

MINIREVIEW

σ Factors and Global Gene Regulation in *Mycobacterium tuberculosis*

Riccardo Manganeli,¹ Roberta Proveddi,¹ Sebastien Rodrigue,² Jocelyn Beaucher,²
Luc Gaudreau,² and Issar Smith^{3*}

*Department of Histology, Microbiology and Medical Biotechnologies, University of Padua, Padua, Italy*¹; *Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada*²; and *TB Center, The Public Health Research Institute at the International Center for Public Health, Newark, New Jersey*³

Tuberculosis remains a worldwide threat despite the availability of the BCG vaccine and antibiotic treatment. It is estimated that its etiologic agent, *Mycobacterium tuberculosis*, infects almost a third of the human population and kills two million people every year (27). The recent human immunodeficiency virus pandemic, the selection of multidrug-resistant strains of *M. tuberculosis*, and the increased immigration from countries with a high tuberculosis incidence, coupled with increasing poverty and homelessness in these countries, have awakened the developed nations from the widespread apathy toward tuberculosis (36). Indeed, recent years have seen great progress in the molecular characterization of this efficient human pathogen (26, 61). However, much work is still needed to understand how *M. tuberculosis* copes with the numerous environments it encounters in the course of a successful infection. Adaptation to such conditions must require a complex regulation of gene expression.

The main stresses faced during infection can be summarized as follows. The first stress is exposure to oxidizing agents, principally represented by the reactive oxygen intermediates and reactive nitrogen intermediates, produced by activated macrophages. The second is exposure to low pH. Even if *M. tuberculosis* is able to block phagosome acidification, this block is not complete as the mycobacterial phagosome undergoes a slight decrease in pH (21). The third is damage of surface structures. Alveolar surfactant is a mild detergent with antibacterial activity and could damage the structure of its fatty acid-rich cell envelope. In addition, toxic peptides and proteins like granulysin, thought to act at the level of the bacterial surface, are released by activated macrophages and NK cells. Specifically, granulysin has been recently shown to be essential for *M. tuberculosis* killing after apoptosis of infected macrophages induced by NK cells (22). Finally, toxic free fatty acids, secreted from macrophages both inside the mycobacterial phagosome and in the external environment, exhibit their toxicity when interacting with the mycobacterial surface (2). The fourth is hypoxia, especially inside granulomas but also inside the phagosome. This environmental condition is actually the best candidate for the induction of persistence (also called dormancy or latency), a phenomenon of great importance in

M. tuberculosis pathogenesis but still not well understood at the molecular level (68). Recent experiments have implicated the transcriptional regulator DosR (dormancy survival regulator) (10, 51, 71), also known as DevR (18), and the response regulator MprA (72) in mycobacterial persistence. Interestingly, Voskuil et al. (67) recently showed that the DosR regulon is induced following NO-dependent inhibition of aerobic respiration. The fifth is nutrient and essential-element starvation. Inside phagosomes and granulomas, the availability of nutrients and essential elements may be reduced, as was recently shown for iron (31; J. Timm et al., unpublished data) and Mg²⁺ (12; S. Walters and I. Smith, unpublished data). Also, during transmission (between expulsion from an infected patient and inhalation by a new host), *M. tuberculosis* must face other environmental stresses such as nutrient starvation, exposure to UV light, dehydration, and low temperature.

The *M. tuberculosis* genome (14, 29) encodes about 190 transcriptional regulators: 13 σ factors, 11 two-component systems, 5 unpaired response regulators, 11 protein kinases (3), and more than 140 other putative transcriptional regulators (9). Several of these regulators have been characterized; some of them respond to environmental stresses such as cold shock (60), heat shock (41, 63), hypoxia (18, 51, 59), iron starvation (56), surface stress (41), and oxidative stress (42, 55), while others respond to still unknown environmental conditions (28, 52, 72). The resulting picture is still incomplete, but it suggests very complex regulatory systems with overlapping functions and redundancies. For example, the heat shock response is determined by the activation of five overlapping regulons under the transcriptional control of three σ factors (SigB, SigE, and SigH) (41, 42, 43) and two other transcriptional regulators (HspR and HrcA) (63).

In this review, we will principally discuss σ factors. Prokaryotic core RNA polymerase (RNAP) is composed of four distinct subunits: β , β' , ω , and an α dimer. A fifth subunit, the σ factor, reversibly associates with RNAP, forming the RNAP holoenzyme, and provides the promoter recognition function. The number of σ factors encoded in a genome is quite variable and ranges from a minimum of one in *Mycoplasma* sp. (30, 37) to a maximum of 65 in *Streptomyces coelicolor* A3(2) (6). *M. tuberculosis* encodes 13 different putative σ factors (14, 32, 34). It is generally observed that every σ factor has its own specificity, allowing the initiation of transcription of different subsets of genes. Genes belonging to a defined regulon often participate in related cellular functions. Therefore, temporal

* Corresponding author. Mailing address: TB Center, The Public Health Research Institute at the International Center for Public Health, 225 Warren St., Newark, NJ 07103-3535. Phone: (973) 854-3260. Fax: (973) 854-3261. E-mail: smitty@phri.org.

TABLE 1. Sigma factor genes in mycobacteria^a

Gene	Presence in:								
	<i>M. tuberculosis</i>	<i>M. bovis</i>	<i>M. bovis</i> BCG Pasteur	<i>M. leprae</i>	<i>M. avium</i>	<i>M. paratuberculosis</i>	<i>M. marinum</i>	<i>M. smegmatis</i>	
<i>sigA</i>	+	+	+	+	+	+	+	+	
<i>sigB</i>	+	+	+	+	+	+	+	+	
<i>sigC</i>	+	+	+	+	+	+	+	-	
<i>sigC</i> like	-	-	-	-	-	-	+	-	
<i>sigD</i>	+	+	+	P	+	+	+	+	
<i>sigE</i>	+	+	+	+	+	+	+	+	
<i>sigF</i>	+	+	+	P	+	+	+	+	
<i>sigF</i> like	-	-	-	-	+	+	-	-	
<i>sigG</i>	+	+	+	P	+	+	+	+	
<i>sigH</i>	+	+	2+	P	+	+	+	+	
<i>sigI</i>	+	+	-	P	-	-	-	-	
<i>sigJ</i>	+	+	+	P	+	+	+	+	
<i>sigK</i>	+	+	+	P	-	-	+	-	
<i>sigL</i>	+	+	+	-	P?	+	+	+	
<i>sigM</i>	+	P?	2+	P	+	+	+	+	
Other ECFs	-	-	-	-	4+	4+	4+	8+?	

^a +, presence of the gene; -, absence of the gene; P, pseudogene. The analysis was performed by using BLASTX network services. Genes listed as present were located in the same genetic locus as their *M. tuberculosis* orthologs and encoded putative proteins at least 68% similar to their *M. tuberculosis* counterparts.

variation in active σ factor populations may represent a powerful way for *M. tuberculosis* to modulate its gene expression profiles in accordance with physiological requirements and thus achieve a successful infection.

CLASSIFICATION OF σ FACTORS

σ factors can be divided in two groups that are phylogenetically distinct: those related to σ^{70} and those related to σ^{54} (70). While all eubacteria encode at least one σ factor belonging to the σ^{70} class, not all of them encode one belonging to the σ^{54} class. Since the latter family of σ factors is not represented in mycobacteria, it will not be discussed any further. The σ^{70} family can be further divided into three groups, depending on their structure and function: (i) primary σ factors, (ii) non-essential primary factor-like σ factors, and (iii) alternative σ factors (70). All eubacterial genomes encode one primary σ factor. It is usually essential and allows the transcription of housekeeping genes. *Escherichia coli* σ^{70} and *Bacillus subtilis* σ^A are part of this category, and in *M. tuberculosis*, this group is represented by σ^A (23, 33).

The σ factors belonging to the second group (primary factor-like σ factors) are nonessential under standard physiologic growth conditions and are highly similar to primary σ factors. They can be involved in different functions. In enterobacteria, they are usually involved in stationary-phase survival (RpoS); in cyanobacteria, they are involved in the circadian cycle and in carbon and nitrogen utilization (σ^B and σ^C of *Synechococcus* sp.); and they are involved in antibiotic biosynthesis in streptomycetes (HrdD) (70). In *M. tuberculosis*, they are represented by σ^B (23).

The third group, that of alternative σ factors, is the most heterogeneous and can be divided into numerous subgroups. In *M. tuberculosis*, they are represented by σ^F (20), belonging to the subgroup that also contains the stress response-sporulation σ factors in bacilli and streptomycetes, and by σ^C , σ^D , σ^E , σ^G , σ^H , σ^I , σ^J , σ^K , σ^L , and σ^M , belonging to the subgroup of the extracellular function (ECF) σ factors. ECF σ factors

are environmentally responsive regulators, and bacteria usually contain several members of the ECF family that control a variety of functions in response to specific extracellular environmental signals, such as the presence of misfolded proteins in the periplasm, the presence of light, changes in osmolarity or barometric pressure, and the presence of toxic molecules in the external environment (45, 70). Examples are *E. coli* σ^E , which controls the response to extreme heat shock (1); AlgU, which controls alginate biosynthesis in *Pseudomonas aeruginosa*; FecI, which controls iron uptake in *E. coli*; CarQ, which controls carotenoid biosynthesis in *Myxococcus xanthus*; and *P. syringae* HrpL, which controls the synthesis of a virulence factor that functions during plant infections (45, 70).

MYCOBACTERIAL σ FACTOR GENOMICS

In addition to the annotated *M. tuberculosis* H37Rv and CDC1551 genomes (14, 29), almost complete DNA sequence data are now available for several mycobacterial species. Examination of these genomes shows that σ factor genes and their loci are well conserved across the genus, even though there are some exceptions. In Table 1 are listed the orthologs of the 13 *M. tuberculosis* σ factors in other mycobacterial species.

The locus containing the genes encoding the principal and principal factor-like σ factors (*sigA* and *sigB*) is well conserved in the completely sequenced mycobacterial genomes available for analysis (23; R. Provvedi, unpublished data).

Interestingly, *M. leprae* *sigF* is a pseudogene and *M. avium* and *M. paratuberculosis* have two genes encoding a σ^F -like protein in different chromosomal loci. Of the two *sigF*-like genes, only the one encoding a σ^F -like protein with greater similarity to *M. tuberculosis* σ^F is preceded by the gene encoding its own putative negative regulator (anti- σ factor), UsFX, as in *M. tuberculosis* (Provvedi, unpublished).

The genes encoding the ECF σ factors show more variability. As previously reported, massive gene decay has occurred in *M. leprae*, in which only *sigC* and *sigE* have functional ho-

mologs. All of the other ECF σ genes are pseudogenes, with the exception of *sigL*, whose locus is deleted (15). It has recently been proposed that the loss of functional σ factors initiated pseudogene accumulation in this bacterium (4). In contrast, all *M. tuberculosis* ECF σ factor genes have an ortholog in *M. bovis*. The annotated *sigM* locus is a pseudogene, but this could be due to a mistake in the available sequence, which is not yet completely assembled. However, *M. bovis* BCG Pasteur lacks *sigI* as this locus is deleted, while in its genome, the *sigH* and *sigM* loci are duplicated (11).

Other differences we noted in the *M. avium* and *M. paratuberculosis* genomes are the lack of clear orthologs of *sigK* and *sigI* and the presence of four ECF σ factor genes encoding putative proteins similar to SigI/SigJ, suggesting that these species have more ECF σ factors than *M. tuberculosis* (Proveddi, unpublished). In *M. avium*, *sigL* has a frameshift, but as is the case for *sigM* in *M. bovis*, this could also be due to a mistake in the available sequence. In *M. marinum*, we could find clear orthologs for all of the 13 *M. tuberculosis* σ factors, with the exception of *sigI*. As in *M. avium* and *M. paratuberculosis*, we also found four additional ECF σ factors genes. One of these encodes a protein very similar to σ^C (Proveddi, unpublished).

The genome that shows the ECF σ factor gene pattern that is the most different from that of *M. tuberculosis* is that of the fast-growing mycobacterium *M. smegmatis*. It was not possible to find orthologs of *sigC*, *sigI*, and *sigK*, but there are at least seven or eight additional open reading frame showing similarity to ECF σ factor genes in this genome (Proveddi, unpublished). Also in this case, the genome sequence data, reportedly complete, are not yet assembled, preventing a complete and accurate analysis. The data extracted from these genomes support a rough correlation between the number of σ factors encoded in a genome and the diversity of possible niches for a given bacterium.

EXPRESSION OF *M. TUBERCULOSIS* σ FACTORS

All of the 13 *M. tuberculosis* σ factors are expressed during exponential growth (38, 41). Quantitative reverse transcription-PCR showed that the amount of *sigA*-specific mRNA is constant during exponential growth and that it can be used as an internal invariant standard for mRNA quantitation when cells are growing either in broth or in macrophages (25). The mRNA levels of some σ factors change when the cells are subjected to stress; e.g., both *sigB* and *sigE* mRNA levels increase when the cells are exposed to sodium dodecyl sulfate (SDS)-induced surface stress (41, 43). Levels of the same two mRNAs and that of *sigH* also increase after heat shock and exposure to diamide (a thiol-specific oxidizing agent) (41, 42). It is interesting that *sigF*, *sigE*, and *sigH* were induced during infection of macrophages, suggesting their involvement in virulence (35). Other studies have recently shown that *sigB*, *sigF*, *sigE*, and *sigD* were induced after prolonged nutrient starvation (8). Finally, *sigJ* was recently shown to be induced in stationary-phase cultures, and the high level of *sigJ* mRNA was maintained after a 5-day treatment with rifampin (38).

TABLE 2. Consensus promoter sequences of *M. tuberculosis* sigma factors

Sigma factor	Consensus sequence ^a	Reference(s)
SigA	TTGACW-N ₁₇ -TATAMT	66
SigF	GTTT-N ₁₇ -GGGTAT	5, 13
SigC	SSSAAT-N ₁₆₋₂₀ -CGTSSS	W. R. Bishai, personal communication
SigE	GGRMC-N ₁₈ -SGTTG	43
SigH	SGGAAC-N ₁₇₋₂₂ -SGTTS	40, 42, 55

^a W = A/T; S = G/C; R = A/G; M = A/C.

σ FACTOR POSTTRANSLATIONAL REGULATION IN *M. TUBERCULOSIS*

Even though mRNA levels of σ factor genes are frequently induced under a given condition, the activity at the protein level may also be regulated by a family of proteins called anti- σ factors. These proteins can bind to a specific σ factor, keeping it in an inactive form. In the presence of a specific stimulus, the anti- σ factor releases the σ factor, which becomes active. Moreover, another class of proteins, the anti-anti- σ factors, can inhibit anti- σ factor activity (39). *M. tuberculosis* alternative σ factors σ^E , σ^F , σ^H , and σ^L are each closely linked to a gene encoding a putative anti- σ factor. The *M. tuberculosis* genome contains another putative anti- σ factor-encoding gene (Rv0093c), not associated with any σ factor gene, and seven genes encoding putative anti-anti- σ factors. The function of some of these molecules will be discussed later.

σ^A , THE PRIMARY σ FACTOR

σ^A (also known as RpoV) is believed to be the principal σ factor of *M. tuberculosis* because inactivation of its genetic determinant, *sigA*, has not been possible in both *M. smegmatis* and *M. tuberculosis* (33; J. Timms and I. Smith, unpublished data). Its consensus promoter sequence is shown in Table 2.

It was the first mycobacterial σ factor to be associated with virulence. An arginine-to-histidine substitution at amino acid residue 515 (R515H) caused attenuation of *M. bovis* virulence in a guinea pig model of infection (16). This mutation was localized to the C terminus of the protein, in a conserved domain known to interact with transcriptional activators in other bacteria (24). Since the mutant strain grew normally in vitro, it was suggested that the mutant protein was still able to drive the expression of the housekeeping genes but was deficient for binding to some virulence-specific transcriptional activators. It was recently shown that σ^A interacts with the putative transcriptional regulator WhiB3 and that this interaction is lost in the R515H mutant (64). Interestingly, a deletion of *whiB3* in *M. bovis* resulted in attenuation of *M. bovis* virulence as in the original *sigA* R515H mutant, but an *M. tuberculosis whiB3* mutant was only partially attenuated for virulence. Since σ^A is the same in *M. tuberculosis* and *M. bovis*, this different phenotype is probably due to their different genetic backgrounds (64). The WhiB family in *M. tuberculosis* includes seven members. Related proteins in *Streptomyces coelicolor* are involved in sporulation, septation, and cell wall deposition (62).

σ^B , A PRIMARY FACTOR-LIKE σ FACTOR

sigB, the gene encoding σ^B , is almost identical to the last 600 bp of *sigA* and is localized approximately 3 kb downstream of *sigA* in all of the mycobacterial species thus far analyzed (23). In contrast to the latter, *sigB* is dispensable for growth both in *M. smegmatis* and in *M. tuberculosis* (M. Gomez and I. Smith, unpublished data). An *M. tuberculosis sigB* knockout mutant is more sensitive to various environmental stresses, such as SDS-induced surface stress, heat shock, and oxidative stress, but it is still able to grow normally in human macrophages and is not attenuated in mice (Gomez and Smith, unpublished). *sigB* regulation is complex; it is induced following exposure to surface or oxidative stress and after heat shock (41). Moreover, its transcription under physiological conditions and its induction after surface stress are dependent on σ^E , while during heat shock or oxidative stress, its induction is dependent on σ^H (42, 55). In vitro transcription experiments recently showed that the *sigB* promoter can be transcribed by RNAP containing σ^E , σ^H , or σ^L , suggesting the necessity for its induction under very different stress conditions (S. Rodrigue et al., unpublished data).

The subdomains of σ^B that are responsible for promoter recognition are almost identical to those of σ^A (23, 53). It is thus tempting to speculate that σ^A and σ^B recognize similar promoter sequences and that their respective regulons partially overlap, as is the case with RpoS and σ^{70} in *E. coli* (65). σ^B could function as a "backup" to maintain the transcription of essential housekeeping genes during exposure to stress, when σ^A could be inactive or its levels could be lowered. The role of σ^B will be clarified when more genes that require it for their transcription are identified. In this regard, it was reported that overexpression of σ^B in *M. smegmatis* and *M. bovis* BCG caused an increase in *katG* expression, but it is not known whether this is a direct transcriptional effect (46). To generate a better σ^B consensus sequence, we are currently using DNA microarrays to find more genes in the σ^B regulon and preliminary results indicate that several heat shock genes require σ^B for their expression (P. Fontan and I. Smith, unpublished data).

σ^F , A σ FACTOR REQUIRED FOR FULL VIRULENCE

sigF, encoding σ^F , is part of a gene cluster with an organization similar to that of the *B. subtilis sigF* and *sigB* operons. In this locus, the anti-sigma factor-encoding gene *usfX* (originally annotated *rsbW* like in the Tuberculist database; RsbW is the *B. subtilis* ortholog) is directly upstream of the σ factor gene (19). In *B. subtilis*, σ^F is involved in sporulation, while σ^B is a general stress response σ factor whose expression is activated by heat, alcohol, osmotic stress, and entry into stationary phase (70). The *M. tuberculosis* gene encoding σ^F is induced in *M. smegmatis* and *M. bovis* BCG after exposure to several antibiotics, hypoxia, cold shock, oxidative stress, and entry into stationary phase (20, 44). However, its induction was not observed in *M. tuberculosis* after cold shock, hypoxia, oxidative stress, or entry into stationary phase (38, 41). This suggests that *sigF* is regulated differently in *M. bovis* and *M. tuberculosis*, despite the similarity of these organisms. These findings, together with those regarding the difference between the effects of *whiB3* inactivation in *M. tuberculosis* and *M. bovis*, discussed above, suggest that caution should be used when extrapolating results

obtained in one species when coping with phenomena as complex as global gene regulation and virulence.

usfX and *sigF* are transcribed from the σ^F -dependent promoter *usfXP1* (Table 2), located directly upstream of *usfX*. The activity of σ^F is posttranslationally regulated by its cognate anti-sigma factor, UsfX. The latter protein is in turn posttranslationally regulated by two anti-anti-sigma factors, RsfA and RsfB. Both are able to disrupt the UsfX- σ^F complex, releasing σ^F to allow its association with RNAP. The function of RsfA is regulated by redox potential, while it is postulated that the activity of RsfB is controlled by phosphorylation (5).

An *M. tuberculosis* CDC1551 mutant lacking *sigF* was produced to investigate its role in virulence and stress response. Interestingly, the mutant strain reached stationary phase later than the wild-type (WT) parental strain and did not exhibit the typical lag phase after dilution of a dense culture into fresh medium. The mutant had the same sensitivity as the WT parent strain to heat shock, cold shock, hypoxia, and long-term stationary-phase growth; however, it was more sensitive than the WT to rifampin. Also, when used to infect human monocytes, the mutant did not show any difference from the WT. However, it was attenuated for virulence in mice when death was used as a criterion (13).

The σ^F regulon was studied by using DNA arrays in order to identify genes that require σ^F for their expression (W. R. Bishai, personal communication), and a consensus sequence was formulated that closely resembles the *usfX* promoter previously shown to be transcribed by σ^F -RNAP (5).

σ^C , AN ECF σ FACTOR REQUIRED FOR MOUSE LETHALITY

σ^C was recently inactivated in *M. tuberculosis* (Bishai, personal communication). The resulting strain was more susceptible to hydrogen peroxide and diamide stress but was not altered for survival in activated mouse macrophages. However, it was significantly attenuated in time-to-death experiments in the mouse model. Functional genomic studies with DNA arrays showed that at least 38 genes are repressed in the *sigC* mutant at different point of the growth curve. Those genes encode proteins involved in a broad range of cellular processes like fatty acids biosynthesis, phospholipid and cell wall biosynthesis, energy metabolism, and general stress response. A σ^C consensus sequence has been proposed from microarray data (Table 2) (Bishai, personal communication).

σ^E , AN ECF σ FACTOR ESSENTIAL FOR VIRULENCE INVOLVED IN RESPONSE TO SURFACE STRESS

The gene encoding σ^E is induced after exposure to various environmental stresses, such as heat shock and detergent-induced surface stress (41), as well as during *M. tuberculosis* growth in human macrophages (35). Interestingly, Schnappinger et al. (58) recently showed by functional genomics that a set of σ -dependent genes are induced in the phagosomal environment. A mutant of *M. tuberculosis* H37Rv lacking a functional *sigE* gene is more sensitive than the WT parent strain to detergent, high temperature, and oxidative stress. This mutant is attenuated for growth in THP-1-derived macrophages and is more sensitive than the WT strain to the killing

activity of activated murine macrophages (43). Moreover, the *sigE* mutant has reduced virulence both in BALB/C and in SCID mice (R. Manganelli et al., submitted for publication). DNA array experiments comparing the transcriptome of the *sigE* mutant with that of the WT strain showed that 38 genes require σ^E for their full expression during exponential growth, while 13 putative transcriptional units containing 23 genes required σ^E for their induction after exposure to a subinhibitory concentration of SDS (43). Nine of the 13 putative transcriptional units were preceded by a conserved ECF σ factor-like promoter (Table 2), suggesting their direct transcriptional dependence on σ^E . The genes whose expression during exponential growth require σ^E include genes encoding proteins involved in translation, transcriptional control, mycolic acid biosynthesis, electron transport, and oxidative stress response. Interestingly, one of these genes is *sigB*, whose transcription under unstressed conditions is almost totally due to σ^E . Since *sigB* is the only gene of this group to be preceded by an ECF σ factor-like promoter, this suggests that at least some of the other 37 genes downregulated in the *sigE* mutant are in the σ^B regulon. Most of them are housekeeping genes, supporting the hypothesis that σ^B and σ^A have overlapping regulons. This question is currently being investigated.

Genes requiring σ^E for SDS-mediated induction encode heat shock proteins, proteins involved in fatty acid degradation, transcriptional regulators (including σ^B), and surface-exposed proteins with unknown function. The presence in this group of *fadE23* and *fadE24* is of particular interest. They were previously found to be induced after exposure to isoniazid, and it was hypothesized that their protein products could be involved in the degradation of the fatty acids accumulating on the surface as a consequence of the block of mycolic acid biosynthesis (69). The σ^E -dependent induction of these genes (together with others encoding fatty acid degradation enzymes) after exposure to a detergent supports the hypothesis of their role (and that of σ^E) in cell wall physiology and structure.

The gene encoding σ^E is followed by an operon including three genes. The first, *Rv1222*, encodes a σ^E -specific anti- σ factor (RseA) (Rodrigue et al., unpublished). The second, *htrA*, encodes a putative membrane serine protease; the third, *tatB*, encodes a putative protein belonging to the Tween arginine translocator (Tat) secretion system. The Tat secretion system translocates proteins showing at the N terminus a typical signal sequence containing a couple of adjacent arginine residues (7). TatB was suggested to be responsible for the association of the proteins secreted by the Tat system to the membrane (57).

In *E. coli*, the anti- σ factor regulating σ^E is a transmembrane protein and it is degraded by a membrane-located serine protease in the presence of misfolded proteins in the periplasmic space (54). In *M. tuberculosis*, RseA is predicted to be a soluble protein. We recently found that it has a putative Tat consensus sequence at its N terminus. The fact that *rseA* is in the same operon with *tatB* suggests that their protein products could interact and that RseA could be secreted or associated to the membrane through TatB. The presence in the same operon of the gene encoding a membrane-located serine protease (HtrA) suggests that HtrA, with its proteolytic activity, could represent the molecular switch acting (directly or indirectly) on RseA activity. The interactions among RseA, HtrA, and TatB are

currently being investigated to better understand the mechanism of posttranslational regulation of σ^E .

σ^H , AN ECF σ FACTOR INVOLVED IN RESPONSE TO HEAT SHOCK AND OXIDATIVE STRESS

σ^H is very similar to the ECF σ factor σ^R of *S. coelicolor*. The latter responds to intracellular formation of disulfide bonds due to oxidation of cysteine thiol groups (49). σ^R activity is regulated at the posttranslational level by a cysteine-containing anti- σ factor (RsrA) whose gene is adjacent to *sigR*. In a reducing environment, RsrA binds σ^R , keeping it inactive; however, in oxidizing environments, disulfide bonds can form between RsrA cysteine residues and, as a consequence, σ^R is released in its active form from the σ^R -RsrA complex (48). Among the genes recognized by the σ^R -RNAP are *sigR* and the *trx* operon, which encodes thioredoxin and thioredoxin reductase, two proteins involved in disulfide bond reduction. Usually, cells have a second pathway by which to reduce intracellular disulfide bonds, based on glutathione. Actinomycetes are an exception, as they do not synthesize glutathione but use a different compound, mycothiol, for similar functions (47). A *sigR* mutant of *S. coelicolor* produces less mycothiol than the WT parental strain, even if it is not known if this is due to the direct control of mycothiol biosynthetic genes by σ^R -RNAP (50).

The *M. tuberculosis sigH* gene is induced after heat shock, after treatment with the thiol-specific oxidizing agent diamide (42, 55), and during macrophage infection (35). Similar to *sigR* in *S. coelicolor*, the *M. tuberculosis sigH* gene is followed by a gene encoding an anti- σ factor whose activity is regulated by redox potential (Rodrigue et al., unpublished). The gene encoding σ^H was inactivated in three different laboratories (40, 42, 55). The mutants are more sensitive than the WT to high temperature and to diamide exposure. However, they are not restricted for growth in THP-1-derived macrophages and were as sensitive as the WT parental strain to the killing activity of activated murine macrophages (42). Interestingly, the *sigH* mutant has a very subtle phenotype in a mouse model of infection: it is able to reach the same bacterial load as the WT parent strain in mouse organs (40, 42), but there are differences in lung histopathology, including fewer granulomas and a generally decreased pulmonary inflammatory response in mice infected with the *sigH* mutant (40).

DNA array experiments comparing the transcriptome of the *sigH* mutant with that of the WT parent strain do not show any gene requiring σ^H for its expression during exponential growth, while 26 putative transcriptional units including 39 genes require σ^H for their induction after exposure to a subinhibitory concentration of diamide (42). Sixteen of the 26 putative transcriptional units were preceded by a conserved ECF σ factor-like promoter, suggesting their direct transcriptional dependence on σ^H , while 4 were preceded by a potential consensus sequence for an unknown regulatory protein. The genes under σ^H control included some encoding transcriptional regulators (σ^B , σ^E , and σ^H); enzymes involved in thiol metabolism, such as thioredoxin, thioredoxin reductase, and a protein of unknown function with a glutaredoxin active site; and enzymes involved in cysteine and molybdopterin biosynthesis (42). Work from two other laboratories (40, 55) also

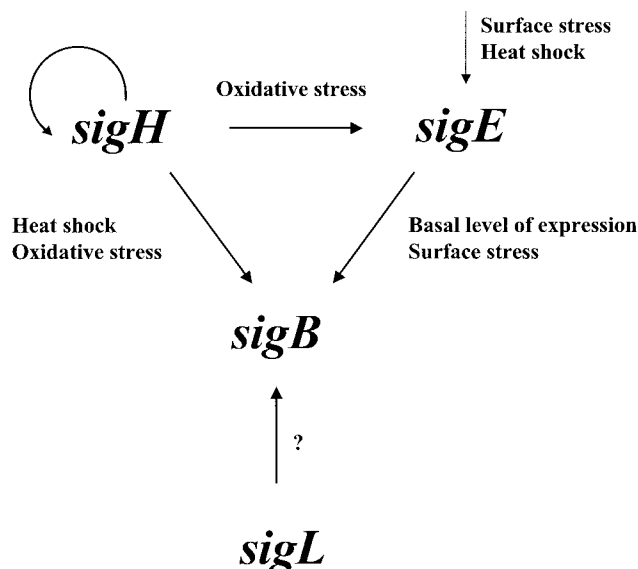


FIG. 1. σ factor regulatory network. Arrows indicate the transcriptional relationships among σ factors. *sigB* can be transcribed by an RNAP containing σ^H , σ^E , or σ^L , depending on the environmental conditions. σ^H also promotes the transcription of its own structural gene and the induction of *sigE* after oxidative stress. The environmental signal activating σ^L is not known.

derived a similar consensus sequence for genes requiring σ^H for their expression (Table 2).

OTHER ECF σ FACTORS

Little information is available about the other seven ECF σ factors encoded by the *M. tuberculosis* genome. *sigD* is induced following total nutrient starvation (8) and in the *M. tuberculosis* Rel mutant (17). The Rel protein has been well studied in *E. coli* and is known to be a key enzyme in the stringent response, a transition process believed to shut down active metabolism. *sigJ* is induced in stationary-phase cultures (38). Of particular interest is *sigL*. Its gene product, σ^L , is the closest *M. tuberculosis* homolog of *S. coelicolor* σ^E . This protein in *S. coelicolor* controls cell wall structure, and its activity is post-translationally regulated by a two-component system encoded by an operon immediately downstream of its structural gene. In *M. tuberculosis*, however, this gene is followed by a gene encoding a transmembrane anti- σ factor, which specifically binds to and reversibly inactivates σ^L (Rodrigue, unpublished data), suggesting its involvement with surface processes.

CONCLUDING REMARKS

σ factors, with their plethora of anti- σ factors and anti-anti- σ factors, are among the major and more complex players in the regulation of gene expression in bacteria. In the last few years, after the publication of the *M. tuberculosis* genome, the 13 σ factors of *M. tuberculosis* have become an important subject of investigation. Mutations in six of the σ factor genes were either made (*sigB*, *-C*, *-E*, *-F*, and *-H*) or identified (*sigA*), and a role in virulence for five of them (all except *sigB*) was demonstrated.

The regulons of four of these σ factors, σ^C , σ^E , σ^F , and σ^H , were characterized by DNA array technology, and this analysis showed that many genes were represented only in one regulon. However, there was some overlap, which is typical in ECF σ factor regulons. As an example of this overlap, some of the *sig* genes are in the regulon of other σ factors: *sigE* induction is σ^H dependent following oxidative stress but not after surface stress or heat shock (42, 55); *sigB* expression, however, is σ^E dependent under standard (unstressed) growth conditions. σ^E is also required for *sigB* induction after surface stress (43), but *sigB* induction after oxidative stress and heat shock is dependent on σ^H (42, 55) (Fig. 1). Our observations, which indicate that *sigB* expression is controlled by RNAPs containing different σ factors, suggest an important role for σ^B in *M. tuberculosis* physiology and perhaps virulence. Otherwise, why would this bacterium go through so much trouble to make sure that this protein is available to control transcription in different environments? However, we have not seen any diminution of pathogenicity in *sigB* mutants, as yet. It is possible that there is a subtle change in virulence that has been missed so far, and these studies are currently being pursued.

The whole question of posttranslational regulation by anti- and anti-anti- σ factors makes the matter even more complicated. The resulting picture is that of a very intricate regulatory network that will become even more complex as other σ factors and other transcriptional regulators are characterized with their regulons. We predict that the understanding of global gene regulation in *M. tuberculosis* will help us to understand its physiology and virulence mechanisms and will help to design new strategies to fight tuberculosis.

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