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Construction and Characterization of *Escherichia coli polA-lacZ* Gene Fusions

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The promoter of the *polA* gene of *Escherichia coli* K-12 was fused to the *lacZ* gene by selecting deletions within a λ*lacZ polA* transducing phage. Four fusions, deleting varying amounts of the *polA* gene, were characterized. The *polA* promoter was found to be approximately 3% as active as the fully induced *lac* promoter. This figure is compatible with the normal intracellular level of deoxyribonucleic acid polymerase I. No evidence was found for autogenous regulation of transcription from the *polA* promoter. Expression from this promoter was influenced by neither *recA* nor mitomycin C, but *uvrD* and *uvrE* mutations reduced expression slightly.

Chromosome replication is vital to the life cycle of all organisms, and therefore studies of the enzymes involved in DNA replication are of particular interest. The first enzyme shown to have DNA polymerase activity was identified in *Escherichia coli* (28). Although this enzyme, now called DNA polymerase I (polI), is not the major polymerase involved in DNA replication, it nevertheless appears to play an essential role (29). Although the mechanism of action of polI has been studied biochemically in great detail (22), the mechanism of regulating expression of the polI structural gene (*polA*) remains obscure.

Genetic analysis of the *polA* gene has been hampered because the *polA* mutations isolated either are conditionally lethal or result in some residual enzyme activity. The absence of any chromosomal deletion mutations in the *polA* gene and the high reversion frequencies of many *polA* alleles have made conventional fine-structure mapping difficult (21). The recent cloning of the *polA* gene in a lambda vector (20) opens new approaches for the genetic study of this gene, particularly an investigation of its regulation.

In contrast to the *polA* gene, the regulation of expression of the *lac* operon (3) is well understood; this reflects, in part, the ease of assay of β-galactosidase in crude cell extracts. Genetic fusions, conveniently joining the *lacZ* structural gene to the regulatory elements of another operon, extend much of the experimental convenience of the β-galactosidase system to analysis of the control system to which the *lacZ* gene has been fused.

Insertion of phage Mu, and thence λp(*lac*:: Mu), into a gene provides an in vivo means for fusion of *lacZ* to the control system of the inactivated gene (5). However, this elegant approach is less readily applicable to a gene whose product is essential for cell viability. In this paper we use an alternative route dependent on the in vitro construction of a λ*lacZ polA* transducing phage and the subsequent isolation of deletion mutants in which transcription of the *lacZ* gene is dependent on the *polA* promoter.

MATERIALS AND METHODS

Bacterial and phage strains. All bacteria used (Table 1) were derivatives of *E. coli* K-12. The genotypes of the phages used, including those constructed during the course of this work, are given in Table 2. Phage JDW36 carries the *lacZ* gene fused to the distal end of the *trp* operon by deletion W205 (32). It is a derivative of JDW19 (48) in which the central part of the *trp* operon, between the *Hin*dIII targets in the *trpB* and *trpD* genes, was deleted in vitro.

Additional information concerning the construction of strains used in this study is contained in the legends to the figures and tables. All bacterial strains carrying λ*lacZ* phages were verified to be singly lysogenic by the ability of the semivirulent phage λcI *c*17 to plaque.

Media. Media were made and used as described by Murray et al. (35). Minimal liquid medium was that of Vogel and Bonner (44) with either 0.2% glucose or 0.2% glycerol as the carbon source. Casamino Acids (0.05%) was added where indicated. Thiamine (1 μg/ml) was added routinely, and other supplements were added where necessary.

General techniques. The methods for preparing phage stocks and making phage crosses have been described (37). The Lac phenotype of phage was detected by using *lacZM15* indicator bacteria and plates supplemented with 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside per ml (7). Phage purification and DNA extraction have been described (45). DNA was digested with restriction endonucleases, and the fragments were analyzed after separation by electropho-

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resis in 1% agarose gels (47). DNA fragments were joined by using T4 polynucleotide ligase and recombinant molecules recovered by transfection of strain AA125 made competent in the uptake of DNA by a slight modification of the calcium chloride procedure (27) in which the first calcium chloride treatment was omitted (46).

TABLE 1. *Bacterial strains*

Strain	Genotype	Reference
C600	*supE tonA thr leu thi lac*	1
AA125	*ΔlacZM15 trpLD102*	A. Anilonis, Ph.D. thesis, University of Edinburgh, Edinburgh, Scotland, 1977
N3098	*lig-7*(Ts) *supF*	40
C38	*ΔlacZM15* (λ*imm*^{21})	This paper
trpA88	*trpA88*	11
ED8365	*trpA23 supF tonA*	50
trpA96	*trpA96*	50
KB8	*polA1 endA thi*	10, 37
C53	*polA*$^+$ *endA thi*	This paper[a]
CM3571	*polA6 thyA lacY14*	21
CM3842	*polA107 thyA lacY14*	21
feb10	*polA10 supE tonA*	36, 51
JG108	*polA*$^+$ *thyA lacY14*	21
W3110	Prototrophic progenitor of JG108	
S159	*uvrA*	18
AB2545	HfrH *thi-1 metE46 relA1*	12
N14-4	*uvrD3 trp-56 galT23 rpsL178*	38
ES245	Hfr PO54 *relA1 ilvD188 uvrE156 thi-1*	43
uvrD	*uvrD3* HfrH *thi-1 relA1*	This paper[b]
uvrD$^+$	*uvrD*$^+$ HfrH *thi-1 relA1*	This paper[b]
uvrE	*uvrE156* HfrH *thi-1 relA1*	This paper[b]
uvrE$^+$	*uvrE*$^+$ HfrH *thi-1 relA1*	This paper[b]
QR47	*supE recA*$^+$	46
QR48	*supE recA*	46

[a] Pol$^+$ P1 transductant of strain KB8.
[b] *metE*$^+$ P1 transductant of strain AB2545.

Enzyme and chemicals. Enzymes were provided by J. Boothroyd, K. Brown, S. A. Bruce, S. G. Hughes, K. Murray, B. Sain, and P. J. Southern. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside was purchased from Bacham Inc., Marina Del Ray, Calif., and methyl methane sulfonate was purchased from Eastman Kodak Co., Rochester, N.Y. Whenever possible, chemicals used were of analytical reagent grade.

Selection of deletion mutants of λ*lac pol* phages. Deletion derivatives of lambda were selected by their increased resistance to chelating agents (39). Single plaques were each picked into 1 ml of phage buffer from which a 0.1-ml sample was preadsorbed to 0.2 ml of C600 plating cells before plating on Trypticase agar (BBL Microbiology Systems, Cockeysville, Md.) containing sodium pyrophosphate (pH 7.0). For phage D48, whose genome size is 97% wild type, a concentration of 7 to 7.5 mM was found to be optimal. Only one plaque from each selection plate was picked.

Specialized transduction by lambda. A fresh exponential culture of bacteria was starved in 10 mM magnesium sulfate for 30 min and infected with the transducing phage at a multiplicity of 5. Fifteen minutes at room temperature was allowed for absorption, after which the infected cells were pelleted and washed with 0.1 M trisodium citrate, pH 7. Dilutions were spread onto selective plates. In transductions of *polA* hosts, 30 min of incubation in L broth at 37°C was allowed for phenotypic expression before selection.

TrpA$^+$ transductants were selected on glucose minimal agar supplemented with Casamino Acids (0.05%). For the selection of PolA$^+$ transductants, 0.4% methyl methane sulfonate was added to the minimal medium supplemented with thymine (40 μg/ml).

β-Galactosidase assay. The differential rate of β-galactosidase synthesis was determined by growing the bacteria in minimal media supplemented where necessary. At least four samples were taken at intervals and assayed. β-Galactosidase activity was plotted against sample optical density at 600 nm. The gradient defines the rate of enzyme synthesis or steady-state

TABLE 2. *Phage strains*

Designation	Genotype	Comments
JDW36	λ*lacZ trpA* Δ(*shn trpB-shn trpD*) *trpE imm*^{21}ts *ninR5 shn*λ6^0	*trp-lac* fusion W205[a]
NM920	λ*W*am *polA1*^{1} Δ(*att-red*) *imm*^{21} *ninR5 shn*λ6^0	
NM822	λ*polA*^{r} Δ(*att-red*) *imm*^{21} *ninR5 shn*λ6^0	
NM74	*h*^{80} *att*^{λ} *gam*am210 *imm*^{λ}	
NM125	*h*^{80} *att*^{80} *imm*^{λ} cI857	S$^+$ derivative of MH480 (33)
NM540	λΔ(*srλ1-srλ2*) *att*$^+$ *imm*^{21} *ninR5 shn*λ6^0	*att*$^+$ λ vector (4)
	λcI *ind*$^-$	—[a]
D42	λ*lacZ trpA polA1*^{1} Δ(*att-red*) *imm*^{21} *ninR5 shn*λ6^0	See Fig. 1
D44	λ*lacZ trpA polA*^{1} Δ(*att-red*) *imm*^{21} *ninR5 shn*λ6^0	*polA*$^+$ derivative of D42
D46	λ*lacZ trpA polA*^{1} *att*$^+$ *red*$^+$ *gam*am *imm*^{λ}	From cross D44 × NM74[b]
D48	λ*lacZ trpA polA*^{1} Δ(*att-red*) *imm*^{λ} cI857	From cross D44 × NM125[b]
D52-D55	Fusion derivatives 3, 13, 14, and 24, all from D48	
D59	λ*lacZ polA* fusion 14 *att*$^+$ *imm*^{21} *ninR5 shn*λ6^0	D54 × NM540, see Table 5
D60	λ*lacZ polA* fusion 24 *att*$^+$ *imm*^{21} *ninR5 shn*λ6^0	D55 × NM540, see Table 5
D61	λ*lacZ polA* fusion 14 *att*$^+$ *imm*^{λ} *ind*$^-$	From cross D59 × λ*ind*$^-$[c]
D62	λ*lacZ polA* fusion 24 *att*$^+$ *imm*^{λ} *ind*$^-$	From cross D60 × λ*ind*$^-$[c]

[a] JDW36 was provided by J. Windass; λcI *ind*$^-$ was provided by D. Mount.
[b] *h*^{λ} *imm*^{λ} recombinants selected on C600(λ*imm*^{21}).
[c] Lac$^+$ *imm*^{λ} recombinants selected on strain C38.

specific activity. β-Galactosidase was assayed essentially as described by Miller (31) except that samples were diluted with 1× Vogel and Bonner salts and assayed at 37°C. Enzyme activity was expressed as nanomoles of *o*-nitrophenol per minute per milliliter. One enzyme unit equals:

$$241 \times \frac{(A_{420} - 1.75 \times A_{550})}{\text{assay time in minutes}}$$

where A_{420} is absorbancy at 420 nm and A_{550} is absorbance at 550 nm. One optical density unit at 600 nm was taken as equal to 220 μg of protein per ml.

Analysis of polypeptides after infection of UV-irradiated cells. Protein synthesis, directed by phage infection of *E. coli* S159 previously irradiated with a high dose of UV light, was analyzed by L-[^{35}S]methionine pulse-labeling (18). Samples (usually 10 μl, containing 10^5 to 10^6 ^{35}S cpm) were analyzed by electrophoresis (20 mA, overnight) in gels with a gradient acrylamide concentration (10 to 20%, wt/vol) made in 0.38 M Tris-hydrochloride (pH 8.8)–0.1% sodium lauryl sulfate (25). The gels were dried and radioautographed.

RESULTS

Construction of λ*lacZ polA* phages. A λ transducing phage which carries both the *polA* and *lacZ* genes in the same orientation with respect to direction of transcription was a prerequisite for the isolation of genetic fusions in which expression of the *lacZ* gene was controlled from the promoter of the *polA* gene. Orientation of a gene in a λ transducing phage is defined by the transcribed DNA strand; when the sense strand is the phage *l* strand the gene is said to be in the *l* orientation (15). The *lacZ* gene in the λ*plac*5 (17) is in the *l* orientation and can be transcribed from the λ promoter p_L.

The left arm of the λ*lacZ polA* phage, including the *lacZ* gene, was from a derivative of λ*plac*5 in which the *lacZ* structural gene had been fused to the end of the *trp* operon (2). This *trp-lac* fusion, W205 (32), removes not only the *lac* promoter but also the transcriptional termination sites between the *lacZ* gene and the *trp* operon. A λ*polA*^{1} phage (NM920) served as donor of both the *polA* gene and the right arm of phage λ.

The steps in the construction of the λ*lacZ polA* phage are illustrated in Fig. 1. Three recombinants were recovered with the genotype λ*lacZ trpA polA* Δ(*att-red*) *imm*^{21} *ninR*5. These recombinants, like the λ*polA*^{1} donor phage, carried the *polA1* amber allele as shown by their ability to form plaques on a *supE polA10* host but not on a suppressor-free *polA1* strain. *polA*$^+$ derivatives of the three recombinants were isolated after growth of the λ*polA*1 phages in the Pol$^+$ bacteria, C600. A significant proportion (10^{-3} to 10^{-4}) of the phage in such a lysate are

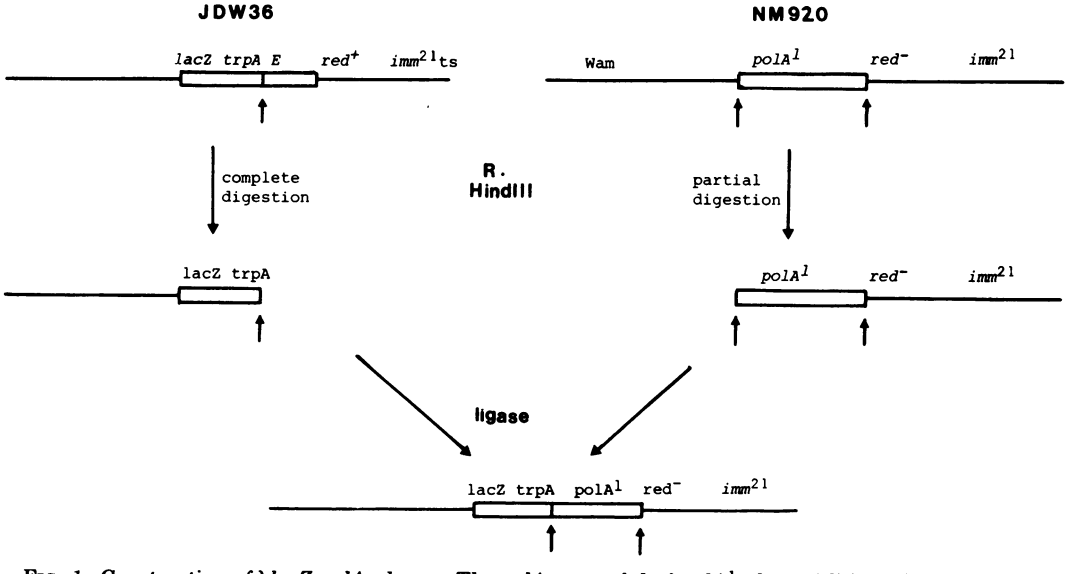

FIG. 1. *Construction of λlacZ polA phages. The polA gene of the λpolA*^{1} *phage (NM920) is within a 5-kb fragment of DNA defined by two HindIII targets (↑). Digestion of the DNA of the lacZ donor phage (JDW36) with HindIII produced only two fragments, one of which was the left arm of phage λ including the trpA-lacZ fusion. The restricted phage DNAs were mixed and treated with T4 DNA ligase, and recombinants were recovered by transfection of the suppressor-free strain AA125. The use of this host precluded the recovery of phages with the left arm (Wam) of the λpolA phage. Recombinant phages with the right arm of the λpolA donor were recognized as Red*$^-$ *by their inability to form plaques on a lig(Ts) host at 37°C (51). Pol*$^+$ *Red*$^-$ *phages will, however, form plaques on PolA*$^-$ *(20).*

polA$^+$, presumably as the result of recombination with the host chromosome. Such derivatives were selected by their ability to form plaques on the suppressor-free *polA1* bacteria.

All three phages included the *lacZ* gene since they retained the ability to produce β-galactosidase as evidenced by the hydrolysis of the chromogenic galactoside, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (7). The cautious approach of selecting against the left arm of the λ*polA* phage, rather than selecting directly for the left arm of the λ*lacZ* phage, was adopted to avoid any selective pressure for the expression of the *lacZ* gene despite the expectation that it would be transcribed from the λ promoter, p_L.

Restriction targets and the orientation of the *polA* gene. DNA was prepared from each of the three λ*lacZ polA* phages and digested with *Hin*dIII, and the presence of the 5-kilobase pair (hb) fragment that carries the *polA* gene was confirmed. The simplest method of determining the orientation of this DNA fragment within the λ vector requires digestion with a second restriction endonuclease that cuts the fragment asymmetrically. The resulting pattern of DNA fragments will then depend on the orientation of the fragment.

DNA was isolated from each of two λ*polA* phages differing in the orientation of the *polA* gene (NM822 and NM920; see Table 2). The direction of transcription of the *polA* gene in these two phages was known (36). The restriction enzymes *Eco*RI, *Bam*HI, *Hpa*I, *Sal*I, and *Kpn*I did not cut the fragment containing the *polA* gene. *Ava*I (16, 34) cut the fragment twice, giving fragments of DNA diagnostic of the orientation of the included gene (Fig. 2). *Xho*I (14), whose recognition sequence is a subset of those recognized by *Ava*I, was shown to cleave both of the *Ava*I targets within the *polA* fragment, and *Bgl*II has a site within the promoter-proximal portion of the fragment.

DNA from each of the three λ*lacZ polA* recombinants was digested with *Ava*I, and the resulting fragment was compared with those obtained from the λ*polA*^{1} and λ*polA*^{r} phages. Two of the recombinant phages had the 3.9-kb fragment characteristic of phages in which the *polA* gene is in the *l* orientation (see Fig. 2). The Pol$^+$ derivative of one of these two phages (D44) was the progenitor of all of the λ*lacZ polA* derivatives described in this paper.

Construction of gene fusions. Deletion derivatives of the λ*lacZ polA* phage were selected with the aim of making new fusions in which maximal expression of the *lacZ* gene would be achieved under the control of the *polA* promoter.

A derivative of D44 with an almost wild-type complement of DNA was constructed (D48; see Table 2). Forty-four independent deletion derivatives of this phage were selected and screened for the PolA$^-$ phenotype which would result from a deletion extending into the *polA* gene. Four Lac$^+$ PolA$^-$ deletion derivatives were isolated, and these fusions (no. 3, 13, 14, and 24) were analyzed further.

The markers in phage λ are conventionally described from left (gene *A*) to right (gene *R*), and therefore the genotype λ*lacZ polA* indicates that the *lacZ* gene is left of *polA*. Fusions in which the *lacZ* gene is transcribed from the *polA* promoter are, however, referred to as *polA-lacZ* fusions, in keeping with previous conventions (32).

Expression of β-galactosidase in *polA-lacZ* fusions. Lysogens of the four fusion phages were isolated in C38, a *lacZ* deletion host lysogenic for λ*imm*^{21}. The presence of the λ*imm*^{21} prophage provides homology to facilitate

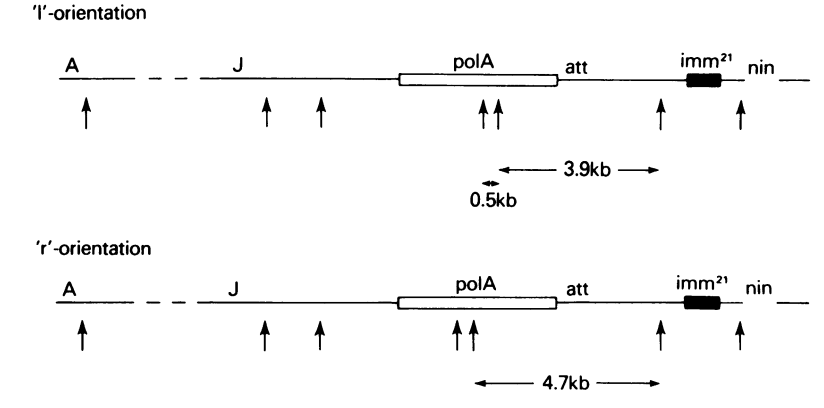

FIG. 2. *Orientation of the polA gene. Digestion of the DNAs of λpolA phages with AvaI indicates a 3.9-kb fragment diagnostic of the l orientation. This fragment is replaced by a 4.7-kb fragment in the λpolA^r phage.*

integration of the fusion phages.

The steady-state level of β-galactosidase in these dilysogens was measured (Table 3). All four fusions gave higher rates of expression of *lacZ* than the original λ*lacZ polA* phage, but there was some variation in the rates of enzyme synthesis. This was also the case for fusions between the *trp* and *lac* operons, where a 60-fold variation in the rate of expression of β-galactosidase was observed (42); this is attributed to polarity resulting from fusions which are out of frame. Fusions 14 and 24 gave the highest levels of β-galactosidase and were therefore preferred for further study.

The possibility that transcription from promoters, other than that of *polA*, could be significantly affecting β-galactosidase synthesis seems unlikely. There does not appear to be any major promoter preceding *polA*; infection of a UV-irradiated lysogenic host by λ*polA* failed to reveal any additional polypeptides which would indicate the presence of a promoter potentially capable of transcribing in the same direction as *polA* (A. Newman, personal communication). Similarly, no appreciable transcription could originate at a bacterial promoter outside of the integrated transducing phage since a λ*trpA* transducing phage, of similar structure to these λ*lacZ polA* fusion phages, fails to express *trpA* in the lysogenic state (15).

Genetic characterization of the gene fusions. The objective in selecting deletions that fuse the *lacZ* gene to the *polA* promoter was to use the simple β-galactosidase assay to monitor the level of transcription from the *polA* promoter. It was therefore necessary to establish that expression of the *lacZ* gene is directed by the *polA* promoter and that the rate of expression of *lacZ* is not susceptible to regulatory influences other than at the *polA* promoter. The endpoints of the deletions were therefore determined with respect to both markers in the intervening *trpA* gene and markers in the *polA* gene.

TABLE 3. *Expression of β-galactosidase in λlacZ polA phages*[a]

Strain	Differential rate of enzyme synthesis
C38	0.4
C38 (λ*lacZ polA* cI857) parent	93
C38 (λ*lacZ polA* cI857) fusion 3	290
C38 (λ*lacZ polA* cI857) fusion 13	410
C38 (λ*lacZ polA* cI857) fusion 14	730
C38 (λ*lacZ polA* cI857) fusion 24	920

[a] Lysogens of the integration-defective λ*lacZ polA* phages were made in strain C38 [*lacZΔM15* (λ*imm*^{21})], using the homology provided by the resident prophage. The parent phage (D48) was made by crossing the original λ*lacZ polA imm*^{21} (strain D44) with λ*h*^{80} cI857 and selecting for *h*$^{\lambda}$ *i*$^{\lambda}$ recombinants. Phage D48 has almost a full complement of DNA and was used as the starting point for the selection of deletion derivatives (see text). Strains were grown in glucose-Casamino Acids-thiamine Vogel and Bonner salts (44) at 32°C, and the steady-state level of β-galactosidase was determined. Enzyme level is expressed as the differential rate of enzyme synthesis in nanomoles of *o*-nitrophenol per minute per milligram of protein.

(i) Mapping with respect to *trpA* markers. Fusion 24 not only transduced *trpA* alleles to Trp$^+$ (Table 4) but also complemented *trpA88*. It therefore retains a functional *trpA* gene. The other fusions transduced *trpA96*, which maps very close to the C-terminus of the gene (50), to *trp*$^+$ but gave no *trp*$^+$ transductants with *trpA88*, a mutation close to the N-terminus (11). All of these deletions must end within the *trpA* gene. Fusion 13 failed to transduce *trpA23* (50), indicating that it extends closer to the distal end than the others.

(ii) Mapping with respect to *polA* alleles. Transductional analyses with fusions 13 and 24 failed to detect recombination with either the *polA1* or the *polA10* allele, whereas the deletions in fusions 3 and 14 stopped short of these markers (Table 4). Unfortunately, very high reversion frequencies prevented mapping the deletions with respect to *polA6* and *polA107*. These mutations define the C- and N-terminal segments of the *polA* gene, respectively (21). Although all four fusion strains are PolA$^-$, it is not proven that the deletions end within the *polA* gene.

An alternative, more sensitive recombinational analysis was undertaken. Various PolA$^-$ hosts were lysogenized by integration-proficient derivatives of the fusion phages 14 and 24. The frequencies with which Pol$^+$ cells arose were estimated. A *pol*$^+$ gene could be generated by a process analogous to homogenotization. The data (Table 5) are consistent with deletion of the entire *polA* gene in fusion 24 and retention of even the *polA6*$^+$ allele in fusion 14. Although these genetic data fail to localize the right-hand ends of these deletions to sites within the *polA* gene, all the physical evidence suggests that this must be so.

Physical characterization of gene fusions. (i) Analysis of DNA with restriction endonucleases. DNA was isolated from each of the four fusion strains, and fragments resulting from digestion with *Hin*dIII and with *Ava*I were analyzed. As expected, the *Hin*dIII target linking the *polA* fragment to the *trp-lac* fusion had been deleted in each of the fusions, but no deletion extended beyond the right-hand *Hin*dIII target. All the deletions must, therefore,

TABLE 4. *Transduction frequencies for the polA-lacZ fusions*[a]

Strain	Transduction frequency				
	trpA96	*trpA23*	*trpA88*	*polA1*	*polA10*
λ*lacZ polA* cI857 parent	>1,000	>1,000	>1,000	42,000	75,000
λ*lacZ polA* cI857 fusion 3	~500	60	0	70	50
λ*lacZ polA* cI857 fusion 13	~400	0	0	0	1
λ*lacZ polA* cI857 fusion 14	~600	70	0	150	70
λ*lacZ polA* cI857 fusion 24	>1,000	>1,000	>1,000	0	0

[a] Frequency of transduction of either *trpA* or *polA* mutations by these λ*lacZ polA* phages was measured; the frequency is expressed per 10^9 phage. The parent phage (strain D48) is *trpA*$^+$ *polA*$^+$.

TABLE 5. *Recombination frequencies with polA mutations*[a]

Host	Recombination frequency	
	Fusion 14	Fusion 24
polA107	18,000	4
polA1	1,400	3
polA6	420	5

[a] Integration-proficient derivatives of λ*lacZ polA* fusions 14 and 24 were constructed in vitro (strains D59 and D60). The left arm of these phages up to *shn*λ3 and containing the *polA-lacZ* fusion was combined with the right arm of phage NM540 from *shn*λ3 including a functional *att* site and integration gene. Lysogens were made in the PolA$^-$ hosts, and the frequency with which PolA$^+$ bacteria arose was measured. This process occurs by a similar mechanism to homogenotization.

enter the fragment carrying the *polA* gene and stop within it.

Two targets for *Ava*I, within the fragment of DNA containing the *polA* gene (see Fig. 2), divided the region of interest into three sectors and allowed approximate physical mapping of the right-hand endpoints of these fusions. In fusions 3 and 14, the deletion stopped short of both the *Ava*I targets, that in fusion 13 ended between the two *Ava*I targets, and that in fusion 24 removed both of these targets.

From the sizes of the fragments missing in the *Ava*I digests and the sizes of the new fusion fragments, estimates of the extent of each deletion could be made. The deletions ranged in size from 2.9 to 4.6 kb. The genetic and physical data were used to construct the maps of the fusions (Fig. 3).

(ii) Heteroduplex analysis. The positions of the right-hand endpoints of deletions 14 and 24 were determined by heteroduplex analysis. λ*lacZ polA att*$^+$ *imm*$^\lambda$ *nin*R5 (strain D46) is an *att*$^+$ derivative of the original λ*lacZ polA* Δ(*att-red*) *imm*[21] (strain D44) progenitor of the fusion phages. Heteroduplexes of D46 with the fusion phages therefore gave a structure with three single-stranded loops (Fig. 4). The first of these was formed by the *nin*R5 deletion and served as the internal standard. The size of this loop was assumed to be 2.9 kb of single-stranded DNA, and the distance from the phage right-hand *cos* site was assumed to be 5.1 kb of double-stranded DNA (N. Willetts, personal communication). The second loop, due to the *att-red* deletion present in the fusion phages, served to mark the position of the phage attachment site, whereas the *polA-lacZ* fusion caused the third loop. The distance between the second and third loops therefore defines the distance between *att* and the right-hand end of the fusion.

Heteroduplex molecules were prepared and analyzed by P. Beattie according to the method of Davis et al. (8). At least 10 molecules for each heteroduplex were measured. The distance from *att* to the endpoint of fusion 14 was 3.9 ± 0.2 kb (errors expressed are standard deviations); for fusion 24 it was 1.7 ± 0.1 kb. Since the fragment of DNA carrying the *polA* gene was inserted some 260 base pairs left of the phage attachment site at the *Hin*dIII target (9, 26), fusion 14 extends to within approximately 3.6 kb of the end of the fragment and fusion 24 to within 1.4 kb. The coding sequence required for the *polA* gene is 3 kb, and these data are therefore quite consistent with one deletion ending in the distal part of the *polA* gene, the other in the proximal part. At least one of these deletions must end in the *polA* gene, and the heteroduplex data are in agreement with the map shown in Fig. 3.

(iii) Analysis of fusion polypeptides. The polypeptides synthesized after infection of UV-irradiated cells (18) with the λ*lacZ polA* fusion phages were labeled with [^{35}S]methionine and separated by electrophoresis through a sodium dodecyl sulfate-polyacrylamide gel (Fig. 5). The *lacZ* polypeptide, of molecular weight 116,000 (13), was readily resolved from polI, molecular weight 109,000 (19). None of the fusion phages produced a polypeptide of the same mobility as the wild-type *polA* product, but each produced a new polypeptide of a size consistent with the predicted fusion product.

The presumed fusion polypeptide from the *polA-lacZ* fusion 14 is greater than the wild-type polypeptide. Assuming that this polypeptide terminates at the normal *trpA* termination site, little of the *polA* gene is deleted by this fusion. The endpoint of this deletion should therefore be close to the terminus of the *polA* gene and, since the *polA* gene requires 3 kb of DNA, the promoter should be ~1 kb from the right-hand *Hin*dIII target. This indicates that the promoter

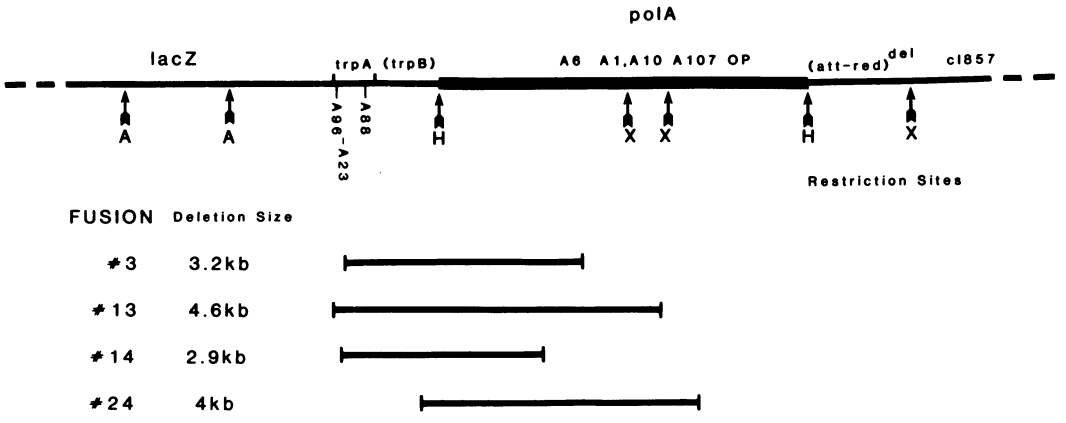

FIG. 3. *Genetic and physical map of λlacZ polA fusion phages. Composite map of the polA-lacZ region shows: restriction endonuclease targets ([A] AvaI, [H] HindIII, [X] R.XhoI); approximate physical locations of genetic markers used for mapping; and extent and position of the polA-lacZ gene fusions based on the genetic and physical mapping described in the text.*

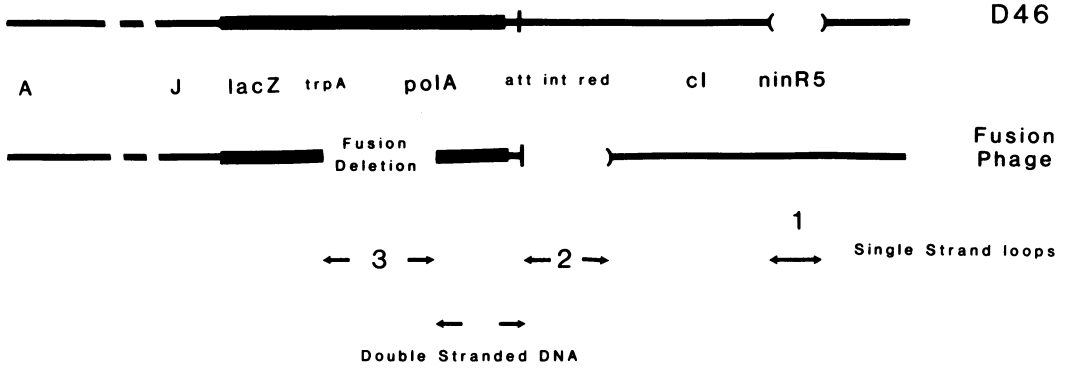

FIG. 4. *Heteroduplex analysis of λlacZ polA fusion phages. Heteroduplexes between λlacZ trpA polA att⁺ cI857 ninR5 (D46) and the fusion phages give three single-stranded regions: (1) ninR5 deletion; (2) att-red deletion; and (3) DNA deleted by the polA-lacZ fusion. The double-stranded region between loops 2 and 3 defines the distance between att and the right-hand endpoint of the fusions.*

would be present in fusion 24.

Effect of cellular level of DNA polI on expression of *polA*. The *polA-lacZ* fusions within a λ phage provide a simple method of examining what effect the level of polI has on the rate of transcription of the *polA* gene. The expression of the *polA* gene can be measured under steady-state conditions in the absence of phage lytic functions by using lysogens of the fusion phages. Since these phages are PolA⁻, they should not contribute to the intracellular level of polI. Fusions 14 and 24 were used since these gave maximal expression of the *lacZ* gene. The left-hand endpoints of both fusions are within the *trp* genes; the right-hand endpoint of fusion 14 must lie just inside the distal end of the *polA* gene, and that of fusion 24 must lie in the promoter-proximal region.

Integration-proficient, imm^{λ} ind^{-} derivatives of the fusion phages were isolated (strains D61 and D62), and monolysogens were made in otherwise isogenic $polA^{+}$ and *polA* strains.

The steady-state levels of β-galactosidase (Table 6) in the lysogenic cells showed that expression from the *polA* promoter was elevated in the *polA1* host. The magnitude of the increase was very low despite the 100-fold difference in the polymerase activity in the PolA⁺ and PolA⁻ hosts (10). In contrast, the levels of β-galactosidase (Table 6) in the lysogens of the *polA6* and *polA107* strains were slightly lower than in the control $polA^{+}$ host. These results provide no support for the self-regulation of polI.

Comparison of the level of β-galactosidase expression from these *polA-lacZ* fusions with that for a fully induced *lac* operon showed that the *polA* promoter was about 3% as active as the *lac* promoter. Assuming that there are 20,000

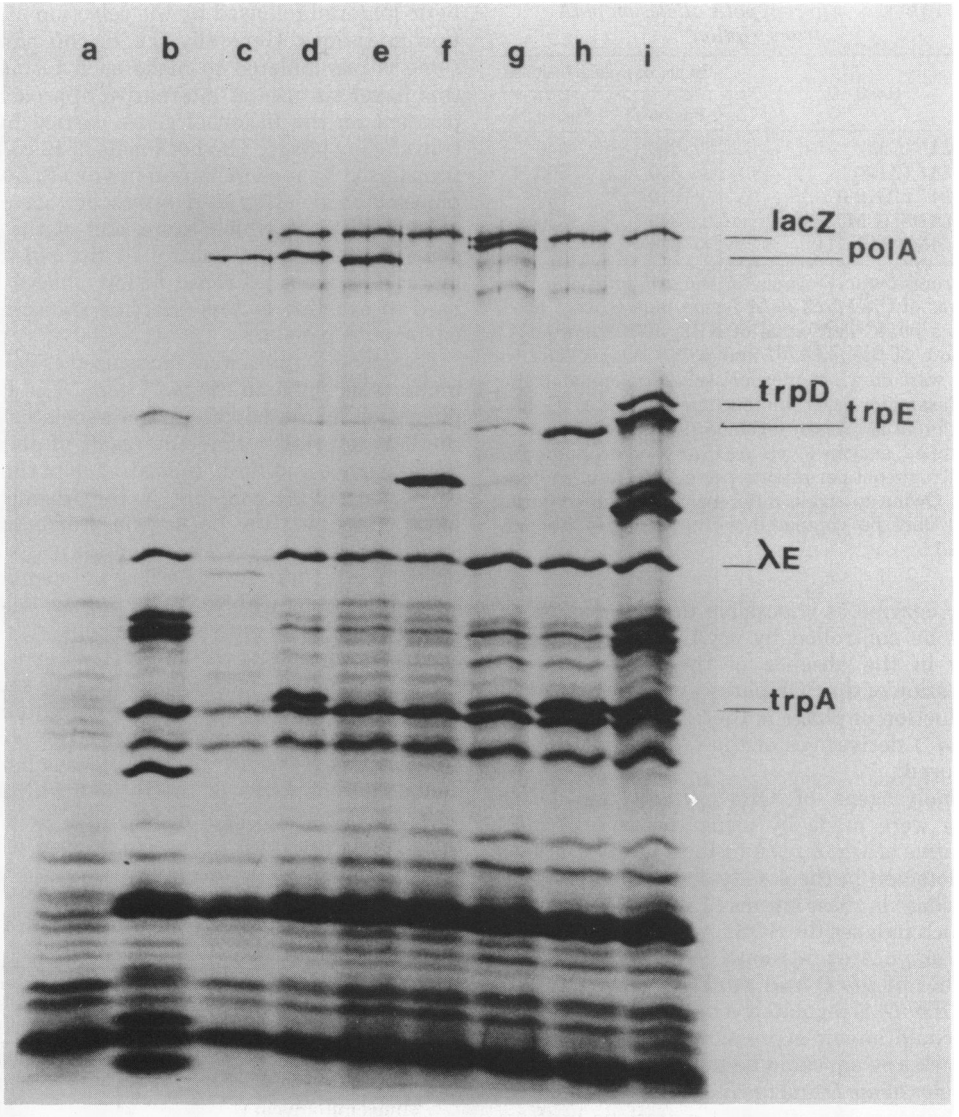

FIG. 5. *Radioautographic analysis of the ^{35}S-labeled polypeptides after electrophoresis through a 10 to 20% polyacrylamide gel containing sodium lauryl sulfate. The cells (S159) were labeled from 3 to 13 min after infection. The genes whose products are identified are indicated to the right of the gel. (a) No phage; (b) λtrpE; (c) λpolA; (d) λlacZ trpA polA (D48); (e) λlacZ polA fusion 3 (D52); (f) λlacZ polA fusion 13 (D53); (g) λlacZ polA fusion 14 (D54); (h) λlacZ polA fusion 24 (D55); (i) λlacZ trpABCDE. For each of the fusion phages, the polI polypeptide is missing and a novel polypeptide, presumed to be a fusion product, is detected. The trpA polypeptide is missing in fusion phage D52, D53, and D54. The weak band in track g (D54) moving slightly slower than the trpA polypeptide is a λ product not detected in other experiments with this phage. The relative abundance of the λ E protein also detected in this infection indicates more labeling of λ late proteins than in those with D52 and D53.*

molecules of β-galactosidase in a cell when *lacZ* expression is fully induced (6), this would be equivalent to 600 molecules per cell for the *polA-lacZ* fusions. If *polA* mRNA is translated with the same efficiency as that of *lacZ*, this would indicate a steady-state level of about 600 molecules of polI per cell, which is compatible with the estimated 400 molecules per cell (23).

Effect of *recA* and mitomycin C on *polA* expression. *recA* is a component of the "SOS" repair pathway (41) responsible for repairing damaged DNA. Since polI functions as a DNA

TABLE 6. *Effect of polA alleles on polA transcription*[a]

Host	Sp act of β-galactosidase	
	Fusion 14	Fusion 24
polA$^+$ (C53)	280	340
polA1 (KB8)	350	500
polA$^+$ (JG108)	610	640
polA107 (CM3842)	340	350
polA6 (CM3571)	520	530

[a] Strains monolysogenic for the *att*$^+$ *imm*$^\lambda$ *ind*$^-$ derivatives of the λ*lacZ-polA* fusion phages were used. C53 is a *polA*$^+$ derivative of KB8; JG108 is a *polA*$^+$ isogenote of the *polA107* and *polA6* strains. The lysogens were grown in appropriately supplemented glucose-Casamino Acids minimal medium at 37°C, and the differential rates of β-galactosidase synthesis were determined; enzyme levels are expressed in nanomoles of *o*-nitrophenol per minute per milligram of protein. (Note: Owing to strain differences, specific activities should only be compared within a set of isogenic strains.)

repair enzyme, it is possible that its expression might be controlled by *recA* or affected indirectly in the absence of the *recA* pathway. Expression of the SOS pathway normally results in induction of phage λ; therefore, noninducible (cI *ind*$^-$) derivatives of the λ*lacZ polA* phages were used.

Monolysogens of QR47 (*rec*$^+$) and QR48 (*recA*) were made by using the *att*$^+$ cI *ind*$^-$ derivative of λ*lacZ-polA* fusion 24. No difference was detected in the steady-state levels of β-galactosidase in these strains (Table 7). Mitomycin C, which induces the SOS functions, also had no effect on *polA* expression (Table 7).

Effect of *uvrD* and *uvrE* on *polA* expression. If *polA* is regulated, a mutation in a control gene could impair expression of the *polA* gene, and such a strain could be sensitive to radiation. The roles of *uvrD* and *uvrE* are not documented, and therefore the effects of mutations in these genes on expression from the *polA* promoter were examined. Monolysogens of the *polA-lacZ* fusion phages were constructed in otherwise isogenic pairs of hosts.

The steady-state level of β-galactosidase in these strains (Table 7) showed that the rate of expression of the *lacZ* gene, and hence by inference the rate of transcription from the *polA* promoter, was approximately twofold lower in the mutant hosts than in the isogenic wild-type hosts. This effect was too small to warrant the conclusion that *uvrD* and *uvrE* are involved in the regulation of the *polA* gene.

DISCUSSION

Gene fusions are usually constructed in a two-step process involving the transposition of genetic material followed by the selection of deletion mutations. Generally the *E. coli* chromosome is manipulated to make such fusions. In this paper we use an alternative approach dependent on the fusion of genes carried by a λ transducing phage. The *polA* gene of *E. coli* was transferred by recombination in vitro to a λ*lacZ* phage, and deletion derivatives of this phage were isolated in which expression of the *lacZ* gene was under the control of the *polA* promoter. Lysogens of these fusion phages were used to examine factors affecting transcription of the *polA* gene.

Fusion derivatives were recognized as deletion mutants defective in the *polA* gene. The PolA$^-$ phenotype is most likely to be associated with the deletion rather than the result of an additional chance mutation. Indeed, each of the four deletions had an endpoint in the fragment of DNA containing the *polA* gene. Furthermore, for each fusion the *polA* polypeptide was shown to be missing, but always a new polypeptide of a size in keeping with the extent of the deletion was detected. The data are consistent with each of the fusions ending within the *polA* gene.

Genetic analysis of the fusion phages showed that they could be used as deletion mutants in the mapping of *polA* mutations. In the present analysis, too few *polA* alleles were available to define the endpoints of the fusions within the *polA* gene, but a series of fusions with endpoints throughout the *polA* gene would allow a fine-structure mapping of this gene.

TABLE 7. *Effect of host mutations on polA transcription*[a]

Host	Fusion no.	Enzyme level (sp act)
recA$^+$	24	520
recA	24	470
Minus mitomycin C	24	320
Plus mitomycin C	24	350
uvrD$^+$	14	690
uvrD	14	400
uvrD$^+$	24	700
uvrD	24	400
uvrE$^+$	24	720
uvrE	24	330

[a] Isogenic pairs of strains monolysogenic for the *att*$^+$ *imm*$^\lambda$ *ind*$^-$ derivatives of the λ*lacZ-polA* fusion phages were used. *recA* = QR48; *uvrD* = *uvrD3*; *uvrE* = *uvrE156* (*mutU4*). The effect of mitomycin C (10 μg/ml) was tested by using a lysogen of W3110. Strains were grown in glucose-Casamino Acids minimal medium at 37°C, and the differential rates of β-galactosidase synthesis were determined; enzyme levels are expressed in nanomoles of *o*-nitrophenol per minute per milligram of protein. (Note: Owing to strain differences, specific activities should only be compared within a pair of isogenic strains.)

Two *Xho*I targets were located within *polA*, and one *Bgl*II target must be close to the promoter of this gene. These targets should be useful for DNA sequence analysis.

Although polI was isolated more than two decades ago and has been extensively studied biochemically, little is known about the regulation of its production. The *polA-lacZ* gene fusions were used in a preliminary investigation of this problem. By measuring steady-state β-galactosidase expression in lysogens of these fusion phages, the rate of *polA* transcription could be determined in different genetic backgrounds. Lysogens were used, as opposed to lytic infection, to obviate any effects λ infection might have.

If *polA* expression is regulated, then the simplest mechanism would be for the gene product to regulate its own expression. Comparison of the β-galactosidase levels in $PolA^-$ and Pol^+ hosts provided no evidence for such autogenous control. Since the rate of *polA* transcription, as estimated in these fusions, corresponds to a steady-state level of about 600 molecules of β-galactosidase per cell, and since there are about 400 molecules of polI per Pol^+ cell, this implies that the *polA* mRNA is translated efficiently and suggests that translational control may not be significant.

One characteristic phenotype of *polA* strains is sensitivity to UV light. The effect of increasing DNA damage by a radiomimetic agent or by blocking the SOS repair pathway was therefore examined. Neither mitomycin C treatment nor a block in the *recA* gene itself had any effect on transcription from the *polA* promoter. Mutations in the *uvrD* and *uvrE* genes were associated with only a modest reduction in the expression of the *polA* gene, too small an effect to imply a direct regulatory role for either of these genes. Recent experiments (24) indicate that *uvrD* and *uvrE* mutations define a single gene. This would explain the similar effect of these mutations on *polA* transcription. Kushner and co-workers (24) have suggested that the product of the *uvrD* (or *uvrE*) gene may be involved in the regulation of either DNA polymerase I or polymerase III.

Although it would be possible to screen many more genes for their effect on transcription of the *polA* gene, a better approach would be to use the *polA-lacZ* fusions to isolate mutations that alter the control of *polA* expression.

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