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DISCONTINUOUS DNA REPLICATION IN A lig-7 STRAIN OF Escherichia coli IS NOT THE RESULT OF MISMATCH REPAIR, NUCLEOTIDE-EXCISION REPAIR, OR THE BASE-EXCISION REPAIR OF DNA URACIL

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Summary. After pulse-labeling with ^{3}H-thymidine for 30 s at 42°C, the newly-synthesized DNA from uvrB5 lig-7, uvrB5 lig-7 ung-1 (or ung152), uvrB5 lig-7 mutL218 (or mutS215), and uvrB5 lig-7 ung-1 mutL218 (or mutS215) cells sedimented very slowly in alkaline sucrose gradients. The bulk of these DNA molecules were smaller than 2,000 nucleotides long (i.e., about the size of Okazaki fragments), and none of the ^{3}H-radioactivity was found to sediment as high-molecular-weight DNA. These results indicate that the apparent discontinuous DNA replication observed in lig-7 strains is not the result of mismatch repair, nucleotide-excision repair, or the base-excision repair of DNA uracil.

The question of whether discontinuous DNA replication operates only for the lagging strand or for both strands in Escherichia coli is still not resolved (10). Most in vitro data indicate that DNA replication is semi-discontinuous, i.e., continuous synthesis in the leading strand and discontinuous synthesis in the lagging strand (5, 13; see 6 for a review). In contrast, the in vivo data suggest that DNA replication is discontinuous in both strands (see 10). Evidence supporting discontinuous synthesis in both DNA strands in vivo comes mainly from studies with temperature-sensitive ligase (lig) mutants (4, 8, 9), and with DNA polymerase I (polA) mutants (5, 11, 14, 16), i.e., after pulse labeling these mutants with ^{3}H-thymidine, virtually all of the radioactivity is found in short DNA chains. However, since ligase and DNA polymerase I are also required for the completion of several DNA repair processes (such as mismatch repair and excision repair), these results can also be interpretated

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to suggest that DNA replication is really continuous in the leading strand, but the observed strand interruptions result from some ongoing repair process. The inhibition of uracil-DNA glycosylase by an *ung* mutation did not eliminate the accumulation of short DNA chains in a *polA* strain (15), indicating that the excision of uracil residues in DNA plays little role in the observed discontinuous DNA synthesis in a *polA* strain.

It is not known, however, whether mismatch repair and other excision repair processes may account for the apparent discontinuous DNA replication observed in *polA* and *lig* mutants. In this communication, we examine the role of mismatch repair, nucleotide-excision repair, and the base-excision repair of DNA uracil in discontinuous DNA replication in a *lig-7* strain of *E. coli*.

The bacterial strains used in this work are listed in Table 1. The *mutL* and *mutS* mutants are deficient in mismatch repair (see 19), which removes mismatched bases from nascent DNA. The *uvrB5* mutant is deficient in the incision step of nucleotide-excision repair. The *ung-1* and *ung152*::Tn*10*

TABLE 1. *E. coli* K-12 strains used[a]

Strains	Genotype	Source or derivation (strain no.)
BD2328	Hfr KL16 *ung152*::Tn*10* *pheA97* *nadB7*	B. Duncan
SR292	F⁻ *lig-7* *proC* *hsp* *rpsL*	K.B. Konrad (KS252)
SR294	F⁻ *uvrB5* *lig-7* *leuB19* *metE70* *deo(C2?)* *lacZ53* *malB45* *rha-5* *rpsL151* λ⁻	D. Youngs
SR686	*ung-1* *argH1* *ara-13* *gal-6* *lacY1* *malB1* *rha-7* *xyl-7* *thi-1* *tonA2* *rpsL9* *supE44* λ^r	B. Duncan (BD1153)
SR990	HfrH *pheA18*::Tn*10* Δ(*gpt-lac*)5 *thi* *relA1* *spoT1*	N. Kleckner (NK6024)
SR1689	F⁻ *mutS215*::Tn*10* *thy* *metB1* *lacY14*	R. Fowler (ES1481)
SR1690	F⁻ *mutL218*::Tn*10* *trpA78* *his* *leu* *arg* *thr*	R. Fowler (KD1073)
SR1829	As SR294, also *pheA18*::Tn*10*	SR294 x P1vira·SR990, select Tc^r
SR1830	As SR294	SR1829 x T4GT7·SR686, select Phe$^+$
SR1831	As SR294, also *ung-1*	SR1829 x T4GT7·SR686, select Phe$^+$
SR1832	As SR294, also *mutS215*::Tn*10*	SR1830 x P1vira·SR1689, select Tc^r
SR1833	As SR294, also *mutL218*::Tn*10*	SR1830 x P1vira·SR1690, select Tc^r
SR1834	As SR294, also *ung-1* *mutS215*::Tn*10*	SR1831 x P1vira·SR1689, select Tc^r
SR1835	As SR294, also *ung-1* *mutL218*::Tn*10*	SR1831 x P1vira·SR1690, select Tc^r
SR2011	As SR294, also *ung152*::Tn*10*	SR1830 x P1vira·BD2328, select Tc^r

[a]Genotype symbols are those used by Bachmann (1). Tc^r means tetracycline-resistance. P1vira was a reisolate of P1vir obtained from Dr. A.J. Clark.

mutants are deficient in uracil-DNA glycosylase (see 3), and fail to excise uracil residues from the DNA. To evaluate whether the initiation of these repair processes may produce short DNA fragments in nascent DNA, and thus account for the appearance of short DNA pieces in a lig(Ts) mutant, which can not complete these repair processes, the size of newly-synthesized DNA at 42°C from uvrB lig-7, uvrB lig-7 mutL(S), uvrB lig-7 ung-1, uvrB lig-7 ung152::Tn10 and uvrB lig-7 ung-1 mutL(S) cells was analyzed in alkaline sucrose gradients. After pulse-labeling with ^{3}H-thymidine for 30 s at 42°C, the newly-synthesized DNA from a uvrB lig-7 strain sedimented very slowly in an alkaline sucrose gradient (Fig. 1), and none of the ^{3}H-radioactivity was found to sediment as high-molecular-weight DNA. The bulk of this nascent DNA is smaller than 2,000 nucleotides long, in agreement

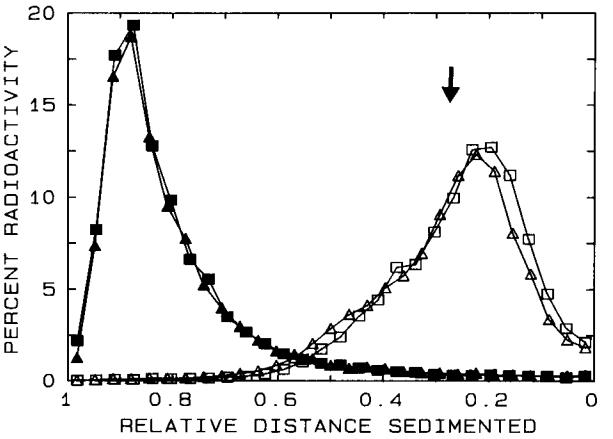

Figure 1. Effects of the uvrB5, ung and mutL(S) mutations on the size distribution of DNA synthesized at 42°C in a lig-7 strain. Cells were grown in minimal medium (16) at 30°C. For prelabeling the DNA, [^{14}C]thymidine (51 Ci/mol) was added to logarithmic cultures at 1 μCi/ml, and the cultures were incubated at 30°C for 2 h before they were filter-washed and resuspended in the original volume of minimal medium. The cultures were grown to 5 x 10^8 cells per ml at 30°C, and then incubated at 42°C for 5 min before they were pulse-labeled with [^{3}H]thymidine (79.9 Ci/mmol) at 8.3 μCi/ml for 30 s. The pulses were terminated by the addition of an equal volume of an ice-cold solution composed of ethanol at 75%, phenol at 2%, sodium acetate (pH 5.3) at 20 mM and EDTA at 2 mM. Incorporation of [^{3}H]thymidine into DNA is about 5 x 10^{-4} cpm per cell. The resulting suspension was centrifuged, and the pellet was resuspended in 0.2 M NaOH containing EDTA at 10 mM. After incubation at 37°C for 1 h, the mixture was centrifuged to remove insoluble material. An aliquot (50 μl) of the supernatant was layered on top of an alkaline sucrose gradient (5 to 20% in 0.1 N NaOH on top of a 0.5 ml cushion of 50% sucrose), and was centrifuged at 20 krpm for 16 h at 20°C. Gradients were collected and processed as previously described (17). Solid symbols are for prelabeled [^{14}C]DNA, while the open symbols are for [^{3}H]DNA. Squares (□,■) are for uvrB lig-7 (SR1830) while triangles (Δ,▲) are for uvrB5 lig-7 ung-1 mutL218::Tn10 (SR1835). The profiles of [^{3}H]DNA for strains SR292 (lig-7), SR1831 (uvrB5 lig-7 ung-1), SR2011 (uvrB5 lig-7 ung152::Tn10), SR1832 (uvrB5 lig-7 mutS215::Tn10), SR1833 (uvrB5, lig-7 mutL218::Tn10), and SR1834 (uvrB5 lig-7 ung-1 mutS215::Tn10) (not shown) are congruent with those for SR1830 and SR1835. The arrow indicates the position of a marker DNA of about 2,000 nucleotides in length, derived by digesting plasmid pACYC184 (2) with restriction endonucleases EcoRI and SalI.

with earlier data on a single lig-7 mutant (4, 8, 9). The presence of ung-1, ung152::Tn10, mutL(S), or a combination of the ung-1 and mutL(S) mutations in the uvrB lig-7 strain had no effect on the size of newly-synthesized DNA (Fig. 1), and there was an absence of ^{3}H-radioactivity in high-molecular-weight DNA.

If DNA replication is semidiscontinuous (i.e., continuous in the leading strand and discontinuous in the lagging strand), and there is no postreplicative repair processes operating on the nascent DNA strand, one would predict that about half of the ^{3}H-radioactivity in newly-synthesized DNA should sediment in alkaline sucrose gradients as high-molecular-weight DNA, and half as short Okazaki fragments in a lig mutant. Since none of the pulse-labeled DNA from uvrB5 lig-7, uvrB5 lig-7 ung, uvrB5 lig-7 mutL(S), and uvrB lig-7 ung mutL(S) cells appeared in high-molecular-weight form, this indicates that the apparent discontinuous DNA synthesis observed in lig mutants is not the result of mismatch repair, nucleotide-excision repair, or the base-excision repair of DNA uracil.

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