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Bacillus subtilis extracytoplasmic function (ECF) sigma factors and defense of the cell envelope

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Summary

Bacillus subtilis provides a model for investigation of the bacterial cell envelope, the first line of defense against environmental threats. Extracytoplasmic function (ECF) sigma factors activate genes that confer resistance to agents that threaten the integrity of the envelope. Although their individual regulons overlap, σ^W is most closely associated with membrane-active agents, σ^X with cationic antimicrobial peptide resistance, and σ^V with resistance to lysozyme. Here, I highlight the role of the σ^M regulon, which is strongly induced by conditions that impair peptidoglycan synthesis and includes the core pathways of envelope synthesis and cell division, as well as stress-inducible alternative enzymes. Studies of these cell envelope stress responses provide insights into how bacteria acclimate to the presence of antibiotics.

The transcriptional specificity of RNA polymerase (RNAP) can be modified by replacement of the primary sigma (σ) subunit with alternative σ factors that modify promoter selectivity [1]. The extracytoplasmic function (ECF) family of σ factors were originally described as a group of related alternative σ factors from diverse bacteria [2]. Structurally, they are smaller in size than the primary σ factor and contain only two of the four major conserved sequence regions of bacterial σ factors. Regions 2 and 4 correspond to the domains that bind the σ factor to the core RNAP and mediate recognition of the −35 and −10 promoter elements. Detailed phylogenomic analyses have revealed that the ECF σ factor family is extremely diverse [3,4]. In many species, ECF family proteins are the most numerous alternative σ factors with >50 paralogs in a single genome.

As reflected in their name, ECF σ factors most commonly regulate functions related to the cell envelope. The two representatives in *Escherichia coli*, σ^E and σ^{FecI}, are amongst the best characterized and control functions related to outer membrane homeostasis and ferric citrate uptake, respectively [5]. Comparable homologs are widespread in the proteobacteria. *Bacillus subtilis* encodes seven ECF σ factors and provides a contrasting view of ECF function in a Gram-positive model organism [6]. In *B. subtilis*, the σ^M, σ^W, σ^X and σ^V regulators each have roles in cell envelope homeostasis. In contrast, the other three ECF σ factors (σ^Y, σ^Z, and σ^{YlaC}) are still poorly understood and will not be further considered.

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Here, I review the roles of the best characterized ECF σ factors in *B. subtilis* with an emphasis on recent insights into the nature of the σ^M envelope stress response.

The roles of ECF σ factors in *Bacillus subtilis*

The ECF σ factors of *B. subtilis* play an integral part in regulating the cell envelope stress responses (CESR) in this organism. CESR has been defined as that set of genetic responses induced by the presence of cell envelope active compounds [7], including the many antibiotics and bacteriocins commonly produced by members of the soil microbial community [8]. The responses regulated by ECF σ factors can therefore be reviewed within the larger context of CESR which additionally include systems activated by two-component systems and other regulators that regulate cell wall homeostasis. Well-characterized examples include the LiaRS, YtrA, WalKR, BceRS, and PsdRS systems [9-11].

Insights into the roles of the seven ECF σ factors encoded in the *B. subtilis* genome have been obtained primarily by studies of (i) phenotypes of mutant strains lacking one or more σ factor, (ii) the set of genes (regulon) controlled by each σ factor, and (iii) those stress conditions that activate each regulon. These studies have revealed that the seven ECF σ factors are individually and collectively dispensable for growth and sporulation [12,13]. A mutant strain lacking all seven ECF σ factors is more sensitive to numerous cell envelope stresses, including that elicited by antibiotics. This sensitivity is due primarily to the lack of σ^M, σ^W, σ^X and/or σ^V [13], which will therefore be the focus of this review. The critical role of these ECF σ factors in conferring resistance to antibiotics is also apparent from forward genetic experiments; selection for resistance to the β-lactam antibiotic cefuroxime led to recovery of a mutation in *rpoC*, encoding the β'-subunit of RNAP, that results in an increased activity of ECF σ factors [14].

General features of ECF σ factor regulons

The σ^M, σ^W, σ^X and σ^V factors are each encoded as the first gene of an operon in which the downstream gene encodes a membrane-localized anti-σ factor [3]. When cells experience an appropriate envelope stress the anti-σ factor is inactivated which leads to release of the σ and activation of the regulon [3,15,16]. The mechanisms of signal perception and the basis for anti-σ inactivation are not yet well understood, although substantial progress has been made in this area for both σ^W and σ^V [16,17], as reviewed below. Once released from the anti-σ, there is an autoregulatory promoter in front of the σ factor operon which serves to amplify the signal (positive autoregulation) and also increases expression of the anti-σ, presumably to allow a rapid shutoff of the response once the stress is relieved.

The regulons controlled by each of these four σ factors have been defined using transcriptomics (profiling of mRNA populations) in cells containing or lacking specific ECF σ factors in both unstressed and stressed conditions. These studies, complemented by *in vitro* transcription experiments, have allowed the definition of promoter consensus sequences for each ECF σ (*Figure 1*). The promoters recognized by each ECF σ factor are similar and are defined by characteristic sequence motifs near −35 and −10 relative to the transcription start site [18,19]. The regulons controlled by these four ECF σ factors overlap at the level of promoter recognition: some promoter sequences are specific for a single ECF

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σ factor whereas others can be recognized by more than one [13,20]. The rules governing promoter selectivity are emerging and have been an active topic of investigation, in part motivated by the possibility of using ECF σ factors as tools for synthetic biology [21]. In the case of the *B. subtilis* σ factors it has been shown, for example, that the sequence of the −10 element can define a promoter as specific for σ^X, σ^W, or both [22]. A role of the spacer region in promoter selectivity has been inferred in the case of σ^V [23].

For several of the ECF σ factors, there is a significant basal activity even in the absence of a specific stimulus. As a result, many genes regulated by ECF σ factors could be discriminated by a large transcriptomic analysis of *B. subtilis* gene expression across 104 different conditions (including a variety of stresses, but none known to activate specifically ECF σ regulons) [24]. Genes associated with an ECF-type promoter (assigned as σ^M, σ^X, σ^W, σ^Y or some combination) were co-clustered in terms or overall expression pattern, but the individual regulons could not be distinguished. More complete definition of each regulon has required the conditional overexpression of individual σ factors or the use of specific inducing conditions.

σ^W and adaptation to membrane-active agents

The σ^W regulon comprises ~60-90 genes, although the precise composition of the regulon depends on experimental conditions [25]. In early studies, many of the strongest σ^W-dependent promoters were identified by sequence similarity to the known, σ^W-dependent autoregulatory promoter of the *sigW* operon [26,27] and further members were added by monitoring σ^W-dependent mRNAs resulting from *in vitro* transcription of genomic DNA with purified σ^W holoenzyme [28]. Further refinements have emerged from meta-analysis (hierarchical clustering) of multiple transcriptional profiling experiments, including those conducted under various cell envelope stress conditions [29]. These studies have revealed that membrane-active compounds such as detergents are amongst the strongest inducers of the σ^W regulon. Other conditions known to elicit a strong, σ^W-dependent response include some peptidoglycan synthesis inhibitors and alkali stress [30]. Induction by alkali stress does not appear to be adaptive, suggesting that high pH may interfere with envelope integrity or function and thereby trigger the σ^W stress response.

The σ^W regulon is expressed at a low, basal level even in unstressed cells growing in rich medium at 37° C [31]. In response to stress, the regulon is activated. Mechanistically, this involves inactivation of the RsiW (regulator of *sigW*) anti-σ by a proteolytic cascade initiated by PrsW, which cleaves the anti-σ exterior to the membrane, followed by RasP, an intramembrane protease [16,32-35]. The precise signals that activate this protease cascade are not yet understood. One possibility is that destabilization of the membrane enhances cleavage of RsiW by PrsW.

Insights into the role of the σ^W in cell envelope homeostasis have emerged from characterization of the σ^W regulon and phenotypic characterization of mutant strains lacking either *sigW* or one or more target genes [25]. The characterization of the σ^W regulon has revealed a large number of genes encoding a variety of functions implicated in resistance to antimicrobial agents (*Table 1*) [27,28]. For example, σ^W is required for the expression of a

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cytosolic enzyme, FosB, that functions in the bacillithiol-dependent inactivation of fosfomycin, a small epoxide-containing antibiotic that inhibits MurA, an early cytosolic step in peptidoglycan biosynthesis [36]. It is likely that this enzyme also helps detoxify other antimicrobials containing reactive electrophilic centers as found, for example, with compounds made by *B. amyloliquifaciens* FZB42 [•37]. In addition, σ^W-dependent functions are important for resistance against nisin and related antimicrobial peptides (lantibiotics) (*Table 1*). *Bacillus* species produce many lantibiotics, and in general producer strains have specific immunity systems to protect against the lantibiotics that they produce [38]. In contrast, ECF σ regulated functions provide a more generic level of protection [39]. Nisin binds to the lipid II intermediate of peptidoglycan (PG) synthesis and induces pore formation. The σ^W stress response is specifically protective against lantibiotics that form pores in the membrane but contributes little to protection against a nisin variant that binds lipid II but does not form pores [39]. Protection against the membrane-destabilizing effects of nisin is due, in part, to induction of PspA, a phage-shock protein homolog that stabilizes the membrane [40], and has been proposed as a general biomarker for membrane-disrupting antimicrobials [41]. Other protective functions include YvlC (a PspC homolog), SppA (a signal-peptide peptidase that cleaves peptides inside the membrane), and the YceGHI operon [39]. σ^W also plays a role in protecting *B. subtilis* against several other antimicrobial peptides (bacteriocins) made by *Bacillus* sp. including sublancin (a peptide antibiotic encoded on the SPβ prophage), the cannibalism toxin SdpC, and amylocyclicin, a small hydrophobic peptide made by *B. amyloquifaciens* FZB42 [•37,42]. In several of these examples, the σ^W-dependent resistance determinants have been identified (*Table 1*).

Collectively, these results suggest that σ^W controls genes that are activated by envelope stress and defend the cell against antibiotics and bacteriocins, particularly those with membrane-active properties. Indeed, σ^W also alters the membrane lipid composition by activating transcription from an intraoperonic promoter site in the *fabHa-fabF* fatty acid biosynthesis operon [43]. The net effect of this promoter is to upregulate FabF and downregulate FabHa, which together leads to changes in membrane lipid composition (longer fatty acyl chain length and increased proportion of straight chain fatty acids) that decrease membrane fluidity. Remarkably, strains that can still activate the σ^W regulon but which are altered so that the promoter inside the *fabHa-fabF* operon is no longer functional show an increased sensitivity to growth inhibition by a variety of other *Bacillus* spp. [43].

Although the precise role of many σ^W regulated genes remains to be elucidated, the results to date support a model in which the major role of this stress response is to defend the cell against the myriad bacteriocins and other antimicrobials present in the soil community, including many made by *Bacillus* spp. [38,44].

σ^X contributes to resistance to cationic antimicrobial peptides

The σ^X regulon is comparatively small and overlaps extensively with those of other ECF σ factors. A *sigX* mutant strain is sensitive to heat and oxidative stress, but the basis of these effects is not clear [45]. In several cases, σ^X has an overlapping protective function with other ECF σ factors. For example, σ^X plays a secondary role (to σ^M) in resistance to β-lactam antibiotics [46], and also plays a role in protection against the lantibiotic nisin and

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the peptidoglycan synthesis inhibitor bacitracin (*Table 1*). The operons most strongly activated by σ^X include the *dltA* operon, encoding enzymes for the D-alanylation of teichoic acids, and the *pssA* operon, encoding enzymes for the synthesis of the phosphatidylethanolamine, a neutral lipid [47]. The common feature of these two systems is that they decrease the net negative charge of the cell envelope, and this has been suggested to account for the protective role of σ^X in resisting the action of cationic antimicrobial peptides [47]. The σ^X regulon has been found to contribute to β-lactam resistance [48] and to the synthesis of sublancin [49], and in both cases this has been linked to the induction of the regulatory protein Abh by σ^X (*Table 1*).

σ^V mediates resistance to lytic enzymes

The regulon controlled by σ^V was defined by transcriptomic analyses of strains engineered to inducibly express σ^V protein [23,50]. The results indicate a strong autoregulatory induction of the *sigV* operon together with the activation of several operons also known to be controlled by other ECF σ factors. The *sigV* operon itself encodes σ^V, the RsiV anti-σ factor, a peptidoglycan O-acetyltransferase (OatA), and an uncharacterized protein (YrhK). The modification of peptidoglycan by O-acetylation is known to be associated with resistance to lytic endoglycosidases such as lysozyme [51], which motivated studies to test the role of σ^V in lysozyme resistance. Indeed, σ^V is strongly and specifically induced by lysozyme and activation confers lysozyme resistance through activation of OatA-dependent peptidoglycan modification and the Dlt system which, as noted above, modifies teichoic acids by D-alanylation [17,23]. A role for σ^V in lysozyme resistance has also been demonstrated in *Enterococcus faecalis* and *Clostridium difficile* [•52,53].

The induction of the σ^V regulon by lysozyme suggests that perhaps this system responds directly to damage to the cell wall. However, the amount of lysozyme needed to activate this system is orders of magnitude below the amount needed to lyse cells [23]. This conundrum was resolved when it emerged that the RsiV regulatory protein can bind directly to lysozyme leading to proteolytic cleavage of the anti-σ [••54]. An unidentified site protease mediates the initial cleavage of RsiV in the extracytoplasmic portion of the protein, followed by intramembrane cleavage by RasP [55]. These observations suggest that the σ^V stress response has evolved to detect and defend against lytic enzymes. It is known that some predatory soil bacteria deploy lysozyme-like enzymes to help lyse their prey, and this may have provided the selective pressure that led to the development of this inducible system. In human pathogens, systems orthologous to σ^V may now function to guard against the lytic activity of mammalian lysozymes deployed as part of the innate immune defenses.

σ^M and adaptation to inhibitors of peptidoglycan synthesis

The σ^M regulon includes at least 30 distinct promoter sites that elevate the expression of 60 or more genes [29]. In marked contrast to σ^W, where the majority of target genes encode proteins of rather specialized function mediating resistance to antimicrobial peptides and membrane-active compounds, the effects of σ^M are directed at modulating expression of the core machinery for cell wall biosynthesis and cell division. Many genes activated by σ^M encode functions essential for cell survival (under most growth conditions), a marked

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contrast with the other ECF σ regulons. Consistent with this central role, inactivation of the anti-σ factor that controls σ^M leads to lethality, presumably due to dysregulation of essential cell processes [56].

The central role of σ^M in helping maintain the integrity of the cell wall was first noted when it was found that *sigM* mutants display cell wall defects (distorted cell morphology and bulging from the division septum) when grown in the presence of high salt [56]. Indeed, the *sigM* regulon is activated by high salt, acidic pH, and heat stress. However, in light of the composition of the σ^M regulon, it is likely that the common feature of these diverse stresses is impairment of cell wall synthesis or function (analogous to the induction of the σ^W regulon by alkali stress, as noted above). A central role in coordinating cell wall biogenesis is also evident from the antibiotic sensitivity of *sigM* mutants which are, for example, highly sensitive to β-lactam antibiotics [46]. For reasons yet unclear, this defect can be suppressed, to a significant degree, by mutations in *gdpP* which encodes a hydrolase for cyclic-di-AMP, an essential second messenger implicated in cell envelope homeostasis [57].

Characterization of the σ^M regulon

The σ^M regulon has been defined by a combination of transcriptomics, promoter consensus searches, and *in vitro* transcription [58-61]. The most comprehensive analysis to date took advantage of the ability of the peptidoglycan synthesis inhibitor vancomycin to induce the σ^M regulon [29]. Comparison of the vancomycin stimulon in wild-type vs. *sigM* mutant cells together with hierarchical clustering of genes coordinately regulated across a spectrum of cell envelope active compounds identified those genes that form the core of the regulon. Finally, the direct targets of σ^M-dependent transcription were revealed by comparing the *in vivo* transcriptomic results with the results from *in vitro* transcription [29]. Promoters activated by σ^M can be conceptually divided into three functional classes (*Table 2*), together with others of still undefined function.

The first class includes promoters that up-regulate the core biosynthesis pathways for assembly of the cell envelope. For example, σ^M activates transcription of enzymes for the synthesis of PG precursors (Ddl, MurB, MurF), for peptidoglycan assembly or modification (penicillin binding proteins PonA and PbpX), and key components of the macromolecular complexes that coordinate PG synthesis, the elongasome (MreBCD, RodA) and the divisome (DivIB, DivIC, MinCD). In *B. subtilis* W23 strains, wall teichoic acid (WTA) synthesis is activated directly by σ^M [62]. In several cases, these core biosynthetic enzymes are encoded in complex operons with multiple promoters; σ^M is not required for their expression but instead serves to increase expression in times of stress [29]. The selective pressures leading to inclusion of these particular enzymes in the σ^M regulon are not well understood. Perhaps these enzymes are rate-limiting for function, particularly in cells exposed to antimicrobial compounds and bacteriocins.

The second class of σ^M-dependent promoters activates genes encoding stress-induced replacement enzymes that provide a backup for key steps in cell envelope synthesis. For example, σ^M (together with two other stress-responsive σ factors, σ^X and σ^I) can activate expression of *bcrC* [63], encoding a phosphatase that converts undecaprenyl-pyrophosphate (UPP) to the monophosphate (UP), the lipid carrier for both PG and WTA synthesis [64].

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BcrC is functionally redundant with another, structurally unrelated, phosphatase, UppP (H. Zhao and JDH, unpublished). By catalyzing UPP dephosphorylation on the external face of the cell membrane, BcrC rapidly converts surface exposed UPP, which is the target molecule for the antimicrobial peptide bacitracin, to UP. Interestingly, the σ^M regulon is also induced by friulimicin, which binds specifically to the UP lipid carrier for PG synthesis [65].

More recently, the σ^M activated YdaH protein was shown to be functionally redundant with a MurJ homolog and therefore renamed as an alternate to *murJ* (Amj) [••66]. The cell requires either MurJ or Amj for cell wall synthesis. The proposed function of MurJ/Amj is translocation of the lipid II PG precursor across the inner membrane (a function also ascribed to the RodA/FtsW family of proteins; [67]). Presumably, induction of Amj provides a mechanism for cells to continue PG synthesis even when faced with molecules that might inhibit the function of MurJ. An additional example is provided by the σ^M-dependent induction of LtaSa [29,60], an alternative synthase that catalyzes the elongation of lipoteichoic acid (LTA) polymers associated with the cell envelope [68]. The major synthase, LtaS, functions in unstressed cells, but can be replaced by activation of the σ^M-dependent paralog in times of stress. Finally, σ^M strongly activates TagT, one of three redundant LytR-CpsA-Psr (LCP) family enzymes that function in the final step of WTA synthesis to attach the lipid-linked precursor to PG [69]. One of the other LCP enzymes (TagU) is a member of the σ^X regulon [70]. These results suggest that this final extracellular step in WTA synthesis may also be targeted by antimicrobial compounds, and the induction of alternative enzymes may have emerged as a resistance mechanism.

A common feature of all four of these σ^M-activated enzymes (BcrC, Amj, LtaSa, and TagT) is that they are seemingly redundant in function with constitutively expressed enzymes. This leads to the general notion that antibiotic inhibition of the constitutively expressed enzymes may lead to the σ^M-dependent induction of substitute enzymes that may help the cell to evade antibiotic inhibition. What is not known is whether these alternate enzymes function solely to replace their constitutively expressed counterparts, or whether they have distinct or alternative activities not yet apparent. For example, the LTA polymer synthesized by LtaSa appears to differ, as observed in electrophoresis, from that made by LtaS [68] and distinct functions can also be envisioned for the LCP enzymes.

Finally, the third class of σ^M-activated promoters encodes proteins with regulatory roles. These include the anti-σ factor for σ^M, the Spx transcription factor (most closely associated with the response to disulfide stress and reactive oxygen species; [71]), synthases for nucleotide second messengers (the ppGpp synthase YwaC and the cyclic-di-AMP synthase DisA), and the transition state regulator Abh (which is also activated by other ECF σ factors). The induction of this diversity of regulatory proteins suggests that the full extent to which σ^M coordinates acclimation of the cell to antibiotic stress is yet to be appreciated. Of these target genes, *ywaC* is notable since its strong induction by σ^M led to its definition as biomarker for inhibition of cell wall synthesis [72], a tool subsequently used to screen for new antibiotics [•73].

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Induction of the σ^M regulon by antibiotics and by defects in cell envelope biogenesis

Several different cell wall active antibiotics are known to induce σ^M, either specifically or in combination with other cell envelope stress responses [61,74,75]. These include both early-stage (e.g. fosfomycin) and late-stage (e.g. vancomycin, moenomycin) inhibitors of PG synthesis (*Figure 2*). Moenomycin, which blocks the active site of the transglycosylase involved in the assembly of PG is a very specific inducer of the σ^M stress responses [11]. In contrast, ramoplanin, which blocks the same step but by binding the lipid II substrate, induces σ^M together with other envelope stress responses [76]. A comprehensive characterization of the sensitivity and response specificity of the *ywaC* promoter confirms the selectivity for PG synthesis inhibitors [•73]. In addition, σ^M is also activated by an inhibitor of WTA synthesis, targocil [•77]. Targocil is a specific inhibitor of the *S. aureus* TagGH efflux channel for WTA and is not normally active against *B. subtilis*. However, *B. subtilis* strains expressing the *S. aureus* TarGH proteins are sensitized to targocil, and inhibition activates the σ^M regulon. However, inhibition of TarGH is likely to also impact PG synthesis since both WTA and PG use the common carrier molecule UP, and blocking WTA synthesis is thought to titrate the limiting pools of this lipid carrier [72].

Activation of the σ^M regulon has also been noted in strains carrying mutations that affect specific steps in cell envelope biogenesis. For example, in a selection for vancomycin resistance a mutation in the ribosome-binding site of UppS was recovered [78]. This mutation reduces the expression of the enzyme required for UPP synthesis and leads to a modest induction of the σ^M regulon. Up-regulation of the σ^M regulon was also noted in strains affected in WTA biogenesis (conditional depletion of *tagD*), presumably due to sequestration of UP [72]. A similar sequestration effect has been proposed to account for the up-regulation of σ^M by disruption of *yfhO* [79]. YfhO is postulated to function as a flippase for polymer synthesized (perhaps using UP as a lipid carrier) by the CsbB glycosyl-transferase (which is itself partially under σ^X control; [26]).

Although the spectrum of compounds and mutations that induce the σ^M regulon has been relatively well defined, the nature of the inducing signal is not obvious. One suggestion is that the regulator(s) controlling σ^M activity might respond to changes in the availability or abundance of UP or the lipid II intermediate in PG synthesis [••66]. However, this notion is challenged by that observation that impairment of LTA synthesis also activates σ^M. Indeed, σ^M is upregulated in strains lacking the major LTA elongation enzyme, LtaS (which does not use UP), enabling synthesis of the alternate enyzme LtaSa [80]. Up-regulation of σ^M was also noted in strains with reduced levels of UgtP, a glycosyltansferase important for synthesis of the lipid carrier of LTA [81,82], and PgsA, which synthesizes phosphatidylglycerol, the glycerol-phosphate donor required for LTA elongation [83]. These results suggest that disruption of LTA biogenesis also generates an activating signal for the σ^M regulon. It is presently unclear whether this signal is distinct from that produced by conditions that impair PG synthesis.

Perspective

B. subtilis is a ubiquitous soil and plant-associated bacterium that produces a variety of antibiotics and other secondary metabolites [38,44]. It shares its environment with many

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other soil bacteria, including actinomycetes which are also notable for the tremendous variety of antimicrobial compounds that they produce. In this chemically complex and variable environment, the ability to modulate the composition of the cell envelope in response to antimicrobial agents has no doubt proven adaptive [8]. Numerous challenges remain as we seek to understand the nature of the inducing signals that activate each ECF σ regulon and the precise ways in which regulon activation helps counter environmental threats. Future efforts will be directed towards clarifying these inducing signals and the variety of mechanisms that allow cells to acclimate to the presence of the antimicrobial compounds ubiquitous in their environment.

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• of special interest

•• of outstanding interest

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HIGHLIGHTS

- *B. subtilis* encodes seven ECF σ factors that activate envelope stress responses
- σ^W coordinates resistance to bacteriocins and other membrane-active agents
- σ^X contributes to cationic antimicrobial peptide resistance
- σ^V is induced by and protects against peptidoglycan lytic enzymes
- σ^M is strongly induced by conditions that impair peptidoglycan synthesis
- σ^M upregulates core pathways of envelope synthesis and cell division
- σ^M upregulates stress-inducible pathways to overcome inhibitors

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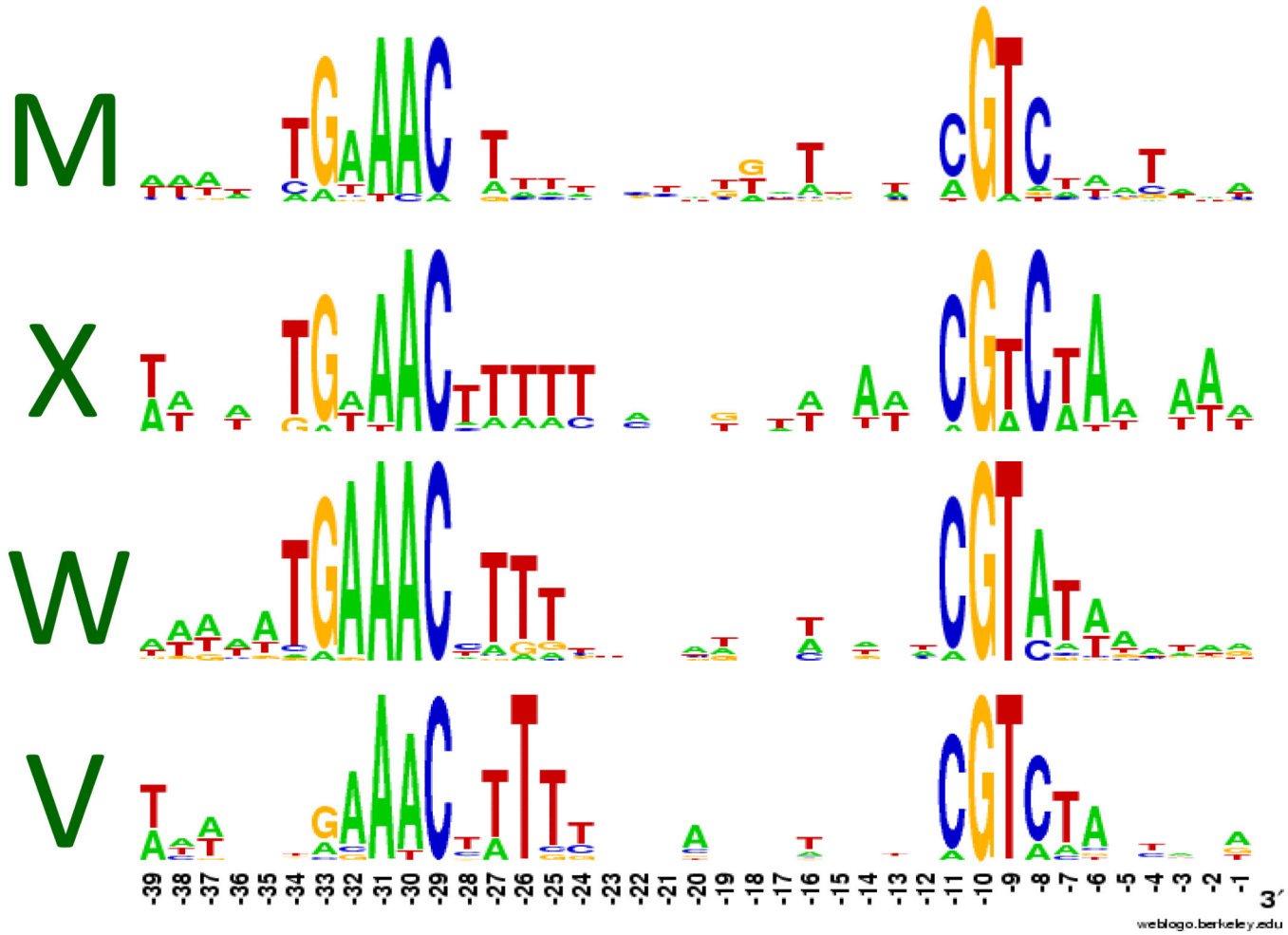

Figure 1. Promoter consensus sequences for σ^{M}, σ^{X}, σ^{W}, and σ^{V}

Promoters recognized by ECF family σ factors are characterized by conserved sequences near the −35 and −10 regions relative to the transcription start site. The consensus sequences shown here share the characteristic “AAC” motif common to many ECF family σ factors [15]. The consensus sequences shown are derived from published datasets: σ^{M} [29], σ^{X} [47], σ^{W} [26-28, 31], and σ^{V} [23]. The key role of the −10 element in discrimination of promoters by σ^{X} and σ^{W} has been previously explored by mutagenesis [22].

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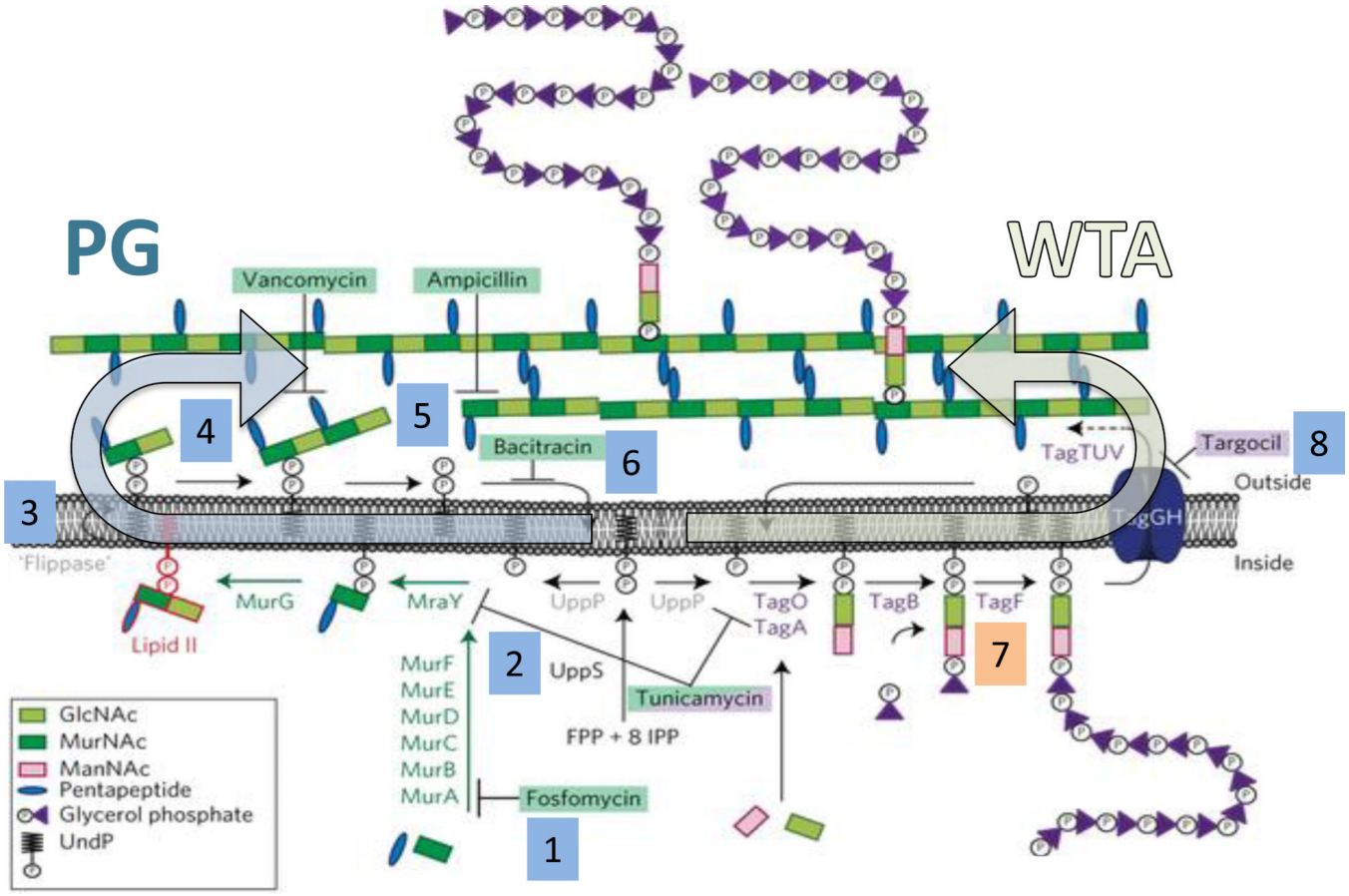

Figure 2. Schematic of the core biosynthetic pathways for PG and WTA

PG biosynthesis and WTA synthesis require the common lipid carrier, undecaprenyl-phosphate (UP) which is synthesized as UPP by UppS. Inhibition of PG biosynthesis (left arrow), either by antibiotics or by reduced expression of key enzymes, induces the σ^M regulon. Steps affected include the early cytosolic steps (box 1) (e.g. MurA; inhibited by fosfomycin; [•73]), UppS (box 2) [72,78], the lipid II flippase (box 3) [••66], the extracellular transglycosylase (box 4) and transpeptidase (box 5) reactions and dephosphorylation of UPP to UP (box 6) [11,65,•73,74]. Activity of σ^M is induced by inhibition of WTA synthesis (right arrow), either by depletion of TagD or other late steps (box 7) [72] or by inhibition of the TarGH transporter (box 8) [•77]. Figure adapted from [•77].

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Table 1

Major members of the σ^W, σ^X and σ^V regulons with assigned functions

Operon[1]	Other Regulators	Function
σ^W regulon		
sigWrsiW		Positive autoregulation; RsiW is anti-σ^W factor
pbpEracX		LMW PBP and amino acid racemase; PbpE (PBP4*) has PG hydrolase activity and contributes to resistance to PG inhibitors, particularly in high salt growth conditions [84]
(fabHa)fabE		Alters membrane fatty acyl chain composition; Decreases fluidity and increases resistance to membrane disrupting agents [43]
fosB		Fosfomycin resistance [36]; Contributes to resistance against amylocyclicin [•37,42]
ydbST		Contributes to resistance against amylocyclicin [•37,42]
sppA		Signal peptide peptidase; contributes to lantibiotic resistance [39]
pspA		PspA (phage-shock protein) homolog; contributes to lantibiotic resistance [39]
yvlABCD		YvlC=PspC homolog; contributes to lantibiotic resistance [39]
yceCDEFGHI		Tellurite resistance gene homologs; contributes to lantibiotic resistance [39]
yqeZfloAyqfB		FloA is a flotillin involved in regulating membrane fluidity; resistance to sublancin [42]
yfhLM		resistance to SdpC* (toxic peptide) [42]
yknWXYZ		resistance to SdpC* (toxic peptide) [42]
yuaFfloTyuaI		FloT is a flotillin involved in regulating membrane fluidity; contributes to cefuroxime resistance [85]
σ^X regulon		
sigXrsiX	σ^A	Positive autoregulation; RsiX is anti-σ^X factor
dltABCD	σ^V	D-alanylation of teichoic acids; resistance to cationic antimicrobial peptides (CAMPs) [39,47]
pssAybfMpsd	σ^A	Phosphatidylethanolamine biosynthesis; CAMP resistance [39,47]
pbpX	σ^V	LMW PBP; PG modification, contributes to lysozyme resistance [17]
abh	σ^V	Transition-state regulator (DNA-binding protein); increases β-lactam resistance [48] and activates expression of the glycopeptide antibiotic sublancin [49]
tagU		Formerly lytR; one of three redundant wall-teichoic acid attaching enzymes [69]
csbByfhO	σ^B	CsbB=Glucosyltransferase; involved in cell envelope polymer synthesis? [79]
rapD	RghR	Putative response-regulator aspartate phosphatase; negatively regulates ComA, an activator of genetic competence [86]
[yabE]	σ^M	Negatively regulated by an ECF o activated antisense; encodes a PG hydrolase [87]
σ^V regulon		
sigVrsiVoatAyrhK		Autoregulation and O-acetylation of PG (OatA); contributes to lysozyme resistance [17,23]
dltABCD	σ^X	resistance to cationic antimicrobial peptides (CAMPs); lysozyme resistance [23,47]
pbpX	σ^X	PG modification, contributes to lysozyme resistance [17]

[1]Parentheses indicate a promoter inside a gene; brackets indicate a gene on the opposite strand (antisense) relative to an upstream promoter

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Table 2

Major members of the σ^M regulon with assigned functions

Operon[1]	Other Regulators	Function
Core Cell Envelope Biogenesis Functions[2]		
(maf)ysxAmreBCDminCD	σ^A	MreBCD function in the elongasome; MinCD regulates divisome function
(murG)murBdivlBylxXWsbp	σ^A	MurB is essential for PG synthesis; DivIB is an essential cell division protein
divIC	σ^A	Essential cell division protein; interacts with DivIB as part of divisome
recUponA	σ^A	PonA=PBP1; a PBP with both transglycosylase and transpeptidase activity
(ydbO)[ydbP]ddlmurF	σ^A	Ddl is essential D-Ala D-Ala ligase for PG synthesis, MurF is essential for PG synthesis [29]
rodA	σ^A	Essential; component of elongasome; putative lipid flippase [29,88]
tarABIJKL	σ^X, PhoPR	Ribitol teichoic acids (in B. subtilis W23 strains) [62]
Stress-induced Substitute Enzymes		
bcrC	σ^X	A UPP phosphatase; redundant with UppP (H Zhao and JDH, unpublished); contributes to bacitracin resistance [63,64]
amj		Amj(YdaH); redundant with MurJ(YtgP); lipid II flippase for PG synthesis [••66]
ltaSa		LtaSa(YfnI), redundant with LtaS; functions as lipoteichoic acid synthase [68,89]
tagT		TagT(YwtF), redundant with TagU and TagV; required for wall teichoic acid attachment to PG [69]
Regulatory Proteins		
sigMyhdLK	σ^A	Autoregulation; YhdL is essential due to lethal effects of unrestrained σ^M activity [29,56]
spx	σ^A, σ^W, σ^X, σ^B	Spx activates a large regulon of genes in response to disulfide stress [90]
ywaC		ppGpp synthase; used as a bioreporter for cell envelope stress [72,•73]
(sms)disAyacLM		DisA = cyclic-di-AMP synthase; regulated by DNA damage [57]
abh	σ^X	Transition-state regulator (DNA-binding protein); increases β-lactam resistance [48] and activates expression of the glycopeptide antibiotic sublancin [49]
Other Regulon Members		
yqjL		A putative hydrolase; contributes to resistance to paraquat [59]
ypbG		Uncharacterized phosphoesterase; proposed as a bioreporter for PG synthesis inhibitors [91]
ypuA		Unknown function protein; proposed as a bioreporter for cell envelope stress [92]
ytpAB		YtpB involved in C_{35} terpenoid synthesis [93,94]

[1] Parentheses indicate a promoter inside a gene; brackets indicate a gene on the opposite strand (antisense) relative to an upstream promoter.

[2] Bold indicates genes encoding proteins that are implicated in cell envelope synthesis and cell division.