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Conditional-Lethal Deoxyribonucleic Acid Ligase Mutant of *Escherichia coli*

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A new *Escherichia coli* deoxyribonucleic acid (DNA) ligase mutant has been identified among a collection of temperature-sensitive DNA replication mutants isolated recently (Sevastopoulos, Wehr, and Glaser, Proc. Natl. Acad. Sci. U.S.A. 74:3485-3489, 1977). At the nonpermissive temperature DNA synthesis in the mutant stops rapidly, the DNA is degraded to acid-soluble material, and cell death ensues. This suggests that the mutant may be among the most ligase-deficient strains yet characterized.

DNA ligase plays an important role in several aspects of DNA metabolism. The enzyme is required during DNA replication for joining of nascent DNA chains, the so-called Okazaki fragments (12, 13, 16), and it also is probably needed for one of the final steps in DNA repair and recombination.

Several *Escherichia coli* mutants defective in DNA ligase activity have been isolated, including two that are conditionally lethal (2, 11, 15). We now describe the properties of a new conditionally lethal DNA ligase mutant, SG251, found among a group of temperature-sensitive DNA replication mutants [*dna*(Ts)] isolated recently by Sevastopoulos et al. (17).

Genetic studies. Our initial interest in the temperature-sensitive DNA replication mutant SG251 (Table 1) was based on the report that the mutant mapped in the region of *nalA* (17). However, we were unable to find any cotransduction of *nalA* with the temperature-sensitive mutation in SG251, contrary to the published report (17). Interrupted mating experiments had located the mutation between 44 and 61 min on the *E. coli* genetic map (D. Glaser, personal communication). Since the gene for DNA ligase (*lig*) is at 51.5 min, we tested the possibility that SG251 might be a *lig* mutant by testing its ability to support the growth of phage T7 ligase mutants. T7 ligase mutants can grow on normal hosts but not on ligase-deficient hosts (2, 19). We found that the T7 ligase mutants LG3, LG26, JS39, and NA1 could not grow on SG251 but could grow on a temperature-resistant revertant, whereas wild-type T7 could grow on both hosts. The efficiency of plating of the T7 ligase mutants on SG251 relative to plating on the revertant was $<5 \times 10^{-3}$, not only at 42 but also at 25°C. These results suggested that SG251 is deficient in DNA ligase.

P1 transduction was used to verify that the temperature-sensitive mutation maps in or very near the *lig* gene. It is known that *lig* and *ptsI* cotransduce about 77 to 95% (3). A P1 lysate grown on SG251 was used to transduce a *ptsI* mutant (CHE30) and *ptsI*$^+$ transductants were checked for temperature sensitivity. Out of 300 *ptsI*$^+$ transductants, 82% were temperature sensitive. Ten temperature-sensitive and nine temperature-resistant transductants were tested for their ability to support the growth of phage T4 30^- rII$^-$ (*amH*39r59), a phage which cannot grow on ligase-deficient hosts but can grow on *lig*$^+$ hosts (2). All 9 of the temperature-resistant transductants were able to support the growth of T4 30^- rII$^-$, whereas none of the 10 temperature-sensitive transductants was able to support growth of the phage at 30 or 42°C. Thus, all of the genetic evidence points to the *lig* gene as the site of the temperature-sensitive lesion in SG251. We call this particular allele *lig-251.*

DNA synthesis. As mentioned previously, SG251 was selected and characterized as a *dna*(Ts) mutant (17). Figure 1 compares the rate of DNA synthesis of SG251 and a temperature-resistant revertant of SG251. The mutant shows normal DNA synthesis at 30°C, but at 42°C the rate of synthesis drops rapidly, reaching a low residual level after 20 min of exposure to the restrictive temperature. The shutdown of DNA synthesis is as rapid as for typical immediate-stop *dna*(Ts) mutants such as most *dnaB*, *dnaE*, and *dnaG* mutants (20). The revertant of SG251, selected for its ability to grow at 42°C, has the same rate of DNA synthesis at 42°C as at 30°C. Figure 1 also shows the rate of DNA synthesis for one of the temperature-sensitive *ptsI*$^+$ transductants, GR501 (see above). It can

TABLE 1. *Strains of E. coli K-12*

Strain	Genotype	Source (reference)
SG251	F⁻ *leu his argG metB thyA thi lac malA xyl gal ton rpsL*[a] λ⁻ λʳ *sup lig-251*	D. Glaser (17)
CHE30	Hfr *ptsI thi*	W. Epstein
GR501	CHE30 *ptsI⁺ lig-251*	P1*vir* transduction from SG251; temperature sensitive
GR523	CHE30 *ptsI⁺ lig⁺*	As above; temperature resistant
CR34	*thr leu thi thyA*	Laboratory collection

[a] Formerly *strA*.

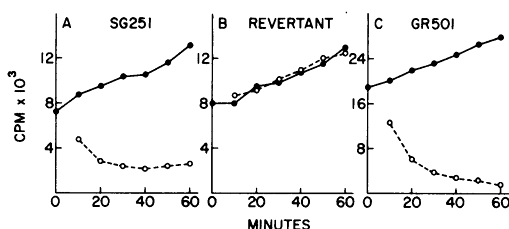

FIG. 1. *Rate of DNA synthesis as a function of time at 30 or 42°C for (A) SG251, (B) revertant of SG251, and (C) GR501. Cultures were grown at 30°C to 2 × 10⁸ cells/ml in M9 medium (8) supplemented with 0.5% Casamino Acids, 2 μg of thymine, and 1 μg of thiamine per ml. At zero time, half of the culture was shifted to 42°C. At 10-min intervals, 1-ml portions were withdrawn from the 30 and 42°C cultures and pulse-labeled. The pulse involved exposing 1 ml of cells to 10 μl of 100 μCi of [³H]thymidine per ml for 1 min at 30°C (●) or 42°C (○). The pulse was terminated by the addition of 1 ml of 15% trichloroacetic acid. The amount of radioactivity incorporated into DNA during the 1-min pulse, determined as described previously (1), is defined as the rate of DNA synthesis.*

be seen that DNA synthesis in GR501 is just as temperature sensitive as that in the original mutant, SG251.

Joining of Okazaki fragments. It is known that DNA is replicated discontinuously in the form of Okazaki fragments and that DNA ligase is required for joining of the nascent DNA chains (12, 13, 16). The ability of two isogenic *ptsI⁺* transductants, GR501 (*lig-251*) and GR523 (*lig⁺*), to join Okazaki fragments was compared. Cells were grown at 30°C, shifted to 42°C for 15 min, and then pulse-labeled at 42°C with [³H]-thymidine. Figure 2A shows that all of the labeled DNA is in the 10S Okazaki fragment peak for the mutant, GR501, whereas the *lig⁺* strain shows a significant amount of large DNA, indicative of normal joining of nascent chains. Pulse-chase experiments also show that GR501 is defective at joining Okazaki fragments. The results

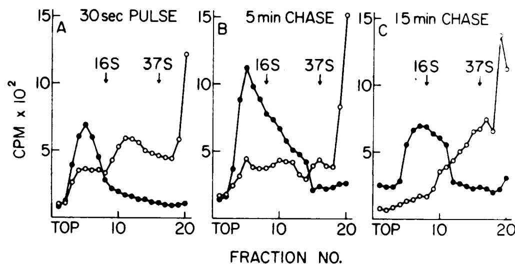

FIG. 2. *Alkaline sucrose gradients of (A) pulse-labeled DNA from GR501 (lig-251) and GR523 (lig⁺) and (B) and (C) pulse-chased DNA from GR501. Cultures of GR501 and GR523 were grown at 30°C in M9 medium (8) supplemented with 0.5% Casamino Acids to 2 × 10⁸ cells/ml, shifted to 42°C for 15 min, and pulse-labeled with [³H]thymidine (1 μCi/ml, final concentration) for 30 s at 42°C, and 5-ml portions were removed and added to 20 ml of −10°C acetone to terminate the pulse (A); GR501 (●); GR523 (○). An excess of nonradioactive thymidine (50 μg/ml final concentration) was then added to the GR501 culture for the chase experiment. The culture was divided into two portions, one incubated at 42°C and the other incubated at 30°C. After a 5-min chase (B) and a 15-min chase (C) at both 30 (○) and 42°C (●), 5-ml amounts were removed and the pulse-chases were terminated by 20 ml of −10°C acetone. Lysis of the cells and centrifugation conditions have been described previously (18).*

of a 30-s pulse at 42°C followed by 5- and 15-min chases at 30 or 42°C are shown in Fig. 2B and C. It can be seen that very little pulse-labeled DNA is joined into high-molecular-weight DNA even after a 15-min chase at 42°C. On the other hand, normal joining occurs if the chase is performed at 30°C. This shows that the mutant's ability to join Okazaki fragments can be regained, at least after only 15 min of exposure to the restrictive temperature.

Degradation of DNA. Nagata and co-workers observed that parental DNA was degraded in the temperature-sensitive ligase mutant KN321 at the restrictive temperature (4). We found the same behavior for strains carrying the *lig-251* mutation. Fig. 3A shows that there is extensive degradation of prelabeled DNA in SG251 at 42, but not at 30°C. No degradation was observed in the revertant at either temperature (Fig. 3B).

In vitro DNA ligase assays. Crude extracts of SG251 and its revertant and wild-type strain CR34 were assayed for DNA ligase activity at 25 and 40°C. The results are shown in Table 2. CR34 and the revertant have ligase activity at both temperatures, whereas the SG251 extract has normal activity at 25°C but no detectable activity at 40°C.

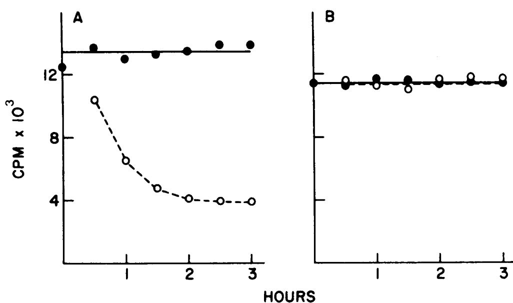

FIG. 3. *Stability of uniformly labeled DNA as a function of time at 30 or 42°C for SG251 (A), and the temperature-resistant revertant of SG251 (B). Cultures (0.5 ml) were grown overnight in the M9 medium described in the legend to Fig. 1 containing 100 μl of 10 μCi of [^{14}C]thymine per ml. Cells were collected on filters (Millipore HA, 25 mm), washed twice with 5 ml of fresh medium plus 50 μg of thymine per ml, and resuspended in 10 ml of fresh medium plus 50 μg of thymine per ml. After 90 min of growth at 30°C, the cultures were divided into two portions, one remaining at 30°C (●) and the other placed at 42°C (○). This was zero time for the experiment. At 30-min intervals, 1-ml portions were removed and added to 1 ml of 15% trichloroacetic acid. The precipitates were collected on filters and counted to determine the amount of radioactive DNA which remained acid precipitable.*

The first step in the ligase reaction is the formation of an enzyme-AMP complex (7). We tested whether SG251 extracts were defective in forming this complex. Extracts were incubated with excess nicotinamide mononucleotide to remove any AMP already attached to the enzyme, dialyzed, and incubated with [^{14}C]nicotinamide adenine dinucleotide, and the amount of [^{14}C]-AMP-ligase complex formed was measured. Table 3 shows that the SG251 extract does not form significant amounts of enzyme-AMP complex at either 25 or 40°C, whereas the revertant extract forms the complex at both temperatures. Thus, the *lig* defect of SG251 prevents the formation of the ligase-AMP complex at least under our assay conditions.

Comparison with other ligase mutants. Several *E. coli* ligase mutants have been isolated previously (2, 11, 15). All are defective in the joining of Okazaki fragments in vivo and all show decreased enzymatic activity in vitro. A comparison of the different *lig* mutants shows that they can be placed into categories of increasing defectiveness (4). Strains carrying mutations such as *lig-4* or *lig-307* are not lethal alone but become so in conjunction with other mutations such as *polA, recA*, or *recB*. A more severe ligase deficiency is shown by strains carrying the well-studied temperature-sensitive mutation *lig-7*(Ts) (3, 5, 15). These strains show no DNA degradation and a normal rate of DNA synthesis for several hours at 42°C (3, 5; our unpublished data). On the other hand, a *lig-7*(Ts) *polA12* double mutant stops DNA synthesis rapidly at 42°C, and there is extensive DNA degradation (6).

TABLE 2. *DNA ligase activity in crude extracts*[a]

Extract from:	Relative activity at:	
	25°C	40°C
CR34	0.69	0.49
SG251	0.71	<0.001
Revertant of SG251	1.0	0.65

[a] Extracts were prepared essentially as described by Wickner et al. (21). One-liter cultures were grown in LB broth (8) to 5 × 10^8 cells/ml, spun down, and resuspended in 4 ml of 10% sucrose-50 mM Tris (pH 8)-1 mM β-mercaptoethanol. Lysozyme and NaCl were added at 0.5 mg/ml and 0.1 M final concentration, respectively. The cells were left on ice for 1 h and then warmed to 37°C for 2 min. The lysed cells were spun at 118,000 × *g* for 1 h at 2°C. The supernatant (fraction A) was assayed for DNA ligase by using a method similar to the one described by Modrich and Lehman (9). Linear [^{3}H]dAT alternating copolymer was incubated with fraction A, and the ligase activity was measured by determining the amount of [^{3}H]dAT which became resistant to an exonuclease. In these experiments we used the exonuclease activity of *E. coli* DNA polymerase I. Extracts were incubated with 2 × 10^4 cpm of [^{3}H]dAT (1.2 × 10^{10} cpm/mmol nucleotide) at 25 or 40°C for 60 min. The reaction was terminated by placing the reaction tubes in boiling water for 2 min. A total of 4 units of DNA polymerase I (Boehringer Mannheim) was added, and the mixture was incubated for 2 h at 37°C. Samples were precipitated with 15% trichloroacetic acid, the precipitates were collected and washed on Whatman GF-A filters, and the radioactivity on the filters was determined. The activity has been normalized relative to the value found for the extract from the revertant strain. The amount of [^{3}H]dAT resistant to exonuclease for that sample was 4747 cpm.

The other previously studied conditionally lethal ligase mutant is KN321 (4, 11). This strain carries an amber mutation in the *lig* gene (*lig-321*) as well as a temperature-sensitive amber suppressor (*sup-126*), which renders the strain temperature sensitive. In the absence of a functional amber suppressor, no ligase is produced and the *lig-321* defect is lethal. KN321 shows both extensive DNA degradation and shutdown of DNA synthesis at 42°C, even in a *polA*$^+$ background (4; our unpublished data). This is taken as evidence that KN321 has a more severe ligase deficiency than strains with the *lig-7*(Ts) mutation (4).

Strain SG251, carrying the mutation we call *lig-251*, is the first ligase mutant isolated by

TABLE 3. *DNA ligase-AMP formation*[a]

Extract from:	pmol of ligase-AMP formed at:	
	25°C	40°C
SG251	<1	<1
Revertant of SG251	429	241

[a] To assay for ligase-AMP formation fraction A (Table 2) had to be further purified (14). A 1-ml portion of 5% streptomycin sulfate was added to 5 ml of fraction A, and the mixture was stirred for 30 min at 0°C and centrifuged at 10,000 × *g* for 20 min. Solid $(NH_4)_2SO_4$ (1.7 g) was added to the supernatant, and it was stirred for 30 min at 0°C and centrifuged as above. $(NH_4)_2SO_4$ (0.8 g) was added to the supernatant, and it was stirred and centrifuged as above. The pellet was dissolved in 0.2 ml of 25 mM Tris (pH 8)-1 mM β-mercaptoethanol and incubated with 50 mM $MgCl_2$ and 22 μM nicotinamide mononucleotide at 25°C for 5 min to dissociate endogenous ligase-AMP complexes (10). After removal of nicotinamide mononucleotide by dialysis, this partially purified fraction was used for measuring ligase-AMP formation by incubating with [^{14}C]nicotinamide adenine dinucleotide (Amersham, 280 mCi/mmol) essentially as described by Modrich and Lehman (10).

screening *dna*(Ts) mutants. Indeed, none of the previously isolated *lig* mutants except KN321 would have been selected by such a screening procedure. Strains carrying the *lig-251* mutation shut down DNA synthesis rapidly (Fig. 1) and show extensive DNA degradation (Fig. 3) at 42°C. By these criteria SG251 may be among the most defective ligase mutants yet characterized. It is clearly more defective than the *lig-7*(Ts) strain, and it is at least as defective as KN321. SG251 has an additional advantage over KN321. The latter mutant contains a *lig*(Am) mutation which needs to be suppressed to be viable and is only temperature sensitive due to the presence of the temperature-sensitive suppressor, *sup-126*.

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