Induction of the SOS System by DNA Ligase-Deficient Growth of *Escherichia coli*

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When *Escherichia coli* carrying a thermosensitive mutation in DNA ligase was grown at the restrictive temperature, several functions associated with the SOS system were induced. These included λ prophage induction, W-reactivation and W-mutagenesis of ultraviolet-irradiated λ phage, and *recA* protein synthesis, all of which were *lexA*⁺ *recA*⁺ *recB*⁺ dependent and chloramphenicol sensitive, and *lexA*⁺-dependent filamentation. These results indicate that ligase-deficient growth leads to the induction of the SOS system, and that all the above functions may respond to common induction signals.

INTRODUCTION

When *Escherichia coli* is subjected to treatments that damage DNA or interfere with DNA replication, numerous processes known as the SOS functions are induced. These include prophage induction in lysogens, induced error-prone DNA repair activity, elevated *recA* protein synthesis, the inhibition of respiration and cell division, and the synthesis of an exonuclease V inhibitor. The expression of these functions is abolished by mutations at the *recA* or *lexA* loci (reviewed by Kimball, 1978; Witkin, 1976).

Numerous stimuli have been reported to trigger the induction of the SOS system. In general, treatments that lead to extensive DNA damage are effective SOS inducers. These include ultraviolet (u.v.) irradiation (Witkin, 1976), treatment with nalidixic acid, bleomycin, or mitomycin C (Gudas & Pardee, 1975, 1976; Smith & Oishi, 1978), temperature elevation in temperature-sensitive *dnaB* mutants (Caillet-Fauquet & Defais, 1977; Witkin, 1975), and elevated dNTP concentrations in permeabilized cells (Oishi & Smith, 1978). The induction of the SOS system by treatments that damage DNA may involve DNA degradation by the *recBC* enzyme, exonuclease V (Smith & Oishi, 1978).

Several phenomena associated with the SOS system appear to be induced when temperature-sensitive DNA ligase mutants are incubated at the restrictive temperature. These include W-reactivation and W-mutagenesis of u.v.-irradiated bacteriophage (Morse & Pauling, 1975), and λ prophage induction in lysogenic cells (Gottesman *et al.*, 1973). However, Morse & Pauling (1975) have reported the inhibition of W-mutagenesis, but not of W-reactivation, when ligase-deficient cells were incubated at the restrictive temperature in the presence of chloramphenicol (CAM; 3 $\mu\text{g ml}^{-1}$). This concentration of CAM reduces the DNA degradation, strand breakage, and lethality associated with DNA ligase deficiency without significantly inhibiting protein synthesis (Morse *et al.*, 1976; Morse & Pauling, 1975; Pauling *et al.*, 1976). A concentration of CAM sufficient to give maximal inhibition of protein synthesis (20 $\mu\text{g ml}^{-1}$), however, was shown to eliminate W-reactivation of λ and cellular mutagenesis as well (Morse & Pauling, 1975).

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It has been shown that *lig recB* double mutants resemble CAM-treated *lig* mutants in several respects. When incubated at 40 °C, DNA strand breakage is eliminated in *lig recB* cells and the DNA degradation profile resembles that of *lig* cells in 3 µg CAM ml⁻¹ (Morse *et al.*, 1976). W-mutagenesis of λ is eliminated, although W-reactivation of λ has been reported to continue at a level comparable to that in *lig* single mutants (Morse & Pauling, 1975). This apparent separation of W-reactivation from W-mutagenesis suggested the possibility that they are distinct processes, at least in CAM-treated *lig* mutants or in *lig recB* double mutants, and that mutagenesis is not an essential component of the DNA repair processes induced by DNA ligase deficiency.

Accordingly, we have further characterized the patterns of SOS induction in *lig* strains incubated at the restrictive temperature. In the present paper, we report that DNA ligase deficiency results in *recA*⁺ *lexA*⁺-dependent λ prophage induction, W-reactivation and W-mutagenesis of λ , *recA* protein synthesis, and cellular filamentation.

In contrast to previous results (Morse & Pauling, 1975), however, we find no evidence for elevated cellular mutagenesis in these strains. Moreover, we find W-reactivation and W-mutagenesis of λ , λ prophage induction, and *recA* protein synthesis to be abolished by a *recB* mutation or by treatment with 3 µg CAM ml⁻¹. These results are therefore consistent with models in which the expression of all SOS functions is controlled by a single regulatory system.

METHODS

Bacterial growth media. Tryptone plus thymine (TT) broth was used for liquid growth; it consisted of 10 g Bacto-Tryptone (Difco), 5 g NaCl and 2 mg thymine per litre of distilled water. Viable counts were done on the above medium solidified with 1.5% (w/v) Bacto-Agar (Difco), using TT top agar (solidified with 0.6% agar). When plating for λ plaques, TT plates containing 1.2% agar were used. When minimal medium was used in strain construction or for mutant screening, CM medium was used (Morse *et al.*, 1976). This was routinely supplemented with 0.2% (w/v) glucose, 20 µg ml⁻¹ each of L-proline, L-histidine, L-threonine, and L-leucine, 2 µg thymine ml⁻¹, and 1 µg thiamin ml⁻¹.

Bacterial strains. All experiments were performed in strains of *E. coli* K12 which were obtained as described in Table 1, using DM1413, a derivative of AB1157 (Castellazzi *et al.*, 1972; Mount, 1977) as the starting strain. The methods used for P1*vir*a transduction, bacterial conjugation, HNO₂ mutagenesis, and trimethoprim selection were those described by Miller (1972).

Isolation of *ptsI* mutants was performed by fosfomycin selection (Castro *et al.*, 1976). One *ptsI* mutant was then transduced to *pts*⁺ with P1*vir*a previously grown on the *ligts7 pts*⁺ strain, KS268, selecting for growth on minimal mannitol medium at 30 °C. *lig* and *lig*⁺ transductants were then transduced to *malB*⁺ (*lexA*⁺ or *lexA* by cotransduction) from DM953. *recA* or *recB* mutations were introduced by conjugation with JC5088 or JC5412, respectively, selecting for *thyA*⁺, followed by trimethoprim selection to restore the Thy⁻ phenotype.

Survival of bacteria at 40 °C. Exponential phase cells growing aerobically at 30 °C in TT broth were diluted in TT broth to $A_{525} = 0.16$ (about 5×10^7 viable cells ml⁻¹ for CP403) and shifted to 40 °C. At intervals samples were removed, diluted as appropriate, and plated in TT top agar on TT agar plates. Colonies were counted after 24 h incubation at 30 °C (or after 48 h for CP410 and CP414).

Thermal λ prophage induction. Bacteria lysogenic for λ were grown and diluted as in survival experiments, and shifted to 40 °C. Samples were removed at intervals, diluted as appropriate, and plated with CP403 indicator bacteria on TT agar using TT top agar. At zero time, cells were also plated for viability on the same medium without indicator. Plaques were counted after 18 h and colonies were counted after 24 h (48 h for CP410 and CP414) incubation at 30 °C.

To determine the maximum level of potential inducibility for these lysogens, cells were lysogenized with λ cI857, which carries a thermolabile repressor. Because thermal induction of this phage does not require a functional SOS system, the ratio of infective centres at 40 °C per viable cell (at 30 °C), represents the maximum level of proficiency for the support of λ growth. These lysogens were therefore plated and incubated at 40 °C with indicator bacteria to measure induction, and at 30 °C without indicator to assay viability. The values obtained for thermal induction of wild type λ were divided by these efficiencies of plating for λ , expressed as λ cI857 plaque-forming units (p.f.u.) per viable cell.

W-Reactivation and W-Mutagenesis of λ . Exponentially growing cultures at 30 °C in TT broth were diluted to $A_{525} = 0.16$ in TT broth and divided; the resulting two cultures were incubated for 60 min at 30 °C and 40 °C,

Table 1. *Strains of E. coli K12*

Strain	Sex	Relevant genotype	Origin
KS268	F ⁻	<i>ligts7 strA thyA thyR</i>	I. R. Lehman
KS269	F ⁻	<i>strA thyA thyR</i>	I. R. Lehman
DM953	F ⁻	<i>lexA3 thyA thyR</i>	D. W. Mount
DM1412	F ⁻	<i>tif-1 thr-1 leu-6 proA2 his-4 thi-1 strA-31</i>	D. W. Mount
DM1413	F ⁻	<i>thr-1 leu-6 proA2 his-4 thi-1 strA-31</i>	D. W. Mount
JC5088	Hfr PO45	<i>recA56 thr-300 ilv-318 thi-1</i>	A. J. Clark
JC5412	Hfr PO45	<i>recB21 sbcA8 thr-300 ilv-318 thi-1</i>	A. J. Clark
AB2480	*	<i>recA13 uvrA6 proA2</i>	P. Howard-Flanders
CP403	F ⁻	<i>thr-1 leu-6 proA2 his-4 thi-1 thyA thyR strA-31</i>	This work
CP404	F ⁻	as CP403, <i>ligts7</i>	This work
CP405	F ⁻	as CP403, <i>lexA3</i>	This work
CP406	F ⁻	as CP403, <i>ligts7 lexA3</i>	This work
CP409	F ⁻	as CP403, <i>recB21</i>	This work
CP410	F ⁻	as CP403, <i>ligts7 recB21</i>	This work
CP413	F ⁻	as CP403, <i>recA56</i>	This work
CP414	F ⁻	as CP403, <i>ligts7 recA56</i>	This work

* May be defective male.

respectively. Cells were harvested by filtration, resuspended to $A_{525} = 1.0$ in TM buffer (0.01 M-Tris/HCl, pH 7.4 and 0.01 M-MgSO₄), and infected with u.v.-irradiated λ phage in TM buffer at a multiplicity of infection less than 0.1. After 20 min adsorption at 30 °C, the infected cells were diluted as appropriate and plated in TT top agar on TT agar plates, using strain AB2480 (*uvrA recA*) as the indicator to prevent rescue of unadsorbed phage. After this adsorption period, fewer than 0.01% of the plaque-forming units remained CHCl₃ resistant, indicating virtually complete adsorption.

Plaques were counted after 18 h incubation at 30 °C. Clear-plaque mutant frequencies were determined by plating approximately 10^4 p.f.u. per plate as described above; clear plaques were counted independently by two observers whose values were averaged.

The reactivation factor is the fractional u.v. survival of phage infecting cells previously grown for 60 min at 40 °C, divided by the u.v. survival of the same phages infecting cells of the same strain previously grown for 60 min at 30 °C. Thermally induced W-reactivation, therefore, is indicated by a reactivation factor greater than 1.0.

U.v. irradiation was performed as previously described (Morse & Pauling, 1975).

recA protein synthesis. Cells were grown, diluted, and shifted to 40 °C as in survival experiments. At intervals, samples containing 1×10^8 cells were removed and labelled for 10 min with [³⁵S]methionine (Schwarz/Mann: 2.1 Ci mmol⁻¹; 78 GBq mmol⁻¹) at 2.5 μ Ci ml⁻¹ in TT broth at 30 °C. Incorporation was stopped by adding an equal volume of ice-cold 0.85% (w/v) NaCl containing 2 mg L-methionine ml⁻¹ and quick-chilling in an ice/salt bath. Cells were harvested by centrifugation and washed with ice-cold 0.01 M-Tris/HCl, pH 6.8. Harvested packed cells were stored at -20 °C.

Electrophoresis on 12% polyacrylamide-sodium dodecyl sulphate slab gels (SDS-PAGE) was performed as described by Laemmli & Favre (1973). Frozen whole cells were resuspended in sample buffer and incubated at 100 °C for 2 min. The radioactivity in 5 μ l of each sample was then determined by liquid scintillation counting, and an equal amount of radioactivity was loaded on each lane of the gel.

Autoradiography was performed on Kodak Tri-X Pan Professional film #4164 (Estar thick base). Films were developed with Kodak Microdol-X and scanned with an E-C Densitometer equipped with a 540 nm filter.

The rate of synthesis of the *recA* protein was determined in each sample by integrating the appropriate peak in the densitometer trace of the autoradiogram. In all cases, the density of the film was proportional to the amount of radioactivity applied to the gel.

The *recA* protein was identified as a 40000 molecular weight band relative to the standards: bovine serum albumin (68000), ovalbumin (43000), chymotrypsinogen (25700), and lysozyme (14300).

RESULTS

Survival of bacterial strains at 40 °C

When shifted to 40 °C, exponential growth continued in all the *lig*⁺ strains over a 120 min period, whereas all *lig* cultures lost viability (Fig. 1). The *lig* strain showed 17% survival after

Fig. 1. Survival of bacterial strains at 40 °C. Exponential cultures were grown at 30 °C, diluted, and transferred to 40 °C. Strains: ●, CP403 (*lig*⁺); ○, CP404 (*lig*); ■, CP405 (*lig*⁺ *lexA*⁺); □, CP406 (*lig lexA*⁻); ▲, CP409 (*lig*⁺ *recB*⁺); △, CP410 (*lig recB*⁻); ▼, CP413 (*lig*⁺ *recA*⁺); ▽, CP414 (*lig recA*⁻).

120 min, whereas the *lig recA* and *lig recB* mutants showed 0.96% and 0.17% survival, respectively. These survival kinetics closely resemble those reported for *lig*, *lig recA*, and *lig recB* strains in another genetic background (Morse & Pauling, 1975).

The *lig lexA* strain exhibited a survival of 6% after 120 min at 40 °C, intermediate between the viabilities of *lig* and *lig rec* strains. Since *recA* and *lexA* strains are deficient in SOS repair, the different survival kinetics of the *lig recA* and *lig lexA* mutants cannot be explained on that basis; the lower viability of the *lig recA* strain might result from impaired recombination ability, as *lexA* mutants are recombination proficient (Mount *et al.*, 1972).

λ prophage induction

It has been shown that DNA ligase deficiency leads to prophage induction in *λ* lysogens (Gottesman *et al.*, 1973). If this lysogenic induction is a manifestation of the SOS system, it should be *recA*⁺*lexA*⁺ dependent, should be inhibitable by CAM, and may be *recB*⁺ dependent.

Accordingly, bacterial strains were lysogenized with wild-type *λ*, purified, and tested for prophage induction at 40 °C. The results in Fig. 2 show that upon shifting cultures to 40 °C, the *lig* strain exhibited complete lysogenic induction within 60 min at 40 °C. In contrast, no evidence of thermal prophage induction was observed in the *lig*⁺, *lig recA*, *lig recB*, or *lig lexA* strains, or when *lig* cells were incubated at 40 °C in the presence of 20 µg CAM ml⁻¹.

Therefore, prophage induction resulting from DNA ligase deficiency depends upon functional *recA*⁺, *recB*⁺ and *lexA*⁺ genes and appears to require protein synthesis.

Induction of *recA* protein synthesis

The hypothesis that DNA ligase deficiency induces the SOS system predicts that shifting *lig* cells to 40 °C should increase the rate of *recA* protein synthesis and that this induction should require the *recA*⁺ *lexA*⁺ *recB*⁺ genotype.

Fig. 2. Induction of λ prophage during DNA ligase deficiency (at 40 °C). Induction results were corrected for efficiencies of plating (e.o.p.) for the appropriate strains as described in Methods, and these e.o.p. values are given in parentheses. Data are expressed as infective centres per induction-proficient cell in the culture. Strains: ●, CP403 (0.97); ○, CP404 (0.81); □, CP404 + 20 μ g CAM ml⁻¹ (0.81); ■, CP406 (1.02); △, CP410 (5.19); ▲, CP414 (1.29). (The relevant genotypes of these strains are shown in the legend to Fig. 1.)

Fig. 3. Synthesis of *recA* protein during DNA ligase-deficient growth (at 40 °C). Results are expressed as the percentage of total protein synthesized as *recA* protein, as determined by SDS-PAGE followed by quantitative analysis of radioactivity in the 40000 mol. wt band. Strains: ●, CP403 (*lig*⁺); ○, CP404 (*lig*); □, CP406 (*lig lexA*); △, CP410 (*lig recB*); ▲, CP414 (*lig recA*).

The kinetics of *recA* protein induction by ligase deficiency are shown in Fig. 3. When *lig* cells were transferred to 40 °C, *recA* protein synthesis was induced, reached a maximum rate after about 60 min. These kinetics are in good agreement with the kinetics of λ prophage induction observed under these conditions. The maximum rate of *recA* protein synthesis in these cells, 4 to 5% of total protein synthesized, also agrees well with the value of 3 to 4% previously observed after nalidixic acid treatment of *E. coli* (Gudas & Pardee, 1976).

In contrast to the *lig* strain, the *lig*⁺, *lig recA*, *lig recB* and *lig lexA* strains showed no increased rates of synthesis of the *recA* protein after a shift to 40 °C (Fig. 3). This indicates that the synthesis of *recA* protein is induced as a consequence of DNA ligase deficiency and that this induction depends upon the *recA*⁺ *recB*⁺ *lexA*⁺ genotype.

Table 2. *Thermally induced W-reactivation of λ*

Cells growing exponentially at 30 °C in TT broth were diluted into fresh broth, and CAM was added where indicated. Cultures were then divided and incubated for 60 min at 30 °C or 40 °C before infection with λ (see Methods). Reactivation factors were calculated as the u.v. survival of λ infecting cells grown at 40 °C divided by the u.v. survival of λ infecting cells of the same strain grown at 30 °C. Results are expressed as means $\pm$ s.e.

Strain	CAM	Reactivation factor	
		u.v. dose to phage:	
		100 J m ⁻²	200 J m ⁻²
CP403 (<i>lig</i> ⁺)	—	1.58 $\pm$ 0.27	2.02 $\pm$ 0.33
CP404 (<i>lig</i>)	—	5.67 $\pm$ 0.42	18.2 $\pm$ 3.7
CP404 (<i>lig</i>)	3 μ g ml ⁻¹	1.83 $\pm$ 0.36	4.08 $\pm$ 0.85
CP404 (<i>lig</i>)	20 μ g ml ⁻¹	1.20 $\pm$ 0.03	1.50 $\pm$ 0.08
CP406 (<i>lig lexA</i>)	—	1.03 $\pm$ 0.09	1.22 $\pm$ 0.03
CP410 (<i>lig recB</i>)	—	1.44 $\pm$ 0.17	1.34 $\pm$ 0.55
CP414 (<i>lig recA</i>)	—	1.53 $\pm$ 0.30	1.50 $\pm$ 0.02

Table 3. *Thermally induced W-mutagenesis of λ*

Experiments were performed as described in the legend for Table 2 and the frequency of clear-plaque mutants among surviving phages was measured. Results are expressed as means $\pm$ s.e.

Strain	CAM	Temperature (°C)	U.v. dose to phage (J m ⁻²)	No. of clear-plaque mutants per 10 ⁴ c.f.u.
CP403 (<i>lig</i> ⁺)	—	30	0	4.7 $\pm$ 0.6
		30	100	4.5 $\pm$ 0.1
		40	0	6.3 $\pm$ 0.4
		40	100	4.8 $\pm$ 0.8
CP404 (<i>lig</i>)	—	30	0	6.6 $\pm$ 1.0
		30	100	3.0 $\pm$ 0.6
		40	0	7.3 $\pm$ 1.0
		40	100	12.4 $\pm$ 1.1
CP406 (<i>lig lexA</i>)	—	30	0	6.4 $\pm$ 0.5
		30	100	2.7 $\pm$ 1.3
		40	0	6.9 $\pm$ 0.3
		40	100	6.8 $\pm$ 1.8
CP410 (<i>lig recB</i>)	—	30	0	4.1 $\pm$ 0.1
		30	100	3.9 $\pm$ 0.6
		40	0	3.6 $\pm$ 0.2
		40	100	4.9 $\pm$ 0.5
CP414 (<i>lig recA</i>)	—	30	0	2.0 $\pm$ 0.1
		30	100	2.6 $\pm$ 0.3
		40	0	1.8 $\pm$ 0.1
		40	100	2.9 $\pm$ 0.5
CP404 (<i>lig</i>)	20 μ g ml ⁻¹	30	0	3.5 $\pm$ 0.3
		30	100	3.2 $\pm$ 0.7
		40	0	4.2 $\pm$ 0.3
		40	100	3.3 $\pm$ 0.1

W-reactivation and W-mutagenesis of λ

Morse & Pauling (1975) reported that *lig* strains incubated at 40 °C expressed a *recA*⁺-dependent DNA repair function acting on u.v.-irradiated bacteriophage. The results in Table 2 show that after 60 min incubation at 40 °C, the *lig* mutant exhibited a pronounced W-reactivation of u.v.-irradiated λ . In contrast, no significant W-reactivation was observed under these conditions in *lig*⁺ or *lig recA* strains, confirming the earlier studies.

Fig. 4. Filamentous growth during DNA ligase deficiency. Bacterial cultures were grown at 30 °C as in survival experiments, and the distribution of cell lengths was determined before and after a 120 min incubation at 40 °C. Formalin-fixed cells were photographed using a phase-contrast microscope, and photographs of cells were measured and calibrated against photographs of a stage micrometer made at the same magnification. The length distribution of 100 cells in each sample was determined, and histograms were constructed with the aid of a computer program. (a, b) CP403 (*lig*⁺) at *t* = 0 and 120 min, respectively; (c, d) CP404 (*lig*) at *t* = 0 and 120 min, respectively; (e, f) CP406 (*lig lexA*) at *t* = 0 and 120 min, respectively.

In contrast to previous findings, however, the *lig recB* double mutant showed no detectable W-reactivation after incubation at 40 °C. In addition, treatment of *lig* cells with 3 μg CAM ml⁻¹ reduced the extent of W-reactivation by 70 to 80%, and 20 μg CAM ml⁻¹ caused complete inhibition of this form of repair. These concentrations of CAM inhibited incorporation of [³⁵S]methionine into acid-precipitable material by 60% and 80%, respectively.

We also examined the requirement for the *lexA* gene product in thermal induction of W-reactivation. This repair was absolutely dependent upon the *lexA*⁺ genotype (Table 2).

Accompanying the W-reactivation of λ, we also observed W-mutagenesis, as measured by an increase in the frequency of clear-plaque mutants of u.v.-irradiated phage infecting *lig* cells grown at 40 °C, when compared with the same cells grown at 30 °C prior to infection (Table 3). However, no significant mutagenesis of unirradiated phage was observed. W-mutagenesis did not occur in *lig*⁺ cells, *lig* cells treated with CAM, *lig recA*, or *lig recB* cells, confirming earlier findings (Morse & Pauling, 1975). In addition, this W-mutagenesis was absent in the *lig lexA* double mutant.

Inhibition of cell division

The induction of the SOS system is also accompanied by the inhibition of cell division, resulting in filament formation. When induced by u.v., this filamentation is *recA*⁺*lexA*⁺ dependent (Witkin, 1976).

The distributions of cell lengths of *lig*⁺, *lig* and *lig lexA* strains before and after 120 min incubation at 40 °C are shown in Fig. 4. Whereas the average length of *lig*⁺ cells was unaffected by the 40 °C growth, the *lig* strain showed a dramatic increase in cell length by 120 min at the restrictive temperature, as well as a much more heterogeneous length distribution. In contrast, the *lig lexA* strain showed no filamentous growth, indicating that the inhibition of cell division by ligase deficiency is *lexA*⁺ dependent.

The *lig recA* and *lig recB* strains exhibited extensive filamentation even during growth at the permissive temperature and their length distributions did not change significantly after 120 min at 40 °C (data not shown). It was not possible, therefore, to determine the possible involvement of the *recA* or *recB* genes in this process.

DISCUSSION

Morse & Pauling (1975) proposed that the inducible, error-prone DNA repair (SOS repair) system is induced by growth of *E. coli* temperature-sensitive DNA ligase mutants at the restrictive temperature. They reported that ligase deficiency leads to elevated cellular mutability, W-reactivation and W-mutagenesis of u.v.-irradiated bacteriophage that requires protein synthesis and depends upon the *recA*⁺ genotype.

The results presented here confirm these findings and support the hypothesis that ligase deficiency induces the SOS system. We have also shown that growth of temperature-sensitive ligase mutants at the restrictive temperature leads to *recA*⁺*lexA*⁻ and protein synthesis-dependent λ prophage induction, *recA* protein synthesis, W-reactivation, and W-mutagenesis of λ , as predicted by the SOS hypothesis (Radman, 1974; 1975; Witkin, 1976).

Morse & Pauling (1975) suggested that inducible DNA repair and inducible mutagenesis are separable, based upon the apparent separation of these two processes in *lig* strains in the presence of CAM and in *lig recB* double mutants. The present results, however, show no such separation of repair from mutagenesis. In this study, the extent of inhibition of SOS repair by CAM parallels its inhibition of cellular protein synthesis. Furthermore, both the repair and the mutagenesis are absolutely dependent on the *recB*⁺ genotype. Accordingly, there is no convincing evidence to suggest that ligase deficiency induces multiple pathways of inducible repair. Rather, our evidence is consistent with the induction by ligase deficiency of a single inducible, error-prone DNA repair pathway acting on bacteriophage DNA.

The hypothesis that ligase deficiency induces the SOS system also predicts that the rate of occurrence of spontaneous mutations should rise under conditions promoting λ prophage induction, elevated *recA* protein synthesis, W-reactivation, and W-mutagenesis. Elevated cellular mutagenesis was reported to occur in *lig* cells incubated at the restrictive temperature (Morse & Pauling, 1975), supporting this prediction. However, Witkin (1976) has argued that these earlier results might also be explained by crowding recovery, in which artificially high apparent mutation rates can result from increased survival of filamenting cells at the high plating densities necessary to detect mutants.

We have verified that crowding recovery does, indeed, occur when *lig* cells are incubated at 40 °C and plated at 30 °C at high cell densities (data not shown). When this artefact of plating density was eliminated by allowing prolonged outgrowth of cells at the permissive temperature prior to mutant selection, no evidence of cellular mutagenesis was detected. This was true in measurements of reversion of the SOS-revertible *his-4* allele to histidine prototrophy, and also for forward mutations to rifampicin or nalidixic acid resistance (data not shown).

Our inability to detect cellular mutator activity associated with DNA ligase deficiency is not surprising in view of the loss of cell viability experienced during 40 °C incubation, as seen in Fig. 1. Since the lethality at 40 °C limited the length of time *lig* cultures could be incubated under SOS-inducing conditions, the apparent lack of cellular mutagenesis may reflect the insensitivity of the method used to detect it. The failure to observe elevated cellular mutagenesis, therefore, is not inconsistent with the induction of the SOS system in these cells.

We have shown that induction of the SOS system during ligase-deficient growth of *E. coli* requires the action of the *recBC* enzyme, exonuclease V. Consistent with this finding, this nuclease has been implicated in SOS induction following other DNA-damaging treatments such as bleomycin, mitomycin C, and thymine starvation (Gudas & Pardee, 1976; Smith & Oishi, 1978). In the case of permeabilized, dNTP-treated cells, *recBC*-mediated DNA

degradation has been found to occur preferentially at replication forks (Oishi & Smith, 1978).

Because DNA replication under ligase deficiency leads to the accumulation of Okazaki fragments (Pauling & Hamm, 1969; Gottesman *et al.*, 1973; Konrad *et al.*, 1974), and *recB*⁺-dependent DNA degradation and strand breakage occur in these cells (Morse *et al.*, 1976), it seems likely that the *recB*⁺-dependent event(s) in SOS induction observed here are a consequence of chromosome replication. This interpretation is supported by our finding (unpublished results) that the antibiotic novobiocin, which interferes with chromosome replication, inhibits both DNA degradation and SOS induction during DNA ligase-deficient growth.

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