Proc. Nat. Acad. Sci. USA
Vol. 71, No. 5, pp. 2048–2051, May 1974

A Conditional Lethal Mutant of *Escherichia coli* K12 Defective in the 5′ → 3′ Exonuclease Associated with DNA Polymerase I

(DNA synthesis/UV sensitivity/genetic recombination)

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Communicated by Arthur Kornberg, February 4, 1974

ABSTRACT **A mutant strain of *E. coli*, initially identified by an abnormally high frequency of recombination, has been found to be defective in the 5′ → 3′ exonuclease associated with DNA polymerase I, but not in the polymerase activity. This defect is tolerated at 30°, but is lethal at 43°. Like other polymerase I mutants, the strain is unusually sensitive to methyl methanesulfonate and to ultraviolet irradiation; it is also unable to support the growth of phage λ defective in general recombination, and shows a reduced rate of joining of 10S "Okazaki fragments." These results demonstrate that a functional DNA polymerase I is essential for normal growth and viability in *E. coli* K12.**

Escherichia coli K12 strains defective in the structural gene for DNA polymerase I (*polA*) have been extensively described (1–5). These mutants are abnormally sensitive to ultraviolet irradiation and to the alkylating agent methyl methanesulfonate, and show retarded joining of the 10S "Okazaki fragments" (6–8). A recent analysis of partially purified fractions derived from several of the *polA* mutants has demonstrated that although they contain much reduced levels of DNA polymerase I activity the 5′ → 3′ exonuclease associated with the polymerase I is present in nearly normal amounts (9).

We have found that *polA* mutants are also characterized by an increased frequency of recombination at least under some conditions (7). By screening mutagenized cells for this phenotype, we have isolated a novel *polA* mutant (*polA*ex1) that we describe in this paper. This mutant has a normal level of polymerase I activity but a greatly reduced level of 5′ → 3′ exonuclease. Unlike other *polA* mutants, it is conditionally lethal.

MATERIALS AND METHODS

Bacterial and Phage Strains. All bacterial strains are derived from *E. coli* K12. Their origin and genotypes are given in Table 1. Phage λ defective in general recombination (λ *red* 3) was provided by D. Freifelder.

Microbiological Procedures. P1*vir* phage stocks were prepared and used for transduction, and Hfr crosses were performed as described by Miller (10). Sensitivity to ultraviolet irradiation was determined as follows. Cells grown at 30° in H broth (11) to A_{595} = 0.5 were chilled, harvested, and resuspended in an equal volume of chilled M63 medium (10); 10 ml of a 5-fold dilution of this suspension in a glass petri plate was irradiated (25 ergs per mm² per sec) with a General Electric germicidal lamp; and samples were removed at various times, diluted in chilled H broth, and plated on yeast extract-tryptone plates (10) that were incubated at 30° in the dark. Sensitivity to methyl methanesulfonate was determined on fresh yeast extract-tryptone plates supplemented with 0.04% methyl methanesulfonate. Cultures were treated with ethyl methanesulfonate as described by Miller (10). Lactose-tetrazolium indicator plates were prepared according to Miller (10). Ability to form plaques of phage λ was determined on tryptone plates as described by Miller (10). Unless otherwise noted, *E. coli polA*ex1 strains were grown at 30°.

Alkaline Sucrose Gradients. Pulse-labeled DNA was prepared as follows. A 6-ml culture was grown to A_{595} = 0.5 at 30° in M63 medium supplemented with 0.2% glucose, 0.5% casamino acids, 2 μg/ml of thymine, 1 μg/ml of thiamine, and 20 μg/ml of methionine. The culture was shifted to 43° for 5 min with agitation and then 200 μl of a solution of [³H]thymidine (24 Ci/mmole, 0.5 mCi/ml, Schwarz/Mann) was added. The pulse was quenched by adding 6 ml of a 75% ethanol solution containing 2% phenol, 20 mM sodium acetate (pH 5.3), and 2 mM EDTA. The suspension was then chilled and cen-

TABLE 1. *E. coli K12 strains used in the isolation of polAex1*

Strain	Genotype	Source or construction
KS391	Hfr Hayes lacMS286 φ80dIIlacBK1 thi⁻	Konrad
RS480	Hfr Hayes lacMS286 φ80dIIlacBK1 thi⁻ polAex1	KS391 mutagenized by EMS
KL209	Hfr KL209 malB⁻	B. Low
H1012	Hfr KL209 malB⁻ rha⁻	KL209 mutagenized by EMS
RS5049	Hfr KL209 malB⁻ polAexl	P1 transduction: RS480 × H1012
KS439*b*	F⁻lacMS286 φ80dIIlacBK1 metB⁻ara⁻thyA⁻ thi⁻	Konrad
RS5052	F⁻lacMS286 φ80dIIlacBKI polAex1 ara⁻thyA⁻ thi⁻	Hfr cross: RS5049 × KS439*b*

Genetic nomenclature is from Taylor and Trotter (14).

φ80*d*IIlac is a defective transducing prophage that carries the *lac* operon (21). but not φ80 *imm*.

*lac*MS286 is a deletion including *lacY* and part of *lacZ*, furnished by M. Malamy.

*lac*BK1 is a deletion of part of *lacZ* that does not overlap *lac*MS286.

trifuged for 10 min at 15,000 × *g*, and the pellet was resuspended in 0.6 ml of 0.2 M NaOH–10 mM EDTA and placed at 37° for 1 hr. After centrifugation for 15 min at 15,000 × *g*, the supernatant solution was removed and stored at −20°. Alkaline sucrose gradients (5–20%) were run as described by Konrad *et al.* (12), except that an SW 41 rotor was used and gradients were formed over a 1-ml shelf of alkaline 80% sucrose. A portion (250-μl) of DNA extract was layered onto the gradient and covered with mineral oil. [^{32}P]M13 DNA, kindly provided by M. Jazwinski, was included as a sedimentation marker.

Partial Purification of DNA Polymerase I. DNA polymerase I was partially purified from French-pressure-cell extracts of *E. coli polA*ex1 and its wild-type parent by ammonium sulfate fractionation followed by sucrose gradient sedimentation (9). Assays of DNA polymerase I and 5′ → 3′ exonuclease activity were performed at 30° as described previously (9).

RESULTS

*Isolation of E. coli polAex1. E. coli polA*ex1 was one of 52 temperature-sensitive mutants, induced by ethyl methanesulfonate, that showed an increased frequency of recombination. The recombination measured was between a *lac*$^-$ mutation at the *lac* region of the chromosome and a different *lac*$^-$ mutation carried on a *ϕ80dlac* prophage (Table 1). Single colonies from the mutagenized culture which displayed this "hyper rec" phenotype were recognized on lactose tetrazolium indicator plates by a larger number of *lac*$^+$ papillae on their surface. Details of this procedure will appear in a subsequent publication.

Mapping of polAex1. The *polA*ex1 mutation was mapped between 74 and 78 min on the *E. coli* chromosome by determining that the locus transferred as an early marker by Hfr R1 but not by Hfr Ra2 (13). With phage P1*vir*, the locus showed cotransduction at a frequency of 4% (3/69) with *metE*, 13% (26/195) with *rha*, and 0% (0/110) with *ilv*. Thus, it lies between *metE* and *rha*, the interval in which *polA* is situated (14).

E. coli polAex1 Contains Normal Polymerase I but Defective 5′ → 3′ exonuclease. Crude extracts of *E. coli polA*ex1 contained approximately normal levels of polymerase I activity when assayed with nicked calf-thymus DNA as template-primer. Sucrose gradient sedimentation of the enzyme after ammonium sulfate fractionation confirmed that its level was comparable to that of wild-type DNA polymerase I (Fig. 1 and Table 2). However, the sedimentation coefficient of the mutant enzyme was consistently somewhat lower than the wild-type enzyme and its activity measured with the $(dA)_{800}\cdot(dT)_{10}$ homopolymer pair ranged from one-third to one-half that of the wild type.

The 5′ → 3′ exonuclease activity measured at 30° was approximately 3% of the wild type, and this low level of activity was thermolabile. Since the exonuclease cosedimented with the polymerase I activity, and was insensitive to *N*-ethyl maleimide and inhibited by antiserum to polymerase I, we conclude that it represented the residual 5′ → 3′ exonuclease

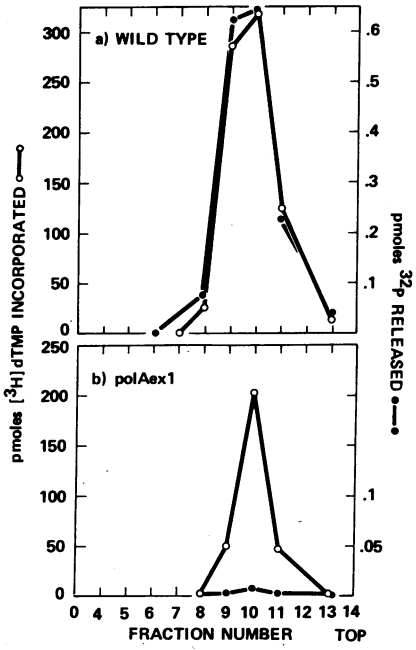

FIG. 1. Sedimentation profile in sucrose gradient of ammonium sulfate fraction II (9) of (*a*) wild type (KS439b) and (*b*) *E. coli polA*ex1 (RS5052). O——O, pmoles of [H^3]dTMP incorporated by polymerase; ●——● pmoles of ^{32}P released from [5′^{32}P]$d(T)_{300}\cdot(dA)_{5000}$ by 5′ → 3′ exonuclease. Polymerase I and 5′ → 3′ exonuclease activities were >90% inhibited by antiserum directed against homogeneous DNA polymerase I.

TABLE 2. *Polymerase I and 5′ → 3′ exonuclease activities in sucrose gradient fractions of E. coli polAex1 and its wild-type parent*

Strain	Polymerase I activity (pmoles dTMP incorporated) $d(A)_{800}\cdot d(T)_{10}$	Polymerase I activity (pmoles dTMP incorporated) Nicked calf-thymus DNA	Activity: 43°/30°	S_{20w}	Exodeoxyribonuclease II activity (pmoles ^{32}P released)	Activity: 43°/30°
E. coli wild type (KS439*b*)	141,000	18,600	1.3	5.54	230	1.6
*E. coli polA*ex1 (RS5052)	54,900	16,800	0.9	5.04	6.3	0.75
Methyl methane-resistant revertant of RS5052	95,400	—	—	5.35	125	1.5

The values shown represent the total DNA polymerase I and 5′ → 3′ exonuclease activities determined at 30° in the gradients shown in Fig. 1, and a similar gradient (not shown), for the methyl methane sulfonate- and temperature-resistant revertant.

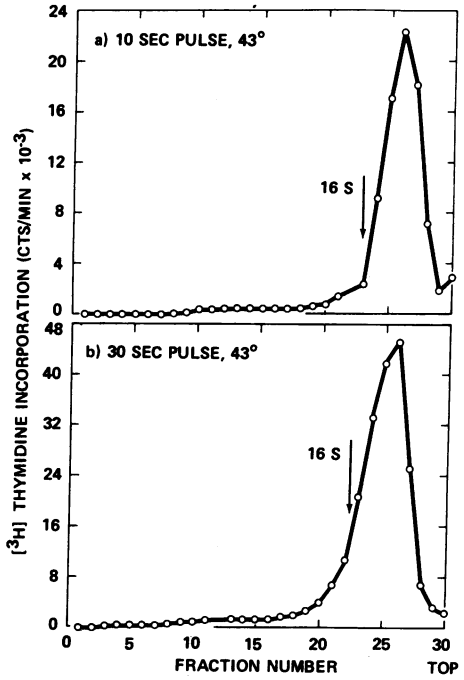

FIG. 2. Sedimentation profile in alkaline sucrose gradients of labeled DNA extracted from *E. coli polA*ex1 (RS5052) following (*a*) a 10-sec pulse at 43° and (*b*) a 30-sec pulse at 43°.

activity of the mutant polymerase I speciffied by the *polA*ex1 locus.

Phenotypic Traits of E. coli polAex1. *E. coli polA*ex1 is conditionally lethal to the extent that about 1% or less of logarithmically growing cells survive to form colonies on tryptone-yeast extract plates at 43°. On tryptone plates or glucose-minimal plates, where the growth rate is lower, the proportion of survivors at this temperature is increased. Mutant cells in broth cultures shifted to 43° form long filaments. These results were found with five different *E coli* K12 *polA*-ex1 strains, including W3110 *polA*ex1 (22).

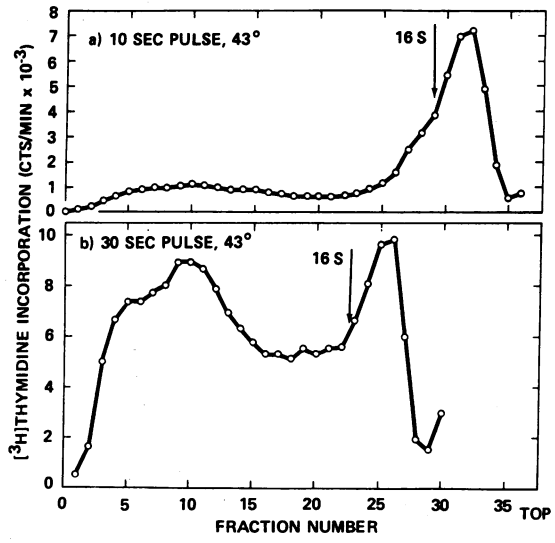

FIG. 3. Sedimentation profile in alkaline sucrose gradients of labeled DNA extracted from wild-type *E. coli* (KS439b) following (*a*) a 10-sec pulse at 43° and (*b*) a 30-sec pulse at 43°.

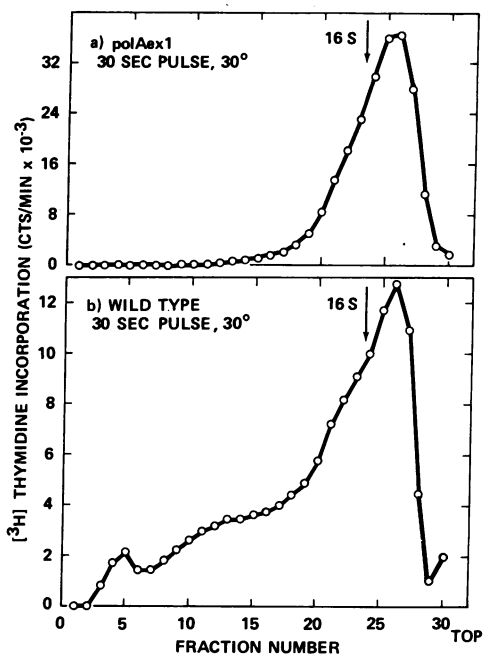

FIG. 4. Sedimentation profile in alkaline sucrose gradients of labeled DNA extracted after a 30-sec pulse at 30° from (*a*) *E. coli polA*ex1 (RS5052) and (*b*) wild-type *E. coli* (KS439b).

Sedimentation profiles in alkaline sucrose of DNA from *E. coli polA*ex1 pulse-labeled at 43° with [^{3}H]thymidine for 10 or 30 sec are shown in Fig. 2. In contrast to the wild-type strain (Fig. 3), the mutant displayed a greatly increased accumulation of 10S fragments in both the 10- and 30-sec pulses. The appearance of some rapidly sedimenting DNA in the 30-sec pulse, and more such material when the pulse was lengthened to 5 min (data not shown), suggests that the 10S fragments are precursors of larger DNA. This finding also indicates that *polA*ex1 may be somewhat leaky at 43°.

When a wild-type culture was pulsed for 30 sec at 30°, there was a greater proportion of slowly sedimenting DNA (Fig. 4*b*) than there was in the corresponding 30-sec pulse at 43° (Fig. 3*b*). In contrast, *E. coli polA*ex1 showed somewhat less 10S material at 30° than at 43°. (Compare Figs. 2*b* and 4*a*.) This result is consistent with the thermolability of the mutant 5′ → 3′ exonuclease activity detected *in vitro* (Table 2).

*E. coli polA*ex1 did not form colonies on tryptone-yeast extract plates supplemented with methyl methane sulfonate. It also showed reduced survival on exposure to ultraviolet irradiation, even at 30° (Fig. 5). Under the same conditions, a *polA*1 strain (JG112) was more sensitive to ultraviolet irradiation than *E. coli polA*ex1 (data not shown).

Like other *polA* mutants, *E. coli polA*ex1 did not support plaque formation by λ mutants deficient in general recombination (λ *red*$^-$), although it did support the growth of wild-type λ phage (5) (data not shown).

polAex1 Is a Single Mutation. Thirty-five phage P1–mediated transductants that had received *polA*ex1 showed no segregation of the traits of sensitivity to methyl methane sulfonate and temperature-sensitive conditional lethality. Moreover, revertants of *polA*ex1 selected for resistance to methyl methane sulfonate also acquired the capacity for normal growth at 43°. One of these revertant strains was

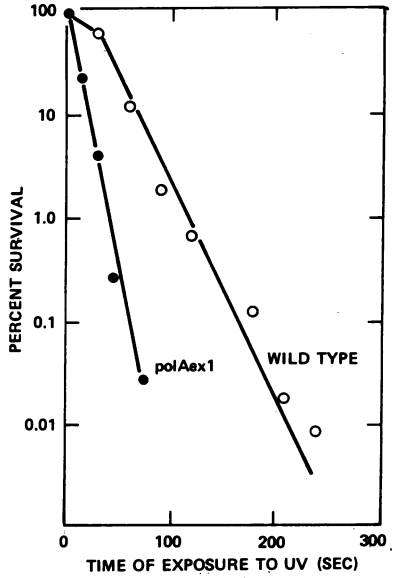

FIG. 5. Survival at 30° of wild-type *E. coli* (H1012) and *E. coli polA*ex1 (RS5049) exposed to ultraviolet (UV) irradiation. Cells were irradiated and the viable titer was measured as described in *Materials and Methods*.

examined more carefully, and found to have levels of 5′ → 3′ exonuclease activity comparable to wild type (Table 2), and to join 10S fragments at approximately the normal rate (data not shown). These results strongly indicate that mutation of the *polA* gene is solely responsible for the abnormal phenotype of *E. coli polA*ex1.

DISCUSSION

We have shown that a new mutation, *polA*ex1, in the structural gene for polymerase I drastically reduces the 5′ → 3′ exonuclease activity but does not markedly alter its polymerase activity. The functional lesion in polymerase I determined by *polA*ex1 is thus distinct from that in other *polA* mutants, which are deficient in polymerase, but not in 5′ → 3′ exonuclease (9). This difference may reflect the enzymic discreteness of these two activities, which can be separated by proteolytic cleavage of polymerase I *in vitro* (15, 16). Glickman *et al.* (17) and Heijneker *et al.* (18) have very recently observed a mutation (*polA'*107) that may be similar to *polA*ex1; however, the extent of the enzymatic defect is not completely clear, and the *polA'*107 mutation is not conditionally lethal.

*E. coli polA*ex1 is similar to other *polA* mutants in its retarded sealing of "Okazaki fragments," its sensitivity to methyl methanesulfonate and to ultraviolet irradiation, and its inability to form plaques of λ *red*⁻ phage. Thus, both polymerase and 5′ → 3′ exonuclease activities may be required *in vivo* for normal DNA replication and repair. Recent experiments indicate that both are in fact necessary for efficient excision of thymine dimers *in vitro* (19). Furthermore, coordinated removal of the RNA primer of an "Okazaki fragment" (20) by the 5′ → 3′ exonuclease associated with polymerase I and extension of the 3′ terminus of the abutting fragment by the polymerase might be necessary for discontinuous DNA replication. Alternatively, the *polA* and *polA*ex1 mutations may exert their effect on the cell through some other alteration in polymerase I activity that has not been detected. Purification and study of the mutant enzyme may show whether this is indeed the case.

The conditional lethality of *polA*ex1 is novel and establishes that polymerase I is essential for viability. The findings reported in this paper do not establish the reason for this essentiality, however. An explanation of the nonlethality of other *polA* mutations will require the isolation of mutations less leaky than these, or a better understanding of the role *in vivo* of the various activities that are associated with polymerase I.

*E. coli polA*ex1 was originally identified by its "hyper rec" phenotype. We have found this phenotype to be characteristic of several different types of mutants that accumulate "Okazaki fragments," including ones defective in DNA ligase (7). Although we have shown that the increased number of papillae is correlated with an increased rate of formation of lactose positive recombinants in our *lac* diploid strains, we have not yet determined whether it is also correlated with an increased frequency of recombination in other types of genetic crosses.

This work was supported in part by a grant from the National Institutes of Health (GM-06196). E.B.K. is a Postdoctoral Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. We are very grateful to Janice R. Chien for her help with the DNA polymerase I and exodeoxyribonuclease II assays. Preliminary work with "hyper rec" mutants was done by E.B.K. in the laboratory of J. R. Beckwith and supported by a grant to J. R. Beckwith from the United States Public Health Service.

1. De Lucia, P. & Cairns, J. (1969) *Nature* **224**, 1164–1166.
2. Kato, T. & Kondo, S. (1970) *J. Bacteriol.* **104**, 871–881.
3. Bazill, G. W., Hall, R. & Gross, J. D. (1971) *Nature New Biol.* **233**, 281–283.
4. Monk, M. & Kinross, J. (1972) *J. Bacteriol.* **109**, 971–978.
5. Zissler, J., Signer, E. & Schaefer, F. (1972) in *The Bacteriophage Lambda*, ed. A. D. Hershey (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 455–468.
6. Okazaki, R., Arisawa, M. & Sugino, A. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2954–2957.
7. Konrad, E. B., Modrich, P. & Lehman, I. R., unpublished results.
8. Kuempel, P. L. & Veomett, G. E. (1970) *Biochem. Biophys. Res. Commun.* **41**, 973–980.
9. Lehman, I. R. & Chien, J. R. (1973) *J. Biol. Chem.* **248**, 7717–7723.
10. Miller, J. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
11. Steinberg, C. M. & Edgar, R. S. (1972) *Genetics* **47**, 187–208.
12. Konrad, E. B., Modrich, P. & Lehman, I. R. (1973) *J. Mol. Biol.* **77**, 519–529.
13. Low, K. B. (1972) *Bacteriol. Rev.* **36**, 587–607.
14. Taylor, A. L. & Trotter, D. (1972) *Bacteriol. Rev.* **36**, 504–524.
15. Brutlag, D., Atkinson, M. R., Setlow, P. & Kornberg, A. (1969) *Biochem. Biophys. Res. Commun.* **37**, 982.
16. Klenow, H. & Henningsen, I. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 168.
17. Glickman, B. W., von Sluis, C. A., Heijneker, H. L. & Rörsch, A. (1973) *Mol. Gen. Genet* **124**, 69–82.
18. Heijneker, H. L., Ellens, D. J., Tjeerde, R. H., Glickman, B. W., van Dorp, B. & Pouwels, P. H. (1973) *Mol. Gen. Genet.* **124**, 83–96.
19. Friedberg, E. & Lehman, I. R., manuscript in preparation.
20. Hirose, S., Okazaki, R. & Tamanoi, F. (1973) *J. Mol. Biol.* **77**, 501–518.
21. Beckwith, J. R. & Signer, E. R. (1966) *J. Mol. Biol.* **19**, 254.
22. Bachman, B. J. (1972) *Bacteriol. Rev.* **36**, 525–557.