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# Signaling diversity and evolution of extracytoplasmic function (ECF) σ factors

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Extracytoplasmic function σ factors (ECFs) represent a fundamental and widely distributed principle of bacterial signal transduction that connects the perception of a stimulus (input) with the induction of an appropriate set of genes (output). In recent years, comparative genomics analyses have not only allowed a systematic and functional classification of ECFs but also indicated the presence of numerous novel and widely conserved mechanisms of ECF-dependent signaling. Some of these novel systems have been experimentally characterized and uncovered unique features not previously observed. These studies demonstrate that ECF-dependent signaling is much more versatile and diverse than has been appreciated before. They also indicate that the majority of mechanisms that regulate ECF activity still remain to be discovered and characterized.

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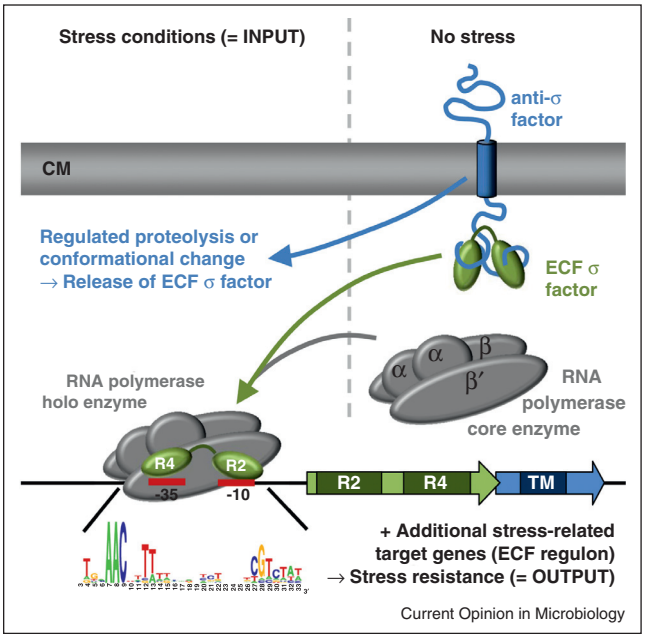
## Introduction

An organism's ability to accurately respond to changing environments is a prerequisite for its survival in the struggle with competitors aiming at the same ecological niche. To mediate such adaptation processes, bacteria possess a number of different means that connect an extracellular input with an appropriate cellular response. Extracytoplasmic function σ factors (ECFs) represent the third most abundant fundamental principle of bacterial signal transduction, outnumbered only by one-component (1CS) and two-component systems (2CSs) [1]. These alternative σ factors belong to the $\sigma^{70}$ protein family that also includes the housekeeping σ factors present in all bacterial genomes and other more closely related alternative σ factors [2,3].

The hallmark features of typical ECFs [4,5] are summarized in Figure 1: First, a characteristic protein domain architecture with only two of the four regions of $\sigma^{70}$ proteins remaining, namely regions $\sigma_2$ and $\sigma_4$. These two regions are sufficient for both promoter recognition and binding to the RNA polymerase core enzyme. Second, regulation of their activity by cognate anti-σ factors, which are often membrane-anchored proteins encoded in an operon with their σ factor. In the absence of a signal, the anti-σ factor tightly binds the σ factor, thereby keeping it inactive. Once stimulated, the anti-σ factor releases the σ factor, which then becomes active and recruits RNA polymerase core enzyme to redirect transcription initiation to its target promoters. Third, recognition of alternative promoter sequences that typically contain a highly conserved 'AAC' motif in the −35 region. Finally, presence of this promoter motif upstream the ECF-encoding operon. Hence, most ECFs are subject to positive autoregulation, thereby enhancing their activating effect as long as inducing conditions prevail. Once the stimulus ceases, the simultaneous upregulation of the co-expressed anti-σ factor then ensures a swift shut-off of σ factor activity.

On an average, bacterial genomes harbor about four ECFs per megabase of sequence, but their distribution does not correlate in a linear fashion with genome size: ECFs seem to be underrepresented and often absent in smaller genomes, while they are overrepresented in more complex bacteria, with some genomes harboring more than 100 ECFs (Huang & Mascher, unpublished). In contrast to the wealth of knowledge on 1CS and 2CS, the importance and diversity of ECFs has only been recognized very recently in the course of a first comprehensive phylogenetic and comparative genomics study [6]. This work not only demonstrated the abundance and conservation of ECFs in the bacterial world, but also allowed their systematic classification, including the identification of conserved groups of ECFs that seem to be regulated by novel mechanisms of signal transduction. Currently, 50 major and numerous smaller ECF groups can be discriminated based on sequence similarity of the ECF σ/anti-σ pair, genomic context conservation and target promoter motif [6,7•]. Collectively, these groups covered about two-thirds of the ECF sequences in the dataset, with the remaining unclassified third indicative of the overall wide variation within this protein family. A significant part of the initially unclassifiable ECFs are derived from phyla underrepresented in the genome sequence database [7•]. The features of the groups relevant to this review are summarized in Table 1.

**Figure 1**




Overview of typical features of ECF-dependent signal transduction. The ECF σ factor is highlighted in green; the cognate anti-σ factor and associated processes are highlighted in blue. The RNA polymerase core enzyme with its four subunits is shown in grey. The promoter is represented by the −35 boxes and −10 boxes, a typical ECF-dependent promoter signature is shown below. R2/R4, conserved signature regions (regions $\sigma_2$ and $\sigma_4$, respectively) of $\sigma^{70}$ proteins. CM, cytoplasmic membrane; TM, transmembrane region. See text for details.

A number of excellent and comprehensive reviews were published on ECFs, mostly concentrating on the ECF paradigms [4,5,8–10]. In contrast, the purpose of this article is to provide an overview on the diversity of mechanisms activating ECFs, based both on experimental evidence and comparative genomics predictions. Our current knowledge strongly suggests that ECF-based signal transduction is far more complex and diverse than has previously been recognized and appreciated. Moreover, our increasingly comprehensive understanding of the phylogenetic relationship of ECFs also allows a first insight into their evolutionary history, the implications of which will be discussed.

## Mechanisms of ECF-dependent signal transduction and σ factor activation

**Regulated proteolysis of membrane-anchored anti-σ factors** represents the best-understood mechanism of releasing an ECF from the inhibitory grip of its cognate anti-σ factor in response to extracellular cues (Figure 2a). It is characteristic for groups ECF01-04, but is presumably also used in a number of so far uncharacterized ECF groups [6]. The underlying mechanism has been studied in great detail for two of the paradigmatic ECF/anti-σ factor pairs, $\sigma^E$-RseA from *Escherichia coli* (group ECF02), and $\sigma^W$-RsiW of *Bacillus subtilis* (group ECF01) [11]. In both cases, a series of three successive proteolytic steps is involved in cleaving first the extracellular domain of the anti-σ factor (site-1) and then its single transmembrane helix (site-2) by regulated intramembrane proteolysis, thereby releasing the σ/anti-σ pair into the cytoplasm, where the remaining anti-σ factor is finally degraded by the ClpXP protease [12,13]. Accordingly, the first protease represents the true sensor of this signal transduction cascade, as it has been demonstrated for DegS, responsible for site-1 proteolysis of the anti-σ factor RseA in *E. coli*. This serine protease only becomes proteolytically active in the presence of misfolded outer membrane porins (OMPs) in the periplasm that trigger the $\sigma^E$-dependent response. Unassembled OMPs are bound by the input (PDZ) domain of the protease, thereby inducing its catalytic activity [14,15]. A similar sensing mechanism involving activation of the site-1 proteases by direct perception of the input signal can also be assumed for the inactivation of other anti-σ factors subject to regulated proteolysis. But this process can also involve additional regulatory proteins: for the *E. coli* $\sigma^E$-RseA pair, a second negative regulator, the periplasmic protein RseB, is also required for RseA-inactivation and functions as an additional signal input gate required to trigger $\sigma^E$-dependent transcription [16].

**Conformational changes of (soluble) anti-σ factors**. A small subset of ECF groups is linked to cytoplasmic anti-σ factors that nevertheless show a significant degree of sequence and structural similarity to membrane-anchored anti-σ factors, at least in the domain that binds the cognate σ factor [17]. But the mechanism of their release is fundamentally different (Figure 2b). On the basis of the two paradigms, SigR-RsrA from *Streptomyces coelicolor* (ECF12) and RpoE-ChrR from *Rhodobacter sphaeroides* (ECF11), such soluble anti-σ factors — which contain a number of highly conserved cysteine residues — seem to perceive redox or oxidative stress. In the case of RsrA, this leads to the formation of intramolecular disulfide bridges, resulting in conformational changes of the anti-σ factor, which ultimately releases the cognate σ factor to initiate gene expression [18,19]. Overall, the RpoE-mediated response of *R. sphaeroides* to singlet oxygen follows a comparable mechanism [17,20]. But in contrast to RsrA, disulfide bridge formation does not play a role in this process, since the highly conserved cysteine residues of the anti-σ factor ChrR are not required to induce RpoE activity [21].

Recently it was demonstrated that conformational changes can also regulate membrane-anchored anti-σ factors. In the CnrXYH cascade of *Cupriavidus metallidurans*, binding of cobalt and nickel ions to the extracellular sensor protein CnrX leads to conformational changes that are transduced via the transmembrane

**Table 1**

**Overview of the signaling diversity of ECF σ factors**[a]

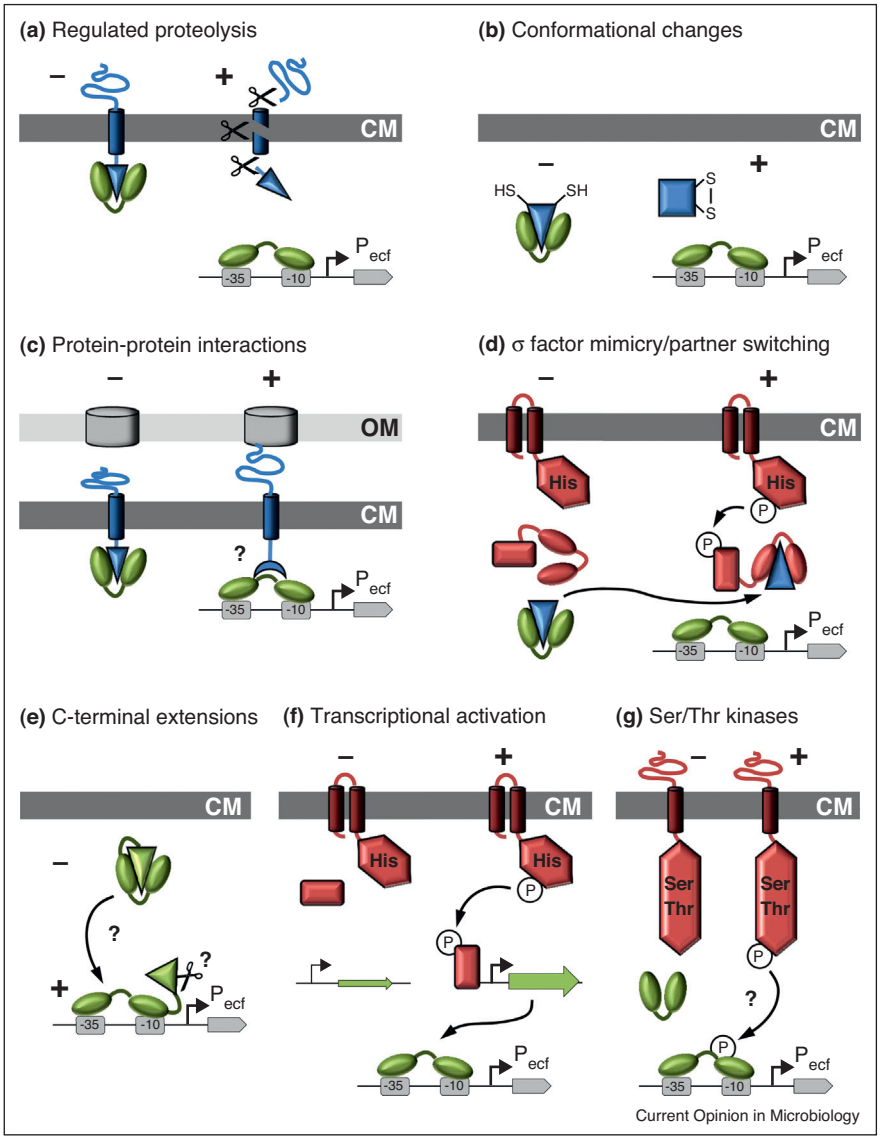
| ECF group[b] | | | (Putative) signaling mechanism | Genomic context conservation; putative function; remarks[f] |
|---|---|---|---|---|
| Name | No. | Phyla | (Effect on anti-σ factor) | |
| Membrane-anchored anti-σ factors | | | | |
| $RpoE_{Eco}$-like | ECF01-04 | 10 | Regulated proteolysis | Diverse (e.g. envelope) stress responses |
| FecI-like | ECF05-07 | 1 | Protein–protein interaction | OMPs; metal ion/siderophore uptake |
| FecI-like | ECF08 | 1 | Protein–protein interaction? | Unknown |
| FecI-like | ECF10 | 1 | Protein–protein interaction | OMPs; polysaccharide uptake/metabolism |
| SigF-like | ECF16 | 1 | Unknown (unique 6TMR anti-σ's) | Oxidative stress response |
| SigU-like | ECF17 | 1 | Regulated proteolysis? | Protein secretion; envelope functions |
| RpoT/SigK | ECF18/19 | 3 | Unknown (unique anti-σ factors) | Stress response/virulence |
| CnrH-like | ECF20 | 2 | Conformational change | CnrXY-like; metal ion resistance |
| n.a. | ECF26 | 2 | Unknown (various anti-σ factors) | Large combinatorial complexity in anti-σ factor architecture & genomic context conservation |
| Soluble anti-σ factors | | | | |
| $RpoE_{Rsp}$-like | ECF11 | 1 | Conformational change | Singlet oxygen stress response |
| SigR-like | ECF12 | 4 | Conformational change | Redox homeostasis |
| n.a. | ECF13 | 1 | Conformational change? | Oxidative stress response |
| n.a. | ECF14 | 1 | Conformational change? | *O*-Methyltransferases; stress response |
| EcfG-like | ECF15 | 1[c] | Partner switch/σ-factor mimicry | PhyR-like RR; $HK_{HWE}$; general stress response |
| No apparent anti-σ factors | | | | |
| FecI-like | ECF09 | 1 | Unknown | Metal ion uptake; siderophore biosynthesis |
| HrpL-like | ECF32 | 1 | Transcriptional control | (HK/RR); type III secretion system expression |
| $SigE_{Sco}$-like | ECF39 | 1 | Transcriptional control[d] | (HK/RR); cell envelope integrity |
| Conserved C-terminal regulatory extensions[e] | | | | |
| n.a. | ECF41 | 10 | 100 aa; conformational change? | COE proteins; oxidative stress response? |
| n.a. | ECF42 | 6 | 200 aa; protein interactions? | DGPF proteins (unknown function); unknown; |
| CorE-like | ECF44 | 1? | 50 aa; conformational change? | Metal ion resistance |
| n.a. | ECF01-Gob | 1[c] | ~1000 aa; protein interactions? | Large/complex C-term. Extensions; unknown |
| Associated with Ser-/Thr-protein kinases | | | | |
| n.a. | ECF43 | 4 | Phosphorylation? | Unknown; ECF-like proteins |
| n.a. | ECF-STK01-04 | 1 | Phosphorylation? | Unknown; all Planctomycetes-specific ECFs |

[a] Only ECF-groups with at least some published evidence and/or unique features are listed. For a complete list and a detailed description of all ECF groups, see [6,7•].
[b] The name usually derives from the best-understood example, the No. is based on the ECF classification [6,7•] or Huang & Mascher, unpublished; 'Phyla' gives the number of different phyla, in which a certain group of ECFs can be found.
[c] EcfG-like σ factors can only be found in α-proteobacteria; ECF01-Gob can only be found in one species of the phylum Planctomycetes.
[d] More than half of the members of this ECF-group are associated with classical anti-σ factors; SigE-CseABC seems to be the exception.
[e] For these ECF σ factors, the average size of the C-terminal extensions (in amino acids, aa) is given in column 4.
[f] OMPs, outer membrane porins; HK/RR, histidine kinase/response regulator; COE, carboxymuconolactone decarboxylases/oxidoreductases/epimerases.

anti-σ factor CnrY to ultimately release the cytoplasmic ECF CnrH, which in turn induces genes mediating resistance against these metal ions [22•,23]. A potentially similar mechanism might regulate σ factors of group ECF16, which are associated with unusual and group-specific anti-σ factors containing six transmembrane helices. In *Bradyrhizobium jabonicum*, the activation of EcfF in response to reactive oxygen species requires two highly conserved cysteine residues in the membrane-anchored anti-σ factor OsrA, indicating that the formation of disulfide bonds might trigger the release of this ECF [24].

**Protein–protein interaction cascades** based on FecI/FecR protein pairs are the third well-described mechanism of activating ECFs (groups ECF05-09) that are usually involved in regulating iron-siderophore uptake (Figure 2c) [6]. In the absence of extracellular $Fe^{3+}$, FecI-like σ factors are kept inactive by their cognate FecR-like anti-σ factors. But the latter seem to play a dual role, since they are also required for ECF activity in the presence of extracellular citrate-bound $Fe^{3+}$ [8,25,26]. The mechanism of this positive role of FecR-like proteins is not fully understood. It could be based on a transient association of FecR with FecI to facilitate binding of the σ factor to RNA polymerase core enzyme. Alternatively, FecI could simply be unstable in the absence of its cognate anti-σ factor and the subject to proteolytic degradation [27]. A positive role of FecR on FecI activity is not established for all FecI-FecR pairs and might in fact be

**Figure 2**




Schematic illustration of the different modes of regulating ECF σ factor activity, as discussed in this review. ECF σ factors are highlighted in green, anti-σ factor in blue, regulatory proteins involved in phosphorylation-dependent signaling processes are shown in red. '–' or '+' indicate absence or presence of inducing conditions, respectively. See text for details.

the exception rather than the rule. But it is established that FecI-like σ factors are activated by a protein–protein interaction cascade that signals the presence of substrate bound to the OMP FecA, via FecR, through the cytoplasmic membrane to the intracellular σ factor FecI. This process also requires the inner membrane receptor protein TonB, which senses the energy state of the cytoplasmic membrane and signals this information to FecA to trigger the uptake of diferric citrate into the periplasm. Ultimately, this cascade induces the expression of an iron-siderophore uptake system, encoded by the *fecABCDE* operon [25]. The operon encoding FecIR is located next to this target locus and is not autoregulated, in contrast to the examples described above.

Remarkably, the same signaling principle has been adopted in the phylum Bacteroidetes for controlling the uptake of complex polysaccharides in the gut environment: FecIR pairs of group ECF10 are associated with putative polysaccharide-binding OMPs and enzymes involved in sugar degradation [28,29]. The FecIR pair therefore seems to represent a versatile, substrate-independent signaling module capable of connecting the input signal of a substrate bound to an OMP (as a measure

of its extracellular availability) to the cytoplasmic output of regulating its uptake and degradation.

**Partner-switching based on σ factor mimicry** is at the heart of the EcfG-dependent general stress response in α-proteobacteria (group ECF15) [30]. This mechanism also represents a regulatory link between 2CS-dependent and ECF-dependent signal transduction (Figure 2d). The signature proteins of such cascades are PhyR-like response regulators that have a very unique domain architecture: the receiver domain, which interacts with the cognate histidine kinase, is located in the C-terminal part of the protein, while the N-terminus of PhyR-like proteins shows significant and specific sequence similarity to EcfG-like σ factors [6,31]. Instead of functioning as DNA-binding proteins, these regulators participate in a partner-switching mechanism based on σ factor mimicry [31,32••,33,34]. As long as PhyR-like response regulators are inactive (unphosphorylated), the ECF-like domain is closely folded onto the receiver domain of PhyR. At the same time, EcfG-like σ factors are kept inactive by their cognate soluble NepR-like anti-σ factors. Activation (phosphorylation) of PhyR by unusual HWE-type histidine kinases [35] under stress conditions results in an opening of the PhyR structure, which allows the EcfG-like N-terminal domain to now bind to NepR, thereby ultimately releasing the EcfG-like σ factor. Depending on the organism, multiple candidate sensor kinases and EcfG-like σ factors may exist, but in most cases, only one (rarely two) PhyR-like and NepR-like protein(s) are encoded in a genome. Such a regulatory architecture would allow not only signal integration from different sensors onto PhyR, but also activation of subregulons by alternative EcfG paralogs, based on stress quality and strength [35]. While most pieces of the puzzle have been solved, phosphorylation of PhyR by the putative partner kinases still awaits experimental proof, despite the fact that a number of histidine kinases have recently been linked to the PhyR-NepR-EcfG cascades from different α-proteobacteria [36–38].

**Conserved C-terminal extensions** are found in some ECF groups that lack an obvious anti-σ factors (Figure 2e). Recently, a regulatory function of these C-terminal domains was demonstrated for two examples. Members of the widely distributed group ECF41 contain a conserved C-terminal domain of about 100 amino acids that seems to be critical for both σ factor activation and inactivation: a partial truncation of this domain leads to hyperactive ECF41 alleles, while deletion of the complete C-terminal extension results in the complete loss of promoter activity [39•].

In the case of CorE-like proteins (group ECF44) involved in copper homeostasis, it was demonstrated that these σ factors are activated in the presence of $Cu^{2+}$ and other divalent metal ions. Binding of $Cu^{2+}$ was necessary to activate CorE-binding to DNA *in vitro*, while $Cu^{1+}$ inhibited this reaction. Both activation and repression of σ factor activity depend on a short and conserved C-terminal extension of about 20 amino acids that contains four invariant cysteine residues, which are necessary for the metal ion-dependent gene regulation mediated by CorE [40•].

Both studies strongly suggest that such C-terminal extensions can exhibit anti-σ factor-like functions, but the exact mechanism of how such extensions affect ECF activity remains to be investigated. A conformational change in response to an appropriate stimulus is very likely (Figure 2e), but — at least for ECF41 — this could also lead to unmasking a proteolytic cleavage site, which would trigger activation of the σ factor by regulated proteolysis.

**Transcriptional activation of σ factor expression** is another, although not very common anti-σ factor-independent mechanism of inducing an ECF response (Figure 2f). While a number of conserved ECF groups lack a recognizable anti-σ factor, only two ECFs have so far been described as being subject to transcriptional control. Expression of *sigE* of *S. coelicolor* (ECF39) is induced by the two-component systems CseBC in response to cell envelope stress. As a result, SigE upregulates expression of genes encoding the enzymes for the biosynthesis of a cell wall glycan [41,42].

A comparable, yet more complex mechanism triggers induction of HrpL-like ECFs of group ECF32. In the plant pathogen *Pseudomonas syringae*, this ECF primarily regulates virulence-associated functions [43,44]. HrpL-like proteins are genomically and functionally linked to HrpXY-like two-component systems, which are responsible for *hrpL* transcription via a regulatory cascade: HrpXY affects HrpL indirectly by regulating the expression of the *hrpRS* operon, encoding two homologous DNA-binding proteins that induce *hrpL* expression [45,46].

**Serine/threonine kinases and other novel mechanisms of regulating ECF activity.** In addition to the experimentally supported mechanisms described above, comparative genomics studies provide evidence for numerous additional mechanisms of activating ECFs. A number of conserved ECF groups (ECF43, and ECFSTK1-4) lack obvious anti-σ factors but instead show microsynteny to neighboring genes encoding serine/threonine protein kinases [6,7•]. This observation indicates a signaling mechanism involving protein phosphorylation, presumably of the ECF itself (Figure 2g) — a unique and rather abundant, yet so far completely unexplored mechanism of regulating ECF activity.

A number of additional, potentially novel mechanisms of activating ECFs have been proposed for some conserved

ECF groups, based primarily on comparative genomics data [6]. This includes unusual and group-specific types of anti-σ factors or 'orphan' ECFs lacking apparent anti-σ factors, but instead containing C-terminal extensions and/or conserved genomic neighborhood. For example, ECF42 proteins contain a large C-terminally fused domain of 200 amino acids and are linked to DGPF proteins of unknown function (Table 1).

Most of these groups still await experimental exploration. But some recent studies on members of novel ECF groups provide first insights into their biology and gene regulation and will surely pave the way for a mechanistic understanding of signal transduction in the future. Examples include work on two novel ECFs, EcfF (ECF16) and EcfQ (ECF33), which are involved in the response of *Bradyrhizobium japonicum* towards reactive oxygen species [24]. Moreover, a paper on a member of a minor ECF group from Actinobacteria identified BldN as an important regulator of development in *Streptomyces venezuelae* that is regulated by a novel type of RsbN-like anti-σ factors [47]. These few examples bear testimony to the hidden potential that still lies in the unexplored majority of ECF-dependent signal transduction.

## Evolution of ECF σ factors

It is assumed that ECFs and other alternative σ factors from the $\sigma^{70}$ protein family are derived from the primary σ factor by duplication and reductive evolution [48]. In addition to the functional relevance and predictive power, the ECF classification may also provide some first insight into the evolutionary history of these σ factors, based on the analysis of the phyletic distribution of orthologous σ factors from conserved ECF groups.

Some ECF groups are widely distributed in many diverse bacterial phyla, indicative of a long evolutionary history that dates back to the time before individual phyla separated. Examples include the group ECF41, which can be found in at least 10 distantly related bacterial phyla [39•]. Other examples of evolutionarily old ECF groups include the RpoE-like group ECF01, and the poorly characterized groups ECF22, ECF24, ECF42 and ECF43 [6].

But the majority of ECF groups, especially in proteobacteria and actinobacteria, are phylum-specific, reflecting the similar life style and physiology of a given group of related organisms. Such ECFs seem to have evolved early in the history of a phylum to facilitate a phylum-specific response to environmental or cellular conditions. This observation also indicates that detailed studies of the ECF repertoire from bacterial phyla currently underrepresented in the available genome sequence space will most likely identify numerous novel conserved groups, as it has recently been demonstrated for the phylum Planctomycetes [7•].

Some ECF groups seem to be evolutionary even younger: About 60 closely related ECFs in the Planctomycete *Gemmata obscuriglobus* are derived from one common ancestor, which then multiplied and diversified only within this species. While the N-terminal core part of these proteins (i.e. regions 2 and 4) are highly conserved, the C-terminal part has dramatically diversified and contains none to three putative transmembrane helices followed by up to 1000 amino acids with implicated functions in protein–protein interactions [7•]. Given that *G. obscuriglobus* contains a nucleus-like compartment surrounded by a membrane layer, it was speculated that this unique type of transmembrane-signaling ECFs is necessary for transducing information from the cytoplasm (input) to the chromosome (output).

While these speculations have to be taken with caution, the examples nevertheless indicate that conserved ECF groups sharing a common signaling mechanism developed at different time points during evolution. Some blueprints represent early success stories that were maintained as bacterial phyla diversified, while other ECF mechanisms developed much later in evolutionary history to facilitate a more specific life style within a phylum or even species.

## Conclusions and outlook

The last few years have seen an increasing number of experimental studies on unusual ECFs with altogether novel mechanisms of signal transduction that clearly deviate from the paradigms established by RpoE-like, SigR-like or FecI-like σ factors. The ECF classification also identified a number of additional and potentially novel mechanisms that go far beyond what is currently known [6,7•].

More recent and ongoing comparative genomics analyses of ECFs, especially when focusing on 'rare' (with regard to genome sequence availability) phyla, identify more and more groups with novel, conserved and unusual features, as demonstrated for the Planctomycetes [7•]. But in order to escape the relatively narrow range of sequence space derived from individual genome sequencing projects, which is heavily biased towards model organisms and those of medical or biotechnological relevance, we ultimately have to dig into the available metagenome datasets that reflect a habitat in a much more unbiased way. It makes me wonder how many additional mechanisms of ECF-dependent signal transduction might be out there, orchestrating an increasingly diverse set of alternative σ factors that were originally grouped together to form the extracytoplasmic function subfamily. From all that we can judge right now, we have hardly seen the tip of the iceberg, let alone what's underneath.

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## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest


1. Staroń A, Mascher T: **Extracytoplasmic function σ factors come of age**. *Microbe* 2010, **5**:164-170.

2. Lonetto MA, Brown KL, Rudd KE, Buttner MJ: **Analysis of the *Streptomyces coelicolor* sigE gene reveals the existence of a subfamily of eubacterial RNA polymerase sigma factors involved in the regulation of extracytoplasmic functions**. *Proc Natl Acad Sci U S A* 1994, **91**:7573-7577.

3. Paget MS, Helmann JD: **The $\sigma^{70}$ family of sigma factors**. *Genome Biol* 2003, **4**:203.

4. Butcher BG, Mascher T, Helmann JD: **Environmental sensing and the role of extracytoplasmic function (ECF) sigma factors**. In *Bacterial physiology — a molecular approach*. Edited by El-Sharoud WM. Berlin, Heidelberg: Springer-Verlag GmbH; 2008: 283-301.

5. Helmann JD: **The extracytoplasmic function (ECF) sigma factors**. *Adv Microb Physiol* 2002, **46**:47-110.

6. Staroń A, Sofia HJ, Dietrich S, Ulrich LE, Liesegang H, Mascher T: **The third pillar of bacterial signal transduction: classification of the extracytoplasmic function (ECF) σ factor protein family**. *Mol Microbiol* 2009, **74**:557-581.

7. • Jogler C, Waldmann J, Huang X, Jogler M, Glöckner FO, Mascher T, Kolter R: **Identification of proteins likely to be involved in morphogenesis, cell division, and signal transduction in Planctomycetes by comparative genomics**. *J Bacteriol* 2012, **194**:6419-6430.

This publication extends the ECF classification (see Ref. [6]), which established the importance and abundance of ECFs in the microbial world. This comprehensive analysis allows the identification of novel conserved ECF groups and provides the basis for functional predictions of ECF-dependent signal transduction and gene regulation.

8. Brooks BE, Buchanan SK: **Signaling mechanisms for activation of extracytoplasmic function (ECF) sigma factors**. *Biochim Biophys Acta* 2008, **1778**:1930-1945.

9. Missiakas D, Raina S: **The extracytoplasmic function sigma factors: role and regulation**. *Mol Microbiol* 1998, **28**:1059-1066.

10. Raivio TL, Silhavy TJ: **Periplasmic stress and ECF sigma factors**. *Annu Rev Microbiol* 2001, **55**:591-624.

11. Ho TD, Ellermeier CD: **Extracytoplasmic function σ factor activation**. *Curr Opin Microbiol* 2012, **15**:182-188.

12. Chaba R, Grigorova IL, Flynn JM, Baker TA, Gross CA: **Design principles of the proteolytic cascade governing the $\sigma^E$-mediated envelope stress response in *Escherichia coli*: keys to graded, buffered, and rapid signal transduction**. *Genes Dev* 2007, **21**:124-136.

13. Heinrich J, Wiegert T: **Regulated intramembrane proteolysis in the control of extracytoplasmic function sigma factors**. *Res Microbiol* 2009, **160**:696-703.

14. Sohn J, Grant RA, Sauer RT: **Allosteric activation of DegS, a stress sensor PDZ protease**. *Cell* 2007, **131**:572-583.

15. Wilken C, Kitzing K, Kurzbauer R, Ehrmann M, Clausen T: **Crystal structure of the DegS stress sensor: how a PDZ domain recognizes misfolded protein and activates a protease**. *Cell* 2004, **117**:483-494.

16. Chaba R, Alba BM, Guo MS, Sohn J, Ahuja N, Sauer RT, Gross CA: **Signal integration by DegS and RseB governs the $\sigma^E$-mediated envelope stress response in *Escherichia coli***. *Proc Natl Acad Sci U S A* 2011, **108**:2106-2111.

17. Campbell EA, Greenwell R, Anthony JR, Wang S, Lim L, Das K, Sofia HJ, Donohue TJ, Darst SA: **A conserved structural module regulates transcriptional responses to diverse stress signals in bacteria**. *Mol Cell* 2007, **27**:793-805.

18. Li W, Stevenson CE, Burton N, Jakimowicz P, Paget MS, Buttner MJ, Lawson DM, Kleanthous C: **Identification and structure of the anti-sigma factor-binding domain of the disulphide-stress regulated sigma factor $\sigma^R$ from *Streptomyces coelicolor***. *J Mol Biol* 2002, **323**:225-236.

19. Zdanowski K, Doughty P, Jakimowicz P, O'Hara L, Buttner MJ, Paget MSB, Kleanthous C: **Assignment of the zinc ligands in RsrA, a redox-sensing ZAS protein from *Streptomyces coelicolor***. *Biochemistry* 2006, **45**:8294-8300.

20. Dufour YS, Landick R, Donohue TJ: **Organization and evolution of the biological response to singlet oxygen stress**. *J Mol Biol* 2008, **383**:713-730.

21. Greenwell R, Nam T-W, Donohue TJ: **Features of *Rhodobacter sphaeroides* ChrR required for stimuli to promote the dissociation of $\sigma^E$/ChrR complexes**. *J Mol Biol* 2011, **407**:477-491.

22. • Trepreau J, de Rosny E, Duboc C, Sarret G, Petit-Hartlein I, Maillard AP, Imberty A, Proux O, Coves J: **Spectroscopic characterization of the metal-binding sites in the periplasmic metal-sensor domain of CnrX from *Cupriavidus metallidurans* ch34**. *Biochemistry* 2011, **50**:9036-9045.

This study combined with Ref. [23] provide structural and biochemical evidence for transmembrane signal transduction based on conformational changes rather than regulated proteolysis of an anti-σ factor in response to extracellular metal ions.

23. Trepreau J, Girard E, Maillard AP, de Rosny E, Petit-Haertlein I, Kahn R, Coves J: **Structural basis for metal sensing by CnrX**. *J Mol Biol* 2011, **408**:766-779.

24. Masloboeva N, Reutimann L, Stiefel P, Follador R, Leimer N, Hennecke H, Mesa S, Fischer HM: **Reactive oxygen species-inducible ECF sigma factors of *Bradyrhizobium japonicum***. *PLoS ONE* 2012, **7**:e43421.

25. Braun V, Mahren S: **Transmembrane transcriptional control (surface signalling) of the *Escherichia coli* Fec type**. *FEMS Microbiol Rev* 2005, **29**:673-684.

26. Mettrick KA, Lamont IL: **Different roles for anti-sigma factors in siderophore signalling pathways of *Pseudomonas aeruginosa***. *Mol Microbiol* 2009, **74**:1257-1271.

27. Braun V, Mahren S, Sauter A: **Gene regulation by transmembrane signaling**. *Biometals* 2006, **19**:103-113.

28. Karlsson FH, Ussery DW, Nielsen J, Nookaew I: **A closer look at Bacteroides: phylogenetic relationship and genomic implications of a life in the human gut**. *Microb Ecol* 2011, **61**:473-485.

29. Xu J, Chiang HC, Bjursell MK, Gordon JI: **Message from a human gut symbiont: sensitivity is a prerequisite for sharing**. *Trends Microbiol* 2004, **12**:21-28.

30. Francez-Charlot A, Frunzke J, Vorholt JA: **The general stress response in alphaproteobacteria**. In *Bacterial Stress Responses*. Edited by Storz G, Hengge R. Washington, DC: ASM Press; 2011:291-300.

31. Francez-Charlot A, Frunzke J, Reichen C, Ebneter JZ, Gourion B, Vorholt JA: **Sigma factor mimicry involved in regulation of general stress response**. *Proc Natl Acad Sci U S A* 2009, **106**:3467-3472.

32. •• Campagne S, Damberger FF, Kaczmarczyk A, Francez-Charlot A, Allain FH, Vorholt JA: **Structural basis for sigma factor mimicry in the general stress response of alphaproteobacteria**. *Proc Natl Acad Sci U S A* 2012, **109**:E1405-E1414.

This paper, together with Ref. [34], provides a structural and mechanistic explanation for how an elegant partner switching mechanism based on σ factor mimicry orchestrates the long-elusive general stress response in α-proteobacteria (group ECF 15).

33. Herrou J, Foreman R, Fiebig A, Crosson S: **A structural model of anti-anti-σinhibition by a two-component receiver domain: the PhyR stress response regulator**. *Mol Microbiol* 2010, **78**:290-304.

34. Herrou J, Rotskoff G, Luo Y, Roux B, Crosson S: **Structural basis of a protein partner switch that regulates the general stress response of alpha-proteobacteria**. *Proc Natl Acad Sci U S A* 2012, **109**:E1415-E1423.

35. Staroń A, Mascher T: **General stress response in α-proteobacteria: PhyR and beyond**. *Mol Microbiol* 2010, **78**: 271-277.

36. Foreman R, Fiebig A, Crosson S: **The LovK-LovR two-component system is a regulator of the general stress pathway in *Caulobacter crescentus***. *J Bacteriol* 2012, **194**: 3038-3049.

37. Kaczmarczyk A, Campagne S, Danza F, Metzger LC, Vorholt JA, Francez-Charlot A: **Role of *Sphingomonas* sp. strain Fr1 PhyR-NepR-$\sigma^{EcfG}$ cascade in general stress response and identification of a negative regulator of PhyR**. *J Bacteriol* 2011, **193**:6629-6638.

38. Lourenco RF, Kohler C, Gomes SL: **A two-component system, an anti-sigma factor and two paralogous ECF sigma factors are involved in the control of general stress response in *Caulobacter crescentus***. *Mol Microbiol* 2011, **80**:1598-1612.

39. • Wecke T, Halang P, Staron A, Dufour YS, Donohue TJ, Mascher T: **Extracytoplasmic function σ factors of the widely distributed group ECF41 contain a fused regulatory domain**. *MicrobiologyOpen* 2012, **1**:194-213.

ECFs of the widely distributed group ECF41 contain a C-terminal extension that functions as a fused anti-σ like domain, which in addition is also required for σ factor activity. These ECFs neither require an anti-σ factor nor transcriptional induction for regulating their output (see also Ref. [40•]).

40. • Gómez-Santos N, Pérez J, Sánchez-Sutil MC, Moraleda-Muñoz A, Muñoz-Dorado J: **CorE from *Myxococcus xanthus* is a copper-dependent RNA polymerase sigma factor**. *PLoS Genet* 2011, **7**:e1002106.

The activity of CorE-like σ factors (group ECF44) is also regulated by a C-terminal extension, which in this case positively or negatively regulates the activity of these metal-responsive σ factors, based on the presence of Copper(II) or Copper(I) ions, respectively.

41. Hong HJ, Paget MS, Buttner MJ: **A signal transduction system in *Streptomyces coelicolor* that activates the expression of a putative cell wall glycan operon in response to vancomycin and other cell wall-specific antibiotics**. *Mol Microbiol* 2002, **44**:1199-1211.

42. Paget MS, Chamberlin L, Atrih A, Foster SJ, Buttner MJ: **Evidence that the extracytoplasmic function sigma factor $\sigma^E$ is required for normal cell wall structure in *Streptomyces coelicolor* A3(2)**. *J Bacteriol* 1999, **181**:204-211.

43. Ferreira AO, Myers CR, Gordon JS, Martin GB, Vencato M, Collmer A, Wehling MD, Alfano JR, Moreno-Hagelsieb G, Lamboy WF *et al.*: **Whole-genome expression profiling defines the HrpL regulon of *Pseudomonas syringae* pv. tomato DC3000, allows de novo reconstruction of the Hrp cis clement, and identifies novel coregulated genes**. *Mol Plant Microbe Interact* 2006, **19**:1167-1179.

44. Vencato M, Tian F, Alfano JR, Buell CR, Cartinhour S, DeClerck GA, Guttman DS, Stavrinides J, Joardar V, Lindeberg M *et al.*: **Bioinformatics-enabled identification of the HrpL regulon and type III secretion system effector proteins of *Pseudomonas syringae* pv. phaseolicola 1448A**. *Mol Plant Microbe Interact* 2006, **19**:1193-1206.

45. Lan L, Deng X, Zhou J, Tang X: **Genome-wide gene expression analysis of *Pseudomonas syringae* pv. tomato DC3000 reveals overlapping and distinct pathways regulated by *hrpL* and *hrpRS***. *Mol Plant Microbe Interact* 2006, **19**:976-987.

46. Merighi M, Majerczak DR, Stover EH, Coplin DL: **The HrpX/HrpY two-component system activates *hrpS* expression, the first step in the regulatory cascade controlling the *hrp* regulon in *Pantoea stewartii* subsp. *stewartii***. *Mol Plant Microbe Interact* 2003, **16**:238-248.

47. Bibb MJ, Domonkos A, Chandra G, Buttner MJ: **Expression of the chaplin and rodlin hydrophobic sheath proteins in *Streptomyces venezuelae* is controlled by $\sigma^{BldN}$ and a cognate anti-sigma factor, RsbN**. *Mol Microbiol* 2012, **84**:1033-1049.

48. Wösten MM: **Eubacterial sigma-factors**. *FEMS Microbiol Rev* 1998, **22**:127-150.