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Molecular Analysis of the *Escherichia coli phoP-phoQ* Operon

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The *phoP-phoQ* operon of *Salmonella typhimurium* is a member of the family of two-component regulatory systems and controls expression of the *phoN* gene that codes for nonspecific acid phosphatase and the genes involved in the pathogenicity of the bacterium. The *phoP-phoQ* operon of *Escherichia coli* was cloned on a plasmid vector by complementation of a *phoP* mutant, and the 4.1-kb nucleotide sequence, which includes the *phoP-phoQ* operon and its flanking regions, was determined. The *phoP-phoQ* operon was mapped at 25 min on the standard *E. coli* linkage map by hybridization with the Kohara mini set library of the *E. coli* chromosome (Y. Kohara, K. Akiyama, and K. Isono, Cell 50:495–508, 1987). The predicted *phoP* and *phoQ* gene products consist of 223 and 486 amino acids with estimated molecular masses of 25,534 and 55,297 Da, respectively, which correspond well with the sizes of the PhoP and PhoQ proteins identified by the maxicell method. The amino acid sequences of PhoP and PhoQ of *E. coli* were 93 and 86% identical, respectively, to those of *S. typhimurium*.

The *phoP* and *phoQ* genes of *Salmonella typhimurium* are members of the family of two-component regulatory systems; PhoP is a regulator protein and PhoQ is a sensor protein (17), and they regulate expression of the *Salmonella* virulence gene (*pagC*) (20) and the nonspecific acid phosphatase gene (*phoN*) (11). The *phoN* gene is induced by carbon, phosphate, nitrogen, and sulfur limitation (10), as well as by a low pH (17). The *phoP* and *phoQ* gene products have been shown to be involved in the transcriptional regulation of a number of the *phoP*-activated genes (*pag*) and the *phoP*-repressed genes (*prg*) (18). The *phoP-phoQ* operon has been mapped at 25 min on the *Salmonella* linkage map (11), and its nucleotide sequence has been reported previously (6, 17).

Escherichia coli has no *phoN* gene which is regulated by the *phoP-phoQ* operon (8), but a DNA sequence similar to that of the *phoP* gene was detected in *E. coli* (8) and several gram-negative species (6). Further, the *phoN* gene of *S. typhimurium*, when introduced into *E. coli*, is induced by phosphate starvation, as in *S. typhimurium* (8). These results suggest that functional *phoP* and *phoQ* genes exist in *E. coli*. We adopted *E. coli*, which is genetically better understood than *S. typhimurium*, for the study of mechanisms of signal transduction and physiological roles of the *phoP-phoQ* regulon. As a step toward these goals, we have cloned and determined the nucleotide sequence of the *phoP-phoQ* operon of *E. coli*.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 and *S. typhimurium* strains used in this study are listed in Table 1.

Media. LB broth, LB agar, T broth, and T agar were as described by Miller (16). The media used for the routine preparation of M13 phage and for the maxicell method were as previously described (1). Ampicillin was added to LB broth or T broth at 100 μg/ml. Chloramphenicol was added to LB agar at 20 μg/ml. So that synthesis of acid phosphatase by bacterial colonies could be examined, 5-bromo-4-chloro-3-indolyl phosphate (X-P) was added to agar plates to a final concentration of 40 μg/ml.

Recombinant DNA methods. Standard methods for recombinant DNA manipulation as described by Sambrook et al. (22) were generally used.

DNA sequencing. The manipulation of M13mp18 phage (27) was as described by Messing et al. (15). A series of M13 phage derivatives with cloned DNA fragments with one end fixed and the other end formed by successive deletions at the 5′ end was prepared as described by Henikoff (7). The DNA sequences were analyzed by the method of Sanger et al. (24).

Assay of chloramphenicol acetyltransferase. Chloramphenicol acetyltransferase in the extracts of sonicated cells was assayed by the method of Shaw (25). The specific activity was expressed as nanomoles of 5-thio-2-nitrobenzoate liberated per minute per unit of optical density of the cell culture at 450 nm.

Identification of the protein encoded by plasmids. The *phoP* and *phoQ* gene products were identified by the maxicell method, which allows isotope labeling of protein encoded by plasmids (23).

Southern hybridization. The DNA fragments of *S. typhimurium* and *E. coli* used as *phoP* probes were radiolabeled by the random primer method (4) and used to examine the presence of similar DNA sequences in the chromosomes by DNA-DNA hybridization as described by Southern (26).

Enzymes and radioisotopes. The restriction endonucleases, T4 DNA ligase, T4 DNA polymerase, Klenow fragment of DNA polymerase I, exonuclease III, mung bean nuclease, bacterial alkaline phosphatase, a random primer DNA labeling kit, and an M13 sequencing kit were obtained from Takara Shuzo (Kyoto, Japan). [α-^{32}P]dCTP (>400 Ci/mmol) and [^{35}S]methionine were purchased from Amersham Japan (Tokyo, Japan).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under the accession number D90393.

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TABLE 1. Bacterial strains used in the study

Strain	Characteristic(s)	Reference or source
E. coli K-12		
ANCK10	F⁻ *leu lacY trp his argG rpsL ilv metA* (or *metB*) *thi*	14
CSR603	*recA1 uvrA6 phr-1*	23
JC7623	*thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rspL-31 tsx-33 sup-37 recB21 recC22 sbcB15*	19
JM103	Δ(*pro-lac*) *supE thi* (F′ *traD36 proAB lacI^qZ M15*)	15
S. typhimurium		
SJ10002	*hsdR*	K. Kutsukake
TA2362	*phoP12*	11

RESULTS AND DISCUSSION

Cloning of the *E. coli phoP* gene and mapping of the gene on the chromosome. We cloned the *E. coli phoP* gene by functional complementation of an *S. typhimurium phoP* mutant with recombinant plasmids carrying *E. coli* chromosomal fragments. The gene library of *E. coli* K-12 strain ANCK10 was constructed by digesting the chromosomal DNA with *Eco*RV enzyme and ligating it with *Eco*RV-digested pBR322 (2). The gene library was amplified in an *S. typhimurium hsdR* strain, SJ10002. Recombinant plasmids that transformed white, X-P⁻ colonies of TA2362 (*phoP12*) into blue colonies were selected. One such plasmid, pMK200, contained a 4.1-kb *Eco*RV fragment. The physical map of this fragment is shown in Fig. 1B, and it hybridized with the DNA fragment containing the *phoP* gene of *S. typhimurium* (data not shown).

To identify the location of the *phoP* gene on the *E. coli* chromosome, hybridization analysis was performed with Kohara clones (12) E3G11, 14C1, E9G1, 15A8, E4C2, 7F9, 20E6, 3E11, 4D1, 2A3, and 7C10 of the *E. coli* genome library, which contain the chromosomal regions around 25 min, which is the locus of the *phoP* gene on the *S. typhimurium* linkage map. The 1.8-kb *Eco*RV-*Sca*I fragment of pMK200 and the 514-bp *Eco*RV fragment internal to the *phoP* gene of *S. typhimurium* were used as probes. Both probes hybridized with clone 7F9 (data not shown). These results suggest that the *phoP* gene is carried on pMK200 and located at 25 min on the *E. coli* linkage map as detailed in Fig. 1.

Nucleotide sequence of the *phoP* locus and identification of the *phoP* and *phoQ* genes. We have determined the DNA sequence of the 4.1-kb *Eco*RV chromosomal fragment carried on pMK200 which contains the *E. coli phoP* gene and its flanking regions. The entire nucleotide sequence is shown in Fig. 2. Two complete open reading frames (ORFs) whose deduced sequences are very similar to those of the *S. typhimurium* PhoP and PhoQ proteins (17) are identified, and they appear to constitute an operon, as do the *S. typhimurium phoP* and *phoQ* genes. Therefore, we conclude that these ORFs are coding regions of the *E. coli phoP* and *phoQ* genes. The *phoP* coding region is located between nucleotides 1051 and 1719, begins with an ATG codon, ends with a TGA codon, and is preceded by a Shine-Dalgarno sequence, GGAG, at an appropriate position. The *phoQ* coding region is located between nucleotides 1722 and 3179, begins with an ATG codon which overlaps the stop codon of *phoP* by 1 nucleotide, and ends with a TAA codon. The putative −10 sequence, cATAAT, is at nucleotides 1003 to 1008, and 12 nucleotides upstream of the −10 region, a direct repeat of hexanucleotide sequence GTTTA(T or C) is found, as with *S. typhimurium* (6). No sequence substantially similar to the consensus −35 sequence is found in this region. These regions were highly conserved between *E. coli* and *S. typhimurium*, while the other parts of the upstream region were not conserved. Functionally important sequences in

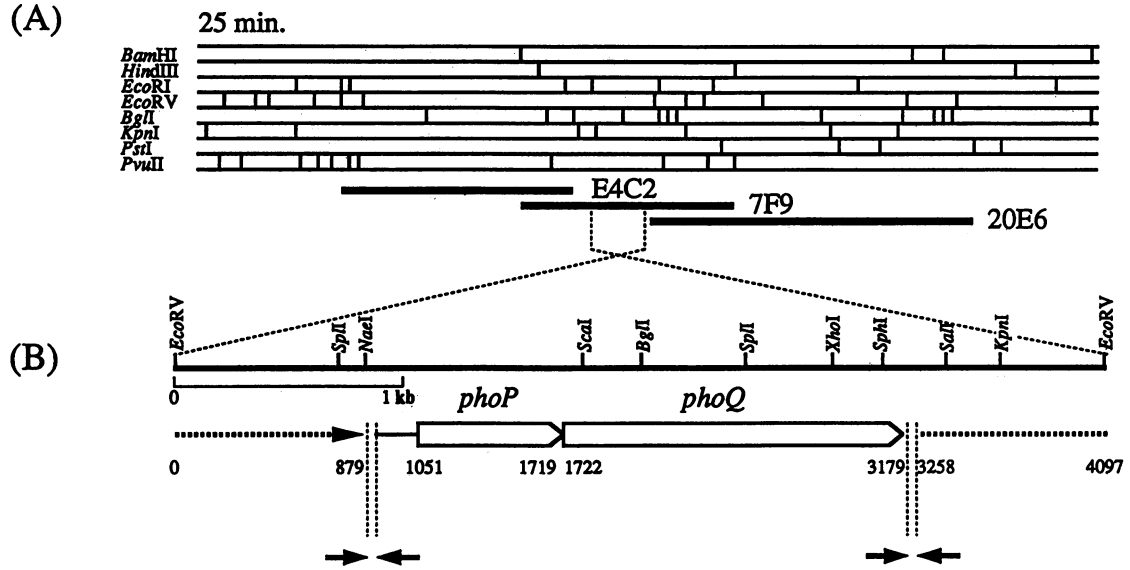

FIG. 1. Physical maps of the DNA fragment containing the *E. coli phoP-phoQ* operon. (A) Restriction map of the *E. coli* chromosome segment that contains the *phoP-phoQ* region carried by λ clones E4C2, 7F9, and 20E6, as described Kohara et al. (12). (B) Enlarged restriction map of the *phoP-phoQ* region. Arrows indicate the inverted repeats.

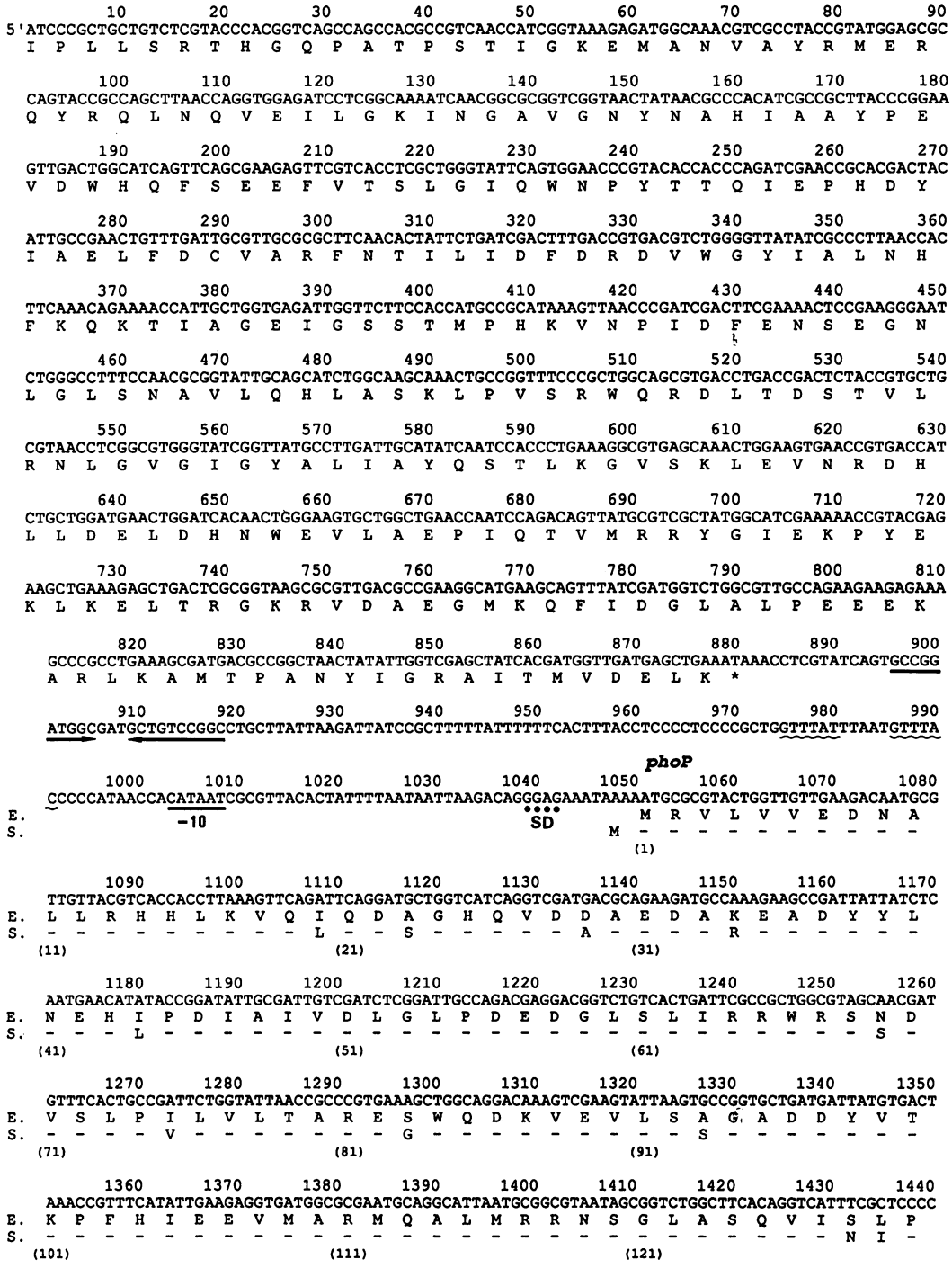

FIG. 2. Nucleotide sequence of the *Eco*RV fragment containing the *E. coli phoP-phoQ* operon and its flanking regions and the deduced amino acid sequences. The amino acid sequences of the PhoP and PhoQ proteins of *E. coli* and *S. typhimurium* were compared. SD and dots mark the ribosome-binding site. A putative −10 sequence is indicated. Hexanucleotide repeated sequences are indicated with wavy lines. The two convergent arrows indicate inverted repeat sequences that may serve as transcriptional terminators. Two hydrophobic transmembrane stretches in PhoQ are indicated with double underlines. ⩚, extra serine residue in the *S. typhimurium* PhoQ protein. Asterisks indicate termination codons. The amino acid sequences of *S. typhimurium* PhoP and PhoQ are as described by Miller et al. (17).

the regulatory regions are highly conserved (13). Therefore, the repeated hexanucleotide sequences and the putative −10 region may be very important for the *phoP* promoter. Sequences that may form stem-loop structures are located upstream of *phoP* and downstream of the TAA translational termination codon of *phoQ*. They may function as transcriptional terminators or regulatory elements of the downstream genes.

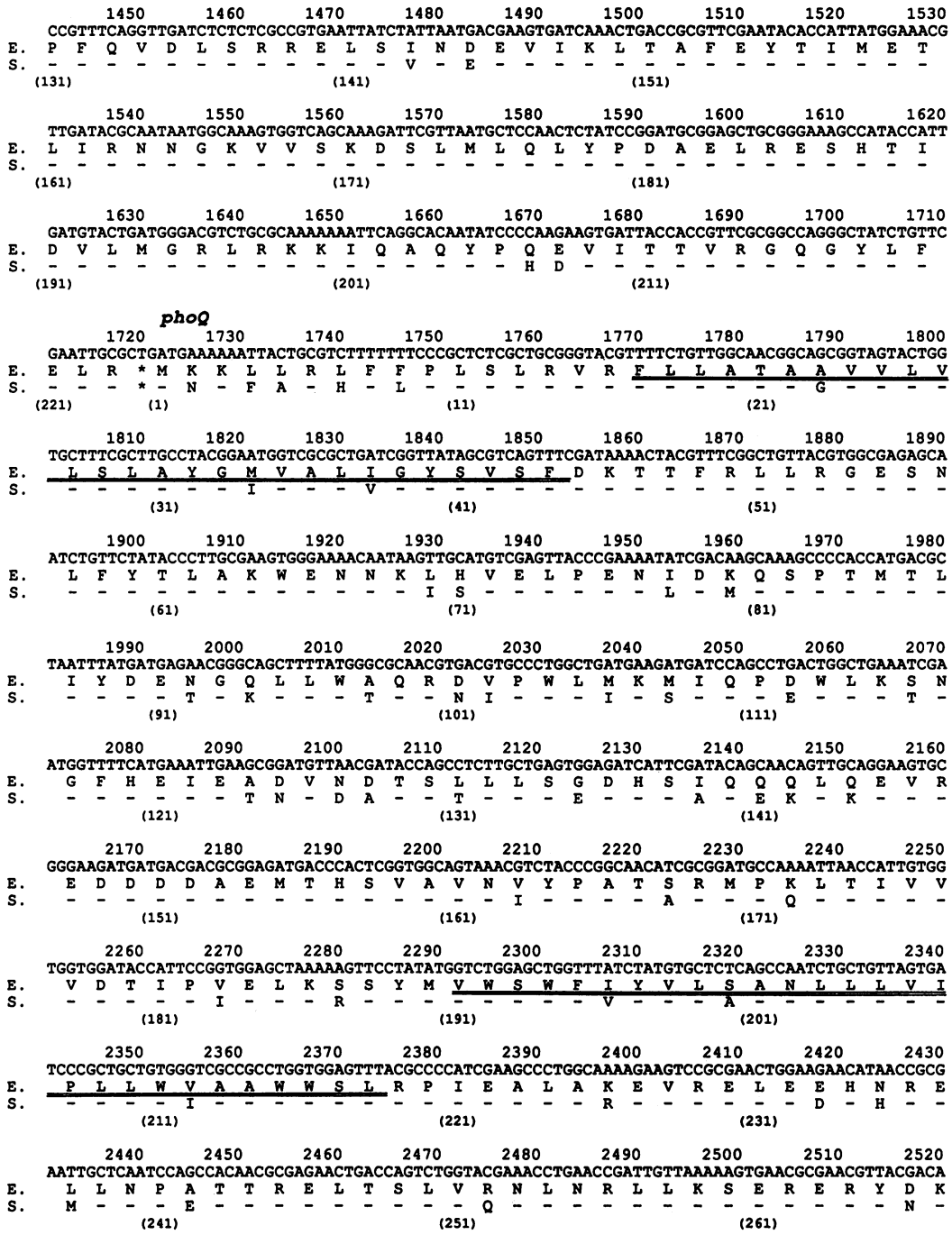

FIG. 2—*Continued.*

The *phoP* and *phoQ* genes could code for proteins of 223 and 486 amino acids with predicted molecular weights of 25,534 and 55,297, respectively. The deduced amino acid sequences of PhoP and PhoQ of *S. typhimurium* (17) and those of *E. coli* are 93 and 86% identical, respectively. There are 15 amino acid differences between the PhoP sequence of *E. coli* and that of *S. typhimurium*, while the PhoQ sequence of *E. coli* differs from that of *S. typhimurium* in 70 amino acids. Met-2 of PhoP and Ser-330 of PhoQ of *S. typhimurium* were absent in the corresponding positions in PhoP and PhoQ of *E. coli* (Fig. 2). The *E. coli* PhoQ protein has two putative transmembrane sequences in the N-terminal regions as predicted by the hydropathy profile, a phenomenon which is often found in the sensor proteins belonging to members of the family of the two-component regulatory systems (21). The features of the *E. coli phoP-phoQ* region mentioned above are very similar to those of the *S. typhimurium phoP-phoQ* regulon (17). Two ORFs were found, one upstream of the *phoP* gene and the other downstream of the *phoQ* gene, both of which extend further into unsequenced

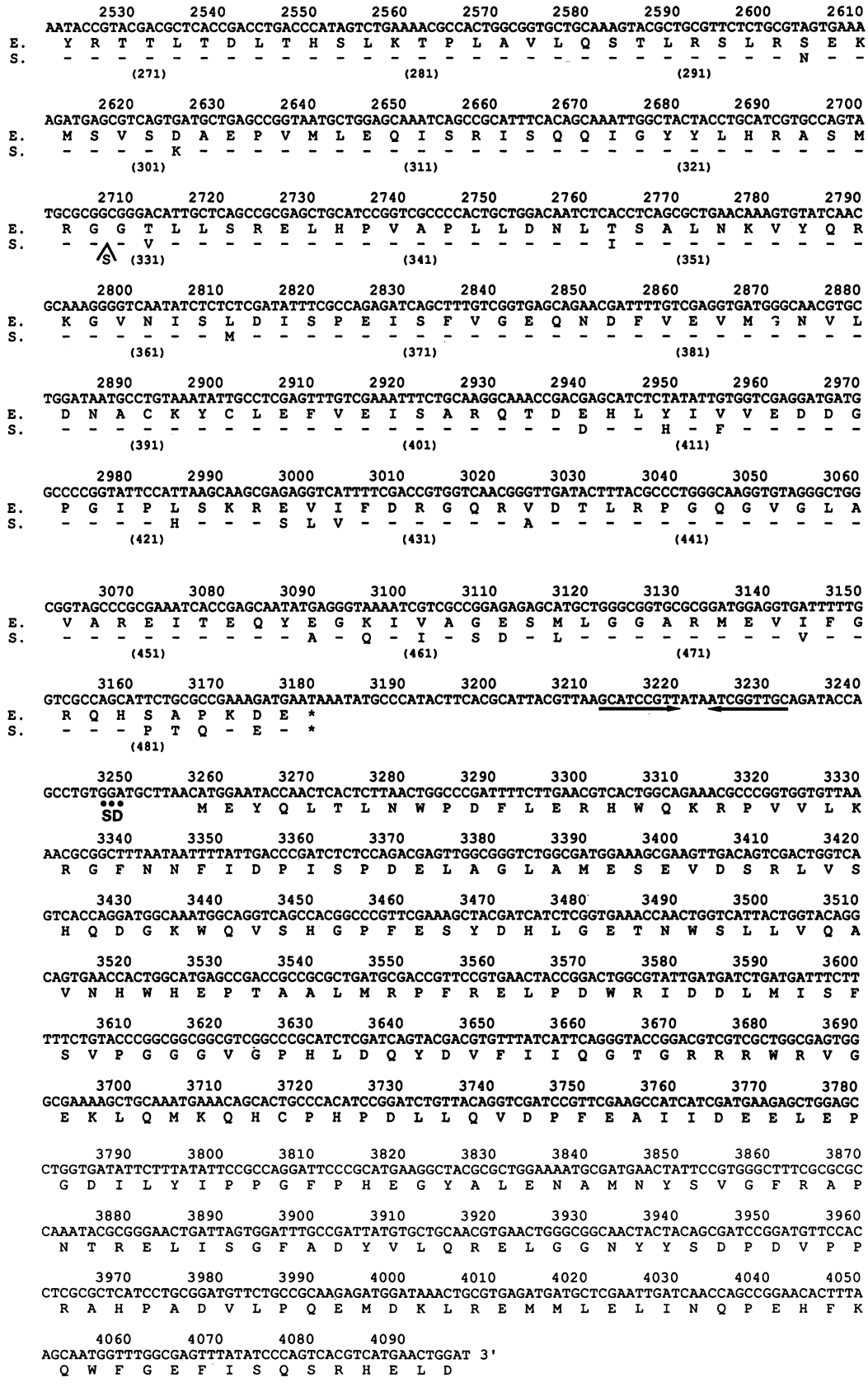

FIG. 2—*Continued.*

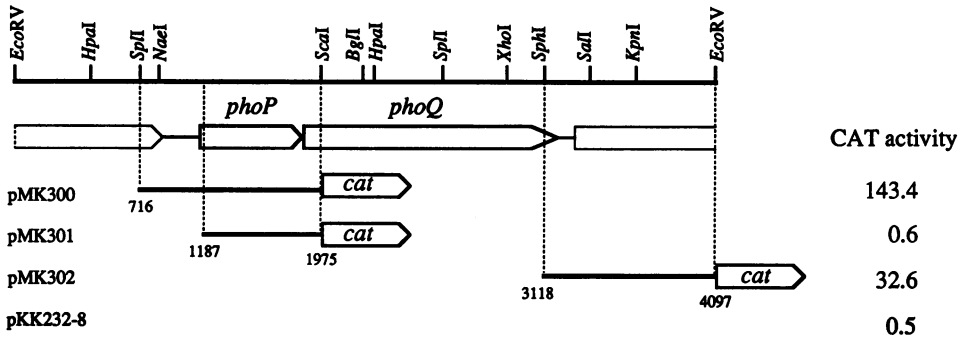

FIG. 3. Assay of the promoter activity for the *phoP* and *phoQ* genes. Bold lines indicate the chromosomal fragments of the *phoP-phoQ* region in the operon fusion plasmids. Arrows indicate the direction of the *cat* gene orientation. Chloramphenicol acetyltransferase (CAT) activity in extracts of sonicated cells of ANCK10 carrying plasmids with the operon fusions was assayed.

regions. Neither flanking ORF appears to be transcriptionally coupled to the *phoP-phoQ* operon.

Analysis of promoter activity in the cloned *phoP-phoQ* region by operon fusions. To locate the promoter of the *phoP* and *phoQ* genes, we constructed a series of operon fusion plasmids. DNA fragments containing nucleotides 716 to 1975, 1187 to 1975, or 3118 to 4097 were recloned into a promoter cloning vector, pKK232-8 (3), to construct fusions with the *cat* gene, and the resultant plasmids (pMK300, pMK301, and pMK302) were introduced into strain ANCK10. The promoter activities of the cloned DNA fragments in the fusion plasmids were measured by monitoring the chloramphenicol acetyltransferase in the extracts of the cells carrying these plasmids (Fig. 3). ANCK10 carrying pMK300 or pMK302 showed high levels of the enzyme activity, while ANCK10 carrying pMK301 showed no activity. This result suggests that a common promoter for *phoP* and *phoQ* is located upstream of the *phoP* gene and that another promoter for the gene distal to *phoQ* is located downstream of the *phoQ* gene. From these results, we conclude that the *phoP* and *phoQ* genes constitute a single operon. These results are also consistent with the hypothesis that the inverted sequences found upstream and downstream of the *phoP-phoQ* operon function as terminators.

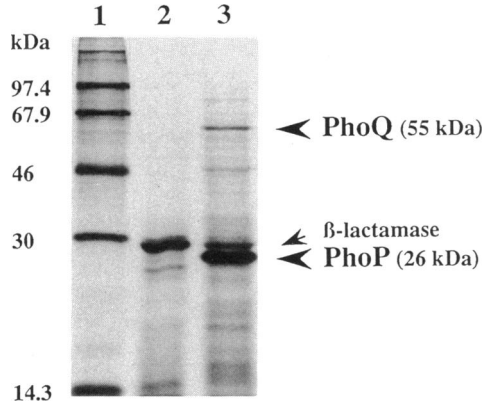

FIG. 4. Identification of the products of *phoP* and *phoQ* by the maxicell method. An autoradiogram of ^{35}S-labeled proteins produced in maxicells with *phoP*$^+$-*phoQ*$^+$ plasmid, which carries nucleotides 909 to 3232 of the chromosomal fragment (lane 3), and with pUC18 (lane 2) is shown. Isotope-labeled protein size markers (Amersham Japan, Tokyo) were run on lane 1.

Identification of the *phoP* and *phoQ* gene products. To identify the *phoP* and *phoQ* gene products, the plasmid-encoded proteins were labeled with [^{35}S]methionine by the maxicell method. Two proteins with approximate molecular masses of 26 and 55 kDa were found in the maxicells carrying the *phoP*$^+$-*phoQ*$^+$ plasmids (Fig. 4, lane 3). The 46-kDa protein found in the maxicells carrying the *phoP*$^+$-*phoQ*$^+$ plasmids may be the degradation product of PhoQ. In the maxicells carrying the *phoP*$^+$ plasmids, only the 26-kDa protein was detected (data not shown). These proteins were not detected in the cells carrying the vector plasmid, pUC18 (Fig. 4, lane 2). The molecular masses of these products agreed very well with those predicted from the nucleotide sequences of the *phoP* and *phoQ* genes.

The *phoP-phoQ* operon of *S. typhimurium* has been shown to be necessary for *Salmonella* virulence (5). *E. coli* K-12 has no such virulence phenotype and lacks the *phoN* gene, but it has the *phoP-phoQ* operon. We recently constructed a Δ(*phoP-phoQ*) strain, compared the whole-cell proteins of the strain with those of the wild-type strain by two-dimensional gel electrophoresis, and found that expression of at least 50 protein species changed as a result of this deletion mutation (9). Therefore, the *phoP* and *phoQ* genes of *E. coli* appear to regulate as many genes as their counterparts in *S. typhimurium* (18). Further work will be necessary to understand the physiological roles of the *phoP-phoQ* regulon in *E. coli*.

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