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Review

Mycobacterial Ser/Thr protein kinases and phosphatases: Physiological roles and therapeutic potential

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Abstract

Reversible protein phosphorylation is a major regulation mechanism of fundamental biological processes, not only in eukaryotes but also in bacteria. A growing body of evidence suggests that Ser/Thr phosphorylation play important roles in the physiology and virulence of *Mycobacterium tuberculosis*, the etiological agent of tuberculosis. This pathogen uses 'eukaryotic-like' Ser/Thr protein kinases and phosphatases not only to regulate many intracellular metabolic processes, but also to interfere with signaling pathways of the infected host cell. Disrupting such processes by means of selective inhibitors may thus provide new pharmaceutical weapons to combat the disease. Here we review the current knowledge on Ser/Thr protein kinases and phosphatases in *M. tuberculosis*, their regulation mechanisms and putative substrates, and we explore their therapeutic potential as possible targets for the development of new anti-mycobacterial compounds.

Keywords: Mycobacterium tuberculosis; Ser/Thr protein phosphorylation; Protein kinases; Protein phosphatases; Drug design

1. Introduction

Mycobacterium tuberculosis is the causative agent of tuberculosis, a major health problem worldwide that is responsible for the deaths of over two million people every year. A better understanding of the physiopathology of the tubercle bacillus, with the goal of discovering and validating new therapeutic targets, is an imperative need to improve the treatment of tuberculosis. Emerging evidence suggests that signaling elements, in particular those involved in Ser/Thr

protein phosphorylation, may represent a new class of promising targets. Recent studies are starting to unveil the important roles of Ser/Thr protein kinases (STPKs) and phosphatases in mycobacterial physiology and virulence. Furthermore, the low sequence identities (<30%) between mycobacterial and human STPKs, and the expertise available in the design of specific inhibitors for eukaryotic STPKs, currently one of the most active therapeutic areas [1], may be exploited to develop novel antibiotics targeting this enzyme family in mycobacteria.

Abbreviations: STPK, Ser/Thr protein kinase; FHA, forkhead-associated domain; PASTA, penicillin-binding protein and Ser/Thr kinase-associated domain; PTP, protein tyrosine kinase; pThr, phospho-threonine; pSer, phospho-serine; MS, mass spectrometry

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The initial analysis of predicted protein kinases in *M. tuberculosis* was described by Av-Gay and co-workers [2] and an excellent review on current structure/function studies of Ser/ Thr and Tyr protein phosphorylation has been published recently by Alber and co-workers [3]. Here, we will focus on the physiological properties and therapeutic potential of mycobacterial Ser/Thr protein kinases and phosphatases as deduced from recent genetic, biochemical and structural studies.

2. Signaling mechanisms in prokaryotes

Signal transduction involving reversible protein phosphorylation in prokaryotes is primarily conducted by two-component systems. In their simplest form, these systems consist of a sensor histidine kinase and a response regulator [4], although signaling cascades involving several histidine kinases and/or response regulators may function in a phospho-relay mechanism to control complex developmental processes such as sporulation [5,6] and [7]. The availability of complete genome sequences has confirmed the prevalence of two-component systems in bacteria. Although the number of these proteins differs greatly (ranging from none in Mycoplasma genitalium, [8] and [9], to 80 in Synechocystis sp. [10]), the genes coding for two-component systems typically represent 1-2% of the bacterial genome (Fig. 1). The M. tuberculosis genome encodes 11 complete twocomponent systems, several of which contribute to the virulence of *M. tuberculosis* [11–14] and [15], but only one system (MtrA, MtrB) was reported to be essential for cell growth [16].

Interestingly, mycobacterial genomes contain a relatively low number of two-component systems when compared to other bacterial genomes of similar size. This reduced number of histidine kinases and response regulators appears to be offset by alternative signal transduction mechanisms involving Ser/Thr phosphorylation (Fig. 1). Indeed, the *M. tuberculosis* genome has as many genes coding for STPKs as those coding for complete two-component systems [17,18] and [19]. Other myco-bacterial genomes have an even greater number of STPK genes (24 in the *Mycobacterium marinum* genome [20]), suggesting that Ser/Thr phosphorylation mediates the bulk of signal transduction.

3. The eukaryotic-like protein kinases in M. tuberculosis

The *M. tuberculosis* genome includes 11 STPK genes, namely *pknA* to *pknL* [17], and their domain organization indicates that only two enzymes (PknG and PknK) are soluble proteins. All other mycobacterial STPKs are predicted to be transmembrane (receptor-like) proteins [2], with their N-terminal kinase domain connected through a single transmembrane helix to one or more C-terminal domains that presumably serve as signal sensors.

The catalytic domains of all mycobacterial STPKs include the 12 conserved motifs defining the 'signature' of eukaryotic protein kinases [2] and [21]. The comparative sequence analysis of the mycobacterial kinase domains with those of other prokaryotic STPKs shows that they can be grouped in well-defined homology clusters (Fig. 2A). Thus, the nine *M. tuberculosis* receptor-like STPKs belong to three distinct groups (PknA/PknB/PknL, PknF/PknI/PknJ and PknD/PknE/PknH), while the two soluble enzymes (PknG and PknK) stand clearly apart from all the others [20]. Despite these differences, however, bacterial STPK sequences are more similar to each other than to their human homologues, with which they share less than 30% sequence identity. A distance tree (Fig. 2B) shows



Fig. 1. Signaling elements in bacteria. The figure shows genome sizes in Mb (grey bars) and the number of genes encoding two-component systems (His kinases, blue dots; response regulators, green dots) and STPKs (red dots), as defined by homology searches in selected bacterial genomes.

3.1. Crystal structures of the catalytic domains. Insights into kinase regulation

The crystallographic studies of the kinase domain of PknB in complex with ATP analogues yielded the first structure of a bacterial STPK [23] and [24] and confirmed the remarkable conservation of the overall protein fold and catalytic machinery between eukaryotic and prokaryotic STPKs (Fig. 3A). However, unlike many eukaryotic STPKs, the activation loop of PknB, having at least two fully phosphorylated threonine residues (Thr171 and Thr173) [24] and [25], was found to be disordered in the available structures, suggesting an induced-fit mode of binding for the acceptor substrate(s) or other modulating factors. On the other hand, the phosphorylation state of this loop was shown to regulate kinase activity, in much the same way as their eukaryotic counterparts, because dephosphorylation of PknB by the cognate Ser/Thr protein phosphatase, PstP, or mutation of the autophosphorylation sites Thr171 and Thr173, significantly decreased kinase activity [25].

Alber and co-workers [3] and [24] noted that bacterial PknBlike STPKs have a conserved site on the opposite side of the Nterminal lobe relative to the active site, which might be functionally important. This conserved surface forms the dimerization interface in three independent crystal forms of PknB [23,24] and [26] and the equivalent region was also involved in dimerization of the PknE structure [27]. Furthermore, the formation of a very similar 'back-to-back' dimer was recently shown to activate the RNA-dependent antiviral protein kinase PKR [28] and [29], suggesting that mycobacterial enzymes use an analogous mechanism of regulation [26]. According to this model, monomeric PknB would be inactive due for instance to a misplacement of helix αC , whose Cterminal end contributes to the dimer interface. Ligand binding to the extracellular sensor domain would then promote 'back-toback' dimerization of the kinase domain and subsequent activation. A direct effect of the extracellular domain on kinase dimerization was indeed demonstrated for the PknB-like protein kinase PrkC from *Bacillus subtilis* [30], and recent work on M. tuberculosis PknD has confirmed the major features of this model, demonstrating that dimer formation allosterically activates the kinase in vitro [31]. Interestingly, the dimer interface is well conserved within each clade of receptor-like kinases (PknA/PknB/PknL, PknF/PknI/PknJ and PknH/PknE/ PknD) [20], suggesting the possibility of hierarchical signaling networks regulated by ligand-induced heterodimer formation [31].

3.2. The extracellular 'sensor' domains of receptor-like STPKs

The functional properties of the putative sensor domains from transmembrane STPKs remain poorly understood. The extracellular domain of PknD has been structurally characterized and found to fold into a highly symmetric six-bladed β propeller [32]. The authors suggested that PknD could bind a multivalent ligand that signals by changing the quaternary structure of the intracellular kinase domain, although the structure failed to provide strong clues about the nature of the



Fig. 2. Dendograms of a multiple sequence comparison of kinase domains. (A) Distance tree of 1311 prokaryotic STPKs identified from sequence homology searches using hidden Markov models for profile analysis [100]. *M. tuberculosis* STPKs and closely related sequences are shown in red and labeled. (B) Distance tree of all mycobacterial STPKs (Mtb, in red) and human STPKs representative of the different groups proposed by Manning and co-workers [22] (*AGC*, containing PKA, PKG, PKC families; *CAMK*, calcium/calmodulin-dependent protein kinases; *CK1*, casein kinase 1; *CMGC*, containing CDK, MAPK, GSK3, CLK families; *STE*, homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases; *TK*, tyrosine kinases; *TKL*, tyrosine kinase-like).



Fig. 3. Conserved structures and catalytic machineries of bacterial STPKs and phosphatases. (A) Structural superposition of PknB (pale yellow) and human phosphorylase kinase (blue). The bound nucleotide and particular residues critical for catalysis are shown in stick representation. (B) Structural superposition of PstP (yellow) and human PP2C α (blue). The metal ions are shown as spheres, the bound phosphate in each structure and conserved metal-coordinating residues in stick representation.

regulatory ligand(s) [32]. Other STPK sensor domains have yet to be characterized experimentally. In the case of PknB, the Cterminal domain consists of four repeated PASTA domains (named for Penicillin-binding protein And Serine/Threonine kinase Associated) [24] and [25]. These small domains are frequently found in bacterial STPKs and high molecular weight penicillin-binding proteins [33]. A possible clue to their function came from the structure of the penicillin-binding protein 2x (Pbp2x) from Streptococcus pneumoniae, which contains two PASTA domains and was found to bind the β lactam antibiotic cefuroxime [34]. This observation led Yeats and co-workers [33] to propose that PASTA domains might specifically recognize stem-peptides from unlinked peptidoglycan, suggesting a role of STPKs in the control of peptidoglycan remodeling during cell growth [35] and the reactivation of dormant bacteria [36]. However attractive, these hypotheses remain highly speculative at this stage, not least because the peptidoglycan-binding specificity of PASTA domains has yet to be proven.

4. The eukaryotic-like protein phosphatases in *M. tuberculosis*

In addition to the STPKs, the *M. tuberculosis* genome also includes three genes (*pstP*, *ptpA*, *ptpB*) encoding eukaryotic-like protein phosphatases. PstP is a metalloenzyme that belongs to the PPM family of Ser/Thr protein phosphatases [37] and has been found to dephosphorylate various mycobacterial STPKs and their substrates [25,38] and [39]. Recent structural studies of the catalytic domain of PstP [40] and *Mycobacterium smegmatis* MspP (a related phosphatase lacking any transmembrane segment [41]) confirmed the conservation of the overall fold and the dinuclear metal center of mycobacterial phosphatases when compared to human PP2C α [42], the most representative and well-characterized member of the PPM family (Fig. 3B). However, the atomic resolution structures of MspP in complex with different ligands support an associative reaction mechanism, which differs in some important aspects from the PP2C α model but resembles closely that proposed for the PPP family, the second large group of eukaryotic Ser/Thr phosphatases [41]. Another major difference of bacterial PPM phosphatases with PP2C α is the presence of a third metal ion in the active site, first observed in PstP [40] and conserved in the structures of MspP [41] and the homologous SaSTP from *Streptococcus agalactiae* [43]. Recent data from our group and others suggest that this third metal is not directly involved in catalysis but could participate in substrate binding by modulating the overall conformation of the flap region [40,41] and [43] (AW, MB and PMA, unpublished).

The two other eukaryotic-like phosphatases in M. tuberculosis, PtpA and PtpB, are secreted proteins thought to function exclusively in infected host cells, where they could interfere with unidentified signals in host signal transduction pathways, as there are no detectable tyrosine protein kinase genes in the mycobacterial genome [44] and [45]. The PtpA structure is similar to that of eukaryotic low-molecular-weight protein tyrosine phosphatases [46], while PtpB resembles the dualspecificity (Ser/Thr and Tyr) protein phosphatases [47] although it was also found to dephosphorylate phosphoinositides in vitro [48]. Disruption of the *ptpB* gene severely impaired growth and virulence of *M. tuberculosis* in IFN- γ activated macrophages and guinea pigs [49], suggesting possible roles of PtpB in phosphatidylinositol metabolism and/or the IFN- γ pathway in the host [48] and [49]. Alber and co-workers [50] have developed a competitive PtpB inhibitor that showed >60-fold selectivity for PtpB over a panel of human PTPs, but the inhibitory effects of this compound in vivo have not been reported.

5. Genetic and inhibition studies of Ser/Thr protein phosphorylation in bacterial physiology and virulence

Recent studies have started to unveil the actual involvement of Ser/Thr protein phosphorylation in bacterial physiology and virulence. Comparative genomics suggests that many STPKs may control redundant physiological processes. Indeed, only four of the eleven M. tuberculosis kinases (PknA, PknB, PknG and PknL) are conserved in the close relative Mycobacterium leprae, which has undergone extensive gene decay leading to removal or inactivation of over 2,400 genes [51], as well as in Corvnebacterium glutamicum, a more distantly related actinomycete [3]. In agreement with this analysis, genome-wide transposon mutagenesis suggested that most STPKs and the three phosphatases were dispensable for growth of M. tuberculosis in culture or in vivo, whereas only PknA, PknB and PknG appeared to be individually required to sustain growth in culture [52] and [53]. The effects of deleting the only recognizable Ser/Thr protein phosphatase, PstP, in M. tuberculosis have not been reported. However, the essentiality of one or more STPKs [52] and [54] is difficult to reconcile with the predicted non-essentiality of the *pstP* gene [52], suggesting that mycobacteria may produce additional Ser/Thr protein phosphatases that remain to be characterized.

Individual gene knockouts of STPK genes have partially confirmed the above results. Deletion of the pknD, pknE and *pknH* genes produced no growth phenotypes in culture [55] and [56]. However, $\Delta pknH$ strains (but not $\Delta pknD$ or $\Delta pknE$) showed a hypervirulent phenotype in mice. In samples from lung and spleen, the $\Delta pknH$ strain grew to higher densities than the parental strain, suggesting a role of PknH in regulating the bacillary load in mouse organs [55]. In the case of PknF, its function was investigated by knocking down expression with antisense RNA in *M. tuberculosis* and by ectopic expression in *M. smegmatis* as a surrogate host [57]. In both cases, the authors observed changes in growth rate as well as defects in septation and cell morphology. Since the PknF gene is known to phosphorylate the ABC transporter Rv1747 [58] and [59], the effect of PknF depletion on nutrient uptake was also investigated. A significant increase of glucose uptake (but not of glycerol, leucine or oleic acid) suggests a possible involvement of PknF in the regulation of sugar transport [57].

5.1. Protein kinase PknG

Since gene essentiality is an important criterion for antibacterial drug design, the above results indicate that only a few mycobacterial STPKs might represent promising therapeutic targets for the development of novel antibiotics. One of these putative targets is PknG, whose gene was predicted to be essential by transposon insertion experiments [52]. However, the experimental data about the physiological function of this kinase are controversial. The inactivation of the *pknG* gene by allelic exchange in *M. tuberculosis* was first reported to decrease both the viability *in vitro* as well as the bacillary load in BALB/c mice, and suggested the involvement of the kinase in the regulation of intracellular glutamate/glutamine levels [60]. In contrast, the same deletion in *Mycobacterium bovis* BCG was independently reported to produce no growth phenotype, nor to affect glutamine uptake or intracellular amino acid concentrations [61]. Furthermore, it has been reported that deletion of *pknG* in the related actinobacterium *C. glutamicum* causes a glutamine utilization defect and the intracellular accumulation of glutamate but not glutamine, suggesting a role of PknG in the regulation of glutamate catabolism [62].

Although the physiological role of PknG inside mycobacterial cells remains unclear, the protein appears to play a key role in infection. Secretion of PknG was found to enhance mycobacterial survival within infected macrophages by inhibiting the phagosome-lysosome fusion [63]. In this work, Pieters and co-workers showed that, in contrast to the wild-type strain, M. bovis BCG lacking PknG is readily transferred to lysosomes and cleared from infected macrophages. Furthermore, the PknG-specific inhibitor, AX20017, increased the lysosomal localization of M. bovis BCG in a dose-dependent way [63]. More recent work from the same team [64] described the crystal structure of a truncated form of *M. tuberculosis* PknG in complex with AX20017. The protein has an N-terminal rubredoxin-like domain adjacent to the canonical kinase domain and a C-terminal tetratricopeptide domain involved in protein dimerization. The ATP-competitive inhibitor binds deep within a pocket shaped by a unique set of amino acid side-chains that are not found in human kinases [64]. Although the molecular mechanisms of PknG secretion, regulation and virulence are still unknown, these results demonstrate that mycobacterial STPKs that interfere with host signaling pathways can be selectively targeted to block the proliferation of M. tuberculosis.

5.2. Protein kinases PknA and PknB

The two other STPK genes (*pknA*, *pknB*), required for growth of *M. tuberculosis*, are found in an operon, which is highly conserved in actinobacteria [25] and [54]. This operon also includes *pstP*, which codes for the only recognizable Ser/Thr protein phosphatase in the genome, and the genes rodA and *pbpA* encoding proteins implicated in the control of cell growth and division [65]. PbpA belongs to the family of penicillinbinding proteins, membrane proteins that play key roles in cell wall synthesis, and RodA is known to control bacterial cell shape and elongation [66] and [67]. The composition of the operon and its location near the origin of replication in actinobacteria suggests that PknA, PknB and PstP could regulate processes linked to cell division and growth [2] and [54]. Indeed, the transcription of the *pknA* and *pknB* genes increases during the exponential growth phase [68] or upon infection within macrophages [69] and [70], whereas pknB expression is downregulated in a nutrient starvation model of M. tuberculosis persistence [71]. Ectopic expression of pknA in Escherichia coli was observed to produce an elongated cell morphology [72], and similar morphological changes were observed upon overexpression of *pknA* or *pknB* in *M*. smegmatis and *M*. bovis BCG [68], suggesting defaults in cell wall synthesis and cell division processes. Possibly linked to this phenotype, recombinant PknA

was shown to phosphorylate and inhibit the GTPase activity of FtsZ, a protein homologous to eukaryotic tubulin, involved in septum formation [73].

Recent gene inactivation studies provided the first direct evidence that essential metabolic processes in bacterial physiology are controlled by Ser/Thr protein phosphorylation. The *pknB* gene in the conserved operon could only be disrupted by allelic replacement in *M. tuberculosis* and *M. smegmatis* upon the introduction of a second functional copy of the gene. Since *pknB* is the distal gene of the operon, the above results indicate that PknB is essential to sustain mycobacterial growth [54]. Conserved PknB-like STPKs (including the extracellular PASTA domains) are also found beyond actinobacteria, in a large number of more distantly related Gram-positive bacteria [25]. However, in contrast to mycobacteria, deletion of the pknB homologous genes in B. subtilis and Enterococcus faecalis led to no differences in growth compared with the wild-type strain [30] and [74], suggesting that bacterial *pknB*-like genes may be functionally unrelated, or alternatively that they have an ancient evolutionary origin, so that the biological processes under STPK control have significantly diverged in response to specific bacterial adaptation to the environment.

Prompted by the essentiality of *pknB* in *M. tuberculosis*, the effect of kinase inhibitors was tested on mycobacterial growth. ATP-competitive PknB inhibitors were found to kill different slow- and fast-growing mycobacteria (*M. tuberculosis*, *M. smegmatis* and *M. aurum*) with minimal inhibitory concentrations in the micromolar range [26] and [54]. Furthermore, the inhibitory effect could be partially reversed in PknB over-expressor strains of *M. smegmatis*, supporting the hypothesis that the inhibition of bacterial growth is related to PknB inactivation [54]. The evidence that PknB, a structurally and mechanistically eukaryotic-like STPK, is an essential enzyme in *M. tuberculosis* and that PknB inhibitors have a significant antibacterial effect, strongly suggest that STPKs represent promising therapeutic targets for anti-tuberculosis drug design.

6. Identification of mycobacterial STPK substrates

Recent studies have identified a substantial number of phosphorylated proteins in bacteria, supporting the emerging view that Ser/Thr and Tyr protein phosphorylation is a general and fundamental regulatory process, not restricted to eukaryotes. Using radioactive labeling and immunostaining, 41 different phosphoproteins could be identified in the actinomycete C. glutamicum after in-gel digestion and mass spectrometry (MS) analysis [75]. Using a similar approach, 29 proteins were found to be phosphorylated in early stationary-phase B. subtilis cells [76]. More recently, Mann and co-workers [77] were able to identify 103 unique phosphopeptides from 78 B. subtilis proteins using high accuracy MS combined with biochemical enrichment of phosphopeptides from digested cell lysates. In this study, phosphorylation sites were found in several enzymes involved in the glycolytic and tricarboxylic acid cycle, as well as in kinases and members of the phosphoenolpyruvate-dependent phosphotransferase system [77]. While these initial studies provide a lower estimate for the size of the bacterial phosphoproteome,

they already support the notion that STPKs regulate many metabolic processes in bacterial cells.

The identification of STPK substrates in *M. tuberculosis* has been the focus of many recent studies. The availability of purified catalytic domains for most kinases [25,69,72,78-81] and [82] assisted the identification of several STPK substrates in vitro. However, the physiological relevance of many of these substrates has yet to be confirmed, given the known promiscuity of STPKs [83] and the high enzyme:substrate ratios (up to 1:1) that were required to detect phosphorylation in some studies. All characterized STPKs were shown to have autophosphorylation activity. PknB was reported to contain up to four phosphorylated residues in its activation loop [24] and [25], and detailed studies of five receptor-like STPKs (PknB, PknD, PknE, PknF, PknH) revealed the presence of several autophosphorylation sites, with two recurrent clusters of pThr/pSer residues respectively in the activation loops and juxtamembrane regions [39] and [84]. As shown for PknB and the homologous PrkC from B. subtilis, phosphorylation of the activation loop provides a mechanism of kinase regulation whereas phosphorylation of the juxtamembrane region does not appear to affect the catalytic activity [39] and [85].

Some *M. tuberculosis* STPKs were shown to phosphorylate in vitro a protein substrate encoded by a gene adjacent to the kinase gene. Thus, PknH was shown to phosphorylate the transcriptional regulator EmbR, PknF the Rv1747 ABC transporter and PknB the penicillin-binding protein PbpA [59,81] and [86]. Both EmbR and Rv1747 are proteins containing predicted FHA (ForkHead-Associated) domains, which are small pThr-binding protein modules involved in eukaryotic signaling pathways [87,88] and [89]. The structure of M. tuberculosis EmbR in complex with a phosphopeptide confirmed that the bacterial FHA domain, which mediates the interaction of EmbR with PknH [81], binds pThr residues in a similar way as the eukaryotic proteins [90]. Extending these observations, subsequent studies reported that different FHA domain-containing proteins are substrates of various M. tuberculosis STPKs [91,92] and [93]. Although the physiological significance of these findings remains unclear, they suggest a web of interactions between STPKs and FHA proteins in mycobacterial signaling pathways [91].

Kremer and co-workers [94] reported that the β -ketoacyl AcpM synthases KasA and KasB, two enzymes of the fatty-acid synthase II (FAS-II) complex, are substrates of various mycobacterial STPKs in vitro. Different phosphorylation isoforms of KasA and KasB were also identified in soluble protein extracts from M. bovis BCG, suggesting that the condensing activities of the FAS-II system can be differentially regulated by phosphorylation in vivo [94]. Recent genetic studies also reported two possible physiological substrates of PknD. Phosphorylation of the cell wall fraction from a *pknD* knockout of a clinical *M. tuberculosis* isolate allowed the identification of MmpL7, which is the transporter of phthiocerol dimycocerosate, a lipid essential for virulence [56]. On the other hand, Alber and co-workers [95] reported that PknD overexpression in M. tuberculosis alters transcription of numerous genes. The authors showed that PknD was able to efficiently phosphorylate an antianti-sigma factor homologue, Rv0516, both *in vitro* and *in vivo* at a single N-terminal Thr residue, and that this phosphorylation inhibited the interaction of Rv0516 with a putative homologous partner, Rv2638 [95].

In a different approach, Kang et al. [68] used peptide specificity screening to find the preferred substrate motifs of PknA and PknB, which led them to identify two STPK substrates: Wag31, a homolog of the cell division protein DivIVA, and Rv1422, a conserved hypothetical protein. While Rv1422 was phosphorylated *in vitro* by both kinases, Wag31 was not phosphorylated by PknB and only weakly by PknA, raising the possibility that it could be the substrate of other mycobacterial STPKs *in vivo*. However, Wag31 phosphorylation was enhanced when PknA and PknB acted in concert, and the expression of different alleles of the protein *in vivo* produced a similar cell shape phenotype to that observed for PknB overexpression [68], lending some support to the involvement of Wag31 in PknA/PknB-mediated signaling pathways.

In our laboratory, we used an unbiased proteomic approach to look for optimal substrate(s) of PknB in native cell-free extracts and culture filtrates of *M. tuberculosis* and *M. smegmatis* [92]. By optimizing the kinase assay, PknB was found to efficiently phosphorylate only one protein substrate, GarA (Rv1827), at a single N-terminal Thr residue. GarA (for Glycogen Accumulation Regulator) is a FHA domain-containing protein that has been proposed to play some role in the regulation of glycogen recycling during the exponential phase of M. smegmatis cell growth [96]. More recent work on *odhI*, the *garA* ortholog in C. glutamicum, demonstrated that OdhI inhibits the 2-oxoglutarate dehydrogenase (ODH), a key enzyme of the tricarboxylic acid cycle [62]. This inhibition is reversed upon OdhI phosphorylation by PknG (also at a single N-terminal Thr residue), suggesting that OdhI (GarA) could function as a phosphorylation-dependent molecular switch in the regulation of different metabolic processes.

Binding studies of PknB-GarA interactions led to a model of STPK substrate recruitment, in which the phosphorylated activation loop of PknB provides a secondary docking site for FHA-mediated substrate binding [92]. The model suggests a possible effect of substrate binding in enzyme activation and might account in part for the observation that FHA domain proteins are substrates of various STPKs with multiple autophosphorylation sites in their activation loop [39,91,92] and [93]. The PknB–GarA interaction model evokes the eukaryotic STPKs that rely on docking interactions with their substrates, using sites distinct from the active site cleft, to achieve specificity [97]. In particular, the accessibility of the activation-loop phosphates of PknB to engage the FHA domain is closely analogous to the SH2-mediated recruitment of the adaptor protein APS to the activated insulin receptor [98], where kinasesubstrate interactions also occur primarily via the phosphorylated activation loop.

7. Concluding remarks

There is a growing body of evidence to indicate that many metabolic processes in *M. tuberculosis* are regulated by STPK-

mediated phosphorylation, and these range from cell wall biogenesis and cell division to central metabolic processes like the tricarboxylic acid cycle and glycogen utilization. Interfering with the signal transduction pathways governing these pathways by inhibition of the corresponding STPK activity should therefore result in marked metabolic perturbation that, in some cases, could result in cell death. Support for this notion is provided by the finding that *pknB*, and possibly the *pknA* and *pknG* genes, are essential for cell growth.

In the past 15 years, the field of oncology has witnessed tremendous drug discovery activity with the realization that uncontrolled cell division, the cause of many human cancers and neoplasms, can be reversed or prevented by targeting the aberrant signal transduction processes with appropriate inhibitors. This has resulted in many pharmaceutical companies developing extensive libraries of compounds that block serine/ threonine and tyrosine protein kinase activity. As a result, several clinically approved anticancer drugs have been discovered and produced, such as Gleevec [99], and are now finding broad application in the treatment of life-threatening conditions, like chronic myeloid leukemia. Given the importance of tuberculosis as a global public health problem, we hope that these kinase inhibitor libraries will also be screened in order to find lead compounds for the development of new anti-TB drugs.

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