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Outer Membrane Protein e of *Escherichia coli* K-12 Is Co-Regulated with Alkaline Phosphatase

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Outer membrane protein e is induced in wild-type cells, just like alkaline phosphatase and some other periplasmic proteins, by growth under phosphate limitation. *nmpA* and *nmpB* mutants, which synthesize protein e constitutively, are shown also to produce the periplasmic enzyme alkaline phosphatase constitutively. Alternatively, individual *phoS*, *phoT*, and *phoR* mutants as well as *pit pst* double mutants, all of which are known to produce alkaline phosphatase constitutively, were found to be constitutive for protein e. Also, the periplasmic space of most *nmpA* mutants and of all *nmpB* mutants grown in excess phosphate was found to contain, in addition to alkaline phosphatase, at least two new proteins, a phenomenon known for individual *phoT* and *phoR* mutants as well as for *pit pst* double mutants. The other *nmpA* mutants as well as *phoS* mutants lacked one of these extra periplasmic proteins, namely the phosphate-binding protein. From these data and from the known positions of the mentioned genes on the chromosomal map, it is concluded that *nmpB* mutants are identical to *phoR* mutants. Moreover, some *nmpA* mutants were shown to be identical to *phoS* mutants, whereas other *nmpA* mutants are likely to contain mutations in one of the genes *phoS*, *phoT*, or *pst*.

Two proteins of the outer membrane of *Escherichia coli* K-12, the products of the genes *ompC* and *ompF*, are involved in the formation of aqueous pores through which small hydrophilic molecules can pass this membrane (5, 25, 28, 34, 35). Mutants lacking these two porins are sensitive to 3% sodium dodecyl sulfate (SDS) (24, 33). From those mutants, SDS-resistant pseudorevertants can be isolated which contain a new outer membrane protein, which in our laboratory has been designated protein e (35) and by others as protein Ic or E (11, 17). Also, this new protein has porin properties (24, 29, 35). Mutations leading to the constitutive synthesis of protein e have been localized in either one of two genes, *nmpA* and *nmpB*, at min 82 (12, 29) and 8 (20, 29), respectively, of the genetic map of *E. coli* K-12 (4). The exact function of these genes is not known.

A search for growth conditions that result in the induction of protein e in wild-type cells of *E. coli* K-12 resulted in the observation that the synthesis of this protein is derepressed by growth in limiting concentrations of P_i (28a).

Phosphate limitation also results in derepression of the synthesis of several periplasmic proteins designated as P1, P2, P3, and P4 by Morris et al. (27). P4 was shown to consist of two proteins designated as P4a and P4b (39). P1 and P4a represent alkaline phosphatase (27) and the phosphate-binding protein (39), respectively. Either P2 or P3 corresponds with band GP2 (3), which functions as a glycerol-3-phosphate binding protein. The functions of the proteins P2 or P3 and those of P4b have not been elucidated. Protein band P3 was not always observed by Morris et al. (27), whereas it was not detected at all by others (39).

A genetic analysis of the regulation of alkaline phosphatase suggested the possibility that two regulatory genes, *phoR* and *phoB*, both located at min 8.5, might be involved in the regulation by phosphate (7, 10, 13). Morris et al. (27) propose that the *phoB* gene codes for an activator protein which is necessary for the expression of the *phoA* gene, the structural gene for the enzyme, at min 8. According to this interpretation, phosphate exerts its effect through the product of the *phoR* gene by interfering with the action of the *phoB* product (27). *phoR* mutations result in the constitutive synthesis of alkaline phosphatase (32); of the periplasmic proteins P2, P4a, and P4b; and possibly also of P3 (27). *phoB* mutations prevent the synthesis of alkaline phosphatase (7) and of the mentioned periplasmic proteins (27) even during growth under phosphate limitation (8, 27), presumably because the regulatory protein is missing or inactive (8, 27, 40). Several other classes of mutants have been described which synthesize alkaline

phosphatase constitutively, namely *phoS* (14) and *phoT* (38) mutants and also *pit pst* double mutants (38, 39). The latter four genes seem to have a primary role in P_i transport and only an indirect role in the regulation of alkaline phosphatase. It has been suggested that the three mutants mentioned synthesize alkaline phosphatase constitutively because the internal levels of P_i are decreased (38), but this theory seems unlikely, since strains carrying *phoS* or *phoT* mutations in a *pit*$^+$ background have fully derepressed alkaline phosphatase levels while maintaining normal rates of phosphate transport through the pit system (30).

phoS (min 82) codes for the phosphate binding protein P4a, whereas *pit* (min 76) and *pst* (min 82) code for cytoplasmic membrane proteins involved in transport of P_i. The exact role and localization of the product of the *phoT* gene (min 82), which is also involved in the uptake of P_i (38), are not known. *phoT* mutations are distinguishable from *pst* mutations by P1 transduction (39).

Since outer membrane protein e and alkaline phosphatase are both derepressed under phosphate limitation and since the mutations *nmpA* and *nmpB* have been localized at almost the same positions at the chromosomal map as mutations leading to the constitutive synthesis of alkaline phosphatase, we considered the possibility that the *nmp* genes might be identical to some of the known *pho* genes. Experiments described in this paper show that this is indeed true.

MATERIALS AND METHODS

Strains and growth conditions. All bacterial strains are derivatives of *E. coli* K-12. The sources and relevant characteristics of most strains are listed in Table 1. Protein e constitutive mutants of strain CE1175 were obtained as follows. A *malT* derivative of strain CE1175 was isolated as a bacteriophage λ *vir*-resistant clone which was unable to use maltose as the only carbon source. This strain was used as a recipient in a P1 transduction experiment (37) with a P1 suspension grown on the *ompB* strain CE1108. Maltose-fermenting transductants were examined for cotrans-

TABLE 1. *Characteristics of bacterial strains*[a]

Strain	Characteristics	Source,[b] references
PC0479	*thr leu thi pyrF thy ilvA his lacY argG tonA tsx rpsL cod dra vtr glpR*	PC (36)
CE1107	*ompB471* derivative of PC0479 lacking both the *ompC* and *ompF* proteins	(36)
CE1108	*nmpA* derivative of CE1107	(24)
CE1181	NG[c]-induced *rbs* mutant of CE1108	This paper
CE1174	Spontaneous TC45-resistant *phoB* derivative of CE1108	This paper
W620Ic$^+$	*thi pyrD gltA galK str trp his nmpA*	(17)
JF694	*ilv his purE proC aroC str cyc xyl lacY tsx ompA nmpA* TuIb resistant	(11)
CE1175[d]	*thi*, (λ *cI857 S7*)	This paper
CE1175 *malT*	Spontaneous *malT* derivative of CE1175	This paper
CE1175 *ompB*	mal$^+$ *ompB471* derivative of CE1175 *malT*	This paper
CE1176 and CE1178	Spontaneous SDS-resistant *nmpA* derivatives of CE1175 *ompB*	This paper
CE1179 and CE1180	Spontaneous SDS-resistant *nmpB* derivatives of CE1175 *ompB*	This paper
K10[e]	HfrC *relA1 tonA22 pit-10 spoT1* T2^r	CGSC 5023
K10[e]	*HfrC relA1 tonA22 pit-10 spoT1* T2^r	CGSC 4234
LEP-1	*proC34 phoB23 purE42 trpE38 thi-1 lacZ73 lacI22 xyl-5 mtl-1 azi-6 tonA23* ? *tsx-67 rpsL109* P1$^+$?*supE44*	CGSC 5681
C5	*phoR17* derivative of K10	CGSC 4934
C9	*phoR18* derivative of K10	CGSC 4935
C29	*phoR19* derivative of K10	CGSC 4936
C78	*phoS28* derivative of K10	CGSC 5651
C86	*phoS21* derivative of K10	CGSC 5009
C90	*phoT9* derivative of K10	CGSC 4680
C101a	*phoT32* derivative of K10	CGSC 5656
C112a	*phoT34* derivative of K10	CGSC 5658
U9	*phoA12* derivative of K10	CGSC 4831
E15	*phoA8* derivative of K10	CGSC 4829
GS5	*proC24 pyrF30 his53 thyA-25 pit-1 pst-2 metB1 nalA2 tsx-63* ? *rpsL97*	CGSC 5507
10B5	*pit-1 pst-2 glpR2 glpD3 phoA8 relA1 tonA22* T2^r	CGSC 5506
Lin8	*glpR2 glpD3 phoA8 relA1 tonA22 pit-10 spoT1* T2^r	CGSC 4681

[a] Genotype descriptions follow the recommendations of Bachmann et al. (4).

[b] PC, Phabagen Collection, Department of Molecular Cell Biology, Section Microbiology, State University of Utrecht, Utrecht, The Netherlands; CGSC, *E. coli* Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, Conn. (B. J. Bachmann, Curator).

[c] NG, *N*-methyl-*N*-nitroso-*N'*-nitroguanine.

[d] This strain is a derivate of CM848, obtained from K. von Meyenburg. In contrast to strain CM848, this strain does not carry the specialized transducing phage λ *asn*132. Since the strain grows well on minimal medium without asparagine, the original *asn* mutation from strain CM848 must be reverted.

[e] Both K10 strains are probably identical (B. J. Bachmann, personal communication).

duction of *ompB* and *malT* by testing for sensitivity to 3% SDS (33) and resistance (16) to both the *ompC* protein-specific phage Me 1 (36) and the *ompF* protein-specific phage Tula (17). From one of these transductants, strain CE1175 *ompB*, 3% SDS-resistant mutants were isolated and tested for the protein e-specific phage TC45 (9). All TC45 sensitive derivates produced protein e as judged by SDS-polyacrylamide gel electrophoresis (22).

Except where noted, cells were grown in yeast broth (23), which contains excess phosphate. Low- and high-phosphate-containing minimal media were obtained by adding a solution of K_2HPO_4 to final concentrations of 41 and 660 μM, respectively, to a medium containing (per liter): HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 29.75 g; NaCl, 4.65 g; KCl, 1.5 g; NH_4Cl, 1.08 g; Na_2SO_4, 0.425 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 29.5 mg; $FeCl_3$, 0.54 mg; and glucose, 4.0 g. Growth requirements due to auxotropic mutations were added in appropriate concentrations. The final pH was 7.2. Cells were grown overnight under vigorous aeration at 37°C, except that a growth temperature of 30°C was used (i) to prevent induction of the thermoinducible phage for strains containing phage λ *c*I857S7 and (ii) to check for the presence of protein e in cell envelopes, because at this growth temperature protein a, which is another outer membrane protein with the same electrophoretic mobility as protein e in the gel system used (24), is hardly produced (23, 26).

Assays for alkaline phosphatase. A semiquantitative assay was used for screening clones which produce alkaline phosphatase constitutively on solid medium by spraying the colonies at room temperature with a solution of *para*-nitrophenyl phosphate (20 mg/ml) in 0.2 M Tris buffer, pH 8.0. Colonies of constitutive mutants become yellow within a few minutes. A quantitative assay for alkaline phosphatase was carried out as follows. The cells of a 9.0-ml portion of an overnight culture were harvested and resuspended in 1 volume of demineralized water. Toluene (0.25 ml) was added, and the suspension was shaken at room temperature for 30 min. Assays were performed at 30°C in a mixture containing an appropriate sample of the cell suspension and Tris buffer, pH 8.0, and *para*-nitrophenyl phosphate in final concentrations of 0.1 M and 1 mg/ml, respectively. The final volume was 3.0 ml. The reaction was stopped by the addition of 3.0 ml of 1 N NaOH. After centrifugation, the amount of *para*-nitrophenol released was determined by measuring the adsorbance of the supernatant fluid at a wavelength of 420 nm against a blank derived of a reaction mixture treated identically except that it contained no bacterial cells. In this paper, the activity of alkaline phosphatase is given in units, defined as nanomoles of *para*-nitrophenol released per minute of reaction time per milligram of cells (dry weight).

Isolation and characterization of cell fractions. Cell envelopes were isolated by differential centrifugation after disintegration of cells by ultrasonic treatment (22). Protein-peptidoglycan complexes were isolated by ultracentrifugation after extraction of cell envelopes at 60°C in a buffer containing 2% SDS (21, 36). For the isolation of periplasmic proteins the EDTA-lysozyme method of Willsky and Malamy (39) was slightly modified. Cells of 50 ml of an overnight culture were harvested at 4°C and washed with 20 ml of a cold buffer solution containing 10 mM Tris-hydrochloride (pH 8.0), 1 mM $MgCl_2$, and 10 μM $ZnCl_2$. The cells were resuspended in 0.8 ml of a solution containing 25% sucrose and 10 mM Tris-hydrochloride, pH 8.0. After the addition of 100 μl of a solution of lysozyme (5 mg/ml) and 100 μl of EDTA (20 mM, pH 8.0), the suspension was incubated for 15 min at 25°C. The supernatant fluid obtained after centrifugation of the suspension at 4°C for 15 min at 10,000 $\times$ *g* was used as the periplasmic protein fraction. The protein patterns of the cell fractions were analyzed by SDS-polyacrylamide gel electrophoresis as described previously (22). In this paper, several protein bands are indicated by their molecular weights multiplied by 10^{-3} and followed by the letter K.

RESULTS

Outer membrane protein e and alkaline phosphatase are induced at the same phosphate concentration. If protein e and alkaline phosphatase are co-regulated, one would assume that induction of both proteins would occur below a certain critical phosphate concentration. Cells of strains PC0479 and K10, both inducible for proteins e and alkaline phosphatase, were grown at 37°C in minimal medium containing various concentrations of P_i (40 and 80 μM and 0.16, 0.33, and 0.66 mM). The cells were harvested in the stationary phase and analyzed for the activity of alkaline phosphatase and for the presence of protein e in protein-peptidoglycan complexes. The results showed that in all cases in which induction of alkaline phosphatase was measured protein e also was induced and vice versa. These results support the notion that the two proteins are co-regulated. Moreover, they show that protein e can be induced not only in chemostat cultures but also in batch cultures.

e^+ mutants synthesize alkaline phosphatase constitutively. To investigate the possibility that *nmp* mutations are actually mutations in *pho* genes, we tested the alkaline phosphatase activity of strains PC0479, its *ompB* derivative CE1107 and the *nmpA* derivative of the latter strain, strain CE1108, after growth on minimal medium containing various concentrations of P_i. The results (Table 2) show that, whereas strains PC0479 and CE1107 are inducible for this enzyme, the *nmpA* mutant strain CE1108 produces the enzyme constitutively. Similar results were found for the *nmpA* e^+ strains W620Ic$^+$ and JF694 (not shown). To test whether e^+ strains in general produce alkaline phosphatase constitutively, 32 independent SDS-resistant protein e^+ revertants were isolated from strain CE1175 *ompB*. The mutations causing the constitutive synthesis of protein e were localized by P1 transduction in 11 mutants. Nine strains were of the

TABLE 2. *Alkaline phosphatase activity of outer membrane protein mutants after growth in media containing various concentrations of P_i*[a]

Phosphate concn (μM)	Alkaline phosphatase activity in:			
	PC0479 (wild type)	CE1107 (*ompB*)	CE1108 (*ompB nmpA*)	CE1174 (*ompB nmpA* TC45r)
660	<1	<1	67	<1
160	<1	4	88	<1
40	21	56	100	<1
10	47	50	121	<1

[a] Cells were grown overnight at 37°C in minimal medium containing various phosphate concentrations. The specific activity of alkaline phosphatase is expressed as nanomoles of *para*-nitrophenol produced per milligram of cells (dry weight) under standard conditions.

nmpA type and two of the *nmpB* type, since they were cotransducible with *ilvA* and *lacY*, respectively. All 32 strains produced alkaline phosphatase constitutively, in contrast to the parent strain. It therefore can be concluded that, as a rule, strains that produce outer membrane protein e constitutively also produce the periplasmic enzyme alkaline phosphatase constitutively.

Synthesis of outer membrane protein e in alkaline phosphatase regulatory mutants. To test whether mutants that are known to synthesize alkaline phosphatase constitutively also produce outer membrane protein e constitutively, cells of known individual *phoR*, *phoS*, and *phoT* mutants and of *pst pit* double mutants were grown in yeast broth at 30°C. In addition, *phoA* and *phoB* mutants, both of which are alkaline phosphatase negative even when grown on media containing limiting phosphate concentrations, were tested. With respect to the cell envelope protein patterns of parent strain K10 and the *pho* mutants which are shown in Fig. 1, we found that the *ompF* protein is missing in strains resistant to phage T2. This observation is consistent with the results of Hantke, who concluded that this phage uses the *ompF* protein as part of its receptor (15). Figure 1 shows that, in contrast to the parent strain K10 (slots a and b), strains that produce alkaline phosphatase constitutively, namely *phoR* strains (slots c, d and e), *phoS* strains (slots f and g), *phoT* strains (slots h, i and j), and a *pst pit* double mutant (slot k), contain a heavy protein band in the position of protein e (slot o). This protein is, like protein e, associated with peptidoglycan as was shown by isolation and characterization of protein-peptidoglycan complexes (data not shown). The protein e-specific phage TC45 plated on all the alkaline phosphatase-constitutive strains except on the *phoT* strain C112a. The efficiency of plating of phage TC45 on *phoR* mutants, which clearly produce less protein e than the other alkaline phosphatase-constitutive strains (Fig. 1), is only 10^{-5}. The sensitivity of strains towards phage TC45 is not always a good measure for the presence or absence of protein e. The efficiency of plating of the phage is very much dependent on the amount of protein e present and on the genetic background. Mutations in the genes *phoA*, the structural gene for alkaline phosphatase, and *phoB*, the gene coding for the positive regulation product, do not produce significant amounts of protein e (slots l, m and n). These strains were resistant to phage TC45. Thus, the results show that alkaline phosphatase-constitutive mutants produce protein e constitutively.

Periplasmic proteins of *nmp* and *pho* mutants. *nmp* mutants have several properties in common with certain *pho* mutants, namely the constitutive synthesis of both alkaline phosphatase (see text and Table 2) and outer membrane protein e (Fig. 1). Moreover, *nmp* mutations have genetically been localized in regions where known *pho* genes are located (1, 6, 7, 10, 12, 29, 38). The periplasmic proteins of *nmp* and *pho* mutants were isolated and compared by SDS-polyacrylamide gel electrophoresis (Fig. 2). Comparison of the periplasmic proteins of strain CE1107 grown in minimal medium with excess phosphate (slot a) and with low phosphate (slot b) shows that three extra protein bands are found in the latter case with apparent molecular weights of 51K, 48K and 37K. The same proteins are synthesized even during growth in medium containing excess phosphate by *phoT* strain C90 (slot d), *phoR* strain C5 (slot e), and the *pit pst* double mutant GS5 (slot f), but not by the parent strain K10 (slot c). Because the 51K band is weak in the *pit pst phoA* strain 10B5 (slot g), this band must contain the monomer of alkaline phosphatase, previously designated as band P1 or GP1 by Morris et al. (27) and Argast et al. (3), respectively. *phoS* mutant

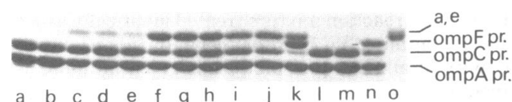

FIG. 1. *SDS polyacrylamide gel electrophoresis patterns of cell envelope proteins of two K10 strains, CGSC5023 and CGSC4234 (a and b); phoR mutant strains C5, C9, and C29 (c, d, and e); phoS strains C78 and C86 (f and g); phoT strains C90, C101a, and C112a (h, i, and j); pit pst double mutant strain GS5 (k); phoA strains U9 and E15 (l and m); and phoB strain LEP-1 (n) and purified protein e (o). Only the relevant part of the gel is shown. Cells were grown overnight in yeast broth at 30°C (see text).*

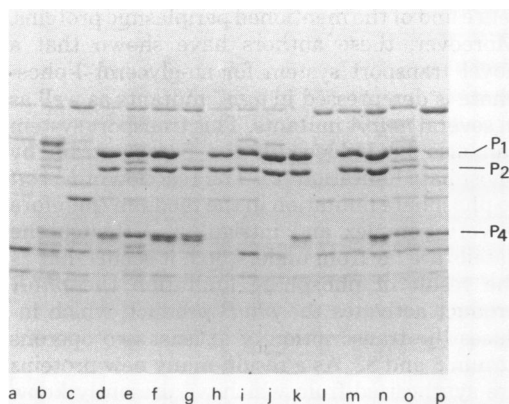

FIG. 2. *SDS-polyacrylamide gel electrophoresis patterns of the periplasmic proteins of strains CE1107 grown in minimal medium with excess phosphate (a), CE1107 grown in minimal medium with low phosphate (b), strain K10 (c), phoT strain C90 (d), phoR strain C5 (e), pit pst double mutant strain GS5 (f), pit pst phoA strain 10B5 (g), phoS strain C86 (h), nmpA strains CE1108, W620Ic⁺, and JF694 (i, j, and k), CE1175 ompB (l), nmpA strains CE1176 and CE1178 (m and n), and nmpB strains CE1179 and CE1180 (o and p). Except for CE1107, all strains were grown overnight in yeast broth (containing excess phosphate). We never observed band P3, whereas band P4b is a weak band, hardly visible on this photograph.*

strain C86 (slot h) produces the 51K and 48K proteins constitutively. However, it has very reduced amounts of the 37K band, which therefore must contain the phosphate-binding protein, corresponding with bands P4a or GP3 (2, 39). The residual amount of protein in band P4 (slot h), designated as P4b (39), is probably identical to the protein designated as GP4 by Argast and Boos, since these authors report that GP4 cannot be separated from the phosphate-binding protein by normal slab gel electrophoresis (2). Because Morris et al. (27) reported that protein P3 cannot always be observed, the 48K protein is probably identical to their P2 band. Either band P2 or P3 corresponds to GP2, which has been identified as a glycerol-3-phosphate-binding protein (3).

In contrast to its parent strain CE1107 (slot a) but like the *phoS* mutant strain C86 (slot h), the *nmpA* mutant strain CE1108 (slot i) produced proteins P1 and P2 constitutively but hardly synthesized protein P4a, the phosphate-binding protein. Nor was the latter protein produced by growing strain CE1108 in low phosphate medium (data not shown). Another *nmpA* mutant, strain W620Ic$^+$ (slot j) has a periplasmic protein pattern similar to that of strain CE1108 (slot i). However, the *nmpA* mutant JF694 (slot k) contains all three bands P1, P2, and P4. In contrast to the parent strain (slot 1), all tested *nmpA* derivatives of strain CE1175 *ompB* contained proteins P1 and P2 constitutively, whereas some (e.g., strain CE1176, slot m) lack P4a, which was present in others (e.g., strain CE1178, slot n). The two *nmpB* derivatives of strain CE1175 *ompB* (strains CE1179 and CE1180, slots o and p) contained, like *phoR* strains, all these bands constitutively.

Synthesis of protein e is under control of the *phoB* gene product. The results described so far are best explained by assuming that *nmpA* and *nmpB* are genes which affect the regulation of both protein e and alkaline phosphatase. If the synthesis of protein e is under control of the *phoB* gene product, which is the positive regulator protein of the regulon, *phoB* mutants can be expected among phage TC45-resistant, protein e-negative mutants of *nmpA* mutant strain CE 1108.

Twenty-one independent, phage TC45-resistant, protein e-negative mutants of strain CE1108 were isolated. One mutant, strain CE1174, was alkaline phosphatase negative even after growth in low phosphate medium (Table 1); it still contained the original *nmpA* mutation, it lacked the periplasmic proteins P1 and P2 in addition to P4, and the mutation is localized in the 8-min region of the chromosome (data not shown). Therefore, it must be of the *phoB* type.

DISCUSSION

The notion that the synthesis of outer membrane protein e and alkaline phosphatase could be subject to a common regulation mechanism arose both from the observation that protein e is, like alkaline phosphatase and some other periplasmic proteins (18, 27, 31), induced by growth in the presence of low levels of P_i (28a) and from the similar genetic localization of *nmp* genes (12, 20, 29) and *pho* genes (1, 6, 7, 10, 38). Our observation that both proteins are induced at the same low phosphate concentration in batch cultures supported this idea. Therefore, *nmp* and *pho* mutants were compared with respect to (i) the constitutive synthesis of alkaline phosphatase and protein e, respectively, and (ii) the constitutive synthesis of a number of periplasmic proteins.

Both *nmpA* and *nmpB* mutants produced alkaline phosphatase constitutively, whereas *phoR*, *phoS*, and *phoT* mutants and *pit pst* double mutants produced protein e constitutively (Fig. 1). With respect to the pattern of periplasmic proteins (Fig. 2), one class of *nmpA* mutants (e.g., CE1108 and W620Ic$^+$) behaved like *phoS* mutants since P1 and P2 were overproduced, whereas P4a was not produced. Be-

cause these *nmpA* mutants are indistinguishable from *phoS* mutants according to all criteria mentioned, it must be concluded that they are changed in the same gene. A second class of *nmpA* mutants, among which was strain JF694, was indistinguishable from *phoT* mutants and from *pit pst* double mutants. All these strains produced P1, P2, and P4a constitutively. The same proteins were produced by the *nmp*$^+$ *pho*$^+$ strain CE1107 only under phosphate limitation (Fig. 2). Mutants of this class therefore can (i) be defective in the *phoT* gene, (ii) carry a missense mutation in the *phoS* gene, which impairs the activity of the phosphate-binding protein without influencing its synthesis or electrophoretic mobility in SDS gels, or (iii) carry a *pst* mutation if for the constitutive synthesis of protein e and the mentioned periplasmic proteins in *pst* mutants a *pit* background is not necessary. Since the *pst* mutation of strain GS5 could be transduced to *pit*$^+$ strain PC0479 and the resulting transductants are constitutive for alkaline phosphatase and protein e (unpublished data), this possibility exists.

Mutants in the genes *nmpB* and *phoR* could not be distinguished: (i) both types synthesized protein e as well as alkaline phosphatase constitutively, and (ii) both types produce the periplasmic proteins P1, P2, and P4a constitutively. Because the genetic localization of these mutations is not significantly different (6, 29), we conclude that the *nmpB* gene is identical to the *phoR* gene.

Our results clearly show that the synthesis of protein e is controlled by the same regulation mechanism as the syntheses of alkaline phosphatase, protein P2, and the phosphate-binding protein. The mutations *phoS*, *phoT*, and *pst* cause the constitutive synthesis of the proteins mentioned. The function of the *phoS*, *phoT*, and *pst* genes in the control of the synthesis of these proteins is not clear. *phoR* mutations are assumed to result in activation of the *phoB* protein, the positive regulator protein of the regulon, and therefore cause the constitutive synthesis of the mentioned proteins. *phoB* mutations are proposed to inactivate the activator and therefore prevent the synthesis of all mentioned proteins. Our observation that a *phoB* mutant was found among phage TC45-resistant, protein e-deficient mutants once more shows that also the synthesis of protein e is under control of the *phoB* product.

As was shown in the accompanying paper (2), the regulation system is even more complex because a mutation in the *ugp* gene, which has been localized at min 1 (3) and thus is clearly different from the other known regulatory genes, can also cause the constitutive synthesis of protein e and of the mentioned periplasmic proteins. Moreover, these authors have shown that a novel transport system for sn-glycerol-3-phosphate is derepressed in *ugp*$^+$ mutants as well as in several *nmpA* mutants. This transport system can also be induced in the parent strain by phosphate limitation (2). The reaction of *E. coli* to phosphate limitation in the medium therefore is very complex and intriguing. Although the details are far from understood, it seems that as the result of phosphate limitation the *phoR* product activates the *phoB* product, which induces the transcription of at least two operons at min 8 and 82. As a result, many new proteins are synthesized from which we presently know (i) in the cytoplasmic membrane: transport systems for inorganic phosphate (30) and for glycerol-3-phosphate or a related compound (2), (ii) in the outer membrane: protein e and, (iii) in the periplasmic space: alkaline phosphatase (= P1 = GP1), binding proteins for P_i (P4a = GP3) and for glycerol-3-phosphate (GP2 = P2 or P3) and the proteins P4b and P2 or P3 with unknown function. The enzyme polyphosphate depolymerase, which is also induced by phosphate limitation (19), could be responsible for one of the proteins with unknown function.

As it has been shown already that many of these proteins are involved in the uptake and degradation of phosphorus-containing nutrients and in the uptake of inorganic phosphate, it is clear that the cell reacts heavily to phosphate limitation by taking measures directed to scavenge the last traces of inorganic phosphate or phosphate-containing nutrients from the surrounding medium.

We are interested especially in the functioning of outer membrane protein e. As mentioned in an earlier paper (28a), we assume that, although the e pore is synthesized as a reaction on phosphate limitation, it is especially active in facilitating the diffusion of negatively charged compounds in general through the outer membrane. Obviously, strong evidence for such a function has recently been obtained by Benz and Henning (cited in reference 2).

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