Supplementary information

Metabolic switching of *Mycobacterium tuberculosis* during hypoxia is controlled by the virulence regulator PhoP

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Running title: Metabolic switching in mycobacteria

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Key Words: Hypoxia regulator; metabolic switching; *M. tuberculosis* PhoP; Protein - protein interactions; virulence regulator

Table S1

Oligonucleotide primers used in RT-qPCR and ChIP experiments of this study

Primers[a]	Sequence or description (5'-3')	Reference
FPnarK1RT	ATCGTGTTGCTAATTCCGGC	This study
RPnarK1RT	TTGCCAACAACCCAACTAGC	This study
FPnirBRT	GTCAGCGGATCGACTTGTTC	This study
RPnirBRT	GCTTTATTTTGTGCGGTGCC	This study
FPnarGRT	TCGAGCATCTTAAGCCGTTT	This study
RPnarGRT	TCGAGTGAAACGACCACTTG	This study
FPglnRRT	GAGCTGCTGAAATACCTGGC	This study
RPglnRRT	AGCTTTGTATCCGACGTTGC	This study
FPdosRRT	CTATCAGGCCTTACCGACCA	This study
RPdosRRT	AACCGCGACACGTAGTTCTT	This study
FPnarK2RT	GTGACCTGGGAGATGTCGTT	This study
RPnarK2RT	AGAACCCGTAGATCGTGGTG	This study
FPhspXRT	CGACAAGGACGTCGACATTA	This study
RPhspXRT	CCTTGTCGTAGGTGGCCTTA	This study
FPnarK1	GTACTCGAGCACAATAGCTTTC	This study
RPnarK1	CGAAGGGGCCGCGGGACTGC	This study
FPnirB	TGGTTATCTCCTCATGCTTCGT	This study
RPnirB	GCCGACCACGACGATCTCGCGAGCCG	This study
FPnarG	ACGGTGTGGTTGACGGTGGCC	This study
RPnarG	GCGCCCGCTGCGCTCCAGCAG	This study
FPglnR	CGAAGGGGCCGCGGGACTGC	This study
RPglnR	GGCAGGACCGGATCCGGATA	This study
FPdosR	AATAATGGATCCGTCCACAACCAT	This study
RPdosR	AATAATGGTACCTGGGCGGGCAG	This study
FPnarK2	CCCTTTCCAGTGGCGACCAGGCT	This study
RPnarK2	CCAGAAGTTGACCACCGAGATCC	This study
FPhspX	TGATCAACCTCCGCTGTTCGAT	This study
RPhspX	CTCAGAAAACTCGGGGAAGA	This study
FP16SrDNA	CTGAGATACGGCCCAGAGCTC	This study
RP16SrDNA	CGTCGATGGTGAAAGAGGTT	This study
FPpks2	GTTGTGGAAGGCGTTGTTAC	This study
RPpks2	GTCGTAGAACTCGTCGCAAT	This study

[a]FP, forward primer; RP, reverse primer

Table S2

Oligonucleotide primers used for cloning and amplifications in this study

Primers		
[a]FPdosRstart	AATAATCATATGATGGGAAGCGCCGA	This study
RPdosRstop	AATAATGGATCCTCATCGAGCACCCA	This study
FPhrcAstart	AATAATCATATGATGGGAAGCGCCGA	This study
RPhrcAstop	AATAATGGATCCTCATCGAGCACCCA	This study
RPdosR-N	AATAATGGATCCATGGGAAGCGCC	This study
FPdosR-C	AATAATTCACTTGTCGTCATCGTCTTTGTAGT CTCGAGCACCCAG	This study
FPphoPD71N	GTGATCCTCAACGTGATGATGCCC	(1)
RPphoPD71N	CATCATCACGTTGAGGATCACCGC	(1)
FPdosR54E	GTCGCGGTGCTGGAGGTCCGGTT	This study
RPdosR54E	AACCGGACCTCCAGCACCGCGAC	This study
FPkidosR	AATAATCATATGATGGCGAAGAAC	This study
RPkidosR	AATAATGGATCCCCGGCGCGGTT	This study
FPphoPstart	AATAATGGATCCATGCGGAAAGGGGT	This study
RPphoPFLAG	AATAAGCTTTCACTTGTCGTCATCGTCTTTGT AGTCTCGAGGCTCCCGCA	This study
[b]FPdosRLHS	CACCTTTTCCATAAATTGGGATATCCCGCAG GTCTGACCGCCGCG	This study
RPdosRLHS	TTTTTTTTCCATTTCTTGGCTCAAAGCGACGC TCGTCTC	This study
FPdosRRHS	CACCTTTTCCATAGATTGGTATATCGGCGACG TCCTGGG	This study
RPdosRRHS	TTTTTTTTCCATCTTTTGGGATATCATCACCG AACCTGGCTGCGA	This study
FPmdosR	AATAATGGATCCATGGGAAGCGCC	This study
RPmdosR	AATAATCTGCAGTCATCGAGCACC	This study
FPmglnR	AATGGATCCATGCCTACGGGCCCCACG	This study
RPmglnR	AATAAGCTTTCACTTGCAACGGTTTAG	This study
FPdosRup	TGCCGCCCTTGACCCGT	This study
RPdosRup	GGTAAGAACGCGTAGTCCA	This study
FPdosRupmut	CGTACTACGATTTCATTACGCTGGTTCGGC	This study
RPdosRupmut	TAATGAAATCGTAGTACGATCGCTTGACCC	This study

[a]FP, forward primer; RP, reverse primer; [b]LHS, left hand sequence; RHS, right hand sequence

Table S3

Plasmids used in this study

Plasmids	Relevant genotype or sequence (5'-3')	Source or Reference
pET28b	*E. coli* cloning vector, Amp^{r} [a]	Novagen
pET-*dosR*	DosR residues 1–217 cloned in pET28b	This study
pET-*dosR-N*	DosR residues 1-193 cloned in pET28b	This study
pET-*dosR-C*	DosR residues 143-217 cloned in pET28b	This study
pET-*phoP-N*	PhoP residues 1-141 cloned in pET15b	(2)
pET-*phoP-C1*	PhoP residues 141-247 cloned in pET15b	(2)
pET-*phoP-C2*	PhoP residues 150-247 cloned in pET15b	(2)
pGEX-4T-1	*E. coli* cloning vector, Amp^{r} [a]	GE-Healthcare
pGEX-*dosR*	DosR residues 1–217 cloned in pGEXT-4T-1	This study
p19Kpro	Mycobacterial expression vector, Hyg^{r} [b]	(3)
p19Kpro-*phoP*	PhoP residues 1–247 cloned in p19kpro	(4)
p19Kpro-*dosR*	DosR residues 1–217 cloned in p19kpro	This study
pST-Ki	Integrative mycobacterial expression vector, Kan^{r} [c]	(5)
pST-Ki-*dosR*	DosR residues 1–217 cloned in pST-Ki	This study
pSM128	Integrative promoter probe vector for mycobacteria [d]	(6)
pSM-dosRup	dosRup3-*lacZ* fusion in pSM128	This work
pSM-dosRupmut	PhoP binding site mutated in pSM-dosRup	This work
pME1mL1	Mycobacterial protein expression vector [b]	(7)
pME1mL1-*phoP*	PhoP residues 1-247 cloned in pME1mL1	(8)
pUAB300[b]	Episomal mycobacteria-*E. coli* shuttle plasmid	(9)
pUAB300-*dosR*	DosR residues 1–217 cloned in pUAB300	This study
pUAB300-*dosR-N*	DosR residues 1–193 cloned in pUAB300	This study
pUAB300-*dosR-C*	DosR residues 143–217 cloned in pUAB300	This study
pUAB300-*dosRD54E*	Asp54 mutated to Glu in *dosR* of pUAB300-*dosR*	This study
pUAB400[c]	Integrative mycobacteria-*E. coli* shuttle plasmid	(9)
pUAB400-*phoP*	PhoP residues 1–247 cloned in pUAB400	(10)
pUAB400-*phoP-N*	PhoP residues 1–141cloned in pUAB400	This study
pUAB400-*phoP-C*	PhoP residues 141–247 cloned in pUAB400	This study
pUAB400-*phoPD71N*	Asp71 mutated to Asn in *phoP* of pUAB400-*phoP*	This study
pUAB300-*glnR*	GlnR residues 1-252 cloned in pUAB300	This study

[a] ampicillin resistance; [b] hygromycin resistance; [c] kanamycin resistance; [d] streptomycin resistance

Figure S1

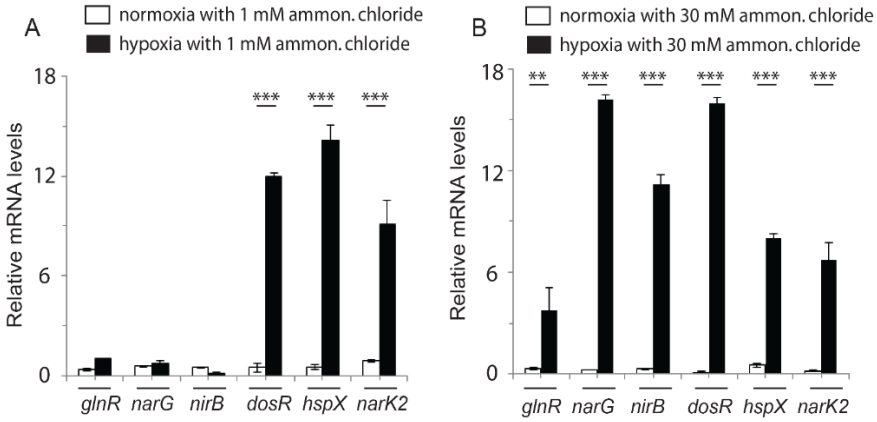

Fig. S1: Regulation of hypoxia-inducible mycobacterial genes. (A-B) RT-qPCR was carried out to compare expression levels of indicated hypoxia-inducible genes in the WT bacilli under specific conditions of growth (normoxic or hypoxic) coupled with either limiting nitrogen (1 mM ammonium chloride) or surplus nitrogen (30 mM ammonium chloride) conditions. The difference of mRNA levels with standard deviations were determined from at least three independent RNA preparations (***P*<0.01; ****P*<0.001).

Figure S2

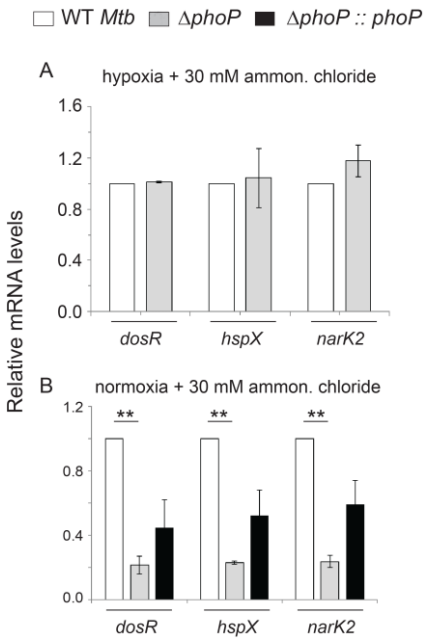

Fig. S2: Regulation of hypoxia-inducible Mtb genes by the *phoP* locus under indicated growth conditions. (A-B) RT-qPCR was carried out to compare relative expression of hypoxia-inducible genes in the WT, *ΔphoP* and the complemented mutant under hypoxia and normoxia, respectively, coupled with surplus (30 mM) ammonium chloride. The results show average mRNA levels of hypoxia-inducible genes with standard deviations derived from replicate experiments using at least 3 independent RNA preparations.

Figure S3:

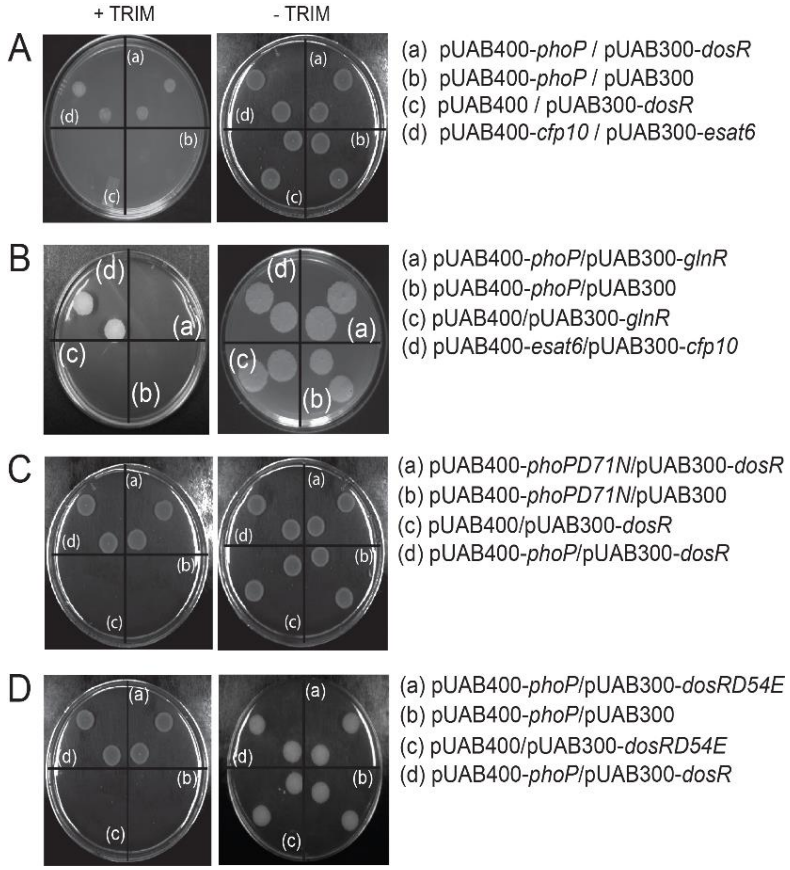

Fig. S3: M-PFC experiments examined (A) PhoP-DosR, and (B) PhoP-GlnR interactions in *M. smegmatis* as described in the Methods. Growth of transformants on 7H11/Kan/Hyg in presence of TRIM suggests *in vivo* protein-protein association between the regulator pairs. Co-expression of empty vectors and *esat6*/*cfp10* pair, were included as negative and positive control, respectively. (C-D) To examine the effect of phosphorylation of regulators on PhoP-DosR interactions, M-PFC experiments were carried out using phosphorylation defective mutants of PhoP and DosR, respectively. Results of the M-PFC experiments using regulator pairs (C) PhoPD71N-DosR and (D) PhoP-DosRD54E suggest that phosphorylation do not appear to have a role on PhoP-DosR protein-protein interactions.

Figure S4

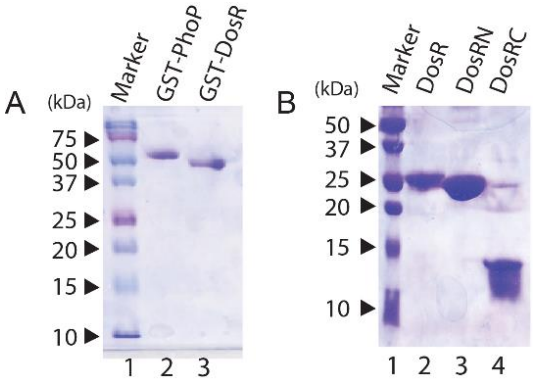

Fig. S4: Recombinant proteins, expressed and purified as described in the Methods, were analysed by 12% SDS-PAGE, and visualized by Coommassie blue staining. While panel A resolves GST-tagged PhoP (lane 2) and DosR (lane 3) (≈3 μg each), panel B analysed His_6-tagged DosR (lane 2), N-domain of DosR (DosRN; lane 3) and C-domain of DosR (DosRC; lane 4), respectively (≈5 μg each). Domain constructs of DosR are described in the 'Results' section. As a reference, molecular mass markers are resolved in lane 1 of both panels, and sizes of proteins in kDa are indicated to the left.

Figure S5

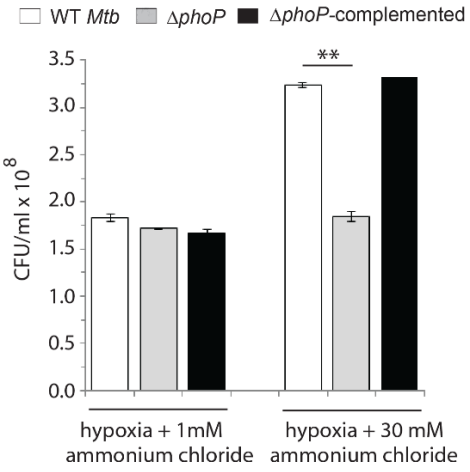

Fig. S5: CFU/ml data of the WT and the mutant strains grown for 12 days under indicated hypoxic conditions. *ΔphoP* shows limited growth as that of the WT bacilli under hypoxia coupled with limiting nitrogen conditions. However, under hypoxia coupled with nitrogen surplus conditions, WT bacilli is capable to overcome the growth defect. In contrast, *ΔphoP* mutant is unable to restore growth as that of the WT bacteria. Importantly, growth defect of the mutant under identical conditions is fully restored in the complemented mutant.

References

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