M. *tuberculosis* σ^E Protects against Environmental Stress, Immune Responses

σ^E master regulator is important for virulence and for responding to environmental stresses, many of which affect the bacterial cell envelope

Patricia A. Fontán and Issar Smith

Bacterial sigma (σ) factors bind to RNA polymerase and provide this enzyme with specificity for particular promoters. This interaction generates a mechanism for regulating gene expression. Thus, different σ factors bind to RNA polymerase, allowing different groups of genes to be expressed in response to changing conditions.

More specifically, σ factors within the extracytoplasmic function (ECF) class regulate genes that allow microorganisms to respond to environmental changes. In some cases, genes controlled by ECF σ factors are involved in bacterial virulence. *Mycobacterium tuberculosis (Mtb)* carries 13 σ factor genes, including 10 of the ECF σ subgroup, reflecting the versatility of this microorganism to adapt to many types of stress and to succeed as a pathogen. Our research group and others are studying the role of σ^E, one of the *Mtb* ECF σ factors, in mycobacterial physiology and virulence. The σ^E regulon is activated during macrophage infection, and the *Mtb* components that are produced exert global

effects on host responses, as well as within epistatic pathways by which σ^E is regulated.

The σ^E Regulon during *M. tuberculosis* In Vitro Growth and in Macrophages

The *Mtb* ECF σ^E is a master regulator that is important for virulence and for responding to environmental stresses, many of which affect bacterial cell envelopes. Although other *Mtb* σ factors, including σ^F and the ECF σ factors σ^E and σ^L, control expression of cell envelope components, only σ^E is essential for this microorganism to survive in macrophages and for its maximal growth in mouse lungs. The *sigE* mutant strain, in addition to being attenuated, is sensitive to several membrane-disrupting agents, including the detergent sodium dodecyl sulfate (SDS), the cationic peptide polymyxin B, and the protonophore carbonyl cyanide chlorophehylhydrzone (CCCP).

SDS induces expression of several *Mtb* genes in a σ^E-dependent manner. One of them encodes a putative PspA protein (*Rv2744c*), which is also induced during macrophage infection, and this upregulation requires σ^E (see table). In *Escherichia coli*, membrane-damaging agents as well as defects in secretion induce PspA. This protein protects bacteria from the action of cationic peptides and CCCP by restoring electron transport. The decreased expression of PspA in the *Mtb sigE* mutant may be the cause of its increased sensitivity to polymyxin B and CCCP.

In addition, σ^E controls the expression of several genes encoding regulatory proteins

Summary

- *M. tuberculosis* σ^E regulates expression of several bacterial components, including those that maintain the cell envelope and several genes encoding regulatory proteins.
- σ^E is important for *M. tuberculosis* to suppress host immune responses and to survive in host macrophages.
- Several components of the σ^E regulon downmodulate the host innate immune response.

Patricia A. Fontán is Assistant Professor and Issar Smith is Professor at The Public Health Research Institute and the Department of Medicine, UMDNJ-New Jersey Medical School, Newark, N.J.

Mtb genes under sigE control during macrophage infection

ID	Gene Name	Functional annotation
Rv0251c	hsp	Cell Processes→ Chaperones/Heat shock
Rv0479c	Rv0479c	Macromolecule metabolism→ Cell envelope→ Other membrane proteins
Rv0563	htpX	Cell Processes→ Chaperones/Heat shock
Rv0896	gltA2	Small-molecule metabolism→ Energy Metabolism→ TCA cycle
Rv1072	Rv1072	Macromolecule metabolism→ Cell envelope→ Other membrane proteins
Rv1630	rpsA	Macromolecule metabolism→ Synthesis and modification of macromolecules→ Ribosomal protein synthesis and modification
Rv2094c	tatA	Macromolecule metabolism→ Cell envelope→ Other membrane proteins
Rv2276	Rv2276	Other→ Cytochrome P450 enzymes
Rv2519	PE 26	Other→ PE and PPE families→ PE family→ PE subfamily
Rv2684	arsA	Cell Processes→ Transport/binding proteins→ Anions
Rv2710	sigB	Macromolecule metabolism→ Synthesis and modification of macromolecules→ RNA synthesis, RNA modification and DNA
Rv2744c	35kd_ag	Putative PspA protein
Rv2745c	Rv2745c	Small-molecule metabolism→ Broad regulatory functions→ Repressors/activators
Rv2971	Rv2971	Small-molecule metabolism→ Energy Metabolism→ Miscellaneous oxidoreductases and oxygenases
Rv3050c	Rv3050c	Small-molecule metabolism→ Broad regulatory functions→ Repressors/activators
Rv3140	fadE23	Small-molecule metabolism→ Degradation→ Fatty acids
Rv3592	Rv3592	Putative sugar binding protein
Rv3634c	rmlB2	Small-molecule metabolism→ Central intermediary metabolism→ Sugar nucleotides
Rv3767c	Rv3767c	Putative S-adenosyl-L-methionine-dependent methyltransferase
Rv3826	fadD23	Small-molecule metabolism→ Degradation→ Fatty acids

such as *sigB*, which encodes σ^B, a general stress sigma factor that is very similar to σ^A, the major housekeeping mycobacterial σ factor. σ^E also controls the expression of *tatA*, which codes for one of the proteins of the Sec-independent TAT system of secretion. In gram-negative bacteria, this secretion system is an important virulence factor. The components of the TAT system are also essential in *M. tuberculosis*. Moreover, a mutant whose gene encoding a substrate for the TAT system is disrupted is no longer virulent.

Transcriptional profile analysis of *M. tuberculosis* wild type and *sigE* mutant strains during infection of macrophages reveals that σ^E regulates genes encoding several distinct classes of proteins, including some that are annotated to be involved in making galactofuran that is essential for linking peptidoglycan and mycolic acid, making mycolic acids, metabolizing and detoxifying fatty acids; some that are annotated as encoding endopeptidases, amidohydrolases, oxidoreductases, oxidases; and trans-membrane proteins (see table). These observations suggest that *M. tuberculosis* σ^E helps to control expression of bacterial components that are involved in maintaining cell envelope integrity, transporting substances across the cell membrane, and detoxifying cells.

The σ^E Regulon Dampens Host Immune Responses

The importance of σ^E in the *Mtb* response to cell envelope stress and in its ability to grow in macrophages suggests that this σ factor controls events that may occur early during the establishment of infection. It is at this time that the host immune system becomes activated and ultimately results in the formation of a granuloma that controls bacterial dissemination.

However, *Mtb* has evolved mechanisms to evade the antibacterial immunological mechanisms of the infected host. For example, the cell envelope phenolic-glycolipid PGL-Tb down regulates the host Th1 innate immune response. Also, the mannose-capped, lipoarabinomannan (ManLAM) cell-surface component exerts a strong immunosuppressive effect.

Similarly, we postulate that σ^E regulates many factors that play a role in the interactions between *M. tuberculosis* and its hosts, including several that directly affect host immune responses. To provide evidence for this hypothesis, we compared the transcriptional

response of human and murine macrophages infected with wild-type *M. tuberculosis* H37Rv and the *sigE* mutant. At the same time we also analyzed the transcriptome of these two bacterial strains during macrophage infections. Our results indicate that components of the σ^E regulon down-modulate the host innate immune response.

Specifically, the *sigE* mutant up-regulates several genes in macrophages that it infects compared with macrophages that the wild-type *M. tuberculosis* infects. The macrophage components encoded by these genes are part of the common, or innate immune, defense program that the host elicits immediately after being infected by many pathogens (Fig. 1). In particular, the *sigE* mutant up-regulates genes encoding proteins of the acute-phase innate immune response, the toll-like receptors TLR1 and TLR2, pro-inflammatory cytokines and chemokines, and defensins and prostoglandins.

The lipid bodies that characterize foamy macrophage in mouse lungs infected with BCG are particularly rich in prostaglandins. When mouse lungs are infected with the *sigE* mutant, numbers of foamy macrophages increase. Other experiments have shown that when dendritic cells are infected with the *sigE* mutant, interleukin (IL)-10 increases while production of the chemokine CXCL10 is reduced. Overall, these changes may limit migration of lymphocytes and reduce tissue damage. Also, increased IL-10 may boost production of interferon (INF)-γ, which is bacteriostatic.

FIGURE 1
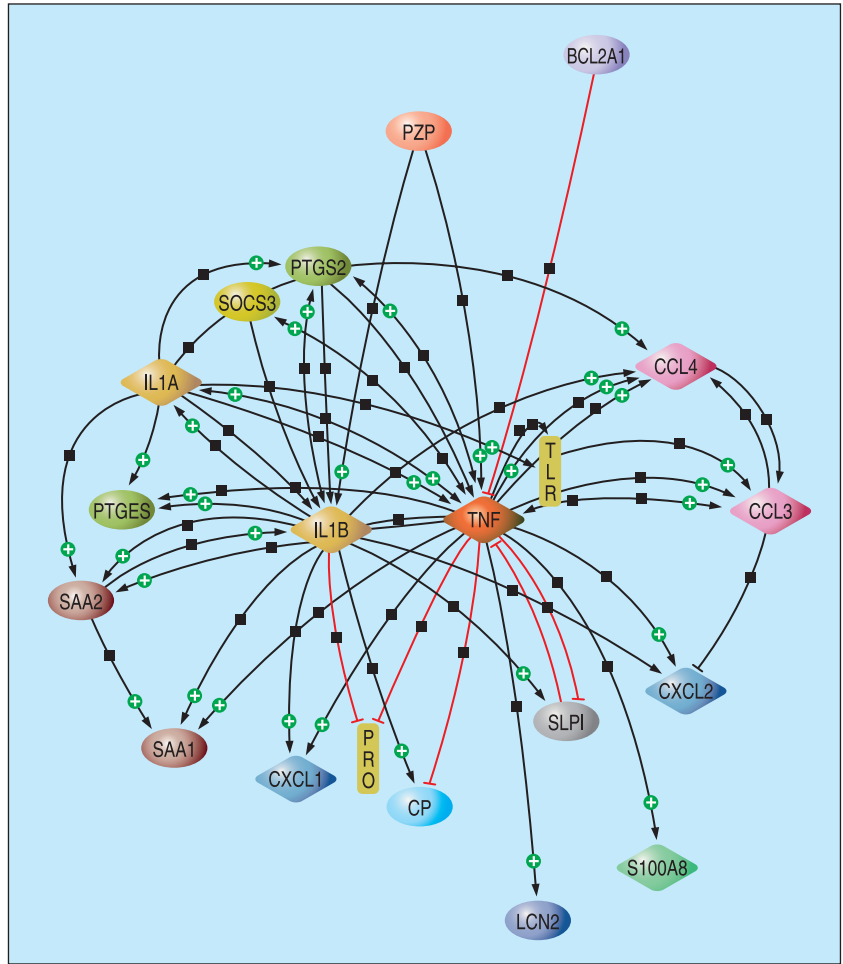
Pathway analysis of differential gene expression in response to infection by H37Rv or the *sigE* mutant. Using Pathway Assist, a biological pathway was built by searching for nodes and links in common from macrophages genes differentially regulated after infection with H37Rv or the *sigE* mutant. The links and nodes among genes represent literature-based findings retrieved from ResNet database 3.0 (Medline as of August 24, 2004). The grey dotted line arrow with "+" depicts positive regulation, the dotted grey line ending with "−" depicts negative regulation. Ligands, receptors, transcription factors, kinases, phosphatases, and proteins are represented by rhombus, stick shapes, O shapes, sickle shapes, concave shape, and ovals, respectively.

## Regulation of σ^E Synthesis and Function

The ECF sigma factors are regulated at several levels. For instance, several environmental conditions induce *M. tuberculosis sigE* expression. Moreover, two genes, one encoding a putative serine protease (*htrA*) and the other a putative anti-sigma factor (*rseA*), are found downstream of *sigE,* suggesting posttranscriptional regulation. In fact, purified RseA specifically binds to σ^E, preventing its function in transcription.

Expression of the gene encoding RelA, a ppGpp synthase, partially depends on σ^E in *Mycobacterium smegmatis*, a nonpathogenic relative of *M. tuberculosis*. This dependence requires the MprAB two-component system, whose expression, in turn, depends on a functional polyphosphate kinase (PPK1). Thus, σ^E in *M. smegmatis* may regulate the alarmone ppGpp, which is part of the stringent response to starvation.

M. tuberculosis appears to encode a similar regulatory pathway. Thus, for example, de-

FIGURE 2
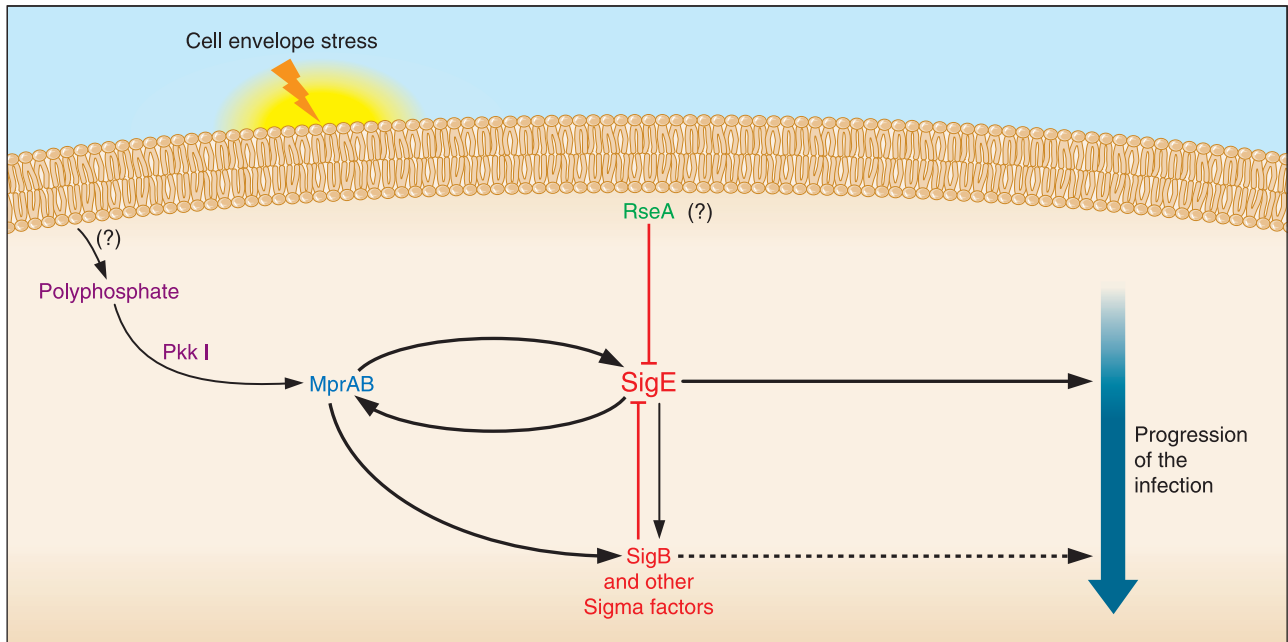
M. tuberculosis σ^E response to cell envelope stress during macrophage infection. The σ^E network of activation during cell envelope stress and during *Mtb* infection of macrophages elicits similar bacterial responses. Filled arrows indicate effects and interactions. Dotted arrows indicate suggested but not proved pathways later in infections. Question marks indicate unknown activation mechanisms.t.

creasing the levels of PPK1 in *M. tuberculosis* by antisense technology reduces expression of *mprAB, sigE*, and *relA*. Moreover, MprAB controls the expression of *sigE* as well as *sigB* in *M. tuberculosis*. However, RelA cannot be responsible for the *sigE* mutant phenotypes since a *relA* mutant is not attenuated during macrophage infection and it survives less well than does the *sigE* mutant during the chronic phase of the mouse lung infection. Moreover, there is no experimental evidence that σ^E regulates expression of *relA*.

In *E. coli*, ppGpp regulates expression of *sigE* in an RseA-independent way as cells enter stationary phase. Thus, σ^E can respond to intracellular signals in addition to responding to cell envelope stress. This regulation might enable *E. coli* to change the cell envelope in response to stress induced during stationary phase.

However, in *M. smegmatis,* ppGpp does not regulate the expression of *sigE*. Although *sigE* expression increases when *M. smegmatis* cells enter stationary phase, the same does not occur in *M. tuberculosis*. Whether ppGpp regulates the expression and activity of *Mtb* sigma

factors remains to be determined. When *M. tuberculosis* cells are starved, they induce expression of *sigE, sigB, sigF,* and *sigD,* whereas for *relA* mutants expression of only *sigD* is affected. Moreover, strains with deletions in these regulators do not show an attenuated growth phenotype during chronic infection as do *relA* mutants.

Role of σ^E during Later Stages of Infection

During a chronic infection, *M. tuberculosis* may modify its cell envelope. Thus, σ^E may be relevant for *M. tuberculosis*'s entrance to a persistence state. Although σ^E plays no apparent major role in *M. tuberculosis* survival during persistence, this protein might be necessary at several other stages of infection when σ^B and other sigma factors may amplify or sustain its signal (Fig. 2). A conditional gene silencing system that shuts down *sigE* expression at various times during *M. tuberculosis* infection could help in determining the role, if any, of σ^E during chronic infections and the hierarchical roles of transcriptional

components in the regulatory network that enables *M. tuberculosis* to survive in its host.

σ^E regulation is complex, and only partly understood (Fig. 2). For example, little is known about the circuitry by which Pkk1 is activated to synthesize polyphosphate and the way in which this compound interacts with the MprAB two-component system. What prevents the anti-sigma factor RseA from binding to σ^E and inhibiting its function is also not understood.

Such information could help in developing new antitubercular therapies. Because the *M. tuberculosis sigE* mutant strain stimulates the host immune system during macrophage infections, that mutant might be evaluated for use as a vaccine strain and whether it can protect against a subsequent virulent *M. tuberculosis* challenge.

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