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

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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 are 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.

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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.

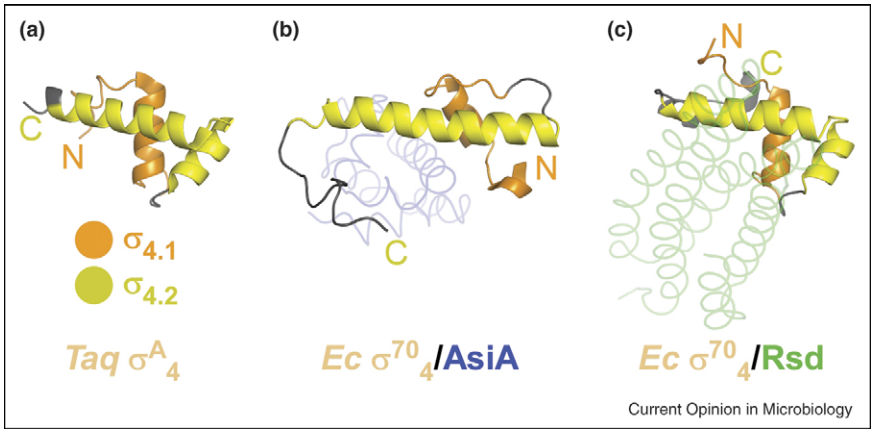
All bacteria have at least one essential σ factor that serves to transcribe the genes required for cell viability, and most bacteria harbor alternative σ factors that transcribe operons in response to specific stimuli. The availability and activity of σ factors are controlled, in part, by at least two 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.

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_4^{70} [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_4^{70} 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_4^{70}/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_4^{70}; Figure 1a). Remarkably, the binding of AsiA rearranges σ_4^{70} 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].

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)** *E. coli* σ_4^{70} from the σ_4^{70}/AsiA complex [16], with AsiA shown as a transparent blue worm. The fold of σ_4 is altered (compare with **(a)**). **(c)** *E. coli* σ_4^{70} from the σ_4^{70} /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)**).

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 access RNAP [26–29]. Structural studies of the σ_4^{70} /Rsd complex reveal that Rsd binds to residues of σ_4^{70} that are important for both RNAP and – 35 element recognition. Unlike AsiA, however, this binding does not alter the structural core of σ_4^{70} (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 protomers 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/AB_2 complex (AB1 of Figure 2a; for more details see Figures 7 and 9 of Ref. [35]) is more accessible to AA. Upon docking to AB1, the AA molecule displaces σ^F because of 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 before 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*