JOURNAL OF BACTERIOLOGY, Feb. 1982, p. 434–439
0021-9193/82/020434-06$02.00/0

Vol. 149, No. 2

Signal Sequence of Alkaline Phosphatase of *Escherichia coli*

HIROSHI INOUYE,[1] WAYNE BARNES,[2] AND JON BECKWITH[1]*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115[1]; and Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110[2]

Received 10 July 1981/Accepted 3 October 1981

The amino acid sequence of the signal sequence of *phoA* was determined by DNA sequencing by using the dideoxy chain termination technique (Sanger et al., Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467, 1977). The template used was single-stranded DNA obtained from M13 on f1 phage derivatives carrying *phoA*, constructed by in vitro recombination. The results confirm the sequence of the first five amino acids determined by Sarthy et al. (J. Bacteriol. **139**:932–939, 1979) and extend the sequence in the same reading frame into the amino terminal region of the mature alkaline phosphatase (Bradshaw et al., Proc. Natl. Acad. Sci. U.S.A., **78**:3473–3477, 1981). As was predicted (Inouye and Beckwith, Proc. Natl. Acad. Sci. U.S.A. **74**:1440–1444, 1977), the signal sequence was highly hydrophobic. The alteration of DNA sequence was identified for a promoter mutation that results in the expression of *phoA* independent of the positive control gene *phoB* and in insensitivity to high phosphate.

Alkaline phosphatase of *Escherichia coli* is a periplasmic protein (15) whose synthesis is subject to complex regulatory mechanisms. Both positive and negative regulatory genes control the expression of the structural gene for this enzyme, *phoA* (23, and references therein). In accordance with the signal hypothesis for secreted proteins, the initial translation product of the *phoA* gene in vitro (11) and in vivo (13) is larger than the mature product found in the periplasm. This precursor of alkaline phosphatase can be processed in vitro by membrane vesicles (8). By use of gene fusions which produce hybrid proteins comprised of portions of alkaline phosphatase and β-galactosidase, the amino-terminal portion of the alkaline phosphatase signal sequence was shown to be Met-Lys-Gln-Ser-Thr (19).

The DNA sequence of the early portion of the *phoA* gene is presented in this paper. A comparison of our DNA sequence with the partial signal sequence above and with the known sequence of the mature protein (6) has allowed us to determine the complete signal sequence. Further, we report the alteration of DNA sequence caused by a promoter mutation *pho-1003*(Bin) (24) which confers a drastic change in the regulation of the expression of the *phoA* gene.

MATERIALS AND METHODS

Bacterial strains, phage strains, and plasmids. Bacterial strains and M13, f1 and λ transducing phages used in these studies are listed in Table 1. Nomenclature is as in Bachmann and Low (1). MZ9/F' Amp^r was constructed by conjugation of MZ9 with WB351 in LB broth (16) for 45 min and selection of His$^+$ Amp^r on minimal M9 glucose plates supplemented with tryptophan and ampicillin (20 μg/ml).

Media and chemicals. M9 glucose minimal medium and rich media were used in most experiments. These and other media used were described by Miller (16). The use of indicator media containing 5-bromo-4-chloro-3-indolyl-phosphate (Bachem Chemical) was described previously (7). Restriction enzymes were either purchased from New England Biolabs or purified in our laboratory.

Preparation of DNA and cloning techniques. The preparation of pBR322-derived plasmid DNA, ligation, transformation, and agarose gel electrophoresis were described previously (12). The technique for preparing the replicative form of M13 DNA was described previously (3). The techniques for estimating the size of plasmid and phage DNA (agarose gel electrophoresis) obtained directly from bacterial colonies and for analysis of annealing between phage DNA molecules carrying a cloned fragment are those of Barnes (2, 5). Transfection with DNA from the replicative form of M13 or f1 derivatives was carried out by using "competent cells" prepared by the method of Cohen et al. (9).

Preparation of primers. Preparation of primers from a *Hin*cII-*Eco*RI complete digest of pHI-1 was carried out as follows. pHI-1 DNA (400 μg) was digested to completion by *Hin*dII (isochizomer of *Hin*cII) and *Eco*RI. The DNA fragments were separated by polyacrylamide concentration gradient gel electrophoresis (14). Each fragment was electroeluted from the gel slab and was precipitated by ethanol twice. The *Hin*dIII$_4$-*Pvu*II$_2$ fragment (~190 base pairs [bp]) was prepared in the same way by using an homogenous 8% polyacrylamide gel.

Sequencing techniques. DNA sequencing was carried out by the method of Sanger et al. (18) as modified

TABLE 1. Bacterial strains, phage strains, and plasmids

Organism	Genotype	Source or reference
E. coli		
XPh90A	F⁻ *lacZ*(Oc) Δ*phoA* E15 *phoB*⁺ *phoR*⁺	Beckwith
WB313	F⁻ Δ(*his-gnd*) *phoA*⁺ *phoR*⁺	Barnes
WB351	F′ Tn*3*/Δ(*his-gnd*) *phoA*⁺ *phoR*⁺	Barnes
MZ9	F⁻ Δ*lac* Δ*phoA20 phoR trp rpsL*	Beckwith
λ phage	λp(*phoA-proC*)	Sarthy, Michaelis, Beckwith (19)
	λp[*pho-1003*(Bin)-*proC*]	
M13 phage		
M13 Hol76	λ(M13-*hisGCD*)	Barnes (4)
φHI-1	(M13-*his-phoA-bin*)	This study
f1 phage		
f1R243	(f1-with *Hin*dIII linker)	Vovis and Model
φHI-2	(f1-R243-*phoA*)	This study
Plasmids		
pHI-1	(PBR322 Tet^r *phoA*⁺)	Inouye et al. (12)
pHI-7	(PBR322 Amp^r *phoA*⁺)	Inouye et al. (12)

by Barnes (3) by using dideoxyribonucleotide triphosphatases as premature chain terminators in enzymatic extension of the primers obtained as restriction fragments of pHI-1 (12) as specified below.

RESULTS

Cloning of the *phoA* gene into single-stranded DNA phages. We chose to clone the *phoA* gene into the single-strand phages M13 and f1 to determine portions of its DNA sequence by the dideoxy sequencing method (18). We first used our previous finding that a *Pst*I fragment of a λp(*phoA-proC*) phage (20) contained an intact *phoA* gene (12). An M13 derivative which carries part of the *his* operon including the *hisD* gene (M13-Hol76) (4) has only one *Pst*I site located outside the *hisD* gene (Barnes, unpublished results). Bacterial strains which carry deletions of the *his* genes and which are transformed with *hisD*⁺ phage DNA can be selected for their ability to use histidinol as a histidine source. When we began these studies, the only *his*⁻ strains available were *phoA*⁺. Therefore, we used as a source of the *phoA* gene a λp(*phoA-proC*) which carried a mutation *pho*-1003(Bin) rendering the gene constitutive. Thus, alkaline phosphatase was made at high levels in the presence of inorganic phosphate. The *pho*-1003(Bin) mutation causes the *phoA* gene to be independent of the positive control gene, *phoB* (24). In this way, we could detect the *phoA* gene cloned from the λ phage on high-phosphate media since the chromosomally located *phoA* gene was normally repressed.

The λp(*pho*-1003(Bin)-*proC*) phage and the replicative form of M13-Ho176 were digested with *Pst*I, the fragments ligated together, and the DNA transformed into WB313. Transformants capable of growing on histidinol as a histidine source and which gave dark blue colonies on XP high-phosphate plates (M9) were chosen as likely candidates for the appropriate clones. These clones were purified and shown to release active *phoA*⁺ phage by the appearance of blue plaques on MZ9/F′ Amp^r growing on media containing 5-bromo-4-chloro-3-indolyl-phosphate. One of these phages was chosen for sequencing work and was designated φHI-1.

While we used the single stranded DNA obtained from φHI-1 for some of the sequencing works, several disadvantages were noted. The level of production of phage was extremely low (at least 10^3-fold lower than the parental M13-Ho176). Moreover, when WB313 (Δ*his*) cells persistently infected with φHI-1 were grown in minimal M9 media supplemented with histidinol, accumulation of deletion mutants was occasionally observed in the analysis of the phage DNA by electrophoresis. Thus, we eventually decided to seek more suitable phage derivatives.

We presumed that the problems of low titer and genetic instability in φHI-1 were due to the large fragments (3.3 kilobases [kb] from the his region and 6.6 kb from the *phoA* region) incorporated into the phage. Therefore, we constructed a *phoA* phage which contained a smaller cloned fragment.

One of the *Hin*dIII fragments in the *phoA*⁺ plasmids, pHI-7, carries the entire *phoA* gene on a fragment of 3.1 kb (12). This fragment also carries a portion of pBR322, but not its origin of replication. Since a derivative of f1 carrying an *Hin*dIII site was available (f1 R243; Vivos and Model, unpublished results) we cloned the fragment carrying the *phoA* region from an *Hin*dIII digest of pHI-7 into the replicative form of f1

R243 digested by the same enzyme. The ligation mixture of two digests was used to transfect MZ9/F' Amp^r on tryptone–yeast extract–5-bromo-4-chloro-3-indolyl-phosphate plates. About 15% of the plaques were blue. Five independent blue plaques were purified.

Agarose gel analysis of the single-strand DNA from the phages which had been annealed with ϕHI-1 DNA showed that four of them have *phoA* in the same orientation as ϕHI-1, whereas one has the *phoA* gene in the opposite orientation from ϕHI-1. One of those that has the same orientation as ϕHI-1 was designated as ϕHI-2. Unlike ϕHI-1, ϕHI-2 did not appear to have serious growth problems, as high-titered lysates (0.5×10^{11} phage per ml) were readily obtainable.

Choice of the appropriate primers. To determine the most appropriate primers for obtaining the *phoA* signal DNA sequence, we performed initial DNA sequencing in other portions of the *phoA* gene. This has allowed us to establish the orientation of the *phoA* gene on the phages and to locate more precisely certain restriction enzyme sites. To obtain primer DNA we treated the *phoA*$^+$ plasmid with various restriction enzymes. The previously determined restriction map of the relevant portions of the *phoA* gene in this plasmid is shown in Fig. 1.

The internal *phoA* *Eco*RI$_2$-*Eco*RI$_3$ fragment (0.33 kb) was used as a primer in a sequencing run with ϕHI-1. The sequencing reaction mixture was then digested with *Eco*RI to remove the primer. The sequence obtained (Fig. 1) corresponds to the DNA coding for amino acids 363 to 377 of mature alkaline phosphatase (6). This result indicates that the strand present in the mature ϕHI-1 phage is complementary to (and not homologous with) mRNA of *phoA*. As there is a potential *Eco*RI site predicted from the amino acid sequence nearby (amino acids 354 to 355) (Glu-Phe), it seemed likely that this corresponds to one of the *Eco*RI sites. When the *Hpa*I$_2$-*Hinc*II$_4$ fragment (Fig. 1) (~780 bp) was used as a primer, the sequence corresponding to amino acids 140 to 171 was obtained. There is a potential *Hinc*II site in the region corresponding to amino acids 122 and 123 (Val-Asp). These results enabled us to correlate the restriction map and amino acid sequence as indicated in Fig. 1.

This correspondence was further supported by the presence of the potential *Eco*RI site at amino acids 254 to 255 (Asn-Ser) and the pres-

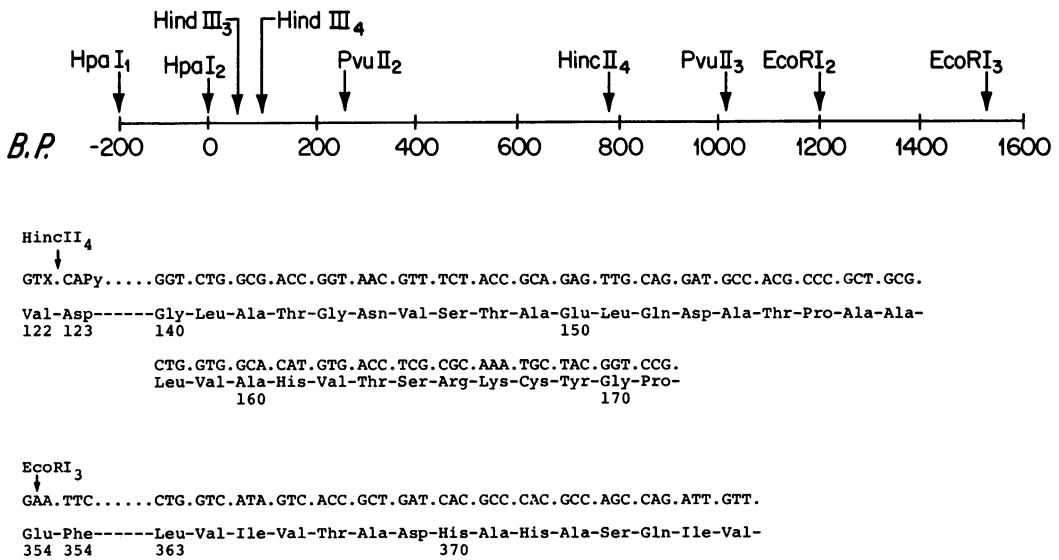

FIG. 1. Correspondence of the restriction map of *phoA* and the amino acid sequence of alkaline phosphatase. The top line represents the restriction map of the *phoA* region with the nomenclature described previously (12). The *phoA* promoter is placed on the left. The numbers under the line represent the number of base pairs distant from the *Hpa*I$_2$ site which was arbitrarily chosen as the zero point. The lower part of the figure gives the nucleotide sequences obtained by using the *Hpa*I$_2$-*Hinc*II$_4$ fragment and the *Eco*RI$_2$-*Eco*RI$_3$ fragment as primers, respectively. The translation of the codons into amino acids in a reading frame which results in the known amino acid sequence of alkaline phosphatase (6) is also shown. The arabic numbers below the amino acids indicate the position beginning with the N terminus of the mature protein. The approximate positions of the *Hinc*II$_4$ and *Eco*RI$_3$ sites were determined from the restriction map and from the positions at which the DNA sequence began. The exact positions of these sites were deduced from the specificity of the restriction enzymes and from the amino acid sequence (6) in the vicinity of the approximate position.

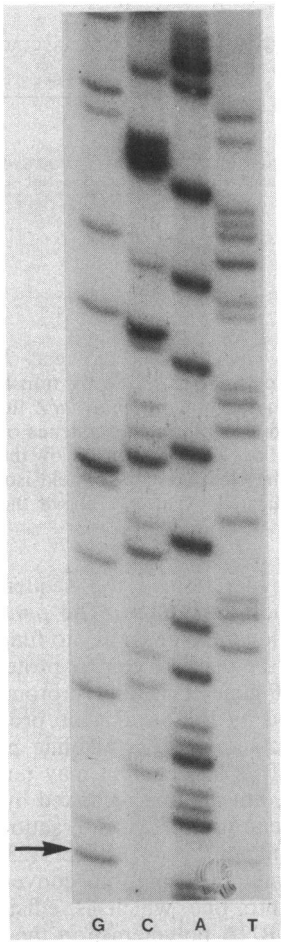

FIG. 2. Autoradiography of the sequencing gel, yielding the signal sequencing of *phoA*. Each channel corresponds to G, C, A, and T from the left to the right. The arrow indicates the first G residue of the GTG triplet coding for the initiator formyl methionine.

ence of a potential *Pvu*II site at amino acids 194 to 195 (Gln-Leu) as predicted from the restriction map. This analysis also provided critical information for the determination of the signal sequence of *phoA*. From the size of the *Pvu*II$_2$-*Pvu*II$_3$ fragment and from the presumed position of the *Pvu*II$_3$ site internal to the *phoA* gene, the other *Pvu*II$_2$ site would appear to be about 100 bp before the beginning of the structural gene for alkaline phosphatase. Furthermore, the *Pvu*II$_2$-*Pvu*II$_3$ fragment (0.77 kb) from pHI-1 which includes an early portion of the *phoA* gene has been fused to the *lacZ* gene in vitro. The expression of the resultant *phoA-lacZ* hybrid gene was regulated by inorganic phosphate (Michaelis, Guarente, Inouye, and Beckwith, unpublished results), indicating that the control region of *phoA* is located within the *Pvu*II$_2$-*Pvu*II$_3$ fragment (0.77 kb).

Further useful information was obtained from the sequence of the *Hpa*I$_1$-*Hpa*I$_2$ fragment. The DNA sequence could not be correlated with any known amino acid sequence within the *phoA* gene, but did establish the existence of the doublet *Hin*dIII sites (~50 bp apart) close to the *Hpa*I$_2$ site on the promoter side of the *phoA* gene. From these results, it seemed likely that the *Hin*dIII$_4$-*Pvu*II$_2$ fragment (0.19 kb) would be an appropriate primer for the sequencing of the promoter and the N-terminal region of *phoA*.

Determination of the signal sequence. The *Hin*dIII$_4$-*Pvu*II$_2$ (Fig. 1) fragment was used as a primer to determine the DNA coding for the signal sequence. The results of the sequencing (Fig. 2) were identical for φHI-1 *pho-1003*(Bin) (promoter-bin) and φHI-2 (wild-type promoter) templates. The use of an *Hpa*I$_2$-*Pvu*II$_2$ primer with φHI-1 also gave identical results (data not shown). It should be noted that the sequence includes the codons for Met-Lys-Gln-Thr-Ser which further extends in the same reading frame into the mature alkaline phosphatase sequence, Arg-Thr-Met-Pro-etc. This initial pentapeptide sequence was previously identified by Sarthy et al. (19). Beginning with that methionine as the N terminus the size of the amino terminal segment of alkaline phosphatase not found in the mature protein is 21 amino acid residues and contains a high proportion of hydrophobic residues as predicted (11).

A *phoA* promoter mutation, *pho-1003*(Bin). From the same sequencing experiment the comparison of φHI-1 *pho-1003*(Bin) and φHI-2 wild-type *phoA* allowed the identification of the promoter mutation. The sequences obtained were identical for the two, except for one residue located 45 bases upstream from the GTG initiator codon. The wild-type G residue was replaced by a T in the promoter mutation.

DISCUSSION

We have determined the DNA sequence of portions of the *phoA* gene and its controlling elements. Certain of these sequences have allowed us to pinpoint the sites of specific restriction enzyme cleavages (*Rsa*I, *Hpa*II, *Fnu*4HI, *Bvd*I, and *Dde*I sites; Fig. 3). Further, we have shown that the precursor of mature alkaline phosphatase contains a signal sequence of 21 amino acids. A number of features of this signal sequence are worth noting. (i) The first five amino acids correspond to those previously determined from the sequence of a hybrid protein containing only this portion of alkaline phosphatase fused to β-galactosidase (19). (ii) The codon for the initiating N-formyl methinone is GUG. This codon is found infrequently among initiating codons in *E. coli*. Other structural genes

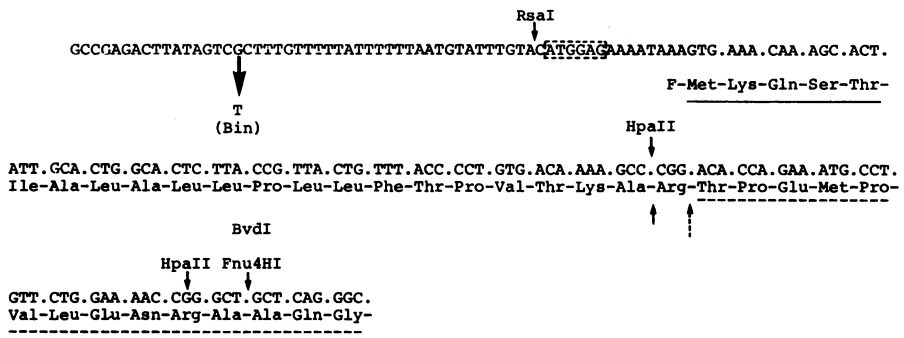

FIG. 3. DNA sequence of the promoter-proximal portion of the *phoA* gene. The 5′ end of the strand homologous to the mRNA is placed at the left hand. The coding region is indicated by translation of the corresponding triplets into amino acid sequence. The N-terminal sequence of the *phoA-lacZ* fusion protein determined by Sarthy et al. (19) is indicated by the full underline, and the N-terminal sequence of the mature protein obtained by Bradshaw et al. (6) is indicated by the dotted underline. The full arrow below the amino acid sequence shows the processing sites, and the dotted arrow shows the cleavage site to yield isozymes. The probable restriction sites deduced from the DNA sequence are indicated by arrows above the nucleotide sequence.

found to begin with GUG are *lacI* (10), *tufA* (24), and *rpsM* (17). It seemed possible that GUG is a less efficient initiation codon than AUG. However, since the *phoA* gene and the *tufA* gene (25) and the *rpsM* gene (16) are expressed at very high rates, this now seems unlikely. (iii) In contrast to most procaryotic signal sequences, which have more than one basic residue at the N-terminal region, there is only one positively charged amino acid near the amino terminus of the alkaline phosphatase signal sequence. The signal sequence of β-lactamase has the same feature (22). (iv) The most unusual feature of the alkaline phosphatase signal sequence is the lysine present at position 20. Ordinarily the last 10 to 20 amino acids of signal sequences are entirely nonpolar and highly hydrophobic. There is, preceding the lysine, a stretch of 16 amino acids which include no charged amino acids. (v) The C-terminal amino acid residue of the signal sequence is Ala. Other signal sequences also contain terminal amino acids with small side chains (Gly, Ala, or Ser).

At this point, we can define neither the precise features of the promoter nor the ribosome binding site of the *phoA* gene. However, there is a sequence in the appropriate position relative to the initiating GUG comparable to a Shine-Dalgarno sequence (21) involved in ribosome binding (Fig. 2). The GGAG sequence of the −12 to −9 position (0 being the first G of the initiator GTG) is the exact complement of the CUCC sequence of the 3′ terminal region of the 16S RNA. This sequence is preceded by one U-U mismatch (−13 position) and then an A-U match (−14 position) with the 16S RNA sequence. Thus, we believe it likely that this region corresponds to the ribosome binding site on the *phoA* mRNA.

We have determined the sequence of one *phoA* promoter mutation. The *pho-1003* (Bin) mutation allows the promoter to function in the absence of the positive control protein encoded by the *phoB* gene. This altered promoter is also less affected by the *phoR* gene product which normally acts to repress alkaline phosphatase synthesis. This latter effect may not be at the DNA level, but may be mediated by effects on the *phoB* gene product or interactions between the *phoR* and *phoB* gene products (23). The *pho-1003* (Bin) mutation may thus convert the *phoA* promoter into one which is efficiently transcribed by RNA polymerase in the absence of added factors. The extent of the *phoA* promoter is presumably less than 90 bp. This estimate is based on the finding that the *PvuII*$_2$-*PvuII*$_3$ fragment contains the entire *phoA* promoter (Michaelis, Guarente, Inouye, and Beckwith, unpublished data) and that the *PvuII*$_2$ site is at around the −90 position.

The sequence has allowed us to determine the restriction sites which lie in this region (Fig. 3). One restriction enzyme site, in particular, is of considerable potential usefulness. A *HpaII* site can be seen at a position exactly between the DNA coding for the alkaline phosphatase signal sequence and that coding for the mature protein. A *PvuII-HpaII* fragment from this region could be used to fuse the genes for other potentially exportable proteins to the *phoA* gene so as to promote the secretion of such proteins. Such an approach may be particularly fruitful in this system since the *phoA* gene on plasmids is expressed at extremely high rates (12).

ACKNOWLEDGMENTS

We are grateful to R. A. Bradshaw for informing us of the amino acid sequence of alkaline phosphatase before its publi-

cation as well as for the partial support of H.I.'s stay in St. Louis during which a part of this work was carried out. We also thank Peter Model for supplying f1-R243, Anthony Beckwith for computer survey of the restriction sites in the region coding for the signal sequence, S. C. Baver for purification of several restriction enzymes, and Ann McIntosh for assistance in the preparation of this manuscript.

The work was supported by grant JFRA-1 to J.B. and W.B. from the American Cancer Society and by Public Health Service grant GM24956 to W.B. from the National Institutes of Health. W.B. acknowledges a Junior Faculty Research Award from the American Cancer Society.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Barnes, W. M.** 1977. Plasmid detection and sizing in single colony lysates. Science **195**:393–394.
3. **Barnes, W. M.** 1978. DNA sequence from the histidine operon control region: seven histidine codons in a row. Proc. Natl. Acad. Sci. U.S.A. **75**:4281–4285.
4. **Barnes, W. M.** 1979. Construction of an M13 histidine transducing phage: a single stranded cloning vehicle with one *Eco*RI site. Gene **5**:127–139.
5. **Barnes, W. M.** 1980. DNA cloning with single strand phage vectors, p. 185–200. *In* J. K. Setlow and A. Hollaender (ed.), Genetic engineering II. Plenum Publishing Corp., New York.
6. **Bradshaw, R. A., F. Cancedda, L. J. Ericsson, P. A. Newmann, S. P. Piccoli, M. J. Schlessinger, K. Schriefer, and K. A. Walsh.** 1981. Amino acid sequence of *E. coli* alkaline phosphatase. Proc. Natl. Acad. Sci. U.S.A. **78**:3473–3477.
7. **Brickman, E., and J. Beckwith.** 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletions and ø80 transducing phages. J. Mol. Biol. **96**:307–316.
8. **Chang, C. N., H. Inouye, P. Model, and J. Beckwith.** 1980. Processing of alkaline phosphatase precursor to the mature enzyme by an *Escherichia coli* membrane preparation. J. Bacteriol. **142**:726–728.
9. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
10. **Farabaugh, P. J.** 1978. Sequence of the *lacI* gene. Nature (London) **274**:765–769.
11. **Inouye, H., and J. Beckwith.** 1977. Synthesis and processing of alkaline phosphatase precursor *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74**:1440–1444.
12. **Inouye, H., S. Michaelis, A. Wright, and J. Beckwith.** 1981. Cloning, restriction map and generation of deletion mutants *in vitro* of the alkaline phosphatase structural gene of *E. coli*. J. Bacteriol. **146**:668–675.
13. **Ito, K., P. J. Bassford, Jr., and J. Beckwith.** 1981. Protein localization in *E. coli*: Is there a common step in the secretion of periplasmic and outer membrane proteins? Cell **25**:143–150.
14. **Jeppesen, R. G. N.** 1974. A method for separating DNA fragments by electrophoresis in polyacrylamide concentration gradient slab gels. Anal. Biochem. **58**:195–207.
15. **Malamy, M., and B. Horecker.** 1961. The localization of alkaline phosphatase in *E. coli* K12. Biochem. Biophys. Res. Commun. **5**:104–108.
16. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Post, L. E., A. E. Arfsten, G. R. Davis, and M. Nomura.** 1980. DNA sequence of promoter region for the α-ribosomal protein operon in *Escherichia coli*. J. Biol. Chem. **255**:4653–4659.
18. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
19. **Sarthy, A., A. V. Fowler, I. Zabin, and J. R. Beckwith.** 1979. Use of gene fusions to determine the partial signal sequence of alkaline phosphatase. J. Bacteriol. **139**:932–939.
20. **Sarthy, A., S. Michaelis, and J. Beckwith.** 1981. Deletion map of the *Escherichia coli* structural gene for alkaline phosphatase *phoA*. J. Bacteriol. **145**:293–298.
21. **Shine, J., and L. Dalgarno.** 1974. The 3′ terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. U.S.A. **71**:1342–1346.
22. **Sutcliffe, J. G.** 1978. Nucleotide sequence of ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. U.S.A. **75**:3737–3741.
23. **Wanner, B. L., and P. Latterell.** 1980. Mutants affected in alkaline phosphatase expression: evidence for multiple positive regulators of the phosphate regulon in *Escherichia coli*. Genetics **96**:353–366.
24. **Wanner, B. L., A. Sarthy, and J. Beckwith.** 1979. *Escherichia coli* pleiotropic mutant that reduces amount of several periplasmic and outer membrane proteins. J. Bacteriol. **140**:229–239.
25. **Yokota, T., H. Sugisaki, M. Takanami, and Y. Kaziro.** 1980. The nucleotide sequence of the cloned *tufA* gene of *Escherichia gene*. Gene **121**:25–31.