Molecular Microbiology (1988) 2(3), 347–352

Regulation of components of the *Pseudomonas aeruginosa* phosphate-starvation-inducible regulon in *Escherichia coli*

R. J. Siehnel, E. A. Worobec and R. E. W. Hancock*
Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.

Summary

Plasmids pPBP and pRS-XP containing the cloned genes for the *Pseudomonas aeruginosa* phosphate-starvation-inducible periplasmic phosphate-binding protein and outer membrane porin P (*oprP*), respectively, were introduced into various *Escherichia coli* Pho-regulon regulatory mutants. Using Western immunoblots and specific antisera, the production of both gene products was observed to be under the control of regulatory elements of the *E. coli* Pho regulon. Sequencing of the region upstream of the translational start site of the *oprP* gene revealed a 'Pho box' with strong homology to the *E. coli* consensus 'Pho box', the putative binding site of the PhoB activator. Since *P. aeruginosa* and *E. coli* belong to different families and have quite different GC contents, these data suggest strong evolutionary conservation of regulatory elements of the Pho regulon.

Introduction

The control of gene expression of the components of the *E. coli* high-affinity phosphate transport system (Pst) is very complex, involving at least four regulatory genes, *phoB, phoR, phoM* and *phoU*, and their gene products PhoB, PhoR, PhoM and PhoU, respectively (see Wanner, 1987 for a review). The ultimate activator is PhoB since mutations in *phoB* result in a pleiotropically negative phenotype with respect to the components of the phosphate-starvation-inducible (Pho) regulon. The PhoB activator is either transcriptionally activated by PhoR when phosphate is limiting, or transcriptionally repressed by PhoR when phosphate is non-limiting. Because phosphate limitation favours production of the PhoB activator, mutants in Pst have constitutive phenotypes for Pho-regulon products. PhoU is thought to function in the conversion of PhoR from the activator form to the repressor form. Mutations in *phoU* or *phoR* result in the constitutive production of the structural elements of the Pho regulon since, in such mutants, another regulatory gene product, PhoM, will transcriptionally activate PhoB. The PhoM activator is not controlled by changes in phosphate levels and is repressed when PhoR is functional.

Conditions of low environmental phosphate stimulate the co-induction, in *E. coli*, of an outer membrane porin (PhoE), a periplasmic phosphate-binding protein (PhoS), and a periplasmic alkaline phosphatase (PhoA). The *phoB* gene product appears to activate the transcription of each of these genes by interacting with specific regulation sequences located in analogous regions upstream from the structural genes. These sequences are highly homologous and have been termed the 'Pho box' (Shinagawa *et al.*, 1987).

Phosphate transport has been examined in several other bacterial species and recent evidence suggests that a phosphate-starvation-inducible regulon, similar to that found in *E. coli*, exists in these other species. For example, a PhoB analogue has been identified in *Bacillus subtilis*. This gene product regulates components of this organism's Pho regulon in a similar fashion to that of *E. coli* (Seki *et al.*, 1987). The genes for the phosphate-regulated porins of *Vibrio parahaemolyticus* (McCarter and Silverman, 1987), *Klebsiella pneumoniae,* and *Enterobacter cloacae* (van der Ley *et al.*, 1987) are all regulated in *E. coli* in the same manner as the *E. coli* phosphate-regulated porin PhoE, with the latter two genes having distinct Pho boxes. *P. aeruginosa* also has a high-affinity phosphate-starvation-inducible regulon (Gray *et al.*, 1982; Poole and Hancock, 1983; 1984), which appears to have several components analogous to those found in *E. coli*. Under conditions of phosphate limitation, *P. aeruginosa* demonstrates induction of an outer membrane phosphate-specific porin protein P (Hancock *et al.*, 1982), which immunologically cross-reacts with PhoE trimers (Poole and Hancock, 1986a) but which has substantially different channel properties (Hancock and Benz, 1986), a periplasmic phosphate-binding protein (Poole and Hancock, 1984), an alkaline phosphatase, and several other polypeptides (Gray *et al.*, 1982). The *P. aeruginosa* phosphate-starvation-inducible products also include two haemolysins, a rhamnolipid, and a phospholipase C (Liu, 1979), which are not produced in *E. coli*. The regulation of this system has not been examined in detail but mutant strains that are either constitutive (Gray *et al.*, 1982; Poole

Received 21 December, 1987. *For correspondence.

and Hancock, 1984) for all the phosphate-starvation-inducible gene products (i.e. PhoU-, PhoS- or PhoR-like mutations) or pleiotropically negative (Poole and Hancock, 1986b) for all these components (i.e. PhoB-like mutation) have been identified.

In this communication we have addressed the analogy between the *P. aeruginosa* and the *E. coli* Pho regulons. We introduced plasmids containing the cloned structural genes for the *P. aeruginosa* phosphate-specific porin P and the periplasmic phosphate-binding protein into wild-type *E. coli* and several *E. coli* strains bearing mutations in the regulatory components of the Pho regulon and found that the expression of these genes was regulated by the *E. coli* Pho-regulon control elements. The identification of a Pho box upstream of the porin P structural gene suggested strong evolutionary conservation of Pho regulon regulation elements.

Results

Regulation of P. aeruginosa *porin P and phosphate-binding protein gene products in* E. coli

The plasmids containing the cloned *P. aeruginosa* genes for the phosphate-porin protein P (pRS-XP) and the phosphate-binding protein (pPBP) were transformed into *E. coli* wild-type strains and several strains with regulatory mutations (Table 1). These *E. coli* strains produce the components of the *E. coli* Pho regulon constitutively in the case of *phoU, phoS* and *phoR* mutants, whereas strains with mutations in *phoB* or *phoR* plus *phoM* are pleiotropically negative for the same components. The strains transformed with plasmids pRS-XP and pPBP were grown in phosphate-sufficient and phosphate-deficient media; cell pellets were collected and cell lysates examined by SDS-polyacrylamide gel electrophoresis and Western immunoblots using antisera specific for protein P or the phosphate-binding protein. Phosphate-binding protein, with the same molecular weight and antigenic properties as authentic *P. aeruginosa* phosphate-binding protein, was found to be produced and regulated by the control elements of *E. coli* phosphate regulon (Table 2). Examples of this regulation are demonstrated in Fig. 1 for a wild-type *E. coli*, a *phoU* constitutive mutant, and a pleiotropically negative *phoB, phoR* double mutant.

Protein P monomer was only detected in whole cell lysates of strains with mutations preventing production of the equivalent *E. coli* outer membrane protein PhoE. In PhoE$^+$ strains, protein P was more readily identified in outer membrane fractions. As with the *P. aeruginosa* phosphate-binding protein, production of porin protein P was also found to be controlled by the *E. coli* regulatory components (Table 2).

All *E. coli* strains with and without plasmids pRS-XP and pPBP were examined for alkaline phosphatase production to confirm the phenotypes of the strains used and the inability of the plasmids' insert DNA to complement the *E. coli* mutations (Table 2).

Table 1. *Escherichia coli* strains and plasmids.

Strain/ Plasmid	Description[a]	Source/ Reference
Bacterial strains		
LE392	F$^-$, *hsdR*-514, (r_k-, m_k-) *supE*-44 *supF*-58, *lacY*1 or Δ(*lacIZY*)6, *galK*-2, *galT*-22, *metB*-1, *trpR*-55, λ$^-$	(Murray *et al.*, 1977)
K10	HfrC, *relA*1, *tonA*22, *pit*-10, *spoT*1, T_2w^r	B. Bachmann
C86	*pho*-21 derivative of K10	J. Tommassen
AB1157	F$^-$, *thr, leu, proA*2, Δ(*proA-phoE-gpt*) *his, thi, argE, lacY, galK, xyl, rpsL*	J. Tommassen
CE1194	*pho*-21 derivative of AB1157	J. Tommassen
S3	*phoB*63 derivative of K10	B. Bachmann
BW256	*thi, crp*-72, *rpsL pho*-510	B. Wanner
BW255	*phoR*68 derivative of BW 256	B. Wanner
BW705	*lac proL*::Tn*5 phoR*68 *phoM* 453 derivative of BW256	B. Wanner
BW3908	*thi, lac*-169, *pho*-510, *rpsL*267	B. Wanner
BW3212	Δ(*psiF proC avoLM phoBR*) derivative of BW3908	B. Wanner
BW6504	*phoU*35 derivative of BW3908	B. Wanner
Plasmids		
pPBP	IncP1:Tc^r *rlx* λcos . pLAFr1 derivative containing the cloned *P. aeruginosa* phosphate-binding protein gene	This laboratory
pRS-XP	Amp^r, pUC18 derivative containing the cloned *P. aeruginosa oprP* gene	This laboratory

a. Abbreviations: Tc^r, tetracycline resistance; Amp^r, ampicillin resistance; IncP1, incompatibility group P1.

Regulatory region of porin protein P

In the *E. coli* Pho regulon, gene expression is dependent on the PhoB activator (Makino *et al.*, 1986). It has been postulated that the PhoB protein interacts with a family of 18 nucleotide sequences termed the phosphate box or Pho box, which are located exactly 10 nucleotides upstream from the deduced Pribnow (−10) boxes (Makino *et al.*, 1986; Shinagawa *et al.*, 1987). The amino-terminus of the protein P (*oprP*) gene had been identified by hybridization of subclones of plasmid pRS-XP with an oligonucleotide probe synthesized on the basis of the known amino-terminal amino acid sequence of the mature P (R.J. Siehnel *et al.*, submitted manuscript). The direction of transcription of the *oprP* gene was deduced from the site of insertion of a transposon (Tn*501*) in a protein-P-deficient mutant and from fusion of downstream sequences to a translational fusion vector pUC18 (R.J. Siehnel *et al.*,

Table 2. Regulation of the cloned *P. aeruginosa* porin protein P and phosphate-binding protein in various *E. coli* phosphate-regulation mutant strains.

Strain	Relevant genotype and phenotype[a]	*P. aeruginosa* phosphate-binding protein expression[b]		*P. aeruginosa* protein P expression[b]		*E. coli* alkaline phosphatase activity	
		+Pi[d]	−Pi	+Pi	−Pi	+Pi	−Pi
K10[e]	wild type	−	+	−	+	< 0.01	1.0
C86	*phoS*; constitutive	+	+	NT[f]	NT	0.5	3.8
AB1157	*phoE*	−	+	−	+	< 0.01	4.4
CE1194	*phoE phoS*; constitutive	+	+	+	+	3.5	2.4
S3	*phoB*; pleiotropically negative	−	−	NT	NT	< 0.01	< 0.01
BW255	*phoR*; constitutive	+	+	NT	NT	2.0	1.0
BW705	*phoR phoM*; pleiotropically negative	−	−	NT	NT	< 0.01	< 0.01
BW3212	*phoB phoR*; pleiotropically negative	−	−	−	−	< 0.01	< 0.01
BW6504	*PhoU*; constitutive	+	+	+	+	0.5	2.4

a. Genotype and phenotype of strain with respect to the constituents of the phosphate-starvation-induced regulon. *phoS*-deficient strains contain the *pho*-S21 mutation.

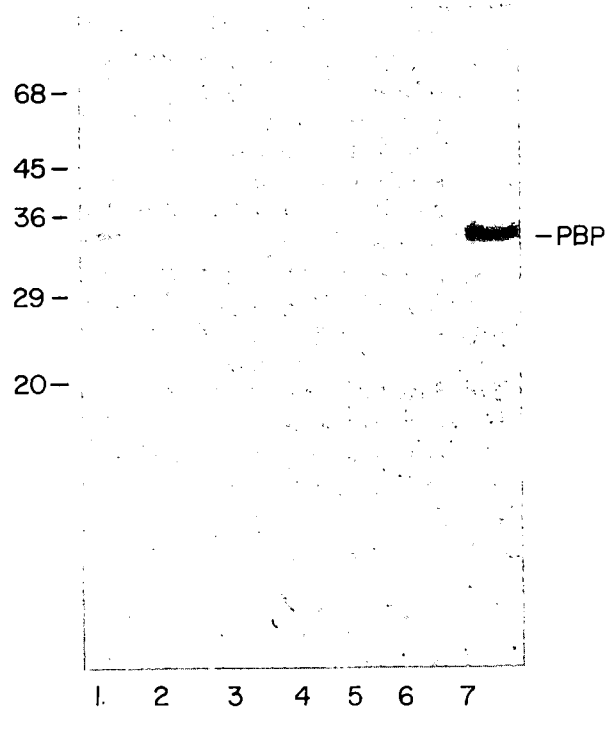
b. Protein P and phosphate-binding protein expression, in the indicated strains containing either plasmid pRS-XP or pPBP, respectively, was determined by Western immunoblot analysis. Protein P expression was relatively weak in *phoE*$^+$ strains, especially the wild-type strains. (−) signifies no expression, (+) signifies expression of a band aligning with authentic protein P or phosphate-binding protein, respectively, and staining with specific antisera. Plasmid stability was examined for all strains and conditions where no expression was observed.

c. Enzyme activity expressed as μmoles substrate hydrolysed min^{-1} mg^{-1} protein.

d. +Pi: phosphate-sufficient medium containing 660 μM phosphate; −Pi: phosphate-deficient medium containing 41 μM phosphate.

e. Similar data were obtained for wild-type strains BW256 and BW3908.

f. NT: not tested.



submitted manuscript). Therefore we cloned and sequenced a *Hin*dIII-*Eco*RI fragment from the upstream sequences flanking the *oprP* gene and overlapping the amino-terminal portion of the structural gene (Fig. 2). The reading frame of the *oprP* gene was confirmed by aligning the DNA sequence to the deduced codons required to give rise to the known amino-terminal sequence of protein P. As with other outer membrane proteins, protein P apparently contained an amino-terminal signal sequence that was 29 amino acids long. A search was made for sequences homologous to the Pho box and one was found 104 nucleotides upstream from the first nucleotide encoding the signal peptide of protein P. This can be compared to the analogous *E. coli* outer membrane protein *phoE* gene for which the Pho box is placed 82 nucleotides prior to the first nucleotide of the coding sequence (van der Ley *et al.*, 1987).

Fig. 1. Immunoblot of whole-cell lysates of various *E. coli* strains containing plasmid pPBP. The immunoblot was probed with phosphate-binding-protein-specific polyclonal sera at a 1:200 dilution. Lanes 1, 2: strain BW6504 (constitutive) grown in phosphate-deficient (1) and phosphate-sufficient (2) media. Lanes 3, 4: strain K10 (wild type) grown in phosphate-deficient (3) and phosphate-sufficient (4) media. Lanes 5, 6: strain BW3212 (pleiotropically negative) grown in phosphate-deficient (5) and phosphate-sufficient (6) media. Lane 7: purified phosphate-binding protein. Molecular weight markers (in kiloDaltons) are indicated on the left. The position of phosphate-binding protein is indicated by PBP.

```
-134  -130        -120        -110        -100        -90
AAGC  TTTCGCGCTT  TGCAGTCTCG  CTGTCACAAC  CCATCCAGAT  GATCCCCCTC

-80         -70         -60         -50         -40         -30
CAGCAGCGCC  GGCCGGCGAC  AGAACCGGCC  GTTTCCGCGC  TGCTCAAGAC  CATTCGAAAT

-20         -10                              15                  30
CCGAACAGGG  GACTTACCTG  ATG ATT CGC AGA CAC TCG TGC AAA GGG GTG GGG AGC
                        Met Ile Arg Arg His Ser Cys Lys Gly Val Gly Ser

         45                  60                  75                  90
AGT GTT GCC TGG AGT TTG CTG GGC CTG GCG ATT TCC GCG CAG AGC CTG GCC GGG
Ser Val Ala Trp Ser Leu Leu Gly Leu Ala Ile Ser Ala Gln Ser Leu Ala Gly

                105                 120                 135
ACC GTG ACC ACC GAC GGT GCC GAC ATC GTG ATC AAG ACC AAG GGC GGC CTC GAA
Thr Val Thr Thr Asp Gly Ala Asp Ile Val Ile Lys Thr Lys Gly Gly Leu Glu

    150                 165
GTC GCC ACC ACC GAC AAG GAA TTC
Val Ala Thr Thr Asp Lys Glu Phe
```

Fig. 2. Nucleotide sequence of the *N*-terminal and upstream regions of the *P. aeruginosa* protein P (*oprP*) gene. The Pho box, possible Pribnow box and Shine-Dalgarno sequences are underlined. The reading frame utilized predicted an *N*-terminal sequence of protein P starting at nucleotide +88 that matched the known *N*-terminal amino acid sequence. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00553.

The Pho box upstream from the *oprP* gene closely matched the consensus *E. coli* Pho box (11/18 identical bases and 3 others which were observed substitutions in at least one of the 5 previously sequenced *E. coli* Pho boxes; Fig. 3). Furthermore, as with all *E. coli* Pho boxes, the putative Pho box of the *oprP* gene was exactly 10 nucleotides from a −10 site which had a 4/6 match to the *E. coli* consensus −10 site.

Discussion

In this communication we have demonstrated the regulation, by the control elements of the *E. coli* Pho regulon, of two major components of the *P. aeruginosa* phosphate-starvation-inducible regulon, the periplasmic phosphate-binding protein, and the outer membrane phosphate-specific porin protein P. The expression of these two genes was controlled by phosphate levels in the wild-type strains, constitutively expressed in strains with *phoU, phoS* or *phoR* mutations, and not activated in strains with *phoB* mutations or *phoR phoM* double mutations (Table 2). The identification of a sequence preceding the protein P structural gene, with substantial homology to the *E. coli* consensus Pho box, emphasizes the close relationships between Pho-regulon regulation in these widely divergent organisms.

The phenomenon of inter-species control of regulons is unusual but not novel. The best-studied examples have been observed for nitrogen metabolism regulatory systems in *Klebsiella pneumoniae, Azotobacter vinelandii, Rhizobium meliloti* and *E. coli* (Gussin *et al.*, 1986); the only other well-characterized examples occur for phosphate regulatory systems. Regulation of the *Vibrio parahaemolyticus ompP* gene by the *E. coli* system (McCarter and Silverman, 1987) has been observed recently. In addition, van der Ley *et al.* (1987) observed regulation in *E. coli* of the *PhoE* genes from other enterobacterial species (*K. pneumoniae* and *Enterobacter cloacae*) and observed the presence of a Pho box in the upstream sequences of these genes. In our studies we have provided additional information to support this cross-species control by showing the control by *E. coli* of two *P. aeruginosa* phosphate-regulated genes, namely the porin protein P and the phosphate-binding protein.

Interestingly, protein P differs substantially from the enterobacterial PhoE porins with respect to function (Hancock and Benz, 1986) and certain physical properties (Poole and Hancock, 1986). The PhoE channels are large

```
                                  Pho box          -10
E. coli phoA                CTGTCATAAAGTTGTCAC--10bp--TATAGT
E. coli phoB                TTTTCATAAATCTGTCAT--10bp--CATAAT
E. coli phoS                CTTACATATAACTGTCAC--10bp--TATTTT
E. coli phoE                CTGTAATATATCTTTAAC--10bp--TAAAAA
K. pneumoniae phoE          TTGTCATAAATATTTAAT--10bp--TAAAAA
E. cloacae phoE             TTGTCATAAAAGTTTCAT--10bp--TAAAAC
P. aeruginosa oprP          TTGCAGTCTCGCTGTCAC--10bp--GATGAT
                                •  •  •              •  •
E. coli
consensus                   CT(G/T)TCATA(A/T)A(A/T)CTGTCA(C/T)--10bp--TATAAT
```

Fig. 3. Comparison of the putative Pho box of *P. aeruginosa* protein P with analogous sequences from the phosphate-starvation-inducible genes of *E. coli* (van der Ley *et al.*, 1987; Makino *et al.*, 1986). The dots below the *P. aeruginosa* sequence indicate nucleotides that do not match nucleotides at that position in any of the known Pho boxes.

channels (Hancock, 1987) with no selectivity for phosphate, although they do appear to be selective for polyphosphates (Dargent *et al.*, 1986), as does the *V. parahaemolyticus ompP* protein (McCarter and Silverman, 1987). In contrast, protein P forms constricted channels containing a phosphate-binding site, thus making protein P channels phosphate-selective (Hancock and Benz, 1986). Despite these functional differences, the protein P gene, like the *phoE* genes, is preceded by a Pho box for PhoB binding which probably directs the regulation of this gene in *E. coli*. We have not yet identified a Pho box with respect to the *P. aeruginosa* phosphate-binding protein gene, but the results presented here predict the presence of one.

Experimental procedures

Bacterial strains and media

The bacterial strains and plasmids used in this study are listed in Table 1. Strains were maintained on Luria broth media (Maniatis *et al.*, 1982). The *E. coli* low (41-μM) and high (660-μM) phosphate-containing minimal media were prepared according to Tommassen and Lugtenberg (1980). Growth requirements were added at concentrations of 50 μg ml^{-1} when required. Tetracycline at 15 μg ml^{-1}, $HgCl_2$ at 15 μg ml^{-1}, and ampicillin at 50 μg ml^{-1} were used in selective media. Media were solidified with 2% (w/v) agar (Difco).

DNA procedures

Standard recombinant DNA procedures (restriction digests, transformation) were performed as described in Maniatis *et al.* (1982).

DNA subcloning and sequencing

A *Hin*dIII-*Eco*RI fragment from plasmid pRS-XP, which contained the *oprP* promoter region (Siehnel *et al.*, 1987, submitted manuscript) was subcloned into the multi-cloning site of the Gene Scribe-Z^{Tm} vector pTZ18R (USB, Cleveland, OH). Single-stranded DNA was prepared by a modification of the procedure of Dente *et al.* (1983) using the NaCl/PEG precipitation step of Zinder and Boeke (1982). DNA was sequenced using the chain-termination DNA sequencing method (Sanger *et al.*, 1977) with Sequenase^{Tm} (USB, Cleveland, OH) enzyme as described by Tabor and Richardson (1987).

SDS polyacrylamide gel electrophoresis and Western immunoblotting

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was performed as described previously (Hancock and Carey, 1979) using a 12% (w/v) acrylamide running gel. The Western immunoblot procedure has been described previously (Mutharia and Hancock, 1983). Antibodies to the purified periplasmic phosphate-binding protein and the monomer form of porin protein P were raised in New Zealand white rabbits according to the immunization protocol described elsewhere (Poole and Hancock, 1984). Whole-cell lysates were obtained by suspending the resultant pellet from 1.5 ml of bacterial cell suspension (from an overnight culture) in 50 μl of 2% (w/v) SDS, 4% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.125 M Tris-HCl (pH 6.5) containing 0.001% (w/v) bromophenol blue. All samples were heated at 100°C for 10 min and loaded (5 μl per well) prior to electrophoresis. Triton X-100 insoluble outer membranes were prepared according to Schnaitman (1971).

Enzyme assays

Alkaline phosphatase activity was measured at an absorbance of 410 nm using the substrate *p*-nitrophenyl-phosphate (Sigma Chemical Co., St. Louis, MO) at a final concentration of 1 mg ml^{-1} in 0.125 M Tris . HCl, pH 8.5 (Tommassen and Lugtenberg, 1980).

Acknowledgements

This work was performed with the assistance of a grant from the Natural Sciences and Engineering Research Council of Canada. E. A. W. was a fellow of the Alberta Heritage Foundation for Medical Research.

References

Dente, L., Casareni, G., and Cortese, R. (1983) pEMBL: a new family of single stranded plasmids. *Nucl Acids Res* **11:** 1645–1655.

Dargent, B., Hoffmann, W., Pattus, F., and Rosenbusch, I.P. (1986) The selectivity of voltage-dependent channels formed by phosphoporin (PhoE protein) from *E. coli*. *EMBO J* **5:** 773–778.

Gray, G.L., Berka, R.M., and Vasil, M.L. (1982) Phospholipase C regulatory mutation of *Pseudomonas aeruginosa* that results in constitutive synthesis of several phosphate-repressible proteins. *J Bacteriol* **150:** 1221–1226.

Gussin, G.N., Ronson, C.W., and Ausubel, F.M. (1986) Regulation of nitrogen fixation genes. *Ann Rev Genet* **20:** 567–591.

Hancock, R.E.W. (1987) Role of porins in outer membrane permeability. *J Bacteriol* **169:** 929–933.

Hancock, R.E.W., and Benz, R. (1986) Demonstration and chemical modification of a specific phosphate binding site in the phosphate-starvation-inducible outer membrane porin protein P of *Pseudomonas aeruginosa*. *Biochim Biophys Acta* **860:** 699–707.

Hancock, R.E.W., and Carey, A.M. (1979) Outer membrane of *Pseudomonas aeruginosa*: heat- and 2-mercaptoethanol-modifiable proteins. *J Bacteriol* **140:** 902–910.

Hancock, R.E.W., Poole, K., and Benz, R. (1982) Outer membrane protein P of *Pseudomonas aeruginosa*: regulation by phosphate deficiency and formation of small anion-specific channels in lipid bilayer membranes. *J Bacteriol* **150:** 730–738.

Liu, P.V. (1979) In *Pseudomonas aeruginosa*. Doggett, R.G. (ed.). New York: Academic Press, Inc., pp. 63–68.

Makino, K., Shinagawa, H., Amemura, M., and Nakata, A. (1986) Nucleotide sequence of the *phoB* gene, the positive regulatory gene for the phosphate regulon of *Escherichia coli* K12. *J Mol Biol* **190:** 37–44.

Maniatis, R., Fritsch, E.F., and Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

McCarter, L.L., and Silverman, M. (1987) Phosphate regulation of gene expression in *Vibrio parahaemolyticus*. *J Bacteriol* **169:** 3441–3449.

Murray, N.E., Brammer, W.J., and Murray, K. (1977) Lamboid phages that simplify the recovery of *in vitro* recombinants. *Mol Gen Genet* **150:** 53–61.

Mutharia, L.M., and Hancock, R.E.W. (1983) Surface localization of *Pseudomonas aeruginosa* outer membrane porin protein F by using monoclonal antibodies. *Infect Immun* **42:** 1027–1033.

Poole, K., and Hancock, R.E.W. (1983) Secretion of alkaline phosphatase and phospholipase C in *Pseudomonas aeruginosa* is specific and does not involve an increase in outer membrane permeability. *FEMS Microbiol Lett* **16:** 25–29.

Poole, K., and Hancock, R.E.W. (1984) Phosphate transport in *Pseudomonas aeruginosa*: involvement of a periplasmic phosphate-binding protein. *Eur J Biochem* **144:** 607–612.

Poole, K., and Hancock, R.E.W. (1986a) Phosphate-starvation-induced outer membrane protein of members of the families Enterobacteriaceae and Pseudomonadaceae: demonstration of immunological crossreactivity with an antiserum specific for porin protein P of *Pseudomonas aeruginosa*. *J Bacteriol* **165:** 987–993.

Poole, K., and Hancock, R.E.W. (1986b) Isolation of a Tn*501* insertion mutant lacking porin protein P of *Pseudomonas aeruginosa*. *Mol Gen Genet* **202:** 403–409.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74:** 5463–5467.

Schnaitman, C.A. (1971) Solubilization of the cytoplasmic membranes of *Escherichia coli* by Triton X-100. *J Bacteriol* **108:** 545–552.

Seki, T., Yoshikawa, H., Takahashi, H., and Saito, H. (1987) Cloning and nucleotide sequence of *phoP*, the regulatory gene for alkaline phosphatase and phosphodiesterase in *Bacillus subtilis*. *J Bacteriol* **169:** 2913–2916.

Shinagawa, H., Makino, K., Amemura, M., and Nakata, A. (1987) Structure and function of the regulatory genes for the phosphate regulon in *Escherichia coli*. In *Phosphate Metabolism and Cellular Regulation in Microorganisms*. Toriani-Guino, A. *et al.* (eds). Washington, D.C.: ASM Press, pp. 20–25.

Tabor, S., and Richardson, C.C. (1987) DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc Natl Acad Sci USA* **84:** 4767–4771.

Tommassen, J., and Lugtenberg, B. (1980) Outer membrane protein e of *Escherichia coli* K12 is co-regulated with alkaline phosphatase. *J Bacteriol* **143:** 151–157.

Wanner, B.L. (1987) Phosphate regulation of gene expression in *Escherichia coli*. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, vol. 2. Neidhardt, F.C. (ed.). Washington, D.C.: ASM Press, pp. 1326–1333.

Van der Ley, P., Bekkers, A., van Meersbergen, J., and Tommassen, J. (1987) A comparative study on the *phoE* genes of three enterobacterial species: implications for structure-function relationships in a pore-forming protein of the outer membrane. *Eur J Biochem* **164:** 469–475.

Zinder, W.D., and Boeke, J.D. (1982) The filamentous phage (Ff) as vectors for recombinant DNA—a review. *Gene* **19:** 1–10.