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Signaling mechanisms of the *Mycobacterium tuberculosis* receptor Ser/Thr protein kinases

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Abstract

Like eukaryotes, bacteria express receptor Ser/Thr protein kinases (STPKs) that initiate a wide variety of signaling networks. Recent biochemical and structural studies of the STPKs of *Mycobacterium tuberculosis* have revealed that bacterial and eukaryotic STPKs adopt common folds and share mechanisms of substrate recognition and regulation. Mycobacterial receptor STPKs are activated by dimerization through two distinct interfaces that promote activation-loop phosphorylation. The active STPKs phosphorylate diverse substrates within the bacterial cell, including other kinases as well as proteins involved in many central physiological processes. In the case of the FHA-domain protein, GarA, the unphosphorylated protein regulates primary metabolism, while phosphorylation mediates GarA autoinhibition. These studies have begun to define the activation mechanisms and the biological regulatory functions of the mycobacterial STPKs.

Keywords

eukaryotic-like kinase; activation mechanism; substrate specificity; autoinhibition; allosteric control; PknB

In all three kingdoms of life, receptor protein kinases play essential roles regulating cell physiology in response to extracellular cues. While two-component systems shuttle chemically labile phosphoryl groups between His and Asp residues, stable phosphorylation that is reversed by cognate phosphatases is mediated by the spatial and temporal regulation of Ser/Thr/Tyr kinases. With more than 10,000 members [1], this protein superfamily is among the largest known. Long associated exclusively with eukaryotes, STPKs were discovered in the last 17 years in bacteria, including many pathogens.

These “eukaryotic-like” STPKs play essential roles in growth, virulence, persistence and reactivation, generally by signaling within bacterial cells. In *Pseudomonas aeruginosa*, for example, Thr phosphorylation by the PpkA kinase regulates assembly of the Type VI secretion system that influences virulence in clinical isolates [2,3]. The *Bacillus* kinase PrkC mediates reactivation of spores in response to fragments of the peptidoglycan [4]. *Mycobacterium tuberculosis* (*Mtb*) expresses 11 STPKs (PknA-PknL), nine of which are predicted transmembrane receptors with the kinase domain located within the mycobacterial cell [5].

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Although the soluble *Mtb* kinase, PknG, has been reported to be secreted by *Mtb* and play an essential role in signaling in host cells [6], this model has not been corroborated by subsequent studies. To the contrary, biochemical studies, the occurrence of orthologs in nonpathogenic bacteria, and characterization of *pknG* knockout mutants of *Mtb* and *Corynebacterium glutamicum* show that PknG instead controls primary nitrogen and carbon metabolism within bacterial cells [7,8,9].

In *Mtb*, PknA and PknB are essential for growth [10,11,12]. Although *Mtb* PknB and *Bacillus* PrkC are orthologous kinases, the deletion of *prkC* results in viable bacteria that are defective in reactivation of spores [4]. These distinct phenotypes indicate that STPKs containing similar extracellular ligand-binding domains can be “wired” to cell physiology in distinct ways in different bacterial species. Here I survey recent work aimed at understanding the fundamental mechanisms of regulation and substrate recognition of the *Mtb* STPKs. Because these kinases show a maximum of 30% sequence identity to human homologs, studies of the bacterial STPKs inform not only the general mechanisms of signaling, they also provide important foundations for developing new antibiotics.

Conserved STPK structures

The Ser/Thr/Tyr kinases are molecular switches. Many structural features in the shared STPK fold influence the equilibrium between more active (“on”) and less active (“off”) states [13, 14]. Nearly universally, the Ser/Thr/Tyr protein kinases exist constitutively in the off state, and signals relieve autoinhibition. In contrast to the activated conformations, in which the catalytic machinery is assembled similarly, the inhibited states are structurally diverse. Several structural elements converge at the active site to control ATP binding, protein-substrate binding and the alignment of the catalytic groups.

A common chemical mechanism for activation involves phosphorylation of one or more sites in the activation loop, a centrally located, 15-33-residue segment flanked by the DFG and APE sequence motifs [13,14]. In the off state, the activation loop generally blocks the active site or remains flexible. In contrast, the phosphorylated activation loops adopt a distinct conformation that assembles a pThr binding site that stabilizes the active conformation. This conformational rearrangement permits ATP access, brings together dispersed elements of the catalytic machinery and forms part of the protein-substrate binding site.

The first crystal structures of a bacterial STPK kinase domain (KD), showing the phosphorylated *Mtb* PknB KD bound to two different ATP analogs [15,16], displayed features largely characteristic of active eukaryotic homologs (Fig. 1). The nucleotide was sandwiched between the N- and C-lobes, engaging the glycine-rich P-loop, a signature β -hairpin with main-chain amides that coordinate the triphosphate moiety. The Lys40-Glu59 ion pair bridged the nucleotide and the C-helix. While the position of the C-helix was reminiscent of inactive c-Src, active eukaryotic kinase structures with the C-helix position resembling PknB also have been reported [13,14]. Thus, the crystal structure of PknB is thought to largely define the active state of bacterial STPKs.

Unlike a typical active STPK, however, the PknB activation loop was disordered. This disorder remains a puzzle, because the PknB KD was phosphorylated on 3–7 sites [15], including both Thr residues in the activation loop essential for activation [17]. With the exception of PknG (which is not switched on by activation-loop phosphorylation), this functional phosphorylation pattern is conserved in mycobacterial receptor kinases [18,19,20,21]. Inhibiting PknD autophosphorylation with a small molecule in *Mtb* limited phosphorylation of cellular proteins, showing that STPK autophosphorylation is a central regulatory strategy *in vivo* [22]. Nonetheless, a disordered activation loop also was found in the structure of phosphorylated apo-PknE KD [23], suggesting that phosphorylation may not stabilize a unique active structure.

However, heterogeneous or hyper-phosphorylation may stabilize multiple conformations. Alternatively, binding to an unidentified cofactor or substrates may be required to fold the activation loop. Ultimately, understanding the changes caused by activation-loop phosphorylation will require structural characterization of an unphosphorylated KD to compare the off state directly to the phosphorylated form.

Activation mechanism

Current evidence indicates that the *Mtb* receptor kinases are activated by reversible interactions through two distinct protein interfaces. These contacts function to promote phosphorylation of the activation loop by different mechanisms. Although the phosphorylated KDs of PknB and PknE removed from the membrane are monomeric at typical concentrations in solution (K_d values in the low mM range), they crystallized as structurally similar dimers (Fig.2) [15,16, 23]. Disparate observations indicate that these “back-to-back” dimers stabilize the active conformation. The N-lobe dimer interface is conserved in orthologs of PknB. Rapamycin-promoted dimerization of unphosphorylated PknD and PknB KD fusion proteins to the FK506 binding protein and FKBP12-rapamycin-associated protein (FRAP) stimulated phosphorylation activity [24]. Mutations in the N-lobe dimer interface reduced this activation, limited autophosphorylation and altered substrate specificity. In contrast, dimerization with an inactive, catalytic-site mutant stimulated the wild-type PknD KD *in vitro* and *in vivo*. Because the inactive subunit in this heterodimer is incapable of phosphorylating the paired wild-type subunit, these results supported the idea that dimer formation allosterically activates unphosphorylated PknD [24]. The generality of this activation was demonstrated recently in *Pseudomonas aeruginosa*, where insertion of FK506 binding protein into an extracellular loop resulted in a PpkA kinase that was activated *in vivo* by addition of a small-molecule dimerizer [3]. Thus, N-lobe dimerization activates different bacterial receptor kinases *in vitro* and/or *in vivo*.

Remarkably, the back-to-back N-lobe interface also mediates allosteric activation of human PKR, the cytosolic double-stranded RNA-dependent protein kinase [25], and Ire1, the transmembrane kinase that mediates the unfolded protein response [26]. While these KD dimers stabilize the active conformations, the Ire1 dimer also mediates assembly of a larger oligomer that stimulates the cleavage of physiological substrates by the appended ribonuclease domains [27]. The conservation of a structurally analogous active dimer in two *Mtb* STPKs and in human PKR implies that this mechanism of protein kinase regulation is ancient and broadly distributed.

It is noteworthy that the kinases thus far found to form back-to-back, N-lobe dimers respond to ancient molecules, including double-stranded RNA (PKR), unfolded proteins (Ire1), and potentially for the bacterial kinases, peptidoglycan fragments (PknB) and disulfide bonds (PknE). In the phylogenetic tree of the human kinome, PKR and Ire1 are not members of the major subfamilies, but instead diverged close to their common ancestor [28]. Combined with the distribution of PknB in diverse bacteria, the occurrence of STPKs in all three kingdoms of life, and the apparently ancient divergence of mycobacterial and human STPKs, these sequence relationships support the idea that the bacterial STPKs arose by divergent evolution.

The idea that the N-lobe interface stabilizes the active conformation left open the question of how the kinase domains are autophosphorylated. This is a general question in protein kinase biochemistry, and both true intramolecular autophosphorylation and intermolecular reactions have been detected. For intermolecular (*trans*) autophosphorylation, it is unknown if the sequence around the phosphorylation sites or structural interfaces between the kinase molecules mediate recognition.

Structural studies of PknB implicated a second interface—including the G-helix and activation loop in the C-lobe—in bacterial KD autophosphorylation. The structure of an ATP-competitive inhibitor bound a PknB KD variant [29] unexpectedly revealed features consistent with an activation complex (Fig. 3). The inhibitor-bound PknB KD formed an asymmetric dimer, with the G-helix and the ordered activation loop of one KD in contact with the G-helix of the other. The activation loop of this putative “substrate” KD was disordered, with the ends positioned at the entrance to the partner KD active site. As expected for an ES complex, single amino-acid substitutions in the G-helix interface reduced activation-loop phosphorylation, and multiple replacements abolished KD phosphorylation and kinase activation. The unaltered consensus PknB phosphorylation motif (TQX $\Phi\Phi$) [11] in the activation loop of the mutants was insufficient to mediate autophosphorylation. These results support a model in which a structurally asymmetric, “front-to-front” association through the G-helix mediates intermolecular autophosphorylation of PknB and homologous kinases.

Once phosphorylated, the monomeric *Mtb* PknD KD is fully active *in vitro*, and dimerization does not further stimulate kinase activity [24]. This property provides a mechanism to propagate and amplify an activation signal to STPK molecules that are not engaged by the extracellular ligand [29] (Fig. 4). In addition, phosphorylation in *trans* allows a signal sensed by one STPK to activate another. *Mtb* PknB phosphorylates PknA, for example, and may activate PknA to phosphorylate distinct substrates [11]. Such crosstalk between STPKs provides a mechanism to integrate pathways, but also raises the problem of establishing signaling specificity. The generality of crosstalk, the specific networks of the *Mtb* STPKs, and the impacts of cross phosphorylation have yet to be defined.

Substrate specificity and metabolic control

The biological roles of the STPKs depend crucially on their substrate specificity. Recent studies demonstrate that the *Mtb* STPKs phosphorylate proteins involved in a large variety of processes, including cell metabolism, transcriptional regulation, translation, cell-wall biosynthesis, and cell division. Demonstrating the functional significance of observed phosphorylations remains an ongoing challenge. A review tabulating reported STPK substrates in *Mtb* was published recently [9], and I will not reprise that list here. A varied combination of *in vitro* and *in vivo* experiments has proven essential to make progress. Biochemical methods, for example, have been critical to identify candidate substrates, quantify modifications, map phosphorylation sites and demonstrate large changes in activity upon phosphorylation. At the same time, studies of the effects of phosphorylation *in vivo* have been critical to establish the biological functions of STPK regulation.

Pioneering work by Husson and coworkers identified Wag31, Rv1422 and PknA as heterologous substrates of mycobacterial PknB [11]. Wag31 is dramatically localized to the cell poles [30], raising the question of whether the STPK shows a similarly restricted spatial distribution. PknD and PknB respectively phosphorylate the σ -factor regulators Rv0516c and RshA [24] on noncanonical sites that do not fit the current paradigm for σ -factor regulation. Nonetheless, RshA phosphorylation was shown recently to regulate SigH *in vivo* [31]. PknD activation in *Mtb* also altered the expression of more than 100 genes regulated by SigF, providing evidence that PknD converts environmental signals into a transcriptional response [22]. Purified *Mtb* STPK KDs except those of PknG, PknI and PknJ phosphorylated the FAS-II components KasA and KasB *in vitro*, and these fatty-acid biosynthetic enzymes are phosphorylated in *M. bovis* BCG [32]. Nearly all the *Mtb* STPKs phosphorylated GarA *in vitro* (see below) [33]. Some reported substrates, however, present unresolved paradoxes. The extracellular cell-wall transpeptidase, PbpA, for example, has been reported to be a substrate of PknB [34], even though the kinase, ATP and the antagonistic phosphatase, PstP, are intracellular. Similarly, the reported PknD phosphorylation site on MmpL7 [35] is predicted

to be extracellular. Overall, many substrates remain to be discovered, and the mechanisms of protein-substrate recognition remain to be determined.

Proteins containing forkhead-associated (FHA) domains comprise an important class of *Mtb* STPK substrates. FHA domains bind pThr peptides [36], triggering assembly of protein complexes. *Mtb* produces five FHA-domain proteins: EmbR, GarA, an ABC transporter (Rv1747), and two proteins (Rv0019c and Rv0020c) co-expressed with PknA and PknB. Molle and coworkers identified the transcriptional regulator, EmbR, as the first cognate substrate of a *Mtb* STPK [20]. This phosphorylation stimulates promoter DNA binding *in vitro* and activates transcription of the *embCAB* genes (involved in arabinan biosynthesis) *in vivo* [37]. PknH autophosphorylation was essential to bind and phosphorylate EmbR, but PknH phosphopeptides, including a phosphorylated activation-loop sequence, failed to bind to the EmbR FHA domain [38]. Indeed, the cocrystal structure of EmbR showed a noncognate pThr peptide from yeast Rad53 bound to the *Mtb* FHA domain. This structure confirmed the canonical mechanism of pThr recognition [38], but left open the nature of the determinants of binding specificity.

In general, a complex network of STPKs phosphorylates the *Mtb* FHA-domain proteins *in vitro* [39]. Alzari and coworkers established the FHA-domain protein GarA as an efficient substrate of several *Mtb* KDs, with phosphorylation occurring at Thr21 (PknG) or Thr22 (other STPKs) in a disordered N-terminal extension [7,33]. Binding of the GarA FHA domain to the PknB KD required the phosphorylated kinase activation loop [33]. Because this interaction would be expected to sequester the activation loop away from the active conformation, however, the basis for phosphorylation of the FHA-domain protein in such a complex remains enigmatic. In contrast, autophosphorylation of sites in the first 65 amino acids of PknG, N-terminal to the kinase domain, recruits GarA and enhances phosphorylation [7].

Exciting recent papers from several groups established not only a new mechanism of GarA regulation of carbon and nitrogen metabolism, but also showed that Thr phosphorylation autoinhibits GarA (Fig. 5). Affinity chromatography of mycobacterial extracts identified NAD⁺-dependent glutamate dehydrogenase (GDH), α -ketoglutarate decarboxylase (KGD), the α subunit of glutamine synthetase (GS), and PknG as soluble GarA binding partners [7,8]. Although it contains a pThr-binding FHA domain, GarA unexpectedly recognized the unphosphorylated forms of GDH, KGD and GS. The pThr-binding site functions instead to recognize phosphorylated STPKs and, once the N-terminal tail is phosphorylated on Thr21 or Thr22, to stabilize the tail in a conformation that inhibits target-enzyme binding [8,40].

Incisive NMR studies of the unphosphorylated and PknB-phosphorylated forms of GarA and the *C. glutamicum* ortholog, OdhI, revealed that this intramolecular binding orders the phosphorylated tail and occludes overlapping surfaces required for binding to all the target enzymes [8,41]. *In vitro* assays demonstrated that GarA binding inhibits the catalytic activities of purified GDH and KGD, and, at the same time, activates GS. These three enzymes act on α -ketoglutarate at the nexus of the TCA cycle and ammonia fixation, and the combined effects of GarA favor glutamate biosynthesis. These results provide a beautifully simple explanation for the increased levels of glutamate in *pknG* knockout bacteria (in which GarA is hyperactive) [7,8].

These studies begin to provide a mechanistic understanding of the biological effects of Ser/Thr phosphorylation in *Mtb*. STPK phosphorylation also exhibits varied downstream effects on many proteins lacking FHA domains. Phosphorylation of the transcription factor VirS (by PknK) [42] increases affinity for promoter DNA sequences, while phosphorylation of Rv2175c (by PknL) inhibits promoter DNA binding [43]. PknD phosphorylation of the anti-anti-sigma factor homolog, Rv0516c, decreases binding of Rv2638 (ref [22]). Phosphorylation (by PknA

or PknF) of FabH, a β -ketoacyl carrier protein involved in mycolic acid biosynthesis, blocks the entrance to the substrate channel and inhibits the enzyme [44]. Structural studies of the phosphorylated substrates of the *Mtb* STPKs promise to continue to reveal diverse mechanisms of allosteric control.

Prospects

Rapid recent progress has begun to define the mechanisms of activation, the cellular substrates and the biologic functions of mycobacterial STPKs. Dimerization and phosphorylation regulate the *Mtb* STPKs in ways that recapitulate and inform the activation mechanisms of eukaryotic family members. While *Mtb* kinase substrates are being discovered rapidly, little is known about how STPKs recognize target proteins. Further studies are needed to test the roles of the phosphorylation-site sequence, structural interactions with cognate kinases and STPK localization in substrate recognition. Much effort is focused on defining how the STPKs regulate bacterial physiology. Recent work on the PknB ortholog (PrkC) in *Bacillus subtilis* has provided an exciting emerging hypothesis that peptidoglycan (PG) fragments activate PknB and regulate PG synthesis, cell growth and division [4]. With 11 STPKs, *Mtb* affords a tractable, medically important system to explore these fundamental issues.

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References

1. Cheek S, Ginalski K, Zhang H, Grishin NV. A comprehensive update of the sequence and structure classification of kinases. *BMC Struct Biol* 2005;5:6. [PubMed: 15771780]
2. Mougous JD, Gifford CA, Ramsdell TL, Mekalanos JJ. Threonine phosphorylation post-translationally regulates protein secretion in *Pseudomonas aeruginosa*. *Nat Cell Biol* 2007;9:797–803. [PubMed: 17558395] Genetic and biochemical analysis showed the central roles of the PpkA STPK, the antagonistic PppA Ser/Thr phosphatase and the Fha1 FHA-domain protein in assembling the type VI secretion apparatus in *P. aeruginosa*.
3. Hsu F, Schwarz S, Mougous JD. TagR promotes PpkA-catalysed type VI secretion activation in *Pseudomonas aeruginosa*. *Mol Microbiol* 2009;72:1111–1125. [PubMed: 19400797] A fascinating study of type VI secretion in *P. aeruginosa* established a cascade of assembly reactions triggered by activation of the PpkA STPK by dimerization *in vivo*.
4. S Shah IM, Laaberki MH, Popham DL, Dworkin J. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* 2008;135:486–496. [PubMed: 18984160] A beautiful demonstration that peptidoglycan fragments signal through PknB orthologs, and the species specificity of signaling is established by the correspondence between the extracellular PASTA domains of the STPK and chemical variations in peptidoglycan.
5. Av-Gay Y, Everett M. The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. *Trends Microbiol* 2000;8:238–244. [PubMed: 10785641] The classic review of the *Mtb* kinome.
6. Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K, Klebl B, Thompson C, Bacher G, Pieters J. Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. *Science* 2004;304:1800–1804. [PubMed: 15155913]
7. O'Hare HM, Duran R, Cervenansky C, Bellinzoni M, Wehenkel AM, Pritsch O, Obal G, Baumgartner J, Vialaret J, Johnsson K, et al. Regulation of glutamate metabolism by protein kinases in mycobacteria. *Mol Microbiol* 2008;70:1408–1423. [PubMed: 19019160] A broad and definitive study that established the molecular mechanism by which PknG regulates glutamate pools by phosphorylating

GarA and inhibiting GarA interactions with target enzymes. This paper also showed that, rather than activating the KD, PknG autophosphorylation provides a GarA binding site. These data significantly resolved the controversy about the biological function of PknG in *Mtb*.

8. Nott TJ, Kelly G, Stach L, Li J, Westcott S, Patel D, Hunt DM, Howell S, Buxton RS, O'Hare HM, et al. An intramolecular switch regulates phospho-independent FHA domain interactions in *Mycobacterium tuberculosis*. *Sci Signal* 2009;2:ra12. [PubMed: 19318624] NMR structural analysis and mutagenesis studies showed how GarA phosphorylation autoinhibits binding of target enzymes. Biochemical studies also identified glutamine synthase as another enzyme regulated by GarA.
9. Chao J, Wong D, Zheng X, Poirier V, Bach H, Hmama Z, Av-Gay Y. Protein kinase and phosphatase signaling in *Mycobacterium tuberculosis* physiology and pathogenesis. *Biochim Biophys Acta*. 2009
10. Sassetti CM, Boyd DH, Rubin EJ. Genes required for mycobacterial growth defined by high density mutagenesis. *Mol Microbiol* 2003;48:77–84. [PubMed: 12657046]
11. Kang CM, Abbott DW, Park ST, Dascher CC, Cantley LC, Husson RN. The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. *Genes Dev* 2005;19:1692–1704. [PubMed: 15985609] The first identification of mycobacterial STPK substrates *in vivo*. PknB phosphorylated Wag31, Rv1422 and PknA, establishing the precedent for STPK cross phosphorylation. Cell morphology phenotypes of depleting or overexpressing PknA or PknB connected these kinases to cell-growth and cell-wall processes.
12. Fernandez P, Saint-Joanis B, Barilone N, Jackson M, Gicquel B, Cole ST, Alzari PM. The Ser/Thr protein kinase PknB is essential for sustaining mycobacterial growth. *J Bacteriol* 2006;188:7778–7784. [PubMed: 16980473]
13. Huse M, Kuriyan J. The conformational plasticity of protein kinases. *Cell* 2002;109:275–282. [PubMed: 12015977] An outstanding review of conformational transitions and regulatory mechanisms of eukaryotic kinases.
14. Nolen B, Taylor S, Ghosh G. Regulation of protein kinases; controlling activity through activation segment conformation. *Mol Cell* 2004;15:661–675. [PubMed: 15350212]
15. Young TA, Delagoutte B, Endrizzi JA, Falick AM, Alber T. Structure of *Mycobacterium tuberculosis* PknB supports a universal activation mechanism for Ser/Thr protein kinases. *Nat Struct Biol* 2003;10:168–174. [PubMed: 12548283] The crystal structure of the PknB KD revealed the bacterial STPK fold, activation-loop phosphorylation and the conserved N-lobe dimer interface.
16. Ortiz-Lombardia M, Pompeo F, Boitel B, Alzari PM. Crystal structure of the catalytic domain of the PknB serine/threonine kinase from *Mycobacterium tuberculosis*. *J Biol Chem* 2003;278:13094–13100. [PubMed: 12551895]
17. Boitel B, Ortiz-Lombardia M, Duran R, Pompeo F, Cole ST, Cervenansky C, Alzari PM. 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* 2003;49:1493–1508. [PubMed: 12950916]
18. Duran R, Villarino A, Bellinzoni M, Wehenkel A, Fernandez P, Boitel B, Cole ST, Alzari PM, Cervenansky C. Conserved autophosphorylation pattern in activation loops and juxtamembrane regions of *Mycobacterium tuberculosis* Ser/Thr protein kinases. *Biochem Biophys Res Commun* 2005;333:858–867. [PubMed: 15967413] Mass spectrometry showed a shared pattern of hyperphosphorylation of purified *Mtb* kinase domains.
19. Molle V, Zanella-Cleon I, Robin JP, Mallejac S, Cozzzone AJ, Becchi M. Characterization of the phosphorylation sites of *Mycobacterium tuberculosis* serine/threonine protein kinases, PknA, PknD, PknE, and PknH by mass spectrometry. *Proteomics* 2006;6:3754–3766. [PubMed: 16739134]
20. Molle V, Kremer L, Girard-Blanc C, Besra GS, Cozzzone AJ, Prost JF. An FHA phosphoprotein recognition domain mediates protein EmbR phosphorylation by PknH, a Ser/Thr protein kinase from *Mycobacterium tuberculosis*. *Biochemistry* 2003;42:15300–15309. [PubMed: 14690440] A groundbreaking study reporting for the first time that activation-loop autophosphorylation is required to activate a bacterial receptor STPK. This paper also identified EmbR as the first heterologous substrate of an *Mtb* STPK, and showed that the EmbR FHA-domain pThr binding site mediates PknH binding and EmbR phosphorylation.
21. Canova MJ, Veyron-Churlet R, Zanella-Cleon I, Cohen-Gonsaud M, Cozzzone AJ, Becchi M, Kremer L, Molle V. 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* 2008;8:521–533. [PubMed: 18175374]
22. Greenstein AE, MacGurn JA, Baer CE, Falick AM, Cox JS, Alber T. M. tuberculosis Ser/Thr protein kinase D phosphorylates an anti-anti-sigma factor homolog. *PLoS Pathog* 2007;3:e49. [PubMed: 17411339] Biochemical studies and transcriptional profiling in *Mtb* established a mechanistic link between STPKs and regulation of transcription via alternative sigma factors. Protein phosphorylation following expression of wild-type and catalytically inactive PknD in *Mtb* suggested that dimerization and autophosphorylation activate the kinase *in vivo*.
 23. Gay LM, Ng HL, Alber T. A conserved dimer and global conformational changes in the structure of apo-PknE Ser/Thr protein kinase from *Mycobacterium tuberculosis*. *J Mol Biol* 2006;360:409–420. [PubMed: 16762364] The second structure of a bacterial receptor KD established the generality of the back-to-back, N-lobe dimer.
 24. Greenstein AE, Echols N, Lombana TN, King DS, Alber T. Allosteric activation by dimerization of the PknD receptor Ser/Thr protein kinase from *Mycobacterium tuberculosis*. *J Biol Chem* 2007;282:11427–11435. [PubMed: 17242402] The first direct biochemical demonstration that dimerization of PknD KD fusion proteins activated auto- and transphosphorylation activities through an allosteric mechanism involving the N-lobe interface.
 25. Dey M, Cao C, Dar AC, Tamura T, Ozato K, Sicheri F, Dever TE. Mechanistic link between PKR dimerization, autophosphorylation, and eIF2alpha substrate recognition. *Cell* 2005;122:901–913. [PubMed: 16179259] Crystal structure a complex of PKR with a protein substrate showed disorder of the substrate phosphorylation site that affords access to the kinase active site and surfaces including the G-helix mediate substrate binding.
 26. Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F. Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. *Cell* 2008;132:89–100. [PubMed: 18191223]
 27. Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, Stroud RM, Walter P. The unfolded protein response signals through high-order assembly of Ire1. *Nature* 2009;457:687–693. [PubMed: 19079236]
 28. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science* 2002;298:1912–1934. [PubMed: 12471243]
 29. Mieczkowski C, Iavarone AT, Alber T. Auto-activation mechanism of the *Mycobacterium tuberculosis* PknB receptor Ser/Thr kinase. *Embo J* 2008;27:3186–3197. [PubMed: 19008858] A PknB:inhibitor cocrystal structure and mutational analysis suggested that asymmetric G-helix contacts mediate intermolecular autophosphorylation of the activation loop. This reaction provided a mechanism to amplify and propagate signals to other kinases.
 30. Kang CM, Nyayapathy S, Lee JY, Suh JW, Husson RN. Wag31, a homologue of the cell division protein DivIVA, regulates growth, morphology and polar cell wall synthesis in mycobacteria. *Microbiology* 2008;154:725–735. [PubMed: 18310019]
 31. Park ST, Kang CM, Husson RN. Regulation of the SigH stress response regulon by an essential protein kinase in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2008;105:13105–13110. [PubMed: 18728196] Studies in several mycobacterial species established that PknB phosphorylation of RshA reduces binding to SigH *in vitro* and increases SigH-mediated transcriptional responses to stress *in vivo*.
 32. Molle V, Brown AK, Besra GS, Cozzzone AJ, Kremer L. The condensing activities of the *Mycobacterium tuberculosis* type II fatty acid synthase are differentially regulated by phosphorylation. *J Biol Chem* 2006;281:30094–30103. [PubMed: 16873379]
 33. Villarino A, Duran R, Wehenkel A, Fernandez P, England P, Brodin P, Cole ST, Zimny-Arndt U, Jungblut PR, Cervenansky C, et al. Proteomic identification of *M. tuberculosis* protein kinase substrates: PknB recruits GarA, a FHA domain-containing protein, through activation loop-mediated interactions. *J Mol Biol* 2005;350:953–963. [PubMed: 15978616]
 34. Dasgupta A, Datta P, Kundu M, Basu J. The serine/threonine kinase PknB of *Mycobacterium tuberculosis* phosphorylates PBPA, a penicillin-binding protein required for cell division. *Microbiology* 2006;152:493–504. [PubMed: 16436437]

35. Perez J, Garcia R, Bach H, de Waard JH, Jacobs WR Jr, Av-Gay Y, Bubis J, Takiff HE. Mycobacterium tuberculosis transporter MmpL7 is a potential substrate for kinase PknD. *Biochem Biophys Res Commun* 2006;348:6–12. [PubMed: 16879801]
36. Durocher D, Taylor IA, Sarbassova D, Haire LF, Westcott SL, Jackson SP, Smerdon SJ, Yaffe MB. The molecular basis of FHA domain:phosphopeptide binding specificity and implications for phospho-dependent signaling mechanisms. *Mol Cell* 2000;6:1169–1182. [PubMed: 11106755]
37. Sharma K, Gupta M, Pathak M, Gupta N, Koul A, Sarangi S, Baweja R, Singh Y. Transcriptional control of the mycobacterial embCAB operon by PknH through a regulatory protein, EmbR, in vivo. *J Bacteriol* 2006;188:2936–2944. [PubMed: 16585755]
38. Alderwick LJ, Molle V, Kremer L, Cozzzone AJ, Dafforn TR, Besra GS, Futterer K. Molecular structure of EmbR, a response element of Ser/Thr kinase signaling in Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A* 2006;103:2558–2563. [PubMed: 16477027]
39. Grundner C, Gay LM, Alber T. Mycobacterium tuberculosis serine/threonine kinases PknB, PknD, PknE, and PknF phosphorylate multiple FHA domains. *Protein Sci* 2005;14:1918–1921. [PubMed: 15987910]
40. England P, Wehenkel A, Martins S, Hoos S, Andre-Leroux G, Villarino A, Alzari PM. The FHA-containing protein GarA acts as a phosphorylation-dependent molecular switch in mycobacterial signaling. *FEBS Lett* 2009;583:301–307. [PubMed: 19114043] Biophysical studies that demonstrated that phosphorylation of the N-terminal tail of GarA stabilizes an intramolecular interaction with the pThr binding site.
41. Barthe P, Roumestand C, Canova MJ, Kremer L, Hurard C, Molle V, Cohen-Gonsaud M. Dynamic and structural characterization of a bacterial FHA protein reveals a new autoinhibition mechanism. *Structure* 2009;17:568–578. [PubMed: 19368890] NMR solution structures of the unphosphorylated and phosphorylated forms of OdhI, the GarA homolog in *C. glutamicum*, showed that phosphorylation triggers intramolecular binding of the of the N-terminal tail to the pThr binding site in the FHA domain. With references 8 and 40, these studies established an elegant new mechanism of FHA-domain regulation.
42. Kumar P, Kumar D, Parikh A, Rananaware D, Gupta M, Singh Y, Nandicoori VK. 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* 2009;284:11090–11099. [PubMed: 19251699]
43. Cohen-Gonsaud M, Barthe P, Canova MJ, Stagier-Simon C, Kremer L, Roumestand C, Molle V. The Mycobacterium tuberculosis Ser/Thr kinase substrate Rv2175c is a DNA-binding protein regulated by phosphorylation. *J Biol Chem* 2009;284:19290–19300. [PubMed: 19457863] A compelling structure-function mystery story focusing on how *Mtb* STPKs regulate cellular substrates. NMR and biochemical studies showed that Rv2175c is a DNA-binding protein whose affinity for DNA is reduced upon Thr9 phosphorylation by PknL.
44. Veyron-Churlet R, Molle V, Taylor RC, Brown AK, Besra GS, Zanella-Cleon I, Futterer K, Kremer L. The Mycobacterium tuberculosis beta-ketoacyl-acyl carrier protein synthase III activity is inhibited by phosphorylation on a single threonine residue. *J Biol Chem* 2009;284:6414–6424. [PubMed: 19074144]
45. Madhusudan, Akamine P, Xuong NH, Taylor SS. Crystal structure of a transition state mimic of the catalytic subunit of cAMP-dependent protein kinase. *Nat Struct Biol* 2002;9:273–277. [PubMed: 11896404]

Figure 1. The PknB KD:ATP γ S structure resembles the transition-state analog complex of cAMP-dependent protein kinase (PKA)

Superimposed backbone ribbons show the similarity of the classic kinase folds of PknB (blue) and PKA (yellow), including the two-domain structure and the P-loop covering the nucleotide. The bound nucleotides and several active-site residues in PknB:ATP- γ -S (blue) and the PKA:ADP:AlF₃:peptide transition-state-analog complex [45] shows that conserved residues contact the nucleotides in similar conformations.

Figure 2. *Mtb* PknB, PknE and human PKR KDs form structurally similar activated dimers
Ribbon drawings of PknB-ATP γ S (left; C-helix yellow, nucleotide in spheres), apo-PknE (middle), and a superposition (right) of PknB (blue) and PKR (orange) reveal the conserved architecture. The N-lobe interaction holds the catalytic sites away from each other and activates the unphosphorylated kinases by an allosteric mechanism. The back-to-back interface communicates to the active site over 25 Å across the protein.

Fig. 3. Inhibitor complex of a PknB KD mutant resembled an autophosphorylation complex

A. PknB monomers formed an asymmetric offset dimer with contacts between the G-helices. Identical interface residues occur in distinct environments in the presumptive substrate (yellow) and enzymatic (blue). The activation loop was disordered in the substrate KD (yellow dashed line) and ordered (red line) in the enzyme KD.) **B.** Surface representation showing the contacts between kinase molecules and the ordered activation loop (red) in the enzyme KD.

Fig. 4. Model for *Mtb* receptor STPK regulation [24]

The inactive, unphosphorylated kinase monomer (left) is paired by an extracellular signal. The dimer adopts a conformation that is activated for autophosphorylation and substrate phosphorylation. The autophosphorylated monomers are active and amplify the signal (right). After the signal dissipates, the PstP phosphatase returns the kinase to the unphosphorylated inactive form.

Figure 5. Phosphorylation autoinhibits GarA

A. Schematic model of *Mtb* GarA inhibition [8]. The FHA domain (orange) engages the N-terminal tail (red) phosphorylated on Thr21 (by PknG) or Thr22 (by PknB), stabilizing a conformation that blocks binding of the STPKs and target enzymes GDH, KGD and GS. The N-terminal tail adopts many conformations in the on state. **B.** Ribbon diagram of the average NMR structure of GarA [8] showing the N-terminal tail (red) occluding the enzyme-binding surface. Spheres show pThr22 (atom colors) and sites where mutations block binding of all three (blue) or two of the three (light blue) unphosphorylated target enzymes. **C.** Superposition of the average NMR structures of OdhI [41], the GarA homolog in *C. glutamicum*, unphosphorylated (cyan) and phosphorylated on Thr15 (blue). Folding of the N-terminal tail into the pThr binding site causes little change in the core FHA domain structure.