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Genetics

Salmonella typhimurium phoP virulence gene is a transcriptional regulator

(phagocytic cells/two-component regulatory systems/phosphate starvation/signal transduction)

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ABSTRACT **_Salmonella typhimurium_ is a facultative intracellular pathogen capable of surviving within host phagocytic cells. _Salmonella_ strains carrying _phoP_ mutations are avirulent, unable to survive in macrophages, and extremely sensitive to peptides having antimicrobial activity such as the host-derived defensins. We present here the DNA sequence of the _phoP_ gene and show that the deduced amino acid sequence of _phoP_ has extensive homology with the _Escherichia coli_ transcriptional regulators PhoB and OmpR, which control the expression of loci in response to different environmental stimuli. The _psiD_ locus, which is regulated by phosphate availability, was found to be under the control of the _phoP_ gene product. Sequences homologous to _phoP_ were found in several Gram-negative species and in the yeast _Saccharomyces cerevisiae_.**

Facultative intracellular pathogens are organisms that can survive and replicate in phagocytic cells. Because of this property, they usually cause long and debilitating diseases and in many cases fatal infections if untreated. Facultative intracellular pathogens, which include the protozoa *Trypanosoma cruzi* and *Leishmania* and bacterial species such as *Mycobacterium tuberculosis, Mycobacterium leprae, Listeria monocytogenes, Brucella abortus, Legionella pneumophila*, and *Salmonella typhimurium*, utilize different strategies to survive within phagocytic cells (1). These strategies, which include inhibiting the fusion of lysosomes with the phagocytic vacuole and escape from or survival within the phagolysosome, have molecular mechanisms that remain largely unknown. *S. typhimurium* causes a typhoid-like syndrome in mice and is frequently used as a model system for human typhoid fever, a worldwide problem with over 30 million cases annually (2). Our laboratory has been studying the molecular mechanisms by which *S. typhimurium* is able to survive and replicate in murine macrophages (3, 4).

We have recently identified the *phoP* locus as a crucial virulence determinant and found that *Salmonella phoP* strains are extremely sensitive to defensins (3). Defensins are microbicidal peptides present in granules of host phagocytic cells. This is an example of a *Salmonella* gene that is responsible for resistance to a well-characterized host antimicrobial mechanism. The *phoP* locus was originally described by virtue of its involvement in the production of nonspecific acid phosphatase (the *phoN* gene product; ref. 5). However, mutants in *phoN* behaved like the wild-type strain with respect to their virulence *in vivo* and in their sensitivity to defensins *in vitro* (3). These results suggest that the *phoP* gene product might be a regulator of gene expression controlling other loci besides *phoN*.

A plasmid clone of the *phoP* gene harboring 5.0 kilobase pairs (kbp) of *Salmonella* DNA could complement *phoP* mutants for all phenotypes tested: virulence *in vivo*, resistance to defensins *in vitro*, and production of nonspecific acid phosphatase (ref. 3; also unpublished data). Analysis of deletions and transposon insertions in the plasmid clone indicated that only ≈900 bp were necessary for PhoP activity. In this paper we propose a regulatory role for the *phoP* gene product based on our findings that it acts as an activator of the phosphate starvation-inducible gene *psiD* and that the derived amino acid sequence of the *phoP* gene is homologous to several known prokaryotic transcriptional regulators.

MATERIALS AND METHODS

Bacterial strains are listed in Table 1. Strain JM103 (10) was used as a host for bacteriophages M13mp18 and M13mp19 (11). The construction of plasmids pEG5381 and pEG5433 (Fig. 1*A*) and the isolation and mapping of transposon insertions in these plasmids (Fig. 1*B*) will be described elsewhere. Media composition (7, 12) and general bacterial genetic techniques (13) have been described. 5-Bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal; Boehringer Mannheim) was added to agar plates to a final concentration of 60 μg/ml. M13 phage was manipulated as described (10). The nonspecific acid phosphatase staining protocol has been described (5). β-Galactosidase assays were carried out as described (12).

Restriction endonucleases and phage T4 ligase were purchased from Amersham, Bethesda Research Laboratories, Boehringer Mannheim, New England BioLabs, or P-L Biochemicals and were used according to the suppliers' specifications. Large-scale isolation of plasmid DNA was done by the procedure described by Kupersztoch and Helinski (14). For nucleotide sequencing, DNA restriction fragments were purified from acrylamide gels and cloned in the phages M13mp18 and M13mp19. Clones were sequenced on both strands by the dideoxy chain-termination method with the Sequenase kit (United States Biochemical) using the universal primer and ^{35}S-labeled dATP (15, 16). Double-stranded plasmid DNA sequencing was carried out with subclones of plasmid pEG5381 and with plasmids pEG5381 or pEG5433 harboring mini-Mu insertions by using the universal primer, the reverse primer, or a primer complementary to the ends of bacteriophage Mu. Homology searches were carried out by using the program of Pearson and Lipman (17) in BIONET. Southern hybridization analysis was performed with Nytran nylon membranes (Schleicher & Schuell) as suggested by the manufacturer. Other protocols were taken from Maniatis *et al.* (18).

RESULTS AND DISCUSSION

To understand the nature of the molecular defect present in the *phoP* mutants, we have cloned (Fig. 1*A*; unpublished data) and determined the DNA sequence of a 960-bp segment

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Abbreviation: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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Table 1. Bacterial strains

Strain*	Description	Ref. or source
S. typhimurium†		
14028s	wild type	4
EG5086	14028s *phoP5086*::MudI1734	This work
EG5110	JF511 *phoP*::Tn*10*	This work
EG5112	JF512 *phoP*::Tn*10*	This work
EG5114	JF514 *phoP*::Tn*10*	This work
EG5116	JF515 *phoP*::Tn*10*	This work
EG5118	JF570 *phoP5086*::MudI1734	This work
EG5120	JF663 *phoP5086*::MudI1734	This work
EG5122	14028s *psiD19*::Mud1-8	This work
EG5124	7953s *psiD19*::Mud1-8	This work
JF511	(Δ*nadA100 psiA9*::Mud1)	6
JF512	(Δ*nadA100 psiB12*::Mud1)	6
JF514	(Δ*nadA100 psi-15*::Mud1)	6
JF515	(Δ*nadA100 psiC17*::Mud1)	6
JF570	(*psi-18*::Mud1-8, *leu-515* (Am) *sup-19 nadA*::Tn*10*)	6
JF663	(*psiD19*::Mud1-8, *leu515* (Am) *sup-19 nadA*::Tn*10*)	6
MS7953s	*phoP*::Tn*10*	3
E. coli		
JM103	(Δ[*pro-lac*], *supE, thi*/F' *traD36, proAB, lacI*^{Q}ZΔM15)	10

S. typhimurium gene designations are summarized by Sanderson and Roth (8). *E. coli* gene designations are summarized by Bachmann (9).

†All *S. typhimurium* strains are derivatives of strain LT2, with the exception of 14028s, EG5122, EG5124, and MS7953s, which are derivatives of strain 14028s.

containing the wild-type *phoP* gene and its flanking regions (Fig. 2).‡

Coding Region of the *phoP* Gene Has Homology with PhoB and OmpR Proteins. A reading frame is present between nucleotides 151 and 822 that would code for a protein product

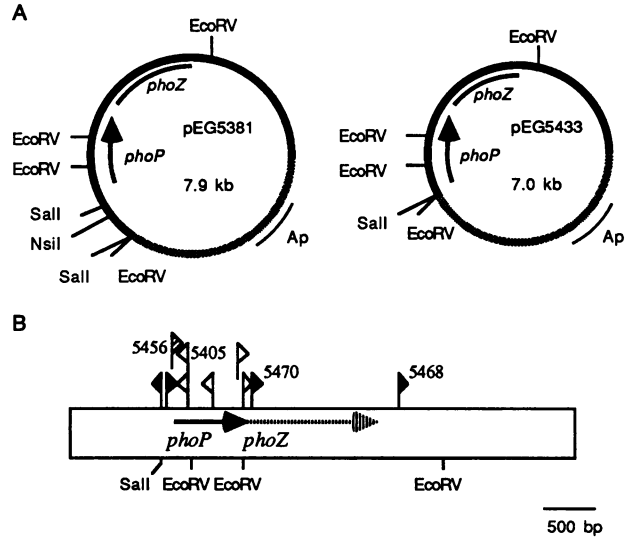

FIG. 1. (*A*) Maps of plasmids pEG5381 and pEG5433. The black line corresponds to *S. typhimurium* DNA, and the stippled line, to pIBI25 vector DNA. pIBI25 is a derivative of plasmid pUC19 (11). (*B*) Localization of the *phoP* gene as determined by transposon mutagenesis of plasmids pEG5381 and pEG5433 using mini-Mu elements MudII1734 and Mud4041. White flags indicate transposon insertions that resulted in a PhoP^{-} phenotype; a striped flag (insertion 5456) resulted in a PhoP$^{+/-}$ phenotype (its precise location is presented in Fig. 2); and black flags indicate transposon insertions that resulted in a PhoP^{+} phenotype with the exception of insertion 5470 (but not 5468), which gave a PhoP^{++} phenotype.

of 224 amino acids with a predicted molecular weight of 25,632 (Fig. 2). This frame begins with an ATG codon, ends with two stop codons (TAA followed by a TGA), and is preceded by a Shine–Dalgarno sequence, AGG, at an appropriate position. The evidence that the DNA segment shown contains at least the coding region of the *phoP* gene is as follows. We have mapped to this region five different mini-Mu insertions in plasmids pEG5381 and pEG5433 that result in lack of *phoP* activity and one mini-Mu insertion that results in reduced levels of *phoP* activity (Fig. 1*B*). Three chromosomal transposon Tn*10* insertions that result in lack of *phoP* activity also map to this fragment (ref. 3; unpublished data). The direction of transcription of the gene is in agreement with that predicted from the phenotype of *phoP-lac* gene fusions.

We searched for proteins with homology to the PhoP protein (as deduced from its nucleotide sequence), using the programs of Pearson and Lipman (17) in both the Protein Identification Resource (Release no. 19) and the Swiss-Prot (Release no. 9) data bases. Extensive homology was found with the *E. coli* transcriptional activator protein PhoB (19), which when aligned to the PhoP sequence showed 35% identical and 41% similar amino acids (see Fig. 3). PhoP is also homologous to the deduced amino acid sequence of the second open reading frame present in the *phoM* operon (20) and the OmpR protein (21) and to regulators from other two-component systems (22), some of which are implicated in virulence (23). In these two-component systems, such as the PhoB-PhoR pair that responds to phosphate availability (19, 24) or the OmpR-EnvZ pair that responds to osmolarity changes (21), there is usually an inner membrane protein (PhoR or EnvZ) that can sense particular environmental changes and modify the second component (PhoB or OmpR) in ways that affect its ability to modulate transcription. Regulation of the nitrogen-utilization genes in enteric bacteria involves the modification of the second component through phosphorylation or dephosphorylation. This modification has been shown to alter the ability of the bacterium to regulate the levels of transcription initiation (25, 26). The *Vibrio cholerae* ToxR protein presents an example of a one-component derivative of a two-component system. In this case, a single polypeptide that has the ability to bind DNA is found in the inner membrane (27). The *vir* region of *Bordetella pertussis* has an open reading frame with a predicted transmembrane domain and homology to both sensors and regulators (23).

To determine whether the PhoP protein had a transmembrane domain and was similar to ToxR and the *B. pertussis* product or whether it lacked one like the PhoB protein, we compared the distribution of hydrophobic groups along PhoP and PhoB using the method of Kyte and Doolittle (28). Their hydropathy profiles were very similar, implying that their conformations were similar and that their mode of action may be the same. Moreover, no transmembrane segments were predicted by the method of Kyte and Doolittle (28) or by the membrane propensity technique of Kuhn and Leigh (29). These results suggested the existence of a second component, the integral membrane sensor protein, required to transduce the signal to PhoP. Consistent with this hypothesis was the phenotype of strains harboring plasmids with transposon insertions or deletions immediately downstream of the coding region of *phoP* that resulted in constitutive expression of nonspecific acid phosphatase (Fig. 1*B*; unpublished data). This phenotype is reminiscent of the one described for some *E. coli phoR* alleles that result in constitutive expression of alkaline phosphatase (30). Kier *et al.* (5) reported a similar phenotype for a chromosomal constitutive mutant mapped in

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M25241).

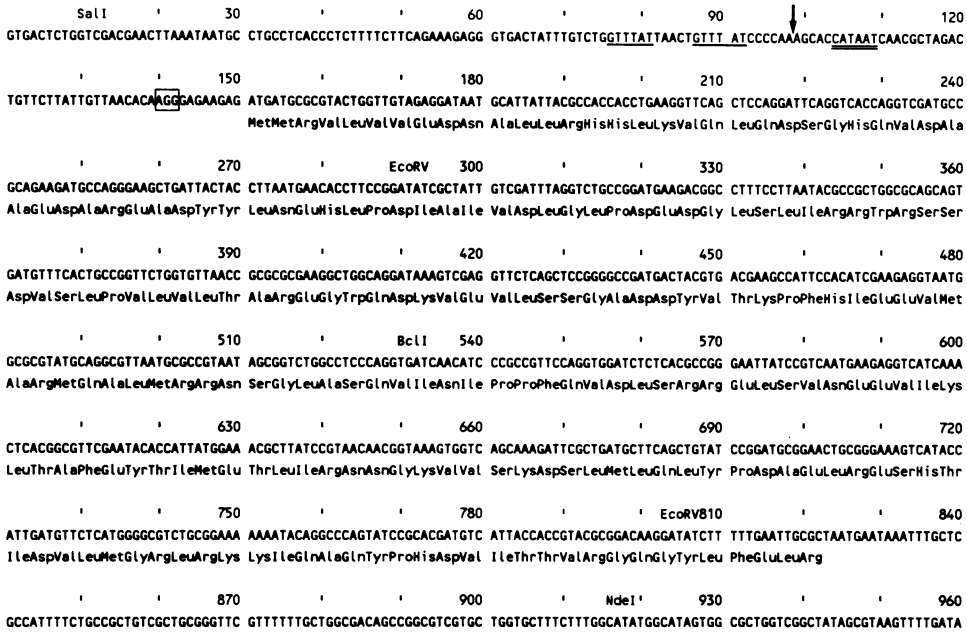

FIG. 2. Nucleotide sequence of the *phoP* gene, its regulatory region, and deduced amino acid sequence of its product. Sequence is shown from nine nucleotides upstream of the *Sal* I site shown in Fig. 1*B*. The putative Pribnow box (nucleotides 104–109) is shown by a double underline; the Shine–Dalgarno sequence (nucleotides 140–142) is shown inside a square; and an hexanucleotide repeat (nucleotides 76–81 and 87–92) is shown underlined. The arrow indicates the position of the mini-Mu transposon insertion 5456 (see Fig. 1*B*) that resulted in an intermediate level of nonspecific acid phosphatase activity.

or near the *Salmonella phoP* region. Strains harboring this chromosomal *phoP*-linked mutation behaved as the wild-type strain in their sensitivity to defensins, suggesting that the level of PhoP made was enough to confer resistance to these peptides (E.A.G. and F.H., unpublished results). This second component (*phoZ*) may be present downstream of *phoP* as found for the *phoBphoR* (19, 24) and *ompRenvZ* (21) operons.

Regulatory Region of the *phoP* Gene. The nucleotide sequence upstream of the coding region of *phoP* was examined for structures that could be relevant for the transcriptional regulation of the gene (Fig. 2). The sequence c-A-T-A-A-T found at nucleotides 104–109 has extensive homology to the consensus sequence for RNA polymerase binding sites (Pribnow box, T-A-T-A-A-T; lower case c indicates nonidentity) and is identical to the one found upstream of the coding region of the *E. coli phoB* gene. No sequence homologous to the consensus sequence of RNA polymerase recognition sites (T-T-G-A-C-A) is found in the −35 region, 15–21 nucleotides upstream from the −10 region. We found, however, that the hexanucleotide sequence G-T-T-T-A-T is present twice, 11 nucleotides upstream of the Pribnow box. Short direct repeats, different from this hexanucleotide, have been found upstream of genes controlled by other transcriptional activators in prokaryotes such as PhoB (31), ToxR (27), and OmpR (32).

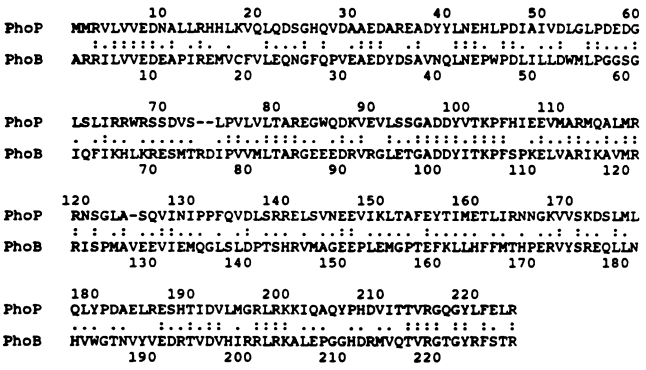

FIG. 3. Comparison of the deduced amino acid sequences of the *phoP* and *phoB* gene products as deduced from their nucleotide sequences. Identical and similar amino acids are indicated by colons and dots, respectively. Amino acid residue numbers are indicated with the first methionine residue designated 1.

PhoB, which controls the expression of genes in response to phosphate availability, has been shown to be a DNA binding protein that is necessary for activation of *in vitro* transcription together with the normal σ factor and core RNA polymerase (31). It binds to the so-called "pho-box," a prokaryotic enhancer sequence found at the regulatory regions of several genes of the *E. coli* phosphate regulon including *phoB* itself (31). The regulatory region of the *phoP* gene does not have a "pho-box," but at an equivalent position the hexanucleotide direct repeat is found, which could correspond to a potential binding site for a transcriptional regulatory factor. The importance of this repeat for the expression of *phoP* is supported by the observation that a mini-Mu transposon insertion present between the repeat and the putative −10 region of *phoP* results in an intermediate level of nonspecific acid phosphatase as indicated by the presence of pink rather than red colonies when stained for nonspecific acid phosphatase activity (see Figs. 1*B* and 2). The intermediate level of expression of this mutant could be due to the presence of the G-T-T-T-t-T sequence in the mini-Mu end, which is very similar to the hexanucleotide repeat (lower case t indicates nonidentity). Further mutational analysis of this region will be necessary to assess the role of this sequence in the control of *phoP* expression. Analysis of the regulatory regions of the *phoN* and *psiD* genes, which are under the control of *phoP* (see below), may reveal similar repeats and suggest that PhoP can regulate its own transcription.

PhoP-Regulated Genes. The *phoP* locus was originally defined genetically as necessary for the production of nonspecific acid phosphatase (5), an enzyme which is known to be induced under phosphate starvation conditions (33). We investigated whether PhoP was involved in the regulation of other loci whose expression is known to be also modulated by phosphate availability (6). Pairs of isogenic strains were constructed harboring *lac* gene transcriptional fusions to phosphate-starvation-inducible genes (originally isolated by Foster and Spector; see ref. 6) and either the wild-type *phoP*$^+$ gene or mutant alleles of this locus (*phoP*::Tn*10* gene of strain MS7953s or *phoP*::MudI1734 of strain EG5086; see Table 1). The strains were then tested for their Lac phenotype (*i*) on lactose MacConkey indicator plates and (*ii*) in minimal M121 salts medium containing glucose as the carbon source, either 50 mM (excess) or 0.1 mM (limiting) phosphate, and the chromogenic substrate X-Gal. Strain JF663, which harbors a

lac gene fusion to the *psiD* gene, showed a weak Lac$^+$ phenotype as indicated by the appearance of fish-eye colonies in lactose MacConkey indicator plates and by a light blue color in excess phosphate and a blue color in limiting phosphate in minimal salt medium containing X-Gal. The *phoP* derivative of JF663, strain EG5120, did not produce β-galactosidase as indicated by a white (Lac$^-$) phenotype in all three media described above. No noticeable differences were found in the Lac phenotype between isogenic *phoP*$^+$ (JF511, JF512, JF514, JF515, and JF570) and *phoP*$^-$ (EG5110, EG5112, EG5114, EG5116, and EG5118) pairs of strains. The levels of β-galactosidase activities (see Table 2) indicated that PhoP plays a positive regulatory role at the *psiD* locus, being necessary for both basal and induced levels of expression. These activities also showed that PhoP probably does not regulate *psiB12*, *psi-15*, and *psiC17*, but it may be necessary for repression of *psiA9* and *psi-18* during growth in high phosphate.

The *phoP* gene has been shown to be necessary for *Salmonella* virulence *in vivo*, for survival in macrophages *in vitro*, and for resistance to defensins (3). We tested whether these phenotypes, which are not mediated by the nonspecific acid phosphatase (3), were in part caused by the *psiD* gene product. Isogenic derivatives of strains 14028s and MS7953s were constructed harboring the *psiD19*::Mud1-8 allele and named EG5122 and EG5124, respectively (see Table 1). We determined that the median lethal dose, LD_{50}, for intraperitoneal injection in BALB/c mice was <10 microorganisms for both EG5122 and 14028s, and >10^5 microorganisms for EG5124 and MS7953s. The sensitivity to defensins of EG5122 and EG5124 was found to be the same as that of their isogenic *psiD*$^+$ parent strains (data not shown). These results indicated that while *psiD* is indeed under the control of PhoP it does not seem to play a role in virulence.

Presence of *phoP* in Other Species. To investigate whether *phoP* homologous DNA sequences were present in other organisms, Southern hybridization analysis was carried out with the 514-bp *Eco*RV fragment internal to the *phoP* gene as probe (see Figs. 1*B* and 2) under stringent hybridization conditions. *phoP* homologs were detected in *E. coli*, *Shigella flexneri*, *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia marsescens*, *Proteus mirabilis*, *Erwinia herbicola*, and *Yersinia pestis*, but for *Proteus vulgaris*, the signal was much weaker than for other members of the family Enterobacteriaceae tested (Fig. 4). A positive signal was also found for *Neisseria gonorrhoeae*, but no hybridization was detected with other

Table 2. β-Galactosidase activities of *psi-lacZ* transcriptional fusions

Strain	Relevant genotype	β-Galactosidase, specific activity* 50 mM PO_4	0.13 mM PO_4
JF511	*psiA9*::Mud1	36	159
EG5110	*psiA9*::Mud1 *phoP*::Tn*10*	129	213
JF512	*psiB12*::Mud1	5	174
EG5112	*psiB12*::Mud1 *phoP*::Tn*10*	14	151
JF514	*psi-15*::Mud1	9	180
EG5114	*psi-15*::Mud1 *phoP*::Tn*10*	13	73
JF515	*psiC17*::Mud1	8	392
EG5116	*psiC17*::Mud1 *phoP*::Tn*10*	16	439
JF570	*psi-18*::Mud1-8	9	39
EG5118	*psi-18*::Mud1-8 *phoP5086*::MudI1734	30	31
JF663	*psiD19*::Mud1-8	198	531
EG5120	*psiD19*::Mud1-8 *phoP5086*::MudI1734	40	41

*Expressed as Miller units (12). Assay was with cells grown in minimal M121 salts medium with glucose, 10 μM nicotinic acid, and 50 mM or 0.13 mM phosphate.

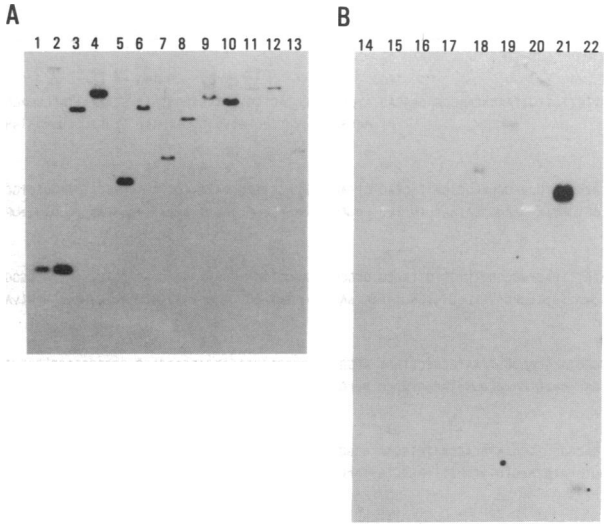

FIG. 4. Hybridization of *S. typhimurium phoP* probe to different species. Chromosomal DNA was prepared, digested with *Eco*RV, and separated on a 0.8% agarose gel as described. Between 50 and 500 ng of DNA was applied per lane except for lane 1 with the *phoP* gene-containing plasmid pEG5381, where 1/1000th that amount was applied. Transfer to membranes and hybridization were performed as described by Maniatis *et al.* (18) with two changes: (*i*) DNA was transferred to nylon membranes (Nytran from Schleicher & Schuell) instead of nitrocellulose, and (*ii*) EDTA was omitted from the hybridization solution. The membrane was prehybridized for 4 hr at 65°C, and hybridization was carried out at 65°C overnight with the ^{32}P-labeled probe corresponding to the *Eco*RV restriction fragment internal to the *phoP* gene (see Fig. 2) purified from a polyacrylamide gel. The blotted and hybridized membranes were washed twice in 0.15 M NaCl/0.015 M sodium citrate/0.1% sodium dodecyl sulfate for 1 hr at 65°C and then exposed to X-AR film (Kodak) for 22 hr (*A*) or 4 hr (*B*). Lanes: 1, plasmid pEG5381; 2, *S. typhimurium* strain 14028s; 3, *E. coli* strain BH66; 4, *Shi. flexneri* strain ATCC 12022; 5, *C. freundii* strain ATCC 8090; 6, *K. pneumoniae* strain ATCC 13883; 7, *Ent. aerogenes* strain ATCC 13048; 8, *Ent. cloacae* strain ATCC 23355; 9, *Ser. marscesens* strain ATCC 8100; 10, *Pro. mirabilis* strain PRM1; 11, *Pro. vulgaris* ATCC 13315; 12, *Erw. herbicola* strain EA1; 13, *Y. pestis* strain KIM6; 14, *V. parahemolyticus* strain BB22; 15, *Pse. aeruginosa* strain PAO1; 16, *Pse. putida* Pps338; 17, *A. tumefaciens* strain 15955; 18, *N. gonorrhoeae* strain MS11; 19, *Sac. cerevisiae* derivative of strain YP52; 20, *T. cruzi* strain Y; 21, *E. coli* strain W3110; and 22, *S. typhimurium* strain 14028s.

Gram-negative species tested such as *Agrobacterium tumefaciens*, *Vibrio parahemolyticus*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*. The yeast *Saccharomyces cerevisiae* but not the protozoa *Trypanosoma cruzi* gave a positive signal as well. Hybridization experiments were repeated with yeast DNA prepared from a different strain (IVY497) digested with two additional restriction enzymes; a single band was detected when the chromosomal DNA was digested with *Bam*HI, and two bands were detected when it was digested with either *Eco*RV or *Hin*dIII (data not shown). No hybridization was detected to DNA prepared from the Gram-negative species *Brucella abortus*, from the yeast *Candida albicans*, or from mouse (data not shown). The *phoP* genes of *E. coli* and *Shigella flexneri* have been cloned and shown to be functionally expressed and able to complement *S. typhimurium phoP* mutants for the production of nonspecific acid phosphatase (E.A.G. and F.H., unpublished results).

The Regulatory Role of PhoP. *S. typhimurium* is a highly adapted microorganism that can respond to a changing environment by modifying the repertoire of expressed genes. It is very likely that PhoP, together with the postulated inner membrane component PhoZ can sense the intracellular environment of the phagocyte and respond accordingly by modulating the expression of different genes, some of which

are probably responsible for conferring resistance to defensins and potentially to other harmful compounds present in the phagolysosome. The *phoP* gene product is the second regulator of gene expression so far described in *Salmonella* to be involved in virulence (ref. 3; this work), the other being the cAMP receptor protein (CRP; ref. 34).

The presence of phosphatase activity seems to be a constant feature of most isolates of the family Enterobacteriaceae (35), although they may differ in the individual phosphatases they encode. For example, *Salmonella* has the *phoBR* operon even though it is missing *phoA* (the structural gene for alkaline phosphatase), and *phoP* is present in most enterics, including those that do not show any nonspecific acid phosphatase activity such as *E. coli* (E.A.G. and F.H., unpublished results). There is evidence that the DNA region corresponding to the *S. typhimurium phoN* locus is absent from the *E. coli* chromosome (ref. 36; E.A.G. and F.H., unpublished results). In *E. coli, phoP* defines a new locus different from *appR*, which regulates the expression of the periplasmic acid phosphatase (the *appA* gene product; ref. 37).

Three regulatory genes that control the expression of genes as a function of phosphate levels have been described in *S. typhimurium*: *psiR*, which controls the expression of *psiC* (6); *phoP*, which controls the expression of *phoN, psiD* and potentially *psiA* and *psi-18* (ref. 5; this work); and *phoBR* as deduced by the regulated expression of the *E. coli phoA* gene when introduced via an episome (38). It is curious that *Salmonella* needs three separate phosphate sensor/regulators. In fact, mutations in *phoP* are pleiotropic, affecting the ability of *S. typhimurium* to use succinate (but not pyruvate or maltose) as a sole carbon source (E.A.G. and F.H., unpublished results). This growth inability, which may be due to an effect on the BF_0F_1 ATPase (39) is presently under investigation. Over 10 differences are found between wild-type and *phoP S. typhimurium* strains in two-dimensional protein gels (N. Buchmeier and F.H., unpublished results). These observations raise the possibility that the PhoZ–PhoP pair may sense and respond to signals other than phosphate starvation. Perhaps compounds such as sulfate that are chemically similar or ions that are chelated or precipitated by phosphate are the actual chemicals that the PhoZ–PhoP pair senses. Further work will be necessary to understand the signals by which the host intracellular environment modifies that pattern of expressed genes in *Salmonella* and the molecular basis of resistance to defensins.

Note Added in Proof. The *phoP* DNA sequence reported here is identical to the one in a recent report (40) where the sequence of *phoZ* (called *phoQ*) is also presented.

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