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Different Requirements for σ Region 4 in BvgA Activation of the *Bordetella pertussis* Promoters P_{fim3} and P_{fhaB}

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Bordetella pertussis BvgA is a global response regulator that activates virulence genes, including adhesin-encoding *fim3* and *fhaB*. At the *fhaB* promoter, P_{fhaB}, a BvgA binding site lies immediately upstream of the −35 promoter element recognized by Region 4 of the σ subunit of RNA polymerase (RNAP). We demonstrate that σ Region 4 is required for BvgA activation of P_{fhaB}, a hallmark of Class II activation. In contrast, the promoter-proximal BvgA binding site at P_{fim3} includes the −35 region, which is composed of a tract of cytosines that lacks specific sequence information. We demonstrate that σ Region 4 is not required for BvgA activation at P_{fim3}. Nonetheless, Region 4 mutations that impair its typical interactions with core and with the −35 DNA affect P_{fim3} transcription. Hydroxyl radical cleavage using RNAP with σD581C–FeBABE positions Region 4 near the −35 region of P_{fim3}; cleavage using RNAP with α276C–FeBABE or α302C–FeBABE also positions an α subunit C-terminal domain within the −35 region, on a different helical face from the promoter-proximal BvgA~P dimer. Our results suggest that the −35 region of P_{fim3} accommodates a BvgA~P dimer, an α subunit C-terminal domain, and σ Region 4. Molecular modeling suggests how BvgA, σ Region 4, and α might coexist within this DNA in a conformation that suggests a novel mechanism of activation.

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Introduction

Pertussis is a reemerging disease in developed nations and a continued presence in the developing world. Although vaccines are available, they do not provide lifetime coverage; outbreaks of pertussis have occurred in populations that were vaccinated a few years earlier.[1–3] *Bordetella pertussis*, the causative agent of pertussis, employs a simple yet elegant system to regulate its virulence genes. In the environment of the human host, the BvgS–BvgA two-component system is active, resulting in the phosphorylation of BvgA (BvgA~P) (reviewed in Ref. 4). BvgA~P is a global activator, which binds to promoters of virulence genes with varied stoichiometry and affinity. Thus, upon entry into an inducing environment, the increasing intracellular concentration of BvgA~P orchestrates a temporal program of virulence gene expression.[5] Genes that are activated

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Abbreviations used: CTD, C-terminal domain; RNAP, RNA polymerase; wt, wild type; PDB, Protein Data Bank; BG, Bordet–Gengou; MCS, multi-cloning site; EDTA, ethylenediaminetetraacetic acid; GTP, guanosine triphosphate; TBE, Tris/borate/EDTA.

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early include those encoding adhesive proteins, such as *fhaB* (filamentous hemagglutinin) and *fim2* and *fim3* (fimbrial subunits). Late-activated genes include those encoding toxic virulence factors such as adenylate cyclase and pertussis toxin.[6] The temporal program of gene expression is thought to be crucial for the virulence of the pathogen,[7,8] as demonstrated in a murine model of *B. pertussis* respiratory infection.[7] Thus, understanding the molecular mechanism of *B. pertussis* virulence gene expression and its regulation by BvgS–BvgA will help us to develop better strategies to fight the disease.

As in all bacteria, a multisubunit RNA polymerase (RNAP) consisting of a core of subunits (α_1, α_2, β, β′, and ω) and a specificity factor (σ) transcribes the *B. pertussis* genome. Promoter recognition involves multiple interactions between regions of σ and the promoter DNA. For primary σ factors, such as σ^{Bp} in *B. pertussis* or σ^{70} in *Escherichia coli*, these contacts can include Region 2 with the −10 element, Region 3 with the sequence $^{-15}$TG^{-14} (extended −10 element), and Region 4 with the −35 element (reviewed in Ref. 10 and 11). As good matches to two out of three of these elements are typically sufficient for promoter activity, many promoters can be classified as belonging to the −35/−10, TGn/−10 (extended −10), or −35/TGn class. In addition, the C-terminal domains (CTDs) of the α subunits of RNAP contribute to promoter binding through their interaction with DNA sequences (UP elements) upstream of position −40 (reviewed in Ref. 12).

Regulated promoters require additional factors besides RNAP, usually to compensate for weak σ/DNA element interactions. In Class I activation, the activator may bind to several locations upstream of the −35 element and interact directly with the αCTDs; in Class II activation, the activator binds to a site adjacent to or overlapping the −35 element and contacts an αCTD and/or σ Region 4 (reviewed in Refs. 13 and 14). Class I and Class II activation may also function in combination with each other, but in each case, σ Region 4 is thought to be required in order to maintain contacts with the −35 region of the DNA.

σ appropriation is a different type of activation used by bacteriophage T4, in which the binding site for the T4 activator MotA includes the −35 region of the DNA (reviewed in Ref. 15). Thus, MotA and σ Region 4 are essentially competing for the DNA. In this case, the T4-encoded co-activator AsiA binds to Region 4, remodeling its structure and preventing its interaction with the −35 portion of the promoter. However, σ Region 4 is still required because of its needed interactions with AsiA and MotA.

Most of the virulence genes in *B. pertussis* have an organization that resembles that seen at Class I/Class II-activated promoters.[16,17] For example, the *fhaB* gene is activated by three dimers of BvgA~P, which bind from −95 to −38 relative to the transcription start site[18,19] (Fig. 1a). In contrast, at the promoters for *fim2* (P_{fim2}) and *fim3* (P_{fim3}; Fig. 1a), the promoter-proximal BvgA~P site fully includes the −35 region of the DNA.[20] Such a position is similar to the binding sites of some class II activators.[21–23] In addition, both P_{fim2} and P_{fim3} contain a tract of poly-cytosines (C), which is located upstream of the −10 element and includes the −35 region.[20] The length of this C-tract regulates BvgA~P activation.[24] We have recently shown that the C-tract is needed to position BvgA~P correctly relative to the bound RNAP; however, there is little, if any, specific sequence information imparted by the tract.[20] The second unusual feature of P_{fim3} is that it contains both the $^{-15}$TG^{-14} motif, which is recognized by σ Region 3, and a good match ($^{-12}$TATTCT^{-7}) to the −10 element, which is recognized by σ Region 2. Thus, P_{fim3} appears to be a TGn/−10 (extended −10) promoter, and we would predict that it would exhibit some activity with RNAP alone.[25–27] However, P_{fim3} displays very little activity in the absence of BvgA~P.[20]

In this study, we have investigated BvgA~P activation at P_{fhaB} and P_{fim3}. We show that σ Region 4 is required for BvgA~P activation at P_{fhaB}, providing further evidence that the promoter-proximal BvgA~P functions as a Class II activator at this promoter. In contrast, BvgA~P activation at P_{fim3} is different. Although we find that it does not require σ Region 4, we also find that Region 4 is still located close to the P_{fim3} −35 DNA. Furthermore, using RNAP reconstituted with FeBABE-conjugated α subunits, we find that each α contacts the same region of P_{fim3} as BvgA, but on a different helical face. Taken together, we speculate that within the activated complex at P_{fim3}, σ Region 4, BvgA~P, and an αCTD are all located within the −35 region C-tract of the DNA.

Results

Experimental design and description of σ mutants

The C-terminal portion of primary σ factors, such as *E. coli* σ^{70}, is composed of five α helices (H1–H5) with a turn (T) between H3 and H4 (Fig. 1b).[28–30] Residues within σ^{70} Region 4 (H1–H4) and H5 perform several important functions, including interaction with the β-flap of core polymerase, recognition of the −35 element promoter DNA, and interactions with regulators that are needed to activate promoters by Class II activation and by σ appropriation (Fig. 1b) (reviewed in Refs. 15, 31, and 32). The interaction with the β-flap positions Region 4 so that it is located close to the −35 DNA, which then allows residues within the second helix of the helix–turn–helix (H3–T–H4) to make specific contact with base determinants within the −35 DNA (Fig. 1b).[28,33–37]

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