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Genetic Analysis of a Large-Cell, Radiation-Resistant Strain of *Escherichia coli*[1]

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A genetic analysis of *Escherichia coli* P6, a large-cell, radiation-resistant strain of *E. coli*, established that it originated as the result of a mutational event. The gene responsible for the complex P6 phenotype was located at 61 ± 0.5 min on the *E. coli* linkage map. The close resemblance of conjugal and transductional recombinants to one or the other parent without indication of an intermediate class suggests that only a single gene may be involved.

Escherichia coli P6 was isolated by Ogg and Zelle (16) after camphor treatment of *E. coli* 82/r, an adenine-dependent derivative of *E. coli* B/r. P6 cells were larger, weighed about three times more, contained approximately three times more deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and were more resistant to X-rays than the parent (16, 21). Preliminary genetic experiments ($F^+ \times F^-$ crosses) showed that colonial morphology, cell size, radiation resistance, and an unselected property, T1 resistance, segregated as a unit (21). These results suggested that a pleiotrophic mutation was responsible for the complex phenotype of P6.

The results of experiments designed to establisht the genetic constitution of *E. coli* P6 are reported here. Using both conjugation and transduction techniques, we mapped the P6 gene, *lar-1*, at 61 ± 0.5 min on the *E. coli* linkage map. Recombinants characterized in terms of the properties originally used to describe the two parental strains resembled either one or the other parent. There were no indications of intermediate-type recombinants. Thus, the complex phenotype of *E. coli* P6 appears to be due to the mutation of a single gene.

MATERIALS AND METHODS

Symbols. The recommendations of Demerec et al. (6) with regard to genotypic and phenotypic designations are followed with two exceptions. Streptomycin- and bacteriophage T6-resistant and -sensitive phenotypes are referred to as Sm^R or Sm^S and $T6^R$ or $T6^S$, respectively. The gene responsible for the complex phenotype of *E. coli* P6 is designated *lar* for large cell. The *E. coli* P6 phenotype is Lar^-, and *E. coli* 82/r and other wild-type strains are Lar^+.

Bacterial strains. The sources and relevant characteristics of *E. coli* strains used in this study are listed in Table 1. The genetic map of *E. coli* [modified from Taylor and Trotter (19)], indicating the positions of markers of interest here and the origins and directions of chromosome transfer of pertinent Hfr donor strains, is shown in Fig. 1. The P1-sensitive indicator strain, *Shigella* 60, was supplied by A. Markovitz.

Phage strains. Bacteriophage P1*bt*, a generalized transducing phage adapted to grow on *E. coli* B derivatives, was obtained from R. Helling. H. Kubitschek supplied the virulent bacteriophage T6. The T6 stocks were replenished by infecting appropriate bacterial strains in liquid cultures (1). Titers of 10^9 to 10^{10} plaque-forming units per ml were obtained with this procedure.

Media and cultural conditions. Cultures were grown and maintained routinely on Difco nutrient broth and agar supplemented with NaCl (5 g/liter). Minimal M9 medium (3) was used for genetic selections with appropriate supplements added to fulfill auxotrophic requirements.

Response to streptomycin was determined on nutrient agar supplemented with streptomycin sulfate (150 μg/ml). The L-broth, agar, and soft agar used in the transduction studies were described by Lennox (9).

Stationary cultures were incubated overnight at 37 C on a reciprocating platform shaker. Exponentially growing cultures were prepared by diluting stationary cultures 1:100 into fresh broth and incubating them at 37 C for an additional 3 to 4 hr until the cell density reached 2×10^8 to 5×10^8 cells/ml.

Cell parameters. DNA, RNA, and protein were determined on perchloric acid-extracted cells (17).

[1] Part of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, Illinois Institute of Technology, Chicago. A summary of this work was presented at the 68th Annual Meeting of the American Society for Microbiology, Detroit, Mich., 5-10 May 1968.

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TABLE 1. *Escherichia coli strains*

Culture no.	Other designations	Strain	Mating type[a]	Genetic constitution[b]	Source
C57		P6	F⁻	*pur-1, lar-1*	M. Zelle
C59		P6	F⁺	*pur-1, lar-1*	M. Zelle
MK45	HfrP6-1	P6	Hfr	*pur-1, (sup-2), lar-1.* (origin···*trp*···*his*···*tyr*···)	M. Kvetkas
MK50	HfrP6-2	P6	Hfr	*pur-1, lar-1* (origin···*leu*···*arg*···*ilv*···)	M. Kvetkas
MK52	HfrP6-3	P6	Hfr	*pur-1, lar-1* (origin···*leu*···*arg*···*ilv*···)	M. Kvetkas
MK54	HfrP6-4	P6	Hfr	*pur-1, lar-1* (origin···*leu*···*arg*···*ilv*···)	M. Kvetkas
MK56	HfrP6-5	P6	Hfr	*pur-1, lar-1* (origin···*leu*···*arg*···*ilv*···)	M. Kvetkas
MK57	HfrP6-6	P6	Hfr	*pur-1, lar-1* (origin···*leu*···*arg*···*ilv*···)	M. Kvetkas
MK120		P6	F⁻	*pur-1, lar-1, arg-10, ilv-2, met-8, strA2, tfrA2, tsx-2*	M. Kvetkas
MK123		P6	F⁻	*pur-1, lar-1, arg-13, ilv-2, met-8, strA2, tfrA2, tsx-2*	M. Kvetkas
C22		82/r	F⁻	*pur-1*	M. Zelle
C28		82/r	F⁻	*pur-1, (sup-1), lac-1, gal-1, mtl-1, strA1, trfA1, tsx-1*	M. Zelle
MK74		82/r	F⁻	*pur-1, (sup-1), arg-3, his-2, (sup-2), ilv-1, leu-1, lysA1, met-3, lac-1, gal-1, mtl-1, strA1, tfrA1, tsx-3*	M. Kvetkas
AC2523	HfrB2	B/r	Hfr	*met-4, thr-4, mal-2* (origin···*met*···*ilv*···*mtl*···)	H. Boyer
MK135	B47	B	F⁻	*argG1*	G. Jacoby

[a] Mating type: Hfr-high frequency of recombination male donor; F⁺-low frequency of recombination male donor; F⁻-female recipient.

[b] Unlisted genetic characters are assumed to be wild type. Nutritional requirements: *pur-*, purine; *arg-*, arginine; *his-*, histidine; *ilv-*, isoleucine-valine; *leu-*, leucine; *lysA-*, lysine; *met-*, methionine; *thr-*, threonine; *trp-*, tryptophan. Utilization of sugars: *gal-*, galactose; *lac-*, lactose; *mal-*, maltose; *man-*, mannose; *mtl-*, mannitol; *xyl-*, xylose. Resistance to agents: *strA-*, streptomycin; *tfrA-*, bacteriophage T4; *tsx-*, bacteriophage T6. Other: *lar-*, large cell; *sup-*, suppressor.

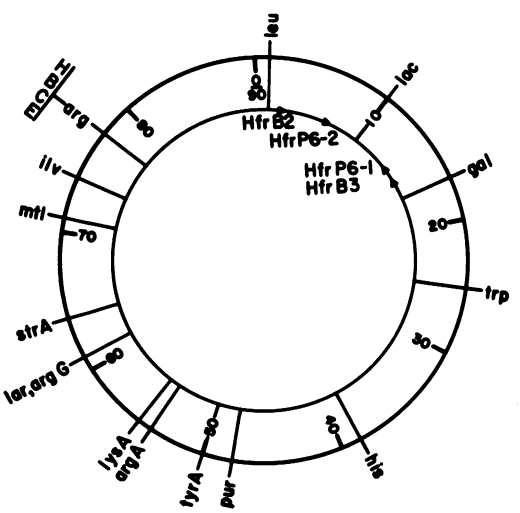

FIG. 1. *E. coli genetic linkage map, redrawn from Taylor and Trotter (19). The origins and directions of chromosome transfer of Hfr donor strains are shown by arrowheads. See Table 1 for symbols.*

DNA was determined by Burton's procedure (5), RNA by the method of Kerr and Seraidarian (7), and protein by the method of Lowry et al. (12).

Colonial morphologies were scored with a Stereoscopic Wide-Field microscope (Bausch and Lomb Optical Co.) with oblique illumination (16). Cell morphologies and the proportion of double cells in the cultures were determined by phase-contrast microscopy with a model GSL microscope (Carl Zeiss, Inc.).

Irradiations. The X-ray equipment and exposure procedures were described previously (21).

A 15-w germicidal lamp (General Electric) mounted in a stainless steel box fitted with a Howard Electric Shutter (Burke and James, Inc.) was the source of ultraviolet light. A thin layer of cell suspension diluted in phosphate buffer was placed in a 2-inch (5-cm) diameter glass petri dish. The dish was rotated at low speed on a clinical rotator (A. H. Thomas Co.) at a distance of 34 cm from the lamp (dose rate of 6.45 ergs per mm² per sec).

Construction of strains. Hfr P6 strains were produced and isolated by using a grid-plate modification (B. Low, Ph.D. Thesis, Univ. of Pennsylvania, Philadelphia, 1965) of the Taylor and Adelberg (18) procedure. Colonies suspected of containing Hfr cells were purified and confirmed by standard mating procedures described below. Newcombe's (14) delayed challenge technique was used to obtain streptomycin-resistant strains.

The leucine requirement was introduced into MK74 by a modification of the penicillin-enrichment procedure (13). Other auxotrophic markers were introduced into recipient strains by treating them with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co.) essentially as described by Adelberg, Mandel, and Chen (2).

Conjugal matings. Exponentially growing donor and recipient cells were mixed in a ratio of 1:10 and were rotated slowly in test tubes on a model 150V multipurpose rotator (Scientific Industries). In uninterrupted matings, samples were removed after 90 min, diluted, and plated on selective agar. Samples were removed periodically in interrupted matings and were subjected to violent mechanical agitation (11). Bacteriophage T6 was used to counter-select against the donor strains. Recombinant colonies were counted after 40 to 44 hr of incubation at 37 C.

Transductions. The transduction procedures used have been described by Lennox (9).

Genetic analyses. Master grid plates prepared from recombinant colonies were replicated by the velveteen replica plating technique (8). The replicated plates were used to score colonial morphologies on nutrient agar, streptomycin responses on nutrient agar containing streptomycin, and nutritional requirements on selective minimal agar plates.

RESULTS

Isolation of Hfr P6 donor strains. C59, a P6 F^+ strain previously made F^+ by infection from *E. coli* K-12 W1177F^+ (21), was exposed to doses of ultraviolet light that killed 99.0 to 99.9% of the population. Six Hfr P6 donor strains were isolated from 2,060 survivors that had been screened by a plate-mating technique. After purification by a series of single clone isolation steps, the high-frequency-of-recombination donor ability of each of the six strains was confirmed in uninterrupted matings. Hfr P6-1, which transfers *trp* as an early marker, was selected by screening with tryptophan-requiring recipient strains. Recipients carrying markers located in the lower left-hand quadrant of the chromosome near the *lar* marker were used in the later screening programs for Hfr P6 donors. Yet, none of the other five Hfr P6 donors (Hfr P6-2 through -6) isolated here has origins in the desired region.

The two donor strains, Hfr P6-1 and -2, were studied in greater detail. Both were typical Hfr donor strains in view of their mating efficiencies, linkage relationships, and in their orientations, gradients, and kinetics of chromosome transfer (*unpublished data*). Origins and directions of chromosome transfer of Hfr P6-1 and P6-2 are shown in Fig. 1.

Mapping of the lar locus through interrupted matings. The first series of interrupted matings involved crosses of MK50 with a multiply-marked auxotrophic F^- recipient strain, MK74. Except for *lar* and the specific marker genes, these two strains presumably were isogenic. Direct selections were made for each of the amino acid markers carried by the F^- recipient. Because it was not feasible to select directly for Lar^- recombinants, an indirect scoring procedure was chosen. Unselected marker analyses were performed on all of the amino acid-independent recombinants. They were scored as Lar^- (P6-type) or Lar^+ (82/r or wild-type) according to their colonial morphologies. Streptomycin response was also scored as an unselected marker because preliminary results suggested that the *lar-1* site was located near *strA*.

The results of a typical interrupted mating of MK50 with MK74 are presented in Fig. 2. The parallel entrance kinetics and the low frequency of crossovers between the *lar-1* and *straA* markers indicated that they were closely linked. The relative number of recombinants carrying the two markers suggested that the *lar-1* site was distal (with reference to the origin of Hfr P6-2) but very close to the *strA* site.

The reciprocal cross, Hfr B2 (4) × MK120, yielded comparable results but indicated that the origin of Hfr B2 was about 10 min closer to *lar-1* than was that of Hfr P6-2. Based on these data and assuming that 5 min is required to mobilize the chromosome (11), the origins of the two donor strains are located as shown in Fig. 1. This location of the origin of Hfr B2 confirms Boyer's results (4).

Ordering of the lar region of the chromosome by unselected marker analysis. The interrupted mating technique could not be used to map the *lar-1* and *strA* markers accurately because they were too far from the origins of any available Hfr donor strains in the *E. coli* B line. Accordingly, recombinants from an uninterrupted mating of Hfr B2 with MK120 were subjected to an unselected marker analysis to establish the order of *lar-1* and *strA* in relation to one another and to other markers in this region of the chromosome. Pur^+T6^R recombinant colonies were scored for the presence of *arg-10*, *lar-1*, and *strA* markers from the donor; similarly, Arg^+T6^R recombinant colonies were scored for *lar-1* and *strA*. In each analysis, the selected marker was distal to the unselected markers as recommended by Verhoef and DeHaan (20).

The results of these analyses (Table 2) indicated that the relative order of the markers involved here is $\cdots pur\text{-}1 \cdots arg\text{-}10 \cdots lar\text{-}1 \cdots strA2 \cdots$ (as read in the clockwise direction). The maximum linkages of 63 to 65% obtained between *pur-1* and *arg-10*, *arg-10* and *lar-1*, and *lar-1* and *strA* suggested that they are farther apart than the interrupted mating data had indicated.

Fine mapping of the lar locus through cotransductional analyses. A contransductional frequency of 95.5% was obtained for *lar-1* and *argG* when either C57 (carrying *lar-1*) or MK120 (carrying *lar-1* and *arg-10*) was the donor and MK135

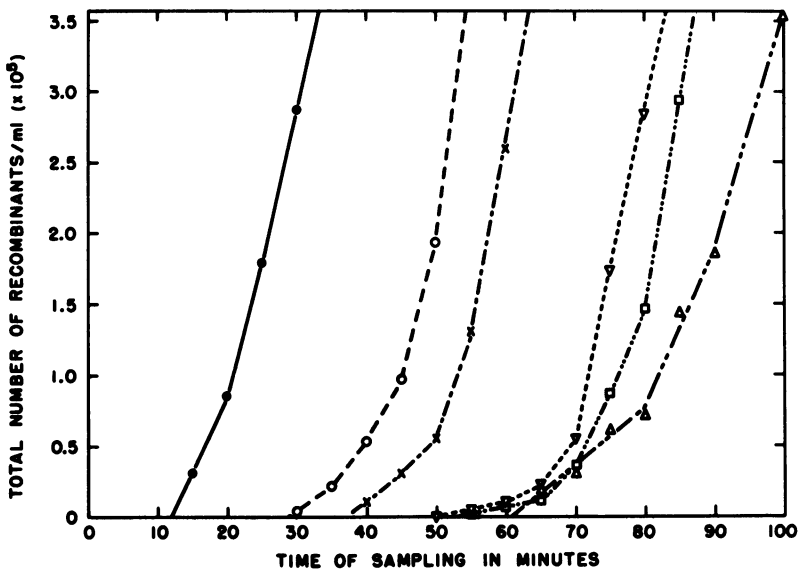

FIG. 2. *Appearance of recombinants from an interrupted mating of Hfr P6-2 (MK30) and an E. coli, 82/r F⁻ recipient strain (MK74). The mating was carried out under standard conditions using bacteriophage T6 to counterselect against the donor. Leu⁺T6ᴿ (●), Arg⁺T6ᴿ (○), Iso⁺T6ᴿ (×) and Lys⁺T6ᴿ (△) recombinants were selected directly on minimal agar plates supplemented appropriately. Two hundred recombinants from each amino acid selection were scored for Lar⁻ on the basis of colonial morphology on nutrient agar plates, and for Smˢ on nutrient agar plates supplemented with streptomycin (150 μg/ml). The numbers of Lar⁺T6ᴿ (□) and SmˢT6ᴿ (▽) recombinants were calculated from these data.*

(*argG*) was the recipient. The *lar-1* marker was not cotransucible with either *strA* or *arg-10* under the same conditions (Table 3). The high frequency of cotransduction for *lar-1* and *argG* locates the *lar-1* site at 61 ± 0.5 min on the *E. coli* linkage map (Fig. 1). No information as to the order of the *lar-1* and *argG* sites was obtained from these crosses.

TABLE 2. *Order of the pur, arg, lar, and strA loci from an uninterrupted mating of HfrB2 (AC2523) and MK120*[a]

Selected recombinants	Recombinants per 10^8 Hfr cells	No. scored	Percentage of recombinants that scored as			
			Pur⁺	Arg⁺	Lar⁺	Smˢ
Pur⁺T6ᴿ	6.8	1,126	100.0	63.1	54.0	36.8
Arg⁺T6ᴿ	8.7	1,112		100.0	64.8	44.1

[a] Uninterrupted mating was carried out under standard conditions; bacteriophage T6 was used to counterselect against the donor; nutritional requirements were scored on selective minimal agar plates supplemented appropriately, streptomycin-sensitivity on nutrient agar plates containing streptomycin (150 μg/ml), and Lar⁻ on the basis of colonial morphology on nutrient agar plates. The donor strain, HfrB2, an *E. coli* B/r derivative, is wild-type for *lar;* the recipient strain, MK120, an *E. coli* P6 strain, carried *lar-1* in addition to *pur-1, arg-10, ilv-1, met-8, strA2,* and *tsx-2.*

Studies of recombinants. A number of repre-

TABLE 3. *Fine mapping of the lar locus by cotransductional analysis*[a]

P1 donor	Recipient	Transductants					
		Selected	Per 10^6 P1	No. scored	Per cent scored		
					Lar⁻	Smᴿ	Arg⁺
C57	MK135	Arg⁺	2.2	4,894	96.5	—[b]	100
MK120	MK135	Arg⁺	2.9	2,334	94.5	—[b]	100
C22	MK120	Arg⁺	32.0	10,890[c]	0.0	0	100
MK120	C22	Smᴿ	7.6	1,538	0.0	100	0

[a] P1*bt* was grown on the donor strain for at least three cycles prior to its use in the standard transduction procedure. The multiplicity of infection ranged from about 0.1 to 1.0 phage per bacterium. Nutritional requirements were scored on selective minimal agar plates supplemented appropriately, streptomycin-sensitivity was scored on nutrient agar plates containing streptomycin (150 μg/ml), and Lar⁻ was scored on the basis of colonial morphology on nutrient agar plates.

[b] Not scored.

[c] *Arg*⁺ transductants were scored directly for Lar⁻ colonial morphology on the arginine-selective agar plates.

sentative conjugal and transductional recombinants were studied in greater detail to demonstrate that recombinants scored solely on the basis of their colonial morphologies also corresponded with their parents in their other properties.

The X-ray responses of three Lar^+ and three-Lar^- recombinant strains isolated in the conjugation experiments were compared to one another and to those of the Hfr P6 donor and 82/r F^- recipient parental strains (Fig. 3). The survival curves for each type of recombinant were very similar to one another and to those for their respective parents.

The macromolecular composition of three recombinants of each type and their parents also were determined (Table 4). As with the X-ray responses, recombinant and parental values were similar. No evidence was obtained for the presence of an intermediate class of recombinants. As shown in Table 4, Lar^- cells contained over three times more DNA, RNA, and protein than Lar^+ cells.

Several transductants were exposed to a single dose (20 kr) of X-rays (Table 5). Comparable values for the donor and recipient cultures also are shown in Table 5. The average survival ratio for Lar^- strains was an order of magnitude greater than that of Lar^+ strains. Thus, transductants also fell into two classes corresponding to the two parents with regard to X-ray survival.

E. coli P6 (Lar^-) cultures tend to be relatively stable under routine culture conditions. However, some conjugal and transductional Lar^- recombinant cultures segregated both types of parental colonies immediately after isolation. This occurred regardless of whether the Lar^- strain was the donor or recipient parent. Most Lar^- recombinant colonies formed in these crosses of *E. coli* B derivatives stabilized after only one or two single clone isolations. All Lar^+ recombinant colonies formed in crosses of P6 with 82/r and other B strains were stable and yielded only wild-type colonies. In contrast, considerable instability was introduced into the Lar^- recombinant

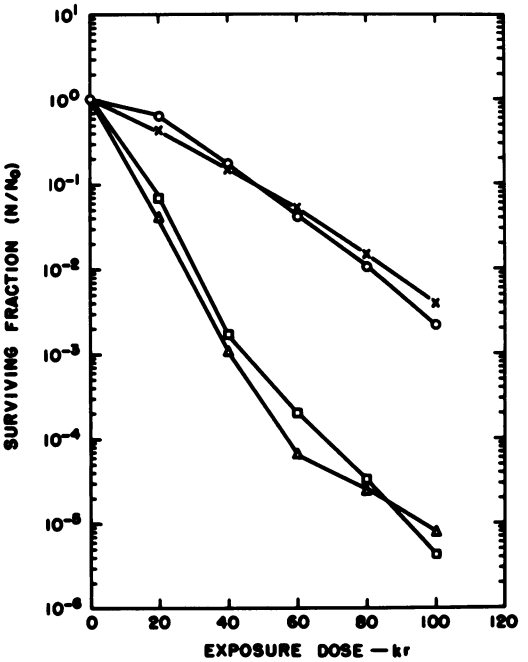

FIG. 3. *X-ray survival curves of Lar^- and Lar^+ recombinant cultures. Stationary nutrient broth cultures of three each Lar^- (×) and Lar^+ (△) recombinants and of their Hfr P6-2 donor (○) and 82/r, F^- recipient (□) parents from the crosses shown in Fig. 2 were exposed to X-rays. The data plotted represent the average of three separate experiments.*

TABLE 4. *Macromolecular composition of Lar^- and Lar^+ recombinant cultures*[a]

Culture	Phenotype	DNA/cell (× 10^{-15} g)	RNA/cell (× 10^{-14} g)	Protein/cell (× 10^{-13} g)
HfrP6 donor (MK50)	Lar^-	23.06 ± 0.44[b]	7.39 ± 0.70[b]	5.87 ± 0.27[b]
P6-type recombinants	Lar^-	28.05 ± 3.19	11.68 ± 0.75	6.68 ± 0.32
82/r F^- recipient (MK74)	Lar^+	7.95 ± 0.74	3.75 ± 0.21	1.70 ± 0.05
82/r-type recombinants	Lar^+	8.43 ± 0.37	3.0 ± 0.29	1.95 ± 0.18
Average for Lar^- strains		26.80 ± 0.10[c]	10.63 ± 0.47[c]	6.50 ± 0.14[c]
Average for Lar^+ strains		8.30 ± 0.02	3.17 ± 0.12	1.88 ± 0.06
Ratio of Lar^-:Lar^+		3.23	3.23	3.43

[a] The cellular parameters of stationary nutrient broth cultures were determined as described in Materials and Methods. The values given represent the averages from two separate experiments. A total of three P6-type and three 82/r-type recombinant strains was tested.

[b] Expressed as the mean ± standard error of the mean based on three individual determinations with a single replication on eight cultures.

[c] Expressed as the mean ± standard error of the mean based on 24 individual determinations for the averages.

TABLE 5. *X-ray responses of ArgG⁺ transductant cultures*[a]

Culture	Phenotype	Surviving fraction ($N/N_0 \times 10^{-2}$)[b]
B47 recipient (MK135)	ArgG⁻ Lar⁺	2.19
B-type transductants	ArgG⁺ Lar⁺	1.61 ± 0.32[b]
P6 donor (C57)	ArgG⁺ Lar⁻	29.00
P6-type transductants	ArgG⁺ Lar⁻	17.10 ± 6.20[b]
Average for Lar⁺ strains		1.61 ± 0.32[b]
Average for Lar⁻ strains		18.30 ± 6.20[b]
Ratio of Lar⁻:Lar⁺		11.40

[a] Representative ArgG⁺Lar⁺ and ArgG⁺Lar⁻ transductants and their Lar⁺ (MK135) and Lar⁻ (C57) parents were grown to stationary phase in nutrient broth. The values shown represent the averages from two separate experiments.

[b] Expressed as the mean ± standard error of the mean considering each of 10 determinations as individuals.

colonies formed when *E. coli* K-12 Hfr donor strains and P6 F⁻ recipient strains were mated. Approximately 7% of apparently Lar⁻ colonies continued to segregate both Lar⁻ and Lar⁺ colonies even after five single clone isolation steps. These observations suggest that the *lar-1* allele is dominant since unstable merodiploids apparently harboring both the mutant and wild-type *lar* alleles express the mutant Lar⁻ phenotype.

DISCUSSION

The results of the mapping studies, i.e., the gene order as determined by unselected marker analyses of uninterrupted matings, the marker entry times, and kinetics obtained from reciprocal crosses in interrupted matings, and the very high cotransductional frequencies between *lar-1* and *argG* measured in transductional analyses, demonstrated that *lar-1* could be assigned a specific locus on the *E. coli* linkage map. The possibility arose, however, that more than a single gene might be involved here when we considered that P6 differed from its parent in several properties and that the transducing phage, P1, can carry the DNA equivalent of some 1 to 2% of the *E. coli* genome, or about 10 or more genes (19). The presence of more than one mutant gene would be indicated by the production of multiple classes of recombinants from genetic crosses. The X-ray response and macromolecular composition data we obtained suggest that this possibility is rather unlikely.

Our ratios of macromolecular contents for *E. coli* P6 to 82/r strains of about 3 for the conjugal recombinants (Table 4) correspond to the values reported by Ogg and Zelle (16). However, Ogg and Humphrey (15) reported smaller ratios of about 2. This may indicate that a range from about 2 to 3 in the ratios of macromolecular cell contents is compatible with the P6 phenotype. Presumably other mutations, possibly some involving the control of cell division by P6 strains (*unpublished data*), can be superimposed upon the *lar* mutation. The X-ray survival curves in Fig. 3 are similar to those presented previously (15, 21).

Two conclusions may be drawn from the successful application of the standard techniques of bacterial genetics to this problem: (i) *E. coli* P6 originated from a mutation in its parent, *E. coli* 82/r, which was induced or selected for by the camphor treatment (16); and (ii) a single gene substitution is probably responsible for the complex *E. coli* P6 phenotype because intermediate-type recombinants were not found.

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