JOURNAL OF BACTERIOLOGY, Sept. 1971, p. 683-689

Vol. 107, No. 3
Printed in U.S.A.

Location and Orientation of the *phoA* Locus on the *Escherichia coli* K-12 Linkage Map

ATSUO NAKATA, GEORGE R. PETERSON, E. LUCILLE BROOKS, AND FRANK G. ROTHMAN

Division of Biological and Medical Sciences, Brown University Providence, Rhode Island 02912, and Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Received for publication 17 May 1971

The order of gene loci in the *phoA-phoR* region of the *Escherichia coli* K-12 linkage map was demonstrated to be *lac p'ioA proC phoR*. The end of the *phoA* locus corresponding to the amino terminus of alkaline phosphatase was shown to be the end nearer *proC*. Translation (and transcription) of *phoA* is therefore in the anticlockwise direction relative to the conventional *E. coli* linkage map.

The alkaline phosphatase gene-protein system of *Escherichia coli* K-12 has proven useful in the study of the genetic determination of protein structure, particularly with respect to the effects of nonsense mutations (14) and the mechanism of intracistronic complementation (11, 15, 46). Alkaline phosphatase (EC 3.1.3.1) is composed of two identical polypeptide chains (41), coded for by the single *phoA* cistron (16) which maps near minute 11 of the *E. coli* linkage map (9, 51). Repression of alkaline phosphatase synthesis is controlled by two regulatory gene loci: *phoR* which is closely linked to *phoA*, and *phoS* which maps near minute 74 (3, 9, 51).

In this paper we describe genetic experiments which establish the location and orientation of *phoA* on the *E. coli* linkage map. We have found the order of gene loci in the *phoA phoR* region, reading clockwise, to be *lac phoA proC phoR*. The same order has been independently determined by Yagil, Bracha, and Silberstein. (5, 53). We have also shown that the end of *phoA* corresponding to the amino terminus of the polypeptide is the end nearer *proC*. Translation (and transcription) of *phoA* is therefore anticlockwise relative to the conventionally drawn *E. coli* linkage map.

MATERIALS AND METHODS

Chemicals. 5-Fluorouracil (5FU) was a generous gift of Hoffmann-LaRoche, Inc., Nutley, N.J. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was obtained from Aldrich Chemical Co., Milwaukee, Wis. *p*-Nitrophenylphosphate, α-naphthylphosphate, and tetrazotized *o*-dianisidine were obtained from Sigma Chemical Co., St. Louis, Mo.

Bacterial strains. The strains of *E. coli* K-12 used in this study are described in Table 1.

Media. L broth: tryptone, 10 g; yeast extract, 5 g; NaCl, 5 g; 1 N NaOH, 1 ml; water, 1 liter. LG broth: L broth containing 2 g of glucose per liter. LG-Ca broth: LG broth containing 5×10^{-3} M $CaCl_2$. TG medium (9), a mineral salt solution buffered with tris-(hydroxymethyl)aminomethane-hydrochloride at *p*H 7.4 and containing 0.2% glucose was supplemented with either 6.4×10^{-4} M KH_2PO_4 (excess phosphate), 6.4×10^{-5} M KH_2PO_4 (limiting phosphate), or 6.4×10^{-5} M β-glycerol phosphate (Pho$^+$ selection plates). For Lac$^+$ selection, 0.2% lactose was substituted for the glucose.

Broth and minimal plates contained 1.2% agar.

Supplements were added to TG phosphate plates (or Lac$^+$ selection plates) as needed in the following concentrations: dihydrostreptomycin, 100 μg/ml; L amino acids, 20 μg/ml each; adenine, 20 μg/ml; thiamine, 5 μg/ml. EMB-lactose-agar contained 10 g of peptone (Difco), 15 g of agar (Difco), 10 g of lactose, 2 g of K_2HPO_4, 0.4 g of Eosine Y, and 0.065 g of methylene blue per liter.

Phage P1 stocks. Phage P1kc was obtained from S. Lederberg. Lysates for transduction were prepared by growth on cells in LG-Ca broth at 37 C with shaking, usually in two stages. (i) Each lysate originated from a single plaque of P1 plated on the bacterial strain which was to serve as recipient in the transduction. An exponentially growing culture of the donor strain (at approximately 5×10^7 cells/ml) was infected with phage which had been picked from the single plaque, suspended in LG-Ca broth, shaken at 37 C for 30 min, and mixed with a drop of chloroform. After 3 to 4 hr of incubation of the infected culture, a few drops of chloroform were added; the culture was chilled and shaken vigorously and was centrifuged at 10,000 × *g* for 10 min to remove bacterial debris. This procedure generally yielded a low-titer lysate containing approximately 10^7 plaque-forming units (PFU)/ml. (ii) High-titer stocks were prepared by growing the phage on 50 ml of the donor strain as described above, by using the low-titer stock to provide a multiplicity of infection (MOI) of 0.05 to 0.1. After centrifugation of bacterial debris, the phage were collected by centrifugation at

TABLE 1. *Bacterial strains*

Escherichia coli K-12 strain	Mutant loci and sites[a]	Mating type when relevant	Source or derivation
K10		Hfr Cavalli	A. Garen (9)
S26	*phoA*-S26[b]	Hfr Cavalli	A. Garen (17)
U12	*phoA*-U12		A. Garen (13)
U18	*phoA*-U18		A. Garen (13)
NS31-11	*proC* (*leu*) (*lacI*) (*thi*)	Hfr Hayes	N. M. Schwartz. The *proC* mutation in NS31-11 is the one used for mapping the elements of *lac* (27)
AN1	*lac proC* (*leu*) (*thi*)		From NS31-11 after treatment with NG.
AN2	*phoA*-U12 (*leu*) (*thi*)		Transduction of AN1 with P1 grown on U12 and selecting Lac$^+$ Pro$^+$
AN3	*phoA*-U12 *proC* (*leu*) (*thi*)		Transduction of AN1 with P1 grown on U12 and selecting Lac$^+$
AN4	*lac phoA*-U12 (*leu*) (*thi*)		Transduction of AN1 with P1 grown on U12 and selecting Pro$^+$
AN5	*phoA*-U18 *proC* (*leu*) (*thi*)		Transduction of AN1 with P1 grown on U18 and selecting Lac$^+$
AN6	*lac phoA*-U18 (*leu*) (*thi*)		Transduction of AN1 with P1 grown on U18 and selecting Pro$^+$
F$^-$ Silver	*lac str* (*purE*) (*thi*)	F$^-$	S. Silver
C2	*phoR*-C2	Hfr Cavalli	A. Torriani (9)
FGR1	*phoA phoR*-C2	Hfr Cavalli	Isolated by B. Rosen from C2 after UV[c] irradiation and screening for Pho$^-$
FGR2	*lac phoA phoR*-C2 *str* (*purE*) (*thi*)	F$^-$	From mating FGR1 × F$^-$ Silver, selecting for streptomycin resistance (Sm^R) and screening for Pho$^-$ Lac$^-$
FGR3	*lac phoR proC leu* (*thi*)	Hfr Hayes	Spontaneous alkaline phosphatase constitutive (Pho^c) mutant from AN1
FGR4	*phoA*-S26 *trp* (*his*) (*met*)	F$^-$	From treatment of an F$^-$ *phoA*-S26 *his met* strain obtained from A. Garen with NG, and penicillin selection for Trp$^-$
FGR5	*lac phoR proC leu str* (*his*) (*met*)	F$^-$	From mating FGR3 × FGR4, selecting Trp$^+$ Sm^R, and screening for a Lac$^-$ Pho^c Pro$^-$ Leu$^-$ recombinant

[a] Symbols used for gene loci are according to Demerec et al. (8). The phenotypes of the mutations in each gene locus used in this study are: *phoA*, alkaline phosphatase negative; *proC*, proline requirement; *leu*, leucine requirement; *lacI*, constitutive for β-galactosidase; *lac*, unable to ferment lactose; *phoS*, alkaline phosphatase constitutive; *str*, streptomycin resistant; *met*, methionine requirement; *purE*, purine requirement; *thi*, thiamine requirement; *phoR*, alkaline phosphatase constitutive; *his*, histidine requirement; *trp*, tryptophan requirement. Loci not relevant to the experiments described are enclosed in parentheses. Mutational sites are indicated after each locus where known. The symbols used for these sites predate the introduction of standardized nomenclature (8) and have been retained.

[b] The S26 mutation is an amber mutation and is phenotypically revertible by 5-fluorouracil.

[c] Ultraviolet.

30,000 × *g* for 2 hr and resuspended in 5 ml of LG-Ca broth. Stocks prepared in this way generally had titers of 3 × 10^9 to 3 × 10^{10} PFU/ml assayed on *Shigella dysenteriae*.

Transduction. The procedure described by Adler and Kaiser (1) was used. Unless stated otherwise, the MOI was about 5. The frequency of selected transductants ranged from 10^{-7} to 10^{-5}.

Scoring of phenotypes. The Lac phenotype was scored on EMB-lactose plates. Pro was scored by replicating from proline-supplemented minimal plates to plates lacking proline. Alkaline phosphatase phenotypes were determined by spraying colonies either with *p*-nitrophenylphosphate (9) or with a mixture of α-naphthylphosphate and tetrazotized *o*-dianisidine (42), which cause colonies containing derepressed levels of alkaline phosphatase to turn yellow and purple, respectively, within seconds. Constitutive phosphatase-positive colonies turn color on plates containing either excess or limiting phosphate; repressed phosphatase-positive colonies turn color on plates containing limiting phosphate, but not excess phosphate; and phosphatase-negative colonies do not turn color on either medium. However, the constitutivity of the phenotypically 5FU revertible *phoA* mutant S26 could be determined by spraying colonies partially grown on excess phosphate-containing plates with 5FU (2.5 mg/ml), incubating overnight, and spraying with α-naphthylphosphate and

tetrazotized *o*-dianisidine. The edges of *phoA*(S26) *phoR* colonies turn purple (40). The *phoA*(S26)*phoR* genotype of such recombinants was confirmed by measuring the synthesis of alkaline phosphatase in liquid medium containing excess phosphate with and without 5FU (5 mg/liter, reference 40).

Conjugation. The Hfr and F^- strains were grown in L broth to approximately 2×10^8 cells/ml each. A 0.5-ml amount of the Hfr culture and 4.5 ml of the F^- culture were mixed. In some experiments the mixture was incubated at 37 C in a 250-ml flask without shaking for 90 min. In others, the mixture was shaken gently for 5 min, after which 1 ml was diluted into 100 ml of broth at 37 C in a 1-liter flask and held at 37 C for 55 min without shaking. The mating cultures were then diluted and plated on appropriate selective plates.

Mutagenesis with NG: (A. Garen, personal communication). Bacteria which had been grown overnight in Penassay Broth (Difco) were washed with and concentrated 10-fold in 0.2 M acetate buffer, *p*H 5.0. To 0.5 ml of this suspension, 0.12 ml of a freshly prepared solution of NG (4 mg/ml) in the same buffer was added. After standing for 3.5 hr at 37 C, the suspension was diluted into 10 ml of Penassay Broth and centrifuged, and the cells were resuspended in 10 ml of Penassay Broth and shaken overnight at 37 C. Specific mutants were isolated by appropriate selection techniques.

RESULTS

Order of lac, phoA, and proC loci. As summarized by Taylor and Trotter (52), the *proC*, *phoA*, and *phoR* loci are all cotransducible at high frequency and are all situated clockwise from the *lacI* locus. The *lac* and *proC* loci are also reported to be cotransducible (7, 33, 47) with a frequency of approximately 0.2 (33). We have confirmed the cotransducibility of these four loci and have found that the frequency of cotransduction of *lac* and *phoA* equals that of *lac* and *proC* The *phoA* and *proC* loci are much more closely linked to each other (cotransduction > 0.84) than either is to *lac* (cotransduction ≤ 0.4; *unpublished experiments*).

We have examined the map order of *lac*, *phoA*, and *proC* by three factor crosses as described in Table 2. In each cross, Pho^+ Pro^+ transductants were selected on Pho^+ selection plates lacking proline. The selected transductants were replica plated onto EMB-lactose and TG-limiting phosphate plates and then restreaked on these two types of plates to determine the Lac phenotype and confirm the Pho^+ phenotype. The restreaking was found to be necessary since the Pho^- and Lac^- phenotypes can be missed in heterogeneous colonies which also contain Pho^+ or Lac^+.

The data of Table 2 are most conveniently analyzed by inspection of the diagrams of the crosses depicted, and comparison of the frequency of the donor Lac phenotype among the selected class in each cross. For the order *lac-phoA-proC* as shown, the frequency Lac^+/Pho^+ Pro^+ in cross A is expected to be smaller than the frequency Lac^-/Pho^+ Pro^+ in cross B, since formation of the former requires two additional crossovers. For the order *lac proC phoA* (not diagrammed), the expected result would be the opposite. The observed results indicate the order to be *lac phoA proC*. We have confirmed this order by the result of two other sets of similar crosses, by using strains carrying a different *phoA* mutation (*unpublished experiments*). The same order has also been found by Yagil, Bracha, and Silberstein (53), by using Hfr $\times$ F^- crosses.

Orientation of phoA on the E. coli linkage map. To orient the *phoA* cistron, we have mapped two sites in *phoA*, U12 and U18, relative to *lac* and *proC*. The U12 and U18 sites are known to be at opposite ends of the fine-structure map of *phoA* (13). The positions of U12 and U18 relative to *lac*, *proC*, and each other were determined by the four-factor crosses described in Table 3. Lac^+ Pro^+ transductants were selected by plating the transduction mixtures on Lac^+ selection plates lacking proline and containing limiting phosphate. The colonies which grew on these plates were sprayed for alkaline phosphatase activity without further purification. Restreaking, which would have been a formidable task, was not necessary since Pho^+ recombinant clones in mixed colonies would have been detected. Crosses E and F served as controls of reversion to Pho^+. U12 and U18 do not show intracistronic complementation, at least not to a sufficient extent for a colony of a heterogenote carrying these two *phoA* mutations to have a Pho^+ phenotype indistinguishable from wild-type (*unpublished experiments*). Therefore, the Pho^+ colonies obtained in crosses C and D cannot be the result of rare heterogenotes formed by transduction by addition and must be true recombinants.

As shown in the diagram of Table 3, more Pho^+ recombinants are expected among the selected class in cross C than in cross D if the order is *lac* U12 U18 *proC*, since two fewer crossovers are required in cross C. The opposite result would be expected if the order were *lac* U18 U12 *proC*. The observed results establish the order *lac* U12 U18 *proC*.

Mapping of phoR. The map position of *phoR* relative to *lac* and *phoA* was determined by the three-factor Hfr $\times$ F^- crosses shown in Table 4. In each cross, the mating mixture was plated on Lac^+ selective plates containing excess phosphate and dihydrostreptomycin to select for Lac^+ Sm^R recombinants. The colonies which grew on these plates were scored for their phosphatase constitutive phenotype without further purification. Re-

TABLE 2. *Order of lac, phoA, and proC*

	Cross	Genotype[a]	No. of Pho$^+$ Pro$^+$ selected	No. of Lac$^+$ among selected class	Frequency of Lac phenotype of donor among selected class
A	Donor: U12 Recipient: AN1	$+\ phoA\ +$ $lac\ +\ proC$	150	13	0.08
B	Donor: AN1 Recipient: AN2	$lac\ +\ proC$ $+\ phoA\ +$	208	152	0.27

[a] Crossovers needed to obtain a Pho$^+$ Pro$^+$ transductant with the Lac phenotype of the donor are indicated.

TABLE 3. *Order of lac, phoA-U12, phoA-U18 and proC*

	Cross	Genotype[a]	Expt	MOI	No. of Lac$^+$ Pro$^+$ selected	Pho$^+$ among selected class	
						No.	%[b]
C	Donor: AN4	$lac\ U12\ +\ +$	1	4.1	122	1	
			2	8.4	1398	47	
	Recipient: AN5	$+\ +\ U18\ proC$	3	3.8	1243	29	
			4	0.8	9	1	
			5	1.6	692	13	
			6	0.4	10,499	315	
			7	4.2	735	22	
			Total		14,698	428[c]	2.9 ± .14
D	Donor: AN6	$lac\ +\ U18\ +$	1	5.0	1387	13	
			2	6.2	871	9	
	Recipient: AN3	$+\ U12\ +\ proC$	3	2.5	1747	23	
			4	0.5	1143	20	
			5	2.7	9958	91	
			7	3.1	802	7	
			Total		15,908	163[c]	1.0 ± .08
E	Donor: AN4	$lac\ U12\ +\ +$	1	2.5	211	0	
			2	5.8	870	0	
	Recipient: AN3	$+\ U12\ +\ proC$	3	2.3	436	0	
			4	0.5	133	0	
			5	1.0	690	0	
			Total		2340	0	
F	Donor: AN6	$lac\ +\ U18\ +$	1	8.3	418	0	
			2	8.9	870	0	
	Recipient: AN5	$+\ +\ U18\ proC$	3	4.1	304	0	
			4	0.8	18	0	
			5	4.3	469	0	
			6	0.6	2278	0	
			Total		4357	0	

[a] Crossovers needed to get Pho$^+$ among the selected class are indicated.

[b] ± Standard deviation.

[c] The maximum reverse mutation frequency to Pho$^+$ calculated from the data in crosses E and F ($P = 0.01$) is 2.0×10^{-8} for U12 and 1.1×10^{-8} for U18. Taking the *lac-phoA* distance to be 20 times the *phoA-proC* distance (as indicated by the results of the cross S26 × FGR5), the calculated maximum Pho$^+$ found among the selected class which could have resulted from reverse mutation is 29 in cross C and 19 in cross D.

TABLE 4. *Order of lac, phoA, phoR*

Cross	Genotype[a]	Expt	No. of Lac$^+$ Sm^R selected	Phosphatase constitutives (*phoA*$^+$ *phoR*$^-$) among the selected class	
				No.	Frequency
G Donor: K10	+ + + +	1	3,445	164	0.048
Recipient: FGR2	*str* / *lac* *phoA* \ *phoR*	2	2,055	117	0.057
		Total	5,500	281	0.051
H Donor: FGR1	+ + *phoA* / *phoR* \	1	6,550	127	0.019
Recipient: F$^-$ Silver	*str* / *lac* \ + / + \	2	4,414	81	0.018
		Total	10,964	208	0.019

[a] Crossovers needed to get a phosphatase constitutive among the selected class are indicated.

streaking was not necessary since a constitutive clone in a mixed colony would have been detected. As shown in the diagram of Table 4, the order *lac phoA phoR* is expected to lead to a higher frequency of constitutive (*phoA*$^+$ *phoR*) recombinants in cross G than in cross H. The opposite result is expected for the order *lac phoR phoA*. The data show that the order is *lac phoA phoR*. The same order has been found by Bracha and Yagil (5).

The map position of *phoR* relative to *proC* was examined by the cross S26 × FGR 5 (Table 5), in which Leu$^+$ Sm^R recombinants were selected and *lac*, *phoA*, *proC*, and *phoR* were each scored as unselected markers. By using the 5FU phenotypically revertible *phoA*-S26 mutation, it was possible to determine the constitutivity of Pho$^-$ recombinants. The selected recombinants were restreaked before scoring the unselected markers, and all progeny which were found to be recombinant for any of the unselected markers were retested by restreaking and checking the phenotype of each marker. Of 1223 colonies tested, 1142 were parental for the unselected markers (748 *lac*$^+$ *phoA proC*$^+$ *phoR*$^+$ and 394 *lac phoA*$^+$ *proC phoR*), 69 resulted from recombination between *lac* and *phoA* (42 *lac phoA proC*$^+$ *phoR*$^+$ and 27 *lac*$^+$ *phoA*$^+$ *proC phoR*), and 12 were recombinant among *phoA*, *proC*, and *phoR*. The genotypes and analysis of these twelve are shown in Table 5. Since we have previously established the order *lac phoA proC* and the order *lac phoA phoR*, the only possible orders are *lac phoA proC phoR* and *lac phoA phoR proC*. The data favor the order *lac phoA proC phoR*: 10 out of these 12 recombinants are the results of a single crossover among the closely linked markers for this order, whereas only four are single-crossover products and eight

TABLE 5. *Order of proC phoR relative to lac phoA: analysis of recombinants among phoA, proC, and phoR*

Cross: Hfr S26 + + + *phoA* + +
F$^-$ FGR5 *str leu lac* + *proC phoR*

Genotype				No. obtained	CO between *phoA*, *proC*, *phoR*	
lac	*phoA*	*proC*	*phoR*		For order *lac phoA proC phoR*	For order *lac phoA phoR proC*
+	−	+	−	4	1	2
−	+	−	+	3	1	2
+	+	+	+	1	1	1
−	−	−	−	1	1	1
+	+	−	+	1	1	2
−	−	−	+	2	2	1

are double-crossover products for the order *lac phoA phoR proC*. The order *lac phoA proC phoR* has also been found by Yagil, Bracha, and Silberstein (53) who carried out a more extensive analysis.

DISCUSSION

The *phoA* cistron is known to code for a polypeptide chain of approximately 391 residues; two of these chains dimerize to form alkaline phosphatase (41). By peptide analysis of fragments of alkaline phosphatase made by nonsense mutants, Suzuki and Garen (50) have oriented the fine-structure genetic map of *phoA* with respect to the polypeptide and have found that the U18 end of the map corresponds to the amino end of the polypeptide. We have shown (Table 3) that the orientation of *phoA* on the *E. coli* map is *lac phoA*-U12 *phoA*-U18 *proC*. These results

taken together demonstrate that the *phoA* cistron is translated (and therefore transcribed) in an anticlockwise direction relative to the *E. coli* linkage map.

The orientation of 18 units of transcription (cistrons and operons) has been determined in *E. coli* by using a variety of methods. As summarized in Table 6, 14 of these are oriented anticlockwise and 4 clockwise. Suggestive but not conclusive evidence has also been presented for the clockwise orientation of *sucA sucB* (22) and for the anticlockwise orientation of each of two operons in the *dra tpp drm pup* loci (2).

Earlier work has demonstrated that operons can function apparently normally when their orientation is inverted (4, 44). However, the probability of finding 4 or less than 4 out of 18 transcription units oriented in one direction is 0.031 if the orientation of each unit is random. The data in Table 6 therefore suggest an evolutionary history for the *E. coli* genome which favors a preponderance of transcription units oriented anticlockwise.

TABLE 6. *Orientation of transcription units in Escherichia coli*[a]

Transcription unit	Map position (min)[b]	Orientation[c]	Evidence[d]
araBAD	1	A	I (30)
araC	1	A	I (45)
leuAB	1	A	I (30)
aceEF	2	C	II (21)
lacOZYA[e]	10	A	I (29); II (12, 26); III (31); IV (28)
lacI	10	A	I (35); III (31)
phoA	11	A	V (this paper)
galETK	17	A	II (1); III (19); IV (6)
bioA	17	A	III (18)
bioBEFGCD	17	C	III (18)
supF	25	A	III (32)
trpEDCBA	25	A	I (29); II (25, 34, 49); V (54, 48); VI (23)
guaAB	48	A	II (36)
malPQ	66	A	II (20)
ilvADE	74	A	II (38); IV (39)
rhaBAD	76	A	II (37)
argE	77	C	I (10)
argCBH	77	C	I (52)

[a] Data for *araBAD*, *araC*, and *leuAB* from *E. coli* B/r; all other data from *E. coli* K-12.

[b] Minutes on time scale drawn by Taylor (51).

[c] A, anticlockwise; C, clockwise.

[d] I, fusion to another operon; II, polar mutants; III, hybridization of messenger ribonucleic acid (mRNA) to deoxyribonucleic acid strand of known orientation; IV, map position of O[c] mutant; V, correlation of amino acid sequence and genetic map; VI, polarity of mRNA synthesis.

[e] *lacO* Is part of the transcription unit (24).

The genome of *Salmonella typhimurium* also contains clockwise-oriented and anticlockwise-oriented operons (43).

ACKNOWLEDGMENTS

We thank Mitsuko Amemura for her assistance; A. Garen, N. M. Schwartz, S. Silver, and A. Torriani for providing bacterial strains; W. Hayes, K. A. Stacey, J. Gross, A. L. Taylor, M. Nei, and B. R. Levin for helpful discussions; E. Yagil for communicating his results prior to publication; and J. Kawamata for his interest and encouragement.

This investigation was supported by grant GB-2237 from the National Science Foundation, by Public Health Service training grant HD-00019 from the National Institute of Child Health and Human Development (A.N.) and by a National Science Foundation Academic Year Institute (G.R.P.).

LITERATURE CITED

1. Adler, J., and A. D. Kaiser. 1963. Mapping of the galactose genes of *Escherichia coli* by transduction with phage P1. Virology **19**:117-126.
2. Ahmad, S. I., and R. H. Pritchard. 1969. A map of four genes specifying enzymes involved in catabolism of nucleosides and deoxynucleosides in *Escherichia coli*. Mol. Gen. Genet. **104**:351-359.
3. Aono, H., and N. Otsuji. 1968. Genetic mapping of regulator gene *phoS* for alkaline phosphatase in *Escherichia coli*. J. Bacteriol. **95**:1182-1183.
4. Beckwith, J. R., E. R. Signer, and W. Epstein. 1966. Transposition of the *lac* region of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **31**:393-401.
5. Bracha, M., and E. Yagil. 1969. Genetic mapping of the *phoR* regulator gene of alkaline phosphatase in *Escherichia coli*. J. Gen. Microbiol. **59**:77-81.
6. Buttin, G. 1963. Mécanismes régulateurs dans la biosynthése des enzymes du metabolisme du galactose chez *Escherichia coli* K12. II. Le determinisme génétique de la régulation. J. Mol. Biol. **7**:183-205.
7. Curtiss, R., III 1965. Chromosomal aberrations associated with mutations to bacteriophage resistance in *Escherichia coli*. J. Bacteriol. **89**:28-40.
8. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61-76.
9. Echols, H., A. Garen, S. Garen, and A. Torriani. 1961. Genetic control of repression of alkaline phosphatase in *E. coli*. J. Mol. Biol. **3**:425-438.
10. Elseviers, D., R. Cunin, and N. Glansdorff. 1969. Reactivation of arginine genes under the influence of polar mutations. FEBS Lett. **3**:18-20.
11. Fan, D. P., M. J. Schlesinger, A. Torriani, K. J. Barrett, and C. Levinthal. 1966. Isolation and characterization of complementation products of *Escherichia coli* alkaline phosphatase. J. Mol. Biol. **15**:32-48.
12. Franklin, N. C., and S. E. Luria. 1961. Transduction of bacteriophage P1 and the properties of the *lac* genetic region in *E. coli* and *S. dysenteriae*. Virology **15**:299-311.
13. Garen, A. 1960. Genetic control of the specificity of the bacterial enzyme, alkaline phosphatase. Symp. Soc. Gen. Microbiol. **10**:239-247.
14. Garen, A. 1968. Sense and nonsense in the genetic code. Science **160**:149-159.
15. Garen, A., and S. Garen. 1963. Complementation *in vivo* between structural mutants of alkaline phosphatase from *E. coli*. J. Mol. Biol. **7**:13-22.
16. Garen, A., C. Levinthal, and F. Rothman. 1961. Alterations in alkaline phosphatase induced by mutations. J. Chim. Phys. **58**:1068-1071.

17. Garen, A., and O. Siddiqi. 1962. Suppression of mutations in the alkaline phosphatase structural cistron of *E. coli*. Proc. Nat. Acad. Sci. U.S.A. **48**:1121-1127.
18. Guha, A., Y. Saturen, and W. Szybalski. 1971. Divergent orientation of transcription from the biotin locus of *Escherichia coli*. J. Mol. Biol. **56**:53-62.
19. Guha, A., M. Tabaczynski, and W. Szybalski. 1968. Orientation of transcription for the galactose operon as determined by hybridization of *gal* mRNA with the separated DNA strands of coliphage λdg. J. Mol. Biol. **35**: 207-213.
20. Hatfield, D., M. Hofnung, and M. Schwartz. 1969. Nonsense mutations in the maltose A region of the genetic map of *Escherichia coli*. J. Bacteriol. **100**:1311-1315.
21. Henning, U., and C. Herz. 1964. Ein Strukturgen-Komplex für den Pyruvatdehydrogenase-Komplex von *Escherichia coli* K-12. Z. Vererbungsl. **95**:260-275.
22. Herbert, A. A., and J. R. Guest. 1969. Studies with α-ketoglutarate dehydrogenase mutants of *Escherichia coli*. Mol. Gen. Genetics **105**:182-190.
23. Imamoto, F., N. Morikawa, and K. Sato. 1965. On the transcription of the tryptophan operon in *Escherichia coli*. III. Multicistronic messenger RNA and polarity for transcription. J. Mol. Biol. **13**:169-182.
24. Ippen, K., J. H. Miller, J. Scaife, and J. Beckwith. 1968. New controlling element in the *lac* operon of *E. coli*. Nature (London) **217**:825-827.
25. Ito, J., and I. P. Crawford. 1965. Regulation of the enzymes of the tryptophan pathway in *Escherichia coli*. Genetics **52**:1303-1316.
26. Jacob, F., and J. Monod. 1961. On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**:193-211.
27. Jacob, F., and J. Monod. 1965. Genetic mapping of the elements of the lactose region in *Escherichia coli*. Biochem. Biophys. Res. Commun. **18**:693-701.
28. Jacob, F., D. Perrin, C. Sanchez, and J. Monod. 1960. L'operon: group de gènes á expression coordonnée par un oppérateur. C. R. Acad. Sci. Paris **250**:1727-1729.
29. Jacob, F., A. Ullmann, and J. Monod. 1965. Délétions fusionnant l'opéron lactose et un opéron purine chez *Escherichia coli*. J. Mol. Biol. **31**:704-719.
30. Kessler, D. P., and E. Englesberg. 1969. Arabinose-leucine deletion mutants of *Escherichia coli* B/r. J. Bacteriol **98**: 1159-1169.
31. Kumar, S., and W. Szybalski. 1969. Orientation of transcription in the *lac* operon and its repressor gene in *Escherichia coli*. J. Mol. Biol. **40**:145-157.
32. Lozeron, H. A., W. Szybalski, A. Landy, J. Abelson, and J. D. Smith. 1969. Orientation of transcription for the amber suppressor gene su_{III} as determined by hybridization between tyrosine tRNA and the separated DNA strands of transducing coliphage $\phi 80ds u_{III}$. J. Mol. Biol. **39**:239-243.
33. Markovitz, A. 1964. Regulatory mechanisms for synthesis of capsular polysaccharide in mucoid mutants of *Escherichia coli* K12. Proc. Nat. Acad. Sci. U.S.A. **51**:239-246.
34. Matsushiro, A., K. Sato, J. Ito, S. Kida, and F. Imamoto. 1965. On the transcription of the tryptophan operon in *Escherichia coli*. I. The tryptophan operator. J. Mol. Biol. **11**:54-63.
35. Miller, J. H., J. Beckwith, and B. Muller-Hill. 1968. Direction of transcription of a regulatory gene in *E. coli*. Nature (London) **220**:1287-1290.
36. Nijkamp, H. J. J., and A. A. G. Oskamp. 1968. Regulation of the biosynthesis of guanosine 5'-monophosphate: evidence for one operon. J. Mol. Biol. **35**:103-109.
37. Power, J. 1967. The L-rhamnose genetic system in *Escherichia coli* K-12. Genetics **55**:557-568.
38. Ramakrishnan, T., and E. A. Adelberg. 1965. Regulatory mechanisms in the biosynthesis of isoleucine and valine. II. Identification of two operator genes. J. Bacteriol. **89**: 654-660.
39. Ramakrishnan, T., and E. A. Adelberg. 1965. Regulatory mechanisms in the biosynthesis of isoleucine and valine. III. Map order of the structural genes and operator genes. J. Bacteriol. **89**:661-664.
40. Rosen, B., F. Rothman, and M. G. Weigert. 1969. Miscoding caused by 5-fluorouracil. J. Mol. Biol. **44**:363-375.
41. Rothman, F., and R. Byrne. 1963. Fingerprint analysis of alkaline phosphatase of *Escherichia coli* K12. J. Mol. Biol. **6**:330-340.
42. Rothman, F., and J. R. Coleman. 1968. Kinetics of transcription and translation of a repressed gene. J. Mol. Biol. **33**:527-531.
43. Sanderson, K. E. 1965. Information transfer in *Salmonella typhimurium*. Proc. Nat. Acad. Sci. U.S.A. **53**:1335-1340.
44. Sanderson, K. E., and C. A. Hall. 1970. F-prime factors of *Salmonella typhimurium* and an inversion between *S. typhimurium* and *Escherichia coli*. Genetics **64**:215-228.
45. Schleif, R., J. Greenblatt, and R. W. Davis. 1971. Dual control of arabinose genes on transducing phage λ-*dara*. J. Mol. Biol., *in press*.
46. Schlesinger, M. J., and C. Levinthal. 1963. Hybrid protein formation of *E. coli* alkaline phosphatase leading to *in vitro* complementation. J. Mol. Biol. **7**:1-12.
47. Schwartz, N. M. 1963. Nature of ethyl methanesulfonate induced reversions of *lac*$^-$ mutants of *Escherichia coli*. Genetics **48**:1357-1375.
48. Somerville, R. L., and C. Yanofsky. 1964. On the translation of the A gene region of tryptophan messenger RNA. J. Mol. Biol. **8**:616-619.
49. Somerville, R. L., and C. Yanofsky. 1965. Studies on the regulation of tryptophan biosynthesis in *Escherichia coli*. J. Mol. Biol. **11**:747-759.
50. Suzuki, T., and A. Garen. 1969. Fragments of alkaline phosphatase from nonsense mutants. I. Isolation and characterization of fragments from amber and ochre mutants. J. Mol. Biol. **45**:549-566.
51. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
52. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**:332-353.
53. Yagil, E., M. Bracha, and N. Silberstein. 1970. Further genetic mapping of the *phoA-phoR* region for alkaline phosphatase synthesis in *Escherichia coli* K12. Mol. Gen. Genet. **109**:18-26.
54. Yanofski, C., B. C. Carlton, J. Guest, D. R. Helinski, and U. Henning. 1964. On the colinearity of gene structure and protein structure. Proc. Nat. Acad. Sci. U.S.A. **51**: 266-272.