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MINIREVIEW

Mycobacterium tuberculosis Transcription Machinery: Ready To Respond to Host Attacks

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Regulating responses to stress is critical for all bacteria, whether they are environmental, commensal, or pathogenic species. For pathogenic bacteria, successful colonization and survival in the host are dependent on adaptation to diverse conditions imposed by the host tissue architecture and the immune response. Once the bacterium senses a hostile environment, it must enact a change in physiology that contributes to the organism's survival strategy. Inappropriate responses have consequences; hence, the execution of the appropriate response is essential for survival of the bacterium in its niche. Stress responses are most often regulated at the level of gene expression and, more specifically, transcription. This minireview focuses on mechanisms of regulating transcription initiation that are required by *Mycobacterium tuberculosis* to respond to the arsenal of defenses imposed by the host during infection. In particular, we highlight how certain features of *M. tuberculosis* physiology allow this pathogen to respond swiftly and effectively to host defenses. By enacting highly integrated and coordinated gene expression changes in response to stress, *M. tuberculosis* is prepared for battle against the host defense and able to persist within the human population.

The survival of any organism relies on its ability to sense and respond to changes in its environment. For bacteria, stress responses are primarily mediated through the regulation of gene expression. By integrating multiple molecular approaches to gene regulation, pathogenic bacteria are able to orchestrate condition-specific patterns that promote survival and pathogenesis in the face of a strong immune response. This minireview focuses on mechanisms of transcription regulation required for stress responses in one of the most successful and deadly pathogens in the world, *Mycobacterium tuberculosis*. *M. tuberculosis* has coexisted with humans for >50,000 years (1) and continues to cause more than 1.5 million deaths a year (2). The coevolution of *M. tuberculosis* with the human host response to infection has resulted in a pathogen that is specialized for long-term infection in people. Tuberculosis is a complex disease that requires the bacteria to multiply within phagocytes, survive extracellularly in hypoxic and necrotic granulomas, and endure a robust immune response to persist in the host. During infection, the host immune response restrains *M. tuberculosis* from proliferating by imposing a battery of defenses, including reactive oxygen and nitrogen stress, hypoxia, acid stress, genotoxic stress, cell surface stress, and starvation (3). Despite this onslaught of attacks, *M. tuberculosis* is able to persist for the lifetime of the host, indicating that this pathogen has highly effective molecular mechanisms to resist host-inflicted damage. In order to enact these defenses and facilitate this specialized lifestyle, *M. tuberculosis* executes a complex, interconnected web of stress responses that rely on changes in gene expression. In fact, *M. tuberculosis* is well suited to respond quickly to diverse stresses in a coordinated fashion. For instance, the RNA polymerase (RNAP) bears kinetic properties that allow it to be easily modulated by accessory factors. Compared to other obligate human pathogens, *M. tuberculosis* encodes the highest ratio of σ factors to genome size (4), which allows the bacterium to tailor its expression profile in response to a given environment. Even during exponential growth in culture, traditionally thought of as a relatively stress-free environment, *M. tuberculosis* expresses its entire complement of σ factors (5–7), indicating that *M. tuberculosis* is poised to quickly respond to stress. *M. tuberculosis* also integrates stress responses into basic cellular processes; as a result, some stress-associated transcriptional regulators are essential in *M. tuberculosis*. In this minireview, we discuss features of the mycobacterial transcription apparatus that position *M. tuberculosis* to be ready to respond to host attacks, the networks of factors that contribute to these responses, and how this culminates in a successful pathogenic strategy. The general strategies to be discussed are illustrated in Fig. 1, and individual factors touched on in this minireview are summarized in Fig. 2.

THE MYCOBACTERIAL RNA POLYMERASE—READY TO RESPOND

Transcription is achieved in all bacteria by a single core RNAP enzyme, consisting of the essential subunits β and β′ and 2 α subunits along with the nonessential ω subunit (8, 9). To recognize and bind promoter sequences upstream from genes, the core RNAP associates with a σ subunit to form an RNAP holoenzyme. Most transcriptional regulation occurs at the level of initiation (10), and transcription factors (TFs) can mediate this regulation by directly affecting the polymerase-promoter interaction, manipulating the equilibrium between closed and open RNAP-promoter complexes (RP_c and RP_o, respectively), or affecting rates of promoter escape (11, 12). The majority of studies on the mechanisms of transcription initiation and its regulation have used *Escherichia coli* as a model system. However, multiple groups have recently shown that *Mycobacterium bovis* RNAP, which differs from the *M. tuberculosis* RNAP by only one amino acid (aa), exhibits an inherently unstable RP_o complex compared to *E. coli* RNAP on the same promoter (13, 14). In these reports, saturating

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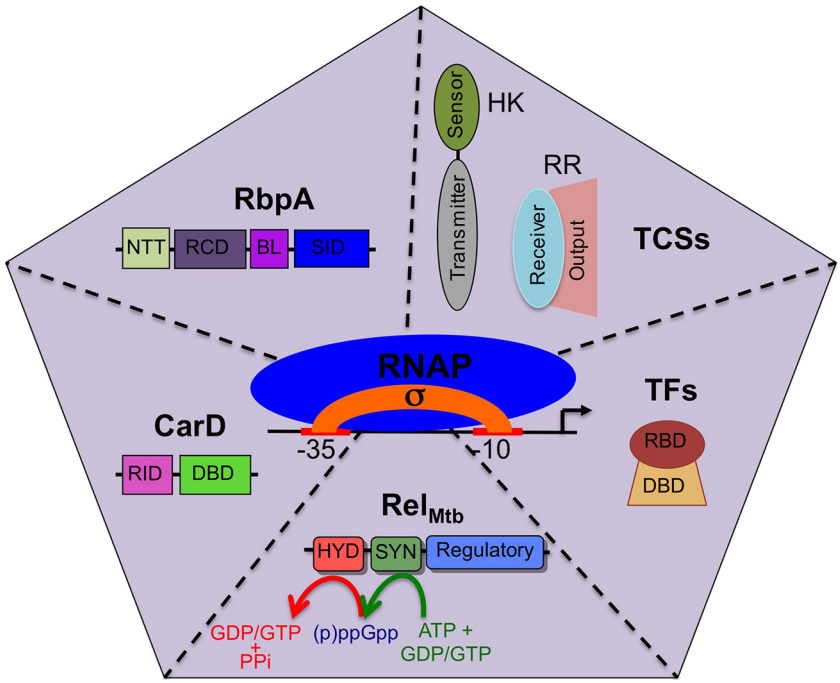

FIG 1 Summary of the branches of transcriptional regulation that are discussed in this minireview. The illustration shows 6 types of factors (σ factors, CarD, RbpA, TCSs, TFs, and Rel_{Mtb}) that modulate RNAP activity at promoters to mediate reprogramming of the expression profile in *M. tuberculosis* in response to different environments. A σ factor associates with the core RNAP to form the RNAP holoenzyme, which is then modified by the other factors shown in the sections of the pentagon. Domains of each protein are shown. For CarD, RID is the RNAP interaction domain and DBD is the DNA binding domain. For RbpA, NTT is the N-terminal tail, RCD is the RbpA core domain, BL is the basic linker, and SID is the sigma interaction domain. For Rel_{Mtb}, HYD is the (p)ppGpp hydrolase domain and SYN is the (p)ppGpp synthetase domain. For TFs, RBD is the RNAP binding domain and DBD is the DNA binding domain. In the presence of a given stress, these factors coordinate their responses to effectively respond to host attacks.

concentrations of *M. bovis* RNAP σ^A holoenzyme were found to be incapable of opening a large percentage of the promoters, leaving the majority of bound complexes in the closed state. It has been proposed (14) that the presence or absence of lineage-specific insertions within RNAP could contribute to the inherent differences in stability of the promoter complexes formed by *M. bovis* versus *E. coli* RNAP. Notably, RNAPs from *Bacillus subtilis*, *Thermus aquaticus*, and *Thermus thermophilus* have also been found to generate relatively unstable open promoter complexes (15–17). Based on these observations, it is worth considering that the properties of *E. coli* RNAP may not be representative of most bacterial RNAPs and that there may be significant lineage-specific variation in enzyme kinetics. The inherent instability of RNAP-promoter complexes would allow the mycobacterial RNAP to be poised to respond to changes in the environment by being easily modified in activity by additional factors.

σ FACTORS: THE GENERALS OF STRESS RESPONSES

The first determinant of gene expression in response to different conditions is the activity of the σ factor repertoire. Each σ factor binds a specific promoter sequence, thus determining what promoters are targeted by the RNAP holoenzyme for transcription. Changes in σ factor activity in response to different stresses and conditions are able to shift a bacterium's expression profile. The σ factor network of *M. tuberculosis* includes one essential housekeeping group 1 σ factor (σ^A), one stress-responsive group 2 σ factor (σ^B), and 11 group 3 and 4 alternative σ factors that also function as environmentally responsive regulators (σ^C to σ^M) (4, 6, 18). This broad panel of σ factors allows *M. tuberculosis* to tune its transcriptional response for a large and diverse set of conditions. All of the σ factors in *M. tuberculosis* belong to the σ^{70} family, whose members in *E. coli* recognize two sequences in the promoter DNA, the −10 element (recognized by sigma region 2.4) and the −35 element (recognized by sigma region 4.2) (19). *M. tuberculosis* promoters contain a conserved −10 sequence that is essential and sometimes sufficient for transcription, while the −35 sequences are less conserved (19–21). The spacer region between the −10 and −35 elements in *M. tuberculosis* also varies dramatically compared to *E. coli* promoters (19, 22, 23). These differences in promoter elements may reflect the sigma diversity in *M. tuberculosis* (19, 23).

The activity of σ factors in *M. tuberculosis* is most often regulated by anti-σ factors that inactivate their cognate σ factors until a signal is received to liberate the σ factor for action. Specifically, σ^B, σ^D, σ^E, σ^F, σ^H, σ^K, σ^L, and σ^M are all regulated by a cognate anti-σ factor (24–32). A putative anti-σ factor has also been proposed for σ^G (33). To investigate under which conditions a particular σ factor is active, the expression levels of σ factors have been studied *in vitro* under many physiologically relevant conditions, but transcriptional upregulation of a given σ factor does not necessarily equate to σ factor activity. Therefore, σ factor gene deletion or overexpression strains have been used to determine the functional role of individual σ factors in response to stress. These data are summarized here and together paint a picture of an intricate circuitry of transcriptional regulation that integrates multiple σ factor regulons under many conditions (Fig. 3 and 4), allowing *M. tuberculosis* to respond to the arsenal of attacks from the host.

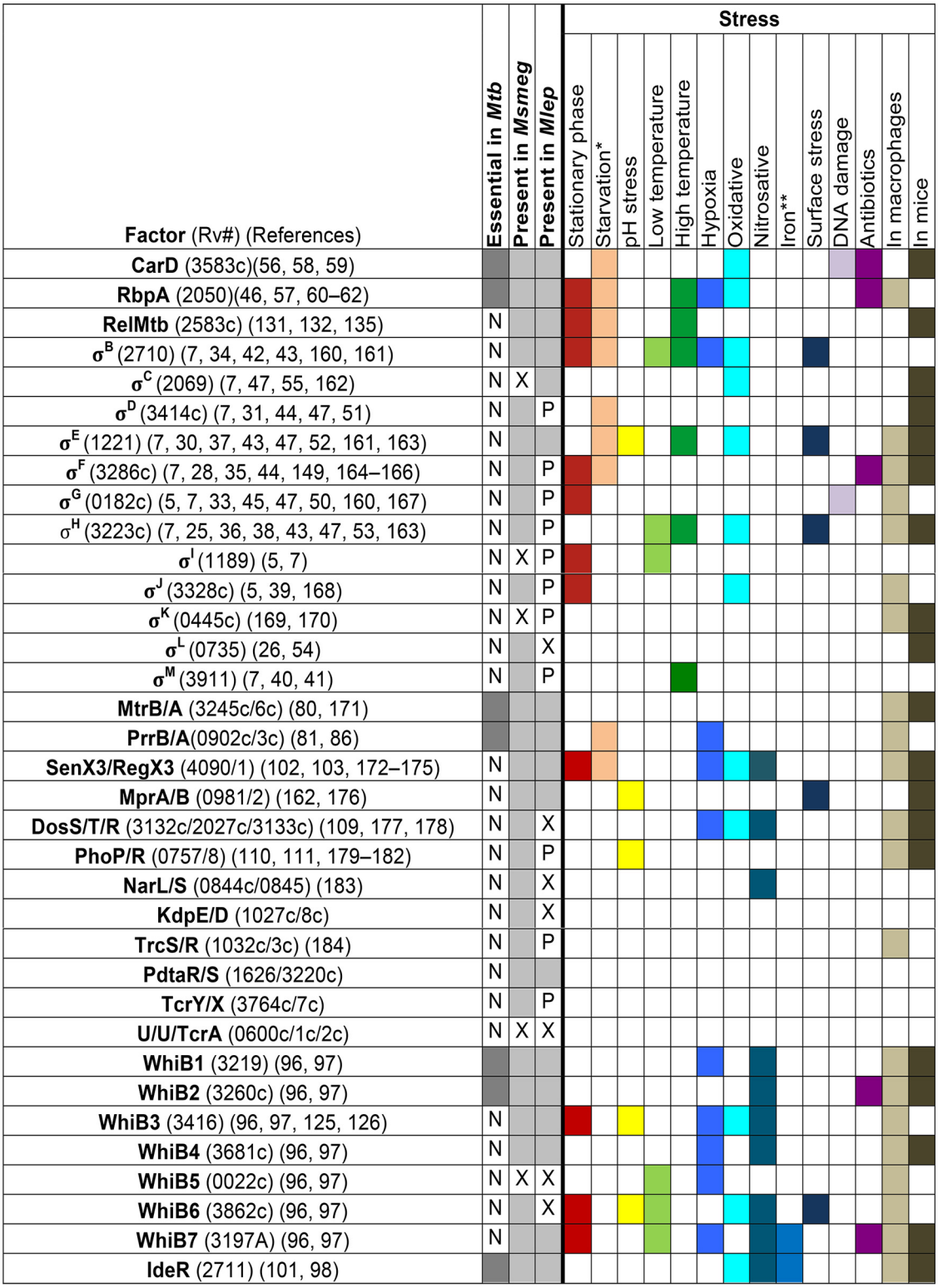

FIG 2 Conservation of *M. tuberculosis* regulatory factors and the stresses that the factors are associated with in *M. tuberculosis* (160–184). The left side of the table designates whether the gene for a transcriptional regulator is essential (shaded) or not essential (N) in *M. tuberculosis* (*Mtb*) and whether that gene is conserved (shaded) or not conserved (X) or exists as a pseudogene (P) in the environmental saprophytic *M. smegmatis* (*Msmeg*) or the obligate pathogen *M. leprae* (*Mlep*). The right side of the table indicates whether a particular stress condition has been associated with a given transcriptional regulator. Involvement in the response to a particular stress is designated by shading of the box and may represent expression profiling data or phenotypic analysis of mutants. An unshaded square indicates that the factor is not induced, is not important for survival, or has not been studied under that particular condition. U, unnamed factor; *, starvation (including nutrient, phosphate, and nitrogen starvation); **, iron-depleted or iron-replete conditions. See specific references for more information.

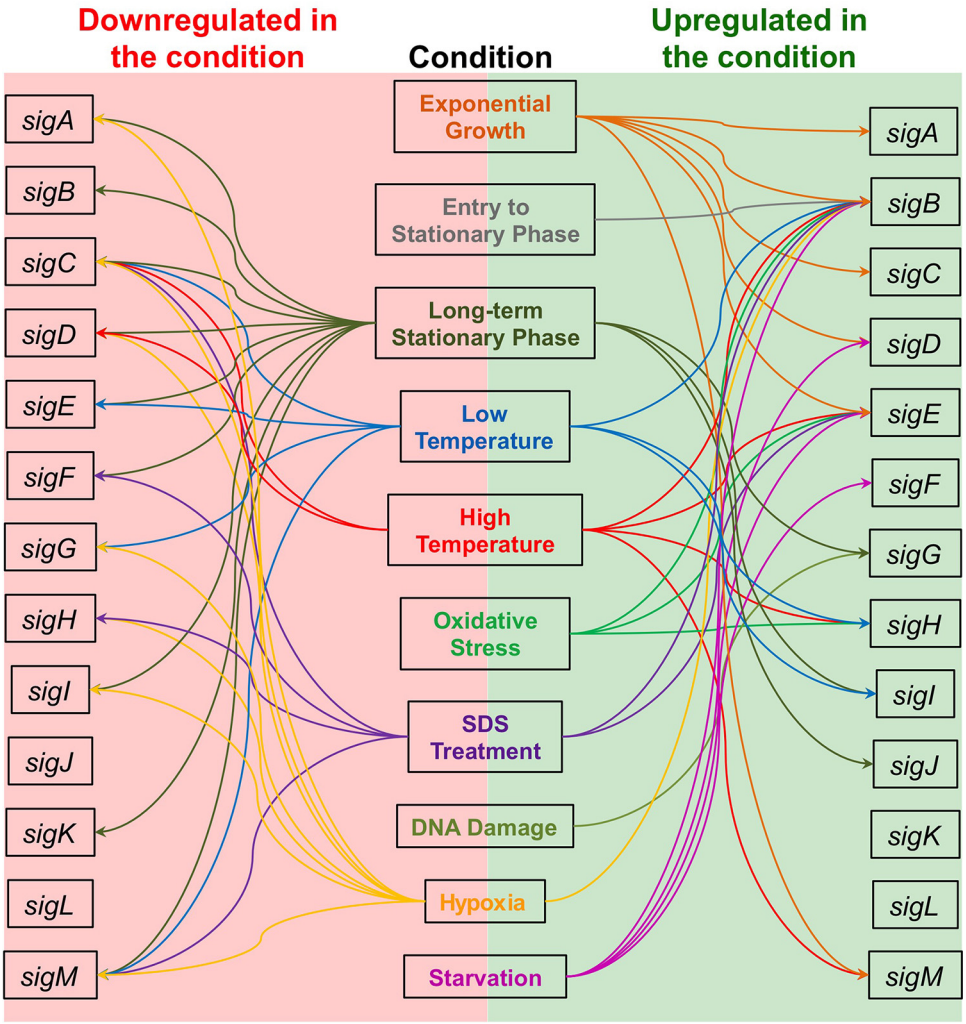

FIG 3 Transcriptional regulation of *M. tuberculosis* σ factor genes in response to various stresses. Transcriptional responses of σ factor genes of *M. tuberculosis* include responses to entry to stationary phase, the long-term stationary phase, mild cold shock (room temperature), heat shock (45°C), oxidative stress, exposure to SDS, DNA damage, hypoxia, and starvation. The σ factor genes that are transcriptionally upregulated in response to a stress are diagramed with arrows to the right, and the σ factor genes that are transcriptionally downregulated are shown with arrows to the left. The σ factor genes that are highly expressed during exponential growth in culture are shown as being upregulated under this condition. Where no arrow is present to connect a σ factor gene to a particular stress, this indicates that expression of the σ factor gene is not significantly changed during exposure to that stress or has not been studied under that particular condition. References are available in the text.

During exponential growth of *M. tuberculosis* in culture, *sigA*, *sigB*, *sigC*, *sigD*, *sigE*, and *sigM* are the most highly expressed σ factor genes (7). Upon entry into stationary phase, levels of *sigB* transcripts increase (34). Strains with a disrupted *sigF* gene grow to a density three times greater than that seen with wild-type cultures in stationary phase, suggesting that σ^F may have a key role in regulating this transition (35). Later in stationary phase, there is a global change in regulation of σ factors resulting in downregulation of most of the σ factor genes, with the exception of *sigG*, *sigI*, and *sigJ*, which are upregulated in long-term stationary cultures (5, 7). σ^H is a central regulator of the response of *M. tuberculosis* to both heat and oxidative stress through regulation of *sigE*, *sigB*, heat shock proteins, thioredoxin reductase/thioredoxin, and synthesis of mycothiol precursors (36). In addition to σ^B, σ^E, and σ^H, survival during oxidative stress is also dependent on σ^C and σ^J (6, 36–39). *sigM* is also induced during exposure to heat in the *M. tuberculosis* CDC1551 strain but not in *M. tuberculosis* H37Rv, indicating strain-specific regulation of σ factor expression (24, 40, 41). Cold temperatures induce expression of *sigB*, *sigH*, and *sigI* while repressing transcription of *sigC*, *sigE*, *sigG*, and *sigM* (7). σ^I is the most highly induced σ factor during cold shock and has been proposed to be important for the bacterium's survival in aerosol particles between hosts (7). Deletion of *sigB*, *sigE*, or *sigH* has been shown to increase *M. tuberculosis*'s sensitivity to cell surface stress (6, 37, 42, 43). Expression of *sigB* is also upregulated under hypoxic conditions (7) and σ^B is the only σ factor shown to impact the sensitivity of *M. tuberculosis* to hypoxia (42). Deletion of *sigF* induces permeability changes in the cell envelope, although this does not affect sensitivity to tested surface stresses (35, 44). *In vitro* studies have shown that *sigG* is induced upon DNA damage but that deletion of *sigG* does not sensitize strains to DNA damage (45). *sigB*, *sigD*, *sigE*, and *sigF* have all been shown to be upregulated during prolonged nutrient starvation (46).

Evidence that alternative σ factors are important in *M. tuber-*

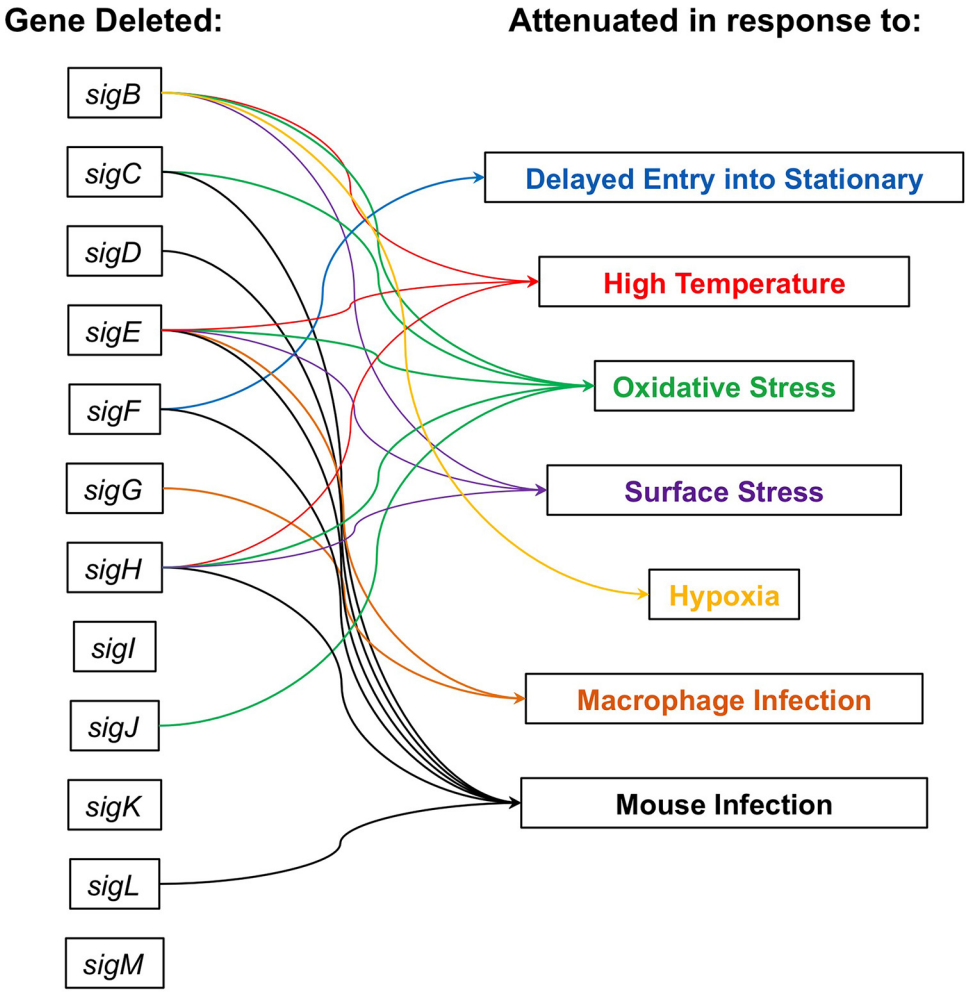

FIG 4 Effects of σ factor gene deletions on stress responses in *M. tuberculosis*. Arrows indicate whether deletion of a σ factor gene causes delayed entry into stationary phase, decreased survival during heat shock, decreased survival during oxidative stress, decreased survival during surface stress or changes in cell permeability, decreased survival during hypoxia, decreased survival in macrophages, or decreased immunopathology during mouse infection. Where no arrow is present to connect a σ factor gene to a particular stress, this indicates that deletion of that σ factor gene did not significantly change survival during exposure to that stress or has not been studied under that particular condition. References are available in the text.

culosis during infection has come from cell culture and animal infection models. *sigE*, *sigF*, *sigG*, *sigH*, and *sigJ* are upregulated during infection of macrophages (47, 48), and both *sigE* and *sigG* are necessary for survival within macrophages (37, 49, 50). Deletion of *sigB*, *sigG*, *sigJ*, or *sigM* has no effect in animal models (6, 39, 40, 50). Deletion of *sigD*, *sigE*, *sigH*, or *sigL* results in a delayed time to death without affecting bacterial burden (51–54), while deletion of *sigC* or *sigF* results in a delayed time to death and a decrease in bacterial burden during acute (*sigC*) or chronic (*sigF*) infection (35, 44, 55). The importance of individual σ factors during infection and for survival under stressful conditions highlights both their central role in guiding *M. tuberculosis*'s stress response and the diverse adverse conditions encountered by *M. tuberculosis* during infection.

CarD AND RbpA—MAINTAINING THE PEACE, BUT READY TO DEFEND

The next branch of transcriptional regulation during stress responses involves RNAP-binding proteins that further modify gene expression from a given holoenzyme. CarD and RbpA are RNAP-binding proteins in *M. tuberculosis* that were each originally identified in experiments looking for genes upregulated in response to stress (56, 57). *carD* expression is upregulated in response to oxidative stress, starvation, and a broad panel of antibiotics. CarD activity is required for survival under the same conditions as well as for virulence in a mouse model of infection (56, 58, 59). *rbpA* is upregulated during oxidative stress, stationary phase, starvation, hypoxia, high temperatures, and treatment with antibiotics and during infection in macrophages (46, 57, 60–62). Overexpression of *rbpA* in mycobacteria also improves resistance to the antibiotic rifampin (63). CarD and RbpA both act by stabilizing the inherently unstable mycobacterial RNAP-promoter complexes, albeit by different mechanisms. While the presence of RbpA is limited to actinobacteria, CarD is present in members of numerous other bacterial phyla (56, 64, 65), including *Bacillus* and *Thermus*, where purified RNAPs also generate relatively unstable open promoter complexes (15–17), but not in *E. coli*, where RNAP generally forms stable open complexes (13, 14) (Fig. 5). *carD* and *rbpA* are essential in *M. tuberculosis* even during growth in nutrient-rich cultures (56, 66–68), indicating a general role in

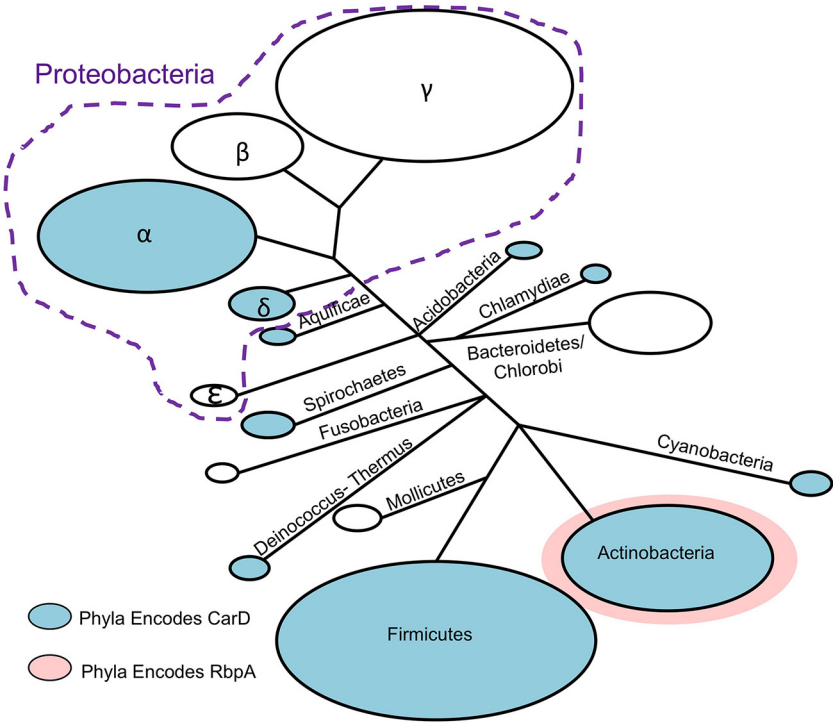

FIG 5 Phylogenetic distribution of CarD and RbpA. The BLAST database of completed genomes was searched for homologs of *M. tuberculosis* CarD and RbpA. Homologs of each protein were schematically drawn on a phylogenetic tree using a previously calculated phylogenetic distribution of bacteria based on the sequence conservation of RNAP subunits (185). Blue-shaded phyla have members that encode CarD homologs, members of the pink-shaded phylum (actinobacteria) encode RbpA, and phyla that are not shaded do not encode CarD or RbpA.

promoting efficient gene expression that also allows the RNAP to optimally respond to stress.

CarD interacts with the RNAP β-subunit β1-lobe through an N-terminal RNAP interaction domain (RID) and with DNA via a C-terminal basic patch (56, 58, 59, 65, 69, 70). In mycobacteria cultured under nutrient-rich conditions, CarD associates with RNAP-promoter complexes throughout the genome to enhance RP_o stability (14, 58, 59, 65). Using a bulk fluorescence assay to measure the effects of CarD on transcription initiation kinetics, it was shown that CarD associates with RP_o with high affinity and slows the rate of DNA closing by preventing bubble collapse and that CarD associates with RP_c with lower affinity and increases the rate of DNA opening (13). Importantly, the concentration of CarD in cells is sufficient for both of these activities to be physiologically relevant (13). These two activities of CarD change the kinetics of open complex formation such that the *M. bovis* RNAP more closely mirrors the *E. coli* RNAP (13, 14). The interactions between CarD and both DNA and RNAP are required for CarD activity (13). In addition, a conserved tryptophan within the C-terminal basic patch is also important for CarD's effects on RNAP-promoter complex stability and, based on structural studies, has been proposed to serve as a wedge at the upstream edge of the transcription bubble that prevents bubble collapse (59, 64, 65). Taken together, the inherently weak transcription initiation activity of *M. bovis* RNAP and CarD's global promoter localization suggest that CarD may be a general member of the mycobacterial transcription machinery.

RbpA consists of a central RbpA core domain (RCD) flanked by an unstructured 26-aa N-terminal tail and a C-terminal σ interaction domain (SID) linked to the RCD by a 15-aa basic linker (BL) (68, 71, 72). RbpA forms a stable binary complex with the σ2-domain of group 1 (σ^A in *M. tuberculosis*) and certain group 2 (σ^B in *M. tuberculosis*) σ factors through its SID (68, 71, 72), with additional contacts made between the N terminus and the σ factor (68). Based on structural modeling, the RbpA BL domain and adjacent residues interact with the DNA phosphate backbone of the nontemplate strand upstream of the −10 promoter element in the RP_o conformation (68). Additional contacts between RbpA and RNAP β have been proposed based on cross-linking experiments (63, 73, 74), but the recent structural modeling of RbpA onto an RNAP-promoter open complex would be incompatible with these interactions (71), suggesting that further analysis will be needed to resolve these inconsistencies. RbpA has been shown to increase the affinity of the σ factor to the core RNAP, increase the affinity of RNAP holoenzyme to promoter DNA, and facilitate the formation of RP_o (71, 75, 76), all of which could contribute to the ability of RbpA to promote RNAP-promoter complex formation and stability. The housekeeping σ factor σ^A has been reported to have an affinity for *M. tuberculosis* RNAP core enzyme similar to that of the alternative σ factor σ^F (74), in which case RbpA may be necessary to improve σ^A affinity and competitiveness for RNAP under conditions that require the activity of σ^A. In *E. coli*, in contrast, σ^{70} has a very high affinity to the RNAP core enzyme and thus can outcompete other σ factors under conditions where it is required without accessary factors such as RbpA. The RbpA SID and BL are important and sufficient to partially activate transcription *in vitro* (71, 72), but full activation of transcription requires the full-length protein, although the function of the N terminus of RbpA remains elusive.

Based on structural modeling performed with the information

currently available, association of CarD and RbpA with the same RNAP holoenzyme is feasible (71), but why *M. tuberculosis* requires both CarD and RbpA activities is unknown. CarD and RbpA transcriptional regulatory activities have thus far been analyzed only on limited promoters under limited conditions. However, their roles and effects at individual promoters likely depend on the kinetic properties of individual RNAP-promoter complexes and the presence of additional transcriptional regulators. The roles for CarD and RbpA during stress responses indicate that their effects on RP_o stability also provide a mechanism for adjusting gene expression during the switch between different physiological states in response to stress. Indeed, RP_o formation and stability comprise a commonly regulated step of transcription initiation during stress responses in bacteria, including during the stringent response (77, 78). It is possible that CarD and RbpA are important in stabilizing transcription complexes activated by stress-responsive transcription factors or alternative σ factors. While the functions of CarD and RbpA in stress responses remain unclear, the diversity of the stresses that they respond to suggests that they are acting at a common point shared among numerous stress responses.

ESSENTIAL TCSs AND TFs—ALWAYS ON THE LOOKOUT FOR HOSTILITY

M. tuberculosis encodes 12 complete two-component systems (TCSs), which are classically recognized as bacterial systems that sense and respond to stress and changes in the environment (79). Each TCS consists of at least one sensor histidine kinase (HK) that responds to specific environmental conditions by autophosphorylation and phosphotransfer to its cognate response regulator (RR), which then binds DNA and activates transcription of a specific regulon (79). Two TCSs in *M. tuberculosis*, MtrAB (80) and PrrAB (81), are essential for growth under unstressed culture conditions and have been integrated into the basic physiology of the bacteria. The HK MtrB colocalizes with cell division machinery at the bacterial septa and poles (82). Upon stimulation by an unknown signal, MtrB phosphorylates its cognate RR MtrA, which then binds DNA and activates transcription of a regulon that includes essential replication and cell division genes *dnaA* and *ripA* as well as the *fbpB* and *rpfB* genes that encode proteins with roles during infection (82–84). Integration of a TCS with the cell division machinery could allow these slowly replicating bacteria to sense environmental stress and abort cell division if unfavorable conditions surface. The second essential TCS in *M. tuberculosis* is the PrrAB system. The RR PrrA can bind DNA in the unphosphorylated state, but its binding affinity increases once phosphorylated by HK PrrB (85). The stimulus that results in activation of the PrrAB TCS has not been characterized, but expression of the *prrAB* operon is induced by nitrogen limitation and growth inside macrophages (81, 86), suggesting a possible role for this TCS under these conditions.

M. tuberculosis also encodes a series of essential iron-binding transcription factors (TF). *M. tuberculosis* does not contain functional homologues of the common redox-sensing TFs, FNR, SoxR, and OxyR, that allow other bacteria to sense and respond to redox state and reactive nitrogen and oxygen species (87–91). Instead, *M. tuberculosis* encodes a 7-member family of WhiB iron-sulfur (Fe-S) cluster TFs that sense the redox state in the cell and regulate gene expression accordingly (92). Of these, *whiB1* and *whiB2* are predicted to be essential, although their regulons have yet to be defined (93–95). *whiB1* is also upregulated during hypoxia and within infected mouse lungs (96, 97). WhiB2 may play a role in cell cycle progression, as a conditional *whiB2* mutant in *Mycobacterium smegmatis* was filamentous during depletion (95). The iron-binding TF IdeR is also essential for *M. tuberculosis* viability (98). IdeR dimerizes when bound to iron (99) and binds DNA as a dimer to inhibit transcription of genes involved in iron uptake and storage in order to promote adaptation to changing levels of iron (100, 101). By reducing levels of intracellular iron that can catalyze formation of reactive oxygen species, IdeR protects *M. tuberculosis* from oxidative and nitrosative stress and is important for survival in macrophages and mice (98, 100, 101). The essentiality of *whiB1*, *whiB2*, and *ideR* indicates a particular need for *M. tuberculosis* to couple redox sensing and iron availability with basic cellular processes to maintain homeostasis.

NONESSENTIAL TCSs AND TFs: SPECIAL FORCES OF THE STRESS RESPONSE TEAM

In addition to the essential TCSs and TFs mentioned above, *M. tuberculosis* maintains 10 nonessential TCSs and a number of nonessential TFs that are not required for bacterial growth *in vitro* but respond to particular stresses.

- The SenX3/RegX3 TCS is activated under low-phosphate conditions to regulate expression of genes encoding proteins involved in phosphate uptake, translation, lipid metabolism, DNA replication, and DNA repair (102, 103). The SenX3/RegX3 TCS is important for optimal *M. tuberculosis* growth during phosphate starvation and for survival in macrophages and mice where the bacteria encounter low phosphate levels (102).
- The DosRST system responds to nitric oxide and hypoxia to activate the "dormancy regulon" in *M. tuberculosis* (104). This TCS contains 2 separate HKs, DosS and DosT, that are both capable of activating the DosR RR. DosS acts as a redox sensor and DosT as a hypoxia sensor, illustrating the integration and differentiation of *M. tuberculosis* stress responses (105). Genetic disruption of the *dosRST* TCS results in reduced bacterial survival under low-oxygen conditions, in mouse models that develop hypoxic lesions, and in a nonhuman primate macaque model of infection (106–109).
- The PhoPR TCS is stimulated by low pH (110). The PhoP regulon includes multiple genes involved in cellular lipid synthesis, *dosR*, *dosS*, and genes involved in the ESX1 secretion system (111, 112). *M. tuberculosis* strains deficient in PhoPR activity display defects in replication in mice and macrophages (111–113). Supporting the idea of a role in *M. tuberculosis* virulence, mutations in *phoPR* in *M. bovis* and *Mycobacterium africanum* are associated with reduced mycobacterial virulence (114). In addition, *M. tuberculosis phoPR* mutants have defects in cell morphology and lipid production in the absence of stress, suggesting that PhoPR is required to maintain normal cell physiology under all growth conditions (113).
- The MprAB TCS regulates expression of a subset of genes in the DosR regulon, the stress-responsive chaperone *pepD*, and the *espA* operon, which encodes ESX-1 substrates (115–118). The MprAB TCS also activates expression of *sigB* and *sigE* in response to envelope stress and indirectly regulates

the stringent response mediator *M. tuberculosis rel* gene (rel_{Mtb}) through σ^E activity (119, 120). Deletion of this TCS compromises *M. tuberculosis* viability during a persistent infection in mice but renders *M. tuberculosis* hypervirulent in macrophages, suggesting a role for this TCS in allowing the bacteria to appropriately respond to their specific *in vivo* niche (80, 121).

- Genes encoding six additional TCSs, KdpDE, TrcRS, TcrXY, NarLS, PtdaRS, and Rv0600c/Rv0601c/TcrA, have been identified in the *M. tuberculosis* genome but have yet to be investigated in detail (79).
- *M. tuberculosis* encodes five nonessential Fe-S cluster WhiB TF family members that have been implicated in a variety of cellular responses (96, 97). In particular, WhiB3, WhiB4, and WhiB5 impact *M. tuberculosis* virulence (122–124). Of these, WhiB3 has been studied in the most detail. WhiB3 promotes mycobacterial lipid regulation, and *whiB3* mutants demonstrate altered macrophage cytokine release and reduced pathology *in vivo*, without directly impacting bacterial titers (125, 126). A model has been proposed in which WhiB3 senses the intracellular redox state and redirects lipid synthesis pathways to cope with reductive stress generated by host lipid catabolism during infection (125).
- *M. tuberculosis* encodes a number of other known and predicted TFs not highlighted in this review. Recently, researchers overexpressed 200 predicted TFs in *M. tuberculosis* and performed chromatin immunoprecipitation sequencing experiments and microarray analyses to catalogue a genome-wide characterization of TF binding events and target gene expression (127–129). These reports describe 16,000 binding sites for 154 TFs and identify regulatory routes for ~70% of the genome. The complex regulatory circuits that were uncovered highlight how much remains to be investigated regarding how *M. tuberculosis* regulates transcription to integrate precise stress responses.

THE STRINGENT RESPONSE: WHEN RATIONS RUN LOW

The stringent response is a conserved global stress response in bacteria that provides an additional layer of gene regulation in harsh environments. The stringent response is best characterized during amino acid starvation, when the Rel_{Mtb} enzyme senses uncharged tRNAs in ribosomes and responds by transferring the pyrophosphate (PPi) group from ATP to GDP and GTP to synthesize hyperphosphorylated guanine nucleotides ppGpp and pppGpp [collectively called (p)ppGpp] (130). (p)ppGpp then coordinates downstream regulation of bacterial physiology and mediates changes in the transcriptional profile to support survival during stress. Deletion of rel_{Mtb} led to differential expression of 159 genes during starvation, including genes involved in coordinating metabolic rate reduction, production of mycobacterial cell wall and lipids, secreted proteins, and cell division machinery (131). (p)ppGpp synthesis by Rel_{Mtb} is required for survival under low-nutrient conditions, in long-term culture, and during infection in animal models, all indicative of a strict requirement for Rel_{Mtb} during exposure to stress (131–135). In *E. coli*, (p)ppGpp directly affects transcription initiation by binding the RNAP (136, 137). In contrast, in a number of Gram-positive bacteria, (p)ppGpp inhibits GTP biosynthesis by directly interacting with GTP synthesis enzymes, which impacts gene expression by altering initiating nucleotide levels (137–140). Although (p)ppGpp has not been demonstrated to directly bind *M. tuberculosis* RNAP or GTP synthesis enzymes, (p)ppGpp has been reported to influence mycobacterial RNAP activity *in vitro*, suggesting that the mechanism of (p)ppGpp action in *M. tuberculosis* transcriptional modulation requires further investigation (136, 138, 141).

Rel_{Mtb} also encodes a second distinct catalytic domain that hydrolyzes (p)ppGpp into PPi and GDP or GTP (142). It was recently shown that (p)ppGpp hydrolysis by Rel_{Mtb} is important for growth and normal physiology in culture and during infection (135). These observations suggest that Rel_{Mtb} constitutively produces (p)ppGpp independently of activation during nutrient limitation and may act continuously to maintain *M. tuberculosis* homeostasis under all growth conditions in addition to its role in survival during stress.

FINAL THOUGHTS

In order to respond to host-derived stresses, *M. tuberculosis* has evolved a complex network of strategies to modify gene expression and promote survival. The responses to different stresses are integrated and coordinated, often resulting in overlapping regulons and stress responders (Fig. 2, 3, and 4). Not only do these highly effective stress response strategies protect *M. tuberculosis* from host immunity, but the resulting changes in physiology also contribute to antibiotic tolerance, which precludes eradication of the infection (143–148). The recalcitrance of *M. tuberculosis* in response to antibiotic therapy has led to an increase in drug-resistant *M. tuberculosis* infections to the point that we are not equipped to successfully battle the *M. tuberculosis* epidemic (2). Therefore, new therapeutic strategies that target *M. tuberculosis* stress responses could increase the susceptibility of the bacteria to both the immune system and antibiotic treatment.

As an obligate pathogen, *M. tuberculosis* is specialized for survival in a mammalian host. Analysis of the conservation of transcriptional regulators across different mycobacterial species reveals some interesting patterns that reflect their respective lifestyles (Fig. 2). *Mycobacterium leprae* is an even more specialized pathogen than *M. tuberculosis* and has undergone a drastic reduction in genetic material to the point that this degenerate genome has retained only 4 functional σ factor genes (*sigA*, *sigB*, *sigC*, and *sigE*) and 5 TCSs. On the other end of the spectrum, environmental mycobacteria such as *Mycobacterium smegmatis* must adapt to a larger diversity of conditions within a larger range of environments. As such, *M. smegmatis* encodes 28 σ factors to facilitate a more versatile lifestyle. In addition, even when a transcriptional regulator is conserved across mycobacterial species, it can be coopted to perform a function specific for a particular species. For example, σ^F homologs are differentially regulated and activated in *M. tuberculosis*, *M. smegmatis*, and *M. bovis* (7, 29, 149).

Finally, this minireview is in no way exhaustive in terms of all of the mechanisms of transcriptional regulation that *M. tuberculosis* employs to respond to stress. In particular, there is a growing area of research into the roles of nucleoid-associated proteins and small RNAs (150–155). *M. tuberculosis* also contains 11 serine/threonine protein kinases (STPKs) that, like TCSs, are involved in signal transduction pathways that aid *M. tuberculosis* in adaptation to its environment (156). However, unlike TCSs that consist of HKs that activate RRs to directly modulate *M. tuberculosis* transcription, STPKs are single proteins that phosphorylate numerous

downstream targets (156). Although STPKs do not directly affect *M. tuberculosis* transcription, they do influence gene expression by modifying the activity of other *M. tuberculosis* proteins with more-direct roles in transcription, such as σ factors, nucleoid-associated proteins, anti-anti-σ factors, and TCSs (24, 154, 157–159). These and other aspects of gene regulation further add to the complexity of stress responses in *M. tuberculosis*.

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