crops, improves the seed replacement rate and variety replacement rates among farmers. Horizontal area expansion of oilseed crops in paddy–fallows, potato–fallows, turmeric–fallows, the North East region and command areas as the second crop and intercropping in pigeon pea, sugarcane, sorghum and cotton. Crop diversification in paddy–paddy systems of the southern states with paddy–oilseeds. Encourage the use of micro-irrigation systems in oilseed crops by providing subsidies on micro-irrigation, specifically for oilseed crops.

Dynamic policy support by an effective procurement mechanism at minimum support price for encouraging farmers to cultivate oilseed crops. Appropriate policy interventions to encourage domestic production of oilseeds and discourage imports by imposing quantitative restrictions, dynamic tariffs and reducing the credit period. Setting up of processing facilities in traditional and non-traditional areas to reduce the supply chain length, creating local demand and encouraging local consumption.

Creating awareness among consumers for optimum use of edible oil to maintain per capita consumption at the recommended levels.

This online survey determined oil consumption by the rural and urban Indian households. Total per capita oil consumption was 14.43 kg per annum. Based on the edible oil consumption survey, the NE region consumed less oil (11.09 kg per annum), followed by the north zone (11.19 kg per annum). The south, central, west and east zones consumed 13.85, 12.27, 15.37 and 14.93 kg per annum respectively.

1. Food and Agriculture Organization of the United Nations. FAOSTAT, USA, 2014.
2. Parcell. J., Kojima, Y., Roach, A. and Cain, W., Global edible vegetable oil market trends. *Biomed. J. Sci. Technol. Res.*, 2018, **2**(1), 2282–2291.
3. Statistics at a glance, DES, 2019.
4. Arya, V., Vikash and Kiran, Consumer behaviour with regard to consumption of edible oil in Hisar. *J. Pharmacogn. Phytochem.* 2021, **10**(1), 350–353.
5. Narayanasamy, R. and Ramasamy, M., A study on consumer brand preference on the consumption of cooking oil of various income groups in Chennai. *SSRN Electron. J.*, 2011; http://dx.doi.org/10.2139/ssrn.1894093.
6. Govindaraj, G. N. and Suryaprakash, S., Determinants of edible oil choice by households in Tamil Nadu, India. *Ecol. Food Nutr.*, 2013, **52**(6), 497–514.
7. Özbek, Z. A., Çelik, K. and Ergönül, P. G., Edible oil and fat consumption patterns among Turkish consumers – a case study of Manisa city. *Novel Tech. Nutr. Food Sci.*, 2020, **5**(1), 413–421.
8. Ali, Z., Aslam, M. and Rasool, R., Factors affecting consumption of edible oil in Pakistan. *IOSR-JBM*, 2013, **15**(1), 87–92.

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In silico evidence for extensive Ser/Thr phosphorylation of *Mycobacterium tuberculosis* two-component signalling systems

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***Mycobacterium tuberculosis* has the innate ability to adapt and survive the intracellular environments during infection. Two-component signalling (TCS) systems and serine (Ser)/threonine (Thr) protein kinases facilitate metabolic and growth adaptation by directing transcriptomic reprogramming in response to environmental stimuli. Presently, little is known about the post-translational regulation of TCS proteins through *O*-phosphorylation. Using the NetPhosBac 1.0 *in silico* tool, we screened components of *M. tuberculosis* TCS systems for potential Ser/Thr phosphosites. We report extensive Ser/Thr phosphorylation of sensor kinases and response regulator proteins, suggesting that it might be a distinct mechanism enabling the co-regulation of pathways impacting adaptive changes in mycobacterial growth and metabolism.**

Keywords: *Mycobacterium tuberculosis*, post-translational modification, serine/threonine protein kinase, two-component systems response regulators.

TUBERCULOSIS is a respiratory disease caused by *Mycobacterium tuberculosis*, an intracellular pathogen responsible for about 1.5 million deaths every year[1]. The pathogenic success of *M. tuberculosis* lies in its ability to adapt and survive the changing growth environments during infection. Signal transduction systems are central to this adaptability and are known to play a vital role in mycobacterial pathogenesis, virulence and persistence. There are two major arms of signalling pathways in mycobacteria, namely the traditional two-component signalling (TCS) systems and the 'eukaryotic-like' serine (Ser)/threonine (Thr) protein kinases (STPKs)[2,3] that regulate diverse cellular pathways like cell division, transport, metabolism, persistence and virulence.

Typically, a TCS system comprises a sensory component – histidine sensor kinase (SK), and a response generating component – response regulator (RR)[4]. This paired system facilitates the adaptation and survival of bacteria under different environmental stress conditions like nutrient starvation, hypoxia, nitrosative and oxidative stress (reviewed in Brett *et al.*[5]). The environmental stimulus is detected by the N-terminal variable 'input domain' of SK, which leads to

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Table 1. *Mycobacterium tuberculosis* Ser/Thr protein kinases (STPKs) and two-component signalling systems (TCS)

	Gene	Known environmental activating signal[a]
STPK		
PknA	*Rv0015c*	ND
PknB	*Rv0014c*	Oxygen-dependent replication
PknD	*Rv0931c*	Osmotic stress
PknE	*Rv1743*	Nitric oxide stress
PknF	*Rv1746*	ND
PknG	*Rv0410c*	Nutritional stress (glutamine/glutamate levels)
PknH	*Rv1266c*	Nitric oxide stress
PknI	*Rv2914c*	Low pH associated with low oxygen availability
PknJ	*Rv2088*	*In vivo* concentrations of Ni^{2+} and Co^{2+}
PknK	*Rv3080c*	Stationary phase stress
PknL	*Rv2176*	ND
TCS		
PhoP/PhoR	*Rv0757*/*Rv0758*	Acid induction
NarL/NarS	*Rv0844c*/*Rv0845*	Nitrate
PrrA/PrrB	*Rv0903c*/*Rv0902c*	Nitrogen limitation, macrophage infection
MprA/MprB	*Rv0981*/*Rv0982*	Detergent, alkaline pH, Triton X-100, nutrient limitation
KdpE/KdpD	*Rv1027c*/*Rv1028c*	K^+ limitation, osmotic stress, turgor pressure
TrcR/TrcS	*Rv1033c*/*Rv1032c*	ND
MtrA/MtrB	*Rv3246c*/*Rv3245c*	ND
TcrX/TcrY	*Rv3765c*/*Rv3764c*	Starvation, low iron
PdtaR/PdtaS	*Rv1626*/*Rv3220c*	Nutrient limitation
RegX3/SenX3	*Rv0491*/*Rv0490*	Phosphate starvation
DevR/DevS, DosT	*Rv3133c*/*Rv3132c*, *Rv2027c*	Hypoxia, nitrate, nitric oxide, carbon monoxide, vitamin C
TcrA/SK1, SK2	*Rv0602c*/*Rv0600c*, *Rv0601c*	Antibiotic stress
Orphan SK/RR		
OSK	*Rv2027c*	
ORR	*Rv0195*	
ORR	*Rv0260c*	
ORR	*Rv0818*	
ORR	*Rv2884*	
ORR	*Rv3143*	

[a]Data compiled from refs 5, 13, 26, 29–37. ND, No data.

phosphorylation of the histidine residue in the autocatalytic kinase domain. SK phosphorylates a conserved aspartate residue in the 'receiver domain' of RR through a phosphotransfer reaction, which in turn modulates the DNA binding ability of the variable 'output domain' of RR. *M. tuberculosis* has 12 completely paired two-component regulatory systems including five orphan regulators and one orphan histidine kinase (Table 1). Among these, multiple TCS systems are known to be involved in virulence[6] and two have been shown to be essential for mycobacterial survival[7,8].

Post-translational modification through Ser/Thr/Tyr phosphorylation, also known as *O*-phosphorylation, is a key regulatory mechanism ubiquitous to living organisms. Phosphorylation of proteins inactivates or activates them, impacting their cellular functions or the downstream regulatory pathways. In comparison to eukaryotic organisms, few prokaryotes, including bacteria, also have STPKs that stimulate a wide variety of signalling networks[3]. The STPKs integrate many cellular or extracellular signals by reversible phosphorylation and dephosphorylation of proteins. In *M. tuberculosis*, there are 11 STPKs, viz. PknA–B, PknD–L that regulate metabolic homeostasis, transportation, transcription, cell growth and division (Table 1)[9–14].

Typically, the paired TCS system is specific since SKs have a kinetic preference for their cognate RRs. This intrinsic preference helps SKs to differentiate their cognate RRs from all other likely substrates. However, recently it has been shown that the absence of either RR or SK generally does not eliminate the associated response of the TCS system, suggesting the possibility of crosstalk amongst TCS proteins *in vivo*[15]. Novel interactions between an SK and a non-cognate RR and between different DNA-binding RR proteins to form heterodimers have been shown to help coregulate the downstream expression of regulon genes[16].

Post-translational regulation via *O*-phosphorylation of TCS proteins is an emerging paradigm of the signalling mechanisms in mycobacteria. Convergence of PknB and RegX3–SenX3 signalling pathways – integrating two different signals, i.e. phosphate limitation and replication state of *M. tuberculosis*[17], suggests that such cross-interactions ultimately lead to a more efficient gene expression and balanced coordination within the cell. The DevR RR of the DevRS two-component system known to play a role in the hypoxic adaptation of mycobacteria[18] is regulated by its cognate SK along with PknB[19] and PknH[20] kinases. Recent findings from our laboratory established PknK, a cytosolic STPK, as the nodal point connecting atypical signalling pathways[21,22].

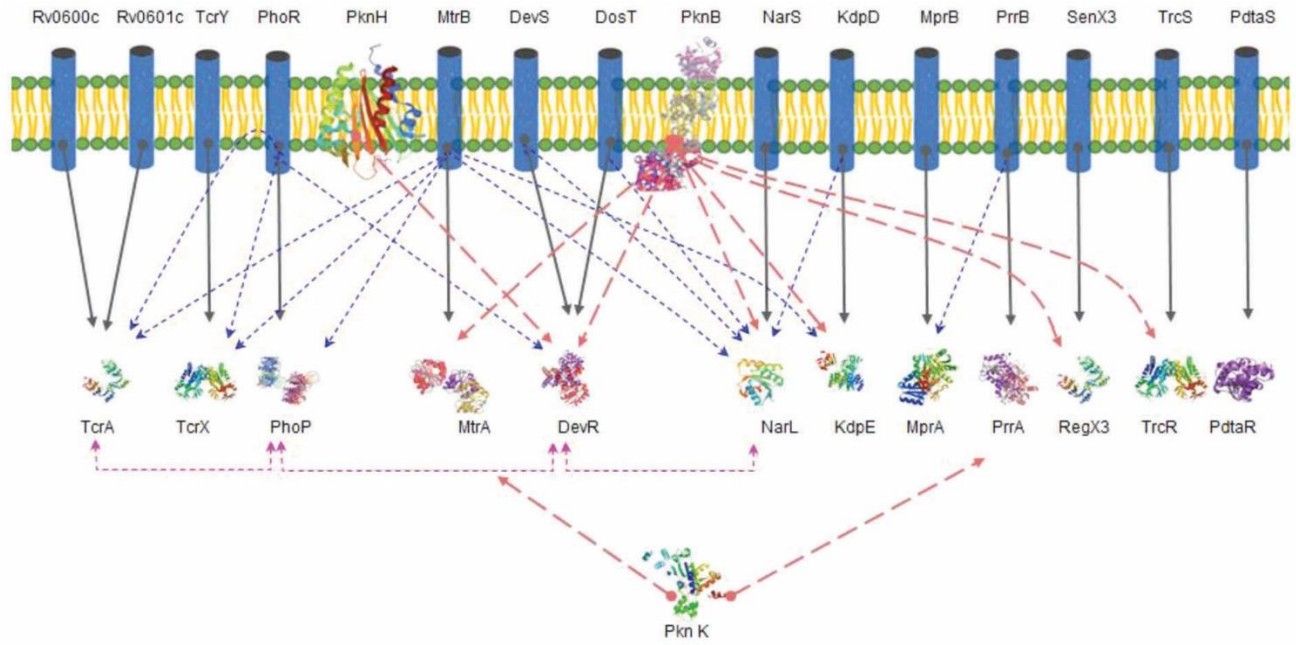

Figure 1. A signalling interactome of *Mycobacterium tuberculosis* two-component signalling (TCS) systems and Ser/Thr protein kinases (STPK) proteins. Cartoon illustrating previously reported interactions between STPKs and TCS components[16,19,20,31,38–40]. The TCS system sensor kinases (SKs) along with STPKs PknB and PknH are shown as membrane receptor kinases, while the response regulators (RRs) are shown as cytosolic proteins. STPK PknK being a soluble kinase is shown as a cytosolic protein. Black solid arrows indicate SK–RR cognate pairs, blue dotted arrows indicate SK–RR non-cognate pairs, pink dotted arrow indicate RR–RR and orange arrows indicate STPK–RR interactions. The 3D structures of specific proteins were obtained from the STRINGS database[41].

Ser/Thr phosphorylation of two essential RRs, viz. MtrA and PrrA by PknK underscore the significance of such interactions increasing the complexity of the underlying regulatory mechanisms.

From an evolutionary point of view, cross-interactions between two distinctively different signalling pathways resulting in multiple intricate and often overlapping regulatory circuits may be responsible for the robust survival fitness of microorganisms. Four main types of interactions have been reported, namely SK–RR (cognate pairs), SK–RR (non-cognate pairs), RR–RR (heterodimers) and STPK-RR interactions[15]. So far, *M. tuberculosis* STPKs PknB and PknH have been shown to phosphorylate multiple RR proteins[19,20], with PknK being a recent addition to the list. However, there is no information on Ser/Thr phosphorylation of TCS sensor kinases (STPK–SK interaction) to date.

Figure 1 shows a compilation of all experimentally validated interactions of TCS systems and STPKs in *M. tuberculosis.* It is noteworthy that out of the 12 paired TCS systems, PdtaS–PdtaR, SenX3–RegX3 and TrcS–TrcR systems are seen to be completely specific and do not interact with any pair other than their cognate partners[15,16]. This, however, does not exclude the possibility of these TCS systems being modified by Ser/Thr phosphorylation, thereby allowing distinctly separate control of the regulatory pathway.

In this study, we screened 31 TCS components listed in Table 1 for potential Ser/Thr phosphorylation sites using NetPhosBac 1.0 (ref. 23). This tool is based on an artificial neural networking model that predicts bacteria-specific *O*-phosphorylation. The web address (http://www.cbs.dtu.dk/services/NetPhosBac/) hosting NetPhosBac 1.0 was used for the identification of bacteria-specific putative Ser/Thr phosphorylation sites[23]. It should be noted that NetPhosBac 1.0 takes into account the full-length of the protein irrespective of any specific domain regions, such as transmembrane regions in its analysis, and only reports phosphosites with scores >0.5.

In addition to the phosphorylation sites predicted by NetPhosBac 1.0, we scored the extent of phosphorylation for any RR or SK protein in terms of the percentage number of Ser or Thr residues that can be potentially phosphorylated. In our analysis, we found that both SK and RR proteins are susceptible to extensive STPK-mediated phosphorylation, in addition to their canonical phosphorylation sites on histidine and aspartate residues respectively. The complete data from the NetPhosBac 1.0 server are provided in Supplementary Table 1.

As shown in Table 2, NetPhosBac 1.0 predicted Ser/Thr phosphosites for all the TCS proteins analysed. While the number of phosphosites varied, the propensity of serine versus threonine sites also varied, with phosphorylation on the former being more prevalent than the latter. Remarkably, KdpD sensor kinase showed the highest number of predicted phosphorylation sites. A large percentage of the serine residues in KdpD (55%) and MtrB (52%) SKs can be potentially phosphorylated by STPKs. The sensor kinase Rv0601c showed the least number of Ser/Thr phosphorylation sites. Amongst the RR proteins, TcrA had the highest percentage

Table 2. Predicted Ser/Thr phosphorylation in sensor kinases (SKs) and response regulators (RRs) of TCS systems

	Gene	Total no. of Ser and Thr residues	Number of potential Ser/Thr phosphorylations	Percentage Ser phosphorylation[a]
SK				
PhoR	*Rv0758*	S-35; T-29	S-16; T-1	S ++
NarS	*Rv0845*	S-25; T-23	S-10; T-1	S ++
PrrB	*Rv0902c*	S-30; T-28	S-10; T-2	S ++
MprB	*Rv0982*	S-39; T-24	S-10; T-2	S ++
KdpD	*Rv1028c*	S-40; T-56	S-22; T-3	S +++
TrcS	*Rv1032c*	S-36; T-38	S-14; T-5	S ++
MtrB	*Rv3245c*	S-38; T-34	S-18; T-1	S +++
TcrY	*Rv3764c*	S-33; T-36	S-12; T-2	S ++
PdtaS	*Rv3220c*	S-32; T-25	S-13; T-4	S ++
SenX3	*Rv0490*	S-30; T-20	S-10; T-2	S ++
DevS	*Rv3132c*	S-26; T-33	S-11; T-1	S ++
DosT[b]	*Rv2027c*	S-25; T-30	S-9; T-3	S ++
SK1	*Rv0600c*	S-6; T-18	S-1; T-1	S +
SK2	*Rv0601c*	S-3; T-18	S-1	S ++
RR				
PhoP	*Rv0757*	S-9; T-17	S-3; T-3	S ++
NarL	*Rv0844c*	S-12; T-5	S-7; T-1	S +++
PrrA	*Rv0903c*	S-14; T-13	S-7; T-2	S ++
MprA	*Rv0981*	S-12; T-12	S-7; T-2	S +++
KdpE	*Rv1027c*	S-8; T-15	S-4; T-2	S ++
TrcR	*Rv1033c*	S-17; T-17	S-9	S +++
MtrA	*Rv3246c*	S-5; T-14	S-1; T-1	S +
TcrX	*Rv3765c*	S-15; T-12	S-7	S ++
PdtaR	*Rv1626*	S-7; T-14	S-2	S ++
RegX3	*Rv0491*	S-13; T-12	S-7; T-1	S +++
DevR	*Rv3133c*	S-10; T- 8	S-4	S ++
TcrA	*Rv0602c*	S-6; T-16	S-5; T-2	S ++++
Rv0195[c]	*Rv0195*	S-12; T-15	S-9; T-2	S +++
Rv0260c[c]	*Rv0260c*	S-26; T-18	S-10; T-3	S ++
Rv0818[c]	*Rv0818*	S-15; T-12	S-5; T-3	S ++
Rv2884[c]	*Rv2884*	S-10; T-17	S-6; T-2	S +++
Rv3143[c]	*Rv3143*	S-7; T-9	S-4	S +++

[a]Percentage of Ser phosphorylation based on the number of potential phosphosites as predicted by the NetPhosBac 1.0 server. Percentage of putative Ser phosphorylation is denoted as follows: + (0–25%), ++ (25–50%), +++ (50–75%) and ++++ (75–100%).
[b]Orphan histidine kinase. [c]Orphan response regulator.

of serine phosphorylation. About 83% of the total number of serine sites in TcrA were potentially amenable for phosphorylation, closely followed by orphan RRs, Rv0195 (~75%) and Rv2884 (~60%) (Table 2).

The results obtained with NetPhosBac 1.0 were compared with recently published data[24], wherein the authors have reported widespread *O*-phosphorylation in *M. tuberculosis* H37Rv strain. We found a distinct overlap between our observations and the data reported by Frando *et al.*[24]. Except for PhoR sensor kinase, there were common phosphosites for all TCS proteins, thus providing validating the *in silico* predictions (Supplementary Table 1). Among all the other experimentally validated phosphosites reported so far in RegX3 (ref. 17), DevR[20] and PrrA[22] RRs, only one phosphosite, Thr151 in RegX3 RR (ref. 17), having a score of 0.621 was common with our analysis. Incidentally, most of the published Ser/Thr phosphosites have low scores and thus, were not identified by NetPhosBac 1.0. Being a prediction tool, these differences are conceivable; however, they cannot undermine the implication of widespread Ser/Thr phosphorylation scored by NetPhosBac 1.0 across all *M. tuberculosis* TCS systems.

Notably, the MtrB sensor kinase exhibits cross-interactions not only with multiple non-cognate RRs[15,16], but was also susceptible to STPK-mediated Ser/Thr phosphorylation. It is not surprising that the MtrAB signalling pathway, known to be essential for mycobacterial growth and cell division[7], is subjected to multiple layers of regulation. SK1–SK2–TcrA forms a three-component system[25]; however, its function is not yet defined. Our analysis revealed that while SK1 and SK2 showed the least number of Ser/Thr phosphorylation sites, TcrA presented most of its Ser/Thr residues as potential sites for phosphorylation. It is possible that the lack of dual phosphorylation of SK1 and SK2 is compensated by *O*-phosphorylation of the TcrA RR. Furthermore, it is interesting to note that TcrA RR is

also a target of MtrB[16], which itself is subjected to regulation by dual phosphorylation. Since SKs and RRs have specific functional domains, such as the histidine kinase and DNA-binding motifs, we mapped the predicted phosphosites onto these domains (data not shown). While no specific pattern was observed in the localization of Ser/Thr phosphosites in RR proteins, most of them were mapped in the histidine kinase domain of the sensor kinases. These observations suggest that STPK-mediated phosphorylation of TCS proteins may play an important role in signal sensing, integration and transduction.

Although the functional significance of these interactions is unknown and warrants further experimentation, it is clear that post-translational modification of both SKs and RR proteins must be taken into account in order to fully understand the dynamics of a signalling pathway. This study triggers several questions. For example, under what conditions does the non-canonical Ser/Thr phosphorylation of TCS systems dominate the traditional His/Asp phosphorylation and does it contribute to the promiscuity observed within the TCS systems? Since 9 out 11 STPKs are membrane receptor kinases, the feasibility of STPK-mediated modification of membrane histidine kinases brings forth the issue about spatial organization of these proteins. It should be noted that the two *M. tuberculosis* STPKs namely PknG and PknK are soluble, cytosolic proteins[13,26,27], with PknK being also associated with the cell wall[13]. It is possible that cytosolic STPKs may be part of a larger scaffold assembly that facilitates protein–protein interactions and post-translational modifications of SKs. Indeed, data suggest that PknK mediates ~86% phosphorylation of RR proteins[24]. Generally, cross-interactions of TCS proteins are considered to be restricted to the TCS systems only. However, recently, it has been shown that DevS SK of the DevR/DevS two-component system can phosphorylate non-TCS proteins[28]. This highlights the possibility of signal transduction through two-component system SKs outside the domain of TCS components. In such a scenario, it will be particularly interesting to determine if STPK-mediated regulation of two-component system SKs is a contributory factor.

Our findings indicate that signalling pathways are best studied in composite rather than in isolation and pave the way for deeper investigations to reveal novel mechanisms for regulating mycobacterial gene expression. In all likelihood, these cross-interactions may well account for the survival fitness of this intracellular pathogen. We envision that uncovering these non-traditional regulatory circuits will facilitate the development of newer therapeutic strategies to combat tuberculosis.

1. Adigun, R. and Singh, R., Tuberculosis. In *StatPearls*, StatPearls Publishing, Treasure Island, Florida, USA, 2021; https://www.ncbi.nlm.nih.gov/books/NBK441916/
2. Cole, S. T. *et al*., Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature*, 1998, **393**(6685), 537–544.
3. Av-Gay, Y. and Everett, M., The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. *Trends Microbiol.*, 2000, **8**, 238–244.
4. Nixon, B. T., Ronson, C. W. and Ausubel, F. M., Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 7850–7854.
5. Bretl, D. J., Demetriadou, C. and Zahrt, T. C., Adaptation to environmental stimuli within the host: two-component signal transduction systems of *Mycobacterium tuberculosis*. *Microbiol. Mol. Biol. Rev.*, 2011, **75**, 566–582.
6. Parish, T., Smith, D. A., Kendall, S., Casali, N., Bancroft, G. J. and Stoker, N. G., Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*. *Infect. Immunol.*, 2003, **71**, 1134–1140.
7. Zahrt, T. C. and Deretic, V., An essential two-component signal transduction system in *Mycobacterium tuberculosis*. *J. Bacteriol*., 2000, **182**, 3832–3838.
8. Haydel, S. E., Malhotra, V., Cornelison, G. L. and Clark-Curtiss, J. E., The prrAB two-component system is essential for *Mycobacterium tuberculosis* viability and is induced under nitrogen-limiting conditions. *J. Bacteriol.*, 2012, **194**, 354–361.
9. Fernandez, P. *et al*., The Ser/Thr protein kinase PknB is essential for sustaining mycobacterial growth. *J. Bacteriol.*, 2006, **188**, 7778–7784.
10. Kang, C.-M., Abbott, D. W., Park, S. T., Dascher, C. C., Cantley, L. C. and Husson, R. N., The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. *Genes Dev.*, 2005, **19**, 1692–1704.
11. Gopalaswamy, R., Narayanan, S., Chen, B., Jacobs, W. R. and Av-Gay, Y., The serine/threonine protein kinase PknI controls the growth of *Mycobacterium tuberculosis* upon infection. *FEMS Microbiol. Lett.*, 2009, **295**, 23–29.
12. Jang, J. *et al.*, Functional characterization of the *Mycobacterium tuberculosis* serine/threonine kinase PknJ. *Microbiology*, 2021, **156**, 1619–1631.
13. Malhotra, V., Arteaga-Cortés, L. T., Clay, G. and Clark-Curtiss, J. E., *Mycobacterium tuberculosis* protein kinase K confers survival advantage during early infection in mice and regulates growth in culture and during persistent infection: implications for immune modulation. *Microbiology (Reading)*, 2010, **156**, 2829–2841.
14. Papavinasasundaram, K. G., Chan, B., Chung, J.-H., Colston, M. J., Davis, E. O. and Av-Gay, Y., Deletion of the *Mycobacterium tuberculosis* pknH gene confers a higher bacillary load during the chronic phase of infection in BALB/c mice. *J. Bacteriol.*, 2005, **187**(16), 5751–5760.
15. Agrawal, R., Sahoo, B. K. and Saini, D. K., Cross-talk and specificity in two-component signal transduction pathways. *Future Microbiol.*, 2016, **11**, 685–697.
16. Agrawal, R., Pandey, A., Rajankar, M. P., Dixit, N. M. and Saini, D. K., The two-component signalling networks of *Mycobacterium tuberculosis* display extensive cross-talk *in vitro*. *Biochem. J.*, 2015, **469**, 121–134.
17. Park, E.-J., Kwon, Y.-M., Lee, J.-W., Kang, H.-Y. and Oh, J.-I., Dual control of RegX3 transcriptional activity by SenX3 and PknB. *J. Biol. Chem.*, 2019, **294**, 11023–11034.
18. Saini, D. K., Malhotra, V., Dey, D., Pant, N., Das, T. K. and Tyagi, J. S., DevR–DevS is a bona fide two-component system of *Mycobacterium tuberculosis* that is hypoxia-responsive in the absence of the DNA-binding domain of DevR. *Microbiology (Reading)*, 2004, **150**, 865–875.
19. Bae, H.-J. *et al*., Inhibition of the DevSR two-component system by overexpression of *Mycobacterium tuberculosis* PknB in *Mycobacterium smegmatis*. *Mol. Cells*, 2017, **40**, 632–642.
20. Chao, J. D. *et al*., Convergence of Ser/Thr and two-component signaling to coordinate expression of the dormancy regulon in *Mycobacterium tuberculosis**[S]. *J. Biol. Chem.*, 2010, **285**, 29239–29246.

21. Malhotra, V. *et al*., *Mycobacterium tuberculosis* PknK substrate profiling reveals essential transcription terminator protein rho and two-component response regulators PrrA and MtrA as novel targets for phosphorylation. *Microbiol. Spectr.*, 2022, **10**, e0135421.
22. Mishra, A. K., Yabaji, S. M., Dubey, R. K., Dhamija, E. and Srivastava, K. K., Dual phosphorylation in response regulator protein PrrA is crucial for intracellular survival of mycobacteria consequent upon transcriptional activation. *Biochem. J.*, 2017, **474**, 4119–4136.
23. Miller, M. L., Soufi, B., Jers, C., Blom, N., Macek, B. and Mijakovic, I., NetPhosBac – a predictor for Ser/Thr phosphorylation sites in bacterial proteins. *Proteomics*, 2009, **9**, 116–125.
24. Frando, A. *et al*., The *Mycobacterium tuberculosis* protein *O*-phosphorylation landscape (preprint). *Microbiology*, 2022.
25. Shrivastava, R., Das, D. R., Wiker, H. G. and Das, A. K., Functional insights from the molecular modelling of a novel two-component system. *Biochem. Biophys. Res. Commun.*, 2006, **344**, 1327–1333.
26. Cowley, S. *et al*., The *Mycobacterium tuberculosis* protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth *in vivo*. *Mol. Microbiol.*, 2004, **52**, 1691–1702.
27. Kumar, P. *et al*., The *Mycobacterium tuberculosis* protein kinase K modulates activation of transcription from the promoter of mycobacterial monooxygenase operon through phosphorylation of the transcriptional regulator VirS. *J. Biol. Chem.*, 2009, **284**, 11090–11099.
28. Gautam, U. S. *et al*., *Mycobacterium tuberculosis* sensor kinase DosS modulates the autophagosome in a DosR-independent manner. *Commun. Biol.*, 2019, **2**, 349.
29. Kundu, M., The role of two-component systems in the physiology of *Mycobacterium tuberculosis*. *IUBMB Life*, 2018, **70**, 710–717.
30. Li, X. *et al.*, Role of two-component regulatory systems in intracellular survival of *Mycobacterium tuberculosis*. *J. Cell. Biochem.*, 2019, **120**, 12197–12207.
31. Malhotra, V., Agrawal, R., Duncan, T. R., Saini, Deepak, K. and Clark-Curtiss, J. E., *Mycobacterium tuberculosis* response regulators, DevR and NarL, interact *in vivo* and co-regulate gene expression during aerobic nitrate metabolism. *J. Biol. Chem.*, 2015, **290**, 8294–8309.
32. Jang, J. *et al.*, Functional characterization of the *Mycobacterium tuberculosis* serine/threonine kinase PknJ. *Microbiology (Reading)*, 2010, **156**, 1619–1631.
33. Chakraborti, P. K., Matange, N., Nandicoori, V. K., Singh, Y., Tyagi, J. S. and Visweswariah, S. S., Signalling mechanisms in Mycobacteria. *Tuberculosis*, 2011, **91**, 432–440.
34. Bhattacharya, M., Biswas, A. and Das, A. K., Interaction analysis of TcrX/Y two component system from *Mycobacterium tuberculosis*. *Biochimie*, 2010, **92**, 263–272.
35. Hatzios, S. K. *et al.*, Osmosensory signaling in *Mycobacterium tuberculosis* mediated by a eukaryotic-like Ser/Thr protein kinase. *Proc. Natl. Acad. Sci. USA*, 2013, **110**, E5069–E5077.
36. Hariharan, V. N. *et al*., Cyclic di-GMP sensing histidine kinase PdtaS controls mycobacterial adaptation to carbon sources. *FASEB J.*, 2021, **35**, e21475.
37. Xu, Y., You, D. and Ye, B.-C., RegX3 controls glyoxylate shunt and Mycobacteria survival by directly regulating the transcription of isocitrate lyase gene in *Mycobacterium smegmatis*. *ACS Infect. Dis.*, 2021, **7**, 927–936.
38. Singh, K. K. *et al.*, Acetylation of response regulator proteins, TcrX and MtrA in *M. tuberculosis* tunes their phosphotransfer ability and modulates two-component signaling crosstalk. *J. Mol. Biol.*, 2019, **431**, 777–793.
39. Lee, H.-N., Jung, K.-E., Ko, I.-J., Baik, H. S. and Oh, J.-I., Protein–protein interactions between histidine kinases and response regulators of *Mycobacterium tuberculosis* H37Rv. *J. Microbiol.*, 2012, **50**, 270–277.
40. Vashist, A., Malhotra, V., Sharma, G., Tyagi, J. S. and Clark-Curtiss, J. E., Interplay of PhoP and DevR response regulators defines expression of the dormancy regulon in virulent *Mycobacterium tuberculosis*. *J. Biol. Chem.*, 2018, **293**, 16413–16425.
41. Szklarczyk, D. *et al*., STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.*, 2015, **43**, D447–D452.

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