

DNA Synthesis in Strains of *Escherichia coli* K12 with Temperature-sensitive DNA Ligase and DNA Polymerase I

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The net DNA synthesis that persists at the restrictive temperature in the conditional lethal DNA ligase mutant *Escherichia coli ligts7* is semiconservative, suggesting that although the rate of joining of 10 S “Okazaki fragments” in the mutant is greatly reduced, it is nevertheless sufficient to permit continued progression of the replication fork and the initiation of new rounds of replication.

DNA synthesis in *E. coli ligts7* of a phage (P2) that replicates its chromosomes unidirectionally is discontinuous on both strands.

A double mutant of *E. coli* K12, (*ligts7 polA12*), has been constructed which bears a temperature-sensitive mutation (*polA12*) in the structural gene for DNA polymerase I in addition to the *ligts7* mutation. Joining of the Okazaki fragments in the double mutant occurs at a slower rate than in either the *ligts7* or *polA12* parents. In contrast to the behavior of the single mutants, DNA synthesis in the double mutant stops abruptly upon shift from 25°C to 42°C.

1. Introduction

Escherichia coli strains with the temperature-sensitive, conditional lethal mutation *ligts7* have an abnormally thermolabile DNA ligase (Pauling & Hamm, 1969; Konrad *et al.*, 1973; Gottesman *et al.*, 1973). Joining of 10 S “Okazaki fragments” in these strains is greatly retarded at a non-permissive temperature. Nevertheless, substantial net synthesis of DNA, predominantly as 10 S fragments, occurs during a period of about 100 minutes after a shift from 25°C to 42°C. To explain this net DNA synthesis, we have suggested that the replication fork continues to advance under these conditions and the rate of joining of Okazaki fragments, although low, is adequate to permit more than one round of replication (Konrad *et al.*, 1973). In this paper we present evidence that DNA synthesis in *E. coli ligts7* at 42°C is semi-conservative, a finding which supports this hypothesis.

Mutants deficient in DNA polymerase I (*polA*⁻) are also defective in joining 10 S fragments (Kuempel & Veomett, 1970; Okazaki *et al.*, 1971). We have constructed a double mutant that carries a temperature-sensitive mutation (*polA12*) in the structural gene for this enzyme (Monk & Kinross, 1972; Lehman & Chien, 1973) in addition to the *ligts7* mutation. The double mutant is more defective in sealing Okazaki fragments than either *E. coli ligts7* or *polA12* and, in contrast to either single mutant, it does not synthesize DNA at 42°C.

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2. Materials and Methods

(a) *Strains and bacteriological methods*

The *E. coli* K12 strains used in this work are described in Table 1. Bacterial matings were carried out according to Miller *et al.* (1968) and transductions with P1vir phage were performed by the procedure of Signer (1966), except that transductants were selected on plates supplemented with 5 mM-sodium citrate. P2vir phage was obtained from Dr R. Calendar, University of California, Berkeley.

TABLE 1

E. coli K12 strains used in the construction of DNA ligase-DNA polymerase I double mutant (*E. coli* ligts7 polA12)

Strain	Genotype	Reference or Construction
KS243	Hfr KL16 <i>thi</i> ⁻ <i>pts</i> 140	Strain CHEp40 from W. Epstein
KS244	Hfr KL16 <i>thi</i> ⁻ <i>ligts</i> 7	KS243 transduced with P1vir grown on KS268
KS268	F ⁻ <i>ligts</i> 7 <i>lac</i> YA482 <i>thyA</i> ⁻ <i>str</i> ^r	Konrad <i>et al.</i> (1973)
KS271	F ⁻ <i>thyA</i> ⁻ <i>rha</i> ⁻ <i>lac</i> Y14 <i>polA</i> 12 <i>str</i> ^r	Monk & Kinross (1972)
KS272	F ⁻ <i>thyA</i> ⁻ <i>rha</i> ⁻ <i>lac</i> Y14 <i>polA</i> 12 <i>pts</i> 140 <i>str</i> ^r	Mating between KS243 and KS271
KS300	F ⁻ <i>ligts</i> 7 <i>polA</i> 12 <i>thyA</i> ⁻ <i>rha</i> ⁻ <i>lac</i> Y14 <i>str</i> ^r	Mating between KS244 and KS272

(b) *Media*

Medium II (Hanawalt & Cooper, 1971) contains 0.1 M-Tris·HCl (pH 7.5), 0.047 M-NH₄Cl, 0.037 M-KCl, 0.5 mM-Na₂SO₄, 1 mM-Na₂HPO₄, 2 mM-MgSO₄, 1.5 mM-CaCl₂, 2.5 μM-FeCl₃, 0.1% glucose, 10 μg thiamine/ml, 10 μg thymidine/ml, and 5 μg each amino acid/ml, except glutamine and asparagine. M63 plates (Pardee *et al.*, 1959) supplemented with 0.2% glucose, 10 μg thiamine/ml, and 10 μg thymidine/ml were used for determining viable titers. Tryptone/yeast extract plates are described by Miller (1972) and H broth by Steinberg & Edgar (1962).

(c) *Density shift experiments*

E. coli ligts7 (KS268) was grown at 25°C in medium II containing ¹⁵NH₄Cl (99.3% ¹⁵N, Biorad), [¹³C]glucose (>50% ¹³C, Merck, Sharp and Dohme), 2.5 μg thymidine/ml and, if indicated, 10 μCi [³H]thymidine/ml (6 Ci/mmol, Schwarz/Mann). The cells were harvested by centrifugation at *A*₅₉₅ = 0.4, washed with an equal volume of medium II containing ¹⁴NH₄Cl and [¹²C]glucose, and suspended in an equal volume of medium II without the heavy isotope supplement; thymidine (2.5 μg/ml), or [³H]thymidine (10 μCi/ml) were added as indicated. The culture was incubated for 5 min at 25°C, and then transferred to 42°C. The *A*₅₉₅ was maintained below 0.8 by dilution with fresh medium (prewarmed to 42°C). Samples (7 to 15 ml) were removed at the indicated times and made 0.025 M in EDTA and 0.0125 M in KCN. The cells were harvested by centrifugation in the cold, and DNA was extracted as described by Sugino *et al.* (1972), except that spheroplast lysis and Pronase digestion was done at 25°C. *E. coli* [³²P]DNA was added as a light density marker, and the DNA samples were adjusted to a weight of 6 g by the addition of 0.02 M-Tris·HCl (pH 8.0), and 10 mM-EDTA. After adding 8 g of CsCl, the samples were centrifuged at 35,000 revs/min for 70 h at 20°C in a type 40 rotor in a Beckman L3-50 ultracentrifuge. Fractions were collected on glass filters (Whatman GF/C, 2.4 cm circles) and radioactivity determined.

(d) *DNA turnover in the double mutant, E. coli ligts7 polA12*

(i) *Culture A*

Cells were grown at 25°C to *A*₅₉₅ = 0.17 in medium II, supplemented with 17 μCi [³H]thymidine/ml (6 Ci/mmol), then harvested and resuspended in an equal volume of medium without [³H]thymidine. After incubation for 10 min at 25°C, the culture was

shifted to 43°C. At the indicated times, [³H]thymidine in DNA was measured by adding 0.3 ml of culture to 0.5 ml of 17% trichloroacetic acid. After 10 min at 0°C, the precipitate was collected on GF/C (Whatman) filters, washed 6 times with 10 ml of chilled 1 N-HCl, and once with 10 ml of chilled ethanol, and the radioactivity was determined.

(ii) *Culture B*

Cells were grown at 25°C to $A_{595} = 0.14$ in medium II with unlabeled thymidine, then harvested and resuspended in an equal volume of fresh medium. After incubation for 10 min at 25°C, the culture was shifted to 43°C. After 10 min, [³H]thymidine (17 μ Ci/ml) was added, and incubation at 43°C was continued for 25 min. The cells were then harvested and resuspended in an equal volume of medium without [³H]thymidine, and incubated again at 43°C. At the indicated times, [³H]thymidine in DNA was measured as described above.

(e) *Preparation of P2vir phage and P2vir phage DNA*

Phage P2vir was grown as described by Lengyel *et al.* (1973). Bacterial debris was removed from the 2-l lysate by centrifugation at 10,000 g for 10 min. The phage were then harvested by centrifugation for 18 h at 10,000 g and resuspended in 30 ml of medium II supplemented with 20 mM-MgCl₂. Five-ml samples of the suspension were layered over a block gradient of CsCl in 0.1 M-Tris·HCl (pH 7.4) (1 ml, 1 ml, 0.5 ml, 1 ml and 1 ml of densities 1.70, 1.60, 1.50, 1.40, and 1.30 g/ml, respectively) and centrifuged for 5 h at 15°C at 30,000 revs/min in a Beckman SW40 rotor. The band of P2vir phage was identified visually and collected by puncturing the side of the tube. The phage suspension was then adjusted to a density of 1.70 g/ml with a saturated solution of CsCl in 0.1 M-Tris·HCl (pH 7.4) and layered under a block gradient of CsCl in 0.1 M-Tris·HCl (pH 7.4) (1 ml each with densities of 1.60, 1.50, 1.40 and 1.30 g/ml) and centrifuged and collected as before. Isolation of P2vir and separation of strands to yield P2(L) and P2(H) was performed by the method of Hradecna & Szybalski (1967), except that poly(U,G) was used instead of poly(I,G). The poly(U,G) was hydrolyzed with 0.5 N-NaOH at 37°C for 120 min, and solutions of the separated strands were dialyzed once against 1.1 of 0.1 M-Tris·HCl (pH 7.4), 1 mM-EDTA for 12 h, then twice against 1.1 portions of 0.02 M-Tris·HCl (pH 8.4), 1 mM-EDTA for 12 h.

(f) *Preparation of pulse-labeled DNA from P2vir-infected E. coli ligts7*

A 25-ml culture of *E. coli ligts7* in M63 buffer supplemented with 0.2% glucose, 0.5% Casamino acids, 10 μ g thymidine/ml, and 10 μ g thiamine/ml was grown at 25°C to $A_{595} = 0.3$ (a viable titer of 8×10^7 cells/ml). CaCl₂ was added to 1 mM, followed by P2vir phage at a multiplicity of 20. The culture was allowed to stand for 5 min and then aerated for 25 min, by which time the viable titer had decreased by 90%. The cells were harvested by centrifugation, resuspended in an equal volume of warmed (43°C) medium lacking thymidine, and aerated for 2.5 min at 43°C. [³H]thymidine (24 Ci/mmol) was then added to a concentration of 16 μ Ci/ml. After 10 s the pulse was quenched by adding an equal volume of 0.02 M-sodium acetate (pH 5.3), 2 mM-EDTA, 2% phenol, and 75% ethanol. The suspension was chilled and centrifuged for 10 min at 15,000 g. The pellet was re-suspended in 1 ml of 0.5 M-NaOH, 10 mM-EDTA and placed at 37°C for 60 min. After centrifugation for 15 min at 15,000 g, the supernatant fluid was removed and saved. The pellet was then re-extracted with 0.2 M-NaOH, 10 mM-EDTA as above; both supernatant solutions were pooled and stored at -20°C. Greater than 96% of the acid-precipitable ³H was recovered in the supernatant solutions by this method.

Pulse-labeled DNA from uninfected *E. coli ligts7* was prepared in the same way except that P2vir phage was omitted. Pulse-labeled DNAs from P2vir-infected and uninfected *E. coli lig*⁺ were also prepared by this procedure except that 6-ml cultures were used, and pulse labeling was done with 8 μ Ci of [³H]thymidine/ml.

(g) *Alkaline sucrose gradients*

5% to 20% alkaline sucrose gradients were run as described by Konrad *et al.* (1973), except that a Spinco SW41 rotor was used at 40,000 revs/min. DNA extract (0.3 ml) was layered onto each gradient.

(h) *DNA hybridization*

DNA hybridization was performed according to Berg, Morrow, Rhodes, Brutlag & Dieckmann (unpublished results). The 1.0-ml annealing mixtures contained: P2(L) or P2(H) DNA at $A_{260} = 0.05$, denatured, pulse-labeled [^3H]DNA from P2vir-infected *E. coli ligts7* (or uninfected *E. coli ligts7*) at $A_{260} = 0.02$, and sonicated, denatured salmon sperm DNA at $A_{260} = 5.0$, in a solution composed of 10 mM-Tris·HCl (pH 7.5), 1 mM-EDTA, and 0.5 M-NaCl. Hybridization was measured as [^3H]DNA made resistant to S1 nuclease (Ando, 1966), kindly provided by Dr Paul Berg.

(i) *Other methods*

Procedures for measuring the rate of [^3H]thymidine incorporation and total DNA content of cultures have been described previously (Konrad *et al.*, 1973). Assays of DNA ligase activity in crude extracts were performed according to Modrich & Lehman (1970). The DNA polymerase I activity of 40 to 60% ammonium sulfate fractions derived from extracts of *E. coli ligts7*, *E. coli polA12*, and *E. coli ligts7 polA12* was assayed as described by Lehman & Chien (1973) using (dA) $_{800}$ ·(dT) $_{10}$ as template primer. Radioactivity was measured by liquid-scintillation counting using a Beckman LS230 spectrometer.

3. Results

(a) *DNA synthesis is sustained in the ligase mutant at the restrictive temperature*

DNA synthesis in a culture of *E. coli ligts7* grown in medium II continued for at least 90 minutes after shift to a non-permissive temperature (Fig. 1). The net increase in DNA during this period was about threefold (cf. Konrad *et al.*, 1973).

When *E. coli ligts7* was labeled at 25°C in "heavy" medium II containing [^{13}C]glucose, $^{15}\text{NH}_4\text{Cl}$, and [^3H]thymidine, and then transferred to "light" unlabeled medium at 42°C (Fig. 2(a)) approximately half of the ^3H -labeled DNA was hybrid in density by the time the total DNA content of the culture had increased twofold (Fig. 3, 40 min). Similarly, when DNA synthesized at 42°C in an identical density shift experiment was labeled with [^3H]thymidine, (Fig. 2(b)) it was also predominantly hybrid in density after 40 minutes at 42°C (Fig. 4). The lag seen before newly synthesized DNA of fully hybrid density appeared (Fig. 4, 0 and 40 min) is presumably due to the time required for exhaustion of the intracellular pool of deoxynucleoside triphosphates labeled with heavy isotopes (Hanawalt & Cooper, 1971).

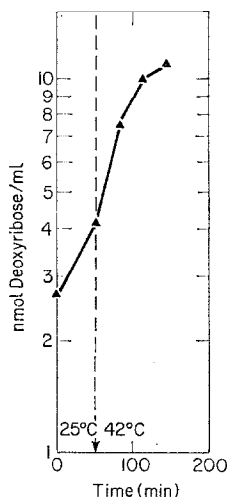


FIG. 1. DNA synthesis in *E. coli ligts7* at 42°C. Cells were grown in medium II.

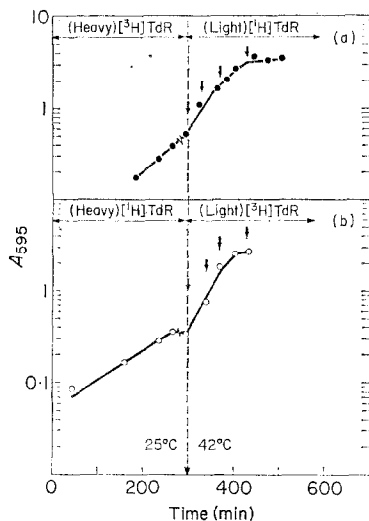


FIG. 2. Protocol for density shift experiments. *E. coli ligts7* was grown in density labeled medium II at 25°C in the presence or absence of [³H]thymidine (Materials and Methods). At 265 min the cells were collected, washed, and resuspended in light medium containing unlabeled or [³H]thymidine as indicated; the discontinuity represents the time required for this operation. After a 5-min incubation in light medium at 25°C, the cultures were transferred to 42°C. Samples were removed at the times indicated by the arrows.

These results are consistent with semi-conservative replication of most of the DNA in the mutant culture for one generation at 42°C. The heavy DNA synthesized at 25°C persisting after prolonged incubation at 42°C (Fig. 3, 145 min) may represent a small population of chromosomes that have stopped replicating, or a portion of some (or all) chromosomes that remain unreplicated at 42°C. By 85 minutes most of the ³H-labeled DNA synthesized at 42°C appeared in the light region (Fig. 4), indicating that some reinitiation of DNA replication must occur on daughter strands synthesized in the ligase mutant at this temperature.

The density shift experiments shown in Figures 3 and 4 were not complicated by the presence of free, single-stranded DNA, since less than 10% of the ³H-labeled DNA was degraded by prolonged incubation with an excess of exonuclease I, a nuclease specific for single-stranded DNA (Lehman, 1960). Similarly there was little, if any, degradation of density-labeled DNA since the [³H]thymidine incorporated into DNA (Figs 3 and 4) remained acid-precipitable (data not shown).

(b) *Discontinuous synthesis of both DNA strands in the ligase mutant at the restrictive temperature*

Since the two strands of phage P2 can be separated (Hradecna & Szybalski, 1967), P2-infected *E. coli ligts7* was examined to determine whether DNA synthesis is discontinuous on one or both of the P2 DNA strands. As shown in Figure 5(a) and (b), the sedimentation profiles of pulse-labeled DNA from uninfected and P2*vir*-infected *E. coli ligts7* were very similar. In both profiles nearly all the pulse-labeled DNA sedimented as a single 10 S peak, while the corresponding profiles for *E. coli lig*⁺ (Fig. 5(c) and (d)) showed a substantial proportion of the pulse-labeled DNA sedimenting faster than 16 S.

The pulse-labeled DNA isolated from P2*vir*-infected *E. coli ligts7* hybridized with

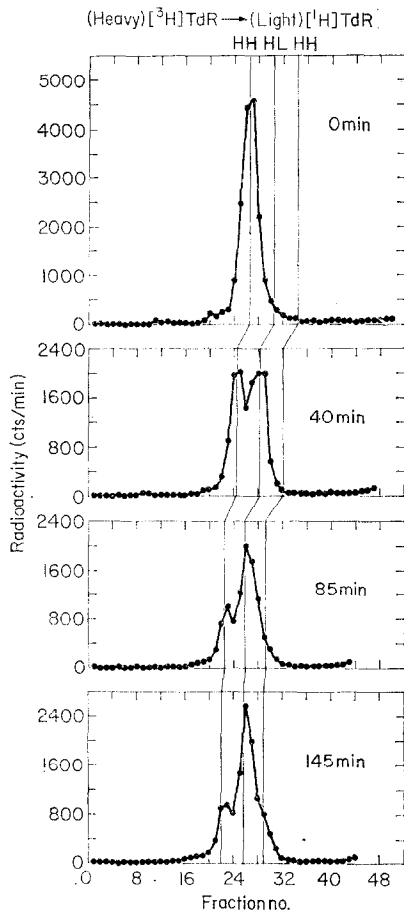


FIG. 3. CsCl equilibrium centrifugation of DNA from density shift experiment shown in Fig. 2(a). The times shown indicate the time of DNA extraction after shift of the culture to 42°C. The position of light DNA was determined from the position of a ^{32}P -labeled internal marker (Materials and Methods); the positions of heavy and hybrid DNA were estimated from the density difference between light and heavy DNA shown in the top panel of this Figure.

both of the separated strands (P2(H) and P2(L)) of P2 DNA (Table 2), demonstrating that they were both synthesized discontinuously.

(c) *Cessation of DNA synthesis and cell death at the restrictive temperature in a DNA ligase-DNA polymerase I double mutant*

A double mutant, *E. coli ligts7 polA12* was constructed by introducing the region of the chromosome containing *pts* and *lig* (Konrad *et al.*, 1973; Gottesman *et al.*, 1973) into an F^- *polA12 pts^-* recipient by means of an Hfr *ligts7 pts^+* donor strain (Table 1). Extracts of one *pts^+* recombinant from this cross (KS300) were assayed and found to be deficient in both DNA ligase and DNA polymerase I (Konrad *et al.*, 1973; Lehman & Chien, 1973).

The double mutant grew more slowly at 25°C than wild-type *E. coli* in medium II or H broth (190 against 120 min and 120 against 70 min doubling times, respectively) and on agar plates containing minimal medium. However, it did not form

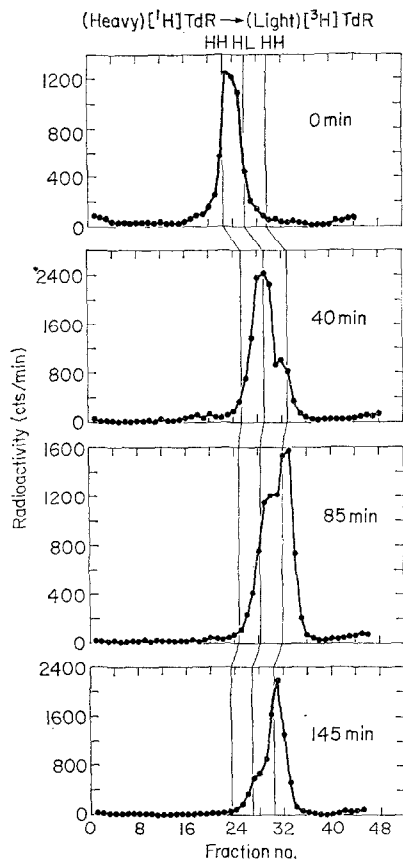


FIG. 4. CsCl equilibrium centrifugation of DNA from the density shift experiment shown in Fig. 2(b). The procedure is described in the legend to Fig. 3.

colonies on tryptone/yeast extract plates. Both the parental *ligts7* and *polA12* strains showed near normal growth rates at this temperature in the above-mentioned media.

After a shift from 25°C to 42°C, the cell mass in a culture of the double mutant in medium II increased about threefold. However, the viable titer declined rapidly (Fig. 6), and net DNA synthesis stopped (Fig. 7). This cessation of net DNA synthesis was not the result of concomitant synthesis and degradation, since the rate of incorporation of [³H]thymidine into DNA with either a short (3 min) pulse at 42°C (Fig. 7), or a long (25 min) pulse (Fig. 8) was reduced. Furthermore, [³H]-thymidine incorporated into DNA at 25°C was stable for 100 minutes after shift of the cells to the restrictive temperature (Figs 7 and 8). In contrast to these results in medium II (minimal medium supplemented with amino acids), in broth cultures, DNA was degraded rapidly very soon after the temperature shift (Fig. 9).

The double mutant incorporated [³H]thymidine predominantly into 10 S fragments even during a relatively long (30 s) pulse at 43°C (Fig. 10(a)). When the pulse

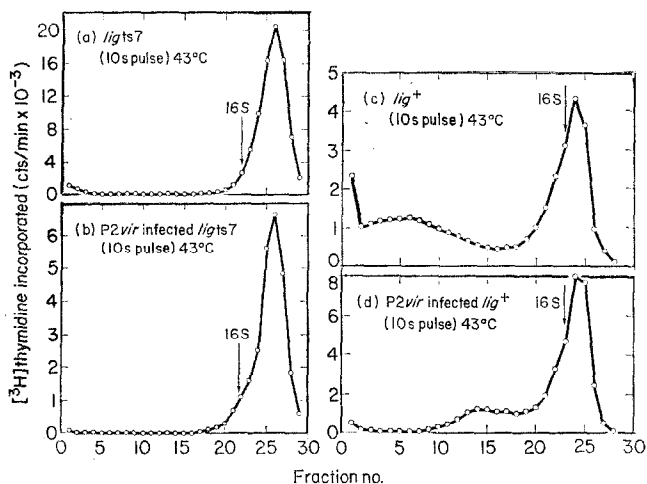


FIG. 5. Sedimentation profile in alkaline sucrose gradients of pulse-labeled DNA extracted from: (a) *E. coli ligts7* after 10 s pulse at 43°C; (b) P2vir-infected *E. coli ligts7* after 10 s pulse at 43°C; (c) *E. coli lig+* after 10 s pulse at 43°C; (d) P2vir-infected *E. coli lig+* after 10 s pulse at 43°C.

TABLE 2

Hybridization of pulse-labeled DNA with separated single strands of Phage P2 DNA

DNA in annealing mixture ³ H-labeled	Unlabeled	S1 nuclease-resistant ³ H		% of Total ³ H hybridized
		0 min	15 min	
		cts/min per ml		
<i>ligts7</i> P2vir-infected	0	0	240	26
	P2(L)†	0	679	47
	P2(H)†	7	683	43
<i>ligts7</i> —uninfected	0	9	128	9.6
	P2(L)	13	51	3.3
	P2(H)	19	39	1.6

The annealing temperature was 68°C. Samples were withdrawn at 0 and 15 min and acid-precipitable ³H, resistant to S1 nuclease, was determined. The percentage of total ³H hybridized is expressed as a percentage of nuclease-resistant acid-precipitable ³H after 15 min at 68°C, less the percentage of nuclease resistant, acid-precipitable ³H at 0 min. Procedures are described in Materials and Methods. DNA from *ligts7* and *ligts7*(P2vir) are pooled fractions with a sedimentation coefficient less than 16 S from alkaline sucrose velocity gradients (Fig. 5) run as described in Materials and Methods.

† Corrected for a value of 26% self hybridization of *ligts7*(P2 vir).

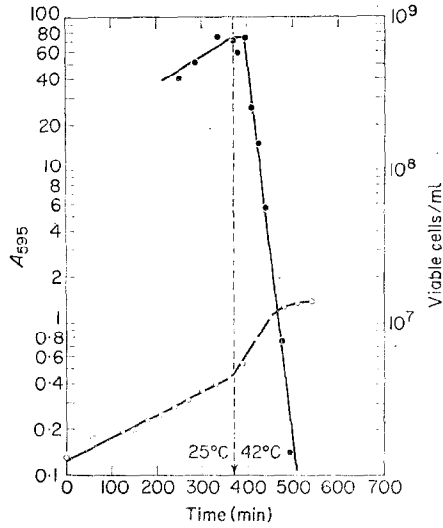


FIG. 6. Viable cell titer (—●—●—) and A_{595} (—○—○—) of the double mutant during the shift from 25°C to 42°C. KS300 was grown at 25°C in medium II and shifted to 42°C at 370 min. Viable titers were determined on M63 glucose plates at 25°C.

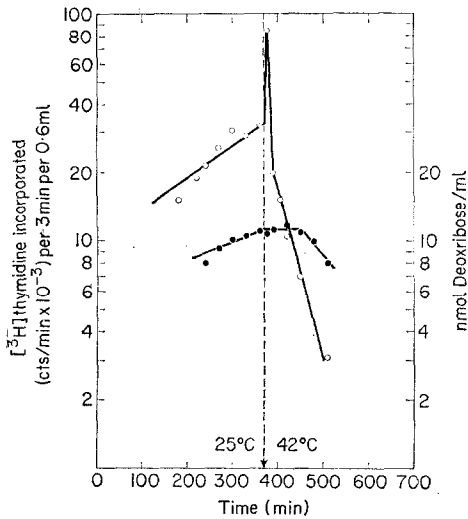


FIG. 7. DNA synthesis in the double mutant shifted from 25°C to 42°C. KS300 was grown as described in the legend to Fig. 6. The rate of [^3H]thymidine incorporation (—○—○—); determined for each point as the amount of incorporation during a 3-min pulse, and DNA content (—●—●—) were determined as described in Materials and Methods.

was followed by a 5-minute chase with unlabeled thymidine (Fig. 10(b)) some of the ^3H appeared in more rapidly sedimenting DNA, suggesting that some joining of 10 S fragments may occur even at the restrictive temperature.

4. Discussion

We have reported previously that DNA synthesized at 42°C in the temperature-sensitive, conditional lethal *E. coli* DNA ligase mutant *ligts7* is predominantly in

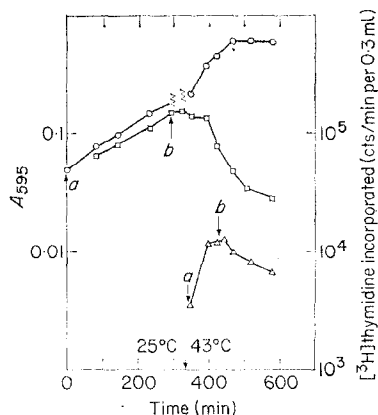


FIG. 8. DNA turnover in the double mutant. —○—○—, A_{595} of culture A (see Materials and Methods); —□—□—, $[^3\text{H}]$ thymidine incorporation in culture A; —△—△— $[^3\text{H}]$ thymidine incorporation in culture B. The letter *a* indicates addition of $[^3\text{H}]$ thymidine, and *b* indicates removal. The A_{595} of culture B was approximately that of culture A. Growth conditions and the assay for incorporation of $[^3\text{H}]$ thymidine into acid-precipitable material are described in Materials and Methods.

the form of 10 S fragments. Our finding that this synthesis is semi-conservative for at least one round of replication suggests that despite the much reduced rate of joining of Okazaki fragments the replication fork continues to progress in the mutant at the restrictive temperature. Thus, the persistence of some ligase activity at 42°C (Konrad *et al.*, 1973; Gottesman *et al.*, 1973) could allow the formation of parental strands sufficiently intact to support another round of replication, and thus account for the greater than twofold increase in DNA that we observed.

Unlike *E. coli*, phage P2 replicates its chromosome unidirectionally (Bird *et al.*, 1972; Schnos & Inman, 1971). Okazaki *et al.* (1973) have suggested that this unidirectional replication might occur asymmetrically with one daughter strand synthesized discontinuously and the other continuously. However, our results show that P2 replication is discontinuous on both strands in an *E. coli* *ligts7* host. Recently, Okazaki *et al.* (1973) have reached a similar conclusion on the basis of studies with a host strain deficient in DNA polymerase I.

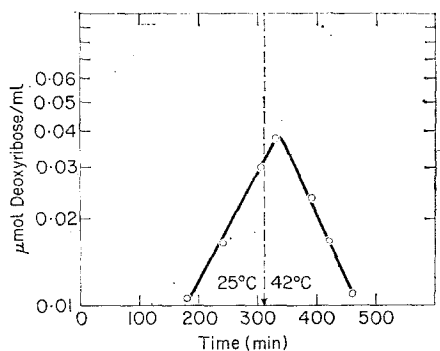


FIG. 9. DNA content of the double mutant in broth culture at 25°C and 42°C. KS300 was grown in H broth at 25°C and then shifted to 42°C as indicated.

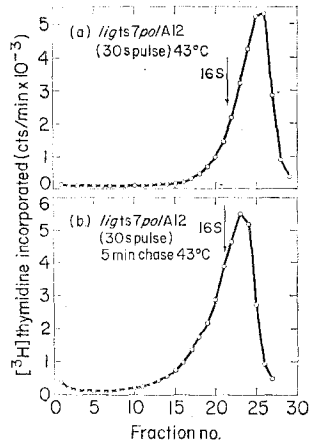


FIG. 10. Sedimentation profile in alkaline sucrose gradients of pulse-labeled DNA extracted from the double mutant: (a) 30-s pulse at 42°C; (b) 30-s pulse at 42°C, followed by a 5-min chase with 100 μg unlabeled thymidine/ml. Pulses were done at $A_{395} = 0.5$ with [^3H]thymidine (6 Ci/mmol), and quenched by adding 0.2 vol. chilled 0.05 M-KCN, 0.1 M-EDTA.

We have found that net DNA synthesis ceases abruptly at 42°C in medium II cultures of a double mutant whose DNA ligase and DNA polymerase I are both abnormally thermolabile. The stability of prelabeled DNA, and the greatly reduced incorporation of [^3H]thymidine during short pulses at this temperature indicate that this is due to cessation of DNA synthesis rather than a steady state between synthesis and degradation; although at later times, DNA degradation does occur. These results are in contrast to the substantial net DNA synthesis we have observed in a mutant defective in ligase alone following a similar temperature shift. Since the rate of joining of Okazaki fragments is more retarded in the double than in the single mutant, this difference might plausibly reflect an earlier appearance of double-strand chromosomal breaks in the double mutant. Alternatively, a deficiency in some other, as yet unidentified, activity associated with DNA ligase or polymerase I may be responsible for this effect.

The culture medium exerts a strong influence on the viability and DNA metabolism of the ligase-defective and the double mutants. Both show a more rapid loss of viability in broth cultures than in minimal media at 42°C, and the double mutant shows a complete loss of colony forming ability on tryptone/yeast extract plates, even at 25°C. Furthermore, in both mutants DNA degradation occurs sooner in broth than in minimal medium. A conjectural explanation for these exacerbations of the mutant phenotypes in broth cultures is that the time interval between rounds of replication is shorter in this medium (Bird & Lark, 1970), and this in turn allows less time for joining on the newly synthesized daughter strands before they must assume a template function.

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