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Genetics

Autogenous regulation of the *Bordetella pertussis bvgABC* operon

(signal transduction/coordinate regulation/two-component system/virulence genes/transcriptional fusions)

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ABSTRACT **The *bvgABC* operon of the bacterial pathogen *Bordetella pertussis* encodes a sensory transduction system that regulates the expression of several virulence genes in response to environmental stimuli. In this study we have examined the transcriptional regulation of the *bvgABC* operon. Transcriptional *bvg::lacZYA* fusions in *Escherichia coli* show that the *bvgABC* operon is autogenously activated. Autoactivation is inhibited by the same environmental stimuli that result in the lack of expression of *bvg*-activated genes in *B. pertussis*. These observations were confirmed in *B. pertussis* using a chromosomal chloramphenicol acetyltransferase transcriptional fusion in *bvgC*. Transcriptional initiation sites upstream of *bvgA* were mapped by primer extension analysis in *E. coli* and *B. pertussis*. Two differentially regulated *bvg* promoters were identified. The bvg_{P1} promoter is a positively autoregulated promoter located 90 base pairs upstream of *bvgA*. The bvg_{P2} promoter is located 141 base pairs upstream of *bvgA* and does not appear to require any positive regulatory factors for activity. These results suggest a model describing the regulatory events that take place upstream of the *bvgABC* operon.**

The *bvgABC* (*vir*) operon is required for the coordinate regulation of numerous virulence-associated factors produced by *Bordetella pertussis*, a respiratory pathogen (1, 2). Expression of these virulence factors is regulated by environmental stimuli through a signal transduction phenomenon called phenotypic modulation (3, 4). Genes that are positively regulated by the *bvgABC* operon are no longer expressed by *B. pertussis* cells grown at low temperature (30°C vs. 37°C) or at 37°C in the presence of high levels of $MgSO_4$ or nicotinic acid; in contrast, under the same conditions the otherwise silent *bvg*-repressed genes are now expressed (5).

Genetic and DNA sequence analyses of the *bvgABC* operon suggest that the *bvgA* and *bvgC* products are members of the two-component family of bacterial signal transduction proteins (6). Prokaryotic organisms utilize two-component systems to regulate cellular functions in response to environmental conditions (7). The BvgA protein is homologous to regulator components as demonstrated by the presence of an N-terminal "receiver" domain, whereas BvgC shares homology with sensor components and contains a C-terminal "transmitter" domain (8, 9).

We have previously described an *in vivo* system to examine cis-acting sequences and trans-acting factors involved in *bvg*-mediated transcriptional regulation and sensory transduction (10). This system was developed in *Escherichia coli* and contains the promoter region of the *bvg*-activated *fhaB* gene attached to a promoterless *lacZYA* operon on a λ prophage. The single-copy *fhaB::lacZYA* fusion is expressed when the *bvgABC* operon is present in trans on a multicopy plasmid. The fusion can also be activated by overexpressing *bvgA* alone, demonstrating that *bvgA* encodes a transcriptional activator (11). Furthermore, using this system we have shown that the products of the *bvgABC* operon mediate the response to $MgSO_4$, nicotinic acid, and temperature.

In this study we have used transcriptional fusions and primer extension in *B. pertussis* and *E. coli* to examine the regulation of the *bvgABC* operon. Our results show that the *bvgABC* operon is autogenously activated in a manner that responds to environmental signals. In addition, the operon is transcribed by two promoters that function under different conditions. A possible mechanism for autogenous regulation of the *bvgABC* operon is presented.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions. The bacterial strains and plasmids used in this study are described in Table 1. *E. coli* strains were grown on L-agar plates (10 g of tryptone per liter, 5 g of yeast extract per liter, 10 g of NaCl per liter, and 15 g of agarose per liter) or in L broth. Antibiotics were supplemented for *E. coli* strains at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 40 μg/ml; tetracycline, 15 μg/ml. *B. pertussis* strains were grown on Bordet–Gengou (BG; Difco) plates containing 15% sheep blood. Broth-grown *B. pertussis* were cultured in Verwey medium (17) (1 g of soluble starch per liter, 0.2 g of KCl per liter, 0.5 g of KH_2PO_4 per liter, 0.1 g of $MgCl_2 \cdot 6H_2O$ per liter, 0.02 g of nicotinic acid per liter, 0.01 g of glutathione per liter, 14 g of Casamino acids per liter, pH 6.8). Antibiotics were supplemented for *B. pertussis* strains at the following concentrations: ampicillin, 50 μg/ml; streptomycin, 400 μg/ml; tetracycline, 10 μg/ml; rifampin, 20 μg/ml.

Recombinant DNA Methods. DNA ligations, restriction endonuclease digestions, and agarose gel electrophoresis were performed as described (18). Bacterial electroporation was used to introduce plasmid DNA into *E. coli* (19). A Sequenase kit (United States Biochemical) was used to determine the DNA sequence of double-stranded plasmid templates as described by the manufacturer. Enzymes were purchased from New England BioLabs and Bethesda Research Laboratories.

β-Galactosidase (β-gal) Assay. *E. coli* strains containing *lacZYA* fusions were grown in L broth to midlogarithmic phase as described (11). β-gal activity was assayed by the method of Miller (20).

Construction of *bvg::lacZYA* Fusions. Restriction fragments containing the 5′ region of the *bvgABC* operon were isolated and inserted upstream of the promoterless *lacZYA* genes in the plasmid pRS551 (16). The *bvg::lacZYA* fusions were then transferred from multicopy plasmids onto the bacteriophage λRS45 by *in vivo* homologous recombination. The recombinant λ phage containing the *bvg::lacZYA* fusions were used to lysogenize the chromosome of *E. coli* MC4101 resulting in a single-copy fusion.

Bacterial Conjugations. Plasmids were conjugatively transferred from the donor strain *E. coli* SM10 to *B. pertussis* as described (21).

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Abbreviations: β-gal, β-galactosidase; CAT, chloramphenicol acetyltransferase.

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Table 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or ref.
Strain		
E. coli		
SM10	RP4-2 Tc::Mu	12
MC4100	Δ(*lacZYA-argF*)U169	10
MC4101	MC4100 *recA1*	10
CR430	MC4101 λCR430 *bvgA::lacZYA*	This study
CR432	MC4101 λCR432 *bvgAB::lacZYA*	This study
CR434	MC4101 λCR434 *bvgABC::lacZYA*	This study
B. pertussis		
BP369	*bvgC1*	13
BP370	Wild type	14
BP3703	BP370 Δ*bvgA*	11
CF1770	BP370 *bvgC*::CAT	6
Plasmid		
pRK290	Broad host-range vector	15
pRS551	*lacZYA* fusion vector	16
pJM26	*bvgABC*$^+$ in pBR322	10
pSS528	*bvgABC*$^+$ in pRK290	2
pUW1004	*bvgABC*$^+$ in pACYC184	2
pCR430	*bvgA' Eco*RI–*Sty* I fragment in pRS551	This study
pCR432	*bvgAB' Eco*RI–*Stu* I fragment in pRS551	This study
pCR434	*bvgABC' Eco*RI–*Bgl* II fragment in pRS551	This study
pCR435	*bvgA*$^+$ in pKS−	11

Chloramphenicol Acetyltransferase (CAT) Assay. *B. pertussis* strains containing transcriptional CAT fusions were grown to midlogarithmic phase in Verwey broth. The bacteria were chilled on ice and pelleted at 5000 × *g* for 10 min at 4°C. The bacterial pellets were resuspended to an OD_{600} of 1.0 in 250 mM Tris·HCl (pH 7.8), and then 2 ml of cells was sonicated at 4°C. The sonicated extracts were spun for 15 min at 4°C in a Microfuge to remove bacterial debris. The supernatants were heated to 65°C for 10 min and spun again in a Microfuge at 4°C. The extracts were then stored at −20°C. Each CAT assay reaction mixture contained 0.5 μg of protein extract, 2 μl of [^{14}C]chloramphenicol (200 μCi/ml; 1 Ci = 37 GBq; Amersham), 20 μl of 4 mM acetyl-coenzyme A in 250 mM Tris·HCl (pH 7.8) (Boehringer Mannheim), and 250 mM Tris·HCl (pH 7.8) to bring the final volume to 172 μl. The reaction mixtures were incubated for 1 hr at 37°C. The acetylated products were separated by thin-layer chromatography (TLC) as described by Gorman *et al.* (22). Percent acetylation was determined for each reaction by scanning the TLC plates on an Ambis radioanalytic imaging system (Ambis Systems, San Diego) to determine the specific activity of acetylated and nonacetylated species of [^{14}C]chloramphenicol. CAT activity is represented as the percentage of [^{14}C]-chloramphenicol acetylated after 1 hr of incubation at 37°C with 0.5 μg of protein from a sonicated *B. pertussis* extract. Values given are the mean ± SD for three experiments.

Primer Extension. Total cellular RNA was isolated from midlogarithmic phase cultures of *B. pertussis* and *E. coli* by extraction with hot phenol as described (23). The primer used was a 19-mer oligonucleotide (5′-GAGGACTTTGTTGTA-CATG-3′; Operon Technologies, Alameda, CA) complementary to the 5′ end of the *bvgA* transcript. The protocol used for primer extension is described in detail elsewhere (24).

RESULTS

Transcriptional *bvg::lacZYA* Fusions in *E. coli*. To study the regulation of the *bvgABC* operon of *B. pertussis* we have constructed single-copy *bvg::lacZYA* transcriptional fusions in *E. coli*. Three fusions, extending from the *Eco*RI site upstream of *bvgA* into each of the *bvg* open reading frames (Fig. 1), were constructed in the multicopy plasmid pRS551. The fusions were recombined onto bacteriophage λRS45 and integrated at single copy into the chromosome of *E. coli* MC4101. The resulting *E. coli* lysogens CR430 (*bvgA::lacZYA*), CR432 (*bvgAB::lacZYA*), and CR434 (*bvgABC::lacZYA*) were grown to midlogarithmic phase in L broth. β-gal activity was then assayed to determine the basal level of transcription of each fusion.

Strain CR430, which does not produce a functional BvgA product, expressed 73 units of β-gal. This suggests that there is a functional promoter directing transcription of the *bvgA::lacZYA* fusion in *E. coli*. When β-gal activity was determined for CR432 and CR434, which contain fusions with *bvgB* and *bvgC*, respectively, β-gal activity dropped to 10 units for CR432 and 5 units for CR434. This decrease in β-gal activity, as compared to CR430, could result from transcriptional polarity along the *bvgABC* operon. Alternatively, the presence of an intact copy of the *bvgA* gene in CR432 and CR434 might account for reduced β-gal activity if BvgA were capable of autogenous repression.

A plasmid containing the *bvgA* gene was transformed into CR430 to determine if BvgA can repress its own expression. Introduction of the plasmid had no measurable effect on expression of the *bvgA::lacZYA* chromosomal fusion (data not shown). This suggests that the decrease in the basal level of β-gal activity detected in CR432 and CR434, as compared to CR430, is the result of transcriptional polarity along the *bvgABC* operon rather than BvgA acting as a repressor. A plasmid that overproduces BvgA (pCR435) was then transformed into CR430. This combination resulted in a >12-fold increase in β-gal activity produced by the *bvgA::lacZYA* fusion in CR430 (Fig. 2). The *bvg* fusions in CR432 and CR434 were also activated upon the introduction of pCR435. These data are similar to earlier results on the transcriptional regulation of the *bvg*-activated *fhaB* gene, which had shown that overproduction of BvgA in *E. coli* results in the transcriptional activation of an *fhaB::lacZYA* fusion (11). From these data we conclude that BvgA functions as a transcriptional activator for the *bvgABC* operon in *E. coli* and that the operon is autogenously activated.

The effects of environmental stimuli on *bvg* autogenous regulation were examined by placing the entire *bvgABC* operon, present on the plasmid pJM26, in trans to the *bvgA::lacZYA* fusion in CR430. β-gal levels were determined for CR430 (pJM26) grown in L broth at 37°C and compared to duplicate cultures grown in the presence of either 40 mM $MgSO_4$ or 5 mM nicotinic acid. The data show that the

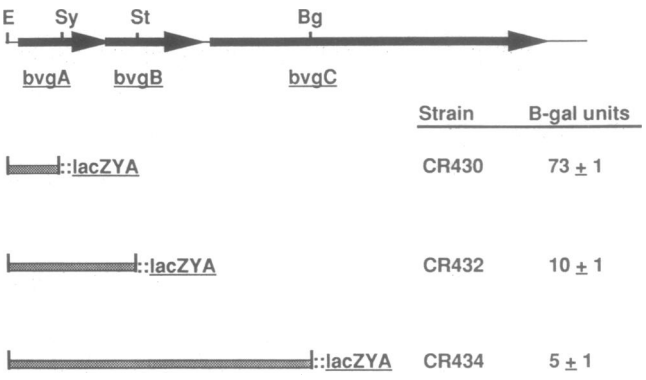

FIG. 1. Schematic representation of the *bvg* restriction fragments contained in three *bvg::lacZYA* fusions used in this study. The fusions were present in *E. coli* on λ prophage. β-gal units expressed by each fusion are given as the mean ± SD for three experiments. Restriction sites: E, *Eco*RI; Sy, *Sty* I; St, *Stu* I; Bg, *Bgl* II.

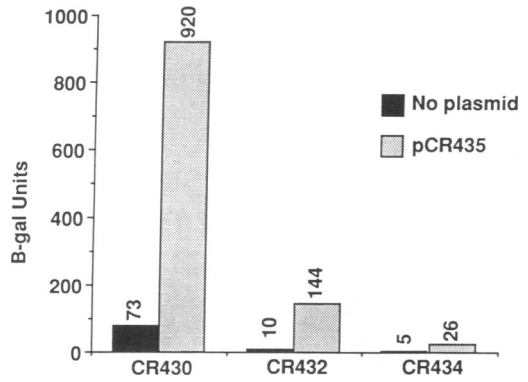

FIG. 2. β-gal activity was measured for three *bvg*::*lacZYA* fusion strains containing a plasmid (pCR435) that overproduces BvgA. These β-gal levels were compared to the levels measured for the strains in the absence of a plasmid. β-gal units represent the mean of three experiments. The SD was <10% of the mean for all experiments.

bvgA::*lacZYA* fusion in CR430 (pJM26) was activated when the bacteria were grown in L broth at 37°C (Table 2). β-gal activity dropped from 665 units to 79 units for CR430 (pJM26) when the bacteria were grown in 40 mM $MgSO_4$ and to 82 units for cultures grown in 5 mM nicotinic acid. The β-gal levels for CR430 (pJM26) grown in the presence of $MgSO_4$ or nicotinic acid are approximately the same as the levels determined for CR430 grown with or without $MgSO_4$ or nicotinic acid. These data suggest that the *bvgABC* operon has a constitutive level of transcription that is insensitive to environmental stimuli as well as an activated level of transcription that requires the *bvgABC* products. Activation of the *bvgABC* operon in *E. coli* appears to be regulated by the same environmental stimuli that control the expression of *bvg*-regulated virulence determinants in *B. pertussis*.

Positive Autoregulation of the *bvgABC* Operon in *B. pertussis*. Regulation of the *bvgABC* operon was investigated in *B. pertussis* using strain CF1770. CF1770, an isogenic derivative of BP370, contains a transcriptional CAT fusion located in the chromosome at a unique *Bgl* II site in *bvgC*. We have previously shown that this strain produces a significant amount of CAT activity, whereas CF1470, which contains the CAT fusion cassette in the reverse orientation, has nearly undetectable levels of CAT activity (6). The insertion of the CAT gene into *bvgC* renders the bacteria Bvg^-. This mutation can be complemented by the introduction of the plasmid pSS528, which contains the entire *bvgABC* operon on the broad host-range vector pRK290.

B. pertussis CF1770 containing either pSS528 or the cloning vector pRK290 was grown in Verwey broth to midlogarithmic phase. The level of CAT expression was approximately the same for CF1770 (pRK290) grown either with or without addition of $MgSO_4$. CF1770 (pRK290) produced 11 ± 2 units of CAT when grown in Verwey broth and 9.5 ± 4 units when 40 mM $MgSO_4$ was present in the medium, indicating that $MgSO_4$ does not affect *bvgABC* expression in bvg^- bacteria. In contrast, the CAT activity detected in CF1770 (pSS528) was 2.3-fold higher when the bacteria were grown in the absence of $MgSO_4$. CF1770 (pSS528) grown in Verwey broth produced 30 ± 10 units of CAT compared to duplicate cultures grown in the presence of 40 mM $MgSO_4$, which produced only 13 ± 5 units. The CAT activity measured for CF1770 (pSS528) grown in the presence of 40 mM $MgSO_4$ was approximately the same as that detected in CF1770 (pRK290) grown either with or without the addition of $MgSO_4$. The moderate level of induction of the *bvgABC* genes observed in CF1770 (pSS528) could be due to the location of the CAT fusion in this strain. In *E. coli*, fusions in *bvgC* are not induced as greatly as fusions in *bvgA* (Fig. 2). From these data it appears that the *bvgABC* operon is autogenously activated in *B. pertussis* and autoactivation can be modulated by environmental stimuli.

Table 2. Effects of different growth conditions on β-gal activity in *E. coli* strains containing a chromosomal *bvgA*::*lacZYA* fusion with and without the *bvgABC* operon in trans

Strain	β-gal activity		
	No addition	40 mM $MgSO_4$	5 mM nicotinic acid
CR430	73 ± 1	77 ± 3	80 ± 1
CR430 (pJM26)	665 ± 56	79 ± 2	82 ± 2

Bacteria were grown in L broth to an OD_{600} of ≈0.5. Cultures were supplemented with $MgSO_4$ and nicotinic acid as indicated. Results represent the mean ± SD for three experiments.

Identification of *bvgABC* Transcriptional Start Sites. To identify the initiation site for transcription of the *bvgABC* operon we performed primer extension on total RNA isolated from several *B. pertussis* strains and from *E. coli* containing the *bvgABC* operon on a multicopy plasmid. RNA was extracted from the *B. pertussis* strain BP370 and the *E. coli* strain MC4101 (pUW1004). The bacteria were grown in broth to midlogarithmic phase with and without the addition of $MgSO_4$. RNA was also extracted from two Bvg^- strains of *B. pertussis*. BP369 contains a frame-shift mutation in *bvgC*, and BP3703 has an internal in-frame deletion in *bvgA*. The primer extension results are shown in Fig. 3.

Two different transcriptional start sites were identified upstream of *bvgA*. A transcript (bvg_{P1}) that initiates 90 base pairs 5′ to the *bvgA* start codon was detected only in $bvgABC^+$ bacteria (Fig. 3, lanes A and E). However, in the presence of 40 mM $MgSO_4$, these bacteria no longer expressed the bvg_{P1} transcript (Fig. 3, lanes B and F). The bvg_{P1} transcriptional initiation site was found to be active and identically regulated in *B. pertussis* and *E. coli*. A second *bvg* transcriptional start site (bvg_{P2}) is located 141 base pairs upstream of *bvgA*. Transcripts originating from this site were identified in $bvgA^-$ and $bvgC^-$ bacteria (Fig. 3, lanes C and D) and also in $bvgABC^+$ organisms grown in the presence of 40 mM $MgSO_4$ (Fig. 3, lanes B and F). Transcriptional initiation from the bvg_{P2} site was not detected in BP370 grown in the absence of $MgSO_4$ (Fig. 3, lane A). This was in contrast to results obtained in *E. coli* containing the *bvgABC* operon on a multicopy plasmid. MC4101 (pUW1004) grown in the absence of $MgSO_4$ expressed both *bvg* transcripts (Fig. 3, lane E). This difference could result from the *bvgABC* operon being present on a multicopy plasmid rather than being encoded on the chromosome.

We conclude that there are at least two separate *B. pertussis* promoters that initiate transcription of the *bvgABC* operon and that these promoters are functional in *B. pertussis* and *E. coli*. The first promoter, bvg_{P1}, appears to be a positively autoregulated promoter that is utilized under growth conditions that favor the expression of *bvg*-activated genes. The second promoter, bvg_{P2}, does not require any *B. pertussis* specific transcription factors for activity. This promoter appears to be repressed in *B. pertussis* when the bvg_{P1} promoter is active.

DISCUSSION

B. pertussis is likely to encounter a number of changes in its surrounding environment from the time of its entry into the host to its subsequent spread throughout the respiratory tract. Some of these changes probably serve as stimuli that elicit a response by the organism. As a result, the bacteria may express a variety of adherence factors, toxins, and other molecules vital for pathogenesis or instead down-regulate the

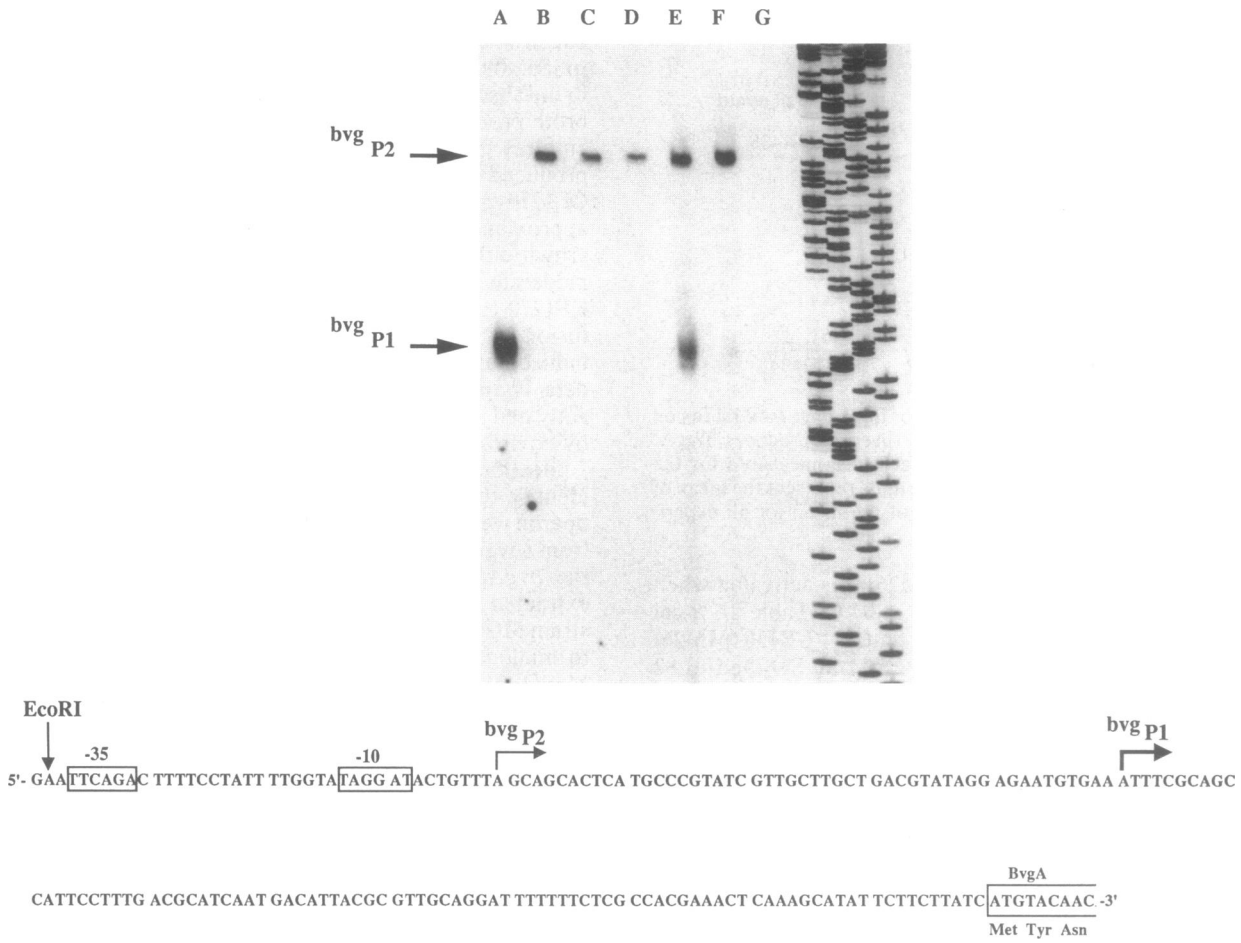

FIG. 3. Primer extension analysis of *B. pertussis* and *E. coli* RNA. Radiolabeled oligonucleotide was annealed to 10 μg of *B. pertussis* RNA (lane A, BP370; lane B, BP370 and 40 mM $MgSO_4$; lane C, BP369; lane D, BP3703) or 40 μg of *E. coli* RNA [lane E, MC4101(pUW1004); lane F, MC4101(pUW1004) and 40 mM $MgSO_4$; lane G, MC4101, no plasmid] and extended with reverse transcriptase. The same oligonucleotide was used as a primer to determine the DNA sequence shown to the right of the primer extension products. The DNA sequence (coding strand) of the *bvgA* upstream region is shown below the primer extension reactions. The locations of the +1 sites for the bvg_{P1} promoter and bvg_{P2} promoter are marked. The putative −10 and −35 regions of the bvg_{P2} promoter are also indicated.

production of these factors. The exact *in vivo* signals responsible for the coordinate regulation of *B. pertussis* virulence factors are not known; however, we are beginning to understand the molecular mechanisms involved in coordinate regulation and sensory transduction.

In this report, we have demonstrated that the *bvgABC* operon of *B. pertussis* is subject to positive autogenous regulation. In *B. pertussis* and *E. coli* expression of *bvg* gene fusions was greater in bacteria that contained a functional *bvgABC* operon than in bvg^- organisms. Autoactivation of the *bvgABC* operon was shown to be sensitive to the same environmental stimuli that inhibit the expression of all previously identified *bvg*-activated genes. The level of expression of *bvg* fusions in $bvgABC^+$ bacteria grown in the presence of 40 mM $MgSO_4$ was approximately the same as that measured in bvg^- bacteria. In addition, expression of *bvg* fusions in the bvg^- bacteria was not affected by environmental stimuli.

Melton and Weiss (3) have reported that transcription of the *bvgABC* operon is repressed in the presence of SO_4^- ions, suggesting autoregulation. Using RNA dot blot hybridization techniques, these investigators do not detect *bvgABC* message in *B. pertussis* cells grown in the presence of $MgSO_4$. Our data demonstrate that the *bvgABC* genes are expressed by *B. pertussis* grown in 40 mM $MgSO_4$, although expression is greater in bacteria grown in the absence of $MgSO_4$ since these conditions favor autogenous activation of the *bvgABC* operon. We conclude from these results that synthesis of the *bvg* products occurs in *B. pertussis* even when the bacteria are grown in an environment that inhibits the expression of *bvg*-activated genes. When the inhibiting stimuli are no longer present, *B. pertussis* then responds to this environmental change with increased expression of the *bvgABC* genes and activation of the *bvg*-regulated virulence genes. It has been proposed that the *bvg*-activated genes may be up-regulated when *B. pertussis* first enters a susceptible host and then down-regulated during the later stages of the disease whooping cough (3). According to this theory, autoactivation of the *bvgABC* operon may facilitate the rapid expression of factors important in the initial stage of *B. pertussis* infection in a new host.

A molecular analysis of the *bvgA* upstream region has identified two different promoters, bvg_{P1} and bvg_{P2}, that initiate transcription of the *bvgABC* operon. The bvg_{P2} promoter does not appear to require positive regulatory factors for activity. This promoter has −10 and −35 regions similar to the consensus sequences for *E. coli* σ^{70} promoters (25). Transcription from this promoter may account for the *bvg*-independent transcriptional activity detected by the *bvg* fusions in *E. coli* CR430 and *B. pertussis* CF1770. In contrast, the bvg_{P1} promoter is an autogenously activated promoter. Transcriptional initiation from this promoter occurs only in bacteria that contain a functional *bvgABC* operon. In addition, bvg_{P1} is not active in $bvgABC^+$ bacteria grown in 40 mM

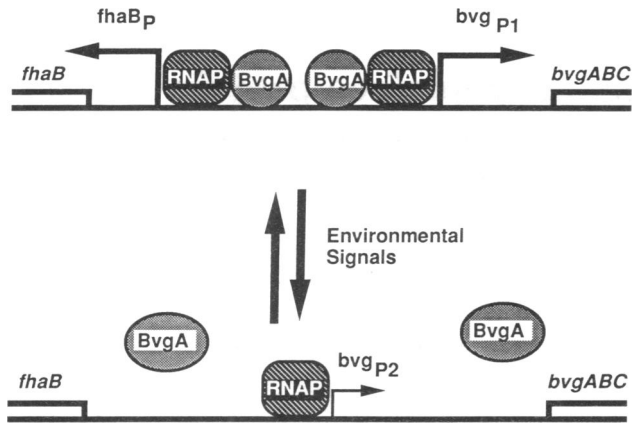

FIG. 4. A potential model for the transcriptional regulation of the *fhaB* and *bvgABC* genes. Under normal growth conditions the *fhaB* promoter and the bvg$_{P1}$ promoter are activated by BvgA in conjunction with RNA polymerase (RNAP). These regulatory events repress transcriptional initiation from the bvg$_{P2}$ promoter. Environmental signals, such as high concentrations of $MgSO_4$, result in an inactive form of BvgA that no longer functions as a transcriptional activator. This leads to a loss of *fhaB* and bvg$_{P1}$ promoter activity. The *bvgABC* genes are now transcribed from the derepressed bvg$_{P2}$ promoter.

$MgSO_4$, demonstrating that transcriptional initiation from bvg$_{P1}$ is sensitive to environmental stimuli.

Our primer extension results suggest that bvg$_{P2}$ may be repressed upon activation of bvg$_{P1}$. A model is presented in Fig. 4 that could explain this finding. In this model binding of the BvgA protein upstream of *bvgA* and *fhaB* results in the activation of bvg$_{P1}$ and the *fhaB* promoter and the simultaneous repression of transcription from the bvg$_{P2}$ promoter. When stimuli that inhibit the expression of *bvg*-activated genes are present, BvgA no longer functions as a transcriptional activator. This results in a loss of bvg$_{P1}$ and *fhaB* promoter activity and derepression of the bvg$_{P2}$ promoter. A similar motif is used in the autogenous regulation of the *glnALG* operon of *E. coli* (26). Further molecular and genetic analysis of bvg$_{P1}$ and bvg$_{P2}$ is required to determine the role of each promoter in the expression of the *bvgABC* genes.

In *E. coli*, the *bvgABC* genes were induced by the introduction of the intact *bvgABC* operon as well as by overexpressing the transcriptional activator BvgA. We have previously shown that the presence of the *bvgABC* operon or overexpression of BvgA induces transcription of the *bvg*-activated *fhaB* gene in *E. coli* but does not activate the *bvg*-regulated *ptx* operon (10, 11). In addition, we have observed a dramatic decrease in transcriptional activation of the *fhaB* gene by the *bvgABC* operon in *E. coli* when either the *fhaB* or *bvgA* upstream region is present on high copy number plasmids, but we have found that the *ptx* upstream region has no effect (unpublished data). This sequence-specific titration is consistent with the idea that BvgA binds to the *fhaB* and *bvgA* promoter regions. It therefore seems likely that there is a common mechanism involved in the activation of *bvgABC* and *fhaB* that differs from that of *ptx*. The *bvgABC* genes and the *fhaB* genes are expressed from divergent promoters. The close proximity of these promoters may facilitate regulatory interactions. An extensive molecular and genetic analysis of the promoter regions for *bvgABC* and *fhaB* is necessary to determine the cis-acting sequences required for transcriptional activation of these genes and the mechanism of transcriptional activation of these genes by BvgA.

It is possible, due to the location and similar regulation of the *bvgABC* and *fhaB* genes, that the *bvgABC* region may have originally evolved to regulate the expression of the filamentous hemagglutinin protein encoded by *fhaB*. The other virulence genes may have later found it advantageous to regulate their expression by networking into the sensitive environmental response capabilities present in the *bvgABC* system. As the number of *bvg*-regulated genes increased, so would the demand for *bvgABC* products, and this increased demand could have resulted in the evolution of a *bvg*-autoactivated promoter. Although this theory is speculative, it will make further investigation into the molecular mechanisms involved in the regulation of *bvg*-activated genes an exciting area of research.

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1. Weiss, A. A. & Falkow, S. (1984) *Infect. Immun.* **43,** 263–269.
2. Stibitz, S., Weiss, A. A. & Falkow, S. (1988) *J. Bacteriol.* **170,** 2904–2913.
3. Melton, A. R. & Weiss, A. A. (1989) *J. Bacteriol.* **171,** 6206–6212.
4. Lacy, B. W. (1960) *J. Hyg.* **58,** 57–93.
5. Knapp, S. & Mekalanos, J. J. (1988) *J. Bacteriol.* **170,** 5059–5066.
6. Arico, B., Miller, J. F., Roy, C., Stibitz, S., Monack, D., Falkow, S., Gross, R. & Rappuoli, R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6671–6675.
7. Stock, J. B., Ninfa, A. J. & Stock, A. M. (1989) *Microbiol. Rev.* **53,** 450–490.
8. Miller, J. F., Mekalanos, J. J. & Falkow, S. (1989) *Science* **243,** 916–922.
9. Nixon, B. T., Ronson, C. W. & Ausubel, F. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7850–7854.
10. Miller, J. F., Roy, C. R. & Falkow, S. (1989) *J. Bacteriol.* **171,** 6345–6348.
11. Roy, C. R., Miller, J. F. & Falkow, S. (1989) *J. Bacteriol.* **171,** 6338–6344.
12. Simon, R., Priefer, U. & Puhler, A. (1983) *Bio/Technology* **1,** 784–789.
13. Stibitz, S., Aaronson, W., Monack, D. & Falkow, S. (1989) *Nature (London)* **338,** 266–269.
14. Stibitz, S., Black, W. & Falkow, S. (1986) *Gene* **50,** 133–140.
15. Ditta, G., Schmidhauser, T., Yakobson, E., Lu, P., Liang, X. W., Finlay, D. R., Guiney, D. & Helinski, D. R. (1985) *Plasmid* **13,** 149–153.
16. Simons, R. W., Houman, F. & Kleckner, N. (1987) *Gene* **53,** 85–96.
17. Verwey, W. F., Thiele, E. H., Sage, D. N. & Schuchardt, L. T. (1949) *J. Bacteriol.* **50,** 127–134.
18. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
19. Dower, W. J., Miller, J. F. & Ragsdale, C. W. (1988) *Nucleic Acids Res.* **16,** 6127–6145.
20. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Weiss, A. A., Hewlett, E. L., Myers, G. A. & Falkow, S. (1983) *Infect. Immun.* **42,** 33–41.
22. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
23. von Gabain, A., Belasco, J. G., Schottel, J. L., Chang, A. C. & Cohen, S. N. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 653–657.
24. Hartz, D., McPheeters, D. S., Traut, R. & Gold, L. (1988) *Methods Enzymol.* **164,** 419–425.
25. Hawley, D. K. & McClure, W. R. (1983) *Nucleic Acids Res.* **11,** 2237–2255.
26. Reitzer, L. J. & Magasanik, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1979–1983.