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Regulation of bacterial RNA polymerase σ factor activity: a structural perspective

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Summary

In bacteria, σ factors are essential for the promoter DNA-binding specificity of RNA polymerase. The σ factors themselves are regulated by anti- σ factors that bind and inhibit their cognate σ factor, and ‘appropriators’ that deploy a particular σ -associated RNA polymerase to a specific promoter class. Adding to the complexity is the regulation of anti- σ factors by both anti-anti- σ factors, which turn on σ factor activity, and co-anti- σ factors that act in concert with their partner anti- σ factor to inhibit or redirect σ activity. While σ factor structure and function is highly conserved, recent results highlight the diversity of structures and mechanisms that bacteria use to regulate σ factor activity, reflecting the diversity of environmental cues that the bacterial transcription system has evolved to respond to.

Introduction

In bacteria, gene expression is regulated primarily at the level of transcription initiation. All bacteria contain a single ~400 kDa multi-subunit core RNA polymerase (RNAP) enzyme that is catalytically competent and can recognize DNA non-specifically, but requires an additional factor, σ , for promoter recognition and initiation [reviewed in [1-3]]. Most σ factors belong to the σ^{70} -family [4], whose members contain at least two domains connected by flexible linkers: σ_2 , which binds the RNAP β' subunit coiled-coil and the promoter -10 element, and σ_4 , which binds the RNAP β subunit flap and promoter -35 elements [5-8]. The σ^{70} -family members share a high degree of sequence and structural conservation within domains σ_2 , and σ_4 [4]. An additional σ family, σ^{54} , does not share significant sequence similarity to the σ^{70} family and is functionally distinct.

All bacteria have at least one essential σ factor that serves to transcribe the genes required for cell viability, and most bacteria harbor alternative σ factors that transcribe operons in response to specific stimuli. The availability and activity of σ factors are controlled, in part, by at least two types of regulatory factors: 1) anti- σ factors that bind and inhibit their cognate σ factor, and 2) appropriators that alter the activity of a specific RNAP holoenzyme. Adding to the complexity is the regulation of the anti- σ factors themselves; either antagonistically by anti-anti- σ 's, cooperatively by co-anti- σ 's, or post-translationally by proteolysis. This review will focus on the structural details of the regulation of σ^{70} -family members by anti- σ , anti-anti- σ , and co-anti- σ factors.

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Regulation of *Escherichia coli* σ^{70} by σ^{70} -binding proteins

The coordinated transcription of the bacteriophage T4 genome is dependent upon T4-encoded regulators that bind to and alter the specificity of the host *Escherichia coli* (*Ec*) RNAP [9]. One regulator expressed early during T4 infection, AsiA, binds specifically and tightly to σ^{70}_4 [10-12], and both inhibits and co-activates transcription. Early during T4 infection, AsiA inhibits the activity of σ^{70} -associated RNAP at host promoters dependent on the -35 element by binding the σ^{70}_4 Helix-Turn-Helix motif (HTH) responsible for -35 element recognition [10,11,13]. Along with reducing transcription from host -35-dependent promoters, AsiA functions as an appropriator by deploying *Ec* RNAP holoenzyme to T4 middle promoters, where it acts in concert with the T4-encoded DNA-binding protein MotA to stimulate middle gene transcription [14,15].

To gain insight into the mechanism of AsiA, the solution structure of the σ^{70}_4 /AsiA complex was determined [16]. Structures of σ_4 have been determined in many different contexts: alone [5], in complex with -35 element DNA [5], in RNAP holoenzyme [6-8], and with anti- σ factors [17,18]. The σ_4 normally forms a structural core of three α -helices (residues 551-599 of *Ec* σ^{70}_4 ; Figure 1a). Remarkably, the binding of AsiA rearranges σ^{70}_4 so that the HTH motif is restructured into one continuous helix (Figure 1b). Not surprisingly, the interaction of AsiA with σ_4 prevents the usual interaction of σ_4 with the β -flap in the RNAP holoenzyme [19,20]. The displacement and restructuring of σ_4 by AsiA are thought to reposition and display elements of σ_4 for interaction with MotA [14,21].

Compared to the alternative σ factors, σ^{70} has the highest affinity for RNAP [22] and is the most abundant σ factor throughout the *Ec* growth cycle [23]. A search for factors that enable alternative σ 's to compete for binding to RNAP yielded a single polypeptide, Rsd, that specifically associates with σ^{70} [24]. The results of subsequent biochemical and genetic experiments suggest that both Rsd and its *Pseudomonas aeruginosa* homolog, AlgQ, a positive regulator of virulence [25,26], sequester σ^{70} in an inactive complex, thus allowing alternative σ 's to exchange with RNAP [26-29]. Structural studies of the σ^{70}_4 /Rsd complex reveal that Rsd binds to residues of σ^{70}_4 that are important for both RNAP and -35 element recognition. Unlike AsiA, however, this binding does not alter the structural core of σ^{70}_4 (Figure 1c; [30]).

Regulation of σ^F activity during sporulation

In *Bacillus* species, σ^F initiates transcription of a cascade of forespore-specific σ factors responsible for the transcription of genes necessary for the morphological development of the spore [31]. The activity of σ^F is regulated by the anti- σ and serine kinase SpoIIAB (AB), and the anti-anti- σ SpoIIAA (AA) [32,33]. Key to the regulation of sporulation is the partner switching of AB from σ^F to AA. The structure of σ^F in complex with AB revealed that AB monomers form a symmetric homodimer that is in complex with a single σ^F molecule, giving rise to an asymmetric complex whereby the RNAP-binding determinants of σ^F are occluded [34]. Several structures of AB in complex with AA have now been solved [35]. These data provided structural evidence for the previously proposed docking model [34,36] that describes how AA induces σ^F release from the σ^F /AB complex (Figure 2a). In this model, one AB protomer of the asymmetric σ^F /AB₂ complex (AB1 of Figure 2a; for more details see Figures 7 and 9 of ref. 32) is more accessible to AA. Upon docking to AB1, the AA molecule displaces σ^F due to electrostatic and steric interactions. The AA molecule can then be phosphorylated by the AB kinase activity and dissociates. This allows for another unphosphorylated AA (if available) to bind to the resulting AB(ADP), forming a stable complex that inhibits the AB kinase and anti- σ activities [37,38]. The model highlights the importance of the asymmetric binding of σ^F to the AB dimer.

Flagellar biosynthesis and the σ^{28} /FlgM complex

In enteric bacteria, the flagellar regulon is divided into a three-tiered transcriptional hierarchy composed of early, middle and late genes. The coordinated assembly of the flagellum is tightly regulated [39], and central to this regulation is the σ /anti- σ pair σ^{28} /FlgM. In response to a plethora of stimuli, the flagellar early genes activate transcription of middle genes. The middle genes encode the components of the hook-basal body (HBB) flagellar substructure, as well as two transcriptional regulators: σ^{28} (encoded by the *fliA* gene), which directs transcription of the extracellular late gene products, and FlgM, a negative regulator of σ^{28} . FlgM binds to and inhibits σ^{28} until completion of the HBB; thereby preventing the expression of the extracellular late gene products prior to the completion of the HBB export apparatus. Once the HBB is completed, FlgM is secreted out of the cell by the HBB, allowing σ^{28} to transcribe the late extracellular gene products.

Biochemical and genetic analyses of the σ^{28} /FlgM complex have now been complemented with the crystal structure of the σ^{28} /FlgM complex from *Aquifex aeolicus* [18]. In this structure, a single FlgM molecule, composed of three α -helices, wraps around a compact σ^{28} molecule (Figure 2b). As a consequence, FlgM sterically occludes the RNAP-binding determinants of σ^{28} and stabilizes extensive interdomain contacts within σ^{28} which mask the σ^{28} promoter-binding determinants.

The extensive interdomain contacts observed within σ^{28} led to the proposal that the conformation of σ^{28} observed in complex with FlgM is stable in solution even in the absence of FlgM, and this proposal was confirmed experimentally using a disulfide crosslinking approach [40]. These findings suggest that the DNA-binding determinants for all σ^{70} -family members may be masked by interdomain contacts in free σ . Core RNAP binding induces large rearrangements of the σ domains, resulting in the exposure of the DNA-binding determinants [18,41].

Response to misfolded proteins in the periplasm: σ^E , RseA and RseB

In *Ec*, response to cell envelope damage is mediated by a Group IV σ factor, σ^E , which is held in an inactive state by interactions with the cytoplasmic domain of the inner membrane-anchored anti- σ^E factor, RseA. Group IV σ factors are alternative σ 's that direct transcription of regulons in response to environmental conditions [42]. The σ^E /RseA interaction is also stabilized by the periplasmic co-anti- σ RseB [43,44]. Release of σ^E from RseA is induced by the presence of misfolded proteins in the periplasm, and is governed by a complex signal transduction pathway involving the regulated proteolysis of RseA [45]. The crystal structure of the cytoplasmic domain of RseA in complex with σ^E , combined with biochemical studies and molecular modeling, revealed that RseA functions as an anti- σ factor by sterically occluding the two major σ^E RNAP-binding determinants (Figure 2c; [17]).

Recently, structural and biochemical analyses of the co-anti- σ^E RseB, a homodimer, show that RseB is composed of two domains; a larger N-terminal domain (D1) linked to a smaller C-terminal domain (D2) [46,47]. Interestingly, the fold of D1 is similar to a class of lipoprotein transporters that have a deep, open hydrophobic β -barrel purported to bind lipids, suggesting that binding of lipids may play a role in the signal transduction cascade activating σ^E . This adds to the complexity of the signaling pathway controlling σ^E activity [45] in ways that have yet to be worked out.

Zinc anti- σ factors

Zinc anti- σ (ZAS) factors bind both Zn^{2+} and their cognate σ factor, and appear to be responsible for regulating the activity of a subset of Group IV σ factors. In *Streptomyces*

coelicolor, the regulatory system that allows the detection and response to thiol-oxidative stress is orchestrated by the σ^R /RsrA complex [48]. Under reducing conditions, σ^R is held in an inactive state by RsrA, a member of the ZAS family. In response to thiol-oxidative stress, RsrA releases the Zn^{2+} ion, which promotes disulphide bond formation between two cysteine residues, Cys11 and Cys44 (formerly coordinated with the Zn^{2+}), leading to a conformational change of RsrA that induces the release of σ^R [49*-51], enabling σ^R to direct the transcription of genes that help mitigate the effects of thiol-oxidative stress [52].

The reactive oxygen species singlet oxygen ($^1\text{O}_2$) is a toxic byproduct of photosynthesis. In the photosynthetic bacterium *Rhodobacter sphaeroides* (*Rsp*), a Group IV σ factor, σ^E , is responsible for the transcription of genes in response to $^1\text{O}_2$ [53]. During the absence of $^1\text{O}_2$, σ^E is held in an inactive 1:1 complex with the ZAS-family member ChrR [54], but upon sensing $^1\text{O}_2$, ChrR releases σ^E . Structural studies of the σ^E /ChrR complex reveal a number of features pertinent to the regulation of the σ^E /ChrR complex [55*]; Figure 2d). First, ChrR comprises two structural domains: an N-terminal domain that interacts with σ^E (termed ASD, for Anti- σ Domain), and a C-terminal β -barrel domain with a Cupin fold (termed CLD, for Cupin-Like Domain; [56]. Second, the ChrR-ASD inhibits σ^E activity by occluding the RNAP binding sites in σ^{E_2} and σ^{E_4} . Third, like AsiA, ChrR rearranges the structure of σ^{E_4} , but the details of the rearrangement are different. Finally, the ASD coordinates a structural Zn^{2+} , which is essential for maintaining the overall fold of the ASD by mediating interactions between the N-terminal loop and the first three α -helices. The ChrR-ASD is sufficient to bind and inhibit σ^E , but will not release σ^E in response to $^1\text{O}_2$. Subsequent experiments revealed that ChrR $^1\text{O}_2$ sensitivity is dependent on the CLD. A second Zn^{2+} ion coordinated by the CLD may play a role in the $^1\text{O}_2$ response [55*].

Surprisingly, the fold of the ChrR-ASD is similar to the portion of RseA that interacts with *Ec* σ^E [17]. Spurred by these findings, the authors used an exhaustive bioinformatic analysis to define the conservation of the ASD. A total of 1,264 open reading frames adjacent to Group IV σ factors were found to contain an ASD, implying that this structural motif has been widely conserved in bacteria as a σ binding domain (Figure 3; [55*]).

Conclusions

The regulation of many key biological processes in bacteria is effected by the interplay between σ factors and their cognate anti- σ factors. The structure/function studies highlighted in this review indicate that anti- σ 's function by directly occluding important core RNAP binding determinants of their cognate σ , as well as by holding the flexibly-linked σ domains in relative orientations that are not compatible with holoenzyme formation and/or promoter binding. This is achieved by multipartite interactions, where the anti- σ interacts with two or more of the σ structural domains simultaneously. Thus, ChrR, FlgM, and RseA not only obscure the core binding determinants of their cognate σ factors, they also tie up the σ 's in a conformation that could not form holoenzyme. Although not visualized in the structure, genetic studies indicate that SpoIIAB simultaneously binds three structural domains of σ^F [57], suggesting SpoIIAB operates through this mechanism as well.

The multipartite interactions between σ 's and anti- σ 's may appear redundant. However, because all the domains of σ interact with core RNAP [2,6,8], occlusion of only one domain by an anti- σ could allow the other domains of σ to bind to RNAP. Although unlikely to allow holoenzyme function, such complexes would be undesirable since they would titrate functional core RNAP away from the transcription cycle. On the other hand, appropriators like T4-AsiA might be expected to interact with only a single domain of σ (such as σ_4) so that they can function as a component of the RNAP holoenzyme.

All σ^{70} family members contain at least two main domains (σ_2 and σ_4) that are highly conserved structurally despite significant sequence variability [17]. The σ_2 domain forms the primary binding interface with core RNAP, recognizes the -10 promoter element, and plays a key role in melting the promoter DNA to form the open complex, while σ_4 recognizes the -35 element and serves as a target for transcription activators [3]. Thus, these two domains perform all of the most critical σ factor functions and represent the elements for basic function common to all σ factors. The structural conservation of these domains between all members of the σ^{70} family indicates that functional constraints allow little room for structural evolution of σ factors.

Unlike the σ factors, the anti- σ 's are highly diverse in sequence and structure (Figure 2). Alternative σ 's must modulate the activity of a common core transcriptional apparatus in response to essentially all possible environmental conditions that a bacterium may encounter. The diversity of anti- σ structure and regulation reflects the wide variety of stimuli that anti- σ 's respond to, and the wide variety of regulatory mechanisms that control their function. The evolution of structurally and functionally diverse anti- σ 's provides much more flexibility for regulation of transcription initiation. Thus, the functional and structural diversity of anti- σ 's reflect the need of bacteria to relay a wide variety of environmental cues to the core transcriptional apparatus via regulation of the structurally conserved σ factors.

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References and recommended reading

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

- of special interest
- of outstanding interest

1. Paget MS, Helmann JD. The sigma 70 family of sigma factors. *Genome Biology* 2003;4:203. [PubMed: 12540296]
2. Murakami K, Darst SA. Bacterial RNA polymerases: the whole story. *Current Opinion in Structural Biology* 2003;13:31–39. [PubMed: 12581657]
3. Gross CA, Chan C, Dombroski A, Gruber T, Sharp M, Tupy J, Young B. The functional and regulatory roles of sigma factors in transcription. *Cold Spring Harbor Symposia on Quantitative Biology* 1998;63:141–155.
4. Lonetto M, Gribskov M, Gross CA. The σ^{70} family: Sequence conservation and evolutionary relationships. *Journal of Bacteriology* 1992;174:3843–3849. [PubMed: 1597408]
5. Campbell EA, Muzzin O, Chlenov M, Sun JL, Olson CA, Weinman O, Trester-Zedlitz ML, Darst SA. Structure of the bacterial RNA polymerase promoter specificity sigma factor. *Molecular Cell* 2002;9:527–539. [PubMed: 11931761]
6. Murakami K, Masuda S, Darst SA. Structural basis of transcription initiation: RNA polymerase holoenzyme at 4 Å resolution. *Science* 2002;296:1280–1284. [PubMed: 12016306]
7. Murakami K, Masuda S, Campbell EA, Muzzin O, Darst SA. Structural basis of transcription initiation: An RNA polymerase holoenzyme/DNA complex. *Science* 2002;296:1285–1290. [PubMed: 12016307]

8. Vassylyev DG, Sekine S, Laptenko O, Lee J, Vassylyeva MN, Borukhov S, Yokoyama S. Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 Å resolution. *Nature* 2002;417:712–719. [PubMed: 12000971]
9. Nechaev S, Severinov K. Bacteriophage-induced modifications of host RNA polymerase. *Annual Review of Microbiology* 2003;57:301–322.
10. Colland F, Orsini G, Brody E, Buc H, Kolb A. The bacteriophage T4 AsiA protein: a molecular switch for sigma 70-dependent promoters. *Molecular Microbiology* 1998;27:819–829. [PubMed: 9515707]
11. Severinova E, Severinov K, Darst SA. Inhibition of *Escherichia coli* RNA polymerase by bacteriophage T4 AsiA. *Journal of Molecular Biology* 1998;279:9–18. [PubMed: 9636696]
12. Stevens A, Rhoton RC. Characterization of an inhibitor causing potassium chloride sensitivity of an RNA polymerase from T4 phage-infected *Escherichia coli*. *Biochemistry* 1975;14:5074–5079. [PubMed: 1103966]
13. Minakhin L, Camarero JA, Holford M, Parker C, Muir TW, Severinov K. Mapping the molecular interface between the sigma(70) subunit of *E. coli* RNA polymerase and T4 AsiA. *Journal of Molecular Biology* 2001;306:631–642. [PubMed: 11243776]
14. Baxter K, Lee J, Minakhin L, Severinov K, Hinton DM. Mutational analysis of sigma 70 region 4 needed for appropriation by the bacteriophage T4 transcription factors AsiA and MotA. *Journal of Molecular Biology* 2006;363:931–944. [PubMed: 16996538]
15. Hinton DM, March-Amegadzie R, Gerber JS, Sharma M. Characterization of pre-transcription complexes made at a bacteriophage T4 middle promoter: Involvement of the T4 MotA activator and the T4 AsiA protein, a sigma 70 binding protein, in the formation of the open complex. *Journal of Molecular Biology* 1996;256:235–248. [PubMed: 8594193]
16. Lambert LJ, Wei Y, Schirf V, Demeler B, Werner MH. T4 AsiA blocks DNA recognition by remodeling sigma70 region 4. *EMBO Journal* 2004;23:2952–2292. [PubMed: 15257291]
17. Campbell EA, Tupy JL, Gruber TM, Wang S, Sharp MM, Gross CA, Darst SA. Crystal structure of *Escherichia coli* σ^E with the cytoplasmic domain of its anti- σ RseA. *Molecular Cell* 2003;11:1067–1078. [PubMed: 12718891]
18. Sorenson MK, Ray SS, Darst SA. Crystal structure of the flagellar σ /anti- σ complex σ^{28} /FlgM reveals an intact σ factor in an inactive conformation. *Molecular Cell* 2004;14:127–138. [PubMed: 15068809]
19. Simeonov MF, Bieber Urbauer RJ, Gilmore JM, Adelman K, Brody EN, Niedziela-Majka A, Minakhin L, Heyduk T, Urbauer JL. Characterization of the interactions between the bacteriophage T4 Asia protein and RNA polymerase. *Biochemistry* 2003;42:7717–7726. [PubMed: 12820881]
20. Gregory BD, Nickels BE, Garrity SJ, Severinova E, Minakhin L, Urbauer RJ, Urbauer JL, Heyduk T, Severinov K, Hochschild A. A regulator that inhibits transcription by targeting an intersubunit interaction of the RNA polymerase holoenzyme. *Proceedings of the National Academy of Sciences USA* 2004;101:4554–4559.
21. Pande S, Makela A, Dove SL, Nickels BE, Hochschild A, Hinton DM. The bacteriophage T4 transcription activator MotA interacts with the far-C-terminal region of the sigma70 subunit of *Escherichia coli* RNA polymerase. *Journal of Bacteriology* 2002;184:3957–3964. [PubMed: 12081968]
22. Maeda H, Fujita N, Ishihama A. Competition among seven *Escherichia coli* sigma subunits: Relative binding affinities to the core RNA polymerase. *Nucleic Acids Research* 2000;28:3497–3503. [PubMed: 10982868]
23. Jishage M, Iwata A, Ueda S, Ishihama A. Regulation of RNA polymerase sigma subunit synthesis in *Escherichia coli*: intracellular levels of sigma 70 and sigma 38. *Journal of Bacteriology* 1996;178:5447–5451. [PubMed: 8808934]
24. Jishage M, Ishihama A. A stationary phase protein in *Escherichia coli* with binding activity to the major sigma subunit of RNA polymerase. *Proceedings of the National Academy of Sciences USA* 1998;95:4953–4958.
25. Deretic K, Konyecsni WM. Control of mucoidy in *Pseudomonas aeruginosa*: Transcriptional regulation of *algR* and identification of the second regulatory gene, *algQ*. *Journal of Bacteriology* 1989;171:3680–3688. [PubMed: 2544550]

26. Ambrosi C, Tiburzi F, Imperi F, Putignani L, Visca P. Involvement of AlgQ in transcriptional regulation of pyoverdine genes in *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology* 2005;187:5097–5107. [PubMed: 16030202]
27. Jishage M, Ishihama A. Transcriptional organization and *in vivo* role of the *Escherichia coli* *rsd* gene, encoding the regulator of RNA polymerase sigma D. *Journal of Bacteriology* 1999;181:3768–3776. [PubMed: 10368152]
28. Jishage M, Kvint K, Shingler V, Nystrom T. Regulation of sigma factor competition by the alarmone ppGpp. *Genes & Development* 2002;16:1260–1270. [PubMed: 12023304]
29. Laurie AD, Bernardo LM, Sze CC, Skarfstad E, Szalewska-Palasz A, Nystrom T, Shingler V. The role of the alarmone (p)ppGpp in sigma N competition for core RNA polymerase. *Journal of Biological Chemistry* 2003;278:1494–1503. [PubMed: 12421818]
30. Patikoglou GA, Westblade LF, Campbell EA, Lamour V, Lane WJ, Darst SA. Crystal structure of the *Escherichia coli* regulator of sigma70, Rsd, in complex with sigma70 domain 4. *Journal of Molecular Biology* 2007;372:649–659. [PubMed: 17681541]
31. Losick R, Pero J. Cascades of sigma factors. *Cell* 1981;25:582–584. [PubMed: 6793235]
32. Duncan L, Losick R. SpoIIAB is an anti- σ factor that binds to and inhibits transcription by regulatory protein σ^F from *Bacillus subtilis*. *Proceedings of the National Academy of Sciences USA* 1993;90:2325–2329.
33. Min KT, Kilditch CM, Deiderich B, Errington J, Yudkin MD. σ^F , the first compartment-specific transcription factor of *B. subtilis*, is regulated by an anti- σ factor that is also a protein kinase. *Cell* 1993;74:735–742. [PubMed: 8358793]
34. Campbell EA, Masuda S, Sun JL, Muzzin O, Olson CA, Wang S, Darst SA. Crystal structure of the *Bacillus stearothermophilus* anti-sigma factor SpoIIAB with the sporulation sigma factor sigmaF. *Cell* 2002;108:795–807. [PubMed: 11955433]
35. Masuda S, Murakami KS, Wang S, Olson CA, Donigian J, Leon F, Darst SA, Campbell EA. Crystal structures of the ADP and ATP bound forms of the *Bacillus* anti-s factor SpoIIAB in complex with the anti-anti-s SpoIIAA. *Journal of Molecular Biology* 2004;340:941–956. [PubMed: 15236958]
36. Ho MS, Carniol K, Losick R. Evidence in support of a docking model for the release of the transcription factor sigma F from the antisigma factor SpoIIAB in *Bacillus subtilis*. *Journal of Biological Chemistry* 2003;278:20898–20905. [PubMed: 12676949]
37. Diederich B, Wilkinson JF, Magnin T, Najafi SMA, Errington J, Yudkin MD. Role of Interactions between SpoIIAA and SpoIIAB in regulating cell-specific transcription factor σ^F of *Bacillus subtilis*. *Genes and Development* 1994;8:2653–2663. [PubMed: 7958923]
38. Duncan L, Alper S, Losick R. SpoIIAA governs the release of the cell-type specific transcription factor σ^F from its anti-sigma factor SpoIIAB. *Journal of Molecular Biology* 1996;260:147–164. [PubMed: 8764397]
39. Chilcott GS, Hughes KT. Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar typhimurium and *Escherichia coli*. *Microbiology and Molecular Biology Reviews* 2000;64:694–708. [PubMed: 11104815]
40. Sorenson MK, Darst SA. Disulfide cross-linking indicates that FlgM-bound and free σ^{28} adopt similar conformations. *Proceedings of the National Academy of Sciences USA* 2006;103:16722–16727.
41. Callaci S, Heyduk E, Heyduk T. Core RNA polymerase from *E. coli* induces a major change in the domain arrangement of the sigma70 subunit. *Molecular Cell* 1999;3:229–238. [PubMed: 10078205]
42. Helmann JD. The extracytoplasmic function (ECF) sigma factors. *Advances in Microbial Physiology* 2002;46:47–110. [PubMed: 12073657]
43. De Las Penas A, Connolly L, Gross CA. The σ^E -mediated response to extracytoplasmic stress in *Escherichia coli* is transduced by RseA and RseB two negative regulators of σ^E . *Molecular Microbiology* 1997;24:373–385. [PubMed: 9159523]
44. Missiakas D, Mayer MP, Lemaire M, Georgopoulos C, Raina S. Modulation of the *Escherichia coli* σ^E (RpoE) heat-shock transcription-factor activity by the RseA, RseB and RseC proteins. *Molecular Microbiology* 1997;24:355–371. [PubMed: 9159522]
45. Ades SE. Control of the alternative sigma factor σ^E in *Escherichia coli*. *Current Opinion in Microbiology* 2007;7:156–162.

46. Wollmann P, Zeth K. The structure of RseB: a sensor in periplasmic stress response of *E. coli*. *Journal of Molecular Biology* 2007;372:927–941. [PubMed: 17692869]
47. Kim DY, Jin KS, Kwon E, Ree M, Kim KK. Crystal structure of RseB and a model of its binding mode to RseA. *Proceedings of the National Academy of Sciences USA* 2007;104:8779–8784.
48. Kang J-G, Paget MS, Seok Y-J, Hahn M-Y, Bae J-B, Hahn J-S, Kleanthous C, Buttner MJ, Roe J-H. RsrA, and anti-sigma factor regulated by redox change. *EMBO Journal* 1999;18:4292–4298. [PubMed: 10428967]
- *49. Zdanowski K, Doughty P, Jakimowicz P, O'Hara L, Buttner MJ, Paget MS, Kleanthous C. Assignment of the zinc ligands in RsrA, a redox-sensing ZAS protein from *Streptomyces coelicolor*. *biochemistry* 2006;45:8294–8300. [PubMed: 16819828]• This paper describes the thorough analysis of the Zn^{2+} ion ligands in RsrA. Using extended X-ray absorption fine structure (EXAFS) spectroscopy and mutagenesis, the authors define those residues that coordinate the RsrA Zn^{2+} ion, and which residues, upon Zn^{2+} expulsion, lead to disulfide formation.
50. Li W, Bottrill AR, Bibb MJ, Buttner MJ, Paget MS, Kleanthous C. The role of zinc in the disulphide stress-regulated anti-sigma factor RsrA from *Streptomyces coelicolor*. *Journal of Molecular Biology* 2003;333:461–472. [PubMed: 14529630]
51. Bae JB, Park JH, Hahn MY, Kim MS, Roe J-H. Redox-dependent changes in RsrA, and anti-sigma factor in *Streptomyces coelicolor*: zinc release and disulfide bond formation. *Journal of Molecular Biology* 2004;335:425–435. [PubMed: 14672653]
52. Paget MS, Molle V, Cohen G, Aharonowitz Y, Buttner MJ. Defining the disulphide stress response in *Streptomyces coelicolor* A3(2): Identification of the sigmaR regulon. *Molecular Microbiology* 2001;42:1007–1020. [PubMed: 11737643]
53. Anthony JR, Warczak KL, Donohue TJ. A transcriptional response to singlet oxygen, a toxic byproduct of photosynthesis. *Proceedings of the National Academy of Sciences USA* 2005;102:6502–6507.
54. Newman JD, Anthony JR, Donohue TJ. The importance of zinc-binding to the function of *Rhodobacter sphaeroides* ChrR as an anti-sigma factor. *Journal of Molecular Biology* 2001;313:485–499. [PubMed: 11676534]
- *55. 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. *Molecular Cell* 2007;27:793–805. [PubMed: 17803943]• This paper describes the first structure of a ZAS anti- σ factor. The authors define a conserved structural motif, the ASD, found in many Group IV anti- σ 's. An extensive bioinformatic analyses of Group IV anti- σ 's identifies putative ASD's in a large number of Group IV anti- σ 's.
56. Dunwell JM, Purvis A, Khuri S. Cupins: the most functionally diverse protein superfamily? *Phytochemistry* 2004;65:7–17. [PubMed: 14697267]
57. Decatur AL, Losick R. Three sites of contact between the *Bacillus subtilis* transcription factor σ^F and its antisigma factor SpoIIAB. *Genes & Development* 1996;10:2348–2358. [PubMed: 8824593]

Figure 1. The conformation of σ_4 when bound to bacteriophage T4 AsiA or Rsd

The σ_4 's are shown as ribbon diagrams.

(A) On the left is *Thermus aquaticus* (*Taq*) σ_4^A alone [5], showing the native conformation of σ_4 .

(B) *Ec* $\sigma_{70_4}^{\sigma_{70_4}}$ from the σ_{70_4} /AsiA complex [16], with AsiA shown as a transparent blue worm. The fold of σ_4 is altered (compare with A).

(C) *Ec* $\sigma_{70_4}^{\sigma_{70_4}}$ from the σ_{70_4} /Rsd complex [30], with Rsd shown as a transparent green worm. Each view is oriented so that the recognition helices are aligned. The fold of σ_4 is identical to σ_4 alone (part A).

Figure 2. Versatility of classical σ /anti- σ structures and mechanisms

(A) Superimposition of AB₂ in complex with σ^F with AB₂ in complex with AA [34,35]. This figure illustrates that the asymmetric binding of σ^F_3 (blue surface representation, the other σ domains were present but disordered in the σ^F /AB₂ crystal structure) to the AB₂ results in one AB protomer (AB1, on the right, colored magenta) being more accessible for binding to AA (colored green). The nucleotides bound to AB are drawn in stick and the ATPase active site Mg²⁺ ions are drawn as green spheres.

(B) Structure of the *Aae* σ^{28} (FliA)/FlgM complex [18]. FlgM (colored red) binds to and occludes the σ^{28} RNAP-binding domains (shaded gray) on σ^{28}_2 (colored green) and σ^{28}_4 (colored yellow). σ^{28}_3 is colored blue.

(C) Structure of the σ^E /RseA complex [17]. σ^E (surface representation) regions are colored σ^E_2 , green; σ^E_{linker} , orange; σ^E_4 , yellow. Surfaces that bind RNAP are shaded gray. RseA, colored red, occludes both major RNAP binding surfaces and is sandwiched between the σ^E_2 and σ^E_4 . RseB binds to RseA in the periplasm, forming a heterotetramer of RseA₂:RseB₂.

(D) The anti- σ ChrR is composed of two functionally and structurally modular domains [55*]. The structure illustrates that σ^E (colored similarly as σ^E (B)) interacts exclusively with the ChrR-ASD (colored bright pink). The second domain, ChrR-CLD (colored light pink), which is required to respond to 1O_2 , is connected to the ASD via a partially disordered linker (drawn as magenta dots). Both domains contain Zn²⁺ (shown as grey spheres): The ASD Zn²⁺ serves an essential structural function, although other functions have not been ruled out.

The roles of both the ASD-Zn²⁺ and CLD-Zn²⁺ are currently under investigation (Tim Donohue, personal communication).

Figure 3. Structural conservation of the N-terminal domains of ECF anti- σ 's

(top) Structural alignments of the N-terminal domains of RseA and ChrR reveal a similar fold for the first three helices (termed the ASD). Both molecules are rendered as α -carbon backbone worms, with RseA-ASD colored green and ChrR-ASD colored pink. The ChrR-Zn²⁺ is rendered as a grey sphere. (bottom) Sequence alignments were generated from the structural alignment and optimized using known Zn²⁺ ligands and secondary structure predictions [55*]. The α -helices from the anti- σ crystal structures are shown schematically above (ChrR, pink cylinders [55*]) and below (RseA, green cylinders [17]) the sequences.