

Two-Component System of *Mycobacterium Tuberculosis*

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INTRODUCTION

Two Component Systems (**TCSs**), are widespread signal transduction devices in prokaryotes that enable these organisms to elicit an adaptive response to environmental stimuli, primarily through changes in gene expression [1]. They are typically composed of a membrane-located sensor with histidine kinase activity and a cytoplasmic transcriptional regulator. Generally, stimuli detected by TCS autophosphorylate sensor proteins at a conserved histidine residue. The sensor proteins then transfer phosphorylation to an aspartic acid residue of the transcription regulator. The active regulatory proteins then induce conformational changes in virulence, metabolism, mobility, etc.

TCSs are able to detect chemical and physical parameters, such as different ions, temperature, pH, oxygen pressure, the redox state of electron carriers, and contact with host cells. They are primarily noted for the role played in bacterium virulence. Attenuation of the virulent properties has been reported in TCS mutants of *Salmonella enteric* [2], *Shigella flexneri* [3], *Vibrio cholera* [4], *Helicobacter pylori* [5], *Pseudomonas aeruginosa* [6], *Streptococcus pneumonia* [7], and

Staphylococcus aureus [8]. Currently, over 4000 TCSs have been identified in 145 sequenced bacterial genomes [9]. In *M. tuberculosis*, there are 11 complete pair of sensor histidine kinases and response regulators (phoPR, narLS, senX3-regX3, tcrXY, devSR, mtrAB, kdpDE, trcRS, prrAB, mtrAB and mprAB), 4 isolated kinases (Rv2027, Rv3220, Rv1363c and Rv3765), and 2 isolated regulatory genes (Rv1626, Rv3143) [10]. The manners in which the TCSs can serve to regulate virulence and drug-resistance in *M. tuberculosis* will be discussed in this chapter.

DOSS/DOST—DOSR (RV3134C/RV3133C-RV3132C)

The gene Rv3132c and Rv3133c encode a 578 amino acid histidine kinase protein (termed DevS) and a 217 amino acid response regulator protein (termed DevR), respectively. Thus, the two-component system was named devR-devS. Rv3133c, was named DevR for differentially expressed gene between H37Rv and H37Ra in the *M. tuberculosis* (dev genes) [11], or DosR for dormancy survival regulator, found for the first time by Calvin Boon and Thomas Dick [12]. DosT encodes a protein that is 62.5% identical to DevS (DosS), is also a sensor kinase that autophosphorylate one of their own histidines and subsequently use the phosphorylated histidine to phosphorylate an aspartate residue of DosR, resulting in binding of DosR to DNA upstream of hypoxic response genes and thus activating the dosR regulon [13]. But unlike DevS, DosT is not upregulated during hypoxia [14].

DevRS is the key of the dormancy regulon that includes genes that are positively regulated in response to hypoxia and/or low levels of NO (Figure 1). 48 genes has been demonstrated to be induced by DevRS, that are accompanied by growth arrest and a dormant drug-tolerant phenotype under in vitro and ex vivo conditions[15,16]. For example, It could up-regulate the expression of a 16 KDa α -crystallin homolog protein (Acr, HspX) [17], which is a small heat shock protein with a chaperonin activity that is required for the growth of *Mtb* in macrophages [18]. HspX functions as an ATP independent molecular chaperone by preventing improper folding and unfolding of other proteins within the cell. During *Mtb* infection, HspX can be found in aggregates on the inner side of the cell wall, has been linked to cell wall thickening [19].

Structure analysis suggested that DosS might have three transmembrane domains, GAF-B domain, C-terminal histidine kinase domain and N-terminal GAF-A domain. The resulting soluble protein was shown that N-terminal GAF-A domain was responsible for binding heme with a 1:1 stoichiometry [20,21]. UV-visible and resonance Raman studies of the truncated GAF-A domain and full-length protein showed that DosS reversibly binds O₂, CO, and NO [22]. The kinase domain is intrinsically catalytically active, and thus response to gases involves interactions with DosS protein. On the other hand, the crystal structure of DevRC-DNA complex has been solved [23]. A dimer of the DevR C-terminal domain (DevRC) interacts with G4G5G6A7C8T9 motif present in each half of a palindromic consensus sequence [24]. The protein binding was happen to two distinct regions in promoter of hspX, a more distal 33 bp stretch from -111 to -79 and a more proximal 33 bp stretch from -66 to -34 [19].

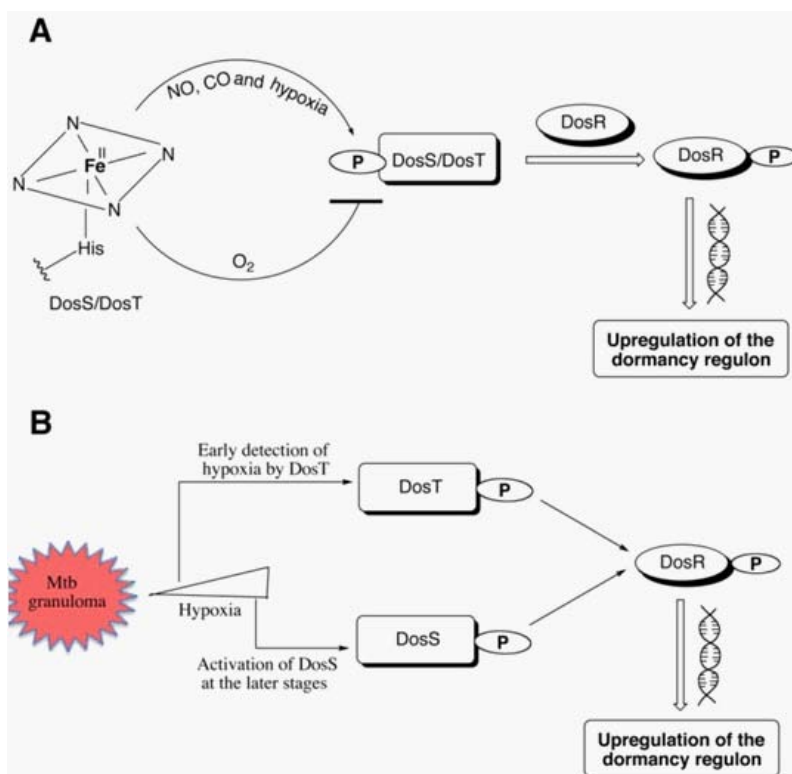


Figure 1: (A) The deoxy ferrous form of DosS/DosT is autophosphorylated under hypoxia or upon binding CO or NO, while no phosphorylation is observed when the proteins bind oxygen. The phosphorylated protein transfers the phosphate group to DosR, which then binds DNA resulting in downstream signaling leading to the upregulation of the 47 other genes necessary for dormancy. (B) DosT is sensitive to low oxygen concentrations hypoxic induction peaked at 72-120 h, and they are temporally a inside the cell and responds during the early transition from aerobic to hypoxia by autophosphorylation and induction of the dosR regulon. DosS detects hypoxia at later stages and is responsible for maintaining the dormancy regulon induction [25].

DevR binding is required for hypoxic gene induction. For most of the genes, and differentially regulated [15]. The loss of *dosR* did not affect the growth of the bacillus in normal condition. But in phenotypes exhibited by BCG Δ *dosR*: km, the extended transition phase and hypoxic death will be lost in oxygen-starved culture [12].

DevR is expressed in *M.bovis* BCG and *M. tuberculosis*, and found to be constitutively over expressed in the hyper-virulent W-Beijing strain of *M. tuberculosis* [26], and more highly expressed in multiple-drug resistant *M. tuberculosis* (MDR) strains [27]. But it is not presented in *M. smegmatis*. Homologs of DevR regulon proteins were found in many environmental bacteria. They are present in various classes such as high GC Gram positives, *archaea*, *Thermotogae*, *Epsilonproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Betaproteobacteria* and

Alphaproteobacteria, *Firmicutes*, *Chlorobi*, *Chloroflexi*, *Aquificae* and *Cyanobacteria*. Based on annotation derived from databases and literature reports, Suganya Selvaraj have categorized DosR regulon proteins into eight functional categories: redox balance, metabolism and energy, nitrogen metabolism, nucleotide metabolism and repair, protein synthesis and cell wall synthesis, sensors and transcriptional regulators, host-pathogen interactions, membrane proteins (proteases and transport proteins) and stress response proteins [16]. Moreover, it has been reported that disruption of response regulator gene *devR* leads to attenuation of virulence in *M. tuberculosis* [28]. It is suggested that DevRS have a very important role in survival and infection of *M. tuberculosis*.

Thus, DevR regulon proteins could be potentially novel therapeutic target in the future. DevS binding peptides have been identified from a phage display library. Two DevR mimetic peptides were found to specifically inhibit DevR-dependent transcriptional activity and restrict the hypoxic survival of *M. tb* [29]. In addition, DevR has been proposed to be diagnostic marker of *Mtb* infection [30], but it is not good enough in PCR methods at least with sensitivity 64% and specificity 100% [31].

PHOP-PHOR (RV0757-RV0758)

Under phosphate-starvation-induction conditions, the Response Regulator (**RR**) PhoP, and the histidine protein kinase (**HK**) PhoR, are involved in the induction of Pho-regulon genes including the *phoPR* operon and genes encoding the major vegetative alkaline phosphatases, *phoA* and *phoB*. The PhoP protein belongs to OmpR/PhoB subfamily, which is considered as the largest of the response regulators [32]. It contains two distinct domains: an N-terminal regulatory domain that highly conserved a phosphorylation site which receives a phosphate group from the cognate HK PhoR and its C-terminal DNA binding domain that structurally characterized with a winged helix-turn-helix DNA binding motif that involved in DNA binding [33,34]. The C-terminal DNA binding domain, which is also called effector domain, binds to specific DNA sequences of the target promoters and interacts with the cellular transcription machinery. The target sequence contains a direct repeat of two 7bp motifs separated by a 4 bp spacer, TCACAGC(N4)TCACAGC [35]. PhoP binds to the direct repeat as a dimer in a highly cooperative manner. Although the stimuli sensed by PhoR has not been identified in MTB, it is considered as nutrient-limitation stress, low PH or oxidative stress in *Bacillus subtilis* and *Enterococcus faecalis* [36,37].

Genetic and biochemical studies indicate that *PhoP* regulates the expression of more than 110 genes, in which 44 genes are upregulated and another 70 genes are downregulated by PhoP in *M. tuberculosis* [38,39]. A global gene expression profiling indicates that. The *PhoP* mutants lack sulfatides, diacyltrehaloses, and polyacyltrehaloses in the cell envelope, suggesting the involvement of the regulatory system in complex lipid biosynthesis [38,40]. Other studies showed that the absence of *phoP* gene could cause attenuation of virulence [38,41]. Also a point mutation in PhoP contributes to avirulence of *M. tuberculosis* H37Ra [42], and accounts for the absence of

polyketide-derived acyltrehaloses in *M. tuberculosis* H37Ra [43]. It is in the Early Antigenic Target (ESAT-6) secretion and specific T-cell recognition during virulence regulation of *M. tuberculosis* [44]. Now we know that the MTB *phoP* gene is responsible for virulence and plays crucial role in the process of infecting host cell [45]. This led to the construction of live candidate vaccines against tuberculosis based on the inactivation of *phoP*. Also the PhoP-PhoR system of *M. tuberculosis* also represents a potential drug target.

In animal models [46], *phoP-phoR* and *mce2* operons knockout strain in *M. bovis* was tested as an antituberculosis experimental vaccine in animal models. The double mutant strain was significantly more attenuated than the wild type strain in immunocompetent and immunodeficient mice. More recently, a three mutations strain has been demonstrated affecting the PhoP/PhoR virulence regulation system in *M. bovis* and in the closely linked *Mycobacterium africanum* lineage 6 (L6) that likely account in human-to-human transmission [47]. The mutations resulted in down-regulation of the PhoP regulon, with loss of biologically active lipids, reduced secretion of the 6-kDa early antigenic target (ESAT-6), and lower virulence. These findings ultimately explain the long-term epidemiological data, suggesting that *M. bovis* and related *phoPR*-mutated strains pose a lower risk for progression to overt human TB, but with major impact on the evolutionary history of TB.

MPRA-MPRB (RV0981-RV0982)

Based on sequence alignment, the response regulator, MprA, and the histidine kinase, MprB, are grouped into the *E. coli* EnvZ-OmpR subfamily of two-component signaling systems [48]. Purified recombinant MprB possesses kinase activity and undergoes autophosphorylation *in vitro* at a conserved histidine residue (His249). Its autophosphorylation is stimulated in the presence of Mg^{2+} . However, phosphorylation also proceeds efficiently in the presence of Mn^{2+} , a characteristic limited to a subset of sensor kinase proteins, including TrcS of *M. tuberculosis* [49], FrzE and ApgA of *Myxococcus Xanthus* [50,51], and FixL of *Rhizobium meliloti* [52]. Purified recombinant MprA undergoes phosphorylation at a conserved aspartic acid residue (Asp48) *in vitro* when incubated in the presence of its recombinant cognate sensor kinase partner MprB or acetyl phosphate. Interestingly, identification of residue 48 as the site of MprA phosphorylation is not consistent with predictions specifying the phosphorylated residue based on sequence alignment of MprA with OmpR and other response regulators from the ROII subclass. In these proteins, the invariant aspartic acid residue at position 53, an amino acid that is also conserved in MprA, defines the phosphorylation site [53]. The ability of recombinant MprA to be phosphorylated in the absence of its cognate sensor kinase partner adds this protein to the growing list of response regulators that can be phosphorylated by small phosphodonor compounds.

It is found high-level expression of MprA in the avirulent strain *M. bovis* bacillus Calmette-Guérin and its silencing or low-level expression in the virulent *M. tuberculosis* strain H37Rv during growth in macrophages. The pattern of MprA expression observed indicates that that it may play

a role in persistent infection [54]. Then investigations have shown that, under SDS stress, the MprAB TCS activates the SigE, which is one of the most studied sigma factors of *Mycobacterium tuberculosis* [55]. It contributes to maintaining basal expression levels of these genes during exponential growth. Moreover, MprA directly regulates *acr2* and that the interactions of MprA with the *acr2* promoter are complex. Depending on the stress condition, MprAB can have either positive or negative effects on *acr2* expression [56,57]. Similar to DevRS, it may represent general stress-responsive elements that are necessary for aspects of *M. tuberculosis* virulence, but the role of MprA in survival of TB is unclear.

In addition, MprAB is required for ESX-1 function, which is the prototype of type VII secretion systems found in some Grampositive bacteria, and the ESX-1 substrate ESAT-6 is a major virulence factor. MprAB have a role in regulation of Rv1057, which encodes the only seven-bladed b-propeller protein in *M.tuberculosis*. b-propeller proteins have diverse functions, ranging from cell-to-cell signaling and chromatin condensation in mammalian systems, to photoreception in plants, and antibiotic resistance and envelope maintenance in bacteria.

PRRA-PRRB (RV0903C-RV09802C)

As other TCSs, the PrrAB system is composed of a response regulator and a sensor histidine kinase which were, respectively, encoded by *prrA* and *prrB* [58,59]. It is present (expressed) in all mycobacteria species [60].

PrrA is one of the OmpR family members, and it contains a characteristic winged helix-turn-helix DNA binding domain, which exists in either a “closed” or “open” form, to control the binding between DNA sequences (promoter regions) and the PrrA [59]. Under Mn^{2+} or Mg^{2+} conditions, PrrB’s cytoplasmic domain can autophosphorylate and transfer phosphate group to PrrA for activating PrrA [61]. Activation of PrrA can convert close form into open form, and increase its affinity for binding DNA and regulating expression of target genes, although nonphosphorylated PrrA also can binding DNA [59].

PrrAB is known to play a role in *M.tuberculosis* virulence. The expression of *prrA* and *prrB* are increased in H37Rv strains during growth in peripheral blood monocytederived macrophages, but no increased during *in vitro* culture. It indicated that PrrAB may participated in adaptation to intracellular infection [62]. Ewann, F., et al. found that with murine bone marrow-derived macrophages being infected by *M.tb*, *prrA* was transiently upregulated at 4 h post infection, and then following infection bone marrow-derived macrophages *in vitro* of murine, the strains with *prrA* and *prrB* ‘deletion’ was attenuated during initial time points [63,64]. It suggests that PrrAB may be important only for early stages of infection *in vitro*. However, in BALB/c mice model, the mutant strains shown differential virulence levels in lungs, spleens, or livers following either intravenous or aerosol infection. It means that the roles and mechanism of PrrAB in *M. tb* pathogenesis need deeply explored.

Several studies indicate that PrrAB also is essential for *M.tb* in vitro survival [62,65]. The expression of PrrA and PrrB is in low levels during in vitro culture, and it can be induced by nitrogen limitation [65]. However, under carbon starvation, hypoxia, or acidic pH conditions cannot increase the expression of *prpA* and *prpB*. It means that PrrA function involves nitrogen-derived metabolism. Previous study shown nitrogen starvation is a key factor of *M.tb* persistence [66]. In this condition, *Bacillus* shown little or no replication, a low respiration rate, and no loss of viability, and aerobic respiration, translation, cell division, and lipid biosynthesis are decreased.

PrrAB may be a potential therapeutic target of *M.tb*. Interestingly, Diarylthiazole (DAT) is a B-raf inhibitor, which can inhibit tumor growth and mitigate [67], but DATs also have a potent antimycobacterial activity, more than 10 compounds of DATs exhibited excellent bactericidal activity and was active against drug-sensitive and resistant *Mtb* [68]. When mutations occurred in *prpB*, the mutant *M.tb* can resist to DAT compounds [68]. However, the mechanism of bactericidal of DAT remains unclear.

SENX3-REGX3(RV0490-RV0491)

SenX3-RegX3 is one of paired TCSs that are conserved in mycobacteria [69]. It is originally identified by degenerate PCR, was the first reported by Wren B.W. [70]. The two genes are separated by a small intergenic region which contains three repeats of a MIRU (mycobacterial interspersed repeat unit). The *senX3-regX* is found to be polycistronic with the promoter region preceding *senX3* [71]. The cytoplasmic portion of SenX3 is able to autophosphorylate. The phosphate group is then transferred onto RegX3. This phosphorelay involves the conserved His-167 and Asp-52 residues of the transmitter domain of SenX3, and the receiver domain of RegX3 bind the *senX3* promoter region, and overproduction of RegX3 increases the expression of the *senX3-regX3* operon in *Mycobacterium smegmatis* [72].

In *M.smegmatis*, phosphor-RegX3 regulates the expression of *phoA*, *pstS* and *senX3-regX3*. This TCS also responds to phosphate starvation in *M.tuberculosis* [73]. SenX3 has a PAS domain, which commonly senses oxygen or redox states [74]. In addition, genes associated with low oxygen environments are differentially expressed in a RegX3 mutant [75]. More recently, global gene expression profiles determined in a *regX3* deletion strain under various growth conditions have confirmed the differential expression of a number of genes, and conclusively demonstrated that the genes *ald*, *cydAB* and *gltA1* are regulated by RegX3 [76]. However, the study still lack comprehensive knowledge of the RegX3 regulon under different conditions of growth [77]. Parish have performed microarray analyses of *M.tuberculosis* H37Rv with a deletion in the *regX3* RR and identified approximately 100 differentially regulated genes involved in diverse cellular pathway [75]. However, no activation of *senX3-regX3* expression was observed in response to a variety of stress conditions, including pH extremes, nutrient deprivation, antibiotics, sodium dodecyl sulfate (SDS), and H₂O₂ [77]. Thus, the genes that directly require *senX3-regX3* for their regulation and the governing environmental stimuli remain to be determined [78].

The role of *senX3-regX3* in virulence of *M.tuberculosis* has been tested. A transposon insertion in the *regX3* gene in *M.tuberculosis* has been created and the mutants ability to survive in bone marrow macrophages and mice proved to be similar to wild type [79]. Moreover, the *M.tuberculosis* *senX3* promoter expression during an infection of BCG in macrophages showed no increase in expression during 14 days of infection.

TRCR-TRCS(RV1033C-RV1032C)

It has previously been shown that the *M.tuberculosis* TrcR-TrcS two-component system communicates via cognate phosphorylation and thus constitutes a functional signal transduction circuit [49]. However, the genes regulated by the TrcRS proteins have not yet been identified. The *trcR* system is usually induced in early exponential phase [80]. The histidine kinase TrcS can directly phosphorylate the response regulation TrcR autoregulates its own gene expression though binding of the phosphorylate TrcR to the *trcR* promoter region. Mutational analyses of this interaction and DNase footprinting have identified an A-T rich sequence that is essential for TrcR binding and *trcR* regulation.

TrcR belongs to the response regulator protein subfamily that also includes OmpR, PhoB, PhoP, and VirG [81]. A number of two-component system genes from this group of response regulators have previously been shown to be auto regulated. It is likely that TrcR can regulate its own expression independent of phosphorylation. However, it is currently unknown if TrcR is subjected to low levels of phosphorylation via cross talk with another native *E. coli* histidine kinase or by a small-molecule phosphodonor, such as acetyl phosphate.

The stimulatory signal for activation of the TrcR-TrcS system is currently unknown, but one study comparing the global transcription profile of an *M.tuberculosis* *trcS* mutant with MprAB H37Rv growing exponentially in 7H9 medium, found 36 genes expressed at a higher level in the wildtype, while 14 genes were overexpressed in the mutant [82]. Besides, the *trcS* mutant showed an increase in virulence, with significantly shorter survival times ($P<0.001$) [83]. Haydel and Clark-Curtiss initially characterized Rv1057 as a regulatory target of TrcRS [84]. So, it may be served as regulator combined with MprAB in pathogenesis of *M.tuberculosis*.

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