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Mycobacterium tuberculosis
Adaptation to Survival in a Human Host

Beatrice Saviola

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1. Introduction

Mycobacterium tuberculosis exists exclusively as a pathogen of humans and in some cases of animals. It is not thought to exist in the environment other than for brief periods during transfer from an infected host to an uninfected contact. Thus *M. tuberculosis* must adapt to an *in vivo* environment by modifying gene expression. Differential expression can occur in immune cells such as macrophages, larger immune structures such as granulomas, and within liquefied lesions of the lung. Within the human body tubercle bacilli experience reactive oxygen intermediates as well as acidity within the phagosomes of macrophages. In addition within the centers of caseating granulomas bacilli experience low oxygen tension as well as toxic lipases and proteases released by dead immune cells. High temperature is present within the body of a person with active tuberculosis in the form of a fever. There may be other unrecognized signals and stresses that modulate gene expression within invading *M. tuberculosis* bacilli as well. Examination of gene expression during *in vivo* growth, within macrophages, or during application of specific stresses can illuminate which critical pathways in the mycobacterium are upregulated that lead to an *M. tuberculosis* bacillus exquisitely adapted to *in vivo* survival.

2. Adaptation to growth in the phagosomal compartment of macrophages

Macrophages are the preferred intracellular location for *M. tuberculosis in vivo*. Infected individuals cough and expel droplet nuclei which contain *M. tuberculosis* bacilli and remain suspended in the air. After inhalation and within the body, the bacilli are transported to the small alveoli in the lungs where they encounter alveolar macrophages which are relatively nonactivated (Dannenberg, 1993; Dannenberg, 1997). These nonactivated macrophages are not

efficient at killing or retarding growth of invading microbes. Initially bacilli are taken up into phagosomal compartments and may replicate. As the immune system becomes activated, macrophages are stimulated with INF-γ to increase their efficiency of mycobacterial killing, becoming more efficient at producing reactive oxygen intermediates and acidic stress. In response, *M. tuberculosis* pushes back against the macrophages and differentially regulates key genes. Within macrophages *M. tuberculosis* increases its lipid metabolism which may reflect an environment in the phagosome which lacks available carbohydrates (Table. 1). In addition the enzyme isocitrate lyase (*icl*) is strongly induced *in vivo*, and *icl* is upregulated in all macrophage models. Icl is a key enzyme in the glyoxylate shunt and utilizes fatty acids as an energy source. When *icl* and other genes in the glyoxylate shunt are mutated this results in attenuation *in vivo*. In addition within macrophages, genes involved in stress responses, cell wall component production, anaerobic respiration, siderophore production to scavange iron, diverse sigma factor production, and tranposases that may mutate the genome are all upregulated (Schnappinger et al, 2003, Beste et al, 2007, Ward et al, 2010).

3. Adaptation to granulomas and caseation

Once infection has progressed, tubercle bacilli replicate within incompletely activated macrophages. Additional macrophages arrive to the site of infection, and engulf newly liberated mycobacteria. The immune cells, T-cells, arrive to this location and an immune structure, the granuloma, composed of macrophages and a mantel of T-cells develops. If the host is resistant, and can robustly activate the body's macrophages, then *M. tuberculosis* infection is likely controlled. If the host immune system is weak, or is weakened, *M. tuberculosis* can replicate in the incompletely activated macrophages. Genes of *M. tuberculosis* required to resist macrophages will be important in resisting the environment of the granuloma as well. As the infection progresses in susceptible individuals, the centers of the granulomas degenerate and form a caseous, or cheesy, center. At the heart of this is an elevated lipid metabolism of the host that produces a variety of lipids including cholesterol, cholesteryl esters, triacyglycerol and others (Kim et al, 2010). Interestingly *M. tuberculosis* infection has been shown to induce elevated lipid metabolism in the host (Table 1.). The cell wall lipid of *M. tuberculosis*, trehalose dimycolate or cord factor, induces a granulomatous response in mice, and this was accompanied by foam cell formation which contains elevated lipids (Kim et al, 2010). It is intriguing to speculate that *M. tuberculosis* infection can induce elevated host lipid metabolism, and as discussed previously as part of adaptation to *in vivo* growth, *M. tuberculosis* also switches to lipid metabolism and lipids as a preferred carbon source (Eisenreich et al, 2010). Thus *M. tuberculosis* induces the host to produce what the microbe has evolved to utilize as an energy source.

4. Liquefied lesions and sputum

Later in infection caseating granulomas continue to breakdown. At a certain point these granulomas begin to liquefy, and host lipases and proteases are present which damage host

tissues. Dead macrophages release lytic enzymes, and bacterial products may also result in host tissue damage and liquefaction ensues. As tissue is damaged, a cavity erodes into the lung airspace. In rabbit studies, *M. tuberculosis* can replicate to extremely high levels in this liquefied environment (Dannenberg 1993, Dannenberg et al 1997, Dannenberg 2006). For the first time *in vivo M. tuberculosis* is capable of replicating extracellularly. Liquid containing free *M. tuberculosis* is expelled through cavities in the lung by coughing.

M. tuberculosis within sputum contains elevated levels of lipid bodies and tends to be inhibited in its replicative process (Table 1.) (Garton et al, 2008). In addition, sputum transcriptome analysis of *M. tuberculosis* reveals that triacylglycerol synthase, *tgs1* part of the DosR regulon, is induced and lipid bodies may be composed of increased stores of triacylglycerol (Garton et al, 2008). Lipid bodies are correlated *in vitro* with nonreplicating persistence, and may help *M. tuberculosis* survive the harsh environment *ex vivo* before it encounters another human host.

5. *Mycobacterium tuberculosis* and dormancy

One third of the world's population is infected with *M. tuberculosis* in part because it causes a latent or dormant infection in a majority of those infected. If therapies are to be developed which can eradicate *M. tuberculosis,* a better understanding of dormancy is required. *M. tuberculosis* can persist for decades in a dormant state within hypoxic granulomas in the lung. Studies have suggested that in a dormant state *M. tuberculosis* is occupied mainly with maintaining cell wall integrity, membrane potential, and protecting its DNA structure. The mycobacterium must also resist the host's immune system. A number of *in vivo* and *in vitro* models have been used to investigate dormancy. These models include exposing mycobacteria to environments that are likely encountered within the host. In one model cultures are stirred slowly and sealed so that oxygen is gradually consumed. In another model nutrient starvation of the bacteria may induce dormancy. In addition, infection of mice, partial treatment with antibiotics, and exposure to immune suppression can lead to dormancy and reactivation (Murphy and Brown, 2007).

The gene encoding a transcriptional regulator, *dosR* (*devR*), part of a two component system that responds to low oxygen seems to be very important in a shift from replicating *M. tuberculosis* to a nonreplicating form (Table 1.). Carbohydrate limitation also upregulated *dosR* and there is indeed an overlap of genes upregulated in phagosomes of macrophages and low carbohydrate availability. In dormancy models aerobic respiratory metabolism was down regulated while anaerobic respiration was upregulated as were DosR controlled genes (Murphy and Brown, 2007). Amino acid and carbon starvation results in the activation of the stringent response. RelA (Rv2583c) mediates this stringent response in *M. tuberculosis* and can globally down regulate components necessary in protein translation, and thus conserve badly needed resources in the mycobacterium during times of stress. RelA may be a target to prevent *M. tuberculosis* from entering dormancy or a target to force *M. tuberculosis* out of dormancy (Murphy and Brown, 2007).

The ability of *M. tuberculosis* to survive in a dormant state relies on maintaining cell integrity, viability, and a proton motive (Rustad et al, 2008). Entry into a dormant state may be followed later by reactivation and growth of this microorganism, and may occur due to waning immunity, age, or disease. T-cells originally controlling infection may become less activated and numbers of T-cells may decrease allowing mycobacteria increased ease of replication in host macrophages. *M. tuberculosis* needs energy to exit this dormant phase, and this may be found in the form of triacylglycerol which is known to accumulate in response to acidic stress, nitric oxide exposure, and lowered oxygen tension (Table 1.) (Sirakova et al, 2006; Garton et al, 2008). In fact triacylglycerol has been shown to be important to transition from dormancy to active growth (Low et al, 2009). The highly pathogenic strain of *M. tuberculosis*, the Beijing lineage strain, over produces triacylglycerol perhaps giving the microorganism a competitive edge in resisting hypoxic stress and dormancy (Fallow et al, 2010).

6. *Mycobacterium tuberculosis* responses to acidic stress

M. tuberculosis encounters acidity in the body in a number of locations including within immune cells, macrophages. When macrophages phagocytose tubercle bacilli, phagosomes of unactivated macrophages are limited in their ability to acidify due to the presence of live *M. tuberculosis*. Bacilli can inhibit phagosomal maturation and also inhibit phagosome lysosome fusion (Armstrong and Hart, 1971; Sturgill-Koszycki et al, 1994; Huynh and Grinstein, 2007). Virulent *M. tuberculosis* can exclude a proton ATPase from the phagosome in non-activated macrophages. Exposure to the cytokine INF-γ can result in increased activation of macrophages and these macrophages that phagocytose live virulent *M. tuberculosis* can lower the intra phagosomal pH (Schaible et al,1998; Via et al, 1998; MacMicking et al, 2003; Ehrt and Schnappinger, 2009). This pH's can be toxic to bacilli either killing them, or inhibiting their growth. The robustness of the response seems to lie in the activation and efficiency of the host's immune response. Anything that interferes with the host's immune status can negatively impact acidic modulation within phagosomes, and lead to more mycobacterial replication. In addition, the tubercle bacillus' ability to respond to acidic stress will likely affect the outcome of the infection.

Mycobacteria seem to bear an intrinsic ability to resist acidic stress. They have a thick waxy cell wall as well as an outer membrane that can resist acidic stress. This physical barrier may serve to inhibit entry of toxic protons, and anything that interferes with this barrier could increase acid susceptibility. Many mutants that are acid susceptible lie in genes that affect cell wall and lipid metabolism (Table 1.). Environmental mycobacteria are found in conditions that may be acidic and can grow at pHs as low as 4.0 (Santos et al, 2007). Pathogenic mycobacteria have evolved to resist acidic stress, and potentially share similar mechanisms with their environmental cousins (Kirschner et al, 1992; Kirschner et al, 1999).

Although *Mycobacterium smegmatis* has been found to have an acid tolerance system it is not known if *M. tuberculosis* also possesses one. However, a large number of genes are upregulated due to acidic stress in *M. tuberculosis*. Interestingly when *M. tuberculosis* is engulfed by the

phagosomes of macrophages many genes are upregulated, and when cocanamycinA is added which interferes with the development of acidity, 80% of genes in *M. tuberculosis* that are normally upregulated in the phagosomes fail to do so (Rohde et al; 2007). This is an indication that acidity is one of the main environmental signals *M. tuberculosis* experiences *in vivo*.

A number of genes that are upregulated by acidic stress have been identified in previous studies. Looking at rapid response to acidity at 15 or 30 minutes it was found that genes involved in cell wall ultrastructure were induced (Fisher et al, 2002). The *mymA* operon was induced in this study, and is under the control of VirS which is an AraC/XylS family transcription factor (Singh et al, 2005). The *lipF* promoter of *M. tuberculosis* is upregulated, but requires a longer time frame (Saviola et al, 2001). It fails to be upregulated at 30 minutes, instead needing more extended exposure to acidic stress of 1.5 hours. LipF is annotated to be an esterase and may also function to alter the cell wall structure. LipF has been shown to be part of the two component system *phoP/R* regulon. In fact many genes involved in the PhoP/PhoR regulon including *pks2*, *pks3*, and *pks4* are responsive to acidic stress (Table 1.) (Gonzalo-Asensio et al, 2009; Rohde et al, 2007). Thus PhoP/R may be responding to acidic stress or conversely PhoP/R controls a downstream regulator that responds to acidity. The *ompATb* gene encodes a porin that is active specifically at low pH and functions to pump ammonia into the phagosomal environment which serves to neutralize acidity (Song et al, 2011). Longer term exposure to acidic stress seems to stimulate production of triacylglycerol. *Tgs1* is not upregulated by short term acid exposure but exposure of three weeks duration or more (Sirakova et al, 2006; Low et al, 2009; Deb et al, 2009). Triacylglycerol production may be important for mycobacteria to resist stress and survive a dormant period which is induced by stress conditions. An energy source such as triacylglycerol may be needed to reanimate from dormancy once stresses such as acidity are removed. Mutatagenesis studies also revealed genes involved cell wall/cell envelope synthesis when mutated resulted in mycobacteria which were unable to maintain neutral pH within their microbial cytoplasm in the presence of acidic stress (Vandal et al, 2008; Vandal et al, 2009, Biswass et al, 2010).

The type VII secretion system, Esx-1, may also may be involved in response to acid stress (Abdallah et al, 2007). The 6 kDa early secreted antigenic target (Esat-6) and the 10kDa culture filtrate protein (CFP-10) are secreted by Esx-1. These two proteins form a heterodimer that can dissociate at acidic pH. Esat-6 is capable of lysing membranes, and *M. tuberculosis* has been identified to reside extraphagosomally in the cytoplasm of macrophages in some cases. In addition when the *esx-1* gene was mutated it could result in an *M. tuberculosis* strain that fails to escape from the phagosomal compartment into the cytoplasm (Simeone et al, 2009). Thus Esat-6 may be involved in mycobacterial responses to acidity and adaptation to *in vivo* stressors.

7. Response to oxidative damage

Inside phagosomes of activated macrophages tubercle bacilli are exposed to reactive oxygen intermediates. *M. tuberculosis* traffics to phagosomes, and a large number of genes are upre-

gulated by oxidative stress indicating this is an important stress *in vivo* (Wu et al, 2007). In addition nutrients are limited in the phagosome which may cause *M. tuberculosis* to enter a stationary phase of growth, which has been shown to induce internal oxidative damage. The gene *whiB1* is more active during stationary phase, and the protein produced by this gene has been shown to reduce cellular disulphide bridges that may predominate during this adaptational phase (Garge et al, 2009).

Mycobacteria contain a unique substance, mycothiol, which combats oxidative stress. Other bacterial species utilize glutathione which can also neutralize oxidative stress. Mycothiol contains cysteine residues which are oxidized when that condition predominates thus forming disulfide bonds, creating mycothione, and preventing other molecules in the mycobacterial cell from becoming oxidized (Table 1.). Human cells produce glutathione to combat oxidative damage, and glutathione is toxic to mycobacterial cells perhaps due to a redox imbalance generated by this substance in the mycobacteria (Venketaraman et al, 2008; Connell et al, 2008)). Mycobacteria also contain other molecules to detoxify oxidative damage including superoxide dismutase (SOD) and catalase (KatG) which can inactivate superoxide (Table 1.) (Shi et al, 2008). SOD and KatG are upregulated early in infection indicating an increase in oxidative damage due to superoxide. Oxidative damage is capable of harming DNA, and histone like proteins (LSR2) can protect against damage by compacting DNA and acting as a physical barrier. UvrB which repairs mycobacterial DNA damage also protects against oxidative damage (Darwin and Nathan, 2005; Colangeli et al, 2009).

8. Heat shock

One of the hallmarks of tuberculosis is fever and night sweats in which body temperature increases and is suboptimal for *Mycobacterium tuberculosis* replication and survival. This allows the immune system a competitive edge over the invading microbes. Heat stress can cause damage to *M. tuberculosis* by causing proteins to unfold which may then be degraded. In response, *M. tuberculosis* can upregulate chaperonins which complex with unfolded proteins and help them refold (Table 1.). The α-crystalline protein, or Acr-2, is activated by heat shock, and has demonstrated chaperonin activity (Pang and Howard, 2007).

Many proteins that are upregulated in *M. tuberculosis in vivo* are heat shock proteins that have chaperonine activity. While these proteins may benefit the organism by complexing with and refolding heat damaged proteins, they are also recognized by the immune system. Both the 65Kd heat shock protein and the HSP70 protein can be found extracellularly to *M. tuberculosis*, and are potent stimulators of an inflammatory response (Anand et al, 2010).

9. Low iron

Normally iron taken up by intestinal epithelial cells and bound to transferrin circulates within the body. This complex binds to cell surface receptors, and is internalized where it releases its

iron to be bound by the host cellular factor ferritin. Infection and inflammation are natural signals to the host to limit availability of iron. Proinflammatory cytokines stimulate hepcidin production, decrease iron uptake from the gut, and inhibits the iron efflux protein ferroportin (Johnson and Wessingling-Resnick, 2012). Inflammation thus inhibits iron uptake by the intestinal epithelium thus preventing iron from being loaded onto transferrin. Interfering with uptake limits iron availability in the host, and *M. tuberculosis* has been shown to be severely growth restricted in a low iron environment. It has been demonstrated in African studies that iron supplementation increases incidence of tuberculosis. Thus being anemic may be protective against infectious processes. Within human macrophages, Nramp1 (natural resistance associated macrophage protein) is produced and localizes to the phagosomal compartment where it reduces iron within this site possibly by extrusion. This function confers resistance to *M. tuberculosis* infections and mutations in the *nramp1* gene can result in increased susceptibility to active disease due to *M. tuberculosis* infection (Johnson and Wessingling-Resnick, 2012).

Mycobacteria have a variety of systems which aid in the uptake of iron and the regulation of iron responsive genes. As mycobacteria have been shown to be somewhat novel among gram positive bacteria, they possess an outer mycolic acid based membrane, as well as an inner membrane and periplasmic space. Porins in the outer membrane appear to transport iron in the presence of high iron conditions (Jones and Niederweis, 2010). *M. tuberculosis* under low iron conditions can produce the siderophore carboxymycobactin as well as mycobactin (Table 1.) (Banerjee et al, 2011). These molecules bind with a higher affinity to iron than the human host's storage proteins and steal iron from the host. Mycobactin is present within the inner membrane and thus can only bind iron imported into the periplasmic space. Interestingly lipid membranes with associated mycobactins may diffuse out, travel to lipid vesicles in the host cell, and sequester iron. These structures may recycle back to interact with the mycobacterium. Disruption of the genes responsible for production of mycobactins can cause these mutant mycobacteria to replicate less well in macrophages (Banerjee et al, 2011). Carboxymycobactins are excreted possibly by the type VII secretion or ESX system. Externally the carboxymycobactins bind available iron from transferrin (Banerjee et al, 2011). Porins and also ABC transporters may allow import of these iron loaded carboxymycobactins (Banerjee et al, 2011). The host cell, in response to infection and inflammation, produces siderocalins such as lipocalin-2 that can bind to and inactivate mycobactin from *M. tuberculosis* thus interfering with mycobacterial iron acquisition (Johnson and Wessingling-Resnick, 2012). In fact mice deleted for genes involved in production of siderocalin are much more susceptible to mortality due to *M. tuberculosis* infection (Johnson and Wessingling-Resnick, 2012). Inside the mycobacterial cell, iron is stored in bacterioferritin and a ferritin like protein. These proteins are required for replication in human macrophages and guinea pigs, act to store iron, and also to limit excess iron in the cells that can lead to iron mediated oxidative damage due to the Fenton reaction (Reddy et al, 2011).

Iron responsive genes in *M. tuberculosis* are controlled in part by the iron dependent regulator IdeR. This protein can act both as an activator and a repressor depending on where it binds within a mycobacterial promoter region (Manabe et al, 1999; Banerjee et al, 2011). Within

promoters of genes involved in mycobactin synthesis it acts as a repressor, inhibiting expression of these genes at high iron concentrations. In promoters of iron storage proteins it acts as an activator, stimulating expression of these genes at high iron concentrations and thus avoiding iron stimulated oxidative damage.

10. Hypoxic growth

In vivo M. tuberculosis experiences low oxygen tension that may be encountered in the centers of granulomas as previously described. Studies have shown that tuberculous granulomas are hypoxic in a variety of animal models including rabbits, guinea pigs, and nonhuman primates (Via et al, 2008). The response to low oxygen tension is biphasic. There is an initial response that predominates and is controlled by the two component system DosS/DosT-DosR (Table 1.). This two component system upregulates genes that are known to be part of the "dormancy regulon". DosR is the transcriptional regulator, and Dos T and DosS are the sensor kinases that respond to low oxygen tension as well as nitric oxide (Park et al, 2003; Kumar et al, 2007). *hspX* (*acr*, *Rv2031c)* is upregulated by low oxygen, is regulated by DosR, and has chaperonin activity that may aid in refolding proteins which are damaged by low oxygen tension (Vasudeva-Rao and McDonough, 2008; Florczyk et al, 2003). It is known that this protein is expressed *in vivo* as latently infected individuals possess T-cells that are reactive to the HspX protein (Geluk et al, 2007). Interestingly one half of the genes in the DosR regulon return to their baseline level after 24 hours. After this initial 24 hour period other regulators play a role in hypoxic responses such as sigE and sigC (Table 1.). An enduring hypoxic response begins after the initial response, and this may be important for *M. tuberculosis* to enter and stay in a dormant state (Rustad et al, 2008).

11. Toxin-antitoxin systems

Interestingly there are many toxin-antitoxin systems within the *M. tuberculosis* genome. These systems seem to provide a mechanism by which bacteria can alter growth rate rapidly, potentially in response to environmental stressors. The toxin is not a protein secreted and targeted against the human host, but targeted against mycobacterial cellular components. The toxin is a stable protein which may be complexed with an antitoxin forming a toxin-antitoxin pair. The antitoxin is relatively unstable and environmental stressors can inactivate it causing release of a free toxin. The toxin is then available to interact with cellular components, and may function to cleave mRNA thus inhibiting subsequent translation and rapidly halting growth of the bacterium. As static bacteria are more resistant to environmental stressors and antibiotics, this system may allow *M. tuberculosis* to survive in the face of external stressors. *M. tuberculosis* possesses 88 toxin-antitoxin systems and four of these have been shown to be activated by phagocytosis of bacilli, by macrophages, or hypoxia (Table 1.). It appears that the toxin in these systems acts by cleaving mRNA (Rapage et al, 2009).
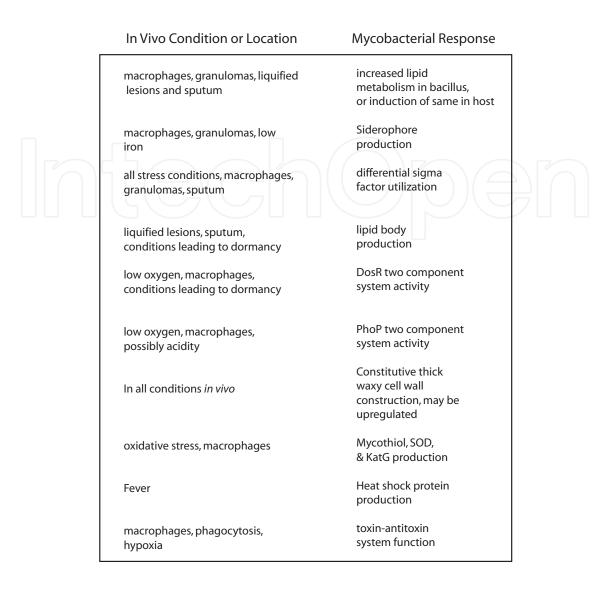
In Vivo Condition or Location	Mycobacterial Response
macrophages, granulomas, liquified lesions and sputum	increased lipid metabolism in bacillus, or induction of same in host
macrophages, granulomas, low iron	Siderophore production
all stress conditions, macrophages, granulomas, sputum	differential sigma factor utilization
liquified lesions, sputum, conditions leading to dormancy	lipid body production
low oxygen, macrophages, conditions leading to dormancy	DosR two component system activity
low oxygen, macrophages, possibly acidity	PhoP two component system activity
In all conditions *in vivo*	Constitutive thick waxy cell wall construction, may be upregulated
oxidative stress, macrophages	Mycothiol, SOD, & KatG production
Fever	Heat shock protein production
macrophages, phagocytosis, hypoxia	toxin-antitoxin system function

Table 1. Mycobacterial responses to in vivo stressors and conditions.

12. Two component systems

Two components systems are common in many bacteria. These systems are comprised of a sensor kinase which phosphorylates the response regulator as a result of an environmental signal, which is often a stress. The sensor kinases are trans membrane proteins which are embedded into membranes. They sense external stresses and transmit these signals internally into the bacterial cell by phosphorylating a response regulator that binds to its cognate promoter DNA, and regulates transcription. The mycobacterial genome contains 11 two component systems (Hett and Rubin, 2008). The large number of these systems in the mycobacterial coding regions is likely the result of evolution to accommodate bacterial responses to diverse stresses.

DosS/DosT-DosR was previously described, and responds to initial hypoxic stress (Table 1.) (Park et al, 2003). Some of the genes controlled by the transcriptional regulator DosR are upregulated by hypoxic stress, and are also part of the transcriptional regulator PhoP regulon, a member of the PhoP/R two component system. While it is unknown what environmental signal PhoP or the sensor kinase PhoR are responding to, genes controlled by PhoP either directly or indirectly are upregulated by such stresses as acidity and low oxygen (Table 1.) (Gonzalo-Asensio et al, 2008).

13. Sigma factors

Mycobacterial RNA polymerase catalyzes RNA synthesis from specific promoter sequences. This RNA polymerase is composed of subunits that comprise the core holoenzyme, and include two α subunits, a β, a β' and a ω subunit. The core enzyme, however, cannot target specific promoter sequences. A sigma factor is required for this function, and can bind and recognize specific -10 and -35 promoter sequences. As the mycobacterial genome possesses many different sigma factors, these RNA polymerase components can recognize diverse mycobacterial promoter sequences to activate a whole class of genes. This activity is in addition to specific transcription factors which bind to promoters, regulate transcription, and are not part of the RNA polymerase enzyme.

The mycobacterial genome possesses many different sigma factors that belong to different categories. The *M. tuberculosis* σ^A is responsible for regulating housekeeping genes, and is also an essential gene for mycobacterial growth *in vitro* and *in vivo*. While the sigma factor σ^B is highly similar to σ^A, it is nonessential and is induced by a variety of stresses including oxidative stress, heat shock, cold shock, stationary phase, and low aeration (Lee et al, 2008). There are a number of sigma factors designated to have extracellular function, and some respond to environmental stresses and are involved in the synthesis of the mycobacterial envelope. These sigma factors are SigC, SigE, SigF, SigG, SigH, SigI, SigJ, SigK, SigL, and SigM. One sigma factor that is known to respond to nutrient starvation is SigF. The sigma factor SigE is involved in response to heat shock and SDS exposure (Manganelli et al, 2004). Both SigJ and SigF are induced in response to antibiotic exposure (Manganelli et al, 2004). The sigma factor SigH also responds to heat shock and oxidative stress (Manganelli et al, 2004). Thus the use of sigma factors by the mycobacterial cell is a manner in which "master regulators" can control whole classes of genes to rapidly facilitate gene regulation in response to specific environmental stresses (Table 1.).

14. Summary

As mycobacteria invade their human hosts they must respond to a plethora of stresses many of which are generated by the host's immune system. Under this selective pressure, *M. tuberculosis* has evolved mechanisms to combat the toxic insults of the host. Although myco-

bacteria are inherently resistant to environmental stresses due to their thick waxy cell envelope, upregulation of genes further reinforce this defense. In addition there are proteins upregulated by environmental stressors which can detoxify the mycobacterial cell as is the case of acidic stress and upregulation of ammonia extruding pumps that neutralize acidic pH of the macrophage phagosome. Thus inducible systems allow *M. tuberculosis* to resist environmental stresses and persist in the human body to cause active or latent disease.

Understanding the specific steps in infection, the stresses associated with each step, and the mycobacterial response may be of clinical relevance. The knowledge that oxidative stress and acidic stress may predominate as adaptive immunity makes the host's macrophages more activated, may lead to the development of chemotherapeutic agents that target mycobacterial components produced by these stressors during this infective stage. In addition, the knowledge that mycobacteria may utilize toxin-antitoxin systems to slow their growth and to enhance their innate antibiotic resistance may spur the development of therapies that target these systems which could be used in conjunction with traditional antibiotic treatments. Chemotherapeutic agents given to decrease activity of triacylglycerol synthase may decrease infectivity of sputum positive individuals by inhibiting lipid body production in the bacilli while antibiotic treatment lags in its sterilizing activity. Ultimately treatments may be developed which target inducible systems upregulated by stresses, and may interfere with mycobacterial responses to these stressors. By thwarting these adaptive responses potentially with chemotherapeutic agents, mycobacteria may be rendered more fragile and susceptible to the host's immune system. In addition a greater understanding of how *M. tuberculosis* enters a latent state of persistence could lead to treatments that prevent this microbe from reactivating from the dormant state, or from becoming dormant to begin with. Greater understanding of *M. tuberculosis* responses to *in vivo* growth will hopefully lead to the development of technologies that lessen *M. tuberculosis'* global impact on human health.

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