphatase assay	16
Streptococcus agalactiae	SaSTP	PP2C		PpaC	Family II inorganic pyrophosphatase		*In vitro* phosphatase assay	144
				PurA	Purine biosynthesis	NA	*In vitro* phosphatase assay	
Streptococcus pneumoniae	PhpP	PP2C	StkP	StkP-P	eSTK		Coimmunoprecipitation	131
				RitR	Transcriptional regulator		*In vitro* phosphatase assay	184
Streptococcus pyogenes	SP-STP	PP2C	SP-STK	SP-HLP	Histone-like protein	Thr(s)	*In vitro* phosphatase assay Phospho-amino acid analysis	72
				SP-STK-P	eSTK	NA	*In vitro* phosphatase assay	72

[a] NA, not available.

In the human PP2C structure, a 60-residue segment between β7 and β8 contacts a large flap comprised of a helix followed by an irregular loop. This loop does not appear to be involved in enzyme catalysis but may assist with protein substrate specificity (31).

The bacterial eSTPs *M. tuberculosis* PstP (192), *Mycobacterium smegmatis* MspP (192), *Streptococcus agalactiae* STP (147), and *Thermosynechococcus elongatus* PphA (154) have been crystallized. Their catalytic domains are structurally nearly identical to that of human PP2C and contain the highly conserved active site residues (166) (Fig. 6A). However, there are several key differences in the structures, namely, the coordination of a third metal ion, the lack of the His62 residue shown to function in splitting of the P-O bond, and the repositioning of the flap region (11, 15, 143, 147, 166) (Fig. 6C). While metal ions 1 and 2 in the bacterial structures are positioned exactly at the locations of the two metal ions present in the human PP2C structure, bacterial enzymes have an additional metal ion in the active site. Likely due to different crystallization buffers, the third ion is magnesium in the PphA and STP structures and manganese in the PstP and MspP structures. Another important difference is the absence of the His62 residue in the bacterial structures. This residue has been shown to function as an acid splitting the phosphate oxygen bond in human PP2C (31). His62 is replaced with a methionine residue in PphA, PstP, and STP and with a phenylalanine residue in MspP. The most striking structural difference between the bacterial and human structures corresponds to the flap subdomain. In the bacterial structures, this region is shifted further away from the active site, being located comparatively closer in PstP and MspP than in PphA and STP. Mutational analysis of the flap region of PphA revealed that Arg169, which seems to be involved in structural organization of the flap domain, causes a pronounced effect on enzymatic activity (154). This suggests that the flap region serves as a mobile element that may facilitate substrate binding and turnover and, furthermore, may introduce specificity to the dephosphorylation of substrates.

Physiological roles of eSTPs. The physiological functions of eSTPs are not as well understood as those of eSTKs, and Table 2 is a comprehensive list of their identified substrates. *S. pyogenes* eSTP and *S. pneumoniae* PhpP deletion mutants are nonviable, highlighting the importance of eSTPs in bacterial physiology (72, 131). However, a deletion of *S. pneumoniae* PhpP could be obtained when the eSTK (StkP) located in the same operon was also deleted, suggesting that an important role of these phosphatases is to modulate the autophosphorylation state of their partner kinases (131). For example, autophosphorylated StkP is a substrate for PhpP (125), the kinase activity of autophosphorylated *M. tuberculosis* PknB is reduced by PstP-mediated dephosphorylation (18, 26), *B. subtilis* PrpC dephosphorylates PrkC *in vitro* (127), the enzymatic function of the *Bacillus anthracis* eSTK Ba-Stk1 is reduced after dephosphorylation by the eSTP Ba-Stp1 (161), and a strong interaction was reported between the *M. xanthus* eSTP Pph1 and the eSTK Pkn5 (180). In some cases, however, the eSTP target is a noncognate kinase. *S. pneumoniae* RitR is an orphan TCS response regulator that is phosphorylated by StkP and forms a ternary complex with the eSTP PhpR that is necessary for regulation of RitR activity (184).

Since eSTKs regulate cell division and peptidoglycan synthesis in *Mycobacteria*, *Corynebacterium*, and *Streptomyces* (110), it is not surprising that deletions in eSTPs also alter normal cell division or growth in diverse bacteria. For example, deletion of *S. aureus* STP results in thickened cell walls and increased resistance to lysostaphin (16) and in other cell wall defects (129), suggesting alterations in peptidoglycan composition and/or structure. Deletion of the *Streptococcus mutans* phosphatase PppL, like its cognate eSTK PknB, results in abnormal cell shape (9). Deletion of *B. subtilis* PrpC also results in prolonged stationary-phase survival, which is the opposite of the case for the kinase null mutant, which has a strong stationary-phase defect, suggesting the importance of modulating the cognate kinase PrkC (50). A number of *in vitro* targets of PrpC are proteins involved in translation, including EF-Tu, EF-G, and CpgA (1, 50). EF-Tu is also a substrate of the eSTP Stp in *Listeria monocytogenes* (4, 5). Thus, while eSTPs appear to modulate processes involved in growth, the mechanistic basis of this regulation is not well understood.

A null mutant of the *M. xanthus* eSTP Pph1 exhibited defects during late vegetative growth, swarming, and glycerol spore formation (167). Under starvation-induced developmental conditions, the Pph1 mutant showed reduced aggregation and failure to form fruiting bodies with viable spores (180). A mutant in the *B. subtilis* eSTP PrpC resulted in defects in spore and biofilm formation (99), although unlike its cognate kinase PrkC, it was not necessary for spore germination in response to peptidoglycan fragments (160).

Although the role of eSTPs in virulence has largely not been examined, deletion of an *L. monocytogenes* STP inhibited growth in a murine model of infection (5). A *B. anthracis* STK/STP double mutant had an impaired ability to survive within macrophages (161), and an *S. agalactiae* STK/STP double mutant was severely attenuated in a neonatal rat sepsis model (144). However, these defects could be due to the absence of both proteins or just of the kinase. Finally, exotoxins, including the hemolysins, play an important role in the pathogenesis of *S. aureus* infections, and inactivation of the eSTP Stp1 reduces hemolysin expression (22).

CONCLUDING REMARKS

The studies described here and listed in Tables 1 and 2 illustrate the important roles that eSTKs and eSTPs play in the physiology of diverse bacteria, as they regulate central processes such as cell division and translation. The mechanistic basis underlying their roles is still far from clear, and most of the evidence for specific substrates is based on *in vitro* phosphorylation experiments that are notoriously subject to false-positive results. However, in the near future, efforts aimed at confirming that these largely *in vitro* observations accurately reflect the *in vivo* situation should bear fruit, and we should be in a position to understand this regulation in mechanistic detail, as well as the physiological conditions responsible for its activation.

ACKNOWLEDGMENTS

This work was supported by the Fundação para a Ciência e Tecnologia, Portugal (SFRH/BPD/65369/2009) (S.F.F.P.), by NIH training

grant GM008224-22 (L.G.), and by NIH grant GM081368-02 and an Irma T. Hirschl Scholar award (J.D.).

REFERENCES

1. **Absalon, C., et al.** 2009. CpgA, EF-Tu and the stressosome protein YezB are substrates of the Ser/Thr kinase/phosphatase couple, PrkC/PrpC, in *Bacillus subtilis*. Microbiology **155:**932–943.

1a. **Absalon, C., et al.** 2008. The GTPase CpgA is implicated in the deposition of the peptidoglycan sacculus in *Bacillus subtilis*. J. Bacteriol. **190:**3786–3790.

2. **Adler, E., A. Donella-Deana, F. Arigoni, L. A. Pinna, and P. Stragler.** 1997. Structural relationship between a bacterial developmental protein and eukaryotic PP2C protein phosphatases. Mol. Microbiol. **23:**57–62.
3. **Alber, T.** 2009. Signaling mechanisms of the *Mycobacterium tuberculosis* receptor Ser/Thr protein kinases. Curr. Opin. Struct. Biol. **19:**650–657.
4. **Alexander, C., et al.** 1995. Phosphorylation of elongation factor Tu prevents ternary complex formation. J. Biol. Chem. **270:**14541–14547.
5. **Archambaud, C., E. Gouin, J. Pizarro-Cerda, P. Cossart, and O. Dussurget.** 2005. Translation elongation factor EF-Tu is a target for Stp, a serine-threonine phosphatase involved in virulence of *Listeria monocytogenes*. Mol. Microbiol. **56:**383–396.
6. **Arora, G., et al.** 2010. Understanding the role of PknJ in *Mycobacterium tuberculosis*: biochemical characterization and identification of novel substrate pyruvate kinase A. PLoS One **5:**e10772.
7. **Av-Gay, Y., and M. Everett.** 2000. The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. Trends Microbiol. **8:**238–244.
8. **Bakal, C. J., and J. E. Davies.** 2000. No longer an exclusive club: eukaryotic signalling domains in bacteria. Trends Cell Biol. **10:**32–38.
9. **Banu, L. D., et al.** 2010. The *Streptococcus mutans* serine/threonine kinase, PknB, regulates competence development, bacteriocin production, and cell wall metabolism. Infect. Immun. **78:**2209–2220.
10. **Barford, D.** 1995. Protein phosphatases. Curr. Opin. Struct. Biol. **5:**728–734.
11. **Barford, D., A. K. Das, and M. P. Egloff.** 1998. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu. Rev. Biophys. Biomol. Struct. **27:**133–164.
12. **Barthe, P., G. V. Mukamolova, C. Roumestand, and M. Cohen-Gonsaud.** 2010. The structure of PknB extracellular PASTA domain from *Mycobacterium tuberculosis* suggests a ligand-dependent kinase activation. Structure **18:**606–615.
13. **Barthe, P., et al.** 2009. Dynamic and structural characterization of a bacterial FHA protein reveals a new autoinhibition mechanism. Structure **17:**568–578.
14. **Belanger, A. E., and G. F. Hatfull.** 1999. Exponential-phase glycogen recycling is essential for growth of *Mycobacterium smegmatis*. J. Bacteriol. **181:**6670–6678.
15. **Bellinzoni, M., A. Wehenkel, W. Shepard, and P. M. Alzari.** 2007. Insights into the catalytic mechanism of PPM Ser/Thr phosphatases from the atomic resolution structures of a mycobacterial enzyme. Structure **15:**863–872.
16. **Beltramini, A. M., C. D. Mukhopadhyay, and V. Pancholi.** 2009. Modulation of cell wall structure and antimicrobial susceptibility by a *Staphylococcus aureus* eukaryote-like serine/threonine kinase and phosphatase. Infect. Immun. **77:**1406–1416.
17. **Bendt, A. K., et al.** 2003. Towards a phosphoproteome map of *Corynebacterium glutamicum*. Proteomics **3:**1637–1646.
18. **Boitel, B., et al.** 2003. PknB kinase activity is regulated by phosphorylation in two Thr residues and dephosphorylation by PstP, the cognate phospho-Ser/Thr phosphatase, in *Mycobacterium tuberculosis*. Mol. Microbiol. **49:**1493–1508.
19. **Bork, P., N. P. Brown, H. Hegyi, and J. Schultz.** 1996. The protein phosphatase 2C (PP2C) superfamily: detection of bacterial homologues. Protein Sci. **5:**1421–1425.
20. **Briken, V., S. A. Porcelli, G. S. Besra, and L. Kremer.** 2004. Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. Mol. Microbiol. **53:**391–403.
21. **Browne, G. J., and C. G. Proud.** 2002. Regulation of peptide-chain elongation in mammalian cells. Eur. J. Biochem. **269:**5360–5368.
22. **Burnside, K., et al.** 2010. Regulation of hemolysin expression and virulence of *Staphylococcus aureus* by a serine/threonine kinase and phosphatase. PLoS One **5:**e11071.
23. **Canova, M. J., L. Kremer, and V. Molle.** 2009. The *Mycobacterium tuberculosis* GroEL1 chaperone is a substrate of Ser/Thr protein kinases. J. Bacteriol. **191:**2876–2883.
24. **Canova, M. J., et al.** 2008. The *Mycobacterium tuberculosis* serine/threonine kinase PknL phosphorylates Rv2175c: mass spectrometric profiling of the activation loop phosphorylation sites and their role in the recruitment of Rv2175c. Proteomics **8:**521–533.
25. **Chao, J. D., et al.** 2010. Convergence of Ser/Thr and two-component signaling to coordinate expression of the dormancy regulon in *Mycobacterium tuberculosis*. J. Biol. Chem. **285:**29239–29246.
26. **Chopra, P., et al.** 2003. Phosphoprotein phosphatase of *Mycobacterium tuberculosis* dephosphorylates serine-threonine kinases PknA and PknB. Biochem. Biophys. Res. Commun. **311:**112–120.
27. **Cohen-Gonsaud, M., et al.** 2009. The *Mycobacterium tuberculosis* Ser/Thr kinase substrate Rv2175c is a DNA-binding protein regulated by phosphorylation. J. Biol. Chem. **284:**19290–19300.
28. **Cole, J. L.** 2007. Activation of PKR: an open and shut case? Trends Biochem. Sci. **32:**57–62.
29. **Cowley, S., et al.** 2004. The *Mycobacterium tuberculosis* protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth in vivo. Mol. Microbiol. **52:**1691–1702.
30. **Dar, A. C., T. E. Dever, and F. Sicheri.** 2005. Higher-order substrate recognition of eIF2alpha by the RNA-dependent protein kinase PKR. Cell **122:**887–900.
31. **Das, A. K., N. R. Helps, P. T. Cohen, and D. Barford.** 1996. Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 A resolution. EMBO J. **15:**6798–6809.
32. **Dasgupta, A., P. Datta, M. Kundu, and J. Basu.** 2006. The serine/threonine kinase PknB of *Mycobacterium tuberculosis* phosphorylates PBPA, a penicillin-binding protein required for cell division. Microbiology **152:**493–504.
33. **Debarbouille, M., et al.** 2009. Characterization of a serine/threonine kinase involved in virulence of *Staphylococcus aureus*. J. Bacteriol. **191:**4070–4081.
34. **Delumeau, O., et al.** 2004. Functional and structural characterization of RsbU, a stress signaling protein phosphatase 2C. J. Biol. Chem. **279:**40927–40937.
35. **Dessen, A., N. Mouz, E. Gordon, J. Hopkins, and O. Dideberg.** 2001. Crystal structure of PBP2x from a highly penicillin-resistant *Streptococcus pneumoniae* clinical isolate: a mosaic framework containing 83 mutations. J. Biol. Chem. **276:**45106–45112.
36. **De Verdier, C. H.** 1952. Isolation of phosphothreonine from bovine casein. Nature **170:**804–805.
37. **Dias, R., D. Felix, M. Canica, and M. C. Trombe.** 2009. The highly conserved serine threonine kinase StkP of *Streptococcus pneumoniae* contributes to penicillin susceptibility independently from genes encoding penicillin-binding proteins. BMC Microbiol. **9:**121.
38. **Didier, J. P., A. J. Cozzone, and B. Duclos.** 2010. Phosphorylation of the virulence regulator SarA modulates its ability to bind DNA in *Staphylococcus aureus*. FEMS Microbiol. Lett. **306:**30–36.
39. **Donat, S., et al.** 2009. Transcriptome and functional analysis of the eukaryotic-type serine/threonine kinase PknB in *Staphylococcus aureus*. J. Bacteriol. **191:**4056–4069.
40. **Duncan, L., S. Alper, F. Arigoni, R. Losick, and P. Stragier.** 1995. Activation of cell-specific transcription by a serine phosphatase at the site of asymmetric division. Science **270:**641–644.
41. **Dutta, R., and M. Inouye.** 2000. GHKL, an emergent ATPase/kinase superfamily. Trends Biochem. Sci. **25:**24–28.
42. **Dutta, S., and R. J. Lewis.** 2003. Crystallization and preliminary crystallographic analysis of the kinase-recruitment domain of the PP2C-type phosphatase RsbU. Acta Crystallogr. D Biol. Crystallogr. **59:**191–193.
43. **Echenique, J., A. Kadioglu, S. Romao, P. W. Andrew, and M. C. Trombe.** 2004. Protein serine/threonine kinase StkP positively controls virulence and competence in *Streptococcus pneumoniae*. Infect. Immun. **72:**2434–2437.
44. **England, P., et al.** 2009. The FHA-containing protein GarA acts as a phosphorylation-dependent molecular switch in mycobacterial signaling. FEBS Lett. **583:**301–307.
45. **Eymann, C., et al.** 2007. Dynamics of protein phosphorylation on Ser/Thr/Tyr in *Bacillus subtilis*. Proteomics **7:**3509–3526.
46. **Faucher, S. P., C. Viau, P. P. Gros, F. Daigle, and H. Le Moual.** 2008. The prpZ gene cluster encoding eukaryotic-type Ser/Thr protein kinases and phosphatases is repressed by oxidative stress and involved in *Salmonella enterica* serovar Typhi survival in human macrophages. FEMS Microbiol. Lett. **281:**160–166.
47. **Fernandez, P., et al.** 2006. The Ser/Thr protein kinase PknB is essential for sustaining mycobacterial growth. J. Bacteriol. **188:**7778–7784.
48. **Fiuza, M., et al.** 2008. The MurC ligase essential for peptidoglycan biosynthesis is regulated by the serine/threonine protein kinase PknA in *Corynebacterium glutamicum*. J. Biol. Chem. **283:**36553–36563.
49. **Fiuza, M., et al.** 2008. From the characterization of the four serine/threonine protein kinases (PknA/B/G/L) of *Corynebacterium glutamicum* toward the role of PknA and PknB in cell division. J. Biol. Chem. **283:**18099–18112.
50. **Gaidenko, T. A., T. J. Kim, and C. W. Price.** 2002. The PrpC serine-threonine phosphatase and PrkC kinase have opposing physiological roles in stationary-phase *Bacillus subtilis* cells. J. Bacteriol. **184:**6109–6114.
51. **Galperin, M. Y., R. Higdon, and E. Kolker.** 2010. Interplay of heritage and habitat in the distribution of bacterial signal transduction systems. Mol. Biosyst. **6:**721–728.
52. **Galyov, E. E., S. Hakansson, A. Forsberg, and H. Wolf-Watz.** 1993. A secreted protein kinase of *Yersinia pseudotuberculosis* is an indispensable virulence determinant. Nature **361:**730–732.
53. **Garnak, M., and H. C. Reeves.** 1979. Phosphorylation of isocitrate dehydrogenase of *Escherichia coli*. Science **203:**1111–1112.
54. **Gay, L. M., H. L. Ng, and T. Alber.** 2006. A conserved dimer and global conformational changes in the structure of apo-PknE Ser/Thr protein kinase from *Mycobacterium tuberculosis*. J. Mol. Biol. **360:**409–420.
55. **Giefing, C., K. E. Jelencsics, D. Gelbmann, B. M. Senn, and E. Nagy.** 2010.

The pneumococcal eukaryotic-type serine/threonine protein kinase StkP co-localizes with the cell division apparatus and interacts with FtsZ in vitro. Microbiology **156:**1697–1707.
56. **Good, M. C., A. E. Greenstein, T. A. Young, H. L. Ng, and T. Alber.** 2004. Sensor domain of the *Mycobacterium tuberculosis* receptor Ser/Thr protein kinase, PknD, forms a highly symmetric beta propeller. J. Mol. Biol. **339:** 459–469.
57. **Grangeasse, C., A. J. Cozzone, J. Deutscher, and I. Mijakovic.** 2007. Tyrosine phosphorylation: an emerging regulatory device of bacterial physiology. Trends Biochem. Sci. **32:**86–94.
58. **Grangeasse, C., R. Terreux, and S. Nessler.** 2010. Bacterial tyrosine-kinases: structure-function analysis and therapeutic potential. Biochim. Biophys. Acta **1804:**628–634.
59. **Greenstein, A. E., N. Echols, T. N. Lombana, D. S. King, and T. Alber.** 2007. Allosteric activation by dimerization of the PknD receptor Ser/Thr protein kinase from *Mycobacterium tuberculosis*. J. Biol. Chem. **282:**11427–11435.
59a. **Greenstein, A. E., et al.** 2007. *M. tuberculosis* Ser/Thr protein kinase D phosphorylates an anti-sigma factor homolog. PLoS Pathog. **3:**e49.
60. **Grundner, C., L. M. Gay, and T. Alber.** 2005. *Mycobacterium tuberculosis* serine/threonine kinases PknB, PknD, PknE, and PknF phosphorylate multiple FHA domains. Protein Sci. **14:**1918–1921.
61. **Halbedel, S., J. Busse, S. R. Schmidl, and J. Stulke.** 2006. Regulatory protein phosphorylation in *Mycoplasma pneumoniae*. A PP2C-type phosphatase serves to dephosphorylate HPr(Ser-P). J. Biol. Chem. **281:**26253–26259.
62. **Hammet, A., et al.** 2003. FHA domains as phospho-threonine binding modules in cell signaling. IUBMB Life **55:**23–27.
63. **Hanks, S. K., and T. Hunter.** 1995. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J. **9:**576–596.
64. **Hoch, J. A.** 2000. Two-component and phosphorelay signal transduction. Curr. Opin. Microbiol. **3:**165–170.
65. **Holub, M., S. Bezouskova, L. Kalachova, and J. Weiser.** 2007. Protein synthesis elongation factor Tu present in spores of *Streptomyces coelicolor* can be phosphorylated in vitro by the spore protein kinase. Folia Microbiol. (Prague) **52:**471–478.
66. **Hsu, F., S. Schwarz, and J. D. Mougous.** 2009. TagR promotes PpkA-catalysed type VI secretion activation in *Pseudomonas aeruginosa*. Mol. Microbiol. **72:**1111–1125.
67. **Huse, M., and J. Kuriyan.** 2002. The conformational plasticity of protein kinases. Cell **109:**275–282.
68. **Hussain, H., P. Branny, and E. Allan.** 2006. A eukaryotic-type serine/threonine protein kinase is required for biofilm formation, genetic competence, and acid resistance in *Streptococcus mutans*. J. Bacteriol. **188:**1628–1632.
69. **Inouye, S., and H. Nariya.** 2008. Dual regulation with Ser/Thr kinase cascade and a His/Asp TCS in *Myxococcus xanthus*. Adv. Exp. Med. Biol. **631:**111–121.
70. **Iwanicki, A., A. Herman-Antosiewicz, M. Pierechod, S. J. Seror, and M. Obuchowski.** 2002. PrpE, a PPP protein phosphatase from *Bacillus subtilis* with unusual substrate specificity. Biochem. J. **366:**929–936.
71. **Jang, J., et al.** 2010. Functional characterization of the *Mycobacterium tuberculosis* serine/threonine kinase PknJ. Microbiology **156:**1619–1631.
72. **Jin, H., and V. Pancholi.** 2006. Identification and biochemical characterization of a eukaryotic-type serine/threonine kinase and its cognate phosphatase in *Streptococcus pyogenes*: their biological functions and substrate identification. J. Mol. Biol. **357:**1351–1372.
73. **Jolly, L., et al.** 1999. Reaction mechanism of phosphoglucosamine mutase from *Escherichia coli*. Eur. J. Biochem. **262:**202–210.
74. **Juris, S. J., A. E. Rudolph, D. Huddler, K. Orth, and J. E. Dixon.** 2000. A distinctive role for the Yersinia protein kinase: actin binding, kinase activation, and cytoskeleton disruption. Proc. Natl. Acad. Sci. U. S. A. **97:**9431–9436.
75. **Kang, C. M., et al.** 2005. The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. Genes Dev. **19:**1692–1704.
76. **Kang, C. M., K. Vijay, and C. W. Price.** 1998. Serine kinase activity of a *Bacillus subtilis* switch protein is required to transduce environmental stress signals but not to activate its target PP2C phosphatase. Mol. Microbiol. **30:**189–196.
77. **Kennelly, P. J.** 1998. Prokaryotic protein-serine/threonine phosphatases. Methods Mol. Biol. **93:**1–21.
78. **Kennelly, P. J.** 2002. Protein kinases and protein phosphatases in prokaryotes: a genomic perspective. FEMS Microbiol. Lett. **206:**1–8.
79. **Kornev, A. P., and S. S. Taylor.** 2010. Defining the conserved internal architecture of a protein kinase. Biochim. Biophys. Acta **1804:**440–444.
80. **Koul, A., et al.** 2000. Cloning and characterization of secretory tyrosine phosphatases of *Mycobacterium tuberculosis*. J. Bacteriol. **182:**5425–5432.
81. **Kravanja, M., et al.** 1999. The hprK gene of *Enterococcus faecalis* encodes a novel bifunctional enzyme: the HPr kinase/phosphatase. Mol. Microbiol. **31:**59–66.
82. **Kristich, C. J., C. L. Wells, and G. M. Dunny.** 2007. A eukaryotic-type Ser/Thr kinase in *Enterococcus faecalis* mediates antimicrobial resistance and intestinal persistence. Proc. Natl. Acad. Sci. U. S. A. **104:**3508–3513.
83. **Krupa, A., and N. Srinivasan.** 2005. Diversity in domain architectures of Ser/Thr kinases and their homologues in prokaryotes. BMC Genomics **6:**129.
84. **Kumar, P., et al.** 2009. The *Mycobacterium tuberculosis* protein kinase K modulates activation of transcription from the promoter of mycobacterial monooxygenase operon through phosphorylation of the transcriptional regulator VirS. J. Biol. Chem. **284:**11090–11099.
85. **Lai, S. M., and H. Le Moual.** 2005. PrpZ, a *Salmonella enterica* serovar Typhi serine/threonine protein phosphatase 2C with dual substrate specificity. Microbiology **151:**1159–1167.
86. **LaPorte, D. C., and T. Chung.** 1985. A single gene codes for the kinase and phosphatase which regulate isocitrate dehydrogenase. J. Biol. Chem. **260:** 15291–15297.
87. **Lee, P. C., T. Umeyama, and S. Horinouchi.** 2002. afsS is a target of AfsR, a transcriptional factor with ATPase activity that globally controls secondary metabolism in *Streptomyces coelicolor* A3(2). Mol. Microbiol. **43:**1413–1430.
88. **Leng, J., A. J. Cameron, S. Buckel, and P. J. Kennelly.** 1995. Isolation and cloning of a protein-serine/threonine phosphatase from an archaeon. J. Bacteriol. **177:**6510–6517.
89. **Leonard, C. J., L. Aravind, and E. V. Koonin.** 1998. Novel families of putative protein kinases in bacteria and archaea: evolution of the "eukaryotic" protein kinase superfamily. Genome Res. **8:**1038–1047.
90. **Levene, P. A., and C. L. Alsberg.** 1906. The cleavage products of vitellin. J. Biol. Chem. **2:**127–133.
91. **Levine, A., et al.** 2006. Analysis of the dynamic *Bacillus subtilis* Ser/Thr/Tyr phosphoproteome implicated in a wide variety of cellular processes. Proteomics **6:**2157–2173.
92. **Liebeke, M., H. Meyer, S. Donat, K. Ohlsen, and M. Lalk.** 2010. A metabolomic view of *Staphylococcus aureus* and its Ser/Thr kinase and phosphatase deletion mutants: involvement in cell wall biosynthesis. Chem. Biol. **17:**820–830.
93. **Lin, W. J., et al.** 2009. Threonine phosphorylation prevents promoter DNA binding of the group B Streptococcus response regulator CovR. Mol. Microbiol. **71:**1477–1495.
94. **Lipmann, F. A., and P. A. Levene.** 1932. Serinephosphoric acid obtained on hydrolysis of vitellinic acid. J. Biol. Chem. **98:**109–114.
95. **Lippmann, C., et al.** 1993. Prokaryotic elongation factor Tu is phosphorylated *in vivo*. J. Biol. Chem. **268:**601–607.
96. **Lomas-Lopez, R., P. Paracuellos, M. Riberty, A. J. Cozzone, and B. Duclos.** 2007. Several enzymes of the central metabolism are phosphorylated in *Staphylococcus aureus*. FEMS Microbiol. Lett. **272:**35–42.
97. **Macek, B., et al.** 2008. Phosphoproteome analysis of *E. coli* reveals evolutionary conservation of bacterial Ser/Thr/Tyr phosphorylation. Mol. Cell. Proteomics **7:**299–307.
98. **Macek, B., et al.** 2007. The serine/threonine/tyrosine phosphoproteome of the model bacterium *Bacillus subtilis*. Mol. Cell. Proteomics **6:**697–707.
99. **Madec, E., A. Laszkiewicz, A. Iwanicki, M. Obuchowski, and S. Seror.** 2002. Characterization of a membrane-linked Ser/Thr protein kinase in *Bacillus subtilis*, implicated in developmental processes. Mol. Microbiol. **46:**571–586.
100. **Madhusudan, P. Akamine, N. H. Xuong, and S. S. Taylor.** 2002. Crystal structure of a transition state mimic of the catalytic subunit of cAMP-dependent protein kinase. Nat. Struct. Biol. **9:**273–277.
101. **Manning, B. D., and L. C. Cantley.** 2002. Hitting the target: emerging technologies in the search for kinase substrates. Sci. STKE **2002:**pe49.
102. **Matsumoto, A., S. K. Hong, H. Ishizuka, S. Horinouchi, and T. Beppu.** 1994. Phosphorylation of the AfsR protein involved in secondary metabolism in Streptomyces species by a eukaryotic-type protein kinase. Gene **146:**47–56.
103. **Mieczkowski, C., A. T. Iavarone, and T. Alber.** 2008. Auto-activation mechanism of the *Mycobacterium tuberculosis* PknB receptor Ser/Thr kinase. EMBO J. **27:**3186–3197.
104. **Mikulik, K., and I. Janda.** 1997. Protein kinase associated with ribosomes phosphorylates ribosomal proteins of *Streptomyces collinus*. Biochem. Biophys. Res. Commun. **238:**370–376.
105. **Mikulik, K., P. Suchan, and J. Bobek.** 2001. Changes in ribosome function induced by protein kinase associated with ribosomes of *Streptomyces collinus* producing kirromycin. Biochem. Biophys. Res. Commun. **289:**434–443.
106. **Mikulik, K., E. Zhoulanova, Q. K. Hoang, J. Janecek, and S. Bezouskova.** 1999. Protein kinase associated with ribosomes of streptomycetes. Folia Microbiol. (Prague) **44:**123–130.
107. **Miller, M., et al.** 2010. Staphylococcal PknB as the first prokaryotic representative of the proline-directed kinases. PLoS One **5:**e9057.
108. **Missiakas, D., and S. Raina.** 1997. Signal transduction pathways in response to protein misfolding in the extracytoplasmic compartments of *E. coli*: role of two new phosphoprotein phosphatases PrpA and PrpB. EMBO J. **16:**1670–1685.
109. **Molle, V., A. K. Brown, G. S. Besra, A. J. Cozzone, and L. Kremer.** 2006. The condensing activities of the *Mycobacterium tuberculosis* type II fatty

acid synthase are differentially regulated by phosphorylation. J. Biol. Chem. **281:**30094–30103.
110. **Molle, V., and L. Kremer.** 2010. Division and cell envelope regulation by Ser/Thr phosphorylation: mycobacterium shows the way. Mol. Microbiol. **75:**1064–1077.
111. **Molle, V., et al.** 2003. An FHA phosphoprotein recognition domain mediates protein EmbR phosphorylation by PknH, a Ser/Thr protein kinase from *Mycobacterium tuberculosis*. Biochemistry **42:**15300–15309.
112. **Molle, V., et al.** 2004. Two FHA domains on an ABC transporter, Rv1747, mediate its phosphorylation by PknF, a Ser/Thr protein kinase from *Mycobacterium tuberculosis*. FEMS Microbiol. Lett. **234:**215–223.
113. **Mougous, J. D., C. A. Gifford, T. L. Ramsdell, and J. J. Mekalanos.** 2007. Threonine phosphorylation post-translationally regulates protein secretion in *Pseudomonas aeruginosa*. Nat. Cell Biol. **9:**797–803.
114. **Munoz-Dorado, J., S. Inouye, and M. Inouye.** 1991. A gene encoding a protein serine/threonine kinase is required for normal development of *M. xanthus*, a gram-negative bacterium. Cell **67:**995–1006.
115. **Nadvornik, R., T. Vomastek, J. Janecek, Z. Technikova, and P. Branny.** 1999. Pkg2, a novel transmembrane protein Ser/Thr kinase of *Streptomyces granaticolor*. J. Bacteriol. **181:**15–23.
116. **Najafi, S. M., A. C. Willis, and M. D. Yudkin.** 1995. Site of phosphorylation of SpoIIAA, the anti-anti-sigma factor for sporulation-specific sigma F of *Bacillus subtilis*. J. Bacteriol. **177:**2912–2913.
117. **Nariya, H., and S. Inouye.** 2002. Activation of 6-phosphofructokinase via phosphorylation by Pkn4, a protein Ser/Thr kinase of *Myxococcus xanthus*. Mol. Microbiol. **46:**1353–1366.
118. **Nariya, H., and S. Inouye.** 2005. Factors that modulate the Pkn4 kinase cascade in *Myxococcus xanthus*. J. Mol. Microbiol. Biotechnol. **9:**147–153.
119. **Nariya, H., and S. Inouye.** 2005. Identification of a protein Ser/Thr kinase cascade that regulates essential transcriptional activators in *Myxococcus xanthus* development. Mol. Microbiol. **58:**367–379.
120. **Nariya, H., and S. Inouye.** 2006. A protein Ser/Thr kinase cascade negatively regulates the DNA-binding activity of MrpC, a smaller form of which may be necessary for the *Myxococcus xanthus* development. Mol. Microbiol. **60:**1205–1217.
121. **Niebisch, A., A. Kabus, C. Schultz, B. Weil, and M. Bott.** 2006. Corynebacterial protein kinase G controls 2-oxoglutarate dehydrogenase activity via the phosphorylation status of the OdhI protein. J. Biol. Chem. **281:**12300–12307.
122. **Nolen, B., S. Taylor, and G. Ghosh.** 2004. Regulation of protein kinases; controlling activity through activation segment conformation. Mol. Cell **15:**661–675.
123. **Nott, T. J., et al.** 2009. An intramolecular switch regulates phosphoindependent FHA domain interactions in *Mycobacterium tuberculosis*. Sci. Signal. **2:**ra12.
124. **Novakova, L., et al.** 2010. Identification of multiple substrates of the StkP Ser/Thr protein kinase in *Streptococcus pneumoniae*. J. Bacteriol. **192:**3629–3638.
125. **Novakova, L., et al.** 2005. Characterization of a eukaryotic type serine/threonine protein kinase and protein phosphatase of *Streptococcus pneumoniae* and identification of kinase substrates. FEBS J. **272:**1243–1254.
126. **Obuchowski, M.** 2005. Serine-threonine protein phosphatases from *Bacillus subtilis*. Postepy Biochem. **51:**95–104.
127. **Obuchowski, M., et al.** 2000. Characterization of PrpC from *Bacillus subtilis*, a member of the PPM phosphatase family. J. Bacteriol. **182:**5634–5638.
128. **O'Hare, H. M., et al.** 2008. Regulation of glutamate metabolism by protein kinases in mycobacteria. Mol. Microbiol. **70:**1408–1423.
129. **Ohlsen, K., and S. Donat.** 2010. The impact of serine/threonine phosphorylation in *Staphylococcus aureus*. Int. J. Med. Microbiol. **300:**137–141.
130. **Ortiz-Lombardia, M., F. Pompeo, B. Boitel, and P. M. Alzari.** 2003. Crystal structure of the catalytic domain of the PknB serine/threonine kinase from *Mycobacterium tuberculosis*. J. Biol. Chem. **278:**13094–13100.
131. **Osaki, M., et al.** 2009. The StkP/PhpP signaling couple in *Streptococcus pneumoniae*: cellular organization and physiological characterization. J. Bacteriol. **191:**4943–4950.
132. **Pancholi, V., G. Boël, and H. Jin.** 2010. *S. pyogenes* Ser/Thr kinase-regulated cell wall hydrolase is a cell division plane-recognizing and chain-forming virulence factor. J. Biol. Chem. **285:**30861–30874.
133. **Papavinasasundaram, K. G., et al.** 2005. Deletion of the *Mycobacterium tuberculosis pknH* gene confers a higher bacillary load during the chronic phase of infection in BALB/c mice. J. Bacteriol. **187:**5751–5760.
134. **Paracuellos, P., et al.** 2010. The extended conformation of the 2.9-A crystal structure of the three-PASTA domain of a Ser/Thr kinase from the human pathogen *Staphylococcus aureus*. J. Mol. Biol. **404:**847–858.
135. **Parikh, A., S. K. Verma, S. Khan, B. Prakash, and V. K. Nandicoori.** 2009. PknB-mediated phosphorylation of a novel substrate, *N*-acetylglucosamine-1-phosphate uridyltransferase, modulates its acetyltransferase activity. J. Mol. Biol. **386:**451–464.
136. **Park, S. T., C. M. Kang, and R. N. Husson.** 2008. Regulation of the SigH stress response regulon by an essential protein kinase in *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. U. S. A. **105:**13105–13110.
137. **Pawson, T., and J. D. Scott.** 1997. Signaling through scaffold, anchoring, and adaptor proteins. Science **278:**2075–2080.
138. **Perez, J., A. Castaneda-Garcia, H. Jenke-Kodama, R. Muller, and J. Munoz-Dorado.** 2008. Eukaryotic-like protein kinases in the prokaryotes and the myxobacterial kinome. Proc. Natl. Acad. Sci. U. S. A. **105:**15950–15955.
139. **Perez, J., et al.** 2006. *Mycobacterium tuberculosis* transporter MmpL7 is a potential substrate for kinase PknD. Biochem. Biophys. Res. Commun. **348:**6–12.
140. **Petrickova, K., and M. Petricek.** 2003. Eukaryotic-type protein kinases in *Streptomyces coelicolor*: variations on a common theme. Microbiology **149:**1609–1621.
141. **Pietack, N., et al.** 2010. In vitro phosphorylation of key metabolic enzymes from *Bacillus subtilis*: PrkC phosphorylates enzymes from different branches of basic metabolism. J. Mol. Microbiol. Biotechnol. **18:**129–140.
142. **Prisic, S., et al.** 2010. Extensive phosphorylation with overlapping specificity by *Mycobacterium tuberculosis* serine/threonine protein kinases. Proc. Natl. Acad. Sci. U. S. A. **107:**7521–7526.
143. **Pullen, K. E., et al.** 2004. An alternate conformation and a third metal in PstP/Ppp, the M. tuberculosis PP2C-family Ser/Thr protein phosphatase. Structure **12:**1947–1954.
144. **Rajagopal, L., A. Clancy, and C. E. Rubens.** 2003. A eukaryotic type serine/threonine kinase and phosphatase in *Streptococcus agalactiae* reversibly phosphorylate an inorganic pyrophosphatase and affect growth, cell segregation, and virulence. J. Biol. Chem. **278:**14429–14441.
145. **Rajagopal, L., A. Vo, A. Silvestroni, and C. E. Rubens.** 2006. Regulation of cytotoxin expression by converging eukaryotic-type and two-component signalling mechanisms in *Streptococcus agalactiae*. Mol. Microbiol. **62:**941–957.
146. **Rajagopal, L., A. Vo, A. Silvestroni, and C. E. Rubens.** 2005. Regulation of purine biosynthesis by a eukaryotic-type kinase in *Streptococcus agalactiae*. Mol. Microbiol. **56:**1329–1346.
147. **Rantanen, M. K., L. Lehtio, L. Rajagopal, C. E. Rubens, and A. Goldman.** 2007. Structure of *Streptococcus agalactiae* serine/threonine phosphatase. The subdomain conformation is coupled to the binding of a third metal ion. FEBS J. **274:**3128–3137.
148. **Robertson, E. S., L. A. Aggison, and A. W. Nicholson.** 1994. Phosphorylation of elongation factor G and ribosomal protein S6 in bacteriophage T7-infected *Escherichia coli*. Mol. Microbiol. **11:**1045–1057.
149. **Saskova, L., L. Novakova, M. Basler, and P. Branny.** 2007. Eukaryotic-type serine/threonine protein kinase StkP is a global regulator of gene expression in *Streptococcus pneumoniae*. J. Bacteriol. **189:**4168–4179.
150. **Sassetti, C. M., D. H. Boyd, and E. J. Rubin.** 2003. Genes required for mycobacterial growth defined by high density mutagenesis. Mol. Microbiol. **48:**77–84.
151. **Sawai, R., A. Suzuki, Y. Takano, P. C. Lee, and S. Horinouchi.** 2004. Phosphorylation of AfsR by multiple serine/threonine kinases in *Streptomyces coelicolor* A3(2). Gene **334:**53–61.
152. **Scheeff, E. D., and P. E. Bourne.** 2005. Structural evolution of the protein kinase-like superfamily. PLoS Comput. Biol. **1:**e49.
153. **Scherr, N., et al.** 2007. Structural basis for the specific inhibition of protein kinase G, a virulence factor of *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. U. S. A. **104:**12151–12156.
154. **Schlicker, C., et al.** 2008. Structural analysis of the PP2C phosphatase tPphA from *Thermosynechococcus elongatus*: a flexible flap subdomain controls access to the catalytic site. J. Mol. Biol. **376:**570–581.
155. **Schmidl, S. R., et al.** 2010. The stability of cytadherence proteins in *Mycoplasma pneumoniae* requires activity of the protein kinase PrkC. Infect. Immun. **78:**184–192.
156. **Schmidl, S. R., et al.** 2010. The phosphoproteome of the minimal bacterium *Mycoplasma pneumoniae*: analysis of the complete known Ser/Thr kinome suggests the existence of novel kinases. Mol. Cell. Proteomics **9:**1228–1242.
157. **Schultz, C., et al.** 2009. Genetic and biochemical analysis of the serine/threonine protein kinases PknA, PknB, PknG and PknL of *Corynebacterium glutamicum*: evidence for non-essentiality and for phosphorylation of OdhI and FtsZ by multiple kinases. Mol. Microbiol. **74:**724–741.
158. **Schumacher, M. A., et al.** 2009. Molecular mechanisms of HipA-mediated multidrug tolerance and its neutralization by HipB. Science **323:**396–401.
159. **Shah, I. M., and J. Dworkin.** 2010. Induction and regulation of a secreted peptidoglycan hydrolase by a membrane Ser/Thr kinase that detects muropeptides. Mol. Microbiol. **75:**1232–1243.
160. **Shah, I. M., M. H. Laaberki, D. L. Popham, and J. Dworkin.** 2008. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. Cell **135:**486–496.
161. **Shakir, S. M., et al.** 2010. Regulatory interactions of a virulence-associated serine/threonine phosphatase-kinase pair in *Bacillus anthracis*. J. Bacteriol. **192:**400–409.
162. **Sharma, K., M. Gupta, A. Krupa, N. Srinivasan, and Y. Singh.** 2006. EmbR, a regulatory protein with ATPase activity, is a substrate of multiple serine/threonine kinases and phosphatase in *Mycobacterium tuberculosis*. FEBS J. **273:**2711–2721.
163. **Sharma, K., et al.** 2006. Transcriptional control of the mycobacterial embCAB operon by PknH through a regulatory protein, EmbR, in vivo. J. Bacteriol. **188:**2936–2944.
164. **Shi, L., and W. W. Carmichael.** 1997. pp1-cyano2, a protein serine/threo-

nine phosphatase 1 gene from the cyanobacterium *Microcystis aeruginosa* UTEX 2063. Arch. Microbiol. **168:**528–531.
165. **Shi, L., M. Potts, and P. J. Kennelly.** 1998. The serine, threonine, and/or tyrosine-specific protein kinases and protein phosphatases of prokaryotic organisms: a family portrait. FEMS Microbiol. Rev. **22:**229–253.
166. **Shi, Y.** 2009. Serine/threonine phosphatases: mechanism through structure. Cell **139:**468–484.
167. **Sickmann, A., and H. E. Meyer.** 2001. Phosphoamino acid analysis. Proteomics **1:**200–206.
168. **Silvestroni, A., et al.** 2009. Identification of serine/threonine kinase substrates in the human pathogen group B streptococcus. J. Proteome Res. **8:**2563–2574.
169. **Singh, K. D., S. Halbedel, B. Gorke, and J. Stulke.** 2007. Control of the phosphorylation state of the HPr protein of the phosphotransferase system in *Bacillus subtilis*: implication of the protein phosphatase PrpC. J. Mol. Microbiol. Biotechnol. **13:**165–171.
170. **Solow, B., J. C. Young, and P. J. Kennelly.** 1997. Gene cloning and expression and characterization of a toxin-sensitive protein phosphatase from the methanogenic archaeon *Methanosarcina thermophila* TM-1. J. Bacteriol. **179:**5072–5075.
171. **Soufi, B., et al.** 2008. The Ser/Thr/Tyr phosphoproteome of *Lactococcus lactis* IL1403 reveals multiply phosphorylated proteins. Proteomics **8:**3486–3493.
172. **Stock, J. B., A. M. Stock, and J. M. Mottonen.** 1990. Signal transduction in bacteria. Nature **344:**395–400.
173. **Sun, H., and W. Shi.** 2001. Genetic studies of mrp, a locus essential for cellular aggregation and sporulation of *Myxococcus xanthus*. J. Bacteriol. **183:**4786–4795.
174. **Sun, X., et al.** 2010. Phosphoproteomic analysis reveals the multiple roles of phosphorylation in pathogenic bacterium *Streptococcus pneumoniae*. J. Proteome Res. **9:**275–282.
175. **Sureka, K., et al.** 2010. Novel role of phosphorylation-dependent interaction between FtsZ and FipA in mycobacterial cell division. PLoS One **5:**e8590.
176. **Sutherland, E. W., Jr., and W. D. Wosilait.** 1955. Inactivation and activation of liver phosphorylase. Nature **175:**169–170.
177. **Tamames, J., M. Gonzalez-Moreno, J. Mingorance, A. Valencia, and M. Vicente.** 2001. Bringing gene order into bacterial shape. Trends Genet. **17:**124–126.
178. **Thakur, M., and P. K. Chakraborti.** 2008. Ability of PknA, a mycobacterial eukaryotic-type serine/threonine kinase, to transphosphorylate MurD, a ligase involved in the process of peptidoglycan biosynthesis. Biochem. J. **415:**27–33.
179. **Thakur, M., and P. K. Chakraborti.** 2006. GTPase activity of mycobacterial FtsZ is impaired due to its transphosphorylation by the eukaryotic-type Ser/Thr kinase, PknA. J. Biol. Chem. **281:**40107–40113.
180. **Treuner-Lange, A., M. J. Ward, and D. R. Zusman.** 2001. Pph1 from *Myxococcus xanthus* is a protein phosphatase involved in vegetative growth and development. Mol. Microbiol. **40:**126–140.
181. **Truong-Bolduc, Q. C., Y. Ding, and D. C. Hooper.** 2008. Posttranslational modification influences the effects of MgrA on norA expression in *Staphylococcus aureus*. J. Bacteriol. **190:**7375–7381.
182. **Udo, H., C. K. Lam, S. Mori, M. Inouye, and S. Inouye.** 2000. Identification of a substrate for Pkn2, a protein Ser/Thr kinase from *Myxococcus xanthus* by a novel method for substrate identification. J. Mol. Microbiol. Biotechnol. **2:**557–563.
183. **Udo, H., J. Munoz-Dorado, M. Inouye, and S. Inouye.** 1995. *Myxococcus xanthus*, a gram-negative bacterium, contains a transmembrane protein serine/threonine kinase that blocks the secretion of beta-lactamase by phosphorylation. Genes Dev. **9:**972–983.
184. **Ulijasz, A. T., S. P. Falk, and B. Weisblum.** 2009. Phosphorylation of the RitR DNA-binding domain by a Ser-Thr phosphokinase: implications for global gene regulation in the streptococci. Mol. Microbiol. **71:**382–390.
185. **Umeyama, T., and S. Horinouchi.** 2001. Autophosphorylation of a bacterial serine/threonine kinase, AfsK, is inhibited by KbpA, an AfsK-binding protein. J. Bacteriol. **183:**5506–5512.
186. **Verma, A., and A. T. Maurelli.** 2003. Identification of two eukaryote-like serine/threonine kinases encoded by *Chlamydia trachomatis* serovar L2 and characterization of interacting partners of Pkn1. Infect. Immun. **71:**5772–5784.
187. **Veyron-Churlet, R., et al.** 2009. The *Mycobacterium tuberculosis* beta-ketoacyl-acyl carrier protein synthase III activity is inhibited by phosphorylation on a single threonine residue. J. Biol. Chem. **284:**6414–6424.
188. **Veyron-Churlet, R., I. Zanella-Cleon, M. Cohen-Gonsaud, V. Molle, and L. Kremer.** 2010. Phosphorylation of the *Mycobacterium tuberculosis* beta-ketoacyl-acyl carrier protein reductase MabA regulates mycolic acid biosynthesis. J. Biol. Chem. **285:**12714–12725.
189. **Villarino, A., et al.** 2005. Proteomic identification of *M. tuberculosis* protein kinase substrates: PknB recruits GarA, a FHA domain-containing protein, through activation loop-mediated interactions. J. Mol. Biol. **350:**953–963.
190. **Vomastek, T., et al.** 1998. Characterisation of two putative protein Ser/Thr kinases from actinomycete *Streptomyces granaticolor* both endowed with different properties. Eur. J. Biochem. **257:**55–61.
191. **Wang, J., C. Li, H. Yang, A. Mushegian, and S. Jin.** 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.
192. **Wehenkel, A., M. Bellinzoni, F. Schaeffer, A. Villarino, and P. M. Alzari.** 2007. Structural and binding studies of the three-metal center in two mycobacterial PPM Ser/Thr protein phosphatases. J. Mol. Biol. **374:**890–898.
193. **Yang, X., C. M. Kang, M. S. Brody, and C. W. Price.** 1996. Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. Genes Dev. **10:**2265–2275.
194. **Yeats, C., R. D. Finn, and A. Bateman.** 2002. The PASTA domain: a beta-lactam-binding domain. Trends Biochem. Sci. **27:**438.
195. **Young, T. A., B. Delagoutte, J. A. Endrizzi, A. M. Falick, and T. Alber.** 2003. Structure of *Mycobacterium tuberculosis* PknB supports a universal activation mechanism for Ser/Thr protein kinases. Nat. Struct. Biol. **10:**168–174.
196. **Zhang, C. C.** 1996. Bacterial signalling involving eukaryotic-type protein kinases. Mol. Microbiol. **20:**9–15.
197. **Zhang, C. C.** 1993. A gene encoding a protein related to eukaryotic protein kinases from the filamentous heterocystous cyanobacterium Anabaena PCC 7120. Proc. Natl. Acad. Sci. U. S. A. **90:**11840–11844.
198. **Zhang, C. C., J. Jang, S. Sakr, and L. Wang.** 2005. Protein phosphorylation on Ser, Thr and Tyr residues in cyanobacteria. J. Mol. Microbiol. Biotechnol. **9:**154–166.
199. **Zhang, W., and L. Shi.** 2004. Evolution of the PPM-family protein phosphatases in Streptomyces: duplication of catalytic domain and lateral recruitment of additional sensory domains. Microbiology **150:**4189–4197.
200. **Zheng, J., C. He, V. K. Singh, N. L. Martin, and Z. Jia.** 2007. Crystal structure of a novel prokaryotic Ser/Thr kinase and its implication in the Cpx stress response pathway. Mol. Microbiol. **63:**1360–1371.
201. **Zheng, X., K. G. Papavinasasundaram, and Y. Av-Gay.** 2007. Novel substrates of *Mycobacterium tuberculosis* PknH Ser/Thr kinase. Biochem. Biophys. Res. Commun. **355:**162–168.

Sandro F. F. Pereira received his Ph.D. in biology from the Instituto de Tecnologiaq Uímica e Biológia at the Universidade Nova de Lisboa, Lisbon, Portugal, in 2008. The last 3 years of his thesis research were conducted in the Laboratory of Microbiology at The Rockefeller University in New York, NY. His thesis focused on cell division and cell wall synthesis in the human pathogen *Staphylococcus aureus.* He is currently a postdoctoral fellow in the Department of Microbiology and Immunology, Columbia University, New York, NY. His postdoctoral research explores the exit from bacterial dormancy, particularly the signaling pathway mediated by PrkC, a serine/threonine kinase in *Bacillus subtilis.*

Lindsie Goss earned her undergraduate degree in molecular and cell biology, with an emphasis in genetics, from the University of California, Berkeley. Before attending graduate school, she was a research assistant for 2 years at the University of California, San Francisco, in the laboratory of Matthias Wabl. She is currently a Ph.D. candidate in Jonathan Dworkin's laboratory at Columbia University in the Department of Microbiology and Immunology. She has been working on her thesis research for 2 years to understand the role of PrkC, one of the eukaryote-like Ser/Thr kinases in *Bacillus subtilis.*

Continued next page

Jonathan Dworkin studied physics as an undergraduate at Swarthmore College and received his Ph.D. with Peter Model at The Rockefeller University in 1998. His postdoctoral training was with Richard Losick at Harvard University, where he worked on aspects of bacterial development and chromosome segregation. He started his independent position in 2004 in the Department of Microbiology and Immunology, Columbia University, and his lab presently examines a range of topics, including exit from dormancy, cell biological and genetic characterization of peptidoglycan synthesis, and the role of the microbiota in the physiology of the bacteriovorous nematode *Caenorhabditis elegans.*