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Regulation of antimicrobial resistance by extracytoplasmic function (ECF) sigma factors

Emily C. Woods^a and Shonna M. McBride^{a,*}

^aDepartment of Microbiology and Immunology, Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta, GA, USA

Abstract

Extracytoplasmic function (ECF) sigma factors are a subfamily of σ^{70} sigma factors that activate genes involved in stress-response functions. In many bacteria, ECF sigma factors regulate resistance to antimicrobial compounds. This review will summarize the ECF sigma factors that regulate antimicrobial resistance in model organisms and clinically relevant pathogens.

Keywords

extracytoplasmic function sigma factors; ECF sigma factors; antimicrobial resistance; regulation

1. Introduction

The Centers for Disease Control and Prevention report, “Antibacterial Resistance Threats in the United States, 2013,” estimated that nearly two million infections and 23,000 deaths occur each year in the United States due to antibiotic resistant organisms [1]. Understanding the mechanisms by which bacteria evade killing by antimicrobials is therefore imperative to address the burden of disease caused by these organisms.

Although bacteria can acquire mutations or novel genes to gain resistance to antimicrobials, many bacteria are intrinsically resistant to some classes of antimicrobials [2–13]. Such intrinsic resistance can either be constitutive or inducible [14–18, 6, 19–21]. In the case of inducible resistance, there are many known mechanisms by which bacteria can sense and respond to the threat of antimicrobials. Two-component regulatory systems have long been a recognized mechanism by which bacteria respond to extracellular signals to alter gene expression, and many two-component systems are known to increase expression of antimicrobial resistance genes [22–29]. Alternative sigma factors are another common mechanism by which bacteria can alter their gene expression in response to a stimulus. The

*Corresponding author mailing address: Department of Microbiology and Immunology, Emory University School of Medicine, 1510 Clifton Rd, Atlanta, GA 30322. Phone: (404) 727-6192. Fax: (404) 727-8250. shonna.mcbride@emory.edu.

Conflict of Interest

The authors do not have any conflicts of interest to declare.

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extracytoplasmic function (ECF) sigma factors are a unique class of alternative sigma factors that also regulate gene expression in response to extracellular signals. ECF sigma factors are present in a wide range of species [30, 31]. This review will provide an overview of the role of ECF sigma factors in regulating resistance to antimicrobials.

After a brief summary of how ECF sigma factors function, we will examine several examples of how ECF sigma factors can regulate resistance to antimicrobial compounds in both Gram-negative and Gram-positive species. Because antimicrobial resistance is primarily a concern for organisms that pose a threat to human health, this review will mostly focus on clinically relevant pathogens, with the exceptions of *Streptomyces coelicolor*, which is included for historical importance, and *Bacillus subtilis*, which is included because it is a well-studied model organism.

2. Overview of ECF sigma factors

This review does not seek to be a comprehensive review of all ECF sigma factors or a review of the pathways that regulate ECF sigma factor function. A number of excellent reviews cover these topics, and the reader is referred to them for further information [30, 32–37]. Nevertheless, a very brief introduction to ECF sigma factors will be necessary before delving into the specifics of how ECF sigma factors regulate antimicrobial resistance.

Core RNA polymerase (RNAP) requires a sigma factor to create the RNAP holoenzyme and initiate transcription. Sigma factors confer promoter specificity and can therefore direct RNAP to transcribe specific sets of genes (Fig. 1C). The ECF sigma factors constitute a subfamily within the larger σ^{70} family of sigma factors. Similar to other sigma factors, the ECF sigma factors contain the σ_2 and σ_4 domains, which are involved in binding the -10 and -35 promoter regions, respectively. However, ECF sigma factors lack the $\sigma_{1.1}$ and σ_3 domains, which are involved in RNAP binding and extended -10 binding, respectively [35, 38, 39, 31, 40]. The name extracytoplasmic function refers to the fact that the common function of these factors is to regulate processes that affect the cell envelope, yet the specific processes that they regulate can vary greatly between different ECF factors. For example, ECF sigma factors have been implicated in regulating processes as diverse as iron uptake, cell wall maintenance, and motility [30, 40–47]. Although a great deal of variation and nuance exists in how ECF sigma factor activity is regulated, the majority of ECF sigma factors are held inactive after translation by a membrane-bound anti-sigma factor [32, 31, 48–54] (Fig. 1A). An extracytoplasmic signal then initiates a proteolytic cascade that results in release of the sigma factor from the anti-sigma factor to initiate transcription [32, 55–59] (Fig. 1B, C).

Finally, a note on the nomenclature of the ECF sigma factors: most Gram-negative bacteria follow the convention established in *E. coli* of using an *rpo* designation for sigma factor genes, whereas most Gram-positive bacteria follow the convention of using a *sig* (σ) gene designation [30].

3. ECF sigma factor-mediated systems in Gram-negative bacteria

3.1 *Escherichia coli*

RpoE is one of two ECF sigma factors in *Escherichia coli*. Although specific RpoE-dependent antimicrobial-resistance genes have not been identified, RpoE bears mention here because RpoE is one of the most-studied ECF sigma factors. RpoE activity is induced in response to misfolded proteins in the outer membrane [60, 61]. It is therefore not surprising that the RpoE regulon contains genes for foldases, proteases, LPS biosynthesis proteins, and chaperone proteins [62]. Although these genes are not implicated directly in antimicrobial resistance, they are necessary for maintaining cell membrane integrity, which has consequences for how well *E. coli* copes with antimicrobial-induced damage. Deletion of *rpoE* is lethal in *E. coli* strain K-12 [63]. Lower levels of RpoE also has consequences in *E. coli*, as overexpressing RseA, the RpoE anti-sigma factor, leads to membrane blebbing and eventual cell death [64]. Unlike laboratory strains, some clinical isolates of *E. coli* can survive *rpoE* deletion. Upon *rpoE* deletion, these strains exhibit increased sensitivity to polymyxin B [65].

3.2 *Salmonella enterica*

The *Salmonella* RpoE is a homolog of *E. coli* RpoE and appears to play a role in regulating resistance to certain antimicrobials in serovar Typhimurium. An *S. Typhimurium rpoE* mutant is more sensitive to polymyxin B, P2 (a derivative of bactericidal and permeability increasing protein produced by neutrophils), and the murine defensin, cryptdin-4 [66, 67]. P2 alters the electron motive force. RpoE may be able to mediate resistance to P2 because it induces expression of *fdhD*, which encodes a formate dehydrogenase that allows *S. Typhimurium* to utilize alternate terminal electron acceptors [68, 67]. Another gene of the RpoE regulon, *smpA*, may also play a role in providing antimicrobial resistance. *smpA* encodes an outer-membrane lipoprotein that plays an essential role in an outer membrane protein assembly complex. An *smpA* mutant is more susceptible to rifampicin, SDS, and EDTA, indicating its important role in maintaining proper outer membrane protein composition [69]. Thus, RpoE is implicated in two different mechanisms that enable *S. Typhimurium* to survive the insults of antimicrobials.

In contrast to the increased sensitivity to antimicrobials seen in the *S. Typhimurium rpoE* mutant, an *S. Typhi rpoE* mutant has increased resistance to several antimicrobials, including penicillins, cephalosporins, aminoglycosides, and ciprofloxacin [70]. The *S. Typhi rpoE* mutant has reduced expression of *ompC* and *ompF*, which encode outer membrane proteins associated with antimicrobial passage into the periplasm [70]. Expression of *ramA*, a gene encoding a transcriptional regulator of the AcrAB-TolC multidrug efflux pump, was increased in the *rpoE* mutant [70]. The lower expression of *ompC* and *ompF* and the increased expression of *ramA* may, at least partially, explain the higher resistance to some antimicrobials observed in the *S. Typhi rpoE* mutant. However, it remains unclear why RpoE has opposite effects on antimicrobial resistance in *S. Typhi* and *S. Typhimurium*.

3.3 *Pseudomonas aeruginosa*

The ubiquitous bacterium, *P. aeruginosa*, encodes an ortholog to the *E. coli* RpoE: the sigma factor, AlgU (also known as AlgT) [71]. AlgU/T shares 78% similarity and 66% identity with RpoE and can initiate transcription from *E. coli* RpoE promoters [72, 71]. Although AlgU/T most prominently controls conversion to the mucoid phenotype by inducing production of the exopolysaccharide, alginate, it also regulates the expression of roughly 35 other genes that are important for virulence and generation of an inflammatory response in the host [73, 74].

There are several lines of evidence that suggest AlgU/T is important for intrinsic antibiotic resistance in *P. aeruginosa* due to its role in alginate production. The mucoid phenotype stimulated by AlgU/T is associated with increased resistance of the bacterium to a variety of antimicrobials [75, 76]. Mucoidy also contributes to biofilm formation, which itself is associated with increased antimicrobial resistance [77–79]. Additionally, alginate binds tobramycin and hinders its diffusion through liquids and agar [80]. This finding suggests that alginate could contribute to antibiotic resistance by impeding antibiotics from reaching their targets, although no differences in diffusion rates of tobramycin were observed in microcolonies or biofilms [80]. Alginate also confers protection against oxidative stress, as demonstrated by increased sensitivity to hydrogen peroxide and paraquat in non-mucoid strains [81]. Many antimicrobials induce oxidative stress, and alginate may prevent the damage caused by these antimicrobials [82]. However, an *algU/T* mutant is more sensitive to hypochlorite than an *algD* (alginate synthesis) mutant, suggesting that AlgU/T contributes to resistance through mechanisms beyond just regulating alginate production [83].

A more specific mechanism by which AlgU/T can regulate antimicrobial resistance is through control of *ampR* transcription [84]. AmpR regulates expression of the *ampC* and *poxB* β -lactamases [84]. Several studies found that alginate production was induced in the presence of a variety of β -lactam antibiotics, suggesting that induction of AlgU/T activity might play a role in responding to these compounds [85, 86]. Similarly, mutations in any of the regulatory components of the AlgU/T system, including *algW*, *mucB*, *mucD*, or *algU/T*, lead to increased sensitivity to the β -lactam antibiotic, imipenim [87]. Based on this evidence, Balasubramanian *et al.* were able to demonstrate that the β -lactamase regulator, *ampR*, is under control of AlgU/T [84]. Although AmpR is active even in an Alg⁻ strain (in which AlgT is held inactive by the MucA anti-sigma factor), auto-induction of the *ampR* promoter in the presence of β -lactams is about three times higher in an Alg⁺ strain (in which AlgT is constitutively active due to a *mucA* mutation) [84]. Thus, AlgU/T plays a positive role in the induction of β -lactamase production.

In addition, AlgU/T impacts antimicrobial resistance by inducing expression of the *mexCD-oprJ* efflux pump. AlgU/T is necessary for induction of *mexCD-oprJ* in response to chlorhexidine exposure, and consequently, an *algU/T* mutant is more sensitive to chlorhexidine [88]. Moreover, AlgU/T is necessary for full induction of *mexCD-oprJ* expression in response to polymyxin B and several other cationic antimicrobial peptides (CAMPs), all of which are potential substrates of this efflux pump [88].

Whereas much progress has been made to elucidate the mechanisms by which AlgU/T confers resistance, much less is currently known about the functions of an additional pseudomonas ECF sigma factor, σ^X . The *P. aeruginosa* σ^X shares 49% similarity to *Bacillus subtilis* σ^W , which is induced by antibiotics that act on the cell wall [82, 58]. A *P. aeruginosa* *sigX* mutant is more sensitive to imipenem and polymyxin B [89]. Several genes of the σ^X regulon have been identified, including ion-gated channels involved in iron homeostasis, a lipid A deacylase, a glucose porin, and the outer membrane porin, *oprF* [90, 89]. *oprF* mutants are more sensitive to lysozyme, suggesting that a lack of this porin results in changes to the cell surface structure [91]. Because all of the genes thus far identified as part of the σ^X regulon are membrane-associated, σ^X is likely involved in maintaining the composition of the membrane and may therefore play a critical role in modulating the ability of antimicrobials to reach their targets in the cell wall.

Although *P. aeruginosa* is predicted to have 19 ECF sigma factors, so far only AlgU/T, σ^X , and the pyoverdine regulator, PvdS, have received much attention [82]. In the related organism, *Pseudomonas putida*, mutants in ECF-10 are more resistant to β -lactams, sulfonamides, and chloramphenicol due to upregulation of an efflux pump [92]. ECF-10 shares 67% similarity with σ^I of *P. aeruginosa* [93]. Whether σ^I plays a similar role in *P. aeruginosa* has not been determined. Nevertheless, it remains possible that some of the other ECF sigma factors also contribute to regulation of antimicrobial resistance mechanisms in *P. aeruginosa*.

4. ECF sigma factor-mediated systems in Gram-positive bacteria

4.1 *Streptomyces coelicolor*

Streptomyces coelicolor is predicted to encode roughly 50 ECF sigma factors, of which σ^E is the most extensively studied [30]. σ^E of *S. coelicolor* was the first ECF sigma factor to be described [94, 95, 40, 30]. Although its initial discovery focused on its role in transcription of the agarase gene, *dagA* [94], further studies tied σ^E to antimicrobial resistance. Paget *et al.* found that a *sigE* mutant is more sensitive to lysozyme and other muramidases [47]. Although their analysis found that mutation of *sigE* does not alter which components are present in the cell wall, a *sigE* mutant does have altered ratios of a number of cell wall components, which may impact the ability of *S. coelicolor* to survive insults from cell wall-active antimicrobials.

One other *S. coelicolor* ECF sigma factor, σ^R , has been tied to antibiotic resistance [96]. In the presence of translation-inhibiting antibiotics such as chloramphenicol and erythromycin, expression of a stable isoform of σ^R increases [96]. A *sigR* mutant is more sensitive than the parent strain to the antibiotics that induce σ^R expression, but is not more sensitive to unrelated antibiotics, such as ampicillin [96]. The σ^R response is therefore likely to induce expression of genes that are important for specifically responding to stalled translation, but antibiotic resistance genes within the σ^R regulon have yet to be identified. Aside from σ^E and σ^R , few of the many ECF sigma factors of *S. coelicolor* have been studied.

4.2 *Bacillus subtilis*

The model Gram-positive organism, *Bacillus subtilis*, has seven ECF sigma factors: σ^M , σ^W , σ^X , σ^V , σ^Y , σ^Z , and σ^{YlaC} [30, 97, 98]. Of these, σ^W , σ^M , and σ^X are the best studied, and appear to be the primary ECF sigma factors in *B. subtilis* that are responsible for conferring antibiotic resistance, based on the phenotype of a σ^{MWX} triple mutant, which is as sensitive to a wide variety of antibiotic classes as a mutant lacking all seven ECF sigma factors [99]. Although their regulons are often overlapping, some of the ECF sigma factors have distinct effects on resistance to specific compounds. For example, σ^W appears to be the main ECF sigma factor that confers resistance to fosfomycin, whereas σ^M appears to be the main ECF sigma factor that confers resistance to moenomycin [97].

Some of the earliest studies on σ^W identified a role for this ECF sigma factor in mediating antimicrobial resistance during stationary phase [100]. By searching for consensus σ^W promoter sequences within the genome, Huang *et al.* identified several genes directly regulated by σ^W that function in cell wall structure and detoxification, including a penicillin binding protein and several ATP-binding cassette (ABC) transporters [101]. More recent studies have identified several genes in the σ^W regulon with direct antimicrobial resistance functions. For example, *fosB*, a metallothiol transferase that confers resistance to fosfomycin, is transcribed by σ^W [102]. Additionally, a σ^W promoter within the *fabHa* coding sequence, a gene necessary for initiation of fatty acid synthesis, reduces FabHa production and increases expression of FabF, a protein responsible for fatty acid elongation. The change in production of these two proteins results in decreased membrane fluidity and greater resistance to detergents [103].

Additionally, σ^W plays a key role in resistance to lantibiotics and other antimicrobials produced by competing bacteria [104, 17]. Genes within the σ^W regulon that confer resistance to the lantibiotic nisin include *sppA*, a signal peptide peptidase, *yvIC* and *pspA*, phage shock protein homologues, and the *yceGHI* operon. The mechanisms by which these genes contribute to nisin resistance remain unverified, yet together they fully account for the increased sensitivity of a *sigW* mutant to nisin [104]. σ^W also provides innate resistance to a variety of antimicrobials that are produced by other *Bacillus* species. For example, through regulation of *fosB* and *ydbST* (membrane proteins of unknown function), σ^W confers resistance to amylocyclicin produced by *Bacillus amyloliquefaciens* FZB42 [17, 105]. Via an unknown mechanism, the σ^W -dependent operon *yqeZyqfAB* provides resistance to sublancin when the SP β resistance genes are absent [17]. Similarly, the σ^W -dependent operons *yfhLM* and *yknWXYZ* confer resistance to the toxic SdpC protein when the SdpI resistance protein is absent [17]. These findings establish σ^W as important for regulating expression of a number of genes that enable *B. subtilis* to resist killing by antimicrobials produced by competitor species in the environment.

Evidence suggests that σ^M also facilitates the ability of *B. subtilis* to respond to insults to the cell surface. Unlike σ^W , which is most important during stationary phase, σ^M has greatest activity during early and exponential growth [106]. σ^M is further induced in the presence of high extracellular salt concentrations, ethanol, heat shock, acidity, paraquat, and the antimicrobials vancomycin, rhamnolipids, bacitracin, fosfomycin, daptomycin, and friulimicin B [106–108]. Given these stimuli, it is not surprising that the σ^M regulon

contains genes involved in cell division, cell membrane composition, DNA repair, and detoxification [109].

More specifically, Luo and Helmann identified several genes in the σ^M regulon that increase resistance to antimicrobials that target the cell wall. For example, in the presence of β -lactams, *abh* expression is increased in a σ^M -dependent manner [110]. Abh is a transcriptional regulator that activates SlrR, which in turn represses transcription of several autolysins [111, 110]. By transcribing *abh* in the presence of β -lactams, σ^M makes *B. subtilis* less susceptible to the autolytic effects of β -lactams and does so independently of the role of Abh in exopolysaccharide production [110, 112]. Additionally, Abh induces biofilm formation, and biofilms themselves contribute to greater resistance to antimicrobials [111, 113]. Another important resistance gene in the σ^M regulon is *spx* [109, 114]. Spx helps cells deal with oxidative stress and is induced in bacitracin and enduracidin [115]. Moreover, σ^M increases expression of *disA*, a diadenylate cyclase (DAC). A mutant lacking *disA* is moderately more sensitive to moenomycin, and c-di-AMP levels influence maintenance of the cell wall [110]. Therefore, σ^M may contribute to resistance to antimicrobials targeting the cell wall by controlling *disA* expression. σ^M also regulates *ltaSa* in response to nisin exposure [104]. LtaSa is a lipoteichoic acid (LTA) synthase that produces longer LTA than the primary synthase, which may impede access of nisin to lipid-II and the membrane, thereby conferring resistance [104].

The mechanism by which σ^M confers resistance to bacitracin has also been examined. The key member of the σ^M regulon that is induced in the presence of bacitracin is *bcrC*. This gene encodes an undecaprenyl pyrophosphate (UPP) phosphatase. BcrC helps cells to overcome the inhibition of cell wall synthesis that occurs due to the inhibition of UPP dephosphorylation caused by bacitracin [116]. Although several two-component systems, including the BceRS, YvcPQ, and LiaRS systems, respond to bacitracin at lower concentrations than are necessary for inducing σ^M , the σ^M -dependent induction of *bcrC* nevertheless remains an important mechanism by which *B. subtilis* can resist killing by bacitracin [116].

The third ECF sigma factor that plays a significant role in regulating antimicrobial resistance in *B. subtilis* is σ^X . There are several genes in the σ^X regulon with clear effects on resistance. For example, σ^X regulates the *dlt* operon, which is responsible for D-alanylation of teichoic acids, a process that decreases the negative charge on the cell surface and thereby provides protection against CAMPs [117]. σ^X has an additional effect on cell surface charge by inducing the *pss* operon, which is responsible for synthesizing phosphatidylethanolamine, a zwitterionic lipid that can reduce the net negative charge of the bacterial surface by decreasing the proportion of negative lipids in the membrane [117]. Like σ^M , σ^X transcribes *abh* transcription, which enhances resistance to β -lactams [100, 118, 119, 112]. Even though σ^M appears to be far more important than σ^X for inducing *abh*, σ^X has an additional unique effect on autolysins by inducing *lytR*, which inhibits autolysin production [119].

Despite the clear importance of the three ECF sigma factors described above, the roles of the remaining four ECF sigma factors in *B. subtilis* remain largely obscure. It appears that the σ^V regulon overlaps considerably with that of σ^M , σ^W , and σ^X and includes *bcrC* and

dltABCDE [120]. In addition, σ^V contributes to lysozyme resistance by inducing the expression of both *dltABCDE* and *oatA*, a peptidoglycan acetylating enzyme [121, 122]. σ^Y , on the other hand, has a unique and limited regulon consisting only of itself and a gene of unknown function, *ybgB* [123]. Additional functions for these remaining ECF sigma factors are hinted at in the study by Luo *et al.* that identified several phenotypes that appear only in a mutant of all seven ECF sigma factors, but not in a σ^{MWX} triple mutant [99]. These phenotypes included decreased production of exopolysaccharide and increased sensitivity to cefuroxime, ciprofloxacin, and ofloxacin. Single σ^V , σ^Y , σ^Z , or σ^{YlaC} mutants, however, did not display any of these phenotypes, making it unclear which factor(s) are responsible for these phenotypes in the heptad mutant [99]. Further investigations will be necessary to tease out the functions of these other ECF sigma factors.

4.3 *Clostridium difficile*

Research on ECF sigma factors in *B. subtilis* laid the groundwork for much of what is known about the ECF sigma factors in the intestinal pathogen, *Clostridium difficile*. Ho and Ellermeier identified three ECF sigma factors in *C. difficile*: σ^T , σ^U , and σ^V (also known as CsfT, CsfU, and CsfV), all of which appear to play a role in regulating antimicrobial resistance [124]. All three ECF sigma factors are induced in the presence of bacitracin and lysozyme [124]. In the presence of bacitracin or lysozyme, the protease, PrsW, releases σ^T and σ^U from their anti-sigma factors, RsiT and RsiU, respectively. A *prsW* mutant, which consequently has inactive σ^T and σ^U , is more sensitive to bacitracin and lysozyme [124]. A similar protease has not been found for regulation of σ^V , but lysozyme binds to the σ^V anti-sigma factor, RsiV, which may allow for direct induction of σ^V activity by lysozyme as it does in *B. subtilis* [125]. Induction of σ^V is necessary for lysozyme resistance, an effect that is partly mediated by one of the genes in the σ^V regulon, *pdaV*. This gene encodes a peptidoglycan deacetylase, which may help *C. difficile* avoid damage from lysozyme by making its peptidoglycans less susceptible to lysozyme cleavage [126]. Moreover, σ^V upregulates *dlt* expression in *C. difficile* in response to lysozyme, and a *dlt* mutant is significantly more sensitive to lysozyme and polymyxin B [127, 128]. Other genes in the regulons of these ECF sigma factors may also contribute to their ability to modulate antimicrobial resistance but have yet to be identified.

4.4 *Enterococcus faecalis*

Enterococcus faecalis is predicted to encode two ECF sigma factors, although to date only σ^V has been extensively studied [30]. Like σ^V in *B. subtilis* and *C. difficile*, σ^V in *E. faecalis* is critical for lysozyme resistance [129]. Le Jeune *et al.* demonstrated that *sigV* is the most important gene for lysozyme resistance, because a *sigV oatA dltA* triple mutant was significantly more sensitive to lysozyme than an *oatA dltA* double mutant, even though *oatA* and *dltA* are the key mediators of lysozyme resistance. Although *oatA* and *dltA* are regulated by the orthologous σ^V in *B. subtilis*, transcription of *oatA* and *dltA* is not controlled by σ^V in *E. faecalis* [129]. This finding suggests that there are additional σ^V -controlled mechanisms that mediate lysozyme resistance in *E. faecalis*.

The *E. faecalis* σ^V regulon has not been fully determined; however, Varahan *et al.* have identified a few mechanisms of σ^V -mediated lysozyme resistance in this bacterium. For one,

they found that overexpression of σ^V (via mutation of its anti-sigma factor, *rsiV*) results in cell chaining, which implies that σ^V regulates autolysin activity [130]. Autolysins play a role in lysozyme-mediated damage, in part because autolysin activity is enhanced in lysozyme [131, 132]. Moreover, both *rsiV* and *sigV* mutants bind more lysozyme, suggesting that σ^V regulates components of the cell surface that can deter lysozyme binding [130]. Additional research is needed to determine additional genes in the σ^V regulon.

5. Discussion

The ECF sigma factors that have demonstrated roles in the regulation of antimicrobial resistance genes are summarized in Table 1. Considering the diverse range of bacterial species described, it is clear that ECF sigma factors have proven useful throughout evolutionary history as a way for bacteria to modulate intrinsic antimicrobial resistance mechanisms. Constitutive expression of intrinsic resistance genes would be the most reliable way to ensure that a bacterium could survive the sudden introduction of antimicrobials into its environment; however, there is a long-established correlation between increased resistance and fitness cost [133]. If there is a fitness cost associated with a particular resistance mechanism, it would be advantageous for a bacterium to be able to exhibit the resistance phenotype only when a threat exists. Even if no fitness cost exists, the production of resistance-mediating proteins could impose an unnecessary metabolic burden on the bacterium if those proteins had no relevance in an antimicrobial-free environment. The regulation of antimicrobial resistance by ECF sigma factors allows a bacterium to quickly sense antimicrobial signals and respond by activating resistance genes and repair mechanisms that are specific to that antimicrobial, only when present.

It is important to note, however, that not all of the ECF sigma factors that play a role in antimicrobial resistance do so through specific, antimicrobial-induced mechanisms. For example, RpoE in *E. coli* is induced by misfolded proteins in the outer membrane rather than by a specific antimicrobial, yet the downstream effects of RpoE activation provide protection against membrane-active antimicrobials such as polymyxin B. In this way, ECF sigma factors can play a role in both specific and broad mechanisms of resistance.

The importance of these regulatory systems is further highlighted by the overlap and redundancy evident in some species. The overlapping regulons of σ^M , σ^W , and σ^X in *B. subtilis* are perhaps the most striking example of ECF redundancy, but *E. coli* also has a measure of regulatory redundancy between RpoE and the CpxAR two-component system [134]. Such redundancy may serve several functions. First, it may simply provide a mechanism so that regulatory systems can respond differently based on antimicrobial concentration. Second, different regulators may be more important at certain times of growth, as seems to be the case in *B. subtilis* [106]. Finally, antimicrobial compounds that are very different structurally (and therefore may trigger different systems) may cause similar types of damage that require expression of a common set of defenses. Induction of *bcrC* expression in *B. subtilis* provides an example of this phenomenon; bacitracin induces expression of only σ^M , yet both σ^M and σ^X can activate *bcrC* transcription [135].

Functional redundancy could be one possible explanation for the absence of ECF sigma factors in some pathogens. For example, *Campylobacter jejuni*, *Chlamydia trachomatis*, *Streptococcus pneumoniae*, and *Helicobacter pylori* do not encode identifiable ECF sigma factors [30]. In these pathogens, other regulatory mechanisms, such as two-component systems may fulfill similar functions to compensate for the lack of ECF sigma factors [136–141]. Other pathogens, such as *Haemophilus influenza*, *Neisseria gonorrhoea*, and *Listeria monocytogenes* have ECF sigma factors that are clearly involved in membrane stress responses, but their specific roles in antibiotic resistance have not been determined [142–145].

Finally, it is notable that ECF sigma factors are not only implicated in regulating resistance to antimicrobials, but they also often play an important role in regulating production of self-made antimicrobials [118, 146–149]. Although it is outside the scope of this review to discuss this function of ECF sigma factors in detail, this additional function emphasizes the close relationship between resistance and antimicrobial production.

6. Conclusion

ECF sigma factors provide a mechanism by which bacteria can respond to extracellular threats, such as antimicrobials, by inducing expression of resistance mechanisms. To date, they have not been as extensively characterized as two-component systems, but it is clear that ECF sigma factors are key factors in the response to antimicrobials for many species of bacteria. Even though sequence homology studies have identified hundreds of ECF sigma factors, few have been studied. It will be important to determine whether these unstudied ECF sigma factors also function in antimicrobial resistance regulation. By characterizing these pathways, we may uncover new therapeutic targets in the ongoing battle against antimicrobial-resistant pathogens.

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Figure 1. Model of ECF sigma factor activation

(A) ECF sigma factors (labeled σ) are typically kept inactive via sequestration at the cell membrane by an anti-sigma factor (labeled anti- σ). (B) An extracellular signal or perturbation initiates a proteolytic cascade that cleaves the anti-sigma factor to release the sigma factor. (C) Upon release, the sigma factor is free to bind to DNA and direct the RNA polymerase (RNAP) to transcribe specific genes.

Table 1
Summary of ECF sigma factors with a demonstrated role in antimicrobial resistance regulation.

Organism	ECF sigma factor(s)	Induced by antimicrobials?	Genes in regulon that affect antimicrobial resistance	Mechanism(s) of resistance	Confers resistance to
<i>E. coli</i>	RpoE	ND ^a	unknown	maintain cell membrane integrity	polymyxin B
<i>S. enterica</i>	RpoE (serovar Typhimurium)	Yes	<i>fidH</i> , <i>snpA</i>	alternate terminal electron acceptor use, outer membrane protein assembly	polymyxin B, P2, cryptidin-4
	rpoE (serovar Typhi)	ND	<i>ompC</i> , <i>ompF</i> , <i>ramA</i>	antimicrobial access to periplasm, antimicrobial efflux	*sensitivity* to β -lactams, quinolones, aminoglycosides
<i>P. aeruginosa</i>	AlgU/T	Yes	<i>algD</i> , <i>ampR</i> , <i>mecCD-ompI</i>	alginate production, β -lactamase production, antimicrobial efflux	β -lactams, tobramycin, chlorhexidine
	SigX	ND	<i>optF</i>	porin	imipenem, polymyxin B
<i>S. coelicolor</i>	σ^E	Yes	unknown	maintain ratios of cell wall components	lysozyme
	σ^R	Yes	unknown	unknown	chloramphenicol, erythromycin, lincomycin, tetracycline
<i>B. subtilis</i>	σ^W	Yes	<i>fosB</i> , <i>fabH</i> , <i>fabF</i> , <i>sppA</i> , <i>yviC</i> , <i>pspA</i> , <i>yceGH</i> , <i>ydhST</i> , <i>yqeZ</i> , <i>yqiAB</i> , <i>yfhLM</i> , <i>yknW</i> , <i>XYZY</i>	regulate membrane fluidity, other unknown mechanisms	fosfomycin, nisin, amphotericin, sublanicin, bacteriocins
	σ^M	Yes	<i>abh</i> , <i>spx</i> , <i>disA</i> , <i>ltaSa</i> , <i>berC</i>	decreased autolysin production, biofilm formation, response to oxidative stress, diadenylate cyclase regulation of cell wall, LTA synthesis, UPP phosphatase	moenomycin, vancomycin, β -lactams, nisim, bacitracin, enduracidin
	σ^X	Yes	<i>dlt</i> , <i>pss</i> , <i>abh</i> , <i>lytR</i>	cell surface charge, decreased autolysin production	β -lactams, nisim
	σ^Y	Yes	<i>dlt</i> , <i>berC</i> , <i>oatA</i>	cell surface charge, UPP phosphatase, peptidoglycan acetylation	lysozyme
<i>C. difficile</i> ^b	σ^T , σ^U	Yes	unknown	unknown	bacitracin, lysozyme
	σ^V	Yes	<i>pdaV</i> , <i>dlt</i>	peptidoglycan deacetylation, cell surface charge	lysozyme
<i>E. faecalis</i>	σ^V	Yes	unknown	cell wall turnover, prevention of lysozyme binding	lysozyme

^aND = Not determined

^b*C. difficile* ECF sigma factors are also annotated as CsfT, CsfU, and CsfV.