Latent tuberculosis: models, computational efforts and the pathogen's regulatory mechanisms during dormancy

Gesham Magombedze[1]*, David Dowdy[2] and Nicola Mulder[3]

[1] National Institute for Mathematical and Biological Synthesis, University of Tennessee, Knoxville, TN, USA
[2] Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[3] Computational Biology Group, Department of Clinical Laboratory Sciences, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

Latent tuberculosis is a clinical syndrome that occurs after an individual has been exposed to the *Mycobacterium tuberculosis* (Mtb) *Bacillus*, the infection has been established and an immune response has been generated to control the pathogen and force it into a quiescent state. Mtb can exit this quiescent state where it is unresponsive to treatment and elusive to the immune response, and enter a rapid replicating state, hence causing infection reactivation. It remains a gray area to understand how the pathogen causes a persistent infection and it is unclear whether the organism will be in a slow replicating state or a dormant non-replicating state. The ability of the pathogen to adapt to changing host immune response mechanisms, in which it is exposed to hypoxia, low pH, nitric oxide (NO), nutrient starvation, and several other anti-microbial effectors, is associated with a high metabolic plasticity that enables it to metabolize under these different conditions. Adaptive gene regulatory mechanisms are thought to coordinate how the pathogen changes their metabolic pathways through mechanisms that sense changes in oxygen tension and other stress factors, hence stimulating the pathogen to make necessary adjustments to ensure survival. Here, we review studies that give insights into latency/dormancy regulatory mechanisms that enable infection persistence and pathogen adaptation to different stress conditions. We highlight what mathematical and computational models can do and what they should do to enhance our current understanding of TB latency.

Keywords: *Mycobacterium tuberculosis*, latency and dormancy regulation, latency models, mathematical and computational modeling

INTRODUCTION

The pathogenesis of *Mycobacterium tuberculosis* (Mtb) is complex and involves an elaborate interaction with the host. Key factors, including the ability to survive in macrophages, the predilection for the lung, the formation of granulomas and long-term persistence, are poorly understood. The Mtb bacterial pathogen generally infects its mammalian host through the aerosol route. Inhalation of Mtb leads to phagocytosis by alveolar macrophages. After the onset of cell-mediated immune response, surviving bacteria are believed to enter a period of non-replicating persistence (NRP) in the phagosome until waning of host immunity results in reactivation from the latent state and the onset of disease. The ability of Mtb to persist for a long time in the latent state has been associated with the ability of the pathogen to maintain resistance against anti-microbial molecules and adaptation to host-induced metabolic constraints such as low nutrients, nitrogen, and oxygen stress (Wayne and Hayes, 1996; Betts et al., 2002; Hampshire et al., 2004; Voskuil et al., 2004b; Deb et al., 2009). In latency, Mtb are less metabolically active, and they have replication rates that are drastically diminished compared to bacilli in an active infection (Lillebaek et al., 2002, 2003). Tubercle bacilli require oxygen for growth; oxygen deprivation is lethal to them unless they have time to adapt to its gradual depletion. In the oxygen deprived

environment if the bacteria are to survive, they need to alter their program of gene expression (Park et al., 2003; Schnappinger et al., 2003; Voskuil et al., 2003, 2004b; Rustad et al., 2008, 2009; Larsson et al., 2012), change their metabolic pathways, and depend on anaerobic respiration or to develop alternative mechanisms for generating energy. All these factors are potential contributors to latent TB infection (LTBI) development in humans.

The interaction between a pathogen and its host occurs on different scales. These range from molecular interactions, including the recognition of specific molecular patterns on innate immune cells by toll-like receptors, to interactions between individual cells, which, in turn, can range from the phagocytosis of bacteria by macrophages to the spread of disease through a host population and the emergence of different strains of pathogens in response to different host conditions. Current approaches to detection, prevention, and treatment of LTBI are inadequate, and rational development of new tools has been limited by poor understanding of the fundamental biology of LTBI, the *Bacillus*-host interaction, the immunologic parameters that are involved in establishing persistent infection, and the mechanisms of pathogen and host that lead to reactivation of Mtb and development of active disease.

LTBI represents a state of equilibrium in which the host shows no apparent symptoms, but is infected and where the immune

system is only robust enough to contain the infection but is unable to clear it. When the immune system gets compromised, this balance is lost, leading to infection reactivation. From the host perspective, LTBI is clinically considered to be an asymptomatic, non-infectious state from which progression to active, infectious disease may occur at any time. Such progression occurs more often soon after the initial infection, and during times of host immune-suppression (e.g., human immunodeficiency virus infection, aging, therapy with tumor necrosis-alpha blockers) (Stead and Lofgren, 1983; Corbett et al., 2003; Gardam et al., 2003). Although classically considered to be a state in which Mtb is either contained or metabolically dormant, it is increasingly understood that clinical LTBI may represent a wide spectrum of host responses (Barry et al., 2009). Our lack of accurate and specific diagnostic tools to differentiate these responses *in vivo* makes the development of corresponding *in vitro* models similarly challenging.

GLOBAL PREVALENCE OF LATENT TB
The global prevalence of LTBI is commonly cited as approximately 1 in 3, although no population-based evidence exists to verify this estimate. Such prevalence is roughly consistent with published population models of TB in globally representative settings (Abu-Raddad et al., 2009; Dowdy et al., 2013). The contribution of LTBI to population-level epidemiology differs widely by region; in high-incidence settings, recent infection contributes the majority of new cases of active TB, whereas in low-incidence settings, the majority of non-imported TB cases arise from reactivation of remote infection. Thus, while diagnosis and treatment of LTBI may substantially reduce the burden of active TB in settings of low or declining incidence (Comstock et al., 1979), it is unlikely to have similar population-level effects in high-burden areas until the ongoing annual risk of TB infection can be reduced. Nevertheless, as discussions regarding TB elimination garner increasing attention (Dye et al., 2012), it is becoming increasingly clear that this goal cannot be achieved without a strategy to diagnose and treat the massive reservoir of individuals with LTBI, in addition to those with active TB disease.

The lifetime risk of developing active TB after latent infection is often quoted as 10%, but modeling analyses suggest that, among individuals infected as adults, this number may be substantially higher (over 15%) (Vynnycky and Fine, 1997), and it is certainly much higher (up to 10% per year) among people living with untreated HIV (Corbett et al., 2003). The majority of this reactivation risk is experienced within the first 2 or 3 years following infection. Preventive therapy (traditionally with 6–9 months of daily isoniazid) can reduce this risk of reactivation by 60–70% (Smieja et al., 2000; Akolo et al., 2010) and is therefore a public health priority among individuals at high-risk of reactivation, including people living with HIV and people initiating biological therapy (e.g., TNF-alpha inhibitors) (Gardam et al., 2003) that affect the body's ability to contain LTBI. However, in many parts of the world, such high-risk individuals comprise a relatively small proportion of the total population that will experience active TB from reactivation of latent infection. Plans for expanding access to preventive therapy among others with risk factors for reactivation (e.g., malnutrition, diabetes mellitus, adult household contacts) are therefore urgently needed and in most places lacking.

MACROPHAGES, GRANULOMA, Mtb PATHOGEN AND LTBI DEVELOPMENT
Macrophages are phagocytes at the frontline of host immune defense against microbial pathogens. They are the primary habitat of Mtb, and the pathogen preferentially targets macrophage vacuoles. Thus, the adaptation of Mtb to the phagosomal compartment of the macrophages is an essential component of its pathogenesis, transmission, and continual survival (Smeulders et al., 1999; Hampshire et al., 2004). This apparent incongruity demands that Mtb either tolerate the macrophage's anti-microbial effectors, that is (i) low pH, (ii) reactive oxygen, and (iii) nitrogen species, or actively subvert normal cellular mechanisms to avoid being killed. It implies that for the pathogen to have extended survival in the host in a state of latency, the pathogen must somehow evade or interfere with the immune surveillance and signaling pathways (Voskuil et al., 2003, 2004b; Bacon et al., 2004; Berney and Cook, 2010; Taneja et al., 2010). Alternatively, the *Bacillus* must tolerate host defense mechanisms, either through mobilization of repair or detoxification pathways or through phenotypic tolerance developed as a result of metabolic adaptation or quiescence. After Mtb phagocytosis by macrophages, phagocytosed micro-organisms are rapidly transferred from phagosomes to lysosomes, where there is fusion of the phagosome with the late endosome and lysosome, and the microbes should be destroyed (Clemens and Horwitz, 1996; Fratti et al., 2001; Dubnau and Smith, 2003). The phagosomal environment is nitrosative, oxidative, functionally hypoxic, carbohydrate-poor, and capable of perturbing the pathogen's envelope (Schnappinger et al., 2003). However, pathogenic mycobacteria resist lysosomal delivery and survive within macrophages inside mycobacterial phagosomes, in an intraphagosomal environment that is friendly to the bacterium. Macrophages, instead of killing the pathogen, have been shown to be manipulated by the bacilli, creating an environment suitable for intracellular replication by progressively translocating from phagolysosomes into the cytosol. The bacilli have been shown to be both anti-apoptotic, keeping the host cell alive to avoid the anti-microbial effects of apoptosis, and pro-necrotic, killing the host macrophage to allow infection of neighboring cells. This Mtb induced macrophage death was shown to be dependent on mycobacterial expression of ESAT-6 (van der Wel et al., 2007; Abdallah et al., 2011; Yu and Xie, 2012). Inhibition of Mtb phagosomal maturation was proposed as another mechanism for the survival of the pathogen in macrophages (Clemens and Horwitz, 1996; Fratti et al., 2001). Mtb is among the micro-organisms most successful at adapting to long-term residence in macrophage phagosomes. Phagosome arrest is a complex process and is not fully understood Taneja et al. (2010) (Clemens and Horwitz, 1996; Fratti et al., 2001, 2003; Anes et al., 2003). It is also speculated that pathogenic mycobacteria (including Mtb) might interfere directly in host trafficking pathways through expression of their own signal transduction molecules. The Mtb genome encodes two eukaryotic-like serine/threonine kinases [reviewed in Av-Gay and Everett (2000)] and protein kinase G (*PknG*), which consists of a kinase domain flanked by a large N- and C-terminal domain, and was shown to inhibit phagosome-lysosome fusion. Rapid lysosomal transfer of mycobacteria lacking *PknG* results in intracellular killing by bactericidal activities present in the

lysosomes. Therefore, the ability of *PknG* to prevent lysosomal transfer suggests that it may affect survival of bacteria that have been internalized in macrophages (Walburger et al., 2004).

In latency, lipids have been found to be a major source of nutrients to the Mtb pathogen (McKinney, 2000; Ehrt and Schnappinger, 2007). Fatty acids have been hypothesized to constitute a significant source of both carbon and energy for non-replicating bacilli and the glyoxylate by-pass may serve in the formation of carbohydrates from fatty acids (Muñoz-Elías and McKinney, 2005; Ehrt and Schnappinger, 2007, 2009). Mtb strain (H37Rv) has a gene that encodes an isocitrate lyse, *icl1,* expressing ICL activity in macrophage infection. Studies by Graham and Clark-Curtiss (1999) and McKinney (2000) showed that a strain of Mtb in which the *icl* gene had been insertionally inactivated, lost the ability to persist in tissues of mice or in activated macrophages even though it did not appear to have diminished ability to survive *in vitro* anaerobiosis (McKinney, 2000). Isocitrate lyases catalyze an essential reaction of the glyoxylate shunt, an anaplerotic pathway that by-passes the CO_2-generating steps of the tricarboxylic acid cycle, and enables bacteria to synthesize carbohydrates and replenish tricarboxylic acid cycle intermediates from fatty acid-derived acetyl-coenzyme A (Bishai, 2000; Ehrt and Schnappinger, 2007). For many bacteria, this pathway is required for growth on fatty acids as a sole carbon source. The genomes of most Mtb strains encode not one but two functional isocitrate lyases: *ICL1* and *ICL2.* Muñoz-Elías and McKinney (2005) showed that both *icl1, icl2* Mtb mutants can grow on different carbon sources (e.g., glycerol, glucose, acetate, propionate).

Another important facet of LTBI development is the granuloma. A granuloma is a multicellular structure comprised of macrophages (resting, activated, and infected), immune effector T cells on the periphery, chemokines, cytokines, adhesion molecules, and a caseous necrotic center (Lin et al., 2006; Lin and Flynn, 2010). Macrophages within a granuloma have two main functions (i) primarily to contain infection and (ii) for bacterial multiplication (Mtb preferred environment for growth). In the event that they fail to control infection, they end up harboring large amounts of bacteria and the bacteria can persist for decades, which is the case in LTBI. An impairment of the immune system will result in the disruption of the granuloma structure and collapse of the granuloma center, hence infection dissemination and potential transmission of the bacteria to other individuals. LTBI in humans comprise of a heterogeneous mixture of granulomas in both lungs and lymph nodes that provide a range of physiological micro-environments associated with bacterial replication and persistence, and therefore the basis for TB clinical latency (Barry et al., 2009).

MATHEMATICAL MODELING AND LATENT Mtb INFECTION

Mathematical and computational efforts just like biological experiments have been key in unveiling many facets of infectious diseases. Their applications have immensely contributed to and shaped our current understanding of TB infection. In the same fashion as biological experiments, constructing, validating and analyzing mathematical, and computational models based on empirical observations can provide new insights into biological systems by making testable predictions and stir new directions for

designing of biological experiments. Computational methods are our hope to handle and analyze vast amounts of high-throughput data. They are an essential tool in predictive studies of disease signatures, biomarkers, and vaccine and drug targets. Several mathematical schemes have been developed to provide a platform to study and understand host immune response mechanisms in active TB infection (Wigginton and Kirschner, 2001; Marino and Kirschner, 2004; Segovia-Juarez et al., 2004; Magombedze et al., 2006b, 2009, 2010; Sud et al., 2006; Ray et al., 2008, 2009). However, there is no substantial effort through mathematical modeling that has been put forth to enhance our understanding of LTBI (Magombedze and Mulder, 2011, 2012; Hegde et al., 2012; Magombedze et al., 2012).

In general, mathematical models have been useful in the evaluation, assessment, planning, and theoretical trial of vaccines and disease control intervention strategies of several infectious diseases and ailments (Scherer and McLean, 2002; Gandon and Day, 2007; Magombedze et al., 2008b; Smith and Schwartz, 2008; Heffernan and Keeling, 2009). The success or failure of a vaccine scheme can easily be predicted by use of such models when they are carefully developed and analyzed. This approach provides cost effective methods to give general insights and specific direction for implementing intervention programs (Long and Owens, 2011; Lugnér et al., 2012), hence providing direction for vaccinations. Application of optimal control and optimization techniques offers a platform to investigate and design optimal treatment, vaccine, and cost effective implementation protocols (Magombedze et al., 2011a,c). On the other hand, the effect of how these vaccines enhance the immune response in shrugging off the infection within the host has not been extensively studied compared to the extent of studies carried out at the population-level. Therapeutic and chemoprophylactic vaccines have been investigated in the studies (Koff et al., 2005; Smith and Schwartz, 2008; Johnson et al., 2011). Currently there is limited understanding for therapeutic vaccines and most of these vaccines offer partial immunity, which wanes over time. There has not been much new discovery for TB vaccines and BCG is still the vaccine that is recommended by WHO in infants. Several theories and debates exist as to what type of TB vaccine will be most effective, either an innate based (dendritic, macrophages, neutrophil) or a T cell based vaccine. A mathematical study (Magombedze et al., 2006a) was used to assess the efficacy of TB chemotherapy first line drug regimen following the directly observed therapy strategy (DOTs). This study was followed by models in which effects of treatment adherence and treatment when there is HIV coinfection were investigated (Magombedze et al., 2010, 2011b; Ramkissoon et al., 2012). This was designed to determine the possible treatment schemes when there are HIV and TB drug interactions, and several sequences of combinations of administering both HIV and TB drug cocktails, hence painting a better understanding of potential treatment schemes.

The current understanding of how the immune system interacts with the Mtb pathogen in TB infection owes credit to several mathematical studies (Wigginton and Kirschner, 2001; Marino and Kirschner, 2004; Magombedze et al., 2006b, 2008a, 2009; Sud et al., 2006; Ray et al., 2008, 2009; Day et al., 2009). However, the contribution of these studies toward the understanding of LTBI is still minimal. The first model of TB infection was in 1994 (Antia and

Koella, 1994), which attempted to explain attributes of mycobacterial evolution and maintenance of micro-parasite virulence. Antia et al. (1996) developed another model on mycobacterial infections, where the mechanisms by which bacteria such as Mtb and *Mycobacterium leprae* persist at low densities for extended periods and attain high densities much later in the presence of an Ag specific immune response were investigated. Comprehensive and detailed modeling of Mtb was carried out in the studies (Wigginton and Kirschner, 2001; Marino and Kirschner, 2004; Segovia-Juarez et al., 2004; Magombedze et al., 2006b, 2008a, 2009, 2010; Sud et al., 2006), which described the interaction of the Mtb pathogen, macrophages, cytokines, and lymphocytes at the site of infection (lung compartment), and lung and lymph node compartments (Marino and Kirschner, 2004; Magombedze et al., 2009). These studies predicted attributes of the pathogen and immune response mechanisms that result in either LTBI or active TB, however without deciphering the mechanisms of LTBI development and persistence. Several other studies were carried, which showed the effects of TNF-alpha in granuloma formation and show how disruption of TNF-alpha mechanisms may enhance infection dissemination (Ray et al., 2008, 2009; Fallahi-Sichani et al., 2011). The mechanisms at the granuloma level that lead to different configurations of cells, however differential containment of the organisms are not well understood (Sharpe et al., 2009). There are current modeling efforts and methods for measuring and modulating TNF-alpha at the granuloma level to determine the effects on local control of Mtb infection using non-human primate data [reviewed in Kirschner et al. (2010)]. The studies (Ray et al., 2009; Fallahi-Sichani et al., 2011) present a way to study a single granuloma and the multi-scale molecular, cellular, and tissue events in the development of a granuloma, and show the critical role of TNF-alpha in control of bacterial growth in the granuloma.

There are studies that have started to incorporate details about signaling pathways into both immune cell and host-pathogen models, with the potential of identifying new drug targets (Goldstein et al., 2004; Franke et al., 2008; see review Kirschner and Linderman, 2009). Our study, Magombedze and Mulder (2011), is a first attempt to combine gene expression data, multiple stress factors, and bacterial cell densities using a mathematical framework to investigate and explain several hypotheses on how persistence and reactivation of LTBI occurs. Results in this study echoes the results in the studies (Hegde et al., 2012; Magombedze and Mulder, 2012), were dynamics of several signaling and metabolic pathways mechanisms were explained using Boolean network modeling (Hegde et al., 2012), which used a bistable switch to help explain a potential mechanism on how the pathogen can switch between two different metabolic states. Our recent study (Magombedze and Mulder, 2012) predicted the regulation of the latency/dormancy program using a systems biology mathematical framework to be orchestrated by dynamic gene regulatory networks constituted of several DosR-regulon genes (Voskuil et al., 2003). This study predicted the role of DosR-regulon genes and other several genes as central in the regulation and maintenance of the latency program. In this study DosR-regulon genes were predicted to cluster with adaptation, detoxification, and virulence genes and several other genes of unknown or putative functions (Rv3131, Rv0569, Rv2032, Rv2530c, and Rv2694c), some of which

were predicted both in the regulation of the stationary and non-replicating phase of the bacteria. This study furthermore predicted that other genes (Rv1133c, Rv2890c, Rv1177, Rv2710, Rv2532c, and Rv0982) to be also involved in the regulation of latency. These genes were shown in other studies (Sassetti and Rubin, 2003; Sassetti et al., 2003) to be essential for H37Rv growth and *in vivo* bacterial survival. Some of the genes predicted to be central in the regulation of latency are essential for bacterial growth (Rv3131), making them an attractive drug target. Other genes are known to have functions that are linked to human immune response mechanisms, such as induction of interferon-c (IFN-c) and interleukin-2 (IL-2) (Rv2032, Rv3133c, Rv3131, and Rv2031c), oxidative stress (Rv1909c), and nitrosative stress (Rv3131, Rv3133c, and Rv2031c). The latter are associated with nitrogen and oxygen reactive intermediate effector mechanisms of the immune response and were shown in other studies (Leyten et al., 2006; Roupie et al., 2007; Black et al., 2009) to have the potential to prime the human immune response. However, these predictions remain to be tested through biological experiments for potential LTBI drug design. With vast generation of data sets from different TB experimental infection models (mouse models, guinea pig, non-human primate models, and culture models) as well as data from real cases of infected people, mathematical and computational modeling (see **Table 1** for current mathematical and computational methods) is our only hope to get sense of all data measured at different scales, through developing virtual models that can integrate data from different scales.

UNDERSTANDING LATENT Mtb AND IDENTIFYING NEW DRUG TARGETS THROUGH COMPUTATIONAL EFFORTS

Several computational models and bioinformatics techniques have revealed network interactions, metabolic, and signaling pathways associated with the development of the latent infection; and with prediction of gene functional groups, hence providing informative data sets for future experiments (Mazandu and Mulder, 2011a,b; Mazandu et al., 2011; Hegde et al., 2012; Magombedze and Mulder, 2012). Mazandu and Mulder (2011a) used a combination of sequence and functional genomics data to generate a functional interaction network for Mtb, which was used for drug target prediction and function prediction for the proteins of unknown function (Mazandu and Mulder, 2011b,c). The data integration method exploited sequence similarity and shared domains, gene synteny, phylogenetic profiles, co-occurrence in abstracts, co-expression and other protein–protein interaction prediction techniques to predict functional interactions between all possible protein pairs in the Mtb proteome. A combined score was attributed to each interaction based on the individual scores from each evidence type. Using only medium to high confidence interactions, a network of 4,136 proteins and 58,000 interactions was generated, and was found to have a small world property, which is common among biological networks. This network was used to predict functions for uncharacterized proteins in Mtb, which constitute a high percentage of the genome. Most of the uncharacterized proteins were found to have metabolic activities, but others were predicted to be involved in transcription regulation, signal transduction, and pathogenesis, suggesting that there are many currently uncharacterized proteins that could play a role in

Table 1 | LTBI mathematical and computational models.

LTBI mathematical and computational models (*in silico*)	Advantages	Disadvantages
Lung and lymph node models	Simplify complicated biological systems that are difficult to study experimentally in the lab Use computer simulation Help to design new experiments and derive new hypotheses Not expensive and flexible	Results are dependent of model parameters and available data Complex to analyze
Granuloma models	Not expensive and flexible Can be used to understand unknown mechanisms of the granuloma with computer simulations	Models the granuloma as an isolated structure Results are dependent on model assumptions and available data
Signaling and gene regulatory models	Can predict gene regulatory mechanisms in Mtb latency/dormancy adaptation Easy to simulated with a computer	Depends on available data and the modeling approach used Difficult to develop and analyze
Multi-scale models	Link different scales: organs, tissues, cells, and molecules Model latency in a more realistic way	Difficult to develop, analyze, and simulate They are complicated
Computational methods (models)	Ability to analyze data from different kinds of experiments Offer a platform to integrate data and mathematical models Predictive tools for identifying vaccine and drug candidates	Computationally intensive Depends on available data Involves a combination of different sophisticated mathematical, statistical, and programing techniques

the survival and persistence of Mtb during infection and reacting to changes in the environment (Mazandu and Mulder, 2011b,c).

Functional interaction networks provide the advantage of enabling a systems level view of an organism. Proteins are not viewed in isolation, and for each protein we can establish its relative importance in the biological system by assessing its network properties. Network properties include degree (number of direct interacting partners), betweenness, closeness, and other measures of how well connected a protein is within the network and whether its removal will have a substantial effect on the rest of the network. Like mathematical modeling where we can model expression or metabolic changes and determine the effect of "knocking out" a gene or protein, in biological networks we can knock out proteins and determine the effect on the network. Mazandu and Mulder (2011a) used their Mtb functional interaction network to identify possible drug targets by ranking proteins by their betweenness and centrality measures. Using this technique, they identified 881 possible drug targets, whose elimination would affect a number of other proteins and interactions. These proteins included 114 previously identified drug targets in either UniProtKB or TDR targets (http://tdrtargets.org), as well as targets for some of the currently used anti-TB drugs (Mazandu and Mulder, 2011a). Zheng et al. (2012) used protein–protein interactions networks from STRING for identifying virulence proteins in several pathogens, including Mtb. Using the interaction network, they identified virulence factors based on number of neighbors and strength of interactions and compared this to a feature selection method and BLAST approaches. Their results were benchmarked against a database of validated virulence factors and the network-based method was found to out-perform the other two methods (Zheng et al., 2012).

These network studies, which are purely computational, allow us to predict effects of perturbations on the whole biological system and can help us to identify genes or proteins that known dormancy genes interact with. For example, the DosR protein has over 75 interactions with other proteins in the DosR-regulon, as well as with other regulators and proteins whose functions are not yet known. The host-pathogen interactions, which link two functional interaction networks, allow us to then determine how these proteins potentially interact with host proteins. Computational tools have also been used to identify new vaccine candidates for TB. For example, there are some new possible vaccine candidates on the horizon, such as the latency antigens α-crystallin (HspX) and Rv2660c of the DosR-regulon, which were identified from studies of gene expression changes during dormancy (Wang et al., 2013). In addition, Gideon et al. (2012), using a bioinformatics and empirical approach, identified several novel TB proteins that elicited an anti-tuberculosis T cell response.

Mtb LATENCY AND DORMANCY MODELS

The bacterial behavior during the growth phase (lag, log, stationary, and death phases) is well known, but little is known about how they behave in LTBI. The ability of the pathogen to enter and exit from different states has associated it with its ability to cause persistent infection. In this state it is not clearly known whether it will be in a persistent slow replicating state or a dormant non-replicating state, thus ultimately causing a latent infection with the potential to reactivate to active disease. The terminology for latent TB state and dormant bacterial state is somehow confusing and is used differently in different studies. TB Latency refers to an *in vivo* situation where the bacteria and the host have established a

balanced state without causing apparent symptoms. Whereas the term dormant refers to the physiological and reversible metabolic shutdown state of the bacteria, which is normally simulated in *in vitro* culture models.

In vitro models have been used to model latency or investigate the adaptive processes of the Mtb to non-proliferating conditions which can be achieved by limiting at least one of the essential conditions required for Mtb growth (for a summary of the latency/dormancy models see **Table 2**). These include: (i) starvation of essential nutrients, such as carbon, nitrogen, or phosphorus and (ii) depletion of oxygen, which prevents aerobic respiration by the obligate aerobe. The adaptation to oxygen is the most widely studied of these conditions. Low-oxygen levels have long been recognized to limit Mtb replication while promoting long-term survival, which is associated with a distinct physiologic adaptation and marked by bacteriostasis in addition to metabolic, chromosomal, and structural changes of the bacilli (Wayne and Hayes, 1996; Wayne and Sohaskey, 2001). These states can be distinguished using a resuscitation reculture failure test (Sun and Zhang, 1999). Resuscitation of non-colony forming cultures only confirms the difference between dead cells and dormant cells. Truly dormant cells were shown to resume replication after stimulation with a resuscitation promoting factor (*Rpf*), a 16-kDa protein derived from a Gram-positive coccus *Micrococcus luteus* (*M. luteus*) [reviewed in Zhang (2004)]. The studies (Mukamolova et al., 2002; Tufariello et al., 2004; Downing et al., 2005; Shleeva et al., 2010) showed over-expression of five of the Mtb *Rpf* genes that could stimulate the growth of tubercle bacilli from an old culture with a small inoculum. It may be concluded that the *Rpf* proteins are directly involved in reactivation of persisting bacteria. However, the mechanism of the activating effect of *Rpf* on dormant cells is still not completely understood.

THE WAYNE MODEL

Wayne and Hayes (1996), established an "*in vivo* model for dormancy" where *in vitro* growth TB cultures were subjected to gradual oxygen depletion to mimic tubercle bacilli *in vivo*. A sealed, standing culture is allowed to incubate over a period of days while the bacteria deplete the available oxygen. The culture becomes progressively more hypoxic with a concomitant shift in Mtb physiology (Wayne, 1977; Wayne and Lin, 1982). Gentle stirring and a defined culture-to-headspin ratio improve reproducibility (Wayne and Hayes, 1996). Two distinct states of NRP that reflect discrete metabolic and drug susceptibility states compared with log phase growth are illustrated. The first stage, designated NRP 1, occurred when the declining oxygen level reached 1% saturation. This stage is characterized by increased production of glycine dehydrogenase and steady ATP generation. The second stage, NRP 2, occurred when the oxygen level reached 0.06% saturation and this stage is characterized by a marked decline of glycine dehydrogenase and susceptibility to metronidazole. The dormant cells in the Wayne model remained responsive to heat shock and were culturable when transferred to fresh aerated medium (Shleeva et al., 2004, 2010). Therefore, it is thought that the Wayne model probably represents the initial stage of formation of a dormant state and essentially reflects bacterial adaptation to low-oxygen level rather than to the dormant state. A study by Shleeva et al. (2002) showed that longer incubation time of several months in the Wayne model can produce some dormant bacilli.

Table 2 | Latency/dormancy *in vitro* and *in vivo* models.

Latency/dormancy model	Advantages	Disadvantages
Wayne model (*in vitro*, culture)	Can simulate different Mtb bacteria physiological dormant states Inexpensive and easy to carry out Easy for expressed genes profiling	Uses a single stress factor (hypoxia) Does not reflect what truly happens *in vivo* Slow simulation of dormancy
Rapid anaerobic model (*in vitro*, culture)	Inexpensive and easy to carry out Rapid simulation of Mtb dormant state Easy for expressed genes profiling	Uses a single stress factor (hypoxia) Difficult to correlate with *in vivo* latency
Multi-stress model (*in vitro*, culture)	Easily achieve the stationary and non-replicating phases of Mtb Not expensive and easy to carry out Easy for expressed genes profiling	Difficult to correlate with *in vivo* latency
Cornell model (*in vivo*, mouse model)	Inexpensive and easy to handle Availability of genetic variant strains Large number of immunological tools and reagents Can simulate latency/dormancy	Latency development does not compare well to human LTBI
Guinea pig/rabbit model (*in vivo*)	Easy to handle Show necrosis and granuloma structure similar to humans	Limited availability of reagents Lack of true latency which resembles human LTBI
Non-human primate model (*in vivo*)	Similar immunological and infection pathology with humans Develop LTBI similar to humans Availability of reagents	Expensive Require trained personnel (veterinary scientists) to handle Ethical issues

THE RAPID ANAEROBIC MODEL

There are several other studies (Honaker et al., 2009; Leistikow et al., 2010) that have improved and modified the Wayne model to yield consistent and more precise results. In these studies, a rapid anaerobic dormancy model is implemented. This model differs from the Wayne model in the larger size of stir bars and faster rate of stirring resulting in a more homogeneous population of bacilli, and even oxygen distribution throughout the culture. The model exhausts available oxygen rapidly ensuring achievement of anaerobiosis in a relatively short time interval compared to the Wayne model.

CORNELL MODEL

The most convincing evidence of dormant Mtb was demonstrated by McCune Jr. et al. (1956, 1966) in a mouse model (Zhang, 2004; Shleeva et al., 2010). Mice were infected with virulent Mtb and the infection was allowed to establish for 2 weeks, followed by treatment with a combination of INH and PZA for 3 months. Initially no bacilli could be detected in the mouse spleen; however, one third of the mice relapsed with culture-positive tubercle bacilli when the treatment was discontinued for 3 months (McCune Jr. et al., 1956; Hu et al., 2000) and more relapses were noticed after administration of immunosuppressive steroids (McCune Jr. et al., 1966). This was an indication that dormant bacilli insensitive to antibiotic treatment persisted. There are variations of the Cornell model that show the same disappearance and reappearance phenomenon of tubercle bacilli in mice with different infectious dose, route of infection (aerosol or intravenous), and different drug combinations (Scanga et al., 1999).

MULTI-STRESS MODEL

The limitation with single stress models, like hypoxia, nutrient starvation, and exposure to NO is that they cannot closely imitate the *in vivo* latency/dormancy stimulating conditions. Unfortunately there is no substantive repertoire of alternative models that can model latency with multiple stresses. To date, the study of Deb et al. (2009) is the only one that has achieved this. This multiple stress model used a combination of low-oxygen (5%), high CO_2 (10%), low nutrients (10% Dubos medium), and an acidic pH of 5.0. This study illustrates how the Mtb pathogen adapts and prepares for the dormancy stage. Under these conditions the pathogen was shown to lose its acid-fastness characteristics, while at the same time it accumulated wax esters and lipids, a process thought to be an energy storing strategy as it shifts from aerobic respiration to anaerobic respiration. Several studies (Hampshire et al., 2004; Deb et al., 2009; Berney and Cook, 2010) have suggested that, continual survival of Mtb in the anaerobic respiration state is sustained through energy generation from metabolism of fatty acids. This model revealed high expression of genes involved in the glyoxylate cycle and energy metabolism, which is a strong indication that the pathogen had switched to anaerobic respiration (Deb et al., 2009; Patel et al., 2011).

NON-HUMAN PRIMATE MODEL

There are several *in vivo* models that are currently used to experimentally explain the development of LTBI. These include, (i) mouse models (for example the Cornell model), (ii) the Guinea pig/rabbit model, with the potential to simulate granulomas similar to those observed in humans. Unfortunately it is difficult to observe LTBI manifestation in Guinea pigs, which are highly susceptible to rapid disease progression proceeding low-dose aerosol infection. Although the pathology in lungs of Guinea pigs and rabbits is reminiscent of human pathology, there is relative lack of immunologic reagents for these animals (Flynn et al., 2003). The most attractive model to date is the non-human primate model. Non-human primates (*Cynomolgus macaque*) have physiological, infection pathology, immunological similarities with humans, and they make it possible to carry out experiments that are difficult or impossible to perform in the human population. Its ability to generate clinical correlates of infection has made the non-human primate model important in the study of LTBI. The downside of this model includes cost, difficulty with handling animals, containment of biohazards, and ethical considerations.

LATENCY AND DORMANCY REGULATION

Survival of Mtb bacilli *in vivo* is attributed to the pathogen's ability to adapt to the changing environment. This is orchestrated by a program of genes that sense changes of stress factors (hypoxia, NO-exposure, heat shock, nutrient starvation, pH change, and low iron) in its environment. The phagosome environment is associated with production of oxidative bursts, the synthesis of inducible nitric oxide (NO) synthase resulting in the production of NO and other reactive nitrogen intermediates, which are responsible for the killing of intracellular bacteria. This changing environment within the macrophages should elicit new programs of gene expression in the pathogen if it is to survive. The Mtb pathogens possess mechanisms to detect the ambient oxygen tension based on the *DosT/DosS/DosR* system and WhiB3 sensory systems, which enable them to adapt to changes in oxygen availability by adjusting their metabolism accordingly (Park et al., 2003; Kumar et al., 2007; Rustad et al., 2008; Honaker et al., 2009).

The *DosS/DosT* and *DosR* response regulator is a two component system, which consists of a sensor kinase (component 1) which autophosphorylates histidine, then transphosphorylates a conserved aspartate of its cognate transcriptional regulator (component 2). Mtb has eleven complete systems and seven orphans belonging to sensor kinase and response regulator families (Cole et al., 1998). *DosR* has been shown to act as a transcriptional regulator, which is responsible for the induction of nearly all hypoxia-induced genes of Mtb (Park et al., 2003; Voskuil et al., 2003, 2004b; Kumar et al., 2007; Rustad et al., 2008). It has been shown that, recombinant sensor kinases *DosS* and *DosT* autophosphorylate to stable phosphoproteins (Malhotra et al., 2006; Murphy and Brown, 2007), but rapidly ransphosphorylate *DosR* under conditions of low-oxygen tension or in the presence of NO or CO (Voskuil et al., 2003; Kumar et al., 2007; Sousa et al., 2007; Shiloh et al., 2008), confirming these as members of the two component family. *DosS* is a redox sensor and *DosT* is a hypoxia sensor. Aerobically, *DosS* is in its inactive Fe^{3+} form, as a result of its rapid oxidation by O_2. When *DosS* is reduced to Fe^{2+}, its autokinase activity is increased and uses this oxidation state to signal *DosR* activation (Honaker et al., 2009). *DosT* exists aerobically in the O_2-bound form, which is inactive and its resistance to oxidation removes the possibility of its functioning as a redox sensor. The study of Honaker et al. (2009)

shows that the *DosR*-regulon is induced in stepwise manner with *DosT* only important in early stages and *DosS* important in the late stages. This is because *DosT* is not able to signal *DosR* expression efficiently under low-oxygen. The early activity by *DosT* is thought to kick start induction of *DosR*-regulon genes, among them *DosS*. As the *DosR*-regulon is expressed, levels of *DosS* become elevated, enabling further induction of the regulon.

The ability of the Mtb bacteria to adapt to changing oxygen concentration has been linked with the role of the *WhiB3* protein implicated in sensing oxygen tension and redox state by mycobacteria. The *WhiB3* gene was found to be strongly induced in Mtb during acute infection of mouse lungs and also during growth in resting bone marrow-derived macrophages, but repressed after IFN-gamma activation of the macrophages (Banaiee et al., 2006). The study of Singh et al. (2007) was able to show that WhiB3 contains a 4Fe–4S cluster, which can bind NO. In the presence of oxygen, the WhiB3 [4Fe–4S]2+ cluster is degraded first to a [3Fe–4S]+ cluster, then to [2Fe–2S]2+, and subsequently lost altogether (Crack et al., 2004; Singh et al., 2007; Alam and Agrawal, 2008). WhiB3-mediated response to the presence of oxygen, therefore, is predicted to occur through direct control of the activity of metabolic proteins or through modification of transcriptional regulators (Alam and Agrawal, 2008). A role for WhiB3 in regulation of the transcriptional machinery in mycobacteria may be supported by the finding that WhiB3 interacts with the major sigma factor, *SigA* (*RpoV*) (Steyn et al., 2002), but the effect of this interaction on *SigA* activity is not known, and further studies are required to understand the precise role of WhiB3 in mediating any adaptation of mycobacteria to changes in oxygen tension.

THE DosR-REGULON

The *DosR*-regulon is a significant regulator of genes in response to hypoxia and NO [see **Table 3** for the list of *DosR*-regulon genes (Park et al., 2003; Kumar et al., 2008)]. This, combined with the production of NO by host immune cells and the likely hypoxic nature of the granuloma, makes a strong prediction that this regulon is a primary trigger of metabolic shift down to achieve the non-replicative state. Latency and dormancy models (Park et al., 2003; Voskuil et al., 2003, 2004b; Honaker et al., 2009) have predicted that LTBI is regulated through the *DosR*-regulon/system. Roberts et al. (2004) showed that deletion of *DosS* or *DosT* in the H37Rv strain causes a reduced expression of hypoxia reporter gene and complete failure of *hspX* stimulation in the double mutant. Studies (Voskuil et al., 2003; Kumar et al., 2007; Sousa et al., 2007; Shiloh et al., 2008) predicted that without the NO signal, the DosR-regulon will not be up-regulated, and cell division will continue. The *DosR*-regulon was shown to be weakly induced in the stationary phase indicating partial stimulation of the dormancy genes. With increased oxygen tension there is increased expression of the transcripts *fdxA*, *pfkB*, *narX*, and *narK2*, which indicate a switch to anaerobic metabolism from aerobic respiration (Bacon et al., 2004; Voskuil et al., 2004b). These genes were also observed to be induced in NO exposure and nutrient starvation (Betts et al., 2002; Bacon et al., 2004; Hampshire et al., 2004; Deb et al., 2009). Dormancy genes expressed in the stationary phase and the NRP were shown (Voskuil et al., 2003, 2004b) to be located in "operon like clusters." One such cluster of genes (*rsbW*, *Rv3288c*, *Rv3289c*, and *lat*) is

seen in the study Hampshire et al. (2004) and has previously been identified as up-regulated in the stationary phase survival study by Betts et al. (2002). Other gene clusters include *Rv0982-Rv0984* (*moaB2*), *Rv1460-Rv1463*, *Rv3138-Rv3141* (*pflA*, *fadE24*, *fadE23*, and *fadB4*, respectively), and *Rv3458c-Rv3460c*. The largest cluster of genes includes *Rv3864*, *Rv3867-Rv3871*, *Rv3876*, and *Rv3878*, the majority of which fall within the RD-1 region deleted from strain BCG Pasteur. A complete list of genes up-regulated and down regulated in hypoxia, NO exposure, nutrient starvation, and other stress conditions are found in the studies (Betts et al., 2002; Park et al., 2003; Voskuil et al., 2003, 2004b; Hampshire et al., 2004; Deb et al., 2009).

LATENCY/DORMANCY STATIONARY PHASE OPERONS

Using hierarchical clustering Voskuil et al. (2004b) showed that most stationary phase-specific induced genes are transcriptionally clustered and thus share a common regulatory profile. These genes include *Rv0106*, *PPE3*, *Rv1535*, *PE20*, *PPE31*, *rpsR2*, *rpsN2*, *rpmG1*, and *rpmB2*. The four ribosome encoding genes are separated from the majority of ribosome encoding genes on the Mtb genome, perhaps indicating a difference in function. By contrast, most ribosomal protein genes are repressed during stationary phase as well as in NRP. It is thought that, the ribosomal proteins encoded by *rpsR2*, *rpsN2*, *rpmG1*, and *rpmB2* are involved in increasing ribosomal fidelity during non-proliferating conditions. The *desA3* gene (encoding a probable fatty acid desaturase) is highly induced in the transition to stationary phase, but not during NRP. Interestingly, the other two fatty acid desaturase genes found in the Mtb genome are induced early during NRP (Bacon et al., 2004; Voskuil et al., 2004b). Also, the *desA* genes are induced, with *desA3* being strongly repressed throughout NRP, while *desA1* and *desA2* are only induced during stationary phase. The fact that all three genes are induced during the transition to non-proliferating conditions while the bacilli are still replicating indicates the bacilli may be changing the degree of saturation of the cell wall mycolic acids in preparation for a non-proliferating state. Genes of the *PE* and *PPE* families (which constitute 8% of the genes in the Mtb genome) are also differentially regulated between stationary phase and NRP. *PE3*, *PE20*, and *PPE31* are induced during stationary phase, while *PE11*, *PE34*, *PE-PGRS11*, *PE-PGRS14*, *PPE17*, *PPE47*, and *PPE48* are induced during NRP, and many PE/PPE genes are repressed under both conditions (Voskuil et al., 2004a). There is speculation on the functions of these proteins that involve their ability to provide antigenic variation and interfere with immune responses, in addition to performing a purely structural role.

In addition to the dormancy regulon, several other genes are strongly up-regulated in the NRP model. Most of these genes are repressed late in the NRP model, with the exception of *hsp*, *Rv0678*, *Rv0841c*, *Rv1734c*, *Rv1874*, *mez*, and *Rv2660c* (Voskuil et al., 2003, 2004b). Interestingly, *Rv1734c* is a dormancy regulon gene that is weakly induced early in NRP but is the only dormancy gene that was noticed to remain expressed in the late stages of NRP. The continued induction of *Rv1734c*, and the induction of a few other dormancy genes, when most dormancy genes are no longer induced, indicates there may be another level of control for some of the dormancy genes other than through activation by DosR. Two strongly induced genes early in NRP (*ahpC* and

Table 3 | Mtb DosR-regulon genes.

Rv number	Gene name	Protein function (probable)	Rv number	Gene name	Protein function (probable)
Rv0079		HP	Rv2028c		Universal stress protein
Rv0080		CHP	Rv2029c	pfkB	Phosphofructokinase
Rv0081		Transcriptional factor	Rv2030c		CHP
Rv0082		Oxidoreductase	Rv2031c	hspX	Heat shock protein
Rv0083		Oxidoreductase	Rv2032	Acg	CHP
Rv0569		CHP	Rv2623	TB31.7	Universal stress protein
Rv0570	nrdZ	Ribonucleotide red.	Rv2624c		Universal stress protein
Rv0571c		CHP	Rv2625c		Leucine rich protein
Rv0572c		HP	Rv2626c	hrp1	Hypoxic response protein
Rv0574c		CHP	Rv2627c		CHP
MT0639		HP	Rv2628		HP
Rv1733c		Transmembrane protein	Rv2629		CHP
Rv1734c		CHP	Rv2630		HP
Rv1736c	narX	Nitrate reductase	Rv2631		CHP
Rv1737c	narK2	Nitrite transporter	Rv2830c	vapB22	Antitoxin VapB22
Rv1738		CHP	Rv3126c		HP
Rv1812c		Dehydrogenase	Rv3127		CHP
Rv1813c		CHP	Rv3128c		CHP
Rv1996		Universal stress protein	Rv3129		CHP
Rv1997	ctpF	Cation trans. ATPase	Rv3130c	tgs1	Triacylglycerol synthase
Rv2003c		CHP	Rv3131		CHP
Rv2004c		HP	Rv3132c	dosS	Sensor hist. Kinase
Rv2005c		Universal stress protein	Rv3133c	dosR	Two-comp. resp. reg.
Rv2006	otsB1	Trehalose phosphatize	Rv3134c		Universal stress protein
Rv2007c	fdxA	Ferredoxin	Rv3841	bfrB	Bacterioferritin
Rv2027c	dosT	Sensor hist. kinase			

A list of dosR-regulon genes. dosT is part of the dosR-regulon but it is not up-regulated in response to many conditions tested to date. HP, hypothetical protein; CHP, conserved hypothetical protein.

ahpD) encode the alkyl hydroperoxide reductase and are strongly repressed throughout the adaptation to stationary phase. *AhpC* is a peroxidase and peroxynitrite reductase is involved in antioxidant defense mechanisms in Mtb. Genes encoding cytochrome *bd* oxidase (*cydA*, *cydB*, *cydC*, and *cydD*) are also expressed early in the NRP model. The cytochrome *bd-1* oxidase is an alternative terminal oxidase for the aerobic respiratory chain that is believed to function at low-oxygen levels due to its higher affinity for oxygen than the primary cytochrome *c* oxidase. Therefore, the induction of these genes is consistent with the depletion of oxygen early in the NRP model (Voskuil et al., 2003, 2004b; Hampshire et al., 2004). Induction of the *bfrB* gene that encodes the iron storage protein bacterioferritin, *bfrB* is repressed when the mycobactin synthesis genes are induced under low iron conditions. The iron-dependent regulation of *bfrB* and the mycobactin genes require the presence of *IdeR*. The fact that the mycobactin and *bfrB* genes are co-induced indicates that *bfrB* can be regulated by a mechanism independent of the IdeR-dependent activation that is

observed during conditions of low iron. During NRP, the bacilli may attempt to increase iron stores for use during long periods of dormancy. Therefore, Mtb appears to induce both uptake and storage functions simultaneously.

Transcriptome analysis of stationary phase Mtb cells incubated under standard conditions (Betts et al., 2002; Voskuil et al., 2004b; Shleeva et al., 2010) revealed activation of some of the DosR-regulon genes, including those encoding the two component regulatory system of stress response, as well as nitrate reductase, nitrite binding protein, and alpha crystalline. It may therefore be suggested that expression of the DosR-regulon not only acts as a marker of a hypoxic state, but is also responsible for development of the universal cellular stress response. However, transcriptome analysis of Mtb under oxygen limitation revealed that enhanced expression of the DosR operon genes under hypoxia was brief and occurred only at the initial stage. A bigger group of genes was then activated, reflecting the "true" cellular response to hypoxia (Rustad et al., 2008; Shleeva et al., 2010).

LATENCY AND Mtb ADAPTATION TO CHANGES IN pH

The macrophage intracellular environment is associated with pH changes that are detrimental to bacterial survival, as such, a change in expression of a large number of Mtb genes over a range of pH values is noticed (Rao et al., 2001; Fisher et al., 2002; Saviola et al., 2003). This implies that mycobacteria are able to perceive the pH of their environment. Two adenylyl cyclases have been suggested to fulfill that role in Mtb. *Rv1264* was observed to have an increased fold change of adenylyl cyclase activity at pH 6 compared to pH 8, mediated through a pH-responsive catalytic domain (Tews et al., 2005). In response to pH, the protein undergoes conformational rearrangements and these may involve an unsaturated fatty acid as a structural element (Tews et al., 2005; Findeisen et al., 2007). In the closely related adenylyl cyclase *Rv2212*, pH-sensing activity is strongly induced by binding of unsaturated fatty acids, but the mechanism of this effect is not fully understood (Motaal et al., 2006). While both *Rv1264* and *Rv2212* have increased activity at acidic pH, the mechanism for activation was reported to be different between the two proteins (Tews et al., 2005; Motaal et al., 2006).

ADAPTATION TO NUTRIENT DEPLETION

A rapid reduction in the ability of bacteria to synthesize proteins leads to the accumulation of the stringent response signaling molecule *(p)ppGpp* which in turn reduces transcription of stable RNA genes (Dahl et al., 2003; Jain et al., 2006). The enzyme *relAb* (the only *ppGpp* synthase homolog in Mtb) is required for survival of mycobacteria under long-term *in vitro* starvation conditions. Microarray studies characterizing the response of Mtb to both a fall in available nutrients and hypoxia, have demonstrated a rise in *relA* (Betts et al., 2002; Hampshire et al., 2004). The study of Hampshire et al., 2004, showed that *relA* is involved in initial nutrient depletion adaptation in facilitating long-term survival (Primm et al., 2000; Chatterji and Kumar Ojha, 2001). The type of regulatory pattern seen for *relA* was also observed for *aceAb*. It has previously been suggested that increasing levels of *(p)ppGpp* in *E. coli* may be the sole inducer of *qor*, a gene shown to be up-regulated in response to conditions of stress, which seems to function by reducing the quinone pool (Chang et al., 2002; Dürrschmid et al., 2008). However, in the study of Hampshire et al. (2004), following the drop in expression of *relA* early on, *qor* stayed up-regulated throughout the time course and even showed a late substantial increase, suggesting that other levels of regulation are present.

Different types of stress tend to up-regulate different sigma factors, however *sigB*, *sigE*, and *sigH* are a subset of sigma factors associated with the entire period of stationary phase adaptation (Betts et al., 2002; Hampshire et al., 2004). Alternative sigma factors are a major category of transcriptional regulators responsible for orchestrating the gene expression responses to non-proliferating conditions in *B. subtilis* and *E. coli*. The sigma factor genes *sigF*, *sigB*, and *sigJ* are the ones that are induced significantly during stationary phase [reviewed in Wang et al. (2011)]. Voskuil et al. (2004b) showed that only *sigB* is induced during stationary phase, while *sigF* and *sigJ* are expressed relative to their levels in exponentially growing bacilli and there is a weak induction of *sigE*. *SigC* is induced early, while *sigH* is induced late in the NRP model. The study (Voskuil et al., 2004b) spells out the need to determine if the

state of Mtb during clinical latency more closely resembles the *E. coli* Sigma S model or the *Bacillus* sporulation cascade paradigm.

The genes involved in Mtb persistence *in vivo* are believed to fall into two groups: those responsible for transition to the persisting state and those responsible for its maintenance (Betts et al., 2002; Shleeva et al., 2010). For example, the Mtb mutant lacking the *relA* gene (its product in *ppGpp*), a "strong response" mediator, regulating cellular activity (Dahl et al., 2003) was capable of normal growth in the macrophages and *in vitro* with citrate and phospholipids as carbon sources, but did not survive transition into the stationary phase or incubation under anaerobic conditions (Primm et al., 2000; Shleeva et al., 2010). The expression of a number of genes in the mutant strain and in the wild type was different, including the genes encoding the reactivating protein: *rpfA* (enhanced expression) and *rpfC* (inhibited expression). This demonstrates that the *relA* product is required for persistence under starvation, oxygen limitation, and in an extensive stationary phase, as well as in the chronic model and in the artificial granuloma in mice *in vivo*; it is one of the candidates for participation in the persistent state in humans.

CONCLUDING REMARKS

Understanding the development and persistence of LTBI infection is complex. Despite many years of research and several strides that have been made in identifying key Mtb persistence factors, gene signatures for vaccine and drug design, eradication of TB still seems to be out of reach. The main short coming of current studies is the inadequacy of the current *in vivo* and *in vitro* latency models [reviewed in Patel et al. (2011)] to closely imitate latency development in humans. Nevertheless, these *in vitro* single stress models have shaped our current understanding on bacilli adaptation to the stationary and non-replicating stages. This has revealed key functional groups of genes that regulate latency and dormancy development. Most of these models are not close to modeling the real human latency development, which in humans can persistent for the entire life span until an opportune moment for reactivation, since they are designed to simulate latency/dormancy over a few hours or days. Development of models that follow the framework proposed in the study Deb et al. (2009), in which several stress factors are considered, has the potential to illuminate and enhance our current understanding of Mtb persistence. The use of the non-human primate model is more encouraging since it simulates latency/dormancy close to that of humans (Flynn et al., 2003), and has become the most attractive model to study LTBI compared to other models. However, it is marred with genotype variability and small sample sizes which affects model results reproducibility. The complexity associated with handling this model makes it expensive and unmanageable for several scientists.

Several studies reviewed here suggest that under each different stress condition the bacteria has a unique set of genes that sense and report changes in its environment, hence facilitating necessary changes for adaptation. These mechanisms seem to be dynamic with the length of time spent in each condition as well as the type of the stress. However, overlaps of genes expressed in hypoxia, NO-exposure, and nutrient starvation are noticed (Betts et al., 2002; Voskuil et al., 2003, 2004b; Hampshire et al., 2004). These kinds

of experiments generate lots of data and this is where we think mathematical and computational modeling should play a crucial role. Studies (Magombedze and Mulder, 2011, 2012; Mazandu and Mulder, 2011a,b,c; Hegde et al., 2012), show how computational and mathematical methods can be used to analyze, learn more, identify traits and properties of protein–protein interactions, gene regulatory mechanisms, and metabolic pathways that are active in latency development and maintenance, which cannot be identified by carrying out basic statistical analysis of the experimental data. A few strides have been made so far to achieve this goal. The study (Fallahi-Sichani et al., 2011) applied a multi-scale approach to study the granuloma using a granuloma model with multiple single cell entities modeled separately to understand TNF trafficking within the granuloma and how this correlates to control of infection. The studies (Magombedze and Mulder, 2011, 2012) are the only recent studies to attempt to model gene cross talk and regulation that occurs in latency adaptation and interface change in gene expression with Mtb cell populations to show how these gene regulations influence the final physiological and metabolic stages of the pathogen. The future should see development of more computational schemes that integrate data from different scales in an endeavor to understand the dynamics of LTBI. A multi-scale model that captures measurements of cells (T cells), cytokines (influence of TNF-alpha and IFN-gamma), signaling mechanisms of immune cells when there is an infection, pathogen mechanisms of interfering with the immune signaling pathways, pathogen gene regulatory mechanisms and how these mechanisms influence the

pathogen phenotypic characteristics and metabolic pathways into one framework will be a good basis to generate and test several hypotheses and has potential to usher the study of LTBI on to a new level.

Existing models of the immune system do not cover much detail at the molecular level such as protein sequences and structures and the effect of polymorphisms. The use of bioinformatics is resourceful in this respect, it allows for analysis of pathogen genome sequences and prediction of protein–protein interactions within the pathogen, and between the pathogen and host (Leung and Cavalieri, 2003; Kendall et al., 2004). Availability of the genome sequence helps in compilation of all the potential gene products encoded by a particular organism, identification of functions that are missing or unique in a particular organism and identifying genes that are common between prokaryotes and eukaryotes. This enhances host-pathogen interaction studies which could provide important clues for development of more rational and specific methods to search for new drug targets, vaccine candidates and diagnostic tests.

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