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Extra Cytoplasmic Function σ Factor Activation

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Abstract

The bacterial cell envelope is essential for cell viability and is a target for numerous antibiotics and host immune defenses. Thus bacteria must sense and respond to damage to the cell envelope. Many bacteria utilize alternative σ factors such as Extracytoplasmic function (ECF) σ factors to respond to cell envelope stress. Although ECF σ factors are utilized by both Gram negative and Gram positive bacteria to respond to cell envelope stress, the mechanisms of sensing differ. In this review, we examine the events and proteins which are required for activation of two model Extracytoplasmic function σ factors, σ^E in *E. coli* and σ^W in *B. subtilis*.

Keywords

Signal transduction; Cell envelope Stress; Regulated intramembrane proteolysis; ECF sigma factor

Introduction

Bacterial gene expression is often controlled at the level of transcription. RNA polymerase recognition of promoters is primarily driven by the σ factor, a subunit of RNA polymerase. Extra-Cytoplasmic Function σ factors (ECF σ factors) represent a diverse family of alternative σ factors [1,2]. ECF σ factors are capable of responding to a number of signals including membrane or cell wall stress, iron levels, and oxidation state [2]. Because of the wide range of ECF σ factors this review will focus on a subset of ECF σ factors whose activity is primarily induced in response to cell envelope stress. We will discuss the features of ECF σ factors and how ECF σ factor activity is controlled by regulated intramembrane proteolysis (RIP).

Common features of ECF σ factors

ECF σ factors belong to the σ^{70} family of σ factors. The majority of ECF σ factors have four common characteristics. First, ECF σ factors contain only σ_2 and $\sigma_{4.2}$ domains which are homologous to the σ^{70} σ_2 and $\sigma_{4.2}$ domains. These domains are responsible for binding to the -35 and -10 regions of their target promoters [1,2]. Second, most ECF σ factors autoregulate their own expression. Third, the activity of many ECF σ factors is controlled by an anti- σ factor which is often encoded within the same operon as the σ factor itself. These anti- σ factors are often, but not exclusively, membrane proteins [1,2]. Finally, the activity of the σ factor is induced by inhibiting activity of the anti- σ factor. This inhibition occurs by

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one of several mechanisms; degradation of the anti- σ factor, a conformational change in the anti- σ factor, or phosphorylation of an anti-anti- σ factor [3–7]. The proteolytic cascades which control ECF σ factor activation are complex and tightly regulated.

ECF σ factors are the largest and most diverse family of σ factors. The number and type of ECF σ factors can vary widely even among closely related bacteria [1,8]. A recent genomic and bioinformatic analysis of ECF σ factors revealed >40 distinct classes of ECF σ factors based on sequence similarity, domain structure of anti- σ factors, and putative promoter conservation [1]. Some ECF σ factors families are more restricted, for example the ECF30 family are present mostly in Firmicutes while the ECF15 are present in exclusively α -proteobacteria [1]. Other classes like ECF01–ECF04 are found in a wide range of bacteria and include two of the ECF σ factors, σ^E from *Escherichia coli* and σ^W from *B. subtilis*, which will be the focus of this review [1].

σ^E in *E. coli*

The best studied ECF σ factor is σ^E from *Escherichia coli*. Homologs of σ^E are present in a number of bacteria and are important for virulence of several Gram negative pathogens including *Salmonella enterica* serovar Typhimurium, *Vibrio cholerae* and *Pseudomonas aeruginosa* [9–14]. In *E. coli*, σ^E is encoded by *rpoE* as part of a four gene operon which includes the genes encoding the anti- σ factor RseA, the negative regulator RseB, and a positive modulator RseC. RseA is a single pass membrane protein and is the anti- σ factor that tethers σ^E to the cytoplasmic membrane inhibiting σ^E activity (Fig. 1) [15,16]. RseB encodes a periplasmic protein which negatively modulates σ^E activity via RseA. RseC is an inner-membrane protein which can positively modulate σ^E activity although the mechanism of this is unclear [15]. In response to cell envelope stress multiple proteases including the site-1 protease DegS, the site-2 protease RseP, and numerous cytosolic proteases are required for the degradation of the anti- σ factor, RseA (Fig 1).

σ^E is activated by cell envelope stress, specifically the accumulation of unfolded outer-membrane beta-barrel proteins [17]. Activation of σ^E is achieved by the step-wise proteolytic destruction of RseA (Fig 1). RseA degradation is initiated by the periplasmic site-1 protease DegS. This initial degradation is recognized as the rate-limiting step in activation of σ^E (Fig 1) [18,19]. DegS is one of the sensors of cell envelope stress. In the absence of stress, the protease activity of DegS is held inactive by a PDZ domain (protein interaction domain) [20]. Deletion of the PDZ domain results in constitutive protease activity and activation of σ^E . In addition to acting as an inhibitor of protease activity, the PDZ domain of DegS is also the sensor of cell envelope stress. The DegS-PDZ domain can bind to the C-termini of unfolded outer membrane proteins (OMPs) [20,21]. This binding alleviates the inhibition of DegS protease activity and results in DegS cleavage of RseA [20,21].

While DegS cleavage of RseA is essential for σ^E activation, it is not sufficient to activate σ^E . To achieve activation of σ^E , RseA must be further degraded by the site-2 protease RseP, a multi-pass transmembrane protein (Fig 1) [3,22]. Once DegS has cleaved RseA, RseA becomes a substrate for RseP which cleaves RseA within or near the transmembrane region [3,22,23]. Earlier work suggested RseP contained a single PDZ domain that inhibits RseP activity [23,24]. However, recent evidence suggests that RseP contains two PDZ domains, RseP-PDZ-N and RseP-PDZ-C, and that most of the negative regulatory effects are due to RseP-PDZ-N [25]. Unlike DegS-PDZ, the RseP-PDZ domains are not involved in sensing cell envelope stress [19,26]. Instead the RseP-PDZ-C domain appears to be important in recognizing DegS-cleaved RseA [27].

Once RseP has cleaved RseA, the N-terminal cytoplasmic portion of RseA (cytoplasmic RseA') bound to σ^E is released into the cytosol. Binding of RseA to σ^E is stronger than the interaction between core RNA polymerase and σ^E ; therefore degradation of cytoplasmic RseA' is required for activation of σ^E [16]. Cytoplasmic RseA' can be degraded by any number of cytosolic proteases including ClpXP, ClpAP, Lon, FstH and HslUV (Fig 1) [19]. The degradation of cytoplasmic RseA' by these proteases appears to be constitutive once the appropriate substrate appears suggesting they do not play a role in cell envelope stress sensing.

The activity of σ^E is further modulated by RseB. RseB is a periplasmic protein which functions as a negative regulator of σ^E activity by directly binding to RseA [28,29]. Recent evidence suggests RseB may protect RseA from cleavage by DegS [29,30]. The structure of RseA bound to RseB shows that RseB may mask the DegS site of cleavage [31][30,31]. It has been proposed that RseB may be capable of sensing a cell envelope stress signal. The nature of this signal has been suggested to be a beta strand motif of OMPs [30,32]. In either case unfolded OMPs are capable of generating both inducing signals. This suggests RseA degradation and σ^E activity are controlled by an "AND" gate, a condition in which the presence of two independent signals are both required for activation of a single system: the C-terminus of OMPs and a beta strand of an OMP. These dual requirements serve to tightly control activation of σ^E in response to OMPs. For a more detailed description of *E. coli* OMP assembly in the outer membrane please refer to the accompanying review by Rigel and Silhavy.

σ^W in *B. subtilis*

B. subtilis encodes seven ECF σ factors which respond to various cell envelope stresses. With respect to molecular mechanisms involved in σ factor activation the most well studied *B. subtilis* ECF σ factors is σ^W . Expression of *sigW* is induced by a number of different extracellular stresses including alkaline stress, peptidoglycan stress (cell wall antibiotics) and membrane stresses [33–36]. However, σ^W does not provide increased resistance to many of these stresses. The most potent inducers of σ^W activity appear to be antimicrobial peptides produced by other bacteria [4,36]. σ^W is encoded by *sigW* as part of a two gene operon which includes the gene encoding the anti- σ factor RsiW [5,37]. In response to cell envelope stress RsiW is degraded by multiple proteases including the site-1 protease PrsW, the site-2 protease RasP, and the cytosolic proteases ClpXP or ClpEP (Fig 2).

Activity of σ^W is inhibited by a membrane-bound anti- σ factor RsiW (Fig. 2) [5,37]. While, RsiW and RseA (anti- σ^E) do not share any sequence similarity they are both single-pass transmembrane proteins with a small cytoplasmic domain and a larger extracytoplasmic domain (Fig 1 and Fig 2) [5]. In the absence of signal, RsiW binds σ^W and inhibits its activity. Activation σ^W is initiated by degradation of the anti- σ factor RsiW. While *B. subtilis* encodes several homologs of *E. coli* DegS, these are not required for site-1 cleavage [5]. Instead the initiation of RsiW cleavage is dependent upon PrsW which represents a novel family of proteases [4,38]. PrsW is a metallo-protease with distant similarity to RCE1 (ras converting enzyme 1), a CAAX prenyl endopeptidase in *Saccharomyces cerevisiae* [4,39]. This is putative glutamic acid metalloprotease which lacks a PDZ domain.

PrsW is required for site-1 mediated cleavage of RsiW and thus activation of σ^W activity (Fig 2). The precise cleavage site of RsiW by PrsW is not known but recent evidence suggests PrsW cleaves near the C-terminus of RsiW [40]. This cleavage is insufficient to allow the site-2 protease RasP to cleave RsiW (Fig 2). Current models suggest that RsiW is subject to further cleavage by an as-yet unidentified protease or multiple proteases [40].

Once RsiW has been cleaved by PrsW and the other protease(s) it becomes a substrate for the site-2 protease, RasP [4,5,40]. RasP, a membrane-embedded metalloprotease homologous to RseP from *E. coli*, is required for degradation of RsiW and activation of σ^W (Fig 2) [5]. RasP is not thought to be responsible for detecting any cell envelope stress signal, since truncations of RsiW are constitutively cleaved by RasP even in the absence of stress [4,5]. RasP, like RseP, also contains two PDZ domains although their role in activation of σ^W is unknown.

The final step required for activation of σ^W requires degradation of the N-terminal RsiW cytoplasmic domain (cytoplasmic-RsiW') which directly interacts with σ^W . Unlike *E. coli* where almost any cellular protease can degrade RseA, the degradation of cytoplasmic-RsiW' domain is heavily dependent upon ClpXP or ClpCP [41]. Either a mutation in *clpP* or overexpression of a *clpXP* substrate are able to block cytoplasmic-RsiW' degradation and thus σ^W activation [41].

While it is clear the cell envelope stress induces σ^W , the precise signal which is responsible is not known. It is assumed that PrsW is the sensor for cell envelope stress in the system which controls σ^W activity. The evidence for this is three fold. First, in the absence of PrsW, full length RsiW is stable even in the presence of stress. Second, truncations of RsiW are constitutively degraded by the unknown protease and RasP even in the absence of cell envelope stress [4,5,40]. Third, several mutants of PrsW were isolated which resulted in a constitutive degradation of RsiW and thus constitutive activation of σ^W [4]. Each of the constitutive mutants resulted in a negatively charged amino acid being changed to a neutral or positively charged amino acid. PrsW lacks a PDZ domain found in DegS thus it is tempting to think that these negatively charged may be involved in sensing cell envelope stress similar to the role of negatively charged residues of PhoQ are involved in sensing antimicrobial peptides in *S. Typhimurium* [42].

Similar to *E. coli*, *B. subtilis* encodes a second negative regulator, YsdB, which appears analogous to RseB [4,43]. Like *rseB*, expression of *ysdB* is controlled by the ECF σ factor it regulates [43,44]. Mutants of *ysdB* cause a modest increase in σ^W activity suggesting that YsdB modulates σ^W activity [4,43]. One major difference between YsdB and RseB however is the presence of a transmembrane domain on YsdB. This transmembrane domain is likely necessary to keep YsdB localized properly in the absence of an outer-membrane. It is not currently known if YsdB interacts with a component of the σ^W signal transduction machinery in a manner analogous to the interaction of RseB with RseA or if YsdB may be able to sense stress itself.

Conservation of regulatory proteases

Regulated intramembrane proteolysis controls a variety of signal transduction pathways. The proteases required for site-1 cleavage differ from one regulatory system to another. PrsW appears to be glutamic acid metalloprotease while DegS (*E. coli*), SpoIVB (*B. subtilis*) and S1P (mammals) are serine proteases but are not homologous to each other, while PerP (*Caulobacter cerentsus*) is an aspartyl protease [18,45–47]. In contrast, the site-2 proteases of all these systems belong to the same family of membrane-embedded, zinc metalloproteases [5,22,47–49]. This further supports the idea that the site-1 proteases are responsible for signal detection since both the inducing signals and the site-1 proteases vary for different systems.

B. subtilis encodes 7 ECF σ factor systems, only one of which is known to be regulated by the site-1 protease PrsW (unpublished data). Similarly *C. difficile* also encodes three ECF σ factor systems of which only *csfT* and *csfU* expression are dependent upon PrsW [50]. *C. difficile* PrsW is able to induce degradation of RsiT but not RsiU suggesting that it directly

regulates CsfT activity but not CsfU [50]. Homologs of PrsW can be found in organisms which do not appear to encode any putative ECF σ factors. This suggests PrsW may have another function independent of ECF signal transduction. Indeed a *B. subtilis* *prsW* mutant accumulates membrane proteins in a manner that is independent of the σ^W pathway it controls [51]. This suggests that even in *B. subtilis* PrsW may play other roles as well.

Mycobacterium tuberculosis species encode 9–10 ECF σ factors several of which are important for virulence including σ^L , σ^H , and σ^E [52–54]. While site-1 proteases controlling activation of these ECF σ factors have not been identified, *M. tuberculosis* encodes a site-2 protease termed Rip1 [55]. In *M. tuberculosis*, Rip1 is required for degradation of 3 anti- σ factors RskA (σ^K), RslA (σ^L), and RsmA (σ^M) [55]. Thus in *M. tuberculosis* several ECF σ factors utilize the same site-2 protease to degrade anti- σ factors leading to σ factor induction.

It is clear that site-2 proteases are involved in a number of different processes independent of their role in ECF σ factor activation. In *E. coli*, RseP is involved in post-liberation cleavage of signal peptides [56]. *B. subtilis* RasP is required for a number of other cellular processes including cleavage of the cell division protein FtsL, and induction of competence [56–58]. A homolog of RseP in *V. cholera* can cleave the membrane-localized regulatory protein TcpP which regulates expression of the toxin co-regulated pilus [59]. Finally in *E. faecalis* a homolog of site-2 proteases, Eep, is required for production of the peptide pheromone cCF10 [60]. These data suggest that the site-2 proteases are likely involved in numerous other cellular processes.

Targets of ECF σ factors

ECF σ factors are the largest and most diverse family of σ factors. Bioinformatics suggest that some ECF σ factors are conserved between organisms and control expression of genes encoding similar functions. For example many gammaproteobacteria encode σ^E homologs which are required for transcription of genes encoding for cell envelope proteases, periplasmic chaperones and numerous lipoproteins [61]. The σ^E homologs also regulate several sRNAs which can promote degradation of OMP transcripts resulting in decreased production of OMPs [62]. Thus, σ^E induces expression of genes involved in protein folding and OM assembly while shutting down production of OMPs presumably to allow the cell to repair the cell envelope damage.

Genomic analysis of bacteria has demonstrated that both the number and type of ECF σ factors can vary widely even within the same bacterial genus. For example the low GC Gram positive bacteria (Firmicutes) do not appear to have a common ECF σ factor. Instead the Firmicutes have a great deal of diversity in the number and type ECF σ factor they encode different strains of the same species contain different complements of ECF σ factors (*B. subtilis* 7; *B. cereus* 10–19; *B. anthracis* 14–16; *C. difficile* 3; *C. perfringens* 1–3 and *C. botulinum* 3–8) [8]. The regulons of the *B. subtilis* ECF σ factors have been determined [44,63,64]. These studies have found that there is overlap between regulons of different ECF σ factors. In particular σ^W , σ^X and σ^M recognize similar promoters [63,65]. This regulatory overlap has phenotypic consequences, for example a mutation in a single ECF σ factor has only a modest increase on sensitivity to lysozyme however deletion of additional ECF σ factors leads to increased sensitivity to lysozyme [66]. This raises the possibility that the roles of ECF σ factors in organisms which encode numerous ECF σ factors may be more difficult to determine due to regulatory overlap.

Conclusions

ECF σ factors have been recognized as one of the largest and most diverse classes of signal transduction systems in bacteria. Overall there is a great deal of similarity in the mechanism of activation of σ^E in *E. coli* and σ^W in *B. subtilis*. However it is also clear that there are several significant differences between these systems. This is not surprising given the very different environments these organisms inhabit and the basic differences in cellular structure. Since *B. subtilis* lacks an outer membrane the cell envelope stresses encountered are likely to be different than those encountered by *E. coli*. In addition further study of different ECF σ factor families will likely uncover other mechanisms of ECF σ factor activation.

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* of special interest

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Highlights

- Here we discuss the common features of ECF sigma factors
- We discuss the mechanisms controlling σ^E from *Escherichia coli*
- We compare what is known about activation of σ^W in *Bacillus subtilis* to σ^E from *Escherichia coli*

Figure 1. Model for activation of σ^E in *E. coli*

(A) In the absence of stress RseA binds to σ^E inhibiting its activity. (B) Unfolded OMPs bind DegS and RseB allowing for DegS to cleave RseA. (C) Cleaved RseA (RseA¹⁻¹⁴⁸) is then cleaved by the site-2 protease RseP. (D) Cytoplasmic-RseA' is degraded by any one of the cytoplasmic proteases (ClpXP, ClpCP, Lon, FtsH, HslUV). Red arrows point to the sites of cleavage on the substrates.

Figure 2. Model for activation of σ^W in *B. subtilis*

(A) In the absence of stress RsiW binds to σ^W inhibiting its activity. YsdB negatively regulates σ^W activity via an unknown mechanism. (B) In response to cell envelope stress, PrsW presumably undergoes a conformational change allowing it to cleave RsiW near the C-terminus. (C) As yet unidentified proteases are required for trimming RsiW to allow recognition by RasP. (D) Cleaved RsiW is then cleaved by the site-2 protease RasP. (E) Cytoplasmic-RsiW' is then degraded by either ClpXP or ClpEP. Red arrows point to the sites of cleavage on the substrates.