MAPPING OF THE *polA* LOCUS OF *ESCHERICHIA COLI* K12: GENETIC FINE STRUCTURE OF THE CISTRON

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ABSTRACT

The close linkage of the *glnA* gene with *polA* was exploited to construct a fine structure map of *polA* by means of generalized transduction with phage P1. Nine different *polA*$^-$ alleles were mapped by recombinational crosses. The results indicate a gene order consistent with previous observations (KELLEY and GRINDLEY 1976a; MURRAY and KELLEY 1979). Three mutations, *polA5*, *polA6* and *polA12* map within the "carboxy-terminal" or "large-fragment" portion of the gene in unambiguous order. Four alleles, known to affect the "amino-terminal" portion of the gene, *polA107*, *polA214*, *polA480*ex and *polA4113*, appear to be closely linked with certain ambiguities in their exact order. All four of these mutations are known to alter the 5′→3′ exonuclease activity of DNA polymerase I and three of them result in the conditional lethal *polA*$^-$ phenotype. The *polA1* nonsense mutation maps between these two groups in a position consistent with its known effect, production of an amber fragment that includes the 5′→3′ exonuclease. The final allele, *resA*1, is another nonsense mutation that maps at the extreme "amino-terminus" of the cistron.——A number of control experiments were conducted to determine the effects of *polA*$^-$ mutations on the P1-mediated recombinational event. These experiments indicated that abortive transduction occurs quite frequently, but the formation of abortive transductants and segregation of unselected transduced markers among daughter progeny is like that observed by other investigators. There was no evidence that any individual *polA*$^-$ allele behaved in an exceptional fashion during recombination.

THE *polA* gene of *E. coli* is the structural gene for DNA polymerase I (KELLEY and WHITFIELD 1971), a single polypeptide with multiple enzymatic functions (KORNBERG 1980). A large number of allelic mutations of *polA* exist, and the enzymatic defects resulting from several of these mutations have been characterized. Such *polA*$^-$ cells are sensitive to methyl methanesulfonate, providing a rapid plating test by which *polA*$^+$ and *polA*$^-$ strains may be distinguished.

The *polA* gene lies in the chromosomal region bounded by the metabolic markers *metE* and *rha* at 85 and 87 minutes, respectively, on the *E. coli* genetic map (BACHMANN, LOW and TAYLOR 1976). These markers were originally used to orient the *polA* cistron by means of transduction with phage P1 (KELLEY and GRINDLEY 1976a). More recently, we have confirmed this orientation by a separate set of experiments utilizing the more closely linked *glnA* marker (MURRAY

and KELLEY 1979). In this communication, I report data used to construct a fine-structure map of the *polA* cistron, utilizing P1-mediated generalized cotransduction of nine different *polA* alleles with the *glnA* marker. The genetic map order derived from these experiments correlates well with the known enzymatic defects resulting from the *polA*⁻ mutations tested.

Of the thirty or more different *polA*⁻ alleles described in the literature, nine were selected for mapping because of their especially interesting phenotypes or because the mutant species of DNA polymerase I that they produce have been well characterized. The polymerase molecule has three enzymatic functions and can be dissected by subtilisin cleavage into two unequal polypeptides. JACOBSEN, KLENOW and OVERGAARD-HANSEN (1974) demonstrated that the small peptide, corresponding to the "amino-terminal" third of the gene, contains the 5′ → 3′ exonuclease active site; the larger fragment, corresponding to the "carboxy-terminal" two-thirds of the enzyme polypeptide contains the polymerase and 3′ → 5′ exonuclease active sites. This has allowed partial assignment of the sites of enzymatic defects when mutant polymerase species can be purified and proteolyzed. The nine alleles chosen for mapping are:

polA1: The first mutation isolated by DE LUCIA and CAIRNS (1969). This is a nonsense mutant that has been shown to make an amber peptide corresponding to the "amino-terminal" third of the enzyme (LEHMAN and CHIEN 1973; FRIEDBERG and LEHMAN 1974).

polA5: A missense mutation from the original CAIRNS collection. The purified mutant enzyme has recently been shown to be defective in polymerizing capacity (MATSON, CAPALDO-KIMBALL and BAMBARA 1978).

polA6: A missense mutation from the original CAIRNS collection. The purified mutant enzyme has been shown to be defective in polymerizing capacity, perhaps in affinity for DNA binding (KELLEY and WHITFIELD 1971; KELLEY and GRINDLEY 1976b).

polA12: A missense mutation isolated by MONK and KINROSS (1972) and characterized on the basis of its temperature-sensitive phenotype *in vivo*. The purified mutant enzyme has a normal 5′ → 3′ exonuclease and polymerase activity at low temperatures, but has an apparent conformational alteration that makes it much more thermolabile than wild-type enzyme *in vitro* (UYEMURA and LEHMAN 1976). DNA binding studies indicate that the enzyme binds poorly to DNA and is sluggish in initiating polymerization (BAMBARA, UYEMURA and LEHMAN 1976).

polA107: A missense mutation characterized by GLICKMAN *et al.* (1973). The polymerase made by this mutation has been purified and shown to be defective in 5′ → 3′ exonuclease activity HEIJNEKER *et al.* 1973). The polymerase fragment liberated from the enzyme by subtilisin proteolysis is normal (HEYNEKER and KLENOW 1975).

polA214: A missense mutation isolated by KINGSBURY and HELINSKI (1973a) *via a* complex two-step mutagenesis and enrichment procedure. Originally characterized as temperature sensitive for the replication of ColEl-type plasmids (KINGSBURY and HELINSKI 1973b), *E. coli* strains containing this mutation

are actually temperature-sensitive conditional lethals for colony formation (KELLEY, REEHL and LEDONNE 1978). The purified polymerase from this strain is extremely labile to endogenous proteases and has a defective 5′ → 3′ exonuclease (KELLEY, REEHL and LEDONNE 1978; LEDONNE and KELLEY, unpublished).

*polA480*ex: A missense mutation isolated by KONRAD and LEHMAN (1974) and characterized as imparting "hyper-rec" phenotype. This mutation was originally designated as *polAex1*, but has been renumbered according to the nomenclature convention of BACHMANN (B. BACHMANN, personal communication). Cells carrying the *polA480*ex allele are deficient in colony formation on rich medium at elevated temperatures (KONRAD and LEHMAN 1974). The purified DNA polymerase I is defective in 5′ → 3′ exonuclease activity at 30° and 43° and loses the ability to carry out coordinated "nick translation" type of DNA synthesis at 43° (UYEMURA, EICHLER and LEHMAN 1976).

polA4113: A missense mutation isolated by OLIVERA and BONHOEFFER (1974). Like *polA214* and *polA480*ex, this mutation confers a temperature-sensitive phenotype on *E. coli*. The polymerase may be temperature-sensitive in *in vitro* assays. However, the proteolytic large fragment appears to have wild-type enzymatic activity when isolated (LUNDQUIST and OLIVERA, personal communication). The mutational defect apparently lies within the 5′ → 3′ exonuclease component of the enzyme.

resA1: A nonsense mutation isolated in *E. coli* B by KATO and KONDO (1970). This allele has been particularly useful to us in experiments with integration proficient *polA* transducing phages since *resA1 E. coli* strains are clearly Pol$^-$ at temperatures between 32° and 42° and do not spawn Pol$^+$ revertant papillae as frequently as *polA1* cell lines (REEHL and KELLEY, unpublished). The *resA1* phenotype is suppressed by *supD*, *supE* and *supF* and cells carrying the non-suppressed mutation are fully viable in all plating tests we have performed on nutrient and minimal medium at temperatures of 25°–42° (COHEN and KELLEY, unpublished).

MATERIALS AND METHODS

Bacteria were grown in L broth (LENNOX 1955) and plated on solid medium containing L broth or M9 salts as previously described (GRINDLEY and KELLEY 1976). When *glnA*$^-$ strains were propagated on minimal plates, the medium was supplemented with filter-sterilized L-glutamine (Sigma Chemicals) at a final concentration of 2 mg/ml.

Bacteriophage stocks: P1vir and P1CMclr100 were obtained from J. L. ROSNER and were used for strain constructions and crosses of *polA* alleles as indicated in the text. P1CMclr100 lysogens were isolated by the methods described by ROSNER (1972). P1vir stocks were prepared from the indicated bacterial strains by infecting log-phase cultures of the cells at an M.O.I. of approximately 0.1 to 0.2, followed by propagation on fresh L broth plates. All nutrient media used for phage growth and transductions contained 0.01 M $CaCl_2$.

Bacterial strains: The primary bacterial strains are described in Table 1a. Utilizing bacteriophage P1vir, a series of *polA*$^-$ *glnA202* stocks were constructed in the JG108 genetic background. Details of the linkages of the *metE*, *polA* and *glnA* markers are described in MURRAY and KELLEY (1979) for construction of *polA1-glnA202*, *polA6-glnA202* and *polA107-glnA202* double-mutant strains. The same methodology was used to construct similar strains carrying the other six *polA*$^-$ alleles used in this study; all of these final recipient strains are listed in Table 1c.

P1CMclr100 lysogens: The construction of P1CMclr100 lysogens of *polA glnA* strains has been described previously (KELLEY and GRINDLEY 1976a; KELLEY, REEHL and LEDONNE 1978). The final lysogenic derivatives that served as donor strains for recombinational crosses are listed in Table 1d. Strains intermediate in their construction have been described previously and are listed in Table 1b.

polA- *phenotype:* The strains carrying the different *polA*- mutations were obtained from the sources indicated in Table 1a-d. All alleles have the same general phenotype—sensitivity to methyl methanesulfonate (MMS)—which allows them to be scored by simple replica-plating tests on MMS-containing nutrient agar. The methyl methanesulfonate was Eastman number 6936, obtained from Fisher Scientific Company. The general test has been described previously (KELLEY and GRINDLEY 1976a). Minor variations in quantities of MMS and in temperature of incubation are necessary in scoring crosses involving the *polA107* mutation (use 0.025% MMS at 42°) and the *polA4113* mutation (use 0.020% MMS at 45°). All other tests were carried out with 0.020% MMS at 42°. No comprehensive complementation testing has been carried out, but complementation between *polA1* and a number of other alleles has been checked and no complementation was observed. The Pol- phenotype is recessive and, because of the apparent nature of the recombinational event in transductional crosses, *polA*- transductants of *polA*+ cells cannot be scored before purification by streaking. Pol+ clones arising from crosses between nonidentical *polA*- alleles are readily recognized in replica plating tests. We have found it most convenient to

TABLE 1a

Primary E. coli *K12 strains*

Designation	Genotype	Source or reference
JG108	*metE70 thyA36 deoC2 lacZ53 lacY14am rha-5 rpsL151* λ-	J. D. GROSS *via* J. WECHSLER
ET1118	*supE44 tonA2 txs68 rpsE rpsL hutC- glnA202*	B. TYLER
CA8000	Hfr Hayes, *thi-1 relA1 spoT1* λ-	J. BECKWITH *via* E. G. MINKLEY
CM1070	JG108 *metE+ polA1* via transduction	KELLEY and GRINDLEY (1976a)
JG110	*metE70 thyA36 deoC2 polA5* λ-	M. PEACEY
JG111	*metE70 thyA36 deoC2 polA6* λ-	J. D. GROSS (KELLEY and WHITFIELD 1971)
MM383	*thyA36 rha-5 lacZ53 deoC2 rpsL151 polA12* λ-	M. PEACEY (MONK and KINROSS 1972)
KMBL1789	*thyA305 argA103 bioA2 pheA97 endA101 deoB301 polA107* λ-	H. J. HEIJNEKER (GLICKMAN *et al.* 1973)
DS602	JG108 *metE+ polA214* λ-	D. SHERRATT *via* N. D. F. GRINDLEY
RS5064	W3110 *trpA33 polA480*ex λ-	I. R. LEHMAN
BT4113	*met+ thy- polA4113*	B. M. OLIVERA (OLIVERA and BONHOEFFER 1974)
KMBL1791	*thyA305 argA103 bioA2 pheA97 endA101 deoB301 resA1* λ-	H. L. HEIJNEKER (GLICKMAN 1974)
CM3225	P1CMclr100 lysogen of RC703 = F- *E. coli* wild type	KELLEY and GRINDLEY (1976a)
CM3762	JG108 *rha+* λ-	CM3225 × JG108

The *E. coli* strains representing the ultimate sources of the transduction donor and recipient strains are listed above.

TABLE 1b

Intermediate E. coli *strains*

Designation	Relevant genes	Construction
CM3574	JG108 *metE*+ *polA1 glnA*+ *rha*+	Met+ transductant of CM1070 with CM3225
CM4050	JG108 *metE*+ *polA5 glnA*+ *rha*+	*polA5* allele *ex* JG110 into CM3762
CM3572	JG108 *metE*+ *polA6 glnA*+ *rha*+	*polA6* allele *ex* JG111 into JG108
CM3593	JG108 *metE*+ *polA6 glnA*+ *rha*-	*polA6* allele *ex* JG111 into JG108
CM3857	JG108 *metE*+ *polA12 glnA*+ *rha*+	*polA12* allele *ex* MM383 into JG108
CM3819	JG108 *metE*+ *polA107 glnA*+ *rha*-	*polA107* allele *ex* KMBL1789 into JG108
CM3842	JG108 *metE*+ *polA107 glnA*+ *rha*+	*polA107* allele *ex* KMBL1789 into CM3762
CM3665	JG108 *metE*+ *polA214 glnA*+ *rha*+	*polA214* allele *ex* DS602 into CM3762
CM4076	JG108 *metE*+ *polA480*ex *rha*+	*polA480*ex allele *ex* RS5064 into CM3762
CM3860	JG108 *metE*+ *polA4113 rha*+	*polA4113* allele *ex* BT4113 into JG108
CM4277	JG108 *metE*+ *resA1 rha*+	*resA1* allele *ex* KMBL1791 into JG108

The various *polA*- alleles were transduced from the original strains into the JG108 genetic background utilizing phage P1CMclr100 induced from lysogens of the primary strains. Transductions were either into CM1062 with Met+ or Rha+ selection followed by a second transduction of Rha+ or Met+ from CM3225 or directly into CM3762 with Met+ selection. For the sake of brevity, the designations of the lysogenic intermediates are omitted here. Strain construction details for the *polA1*, *polA6* and *polA107* derivatives have been detailed in KELLEY and GRINDLEY (1976a).

TABLE 1c

glnA202 *Transduction recipient strains*

Designation	Relevant genes	Construction
CM5294	*metE70 polA*+ *glnA202 rha*+	MURRAY and KELLEY (1979)
CM5500	*metE*+*polA*+ *glnA202*	P1vir *ex* CA8000 × CM5294
CM5321	*metE*+ *polA1 glnA202*	P1vir *ex* CM1070 × CM5294
CM5587	*metE*+ *polA5 glnA202*	P1vir *ex* CM4050 × CM5294
CM5307	*metE*+ *polA6 glnA202*	P1vir *ex* CM3593 × CM5294
CM5580	*metE*+ *polA12 glnA202*	P1vir *ex* CM3857 × CM5294
CM5309	*metE*+ *polA107 glnA202*	P1vir *ex* CM3819 × CM5294
CM5538	*metE*+ *polA214 glnA202*	P1vir *ex* CM3665 × CM5294
CM5532	*metE*+ *polA480*ex *glnA202*	P1vir *ex* RS5064 × CM5294
CM5541	*metE*+ *polA4113 glnA202*	P1vir *ex* CM3860 × CM5294
CM5589	*metE*+ *resA1 glnA202*	P1vir *ex* KMBL1792 × CM5294

Strains used as recipients in the mapping experiments are listed here. Description of the strain construction techniques are found in MURRAY and KELLEY (1979).

TABLE 1d

P1CM1r100 lysogens of glnA+ polA- *donor strains*

Designation	Relevant genes	Parent nonlysogen
CM3579	JG108 *glnA+ polA1*	CM3574
CM4414	JG108 *glnA+ polA5*	CM4050
CM3577	JG108 *glnA+ polA6*	CM3572
CM3895	JG108 *glnA+ polA12*	CM3857
CM3864	JG108 *glnA+ polA107*	CM3842
CM3667	JG108 *glnA+ polA214*	CM3665
CM4089	JG108 *glnA+ polA480ex*	CM4076
CM3896	JG108 *glnA+ polA4113*	CM3860
CM4294	JG108 *glnA+ resA1*	CM4277

Phage P1CMclr100 lysogens were derived from the relevant strains in Table 1b via the methods of ROSNER (1971).

do this *via* gridded master plates, which makes recognition of Pol+ patches easier, especially if there are substantial variations in colony size on the original transductional selective plate.

RESULTS

The experiments described below are a series of analyses of the behavior of nine different allelic mutations of the *polA* gene in crosses between different *E. coli* genotypes carried out by generalized transduction with P1 phages. Although the use of P1 phages in such experiments has long been recognized (LENNOX 1955), very little is known about the actual molecular mechanism by which the transducing fragment recombines with the *E. coli* chromosome. Recent experiments of SANDRI (personal communication) have indicated that the fragment can persist in the *E. coli* cytoplasm as a physical entity for considerable periods of time after phage infection and transfer. DNA polymerase I is an enzyme involved in DNA metabolism, and a number of *polA*$^-$ alleles have been described as "hyper-rec" (KONRAD and LEHMAN 1974; KONRAD 1977). Therefore, genetic experiments designed to study recombinational events within the *polA* cistron could be subject to a number of artifacts resulting from abortive transductional events. Because of these possibilities, a thorough study of the transductional system was carried out as a background to constructing an intracistronic linkage map of these *polA*$^-$ alleles.

Co-transductional linkage of polA *and* metE: The initial genetic characterizations of GROSS and GROSS (1969) relied on the linkage of *polA* to the *metE* gene; more recently, we were able to utilize this linkage for limited fine-structure analysis of *polA* (KELLEY and GRINDLEY 1976a; KELLEY, REEHL and LEDONNE 1978.). However, we have always observed that co-inheritance of *polA*$^-$ alleles with the selected *metE*$^+$ marker varies considerably from allele to allele (Table 2). In this experiment, transduction of each of the nine *polA*$^-$ markers with phage P1CMclr100 produced Met$^+$ pol$^-$ clones at frequencies of 3 to 18%. These values are considerably lower than some previously reported for *polA*$^-$ alleles (*e.g.*, GLICKMAN *et al.* 1973; HOURS and DENHARDT 1979). Although the

TABLE 2

Co-transduction of polA⁻ *alleles with* metE

Donor	Recipient	Met⁺ transductants: PolA⁺ Rha⁻	PolA⁻ Rha⁻	PolA⁻ Rha⁺	(Mixed for PolA) PolA⁺/⁻ Rha⁻	(Mixed for Rha) PolA⁻ Rha⁺/⁻	Percent *metE polA* co-transduction
CM3579 = *metE+ polA1 rha+*	× JG108 = *metE70 polA+ rha5*	90	7	0	3	0	10%
CM4414 = *metE+ polA5 rha+*	× JG108 = *metE70 polA+ rha5*	92	6	0	2	0	8%
CM3577 = *metE+ polA6 rha+*	× JG108 = *metE70 polA+ rha5*	90	10	0	0	0	10%
CM3895 = *metE+ polA12 rha+*	× JG108 = *metE70 polA+ rha5*	91	3	0	0	1	4%
CM3864 = *metE+ polA107 rha+*	× JG108 = *metE70 polA+ rha5*	94	1	1	1	0	3%
CM3667 = *metE+ polA214 rha+*	× JG108 = *metE70 polA+ rha5*	82	16	1	1	0	18%
CM4089 = *metE+ polA480ex rha+*	× JG108 = *metE70 polA+ rha5*	92	6	0	2	0	8%
CM3896 = *metE+ polA4113 rha+*	× JG108 = *metE70 polA+ rha5*	85	12	1	2	0	15%
CM4294 = *metE+ resA1 rha+*	× JG108 = *metE70 polA+ rha5*	96	3	0	1	0	4%

Crosses between P1CMclr100 lysates of *metE+ polA⁻ rha+ E. coli* lysogens and the *metE70 polA+ rha5 E. coli* strain JG108 were carried out as described in MATERIALS AND METHODS. The transductants were selected by plating on minimal medium lacking methionine at 37°. One hundred Met⁺ transductant clones from each cross were picked and streaked on the same selective medium. Two subclones of each were then picked and transferred to a master nutrient plate, allowed to grow overnight and then replica-plated onto minimal medium, nutrient and nutrient plus methyl methanesulfonate and scored for inheritance of nonselected markers. Segregation of a *metE+* transductant clone to yield one *polA+* and one *polA⁻* subclone or one *rha+* and one *rha⁻* subclone was observed in several cases. These segregating clones were scored as under the column heading of "mixed" in the table, but are included in the *metE+ polA⁻* class for the purpose of calculating the frequency of co-transduction of *polA⁻* with *metE*.

absolute values might reflect differences in recipient strains between these experiments and those of others, the linkage variations among the various alleles is disturbing since many investigators have relied on this criterion for genetic authentication and characterization of *polA⁻* mutations.

Segregation of the unselected polA⁻ *marker:* A further concern is the observation that, in such a cross, considerable segregation takes place among the unselected markers. This is apparent when Met⁺ transductant clones are purified and two daughters of each clone are scored for inheritance of the unselected *polA⁻* and *rha+* markers (Table 2, right hand columns). Among siblings arising from a single Met⁺ transductant, segregation yielding Pol⁺ and Pol⁻ or Rha⁺ and Rha⁻ daughters was frequently observed. Segregation behavior was further demonstrated when each of the 76 pairs of Pol⁻ subclones was passed through a second cycle of streaking and testing (Table 3). In the second cycle, ten colonies

TABLE 3

Segregation behavior of Met+ Pol+ transductants

Donor	Recipient	First Pol- subclones	Second set of subclones	Third set of subclones
CM3895 = *metE+ polA12 rha+*	JG108 = *metE70 polA+ rha-5*	2a = Met+ Pol-	9 Met+ Pol+ 1 Met+ Pol-	n.d.*
		2b = Met+ Pol-	4 Met+ Pol+ 6 Met+ Pol-	n.d.
CM3896 = *metE+ polA4113 rha+*	JG108 = *metE70 polA+ rha-5*	88a = Met+ Pol-	10 Met+ Pol-	n.d.
		88b = Met+ Pol+	9 Met+ Pol+ 1 Met+ Pol-	n.d.
CM4089 = *metE+ polA480*ex *rha+*	JG108 = *metE70 polA+ rha-5*	47a = Met+ Pol-	1 Met+ Pol+	Gave 10 Met+ Pol+.
			9 Met+ Pol-	Each gave 10 Met+ Pol- subclones.
		47b = Met+ Pol-	10 Met+ Pol-	n.d.

Each of the two subclones of the 76 original transductant clones that had been scored as Met+ Pol- or as Met+ and mixed for Pol+ and Pol- in Table 2 were restreaked on selective medium at 37°. Ten daughter clones (second subclones) of each were picked, transferred to master nutrient plates, grown up and replica-plated as described in Table 2. Of these 76 pairs of subcloned lines, three pairs showed further segregation of the Pol+ and Pol- phenotype among their daughters as displayed here. One set of ten sub-subclones was restreaked and retested a third time as indicated.

* n.d. = not determined.

of each were tested, and further segregation of the unselected Pol phenotype was observed in three of the daughter lines (two of which had appeared stable on the first cycle). Only one of these sets was passed through a third cycle of streaking and testing and no further segregation was observed.

This experiment raised two obvious possibilities: (1) that the phenomenon was due to lysogenization by the phage; (2) that the phenomenon was due in some way to the nature of the incoming *polA*$^-$ marker, resulting in some subtle counter-selection of hyper-recombinational event. To guard against the possibility of lysogenization artifacts, many experiments have been conducted with the nonlysogenic phage Plvir (SCOTT 1968), and tests of the ability of the transductants to restrict growth of phage lambda have been conducted. The results of some of these experiments are detailed below and show no evidence supporting lysogeny as the cause of this segregation or abortive transduction. Artifacts of selection for or against *polA*$^-$ alleles are difficult to exclude categorically, but the individual Pol$^+$ and Pol$^-$ segregants appear to grow equally well on selective plates. I have observed similar segregation with markers other than *polA*$^-$ alleles. I also have been able to conduct Met$^-$ Pol$^-$ homogenotes by carrying out such a transduction (under Met$^+$ selective conditions) and purifying transductant clones on nutrient rather than selective medium (data not shown). This type of phenomenon has occasionally been reported in the past with other transductional studies (*e.g.*, LENNOX 1955; BLUMENTHAL 1972; STODOLSKY, RAE and MULLENBACH 1972) and seems to be inherent in phage P1 transductions. Experiments by R. SANDRI (personal communication) have provided additional biophysical evidence that P1 transducing DNA can persist within the recipient cell in an unintegrated form following the transductional event. Presumably my observations reflect the persistence of such an unintegrated fragment. Believing that such segregation phenomena are normal to this system, I proceeded with the fine structure analysis described below.

Co-transduction of PolA *and* glnA: The *glnA* gene is more closely linked to *polA* than is *metE*, lying between *polA* and *rha* and has been utilized for the construction of lambda *polAglnA* specialized transducing phages (KELLEY, CHALMERS and MURRAY 1977; MURRAY and KELLEY 1979). The *glnA202* allele is a nonreverting mutation obtained from B. TYLER Details of the linkage of *metE*, *polA* and *glnA* are described in MURRAY and KELLEY (1979).

To test the *glnA-polA* linkage in phage P1-mediated generalized transduction, I carried out a series of transductions in which the nine different *polA*$^-$ alleles were transduced into the *metE*$^+$ *polA*$^+$ *glnA202* derivative of JG108, CM5500. Recipient cells were grown at 37° and infected with the same P1CMclr100 phage stocks utilized in the Met$^+$ transduction experiments described above. These infected cells were then spread on selective plates at both 32° and 37° and allowed to grow. Gln$^+$ transductant clones were picked, purified by streaking at the respective temperatures and scored by replica-plating as before. The results (Table 4) show that co-transduction of *polA* and *glnA* is much more frequent than with *metE* (52–80% *vs.* 3–18%), that the co-transduction of *polA*$^-$ alleles with *glnA* is essentially equal for each of the nine alleles tested, that

TABLE 4

Co-transduction of polA⁻ *alleles with* glnA

Donor	Recipient	Temp.	*glnA*+ Transductants GlnA+ PolA+	GlnA+ PolA⁻	(Mixed) GlnA+ PolA+/-	Percent *glnA polA* co-transduction
CM3579 =	× CM5500 =	32°	48	50	2	52%
polA1 glnA+	*polA*+ *glnA202*	37°	37	56	7	63%
CM4414 =	× CM5500 =	32°	33	63	4	67%
polA5 glnA+	*polA*+ *glnA202*	37°	33	63	4	67%
CM3577 =	× CM5500 =	32°	22	70	8	78%
polA6 glnA+	*polA*+ *glnA202*	37°	27	71	2	73%
CM3895 =	× CM5500 =	32°	n.d.*	n.d.	n.d.	n.d.
polA107 glnA+	*polA*+ *glnA202*	37°	28	65	7	72%
CM3864 =	× CM5500 =	32°	40	55	4	60%
polA107 glnA+	*polA*+ *glnA207*	37°	40	53	7	60%
CM3667 =	× CM5500 =	32°	21	72	7	79%
polA214 glnA+	*polA*+ *glnA202*	37°	20	76	4	80%
CM4089 =	× CM5500 =	32°	31	62	4	67%
*polA480*ex *glnA*+	*polA*+ *glnA202*	37°	31	72	2	71%
CM3896 =	× CM5500 =	32°	40	57	3	60%
polA4113 glnA+	*polA*+ *glnA202*	37°	25	73	2	75%
CM4294 =	× CM5500 =	32°	48	51	1	52%
resA1 glnA+	*polA*+ *glnA202*	37°	43	53	4	57%

Crosses between P1CMclr100 lysates of *polA glnA*+ *E. coli* lysogens and *polA*+ *glnA202* *E. coli* strain CM5500 were carried out as described in MATERIALS AND METHODS. The transductants were selected by plating on minimal medium lacking glutamine at 32° and at 37°. One hundred GlnA+ transductant clones from each cross at each temperature were picked and streaked on the same selective medium at the respective temperature. Two subclones of each were then picked and transferred to a master nutrient patch plate, allowed to grow overnight at 37°, and then replicated onto minimal medium, nutrient and nutrient plus methyl methanesulfonate and scored for inheritance of nonselected markers. Segregation of a *glnA*+ transductant clone to yield one *glnA*+ *polA*+ and one *glnA*+ *polA*⁻ subclone was observed in each cross. These segregating clones are scored as "mixed" in the table, but are included with the *glnA*+ *polA*⁻ class in calculating the frequency of cotransduction of *polA*⁻ with *glnA*.

* n.d. = not determined.

co-transduction occurs as well or better at 37° than at 32° and that some segregation of unselected markers also occurs in this experiment.

Each of these conclusions is interesting, albeit for different reasons. Generally speaking, the practical implication is that intragenic crosses of the type previously carried out utilizing *metE* co-transduction (KELLEY and GRINDLEY 1976a; KELLEY, REEHL and LEDONNE 1978) can now be carried out much more readily since the linkage to *glnA* is much higher and fewer transductants need be scored. With the closely linked *glnA* marker, one can be sure that each *polA*⁻ allele is being brought into the recipient bacterial genome at approximately the same frequency. And, even though three of the alleles (*polA214*, *polA480*ex and *polA4113*) have been described as temperature-sensitive conditional lethals, there does not appear to be any selection against inheritance of *polA*⁻ at 37° as opposed to 32°. Finally, the segregation frequency of Pol+ and Pol⁻ clones is somewhat higher in this experiment than in the *metE* transduction (1–8% *vs.* 0–3%), but

not significantly so. This implies that the segregation phenomenon is normal and not one that is closely linked to the *polA*⁻ locus in particular, nor is it likely to vary substantially over the length of the P1 transducing fragment.

Construction of polA⁻ glnA202 *recipient strains:* A series of *polA*⁻ *glnA202* strains was constructed *via* co-transduction of *metE*+ and each individual *polA*⁻ allele into the *metE70 polA*+ *gln202* strain, CM5294. All transductions for strain construction were carried out using phage P1vir to minimize any possibility of the formation of lysogens. Individual transductant clones were streaked on selective agar and two subclones of each were picked and tested by replica plating. As with the P1CMclr100 transductions, these subclones showed segregation of the *polA*⁻ markers, the *glnA* markers and occasionally both *glnA* and *polA* (see MURRAY and KELLEY 1979, Table 2b). Each Met+ Pol⁻ Gln⁻ clone was carried through two further cycles of subcloning and testing before being given a strain number designation and utilized as a transduction recipient. No further segregation of either *polA* or *glnA* occurred after the first subcloning or when each strain was carefully tested for both the *polA*⁻ and *glnA*⁻ characters during use as a recipient in each subsequent transduction (see below).

Verification of genetic linkages in polA⁻ glnA202 *strains by backcrossing:* To verify the genetic constitution of the *polA*⁻ *glnA202* strains, each was crossed to *polA*+ *glnA*+ as shown in Table 5. In this experiment, the *glnA*+ *polA*+ region was transduced *via* P1vir grown on wild-type *E. coli* of a pedigree different from that of JG108 genetic background. In this cross, the *glnA polA* co-trans-

TABLE 5

Testing of polA glnA *linkage in recipient strains by backcrosses*

Donor	Recipient	GlnA+ Transductants: GlnA+ PolA+	GlnA+ PolA⁻	GlnA+ (Mixed for Pol) PolA+/⁻	Percent *glnA polA* co-transduction
CK8000 = prototroph ×	CM5321 = *polA1 glnA202*	63	34	3	66%
CK8000 = prototroph ×	CM5587 = *polA5 glnA202*	61	35	3	65%
CK8000 = prototroph ×	CM5307 = *polA6 glnA202*	70	29	1	71%
CK8000 = prototroph ×	CM5580 = *polA12 glnA202*	65	31	4	69%
CK8000 = prototroph ×	CM5309 = *polA107 glnA202*	65	32	3	68%
CK8000 = prototroph ×	CM5538 = *polA214 glnA202*	70	27	3	73%
CK8000 = prototroph ×	CM5532 = *polA480*ex *glnA202*	60	35	5	65%
CK8000 = prototroph ×	CM5541 = *polA4113 glnA202*	59	36	5	64%
CK8000 = prototroph ×	CM5589 = *resA1 glnA202*	75	17	3	82%

Crosses between P1vir lysates of the prototrophic strain CK8000 = HfrHayes and each of the *polA*⁻ *glnA202* recipient strains were carried out as described in MATERIALS AND METHODS. The transductants were selected by plating on minimal medium lacking glutamine at 37°. One hundred GlnA+ transductant clones from each were picked and streaked on the same selective medium. Two subclones of each were then picked and transferred to a master nutrient patch plate, allowed to grow overnight at 37°, and then replica-plated onto minimal medium, nutrient and nutrient plus methyl methanesulfonate and scored for inheritance of the nonselected *polA*+ marker. Segregation of GlnA+ transductant clones to yield one GlnA+ PolA+ and one GlnA+ PolA⁻ subclone was observed in each cross. These segregating clones are scored as "mixed" in the Table, but are included with the GlnA+ PolA+ class in calculating the frequency of co-transduction of *polA*+ with *glnA*+.

ductional linkage was once again shown to be high (64–82%) at 37° and about the same number of mixed Pol+ and Pol- clones appeared (1–5%), indicating that there are no *cis-trans* differences between *glnA–polA* crosses dependent on donor and recipient strains (Tables 4 and 5). This is interesting because, in previous experiments with the *metE70* marker, we had noted differences in *metE–polA* linkages depending on the marker and on the direction in which the experiment was carried out (Table 2 above; KELLEY and GRINDLEY 1976a; KELLEY, REEHL and LEDONNE 1978).

Self-transduction: Self-transduction or "selfing" (DEMEREC 1963), *i.e.*, reversion of a mutation *via* crossing into itself, was occasionally observed in earlier *metE polA* crosses, although at a very low frequency. Similar tests were conducted with the *polA- glnA202* recipient strains, but no "selfing" was observed among the nine strains in a total of 104 transductants scored (Table 6).

Segregation in polA- *interallelic transductional crosses:* Although we have no precise molecular mechanism to explain the abortive transductions or segregation events observed above, the most reasonable suggestion would seem to be that the transducing fragment persists within the cell in some form and can be expressed while undergoing recombinational events. If this residence time is long, an extended period of partial diploidy should result. If *polA-* alleles complemented each other, such a partial diploid might appear as a Pol+ clone in the absence of recombination. In complementation studies with F′ merodiploid *E. coli* strains, no such *in vivo* complementation has been observed among the alleles tested (PEACEY and GROSS, personal communication; GLICKMAN *et al.* 1973). Alternatively, if two mutant enzyme molecules could associate to form an active dimer, one might expect interallelic complementation to take place. However, DNA polymerase I is active as a single subunit enzyme and not known to associate or form enzymatically active aggregates *in vitro*. Whatever the mechanism, the effects of this phenomenon or recombination are not obviously incompatible with the use of P1 transduction for genetic analysis. In our previous studies (KELLEY

TABLE 6

Tests for self-transduction

CM3579 = *polA1 glnA+*	× CM5321 = *polA1 glnA202*	0/1435 < 0.07%
CM4414 = *polA5 glnA+*	× CM5587 = *polA5 glnA202*	0/1000 < 0.10%
CM3577 = *polA6 glnA+*	× CM5307 = *polA6 glnA202*	0/970 < 0.10%
CM3895 = *polA12 glnA+*	× CM5580 = *polA12 glnA202*	0/1000 < 0.10%
CM3864 = *polA107 glnA+*	× CM5309 = *polA107 glnA202*	0/1097 < 0.09%
CM3667 = *polA214 glnA+*	× CM5538 = *polA214 glnA202*	0/996 < 0.10%
CM4089 = *polA480*ex *glnA+*	× CM5532 = *polA480*ex *glnA202*	0/679 < 0.15%
CM3896 = *polA4113 glnA+*	× CM5541 = *polA4113 glnA202*	0/1000 < 0.10%
CM4294 = *resA1 glnA+*	× CM5589 = *resA1 glnA202*	0/1000 < 0.10%

Crosses between P1CMc1r100 lysates of *polA glnA+ E. coli* lysogens and *polA- glnA202 E. coli* recipients were carried out at 37°, as described in MATERIALS AND METHODS. Individual colonies were picked onto nutrient master plates, incubated at 37° overnight and replica-plated onto minimal, nutrient and nutrient plus methyl methanesulfonate. No Pol+ clones were detected in any of these crosses.

and GRINDLEY 1976a; KELLEY, REEHL and LEDONNE 1978) we noted segregation among the recombinants and yet were able to obtain an unambiguous gene orientation that has been verified by alternative genetic tests (MURRAY and KELLEY 1979; WARD 1979). Presumably, an extended intracellular life time for the transducing fragment merely extends the period of time during which genetic recombination can occur. Such an effect would not invalidate the results, as long as the event was not spontaneously reversible.

In order to examine segregation during intragenic recombination, the experiment described in Table 7 was carried out. The cross chosen for testing was *polA1 glnA*$^+$ × *polA6 glnA202*, which previous studies had indicated should give positive" results since a wild-type *polA* gene could be generated *via* a two-crossover event. Furthermore, the two allelic mutations had been shown *not* to complement in diploids (KELLEY and WHITFIELD 1971), and the mutant gene products have been characterized and are not expected to complement each other's enzymatic defects (LEHMAN and CHIEN 1973; KELLEY and GRINDLEY 1976b).

These data show that of 10^8 independent transductant clones, 10 produced Pol$^+$ daughters when restreaked on selective medium. Six of these appeared homogeneously Pol$^+$ (all 10 daughters were Pol$^+$) and were not examined further. The remaining four showed segregation into various mixtures of Pol$^+$ and Pol$^-$ daughters, and each daughter was restreaked on selective medium. At that stage, three clones had produced homogeneous daughter lines and were not examined further. One segregated at this stage, and its mixed daughter clones were followed through another cycle (its daughters, which were not mixed, were not examined further). Segregation was complete by the third cycle of restreaking, and each subclone now appeared to cast off homogeneous sibs. Thus, as indicated in Table 3, formation of stable transductants and intragenic recombinants appears to occur during an extended period of time after the initial transductional event.

Two conclusions seem reasonable. First, since the phenomenon occurs with the nonlysogenic P1vir and above the normal lysogenization temperature of P1CMclr100, it does not appear to be due to lysogenization by the phage *via* any obvious "classic" mechanism. Second, if the clones are followed through several cycles of restreaking, they eventually breed true. Assuming that it takes twenty generations to produce a visible colony for manipulation and testing, however, the implication is that the transducing fragment does exist in the cell for at least forty generations. Still, whatever the mechanism, recombinational studies utilizing this system are clearly feasible.

Three-factor glnA polA *intra-allelic crosses:* The 36 possible permutations of pairwise crosses between the nine *polA*$^-$ alleles and *glnA202* were carried out and are summarized in Tables 8 and 9. From each set of data, a probable map order could usually be assigned. The rationale used is exactly that explained in KELLEY and GRINDLEY (1976a), i.e., that two crossover events are more likely than four. Pairs of alleles giving recombinational frequencies differing by less than a factor of two are enclosed in parentheses. Pairs of crosses that gave recombinational values of less than 0.15% are bracketed.

TABLE 7

Segregation patterns of Gln+ Pol+ recombinants in a P1vir Transductional cross of polA1 glnA+ × polA6 glnA202

(1) First test: Potential Gln+ Pol+ transductant clones streaked for single colonies			(2) Second test: Segregant subclones of initial GlnA+ clones C, D, E and K restreaked		(3) Third test: Segregant subclones of GlnA+ subclones from E1 through E9 restreaked	
Initial GlnA+ clone from master plate	Purified GlnA+ primary subclones		GlnA+ subclone from (1)	GlnA+ secondary subclones	GlnA+ subclone from (2)	GlnA+ tertiary subclones
	Pol+	Pol-				
A	10	0	C1 through C8 = GlnA+ Pol+	Each gives 10 GlnA+ Pol+ subclones	E1a = GlnA+ Pol+	Gives 10 GlnA+ Pol+ subclones
B	10	0	C9, C10 = GlnA+ Pol-	Each gives 10 GlnA+ Pol- subclones	E1b through E1j = GlnA+ Pol-	Each gives 10 GlnA+ Pol- subclones
C	8	2	D1 through D3 = GlnA+ Pol-	Each gives 10 GlnA+ Pol- subclones		
D	3	7			E2a through E2i = GlnA+ Pol+	Each gives 10 GlnA+ Pol+ subclones
E	1	8	D4 through D10 = GlnA+ Pol-	Each gives 10 GlnA+ Pol- subclones		
F	10	0	E1 = GlnA+ Pol+	Gives 1 GlnA+ Pol+ and 9 GlnA+ Pol- subclones	E2j = GlnA+ Pol-	Gives 10 GlnA+ Pol- subclones
G	10	0				
H	0	10				
I	10	0	E2 = GlnA+ Pol+	Gives 9 GlnA+ Pol+ and 1 GlnA+ Pol- subclones		
J	10	0				
K	6	4	E3 through E9 = GlnA+ Pol-	Each gives 10 GlnA+ Pol- subclones		

TABLE 7—Continued

(1) First test: Potential Gln+ Pol+ transductant clones streaked for single colonies		(2) Second test: Segregant subclones of initial GlnA+ clones C, D, E and K restreaked		(3) Third test: Segregant subclones of GlnA+ subclones from E1 through E9 restreaked	
Initial GlnA+ clone from master plate	Purified GlnA+ primary subclones	GlnA+ subclone from (1)	GlnA+ secondary subclones	GlnA+ subclone from (2)	GlnA+ tertiary subclones
		K1 through K6 = GlnA+ Pol+	Each gives 10 GlnA+ Pol+ subclones		
		K7 through K10	Each gives 10 GlnA+ Pol- subclones		

The transductional cross between a P1vir lysate grown on the *polA1 glnA*+ strain CM3574 and the *polA6 glnA202* strain CM5307 was carried out as described in MATERIALS AND METHODS. The transductants were selected by plating on minimal medium lacking glutamine; following outgrowth, individual clones were picked and transferred to a master patch plate, allowed to grow overnight at 37° and then replicated onto minimal medium, nutrient and nutrient plus methyl methanesulfonate. Of one thousand patches tested, eleven appeared Pol+. Each of these was retested for Pol+ phenotype through cycles of streaking on selective plates, followed by replica-plating until segregation into Pol+ and Pol- daughter clones ceased.

(1) The original eleven clones were streaked, picked, patched, replica plated and scored. Four (C, D, E and F) gave rise to sets of ten primary subclones exhibiting both Pol+ and Pol- phenotype.

(2) Each of these four sets of primary subclones (40 total) was streaked, picked, patched, replica-plated and scored. Two (E1 and E2) gave rise to sets of ten secondary subclones exhibiting both Pol+ and Pol- phenotype.

(3) Each of these two sets of secondary subclones (20 total) was streaked, picked, patched, replica-plated and scored. No further segregation was observed.

TABLE 8

Three-factor transductional crosses between polA⁻ *alleles* — GlnA⁺ *selection*

	Donor	Recipient	*polA*⁺ Recombinants	Probable map order
1a	CM3579=*polA1*	×CM5587=*polA5*	12/997 =1.20%	*polA1 polA5 glnA*
1b	CM4414=*polA5*	×CM5321=*polA1*	3/1000=0.30%	
2a	CM3579=*polA1*	×CM5307=*polA6*	24/1446=1.66%	*polA1 polA6 glnA*
2b	CM3577=*polA6*	×CM5321=*polA1*	3/999 =0.30%	
3a	CM3579=*polA1*	×CM5580=*polA12*	12/999 =1.20%	*polA1 polA12 glnA*
3b	CM3895=*polA12*	×CM5321=*polA1*	3/1000=0.30%	
4a	CM3579=*polA1*	×CM5309=*polA107*	5/715 =0.70%	*polA107 polA1 glnA*
4b	CM3864=*polA107*	×CM5321=*polA1*	17/897 =1.90%	
5a	CM3519=*polA1*	×CM5538=*polA214*	1/997 =0.10%	*polA214 polA1 glnA*
5b	CM3667=*polA214*	×CM5321=*polA1*	3/900 =0.33%	
6a	CM3579=*polA1*	×CM5532=*polA480*ex	6/900 =0.60%	(*polA480*ex *polA1 glnA*)
6b	CM4089=*polA480*ex	×CM5321=*polA1*	9/960 =0.94%	
7a	CM3579=*polA1*	×CM5541=*polA4113*	4/991 =0.40%	*polA4113 polA1 glnA*
7b	CM3896=*polA4113*	×CM5321=*polA1*	8/999 =0.80%	
8a	CM3579=*polA1*	×CM5589=*resA1*	9/3395=0.27%	*resA1 polA1 glnA*
8b	CM4294=*resA1*	×CM5321=*polA1*	9/1650=0.54%	
9a	CM4414=*polA5*	×CM5307=*polA6*	0/1000=<0.10%	*polA6 polA5 glnA*
9b	CM3577=*polA6*	×CM5587=*polA5*	4/998 =0.40%	
10a	CM4414=*polA5*	×CM5580=*polA12*	5/1000=0.50%	*polA12 polA5 glnA*
10b	CM3895=*polA12*	×CM5587=*polA5*	18/1000=1.80%	
11a	CM4414=*polA5*	×CM5309=*polA107*	16/1000=1.60%	(*polA107 polA5 glnA*)
11b	CM3864=*polA107*	×CM5587=*polA5*	26/998 =2.61%	
12a	CM4414=*polA5*	×CM5538=*polA214*	3/999 =0.30%	*polA214 polA5 glnA*
12b	CM3667=*polA214*	×CM5587=*polA5*	13/998 =1.30%	
13a	CM4414=*polA5*	×CM5532=*polA480*ex	10/998 =1.00%	(*polA480*ex *polA5 glnA*)
13b	CM4089=*polA480*ex	×CM5587=*polA5*	19/1000=1.90%	
14a	CM4414=*polA5*	×CM5541=*polA4113*	5/1000=0.50%	*polA4113 polA5 glnA*
14b	CM3896=*polA4113*	×CM5587=*polA5*	26/996 =2.61%	
15a	CM4414=*polA5*	×CM5589=*resA1*	5/1000=0.50%	*resA1 polA5 glnA*
15b	CM4294=*resA1*	×CM5587=*polA5*	15/700 =2.10%	
16a	CM3577=*polA6*	×CM5580=*polA12*	5/1000=0.50%	(*polA12 polA6 glnA*)
16b	CM3895=*polA12*	×CM5307=*polA6*	6/816 =0.73%	
17a	CM3577=*polA6*	×CM5309=*polA107*	5/795 =0.63%	*polA107 polA6 glnA*
17b	CM3864=*polA107*	×CM5307=*polA6*	12/973 =1.29%	
18a	CM3577=*polA6*	×CM5538=*polA214*	0/995 =<0.10%	*polA214 polA6 glnA*
18b	CM3667=*polA214*	×CM5307=*polA6*	16/2071=0.77%	
19a	CM3577=*polA6*	×CM5532=*polA480*ex	9/2672=0.34%	*polA480*ex *polA6 glnA*
19b	CM4089=*polA480*ex	×CM5307=*polA6*	62/4294=1.44%	
20a	CM3577=*polA6*	×CM5541=*polA4113*	5/995 =0.50%	*polA4113 polA6 glnA*
20b	CM3896=*polA4113*	×CM5307=*polA6*	40/992 =4.03%	
21a	CM3577=*polA6*	×CM5589=*resA1*	2/1000=0.20%	*resA1 polA6 glnA*
21b	CM4294=*resA1*	×CM5307=*polA6*	12/853 =1.41%	
22a	CM3895=*polA12*	×CM5309=*polA107*	6/991 =0.61%	*polA107 polA12 glnA*
22b	CM3864=*polA107*	×CM5580=*polA12*	17/899 =1.89%	
23a	CM3895=*polA12*	×CM5538=*polA214*	2/992 =0.20%	*polA214 polA12 glnA*
23b	CM3667=*polA214*	×CM5580=*polA12*	8/998 =0.80%	
24a	CM3895=*polA12*	×CM5532=*polA480*ex	6/1000=0.60%	*polA480*ex *polA12 glnA*
24b	CM4089=*polA480*ex	×CM5580=*polA12*	14/998 =1.40%	

TABLE 8—Continued

	Donor	Recipient	*polA*+ Recombinants	Probable map order
25a	CM3895=*polA12*	×CM5541=*polA4113*	1/999 =0.10%	*polA4113 polA12 glnA*
25b	CM3896=*polA4113*	×CM5580=*polA12*	13/999 =1.30%	
26a	CM3895=*polA12*	×CM5589=*resA1*	5/998 =0.50%	*resA1 polA12 glnA*
26b	CM4294=*resA1*	×CM5580=*polA12*	21/998 =2.10%	
27a	CM3864=*polA107*	×CM5538=*polA214*	3/2389=0.13%	[*polA107 polA214 glnA*]
27b	CM3667=*polA214*	×CM5309=*polA107*	1/2609=0.04%	
28a	CM3864=*polA107*	×CM5532=*polA480*ex	7/991 =0.71%	(*polA107 polA480*ex *glnA*)
28b	CM4089=*polA480*ex	×CM5309=*polA107*	4/1054=0.38%	
29a	CM3864=*polA107*	×CM5541=*palA4113*	1/2195=0.05%	[*polA4113 polA107 glnA*]
29b	CM3898=*polA4113*	×CM5309=*polA107*	2/1599=0.13%	
30a	CM3864=*polA107*	×CM5589=*resA1*	15/1994=0.75%	(Not resolved)
30b	CM4294=*resA1*	×CM5309=*polA107*	8/1287=0.62%	
31a	CM3667=*polA214*	×CM5532=*polA480*ex	0/2597=<0.04%	[Identity?]
31b	CM4089=*polA480*ex	×CM5538=*polA214*	0/2054=<0.05%	
32a	CM3667=*polA214*	×CM5541=*polA4113*	7/2588=0.27%	(Not resolved)
32b	CM3896=*polA4113*	×CM5538=*polA214*	4/1798=0.22%	
33a	CM3667=*polA214*	×CM5589=*resA1*	9/1997=0.45%	*resA1 polA214 glnA*
33b	CM4294=*resA1*	×CM5538=*polA214*	85/2385=3.48%	
34a	CM4089=*polA480*ex	×CM5541=*polA4113*	2/1882=0.11%	*polA4113 polA480*ex *glnA*
34b	CM3896=*polA4113*	×CM5532=*polA480*ex	5/1695=0.30%	
35a	CM4089=*polA480*ex	×CM5589=*resA1*	5/1990=0.25%	*resA1 polA480*ex *glnA*
35b	CM4294=*resA1*	×CM5532=*polA480*ex	20/1859=1.07%	
36a	CM3896=*polA4113*	×CM5589=*resA1*	8/999 =0.80%	*resA1 polA4113 glnA*
36b	CM4294=*resA1*	×CM5541=*polA4113*	16/997 =1.60%	

Crosses between P1CMclr100 lysates of *polA*− *glnA*+ *E. coli* lysogens and *polA*− *glnA202* *E. coli* recipients were carried out at 37° as described in MATERIALS AND METHODS. Individual colonies were picked onto nutrient master plates, incubated at 37° overnight and replica-plated onto minimal, nutrient and nutrient plus methyl methanesulfonate. Potential Pol+ clones were then streaked on selective medium for single colonies and retested for Pol+ phenotype as described in KELLEY and GRINDLEY (1976a). Probable map orders for the pairs of alleles are indicated in the right hand column. Several pairs of crosses yielded appreciable recombinants in each configuration; these map orders are enclosed by parentheses (crosses 6, 11, 13, 16, 28, 30 and 32). Several other pairs yielded extremely few recombinants in either configuration; these map orders are enclosed in brackets (crosses 27, 29 and 31). Interpretations of these results are discussed in the text.

To make the visualization of these results simpler, a "most reasonable" gene order was assumed, and the data are tabulated in summary form as a matrix (Table 9). For the sake of clarity, this matrix includes only the data from the self-transductions that define the null values at the diagonal and the "positive" percentages derived from the raw data in Table 8. Note that values in parentheses and brackets that represent uncertain determinations fall in the upper left-hand corner of the matrix and mainly near the diagonal, as might be expected for closely linked alleles. The matrix is a representation of genetic order only. Map distances separating alleles should not be inferred from these data.

Relying on knowledge of the enzymological defects found in the mutant enzymes produced by these alleles, the gene order may be represented as in Figure 1 for correlation of the genetic data with the known molecular structure.

TABLE 9

Summary of three-factor crosses of polA⁻ *alleles — GlnA+ selection*

$polA_y$ + / $polA_x$ $glnA^-$	$polA_x$ Allele = Recipient								
	resA1	*polA107*	*polA4113*	*polA480ex*	*polA214*	*polA1*	*polA12*	*polA6*	*polA5*
resA1	<0.10%	(?)	1.60%	1.07%	3.48%	0.54%	2.10%	1.41%	2.10%
polA107		<0.09%	[?]	(0.71%)	[?]	1.90%	1.89%	1.23%	(2.61%)
polA4113			<0.10%	0.30%	(?)	0.80%	1.30%	4.30%	2.61%
polA480ex				<0.15%	[?]	(0.94%)	1.40%	1.44%	(1.90%)
polA214					<0.10%	0.33%	0.80%	0.77%	1.30%
polA1						<0.07%	1.20%	1.66%	1.20%
polA12							<0.10%	(0.73%)	1.80%
polA6								<0.10%	0.40%
polA5									<0.10%

The data of Tables 6 and 8 are presented as a matrix to emphasize the reasons for selection of the map order of the individual alleles. The diagonal values represent the results of crosses of individual alleles into themselves. Only the positive values of the interallelic crosses are represented.

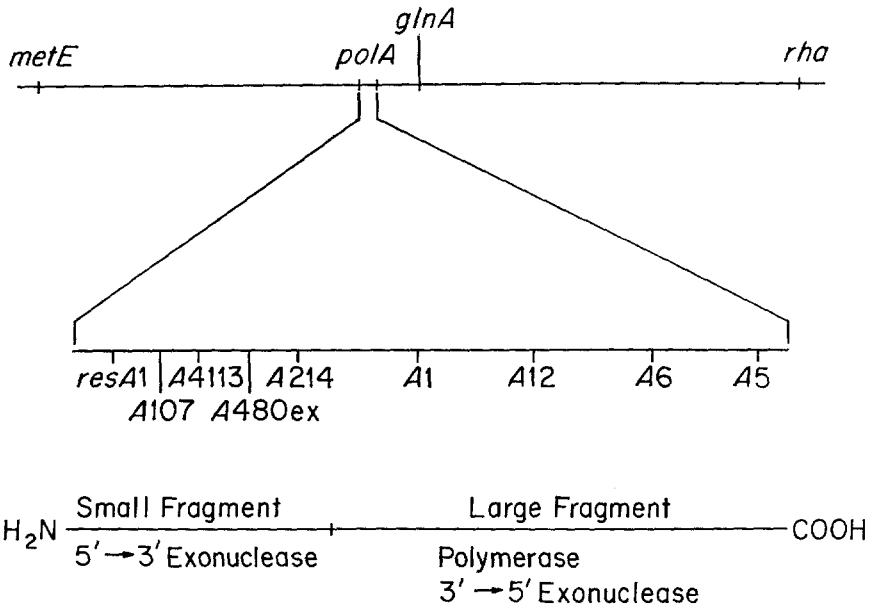

FIGURE 1.—The portion of the *E. coli* genetic map lying between 85 and 87 minutes is represented with the four loci that have been utilized for transductional mapping shown on the upper line. The region representing the *polA* cistron has been expanded and the order of the nine *polA*⁻ alleles is indicated on the middle line. This ordering may be correlated with the representation of the primary structure of DNA polymerase I at the bottom of the figure.

Map positions represent the order derived for the different allelic mutations within the small and large fragment portions of the polymerase structural gene; linear spacings are arbitrary.

DISCUSSION

These experiments were initiated in order to determine the intracistronic map order of a series of *polA*⁻ allelic mutations. The mutations chosen for analysis are those about which the greatest amount of physiological and enzymatic structural detail is known. A number of *E. coli* cistrons and operons have been analyzed by the traditional techniques of bacterial genetics—recombinational crossing *via* conjugation, transduction and partial deletion analysis. Perhaps the most classic case of this sort of fine-structure analysis is that of YANOFSKY *et al.* (1964), in which the *trpA* cistron was analyzed, in part, by co-transductional mapping with phage P1. To apply such an analysis to *polA*, I have utilized a series of co-transductional crosses of different *polA*⁻ allelic mutations *via* linkage to the *glnA202* marker.

One particular concern in such an experimental approach is the possible role of the *polA* gene product itself in the recombinational event. DNA polymerase I is an enzyme of DNA metabolism that has been shown to be capable of carrying out key steps of the excision repair processes *in vitro* by means of its nick translation reaction (KELLY *et al.* 1969). Furthermore, a number of *polA*⁻ allelic mutations have been isolated that are characterized as being "hyper-rec" due to their effects in elevating levels of intrachromosomal rearrangements in *E. coli* (KONRAD and LEHMAN 1974). In addition, there are substantial arguments that at least part of the DNA polymerase I molecule is necessary for replication of the *E. coli* chromosome (KONRAD and LEHMAN 1974; OLIVERA and BONHOEFFER 1974; LEHMAN and UYEMURA 1976). Faced with these realizations, I carried out a series of control experiments to assure that the P1 transductional system obeys traditional behavior patterns in *polA*⁻ cells.

DNA polymerase I is not known to play any significant role in the replication cycle of phage P1, although the effects of *polA*⁻ alleles have not been systematically examined (VAPNEK, personal communication). We have been able to propagate P1vir on strains carrying all of the *polA*⁻ alleles used in this study, although there have been minor variations in efficiencies of phage production. Phage P1CMclr100 lysogens can be easily constructed, and all yield uniformly high-titer lysates upon thermal induction. Thus, there seem to be no reasons for concluding that the *polA*⁻ alleles interfere with phage reproduction *per se*.

Of greater concern to this work is the capacity of these cells to integrate the P1 transducing fragment. Initially, we were concerned with the segregation of nonselected markers in our first co-transductional experiments (KELLEY and GRINDLEY 1976a). However, it is clear from the work of others that analogous phenomena are found in P1 transductional analysis of other *E. coli* genetic systems: LENNOX (1955) was the first to observe such second-generation segregation events in transductions with various metabolic markers. Later, STODOLSKY, RAE and MULLENBACK (1972) were able to demonstrate transduction and maintenance of the *proA-proB-lac* portion of *E. coli* chromosome in a host cell line with a substantial deletion. In similar experiments, BLUMENTHAL (1972) analyzed *metG* and *hisI* co-transduction of linked P2 lysogens *via* P1. The net con-

clusion of these two latter studies is that the incoming DNA fragment can create a partial heterozygote that need not recombine with the chromosome and that can exist for several generations, although it is ultimately unstable. SANDRI (personal communication) has examined the DNA of P1 transducing particles *via* density transfer experiments with purified 5-bromouracil-labeled P1. Her data provide physical evidence for persistence of the transducing DNA in an unreplicated and nonintegrated form in a population of infected cells. Whatever the mechanism of recombination and formation of abortive transductants, it appears that this phenomenon is generalizable to all P1 transductions, so that there is no reason to suspect that *polA* is particularly affected by the vagaries of the P1 system.

The physiological studies of *polA*$^-$ mutations have implied the necessity of DNA polymerase I for chromosome replication. These are further reinforced by the failure of a series of experiments designed to generate partial deletions of the *polA* gene (GROSS, personal communication). Lacking such deletions, the current analysis is confined to an examination of recombination between the various point mutations. Further experiments are underway to extend these data with partial deletions constructed in λ*polA* specialized transducing phages.

The experiments described in this paper were undertaken with the purpose of extending earlier studies of the *polA* genetic structure *via* co-transductional mapping with *metE* and *rha* (KELLEY and GRINDLEY 1976a). Since the *glnA* marker is much more closely linked to *polA* than is either of those two, more mutations could be mapped with a greater assurance and less effort. The linkage of *polA*$^-$ mutations to *metE* is variable, as shown by our data here and by the different results reported in the literature. To some extent, this phenomenon appears to be allele specific, although we have not conducted a systematic study. Linkage to *glnA* is consistently high and relatively invariant from allele to allele. Previous attempts to generate a fine-structure map of the gene had yielded recombination frequencies between *polA*$^-$ alleles of 0.01 to 0.35% in *metE* transductional crosses (KELLEY *et al.* 1978). Recombination frequencies between *polA*$^-$ alleles reported here are often greater than 1% and occasionally as high as 4% of all Gln$^+$ transductants.

In interpreting the results of individual crosses, I have followed the general rule described in KELLEY and GRINDLEY (1976a) that a stable Gln$^+$ transductant is generated by two crossovers, one on either side of *glnA*. Pol$^+$ transductants are assumed to result from crossovers within the interval separating the two *polA*$^-$ markers. Predictably, the two-crossover event diagrammed in the upper left corner of Table 9 should generate *polA*$^+$ recombinants between $polA_y$ and $polA_x$. Should the donor-recipient roles of $polA_y$ and $polA_x$ be reversed, a *polA*$^+$ recombinant could be generated only as the result of four crossovers. In the absence of high negative interference (JACOB and WOLLMAN 1961), these interpretations should be straightforward. In analyzing a system of similar linkage (*proC-lac*) by Hfr × F$^-$ crosses, BECKWITH (1970) pointed out such possibilities, however, and cautions about the interpretation of "positive" *versus* "negative" values when the differences are less than two-fold.

From an examination of Table 9 and the genetic map displayed in Figure 1, correlations between the putative gene order and information about specific *polA*$^-$ enzymological defects are obvious. Assuming that *polA1* lies at or slightly distal to the *in vitro* subtilisin cleavage point that divides the small from the large fragment, the mutations may be divided into two classes. The alleles *polA5*, *polA6* and *polA12* all produce enzymes that, when purified, have demonstrable defects in polymerization capacity but are not defective in 5′→3′ exonuclease. The mutations in *polA107*, *polA214*, *polA480*ex and *polA4113* are known to have some defects in 5′→3′ exonuclease function and appear to be closely clustered.

Recombination levels indicated by brackets represent experiments in which very little recombination was observed between the alleles in either configuration. This might be anticipated in the crosses between very closely linked adjacent markers, represented by the diagonal row of values adjacent to the null values. In these cases, 27 = *polA107* × *polA214* and 29 = *polA107* × *polA4113*, the positive values are low, although not zero (0.13% for each) and essentially zero for 31 = *polA480*ex × *polA214* (<0.05%). These spaces in the matrix are indicated by question marks. The lack of measured recombination between *polA480*ex and *polA214* is striking. This is essentially a reaction of identity. Both mutational alleles code for isolabile polypeptides. *polA480*ex and *polA214* cells are very similar in colony-forming ability at various temperatures (KELLEY *et a.* 1978) and in their effects on the stability of various DNA polymerase I-requiring plasmids (GRINDLEY and KELLEY 1976). However, minor phenotypic differences do appear in both tests, and the alleles seem to recombine with their neighbors in the matrix with slightly different frequencies. The *polA214* mutation was initially isolated following a bromouracil-enrichment regimen (KINGSBURY and HELINSKI 1973), and it is possible that this mutation could be the result of a short in-frame insertion or deletion mutation coinciding with *polA480*ex. It seems unlikely that these uncertainties will be further resolved by this type of analysis.

The positive transductional recombination values for all crosses in which the positive and negative values differ by less than two-fold have been enclosed in parentheses in the matrix. From examination of the data displayed in Table 8, it is clear that the predicted four-crossover event is usually a measurable parameter. Only two such crosses (9a = *polA5* × *polA6* and 18a = *polA6* × *polA214*) gave no recombinants. Thus, either multiple crossover events do appear to occur frequently or certain recombinations of double mutants actually result in a Pol$^+$ phenotype. Values of the pairs of crosses 30a and 30b (*polA1* × *resA1*) and 32a and 32b (*polA214* × *polA4113*) cannot be resolved and are displayed in the matrix as question marks in parentheses; other values in parentheses are shown and fit into the matrix array.

One question about the fate of the transducing fragment and its expression in abortive transductants has not been explained by these experiments. The *polA*$^+$ allele is dominant in tests with merodiploids (GROSS and GROSS 1969) and in lysogens of λ*polA*$^+$ transducing phages in *polA*$^-$ cells (MURRAY and KELLEY

1979). However, the data of Table 3 clearly indicate that Pol$^+$ clones can segregate among the subclones derived from transduction of *polA12* and *polA480*ex into *polA*$^+$ cells, although the primary transductant clones were Pol$^-$. This result has little bearing on the recombinational mapping data, but does imply that the primary transductional event and subsequent segregation of daughter cell lines may include a number of complex transcriptional and recombinational steps.

With respect to the *polA*$^-$ alleles, one important result is the establishment of the *resA1* allele as a mutation mapping in the "amino-terminal" portion of the *polA* cistron. Independent biochemical experiments (KELLEY, unpublished) indicate that this mutation results in the production of an enzymatically active amber fragment containing the 5′→ 3′ exonucleolytic capacity. This fragment is immunologically similar to that produced by the *polA1* mutation (LEHMAN and CHIEN 1973) but appears to be smaller when examined by gel filtration chromatography. Even under nonsuppressing conditions there is some "read-through" of both the *polA1* and *resA1* mutations to produce whole DNA polymerase I at about one percent of wild-type levels. Thus, the hypothesis of LEHMAN and UYEMURA (1976) that DNA polymerase I is an essential *E. coli* replication protein remains unaltered by my results.

An interesting implication of the positioning of the *resA1* mutation is that the *polA107*, *polA4113*, *polA480*ex and *polA214* alleles are positioned between it and the *polA1* allele. Since both *polA1* and *resA1* produce enzymatically active amber fragments, this implies that these four missense mutations that alter the enzyme's 5′→ 3′ exonucleolytic activity do not actually map within that enzymatic active site.

Ultimate resolution of the genetic fine structure of *polA* will rely upon DNA sequencing techniques and deletion mapping analysis utilizing the *polA* chromosomal fragment as cloned on the λ*polA* transducing phages (KELLEY, CHALMERS and MURRAY 1977; MURRAY and KELLEY 1979). We are currently pursuing both objectives.

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LITERATURE CITED

BACHMANN, B. J., K. B. LOW and A. L. TAYLOR, 1976 Recalibrated linkage map of *Escherichia coli* K12. Bac. Rev. **40**: 116–167.

BAMBARA, R. A., D. UYEMURA and I. R. LEHMAN, 1976 On the processive mechanism of *Escherichia coli* DNA polymerase I. J. Biol. Chem. **251**: 4090–4094.

BECKWITH, J. R., 1970 *lac*; The genetic system. pp. 5–26 In: *The Lactose Operon*. Edited by J. R. BECKWITH and D. ZIPSER. Cold Spring Harbor Laboratory, New York.

BLUMENTHAL, T., 1972 P1 transduction: Formation of heterogenotes upon cotransduction of bacterial genes with a P2 prophage. Virology **47**: 76–93.

DE LUCIA, P. and J. CAIRNS, 1969 Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature **224**: 1164–1166.

DEMEREC, M., 1963 Selfer mutants of *Salmonella typhimurium*. Genetics **48**: 1519–1531.

FRIEDBERG, E. C. and I. R. LEHMAN, 1974 Excision of thymine dimers by proteolytic and amber fragments of *E. coli* DNA polymerase I. Biochem. Biophys. Res. Comm. **58**: 132–139.

GLICKMAN, B. W., 1974 The role of DNA polymerase I in pyrimidine dimer excision and repair replication in *Escherichia coli* K12 following ultraviolet irradiation. Biochimica et Biophysica Acta **335**: 115–122.

GRINDLEY, N. D. F. and W. S. KELLEY, 1976 Effects of differing alleles of the *E. coli* K12 *polA* gene on the replication of non-transferring plasmids. Molec. Gen. Genet. **143**: 311–318.

GROSS, J. D. and M. M. GROSS, 1969 Genetic analysis of an *E. coli* strain with a mutation affecting DNA polymerase. Nature **224**: 1166–1168.

HEIJNEKER, H. L., D. J. ELLENS, R. H. TJEERDE, B. W. GLICKMAN, B. VAN DORP and P. H. POUWELS, 1973 A mutant of *Escherichia coli* K12 deficient in the 5′→3′ exonucleolytic activity of DNA polymerase I. II. Purification and properties of the mutant enzyme. Molec. Gen. Genet. **124**: 83–96.

HEYNEKER, H. L. and H. KLENOW, 1975 Involvement of *Escherichia coli* DNA polymerase I associated 5′→3′ exonuclease in excision-repair of U.V. damaged DNA. pp. 219–223 In: *Molecular Mechanisms for the Repair of DNA*. Edited by R. B. SETLOW and P. C. HANAWALT. Plenum Publishing Corporation, New York.

HOURS, C. and D. T. DENHARDT, 1979 "Nick Translation" in *Escherichia coli rep* strains deficient in DNA polymerase I activities. Molec. Gen. Genet. **172**: 73–80.

JACOB, F. and E. L. WOLLMAN, 1961 Chapter XIII. pp. 223–248 In: *Sexuality and the Genetics of Bacteria*. Academic Press, New York.

JACOBSEN, H., H. KLENOW and K. OVERGAARD-HANSEN, 1974 The N-terminal amino acid sequences of DNA polymerase I from *Escherichia coli* and of the large and the small fragments obtained by a limited proteolysis. Euro. J. Biochem. **45**: 623–627.

KATO, T. and S. KONDO, 1970 Genetic and molecular characteristics of X-ray-sensitive mutants of *Escherichia coli* defective in repair synthesis. J. Bact. **104**: 871–881.

KELLEY, W. S., K. CHALMERS and N. E. MURRAY, 1977 Isolation and characterization of a λ*polA* transducing phage. Proc. Natl. Acad. Sci. U.S. **74**: 5632–5636.

KELLEY, W. S. and N. D. F. GRINDLEY, 1976a Mapping of the *polA* locus of *Escherichia coli* K12: Orientation of the amino- and carboxy-termini of the cistron. Molec. Genet. **147**: 307–314. ——, 1976b *polA6*, a mutation affecting the DNA binding capacity of DNA polymerase I. Nucleic Acids Research **3**: 2971–2984.

KELLEY, W. S., J. A. REEHL and N. C. LEDONNE, 1978 Recombinational mapping of the *polA* locus of *Escherichia coli* K12: Genetic fine structure. pp. 455–466 In: *DNA Synthesis Present and Future*. Edited by I. MOLINEUX and M. KOHIYAMA. NATO Advanced Study Institute Series, Vol. A17. Plenum Publishing Corporation, New York.

KELLEY, W. S. and H. J. WHITFIELD, 1971 Purification of an altered DNA polymerase from an *E. coli* strain with a *pol* mutation. Nature (London) **230**: 33–36.

KELLY, R. B., M. R. ATKINSON, J. A. HUBERMAN and A. KORNBERG, 1969 Excision of thymine dimers and other mismatched sequences by DNA polymerase of *Escherichia coli*. Nature **224**: 495–501.

KINGSBURY, D. T. and D. R. HELINSKI, 1973a Temperature-sensitive mutants for the replication of plasmids in *Escherichia coli*. I. Isolation and specificity of host and plasmid mutations. Genetics **74**: 17–31. ——, 1973b Temperature-sensitive mutants for the replication of plasmids in *Escherichia coli*: Requirement for deoxyribonucleic acid polymerase I in the replication of the plasmid Col El. J. Bact. **114**: 1116–1124.

KONRAD, E. B., 1977 Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. J. Bact. **130**: 167–172.

KONRAD, E. B. and I. R. LEHMAN, 1974 A condition lethal mutant of *Escherichia coli* K12 defective in the 5′→3′ exonuclease associated with DNA polymerase I. Proc. Natl. Acad. Sci. U.S. **74:** 2048–2051.

KORNBERG, A., 1980 Chapter 4, pp. 101–166 In: *DNA Replication.* Freeman, San Francisco.

LEHMAN, I. R. and J. R. CHIEN, 1973 Persistance of deoxyribonucleic acid polymerase I and its 5′→3′ exonuclease activity in *polA* mutants of *Escherichia coli* K12. J. Biol. Chem. **248:** 7717–7723.

LEHMAN, I. R. and D. G. UYEMURA, 1976 DNA polymerase I: Essential replication enzyme, coordination of polymerization and 5′→3′ exonuclease is an essential feature of discontinuous DNA replication. Science **193:** 963–969.

LENNOX, E. S., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1:** 190–206.

MATSON, S. W., F. N. CAPALDO-KIMBALL and R. A. BAMBARA, 1978 On the processive mechanism of *Escherichia coli* DNA polymerase I, the *polA5* mutation. J. Biol. Chem. **253:** 7851–7856.

MONK, M. and J. KINROSS, 1972 Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bact. **109:** 971–978.

MURRAY, N. E. and W. S. KELLEY, 1979 Characterization of λ*polA* transducing phages; effective expression of the *E. coli polA* gene. Molec. Gen. Genet. **175:** 77–87.

OLIVERA, B. M. and F. BONHOEFFER, 1974 Replication of *Escherichia coli* requires DNA polymerase I. Nature **250:** 513–514.

ROSNER, J. L., 1972 Formation, induction and curing of bacteriophage P1 lysogens. Virology **48:** 679–689.

SCOTT, J. R., 1968 Genetic studies of bacteriophage P1. Virology **36:** 564–574.

STODOLSKY, M., M. E. RAE and E. MULLENBACK, 1972 The addition of Lac+ chromosome fragments to the *E. coli proA-proB-lac* deletion XIII chromosome. Genetics **70:** 495–510.

UYEMURA, D., D. C. EICHLER and I. R. LEHMAN, 1976 Biochemical characterization of mutant forms of DNA polymerase I from *Escherichia coli*. II. The *polAex1* mutation. J. Biol. Chem. **251:** 4085–4089.

UYEMURA, D. and I. R. LEHMAN, 1976 Biochemical characterization of mutant forms of DNA polymerase I from *Escherichia coli*, I. The *polA12* mutation. J. Biol. Chem. **251:** 4078–4084.

WARD, D., 1979 Fusions of *E. coli* genes *lac*, *trp* and *polA* in bacteriophage lambda. Ph.D. Thesis, Edinburgh University.

YANOFSKY, C., B. C. CARLTON, J. R. GUEST, D. R. HELINSKI and U. HENNING, 1964 On the colinearity of gene structure and protein structure. Proc. Natl. Acad. of Sci. U.S. **51:** 266–276.

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