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Review

Protein kinase and phosphatase signaling in *Mycobacterium tuberculosis* physiology and pathogenesis

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ABSTRACT

Mycobacterium tuberculosis (Mtb), the etiological agent of tuberculosis (TB), evades the antimicrobial defenses of the host and survives within the infected individual through a complex set of strategies. These include active prevention of host cellular killing processes as well as overwhelming adaptive gene expression. In the past decade, we have gained an increased understanding of how mycobacteria not only have the ability to adapt to a changing host environment but also actively interfere with the signaling machinery within the host cell to counteract or inhibit parts of the killing apparatus employed by the macrophage. Mtb is able to sense its environment via a set of phospho-signaling proteins which mediate its response and interaction with the host in a coordinated manner. In this review, we summarize the current knowledge about selected Mtb serine, threonine, and tyrosine kinase and phosphatase signaling proteins, focusing on the protein kinases, PknG and PtkA, and the protein phosphatase, PtpA.

1. Introduction

TB continues to be a leading cause of morbidity and mortality worldwide despite strong efforts to mitigate its toll on humanity since the WHO declared TB a global emergency over 15 years ago. The emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of Mtb is thrusting this "curable" disease back into the pre-antibiotic era. To complicate this problem, no new antituberculosis drugs have been developed in close to 40 years, and therefore drug discovery is a major focus of TB research. Recently, due to the success of kinase inhibitors in the treatment of non-infectious human diseases, Mtb signal transduction has become a prime target for the development of novel therapeutics in the treatment of TB.

In Mtb, signal transduction is co-mediated by five main families of kinases and phosphatases. The first, the classic bacterial signaling machinery, comprises the "two-component" systems. Eleven complete systems have been identified in this category, each consisting of a histidine kinase and a response regulator [1]. The second family contains the "eukaryotic-like" serine/threonine protein kinases (STPKs) [2]. The third contains a sole Ser/Thr phosphatase belonging to the protein phosphatase type 2C (PP2C) family. The fourth family encodes a pair of protein tyrosine phosphatases (PTPs), one of them shown to interfere with host-signaling pathways within infected macrophages; and fifth, a recently discovered Mtb protein tyrosine kinase (PTK), PtkA, which belongs to a newly identified class of tyrosine kinases [3]. These systems provide a means for adaptive gene expression and regulation of metabolic processes in response to external stimuli, as well as disruption of host cellular pathways.

In recent years, efforts in a number of laboratories have led to the identification of a wide range of microbial substrates phosphorylated by Mtb STPKs both *in vitro* (Table 1) and *in vivo* (Fig. 1). The contribution of the PTPs to microbial survival within the human host has been revealed and the role of mycobacterial kinases and phosphatases in regulation of both global microbial physiology pathways as well as cell division and cell-wall formation has been established. As such, Mtb signaling proteins have been reviewed extensively in the past years mainly from bioinformatics and contribution to the bacilli's physiology point of views [1,2,24–26]. Recent findings of cross-talk between mycobacterial and host macrophage signaling systems, in the context of successful infection, stimulated further research efforts in targeting mycobacterial signaling components. More importantly, identification and validation of these proteins as novel drug targets for TB therapeutics have been explored, launching both academic and pharmaceutical industry programs for screening and designing specific inhibitors against Mtb STPKs and protein phosphatases [26].

The Mtb genome contains eleven STPKs named PknA to PknL [2], two of which are soluble proteins (PknG and PknK) and the rest are transmembrane kinases. Apart from PknB, and likely PknA, key

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Table 1
Kinases and substrates.

Kinase	Substrate	Identification	Ref.	Kinase	Substrate	Identification	Ref.
PknA	EmbR	*In vitro*	[4]	PknE	FabD	*In vitro*	[5]
	FabD	*In vitro*	[5]		FabH	*In vivo*/ *in vitro*	[6]
	FabH	*In vivo*/ *in vitro*	[6]		GarA	*In vitro*	[7]
	FtsZ	*In vivo*	[8]		GroEL1	*In vitro*	[9]
	GlmU	*In vivo*/ *in vitro*	[10]		KasA	*In vitro*	[5]
	GroEL1	*In vitro*	[9]		KasB	*In vitro*	[5]
	KasA	*In vitro*	[5]		RsfA	*In vitro*	[11]
	KasB	*In vitro*	[5]		RshA	*In vitro*	[11]
	MurD	*In vitro*	[12]		Rv0516c	*In vitro*	[11]
	Rv1422	*In vivo*	[13]		Rv1747	*In vitro*	[14]
	Wag31	*In vivo*	[13]		Rv1904	*In vitro*	[11]
PknB	EmbR	*In vitro*	[4]	PknF	FabD	*In vitro*	[5]
	FabD	*In vitro*	[5]		FabH	*In vivo*/ *in vitro*	[6]
	GarA	*In vivo*	[7]		GarA	*In vitro*	[7]
	GlmU	*In vivo*/ *in vitro*	[10]		GroEL1	*In vitro*	[9]
	GroEL1	*In vitro*	[9]		KasA	*In vitro*	[5]
	KasA	*In vitro*	[5]		KasB	*In vitro*	[5]
	KasB	*In vitro*	[5]		Rv0020c	*In vitro*	[15]
	PbpA	*In vivo*	[16]		Rv1747	*In vitro*	[14]
	RshA	*In vivo*	[17]	PknG	GarA	*In vivo*	[18]
	Rv0020c	*In vitro*	[15]	PknH	DacB1	*In vitro*	[19]
	Rv0516c	*In vitro*	[11]		EmbR	*In vivo*	[20]
	Rv1422	*In vivo*	[13]		FabD	*In vitro*	[5]
	Rv1747	*In vitro*	[15]		FabH	*In vivo*/ *in vitro*	[6]
	SigH	*In vivo*	[17]		GroEL1	*In vitro*	[9]
	Wag31	*In vivo*	[13]		KasA	*In vitro*	[5]
PknD	FabD	*In vitro*	[5]		KasB	*In vitro*	[5]
	FabH	*In vivo*/ *in vitro*	[6]		Rv0681	*In vitro*	[19]
	GarA	*In vitro*	[7]	PknI	FabD	*In vitro*	[5]
	GroEL1	*In vitro*	[9]	PknK	FabD	*In vitro*	[5]
	KasA	*In vitro*	[5]		VirS	*In vivo*	[21]
	KasB	*In vitro*	[5]	PknL	FabD	*In vitro*	[5]
	MmpL7	*In vivo*	[22]		GroEL1	*In vitro*	[9]
	Rv0516c	*In vivo*	[11]		KasA	*In vitro*	[5]
	Rv1747	*In vitro*	[15]		KasB	*In vitro*	[5]
					Rv2175c	*In vitro*	[23]
				PtkA	PtpA	*In vitro*	[3]

Compilation of Mtb kinases and substrates including method of identification and first report. FabH and GlmU were found to be phosphorylated *in vivo*. However, several possible kinases were found to phosphorylate them *in vitro*; thus, they are designated "*In vivo*/*in vitro*".

members of these families of proteins are not essential for growth *in vitro*. Nevertheless, these non-essential STPKs and PTPs are required for controlled infection and disease progression in models of infection. Specifically, PknG [27,28], PknH [29], PtpA [30], and PtpB [31] play an important role in Mtb virulence, adaptation, and growth within animal models of infection or in host macrophages *in vitro*. As illustrated in Table 1 and Fig. 1, Mtb STPKs share substrates between them, suggesting a complicated temporal and developmental control over key mycobacterial processes. In this review, we summarize the current knowledge about selected Mtb "eukaryotic-like" serine, threonine, and tyrosine kinase and phosphatase signaling proteins, focusing on two protein kinases, PknG and PtkA, and one protein phosphatase, PtpA.

2. PknG

PknG is one of two soluble STPKs in Mtb, containing an N-terminal rubredoxin domain [32], a central kinase domain, and a C-terminal tetratricopeptide repeat (TPR) domain [2]. PknG is classified as a non-RD kinase as it lacks a conserved arginine immediately preceding an invariant aspartate in Hanks domain VIb, corresponding to the catalytic loop of the kinase. PknG adopts a unique quaternary structure compared to the other Mtb kinases for which crystal structures have been solved [32]. In the classical example, PknB dimerizes via its kinase domain, and dimerization mediates trans-phosphorylation of the activation loop, leading to activation of the kinase [33]. PknG, on the other hand, dimerizes via its TPR domain, a structural motif involved in protein–protein interactions [34], positioning the kinase domains of the two monomers distant from each other [32]. Furthermore, PknG lacks autophosphorylation residues in the activation loop. In fact, the autophosphorylation sites, as first determined by O'Hare et al. [18], only occur in the first 73 residues upstream of the rubredoxin domain and do not play any role in activation of the kinase domain. Instead, activation appears to be dependent on the two Cys-X-X-Cys motifs in the N-terminal, rubredoxin domain, which makes contacts with both the N- and C-terminal lobes of the kinase domain. Rubredoxins, which contain an iron ion coordinated by four cysteine residues, are proteins that participate in electron transfer [35]. Mutations in all four cysteine residues of PknG rendered it inactive [32]; however, individual mutations were not analyzed for PknG activity. As such, PknG is likely to be regulated by the redox status of its environment via the rubredoxin domain as suggested previously [2]. So far, the redox mechanism and putative enzymes to catalyze the redox reactions have not yet been elucidated.

PknG is of particular interest because of its role in pathogenicity and survival within the macrophage. Infection of SCID mice with a Δ*pknG* mutant in Mtb resulted in prolonged survival compared to wild type (WT) Mtb [27]. Intravenous infection of immunocompetent BALB/c mice with Δ*pknG* had a significantly reduced bacillary load in the lungs, spleen, and liver; while infection by aerosol route caused a delayed dissemination to the spleen compared to WT and complemented Δ*pknG*. The decreased viability of the mutant in mice is correlated with a diminished ability of the mutant to grow *in vitro* compared to WT and complement, particularly in nutrient depleted media [27]. However, in the vaccine strain, *Mycobacterium bovis* BCG, deletion of *pknG* had no effect on normal growth *in vitro* [36]. Similarly, deletion of *pknG* in the related *Corynebacterium glutamicum* (Cg) did not affect its growth under typical growth conditions. However, when grown on glutamine-supplemented minimal media [37] or in the presence of low levels of penicillin [38], the Cg Δ*pknG* mutant was impaired for growth. Therefore, differences in experimental observations between Mtb and BCG may be due to the differences in growth conditions tested, or possibly due to intrinsic differences between the strains.

In addition to the *in vitro* growth deficiency, the Mtb Δ*pknG* mutant accumulated intracellular levels of glutamate and/or glutamine [27]. Levels of glutamate and glutamine were initially tested due to the location of the *pknG* gene adjacent to and putatively within the same operon as genes involved in glutamine uptake (*glnH* and *glnX*). In the above study, a distinction between glutamate and glutamine levels was not made as their levels were measured simultaneously. Nevertheless, in a separate analysis, glutamine levels were often below detection limit, leading the authors to propose that PknG may be involved in the direct control of glutamate only. While differences in glutamine and glutamate levels were not detected in WT and Δ*pknG* in BCG [36], Cg Δ*pknG* mutant similarly accumulated intracellular glutamate and was severely reduced for growth in minimal media with glutamine as the sole carbon and nitrogen source [37]. Glutamine uptake, however, was relatively unaffected in the Cg Δ*pknG* mutant; rather, the *in vivo* substrate for Cg-PknG was identified to be the forkhead-associated domain (FHA) containing protein, OdhI, an inhibitor of the 2-oxoglutarate dehydrogenase complex (ODH) of the tricarboxylic acid (TCA) cycle. ODH catalyzes the NAD^+-dependent conversion of 2-oxoglutarate (α-ketoglutarate) to succinyl CoA. In the absence of PknG, OdhI is active and inhibits ODH; phosphorylation by PknG inactivates OdhI and thus relieves inhibition of ODH.

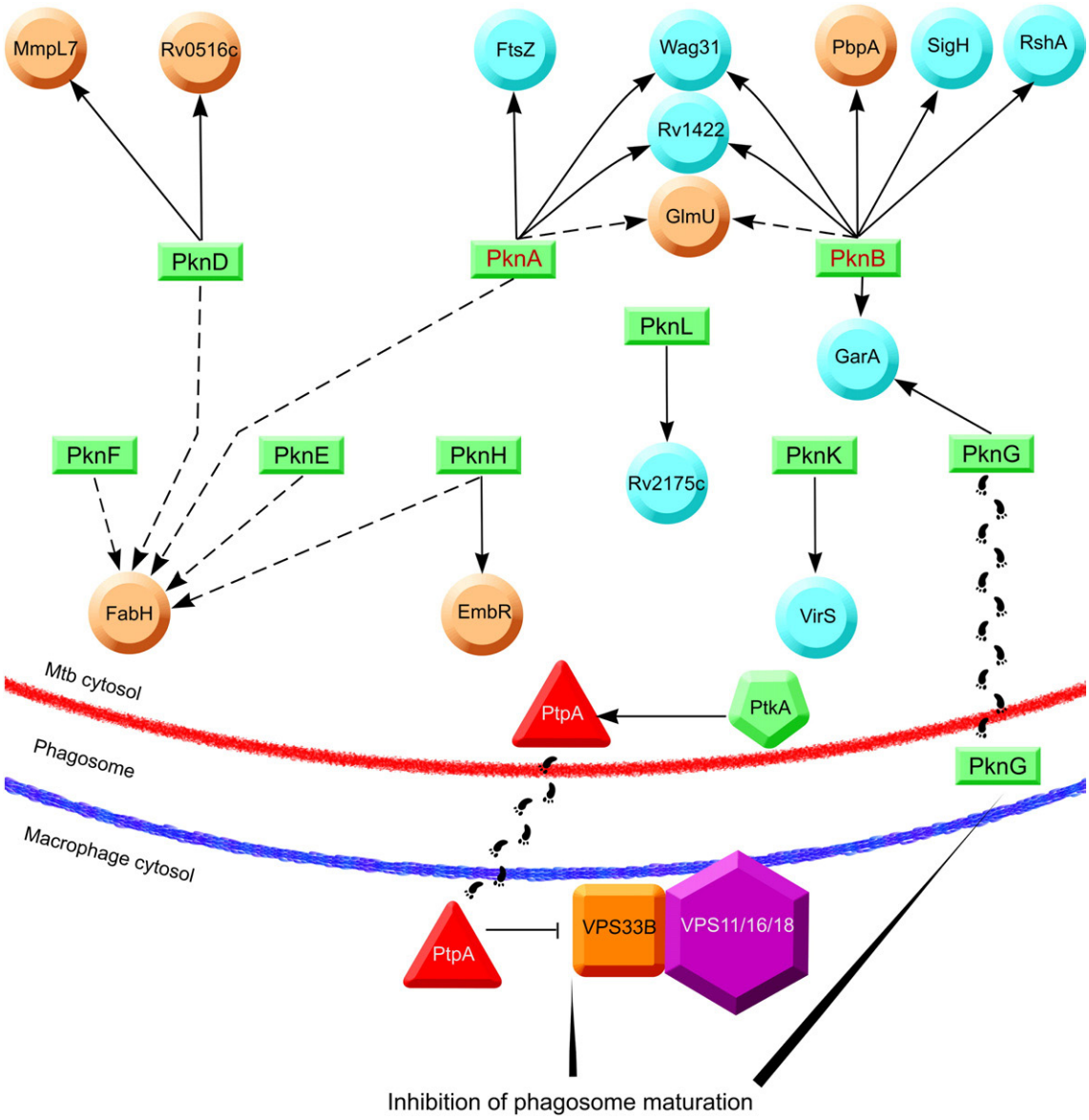

Fig. 1. Mtb *in vivo* interaction. Compilation of signal transduction proteins and their *in vivo* identified substrates. Solid lines with arrowheads denote *in vivo* phosphorylation events that lead to modulation of enzyme activity; dashed lines with arrowheads denote *in vivo* phosphorylation by several possible kinases; and solid lines with perpendicular ends denote dephosphorylation events. Beige circles denote cell-wall-related substrates. PknA and PknB are labeled in red to indicate that they are essential kinases.

Consistent with this finding, deletion of *odhI* in addition to *pknG* restores the ability of Cg to grow on glutamine minimal media [37].

The OdhI homolog in Mtb is GarA (Rv1827). Mtb lacks ODH activity, and instead has a variant TCA cycle where α-ketoglutarate is converted to succinic semialdehyde by α-ketoglutarate decarboxylase (Rv1248c, KGD), and then to succinate [39]. Based on this homology, and as illustrated in Fig. 2, O'Hare et al. [18] demonstrated that GarA is phosphorylated by Mtb-PknG, and that unphosphorylated GarA binding partners are KGD, NAD^+-dependent glutamate dehydrogenase (Rv2476c, GDH), and the α-subunit of the glutamate synthase (GS) complex (Rv3859c, GltB) [40]. However, Scherr et al. [41], ignoring prior literature, have argued that GarA may not be a relevant substrate of PknG *in vivo*. Their claim is based on one report showing deletion of *pknG* in BCG affects neither *in vitro* growth under select conditions nor intracellular glutamine concentrations [36] (as discussed above) and favors a role for PknG in inhibiting phagosome–lysosome fusion (see below). Although GarA was first identified to be the preferred substrate of PknB *in vitro* [7], GarA is phosphorylated on a unique residue, Thr21, by PknG [18], allowing for identification of phosphorylation *in vivo*. While phosphorylation occurs at Thr22 by PknB, PknD, PknE, and PknF [7], Thr21 was unambiguously identified as the predominant phosphorylation site of GarA from extracts from both Mtb and *Mycobacterium smegmatis* [18]. Similarly, Cg-PknA, -PknB, and -PknG are all able to phosphorylate OdhI *in vitro* [42]. Nevertheless, *in vivo* phosphorylation is primarily mediated by Cg-PknG [37]. Mtb-PknG and GarA were shown to interact *in vivo* using a yeast two hybrid-like system in *M. smegmatis*, and immobilized recombinant GarA was able to pull down PknG from Mtb and *M. smegmatis* cell extracts [18]. These findings together with the observation that deletion of *pknG* results in accumulation of internal glutamate levels in Mtb [27], by a mechanism dependent on GarA phosphorylation (Fig. 2), strongly suggest that GarA is the cognate substrate of PknG *in vivo*.

As indicated above, no growth defect was observed in Δ*pknG* BCG grown in culture *in vitro* [36]. Instead, infection of J774A.1 or bone-marrow derived macrophages from BALB/c mice resulted in transfer of Δ*pknG* BCG, but not the parental strain, to lysosomal compartments as observed by immunofluorescence microscopy and

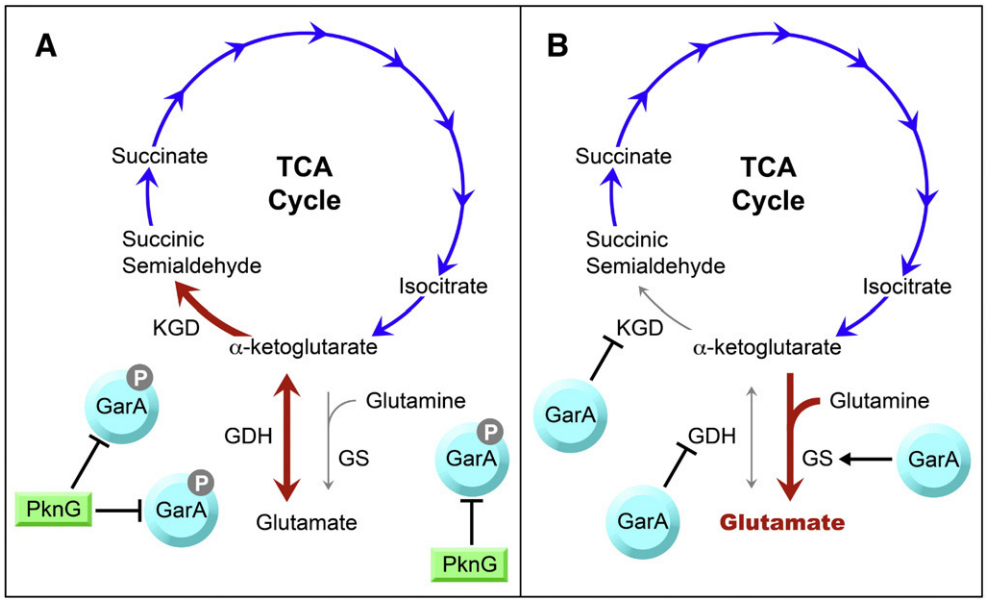

Fig. 2. PknG intracellular mode of action on the TCA cycle, modeled according to Nott et al. [40]. (A) In WT Mtb, GarA is phosphorylated by PknG, causing the phosphorylated N-terminus of GarA to bind intramolecularly with its FHA domain and thereby inhibits binding of GarA to KGD, GDH, and GS. (B) In Δ*pknG* Mtb, GarA is predominantly unphosphorylated, and is thus able to bind and inhibit GDH and KGD, preventing conversion of glutamate to succinic semialdehyde through α-ketoglutarate. At the same time, GarA binds and enhances GS activity to produce glutamate. This dual effect leads to the accumulation of glutamate within the cell.

organelle electrophoresis [28]. Complementation with WT *pknG* but not *pknG* mutated at its autophosphorylation residues restored the parental phenotype [41]. These results confirm the importance of PknG for survival in the host and further suggest that PknG plays a role in inhibiting phagosome–lysosome fusion. In addition, the authors expressed BCG-PknG in *M. smegmatis* and demonstrated that BCG-PknG expressing *M. smegmatis*, but not WT *M. smegmatis*, prevents translocation of the bacteria to lysosomes [28], supporting their observation that PknG inhibits phagosome–lysosome fusion. However, WT *M. smegmatis* encodes its own *pknG* gene, whose product shares 78% identity and 87% similarity to Mtb- and BCG-PknG, casting doubt on the validity of the above experiment. The authors later demonstrated that *M. smegmatis*-PknG is able to complement their Δ*pknG* mutant in BCG, indicating that *M. smegmatis*-PknG is able to prevent translocation of BCG to lysosomes in their experimental system. In order to explain why the non-pathogenic strain of *M. smegmatis* does not prevent phagosome–lysosome fusion, the authors suggested, using western blotting, that *pknG* is transcribed but not translated in *M. smegmatis* due to a translational block in the upstream region of the *pknG* operon [43]. However, an earlier publication by O'Hare et al. [18] contradicts this conclusion as PknG protein was successfully co-precipitated from *M. smegmatis* cell extracts using the PknG substrate GarA as bait. Therefore, based on the experimental data presented above from three independent laboratories [18,27,37], it is possible that the reason for lysosomal transfer observed in the BCG Δ*pknG* mutant is due to physiological changes within mycobacteria and not necessarily a result of direct interference of PknG with host-signaling pathways.

PknG is one of the major targets for inhibitor screening by numerous research groups and pharmaceutical companies. Using combined screening and medicinal chemistry strategies, Axxima developed AX20017, a tetrahydrobenzothiophene compound that specifically inhibits PknG kinase activity *in vitro*. Inhibition of PknG in *M. bovis* BCG by AX20017 promoted phagosome–lysosome fusion in macrophages, leading to bacterial killing in host cells without affecting the viability of the macrophages [28]. AX20017 did not inhibit growth of BCG in culture, which is similar to the *pknG* deletion mutant in BCG [28], supporting a role for specific inhibition of PknG by AX20017. However, off-target inhibition contributing to the cellular effects of AX20017 in phagosome–lysosome fusion has not been ruled out. This hit compound was later used for optimization, resulting in a series of compounds effective in blocking mycobacterial growth within macrophages [44].

3. Protein tyrosine phosphorylation

Protein tyrosine phosphorylation has long been recognized to play a key role in the regulation of numerous fundamental cellular processes in eukaryotes [45] and various bacterial species [46–48]. Thus far, bacterial phosphotyrosine signaling has been shown to be involved in cell division, antibiotic production, capsule synthesis, and host infection [47–52].

The first indication of bacterial protein tyrosine kinase activity was demonstrated in *Escherichia coli*, in which the presence of phosphotyrosine in partial hydrolysates of proteins was shown [53]. Since then, a large and increasing number of tyrosine kinases and phosphatases were identified in various bacterial species. These include two protein kinases from *Streptococcus pneumoniae* that autophosphorylate on tyrosine residues and are involved in the regulation of capsular polysaccharide production [47,49]. Pathogenic *Yersiniae* contain an extrachromosomal virulence plasmid that encode a PTP named YopH, a protein injected into the host through a Type III secretion system encoded on the same plasmid [54]. Once inside the host cells, YopH modulates host-signaling pathways by dephosphorylating $p130^{Cas}$ to inhibit phagocytosis of the bacteria [54].

In Mtb, the presence of tyrosine phosphorylation activity has been predicted since the identification of a 55-kDa protein that was recognized by the 4G10 anti-phosphotyrosine antibody in cell extracts from virulent but not avirulent Mtb strains [55]. Furthermore, Mtb possesses two protein tyrosine phosphatases, PtpA and PtpB. Together with these known PTPs, the recent identification of a PTK, termed PtkA, further confirms the existence of phosphotyrosine signaling activity in Mtb. In this section of the review, the current understanding of these Mtb tyrosine signaling proteins, particularly PtpA and PtkA, and their roles in the modulation of host-signaling pathways and the pathogenesis of TB is described.

4. Protein tyrosine phosphatase A (PtpA)

PtpA, first identified from the genome sequence of Mtb H37Rv through its homology to known PTPs, contains a conserved CX_5R motif typical of functional PTPs. PtpA possesses 37% sequence identity and high structural similarity with the human low molecular weight PTP (LMW-PTP) [56]. However, the absence of a typical PTK in the genome raised questions regarding the functions of PtpA in relation to the physiology of Mtb. Initial studies demonstrated that purified recombinant PtpA is specific for phosphotyrosine residues [57,58]. Expression of *ptpA* is upregulated during entry into host macrophages as determined using a GFP reporter construct [57]. Furthermore, PtpA can be secreted or released from Mtb into the culture medium during *in vitro* growth [57]. The extracellular localization of PtpA was confirmed by western blot analysis of culture filtrate with antibodies against PtpA [57,58], and PtpA in *Mycobacterium avium* subsp. *paratuberculosis* is secreted upon infection of human macrophages [59], suggesting a role for PtpA in the interaction with host macrophages. These observations greatly spurred interest in the elucidation of the cognate substrate of PtpA and its physiological function in host macrophages during infection. Expression of PtpA in RAW 264.7 murine macrophages results in a decrease of phagocytic activity and an increase of F-actin nucleation on phagosomes *in vivo* and *in vitro* [60]. The authors reasoned that the increase in F-actin polymerization might physically block direct contact between donor and acceptor membranes, thereby inhibiting phagosome–lysosome fusion. However, PtpA is not required for Mtb growth in a mouse infection model [61], raising questions as to how PtpA exerts its effects on the phagocytic process in mouse cells.

Nevertheless, PtpA was shown to be essential for successful Mtb infection of human macrophages using a *ptpA* gene deletion mutant [30]. Furthermore, PtpA-neutralizing antibodies expressed in the cytosol of human macrophages are capable of blocking Mtb infection. Specifically, PtpA dephosphorylates the human class C Vacuolar Protein Sorting VPS33B, a key regulator of membrane trafficking and fusion. Inactivation of the host protein results in the arrest of phagosome–lysosome fusion [30]. Along with 5 other proteins (VPS41, VPS39, VPS11, VPS16, and VPS18), VPS33B forms the HOPS complex (homotypic vacuole fusion and vacuole protein sorting) [62]. As illustrated in Fig. 3, the HOPS complex modulates the activity of SNARE proteins promoting vesicle docking and fusion with target membranes [62,63]. The HOPS complex also has the capability to associate with the GTP-bound form of Rab GTPases [62]. Since VPS33B is part of the HOPS complex, it binds to t-SNAREs and can specifically associate with GTPases [63]. Dephosphorylation of VPS33B by PtpA most likely leads to blockage of the macrophage's membrane docking machinery by preventing the association of VPS33B with the small GTPases required for proper membrane fusion.

5. Protein tyrosine phosphatase B (PtpB)

Unlike PtpA, PtpB does not have human orthologues. Koul et al. [58] have shown that Mtb PtpB is secreted into the growth media and multiple reports have suggested that PtpB is specific for phosphotyrosine substrates [58,64]. However, a recent study indicates that Mtb PtpB can also dephosphorylate phosphoserine/threonine and phosphoinositides, thereby exhibiting triple-specificity [65]. Based upon these results, the authors further argued that PtpB might be capable of disrupting host phosphoinositide metabolism and its associated signaling pathways, which are known to have a key role in phagosome maturation.

Crystallographic analysis revealed that PtpB possesses the distinct features of dual phosphotyrosine binding sites and a two-helix lid structure that covers and protects the active site of the enzyme in an oxidative environment [66,67]. Though the cognate substrate of PtpB within the host remains to be elucidated, deletion of *ptpB* from the genome of Mtb led to decreased bacterial survival in activated J774A.1 murine macrophages and 70-fold reduction of bacillary loads in the

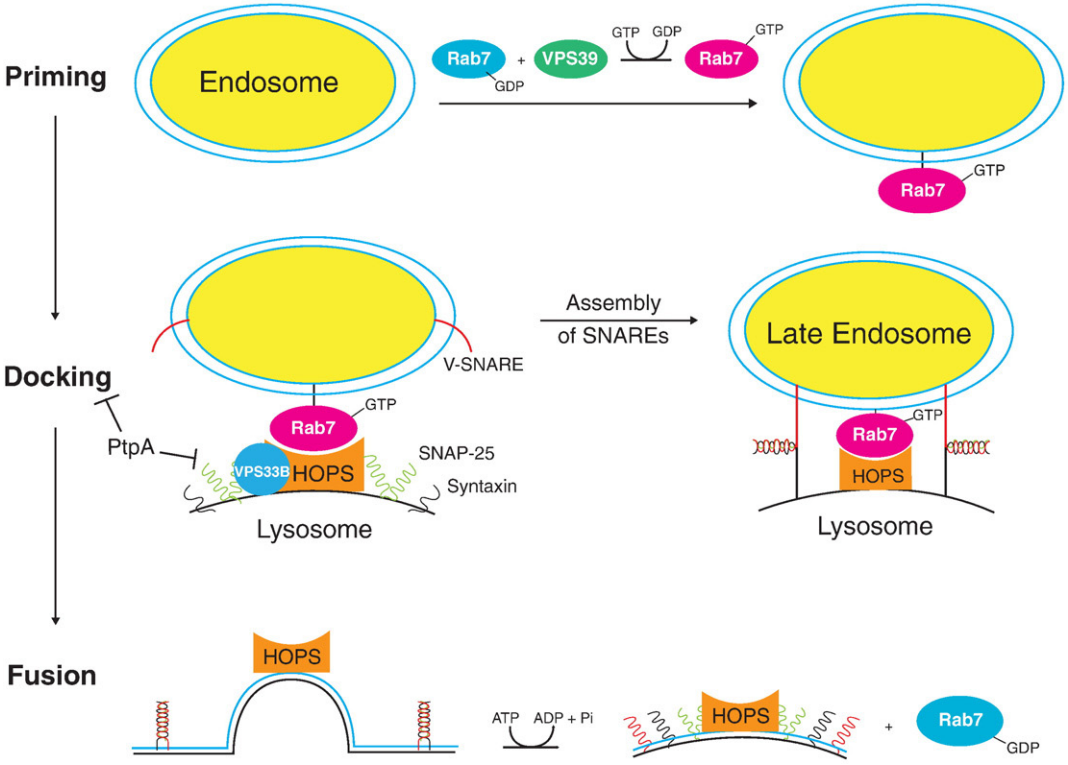

Fig. 3. Macrophage membrane fusion during phagosome maturation. Priming is represented as the activation of Rab7 by VPS39. Activated GTP-bound Rab7 binds to the endosome. Docking is illustrated by the binding of GTP-Rab7 and the HOPS complex linking both organelles. Fusion is illustrated by the joining of the GTP-Rab7-bound endosome and the lysosome. Mtb PtpA blocks the docking step by dephosphorylating VPS33B a member of the HOPS complex.

spleens of infected guinea pigs [31]. The importance of PtpB to the intracellular survival of Mtb was further confirmed by a recent study in which specific inhibitors against PtpB were shown to impair mycobacterial survival within murine macrophages [68]. Yet, further investigations are needed to identify the role of PtpB in the pathogenesis of TB.

6. Inhibitors of Mtb protein tyrosine phosphatases

Both PtpA and PtpB are attractive targets for the development of novel antitubercular drugs due to their role in the intracellular survival of Mtb in various infection models. Platforms adopted from cancer research for the design of PTP inhibitors have been developed, and compounds which can inhibit PtpA and PtpB activity *in vitro* or reduce mycobacterial survival within host macrophages have been successfully identified and characterized [67–72].

In particular, Chiaradia et al. [69] have recently characterized synthetic chalcones, essential intermediate compounds in flavonoid biosynthesis in plants, as inhibitors of PtpA. Manger et al. [70] also reported the discovery of potential inhibitors against PtpA using natural products and a fragment-based approach. However, as PtpA has a high sequence and structural similarity with human PTP, specificity is an issue with the identified compounds as they show inhibition against human PTPs including PTP1B and the dual-specificity phosphatase Cdc25A. Nevertheless, these results can serve as a template for further development to increase inhibitor specificity for PtpA. On the other hand, PtpB, which has no human orthologues, was considered a better candidate for novel antitubercular drug development. Grundner et al. [67] crystallized the highly specific oxalylamino derivative inhibitor (oxalylamino-methylene)-thiophene sulfonamide (OMTS), in complex with PtpB. The crystallographic complex revealed dual inhibitor binding sites, suggesting the possibility that PtpB can recognize substrates with adjacent phosphotyrosine residues. Furthermore, a double-site binding isoxazole-based compound was found to selectively inhibit PtpB and was capable of reducing BCG survival in J774A.1 murine macrophages [68]. Together, these results provide evidence that PTPs can be targeted as a treatment against mycobacterial infections.

7. Protein tyrosine kinase A (PtkA)

Based upon sequence analysis, PtkA, encoded in the open reading frame *Rv2232*, was originally classified as a member of the haloacid dehalogenase (HAD) superfamily. More than 3000 sequenced proteins belong to this superfamily, which consists of phosphatases, dehalogenases, ATPase, phosphonatases, and sugar phosphomutases [73–78].

A large number of HAD enzymes are specialized in phosphoryl transfer, promoting the hypothesis that PtkA also possesses phosphorylation or dephosphorylation activity. Indeed, recombinant PtkA is autophosphorylated in a time- and dose-dependent manner, and phosphorylation occurs on tyrosine residues [3]. Although PtkA does not contain the characteristic bacterial PTK Walker A (GXXGXGK[T/S]) and B (hhhhD) motifs, several lines of evidence support the identification of PtkA as a genuine PTK and thus the classification of PtkA as a novel class of PTKs. First, a large number of members in the HAD superfamily are phosphotransferases capable of transferring phosphates onto a variety of substrates. Therefore, it is not surprising that PtkA is also capable of phosphorylation of tyrosine residues. Second, other bacterial kinases, exemplified by MasK from *Myxococcus xanthus* [79] and WaaP from *Pseudomonas aeruginosa* [46], also do not possess the typical bacterial PTK conserved motifs yet possess tyrosine kinase activity. An interesting observation is that PtkA is capable of utilizing both ATP and GTP as phosphate donors [3], which is in line with a previous report showing that the AlgR2 protein kinase from *P. aeruginosa* possesses similar capability [80]. This, together with the fact that PtkA activity is not inhibited by staurosporine, a general non-selective inhibitor of typical protein tyrosine kinases, further supports PtkA as a novel class of PTK. Although site-directed mutagenesis identified several residues in PtkA to have a critical role in its enzymatic activity [3], the exact mechanism of PtkA phosphorylation remains to be elucidated.

Genes encoding kinases are often located in the proximity or in the same operon with the genes encoding their substrates. Studies on bacterial PTKs and PTPs showed that their corresponding genes are frequently clustered in an operon such that the expression of the PTKs and PTPs can be coordinated and regulated in concert [e.g. 81]. Indeed, *ptkA is* located immediately upstream of *ptpA* in the same operon, and PtpA is phosphorylated by PtkA on adjacent tyrosine residues, Tyr^{128} and Tyr^{129}, in a time- and dose-dependent manner [3]. Although PtpA is itself a PTP, no dephosphorylation activity was exerted on PtkA, which is consistent with the fact that PtpA is a secretory protein that is specific for host macrophage substrates [3].

The functional role of PtkA phosphorylation of PtpA has yet to be determined. In humans, a homolog of PtpA, LMW-PTP, is also phosphorylated on two consecutive C-terminal tyrosine residues, Tyr^{131} and Tyr^{132}, *in vivo* [82,83]. Currently, there are mixed views on the role of this phosphorylation. Two groups have separately reported that PTP activity is increased by tyrosine phosphorylation [82,83]. However, in a more recent study, incorporation of site-specific non-hydrolyzable phosphotyrosine analogues into LMW-PTP resulted in negative regulation of its PTP activity [84]. Alternatively, as PtpA does not carry the typical N-terminal signal peptide for the secretion through the ubiquitous general secretory pathway (Sec) or the twin arginine transport system (Tat), PtpA phosphorylation may regulate its secretion. In *P. aeruginosa*, the STPK PpkA phosphorylates an FHA containing protein known as Fha1 [85]. Phosphorylated Fha1 recruits components of the Type VI secretion system to secrete another protein, Hcp1. Although the target of phosphorylation in this case is not the secreted protein substrate, we should not rule out the possibility that such a mechanism exists in Mtb.

8. Concluding remarks

The ser/thr protein kinase, PknG, and the protein tyrosine phosphatase, PtpA, have joined a growing number of Mtb cellular components shown to cause the hallmark of Mtb pathogenesis—its ability to block phagosome–lysosome fusion. These Mtb macromolecules include the cell-wall components, trehalose dimycolate (cord factor) and mannose capped lipoarabinomanan (LAM) [reviewed in 86], and a secreted lipid phosphatase, SapM [64]. Only SapM and PtpA have been shown experimentally to directly interfere with host physiological processes, namely hydrolysis of host phosphatidylinositol 3-phosphate (PI3P) and dephosphorylation of VPS33B, respectively, resulting in maturation arrest of the mycobacterial phagosome [30,64].

In the course of infection, Mtb encounters a highly hostile environment in the host, which includes changes in the redox balance in part due to the production of reactive oxygen and nitrogen species [87,88]. This has become a point of interest with respect to signal transduction particularly in the case of the Dos two-component system, where the histidine kinases DosS and DosT function as a redox and a hypoxia sensor, respectively [89]. Structural analysis of PknG and PtpB, two secreted proteins, also suggest a role for the STPK and PTP in sensing the redox environment. As discussed above, PknG contains a rubredoxin domain involved in electron transport, which controls its kinase activity, and PtpB contains a cap structure that protects its phosphatase domain from oxidative stress. As for PtpA, the two cysteine residues (Cys^{11} and Cys^{16}) in the active site are predicted to form an intramolecular disulfide bond under oxidative stress, protecting the catalytic Cys^{11} from undergoing irreversible oxidation [90,91]. The redox sensitivity of these secretory signaling

proteins might therefore represent a common regulatory strategy for the adaptation to the hostile host environment. Further research in this direction may unlock novel mechanisms that enable Mtb to survive within the macrophage of the host.

Mtb signaling proteins involved in blocking phagosome maturation are considered attractive drug targets, as this would enable the macrophage to overcome infection as demonstrated by the PknG inhibitors [44]. In contrast to other diseases where treatment with kinase inhibitors must necessarily be highly specific for their targets, the non-specificity among Mtb kinases will likely be beneficial. If a single inhibitor can act to inhibit multiple kinases within Mtb, the therapeutic outcome is more likely. As such, the STPK family, which exhibits high homology among themselves and yet are sufficiently different from their human counterparts, are promising Mtb drug targets.

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