Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 3569–3573, August 1978
Biochemistry

Inactivation of phage repressor in a permeable cell system: Role of *recBC* DNase in induction

(plasmolysis/dNTPs/replication fork/SOS functions)

M. OISHI AND C. L. SMITH

The Public Health Research Institute of The City of New York, Inc., 455 First Avenue, New York, New York 10016

Communicated by Evelyn M. Witkin, April 17, 1978

ABSTRACT **UV light causes inactivation of phage (φ80) repressor molecules in a plasmolyzed, permeable cell preparation of *Escherichia coli*. Induction without UV irradiation occurs when the permeable cells are incubated in the presence of four deoxyribonucleoside triphosphates and ATP. The induction triggered by dNTPs requires a functional *recBC* gene product and is associated with degradation of the DNA replication fork. The role of *recBC* DNase in the induction of prophage and SOS functions in general is discussed.**

When normal cellular DNA replication is inhibited by various treatments (e.g., UV irradiation), numerous processes, collectively known as "SOS functions," are induced in bacteria and possibly in animal cells (1). In lysogenic bacteria, prophage induction is one of the cellular responses to such interference with DNA replication. Induction of λ prophage is accompanied by proteolytic cleavage of λ repressor molecules (2). Roberts *et al.* (3) have demonstrated ATP-dependent cleavage of λ repressor in extracts derived from an induction-constitutive mutant strain. Little is known, however, about the initial molecular events that trigger the SOS functions. Recently, we presented evidence (4) that strand scission is the most immediate DNA structural modification that generates an SOS induction signal and that degradation of the modified chromosomal DNA by certain DNases, including *recBC* DNase, is closely correlated with and may trigger prophage induction.

As an approach to the identification of a postulated DNA degradation product or modified DNA structure that could trigger the onset of SOS functions, we developed a permeable cell system, using our biochemical assay (5) for inactivation of phage repressor molecules, to detect the induction processes. We here present evidence that prophage induction in this system is associated with degradation at the DNA replication fork resulting from *recBC* DNase activity and discuss the role played by the *recBC* DNase in the induction of SOS functions.

MATERIALS AND METHODS

Bacterial Strains, Media, and Chemicals. Bacterial and phage strains used were described recently (4, 5). The SSA minimal (6) medium was supplemented with 0.5% (wt/vol) Casamino acids (Difco), 100 μg of L-tryptophan per ml, 5 μg of vitamin B_1 per ml, and 0.2% (vol/vol) glycerol (instead of glucose). The sources of reagents were: amino acids (Calbiochem); *p*-aminobenzoic acid, pyridoxine, spermidine, all nucleotides, phospho*enol*pyruvate, and glutathione (Sigma Chemical Co.); *Escherichia coli* tRNA mixture and pyruvate kinase (Boehringer Mannheim Biochemicals); and folinic acid (calcium leucovorin, American Cyanamid Company). Chorismic acid was purified by the method of Gibson and Gibson (7).

Plasmolyzed Cell Preparation. Cells grown to a density of 3.5–4.0 × 10^8 cells per ml in SSA medium (usually 50–100 ml) at 37° were harvested by centrifugation (3000 rpm) for 5 min at room temperature, resuspended in 10 ml of buffer (6.0 mM Tris·HCl, pH 7.5/10 mM $MgSO_4$/0.5 mM $CaCl_2$/1 mM KCN/10 μg of gelatin per ml) plus L-tryptophan (50 μg/ml), infected with *φ80ptrp* [*φ80ptrp190h*, multiplicity of infection (m.o.i.) = 6], and incubated for 10 min at 37°. For those preparations that were to undergo UV irradiation, the *φ80ptrp*-infected cells (10 ml) were transferred to a glass petri dish (diameter 9 cm) and irradiated under two General Electric (G15T8, 15 W) germicidal UV lamps. The cells were then centrifuged at 2°, washed once with a cold buffer [10 mM Tris·HCl, pH 7.7/10 mM $MgCl_2$/50 mM KCl/1 mM ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA)/100 μg of L-tryptophan per ml], and resuspended in 0.5 ml of cold buffer (10 mM Tris·HCl, pH 7.7/10 mM $MgCl_2$/60 mM KCl/2 M sucrose/1 mM EGTA) for plasmolysis (8). After a 10 min incubation at 0°, 10 ml of cold RS buffer (10 mM Tris·HCl, pH 7.7/10 mM $MgCl_2$/50 mM KCl/1 mM $CaCl_2$/2.5 mM spermidine/0.2 mM dithiothreitol/100 μg of L-tryptophan per ml) was added. The plasmolyzed cells were then centrifuged (8000 rpm, 5 min) and resuspended in 1.2 ml (50-ml original culture) of RS buffer. All manipulations were at 0°–2°, unless otherwise specified.

Synthesis of Anthranilate Synthase in Plasmolyzed Cells. The plasmolyzed cell preparation in RS buffer (40–200 μl) was incubated at 35° (with constant shaking) in the reaction mixture (total volume 0.2–1.0 ml) whose composition was slightly modified from the original one (9). The components (concentrations) were as follows: 20 mM Tris·HCl, pH 7.7; 100 mM KCl; 1 mM $CaCl_2$; 13 mM $MgCl_2$; 0.1 mM $MnCl_2$; 4 mM spermidine-HCl; 0.25 mM each of 19 L-amino acids except L-tryptophan; 2.5 mM L-tryptophan; 0.1 mM β-NAD; 0.03 mM β-NADP; folinic acid, 25 μg/ml; *p*-aminobenzoic acid, 10 μg/ml; pyridoxine, 25 μg/ml; flavin adenine dinucleotide, 25 μg/ml; 2.5 mM ATP; 0.5 mM GTP; 0.5 mM CTP; 0.5 mM UTP; 10 mM phospho*enol*pyruvate; pyruvate kinase, 25 μg/ml; *E. coli* tRNA mixture, 100 μg/ml; 5 mM glutathione (reduced form); and 62.5 mM glycerol. The ATP, GTP, CTP, UTP, and phospho*enol*pyruvate were all sodium salts.

The plasmolyzed cell concentration in the reaction mixture was approximately 30 × 10^9/ml. The reaction was stopped by chilling in ice water. The samples (usually 200 μl) were then mixed with 100 μl of 50 mM potassium phosphate buffer (pH 7.0) containing 100 μg of chloramphenicol per ml, 0.1 mM EDTA, 5 mM dithiothreitol, and toluene (final concentration 1% vol/vol). After incubation for 10 min at 37°, the cells were incubated for 20 min at 37° for assay of anthranilate synthase (ASase) activity [chorismate pyruvate-lyase (amino-accepting);

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Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; ASase, anthranilate synthase; m.o.i., multiplicity of infection.

EC 4.1.3.27) as described (5). One unit of enzyme activity in the permeable cell system was defined as the amount of enzyme catalyzing the conversion of 1 nmol of chorismic acid to anthranilic acid in 20 min at 37°.

RESULTS

Repressor inactivation in a permeable cell system

By using our biochemical assay for prophage induction (5), which involves readthrough transcription–translation of a tryptophan operon integrated into a phage genome, we have reproduced the *in vivo* induction process (inactivation of phage repressor molecules) in a permeable cell system. We use the appearance of activity of the first tryptophan biosynthetic enzyme, ASase, as a signal that the phage repressor molecules have been inactivated. A φ80 lysogen (MO1513) was infected with φ80*ptrp*, irradiated with UV light (80 J/m^2), and made permeable by plasmolysis. Such plasmolyzed, permeable cells were then incubated in the reaction mixture, which allows transcription and *de novo* protein synthesis. ASase (coded by the *trpED* genes) activity appeared after an approximate 50-min lag period (Fig. 1*A*). This 50-min lag period (at 37°) with the plasmolyzed cells was much longer than the approximately 30 min observed *in vivo* (5). However, once ASase activity did begin to appear, the rate of ASase synthesis in the permeable cell system, which varied between 0.1 and 1 unit/10 min per 10^8 cells, was equivalent to the values observed *in vivo* (approximately 0.4 unit/10 min per 10^8 cells). The synthesis of ASase was completely dependent upon UV irradiation and infection with φ80*ptrp* (Fig. 1*A*). The optimum UV dose for the induction was around 80 J/m^2 (data not shown). With permeable cells of a nonlysogen (MO1512), the ASase activity appeared in φ80*ptrp*-infected cells, with and without UV irradiation (Fig. 1*B*). These patterns of ASase synthesis were similar to those observed with equivalent, intact cells except that the lag period was up to 20 min longer.

ASase synthesis in the plasmolyzed cells was dependent upon the presence of ATP and an ATP-generating system (phospho*enol*pyruvate and pyruvate kinase) as well as GTP, CTP, and UTP (Table 1). ASase synthesis was inhibited in the presence of actinomycin D. The system was also sensitive to pancreatic RNase, but relatively insensitive to DNase. These results suggest that the plasmolyzed cells used here are permeable only to low molecular weight materials such as nucleotides and small proteins (e.g., pancreatic RNase). The presence of deoxyribonucleoside triphosphates produced no effect or a slight inhibition (10–20%). A mutation in the *recA* gene completely eliminates the capacity of a lysogen to undergo prophage induction after UV irradiation (10, 11). Recently we obtained evidence that the *recB* gene product (*recBC* DNase) is also involved in prophage induction (4). In that study, we observed delayed induction of ASase synthesis in a *recB*$^-$ lysogen after treatment with bleomycin, UV, or mitomycin C. No induction of ASase synthesis was observed after UV treatment of φ80*ptrp*-infected lysogenic permeable cells carrying a *recA*$^-$ mutation (MO1522) while immediate synthesis of ASase was observed with the equivalent *recA*$^-$ nonlysogen MO1521 (Fig. 2). When similar experiments were performed with a lysogen carrying a *recB*$^-$ mutation (MO1519), ASase synthesis was detected only after a long lag period of more than 90 min (Fig. 3*A*). Permeable cells of a *recB*$^-$ nonlysogen (MO1518) produced ASase synthesis (Fig. 3*B*) after the same short lag period found with the *rec*$^+$ strain (MO1512). These results parallel similar observations of *in vivo* induction with intact cells (4).

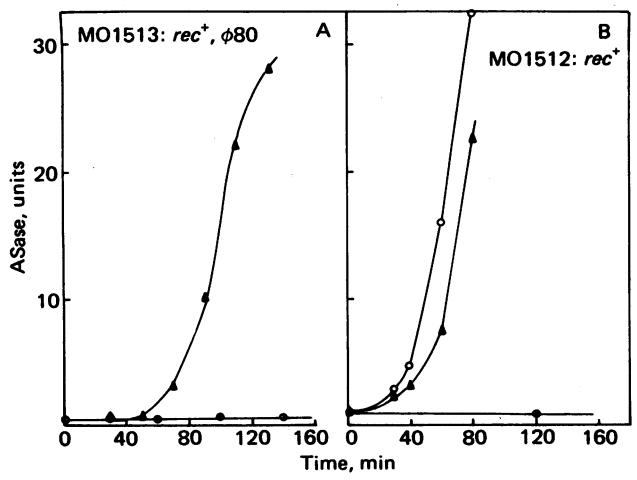

FIG. 1. ASase synthesis in permeable cell preparations from *rec*$^+$ cells. *E. coli* φ80 lysogen MO1513 (*A*) and nonlysogen MO1512 (*B*) were grown in SSA medium, infected with φ80*ptrp* (m.o.i. 6), and irradiated with UV light (80 J/m^2). The cells were made permeable by plasmolysis. Reaction mixtures (200 μl) were incubated at 35°; the reaction was terminated at various time intervals for ASase assay. ▲, UV-irradiated and infected with φ80*ptrp*; ●, UV-irradiated only; ○, infected with φ80*ptrp* only.

Repressor inactivation triggered by deoxyribonucleoside triphosphates and ATP

We explored for factors that initiate the induction process in permeable cells without the usual inducing treatments such as UV irradiation. Inactivation of the repressor molecules, without UV irradiation, was observed when a mixture of four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, and dTTP) was added to the permeable cell reaction mixture. ASase synthesis began 60 min after incubation was started with a mixture of the four deoxyribonucleoside triphosphates (dNTPs) (Fig. 4*A*). The presence of the dNTPs in a reaction mixture with UV-irradiated permeable cells had no effect on the kinetic pattern of the UV-triggered induction process other than to often cause a slight inhibition at the optimum UV dose for ASase induction (Table 1). Addition of the dNTPs also had no effect on ASase synthesis in a nonlysogen (*rec*$^+$ MO1512) with or without inducing treatment (data not shown). The dNTP-triggered inactivation of the repressor molecules did not occur in either a *recA*$^-$ strain (Fig. 4*B*) or in a *recB*$^-$ strain (Fig. 4*C*). The failure to induce any ASase synthesis in the *recB*$^-$ strain

Table 1. ASase synthesis in plasmolyzed cells

Reaction mixture		ASase, units	%
Complete		3.85	100
Minus ATP and PEP*		0.54	14
Minus GTP, CTP, UTP		0.12	3
Plus actinomycin D:	10 μg/ml	0.77	20
	50 μg/ml	0.15	4
	100 μg/ml	0.08	2
Plus RNase:	10 μg/ml	0.81	21
	100 μg/ml	0.58	15
Plus DNase:	10 μg/ml	3.31	86
	100 μg/ml	2.08	54
Plus KCN (1 mM)		3.70	96
Plus dATP, dGTP, dCTP, dTTP (0.5 mM each)		3.15	82

Strain MO1513 (*rec*$^+$, φ80 lysogen) cells were infected with φ80*ptrp* (m.o.i. 6), irradiated with UV light (80 J/m^2), and made permeable by plasmolysis. Reaction mixtures (200 μl) were incubated at 35° for 120 min and ASase activities were assayed.
* PEP, phospho*enol*pyruvate.

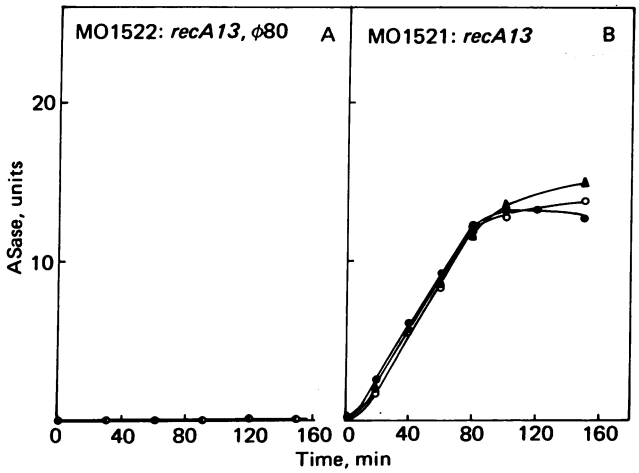

FIG. 2. ASase synthesis in permeable cell preparations from *recA*$^-$ cells. *E. coli recA*$^-$ strains MO1522 (*recA13*, φ80 lysogen) (*A*) and MO1521 (*recA13*, nonlysogen) (*B*) were grown in SSA medium, infected with φ80*ptrp* (m.o.i. 6), and irradiated with UV light: ▲, 80 J/m^2; ●, 13.3 J/m^2; ○, 0 J/m^2. The cells were made permeable by plasmolysis. Reaction mixtures (200 μl) were incubated at 37° and the reaction was terminated at various times for ASase assay.

was quite unexpected and in sharp contrast to the UV-triggered induction in which the synthesis of ASase activity in the permeable cells was delayed but did appear after about 90 min of incubation (Fig. 3A). The observation that the dNTP-triggered induction has an absolute requirement for the *recB*$^+$ gene function suggests that at least one type of induction pathway is completely dependent upon the *recBC* gene product and is consistent with indications of its involvement *in vivo* (4).

Any single deoxyribonucleoside triphosphate alone did not give any appreciable induction of ASase (Table 2). Certain combinations of three deoxyribonucleoside triphosphates triggered significant induction of ASase synthesis but much less than that observed with all four. The inducing activity reached a plateau at concentrations of approximately 0.1 mM of each dNTP (data not shown). The combination of four deoxyribonucleoside diphosphates (dNDPs) produced approximately 50% of the ASase activity induced by the triphosphates, while

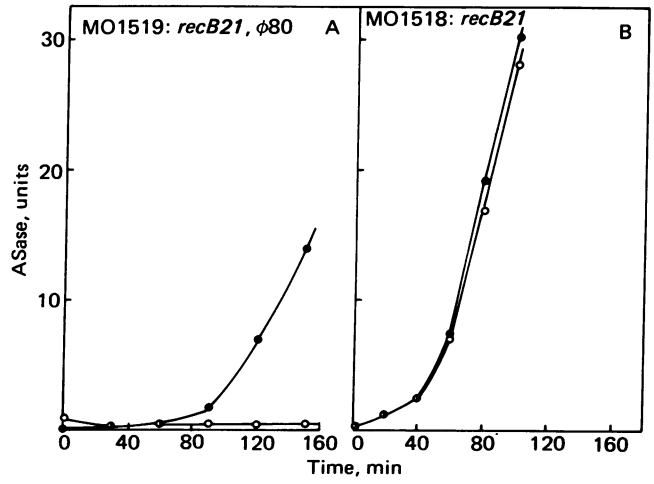

FIG. 3. ASase synthesis in permeable cell preparations from *recB*$^-$ cells. *E. coli recB*$^-$ strains MO1519 (*recB21*, φ80 lysogen) (*A*) and MO1518 (*recB21*, nonlysogen) (*B*) were grown in SSA medium, infected with φ80*ptrp* (m.o.i. 6), and irradiated with UV light (80 J/m^2) (●) or not irradiated (○). The cells were made permeable by plasmolysis. Reaction mixtures (200 μl) were incubated at 35°; the reaction was terminated at various times for ASase assay.

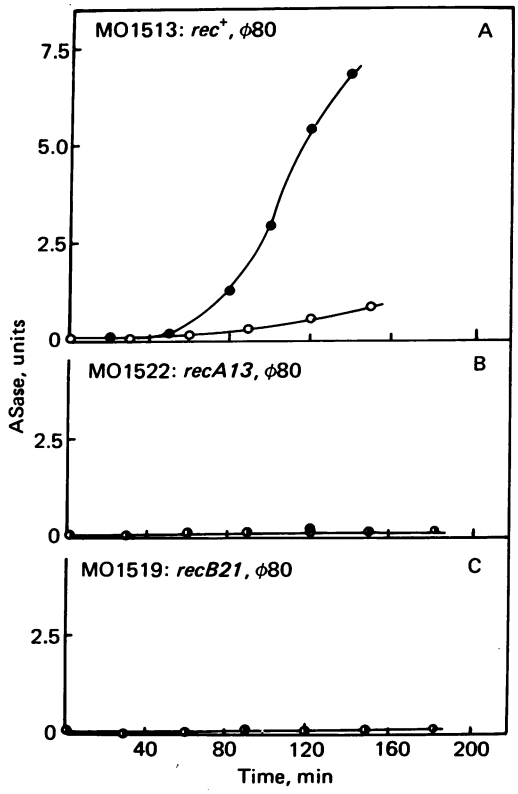

FIG. 4. ASase synthesis in the presence of the four dNTPs. *E. coli* strains MO1513 (*rec*$^+$ φ80 lysogen) (*A*), MO1522 (*recA13*, φ80 lysogen) (*B*), and MO1519 (*recB21*, φ80 lysogen) (*C*) were grown in SSA medium and infected with φ80*ptrp*. The cells were made permeable by plasmolysis. Reaction mixtures (200 μl) were incubated at 35° in the absence (○) and presence (●) of four dNTPs (0.25 mM each). The reaction was terminated at various times for assay of ASase activity.

the four deoxyribonucleoside monophosphates (dNMPs) had essentially no inducing effect.

In order to further analyze the dNTP-dependent induction, we tried to separate the reaction into two steps. Table 3 shows an experiment in which we incubated the permeable cells with and without dNTPs and/or ATP for various time periods (first step). The dNTPs and ATP were removed by washing and the cells were reincubated in the reaction mixture necessary for

Table 2. Induction of ASase by dNTPs

Addition	ASase, units
None	0.15
dATP	0.27
dGTP	0.31
dCTP	0.46
dTTP	0.31
dATP, dGTP, dCTP	0.92
dATP, dCTP, dTTP	2.85
dATP, dGTP, dTTP	0.38
dGTP, dCTP, dTTP	1.23
dATP, dGTP, dCTP, dTTP	6.35
dADP, dGDP, dCDP, dTDP	3.23
dAMP, dGMP, dCMP, dTMP	0.23

Strain MO1513 (*rec*$^+$, φ80 lysogen) was grown in SSA medium and infected with φ80*ptrp* (m.o.i. 6). The cells were made permeable by plasmolysis. Reaction mixtures (200 μl) were incubated at 35° for 120 min with various combinations of deoxyribonucleotides. The concentration of each nucleotide was 0.25 mM. ASase was assayed after 120 min of incubation.

Table 3. Analysis of dNTP-dependent induction of ASase

Preincubation		ASase,
Time, min	Additions	units
0	4 dNTPs, ATP	0.15
5	4 dNTPs, ATP	3.00
10	—	0.15
10	4 dNTPs	0.58
10	ATP	0.69
10	4 dNTPs, ATP	3.10
20	4 dNTPs, ATP	3.15

Strain MO1513 (*rec*$^+$, ϕ80 lysogen) was grown in SSA medium and infected with ϕ80*ptrp* (m.o.i. 6). The cells were made permeable by plasmolysis. For the first incubation period (preincubation), the reaction mixture (1.6 ml) consisted of: 20 mM Tris·HCl (pH 7.7), 100 mM KCl, 4 mM spermidine, 1 mM $CaCl_2$, 13 mM $MgCl_2$, 62.5 mM glycerol, 5 mM glutathione (reduced form), plasmolyzed cells in RS buffer (200 μl, total original cells 4.8×10^9), plus 2 mM ATP and/or 0.25 mM each of the four deoxyribonucleoside triphosphates. After incubation at 37° for various time intervals with gentle shaking, the samples were chilled and twice washed with 10 ml of RS buffer. For the second incubation period, the reaction mixture (800 μl) was the standard one that allows ASase synthesis (see text) and samples (150 μl) were taken at 150 min for ASase assay.

protein synthesis (second step). The highest ASase activity was induced when the cell preparation had first been preincubated with both the dNTPs and ATP. Preincubation with ATP or dNTPs alone gave substantially less ASase activity during the second incubation. The requirement for ATP in addition to the four dNTPs for induction was not detected in the previous experiments because of the presence of ATP as a component of the reaction mixture necessary for protein synthesis. The experiment described above suggests that once the induction process is triggered by the dNTPs plus ATP, the dNTPs are not necessary for any of the subsequent steps. As seen in Table 3, a minimum of 5 min of preincubation with the dNTPs and ATP was sufficient for triggering the induction process. It is clear that within 5 min after addition of the dNTPs plus ATP the permeable cells have become fixed in the induced state.

We attempted to identify the induction-triggering reaction initiated by the presence of the four dNTPs plus ATP. The dependence of this reaction on the *recBC* gene product (*recBC* DNase) (12) prompted us to look for DNA degradation reactions that require the presence of four dNTPs plus ATP and the *recB*$^+$ allele. Among various reactions examined, we found that a significant fraction of pulse-labeled [^{3}H]DNA, but not uniformly labeled [^{14}C]DNA, became acid soluble in the presence of the four dNTPs and ATP in a *rec*$^+$ permeable cell system (Fig. 5*A*). This indicates that newly synthesized DNA near the replicating fork is specifically sensitive to this type of degradation. Approximately 50% of labeled DNA that had been pulsed *in vivo* for 60 sec at 37° became acid soluble when the four dNTPs and ATP were provided. Omission of any one of the four dNTPs substantially reduced or eliminated degradation (80–100% reduction, data not shown). The degradation of pulse-labeled DNA, dependent upon the four dNTPs and ATP, was also observed in *recA*$^-$ cells (data not shown) but not in *recB*$^-$ cells (Fig. 5*B*) nor in *recA*$^-$ *recB*$^-$ cells (data not shown). These results indicate that this degradation of newly synthesized DNA at the replicating fork requires a functional *recBC* gene product (*recBC* DNase). It is clear from these experiments that the two phenomena, ASase induction (phage repressor inactivation) and degradation of pulse-labeled DNA at the replication fork, share two common characteristics, namely, the involvement of the *recBC* gene product and the requirement for the four dNTPs and ATP.

We examined the inducibility of ASase that was dependent

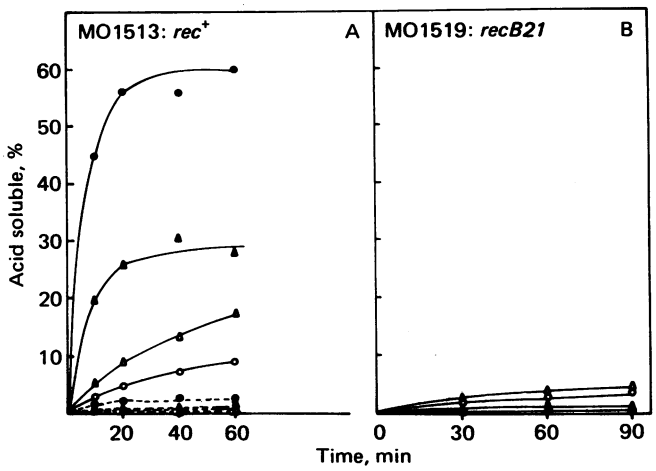

FIG. 5. Degradation of pulse-labeled (^{3}H-labeled) and uniformly labeled (^{14}C-labeled) DNA. Strains MO1513 (*rec*$^+$, ϕ80 lysogen) (*A*) and MO1519 (*recB21*, ϕ80 lysogen) (*B*) were grown in SSA medium (50 ml) supplemented with [2-^{14}C]thymine (New England Nuclear, specific activity 0.34 Ci/mmol, 0.5 μCi/ml) plus deoxyadenosine (200 μg/ml). At a cell concentration of 3×10^8/ml the cells were pulsed for 60 sec with [*5-methyl*-^{3}H]thymidine (Schwarz/Mann, specific activity 18 Ci/mmol, 2.5 μCi/ml with 0.1 μg of carrier thymidine per ml). After the samples were chilled in ice water, the cells were made permeable. The plasmolyzed cells were incubated at 35° with gentle shaking in reaction mixtures (600 μl) whose compositions were identical to the ones used for the preincubation experiments (Table 3). The ATP and dNTP concentrations used here were 2 mM and 0.25 mM (each), respectively. Samples (100 μl) were withdrawn at various time intervals, and radioactivity of trichloroacetic acid (final concentration 8% wt/vol)-soluble material was measured on glass filters (Enzo filter, EGF-R3-25) in a Beckman scintillation counter. ○, Control; △, plus ATP; ▲, plus 4 dNTPs; ●, ATP plus 4 dNTPs. (—) Pulse-labeled [^{3}H]DNA; (- - -) uniformly labeled [^{14}C]DNA.

on dNTPs plus ATP using plasmolyzed cells that had been subjected to amino acid starvation. In such starved cells, any replication in progress is completed but no new replicating forks are initiated (13). After 30–45 min of amino acid (L-tryptophan) starvation, the synthesis of ASase induced by dNTPs plus ATP dropped to approximately 20% of the value obtained with unstarved cells, whereas UV-induced synthesis of ASase (control) increased after amino acid starvation (Table 4). This experiment provides further evidence of a relationship between DNA degradation at the replication fork brought about by the dNTPs plus ATP and the ensuing induction of ASase synthesis. All these

Table 4. Effect of amino acid starvation on induction of ASase

Amino acid	ASase, units	
starvation, min	+ dNTPs	+ UV
0	0.99	2.06
15	0.50	3.55
30	0.23	6.08
45	0.21	5.31

Strain MO1513 (*rec*$^+$, ϕ80 lysogen) was grown in SSA medium (100 ml) with reduced tryptophan supplementation (10 μg/ml). When the cell concentration reached 2.8×10^8/ml, the cells were washed and reincubated in the warmed SSA medium (100 ml) without tryptophan. Samples (20 ml) were taken at 0, 15, 30, and 45 min during amino acid starvation. After ϕ80*ptrp* infection (m.o.i. 6), each sample was further divided into two, and one of each was irradiated with UV light (150 J/m^2). Each preparation (total eight) was then subjected to plasmolysis and incubated in the standard reaction mixture (200 μl) for ASase synthesis. The four dNTPs (0.25 mM each) were also present in the reaction mixtures of only the cells that had not been UV irradiated. At 120 min, the reaction was terminated for assay of ASase activity.

results suggest that there is a strong correlation between the action of the *recBC* DNase at the replication fork and the capacity of the cell to trigger the induction process. The fact that a *recB*$^-$ mutation blocks both DNA degradation and ASase synthesis while a *recA*$^-$ mutation only prevents the ASase synthesis indicates that *recBC* DNase acts first and the *recA* gene product acts at a later step in the prophage induction process and perhaps also in general SOS induction.

DISCUSSION

Recently we presented evidence that *recBC* DNase has a significant role in the prophage induction process and possibly in general SOS induction (4). The previous failure to detect the effect of a *recB* mutation on prophage induction by the plaque assay method is probably due to the presence of an alternate pathway(s) which can substitute for the one provided by a functional *recBC* gene product. The results presented here, disclosing an absolute *recBC* DNase dependence for prophage induction initiated in permeable cells by dNTPs plus ATP, is consistent with this interpretation. These results suggest that even *in vivo* under certain conditions prophage induction may be completely dependent upon a functional *recBC* DNase. Our observation that in permeable cells prophage induction can be associated with a *recBC* DNase-dependent degradation specifically of DNA at the chromosome replication fork suggests further that under certain conditions *in vivo* the DNA structure at the replication fork may become susceptible to *recBC* DNase activity and the resulting degradation provides a signal that triggers prophage induction and possibly other SOS functions. This type of induction mechanism may, in fact, be the basis for induction by thymine starvation or by temperature shift-up of *dna* ts mutants. Supporting this interpretation is the fact that we could not detect any significant induction of ASase synthesis in thymine-requiring *recB*$^-$ mutant strains after thymine starvation (unpublished results). It is possible that under such conditions the level of dNTPs in the cells may increase, thus creating the condition that makes the immobilized DNA replication fork susceptible to degradation by *recBC* DNase. In fact, Neuhard and Thomassen (14) reported that the concentration of dNTPs increased to usually high levels during thymine deprivation or after nalidixic acid treatment. Under normal growing conditions, the level of one or more of the dNTPs may be too low to cause replication fork degradation and/or a replication fork that is actively replicating may not be susceptible to the DNase(s). In thymine starvation which would result in the absence of dTTP, the *recBC* DNase may still be able to attack the immobilized replication fork in the presence of only the three dNTPs and ATP (see Table 2) even though this is less efficient than in the presence of four dNTPs. It is also possible that dUTP can substitute for dTTP.

Since most inducing agents inhibit DNA replication, one might argue that the resulting immobilization of the replicating fork may lead to instability and/or increased levels of dNTPs and thereby provide a general basis for initiation of induction by most types of inducing treatments. If this were the case, the induction process would not be triggered in a cell that has a completed chromosome and thus no replication fork. However, *in vivo* (15), and from the results in Table 4, a lysogen with completed, nonreplicating chromosomes can be induced by UV irradiation. Of course, it is conceivable that many inducing agents such as UV simultaneously cause more than one type of primary change in DNA that leads to induction, and immobilization of the replication fork is one such effect.

We do not know whether the replication fork DNA in the plasmolyzed, permeable cells is altered so that it is more susceptible to degradation than is that of normal cells or if the concentrations of dNTPs we have used would initiate degradation of the normal replicating fork DNA. Also we have not determined the exact role of the dNTPs in degradation at the DNA replication fork. It is possible that the dNTPs provide a substrate for an abortive DNA replication and/or affect, directly or indirectly, the nucleolytic activity of the replication complex. One or both of these effects may result in the conversion of the replication fork to a *recBC* DNase-susceptible structure. The absolute dependence of this dNTP-initiated DNA degradation and induction upon the presence of *recBC* DNase is in accord with *in vivo* (16) and *in vitro* (17) evidence suggesting an association of this enzyme with the DNA replication complex.

The involvement of *recBC* DNase in the prophage induction process described in this and our recent paper and the evidence mentioned above, that it acts at an earlier step than the *recA* gene product, provide a basis for speculation regarding the overall role of the enzyme in bacteria.

Generally the *recBC* DNase is viewed as having a direct role in genetic recombination. We suggest that the *recBC* DNase, although it may have a direct role in recombination, has a role in the regulation of cellular SOS functions. More specifically, we suggest that a primary role of this enzyme is to detect unusual DNA structures, such as scissions in the chromosome or immobilized replication forks, and to create signals that induce the production of higher levels of SOS components. This concept is consistent with the model of Gudas and Pardee (18) for induction of X protein synthesis.

We thank Ms. B. Friefeld for technical assistance and Ms. J. Tamayo for preparing the manuscript. We also thank Dr. P. Margolin for critically reading the manuscript and for helpful discussions throughout the study. This work was supported by Grant GM-21073 from the National Institutes of Health to M.O. C.L.S. is a recipient of U.S. Public Health Service Postdoctoral Fellowship GM-05460.

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