Review

Mycobacterium tuberculosis-secreted phosphatases: from pathogenesis to targets for TB drug development

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Mycobacterium tuberculosis **(Mtb) infects human alveolar macrophages and relies on the inhibition of phagosome acidification and maturation. This is, in part, dependent on the disruption of host signaling networks within the macrophage. In recent years, Mtb-secreted protein- and lipid-phosphatases protein-tyrosine phosphatase A (PtpA), PtpB, and secreted acid phosphatase M (SapM) have been shown to contribute to Mtb pathogenicity. Here, we review the current knowledge on PtpA, PtpB, and SapM focusing on their ability to interfere with host functions. We further explore how these phosphatase-dependent host–pathogen interactions can be targeted for novel tuberculosis (TB) drug discovery and examine the ongoing development of inhibitors against these phosphatases.**

Mtb-secreted phosphatases interfere with host signal transduction

Bacterial pathogens have developed diverse strategies that alter host signaling pathways aiming to either subvert the immune response or create permissive niches for their survival. One such strategy is the secretion of bacterial signaling proteins into the target host cells, directly modulating the phosphorylation status of host signaling networks. Since the discovery of the *Yersinia* PTP YopH [1,2], numerous host–pathogen interactions were found to be dependent on pathogen-secreted phosphatases [3] (Table 1).

In recent years, studies have shown that Mtb also directly alters host signaling through secretory phosphatases, thereby shutting down critical cellular processes and promoting its survival within macrophages [4–9]. New advances in the understanding of the role of these Mtb phosphatases in the pathogenesis of TB have opened up an exciting avenue for TB drug development.

Human alveolar macrophages provide the first line of defense against invading microbes in the lung. Macrophages engulf foreign particles into phagosomes that subsequently interact with the endocytic pathway [10], resulting in changes to membranes that allow the phagosome to acquire antimicrobial properties, including the recruitment of hydrolytic enzymes and the vacuolar H^+-ATPase (V-ATPase) [11]. The presence of V-ATPase on the phagosomal membrane creates an acidic compartment (pH 4.5–5.0), the hallmark of phagosome maturation [12]. Acid-activated hydrolases then mediate the destruction of the invading microorganisms.

Mtb directly infects alveolar macrophages causing phagosome maturation arrest at an early stage. Although the mycobacterial phagosome can continue to interact with early endosomes [13], fusion with lysosomes is blocked [4,5]. This inhibition results in the lack of V-ATPase recruitment, restricting the compartment to a pH of 6.4 and blocking hydrolase activity [5,11]. This creates a favorable environment for Mtb to survive within the human host while evading immune detection [14].

Mtb adaptation to the host milieu is dependent on its wide repertoire of signal transduction systems that includes 11 complete two-component systems, 11 eukaryotic-like serine/threonine-protein kinases (STPKs) (PknA–B, PknD–L), two PTPs (PtpA and PtpB), and a protein-tyrosine kinase (PTK) (PtkA) [4,15–20]. Among the proteins secreted by Mtb into the host, three phosphatases, PtpA, PtpB, and SapM, are essential for Mtb pathogenesis [4,5,7,8,21]. In recent years, major leaps have been made in understanding the mechanisms by which these Mtb phosphatases act in the macrophage during infection (Figure 1). Specifically, SapM is a phosphoinositide phosphatase that inhibits the generation of phosphatidylinositol 3-phosphate (PI3P), which is essential for phagosome biogenesis [7]. PtpA inhibits V-ATPase trafficking to the mycobacterial phagosome and blocks phagosome–lysosome fusion [4,5]. PtpB has a possible role in the subversion of host immune response to infection [9,21].

Despite belonging to distinct families of phosphatases (Figure 2a), the similar roles of PtpA, PtpB, and SapM as key molecules in pathogenesis at the host–pathogen interaction interface warrants their consideration as a unique new class of targets for TB drug discovery. These Mtb-secreted phosphatases, needed only for *in vivo* growth, are distinct from the traditional *in vitro* essential targets, which have been the focus of antibiotic development during the past decades. Targeting phosphatases, particularly PTPs, for drug development has proved challenging as evident from efforts in the search for selective and cell-permeable PTP inhibitors [22]. Advances in the understanding of the structural aspect of Mtb-secreted phosphatases have allowed better drug design

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Table 1. Secreted phosphatases from bacterial pathogens and their roles in host–pathogen interaction

Organism	Phosphatase	Host substrates/targets	Function	Refs
Yersinia sp.	YopH	Focal adhesion kinase (FAK), paxillin, Lck, p85, Fyn-binding protein (FYB), $p130^{Cas}$, SKAP-HOM, SLP-76, LAT	Inhibition of phagocytosis, cytoskeletal rearrangements, and production of reactive oxygen species (ROS)	[1,2]
***Salmonella enterica* serovar Typhimurium**	SptP	AAA+ ATPase valosin-containing protein (VCP/p97)	Modulation of actin reorganization; inhibition of MAPK signaling and IL-8 production; biogenesis of *Salmonella*-containing vacuoles (SCVs)	[64,65]
	SopB	PI(3,4,5)P3, PI(3,5)P2, PI(4,5)P2	Activation of Cdc42 and RhoG to modulate cytoskeletal rearrangements; disrupts tight junctions; activation of PI3K/Akt signaling pathway and Class III PI3K; biogenesis of SCVs	[66–69]
Listeria monocytogenes	LipA	Unknown; targets phosphotyrosines and phosphoinositides *in vitro*	Unknown; plays a role in virulence in mouse infection model	[70]
Shigella flexneri	IpgD	PI(3,4,5)P3, PI(3,4)P2 PI(4,5)P2	Modulation of cytoskeletal reorganization; activation of PI3K/Akt pathway	[71,72]
Francisella tularensis	AcpA	$p47^{phox}$, $p40^{phox}$, $p67^{phox}$	Inhibits NADPH oxidase and oxidative burst	[73]
Legionella pneumophila	Map	Unknown	Unknown	[74]
Legionella micdadei	ACP_2	Unknown	Inhibits superoxide anion production in human neutrophils	[75]
Coxiella burnetti	ACP	$p47^{phox}$, $p60^{phox}$	Inhibits NADPH oxidase and oxidative burst	[76]
Mycobacterium tuberculosis	PtpA	VPS33B, V-ATPase subunit H	Inhibits phagosome acidification and maturation; blocks V-ATPase recruitment to phagosome	[4,5]
	PtpB	Unknown	Inhibits ERK1/2 and p38 signaling pathways and caspase 3; activates Akt	[9]
	SapM	PI3P	Inhibits phagosome maturation	[7]
Pseudomonas syringae	HopAO1	Unknown	Suppresses apoptosis	[77]

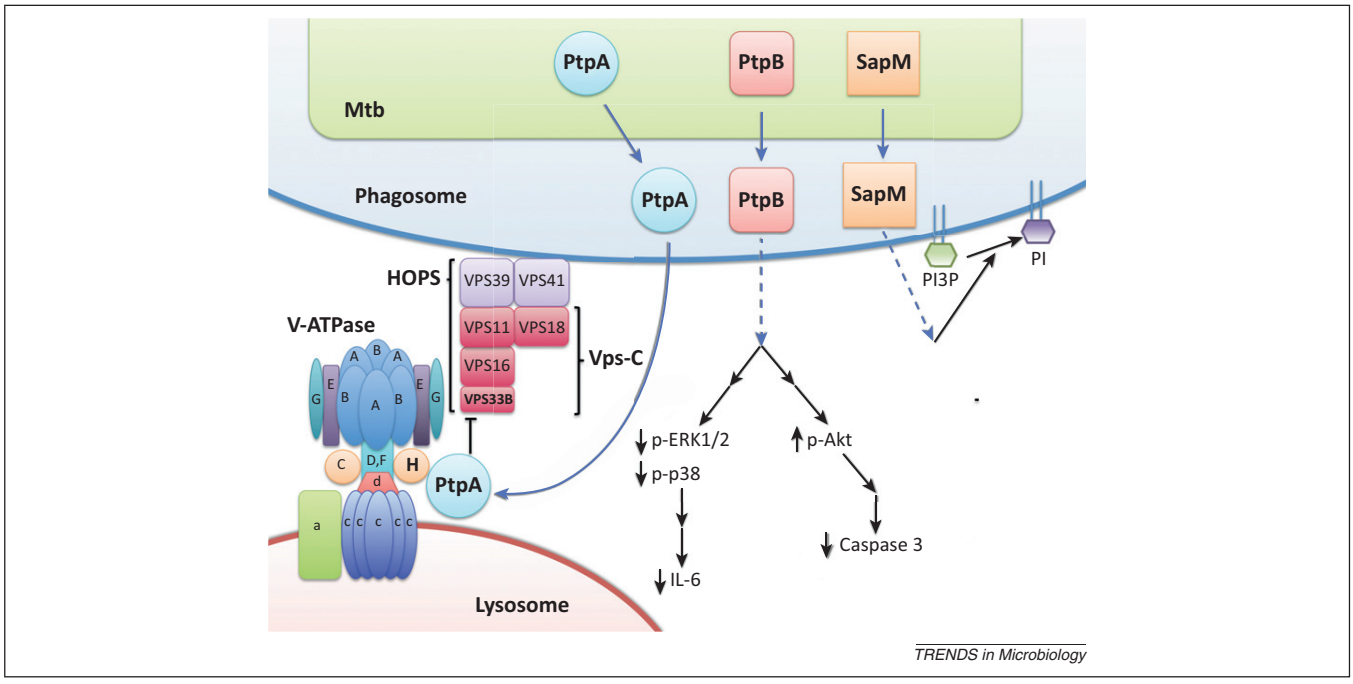

Figure 1. Disruption of host macrophage cellular processes by *Mycobacterium tuberculosis* (Mtb) phosphatases. Protein-tyrosine phosphatase A (PtpA), PtpB, and secreted acid phosphatase M (SapM) are secreted by Mtb during macrophage infection (as shown by blue arrows). Whereas PtpA has been demonstrated to translocate to the macrophage cytosol, the same phenomenon has not been directly observed for PtpB and SapM (broken blue arrows). PtpA binds to subunit H of the vacuolar H^+-ATPase (V-ATPase) in order to specifically localize to its catalytic substrate vacuolar protein sorting 33B (VPS33B) at the phagosome–lysosome fusion interface. VPS33B is a subunit of the class C vacuolar protein sorting complex (Vps-C) that serves as the core of homotypic fusion and protein sorting (HOPS) and regulates membrane trafficking throughout the endocytic pathway. Dephosphorylation of VPS33B ultimately results in the exclusion of V-ATPase from the mycobacterial phagosome. PtpB activity within the host macrophage leads to decreased phosphorylation of extracellular signal-regulated kinase 1/2(ERK1/2) and p38 and increased phosphorylation of Akt, resulting in reduced production of interleukin-6 (IL-6) and decreased apoptotic activity, respectively. SapM dephosphorylates phosphatidylinositol 3-phosphate (PI3P) on the phagosomal membrane, thereby, inhibiting host signaling pathways and recruitment of membrane trafficking proteins to the phagosome.

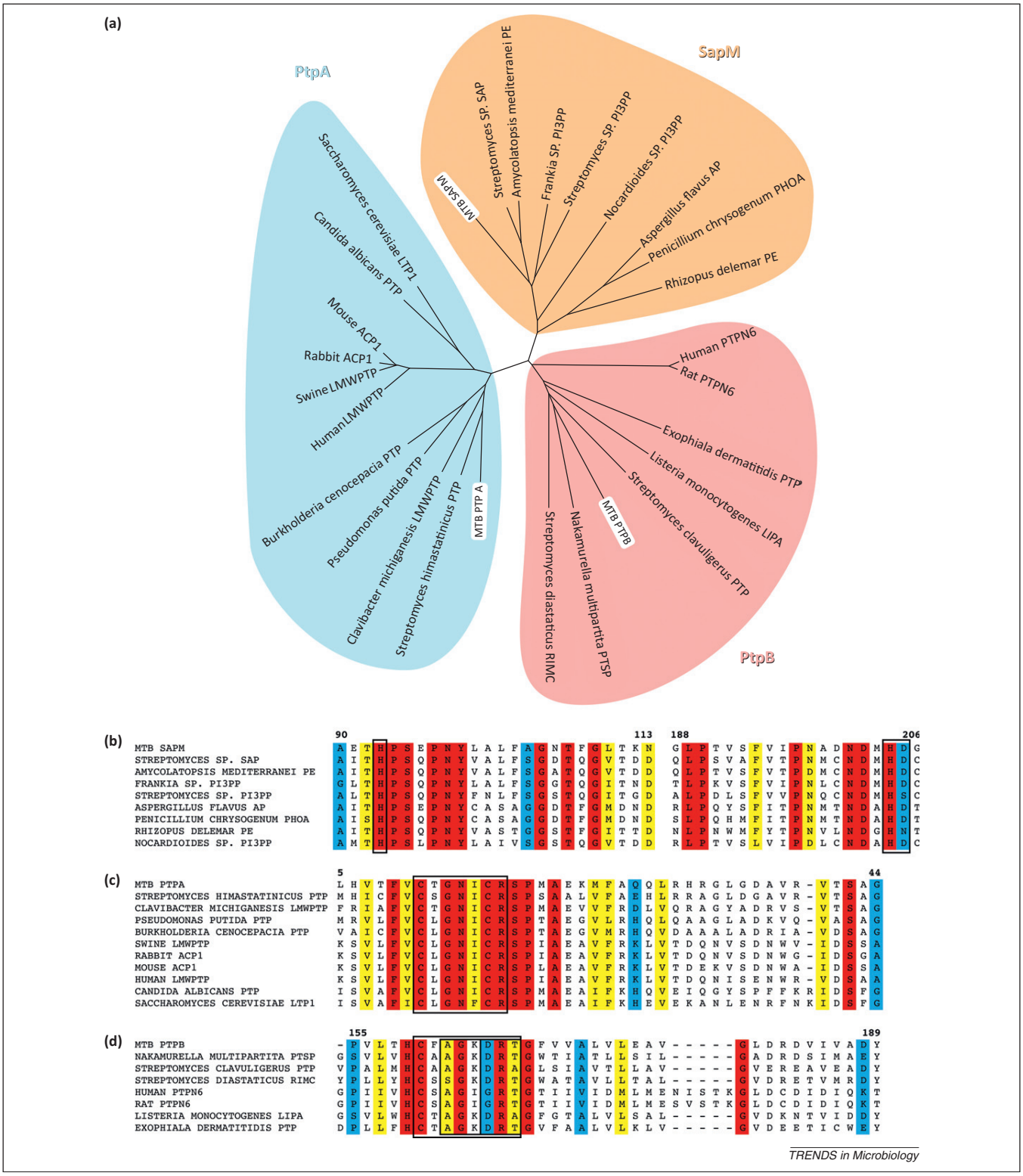

Figure 2. Sequence analysis of *Mycobacterium tuberculosis* (Mtb)-secreted phosphatases. **(a)** Dendrogram of a multiple sequence comparison of protein-tyrosine phosphatase A (PtpA, cyan), PtpB (red), and secreted acid phosphatase M (SapM, orange), with their respective hits identified by BLAST searches of human, mammalian, fungal, and bacterial organisms, excluding other mycobacterial species which clustered together with Mtb (data not shown). Sequences were aligned using ClustalW (gap open 10; gap extension 0.2) and displayed using Molecular Evolutionary Genetics Analysis (MEGA) 5 software. Uniprot identifiers listed in clockwise order beginning with MTB PTPA are: PtpA: P65716, D9WS25, B0RDM3, B0KF44, A2VX28, P24666, P81693, G1T489, Q561M1, O94044, P40347; SapM: O53361, D9V5G2, D8HN03, Q2JF19, D9VVX9, A1SH32, B8NCF7, P37274, I1BTX3; PtpB: P29350, Q499N7, H6C7Y9, Q8Y696, E2PZ53, P96830, C8XE08, Q6T2C5. Abbreviations: PE, phosphoesterase; PI3PP, PI3P phosphatase; AP, acid phosphatase; PTSP, protein-tyrosine/serine phosphatase. **(b–d)** Alignment of the proposed active sites of PtpA, PtpB, and SapM. Full-length SapM (b), PtpA (c), and PtpB (d), with their respective orthologs identified in Figure 2a, were aligned separately using ClustalW (gap open 10; gap extension 0.2). The active site region (displayed) shows highly homologous residues across all organisms. Both regions of SapM that contribute to the active site are shown. Red indicates 100% residue identity; yellow indicates residues with strongly similar properties ($>$ 0.5 in the Gonnet PAM 250 matrix); blue indicates residues with weakly similar properties ($\leq$ 0.5 in the Gonnet PAM 250 matrix). Numbers indicate positions of the first and last amino acids for the Mtb phosphatases. Conserved active site motifs are boxed.

[23–25]. Efforts from numerous laboratories have identified drug candidates that could specifically inhibit PtpA and PtpB and impair Mtb intracellular survival [8,9,26]. It is therefore timely to review the current knowledge on PtpA, PtpB, and SapM in the context of TB pathogenesis and to discuss their druggability with regard to the current development of inhibitors.

SapM

SapM was first identified in culture filtrate of Mtb as a secreted acid phosphatase [27]. SapM lacks any conserved sequence motifs of bacterial phosphatases, however, it does possess two catalytically important histidine residues (positions 93 and 204) highly conserved in fungal acid phosphatases (Figure 2b, boxed). The secretory nature of SapM (Box 1) further suggests that it might function to interact with host molecules during infection.

SapM was initially found to exhibit activity against phosphoenolpyruvate, glycerophosphate, GTP, NADPH, phosphotyrosine, and trehalose-6-phosphate [6]. In a later study, Vergne *et al*. showed that PI3P, a membrane trafficking regulatory lipid that is essential for phagosomes to acquire lysosomal constituents, is retained on phagosomes containing dead mycobacteria but is continuously eliminated from phagosomes with live bacilli [7]. SapM was found to possess PI3P phosphatase activity and was responsible for PI3P removal [7]. This suggested that SapM mediates the arrest of phagosome maturation by disrupting the recruitment of PI3P effector proteins such as early endosome antigen 1 (EEA1). Indeed, addition of SapM to an *in vitro* assay inhibited phagosome fusion with late endosomes [7]. Deletion of *sapM* from *Mycobacterium bovis* BCG resulted in a vaccine strain characterized by longer survival of mice lethally challenged with Mtb [28]. The Δ*sapM* BCG vaccine was more effective in inducing recruitment and activation of $CD11c^{+}$ $MHC\text{-}II^{int}$ $CD40^{int}$ dendritic cells (DCs) to the draining lymph nodes. The increased efficacy of the vaccine may be the result of a lack of SapM inhibition of phagosome maturation, thus allowing successful antigen presentation and activation of adaptive immunity by DCs. A more recent study showed that Δ*fbpA*Δ*sapM* double deletion in Mtb H37Rv results in a highly attenuated and immunogenic strain, further supporting SapM's role in phagosome maturation arrest [29]. However, there is currently no SapM specific inhibitor reported, possibly due to limited study on SapM and industrial know-how in targeting this family of phosphatases.

Box 1. Secretion of Mtb phosphatases

Protein secretion in Mtb is mediated by several different mechanisms. Mtb possesses the general secretory (Sec) pathway (SecA1) and the twin-arginine translocation (Tat) system that are characterized by the recognition of N-terminal signal sequences in secreted substrates (reviewed in [78]). Mtb also utilizes two alternate pathways, SecA2 and ESX/type VII, both of which are known to export proteins lacking N-terminal signal sequences (reviewed in [78]).

Whereas SapM secretion is likely to be SecA1-dependent due to the presence of an N-terminal signal peptide [27], both PtpA and PtpB lack such a sequence for export [17,18]. However, like SapM, both PtpA and PtpB were found to be present in the culture filtrate of Mtb grown *in vitro* [17,18,27]. Although there is no study showing PtpB and SapM translocation into host cell cytosol, PtpA secretion into the host macrophage cytoplasm has been detected directly with immuno-electron microscopy and Western blot analysis of the cytosolic fractions of infected macrophages and indirectly with the expression of neutralizing single-chain anti-PtpA antibodies within macrophages [4]. Yet, the exact mechanism by which PtpA and PtpB are exported out of Mtb and, in the case of PtpA, into the macrophage cytosol remains obscure. The lack of an N-terminal signal sequence rules out the SecA1 and Tat pathways, whereas the SecA2 and ESX/type VII export systems are possible candidates responsible for PtpA and PtpB secretion. In fact, a recent study showed that phagosomes harboring a Mtb Δ*secA2* mutant strain were, similar to the Δ*ptpA* strain, more acidified and exhibited accumulation of lysosomal markers as compared to the parental strain [79]. This indicates that the SecA2 pathway is needed to export Mtb proteins that mediate phagosome maturation arrest and might be responsible for the PtpA secretion. Despite the presence of conflicting reports, there is evidence indicating that molecules less than 70-kDa can cross the mycobacterial phagosome membrane [80,81]. Furthermore, the ESX-1 secretion system with its substrate ESAT-6 was more recently found to perturb the host cell membrane and facilitate the translocation of mycobacterial proteins into the macrophage cytosol [82]. Therefore, it is also possible that both SecA2 and ESX/type VII secretion systems might contribute to PtpA secretion out of Mtb and subsequent translocation into the host cell cytosol. Further investigation is required to determine whether PtpA and PtpB are direct substrates for SecA2, the ESX/type VII secretion system, or an alternate mechanism altogether.

PtpA

PtpA was first identified from the genome sequence of Mtb H37Rv through its homology to known eukaryotic low-molecular-weight PTPs (LMW-PTP). PtpA contains the conserved $C(X)_5R(S/T)$ signature motif typical of functional PTPs (Figure 2c, boxed), where the Cys11 residue serves as the nucleophile that attacks the phosphotyrosine residue of its protein substrate [30].

Interestingly, the presence of Cys16 residue in the active site of PtpA suggests that an intramolecular disulfide bridge can be formed within the active site (Cys11 and Cys16). The formation of the disulfide bond was proposed to protect the nucleophilic Cys11 from oxidation, allowing PtpA to rapidly sense and respond to the redox status of its environment [31]. However, when PtpA was treated with reactive nitrogen species *in vitro*, it was nitrosylated on Cys53, leading to a reduction in activity [32]. This indicates that PtpA could be susceptible to the oxidative environment in the macrophage. It is therefore tempting to speculate that another reduction system to protect PtpA from oxidative damage might exist.

PtpA is secreted from Mtb into the host macrophage cytosol (Box 1) and disrupts key components of the endocytic pathway, resulting in the arrest of phagosome maturation [4,5]. Human vacuolar protein sorting 33B (VPS33B) was identified as the cognate substrate of PtpA using a substrate-trapping approach. VPS33B is a member of the class C vacuolar protein sorting (VPS) complex (Vps-C), which is composed of three other subunits, VPS11, 16, and 18 [33]. Vps-C is a key regulator of endosomal membrane trafficking and fusion, mediating tethering and *trans*-soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (*trans*-SNARE) assembly throughout the endocytic pathway [34]. PtpA dephosphorylation of VPS33B inactivates the host protein, leading to inhibition of phagosome–lysosome fusion [4]. In support of

this, *ptpA* deletion in Mtb H37Rv impaired mycobacterial survival within human THP-1 macrophages, and phagosomes harboring the Δ*ptpA* strain showed increased lysosome fusion and transfer of lysosomal contents as compared to the parental strain [4].

Following this study, PtpA was also found to bind subunit H of the V-ATPase complex during infection [5]. PtpA is capable of blocking phagosome acidification as demonstrated by the failure of the Δ*ptpA* strain to maintain an unacidified phagosome and by the inhibition of phagosome acidification in macrophages transfected with PtpA [5]. Mechanistic study of PtpA-mediated inhibition of phagosome acidification revealed that Vps-C is recruited to the V-ATPase during infection [5]. Vps-C plays a key role in the membrane fusion machinery, suggesting that its interaction with the V-ATPase is required for successful phagosome–lysosome fusion, particularly the trafficking of V-ATPase to the phagosome. Indeed, PtpA binding to V-ATPase subunit H interferes with host protein complex interaction, leading to the exclusion of V-ATPase from the mycobacterial phagosome (Figure 1).

Interestingly, PtpA binding to the V-ATPase is a prerequisite for the dephosphorylation of VPS33B within the macrophage [5]. This suggests that PtpA binding to V-ATPase localizes PtpA to the vicinity of its substrate within the host cytosol. In fact, a mechanism of specific V-ATPase trafficking inhibition to the phagosome is immediately apparent. The mycobacterial phagosome, despite being refractory to fusion with the lysosomes, maintains interaction with the early endosomes, thereby acquiring critical nutrients for intracellular survival [13]. Furthermore, consistent with another report [35], Sun-Wada *et al*. [36] demonstrated that phagosomal V-ATPase originates from tubular lysosomes. These fusion events with endosomes and lysosomes are likely to be mediated by the Vps-C complex. Therefore, through its interaction with the subunit H of V-ATPase, PtpA can specifically localize to the phagosome–lysosome fusion interface, target the Vps-C that mediates fusion with lysosomes, and inhibit V-ATPase recruitment to the mycobacterial phagosome while leaving fusion with early endosomes unaffected.

This proposed model (Figure 1) is compatible with recent advances in the study of membrane trafficking, where vesicle fusion in the yeast (*Saccharomyces cerevisiae*) endocytic pathway depends on two membrane tethering protein complexes, class C core vacuole/endosome tethering (CORVET) and homotypic fusion and protein sorting (HOPS) [37,38]. Vps-C serves as the core of both CORVET and HOPS through reversible association with CORVET-specific (VPS3 and VPS8) and HOPS-specific (VPS39 and VPS41) accessory subunits (reviewed in [39]). Although CORVET, which mediates early to late endosome fusion events in *S. cerevisiae*, has not yet been identified in mammalian cells, it likely exists and has the same functions given the similarity in the transport machinery between yeast and mammals. Mammalian HOPS, which regulates fusion with lysosomes, has been identified and plays the same role as the *S. cerevisiae* homologs [40]. It is therefore likely that V-ATPase participates in the regulation of membrane fusion at the phagosome–lysosome interface by specifically interacting with the HOPS (Figure 1). PtpA localization to the V-ATPase then allows PtpA to distinguish the Vps-C cores of the HOPS and CORVET complexes during Mtb infection. This is supported by a previous study demonstrating that the HOPS-specific subunit VPS41 failed to function in a V-ATPase mutated yeast strain [41]. Interestingly, the integral V_o domain of the V-ATPase has been demonstrated to play a direct role in membrane fusion, where *trans* pairing of two V_o domains on opposing membranes form a fusion pore that facilitate membrane fusion [42]. Thus, it is possible that PtpA binding to the V-ATPase might also disrupt membrane fusion mediated by the V_o domain. However, PtpA interaction with V-ATPase alone is not sufficient to block phagosome–lysosome fusion [5]. Yet, without a clear understanding of the mechanistic details behind V_o-mediated membrane fusion, we cannot rule out such a possibility. Although unanswered questions remain in the proposed model, PtpA is clearly a key protein involved in Mtb inhibition of phagosome acidification and maturation and, ultimately, the pathogenesis of Mtb.

PtpB

Similar to PtpA, PtpB was identified due to its homology with eukaryotic PTPs, particularly the presence of the $C(X)_5R(S/T)$ PTP motif (Figure 2d, boxed). Koul *et al*. [17] showed that PtpB phosphatase activity is specific for phosphotyrosine substrates, and it is secreted into culture media. However, the exact secretion mechanism is not known (Box 1). Crystallographic analysis found that PtpB possesses the features of dual-phosphotyrosine binding sites and a lid structure that protects the active site of the enzyme in an oxidative environment [25]. It has then been postulated that PtpB might be secreted by Mtb into either the phagosome or host cytosol during infection, disrupting macrophage tyrosine kinase signaling pathways. However, a study that re-examined the sequence homology and enzymatic activity of PtpB found that PtpB contains the conserved AGK and DRT motifs found in phosphatase and tensin homolog (PTEN) and myotubularin (MTM) phosphoinositide phosphatase, respectively, within its $C(X)_5R(S/T)$ motif [43] (Figure 2d, boxed). With an active site region that also resembles the eukaryotic dual-specificity phosphatase, PtpB was found to possess the unique property of triple-specificity for phosphoinositides, phosphotyrosine, and phosphoserine/phosphothreonine [43]. Based upon these results, it is reasonable to argue that PtpB might also be capable of disrupting host phosphoinositide and its associated signaling pathways. However, thus far, the cognate substrate of PtpB remains elusive.

Although the target of PtpB within the host macrophage is yet to be identified, there is evidence demonstrating the essentiality of PtpB in Mtb pathogenesis [8,21]. Deletion of *ptpB* from the genome of Mtb H37Rv led to decreased bacterial survival in interferon-γ (IFN-γ)-activated murine macrophages and a 70-fold reduction of bacillary loads in the spleens of infected guinea pigs 6 weeks post-infection [21]. The importance of PtpB to Mtb intracellular survival was also suggested by two other studies in which specific inhibitors against PtpB were shown to inhibit mycobacterial survival within murine macrophages as compared to untreated macrophages [8,9]. However, in both studies, no

reduction in mycobacterial intracellular growth was observed when compared to bacillary load at time zero.

Nonetheless, Zhou *et al.* [9] showed that expression of PtpB in activated murine macrophages resulted in reduced phosphorylation of the extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK), which led to decreased production of interleukin-6 (IL-6) [9]. IL-6 has been implicated in the induction of innate immunity during Mtb infection [44]. Furthermore, PtpB expression resulted in increased Akt phosphorylation and decreased caspase-3 activation associated with apoptosis inhibition [9]. It has been previously observed that mycobacterial infection can induce ERK1/2 and p38 MAPK activation during entry into macrophages; however, this activation is only sustained in attenuated strains whereas pathogenic species quickly diminish the host kinase activities [45]. Although ERK1/2 and p38 are not direct substrates of PtpB, these observations suggest that PtpB disrupts host signal transductions resulting in subversion of host immune response. It is interesting to note that Mtb antigens such as 6 kDa early secretory antigenic target (ESAT-6) and various cell wall components are also known to elicit the MAPK and PI3K-Akt signaling pathways [46,47]. Thus, it is possible that PtpB plays a key role as an immune response damper, balancing host immune detection with evasion during Mtb infection.

Mtb phosphatase inhibitors as TB drugs

Genetic validation suggests the essentiality of PtpA and PtpB Mtb intracellular survival in infection models. The Δ*ptpA* strain was found to be impaired in survival within human THP-1 macrophages [4], and the *ptpB* deletion mutant showed decreased survival within IFN-γ-activated murine macrophages and in a subcutaneous guinea pig infection model [21]. This spurred great interest in exploiting PtpA and PtpB as novel drug targets [4,21], resulting in several studies demonstrating that inhibitors against PtpA and PtpB can effectively arrest Mtb growth in human or murine macrophages, respectively [8,9,26]. Interestingly, *ptpA* deletion did not impact Mtb survival in C57BL/6 mice [48], suggesting that the mouse model is not suitable for PtpA inhibitor study. This variation, in contrast with the phenotype observed in human macrophages, is likely to be a result of the apparent difference in immune response to Mtb in the mouse model as compared to TB progression in humans [49]. Other animal models, such as minipigs, which have a more 'human-like' control of infection [50], are currently being explored.

Mtb-secreted phosphatases, particularly PtpA and PtpB, represent a unique novel class of potential TB drug targets. Traditional antibiotics were discovered for their ability to kill Mtb *in vitro*, targeting intra-bacterial functions such as protein, DNA, and cell wall biosynthesis. By contrast, PtpA and PtpB, which are secreted to the extracellular medium or host cytosol, are both dispensable for *in vitro* growth but essential for *in vivo* viability within the host cell. Several advantages, including commensal flora preservation and reduced selective pressure, of targeting bacterial *in vivo* essential gene functions have been proposed [51]. For Mtb, the extracellular localization of PtpA and PtpB presents a unique opportunity to avoid the drug delivery issue due to Mtb's thick hydrophobic cell envelope that contributes to its intrinsic resistance to conventional antibiotics. Targeting PtpA and PtpB would therefore circumvent the need to deliver drugs across the Mtb cell wall, thereby potentially improving bioavailability. Furthermore, kinases and phosphatases serve as molecular switches that regulate multiple signaling pathways. Inhibiting PtpA and PtpB might therefore restore the macrophage signaling networks, rescuing multiple host defense mechanisms.

The development of clinically useful inhibitors against PtpA and PtpB, however, is complicated by selectivity issues. As mentioned above, PTPs share a common cysteine-catalyzed mechanism characterized by the $C(X)_5R(S/T)$ active site motif. Therefore, inhibitors against PTPs tend to suffer from non-specific inhibition. Clinically useful PTP inhibitors must be highly selective, and this is particularly important for PtpA because it shares 37% identity with its human ortholog (hLMW-PTP) and a high degree of similarity with other mammalian LMW-PTPs (Figure 2a). By contrast, PtpB has a distinct structural fold [25], with no human ortholog and only 6% similarity with human PTP1B (Figure 2a). Selectivity can be improved by several approaches. Non-conserved regions surrounding the active site can be targeted simultaneously using multidentate compounds as illustrated by several studies that have adopted this approach for PtpB [8,52], which was previously found to possess a distinct secondary phosphotyrosine binding site [24]. In the case of PtpA, a recent advance in structural analysis revealed features that might be exploited for improving selectivity [23]. In particular, the Mtb PTK PtkA phosphorylates PtpA at Tyr128 and Tyr129 and binds to a region near the active site [16,23]. This interface could be exploited for inhibitor design, and blocking the interaction between PtpA and PtkA might further subdue PtpA function. Furthermore, the stabilizing electrostatic interaction network within the active site of PtpA shows variations from that of mammalian LMW-PTPs, which could also be exploited [23]. Allosteric inhibition targeting distant non-conserving regions is an alternative to achieve selectivity. A non-competitive inhibitor has been previously identified for PtpB [9]. For PtpA, the discovery of its interaction with V-ATPase subunit H presents an interface where an allosteric inhibitor can be designed to disrupt the binding [5].

Cell permeability is another issue that has slowed the development and introduction of PTP inhibitors as novel therapeutics [22]. Due to the anionic property of the phosphotyrosine substrates for PTPs, inhibitory phosphomimetics tend to also be highly negatively charged in order to stably fit into the PTP active site. This anionic nature renders PTP inhibitors cell-impermeable, preventing access to the target enzyme. The lack of cell permeability is the major reason for the small number of cellular active Mtb PTP inhibitors despite an abundance of hits described in the current literature. Continued development of novel phosphomimetics, such as isothiazolidinone [53], which exhibit high affinity and are less anionic, is required for improving Mtb PtpA and PtpB inhibitor design.

A developing trend in the identification of Mtb PtpA and PtpB inhibitors is to utilize a combination of *in vitro* enzyme

Table 2. PtpA and PtpB inhibitors described in the literature

Inhibitors	PtpA IC_{50} (μM)	PtpB IC_{50} (μM)	Refs
Stevastelins	8.8 ± 5.9		[55]
Roseophilins	9.4 ± 2.1		[55]
Prodigiosins	28.7 ± 9.7		[55]
Hydroxypyrrole benzoic acids	1.6 ± 0.4[a]		[55]
Difluoromethylphosphonic acid (DFMP)	1.4 ± 0.3[a]	>100	[60]
Chalcones	8.4 ± 0.9		[26,54]
Macrolines	>100	4.71 ± 1.14	[56]
Brunsvicamides	>100	7.3	[83]
Indolizines	74.9 ± 8.8	7.5 ± 1.9	[84]
Indoloquinolizidines	>100	0.36 ± 0.12	[62]
Oxalylamino-methylene-thiophene sulfonamide (OMTS)		0.44 ± 0.05	[24]
Isoxazole carboxylate	>50	0.22 ± 0.3	[57]
Isothiazolidinone (isoxazole based)		3.7 ± 0.6	[58]
DFMP (isoxazole based)		0.69 ± 0.21	[58]
Indolin-2-on-3-spirothiazolidinone		0.32 ± 0.05	[63]
Isoxazole azides		0.55 ± 0.3 0.15 ± 0.03[a]	[52]
Isoxazole salicylate		7.0 ± 0.4 1.5 ± 0.8[a]	[8]
Benzofuran salicylate	77.3 ± 5.1	1.26 ± 0.22 1.08 ± 0.06[a]	[9]
2-Oxo-1,2-dihydrobenzo [cd]indole-6-sulfonamide	>50	1.2 ± 0.1 1.1 ± 0.03[a]	[59]
Piperazinyl-thiophenyl-ethyl-oxalamide		4.8 ± 0.1 3.2 ± 0.3[a]	[59]

[a]K_i values.

inhibition and *ex vivo* macrophage infection for high-throughput screening. Traditional screening approaches for *in vitro* growth inhibition would be ineffective for PtpA and PtpB as both are non-essential for *in vitro* growth. Using *ex vivo* high-throughput screening, selective and cell-permeable compounds that can specifically inhibit Mtb PTPs and subdue Mtb survival with low toxicity against the infected host could be rapidly identified. This approach could accelerate the search for clinically useful Mtb PTP inhibitors and build upon drug candidates that have been previously described [8,9,24,26,52,54–61] (Table 2).

PtpA inhibitors

In a pioneering study identifying PtpA inhibitors, Manger *et al.* tested natural products such as stevastelins, obtaining IC_{50} values between 8.8 μM and 28.7 μM [55]. Furthermore, a fragment-based approach identified a novel group of hydroxypyrrol benzoic acids as potent PtpA inhibitors (K_i = 1.6 μM). However, specificity is an issue with the identified compounds as they showed inhibition against human PTPs such as PTP1B. Using a similar approach, a group of aryl difluoromethylphosphonic acid (DFMP) compounds were identified as potent inhibitors of PtpA (K_i = 1.4 μM) [60]. This group of inhibitors, due to stringent design, was reported to have greater than 70-fold selectivity against PtpA as compared to the human PTPs tested. In another study, Chiaradia *et al.* [54] characterized a family of chalcones as inhibitors of PtpA with IC_{50} values between 8.4 μM and 53.7 μM. When these compounds were analyzed for their *ex vivo* efficacy in a macrophage infection model, one inhibitor was able to reduce the bacillary load by 77% at 96 hours post-infection with minimal toxicity against the host cells [26]. This was the first demonstration of the effectiveness of PtpA inhibitors as TB drugs in an infection model.

PtpB inhibitors

Despite a lack of knowledge about the PtpB mode of action and its substrate within host cells, the absence of a human ortholog for PtpB makes it an attractive target for novel TB drug discovery. To date, all IC_{50} values reported in the literature were determined using the artificial substrate *para*-nitrophenylphosphate (*p*NPP). Several studies have identified inhibitors against PtpB with only a few tested for their *in vivo* efficacy in reducing bacillary load [9,21,52,57–59,61,62]. Using biologically pre-validated scaffolds, Nören-Müller *et al.* [62] developed a group of indoloquinolizidines that were highly potent PtpB inhibitors (IC_{50} = 0.34 μM). In an alternative approach, Grundner *et al.* [24] identified (oxalylamino-methylene)-thiophene sulfonamide (OMTS) as a potent inhibitor against PtpB with an IC_{50} of 0.44 μM and 65-fold selectivity for PtpB compared to the human PTPs tested. Soellner *et al.* [57] employed an innovative fragment-based substrate activity screening method to identify a group of highly potent isoxazole derivatives with K_i values in the nanomolar range.

These aforementioned compounds likely suffer from low cell permeability due to highly anionic moieties. Several groups attempted to address this issue [52,58,59,63]. In particular, Vintonyak *et al.* [63] identified indolin-2-on-3-spirothiazolidinones as PtpB inhibitors possessing promising cell permeability. Tan *et al.* [52] used the isoxazole derivative as a template for bidentate inhibitors, resulting in the most potent PtpB inhibitor (K_i = 150 nM) described to date. Bidentate compounds may have improved selectivity due to their capability to target a secondary site on PtpB, and thus have become a major focus of the search for PtpB inhibitors. Beresford *et al.* [8] and Zhou *et al.* [9] have separately developed selective double-site PtpB inhibitors with IC_{50} of 1.26 μM and 7.0 μM, respectively. However, the effectiveness of these inhibitors is not without question. Although both studies demonstrated that treatment of macrophages lowered mycobacterial loads compared to untreated, both the isoxazole-salicylate and benzofuran-salicylate did not show significant reduction of mycobacterial growth when compared to initial time points of infection [9]. In fact, a slight increase in bacillary loads at 72 hours or 7 days post-infection can be observed [8,9]. This is in stark contrast to a study examining Mtb survival in macrophages treated with PtpA inhibitors where bacillary loads were continually reduced over similar timeframes post-infection [26]. Chen *et al.* [59] later identified 2-oxo-1,2-dihydrobenzo[cd]indole-6-sulfonamide and piperazinyl-thiophenyl-ethyl-oxalamide derivatives as PtpB inhibitors with similar cellular activities as the benzofuran-salicylate derivatives. Further development of potential PtpB inhibitors and elucidation of the exact role of PtpB

Box 2. Outstanding questions

- Will the pharmaceutical industry accept 'non-standard' antimicrobial agents inhibiting pathogenesis or growth *in vivo* as opposed to classical antibiotics targeting housekeeping essential targets?
- Can drugs against Mtb-secreted phosphatases be compatible with the current TB drug regimen?
- What is the mechanism of secretion for the Mtb phosphatases?
- What is the direct substrate(s) of PtpB within the host macrophage?
- What are the downstream signaling pathways affected by PtpA, PtpB, and SapM?
- How do these phosphatases cope with oxidative damage in the host cell?
- How are these enzymes regulated? What is the exact role of their redox status?
- With the recent proposal of the dynamic re-infection hypothesis in latent TB infection suggesting that, instead of the traditional static model of dormant Mtb, there is constant endogenous re-infection of the host cells during latency due to drainage of non-replicating Mtb from the granuloma to the alveolar fluid as new growing bacilli [85], will targeting PtpA, which plays a crucial role during host macrophage entry, be effective against latent infection?

in Mtb pathogenesis will be needed to truly understand PtpB druggability.

Concluding remarks

Immune evasion and inhibition of phagosome acidification and maturation are key aspects of Mtb pathogenesis. Research in recent years has demonstrated that Mtb-secreted phosphatases play crucial roles in these phenomena. Thus, Mtb-secreted phosphatases represent a class of novel targets for TB drug discovery, which has been lagging behind for decades. Currently, there is an abundance of promising hits against PtpA and PtpB described. However, there is limited evidence for their efficacies *in vivo*. It is therefore crucial to test these compounds for cellular activity within infection models in order to promote continual development of PtpA and PtpB inhibitors. The key areas for future research directions are summarized in Box 2.

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References

1 Black, D.S. and Bliska, J.B. (1997) Identification of p130Cas as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J.* 16, 2730–2744
2 Persson, C. *et al.* (1997) The PTPase YopH inhibits uptake of *Yersinia*, tyrosine phosphorylation of p130Cas and FAK, and the associated accumulation of these proteins in peripheral focal adhesions. *EMBO J.* 16, 2307–2318
3 Heneberg, P. (2012) Finding the smoking gun: protein tyrosine phosphatases as tools and targets of unicellular microorganisms and viruses. *Curr. Med. Chem.* 19, 1530–1566
4 Bach, H. *et al.* (2008) *Mycobacterium tuberculosis* virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. *Cell Host Microbe* 3, 316–322
5 Wong, D. *et al.* (2011) *Mycobacterium tuberculosis* protein tyrosine phosphatase (PtpA) excludes host vacuolar-H+-ATPase to inhibit phagosome acidification. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19371–19376
6 Saleh, M.T. and Belisle, J.T. (2000) Secretion of an acid phosphatase (SapM) by *Mycobacterium tuberculosis* that is similar to eukaryotic acid phosphatases. *J. Bacteriol.* 182, 6850–6853
7 Vergne, I. *et al.* (2005) Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U.S.A.* 102, 4033–4038
8 Beresford, N.J. *et al.* (2009) Inhibition of MptpB phosphatase from *Mycobacterium tuberculosis* impairs mycobacterial survival in macrophages. *J. Antimicrob. Chemother.* 63, 928–936
9 Zhou, B. *et al.* (2010) Targeting *Mycobacterium* protein tyrosine phosphatase B for antituberculosis agents. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4573–4578
10 Bright, N.A. *et al.* (2005) Endocytic delivery to lysosomes mediated by concurrent fusion and kissing events in living cells. *Curr. Biol.* 15, 360–365
11 Sturgill-Koszycki, S. *et al.* (1994) Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 263, 678–681
12 Tjelle, T.E. *et al.* (2000) Phagosome dynamics and function. *Bioessays* 22, 255–263
13 Vergne, I. *et al.* (2004) *Mycobacterium tuberculosis* phagosome maturation arrest: mycobacterial phosphatidylinositol analog phosphatidylinositol mannoside stimulates early endosomal fusion. *Mol. Biol. Cell* 15, 751–760
14 Hestvik, A.L. *et al.* (2005) Mycobacterial manipulation of the host cell. *FEMS Microbiol. Rev.* 29, 1041–1050
15 Av-Gay, Y. and Everett, M. (2000) The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. *Trends Microbiol.* 8, 238–244
16 Bach, H. *et al.* (2009) *Mycobacterium tuberculosis* PtkA is a novel protein tyrosine kinase whose substrate is PtpA. *Biochem. J.* 420, 155–160
17 Koul, A. *et al.* (2000) Cloning and characterization of secretory tyrosine phosphatases of *Mycobacterium tuberculosis*. *J. Bacteriol.* 182, 5425–5432
18 Cowley, S.C. *et al.* (2002) Expression and localization of the *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpA. *Res. Microbiol.* 153, 233–241
19 Chao, J.D. *et al.* (2010) Protein kinase and phosphatase signaling in *Mycobacterium tuberculosis* physiology and pathogenesis. *Biochim. Biophys. Acta* 1804, 620–627
20 Chao, J.D. *et al.* (2010) Convergence of Ser/Thr and two-component signaling to coordinate expression of the dormancy regulon in *Mycobacterium tuberculosis*. *J. Biol. Chem.* 285, 29239–29246
21 Singh, R. *et al.* (2003) Disruption of mptpB impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs. *Mol. Microbiol.* 50, 751–762
22 Barr, A.J. (2010) Protein tyrosine phosphatases as drug targets: strategies and challenges of inhibitor development. *Future Med. Chem.* 2, 1563–1576
23 Stehle, T. *et al.* (2012) The apo-structure of the low-molecular-weight protein tyrosine phosphatase A (MptpA) from *Mycobacterium tuberculosis* allows for better target-specific drug development. *J. Biol. Chem.* 287, 34569–34582
24 Grundner, C. *et al.* (2007) Structural basis for selective inhibition of *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpB. *Structure* 15, 499–509
25 Grundner, C. *et al.* (2005) *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpB structure reveals a diverged fold and a buried active site. *Structure* 13, 1625–1634
26 Mascarello, A. *et al.* (2010) Inhibition of *Mycobacterium tuberculosis* tyrosine phosphatase PtpA by synthetic chalcones: kinetics, molecular modeling, toxicity and effect on growth. *Bioorg. Med. Chem.* 18, 3783–3789
27 Saleh, M.T. and Belisle, J.T. (2000) Secretion of an acid phosphatase (SapM) by *Mycobacterium tuberculosis* that is similar to eukaryotic acid phosphatases. *J. Bacteriol.* 182, 6850–6853
28 Festjens, N. *et al.* (2011) Disruption of the SapM locus in *Mycobacterium bovis* BCG improves its protective efficacy as a vaccine against *M. tuberculosis*. *EMBO Mol. Med.* 3, 222–234
29 Saikolappan, S. *et al.* (2012) The *fbpA*/*sapM* double knock out strain of *Mycobacterium tuberculosis* is highly attenuated and immunogenic in macrophages. *PLoS ONE* 7, e36198
30 Madhurantakam, C. *et al.* (2005) Crystal structure of low-molecular-weight protein tyrosine phosphatase from *Mycobacterium tuberculosis* at 1.9-A resolution. *J. Bacteriol.* 187, 2175–2181

31 Raugei, G. *et al.* (2002) Low molecular weight protein tyrosine phosphatases: small, but smart. *Cell. Mol. Life Sci.* 59, 941–949
32 Ecco, G. *et al.* (2010) *Mycobacterium tuberculosis* tyrosine phosphatase A (PtpA) activity is modulated by S-nitrosylation. *Chem. Commun. (Camb.)* 46, 7501–7503
33 Peterson, M.R. and Emr, S.D. (2001) The class C Vps complex functions at multiple stages of the vacuolar transport pathway. *Traffic* 2, 476–486
34 Rieder, S.E. and Emr, S.D. (1997) A novel RING finger protein complex essential for a late step in protein transport to the yeast vacuole. *Mol. Biol. Cell* 8, 2307–2327
35 Clarke, M. *et al.* (2010) Retrieval of the vacuolar H-ATPase from phagosomes revealed by live cell imaging. *PLoS ONE* 5, e8585
36 Sun-Wada, G. *et al.* (2009) Direct recruitment of H+-ATPase from lysosomes for phagosomal acidification. *J. Cell Sci.* 122, 2504–2513
37 Peplowska, K. *et al.* (2007) The CORVET tethering complex interacts with the yeast Rab5 homolog Vps21 and is involved in endo-lysosomal biogenesis. *Dev. Cell* 12, 739–750
38 Wurmser, A.E. *et al.* (2000) New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. *J. Cell Biol.* 151, 551–562
39 Nickerson, D.P. *et al.* (2009) Vps-C complexes: gatekeepers of endolysosomal traffic. *Curr. Opin. Cell Biol.* 21, 543–551
40 Kinchen, J.M. *et al.* (2008) A pathway for phagosome maturation during engulfment of apoptotic cells. *Nat. Cell Biol.* 10, 556–566
41 Takeda, K. *et al.* (2008) The vacuolar V1/V0-ATPase is involved in the release of the HOPS subunit Vps41 from vacuoles, vacuole fragmentation and fusion. *FEBS Lett.* 582, 1558–1563
42 Peters, C. *et al.* (2001) Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. *Nature* 409, 581–588
43 Beresford, N. *et al.* (2007) MptpB, a virulence factor from *Mycobacterium tuberculosis*, exhibits triple-specificity phosphatase activity. *Biochem. J.* 406, 13–18
44 Saunders, B.M. *et al.* (2000) Interleukin-6 induces early gamma interferon production in the infected lung but is not required for generation of specific immunity to *Mycobacterium tuberculosis* infection. *Infect. Immun.* 68, 3322–3326
45 Roach, S.K. and Schorey, J.S. (2002) Differential regulation of the mitogen-activated protein kinases by pathogenic and nonpathogenic mycobacteria. *Infect. Immun.* 70, 3040–3052
46 Ganguly, N. *et al.* (2007) *Mycobacterium tuberculosis* 6-kDa early secreted antigenic target (ESAT-6) protein downregulates lipopolysaccharide induced c-myc expression by modulating the extracellular signal regulated kinases 1/2. *BMC Immunol.* 8, 24
47 Jung, S.B. *et al.* (2006) The mycobacterial 38-kilodalton glycolipoprotein antigen activates the mitogen-activated protein kinase pathway and release of proinflammatory cytokines through Toll-like receptors 2 and 4 in human monocytes. *Infect. Immun.* 74, 2686–2696
48 Grundner, C. *et al.* (2008) Protein tyrosine phosphatase PtpA is not required for *Mycobacterium tuberculosis* growth in mice. *FEMS Microbiol. Lett.* 287, 181–184
49 Gupta, U.D. and Katoch, V.M. (2005) Animal models of tuberculosis. *Tuberculosis (Edinb.)* 85, 277–293
50 Gil, O. *et al.* (2010) Granuloma encapsulation is a key factor for containing tuberculosis infection in minipigs. *PLoS ONE* 5, e10030
51 Barczak, A.K. and Hung, D.T. (2009) Productive steps toward an antimicrobial targeting virulence. *Curr. Opin. Microbiol.* 12, 490–496
52 Tan, L.P. *et al.* (2009) High-throughput discovery of *Mycobacterium tuberculosis* protein tyrosine phosphatase B (MptpB) inhibitors using click chemistry. *Org. Lett.* 11, 5102–5105
53 Combs, A.P. *et al.* (2005) Structure-based design and discovery of protein tyrosine phosphatase inhibitors incorporating novel isothiazolidinone heterocyclic phosphotyrosine mimetics. *J. Med. Chem.* 48, 6544–6548
54 Chiaradia, L.D. *et al.* (2008) Synthetic chalcones as efficient inhibitors of *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpA. *Bioorg. Med. Chem. Lett.* 18, 6227–6230
55 Manger, M. *et al.* (2005) Discovery of *Mycobacterium tuberculosis* protein tyrosine phosphatase A (MptpA) inhibitors based on natural products and a fragment-based approach. *Chembiochem* 6, 1749–1753
56 Noren-Muller, A. *et al.* (2008) Discovery of a new class of inhibitors of *Mycobacterium tuberculosis* protein tyrosine phosphatase B by biology-oriented synthesis. *Angew. Chem. Int. Ed. Engl.* 47, 5973–5977
57 Soellner, M.B. *et al.* (2007) Fragment-based substrate activity screening method for the identification of potent inhibitors of the *Mycobacterium tuberculosis* phosphatase PtpB. *J. Am. Chem. Soc.* 129, 9613–9615
58 Rawls, K.A. *et al.* (2010) Design and synthesis of nonpeptidic, small molecule inhibitors for the *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpB. *Org. Biomol. Chem.* 8, 4066–4070
59 Chen, L. *et al.* (2010) Identification and characterization of novel inhibitors of mPTPB, an essential virulent phosphatase from *Mycobacterium tuberculosis*. *ACS Med. Chem. Lett.* 1, 355–359
60 Rawls, K.A. *et al.* (2009) Fragment-based discovery of selective inhibitors of the *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpA. *Bioorg. Med. Chem. Lett.* 19, 6851–6854
61 Chiaradia, L.D. *et al.* (2012) Synthesis, biological evaluation, and molecular modeling of chalcone derivatives as potent inhibitors of *Mycobacterium tuberculosis* protein tyrosine phosphatases (PtpA and PtpB). *J. Med. Chem.* 55, 390–402
62 Noren-Muller, A. *et al.* (2006) Discovery of protein phosphatase inhibitor classes by biology-oriented synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10606–10611
63 Vintonyak, V.V. *et al.* (2010) Identification of thiazolidinones spiro-fused to indolin-2-ones as potent and selective inhibitors of the *Mycobacterium tuberculosis* protein tyrosine phosphatase B. *Angew. Chem. Int. Ed. Engl.* 49, 5902–5905
64 Fu, Y. and Galan, J.E. (1999) A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. *Nature* 401, 293–297
65 Humphreys, D. *et al.* (2009) The *Salmonella* effector SptP dephosphorylates host AAA+ ATPase VCP to promote development of its intracellular replicative niche. *Cell Host Microbe* 5, 225–233
66 Boyle, E.C. *et al.* (2006) *Salmonella enterica* serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function. *Cell. Microbiol.* 8, 1946–1957
67 Norris, F.A. *et al.* (1998) SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14057–14059
68 Patel, J.C. and Galan, J.E. (2006) Differential activation and function of Rho GTPases during *Salmonella*-host cell interactions. *J. Cell Biol.* 175, 453–463
69 Zhou, D. *et al.* (2001) A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization. *Mol. Microbiol.* 39, 248–259
70 Kastner, R. *et al.* (2011) LipA, a tyrosine and lipid phosphatase involved in the virulence of *Listeria monocytogenes*. *Infect. Immun.* 79, 2489–2498
71 Niebuhr, K. *et al.* (2002) Conversion of PtdIns(4,5)P(2) into PtdIns(5)P by the *S. flexneri* effector IpgD reorganizes host cell morphology. *EMBO J.* 21, 5069–5078
72 Pendaries, C. *et al.* (2006) PtdIns5P activates the host cell PI3-kinase/Akt pathway during *Shigella flexneri* infection. *EMBO J.* 25, 1024–1034
73 Mohapatra, N.P. *et al.* (2010) *Francisella* acid phosphatases inactivate the NADPH oxidase in human phagocytes. *J. Immunol.* 184, 5141–5150
74 Aragon, V. *et al.* (2001) *Legionella pneumophila* major acid phosphatase and its role in intracellular infection. *Infect. Immun.* 69, 177–185
75 Saha, A.K. *et al.* (1985) Properties of an acid phosphatase from *Legionella micdadei* which blocks superoxide anion production by human neutrophils. *Arch. Biochem. Biophys.* 243, 150–160
76 Hill, J. and Samuel, J.E. (2011) *Coxiella burnetii* acid phosphatase inhibits the release of reactive oxygen intermediates in polymorphonuclear leukocytes. *Infect. Immun.* 79, 414–420
77 Espinosa, A. *et al.* (2003) The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. *Mol. Microbiol.* 49, 377–387

78 DiGiuseppe Champion, P.A. and Cox, J.S. (2007) Protein secretion systems in Mycobacteria. *Cell. Microbiol.* 9, 1376–1384
79 Sullivan, J.T. *et al.* (2012) The *Mycobacterium tuberculosis* SecA2 system subverts phagosome maturation to promote growth in macrophages. *Infect. Immun.* 80, 996–1006
80 Teitelbaum, R. *et al.* (1999) Mycobacterial infection of macrophages results in membrane-permeable phagosomes. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15190–15195
81 Clemens, D.L. *et al.* (2002) The *Mycobacterium tuberculosis* phagosome in human macrophages is isolated from the host cell cytoplasm. *Infect. Immun.* 70, 5800–5807
82 de Jonge, M.I. *et al.* (2007) ESAT-6 from *Mycobacterium tuberculosis* dissociates from its putative chaperone CFP-10 under acidic conditions and exhibits membrane-lysing activity. *J. Bacteriol.* 189, 6028–6034
83 Muller, D. *et al.* (2006) Brunsvicamides A-C: sponge-related cyanobacterial peptides with *Mycobacterium tuberculosis* protein tyrosine phosphatase inhibitory activity. *J. Med. Chem.* 49, 4871–4878
84 Weide, T. *et al.* (2006) 3-Substituted indolizine-1-carbonitrile derivatives as phosphatase inhibitors. *Bioorg. Med. Chem. Lett.* 16, 59–63
85 Cardona, P.J. (2009) A dynamic reinfection hypothesis of latent tuberculosis infection. *Infection* 37, 80–86