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σ^{ECF} factors of gram-positive bacteria
A focus on *Bacillus subtilis* and the CMNR group

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Abbreviations: σ, sigma factor; σ^{ECF}, extracytoplasmic function sigma factor; A, adenine; ABC transporter, ATP-binding cassette transporter; Ag85A, constituent A of the antigen 85 complex; Ag85C, constituent C of the antigen 85 complex; ATP, adenosine triphosphate; bp, base pair; BHI, brain heart infusion; C, cytosine; CMNR, *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus*; C-terminal, carboxy-terminal; DNA, deoxyribonucleic acid; DT, diphtheria toxin; EDTA, ethylenediaminetetraacetic acid; Enoyl-ACP, enoyl-acyl carrier protein; FBS, fetal bovine serum; Fe-S, iron-sulfur; G, guanine; GlcNAc, *N*-acetyl-glucosamine; H_2O_2, hydrogen peroxide; INH, isoniazid; iNOS, inducible nitric oxide synthase; $iNOS^{-/-}$, iNOS knockout; Kb, kilobase; kDa, kilodalton; mRNA, messenger RNA; MurNAc, *N*-acetylmuramic acid; N, nucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; N-terminal, amino-terminal; N-terminus, amino-terminus; P1, promoter 1; P2, promoter 2; PDIM, phthiocerol dimycocerosate; PE, phosphatidylethanolamine; PGRS, polymorphic GC-rich repetitive sequences; PI, pathogenicity island; Poly(RboP), poly(ribitol phosphate); ppGpp, guanosine tetraphosphate; qRT-PCR, quantitative real time-polymerase chain reaction; RNA, ribonucleic acid; RNAP, RNA polymerase; SDS, sodium dodecyl sulfate; T, thymine; TB, tuberculosis; T $CD4^+$, $CD4^+$ T helper lymphocyte; T $CD8^+$, $CD8^+$ T helper lymphocyte; TSP, transcription start point; WHO, World Health Organization

The survival of bacteria to different environmental conditions depends on the activation of adaptive mechanisms, which are intricately driven through gene regulation. Because transcriptional initiation is considered to be the major step in the control of bacterial genes, we discuss the characteristics and roles of the sigma factors, addressing (1) their structural, functional and phylogenetic classification; (2) how their activity is regulated; and (3) the promoters recognized by these factors. Finally, we focus on a specific group of alternative sigma factors, the so-called σ^{ECF} factors, in *Bacillus subtilis* and some of the main species that comprise the CMNR group, providing information on the roles they play in the microorganisms' physiology and indicating some of the genes whose transcription they regulate.

Introduction

Bacteria are frequently exposed to a range of different circumstances in which the activation of adaptive mechanisms is essential for cell maintenance and survival. The modulation of such activity is intricately driven through gene regulation, involving diverse events at the transcriptional, translational, and posttranslational levels. Thus, transcription initiation could be considered the major step in the control of bacterial genes.[1-4]

In its inactive state, RNAP consists of only its core, which is formed by the subunits α_2, β, β', and ω, whereas in association with a sigma factor, the core transiently becomes an RNAP holoenzyme, whose function is to transcribe DNA into RNA.[1,4-7] In association with the core RNAP, the sigma factors provide a dynamic mechanism for cellular responses performed through redirecting transcription initiation.[8,9] Not only do these factors include many, if not all, of the determinants of promoter recognition,[1,5,7] they also contribute to DNA strand separation during the initiation of transcription.[8,9]

Because these proteins preferentially recognize specific promoters in the genome, the generation of distinct combinations of several of these proteins with RNAP provides an effective means of modulating transcription profiles,[3,10] altering the expression of large groups of genes, or regulons, in response to various environmental stimuli, and changing extracellular and/or intracellular conditions[4] in accord with the physiological requirements of the organism.[6] In some cases, the genes that constitute a specific regulon have a clearly defined primary function, such as those regulated by the sporulation sigma factors of *B. subtilis*,[9,11] while in other cases, these genes contribute to multiple processes, such as those related to the stationary phase of growth and general stress response regulated by the alternative sigma factor σ^B in *Listeria monocytogenes*.[9,12] In the case of bacterial pathogens in particular, the mechanism of changing sigma factors regulates the expression of virulence genes, encoding proteins whose functions are essential for the bacterium to effectively establish an infection, and

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virulence-associated genes, which contribute to survival in the host and other environments.[9]

Most of the sigma factors are not essential for cell viability under optimum growth conditions, and they are therefore referred to as alternative sigma factors. Their functions typically encompass the recognition of the promoters of genes that become active in response to specific stimuli,[4,7,13] such as various stress conditions, in the stationary growth phase, during starvation for various nutrients,[4] and during morphological differentiation.[14] Nevertheless, bacteria (mainly the rapidly growing strains) usually exhibit a single primary sigma factor that is responsible for the transcription of genes required for basic survival, referred to as housekeeping genes.[4,7,13]

The number of alternative sigma factors present in a bacterium may range from two, as in *Streptococcus pyogenes*, a gram-positive bacterium whose habitat is restricted to the human oropharynx, to more than 60, as in *Streptomyces coelicolor*, a soil bacterium whose habitat, unlike that of *S. pyogenes*, is highly variable in terms of nutrients, stresses, and competing microbial flora. In general, bacterial species that have developed differentiation programs, such as sporulation, present higher ratios of alternative sigma factor-encoding genes per a given length of the genome than those that are obligate pathogens or commensal species.[1,3,7]

Overview of the Classification of Bacterial Sigma Factors

Bacterial sigma factors can be primarily classified into two structurally, functionally, and phylogenetically distinct families, designated σ^{54} and σ^{70}.[1,4,6,7,9,15] The sigma factors of the σ^{54} family are structurally similar to the 54 kDa sigma factor of *Escherichia coli*, which drives the transcription of genes related to the metabolism of nitrogen in an ATP-dependent manner and requires the activity of a specific enhancer. They are considered relatively rare, as there no representatives of this family have been found in any GC-rich gram-positive bacterium or cyanobacterium.[1,4,7,15] On the other hand, the sigma factors of the σ^{70} family, which are structurally similar to the 70 kDa primary sigma factor of *E. coli*, have been found in all bacterial species studied to date.[1,7,15] In addition to the primary transcriptional regulators, this family includes their related alternative sigma factors, which can be further categorized according to the physiological processes they control.[9,16]

Generally, groupings by function are correlated with the phylogenetic relationships among sigma factor protein sequences,[9,16] and under this kind of classification, it can be noted that the sigma factors of the σ^{70} family are modular proteins[1,4] that consist of up to four conserved regions,[1,3,4,17] which can be divided into subregions. Region 1 lies in the N-terminal portion of the protein and comprises subregions 1.1 and 1.2, with the former being responsible for preventing other sigma factors that are not associated with RNAP from binding to the target DNA. Region 2 consists of four subregions, 2.1, 2.2, 2.3, and 2.4, where 2.3 is involved in the formation of the transcription bubble, and 2.4 is required for the recognition of the –10 promoter element. Region 3, in turn, consists of subregions 3.0, 3.1, and 3.2, with 3.0 being implicated in the recognition of the extended –10 promoter element. Lastly, region 4 includes subregions 4.1 and 4.2, the latter of which is capable of interacting with various transcription activators and is required for the recognition of the –35 promoter element (**Fig. 1**).[1,3,4,16,18-25] All regions participate in the interaction between sigma factors and core RNAP.[3,26,27]

Depending on their structure and function, the sigma factors of the σ^{70} family can be categorized into four distinct groups. The first group (group 1) is composed of the "primary sigma factors", which present all four above-mentioned regions and are essential for bacterial survival, as they mainly control the transcription of housekeeping genes (**Fig. 1**).[1,3,6,7,15,16,28]

The second group (group 2) is composed of the "primary-like sigma factors", which are closely related to the group 1 sigma factors but are not essential for survival under optimal growth conditions. They do not carry most of region 1 in their structure (**Fig. 1**). The best-characterized factors in this group are involved in the transcription of general stress response genes and genes that are required for late stationary growth in vitro. Nevertheless, these sigma factors are found in a limited number of bacteria, such as Proteobacteria, Cyanobacteria, and gram-positive species with high G+C content in their genomes.[1,3,6,7,16,28]

The third group (group 3) is composed of regulators that are more distantly related to the primary sigma factors, presenting only the conserved regions 2, 3, and 4 in their structure (**Fig. 1**). The sigma factors that belong to this group generally control the transcription of genes involved in the heat shock response, sporulation, and flagellar biosynthesis.[1,3,7,16,28]

Finally, the fourth group (group 4) is the largest and most heterogeneous among the σ^{70} family-related groups. The "extracytoplasmic function sigma factors (σ^{ECF})" that constitute group 4 are characterized by the presence of only regions 2 and 4 in their structure (**Fig. 1**). These factors contribute to controlling the expression of genes whose products exert various functions in the bacterial response to specific extracellular signals and surrounding modifications, such as the presence of denatured proteins or toxic molecules, changes in osmolality or barometric pressure, nutrient limitation, and oxidative and other surface stresses. Additionally, several sigma factors that belong to this group are involved in the regulation of components that are important for virulence in pathogenic bacteria.[1,3,6,7,16,28-30]

Posttranslational Regulation of Bacterial Sigma Factors

To achieve appropriate control of gene expression, bacteria are able to modulate the activity of alternative sigma factors, in accord with their needs.[7,31] The regulation of such transcriptional factors therefore constitutes a key component of eliciting adequate responses to specific external stimuli.[4]

The alternative sigma factors are regulated at the transcriptional, translational, and posttranslational levels.[32] Transcriptional regulation is made possible mainly by the fact that a given sigma factor-encoding gene may have one or more upstream

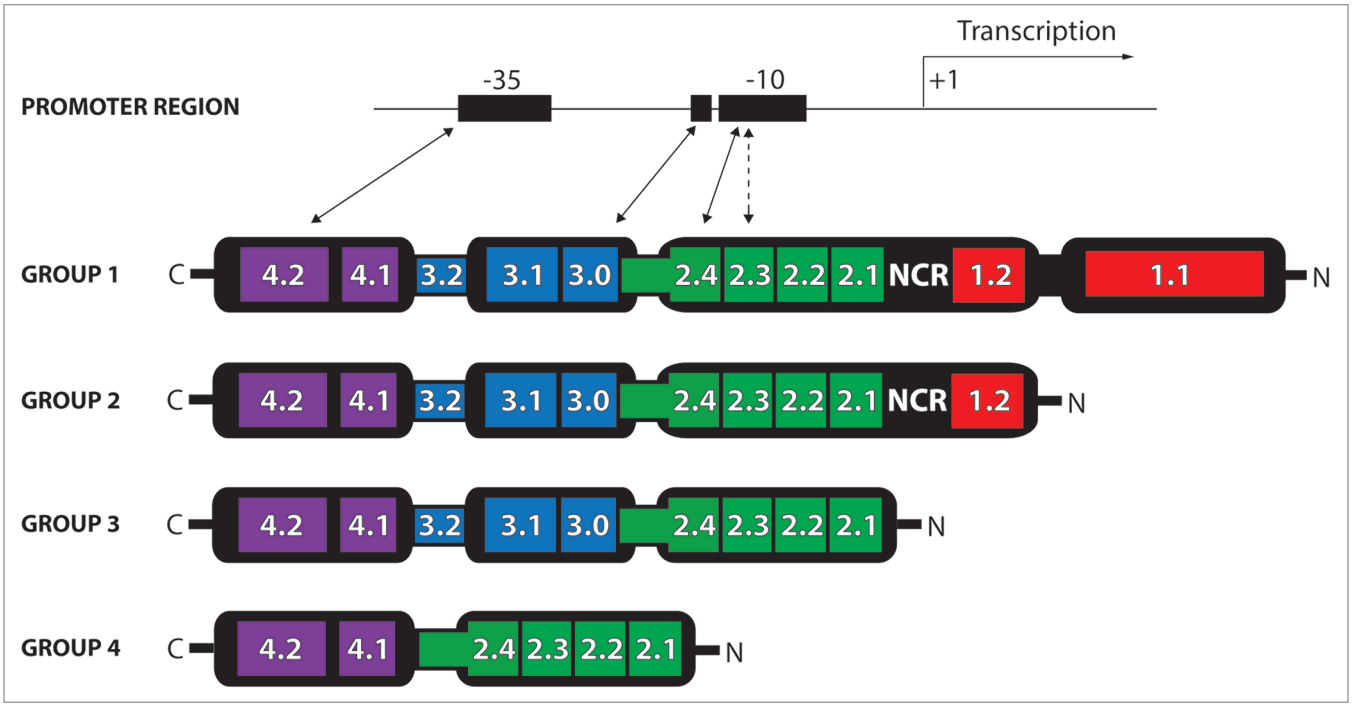

Figure 1. Schematic representation of the structure of the four known groups of sigma factors, emphasizing the four conserved regions that each factor may present and their interactions with the promoter region. Subregion 1.1, which belongs to region 1 (represented in red), is responsible for preventing sigma factors that are not bound to the RNAP from binding to DNA and accelerating the open complex at some promoters. Subregions 2.3 and 2.4, which belong to region 2 (represented in green), are involved in the formation of the transcription bubble and required for the recognition of the –10 promoter element, respectively. Subregion 3.0, which belongs to region 3 (represented in blue), is implicated in the recognition of the extended –10 promoter element. Subregion 4.2, which belongs to region 4 (represented in purple) is responsible for interacting with various transcription activators, in addition to being required for the recognition of the –35 promoter element.[16,28] The arrows between the sigma factor and the promoter region of a gene represent the interactions between the former and the latter. The dashed arrow represents the interaction of subregion 2.3 with single-stranded DNA in the open complex.

promoters that are recognized by different sigma factors and/or the sigma factor it encodes. On the other hand, translational regulation is often accomplished through the action of factors that can either allow or inhibit the translation of a specific sigma factor's mRNA. Interestingly, some specific promoters in sigma factor genes lead to the synthesis of leaderless mRNAs, which are preferentially translated by 70S ribosomes (abundant under adverse conditions such as carbon starvation, stationary growth, and slow growth).[33] Lastly, posttranslational regulation relies on the action of anti-sigma factors,[34] which are constituents of a partner-switching system[7,31] in which the interaction between an anti-sigma factor and its cognate sigma factor prevents the latter from binding to the core RNAP. Interaction with an anti-sigma factor inhibits the sigma factor's influence on gene expression profiles until the appropriate stimulus is detected by the bacterium.[3]

The anti-σ^{ECF} factors are inner membrane proteins with at least one transmembrane domain. In contrast to their C-terminal region, the N-terminal region of anti-sigma factors is highly conserved.[31] These characteristics are not without a purpose: the N-terminal region is located in the cytoplasm, where the anti-sigma factor interacts specifically with its cognate sigma factor, whereas the C-terminal region is projected to the periplasm, where it can interact with a sensor protein. When a specific stimulus is sensed, the sensor protein modifies the C-terminal portion of anti-σ^{ECF}, causing changes in its structural conformation and allowing cleavage by the site-1 protease. Once cleaved, anti-σ^{ECF} becomes a substrate for the site-2 protease, which cleaves within or near the transmembrane region. Finally, the N-terminal portion of anti-σ^{ECF}, still bound to the σ^{ECF} factor, is released and then degraded by cytoplasmic proteases. Thus, the inhibition of the sigma factor is abolished, and the genes constituting the regulon are transcribed (**Fig. 2**). However, the transcription of such genes only lasts while the sensor protein is still able to sense the external stimulus, and once the signal ceases, the regulation cycle is reinitiated.[30,35]

One well-studied example of this type of posttranslational regulation involves *B. subtilis* σ^{W} and its cognate anti-sigma factor, RsiW. In the absence of a stimulus, RsiW binds to σ^{W} and inhibits its activity. In the presence of a stimulus, the site-1 protease PrsW and other unidentified proteases cleave RsiW near its C-terminus. Then, the site-2 protease RasP cleaves RsiW within its transmembrane region, releasing the N-terminal portion of RsiW into the cytoplasm. Finally, either the ClpXP or ClpCP protease degrades RsiW, thus allowing activation of σ^{W}.[35]

Another example concerns *Mycobacterium tuberculosis* σ^{E} and its cognate anti-sigma factor, RseA. Barik et al.[36] demonstrated for the first time that the σ^{E}-RseA interaction is regulated by distinct mechanisms under specific stress conditions, with different

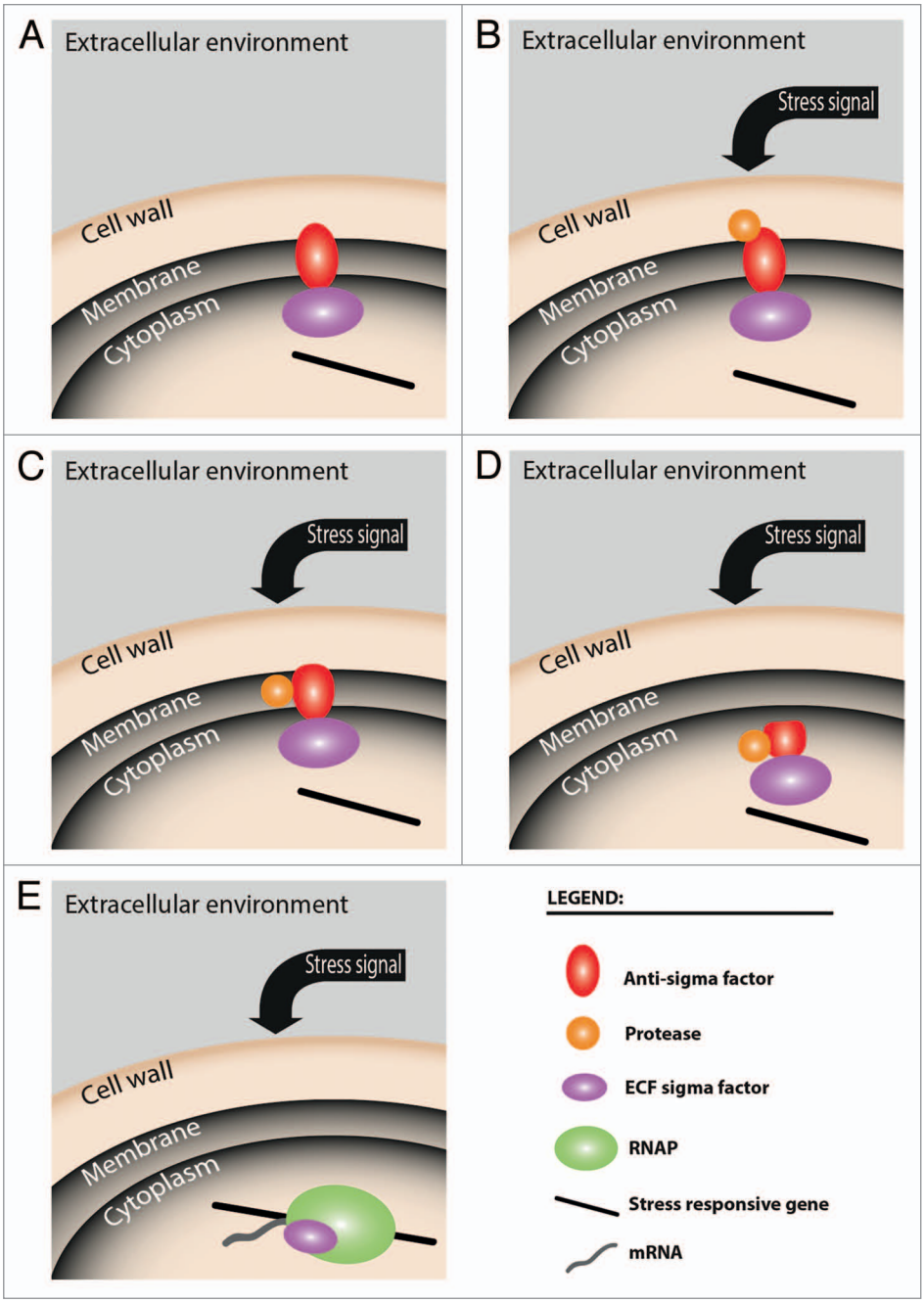

Figure 2. Schematic representation of the posttranslational regulation of σ^{ECF} factors from gram-positive bacteria. (**A**) When no stimulus is sensed by the sensor protein (not represented), the anti-σ^{ECF} factor sequesters its cognate σ^{ECF} factor, inhibiting the sigma factor's activity. (**B**) When a stimulus is sensed by the sensor protein, it interacts with the C-terminal portion of the anti-σ^{ECF} factor, modifying the anti-sigma factor's structural conformation. This, in turn, allows the site-1 protease to cleave the anti-sigma factor near its C-terminus. (**C**) Once the anti-σ^{ECF} factor is cleaved by the site-1 protease, it becomes a substrate for the site-2 protease, which cleaves the anti-sigma factor within or near its transmembrane region. (**D**) The N-terminal portion of the anti-σ^{ECF} factor, still bound to the σ^{ECF} factor, is released into the cytoplasm, where it is degraded by cytoplasmic proteases. (**E**) The inhibition of the σ^{ECF} factor is abolished, and the genes that constitute its regulon are transcribed.[30,35]

pathways of RseA degradation being observed when the microorganism is exposed to cell-surface stresses. In this case, proteolytic degradation is triggered by RseA, which is phosphorylated by PknB, one of the best-studied serine/threonine kinases found in mycobacteria. Additionally, Barik et al.[36] suggested that the RseA degradation pathway consists of two positive feedback loops. After being released into the cytoplasm, RseA is ultimately degraded by the protease ClpC1P2. Free σ^{E} induces the transcription of its regulon, which includes the gene that encodes ClgR. This transcriptional regulator, in turn, induces the transcription of the *clp* regulon and *ppk1*, which is the gene that encodes the enzyme responsible for polyphosphate (polyP) biosynthesis. Consequently, increased amounts of ClpC1P2 lead to more efficient degradation of RseA and to a higher concentration of free σ^{E} in the cell (first positive feedback loop). Also, it appears that increased amounts of PPK1 lead to a higher concentration of polyP in the cell, stimulating the Two Component System (TCS) MprAB constituent MprA phosphorylation by the other TCS MprAB constituent MprB. Then, phosphorylated MprA binds its consensus sequence upstream of *sigE*, disabling the P1 promoter and activating the P2 promoter, which results in the synthesis of the major isoform of σ^{E} (second positive feedback loop).[36,37]

Promoter Recognition by Bacterial Sigma Factors

Distinct sigma factors are capable of recognizing distinct bacterial promoters, which are distributed among sets of genes that are likely to exert correlated functions. Thus, promoters may be considered major components involved in the regulation of gene expression.[4,38,39]

According to studies on several *E. coli* promoters, the classical prokaryotic promoter consists of a DNA sequence (40–50 bp) that specifies the RNAP binding site located upstream of the transcription start point (TSP). The TSP, which is generally either an adenine or a guanine, occupies the +1 position in the core promoter. The –10 sequence (TATAAT), also known as the Pribnow box, is a highly conserved region centered ~10 bp upstream of the TSP and separated by an interhexameric region of ~17 bp from the –35 sequence (TTGACA),

which is centered ~35 bp upstream of the TSP. Additionally, some promoters present a TGN motif located upstream of the –10 sequence (extended –10 sequence) (**Fig. 1**).[38,39]

Although the consensus sequences in promoters recognized by the primary sigma factors in other Eubacteria may appear to be similar to the ones in *E. coli*, there are particular characteristics that contribute to increasing the microorganism's fitness. In *M. tuberculosis*, for example, the TSP (+1 position) is usually a guanine preceded by a cytosine (–1 position) and followed by a thymine (+2 position). However, *M. tuberculosis* is able to use any base as a TSP, even a pyrimidine. Interestingly, this mycobacterium does not share the –35 consensus sequence (TTGACA) found in other bacteria and presents little homology with other mycobacteria. This, in turn, is most likely because different sigma factors recognize different –35 sequences. Nevertheless, promoters with unrecognizable –35 sequences may require the recognition of additional transcriptional activators to initiate transcription. Thus, it has been suggested that in the presence of an unrecognizable –35 sequence, the extended –10 sequence assists RNAP in binding to the promoter, extending its contact with the DNA sequence. Additionally, the TGN motif plays an important part in the determination of promoter strength in mycobacteria.[38]

Another example is provided by *Corynebacterium glutamicum*, whose –10 (TANAAT) and –35 (TTGC/GCA) consensus sequences are quite similar to the –10 and –35 consensus sequences of other eubacterial promoters recognized by the primary sigma factor. However, similar to *M. tuberculosis*, the nucleotides found in the –35 sequence are much less conserved in *C. glutamicum*, making it difficult for –35 hexamers to be recognized. Furthermore, similar to *M. tuberculosis*, additional elements that are not conserved, such as the TGN motif of the extended –10 sequence, may affect promoter activity in *C. glutamicum*.[4,39]

Whereas the primary sigma factor is usually responsible for the transcription of housekeeping genes, the expression of genes involved in cell adaptation to limited growth conditions, such as the stationary growth phase and exposure to various stresses, is mainly controlled by the alternative sigma factors. Thus, unique characteristics meant to increase the fitness of the cell in such situations are found in the promoters recognized by these sigma factors. For instance, in *C. glutamicum*, the –10 (C/T**GTT**G/AA/TA/T) and –35 (G/T**GGAA**T/CA/T) sequences recognized by the σ^{ECF} factor σ^{H} present a highly conserved core (5′-GGAA-N18–21-GTT-3′) that is also found in the promoters of stress response genes in *Mycobacterium* and *Streptomyces*.[4,39]

Most of the genes of a bacterium are transcribed from a single promoter located in the intergenic region upstream of the gene. However, the same gene can be transcribed by more than one promoter, which may be either separated or overlapping, with different efficiencies or under different conditions. These promoters are in turn recognized by an RNAP bound to the same or a different sigma factor. In *C. glutamicum*, for example, the –10 sequence recognized by the primary-like sigma factor σ^{B} is very similar to the –10 sequence recognized by the primary sigma factor σ^{A}. This implies that some σ^{A}-dependent promoters might also be dependent on σ^{B} under specific conditions. Thus, the presence of multiple promoters is important for the fine-tuning of the expression of genes under specific conditions to optimize the mRNA and protein contents of the bacterial cell.[4,38,39]

Model Gram-Positive Bacterium σ^{ECF} Factors

The non-pathogenic bacterium *B. subtilis* is among the best-studied microorganisms and is considered a model for studies on gram-positive bacteria with low G+C contents, which include deadly pathogens, such as *Bacillus anthracis*, and bacteria that are extensively used in industry, such as other bacilli and lactococci.[40] This endospore-forming bacterium is one of the main sources of industrial enzymes, particularly amylases and proteases, and it is therefore also used to study protein secretion and heterologous protein production.[41] Additionally, along with the model gram-negative bacterium *E. coli*, *B. subtilis* was one of the first prokaryotes in which gene regulation studies were performed, the first reports of which date back to the 1960s. This contributed to pioneering studies on transcriptional regulators, such as the sigma factors, in other microorganisms, including those that belong to the CMNR group.

B. subtilis, which is found in association with plants, in water sources, and most commonly, in soil,[41] often faces various harsh environmental conditions, such as oxygen and nutrient starvation and thermal, oxidative, and osmotic stresses. Thus, this bacterium has to make use of certain strategies to maintain its viability under such stresses and to ensure its persistence and regrowth in that particular environment.[42] One of these strategies is activation of alternative sigma factors, such as one of its seven σ^{ECF} factors, σ^{M}, σ^{V}, σ^{W}, σ^{X}, σ^{Y}, σ^{YlaC}, and σ^{Z} (**Table 1**).[43-47]

The σ^{M} factor of *B. subtilis* is encoded by a gene known as *sigM*. This gene is part of an operon located upstream of the genes *yhdL* and *yhdK*, which in turn encode the anti-σ^{M} factor.[48] Because σ^{M} is one of the best-studied σ^{ECF} factors found in *B. subtilis*, significant progress has been made in defining the functions of this sigma factor. Thus, it has been reported by several research groups that σ^{M} contributes to the *B. subtilis* response to cell wall antibiotics, such as vancomycin, bacitracin and phosphomycin, as well as heat shock and osmotic, ethanol, acidic and superoxide stresses.[47-51] Additionally, among the genes that comprise the σ^{M} regulon, the most frequently discussed include *ponA*, which encodes a penicillin-binding protein; *bcrC*, which encodes a bacitracin resistance protein; *yjbD*, which appears to be involved in the development of competence; *ftsH*, which encodes a protease located in the cytoplasmic membrane; *yqjL*, which encodes a putative hydrolase; *yceC*, which belongs to the *yceCDEFGH* detoxification operon and encodes a tellurium resistance protein; *yrhJ*, which encodes a cytochrome P450/NADPH reductase; *yraA*, which encodes an intracellular protease PH1704 of *Pyrococcus horikoshii* homolog that was the first protein found to be essential for acid stress tolerance in this bacterial genus; *yacK,* which is a class III heat shock protein; and *yjbC* and *yacL*, which are members of the heat shock stimulon.[48-51]

σ^{V}, which is one of the least-studied σ^{ECF} factors found in *B. subtilis*, is encoded by the *sigV* gene. This σ^{ECF} factor is a member of an operon localized upstream of the following genes:

Table 1. *Bacillus subtilis* σ^{ECF} factors and their corresponding functions

Sigma factor	Coding gene	Function	References
σ^{M}	*sigM*	-Response to cell wall antibiotics, heat shock and osmotic, ethanol, acid and superoxide stresses	47–51
σ^{V}	*sigV*	-Response to lysozyme stress	47 and 53
σ^{W}	*sigW*	-Response to agents that interfere with the biosynthesis and/or in proper functioning of the cell wall -Detoxification of the bacterium -Synthesis and/or secretion of bacteriocins	55 and 56
σ^{X}	*sigX*	-Control of cell envelope modification processes -Response to heat shock and cationic antimicrobial peptides -Septum and wall synthesis; -Biofilm architecture	58–60
σ^{Y}	*sigY*	-Production of and resistance to the antibiotic sublancin	62
σ^{YlaC}	*ylaC*	-Response to oxidative stress	64
σ^{Z}	*sigZ*	-Not yet determined	65

rsiV (previously known as *yrhM*), which encodes the anti-σ^{V} factor; *oatA* (previously known as *yrhL*), which encodes a peptidoglycan *O*-acetyltransferase; and *yrhK*, whose function is still unknown.[47,52,53] Thus far, only a limited number of studies have described the functions of the σ^{V} factor in the physiology of *B. subtilis*. Nevertheless, it has recently been reported by two different research groups that this σ^{ECF} factor is strongly and specifically induced by lysozyme, an enzyme that hydrolyzes the β-1,4-glycosidic bond between the *N*-acetylmuramic acid (MurNAc) and the *N*-acetyl-glucosamine (GlcNAc) in the peptidoglycan polysaccharide backbone.[47,53] Additionally, Guariglia-Oropeza and Helmann[47] suggested that σ^{V} is the main factor responsible for the *B. subtilis* response to lysozyme stress because the Δ*sigV* mutant and a mutant strain with null mutations in all seven σ^{ECF} factor genes presented almost the same level of sensitivity to the stress in question. The intrinsic ability of *B. subtilis* to resist to lysozyme exposure is most likely because the σ^{V}, sometimes along with other σ^{ECF} factors, is responsible for the upregulation of *oatA*, which encodes a peptidoglycan *O*-acetyltransferase; *dltA*, which is part of the *dltABCDE* operon, which encodes enzymes for teichoic acid D-alanylation; and, apparently to a lesser extent, *pbpX*, which is a penicillin-binding protein.[47,53]

The σ^{W} factor of *B. subtilis*, which is also among the best-studied sigma factors in this bacterium, is encoded by the *sigW* gene, which is located upstream of the *rsiW* gene, encoding the anti-σ^{W} factor.[54] The σ^{W} regulon is relatively large and is most frequently induced when the microorganism is exposed to agents that interfere with cell wall biosynthesis and/or in its proper functioning.[55] Thus, σ^{W} activates the expression of several genes that play an important role in the detoxification of the bacterium, such as *fosB*, which encodes a phosphomycin resistance protein; *ybfO*, which encodes an erythromycin esterase; *ydjP*, which encodes a bromoperoxidase; *yfhM*, which encodes an epoxide hydrolase; *pbpE*, which encodes a penicillin-binding protein; and the already-cited *yceC*, which is also induced by the σ^{M} factor. Additionally, σ^{W} can activate the expression of genes that likely participate in the synthesis and/or secretion of bacteriocins, such as *ywoA*, which encodes a predicted bacteriocin permease; *yqeZ* and *sppA*, which encode protease IV homologs; and the *yknXYZ* operon, which encodes an ABC transporter that is thought to be involved in bacteriocin export.[55,56] Thus, Cao et al.[55] suggested that the σ^{W} factor of *B. subtilis* controls an "antibiosis" regulon, which includes genes encoding both defensive and offensive components that play a role in the survival of the microorganism.

The σ^{X} of *B. subtilis*, which is encoded by the *sigX* gene, is located upstream of the *rsiX* gene (previously known as *ypuN*), which encodes the anti-σ^{X} factor. Interestingly, *sigX* and *rsiX* are homologous to *fecI* and *fecR* from *E. coli*, the former of which is the σ^{ECF} factor responsible for activating the transcription of the *fecABCDE* operon, which encodes the ferric citrate transport genes, while the latter is a transmembrane protein responsible for inducing the transcription of the ferric citrate transport genes when ferric citrate binds to the outer membrane protein FecA. Nevertheless, as the SG64 strain (*trpC2*, *lacR1*, and *lacA17* genotype) does not use ferric citrate as an iron source, studies have shown that neither *sigX* nor *rsiX* is involved in ferric citrate transport in *B. subtilis*.[57] On the other hand, they appear to be involved in controlling processes related to cell envelope modification, such as resistance to heat shock[58] and to cationic antimicrobial peptides,[59] septum and wall synthesis,[58] and biofilm architecture.[60] The genes that make up the σ^{X} regulon include *tarA*, which is related to the synthesis of poly(RboP), the most important teichoic acid in the W23 strain (prototroph, Str^{r} phenotype); *divIC*, whose product is required for the initiation of septum formation; *lytR*, whose product negatively regulates the *lytABC* operon, where LytC is an amidase; *dltABCDE*, which encodes enzymes for teichoic acid D-alanylation; *csbB*, which encodes a membrane-bound glucosyl transferase; *rapD*, which encodes an aspartate phosphatase response regulator; *pssA* and *psd*, which encode enzymes involved in phosphatidylethanolamine (PE) biosynthesis; and *abh*, whose product is an AbrB homolog that regulates biofilm architecture.[58-60]

The σ^{Y} of *B. subtilis*, which is encoded by the *sigY* gene, appears to be regulated by the product of the gene *yxlC*, located immediately downstream of *sigY* in the *sigY-yxlCDEFG* operon.[61,62] The

Table 2. *Corynebacterium* σ^{ECF} factors and their corresponding functions

Species	Sigma factor	Coding gene	Function	References
Corynebacterium glutamicum	σ^{C}	*sigC*	-To be elucidated	4
	σ^{D}	*sigD*	-Response to microaerobic conditions	68
	σ^{E}	*sigE*	-Response to nutritional and cell surface stresses, heat shock, magnesium deficiency, long-term exposure to lactic acid, and microaerobic conditions	68–71
	σ^{H}	*sigH*	-Response to heat shock and oxidative stress	4
	σ^{M}	*sigM*	-Response to heat shock, cold, and oxidative stresses	73
Corynebacterium pseudotuberculosis	σ^{C}	*sigC*	-Not yet determined	-
	σ^{D}	*sigD*	-Putative role in virulence	77
	σ^{E}	*sigE*	-Response to SDS, lysozyme, acid, nitric oxide, and nitric oxide/peroxide stresses	78
	σ^{H}	*sigH*	-Not yet determined	-
	σ^{K}	*sigK*	-Not yet determined	-
	σ^{M}	*sigM*	-Not yet determined	-

functions of this factor, which is another of the least-studied σ^{ECF} factors from this bacterium, have not yet been well defined. Nevertheless, Mendez et al.[62] reported that, in addition to regulating the transcription of its own operon, σ^{Y} is required for maintaining the Spβ prophage, which is a viral genome of 135 Kb that includes genes for the production of and resistance to the antibiotic sublancin and is found in the chromosome of *B. subtilis*. Thus, it was concluded that, similar to other σ^{ECF} factors found in *B. subtilis*, σ^{Y} is responsible for the regulation of genes that contribute to the survival of the bacterium in regard to the necessity of producing and resisting to the action of antibiotics in a certain environment.[62]

The σ^{YlaC} of *B. subtilis* is encoded by a gene known as *ylaC*, which is located upstream of the *ylaD* gene (whose product is the probable anti-σ^{YlaC} factor) in the *ylaABCD* operon.[63,64] This σ^{ECF} factor, similar to σ^{V} and σ^{Y}, does not have well-defined functions. However, Matsumoto et al.[63] and Ryu et al.[64] have suggested that this sigma factor contributes to *B. subtilis* resistance to oxidative stress because YlaD is very similar to RsrA, the anti-σ^{R} factor of *S. coelicolor*, which presents several cysteine residues that, in turn, sense the cytoplasmic thiol-disulphide status. Nevertheless, only Ryu et al.[64] were able to show that σ^{YlaC} does, in fact, contribute to oxidative stress resistance, as they observed that a strain overproducing this factor exhibited peroxidase activity that was almost three times the activity of the control strain under hydrogen peroxide treatment.

Finally, the least-studied σ^{ECF} factor from *B. subtilis*, σ^{Z}, is encoded by the *sigZ* gene. Interestingly, in contrast to the other σ^{ECF} factors found in this bacterium, σ^{Z} appears to be monocistronic, and it does not appear to direct its own transcription. Additionally, σ^{Z} does not seem to regulate many genes, with *gsiB*, a proven σ^{B} target, and *yrpG* being the only genes that have been suggested to be possibly activated by this sigma factor.[65]

σ^{ECF} Factors in the CMNR Group

Within the class Actinobacteria, which is mainly characterized by the high G+C contents of the genomes of its members, there is a group of microorganisms referred to as the CMNR group. This group is in turn characterized by the cell wall organization of its members (based on the presence of a polymer complex composed of peptidoglycans, arabinogalactans, and mycolic acids), which belong to the genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus*.[66] Nevertheless, it must be highlighted that there are no reports addressing the σ^{ECF} factors of the bacteria belonging to the *Nocardia* genus, which is why they were not discussed here.

Corynebacterium σ^{ECF} factors

Corynebacterium glutamicum

Some of the species that constitute the CMNR group stand out due to their potential to be used as biotechnological tools, such as the *C. glutamicum*, which has been widely used in the industrial production of several amino acids and nucleic acids.[67] This bacterium, which is a non-pathogenic species of increasing interest as a model organism with closely related pathogenic species, harbors only 5 σ^{ECF} factors: σ^{C}, σ^{D}, σ^{E}, σ^{H}, and σ^{M} (**Table 2**). Of these factors, only σ^{E}, σ^{H}, and σ^{M} have been studied in further detail, whereas no studies on σ^{C} have been published thus far.[4] Additionally, there is only one available report that potentially provides hints about the functions of σ^{D}, suggesting that this sigma factor contributes to the survival of *C. glutamicum* under low oxygen concentrations.[68]

It has been reported that σ^{E}, encoded by the *sigE* gene, located upstream of *cseE*, which encodes the anti-σ^{E} factor, plays a role in the resistance of *C. glutamicum* to cell surface stresses, such as those caused by the addition of SDS, lysozyme, EDTA and antibiotics to the culture medium as well as magnesium deficiency and heat.[69] Additionally, it appears that σ^{E} contributes to this

bacterium's responses to nutritional stress, long-term exposure to lactic acid and microaerobic conditions.[68,70,71]

It has been demonstrated that the σ^H of *C. glutamicum*, which is encoded by the *sigH* gene, situated upstream of the *rshA* gene, which appears to encode the anti-σ^H factor, directly or indirectly regulates the expression of more than 65 genes,[72] including genes that function in the response of this microorganism to heat shock and oxidative stress. Additionally, this sigma factor is involved in the regulation of the transcription of *sigB*, *sigE*, *sigH*, and *sigM*, which encode alternative sigma factors, and *hspR*, *clgR*, *sufR*, *whcA*, and *whcE*, which encode other stress response regulators. Thus, these data suggest that σ^H occupies a central position in the sigma factors regulatory network, playing a key role in the response of *C. glutamicum* to several types of environmental stresses.[4]

Finally, the σ^M of *C. glutamicum*, which is encoded by the *sigM* gene, takes part in this bacterium's response to cold, heat and oxidative stresses. In regard to the response of *C. glutamicum* to disulfide stress, it appears that σ^M activates the transcription of the *suf* genes *sufR* and *sufBDCUS*, which are involved in the formation of iron-sulfur (Fe-S) clusters and their insertion in proteins; *trxB*, *trxC*, and *trxB1*, which are involved in the cell's response to thiol-oxidizing conditions; and *groES*, *groEL*, and *clpB*, which encode chaperones. Of particular note, as mentioned above, it has also been suggested that σ^H activates the transcription of σ^M when this microorganism is exposed to oxidative stress.[73]

Corynebacterium pseudotuberculosis

C. pseudotuberculosis is a facultative intracellular pathogen that is capable of infecting different types of animals, such as goats, sheep, horses, llamas, buffaloes, camels, antelopes, and primates.[74] Prior to 2005, this bacterium was associated with the occurrence of at least 25 cases of human infection, with the majority of infected people, such as breeders and veterinarians, having had contact with sick animals, which indicates the high occupational zoonotic potential of this bacterium.[75,76]

One of the reasons that *C. pseudotuberculosis* is able to successfully infect such a wide range of hosts is the transient activation of its sigma factors, including its σ^{ECF} factors: σ^C, σ^D, σ^E, σ^H, σ^K, and σ^M (**Table 2**). However, there are no available reports addressing the functions of sigma factors other than σ^D and σ^E in this bacterium.

After generating growth curves of *C. pseudotuberculosis* in brain heart infusion (BHI) broth (a rich medium commonly used for culturing this bacterium) and fetal bovine serum (FBS) (a culture medium that mimics the presence of certain host factors, such as serum proteins, hormones, lipids, and vitamins), it was observed that this bacterium presented an increased growth rate when cultured in FBS, indicating that there had been changes in the physiology of *C. pseudotuberculosis* under those conditions. Thus, to evaluate the differential expression of the genes that encode the alternative sigma factors of this microorganism in the presence of FBS, qRT-PCR (quantitative real-time-polymerase chain reaction) assays were performed. Based on the obtained results, it was possible to observe changes in the expression of the *sigB*, *sigC*, *sigD*, *sigE*, *sigH*, *sigK*, and *sigM* genes. However, only *sigD* presented significantly higher transcriptional levels under the tested environmental conditions. This, in turn, indicates that σ^D represents an important target in the study of the molecular determinants of the virulence of *C. pseudotuberculosis*.[77]

Additionally, it was demonstrated that the σ^E of *C. pseudotuberculosis*, encoded by the *sigE* gene, located upstream of the *cseE* gene, which in turn, likely encodes the anti-σ^E factor, plays a role in the resistance of this bacterium to SDS, lysozyme, acid, nitric oxide, and nitric oxide/peroxide stresses. Of particular note, it was also reported that this sigma factor is required for the resistance of *C. pseudotuberculosis* to nitric oxide stress during infection because, in contrast to the *sigE* null mutant (Δ*sigE*), which persists only in the spleens of mice that are unable to produce nitric oxide in their intraphagosomes (iNOS knockout [$iNOS^{-/-}$] mice), the 1002 wild-type strain persisted in the spleens of C57BL/6 and $iNOS^{-/-}$ mice three days after infection.[78]

Corynebacterium diphtheriae

C. diphteriae secretes the bacteriophage-encoded exotoxin known as diphteria toxin (DT), which is responsible for the characteristic symptoms of diphtheria. This infectious disease, which can be life threatening, has already been controlled in countries such as the United States and Canada, where public health standards require mandatory vaccination. However, small outbreaks of this disease still occur in nonimmunized and immunocompromised people. In contrast, diphtheria remains endemic in South America, Africa, and India, among other countries, even though immunization is relatively widespread.[79]

The pathogenesis of diphtheria depends on the ability of *C. diphtheriae* to colonize the nasopharyngeal cavity and the skin and produce DT. In lysogenic strains, this toxin is encoded by the gene *tox*, whose transcription is regulated by the repressor protein DtxR. The holoenzyme DtxR (iron-bound form) binds the operator sequence of the *tox* promoter region, preventing its transcription. The apoenzyme DtxR (the non-iron-bound form), on the other hand, is unable to bind the operator sequence, allowing the transcription of *tox*.[80]

dtxR, the gene that encodes the DtxR protein, is located downstream of *sigB*, which is predicted to encode a σ^{70} family primary-like sigma factor, and upstream of *galE*, which is predicted to encode a UDP-galactose 4-epimerase,[81] and it has been suggested by Oram et al.[80,82] that *sigB* and *dtxR* as well as *dtxR* and *galE* are cotranscribed. Additionally, Oram et al.[80] demonstrated that the transcription of *sigB-dtxR* is induced by acid, cold, heat, ethanol and SDS-caused stresses, which in turn suggests that *sigB-dtxR* transcription could be activated by the recognition of the promoter upstream of *sigB* by an σ^{ECF} factor.[80]

Furthermore, the *C. diphtheriae* genome harbors the genes *sigE* and *sigH*, which are predicted to encode the σ^{ECF} factors σ^E and σ^H, respectively, and the promoter upstream of *sigB* contains sequences that are homologous to the –35 and –10 consensus sequences for σ^E and σ^H recognition. Thus, it appears that the transcription of *C. diphtheriae sigB-dtxR* is regulated by σ^E and σ^H in response to environmental conditions that cause changes in the cell envelope.[80]

Table 3. *Mycobacterium tuberculosis'* σ^{ECF} factors and their corresponding functions

Sigma factor	Coding gene	Function	Reference
σ^{C}	*sigC*	-Putative role in virulence	87–89
σ^{D}	*sigD*	-Response to nutrient starvation -Promotion of optimum growth at the beginning of infection -Folding of nascent proteins -Roles in ATP biosynthesis, DNA repair, the elongation step of translation, and bacterial virulence	90 and 91
σ^{E}	*sigE*	-Response to heat shock, oxidative stress, vancomycin, and membrane-disrupting agents -Putative role in virulence -Role in fatty acid degradation	37 and 92–94
σ^{G}	*sigG*	-Putative role in DNA repair, virulence, and fatty acid metabolism	87 and 88
σ^{H}	*sigH*	-Response to heat shock and oxidative stress -Putative role in virulence	97–101
σ^{I}	*sigI*	-Response to heat shock and the drug isoniazid -Role in ATP biosynthesis	102
σ^{J}	*sigJ*	-Putative role in the response to oxidative stress	104
σ^{K}	*sigK*	-To be elucidated	105–107
σ^{L}	*sigL*	-Putative role in posttranslational protein modifications and virulence	108
σ^{M}	*sigM*	-Appears to promote successful host-pathogen interactions in the later stages of infection	109 and 110

Mycobacterium σ^{ECF} factors

Mycobacterium tuberculosis

M. tuberculosis is the main etiologic agent of human tuberculosis (TB),[83] which is an infectocontagious disease that is intimately associated with poverty and occurs mostly in developing countries.[84,85] It was estimated by the World Health Organization (WHO)[86] that as of the year 2010, almost 9 million people had contracted this disease and approximately 1.5 million people had died from it worldwide.

One of the reasons that makes *M. tuberculosis* such a successful intracellular pathogen is its ability to respond appropriately to the different stresses to which it is subjected once it has infected the host by transiently activating its alternative sigma factors, which include the σ^{ECF} factors σ^{C}, σ^{D}, σ^{E}, σ^{G}, σ^{H}, σ^{I}, σ^{J}, σ^{K}, σ^{L}, and σ^{M} (**Table 3**).

σ^{C} of *M. tuberculosis*, which is encoded by the *sigC* gene, plays a crucial role in the pathogenicity of this mycobacterium[87-89] through regulating the transcription of virulence genes, such as *hspX*, which encodes an α-crystalline homolog; *senX3*, which encodes a sensor kinase; *mtrA*, which encodes a response regulator; and *fbpC*, which encodes constituent C of the antigen 85 complex, Ag85C.[87]

Encoded by the *sigD* gene, σ^{D} appears to be related to the response of *M. tuberculosis* to nutrient starvation, which is at least partially dependent on the activation of this sigma factor by the ppGpp synthase RelA. Additionally, σ^{D} appears to promote the optimum growth of this microorganism at the beginning of infection, playing an important role in the folding of nascent proteins.[90] Furthermore, during the stationary phase of growth, this σ^{ECF} factor has been suggested to activate the transcription of genes that encode ribosome components and factors that participate in ATP biosynthesis, DNA repair, the elongation step of translation and bacterial virulence, such as *atpC* and *atpD*, which encode subunits of a proton-gradient ATP synthase; *hns*, which encodes a histone-like protein; *recR*, which encodes a DNA repair enzyme; *tsf*, *fusA* and *tuf*, which are elongation factors; *pks10*, which encodes a polyketide-like chalcone synthase; *fbpA*, which encodes constituent A of the antigen 85 complex, Ag85A; and *mce1*, which encodes a protein that plays a role in the uptake of *M. tuberculosis* into non-phagocytic cells.[91]

σ^{E}, which is one of the best-studied σ^{ECF} factors of *M. tuberculosis* and is encoded by the *sigE* gene, appears to be related to the response of this bacterium to heat shock, oxidative stress, vancomycin and membrane-disrupting agents, such as SDS. Additionally, it has been reported that mutants deficient in σ^{E} show some difficulty in growing within inactivated murine and human macrophages and are more sensitive to the microbicidal activity of activated murine macrophages.[37,92-94] Also, this sigma factor appears to regulate the transcription of genes involved in fatty acid degradation, such as *aceA*, which encodes an isocitrate lyase, and *fadB2*, which encodes a 3-hydroxyacyl-CoA dehydrogenase; heat shock proteins, such as *hsp*, which encodes a chaperone, and *htpX*, which encodes a membrane prenyl-protease; and other transcriptional regulators, such as *sigB*, which encodes the primary-like sigma factor σ^{B}.[92]

σ^{G}, which is encoded by the *sigG* gene, is one of the least-studied σ^{ECF} factors of *M. tuberculosis*, along with σ^{I} and σ^{J}. According to Lee et al.,[95] this factor appears to be involved in the response of *M. tuberculosis* to DNA damage and to directly and/or indirectly regulate genes that play a role in the SOS system, such as *lexA*, which encodes a repressor of the activity of other genes that take part in this response. However, according to Smollett et al.,[96] σ^{G} is only part of the RecA-independent regulon and appears to control the transcription of secondary genes that are not directly responsible for the repair of damaged DNA. Nevertheless, this σ^{ECF} sigma factor has been suggested to directly and/or indirectly regulate genes that are induced in a murine model of infection, such as *lat*, which encodes an *L*-lysine-epsilon

aminotransferase; genes that play a role in fatty acid metabolism, such as *aceA*, which encodes an isocitrate lyase that is also part of the σ^E regulon; *fadE5*, which encodes an acyl-CoA dehydrogenase; and *scoA*, which encodes a succinyl-CoA. Additionally, σ^G appears to be involved in the regulation of genes that are part of the regulons of other transcriptional regulators, such as *rpfC*, which encodes a resuscitation-promoting factor regulated by σ^D; *clpB*, *dnaK,* which encode heat shock proteins; and *trxB2*, which encodes a thioredoxin reductase, regulated by σ^H.[95]

The alternative sigma factor σ^H, which is encoded by the *sigH* gene, plays an important role in the response of *M. tuberculosis* to heat shock and oxidative stress[97-101] and presents increased transcriptional levels during macrophage infection in vitro.[97,98] This factor appears to regulate the transcription of genes involved in the heat shock response, such as *dnaK* and *clpB*, which encode heat shock proteins/chaperones; genes involved in the response to oxidative stress, such as *trxB2*, which encodes a thioredoxin reductase, and *trxC*, which encodes a thioredoxin; and other transcriptional regulators involved in the response to the same stresses, such as *sigB*, which encodes the primary-like sigma factor σ^B, and *sigE*, which encodes the alternative sigma factor σ^E.[98] Additionally, infection of resistant (C57BL/6) and sensitive (C3H) mice with the wild-type and mutant (Δ*sigH*) strains of *M. tuberculosis* showed that the former presents increased immunopathology compared with the latter, which appears to be due to the reduced recruitment of T $CD4^+$ and $CD8^+$ cells by the mutant strain.[99] This result in turn suggests that the σ^H regulon is required for the modulation of the host innate immune response against *M. tuberculosis*.[101]

σ^I, one of the least-studied σ^{ECF} factors of *M. tuberculosis*, is encoded by the *sigI* gene. This sigma factor, which appears to directly or indirectly regulate the transcription of the genes encoding an ATP synthase (Rv1304), heat shock proteins (Rv0440, Rv0350, and Rv3417), and the catalase/peroxidase KatG, appears to play a role in the response of this microorganism to isoniazid (INH), which is one of the drugs that are commonly used in the treatment of tuberculosis. However, in contrast to what is usually observed, it is the Δ*sigI* mutant strain, not the wild-type strain, that is resistant to the antibiotic. In fact, this phenomenon arises when the absence of σ^I contributes to decreasing the transcription of the *katG* gene, which encodes the catalase/peroxidase KatG. The reduced levels of this enzyme, in turn, contribute to the decreased activation of INH, and there is therefore a reduction of the inhibition of the enoyl-ACP reductase InhA, which is necessary for mycolic acid biosynthesis. It is also noteworthy that the mutant strain was found to be hypervirulent in a mouse in model of tuberculosis when compared with the wild-type strain.[102]

According to Hu and Coates,[103] the *sigJ* gene, which encodes the poorly studied factor σ^J, showed strongly increased transcription in *M. tuberculosis* cultures in the late stationary phase, whereas the level of *sigJ* transcription was not altered when the antibiotic rifampicin was added to the medium. Thus, at that time, it was concluded that the σ^J factor appeared to be important for the bacterium's survival in the stationary phase, even in the presence of antibiotics. However, a few years later, the same research group showed that in an in vitro microaerophilic model, the survival of the Δ*sigJ* mutant strain was similar to the survival of the wild-type strain. Additionally, it was demonstrated that even when rifampicin was added, the viability of the two strains remained the same. Furthermore, it was shown that the growth of the wild-type and mutant strains was similar in an immune stasis murine model. Thus, it was concluded that σ^J did not contribute to *M. tuberculosis* survival in the stationary phase of growth in either the presence or the absence of antibiotics. Interestingly, it was also concluded that the Δ*sigJ* mutant strain was more sensitive to oxidative stress caused by hydrogen peroxide (H_2O_2) than the wild-type strain.[104]

The σ^K factor of *M. tuberculosis*, encoded by the *sigK* gene, whose activity is regulated by the anti-σ^K factor RskA, exhibits a relatively small regulon compared with the regulons of the other σ^{ECF} factors. σ^K regulates genes at the *sigK* and *mpt70*/*mpt83* loci. However, in *M. tuberculosis*, the transcription of the Mpt70 and Mpt83 proteins, whose functions are still unknown, only increases during infection, and in *Mycobacterium bovis*, the transcription of these proteins is constitutively high due to two mutations in the gene encoding the anti-σ^K factor, *rskA*.[105-107]

σ^L, which is encoded by the *sigL* gene, does not appear to be a stress response regulator like the other σ^{ECF} factors of *M. tuberculosis*. Instead, it has been suggested to regulate the transcription of genes that play a role in polyketide-lipid synthesis and genes that encode membrane-associated proteins that, in turn, are involved in posttranslational protein modifications. In fact, this σ^{ECF} factor exhibits a relatively small regulon that comprises four small operons: *sigL-rslA*, which encode the σ^L and anti-σ^L factors; *mpt53*-Rv2877c, which encode a disulfide oxidoreductase and a thiol disulfide; *pks10-pks7*, which encode a type III polyketide synthase and a multifunctional enzyme; and Rv1139c-Rv1138c, which encode an oxidoreductase and a membrane protein with an isoprenylcysteine carboxylmethyltransferase motif. Additionally, it is possible that σ^L regulates four PE-PGRS genes indirectly. Furthermore, it is noteworthy that the Δ*sigL* strain is highly attenuated in an in vivo mouse model of infection. This finding, together with the data presented above, indicates that σ^L likely contributes to *M. tuberculosis* pathogenicity.[108]

σ^M, the last σ^{ECF} factor of *M. tuberculosis,* is encoded by the gene *sigM*. This sigma factor appears to positively regulate genes such as those encoding the ESAT-6-like proteins *esxU-esxT* and *esxE-esxF*, which are members of the PPE protein family, and a nonribosomal peptide synthase. Additionally, it has been suggested to negatively regulate genes including some genes involved in phthiocerol dimycocerosate (PDIM) synthesis and the *kasA-kasB* operon, which is related to mycolic acid synthesis.[109,110] However, according to Agarwal et al.,[110] the transcripts of the genes regulated by σ^M accumulate at relatively low levels only during the stationary phase of growth and when the bacterium is subjected to heat shock. Additionally, a Δ*sigM* mutant strain showed the same behavior as the wild-type strain in an in vitro macrophage infection and an in vivo mouse infection.[110] Thus, in accordance with Raman et al.,[109] it is likely that σ^M is one of the transcriptional regulators responsible for promoting successful host-pathogen interactions in the later stages of infection.

Rhodococcus σ^{ECF} factors

Rhodoccocus equi

R. equi is a facultative intracellular respiratory pathogen that causes the life-threatening pyogranulomatous pneumonia in foals, causing considerable economic losses to the equine industry.[111] However, it is also capable of infecting pigs, goats, cattle and immunocompromised humans.[112] Thus, this disease presents challenges regarding its epidemiology and therapeutic control due to the complexity of this pathogen, which is not yet fully understood, and the environmental interactions that contribute to the disease process.[111]

The pathogenicity of *R. equi* depends on its ability to survive and replicate in the intracellular environment of the host cell, which is partly due to the presence of 80–90 kb plasmids, including virulence-associated protein (*vap*)-encoding genes on a pathogenicity island (PI) that are essential for intracellular replication and virulence.[112,113] However, bacterial isolates from immunocompromised humans and animals usually lack these plasmids, showing that virulence is not only plasmid associated.[113]

Because most of the chromosomal genes required for this microorganism's virulence are unknown, Raman et al.[113] developed a microarray to identify such genes that are differentially expressed within macrophages and under conditions that mimic the intra-macrophage environment. Interestingly, one of the chromosomal genes that was significantly induced under both experimental conditions was *sigK*, which is the gene that encodes the σ^{ECF} factor σ^{K}.

Conclusion

A large part of bacterial versatility is due to the functions of bacterial sigma factors, which act as positive and/or negative regulators of gene transcription. The σ^{ECF} factors, in special, stand out for their ability to make possible the adaptation of the microorganisms to changes in the surrounding environments.

However, one of the most noteworthy characteristics of sigma factors is the complexity of their regulatory networks. For example, in *M. tuberculosis*, σ^{B} can be transcribed by RNAPs bound to four distinct sigma factors, three of which recognize the same promoter: σ^{E} is involved in the basal expression of *sigB* and in its induction during surface stress; σ^{H} is involved in the induction of *sigB* and *sigE* as well as its own induction during oxidative stress and heat shock; σ^{F} and σ^{L} are involved in the induction of *sigB* and in their own induction under as yet unknown conditions. Additionally, σ^{E}, σ^{H}, σ^{F}, and σ^{L} are regulated by their cognate anti-sigma factors, with anti-σ^{F} being further regulated by two specific anti-anti-σ^{F} factors.[3]

Thus, elucidation of the specific stimuli that activate the σ^{ECF} factors as well as the genes whose expression they regulate and the associated regulatory networks would contribute to a better understanding of the overall modulation of gene expression and, hence, of the physiology of these microorganisms. Additionally, it may assist the identification of new bacterial strains with biotechnological potential and/or about how to improve the capabilities of the currently used strains, such as those of *B. subtilis* and *C. glutamicum*.

Finally, the understanding of the sigma factors and their regulatory networks largely benefits the research on pathogenic bacteria, such as *C. diphtheriae*, *C. pseudotuberculosis*, *M. tuberculosis*, and *R. equi*, facilitating the identification of virulence factors, which could be used as targets for prophylaxis (e.g., vaccine generation), therapies, and diagnostics. Moreover, the outstanding diversity in the underexplored CMNR group certainly provides a rewarding challenge for those willing to better understand the functions of sigma factors and their associated regulation networks.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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