REVIEW ARTICLE

Pathogenesis in tuberculosis: transcriptomic approaches to unraveling virulence mechanisms and finding new drug targets

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Abstract

Tuberculosis (TB) remains a major health problem worldwide. Attempts to control this disease have proved difficult owing to our poor understanding of the pathobiology of *Mycobacterium tuberculosis* and the emergence of strains that are resistant to multiple drugs currently available for treatment. Genome-wide expression profiling has provided new insight into the transcriptome signatures of the bacterium during infection, notably of macrophages and dendritic cells. These data indicate that *M. tuberculosis* expresses numerous genes to evade the host immune responses, to suit its intracellular life style, and to respond to various antibiotic drugs. Among the intracellularly induced genes, several have functions in lipid metabolism, cell wall synthesis, iron uptake, oxidative stress resistance, protein secretion, or inhibition of apoptosis. Herein we review these findings and discuss possible ways to exploit the data to understand the complex etiology of TB and to find new effective drug targets.

Introduction

More than 100 years after Koch's discovery, tuberculosis (TB) still remains a major threat to human existence. An extremely resilient cell wall, opportunistic switching over to latency and adoption of a plethora of cunning strategies to fool the host immune system makes *Mycobacterium tuberculosis* a very successful pathogen. The emergence of multiple drug-resistant (MDR) and extremely drug resistant (XDR) strains severely compromise traditional drug-based intervention, with no new effective anti-mycobacterial drugs available after the discovery of rifampicin more than 40 years ago. Therefore, identification of new drug or vaccine targets is needed to contain this menace.

The bacterium infects its mammalian host primarily in the lungs and only 5–10% of the infected individuals develop clinical disease (Chackerian *et al.*, 2002). On infection, the bacteria encounter alveolar macrophages and are engulfed within these phagocytic cells. However, under normal circumstances, an invading pathogen is usually destroyed in the phagolysosome by a combination of low pH-activated proteolytic enzymes, resulting in the formation of reactive oxygen species (ROS) and, in murine macrophages, synthesis of reactive nitrogen intermediates (RNI) (Nathan & Hibbs, 1991). Since most of the killing occurs inside the phagolysosome (Fenton & Vermeulen, 1996), adept pathogens like *M. tuberculosis* evolved strategies that help it to inhibit phagosome-lyso-

some fusion (Frehel *et al.*, 1986) and to escape acidic environments inside the phagolysosome (Crowle *et al.*, 1991). The mycobacterial phagosome fails to acidify presumably because of lack of incorporation of the proton pumping ATPase complex in the vacuolar membrane (Sturgill-Koszycki *et al.*, 1994). Although inhibition of *M. tuberculosis* phagosomal maturation was initially proposed as a requirement for the survival of the pathogen (Armstrong & Hart, 1971), subsequently it was demonstrated that when *M. tuberculosis* was pretreated with specific anti-mycobacterial antibodies prior to infection of murine peritoneal macrophages, the phagosomes containing bacteria were fused with lysosomes without affecting bacterial survival (Armstrong & Hart, 1975). Stimulation of murine macrophages with lipopolysaccharide or interferon γ (IFN-γ) results in killing of mycobacteria in these cells, during acidification and mycobacterial phagosome maturation (Russell *et al.*, 1996; Schaible *et al.*, 1998). However, it is unclear whether the observed mycobacterial phagosome acidification and maturation is the cause or effect of the killing of *M. tuberculosis*, as these treatments activate macrophages (Dubnau & Smith, 2003).

Unfortunately, not much is known about how the bacterium survives and grows inside the lung. The infection is usually contained in the lung by formation of granulomas where the activated macrophages and other immune cells surround the site of infection to limit further tissue damage and restrict further dissemination of the bacteria (Saunders *et al.*, 1999; Smith, 2003). Although *M. tuberculosis* is postulated to be unable to multiply within the granuloma due to acidic pH, poor oxygen levels and the presence of toxic fatty acids, some of the bacteria may remain dormant but live for decades without any active clinical disease (Smith, 2003). However, if an infected person's immune system is weakened due to HIV infection, use of immunosuppressive drugs, malnutrition or aging, the otherwise dormant bacteria begin active multiplication and spread into other parts of the lung (active pulmonary TB) and even to other tissues (extra-pulmonary TB) via the circulation system (Smith, 2003). However, the metabolic state of the bacilli during human infection is largely unknown.

Virulence factors of *M. tuberculosis*

Interestingly, mycobacteria lack classical virulence factors such as toxins, which are typical of other bacterial pathogens. In the case of mycobacteria, virulence factors may be broadly defined as traits that are important for the progression of the TB disease, usually measured in terms of mortality and morbidity. Mortality can be expressed as the percentage of hosts that die and also as the time taken for a host to die after infection. Another important parameter is the bacterial load or burden, i.e. the number of bacteria found in the host after an initial infection (Smith, 2003). Mycobacterial virulence can be studied *in vitro* using macrophages, dendritic cells (DCs) and pneumocytes (Bermudez & Goodman, 1996; Bodnar *et al.*, 2001; Hickman *et al.*, 2002) as well as *in vivo* using animal models like mice, guinea-pigs and rabbits. While *in vitro* models are easier to work and produce faster results, they are limited to the early stages of infection. On the other hand, experiments involving animal models are time-consuming and require specialized facilities, but have the advantage to include almost all stages of the disease. Mice are the most widely used animal models because of their well-characterized genetics, ease of genetic manipulation, and availability of reagents to measure their immune responses as well as the relatively low cost of maintenance as compared to the other models (Smith, 2003). Since in the lung *M. tuberculosis* primarily infects macrophages, these phagocytes are widely used as an *ex vivo* model to analyze the virulence of *M. tuberculosis* strains and mutants. As human lung alveolar macrophages are difficult to obtain, mouse primary macrophages obtained from bone marrow, lung alveoli or peritoneal cavities as well as various mouse macrophage cell lines are used. Human monocyte-derived macrophages, which are obtained from peripheral blood monocytes, are also widely used (Tsuchiya *et al.*, 1982). However, there are certain caveats in using macrophages for virulence studies as some *M. tuberculosis* mutants which do not show attenuated growth in macrophages may show defective growth in mice and/or exhibit fewer histopathologic changes (Smith, 2003). Thus, genetic screens for *M. tuberculosis* virulence traits based on macrophages alone may miss some attenuated mutants.

In the pregenomics era, the physiology and pathogenicity of *M. tuberculosis* was mostly studied using methods that allowed creation of mutation in specific genes, and this still remains a rational method to determine essential virulence traits. The choice of genes to be inactivated in virulence studies is frequently based on naturally occurring mutations affecting pathogenicity or on intelligent predictions. A number of methods can be employed to create mutant strains, e.g. directed gene disruption with an antibiotic resistance cassette or by allelic replacement (for details see Smith, 2003). Other methods rely on transposable element-mediated mutagenesis, e.g. with Tn*1096*, Tn*5367* or *Himar*1. In addition, genetic complementation can be used to identify *M. tuberculosis* virulence traits: thereby genes that code for potential virulence factors are introduced into nonpathogenic *M. tuberculosis* strains. *In vivo* complementation has become possible owing to integration-proficient vectors that allow stable propagation of genomic libraries or individual genes in bacteria during

animal infections (Lee *et al.*, 1991). A combination of these and further methods has allowed identification of numerous virulence factors that are important for various aspects of *M. tuberculosis* pathogenicity. These factors include secretory proteins, cell wall components, enzymes involved in lipid and fatty acid metabolism or in amino acid and purine biosynthesis, compounds required for metal uptake, oxidative stress proteins and transcription regulators (Supporting Information, Table S1). Some of them are upregulated during infection; however, their importance for virulence has not been established by mutant analysis in each case (reviewed by Smith, 2003).

Lipid metabolism in *M. tuberculosis*

Mycobacteria have evolved an extremely versatile lipid metabolism system that includes homologs of enzymes found in mammals, plants and other bacteria, as well as polyketide products (Table S1). There are approximately 250 distinct enzymes involved in fatty acid metabolism of *M. tuberculosis*, compared with only 50 in *Escherichia coli* (Cole *et al.*, 1998). Mycobacteria are unusual in that they possess both a mammalian type fatty acid synthase (FAS-I), which has all the necessary enzymatic and carrier function on a single polypeptide (Rv2524, *fas*), and a bacterial type FAS-II, in which dissociable enzymes interact with an acyl carrier protein (*acpM*) (Kremer *et al.*, 2001). *Mycobacterium tuberculosis* FAS-I synthesizes fatty acids of intermediate chain length (principally C_{16} and C_{24}), whereas FAS-II is incapable of *de novo* fatty acid synthesis but elongates the C_{16} acyl-CoA primers from FAS-I into long-chain fatty acids via a condensation reaction carried out by β–ketoacyl-ACP-synthase III (*fabH)* (Brown *et al.*, 2005) Long-chain fatty acid products of FAS-II are converted into mycolic acids and their derivatives by a series of enzymatic reactions (Fig. 1). Mycolic acids are key components of the mycobacterial cell wall and may also play a role as an effective barrier to penetration of some antibiotics (Brennan & Nikaido, 1995). Mutations in genes participating in the mycolic acid biosynthesis pathway have been found to result in attenuated virulence *in vitro* and *in vivo* (Takayama *et al.*, 2005); e.g. *M. tuberculosis* Δ*kasB* (FAS-II-negative) mutants are severely attenuated in immunocompetent mice (Bhatt *et al.*, 2007) and deletion of genes involved in methoxy- or keto-mycolate synthesis also leads to significant attenuation in a mouse model of infection (Dubnau *et al.*, 2000). Once full-length functional mycolic acids are synthesized, they are transported for attachment to cell wall arabinogalactan to form mycolyl-arabinogalactan. The transfer and subsequent transesterification are mediated by three well-known immunogenic proteins of the antigen 85 complex (*fbpA*, *fbpB*, *fbpC*) (Belisle *et al.*, 1997). Although FbpC is the most active enzyme, transposon-mediated disruption of *fbpC* did not cause an attenuated phenotype in mouse macrophages (Jackson *et al.*, 1999), as its function may be partially assumed by the functionally redundant *fbpA* and *fbpB* genes (Puech *et al.*, 2002). A *M. tuberculosis* H37Rv mutant with disruption in *fbpA*, but not in *fbpB*, was attenuated in mice, indicating a specific role of FbpA in mycobacterial virulence (Copenhaver *et al.*, 2004). FbpA appears to play a role in protecting mycobacteria against intracellular oxidative stress damage and in inhibiting phagosomal maturation (Katti *et al.*, 2008).

Another interesting feature of the *M. tuberculosis* genome is the presence of a number of genes belonging to the family of polyketide synthases (PKSs) (Cole *et al.*, 1998). The PKSs are structurally and mechanistically related to the FASs. The FASs are involved in the biosynthesis of fatty acids, which are primary metabolites, whereas the PKSs ordinarily catalyze the formation of polyketide secondary metabolites. The PKSs carry out Claisen-like condensations of small- to long-chain carboxylic acid moieties with acetate or branched chain acetate units, which are commonly derived from malonyl-CoA or methylmalonyl-CoA (Khosla *et al.*, 1999). The mycobacterial cell wall contains a number of polyketide-derived complex lipids, which prominently include phthiocerol dimycoserate, sulfolipids, polyacyl trehaloses, diacyl trehaloses and mannosyl-β-1-phosphomycoketides (reviewed by Gokhale *et al.*, 2007). The phthiocerol derivatives are synthesized by a modular system encoded by the large operon *ppsABCDE* (Cole *et al.*, 1998). PpsA is primed by long-chain fatty acids (C_{16}–C_{26}) by FadD26, a fatty acyl-AMP ligase (Trivedi *et al.*, 2004), and extended by a series of condensation reactions with malonyl-CoA and methoxymalonyl-CoA under *ppsABCDE* control (Gokhale *et al.*, 2007). Similarly, mycocerosic acids are synthesized by mycocerosic acid synthase (*mas*), an iterative PKS. Further PKS genes like *pks-1*, *pks-10* (Sirakova *et al.*, 2003a) and *pks-7* (Rousseau *et al.*, 2003) are involved in dimycocerosyl phthiocerol synthesis. *Mycobacterium tuberculosis pks-1*, *pks-7* and *pks-10* mutants were attenuated in mice (Rousseau *et al.*, 2003; Sirakova *et al.*, 2003a). Gene inactivation studies indicate a critical role of iterative PKS2 in sulfolipid biosynthesis (Sirakova *et al.*, 2001). Sulfolipids are thought to suppress the formation of ROS in macrophages (Brozna *et al.*, 1991). Mannosyl-phosphomycoketides are found in low abundance in the cell wall and PKS12 is proposed to be involved in their biosynthesis (Matsunaga *et al.*, 2004). The *pks-12* gene contains the largest open reading frame in *M. tuberculosis* (Sirakova *et al.*, 2003b).

In vivo grown mycobacteria have been suggested to be largely lipolytic rather than lipogenic, because degradation of available host cell lipids is vital for the intracellular life

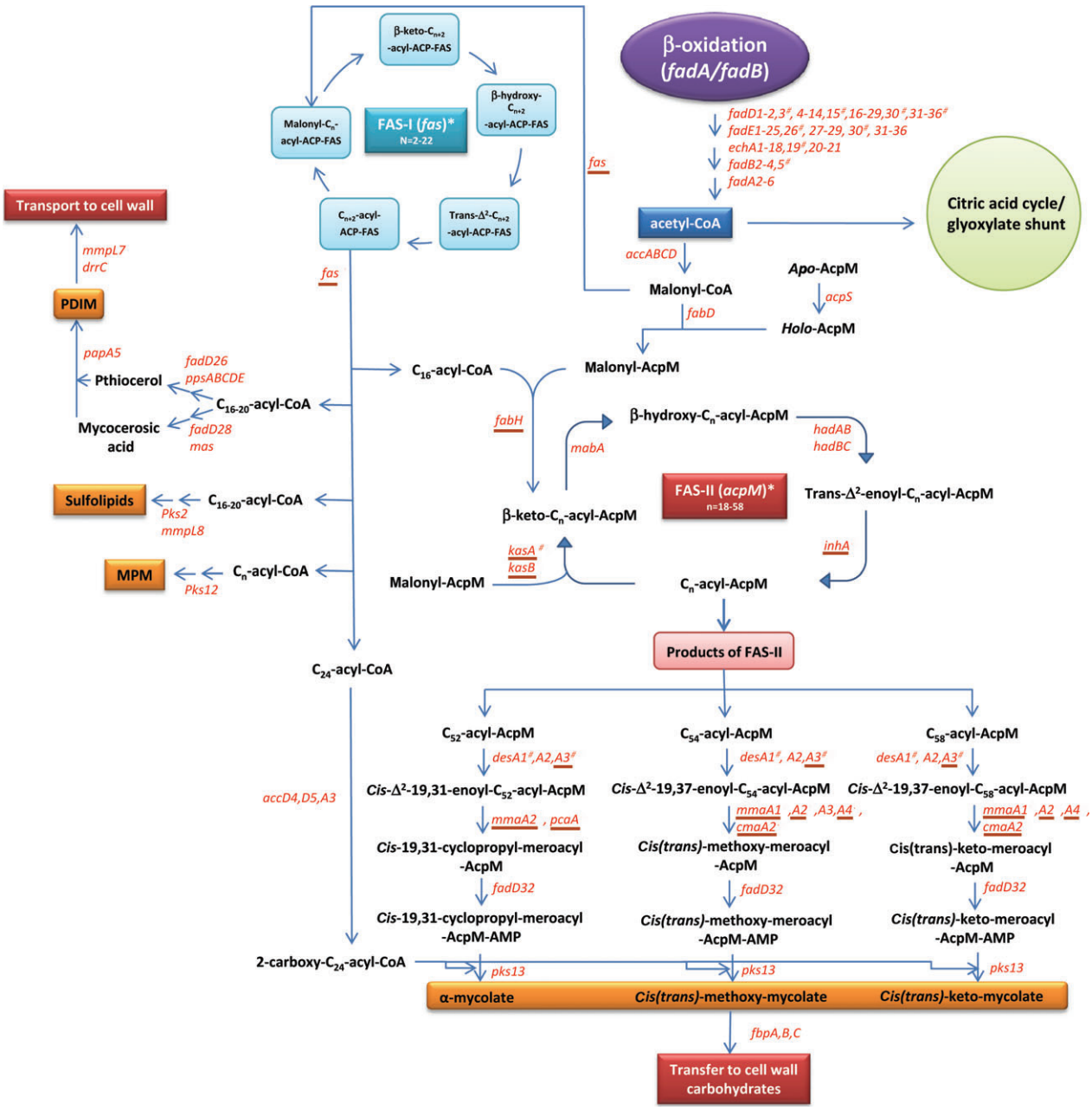

Fig. 1. Schematic diagram of the biosynthetic pathways involved in lipid metabolism in *Mycobacterium tuberculosis*. The gene whose products catalyze different reactions are indicated in italicized fonts. ACP, acyl carrier protein; PDIM, phthiocerol dimycocerosate; MPM, mannosyl-β-1-phosphomycoketides (adapted from Raman *et al.*, 2005; Gokhale *et al.*, 2007). The genes whose expression is found to be significantly changed both *in vivo* and *in vitro* models of virulence are marked by a hash (#) [Based on *in vitro* cell culture experiments by Schnappinger *et al.*, 2003; Rachman *et al.*, 2006a; Tailleux *et al.*, 2008; Fontán *et al.*, 2008a; Homolka *et al.*, 2010 and *in vivo* in human lung (Rachman *et al.*, 2006b) and murine lung (Talaat *et al.*, 2004)]. The genes which are known to be targets of established or experimental drugs are underlined (Raman *et al.*, 2008).

of *M. tuberculosis* (Cole *et al.*, 1998). In addition to genes for the canonical FadA/FadB (Rv0859/Rv0860) β-oxidation complex, the *M. tuberculosis* genome contains multiple copies of genes encoding fatty acid degradation functions (Cole *et al.*, 1998). These include 36 acyl-CoA synthases and a family of 36 related enzymes that could catalyze the first step in fatty acid degradation. There are a number of enzymes homologous to the enoyl-CoA hydratase/isomerase superfamily (*echA1-21*). The genome also codes for four enzymes that may convert 3-hydroxy

fatty acids to 3-keto fatty acids (*fadB2-5*) and five enzymes that may complete the cycle by thiolysis of β-ketoesters (*fadA2-6*). The end product of fatty acid degradation is acetyl-CoA, which can be converted to many different metabolites (Fig. 1) and fuels the citric acid cycle and its glyoxylate shunt to generate energy (Cole *et al.*, 1998).

Iron acquisition in *M. tuberculosis*

Iron is an obligate cofactor for at least 40 different enzymes in *M. tuberculosis* and is essential for its virulence (De Voss *et al.*, 1999). Mycobacteria circumvent the poor availability iron in the intracellular environments by producing low-molecular-weight iron scavengers (siderophores) to sequester iron. Mycobacteria produce two classes of siderophores, the salicylate-derived mycobactins and the exochelins, which are peptidic molecules. Mycobacterial siderophores are synthesized by nonribosomal peptide synthetases. Mycobactins and exochelins are produced by saprophytic mycobacteria, whereas mycobactins are produced only by pathogenic mycobacteria. Pathogenic mycobacteria like *M. tuberculosis*, produce mainly two types of mycobactins, the lipophilic mycobactins that remain cell-associated, and the secreted carboxymycobactins which possess same nuclear structure as mycobactins but have a short carboxy(acyl) side-chain in place of the long alkyl or alkenyl chain. This enables the carboxymycobactin to diminish its lipoidal character and thereby allows it to be secreted into the medium (Ratledge & Ewing, 1996; Rodriguez & Smith, 2003). The *M. tuberculosis* genome contains a cluster of 10 genes (*mbt-1* cluster; Fig. 2), which includes *mbtA*, coding for a salicyloyl-AMP ligase, three peptide synthetase genes (*mbtB*, *mbtE* and *mbtF*), two PKS genes (*mbtC*, *mbtD*), a salicylate synthase gene (*mbtI*) and *mbtG*, a hydroxylase gene (Quadri *et al.*, 1998; Harrison *et al.*, 2006) (Fig. 2; Table S1). Originally, genes encoding enzymes that transfer alkyl substituents of different length to mycobactins were not found in this cluster (Cole *et al.*, 1998). Later, using a cell-free reconstitution system, Krithika *et al.* (2006) identified a second locus (*mbt*-2 cluster; Fig. 2) and delineated its biochemical functions. The four genes in this cluster are annotated as *mbtK* (*rv1347c*), *mbtL* (*rv1344*), *mbtM* (*fadD33*) and *mbtN* (*fadE14*). The *mbtK* and *mbtG* genes were found to be essential for *M. tuberculosis* growth (Sassetti & Rubin, 2003). The genes belonging to these two clusters are regulated by IdeR (iron-dependent repressor). In the presence of iron, IdeR binds to a 19-bp consensus sequence (iron-box) located in the promoter regions of siderophore biosynthetic genes (Fig. 2) to repress transcription of the *mbt* clusters. IdeR also upregulates transcription of a gene (*bfrA*) encoding bacterioferritin, an iron storage protein (Gold *et al.*, 2001; Krithika *et al.*, 2006). Under low-iron conditions, IdeR fails to bind to the iron-box, resulting in derepression of transcription of the *mbt* clusters (Rodriguez & Smith, 2003; Krithika *et al.*, 2006). About one-third of the iron-regulated genes were found to be under the control of IdeR, and IdeR appears to be necessary for an efficient response to oxidative stress (Rodriguez *et al.*, 2002). Recently, it has been demonstrated that *M. tuberculosis* possess a mechanism to use exogenous heme as a source of iron (Tullius *et al.*, 2011). A unique secreted heme-binding protein and several membrane proteins encoded by the region inclusive of Rv0202c-Rv0207c were implicated in sequestering heme iron from the host and transportation across the cell wall and membrane. A model was proposed where a hemophore Rv0203 sequesters the heme from host hemoglobin and delivers it to membrane proteins MmpL11 (encoded by *Rv0202c*) or MmpL3 (encoded by *Rv0206c*), where it is shuttled through the membrane and in the cytoplasm a heme degrading protein, MhuD (encoded by *Rv3592*; Chim *et al.*, 2010) breaks down the heme to release iron. This discovery particularly challenges the long existing paradigm that *M. tuberculosis* obtains iron solely via mycobactins and carboxymycobactins scavenging iron from iron-containing host proteins like transferrin and lactoferrin.

Oxidative stress response in *M. tuberculosis*

Macrophages exert much of their anti-microbial activity by generating ROS and RNI, which have immunoregulatory functions and kill invading bacteria by damaging macromolecules such as DNA and structural lipids. *Mycobacterium tuberculosis* has evolved strategies to counteract these innate effector mechanisms by detoxifying ROS and RNI and by limiting the production of these damaging molecules in macrophages (Nathan & Shiloh, 2000; Khan *et al.*, 2006) (Fig. 3; Table S1). Nitric oxide synthase-2 (*nos2*) and phagocyte oxidase (*phox*) knock-out mice, which are deficient in RNI and ROS production, respectively, exhibit increased sensitivity to *M. tuberculosis* infection (MacMicking *et al.*, 1997; Cooper *et al.*, 2000). Conversely, a *M. tuberculosis katG* mutant is less virulent in mouse models (Ng *et al.*, 2004); the KatG peroxidase helps neutralize RNI (Wengenack *et al.*, 1999) and provides resistance to hydrogen peroxide (Manca *et al.*, 1999). *Mycobacterium tuberculosis* also secretes a superoxide dismutase encoded by *sodA* (Braunstein *et al.*, 2003), which may protect the bacterium from ROS-mediated damage (Teixeira *et al.*, 1998). Similarly, resistance to RNI appears to be a physiological function of AhpC (Chen *et al.*, 1998) and a *M. tuberculosis ahpc* mutant is

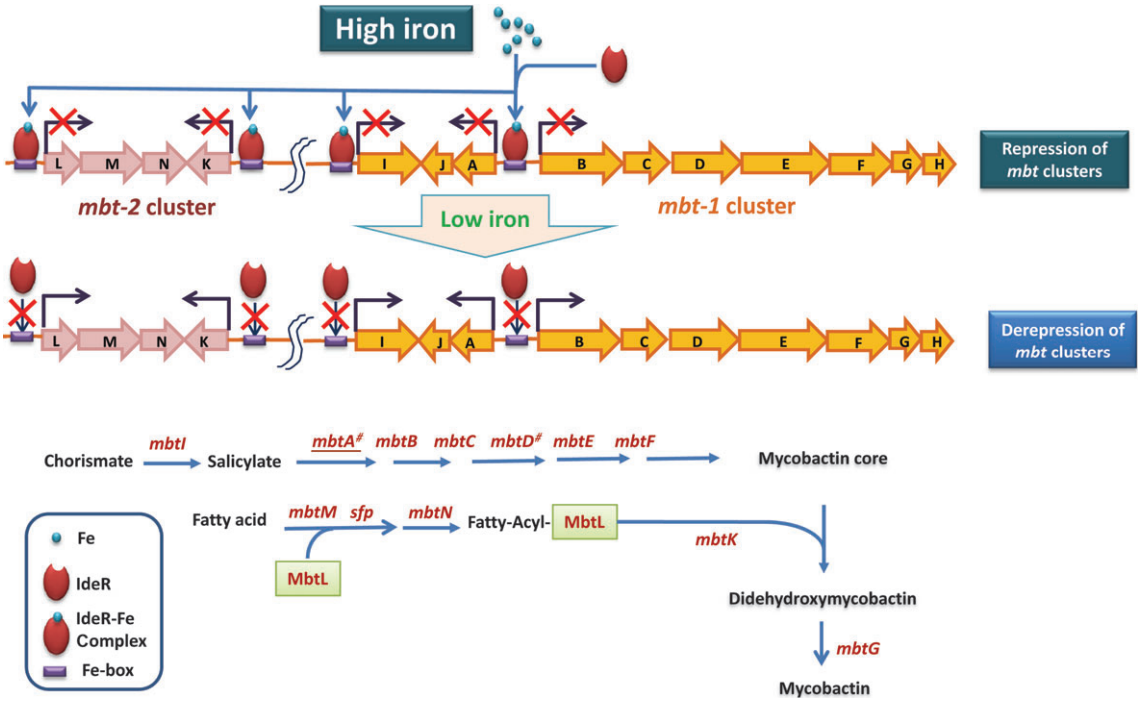

Fig. 2. Schematic diagram of iron-dependent regulatory functions of IdeR and biosynthetic pathways involved in the synthesis of mycobactin in *Mycobacterium tuberculosis*. In iron-rich environments, IdeR-Fe complexes bind to the iron-box (Fe-box) located in the promoter regions of the *mbt* clusters and repress transcription. In iron-poor conditions, IdeR-Fe complexes are not formed, resulting in derepression of the *mbt* clusters. The products from the *mbt*-1 cluster synthesize the mycobactin skeleton from chorismate. The products of the *mbt*-2 cluster synthesize fatty-acyl-ACP intermediates, which are transferred to the mycobactin skeleton to produce didehydroxymycobactin by MbtK (Rv1347c). MbtL (Rv1344) acts as an acyl carrier protein (ACP). The final hydroxylation is carried out by MbtG to generate mycobactin (adapted from De Voss *et al.*, 1999; Krithika *et al.*, 2006). The genes whose expression is found to be significantly changed both *in vivo* and *in vitro* models of virulence are marked by a hash (#) [Based on *in vitro* cell culture experiments by Schnappinger *et al.*, 2003; Rachman *et al.*, 2006a; Tailleux *et al.*, 2008; Fontán *et al.*, 2008a; Homolka *et al.*, 2010 and *in vivo* in human lung (Rachman *et al.*, 2006b) and murine lung (Talaat *et al.*, 2004)]. The genes which are known to be targets of established or experimental drugs are underlined (Raman *et al.*, 2008).

more susceptible to peroxynitrite (Master *et al.*, 2002). FbpA (=Ag85A) (Katti *et al.*, 2008), reductases like Rv3303c (an NADPH-quinone reductase) and CysH (Senaratne *et al.*, 2006) as well as cell wall components like lipoarabinomannan (Chan *et al.*, 1991) participate in defense against oxidative stress. Furthermore, the histone-like Lsr2 protein binds to bacterial DNA shielding it from damages inflicted by Reactive Oxygen Intermediates (ROI) (but not by RNI) (Colangeli *et al.*, 2009).

Mycobacterium tuberculosis deploys several other proteins that indirectly inhibit production of ROI and RNI by the host and prevent activation of macrophages by inhibiting production of proinflammatory cytokines like TNF-α, IL-12 and IFN-γ (Fig. 3). The ESAT-6 (6 kDa early secreted antigenic target) protein of *M. tuberculosis* was found to interact with the Toll-like receptor TLR2 and abrogate NF-κB signaling (Pathak *et al.*, 2007). A member of the proline-proline-glutamic acid (PPE) family proteins, PPE18, also interacts with TLR2 to inhibit production of IL-12 in macrophages (Nair *et al.*, 2009) and heat shock protein 60 (Mtbhsp60), is yet another protein that abrogates IL-12 production in macrophages (Khan *et al.*, 2008). Some cell wall components like lipoarabinomannan (Chan *et al.*, 1991), a mycolylarabinogalactan peptidoglycan complex and a 19-kDa lipoprotein (Fortune *et al.*, 2004) are also known to inhibit transcription of the IFNγ-signaling pathway in macrophages. In murine models of TB, nitric oxide (NO) plays a crucial role in anti-mycobacterial activity. However, it is controversial whether or not NO is critically involved in host defense against *M. tuberculosis* in humans (Yang *et al.*, 2009).

Transcriptional expression profiling of host–pathogen interactions

The availability of the complete genomic sequence of *M. tuberculosis* (Cole *et al.*, 1998), along with DNA microarrays, which provide a snapshot of the global gene expression profile, renew our hopes to identify new suit-

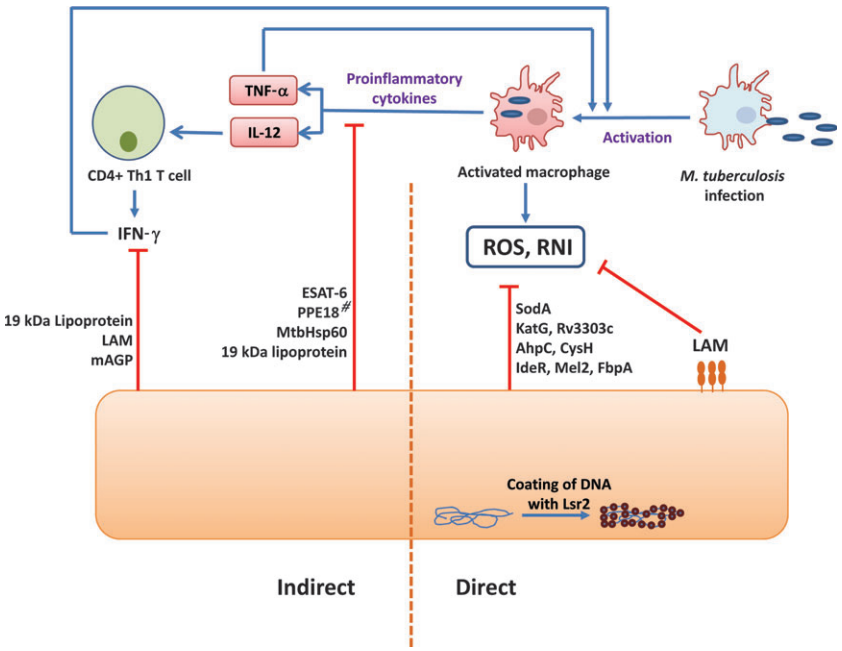

Fig. 3. Schematic diagram of the strategies employed by *Mycobacterium tuberculosis* for protection against oxidative stress. The strategies employed by *Mycobacterium tuberculosis* to counteract ROS and RNI can be broadly classified into direct and indirect mechanisms. In direct mechanisms proteins are directly involved in detoxifying or shielding the bacterial DNA from damage by free radicals, whereas in indirect mechanisms *Mycobacterium tuberculosis* inhibits production of ROS and RNI by modulating macrophage signal transduction pathways and inhibition of production of cytokines like TNF-α and IFN-γ. mAGP, mycolyl arabinogalactan; LAM, lipoarabinomannan. The genes whose expression is found to be significantly changed both *in vivo* and *in vitro* models of virulence are marked by a hash (#) [Based on *in vitro* cell culture experiments by Schnappinger *et al.*, 2003; Rachman *et al.*, 2006a; Tailleux *et al.*, 2008; Fontán *et al.*, 2008a; Homolka *et al.*, 2010 and *in vivo* in human lung (Rachman *et al.*, 2006b) and murine lung (Talaat *et al.*, 2004)].

able targets of intervention. Currently, the number of candidates in the TB drug pipeline is not sufficient to cope with the rapid emergence of multidrug-resistant strains (Casenghi *et al.*, 2007). However, there have been significant advances in understanding the molecular bases of the etiology of the disease, particularly with respect to how the host reacts to mycobacterial infection. The interaction between the pathogen and the host is a dynamic confrontation where the microorganism's survival strategy of expressing virulence factors challenges formidable defenses of the host immune system. *Mycobacterium tuberculosis* exploits and corrupts the early defense systems of the host, i.e. macrophage-mediated innate immune responses, for its survival and multiplication. Comparative analyses of mycobacterial genomes indicate that the pathogenic *Mycobacterium* spp. have acquired numerous genes that are required for virulence. *Mycobacterium tuberculosis* has 13 sigma factors and about 192 regulatory proteins, many of which are likely to enable the pathogen to establish a successful infection and subsequent persistence inside the host (Cole *et al.*, 1998). For instance, an avirulent mycobacterial species *Mycobacterium smegmatis* is predicted to possess 26 sigma factors, orthologs of the sigma factor genes *sigC*, *sigI*, and *sigK* of *M. tuberculosis* are found to be absent in the *M. smegmatis* genome (Waagmeester *et al.*, 2005), indicating that these sigma factors may be important for the virulence of *M. tuberculosis*. To understand *Mycobacterium*-specific expression signatures, e.g. in host macrophages, it is of utmost importance to consider the pathophysiology of TB. It is widely believed that many mycobacterial genes that are expressed *in vivo* may be essential for bacterial survival in the host organism. Essentially, the rationale behind identification of virulence factors through gene expression profiling assumes that virulence genes are often coordinately regulated and unknown virulence genes are likely to be co-regulated along with the known ones (Cummings & Relman, 2000). Also, genes that are specifically expressed during infection or conditions mimicking infection are likely to be candidate virulence genes (Cotter & Miller, 1998; Cummings & Relman, 2000). However, capturing the transcriptome of mycobacteria inside host cells during infection is technically challenging, because of low abundance of bacterial RNAs in the infected tissues (Lucchini *et al.*, 2001). Microarray-based transcript analyses are usually less sensitive than are reporter enzyme assays. Moreover, it is important to point out that not all mycobacterial genes that are

transcribed in macrophages, or within infected tissues, are necessarily required for virulence or for *in vivo* survival of the bacteria. In microarray-based expression data, the constitutively expressed genes should not be ignored as they might be of prime importance as well. At best, the regulated genes form a subset of key genes. Also, in such studies, the absolute levels of expression should not be ignored, induction ratios need to take the basal expression levels into account. Another caveat is that in regulons the genes might be co-regulated, but only some of them may be of crucial importance (Kendall *et al.*, 2004b). Again, *M. tuberculosis* has been found to be present in highly different states within the infected lungs and differences in their transcriptomes are likely to be obscured by isolating the mRNA pools. Only gene deletion studies can confirm that an expressed gene confers a functional phenotype in macrophages or an appropriate animal model.

Initial transcriptomic studies mostly focused on capturing the *M. tuberculosis* transcriptome under various conditions *in vitro* (reviewed in Kendall *et al.*, 2004b; Waddell & Butcher, 2007). Thus, low pH, low nutrients and free radical stress were associated with an intra-macrophage phagosomal environment, whereas hypoxia, long-term stationary phase and starvation were used to mimic *in vivo* persistence. However, these approaches are limited by our poor understanding of the nature of the microenvironment that the bacteria encounter within the cell after phagocytosis. Therefore, a snapshot of the transcriptome of invading bacteria is a more realistic approach to identify a set of genes required during infection. Schoolnik's group was the first to catch a glimpse of the *M. tuberculosis* transcriptome *in vivo* in murine bone marrow macrophages (Schnappinger *et al.*, 2003). The array data revealed that *M. tuberculosis* perceives the phagosome of naïve and activated macrophages as a fatty acid-rich, iron-poor environment containing relatively few carbohydrates, with a potential to damage DNA and the cell envelope, as the bacterium adapts to this environment by upregulating genes required for fatty acid degradation, siderophore synthesis, DNA repair and cell envelope remodeling functions. Generation of NO by IFNγ-activated macrophages triggers additional responses resulting in a phagosomal environment that is indicative of nitrosative and oxidative stress, inhibitory to aerobic respiration and conducive to increased iron uptake by the pathogen (Schnappinger *et al.*, 2003). Later on, Talaat *et al.* (2004) studied the temporal gene expression pattern of *M. tuberculosis* in immune-deficient SCID mice during the first 28 days of infection. This gene expression profile resembled that of *M. tuberculosis* grown in broth, but differed from that in infected, immunocompetent BALB/c mice. A group of 67 genes were over-expressed in BLAB/c mice, but not in SCID mice at 21 days post infection, indicating that the host immune system dictates, at least in part, the expression of genes that are required for the growth of the bacteria in the immunocompetent host. This comparative study provided a starting point to differentiate between host-responsive and phase-dependent gene expression. By combining microarray expression data with computational methods of protein network identification, Rachman *et al.* (2006a) were able to infer functions of previously uncharacterized genes and to identify signatures that are critical for *M. tuberculosis* survival *in vivo*. For example, genes involved in siderophore biosynthesis were upregulated, whereas genes coding for proteins that require iron as cofactor were repressed. Another prominent signature was reflected by the immense changes in certain cell wall components. Thus, microarray-based transcriptome profiling reveals a number of genes whose expression is upregulated in *M. tuberculosis* inside the macrophages, and leads to signature groups defined by similar functions or participation in similar pathways. However, as the array data provide a vast amount of information, it is impractical to list all differentially expressed genes. Our aim herein is to provide an overall idea of how the bacterium responds to the intracellular environment upon infection.

Lipid metabolism

Inside the macrophages, *M. tuberculosis* encounters a nutrient-poor environment resulting in the stringent control response [accompanied by altered RNA polymerase promoter selectivity (Avarbock *et al.*, 1999)], and switches to lipids as the predominant carbon source. Utilization of fatty acids for energy is supported by marked upregulation of expression of genes linked to fatty acid metabolism via the β-oxidation pathway, e.g. genes for fatty acid-CoA synthase (*fadD3*, *fadD9*, *fadD10*, *fadD19*), acyl-CoA dehydrogenase (*fadE5*, *fadE14*, *fadE22-24*, *fadE27-29*, *fadE31*), enoyl-CoA hydratase (*echA19*), hydroxybutyryl-CoA dehydrogenase (*fadB2*, *fadB3*), and acetyl-CoA transferase (Schnappinger *et al.*, 2003; Waddell & Butcher, 2007). In addition, multiple genes (*icl*, *gltA1*, *rv1130*) are highly expressed that are required for subsequent utilization of the degradation products via the citric acid cycle and the glyoxylate shunt (Schnappinger *et al.*, 2003). Isocitrate lyase (*icl*) is essential for mycobacterial persistence (McKinney *et al.*, 2000). In pulmonary TB, expression of *fadD30*, *fadD32*, *fadE2*, *fadE26* was upregulated (Rachman *et al.*, 2006b). In addition, upregulation of a phosphoenol-pyruvate kinase (*pckA*) implies that fatty acids can be converted to sugars via gluconeogenesis (Schnappinger *et al.*, 2003). Rengarajan *et al.* (2005) found that some of

β-oxidation pathway genes (*fadE28*, *fadE29*) were also essential for survival of the bacteria inside the macrophages. The expression of the *icl* and *pckA* genes was also upregulated in the mouse lung after infection (Talaat *et al.*, 2004), along with other fatty acid metabolism genes such as *fadD10*, *fadD19*, *fadD34*, *fadE1*, *fadE10*, and *fadE24* (Karakousis *et al.*, 2004). The importance of the β-oxidation pathway genes was further underscored by the transcription profile of *M. tuberculosis* in human macrophage-like THP-1 cells (Fontán *et al.*, 2008a). Similarly, a number of fatty acid metabolism genes like *fadD9*, *fadD21*, *fadD26*, *fadE5*, *fadE22*, *fadE26*, *fadE28*, *fadE29* were found to be upregulated in several clinical stains of *M. tuberculosis* inside murine bone marrow-derived marcrophages (Homolka *et al.*, 2010). The *mymA* operon, which is expressed upon exposure to low pH (Fisher *et al.*, 2002), was induced in THP-1 cells (Fontán *et al.*, 2008a), suggesting that a low pH may trigger a switch to fatty acids as an energy source. In conclusion, the pattern of fatty acid metabolism-related gene expression appears to be common to most of the infection models tested and to reflect a fundamental adaptation to intracellular growth. Indeed, many FadD and FadE proteins were found to be present in the *M. tuberculosis* proteome from guinea-pig lung granuloma, suggest that the bacteria are able to breakdown host lipids to utilize them as nutrients (Kruh *et al.*, 2010).

Broad transcription regulators

Transcriptional regulators play vital roles in controlling interactions of *M. tuberculosis* with the host. Eukaryotic-like protein kinases (Pkns) such as PknB (Rv0014c) and PknL (Rv2176) belonging to the protein serine/threonine kinase family were upregulated in bacteria living in activated macrophages (Rachman *et al.*, 2006a). Of the 11 Pkns, at least eight including PknA, PknB, and PknG were detected in the *M. tuberculosis* proteome in the lung granuloma of guinea-pig (Kruh *et al.*, 2010). Among the 13 sigma factors of *M. tuberculosis* (Cole *et al.*, 1998), which allow the bacterium to adapt to changing environments, some are important for virulence, as shown by mutant analysis and complementation (Smith, 2003; Manganelli *et al.*, 2004a; Rodrigue *et al.*, 2006). Importantly, expression of *sigE*, a member of the extracytoplasmic functions group of sigma factors, which control the bacterial response to external stimuli, was upregulated in *M. tuberculosis* after phagocytosis (Manganelli *et al.*, 2001; Schnappinger *et al.*, 2003). SigE regulates the expression of genes that are important for maintenance of the cell envelope, which help the mycobacteria cope with environmental stress and the antibacterial responses of the host (Fontán *et al.*, 2008b). A *sigE* mutant was attenuated *in vitro* and in mice (Manganelli *et al.*, 2001, 2004b) and seems to be a very promising live attenuated vaccine candidate (Hernandez Pando *et al.*, 2010). By microarray analysis, it was realized that SigD also plays an important role in regulating *M. tuberculosis*. In a mouse model of infection, a Δ*sigD M. tuberculosis* mutant showed a moderate, but significant decrease in virulence and several members of the PE_PGRS (PE proteins containing polymorphic GC-rich repetitive sequences) family proteins were upregulated (Raman *et al.*, 2004). In a similar experiment, the requirement of SigD for virulence in mice was confirmed (Calamita *et al.*, 2005).

Furthermore, the *sigB*, *sigG*, and *sigH* transcripts were upregulated inside human macrophages (Graham & Clark-Curtiss, 1999; Cappelli *et al.*, 2006), and SigF and SigL may also be necessary for successful intracellular survival (Chen *et al.*, 2000; Li *et al.*, 2004; Dainese *et al.*, 2006). The *sigL* gene is co-transcribed with *rv0736*, which encodes a membrane protein acting as a putative anti-sigma factor for SigL. Microarray analyses have revealed that the *sigL* regulon includes genes like *sigL* itself, *mpt-53*, *pks-7*, and *pks-10* (Hahn *et al.*, 2005). Interestingly, a *pks-10* mutant, which is defective in phthiocerol dimycoserate, was significantly attenuated for growth in mice (Sirakova *et al.*, 2003a).

Upregulation of a number of two-component signal transduction systems *in vivo* includes the DosR (also known as devR) which is comprised of a membrane-bound histidine kinase sensor DosS (also known as DevS) and a cytoplasmic response regulator (DosR). DosR is required for the expression of genes that are induced during hypoxia (Park *et al.*, 2003) and in response to nitric oxide (Voskuil *et al.*, 2003) which are frequently associated with the onset and maintenance of latent TB. DosR regulon is induced in macrophages (Schnappinger *et al.*, 2003) as well as in early (Karakousis *et al.*, 2004) and late mouse infections (Shi *et al.*, 2003). The initial hypoxic response controlled by DosR features powerful induction of as many as 48 genes including *hspX* (Stewart *et al.*, 2002). Interestingly, many of the DosR-regulated genes are conserved hypothetical proteins with as yet still unknown products and functions. Only few genes have been designated a function such as *narX*, *narK2*, *fdxA*, *hspX*, *pfkB* etc. (Lin & Ottenhoff, 2008). The DosS appears to function as both an oxygen sensor and redox sensor and responds to a reduced electron transport system to induce the DosR regulon (Honaker *et al.*, 2010). In addition, a wide network of transcription regulator genes (*ntrA*, *regX3*, *phoP*, *prrA*, *mprA*, *kdpE*, *trcR*, and *trcX)* is expressed in *M. tuberculosis* upon infection (Zahrt & Deretic, 2001; Haydel & Clark-Curtiss, 2004) and understanding this network will hopefully advance our knowledge about the pathogenicity of mycobacteria.

Molecular chaperones

The DNA array results have revealed high expression of genes involved in detoxification and chaperone activity in pulmonary TB, indicating highly toxic and stressful conditions at the infection sites. Some chaperones, in addition to their function as stress proteins, are potential virulence determinants and play roles in modulating host immune responses (Lewthwaite *et al.*, 2001; Khan *et al.*, 2008). One such chaperone, the heat shock protein 60 of *M. tuberculosis* (Mtbhsp60), was overexpressed in infected macrophages (Zügel & Kaufmann, 1997; Monahan *et al.*, 2001) and probably offers a survival advantage within the host (Hu *et al.*, 2008). Mtbhsp60 favors the development of the T helper 2 (Th2)-type response by stimulating expression of TLR2 on macrophages (Khan *et al.*, 2008). Chaperone-related genes of *M. tuberculosis* (*groEL/ES*, *suhB*, *dnaJ1/2*, *dnaK*, *hspR*, *hspX*) were highly expressed during infection of the human lung (Rachman *et al.*, 2006b). Similarly, the *hspX*, *htpX*, and *htpG* genes were also over-expressed during infection of THP-1 cells (Fontán *et al.*, 2008a). *Mycobacterium tuberculosis* mutants lacking *hspX* failed to grow in THP-1 cells or primary mouse bone-marrow-derived macrophages (Yuan *et al.*, 1998).

Iron uptake

Mycobacterium tuberculosis overcomes poor availability of intracellular iron by expressing a set of genes required for sequestration and storage of iron. Some of the genes that are induced in a low-iron medium (e.g. the *mbt* operon) overlap with genes induced in IFN-γ-stimulated macrophages (Rodriguez & Smith, 2003; Rodriguez, 2006). One of these genes, *mbtB*, was upregulated upon infection in murine lungs (Timm *et al.*, 2003), whereas, expression of the bacterioferritin gene *brfA* was down-regulated. In immunocompromised mice, at 21 days post infection, several genes for iron uptake-related proteins (*mbtD*, *hupB*, and *fdxA)* were upregulated (Talaat *et al.*, 2004) and in THP-1 macrophages other iron-regulated genes under IdeR control (*mbtB*, *mbtI*, and *rv3402c*) were also found to be overexpressed (Gold *et al.*, 2001). These data indicate that *M. tuberculosis* induces a number of genes to cope with the iron-poor intracellular environment. By contrast, a *M. tuberculosis zur* (=*furB*) mutant, which lacks a zinc uptake regulator, failed to show a discernable phenotype, possibly due to a redundant mechanism controlling zinc uptake (Maciag *et al.*, 2007). Recently, a type VII secretion system, ESX-3 (whose expression is under Zur and IdeR transcriptional regulation), has been found to be involved in iron and zinc uptake (Serafini *et al.*, 2009; Siegrist *et al.*, 2009). Mutant data suggest that *esx-3* is required for growth of *M. tuberculosis* in iron limiting conditions *in vitro* (Serafini *et al.*, 2009). *Esx3* was also found to be essential for growth both *in vitro* and in macrophages in *Mycobacterium bovis*. ESX-3 appears to be a specialized secretion system required for mycobactin-mediated iron acquisition (Siegrist *et al.*, 2009).

Secretory proteins

Some of the *M. tuberculosis* proteins secreted in the phagosome of infected cells are highly immunogenic. The most prominent proteins belong to the ESAT-6 (6 kDa early secreted antigenic target) family. They are recognized by sera from a large number of TB patients (Andersen *et al.*, 1992). A subunit vaccine made by fusion of ESAT-6 with antigen 85B gave a protective effect in mice (Olsen *et al.*, 2004). Some of the ESAT-6 family member proteins like the products of the *esxH*, *esxO*, and *esxV* genes were expressed in the murine lung (Dubnau *et al.*, 2005).

PE/PPE proteins

The *M. tuberculosis* genome sequence reveals the presence of two gene families, PE and PPE (Cole *et al.*, 1998), which are unique to mycobacteria and are highly expanded in several pathogenic species such as *M. tuberculosis* and *Mycobacterium marinum* (Gey van Pittius *et al.*, 2006). Some of the PE and PPE genes are associated with the ESAT-6 gene cluster region (ESX), which is predicted to encode a novel type VII secretory apparatus (Abdallah *et al.*, 2006; Abdallah *et al.*, 2007; Gey van Pittius *et al.*, 2006). By comparing the secretomes of a *M. marinum esx-5* mutant to that of the wild type, Abdallah *et al.* (2009) found that a number of PPE and PE proteins were dependent on ESX-5 for transport (Abdallah *et al.*, 2009). The *M. marinum* ESX-5 secretion system suppresses TLR-dependent innate cytokine secretion in human macrophages (Abdallah *et al.*, 2008). Expression profiling of *M. tuberculosis* revealed differential regulation of 128 of the 169 PE/PPE genes, suggesting their roles in generating antigenic variations during the course of changing microenvironments within the host (Voskuil *et al.*, 2004). The PE_PGRS protein Rv1818c (PE_PGRS33) was expressed at all sites of pulmonary TB (Rachman *et al.*, 2006b). This observation corresponds well with the immunodominant nature of this protein (Delogu & Brennan, 2001). While the PGRS domain of Rv1818C elicited a strong antibody response, the PE domain conferred protection in a mouse model (Delogu & Brennan, 2001). The PGRS domain of this protein also affected the cell morphology of the bacterium, while the PE domain was necessary for the sub-cellular localization of the protein (Delogu *et al.*, 2004). In another PE_PGRS

protein (Rv1759c), the PGRS domain elicited a strong immune response, which could prevent reactivation in a murine model of chronic TB (Campuzano *et al.*, 2007). According to microarray data, at least one of the PE proteins (Rv2519, PE26) is regulated by SigE in human macrophages (Fontán *et al.*, 2008b).

Using an *in vivo* expression technology approach, which identified macrophage-specific gene expression, Srivastava *et al.* (2007) found one of the PE/PPE genes, *rv3097c* (PE_PGRS63), to be the most highly expressed gene as early as 24 h post infection. The promoters of several other PE/PPE genes (*rv0977*, *rv1361c*, *rv1840c*) were upregulated in THP-1 macrophages (Dubnau *et al.*, 2002). Yet another PPE protein, Rv1168c, was overexpressed under microaerophilic and anaerobic conditions (Bacon *et al.*, 2004; Muttucumaru *et al.*, 2004), nutrient starvation (Betts *et al.*, 2002), and in the presence of palmitic acid (Schnappinger *et al.*, 2003), i.e. conditions that mimic the features of an intraphagosomal environment. In a transposon site hybridization (TraSH) assay, a microarray-based technique, mutations in three genes belonging to the PE/PPE family (*rv1807*, *rv3872*, *rv3873*) had growth-attenuating effects (Sassetti & Rubin, 2003). Failure of mutations in other PE/PPE genes to produce a phenotype can probably be attributed to functional redundancy of these genes. Recently, the genes for PE13 and PPE18 have been shown to be part of the *rv0485* regulon, which encodes a putative transcription regulatory protein. Mutation studies indicate that Rv0485 is required for *M. tuberculosis* virulence (Goldstone *et al.*, 2009). In conclusion, although the physiological functions carried out by the PE/PPE proteins are not well understood, they appear to contribute to mycobacterial pathogenesis (Basu *et al.*, 2007; Nair *et al.*, 2009). Interestingly, analysis of the *M. tuberculosis* proteome in the lungs of guinea-pigs revealed that PE/PPE proteins are the third most abundant category and showed the most consistent expression during the infection. The PE-PGRS53/54, and PE-PGRS56/57, and PPE38 proteins were found to be among the ten most dominant proteins at 90 days postinfection (Kruh *et al.*, 2010).

DNA damage repair enzymes

A number of genes for DNA repair enzymes (*alkA*, *recX*, *recC*, *dinF*, and *radA*) were upregulated in the intra-phagosomal environment of both naïve and activated murine macrophages. In human macrophages (Graham & Clark-Curtiss, 1999) and human lungs (Rachman *et al.*, 2006b), *uvrA* and *dinX*, *dinF* and *gyrAB*, respectively, are examples of highly expressed genes. Induction of DNA damage in macrophages may involve NO-dependent and -independent mechanisms (Schnappinger *et al.*, 2003).

Cell wall synthesis

The mycobacterial cell wall lies at the interface of the mycobacteria–host interaction through which the mycobacteria may transduce specific signals that may be required to communicate with the host during infection. Experiments with mutant strains clearly indicate that cell wall integrity is critical for *M. tuberculosis* to survive intracellularly, to persist and to develop resistance against antibiotic drugs (Berthet *et al.*, 1998; Gao *et al.*, 2006). Consequently, a number of mycobacterial genes involved in lipid biosynthesis and cell wall remodeling were found to be required for growth in mice (Sassetti & Rubin, 2003) and their expression was upregulated in the phagosome of infected mice and in human lung granulomas (Schnappinger *et al.*, 2003; Rachman *et al.*, 2006b). The induced genes (e.g. *desA1*, *desA3*, *umA2*) are likely to be involved in the maintenance of the hydrophobic nature of the cell wall barrier during macrophage infection (Waddell & Butcher, 2007). Genes like *uma-2*, *desA3*, and *mmaA3* were predicted to be required for survival *in vivo* (Sassetti & Rubin, 2003) and an *mmaA4* mutant was attenuated *in vivo* (Dubnau *et al.*, 2000). In THP-1 cells, genes (e.g. *drrB*, *ppsA*, *ppsB*, and *pks-11*) involved in phthiocerol dimycocerosate synthesis and transport to the cell surface (Fontán *et al.*, 2008a) as well as genes (e.g. *cpsY* and *rmlB2*) that are required for the synthesis of galactofuran, an essential link between peptidoglycan and mycolic acids, were upregulated (Weston *et al.*, 1997). The *rmlB2* gene, which codes for a putative galactose epimerase, is under the control of *sigE* (Fontán *et al.*, 2008b). *In vivo* proteomic data were able to identify a total of nine PKSs including PKS4-9, 13, 15, and 17 in guinea-pig lung granuloma. Interestingly, unlike microarray data, where some genes were found to be upregulated 2 h after exposure to lung surfactants, proteomic data suggest their presence throughout the *in vivo* growth, e.g. PpsA-D, Mas, PapA5, DrrA, and MmpL7 (Kruh *et al.*, 2010). Since some of the lipids and cell wall components *M. tuberculosis* are known to modulate host immune responses (Barry, 2001), remodeling the cell wall components could be one of the important strategies employed by *M. tuberculosis* to persist.

Response to oxidative stress

Upon infection, macrophages produce a range of effector molecules to destroy the invading pathogen, i.e. ROS, NO, and NO-derived species such as NO_2, NO_2^-, N_2O_3, N_2O_4, S-nitrosothiols, and peroxynitrite ($ONOO^-$) (Chan *et al.*, 1992; Khan *et al.*, 2006). Upon *M. tuberculosis* infection, macrophages produce this oxidative burst, but it does not seem to affect the viability of the pathogen

(Chan *et al.*, 1992). It is therefore likely that *M. tuberculosis* elicits appropriate protective responses to overcome these challenges. In fact, Schnappinger *et al.* (2003) identified 68 genes that were preferentially induced in IFNγ-activated macrophages after infection, compared to the naïve murine macrophages. Nearly half of these genes are part of the DosR regulon and are also induced under hypoxic conditions (Bacon *et al.*, 2004) and after NO or hydrogen peroxide treatment (Voskuil *et al.*, 2003). Many of these genes were not induced in *NOS2*-deficient macrophages (Schnappinger *et al.*, 2003). Using mutants and microarrays, it was found that the *acr-2* (*rv0251c*) gene, which encodes a member of the α-crystallin protein family, was the most strongly upregulated gene following heat shock (Stewart *et al.*, 2002) and in IFN-γ-activated macrophages (Schnappinger *et al.*, 2003). The *acr-2* gene is a paralog of the DosR-controlled *hspX* (*rv2031c*) gene (Yuan *et al.*, 1998; Sherman *et al.*, 2001; Purkayastha *et al.*, 2002; Florczyk *et al.*, 2003), which is a prominent member of the hypoxia/NO regulon and which has been implicated to contribute to *M. tuberculosis* persistence (Sherman *et al.*, 2001; Voskuil *et al.*, 2003).

Mycobacteria respond to environmental stresses through a variety of sigma factors that transcribe specific sets of genes required for survival, and mutation analyses have established that several sigma factors are essential for virulence (Smith, 2003). SigH is a central regulator of the responses to oxidative, nitrosative, and heat stresses in *M. tuberculosis* (Raman *et al.*, 2001) and is expressed during macrophage infection (Graham & Clark-Curtiss, 1999). *Mycobacterium tuberculosis sigH* mutants display impaired ability to survive under oxidative or heat stress (Raman *et al.*, 2001; Manganelli *et al.*, 2002). Microarray analyses conducted in *M. tuberculosis* identified genes encoding stress-responsive transcriptional regulators (*sigH*, *sigB* and *sigE*), genes involved in thiol metabolism (*trxB* and *trxC*), *rv0142* (for a putative transcriptional regulator), and *dnaK* and *clpB* (for heat shock proteins) (Kaushal *et al.*, 2002; Manganelli *et al.*, 2002).

Inhibition of apoptosis

Induction of programed cell death or apoptosis is an important aspect of the host innate immune defense, which is conserved among the animal and plant kingdoms (Iriti & Faoro, 2007) and where the infected cells are sacrificed for the benefit of the remaining cells. Virulent strains of *M. tuberculosis* induce considerably less apoptosis than do avirulent strains in infected macrophages (Keane *et al.*, 2000). Thus, the capacity of *M. tuberculosis* to inhibit apoptosis is proposed to be a virulence trait (reviewed in Briken & Miller, 2008). Using a 'gain-of-function' genetic screen, Velmurugan *et al.* (2007) identified a *M. tuberculosis* anti-apoptopic gene, *nuoG*, which encodes a subunit of the type I NADH dehydrogenase. Over-expression of *nuoG* in apoptosis-inducing *Mycobacterium kansasii*, conferred the ability to inhibit apoptosis in infected human and murine macrophages. Conversely, deletion of *nuoG* in *M. tuberculosis* ablated its ability to inhibit apoptosis in macrophages (Velmurugan *et al.*, 2007). In addition to *nuoG*, two other *M. tuberculosis* genes, *secA2*, and *pknE*, have been implicated in the inhibition of host cell apoptosis (Hinchey *et al.*, 2007; Jayakumar *et al.*, 2008). The *secA2* gene encodes a mycobacterial secretion system that mediates secretion of superoxide dismutase (SodA) among other proteins. PknE is a serine-threonine kinase induced during NO stress. A *M. tuberculosis pknE* mutant was found to be more susceptible to NO exposure and also capable of inducing a higher level of apoptosis in human macrophages (Jayakumar *et al.*, 2008).

Mycobacterium tuberculosis can inhibit the intrinsic pathway of host cell apoptosis by upregulating host anti-apoptosis genes like *mcl-1* (Sly *et al.*, 2003) and *A1* (Kremer *et al.*, 1997; Kausalya *et al.*, 2001), both of which encode Bcl-2 (B-cell lymphoma-2) like proteins. Upregulation of *A1* mRNA was not observed in macrophages infected with avirulent *M. tuberculosis* H37Ra (Dhiman *et al.*, 2008). Another anti-apoptosis protein, Bcl-w, was present in increased concentrations in cells infected with virulent *M. tuberculosis* H37Rv, but not with *M. tuberculosis* H37Ra (Spira *et al.*, 2003), while Bad, a pro-apoptosis protein, was inactivated following *M. tuberculosis* H37Rv infection (Maiti *et al.*, 2001). *Mycobacterium tuberculosis* also inhibits the extrinsic apoptosis pathway in infected macrophages by decreasing the expression of death receptors such as Fas (CD95) (Oddo *et al.*, 1998) and increasing the secretion of the soluble TNF receptor 2 (sTNFR2) (Balcewicz-Sablinska *et al.*, 1998).

In different mouse strains, mycobacterial infection was found to be associated with the capacity of infected macrophages to undergo either necrosis or apoptotic cell death, with necrosis imparting a susceptible phenotype and apoptosis a resistant phenotype (Pan *et al.*, 2005). This view is corroborated by the finding that virulent *M. tuberculosis* H37Rv induces a higher rate of lung macrophage necrosis than does attenuated H37Ra (Gan *et al.*, 2008). *Mycobacterium tuberculosis* can manipulate the surface of infected macrophages to favor a necrotic outcome rather than apoptotic cell death (Lee *et al.*, 2006). In macrophages infected with virulent *M. tuberculosis* H37Rv, but not with avirulent *M. tuberculosis* H37Ra, the amino-terminal domain of annexin-1 is removed by proteolysis, preventing the formation of the apoptotic envelope (Gan *et al.*, 2008). Similarly, virulent *M. tuberculosis* induces necrosis in infected macrophages by inhibiting

membrane repair mechanisms. By contrast, in the presence of the attenuated strain, plasma membrane microdisruptions are resealed by repair mechanisms, resulting in apoptosis of the infected macrophages (Divangahi *et al.*, 2009).

These studies suggest a dichotomy between apoptosis and necrosis in controlling *M. tuberculosis* infection, wherein apoptosis is clearly beneficial to the host in that the *M. tuberculosis* replicative niche is destroyed, whereas necrosis promotes dissemination and spread of infection. The success of initial infection by virulent *M. tuberculosis* strains depends on their capacity to actively suppress apoptosis of the host macrophage (reviewed in Lee *et al.*, 2009). When apoptosis predominates, infection may be controlled. By contrast, when *M. tuberculosis* is successful in suppressing apoptosis of the macrophages, the bacteria may proliferate to large numbers and subsequently trigger a necrotic mode of macrophage cell death, releasing them to infect new host cells and ultimately to grow as extracellular pathogens in necrotic cavities (Park *et al.*, 2006). Therefore, from a clinical perspective, the anti-apoptosis gene products of *M. tuberculosis* may constitute new drug targets and the corresponding genes may also be promising targets for improving existing and developing new attenuated live vaccine strains (Briken & Miller, 2008).

Comparison between DCs and macrophages as host cells

Tailleux *et al.* (2008) analyzed mycobacterial transcripts in both human DCs and human macrophages as host cells. Both cell types are central to anti-mycobacterial immunity, yet they play distinctive roles during the infection process. Macrophages basically act as sentinel cells that engulf foreign particles by active phagocytosis and mainly play the role of a scavenger. These cells are poor activators of naïve T cells compared to DCs, which play a major role in eliciting an adaptive immune response by processing and presenting the antigens to the naïve lymphocytes and secretion of cytokines (Mbawuike & Herscowitz, 1988; Mehta-Damani *et al.*, 1994; Olazabal *et al.*, 2008). Interestingly, mycobacteria respond differently to these two types of cells, in addition to expressing a common set of genes. The DC phagosome was perceived as a more constraining environment as compared to the macrophage phagosome, reflected by a greater number of stress-responsive genes expressed in DCs.

The core set of genes that is expressed by mycobacteria in DCs corresponds to that expressed in macrophages (Schnappinger *et al.*, 2003; Cappelli *et al.*, 2006; Rachman *et al.*, 2006a; Waddell & Butcher, 2007; Tailleux *et al.*, 2008). These traits include a switch to lipids as the energy source characterized by high expression of genes involved in the β-oxidation pathway like fatty acid-CoA synthase (*fadD3*, *fadD9*), acyl-CoA dehydrogenase (*fadE5*, *fadE14*, *fadE24*, *fadE28*, *fadE30*, *fadE33*, *fadE34*), enoyl-CoA hydratase (*echA6*, *echA7*, *echA12*, *echA19*, *echA20*), hydroxybutyryl-CoA dehydrogenase (*fadB2*), the glyoxylate shunt (*icl*) (McKinney *et al.*, 2000), gluconeogenesis (*gltA1*, *pckA*) (Schnappinger *et al.*, 2003) and cholesterol metabolism, which includes a cluster of 42 genes (Van der Geize *et al.*, 2007). The DosS-DosR system, which regulates coordinated expression of genes in response to hypoxia and free radicals (Kendall *et al.*, 2004a; Roberts *et al.*, 2004; Shi *et al.*, 2005), was induced in mycobacteria infecting both DCs and macrophages. Genes required for iron sequestration (*mbtB*, *mbtD*, *mbtE*, *mbtF*, *mbtI*, *mbtJ*) were also upregulated. Interestingly, several genes encoding enzymes for polyketide synthesis (*papA1*, *papA3*, *pks-2*, *pks-3*, and *pks-4*) were down-regulated in both DCs and macrophages.

Many genes that were more strongly expressed in DCs than in macrophages have also been identified to be associated with dormancy *in vivo* (Karakousis *et al.*, 2004), nutrient starvation (Betts *et al.*, 2002), limiting oxygen (Bacon *et al.*, 2004) or slow replication (Beste *et al.*, 2007). On the other hand, some genes encoding ribosomal proteins (*rplB*, *rplF*, *rplN*, *rpsF*, *rpsN*), DNA biosynthetic proteins (*dnaB*, *dnaN*), *fusA* (elongation factor G and enzymes of phthiocerol dimycocerosate biosynthesis (*papA5*, *ppsC*, *pks-1*, *pks-15*, *fadD22/fadD29*) and export (*drrB-C*, *lppX*) were more weakly expressed in DCs than in macrophages (Tailleux *et al.*, 2008). These data suggest that DCs are more efficient in restricting mycobacteria to nutrients.

Drug-induced alteration of the mycobacterial transcriptome

Since the components of a multi-enzyme pathway are often coregulated in response to cofactors, intermediates or products of the same pathway, compounds including drugs that selectively inhibit a specific pathway enzyme and cause accumulation of precursors and depletion of products, are expected to induce changes in the expression profile of the genes in the affected pathway. The resulting gene expression profile not only serves as a signature of the specific inhibitors used, but may also help to identify the target of an inhibitor whose mode of action is unknown (Wilson *et al.*, 1999). By comparing the expression profiles of drug-sensitive and drug-resistant mycobacteria it may be possible to uncover novel mechanisms of drug resistance (Waddell & Butcher, 2010).

Wilson *et al.* (1999) studied the gene expression profile of *M. tuberculosis* during its exposure to isoniazid (INH),

a drug that is activated by the bacteria and then blocks the mycolic acid biosynthesis pathway by inhibiting the InhA enzyme. INH treatment resulted in the induction of several genes that are physiologically relevant to the mode of action of the drug, including an operonic cluster of five genes encoding type II FAS enzymes (FAS-II) and *fbpC*, which encodes trehalose dimycolyl transferase (Wilson *et al.*, 1999). Other INH-induced genes, *efpA*, *fadE23*, *fadE24*, and *ahpC*, are likely to mediate processes that are linked to toxic consequences of the drug. Interestingly, *efpA* encoding a putative efflux system may play a role in intrinsic drug resistance (Wilson *et al.*, 1999). These observations were corroborated by Fu (2006). Exposure of *M. tuberculosis* to ethionamide, another drug that targets *inhA*, elicited a response pattern that was similar to that of INH (Wilson *et al.*, 1999; Fu, 2006), but alterations in the expression of some genes were found to be specific for the drug used. Thus, drugs that share a similar mode of action may still be distinguished on the basis of their transcriptome signature.

Expression profiling of *M. tuberculosis* treated with drugs also allows a better understanding of mechanisms of action and of genes that confer resistance to a particular drug. For example, the *sigE* regulator was found to confer basal resistance to vancomycin in *M. tuberculosis* (Provvedi *et al.*, 2009). Similarly, it has been possible to uncover the mechanism of action of PA-824, a novel prodrug that is effective against both replicating and hypoxic, nonreplicating *M. tuberculosis*. Treatment of aerobically replicating cells with PA-824 is known to rapidly disrupt the formation of ketomycolates with concomitant accumulation of hydroxymycolates (Stover *et al.*, 2000), a class of mycolic acids that are major constituents of the cell envelope. However, this effect seemed unlikely to be responsible for cell killing under nonreplicating conditions since the bacilli do not extensively remodel mycolic acids under anaerobiosis. Transcriptional profiling suggests that PA-824 which acts as an NO donor causes respiratory poisoning in nonreplicating cells. The released NO possibly reacts with cytochromes/cytochrome oxidase to interfere with the electron flow and ATP homeostasis under hypoxic, nonreplicating conditions. However, like cyanide, NO-releasing effect of PA-824 is not sufficient to kill aerobically replicating cells because NO-mediated inhibition of cytochrome *c* oxidase is reversible in the presence of oxygen (Manjunatha *et al.*, 2009). Similarly, the candidate drug thioridazine, intended for the therapy of MDR- and XDR-TB, was found to modulate the expression of genes encoding membrane proteins, efflux pumps, oxido-reductases, enzymes of fatty acid metabolism, and anaerobic respiration. This study underscores the importance of the *M. tuberculosis* sigma factor network (*sigH*, *sigE*, and *sigB*) in protecting the pathogen against cell-envelope damage (Dutta *et al.*, 2010). Among the known anti-TB drugs, only capreomycin exhibits bactericidal effect against nonreplicating *M. tuberculosis* in anaerobic conditions *in vitro* (Heifets *et al.*, 2005). Capreomycin is known to interfere with translation in mycobacteria and is structurally similar to viomycin which binds to both 50S and 30S ribosomal subunits (Maus *et al.*, 2005). Genome-wide exploration genes related to capreomycin action not only validated the specific molecular target, 16S rRNA, but also led to identification of novel drug targets that included genes operating at the DNA levels such as Rv0054 (*ssb*), Rv3715c (*recR*) as well as genes involved in cell division such as Rv3260c (*whiB2*). In addition, the *nuo* gene cluster and the ATP synthase gene cluster are repressed (Fu & Shinnick, 2007).

However, there are caveats concerning transcriptomic data which primarily identify genes that are subject to transcriptional regulation. For example, the *inhA* gene expression level was found to remain unaltered in response to INH treatment in both studies discussed above (Wilson *et al.*, 1999; Fu, 2006), although InhA is one of the targets of INH. High-level expression of transcripts *in vivo* does not necessarily reflect essentiality for virulence. Some highly expressed genes are also redundant, e.g. the Fbp proteins. Thus, gene expression data alone do not necessarily suffice to identify new drug targets, but can reveal pathways involved in pathogenicity and possible mechanisms of drug action (Fu, 2006).

Impact of DNA microarray studies on the understanding of virulence mechanisms and drug discovery

Whole-genome microarrays, which profile the transcriptional responses of *M. tuberculosis* under different conditions *in vitro* and *in vivo,* generate vast data sets, and screening of such enormous data to identify and select potential drug or vaccine candidates becomes a daunting task. However, integration of microarray data, gene essentiality studies, and information on cellular and humoral immune response may eventually help us prioritizing vaccine and drug target candidates in *M. tuberculosis* (Verkhedkar *et al.*, 2007). For instance, Balázsi *et al.* (2008) identified sets of transcriptional subnetworks (origons) that are required early and late during adaptation of *M. tuberculosis* to hypoxia and stationary phase. The *dosR* origon emerged as the most consistent early and transient responder, whereas the *sigD*, *hrcA*, and *rv0494* origons were condition-dependent initiators of growth arrest. The late responder *nadR*, *sigE*, *sigC*, and *furB* origons, which may be required for maintenance of dormancy, appear to be specifically associated with growth arrest *per se* (Balázsi *et al.*, 2008). Attempts have also been made to integrate

genomic expression data obtained under various experimental conditions along with genome annotation data and analysis tools on a single platform (Raman *et al.*, 2008; Reddy *et al.*, 2009; The Tuberculosis Database (TBDB) (http://www.tbdb.org/).

The observation that *M. tuberculosis* elicits differential gene expression programs in macrophages vs. DCs (Tailleux *et al.*, 2008), indicates an extraordinary plasticity of mechanisms involved in *M. tuberculosis* host-interactions, probably resulting from long co-evolution. So far, the majority of the microarray data is available for macrophages, but is limited for DCs, which are considered to be important for immune evasion by *M. tuberculosis* (Mortellaro *et al.*, 2009). In macrophages, *M. tuberculosis* displays a gene transcription profile that is consistent with an active multiplication phenotype as there is an increased transcription of ribosomal genes. By contrast, in DCs the *M. tuberculosis* transcriptome reflects a non-replicating phenotype and a stress response similar to that found under conditions of nutrient limitation, oxygen deprivation or dormancy (Tailleux *et al.*, 2008). The inability of *M. tuberculosis* to replicate inside DCs probably confers an advantage to the bacteria, because quick replication inside the DCs would lead to destruction of these cells and would induce too strong an inflammatory response, which could compromise the survival of the bacteria. Therefore, strategies that would make the DCs more replication-permissive could possibly mount a strong immune response against *M. tuberculosis* (Mortellaro *et al.*, 2009). Finally, dual microarray studies, which we have not discussed herein, offer excellent opportunities to link the transcriptional responses of mycobacteria to those of their host cells and therefore can shed some light on the host-pathogen interactome as a whole.

Have gene expression profiling efforts made so far led to the identification of potential new drug targets? There are several examples which support the idea that such information can be exploited to find new anti-TB drugs and to design more effective analogs of existing drugs. The *desA3* gene, which codes for linoleoyl-CoA desaturase and is upregulated in human lung granuloma (Rachman *et al.*, 2006b), was found to be a target of an anti-TB thiourea drug isoxyl (Phetsuksiri *et al.*, 2003). Thiolactamycin and analogs target β-keto-acyl-carrier protein synthase encoded by *kasA* and *kasB* (Kremer *et al.*, 2000). Recently, platensimycin has been found to be active against KasA and KasB and is an exciting lead compound against *M. tuberculosis*, with potential development of novel synthetic analogs (Brown *et al.*, 2009). The *mma-4* gene product, which is known to be upregulated in human lung granuloma (Rachman *et al.*, 2006b), has been suggested to be a potential target of thiacetazone analogs like SRI-224 (Alahari *et al.*, 2007). Flavonoids and two pro-drugs, NAS21 and NAS91, have been described as potential anti-TB compounds that target a putative β-hydroxyacyl-ACP dehydratase of mycobacterial FAS-II, Rv0636 (Brown *et al.*, 2007; Bhowruth *et al.*, 2008). A classical anti-TB drug, p-aminosalicylic acid, is thought to interfere with mycobactin synthesis (Berthet *et al.*, 1998). Using molecular modeling, Neres *et al.* (2008) reported novel prototype inhibitors of siderophore biosynthesis that target MbtA. The salicyl-AMP analogs developed act as inhibitors that are specific for *M. tuberculosis* and possibly for other pathogens that require aryl-capped siderophores for their virulence (Neres *et al.*, 2008). Other inhibitors of MbtA, 5′-O-[(*N*-acyl)sulfamoyl]adenosines, have the potential to be a new class of anti-TB drugs as well (Qiao *et al.*, 2007). Recently, Kumar *et al.* (2009), identified a phenylalanine -rich peptide that specifically interacts with ESAT-6 and inhibits growth of *M. tuberculosis* H37Rv in THP1 macrophages (Kumar *et al.*, 2009).

Concluding remarks

If the gene expression patterns of *M. tuberculosis* (and its host cells) are to help us to understand the mechanisms of pathogenesis and to identify potential drug targets, we need to develop tools that integrate this vast amount of data generated from studies on various models (Young *et al.*, 2008). From a simplistic point of view, bacterial genes that are highly and specifically expressed during an early time point of infection may be good prophylactic drug targets, in particular, if they serve critical conserved functions (Talaat *et al.*, 2004). By combining microarray-based expression profiling along with genome-wide protein-protein interaction data, we may arrive at a better understanding of pathogen-host interactions. Bioinformatic approaches may allow us to elucidate compartmentalization of protein regulatory networks and to obtain functional information on hypothetical gene clusters in *M. tuberculosis* (Fu & Fu-Liu, 2007). Integrated studies on the *M. tuberculosis* and host transcriptomes will further improve our understanding of mycobacterial survival inside the host and uncover novel aspects of host cell biology. Ultimately such insight might help us in designing new drugs to control TB.

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References

Abdallah AM, Verboom T, Hannes F *et al.* (2006) A specific secretion system mediates PPE41 transport in pathogenic mycobacteria. *Mol Microbiol* **62**: 667–679.

Abdallah AM, Gey van Pittius NC, Champion PA, Cox J, Luirink J, Vandenbroucke-Grauls CM, Appelmelk BJ & Bitter W (2007) Type VII secretion–mycobacteria show the way. *Nat Rev Microbiol* **5**: 883–891.

Abdallah AM, Savage ND, van Zon M, Wilson L, Vandenbroucke-Grauls CM, van der Wel NN, Ottenhoff TH & Bitter W (2008) The ESX-5 secretion system of *Mycobacterium marinum* modulates the macrophage response. *J Immunol* **181**: 7166–7175.

Abdallah AM, Verboom T, Weerdenburg EM *et al.* (2009) PPE and PE_PGRS proteins of *Mycobacterium marinum* are transported via the type VII secretion system ESX-5. *Mol Microbiol* **73**: 329–340.

Alahari A, Trivelli X, Guérardel Y, Dover LG, Besra GS, Sacchettini JC, Reynolds RC, Coxon GD & Kremer L (2007) Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria. *PLoS ONE* **2**: e1343.

Andersen P, Askgaard D, Gottschau A, Bennedsen J, Nagai S & Heron I (1992) Identification of immunodominant antigens during infection with *Mycobacterium tuberculosis*. *Scand J Immunol* **36**: 823–831.

Armstrong JA & Hart PD (1971) Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J Exp Med* **134**: 713–740.

Armstrong JA & Hart PD (1975) Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. *J Exp Med* **142**: 1–16.

Avarbock D, Salem J, Li LS, Wang ZM & Rubin H (1999) Cloning and characterization of a bifunctional RelA/SpoT homologue from *Mycobacterium tuberculosis*. *Gene* **233**: 261–269.

Bacon J, James BW, Wernisch L *et al.* (2004) The influence of reduced oxygen availability on pathogenicity and gene expression in *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* **84**: 205–217.

Balázsi G, Heath AP, Shi L & Gennaro ML (2008) The temporal response of the *Mycobacterium tuberculosis* gene regulatory network during growth arrest. *Mol Syst Biol* **4**: 225.

Balcewicz-Sablinska MK, Keane J, Kornfeld H & Remold HG (1998) Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-α. *J Immunol* **161**: 2636–2641.

Barry CE III (2001) Interpreting cell wall 'virulence factors' of *Mycobacterium tuberculosis*. *Trends Microbiol* **9**: 237–241.

Basu S, Pathak SK, Banerjee A, Pathak S, Bhattacharyya A, Yang Z, Talarico S, Kundu M & Basu J (2007) Execution of macrophage apoptosis by PE_PGRS33 of *Mycobacterium tuberculosis* is mediated by Toll-like receptor 2-dependent release of tumor necrosis factor-alpha. *J Biol Chem* **282**: 1039–1050.

Belisle JT, Vissa VD, Sievert T, Takayama K, Brennan PJ & Besra GS (1997) Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science* **276**: 1420–1422.

Bermudez LE & Goodman J (1996) *Mycobacterium tuberculosis* invades and replicates within type II alveolar cells. *Infect Immun* **64**: 1400–1406.

Berthet FX, Lagranderie M, Gounon P *et al.* (1998) Attenuation of virulence by disruption of the *Mycobacterium tuberculosis erp* gene. *Science* **282**: 759–762.

Beste DJ, Laing E, Bonde B, Avignone-Rossa C, Bushell ME & McFadden JJ (2007) Transcriptomic analysis identifies growth rate modulation as a component of the adaptation of mycobacteria to survival inside the macrophage. *J Bacteriol* **189**: 3969–3976.

Betts JC, Lukey PT, Robb LC, McAdam RA & Duncan K (2002) Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. *Mol Microbiol* **43**: 717–731.

Bhatt A, Fujiwara N, Bhatt K *et al.* (2007) Deletion of *kasB* in *Mycobacterium tuberculosis* causes loss of acid-fastness and subclinical latent tuberculosis in immunocompetent mice. *P Natl Acad Sci USA* **104**: 5157–5162.

Bhowruth V, Brown AK & Besra GS (2008) Synthesis and biological evaluation of NAS-21 and NAS-91 analogues as potential inhibitors of the mycobacterial FAS-II dehydratase enzyme Rv0636. *Microbiology* **154**: 1866–1875.

Bodnar KA, Serbina NV & Flynn JL (2001) Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect Immun* **69**: 800–809.

Braunstein M, Espinosa BJ, Chan J, Belisle JT & Jacobs WR Jr (2003) SecA2 functions in the secretion of superoxide dismutase A and in the virulence of *Mycobacterium tuberculosis*. *Mol Microbiol* **48**: 453–464.

Brennan PJ & Nikaido H (1995) The envelope of mycobacteria. *Annu Rev Biochem* **64**: 29–63.

Briken V & Miller JL (2008) Living on the edge: Inhibition of host cell apoptosis by *Mycobacterium tuberculosis*. *Future Microbiol* **3**: 415–422.

Brown AK, Sridharan S, Kremer L, Lindenberg S, Dover LG, Sacchettini JC & Besra GS (2005) Probing the mechanism of the *Mycobacterium tuberculosis* beta-ketoacyl-acyl carrier protein synthase III *mt*FabH: factors influencing catalysis and substrate specificity. *J Biol Chem* **280**: 32539–32547.

Brown AK, Papaemmanouil A, Bhowruth V, Bhatt A, Dover LG & Besra GS (2007) Flavonoid inhibitors as novel antimycobacterial agents targeting Rv0636, a putative dehydratase enzyme involved in *Mycobacterium tuberculosis* fatty acid synthase II. *Microbiology* **153**: 3314–3322.

Brown AK, Taylor RC, Bhatt A, Fütterer K & Besra GS (2009) Platensimycin activity against mycobacterial beta-ketoacyl-ACP synthases. *PLoS ONE* **4**: e6306.

Brozna JP, Horan M, Rademacher JM, Pabst KM & Pabst MJ (1991) Monocyte responses to sulfatide from *Mycobacterium tuberculosis*: inhibition of priming for enhanced release of superoxide, associated with increased secretion of interleukin-1 and tumor necrosis factor alpha, and altered protein phosphorylation. *Infect Immun* **59**: 2542–2548.

Calamita H, Ko C, Tyagi S, Yoshimatsu T, Morrison NE & Bishai WR (2005) The *Mycobacterium tuberculosis* SigD sigma factor controls the expression of ribosome-associated gene products in stationary phase and is required for full virulence. *Cell Microbiol* **7**: 233–244.

Campuzano J, Aguilar D, Arriaga K, Leon JC, Salas-Rangel LP, Gonzalez-y-Merchand J, Hernandez-Pando R & Espitia C (2007) The PGRS domain of *Mycobacterium tuberculosis* PE_PGRS Rv1759c antigen is an efficient subunit vaccine to prevent reactivation in a murine model of chronic tuberculosis. *Vaccine* **25**: 3722–3729.

Cappelli G, Volpe E, Grassi M, Liseo B, Colizzi V & Mariani F (2006) Profiling of *Mycobacterium tuberculosis* gene expression during human macrophage infection: upregulation of the alternative sigma factor G, a group of transcriptional regulators, and proteins with unknown function. *Res Microbiol* **157**: 445–455.

Casenghi M, Cole ST & Nathan CF (2007) New approaches to filling the gap in tuberculosis drug discovery. *PLoS Med* **4**: e293.

Chackerian AA, Alt JM, Perera TV, Dascher CC & Behar SM (2002) Dissemination of *Mycobacterium tuberculosis* is influenced by host factors and precedes the initiation of T-cell immunity. *Infect Immun* **70**: 4501–4509.

Chan J, Fan XD, Hunter SW, Brennan PJ & Bloom BR (1991) Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. *Infect Immun* **59**: 1755–1761.

Chan J, Xing Y, Magliozzo RS & Bloom BR (1992) Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J Exp Med* **175**: 1111–1122.

Chen L, Xie QW & Nathan C (1998) Alkyl hydroperoxide reductase subunit C (AhpC) protects bacterial and human cells against reactive nitrogen intermediates. *Mol Cell* **1**: 795–805.

Chen P, Ruiz RE, Li Q, Silver RF & Bishai WR (2000) Construction and characterization of a *Mycobacterium tuberculosis* mutant lacking the alternate sigma factor gene, *sigF*. *Infect Immun* **68**: 5575–5580.

Chim N, Iniguez A, Nguyen TQ & Goulding CW (2010) Unusual diheme conformation of the heme-degrading protein from *Mycobacterium tuberculosis*. *J Mol Biol* **395**: 595–608.

Colangeli R, Haq A, Arcus VL *et al.* (2009) The multifunctional histone-like protein Lsr2 protects mycobacteria against reactive oxygen intermediates. *P Natl Acad Sci USA* **106**: 4414–4418.

Cole ST, Brosch R, Parkhill J *et al.* (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**: 537–544.

Cooper AM, Segal BH, Frank AA, Holland SM & Orme IM (2000) Transient loss of resistance to pulmonary tuberculosis in p47(phox−/−) mice. *Infect Immun* **68**: 1231–1234.

Copenhaver RH, Sepulveda E, Armitige LY, Actor JK, Wanger A, Norris SJ, Hunter RL & Jagannath C (2004) A mutant of *Mycobacterium tuberculosis* H37Rv that lacks expression of antigen 85A is attenuated in mice but retains vaccinogenic potential. *Infect Immun* **72**: 7084–7095.

Cotter PA & Miller JF (1998) *In vivo* and *ex vivo* regulation of bacterial virulence gene expression. *Curr Opin Microbiol* **1**: 17–26.

Crowle AJ, Dahl R, Ross E & May MH (1991) Evidence that vesicles containing living, virulent *Mycobacterium tuberculosis* or *Mycobacterium avium* in cultured human macrophages are not acidic. *Infect Immun* **59**: 1823–1831.

Cummings CA & Relman DA (2000) Using DNA microarrays to study host-microbe interactions. *Emerg Infect Dis* **6**: 513–525.

Dainese E, Rodrigue S, Delogu G *et al.* (2006) Posttranslational regulation of *Mycobacterium tuberculosis* extracytoplasmic-function sigma factor sigma L and roles in virulence and in global regulation of gene expression. *Infect Immun* **74**: 2457–2467.

De Voss JJ, Rutter K, Schroeder BG & Barry CE III (1999) Iron acquisition and metabolism by mycobacteria. *J Bacteriol* **181**: 4443–4451.

Delogu G & Brennan MJ (2001) Comparative immune response to PE and PE_PGRS antigens of *Mycobacterium tuberculosis*. *Infect Immun* **69**: 5606–5611.

Delogu G, Pusceddu C, Bua A, Fadda G, Brennan MJ & Zanetti S (2004) Rv1818c-encoded PE_PGRS protein of *Mycobacterium tuberculosis* is surface exposed and influences bacterial cell structure. *Mol Microbiol* **52**: 725–733.

Dhiman R, Kathania M, Raje M & Majumdar S (2008) Inhibition of bfl-1/A1 by siRNA inhibits mycobacterial growth in THP-1 cells by enhancing phagosomal acidification. *Biochim Biophys Acta* **1780**: 733–742.

Divangahi M, Chen M, Gan H, Desjardins D, Hickman TT, Lee DM, Fortune S, Behar SM & Remold HG (2009) *Mycobacterium tuberculosis* evades macrophage defenses by inhibiting plasma membrane repair. *Nat Immunol* **10**: 899–906.

Dubnau E & Smith I (2003) *Mycobacterium tuberculosis* gene expression in macrophages. *Microbes Infect* **5**: 629–637.

Dubnau E, Chan J, Raynaud C, Mohan VP, Lanéelle MA, Yu K, Quémard A, Smith I & Daffé M (2000) Oxygenated mycolic acids are necessary for virulence of *Mycobacterium tuberculosis* in mice. *Mol Microbiol* **36**: 630–637.

Dubnau E, Fontán P, Manganelli R, Soares-Appel S & Smith I (2002) *Mycobacterium tuberculosis* genes induced during

infection of human macrophages. *Infect Immun* **70**: 2787–2795.

Dubnau E, Chan J, Mohan VP & Smith I (2005) Responses of *Mycobacterium tuberculosis* to growth in the mouse lung. *Infect Immun* **73**: 3754–3757.

Dutta NK, Mehra S & Kaushal D (2010) A *Mycobacterium tuberculosis* sigma factor network responds to cell-envelope damage by the promising anti-mycobacterial thioridazine. *PLoS ONE* **5**: e10069.

Fenton MJ & Vermeulen MW (1996) Immunopathology of tuberculosis: roles of macrophages and monocytes. *Infect Immun* **64**: 683–690.

Fisher MA, Plikaytis BB & Shinnick TM (2002) Microarray analysis of the *Mycobacterium tuberculosis* transcriptional response to the acidic conditions found in phagosomes. *J Bacteriol* **184**: 4025–4032.

Florczyk MA, McCue LA, Purkayastha A, Currenti E, Wolin MJ & McDonough KA (2003) A family of *acr*-coregulated *Mycobacterium tuberculosis* genes shares a common DNA motif and requires Rv3133c (*dosR* or *devR*) for expression. *Infect Immun* **71**: 5332–5343.

Fontán P, Aris V, Ghanny S, Soteropoulos P & Smith I (2008a) Global transcriptional profile of *Mycobacterium tuberculosis* during THP-1 human macrophage infection. *Infect Immun* **76**: 717–725.

Fontán PA, Aris V, Alvarez ME, Ghanny S, Cheng J, Soteropoulos P, Trevani A, Pine R & Smith I (2008b) *Mycobacterium tuberculosis* sigma factor E regulon modulates the host inflammatory response. *J Infect Dis* **198**: 877–885.

Fortune SM, Solache A, Jaeger A, Hill PJ, Belisle JT, Bloom BR, Rubin EJ & Ernst JD (2004) *Mycobacterium tuberculosis* inhibits macrophage responses to IFN-gamma through myeloid differentiation factor 88-dependent and -independent mechanisms. *J Immunol* **172**: 6272–6280.

Frehel C, de Chastellier C, Lang T & Rastogi N (1986) Evidence for inhibition of fusion of lysosomal and prelysosomal compartments with phagosomes in macrophages infected with pathogenic *Mycobacterium avium*. *Infect Immun* **52**: 252–262.

Fu LM (2006) Exploring drug action on *Mycobacterium tuberculosis* using affymetrix oligonucleotide genechips. *Tuberculosis (Edinb)* **86**: 134–143.

Fu LM & Fu-Liu CS (2007) The gene expression data of *Mycobacterium tuberculosis* based on Affymetrix gene chips provide insight into regulatory and hypothetical genes. *BMC Microbiol* **7**: 37.

Fu LM & Shinnick TM (2007) Genome-wide exploration of the drug action of capreomycin on *Mycobacterium tuberculosis* using Affymetrix oligonucleotide GeneChips. *J Infect* **54**: 277–284.

Gan H, Lee J, Ren F, Chen M, Kornfeld H & Remold HG (2008) *Mycobacterium tuberculosis* blocks crosslinking of annexin-1 and apoptotic envelope formation on infected macrophages to maintain virulence. *Nat Immunol* **9**: 1189–1197.

Gao LY, Pak M, Kish R, Kajihara K & Brown EJ (2006) A mycobacterial operon essential for virulence *in vivo* and invasion and intracellular persistence in macrophages. *Infect Immun* **74**: 1757–1767.

Gey van Pittius NC, Sampson SL, Lee H, Kim Y, van Helden PD & Warren RM (2006) Evolution and expansion of the *Mycobacterium tuberculosis* PE and PPE multigene families and their association with the duplication of the ESAT-6 (esx) gene cluster regions. *BMC Evol Biol* **6**: 95.

Gokhale RS, Saxena P, Chopra T & Mohanty D (2007) Versatile polyketide enzymatic machinery for the biosynthesis of complex mycobacterial lipids. *Nat Prod Rep* **2**: 267–277.

Gold B, Rodriguez GM, Marras SA, Pentecost M & Smith I (2001) The *Mycobacterium tuberculosis* IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. *Mol Microbiol* **42**: 851–865.

Goldstone RM, Goonesekera SD, Bloom BR & Sampson SL (2009) The transcriptional regulator Rv0485 modulates the expression of a *pe* and *ppe* gene pair and is required for *Mycobacterium tuberculosis* virulence. *Infect Immun* **77**: 4654–4667.

Graham JE & Clark-Curtiss JE (1999) Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). *P Natl Acad Sci USA* **96**: 11554–11559.

Hahn MY, Raman S, Anaya M & Husson RN (2005) The *Mycobacterium tuberculosis* extracytoplasmic-function sigma factor SigL regulates polyketide synthases and secreted or membrane proteins and is required for virulence. *J Bacteriol* **187**: 7062–7071.

Harrison AJ, Yu M, Gårdenborg T, Middleditch M, Ramsay RJ, Baker EN & Lott JS (2006) The structure of MbtI from *Mycobacterium tuberculosis*, the first enzyme in the biosynthesis of the siderophore mycobactin, reveals it to be a salicylate synthase. *J Bacteriol* **188**: 6081–6091.

Haydel SE & Clark-Curtiss JE (2004) Global expression analysis of two-component system regulator genes during *Mycobacterium tuberculosis* growth in human macrophages. *FEMS Microbiol Lett* **236**: 341–347.

Heifets L, Simon J & Pham V (2005) Capreomycin is active against non-replicating *M. tuberculosis*. *Ann Clin Microbiol Antimicrob* **4**: 6.

Hernandez Pando R, Aguilar LD, Smith I & Manganelli R (2010) Immunogenicity and protection induced by a *Mycobacterium tuberculosis sigE* mutant in a BALB/c mouse model of progressive pulmonary tuberculosis. *Infect Immun* **78**: 3168–3176.

Hickman SP, Chan J & Salgame P (2002) *Mycobacterium tuberculosis* induces differential cytokine production from dendritic cells and macrophages with divergent effects on naive T cell polarization. *J Immunol* **168**: 4636–4642.

Hinchey J, Lee S, Jeon BY *et al.* (2007) Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. *J Clin Invest* **117**: 2279–2288.

Homolka S, Niemann S, Russell DG & Rohde KH (2010) Functional genetic diversity among *Mycobacterium tuberculosis* complex clinical isolates: delineation of conserved core and lineage-specific transcriptomes during intracellular survival. *PLoS Pathog* **6**: e1000988.

Honaker RW, Dhiman RK, Narayanasamy P, Crick DC & Voskuil MI (2010) DosS responds to a reduced electron transport system to induce the *Mycobacterium tuberculosis* DosR regulon. *J Bacteriol* **192**: 6447–6455.

Hu Y, Henderson B, Lund PA, Tormay P, Ahmed MT, Gurcha SS, Besra GS & Coates AR (2008) A *Mycobacterium tuberculosis* mutant lacking the *groEL* homologue *cpn60.1* is viable but fails to induce an inflammatory response in animal models of infection. *Infect Immun* **76**: 1535–1546.

Iriti M & Faoro F (2007) Review of innate and specific immunity in plants and animals. *Mycopathologia* **164**: 57–64.

Jackson M, Raynaud C, Lanéelle MA, Guilhot C, Laurent-Winter C, Ensergueix D, Gicquel B & Daffé M (1999) Inactivation of the antigen 85C gene profoundly affects the mycolate content and alters the permeability of the *Mycobacterium tuberculosis* cell envelope. *Mol Microbiol* **31**: 1573–1587.

Jayakumar D, Jacobs WR Jr & Narayanan S (2008) Protein kinase E of *Mycobacterium tuberculosis* has a role in the nitric oxide stress response and apoptosis in a human macrophage model of infection. *Cell Microbiol* **10**: 365–374.

Karakousis PC, Yoshimatsu T, Lamichhane G, Woolwine SC, Nuermberger EL, Grosset J & Bishai WR (2004) Dormancy phenotype displayed by extracellular *Mycobacterium tuberculosis* within artificial granulomas in mice. *J Exp Med* **200**: 647–657.

Katti MK, Dai G, Armitige LY, Rivera Marrero C, Daniel S, Singh CR, Lindsey DR, Dhandayuthapani S, Hunter RL & Jagannath C (2008) The Delta fbpA mutant derived from *Mycobacterium tuberculosis* H37Rv has an enhanced susceptibility to intracellular antimicrobial oxidative mechanisms, undergoes limited phagosome maturation and activates macrophages and dendritic cells. *Cell Microbiol* **10**: 1286–1303.

Kausalya S, Somogyi R, Orlofsky A & Prystowsky MB (2001) Requirement of A1-a for bacillus Calmette-Guérin-mediated protection of macrophages against nitric oxide-induced apoptosis. *J Immunol* **166**: 4721–4727.

Kaushal D, Schroeder BG, Tyagi S *et al.* (2002) Reduced immunopathology and mortality despite tissue persistence in a *Mycobacterium tuberculosis* mutant lacking alternative sigma factor, SigH. *P Natl Acad Sci USA* **99**: 8330–8335.

Keane J, Remold HG & Kornfeld H (2000) Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages. *J Immunol* **164**: 2016–2020.

Kendall SL, Movahedzadeh F, Rison SC, Wernisch L, Parish T, Duncan K, Betts JC & Stoker NG (2004a) The *Mycobacterium tuberculosis* DosRS two-component system is induced by multiple stresses. *Tuberculosis (Edinb)* **84**: 247–255.

Kendall SL, Rison SC, Movahedzadeh F, Frita R & Stoker NG (2004b) What do microarrays really tell us about *M. tuberculosis*? *Trends Microbiol* **12**: 537–544.

Khan N, Rahim SS, Boddupalli CS, Ghousunnissa S, Padma S, Pathak N, Thiagarajan D, Hasnain SE & Mukhopadhyay S (2006) Hydrogen peroxide inhibits IL-12 p40 induction in macrophages by inhibiting c-rel translocation to the nucleus through activation of calmodulin protein. *Blood* **107**: 1513–1520.

Khan N, Alam K, Mande SC, Valluri VL, Hasnain SE & Mukhopadhyay S (2008) *Mycobacterium tuberculosis* heat shock protein 60 modulates immune response to PPD by manipulating the surface expression of TLR2 on macrophages. *Cell Microbiol* **10**: 1711–1722.

Khosla C, Gokhale RS, Jacobsen JR & Cane DE (1999) Tolerance and specificity of polyketide synthases. *Annu Rev Biochem* **68**: 219–253.

Kremer L, Estaquier J, Brandt E, Ameisen JC & Locht C (1997) *Mycobacterium bovis* Bacillus Calmette Guérin infection prevents apoptosis of resting human monocytes. *Eur J Immunol* **27**: 2450–2456.

Kremer L, Douglas JD, Baulard AR *et al.* (2000) Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. *J Biol Chem* **275**: 16857–16864.

Kremer L, Nampoothiri KM, Lesjean S, Dover LG, Graham S, Betts J, Brennan PJ, Minnikin DE, Locht C & Besra GS (2001) Biochemical characterization of acyl carrier protein (AcpM) and malonyl-CoA:AcpM transacylase (mtFabD), two major components of *Mycobacterium tuberculosis* fatty acid synthase II. *J Biol Chem* **276**: 27967–27974.

Krithika R, Marathe U, Saxena P, Ansari MZ, Mohanty D & Gokhale RS (2006) A genetic locus required for iron acquisition in *Mycobacterium tuberculosis*. *P Natl Acad Sci USA* **103**: 2069–2074.

Kruh NA, Troudt J, Izzo A, Prenni J & Dobos KM (2010) Portrait of a pathogen: the *Mycobacterium tuberculosis* proteome *in vivo*. *PLoS ONE* **5**: e13938.

Kumar K, Tharad M, Ganapathy S *et al.* (2009) Phenylalanine-rich peptides potently bind ESAT6, a virulence determinant of *Mycobacterium tuberculosis*, and concurrently affect the pathogen's growth. *PLoS ONE* **4**: e7615.

Lee MH, Pascopella L, Jacobs WR Jr & Hatfull GF (1991) Site-specific integration of mycobacteriophage L5: integration-proficient vectors for *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, and bacille Calmette-Guérin. *P Natl Acad Sci USA* **88**: 3111–3115.

Lee J, Remold HG, Ieong MH & Kornfeld H (2006) Macrophage apoptosis in response to high intracellular burden of *Mycobacterium tuberculosis* is mediated by a novel caspase-independent pathway. *J Immunol* **176**: 4267–4274.

Lee J, Hartman M & Kornfeld H (2009) Macrophage apoptosis in tuberculosis. *Yonsei Med J* **50**: 1–11.

Lewthwaite JC, Coates AR, Tormay P, Singh M, Mascagni P, Poole S, Roberts M, Sharp L & Henderson B (2001)

Mycobacterium tuberculosis chaperonin 60.1 is a more potent cytokine stimulator than chaperonin 60.2 (Hsp 65) and contains a CD14-binding domain. *Infect Immun* **69**: 7349–7355.

Li MS, Waddell SJ, Monahan IM, Mangan JA, Martin SL, Everett MJ & Butcher PD (2004) Increased transcription of a potential sigma factor regulatory gene Rv1364c in *Mycobacterium bovis* BCG while residing in macrophages indicates use of alternative promoters. *FEMS Microbiol Lett* **233**: 333–339.

Lin MY & Ottenhoff TH (2008) Host-pathogen interactions in latent *Mycobacterium tuberculosis* infection: identification of new targets for tuberculosis intervention. *Endocr Metab Immune Disord Drug Targets* **8**: 15–29.

Lucchini S, Thompson A & Hinton JC (2001) Microarrays for microbiologists. *Microbiology* **147**: 1403–1414.

Maciag A, Dainese E, Rodriguez GM, Milano A, Provvedi R, Pasca MR, Smith I, Palù G, Riccardi G & Manganelli R (2007) Global analysis of the *Mycobacterium tuberculosis* Zur (FurB) regulon. *J Bacteriol* **189**: 730–740.

MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK & Nathan CF (1997) Identification of nitric oxide synthase as a protective locus against tuberculosis. *P Natl Acad Sci USA* **94**: 5243–5248.

Maiti D, Bhattacharyya A & Basu J (2001) Lipoarabinomannan from *Mycobacterium tuberculosis* promotes macrophage survival by phosphorylating Bad through a phosphatidylinositol 3-kinase/Akt pathway. *J Biol Chem* **276**: 329–333.

Manca C, Paul S, Barry CE III, Freedman VH & Kaplan G (1999) *Mycobacterium tuberculosis* catalase and peroxidase activities and resistance to oxidative killing in human monocytes *in vitro*. *Infect Immun* **67**: 74–79.

Manganelli R, Voskuil MI, Schoolnik GK & Smith I (2001) The *Mycobacterium tuberculosis* ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. *Mol Microbiol* **41**: 423–437.

Manganelli R, Voskuil MI, Schoolnik GK, Dubnau E, Gomez M & Smith I (2002) Role of the extracytoplasmic-function sigma factor sigmaH in *Mycobacterium tuberculosis* global gene expression. *Mol Microbiol* **45**: 365–374.

Manganelli R, Provvedi R, Rodrigue S, Beaucher J, Gaudreau L & Smith I (2004a) Sigma factors and global gene regulation in *Mycobacterium tuberculosis*. *J Bacteriol* **186**: 895–902.

Manganelli R, Fattorini L, Tan D, Iona E, Orefici G, Altavilla G, Cusatelli P & Smith I (2004b) The extra cytoplasmic function sigma factor E is essential for *Mycobacterium tuberculosis* virulence in mice. *Infect Immun* **72**: 3038–3041.

Manjunatha U, Boshoff HI & Barry CE (2009) The mechanism of action of PA-824: novel insights from transcriptional profiling. *Commun Integr Biol* **2**: 215–218.

Master SS, Springer B, Sander P, Boettger EC, Deretic V & Timmins GS (2002) Oxidative stress response genes in *Mycobacterium tuberculosis*: role of *ahpC* in resistance to peroxynitrite and stage-specific survival in macrophages. *Microbiology* **148**: 3139–3144.

Matsunaga I, Bhatt A, Young DC *et al.* (2004) *Mycobacterium tuberculosis pks12* produces a novel polyketide presented by CD1c to T cells. *J Exp Med* **200**: 1559–1569.

Maus CE, Plikaytis BB & Shinnick TM (2005) Mutation of tlyA confers capreomycin resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **49**: 571–577.

Mbawuike IN & Herscowitz HB (1988) Relationship between ineffective antigen presentation by murine alveolar macrophages and their immunosuppressive function. *Immunology* **64**: 61–67.

McKinney JD, Höner zu Bentrup K, Muñoz-Elías EJ, Miczak A, Chen B, Chan WT, Swenson D, Sacchettini JC, Jacobs WR Jr & Russell DG (2000) Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* **406**: 735–738.

Mehta-Damani A, Markowicz S & Engleman EG (1994) Generation of antigen-specific CD8+ CTLs from naive precursors. *J Immunol* **153**: 996–1003.

Monahan IM, Betts J, Banerjee DK & Butcher PD (2001) Differential expression of mycobacterial proteins following phagocytosis by macrophages. *Microbiology* **147**: 459–471.

Mortellaro A, Robinson L & Ricciardi-Castagnoli P (2009) Spotlight on Mycobacteria and dendritic cells: will novel targets to fight tuberculosis emerge? *EMBO Mol Med* **1**: 19–29.

Muttucumaru DG, Roberts G, Hinds J, Stabler RA & Parish T (2004) Gene expression profile of *Mycobacterium tuberculosis* in a non-replicating state. *Tuberculosis (Edinb)* **84**: 239–246.

Nair S, Ramaswamy PA, Ghosh S, Joshi DC, Pathak N, Siddiqui I, Sharma P, Hasnain SE, Mande SC & Mukhopadhyay S (2009) The PPE18 of *Mycobacterium tuberculosis* interacts with TLR2 and activates IL-10 induction in macrophage. *J Immunol* **183**: 6269–62681.

Nathan CF & Hibbs Jr JB (1991) Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr Opin Immunol* **3**: 65–70.

Nathan C & Shiloh MU (2000) Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *P Natl Acad Sci USA* **97**: 8841–8848.

Neres J, Labello NP, Somu RV, Boshoff HI, Wilson DJ, Vannada J, Chen L, Barry CE III, Bennett EM & Aldrich CC (2008) Inhibition of siderophore biosynthesis in *Mycobacterium tuberculosis* with nucleoside bisubstrate analogues: structure-activity relationships of the nucleobase domain of 5′-O-[N-(salicyl)sulfamoyl]adenosine. *J Med Chem* **51**: 5349–5370.

Ng VH, Cox JS, Sousa AO, MacMicking JD & McKinney JD (2004) Role of KatG catalase-peroxidase in mycobacterial pathogenesis: countering the phagocyte oxidative burst. *Mol Microbiol* **52**: 1291–1302.

Oddo M, Renno T, Attinger A, Bakker T, MacDonald HR & Meylan PR (1998) Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. *J Immunol* **160**: 5448–5454.

Olazabal IM, Martín-Cofreces NB, Mittelbrunn M, Martínez del Hoyo G, Alarcón B & Sánchez-Madrid F (2008) Activation outcomes induced in naïve CD8 T-cells by macrophages primed via "phagocytic" and nonphagocytic pathways. *Mol Biol Cell* **19**: 701–710.

Olsen AW, Williams A, Okkels LM, Hatch G & Andersen P (2004) Protective effect of a tuberculosis subunit vaccine based on a fusion of antigen 85B and ESAT-6 in the aerosol guinea pig model. *Infect Immun* **72**: 6148–6150.

Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, Kobzik L, Higgins DE, Daly MJ, Bloom BR & Kramnik I (2005) *Ipr1* gene mediates innate immunity to tuberculosis. *Nature* **434**: 767–772.

Park HD, Guinn KM, Harrell MI, Liao R, Voskuil MI, Tompa M, Schoolnik GK & Sherman DR (2003) Rv3133c/*dosR* is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis*. *Mol Microbiol* **48**: 833–843.

Park JS, Tamayo MH, Gonzalez-Juarrero M, Orme IM & Ordway DJ (2006) Virulent clinical isolates of *Mycobacterium tuberculosis* grow rapidly and induce cellular necrosis but minimal apoptosis in murine macrophages. *J Leukoc Biol* **79**: 80–86.

Pathak SK, Basu S, Basu KK, Banerjee A, Pathak S, Bhattacharyya A, Kaisho T, Kundu M & Basu J (2007) Direct extracellular interaction between the early secreted antigen ESAT-6 of *Mycobacterium tuberculosis* and TLR2 inhibits TLR signaling in macrophages. *Nat Immunol* **8**: 610–618.

Phetsuksiri B, Jackson M, Scherman H *et al.* (2003) Unique mechanism of action of the thiourea drug isoxyl on *Mycobacterium tuberculosis*. *J Biol Chem* **278**: 53123–53130.

Provvedi R, Boldrin F, Falciani F, Palù G & Manganelli R (2009) Global transcriptional response to vancomycin in *Mycobacterium tuberculosis*. *Microbiology* **155**: 1093–1102.

Puech V, Guilhot C, Perez E, Tropis M, Armitige LY, Gicquel B & Daffé M (2002) Evidence for a partial redundancy of the fibronectin-binding proteins for the transfer of mycoloyl residues onto the cell wall arabinogalactan termini of *Mycobacterium tuberculosis*. *Mol Microbiol* **44**: 1109–1122.

Purkayastha A, McCue LA & McDonough KA (2002) Identification of a *Mycobacterium tuberculosis* putative classical nitroreductase gene whose expression is coregulated with that of the acr aene within macrophages, in standing versus shaking cultures, and under low oxygen conditions. *Infect Immun* **70**: 1518–1529.

Qiao C, Gupte A, Boshoff HI, Wilson DJ, Bennett EM, Somu RV, Barry CE III & Aldrich CC (2007) 5′-O-[(N-acyl) sulfamoyl]adenosines as antitubercular agents that inhibit MbtA: an adenylation enzyme required for siderophore biosynthesis of the mycobactins. *J Med Chem* **50**: 6080–6094.

Quadri LE, Sello J, Keating TA, Weinreb PH & Walsh CT (1998) Identification of a *Mycobacterium tuberculosis* gene cluster encoding the biosynthetic enzymes for assembly of the virulence-conferring siderophore mycobactin. *Chem Biol* **5**: 631–645.

Rachman H, Strong M, Schaible U, Schuchhardt J, Hagens K, Mollenkopf H, Eisenberg D & Kaufmann SH (2006a) *Mycobacterium tuberculosis* gene expression profiling within the context of protein networks. *Microbes Infect* **8**: 747–757.

Rachman H, Strong M, Ulrichs T, Grode L, Schuchhardt J, Mollenkopf H, Kosmiadi GA, Eisenberg D & Kaufmann SH (2006b) Unique transcriptome signature of *Mycobacterium tuberculosis* in pulmonary tuberculosis. *Infect Immun* **74**: 1233–1242.

Raman S, Song T, Puyang X, Bardarov S, Jacobs WR Jr & Husson RN (2001) The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in *Mycobacterium tuberculosis*. *J Bacteriol* **183**: 6119–6125.

Raman S, Hazra R, Dascher CC & Husson RN (2004) Transcription regulation by the *Mycobacterium tuberculosis* alternative sigma factor SigD and its role in virulence. *J Bacteriol* **186**: 6605–6616.

Raman K, Rajagopalan P & Chandra N (2005) Flux balance analysis of mycolic acid pathway: targets for anti-tubercular drugs. *PLoS Comput Biol* **1**: e46.

Raman K, Yeturu K & Chandra N (2008) targetTB: a target identification pipeline for *Mycobacterium tuberculosis* through an interactome, reactome and genome-scale structural analysis. *BMC Syst Biol* **2**: 109.

Ratledge C & Ewing M (1996) The occurrence of carboxymycobactin, the siderophore of pathogenic mycobacteria, as a second extracellular siderophore in *Mycobacterium smegmatis*. *Microbiology* **142**: 2207–2212.

Reddy TB, Riley R, Wymore F *et al.* (2009) TB database: an integrated platform for tuberculosis research. *Nucleic Acids Res* **37**: D499–D508.

Rengarajan J, Bloom BR & Rubin EJ (2005) Genome-wide requirements for *Mycobacterium tuberculosis* adaptation and survival in macrophages. *P Natl Acad Sci USA* **102**: 8327–8332.

Roberts DM, Liao RP, Wisedchaisri G, Hol WG & Sherman DR (2004) Two sensor kinases contribute to the hypoxic response of *Mycobacterium tuberculosis*. *J Biol Chem* **279**: 23082–23087.

Rodrigue S, Provvedi R, Jacques PE, Gaudreau L & Manganelli R (2006) The sigma factors of *Mycobacterium tuberculosis*. *FEMS Microbiol Rev* **30**: 926–941.

Rodriguez GM (2006) Control of iron metabolism in *Mycobacterium tuberculosis*. *Trends Microbiol* **14**: 320–327.

Rodriguez GM & Smith I (2003) Mechanisms of iron regulation in mycobacteria: role in physiology and virulence. *Mol Microbiol* **47**: 1485–1494.

Rodriguez GM, Voskuil MI, Gold B, Schoolnik GK & Smith I (2002) *ideR*, an essential gene in *Mycobacterium tuberculosis*: role of IdeR in iron-dependent gene expression, iron metabolism, and oxidative stress response. *Infect Immun* **70**: 3371–3381.

Rousseau C, Sirakova TD, Dubey VS, Bordat Y, Kolattukudy PE, Gicquel B & Jackson M (2003) Virulence attenuation of

two Mas-like polyketide synthase mutants of *Mycobacterium tuberculosis*. *Microbiology* **149**: 1837–1847.

Russell DG, Dant J & Sturgill-Koszycki S (1996) *Mycobacterium avium*- and *Mycobacterium tuberculosis*-containing vacuoles are dynamic, fusion-competent vesicles that are accessible to glycosphingolipids from the host cell plasmalemma. *J Immunol* **156**: 4764–4773.

Sassetti CM & Rubin EJ (2003) Genetic requirements for mycobacterial survival during infection. *P Natl Acad Sci USA* **100**: 12989–12994.

Saunders BM, Frank AA & Orme IM (1999) Granuloma formation is required to contain bacillus growth and delay mortality in mice chronically infected with *Mycobacterium tuberculosis*. *Immunology* **98**: 324–328.

Schaible UE, Sturgill-Koszycki S, Schlesinger PH & Russell DG (1998) Cytokine activation leads to acidification and increases maturation of *Mycobacterium avium*-containing phagosomes in murine macrophages. *J Immunol* **160**: 1290–1296.

Schnappinger D, Ehrt S, Voskuil MI *et al.* (2003) Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment. *J Exp Med* **198**: 693–704.

Senaratne RH, De Silva AD, Williams SJ *et al.* (2006) 5′-Adenosinephosphosulphate reductase (CysH) protects *Mycobacterium tuberculosis* against free radicals during chronic infection phase in mice. *Mol Microbiol* **59**: 1744–1753.

Serafini A, Boldrin F, Palù G & Manganelli R (2009) Characterization of a *Mycobacterium tuberculosis* ESX-3 conditional mutant: essentiality and rescue by iron and zinc. *J Bacteriol* **191**: 6340–6344.

Sherman DR, Voskuil M, Schnappinger D, Liao R, Harrell MI & Schoolnik GK (2001) Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding alpha - crystallin. *P Natl Acad Sci USA* **98**: 7534–7539.

Shi L, Jung YJ, Tyagi S, Gennaro ML & North RJ (2003) Expression of Th1-mediated immunity in mouse lungs induces a *Mycobacterium tuberculosis* transcription pattern characteristic of nonreplicating persistence. *P Natl Acad Sci USA* **100**: 241–246.

Shi L, Sohaskey CD, Kana BD, Dawes S, North RJ, Mizrahi V & Gennaro ML (2005) Changes in energy metabolism of *Mycobacterium tuberculosis* in mouse lung and under *in vitro* conditions affecting aerobic respiration. *P Natl Acad Sci USA* **102**: 15629–15634.

Siegrist MS, Unnikrishnan M, McConnell MJ, Borowsky M, Cheng TY, Siddiqi N, Fortune SM, Moody DB & Rubin EJ (2009) Mycobacterial Esx-3 is required for mycobactin-mediated iron acquisition. *P Natl Acad Sci USA* **106**: 18792–18797.

Sirakova TD, Thirumala AK, Dubey VS, Sprecher H & Kolattukudy PE (2001) The *Mycobacterium tuberculosis* pks2 gene encodes the synthase for the hepta- and octamethyl-branched fatty acids required for sulfolipid synthesis. *J Biol Chem* **276**: 16833–16839.

Sirakova TD, Dubey VS, Cynamon MH & Kolattukudy PE (2003a) Attenuation of *Mycobacterium tuberculosis* by disruption of a *mas*-like gene or a chalcone synthase-like gene, which causes deficiency in dimycocerosyl phthiocerol synthesis. *J Bacteriol* **185**: 2999–3008.

Sirakova TD, Dubey VS, Kim HJ, Cynamon MH & Kolattukudy PE (2003b) The largest open reading frame (*pks12*) in the *Mycobacterium tuberculosis* genome is involved in pathogenesis and dimycocerosyl phthiocerol synthesis. *Infect Immun* **71**: 3794–3801.

Sly LM, Hingley-Wilson SM, Reiner NE & McMaster WR (2003) Survival of *Mycobacterium tuberculosis* in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member *Mcl-1*. *J Immunol* **170**: 430–437.

Smith I (2003) *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clin Microbiol Rev* **16**: 463–496.

Spira A, Carroll JD, Liu G, Aziz Z, Shah V, Kornfeld H & Keane J (2003) Apoptosis genes in human alveolar macrophages infected with virulent or attenuated *Mycobacterium tuberculosis*: a pivotal role for tumor necrosis factor. *Am J Respir Cell Mol Biol* **29**: 545–551.

Srivastava V, Rouanet C, Srivastava R, Ramalingam B, Locht C & Srivastava BS (2007) Macrophage-specific *Mycobacterium tuberculosis* genes: identification by green fluorescent protein and kanamycin resistance selection. *Microbiology* **153**: 659–666.

Stewart GR, Wernisch L, Stabler R, Mangan JA, Hinds J, Laing KG, Young DB & Butcher PD (2002) Dissection of the heat-shock response in *Mycobacterium tuberculosis* using mutants and microarrays. *Microbiology* **148**: 3129–3138.

Stover CK, Warrener P, VanDevanter DR *et al.* (2000) A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* **405**: 962–966.

Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J & Russell DG (1994) Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* **263**: 678–681.

Tailleux L, Waddell SJ, Pelizzola M *et al.* (2008) Probing host pathogen cross-talk by transcriptional profiling of both *Mycobacterium tuberculosis* and infected human dendritic cells and macrophages. *PLoS ONE* **3**: e1403.

Takayama K, Wang C & Besra GS (2005) Pathway to synthesis and processing of mycolic acids in *Mycobacterium tuberculosis*. *Clin Microbiol Rev* **18**: 81–101.

Talaat AM, Lyons R, Howard ST & Johnston SA (2004) The temporal expression profile of *Mycobacterium tuberculosis* infection in mice. *P Natl Acad Sci USA* **101**: 4602–4607.

Teixeira HD, Schumacher RI & Meneghini R (1998) Lower intracellular hydrogen peroxide levels in cells overexpressing CuZn-superoxide dismutase. *P Natl Acad Sci USA* **95**: 7872–7875.

Timm J, Post FA, Bekker LG *et al.* (2003) Differential expression of iron-, carbon-, and oxygen-responsive

mycobacterial genes in the lungs of chronically infected mice and tuberculosis patients. *P Natl Acad Sci USA* **100**: 14321–14326.

Trivedi OA, Arora P, Sridharan V, Tickoo R, Mohanty D & Gokhale RS (2004) Enzymic activation and transfer of fatty acids as acyl-adenylates in mycobacteria. *Nature* **428**: 441–445.

Tsuchiya S, Kobayashi Y, Goto Y, Okumura H, Nakae S, Konno T & Tada K (1982) Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res* **42**: 1530–1536.

Tullius MV, Harmston CA, Owens CP *et al.* (2011) Discovery and characterization of a unique mycobacterial heme acquisition system. *P Natl Acad Sci USA* **108**: 5051–5056.

Van der Geize R, Yam K, Heuser T *et al.* (2007) A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into *Mycobacterium tuberculosis* survival in macrophages. *P Natl Acad Sci USA* **104**: 1947–1952.

Velmurugan K, Chen B, Miller JL, Azogue S, Gurses S, Hsu T, Glickman M, Jacobs WR Jr, Porcelli SA & Briken V (2007) *Mycobacterium tuberculosis nuoG* is a virulence gene that inhibits apoptosis of infected host cells. *PLoS Pathog* **3**: e110.

Verkhedkar KD, Raman K, Chandra NR & Vishveshwara S (2007) Metabolome based reaction graphs of *M. tuberculosis* and *M. leprae*: a comparative network analysis. *PLoS ONE* **2**: e881.

Voskuil MI, Schnappinger D, Visconti KC, Harrell MI, Dolganov GM, Sherman DR & Schoolnik GK (2003) Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. *J Exp Med* **198**: 705–713.

Voskuil MI, Schnappinger D, Rutherford R, Liu Y & Schoolnik GK (2004) Regulation of the *Mycobacterium tuberculosis* PE/PPE genes. *Tuberculosis (Edinb)* **84**: 256–262.

Waagmeester A, Thompson J & Reyrat JM (2005) Identifying sigma factors in *Mycobacterium smegmatis* by comparative genomic analysis. *Trends Microbiol* **13**: 505–509.

Waddell SJ & Butcher PD (2007) Microarray analysis of whole genome expression of intracellular *Mycobacterium tuberculosis*. *Curr Mol Med* **7**: 287–296.

Waddell SJ & Butcher PD (2010) Use of DNA arrays to study transcriptional responses to antimycobacterial compounds. *Methods Mol Biol* **642**: 75–91.

Wengenack NL, Jensen MP, Rusnak F & Stern MK (1999) *Mycobacterium tuberculosis* KatG is a peroxynitritase. *Biochem Biophys Res Commun* **256**: 485–487.

Weston A, Stern RJ, Lee RE, Nassau PM, Monsey D, Martin SL, Scherman MS, Besra GS, Duncan K & McNeil MR (1997) Biosynthetic origin of mycobacterial cell wall galactofuranosyl residues. *Tuber Lung Dis* **78**: 123–131.

Wilson M, DeRisi J, Kristensen HH, Imboden P, Rane S, Brown PO & Schoolnik GK (1999) Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. *P Natl Acad Sci USA* **96**: 12833–12838.

Yang CS, Yuk JM & Jo EK (2009) The role of nitric oxide in mycobacterial infections. *Immune Netw* **9**: 46–52.

Young D, Stark J & Kirschner D (2008) Systems biology of persistent infection: tuberculosis as a case study. *Nat Rev Microbiol* **6**: 520–528.

Yuan Y, Crane DD, Simpson RM, Zhu YQ, Hickey MJ, Sherman DR & Barry CE III (1998) The 16-kDa alpha-crystallin (Acr) protein of *Mycobacterium tuberculosis* is required for growth in macrophages. *P Natl Acad Sci USA* **95**: 9578–9583.

Zahrt TC & Deretic V (2001) *Mycobacterium tuberculosis* signal transduction system required for persistent infections. *P Natl Acad Sci USA* **98**: 12706–12711.

Zügel U & Kaufmann SH (1997) Activation of CD8 T cells with specificity for mycobacterial heat shock protein 60 in *Mycobacterium bovis* bacillus Calmette-Guérin-vaccinated mice. *Infect Immun* **65**: 3947–3950.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Virulence factors identified in *M. tuberculosis* in the pretranscriptomic era.

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