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Identification and Characterization of BpH2, a Novel Histone H1 Homolog in *Bordetella pertussis*

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A basic protein, BpH2, with an apparent molecular mass of 18 kDa was purified from *Bordetella pertussis*, and the corresponding gene, *bph2*, was cloned. Sequence analysis revealed some homology to the H1 class of eukaryotic histones and to AlgP protein of *Pseudomonas aeruginosa*. BpH2 binds both single- and double-stranded DNA in a nonspecific manner. Deletion of the corresponding gene in *B. pertussis* generated a BpH2 null mutant with an altered growth rate in which the expression of two virulence factors, adenylate cyclase-hemolysin (CyaA) and filamentous hemagglutinin (FhaB), was reduced. It is suggested that BpH2 may exhibit specific regulatory functions through its interaction with chromosomal DNA.

Eubacteria maintain their DNA in a negatively supercoiled form, achieved by DNA topoisomerase and by architectural elements called histone-like proteins. Among these, the best characterized are HU, HNS, and IHF (10, 20, 24). These chromatin-associated proteins organize the bacterial chromosome and also exert regulatory influence on transcription, recombination, and DNA replication (11, 20, 30, 35). For many pathogenic bacteria, it has been suggested that the expression of certain virulence genes, which are regulated by a specific regulatory element, can also be affected by DNA topology (9). In line with this hypothesis, a new class of chromatin-associated proteins which present homology with the eukaryotic histone H1 protein have been found in some pathogenic bacteria. These proteins include the Hc1 and Hc2 proteins of *Chlamydia* species (15, 16, 23) and the AlgP protein of *Pseudomonas aeruginosa* (8). It has been established that AlgP is involved in the regulation of the *algD* gene, which plays a key role in mucoidy, necessary for virulence of this bacterium (7, 8, 17). Recently, Scarlato et al. (29) have identified and characterized BpH1, a new member of the family with homology to histone H1 which is involved in chromatin formation in *Bordetella pertussis* (29). *B. pertussis*, the etiological agent of whooping cough, encodes a large number of virulence factors, including pertussis toxin (Ptx), filamentous hemagglutinin (FhaB), and adenylate cyclase-hemolysin (CyaA) (6, 36). The expression of virulence factors in *Bordetella* species is coordinately regulated by the *bvg* locus, which encodes two proteins, BvgA and BvgS, members of the histidine-kinase response regulator family of signal transduction systems (2, 26, 33). Mutations in the *bvg* locus abolish the expression of virulence factors and result in avirulent-phase bacteria.

In the present paper, I report the identification of a second protein of *B. pertussis*, with homology to eukaryotic histone H1 protein, named BpH2. The *bph2* gene has been sequenced, and the deduced amino acid sequence showed homology with eukaryotic histone H1 proteins and AlgP of *P. aeruginosa*. The BpH2 protein was purified, and its interaction with DNA in vitro was analyzed. A *B. pertussis* strain lacking the *bph2* gene was constructed, and the effects of the deletion on growth rate and on the expression of two virulence factors (CyaA and FhaB) are presented.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are described in Table 1. *Escherichia coli* strains were grown in Luria broth (LB) or 2× YT media, supplemented when necessary with the appropriate antibiotics (ampicillin, 100 μg/ml; kanamycin, 50 μg/ml). *B. pertussis* strains were grown on Bordet-Gengou (BG) agar plates supplemented with 10% sheep blood or in Stainer-Scholte (SS) (32) medium with minor modifications. For modulating conditions (characterized by loss of virulence factors), 40 mM $MgSO_4$ or 4 mM nicotinic acid was added to SS medium. Antibiotics were added at the following concentrations when necessary: ampicillin, 50 μg/ml; kanamycin, 100 μg/ml; streptomycin, 200 μg/ml.

Recombinant DNA methods. Standard methods were used for the isolation and transformation of plasmid DNA, endonuclease digestions, and DNA ligations (27). Electroporation was used to transform *B. pertussis*. Nucleotide sequences for both DNA strands were determined by the dideoxynucleotide method of Sanger et al. (28), and overlapping deletions were obtained with exonuclease III.

Southern hybridization analyses were performed by using the 5′ ACIAARG CIAARGCICCIGCIAARAARGTIACIAARACIGC (R = A or G; I = inosine) degenerate oligonucleotide labeled with ^{32}P by using T4 polynucleotide kinase according to standard protocols.

Purification of BpH2 and amino acid sequencing. Briefly, 3 g (wet weight) of *B. pertussis* 18323 (25) or recombinant *E. coli* was disrupted by sonication in TE buffer (50 mM Tris, pH 8; 1 mM EDTA) with 500 mM NaCl. Cell debris was removed by centrifugation. The crude extract was diluted to obtain a final NaCl concentration of 200 mM and then loaded on a 1-ml heparin-Sepharose Hitrap column (Pharmacia). Following adsorption to the column and washing, proteins were eluted with a stepwise gradient of NaCl in TE.

The purified protein was subjected to N-terminal amino acid sequence analysis by the Laboratoire de Microséquençage des Protéines, Institut Pasteur, Paris, France.

Southwestern (DNA-protein) blot. Proteins were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) procedures and electrotransferred to nitrocellulose. Filters were saturated with 1% of the blocking agent (Boehringer) for 30 min at room temperature and rinsed with TE buffer. For DNA-binding assays, the membranes were incubated with labeled DNA for 30 min at room temperature. DNA was labeled with digoxigenin (DIG)-UTP by using terminal transferase (DIG oligonucleotide labeling kit; Boehringer). After three washes in TE–100 mM NaCl buffer, the protein-DNA complexes were revealed by using a specific antibody raised against DIG hapten in accordance with the instructions of the manufacturer (Boehringer).

In vitro gel mobility assay for BpH2-DNA interactions. DNA-binding assays were performed with pTZ18 plasmid or M13 single-stranded DNA (ssDNA). Two hundred micrograms of DNA was mixed with purified BpH2 protein in TE buffer. The mixtures were incubated for 10 min at 25°C before addition of dye and running of mixtures in a 0.8% agarose gel in 1× Tris-borate-EDTA (TBE). After electrophoresis, the gel was stained with ethidium bromide.

Chromosomal integration. For chromosomal integration, derivatives of plasmid pRTP1 (34), pNM482 (19), or PRS551 (31), unable to be maintained in *B. pertussis*, were electroporated into *B. pertussis*. Recombinant strains with a single recombination event were selected for ampicillin resistance in the case of

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Strains		
E. coli XL1-Blue	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 lac*[F′ *proAB lacI*^{q} *Z*Δ*M15*Tn*10*]	5
B. pertussis		
18323	Wild type, Str^r	25
$18A_4$	Wild type *cya*::*lacZY*, Ap^r Str^r	13
18F	Wild type *fhaB*::*lacZY*, Ap^r Str^r	This study
$18\Delta H_2$	Δ*bph2*::*km*, Str^r Km^r	This study
$18\Delta H_2A_4$	Δ*bph2*::*km cyaA*::*lacZY*, Ap^r Km^r Str^r	This study
$18\Delta H_2F$	Δ*bph2*::*km fhaB*::*lacZY*, Ap^r Km^r Str^r	This study
$18HZ_1$	*bph2*::*lacZY*, Ap^r Km^r Str^r	This study
Plasmids		
pTZ18R	Cloning vector, Ap^r	38
pTRC99A	Expression vector, Ap^r	1
pNM482	*lacZYA* operon fusion vector, Ap^r	19
pRS551	*lacZYA* operon fusion vector, Ap^r Km^r	31
pRTP1	Gene replacement vector for *B. pertussis*, Ap^r Str^s	34
pSGA4	*cyaA*::*lacZY* fusion on plasmid pNM482, Ap^r	13
pSGF1	*fhaB*::*lacZY* fusion on plasmid pNM482, Ap^r	12
pSG18.1	3-kb *Cla*I fragment carrying the *bph2* gene into pTZ18	This study
pES3	5′ deletion of pSG181 carrying 210 bp upstream from ATG of *bph2* gene	This study
pSGH1	*bph2*::*lacZY* fusion encompassing bp −210 to +273 from *bph2* cloned into pRS551	This study
pES3K	pES3 derivative Δ*bph2*::*km*, Ap^r Km^r	This study
pRTPHK	1-kb *Eco*RI fragment carrying Δ*bph2*::*km* from pES3K subcloned into pRTP1, Ap^r Km^r Str^s	This study
pTN	446-bp *Nco*I-*Aaf*II fragment corresponding to open reading frame of *bph2* subcloned into pTRC99A, Ap^r	This study

pSGA4, pSGF1, or pSGH1. For pRTPHK (derivative of pRTP1), the first recombination event was selected for both ampicillin and kanamycin and the second recombination event, corresponding to the allelic exchange, was selected for kanamycin and streptomycin resistance.

Assay of enzymatic activities. Enzymatic activities were determined in toluenized bacterial suspensions of exponentially growing cultures in SS medium. β-Galactosidase activity was measured as described by Pardee et al. (21). One unit of enzyme corresponds to 1 nmol of substrate formed per min at 28°C.

RESULTS AND DISCUSSION

Identification of DNA-binding proteins of *B. pertussis*. Scarlato et al. (29) have described the isolation of BpH1, a histone H1 homolog in *B. pertussis*, by Southwestern blot techniques; they also detected additional protein bands of lower molecular weights that can also bind to DNA. In order to identify these DNA-binding proteins, crude extracts of *B. pertussis* 18323 were subjected to affinity chromatography on a heparin-Sepharose column as described in Materials and Methods.

Fractions were analyzed by Southwestern blotting with DNA fragments corresponding to the *cyaA* upstream promoter region (positions −130 to −80 with respect to the start of transcription). As shown in Fig. 1, several proteins were identified: the BpH1 protein (which migrates with an apparent molecular mass of 30 kDa) described recently by Scarlato et al. (29) was eluted at 600 mM NaCl; in addition, two proteins (of approximately 16 and 9 kDa) were eluted at 800 mM NaCl and an 18-kDa protein eluted at 1 M NaCl.

We focused our interest on the 18-kDa protein, which had the highest affinity for the heparin column. The N-terminal sequence of this protein was determined. The first amino acid could not be identified, suggesting a posttranslational modification of this residue. The sequence of the following 21 amino acid residues (RTKAKAPAKKVTKTAAKTPAK) showed a high content of lysine and alanine, as does the BpH1 protein (29), and for this reason this protein was named BpH2.

Cloning and sequencing of the *bph2* gene. The N-terminal sequence was used to design a degenerate 41-mer oligonucleotide representing the stretch of amino acids TKAKAPAK KVTKTA. A partial library of *B. pertussis* genomic DNA was screened by hybridization to the ^{32}P-labeled oligonucleotide. One strongly hybridizing recombinant clone, pSG18.1, was isolated and further analyzed. The nucleotide sequence of a 1-kb region (Fig. 2) including the *bph2* gene was determined by the dideoxynucleotide chain method (28).

The insertion element IS*481* of *B. pertussis*, in orientation II (18), was found 180 bp upstream from a possible ATG start codon (data not shown). The N-terminal methionine appeared

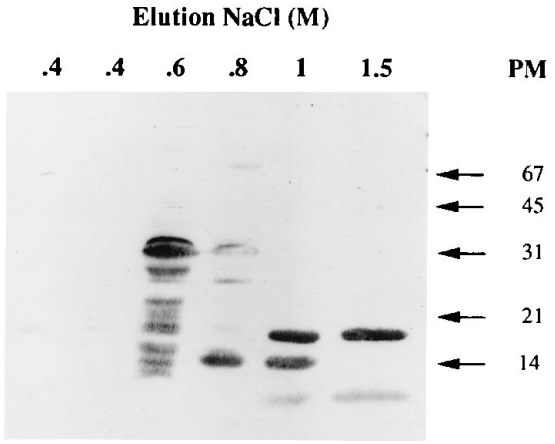

FIG. 1. Identification of DNA-binding proteins by Southwestern blotting. Fractions from heparin affinity chromatography were separated by SDS-PAGE (13% polyacrylamide gel) and then electrotransferred. Membranes were incubated with DIG-labeled DNA, and protein-DNA complexes were revealed by using an antibody raised against DIG coupled with alkaline phosphatase. NaCl concentrations used for the stepwise gradient are listed above the lanes. Molecular mass marker sizes (in kilodaltons) are indicated by arrows. PM, protein markers.

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GCTACCCAAAACCAGCGTCGGCAAGCATTCGTTGCGCTGAAAACAACAACCTGTACCTCT

GCGCGGCGTGAACGCATGCAGCCGGCGCGAGTTTCCGCTATCATCGGGCGCGAGGTGTTG

TTCATCGAATATCCGGCGGAACACGCTGCCTCCATATCCTCACTACTACGGATTTTGACC

ATGGCTACCAAAGCAAAAGCGCCTGCCAAGAAAGTCACGAAGACCGCCGCCAAGACTCCT
M  A  T  K  A  K  A  P  A  K  K  V  T  K  T  A  A  K  T  P

GCCAAGGCTCCCGCCAAGAAGGCTCCCGCCAAACCCGCCGTGAAGAAAACGGCCGCCGCA
A  K  A  P  A  K  K  A  P  A  K  P  A  V  K  K  T  A  A  A

GTGCGCCCCATCAAGGAAGCGCTGAACAAGTCGCAACTGATCGCCTACCTGGTCGAGAAC
V  R  P  I  K  E  A  L  N  K  S  Q  L  I  A  Y  L  V  E  N

ACCGGCGTGGAAGCCAAGTCGGTCAAGGCCGTGCTGGCTGGCCTGGAAAGCTCGGTGCTC
T  G  V  E  A  K  S  V  K  A  V  L  A  G  L  E  S  S  V  L

GCCTCGGTCGACAAGAAGGGCGCTGGCGAATTCACCCTGCCCGGCCTGTTCAAGGTCGCC
A  S  V  D  K  K  G  A  G  E  F  T  L  P  G  L  F  K  V  A

GTGCAGAAGGTTCCCGCCAAGGCCAAGCGCTTCGGCAAGGACCCGTTCACCGGCGAAGAG
V  Q  K  V  P  A  K  A  K  R  F  G  K  D  P  F  T  G  E  E

CGCTGGTTCCCGGCCAAGCCCGCCTCGGTGAAGGTCAAGGTTCGTCCCCTGAAGAAGCTG
R  W  F  P  A  K  P  A  S  V  K  V  K  V  R  P  L  K  K  L

AAGGACGCCGCGCAGTAAGCTGACGTCTTCAGGTCAAACCGCAGAGCCCGCCGCACCGCG
K  D  A  A  Q  *

CTCTTCGTTTATGCGGCGGCGATTTGACCGGGCCGTTTTATCTTGATATAAAGATACTTT

ACGTCAAGACAATAATGAAAGACCACGTCGACCCTGGTGTGTCCAATTGGCGCCACGAAC

GCCCCGACCTGGACGTCTCCGTCCATGGCCTGGCGCGTATTTTCCGGTTCAATACCGCGC

GCGCCTGCATCGAGCCGCTGTTTCGCCGGCCGGCCTGCACCAGGGACAGTTCGACCTGCT

GGCCACGCTGTA
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FIG. 2. Nucleotide sequence of the *B. pertussis bph2* gene and the deduced amino acid sequence of BpH2 (in one-letter code). The asterisk indicates a codon.

to be posttranslationally cleaved and the second amino acid appeared to be modified, since the following 21 deduced amino acids could be aligned with the N-terminal amino acid sequence of the mature protein.

The *bph2* gene consists of 435 nucleotides encoding 145 amino acids with a deduced molecular mass of 15 kDa. Its apparent molecular mass of 18 kDa on SDS-PAGE is likely to be due to the highly charged nature of BpH2. The amino acid composition of BpH2 is unusual in that it consists of a very high content of lysine (20%), alanine (20%), and proline (8.3%) with a predicted isoelectric point of 11.2.

Comparison of the deduced amino acid sequence of BpH2 by using the FASTA program (22) revealed homology with the H1 class of eukaryotic histones (51.8% identity in a 54-amino-acid overlap with the painted sea urchin H1 or 33% identity in a 143-amino-acid overlap with tomato histone H1) and with the AlgP regulatory protein of *P. aeruginosa*. Homology with Hc1 and Hc2 proteins of *Chlamydia trachomatis* could be found, but scores were lower. The alignments of the amino acid sequence of BpH2 with the sequences of sea urchin histone H1 (C-terminal sequence) and AlgP are shown in Fig. 3. However, the amino acid identity among these proteins is mostly limited to alanine, lysine, and proline. A similar observation was made for homology among histone H1 and Hc1, Hc2, and AlgP, suggesting that this conservation of amino acid composition may reflect a convergent evolution of successful DNA binding structures rather than the usual sequence similarity. No significant homology to other prokaryotic histone-like proteins such as HNS or IHF was observed.

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AlgP PATKPAAKAAAKPAVKTVAANAAKPAAKPAAKPAAKTAAAKPAAKPAAKPA-AKPAAKP  225
                                  ||  || : || |||  ||  || | ||||:|
BpH2                           MATKAKAPAKKVTKT-AAKTPAKAPAKKAPAKPAVKK   36
                                | ||| ||||: |  |||  || || | ||| |:||
SUH1 TKARKEKLAAKKAAKKAAKKVKKPAAKAKKPAKKAAKKPAAKKAAKKPAAKKPAKKAAKK  184

AlgP AAKTAAAKPAAKPAAKP--VAKPAANAAAKTAAAKPAAKPAAKPVAKPAAKPAAKTAAAK  283
      |  || :|      |    |    |         |       |     |   |  |
BpH2 TA--AAVRPIKEALNKSQLIAYLVENTGVEAKSVKAVLAGLESSVLASVDKKGAGEFTLP   94
        || :  | |       |
SUH1 KP--AAKKAAKPAKKAAKKPAAKKAAKK.                                  210

AlgP PAAKPAAKHVAKPAAAKPAAK-PAANAAAKPAAKPAAKPVAAKPAAAKPATAPAANAA.   340
       | |:  |  || ||   | |          ||||   |  :|    |
BpH2 GLFKVAVQKV--PAKAKRFGKDPFTGEERWFPAKPASVKVKVRPLKKLKDAAQ.        145
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FIG. 3. Amino acid sequence homology. Alignment of BpH2 with AlgP of *P. aeruginosa* (32.8% identity in a 140-amino-acid overlap) and with the conserved C-terminal sequence of the painted sea urchin H1 (SUH1) (51.8% identity in a 54-amino-acid overlap). Computer-assisted alignment was performed by using the FASTA program (22). Vertical bars represent identity, and pairs of dots indicate a conserved amino acid change.

Binding of BpH2 to double-stranded DNA (dsDNA) and ssDNA. To avoid any contamination with the 16- or 9-kDa proteins and to achieve high-level conditional expression of BpH2 in *E. coli*, a 450-bp *Nco*I-*Act*II fragment corresponding to the *bph2* coding region was subcloned into the expression vector pTRC99A (1). Recombinant BpH2 protein was purified from *E. coli*, by using the same purification protocol as used for *B. pertussis*. The purity was estimated to be more than 90% on SDS-PAGE gels stained with Coomassie brilliant blue (data not shown).

To check the specificity of BpH2 binding to nucleic acids, mobility shift assays were performed by using purified BpH2 with dsDNA or ssDNA (Fig. 4). At a high concentration of BpH2 (1 μg of BpH2 for 0.2 μg of DNA), both dsDNA and ssDNA were trapped at the top of the gel. At lower protein concentrations, the dsDNA migrated into the gel somewhat more slowly than free DNA. On the basis of the relative intensities of the plasmid topoisomer bands, there seemed to be a slight preference of BpH2 for binding to supercoiled DNA. At concentrations between 0.5 and 0.2 μg of BpH2 protein acceleration of ssDNA mobility was observed, presumably because of a compaction of DNA.

The BpH2 protein binds nonspecifically to DNA and pre-

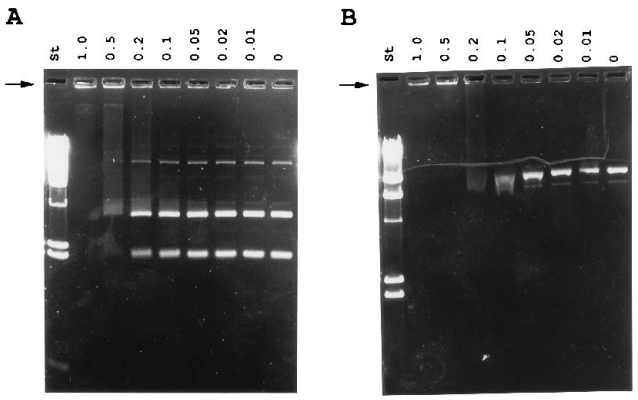

FIG. 4. In vitro analysis of BpH2-DNA interactions by gel mobility shift assay. (A) pTZ18 DNA; (B) M13 ssDNA. DNA (0.2 μg) was mixed with purified BpH2 for 10 min prior to migration on a 0.8% agarose gel. The amounts of BpH2 (in micrograms) are listed above the lanes. Lane st contains *Hin*dIII-cleaved lambda DNA. Arrows indicate the positions of the slots for DNA application.

TABLE 2. Doubling times of the 18323 and $18H_2$ strains at different temperatures

Growth temp (°C)	Doubling time (min)[a]		Final OD_{600}[b]	
	18323 (WT)[c]	$18H_2$ (Δ*bph2*)	18323 (WT)	$18H_2$ (Δ*bph2*)
32	600	600	6.5	6
35	320	320	6	4.5
37	300	360	6	4.0
38	270	390	5	4
39	210	450	4	1.7

[a] Doubling times, determined from growth curves, correspond to the early exponential growth phase and represent the average values obtained for at least three independent experiments that differed by less than 10%.
[b] OD_{600}, optical density at 600 nm.
[c] WT, wild type.

sents the same characteristics as those observed for the *C. trachomatis* histone H1 homolog Hc1 (3, 15). In addition, like the *Chlamydia* histone homologs, which represent 5 to 10% of total proteins in the elementary body, BpH2 can be considered an abundant protein. Indeed, it can be estimated, by measuring the concentration by the Bradford assay, that BpH2 represents approximately 0.5 to 1% of total proteins present in crude extract. Taken together, these results suggest that the two proteins, Hc1 and BpH2, could have similar architectural roles in chromatin formation.

Deletion of *bph2* affects growth rate of *B. pertussis*. To study the role of BpH2 in *B. pertussis*, gene disruption was performed with a double recombination event by using the conventional strategy for gene replacement, by inserting a kanamycin cassette to replace the gene in the chromosome of *B. pertussis*. The growth rates of Δ*bph2* and wild-type strains were measured at different temperatures. The doubling times, corresponding to the early exponential growth phase, are shown in Table 2. For temperatures of 35°C or less, the doubling times of the two strains were indistinguishable, but at temperatures over 35°C, the doubling time for the Δ*bph2* strain increased relative to that for the wild type. At 39°C, the Δ*bph2* strain was not able to grow beyond an optical density of 1.7, corresponding to the point where the wild-type strain enters into the late log phase. More generally, the final optical density obtained for the Δ*bph2* strain was always less than that obtained for the wild-type strain (Table 2). Interestingly, similar observations have been made for *E. coli* strains with mutations in the chromatin-associated *hns* gene, which have doubling times greater than those of the wild-type strain and never reach the final optical density obtained for the wild-type strain (37).

Expression of BpH2 in *B. pertussis*. Scarlato et al. (29) have shown that *bph1* is a growth-regulated gene and is expressed only during the exponential phase. To study the expression of *bph2* in *B. pertussis*, a *bph2*::*lacZY* fusion was constructed on pRS551 (31) and was then introduced into the chromosome of *B. pertussis* by a single recombination event. β-Galactosidase activities were determined during the whole growth phase at different temperatures, and the differential rates of synthesis were calculated. Growth temperature did not influence the expression of the *bph2*::*lacZY* fusion in *B. pertussis*, since no significant variation could be detected between 32 and 39°C: the β-galactosidase activities ranged between 9,000 and 10,000 U of enzyme per mg (dry weight). In the presence of modulating agents such as $MgSO_4$ and nicotinic acid, known to

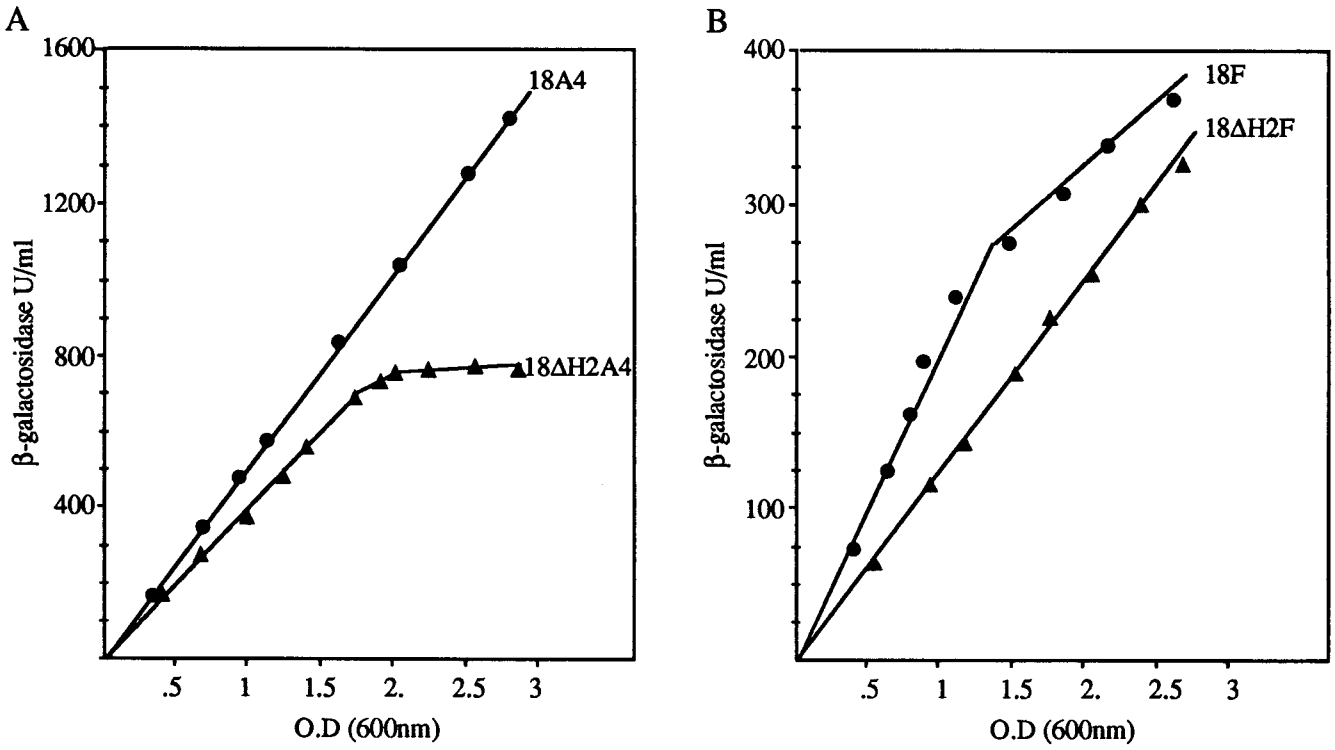

FIG. 5. Expression of *cyaA*::*lacZY* and *fhaB*::*lacZY* fusions at 35°C. (A) 18A4, 18323 wild-type strain with a *cyaA*::*lacZY* fusion integrated in the chromosome; 18ΔH2A4, 18323 Δ*bph2* strain with a *cyaA*::*lacZY* fusion integrated in the chromosome. (B) 18F, 18323 wild-type strain with an *fhaB*::*lacZY* fusion integrated in the chromosome; 18ΔH2F, 18323 Δ*bph2* strain with an *fhaB*::*lacZY* fusion integrated in the chromosome. β-Galactosidase activities were determined in toluenized bacterial suspensions and represent the average values obtained for at least three independent cultures which differed by less than 10%. OD, optical density.

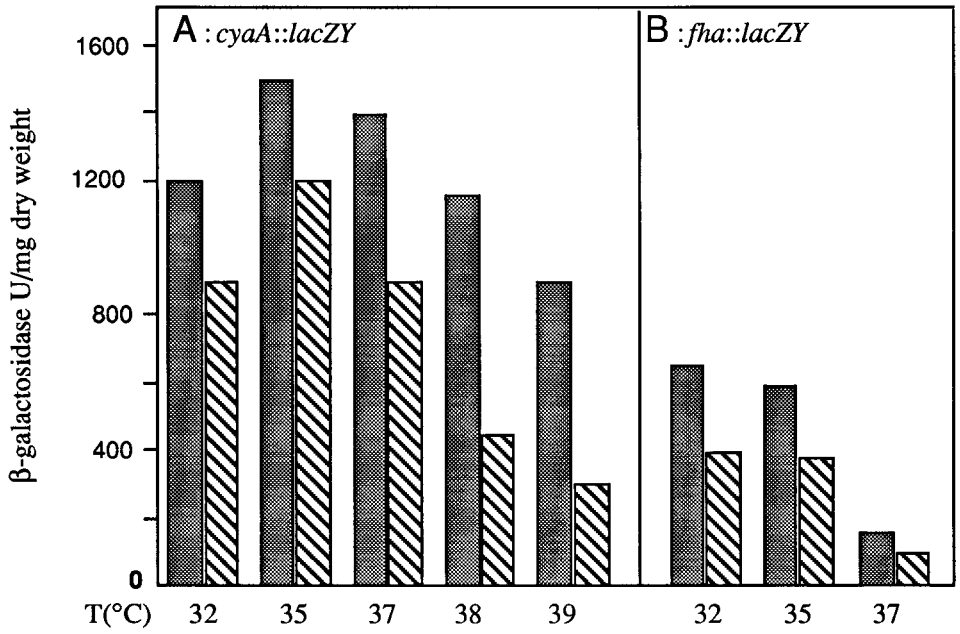

FIG. 6. Differential rates of β-galactosidase synthesis at different temperatures. (A) *cyaA*::*lacZY* fusion; (B) *fhaB*::*lacZY* fusion. Differential rates of β-galactosidase synthesis (units per milligram [dry weight]) were determined from cultures before the bacterial density reached an optical density of 1.6 and represent the average values obtained for at least three independent experiments that differed by less than 10%. Growth temperatures are shown at the bottom. Shaded columns, wild-type strain; hatched columns, Δ*bph2* strain.

abolish virulence gene expression (2), β-galactosidase activities were stimulated (by a factor of 2) rather than inhibited. This shows that *bph2*, like *bph1* (29), is not positively regulated by the *bvg* locus. In contrast to the *bph1* gene, the *bph2* gene appears to be expressed constitutively during the entire growth phase. This absence of temporal control for the *bph2* gene constitutes a major difference from the *Chlamydia* Hc1 and Hc2 histone H1 homolog proteins, which are expressed only during the transition from reticulate to elementary body (3, 15, 23). For this reason, it can be speculated that BpH2 is not involved in gene silencing, as has been suggested for the histone-like proteins of *C. trachomatis* (3, 15, 16, 23).

Expression of *cyaA* and *fhaB* in the wild-type and Δ*bph2* strains. The Δ*bph2* strain formed hemolytic clones on BG agar-blood plates, indicating that the adenylate cyclase toxin was expressed. To study more precisely the expression of virulence factors, *cyaA*::*lacZY* (13) and *fhaB*::*lacZY* fusions (12) were introduced into the chromosome of Δ*bph2* and wild-type strains. β-Galactosidase activities were determined during the whole growth phase for both strains. Figure 5 depicts a typical result obtained for bacteria grown at 35°C, a temperature at which the growth rates of the two strains were comparable. Two conclusions can be drawn from this experiment: (i) the β-galactosidase expressions of the *cyaA*::*lacZY* and *fhaB*::*lacZY* fusions were reduced in the Δ*bph2* strain in comparison with levels for the wild-type strain, and (ii) when the Δ*bph2* strain entered into the beginning of late log phase, the expression of the *cyaA*::*lacZY* fusion, but not that of the *fhaB*::*lacZY* fusion, was severely reduced.

To determine if the growth temperature has an effect on the expression of the two fusions, the recombinant strains were grown at different temperatures and the differential rates of β-galactosidase synthesis were established during the early exponential phase (Fig. 6). Surprisingly, the results show that in the wild-type strain the activity of the *cyaA*::*lacZY* fusion was maximal at 35 to 37°C whereas the expression of the *fhaB*::*lacZY* fusion was maximal between 32 and 35°C. For the Δ*bph2* strain, the *cyaA*::*lacZY* fusion was less active than it was in the wild type, this reduction being more dramatic at temperatures above 35°C. For all temperatures tested, the expression of the *cyaA*::*lacZY* fusion was nearly abolished when the strain entered into the late log phase, as depicted in Fig. 5 for growth at 35°C. The expression of the *fhaB*::*lacZY* fusion in the Δ*bph2* strain was reduced by 40% in comparison with levels for the wild type at all temperatures tested; since activity was reduced by a factor of 4 at 37°C, expression at higher growth temperatures was not tested.

The involvement of DNA-binding proteins by affecting DNA topology in global regulation schemes is now well documented, and this has also been extended to the regulation of virulence genes in pathogenic bacteria (9). In particular, it has been shown that in *P. aeruginosa* the transcription of *algD* depends in part on AlgR, the response regulator of the AlgB/R two-component system (7), but also on AlgP, a histone H1 homolog. Indeed, a strain lacking *algP* does not express AlgD; it has therefore been postulated that AlgP could facilitate the interaction of the distally bound AlgR with RNA polymerase at the *algD* promoter by introducing DNA bends (8).

Prokaryotic chromatin-associated proteins with homology to histone H1 have been identified so far to be present only in pathogenic bacteria, including *Chlamydia* species, *P. aeruginosa*, and *B. pertussis*. In *B. pertussis*, both *bph1* and *bph2* are dispensable genes. Several observations, including the high percentage of BpH2 in the cells, the binding of BpH2 to DNA with apparently no sequence specificity (as shown in Fig. 4), and the requirement of BpH2 for growth at temperatures above 35°C, indicate that BpH2 could have a general regulatory effect on gene expression or an architectural role in participating in the general topology of DNA, as has been proposed for the histone H1 homolog proteins of *Chlamydia* species (15, 16, 23) and other chromatin-associated proteins, such as HU or HNS (10, 24), although a more specific regu-

latory function of BpH2 could also be proposed. Indeed, the expressions of *cyaA* and *fhaB* are reduced in the Δ*bph2* strain in comparison with levels in the wild-type strain and it has been previously shown that the *cyaA* and *fhaB* promoters are affected by changes in DNA supercoiling (14). By analogy with AlgP, it can be speculated that one function of BpH2 in *B. pertussis* could be to facilitate the interaction of the positive regulator BvgA with its target promoter DNA sequences (4, 26) or with the RNA polymerase via topological effects such as bending or looping of DNA.

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