Two-Component Systems of Mycobacterium tuberculosis as potential targets for drug development

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Sistemas de dos componentes de Mycobacterium tuberculosis como objetivos potenciales para el desarrollo de fármacos

Sistemes de dos components de Mycobacterium tuberculosis com objetius potencials pel desenvolupament de fàrmacs

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RESUMEN

La tuberculosis, infección causada por *Mycobacterium tuberculosis,* es un problema global de salud que causa aproximadamente dos millones de muertes anuales. Además, se estima que un tercio de la población mundial está infectada con la forma latente de este bacilo. Las terapias existentes contra la tuberculosis están dirigidas contra bacterias que se replican activamente, mientras que no hay actualmente ningún tratamiento específico para la infección latente de tuberculosis. Los sistemas de dos componentes (*two-component systems*, TCSs) bacterianos juegan un papel central en la adaptación de las bacterias patógenas al medio ambiente que prevalece en los tejidos del huésped. Los TCSs permiten a los microorganismos detectar y responder a los cambios en diferentes condiciones ambientales, y como tales se consideran posibles dianas terapéuticas para el diseño de nuevos fármacos antimicobacterianos. En esta revisión, se describe el conocimiento actual de los TCS de *Mycobacterium tuberculosis*. Se discute el papel que desempeñan en la patogénesis bacteriana y en la virulencia, destacando el sistema DosS/DosT/DosR por su papel en el desarrollo de la tuberculosis latente.

Palabras clave: Tuberculosis, sistema de dos componentes, dianas terapéuticas, latencia.

SUMMARY

Tuberculosis, caused by *Mycobacterium tuberculosis,* is a global health problem with approximately two million deaths every year. Furthermore, up to one-third of the world population is infected with latent form of this bacterium. Existing anti-tuberculosis therapies are directed against actively replicating bacteria, while there is no particular treatment for latent tuberculosis infection.
Bacterial two-component systems (TCSs) are pleiotropic and play a central role in the adaptation of pathogenic bacteria to the environment prevailing within host tissues. TCS allow microorganisms to sense and respond to changes in many different environmental conditions therefore are considered potential pharmacological targets for the development of novel antimycobacterial drugs.
In this work, we review the current knowledge of the TCSs of *Mycobacterium tuberculosis*. We discuss their role in bacterial pathogenesis and virulence. We pay special attention to the DosS/DosT/DosR TCS, emphasizing its importance in latent tuberculosis development.

Keywords: Tuberculosis, two-component system, drug targets, latency

RESUM

La tuberculosi, infecció causada per *Mycobacterium tuberculosis,* és un problema global de salut que causa aproximadament dos milions de morts anuals. A més un terç de la població mundial està infectada amb la forma latent d'aquest bacil. Les teràpies existents contra la tuberculosi estan dirigides contra els bacteris que es repliquen activament, mentre que no hi ha tractament específic per a la infecció latent. Els sistemes de dos components (*two-component systems*, TCSs) bacterians tenen un paper central en l'adaptació dels bacteris patògens al medi ambient que preval dins dels teixits de l'hoste. Els TCS permeten als microorganismes detectar i respondre als canvis en moltes condicions ambientals diferents, i com a tals es consideren potencials dianes farmacològiques per al disseny de nous fàrmacs antimicobacterians. En aquest treball, es revisa el coneixement actual dels TCSs de *Mycobacterium tuberculosis*. Es discuteix el paper que tenen en la patogènesi bacteriana i virulència, amb particular atenció al TCS DosS/DosT/DosR pel seu paper en el desenvolupament de la tuberculosi latent.

Mots clau: Tuberculosi, sistema de dos components, dianes terapèutiques, latència.

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INTRODUCTION

Tuberculosis (TB), the infectious disease caused by *Mycobacterium tuberculosis*, is currently one of the major health problems worldwide. The World Health Organization (WHO) estimates that one third of world's population is currently infected with *M. tuberculosis* and approximately 10% of these people are expected to develop active TB at some point in their lifetime. The development of multidrug-resistant and extensively drug-resistant tuberculosis (MDR-, XDR-TB), together with the spread of risk factors such as human immunodeficiency virus (HIV) and diabetes (Corbett *et al.,* 2003, Restrepo *et al.,* 2007), strengthen the necessity to develop new therapeutic interventions against tuberculosis. The current development of new antibiotics is not keeping pace with the rapid evolution of resistance to almost all clinically available drugs so alternative strategies are required to fight against mycobacterial infections. Amongst these new therapeutic interventions, bacterial two-component systems (TCSs), stimulus-response coupling mechanisms that allow bacteria to sense and respond to changes in many different environmental conditions, appear as promising new targets.

TWO-COMPONENT SYSTEMS IN MYCOBACTERIUM TUBERCULOSIS

Every living organism senses changes in environmental and intracellular signals and responds accordingly to adapt to and survive in these new conditions. For this purpose, bacteria have evolved surface-exposed signal transduction systems, typically comprised of transmembrane proteins that channel the input from sensory modules to intracellular responses. These signaling systems include chemotaxis receptors, serine/threonine protein kinases, and two-component systems.

Bacterial two-component systems (TCSs) are known to respond to a wide variety of environmental conditions, for example starvation, cold/heat shock or the presence of antimicrobial compounds (Aguilar *et al.,* 2001; Jordan *et al.,* 2008; Sun *et al.,* 1996). These extracellular signals are transduced into the cell predominantly by TCSs, allowing bacteria to adapt to these new conditions (Hoch, 2000; Stock *et al.,* 2000; Mascher *et al.,* 2006; Gao *et al.,* 2007). TCSs can regulate a wide variety of cellular processes, including motility and chemotaxis, sporulation, biofilm formation and quorum sensing (Jiang *et al.,* 2000; López *et al.,* 2009; Lyon & Novick, 2004; Szurmant & Ordal, 2004). TCSs have also been shown to play a crucial role in bacterial virulence (Hoch & Silhavy, 1995; Atkinson & Ninfa, 1999).

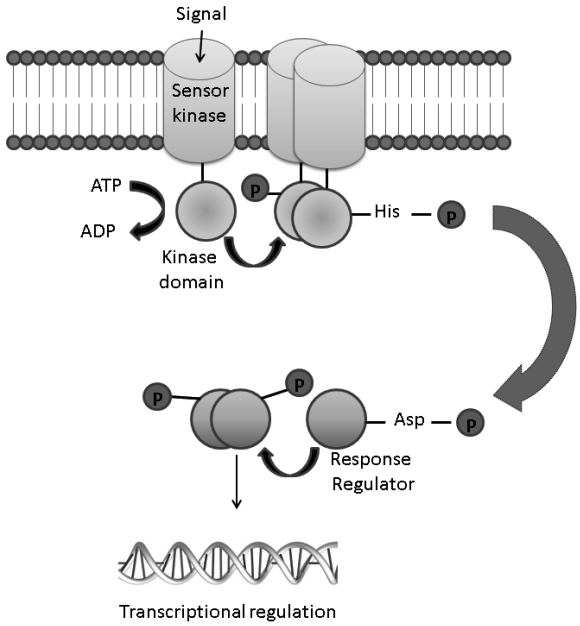

Figure 1. *Illustration of TCS signalling pathway.*

The prototypical TCS consists of a sensor histidine kinase (HK) that responds to a specific signal by modifying the phosphorylated state of its cognate response regulator (RR) (Figure 1). First, upon the perception of environmental or intracellular signal, HK undergoes conformational changes that result in autophosphorylation (using ATP as the phosphate source) within a single, conserved histidine residue in the carboxyl-terminal region of its receiver domain. Subsequently the phosphoryl group is transferred in a magnesium-dependent manner to an aspartate residue in the amino-terminal region of the partner RR protein. Phosphorylation of a RR induces structural changes of its

Table 1. *Complete two-component systems of M. tuberculosis (Bretl et al., 2011).*

TCS (HK/RR)	ORF annotation	Regulation or Effect of inactivation	Reference
SenX3/RegX3	Rv0490/Rv0491	Regulation of phosphate dependent gene expression	(Himpens *et al.,* 2000)
U/U/TcrA	Rv0600c/Rv0601c/ Rv0602c	Unknown	(Hayden & Clark-Curtiss, 2004)
PhoP/PhoR	Rv0757/Rv0758	Implication in regulating production of complex cell wall lipids	(Ludwiczak *et al.,* 2002; Zahrt & Deretic, 2001)
NarL/NarS	Rv0844c/Rv0845	Unknown	(Parish *et al.,* 2003)
PrrB/PrrA	Rv0902c/Rv0903c	Involvement in early intracellular multiplication during macrophage infection	(Ewann *et al.,* 2002, 2004)
MprA/MprB	Rv0981/Rv0982	Regulation of different genes engaged in physiology and pathogenesis	(Zahrt *et al.,* 2003)
KdpE/KdpD	Rv1027c/Rv1028c	Involvement in virulence	(Parish *et al.,* 2003)
TrcS/TrcR	Rv1032c/Rv1033c	Unknown	(Haydel *et al.,* 1999)
DosS-DosT/ DosR	Rv3132c/Rv3133c	Involvement in hypoxic adaptation	(Saini *et al.,* 2004a)
MtrB/MtrA	Rv3245c/Rv3246c	Proliferation in macrophages; essential for *Mtb* viability	(Zahrt & Deretic, 2001)
TcrY/TcrX	Rv3764c/Rv3765c	Involvement in virulence	(Parish *et al.,* 2003)

output domain, which can participate in DNA binding and transcriptional control, catalyse enzymatic reactions, bind RNA, or participate in protein–protein interactions (Gao *et al.,* 2007; Stock *et al.,* 2000; Galperin *et al.,* 2001; Hoch, 1995, 2000).

The *M. tuberculosis (Mtb)* genome encodes about two hundred regulatory proteins, which include over one hundred putative transcriptional regulators, eleven complete TCSs (Table 1), six orphan RRs, and two orphan HKs (Cole *et al.,* 1998; Tekaia *et al.,* 1999). The number of TCSs in *Mtb* is rather low compared with other bacteria, e.g. *E. coli*, which has more than thirty. This relatively small number of TCS probably reflects the intracellular lifestyle of *Mtb*, as the cell environment is less variable than that confronted by soil bacteria or gut microbiota, or a certain degree of overlap in signal processing (Cimino *et al.,* 2012). Comparative genomic analyses of TCSs in *Mtb* indicate that homologues of these genes exist in other representatives of *Mycobacterium* species, including *M. bovis, M*. *avium*, *M. leprae*, and *M. smegmatis* (Cole *et al.,* 1998; Zahrt *et al.,* 2003). All of *Mtb* TCSs are conserved in their genetic arrangement and location within the closely related *M. bovis* bacillus Calmette–Guérin (BCG) vaccine strain.

Evidence suggests that many of the TCSs are engaged in sensing the host environment and adjusting bacterial transcription to adapt to the new environment, including PrrB/PrrA (Ewann *et al.,* 2002), DosRST (Malhotra *et al.,* 2004; Roberts *et al.,* 2004), SenX3/RegX3 (Parish *et al.,* 2003), MprA/B (Zahrt *et al.,* 2003), MtrB/A (Fol *et al.,* 2006), and PhoP/PhoR (Perez *et al.,* 2001).

Constitutive expression of *pdtaR* (coding for an orphaned RR, described as potential phosphorylation-dependent transcriptional antitermination regulator), *dosT*, and *mtrA* during intracellular growth indicates that these genes are likely to be involved in *Mtb* adaptation to life within macrophages. Several studies have analysed the expression profiles during *Mtb* growth in human macrophages (Haydel & Clark-Curtiss, 2004; Zahrt & Deretic, 2001) and mice suggesting the biological role for these signal transduction systems in host-pathogen interactions.

The mutagenesis studies support the role of TCS in growth and survival (Sassetti *et al.,* 2001), indicating that the *senX3, kdpD* and *mtrA* (Sassetti *et al.,* 2003a) gene products are required for survival in mice and that the response regulators PhoP, KdpE, PdatR, and MtrA, as well as the sensor kinases MprB, DosS and Mtr B (Sassetti *et al.,* 2003b) are required for optimal growth *in vitro*.

A large number of experiments have pointed out at several TCS proteins as important regulatory elements for virulence of the tubercle bacillus. This applies to DosR (Malhotra *et al.,* 2004), RegX 3 (Parish *et al.,* 2003), PhoP (Perez *et al.,* 2001), SenX3 (Rickman *et al.,* 2004), MprA (Zahrt and Deretic, 2001) and PrrA (Ewann *et al.,* 2002). The most relevant features of *Mtb* TCSs related to virulence, pathogenesis and host-pathogen interactions are discussed here.

a) RegX3 regulates a large and functionally diverse regulon comprised of 100 genes. Several of these genes are involved in important physiological activities, including energy metabolism, cell envelope maintenance, and regulatory functions (Parish *et al.,* 2003). In *Mtb*, the system is required for virulence, with mutant strains showing attenuation in macrophage and murine infection models (Parish *et al.,* 2003; Rickman *et al.,* 2004; Rifat *et al.,* 2009).

b) PhoP/PhoR is involved in diverse aspects of metabolic physiology and is required for virulence in *Mtb*. This TCS regulates genes associated with the ESX-1 secretion system and synthesis of virulence associated lipids (Frigui *et al.,* 2008; Gonzalo-Asensio *et al.,* 2008). Inactivation of *pho*P results in high attenuation of *Mtb*. The mutant is impaired to grow in macrophages and BALB/c mice; however, it is not completely eliminated and persists in *in vitro* cultured-macrophages and also in mice organs. This mutation, together with a deletion in fadD26, essential for the synthesis of one of the major mycobacterial virulence factors, has led to the construction of the first live-attenuated *M. tuberculosis*-based vaccine to enter clinical trials (Martin *et al.,* 2006).

c) PrrB/PrrA TCS has been shown to be expressed during growth in human macrophages and is required for early intracellular multiplication (Ewann *et al.,* 2002; Graham & Clark-Curtiss, 1999; Haydel & Clark-Curtiss, 2004) and mycobacterial viability (Haydel *et al.,* 2012).

d) MprA/MprB TCS was originally described as being necessary for the establishment and maintenance of persistent infection by *Mtb* in mice and was consequently named *mpr* for mycobacterium persistence regulator (Zahrt & Deretic, 2001). This TCS regulates adaptation programs in response to several environmental stimuli and plays a role in virulence. It has been reported recently that MprAB modulates ESX-1 function (Pang *et al.,* 2013). ESX-1 is the prototype of type VII secretion systems found in some Gram-positive bacteria, and the ESX-1 substrate ESAT-6 is a major virulence factor, implicated in different host-pathogen interactions (Mishra *et al.,* 2010; Samten *et al.,* 2011).

e) *kdp*D is induced in *Mtb* under conditions of starvation (Betts *et al.,* 2002). KdpE/KdpD TCS plays a role in *Mtb* virulence and regulates turgor pressure and potassium homeostasis.

f) Dos (also known as Dev) TCS is composed of two soluble, full-length histidine kinases, DosS and DosT and a single response regulator DosR (Figure 2). Dev TCS proteins were originally identified as DevR (Rv3133c) and DevS (Rv3132c) in a screen for genes differentially expressed in the virulent strain (*dev*) H37Rv compared to the avirulent H37Ra strain (Dasgupta *et al.,* 2000). Subsequent studies demonstrated that Rv3133c was induced in the tubercle bacillus by hypoxia indicating that DevR is a key protein for adaptation of the bacillus to non-replicating survival in hypoxic environments. For this reason the gene was named *dos*R as a regulator of dormancy survival (dos) (Boon & Dick, 2012). Both gene designations remain in use today.

g) MtrA/MtrB TCS regulates essential physiological processes, including DNA replication and cell wall integrity. The *mtr*A gene is constitutively expressed (Haydel and Clark-Curtiss, 2004) and MtrA/MtrB TCS is the only essential system in *Mtb* (Via *et al.,* 1996; Zahrt & Deretic, 2000), while others are required under specific growth conditions. Therefore this TCS may represent a novel therapeutic target (Bretl *et al.,* 2011).

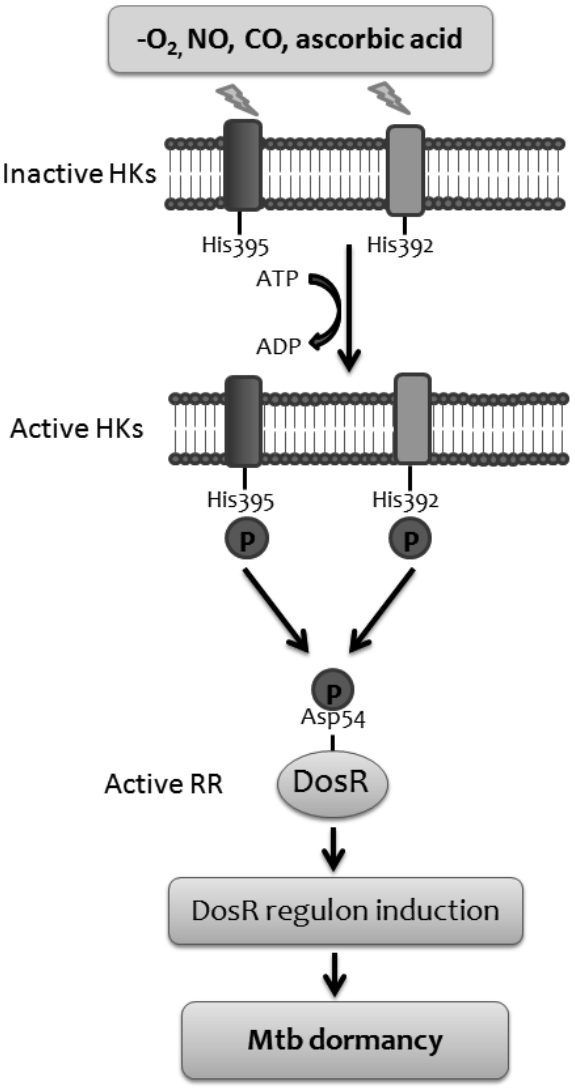

Figure 2. *Mtb dormancy response to hypoxic conditions.*

LATENT TUBERCULOSIS INFECTION: TARGETING DosRST TWO-COMPONENT SYSTEM

Many features make TCSs attractive targets for the development of novel antimicrobial agents. Significant homology is shared among kinase and response regulator proteins of different bacterial species (Parkinson *et al.,* 1992), which could facilitate the design of broad-spectrum antimicrobials. Elucidated crystal structures of several RRs and HKs, which are available in public databases, are a very valuable asset to sustain a platform for structural-based drug development projects.

Pathogenic bacteria, *M. tuberculosis* being a clear example, use TCS signal transduction to regulate expression of virulence factors that are required for survival inside the host (Dziejman & Mekalanos, 1995; Groisman & Heffron, 1995). The inhibition of virulence factors offers an opportunity for specific intervention at the level of host-pathogen interactions (Miller *et al.,* 1989).

In *Mtb*, *dos*R and *dos*S are genetically linked and transcriptionally coupled with each other, as well as with upstream Rv3134c gene (Dasgupta *et al.,* 2000). In contrast, *dos*T does not belong to the core of DosR regulon and is located at the end of a highly induced cluster of genes regulated by DosR (Gerasimova *et al.,* 2011). Genes *dos*R and *dos*S are conserved and tandemly arranged in many mycobacterial species (except *M. leprae* and *M. ulcerans*), while *dos*T appears to be less well conserved. DosS and DosT are capable of autophosphorylating at conserved histidine residues (His-395 and His-392, respectively), and both proteins can transfer the phospho-moiety to Asp-54 of DosR (Roupie *et al.,* 2007, Saini *et al.*, 2004a, 2004b).

DosR upregulates a well-defined regulon of 48 genes in *Mtb* following exposure to hypoxia, nitric oxide (NO), carbon monoxide (CO), and ascorbic acid (Honaker *et al.,* 2009; Kumar *et al.,* 2008, Taneja *et al.,* 2010; Voskuil *et al.,* 2003). The DosR regulon controls survival of the bacilli in an anaerobically-induced state of dormancy and is necessary for optimal transition of *Mtb* back to aerobic growth from an anaerobic or nitric oxide-induced non-respiring state (Leistikow *et al.,* 2010; Rustad *et al.,* 2009).

Existing anti-TB therapies are directed against actively replicating bacteria, while there is no particular treatment for LTBI (latent TB infection). It is believed that the study of the DosRST signaling pathway will improve the understanding of the dormancy response in *M. tuberculosis*. DosRST two-component system was proposed as an attractive target for the development of inhibitors against dormant organisms in different studies (Murphy & Brown, 2007; Lamichhane, 2010; Saini *et al.,* 2005; Vohra, 2006.). Murphy & Brown identified several genome wide trends and used them to guide the selection of targets for therapeutic development. The significant up-regulation of genes controlled by *dos*R was included. They speculated that targeting DosRST TCS may not induce *M. tuberculosis* death directly, but by forcing them to leave the non-replicative state, bacilli would be made susceptible to currently available antimycobacterial treatments.

A homology-based model of DosR was generated and used for the rational design of inhibitors (Gupta *et al.,* 2009). A phenylcoumarin derivative was identified by *in silico* screening and established to be a pathway specific inhibitor that appears to act by locking DosR in an inactive conformation. It is tempting to speculate that this compound is a good starting point for the development of novel compounds targeting DosR with the potentiality of becoming coadjuvants of current antimycobacterial drugs.

CONCLUDING REMARKS

Two-component systems of *Mtb* play essential roles in the virulence and pathogenesis of tuberculosis. These signalling transduction systems show structural features and biochemical activities that make them susceptible to inhibition and amenable to high-throughput screening campaigns for the development of new antimycobacterial drugs. In particular, the inhibition of the DosRST TCS could lead to the development of a novel class of "anti-latent *Mtb*" drugs.

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