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Genetic and Enzymatic Characterization of a Conditional Lethal Mutant of *Escherichia coli* K12 with a Temperature-sensitive DNA Ligase

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TAU *ts7*, an *Escherichia coli* 15 strain with a thermolabile DNA ligase, has previously been shown to be a temperature-sensitive conditional lethal mutant that is sensitive to methyl methane sulfonate and to ultraviolet irradiation; it also accumulates 10 S DNA fragments to an abnormal extent. When the ligase mutation is transferred to a wild-type *E. coli* K12 strain, the strain becomes temperature sensitive for growth and displays the same characteristics as TAU *ts7*. These findings show that a functional DNA ligase is essential for normal DNA replication and repair in *E. coli*.

1. Introduction

In 1968 Pauling and Hamm isolated a conditional lethal mutant of *Escherichia coli* 15, TAU *ts7*, which was unable to grow at 42°C (Pauling & Hamm, 1968). Examination of the DNA ligase activity in the mutant strain showed it to be abnormally low at 25°C and undetectable at 42°C, measured by enzyme–AMP formation or in the over-all joining reaction (Modrich & Lehman, 1971). The mutant was also more sensitive to ultraviolet light and to the alkylating agent methyl methane sulfonate than wild-type *E. coli* 15 even at 25°C, and it accumulated newly replicated DNA in the form of small, 10 S fragments (Pauling & Hamm, 1968,1969). These properties are consistent with DNA ligase of *E. coli* being an essential enzyme that functions in DNA replication (Okazaki *et al.*, 1968) and probably in the repair of damage to DNA induced by u.v. light and by methyl methane sulfonate (Howard-Flanders & Boyce, 1966).

More recently, Gellert and his colleagues have isolated a mutant of *E. coli* K12 with a thermolabile DNA ligase (Gellert & Bullock, 1970; Gottesman *et al.*, 1973). This strain (*E. coli* K12 *lig4*) is similar to TAU *ts7* in that it shows an abnormal accumulation of 10 S fragments and an increased sensitivity to methyl methane sulfonate. However, it is not sensitive to u.v. irradiation, and is completely viable at 42°C (Gottesman *et al.*, 1973). The viability of this strain at elevated temperatures may be due to the presence of sufficient residual ligase activity at these temperatures to permit cell growth. In fact, although extracts of *E. coli* K12 *lig4* show little or no joining activity at 42°C, enzyme–AMP formation proceeds normally at this temperature. An alternative explanation is that DNA ligase is not an essential enzyme

for *E. coli*, and that a secondary mutation, or one of the plasmids or cryptic prophages carried by *E. coli* 15 (Ikeda *et al.*, 1970) is involved in the conditional lethality shown by strain $\overline{\text{TAU}}$ *ts7*. The mutagen used in the isolation of *ligts7*, *N*-methyl-*N'*-nitro-nitrosoguanidine, is known to induce secondary, linked, mutations with a high frequency (Guerola *et al.*, 1971).

In this paper we show that when *ligts7*, the mutation responsible for thermolability of the DNA ligase of $\overline{\text{TAU}}$ *ts7*, is transferred to a wild-type *E. coli* K12 strain, the mutation is still conditionally lethal and has the same phenotype as in strain $\overline{\text{TAU}}$ *ts7*. Similar results have been obtained by Gottesman *et al.* (1973).

2. Materials and Methods

(a) *Bacterial and phage strains*

The *E. coli* K12 bacterial strains used in this work are listed in Table 1. Genetic markers are named after Taylor (1970); their locations on the *E. coli* map are shown in Fig. 1.

P1*vira*, isolated by B. Wolfe, is a derivative of P1*kc* (Lennox, 1955) which does not lysogenize (provided by J. Shapiro).

TABLE 1

Escherichia coli *K12 strains used in the transfer of the* ligts7 *mutation from* $\overline{\textit{TAU}}$ ts7 *into* E. coli *K12*

Strain	Genotype	Origin
KS358	*Hfr Hayes lacΔ U169 thi*$^-$ (P1CM)	Strain CA7027 of F. Jacob lysogenized by phage P1CM
KS359	*F' metB*$^+$/*metB*$^-$ *argG*$^-$ *leu*$^-$ *recA1 mal*$^-$ *xyl*$^-$ *mtl*$^-$ *str*^{r} (P1CM)	Strain KLF 10/B3 of B. Low lysogenized with phage P1CM
KS360	*Hfr Hayes lac YA482 thi*$^-$	F. Jacob strain CA7008
KS230	*F*$^-$ *ptsI140 proC*$^-$ *str*^{r}	W. Epstein strain FF804
KS231	*F*$^-$ *ptsI140 lacYA482 str*^{r}	KS360 × KS230
KS253	*F*$^-$ *lacYA482 ligts7 str*^{r}	Described in Table 2
KS254	*F*$^-$ *lacYA482 lig*$^+$ *str*^{r}	P1*vira* (KS252) × KS231
KS269	*F*$^-$ *lacYA482 lig*$^+$ *thyA*$^-$ *str*^{r}	Trimethoprim-resistant mutant of KS254

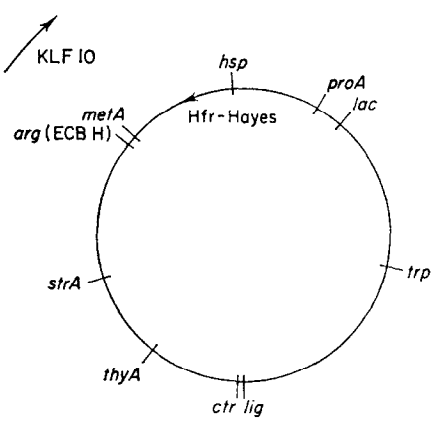

FIG. 1. Genetic loci cited in this paper. Map drawn after Taylor (1970). *ctr* refers to the *ptsI* locus.

(b) *Media*

H broth (Steinberg & Edgar, 1962) was used for liquid cultures. Tryptone/yeast extract plates (Gottesman & Beckwith, 1969) were used for determining viable cell titers. M63 plates (Pardee *et al.*, 1959) supplemented with 0·2% carbon source, 50 μg each of amino acids/ml, 10 μg each of purines and pyrimidines/ml and 150 μg streptomycin sulfate/ml as indicated, were used for selection of prototrophic recombinants. Tryptone/yeast extract, calcium + glucose plates (Adams & Luria, 1958) were used for preparation of P1*vira* phage stocks. H-top agar (Gottesman & Beckwith, 1969) was used for plating on broth plates and F-top agar (Miller *et al.*, 1968) was used for plating on minimal plates. Tryptone/yeast extract plates supplemented with 0·04% methyl methane sulfonate were used for determining sensitivity to methyl methane sulfonate.

(c) *Bacteriological methods*

All procedures were carried out at 37°C, except when strains carrying *ligts7* mutation were used, in which case the temperature was 25°C. Bacterial matings were carried out according to the method of Miller *et al.* (1968).

P1*vira* phage stocks were grown on strain KS252 by the following method. A culture of strain KS252 was grown to a titer of about 2×10^8/ml, made 5 mM in $CaCl_2$, and grown for an additional 5 min. One ml of this culture was mixed with 10^5 P1*vira* phage, incubated 20 min, then plated in 2·5 ml H-top agar. Phage were harvested after 12 h.

The following procedure was used to transduce strain KS231 to *ptsI*$^+$ (*ptsI* is the structural gene for enzyme I of the sugar phosphotransferase system (Epstein *et al.*, 1970)). Strain KS231 was grown to saturation, harvested, resuspended in an equal volume of 0·01 M-$MgSO_4$, 5 mM-$CaCl_2$ and incubated for 10 min. Samples (0·1 ml) were mixed with dilutions of P1*vira*, incubated 20 min, then plated in 2·5 ml F-top agar on M63 plates supplemented with mannitol.

(d) *Measurement of ultraviolet sensitivity*

Cell cultures were grown to an A_{595} of about 0·5, chilled, centrifuged and resuspended in an equal volume of M63 medium. A 6-ml sample was then irradiated in a Petri dish with agitation at an intensity of 28 ergs mm^{-2} s^{-1} at room temperature. At the indicated times, 0·1-ml portions were removed, diluted with H broth and plated on tryptone/yeast extract plates. Colonies were counted after incubation for 24 h at 30°C in the dark.

(e) *Isolation of pulse-labeled DNA*

The following procedure, modified from Okazaki *et al.* (1968), was used for isolation of DNA pulse-labeled with [^{3}H]thymidine. Cell cultures were grown to an A_{595} of about 0·5 in M63 medium supplemented with 0·2% glucose, 0·5% Casamino acids, and 10 μg thymidine/ml. A 6-ml sample was removed, centrifuged and the pellet resuspended in an equal volume of the same medium (without thymidine) that had been pre-warmed to the desired temperature. The suspension was agitated for 2 min, then 200 μCi of [^{3}H]-thymidine (6 Ci/mmol, Schwarz–Mann) were added. The pulse was quenched by adding 1·2 ml of a solution (0°C) containing 0·05 M-KCN and 0·1 M-EDTA, and the culture was quickly chilled to 0°C. The suspension was centrifuged and the pellet resuspended in 0·6 ml of 0·1 M-NaOH, 10 mM-EDTA. A quantity of M13 phage labeled with ^{32}P (provided by M. Jazwinski) was added as a sedimentation marker, and the mixture was incubated at 37°C for 1 h. It was then centrifuged at 20,000 *g* to remove insoluble material. The supernatant was saved and stored at −20°C. Samples of 0·3 ml were used for each gradient.

Alkaline sucrose gradients (5 to 20%) were run (Martin & Ames, 1961) in a Beckman L265B ultracentrifuge using an SW40 rotor for 4 h at 39,000 revs/min at 5°C, in solutions containing 0·9 M-NaCl, 0·1 M-NaOH, and 2 mM-EDTA in addition to the sucrose. At the end of the run, 0·4-ml fractions were collected from the bottom of the tube; 0·1 ml of calf thymus DNA (0·4 mM in nucleotide) was added to each fraction, followed by 0·6 ml of cold 10% trichloroacetic acid. Precipitates were collected on Whatman GF/C glass filters and washed with five 10-ml portions of cold 1 N-HCl. Radioactivity was determined in a Beckman LS230 liquid scintillation counter.

(f) *Other methods*

Procedures for measurement of the rate of [^{3}H]thymidine incorporation into DNA are given by Modrich & Lehman (1970). Total DNA was determined according to the method of Dische (1955). Assays for *E. coli* DNA ligase activity were carried out according to Modrich & Lehman (1970,1971), except that assay mixtures were warmed to the assay temperature for several minutes before the addition of enzyme.

3. Results

(a) *Transfer of* ligts7 *from* $\overline{TAU}$ ts7 *into* Escherichia coli *K12*

The *ligts7* mutation was transferred from $\overline{TAU}$ *ts7* into *E. coli* K12 *via* the generalized transducing phage P1 (Lennox, 1955) using the finding of Gottesman *et al.* (1973) that *lig* is cotransduced by P1 with *ptsI*. This method was chosen since it introduces only about 1% of the chromosome from the donor strain into the recipient strain (Taylor & Trotter, 1967). However, it was necessary to modify $\overline{TAU}$ *ts7* before it could serve as a donor in this cross. First, the DNA restriction and modification locus of *E. coli* K12 (*hsp* K12) was introduced in place of the corresponding *E. coli* 15 locus (*hsp* A) (Stacy, 1965) to make a *hsp* K12 *ligts7* strain (KS250). This strain was then used to construct a *ligts7 hsp* K12 strain with a predominantly *E. coli* K12 constitution (KS252) which, unlike $\overline{TAU}$ *ts7*, could support good vegetative growth of phage P1*vira*. Details of these procedures are given in Table 2.

TABLE 2

Construction of Escherichia coli *K12* ligts 7

Strain	Genotype	Construction or origin
KS221	*F⁻ arg(HBCE)⁻ metA⁻ pro⁻ trp⁻ thy⁻ pyr⁻ ligts7 hsp A*	C. Pauling strain $\overline{TAU}$ *ts7*
KS222	*F⁻ arg(HBCE)⁻ metA⁻ pro⁻ trp⁻ thy⁻ pyr⁻ ligts7 hspA str^r*	Spontaneous *str^r* mutant of KS221
KS250	*F⁻ arg(HBCE)⁻ metA⁻ thy⁻ lacΔ U169 pyr⁻ ligts7 hsp K12 str^r*	KS358 × KS222
KS251	*F′ arg(HBCE)⁺ metA⁺/arg(HBCE)⁻ metA⁻ pyr⁻ thyA⁻ lacΔ U169 ligts7 hsp K12 str^r*	KS359 × KS250
KS252	*F⁻ proC⁻ ligts7 hsp K12 str^r*	KS251 × KS230. *Mt1⁺* recombinant that is temperature-sensitive and supports growth of phage P1.
KS253	*F⁻ lacYA482 ligts7 hsp K12 str^r*	P1*vira* (KS252) × KS231

The locations on the *E. coli* chromosome of the markers used in these crosses are shown in Fig. 1. The *metA⁻* mutation of KS221 was identified by the ability of the strain to grow on *O*-succinylhomoserine. The *arg(HBCE)⁻* mutation of this strain was identified by genetic crosses.

(b) *DNA ligase assays of parental and recombinant strains*

Nine transductants from a P1*vira* cross between strain KS252 (*ligts7 hsp* K12) and an *E. coli* K12 strain, KS321 (*ptsI⁻*) were analyzed. As shown in Table 3, temperature-sensitive enzyme–AMP formation associated with the *ligts7* mutation was cotransduced with *ptsI⁺* in six of the nine transductants. All six transductants that inherited *ligts7* were also sensitive to methyl methane sulfonate at 25°C and were

temperature-sensitive, conditionally lethal strains. On the other hand, all transductants that were *lig*$^+$ were also wild type in their sensitivity to methyl methane sulfonate and temperature. These findings are consistent with temperature-sensitive lethality being due to the *ligts7* mutation.

TABLE 3

Temperature-sensitive growth and methyl methane sulfonate sensitivity as a result of temperature-sensitive DNA ligase activity

	Temperature sensitivity of growth	Methyl methane sulfonate sensitivity (25°C)	Enzyme–AMP formed (pmol/mg) 25°C	40°C
Parents				
ptsI$^-$*lig*$^+$ (Strain KS231)	R	R	1·4	1·2
ptsI$^+$*ligts7* (Strain $\overline{\text{TAU}}$ *ts7*)	S	S	0·30	<0·02
Transductants				
7	R	R	1·2	0·99
9	R	R	0·90	0·77
16	R	R	1·0	0·88
10	S	S	0·20	<0·02
11	S	S	0·14	<0·02
12	S	S	0·19	<0·02
13	S	S	0·15	<0·02
14	S	S	0·18	<0·02
15	S	S	0·09	<0·02

Temperature sensitivity was scored on tryptone/yeast extract plates incubated at 42°C. Enzyme–AMP formation was determined according to the method of Modrich & Lehman (1971). Abbreviations used: R, resistant; S, sensitive.

An additional 227 transductants were examined for sensitivity to methyl methane sulfonate and temperature-sensitive conditional lethality. In none of these did the two traits segregate. This result further indicates that *ligts7* determines temperature-sensitive lethality, since two other mutants deficient in DNA ligase, which were isolated by completely independent methods, are also sensitive to methyl methane sulfonate: the *lig4* mutation described by Gellert & Bullock (1970), and a strain that one of us (Konrad, unpublished results) has isolated as a mutant with an enhanced frequency of recombination.

The DNA joining activity of extracts of the parental strains in the cross and of a temperature-sensitive and a temperature-resistant recombinant were also measured. These assays (Table 4) show that the temperature-sensitive joining activity characteristic of extracts of $\overline{\text{TAU}}$ *ts7* is also present in extracts of the *E. coli* K12 *ligts7* recombinants.

(c) *DNA synthesis and repair in* Escherichia coli *K12* ligts7

One *E. coli* K12 *ligts7* transductant (KS268) was more extensively characterized in the following series of experiments. The survival of *E. coli* K12 *lig*$^+$ (KS269) and *E. coli* K12 *ligts7* (KS268) during u.v. irradiation was measured. As shown in Figure 2,

TABLE 4

DNA joining activity of DNA ligase defective transductants

	Temperature sensitivity of growth	Joining activity (units/mg) 25°C	40°C
Parents			
ptsI$^-$ *lig*$^+$ (strain KS231)	R	1·6	1·4
ptsI$^+$ *ligts7* (strain $\overline{\text{TAU}}$ *ts7*)	S	0·034	<0·004
Transductant no.			
7	R	2·6	1·9
8	S	0·024	<0·004

DNA joining activity was determined by the procedure of Modrich & Lehman (1970). Abbreviations used: R, resistant; S, sensitive.

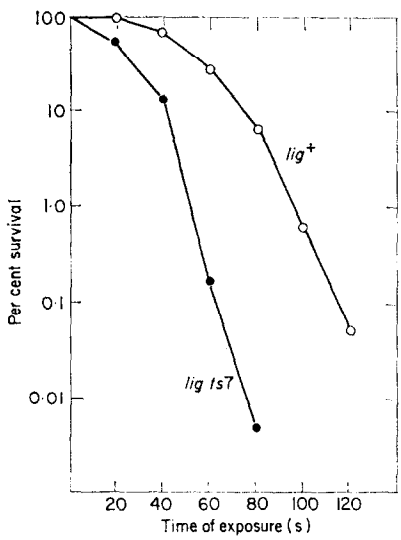

FIG. 2. Survival at 25°C of *E. coli* K12 (KS269) and *E. coli* K12 *ligts7* (KS268) exposed to u.v. irradiation. Cells were irradiated and viable titer was measured as described in Materials and Methods.

E. coli K12 *ligts7* was more sensitive to u.v. light than the *lig*$^+$ strain even at the permissive temperature. This result is consistent with a role for DNA ligase in the repair of u.v.-induced damage to DNA, since enzyme activity in extracts of *E. coli* K12 *ligts7* is low even at 25°C (see Table 3).

The effect on growth and DNA synthesis of shifting logarithmic cultures in H broth of *E. coli* K12 *ligts7* and *E. coli* K12 *lig*$^+$ from a permissive to a non-permissive temperature is shown in Figures 3, 4 and 5. In the case of *E. coli* K12 *lig*$^+$ there was an exponential increase in viable titer, cell density, rate of [^{3}H]thymidine incorporation and total DNA at both temperatures. *E. coli* K12 *ligts7* showed markedly different behavior. Following the shift from permissive to non-permissive temperature, the viable titer increased by about 50% within less than a generation time,

then began to decline. (This decline did not occur in minimal medium where viable titer remained stationary for up to two hours after shift to 42°C.) Cell mass, rate of thymidine incorporation, and total DNA accumulation all continued to increase for several generation times, then became stationary at levels several-fold higher than those existing just before the temperature shift. At this point the labeled thymidine

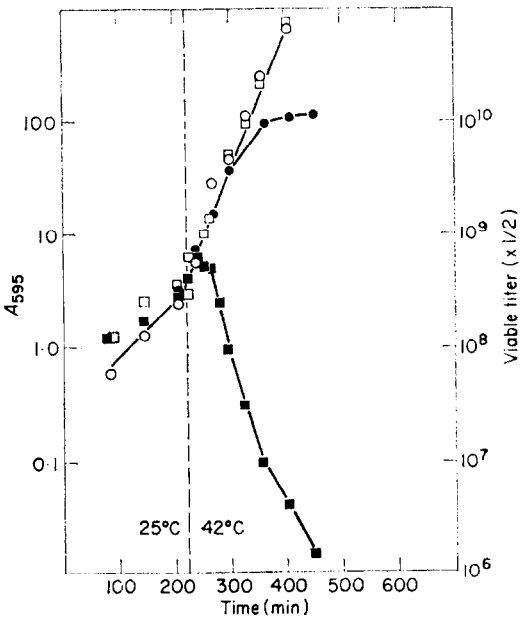

FIG. 3. Viable titer and A_{595} of cultures of *E. coli* K12 *ligts7* (KS268) and *E. coli* K12 *lig*$^+$ (KS269) shifted from 25°C to 42°C. A_{595} of *E. coli* K12 *ligts7*, (●); viable titer of *E. coli* K12 *ligts7*, (■); A_{595} of *E. coli* K12 *lig*$^+$, (○); viable titer of *E. coli* K12 *lig*$^+$, (□).

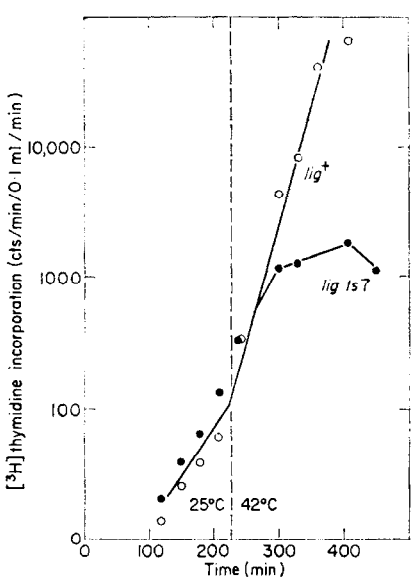

FIG. 4. Rate of incorporation of [^{3}H]thymidine into DNA during 3-min pulses of cultures shifted from 25°C to 42°C. *E. coli* K12 *ligts7*, (●), *E. coli* K12 *lig*$^+$, (○).

incorporated during a five-minute pulse was found predominantly in DNA with a sedimentation coefficient of less than 16 S (Fig. 6), and the cells showed an extremely elongated morphology.

Alkaline sucrose density gradient sedimentation profiles of DNA from *E. coli* K12 *ligts7* and *E. coli* K12 *lig*$^+$ labeled with a short pulse (10 to 30 s) of [^{3}H]thymidine show that the mutant accumulated 10 S fragments to a strikingly greater extent than did the wild-type strain, even at the permissive temperature (Fig. 7). Following a ten-second pulse at 25°C or 42°C, essentially all of the [^{3}H]thymidine incorporated into DNA by K12 *ligts7* had a sedimentation coefficient of about 10 S. Under the same conditions the *lig*$^+$ strain incorporated [^{3}H]thymidine primarily into DNA with a sedimentation coefficient of 24 S or greater.

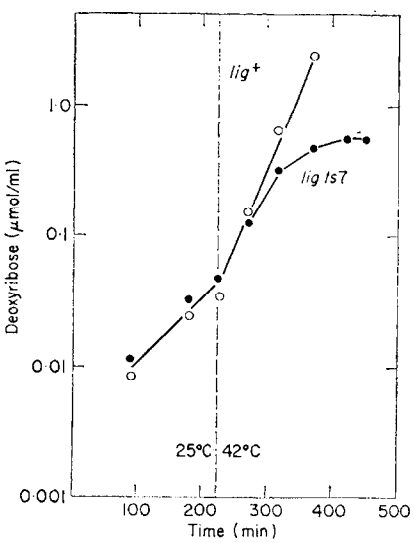

FIG. 5. DNA content of cultures shifted from 25°C to 42°C. *E. coli* K12 *ligts7*, (●); *E. coli* K12 *lig*$^+$, (○).

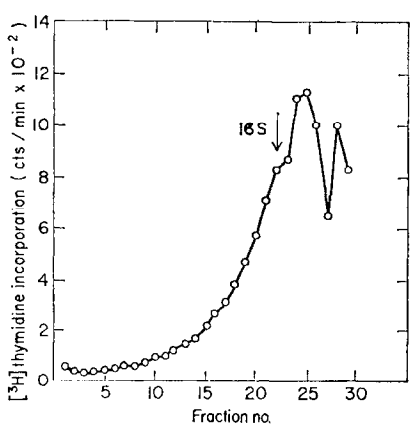

FIG. 6. Sedimentation profile in alkaline sucrose density gradient of labeled DNA extracted from *E. coli* K12 *ligts7* pulsed with [^{3}H]thymidine for 5 min at 250 min after temperature shift in the experiment shown in Fig. 5. The methods for pulse labeling with [^{3}H]thymidine, and isolation and sucrose density gradient centrifugation of the labeled DNA are described in Materials and Methods.

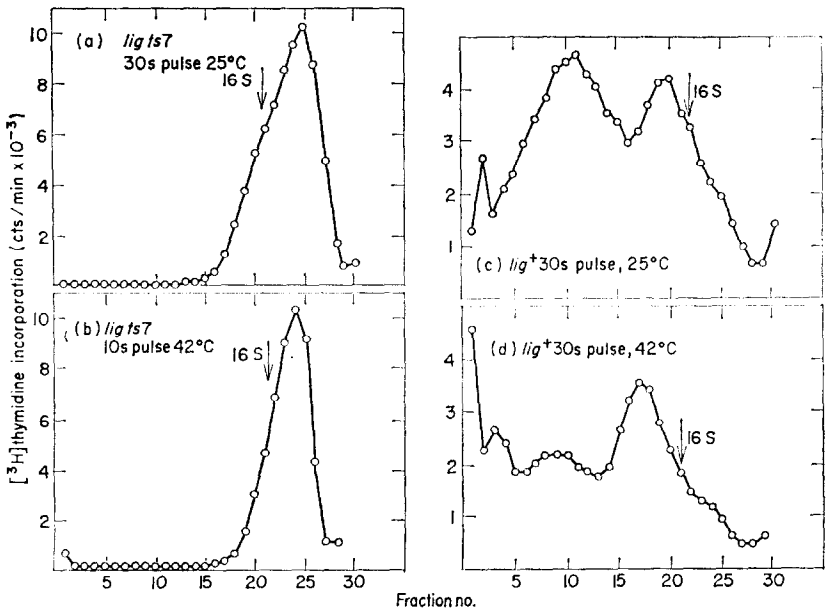

FIG. 7. Sedimentation profile in alkaline sucrose density gradients of labeled DNA extracted from: (a) *E. coli* K12 *ligts7* after 30-s pulse at 25°C; (b) *E. coli* K12 *ligts7* after 10-s pulse at 42°C; (c) *E. coli* K12 *lig*$^+$ after 30-s pulse at 25°C; (d) *E. coli* K12 *lig*$^+$ after 10-s pulse at 42°C. The methods for pulse labeling with [^{3}H]thymidine, and isolation and sucrose density gradient centrifugation of the labeled DNA are described in Materials and Methods.

When the ten-second pulse of K12 *ligts7* at 42°C was followed by a five-minute chase with excess unlabeled thymidine at 25°C, most of the [^{3}H]thymidine incorporated during the pulse period appeared in DNA with a median sedimentation coefficient of 31 S (data not shown). This result suggests that the 10 S material that accumulates in the mutant after pulsing with [^{3}H]thymidine at 42°C is a precursor of high molecular weight DNA. Similar pulse–chase experiments in which the ten-second pulse at 42°C was followed by a five-minute chase at 42°C also resulted in the appearance of much of the [^{3}H]thymidine in rapidly sedimenting DNA (median sedimentation coefficient of 23 S). The finding that a slow joining of 10 S DNA fragments does occur at 42°C indicates that residual DNA ligase activity persists at the restrictive temperature.

4. Discussion

The experiments described here taken together with those published previously (Modrich & Lehman, 1971) clearly permit a distinction between the two hypotheses that have been offered to explain the discrepancy between the phenotypes of $\overline{\text{TAU}}$ *ts7* and *E. coli* K12 *lig4*. Since the phenotype associated with *ligts7* is independent of the genetic background in which the mutation is situated, the hypothesis that the lethality of $\overline{\text{TAU}}$ *ts7* reflects a strain-specific requirement for the ligase can be eliminated. Therefore, we conclude that the viability of *lig4* at 42°C is due to the persistence of significant DNA ligase activity at this temperature. It is noteworthy that in contrast to the *lig4* enzyme, the *ligts7* ligase is temperature-sensitive even when measured by the formation of enzyme–AMP, an assay which is capable of detecting a single enzymatic turnover.

Like TAU *ts7*, *E. coli* K12 *ligts7* strains are abnormally sensitive to u.v. irradiation and to treatment with methyl methane sulfonate, even at permissive temperatures, suggesting that DNA ligase is involved in the repair *in vivo* of u.v. and methyl methane sulfonate-induced damage to DNA. Abnormal DNA replication is a further consequence of the *ligts7* mutation. Thus, when K12 *ligts7* was pulsed briefly (10 to 30 s) with [^{3}H]thymidine at 25°C or 42°C, all of the label appeared in slowly sedimenting (10 S) DNA. This result is consistent with the involvement of *E. coli* DNA ligase in discontinuous DNA replication (Okazaki *et al.*, 1968). Furthermore, the complete absence of rapidly sedimenting label in the mutant would suggest that both strands of the parental duplex in *E. coli* are replicated discontinuously.

The temperature shift experiments carried out with the K12 *ligts7* confirm the results obtained earlier with TAU *ts7* (Modrich & Lehman, 1971). As observed previously, there was an initial increase in viable titer after shift to 42°C (approximately 60%), followed by a rapid loss in viability. Although the reason for the increase in viable titer is not clear, it may reflect a relatively slow rate of inactivation of the ligase *in vivo* at 42°C. Alternatively, damage sustained by the chromosome during the brief period at the high temperature may have been repaired on shifting the cells to 30°C, the temperature at which the viable titer was determined. Again, as observed previously with TAU *ts7*, the rate of DNA synthesis in K12 *ligts7*, as judged by measurements of [^{3}H]thymidine incorporation, increased about tenfold over a period of about 90 minutes after temperature shift-up, and then leveled off. This increase was paralleled by a similar increase in net DNA content. The basis for the net increase in DNA is not known. It may be due to an extremely low, but nevertheless significant, rate of joining of 10 S fragments at restrictive temperatures. As another possibility, movement of the replication fork may not occur when newly synthesized fragments cannot be joined, and as a consequence, the fragments in the vicinity of the fork may be continually synthesized and then rapidly displaced from the parental strands.

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REFERENCES

Adams, J. N. & Luria, S. E. (1958). *Proc. Nat. Acad. Sci., U.S.A.* **44**, 590–594.
Dische, Z. (1955). In *The Nucleic Acids*, ed. by E. Chargaff & J. N. Davidson, vol. 1, pp. 285–305. Academic Press, New York.
Epstein, W., Jewett, S. & Fox, C. F. (1970). *J. Bact.* **104**, 793–797.
Gellert, M. & Bullock, M. L. (1970). *Proc. Nat. Acad. Sci., U.S.A.* **67**, 1580–1587.
Gottesman, S. & Beckwith, J. R. (1969). *J. Mol. Biol.* **44**, 117–127.
Gottesman, M. M., Hicks, M. & Gellert, M. (1973). *J. Mol. Biol.* **77**, 531–547.
Guerola, N., Ingraham, J. L. & Cerda-Olmedo, E. (1971). *Nature New Biol.* **230**, 122–125.
Howard-Flanders, P. & Boyce, R. P. (1966). *Radiation Res.* (Suppl.) **6**, 156–184.
Ikeda, H., Inuzuka, M. & Tomizawa, J. (1970). *J. Mol. Biol.* **50**, 457–470.
Lennox, E. S. (1955). *Virology*, **1**, 190–206.
Martin, R. G. & Ames, B. N. (1961). *J. Biol. Chem.* **236**, 1372–1379.
Miller, J. H., Ippen, K., Scaife, J. G. & Beckwith, J. R. (1968). *J. Mol. Biol.* **38**, 413–420.
Modrich, P. & Lehman, I. R. (1970). *J. Biol. Chem.* **245**, 3626–3631.

Modrich, P. & Lehman, I. R. (1971). *Proc. Nat. Acad. Sci., U.S.A.* **68**, 1002–1005.
Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K. & Sugino, A. (1968). *Proc. Nat. Acad. Sci., U.S.A.* **59**, 598–605.
Pardee, A. B., Jacob, F. & Monod, J. (1959). *J. Mol. Biol.* **1**, 165–178.
Pauling, C. & Hamm, L. (1968). *Proc. Nat. Acad. Sci., U.S.A.* **60**, 1495–1502.
Pauling, C. & Hamm, L. (1969). *Proc. Nat. Acad. Sci., U.S.A.* **64**, 1195–1202.
Stacy, K. A. (1965). *Brit. Med. Bull.* **21**, 211–216.
Steinberg, C. M. & Edgar, R. S. (1962). *Genetics*, **47**, 187–208.
Taylor, A. L. (1970). *Bact. Rev.* **34**, 155–175.
Taylor, A. L. & Trotter, C. D. (1967). *Bact. Rev.* **31**, 332–353.