*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 11806–11809, December 1995
Genetics

# Recovery of respiration following the SOS response of *Escherichia coli* requires RecA-mediated induction of 2-keto-4-hydroxyglutarate aldolase

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*Communicated by Philip C. Hanawalt, Stanford University, Stanford, CA, September 6, 1995 (received for review June 5, 1995)*

**ABSTRACT** **Agents that damage DNA in *Escherichia coli* or interfere with its replication induce DNA repair and mutagenesis via the SOS response. This well-known activity is regulated by the RecA protein and the LexA repressor. Following repair or bypass of the DNA lesion, the cell returns to its resting state by a largely unknown process. We found that 2-keto-4-hydroxyglutarate aldolase (4-hydroxy-2-oxoglutarate aldolase; EC 4.1.3.16) is necessary for the recovery of respiration and that it is regulated by the SOS response. This protein was induced by DNA-damaging agents. Induction required RecA activation. When the LexA regulon was repressed, activation of RecA was not sufficient for induction, indicating the requirement for an additional protein under LexA control. Finally, a mutant in the corresponding *hga* gene was UV sensitive. 2-Keto-4-hydroxyglutarate aldolase also plays a role in respiratory metabolic pathways, which suggests a mechanism for respiration resumption during the termination of the SOS response.**

Exposure of *Escherichia coli* to agents that damage DNA or interfere with its replication induces the expression of a set of genes called the SOS response, which allows the cell to repair or tolerate DNA lesions (1, 2). Following DNA damage, the presence of single-stranded DNA at a stalled replication fork activates RecA protein. Activated RecA protein subsequently catalyzes autocleavage of LexA, the repressor of the SOS regulon (3), leading to expression of SOS functions such as inhibition of cell division, induction of DNA repair, and mutagenesis. If the cell successfully processes the lesion, the level of activated RecA protein decreases and the bacteria returns to its steady state. Although the induction of the SOS system is fairly well understood, the biochemical events leading to the return to steady state and the mechanism of their regulation are largely unknown.

During a search for novel SOS proteins by two-dimensional electrophoresis, we observed a highly inducible protein that was identified as 2-keto-4-hydroxyglutarate (KHG) aldolase (4), also called 2-keto-3-deoxy-6-phosphogluconate aldolase (5). This protein was induced by DNA-damaging agents via a process requiring activable RecA protein. Induction of KHG aldolase and at least one unidentified protein dependent on LexA was indispensable for cells to resume respiration after UV irradiation. These results show that the return to steady state after the SOS response is controlled by proteins of the SOS regulon and suggest a mechanism for the regulation of respiration during the SOS response.

## MATERIALS AND METHODS

**Chemicals.** Ampicillin, kanamycin, Coomassie brilliant blue R-250, isopropyl β-D-thiogalactopyranoside, and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside were from Sigma. Trans$^{35}$S-label was from ICN. [$^{32}$P]dCTP and [$^{35}$S]dATP were from NEN. Phenol/chloroform was from Interchim. *Taq* DNA polymerase was from Tebu. Urea, *N,N,N′,N′*-tetramethylenediamine, sodium persulfate, and low-melting-point agarose were from Bio-Rad. Ampholines, pH 5–7 and pH 3.5–10, were from LKB. Acrylamide and *N,N′*-methylenebisacrylamide were from Fluka. DNA size markers were from GIBCO. The DNA sequencing kit and kanamycin resistance GenBlock were from Pharmacia. The Multiprime DNA labeling kit was from Amersham.

***E. coli* Strains, Phage, and Plasmids.** The strains used are listed in Table 1. pBluescript II KS(+/−) phagemid was from Stratagene.

**Labeling and Two-Dimensional Gel Electrophoresis of Proteins.** Five milliliters of bacteria grown in M63 medium to a concentration of $2 \times 10^8$ cells per ml were irradiated with UV light at 60 J/m$^2$. Aliquots (1 ml), irradiated or not, were aerated for 10 min at 37°C before being pulse labeled for 5 min with 100 μCi of [$^{35}$S] methionine (specific activity, 1000 Ci/mmol; 1 Ci = 37 GBq) and chased by addition of 160 μl of 0.2 M methionine (13). Techniques for extraction of proteins and resolution on two-dimensional polyacrylamide gels were modified from O'Farrell (14) and Hochstrasser *et al.* (15). Three independent experiments with control and irradiated bacteria were carried out.

**Quantification of Protein Induction.** Labeled proteins were separated by two-dimensional PAGE. Gels were exposed to Amersham β-max Hyperfilm for 2, 5, and 7 days to ensure linearity between optical density and incorporated label and accurate quantitation. Autoradiograms were laser scanned and analyzed with the PD QUEST system for computer analysis of two-dimensional gel images (16).

**Quantification of mRNA Induction.** mRNA was detected by Northern blot analysis using the cloned *hga* gene as a probe and quantified with a BioImage whole-band Analyzer.

**Protein Concentration and Purification.** Protein was prepared according to Lesca *et al.* (13). The SOS response was induced in 50-ml cultures by treatment with nalidixic acid (40 μg/ml), and protein extracts (2 mg) were fractionated by preparative isoelectric focusing for 2–6 hr (12 W, 250–1200 V) in a liquid-phase Rotofor system (Bio-Rad). Protein fractions were analyzed on two-dimensional polyacrylamide gels and stained, and the protein corresponding to P24 was cut from the gel. The pieces of gel were neutralized in water, washed, and suspended in 12 mM Tris·HCl, pH 6.8/50 mM dithiothreitol/1% SDS/0.1% bromophenol blue. Proteins were concentrated by SDS PAGE at 200–350 V on a 40-ml stacking gel (5% acrylamide) and 20-ml resolving gel (12.5% acrylamide) as

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Abbreviation: KHG, 2-keto-4-hydroxyglutarate.

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Table 1. *E. coli* strains used

| Strain | Relevant genotype | Ref. |
|---|---|---|
| AB1157 | F⁻ *thr-1 leuB6 proA2 hisG4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mrt-1 supE44 tsx-33 rpsL31* Sm$^r$ | 6 |
| DM1420 | AB1157 *lexA51*(Def) *sfiA11* | 7 |
| GC2281 | AB1157 *lexA3 malB*::Tn*9* | 8 |
| DM2210 | AB1157 *lexA3 recAo98* | 9 |
| JC7623 | AB1157 *recB21 recC22 sbcB15* | 10 |
| TG1 | Δ(*lac–pro*) *supE thi hsdD5*/F′ *traD36 proA*$^+$*B*$^+$ *lacI*$^q$ *lacZ*Δ*M15* | 11 |
| PC1424 | DM1420 *recA430 slr*::Tn*10* | 12 |
| CA1421 | *DM1420 hga*::*kan*$^R$ | * |
| CA1418 | TG1/pBluescript *hga* | * |
| CA1419 | JC7623/pBluescript *hga*::*kan*$^R$ | * |

*This work.

reported (17). The gel was stained and the protein band was cut out, rinsed in water, and used for microsequencing.

**Microsequencing.** Techniques for in-gel protein digestion, HPLC peptide purification, and amino acid characterization were modified from Rosenfeld *et al.* (18). Peptides released after tryptic protein digestion were analyzed by laser desorption ionization mass spectrometry (19). The peptides were separated by reverse-phase HPLC and those present in sufficient amount were sequenced by N-terminal sequence analysis. The protein was identified by comparison of peptides sequences with the Swiss-Prot data base.

**Cloning and Inactivation of the *hga* Gene.** The chromosomal *hga* gene was cloned into pBluescript II KS vector by PCR using two primers, 5′-CTAGCGAATTCATGAAAAACTGGAAAACAAGCGCCGAA-3′ and 3′-CGGGCGCTGCGGCAGCTCCCGCGAGACCCTAGGACTCT-5′. These oligonucleotides were derived from the 5′ and 3′ ends of the *hga* gene (4) and were coupled to synthetic *Eco*RI and *Bam*HI restriction sites for cloning. The sequence was verified by the dideoxy-chain termination method (4, 20). The gene was disrupted by insertion of a kanamycin-resistance gene into the unique *Pst* I site. The vector was linearized at its unique *Kpn* I site and used to transform *E. coli* JC7623 by recombination at its chromosomal location. The presence of the mutation was checked by its high cotransduction with the *ruvAB* operon (83%), both being located between 40 and 41 min on the *E. coli* chromosome. The mutation was transduced into the *lexA*(Def) strain DM1420.

**Oxygen Consumption Measurements.** Cultures were grown at 37°C to a concentration of $2 \times 10^8$ cells per ml in M9 minimal medium (21). The culture was divided into two parts, one of which was UV irradiated. Both cultures were incubated at 37°C and samples were collected, at various times, to measure the oxygen consumption with a Clark electrode at 37°C. The quantity of consumed oxygen was calculated (22) as $(0.4\ \mu\text{mol of O}/N) \times (\text{slot}/V) \times 10^{-3}$, where $N$ = slot between 0% and 100% and $V$ = volume of the cuvette (1.2 ml). These experiments were done twice and the deviation was ≤5%.

## RESULTS

**Characterization of P24 Protein.** In *E. coli* strains carrying insertions in the *lexA* gene or point mutations leading to an inactive LexA protein, most known SOS genes are constitutively expressed. We observed >100-fold induction by UV irradiation of about 20 out of 1800 proteins in such strains by two-dimensional gel electrophoresis. In some cases induction required the presence of an activable RecA protein (13, 23). In particular, P24 protein, which had a pI of 6.3 and a molecular mass 24 kDa, was induced 200-fold 15 min after UV irradiation (Table 2). Less than 5-fold induction of P24 was observed in bacteria carrying a *recA430* mutation, which codes for a RecA protein with deficient coprotease activity (Table 2).

Table 2. UV induction of KHG aldolase protein and mRNA

| | DM1420 | | | PC1424 | | |
|---|---|---|---|---|---|---|
| | Intensity | | | Intensity | | |
| | 0 J/m$^2$ | 60 J/m$^2$ | IF | 0 J/m$^2$ | 60 J/m$^2$ | IF |
| Protein | 1.4 | 279 | 198* | 1.4 | <7 | <5† |
| mRNA | 64 | 58 | 0.9† | 64 | 60 | 0.9† |

Intensities are relative to background and are mean values from three independent experiments. Protein intensity values are from PD QUEST analysis of two-dimensional gels. mRNA intensity values are from quantitation with a BioImage whole-band analyzer. IF, induction factor (irradiated/nonirradiated).
*Significant at $P < 0.001$, Student $t$ test.
†Difference between control and irradiated not significant ($P > 0.3$).

To identify P24 protein, extracts from nalidixic acid-treated *lexA*(Def) bacteria were concentrated in a liquid pH gradient. Fractions between pH 6.1 and 6.5 were analyzed by two-dimensional gel electrophoresis. P24 was recovered from 20 gels and concentrated by SDS/PAGE. A major band corresponding to 20 pmol of a protein of 25 kDa was cut from the gel. Tryptic digestion of the stained protein in the gel gave rise to several peptides that were eluted. After mass spectrometry analysis and HPLC purification, two peptides were sequenced: LQAIAGPFSQV and AESILT. These sequences correspond to amino acid residues 146–156 and 8–13, respectively, of a known *E. coli* protein, KHG aldolase (4), also known as 2-keto-3-deoxy-6-phosphogluconate aldolase (5).

To investigate possible transcriptional regulation of KHG aldolase, we measured mRNA levels after UV treatment. No increased transcription was observed by Northern analysis even though the amount of KHG aldolase protein was strongly increased (Table 2).

**Characterization of an *hga*::*kan*$^R$ Mutant.** In order to understand the role of KHG aldolase in the SOS response, we cloned the *hga* gene and constructed a mutant by insertion of a gene for kanamycin resistance (4). Introduction of the mutation into the bacterial chromosome led to the complete disappearance of P24 on two-dimensional gel electrophoresis, confirming the identity of the protein (data not shown). The *hga*::*kan*$^R$ mutant was sensitive to UV radiation compared with the parental strain (Fig. 1).

**Role of KHG Aldolase in Respiration Recovery After UV Irradiation.** KHG aldolase regulates the level of glyoxylate, a metabolite that can inhibit key enzymes of the Krebs cycle (24). It has also been reported that respiration inhibition after

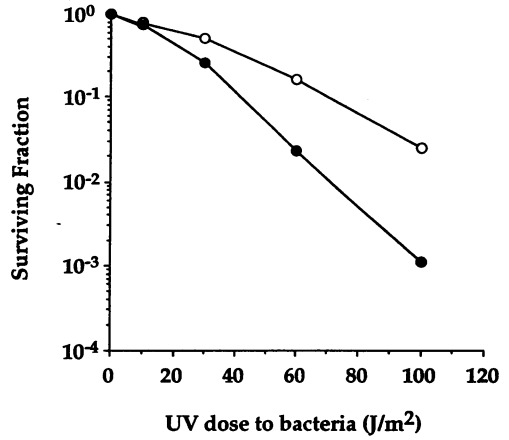


FIG. 1. Survival of exponentially growing cells ($2 \times 10^8$ per ml) after UV irradiation; ○, *lexA*(Def) strain DM1420; ●, *lexA*(Def) *hga* strain CA1421.

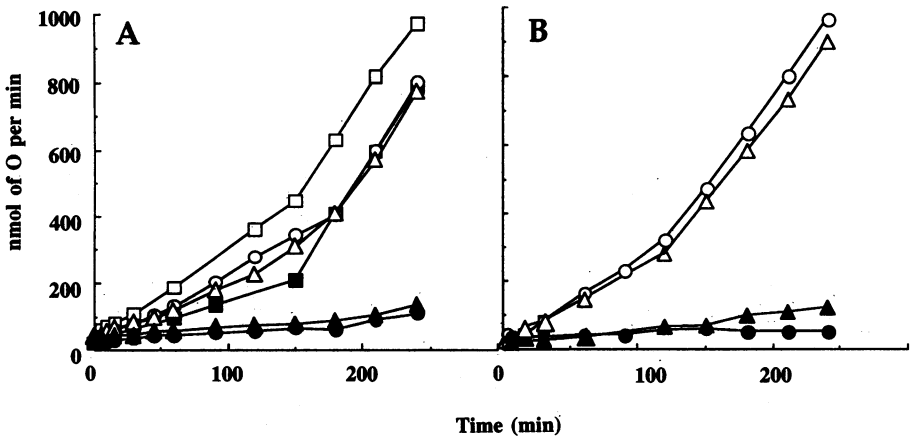


FIG. 2. Effect of UV irradiation on respiration. (*A*) Oxygen consumption of DM1420 [*lexA*(Def)] nonirradiated (□) and irradiated (■) with 30 J/m$^2$, CA1421 [*lexA*(Def) *hga*] nonirradiated (○) and irradiated (●) with 30 J/m$^2$, and PC1424 [*lexA*(Def) *recA430*] nonirradiated (△) and irradiated (▲) with 10 J/m$^2$. (*B*) Oxygen consumption of GC2281 [*lexA*(Ind$^-$)] nonirradiated (○) and irradiated (●) with 10 J/m$^2$ and of DM2210 [*lexA*(Ind$^-$) *recAo98*] nonirradiated (△) and irradiated (▲) with 10 J/m$^2$.

UV irradiation is an SOS function (25). Therefore we investigated the effect of the *hga* gene on oxygen consumption with (filled symbols) or without (open symbols) UV irradiation (Fig. 2).

UV irradiation of *lexA*(Def) bacteria at 30 J/m$^2$ inhibited respiration, which subsequently resumed at the same rate as in nonirradiated bacteria (Fig. 2*A*; ■, □). Identical results were observed with 60 J/m$^2$ (data not shown). UV irradiation also suppressed respiration of *lexA*(Ind$^-$) bacteria (Fig. 2*B*; ●, ○), demonstrating that the SOS response is not required for this inhibition. However, oxygen consumption remained blocked after irradiation in the mutant CA1421, in which the *hga* gene was disrupted (Fig. 2*A*; ●, ○). This result implies that KHG aldolase is required for respiration recovery after DNA damage. Furthermore, resumption of respiration was not observed in the absence of RecA coprotease activity [*lexA*(Def) *recA430*] (Fig. 2*A*; ▲, △), consistent with the observation that induction of KHG aldolase protein is under the control of RecA (Table 2).

Oxygen consumption did not recover when the SOS proteins (including RecA) were expressed at only their basal levels [*lexA*(Ind$^-$)] (Fig. 2*B*; ●, ○). To investigate whether these results were exclusively an effect of the low level of active RecA protein, we measured respiration in a *lexA*(Ind$^-$) *recAo98* strain in which a mutation in the *recA* operator allows the expression of high level of RecA protein while genes regulated by the LexA protein remain repressed. In these conditions respiration was also inhibited after UV irradiation and did not recover (Fig. 2*B*; ▲, △). These results indicate that (*i*) UV damage inhibits respiration without derepression of the SOS regulon and (*ii*) respiration resumption requires the RecA-dependent induction of KHG aldolase and some unidentified LexA-dependent protein(s).

## DISCUSSION

Evidence has accumulated that suggests that the SOS response is composed of two sets of genes (26). One exerts its regulatory role via LexA cleavage (3, 27) and the other depends on activated RecA protein without being directly under LexA control. The protein KHG aldolase, coded by the *hga* gene (4), also known as the *eda* gene (5), was induced 200-fold after DNA damage when an activable RecA protein was present in the cell (Table 2). KHG aldolase induction is not under direct control of LexA, since it was induced in a LexA-deficient strain and it does not have a consensus sequence for LexA binding in its operator (5). Therefore *hga*, encoding KHG aldolase, belongs to the second set of genes, which also includes *dnaA, dnaN, dnaQ, phr*, and *dinY* (23, 26, 28, 29).

UV treatment increased the amount of KHG aldolase (Table 2), although enhanced transcription of the *hga* gene was not observed. This suggests that during the UV response, the protein undergoes posttranscriptional regulation. It is known that mutagenesis requires posttranslational modification of the UmuD protein (30). However, the pI of basal and induced KHG aldolase were identical and the molecular weight of the protein corresponded to the value expected from the sequence of the *hga* gene. Hence translational regulation rather than posttranslational modification most likely leads to KHG aldolase accumulation in UV-irradiated cells.

We observed respiration arrest in bacteria where all classical LexA-dependent SOS functions are blocked [*lexA*(Ind$^-$); Fig. 2*B*]. Hence, in our conditions, respiration inhibition was not dependent on RecA and LexA proteins, in contrast to previous reports (25, 31). On the other hand, the regulation of KHG aldolase by RecA protein (Table 2), the UV sensitivity of an *hga*::*kan*$^R$ mutant (Fig. 1), and the implication of the protein in respiration arrest (Fig. 2*A*) indicate that respiration recovery (mediated by KHG aldolase) is under control of the SOS response.

Respiration in bacteria with functional KHG aldolase recovered after UV irradiation, whereas respiration never resumed in UV-irradiated bacteria without the enzyme (Fig. 2*A*). Furthermore, the arrest in oxygen consumption persisted in bacteria carrying a *recA430* mutation, which prevents the induction of KHG aldolase. These results show that respiration recovery requires KHG aldolase amplification. However, RecA was not the only SOS protein required. As yet uniden-

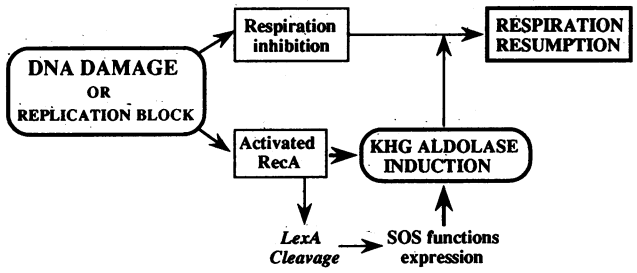


FIG. 3. Model for respiration inhibition and recovery during the SOS response. Respiration inhibition is independent of the SOS regulon. Respiration resumption requires KHG aldolase induction, which depends on activated RecA protein and unknown LexA-controlled protein(s).

tified gene(s) repressed by LexA were also necessary for respiration resumption (Fig. 2*B*).

KHG aldolase is a multifunctional enzyme. It regulates the level of glyoxylate (24), which is used in the glyoxylate bypass pathway, an alternative to the Krebs cycle when bacteria are grown on an acetate carbon source. KHG aldolase is also involved in the Entner–Doudoroff pathway, where it is known as 2-keto-3-deoxy-6-phosphogluconate aldolase (5). This pathway is employed for metabolism of gluconate in *E. coli*. Induction of KHG aldolase in the Entner–Doudoroff pathway is under transcriptional control (5). However, transcription of KHG aldolase mRNA was not increased after irradiation. These observations suggest that respiration recovery may involve the glyoxylate bypass pathway.

Our results suggest the addition of features to the current model of respiration inhibition and recovery in *E. coli* treated with DNA-damaging agents (Fig. 3). DNA damage or inhibition of replication appears to produce a signal that blocks respiration independently of the SOS response. In contrast, respiration recovery is under the control of the SOS response, which induces KHG aldolase, apparently via translational regulation. Induction requires activated RecA protein and at least one unidentified LexA-dependent protein.

We are indebted to Drs. P. Kaiser and C. Thompson for making the PD QUEST system available. We gratefully acknowledge Prof. G. Lanéelle for help with respiration measurements and Dr. N. P. Johnson for critical reading of the manuscript and helpful suggestions. C.C. was a recipient of an Association pour la Recherche contre le Cancer fellowship. This work was partly supported by a grant from the Association pour la Recherche sur le Cancer to M.D.

1. Radman, M. (1974) in *Molecular and Environmental Aspects of Mutagenesis*, eds. Prakash, L., Sherman, F., Miller, M., Lawrence, C. & Tabor, H. W. (Thomas, Springfield, IL), pp. 128–142.
2. Witkin, E. M. (1976) *Bacteriol. Rev.* **40,** 869–907.
3. Little, J. W. & Mount, D. W. (1982) *Cell* **29,** 11–22.
4. Patil, R. V. & Dekker, E. E. (1992) *J. Bacteriol.* **174,** 102–107.
5. Egan, S. E., Fliege, R., Tong, S., Shibata, A., Wolf, R. E. & Conway, T. (1992) *J. Bacteriol.* **174,** 4638–4646.
6. Howard-Flanders, P., Boyce, R. P. & Theriot, L. (1966) *Genetics* **53,** 1119–1136.
7. Mount, D. W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 300–304.
8. Mount, D. W., Brooks Low, K. & Edmiston, S. J. (1972) *J. Bacteriol.* **112,** 886–893.
9. Ginsburg, H., Edminson, S. H., Harper, J. & Mount, D. W. (1982) *Mol. Gen. Genet.* **187,** 4–11.
10. Winans, S. C., Elledge, S. J., Krueger, J. H. & Walker, G. C. (1985) *J. Bacteriol.* **161,** 1219–1221.
11. Debarbouillé, M., Kuntz, F., Klier, A. & Rapoport, G. (1987) *FEMS Microbiol.* **41,** 137–140.
12. Calsou, P. & Defais, M. (1985) *Mol. Gen. Genet.* **201,** 329–333.
13. Lesca, C., Petit, C. & Defais, M. (1991) *Biochimie* **73,** 407–409.
14. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
15. Hochstrasser, D. F., Patchornik, A. & Merril, C. R. (1988) *Anal. Biochem.* **173,** 412–423.
16. Garrels, J. I. (1989) *J. Biol. Chem.* **264,** 5269–5282.
17. Rasmussen, H. H., Van Damme, J., Bauw, G., Puype, M., Gesser, B., Celis, J. E. & Vandekerckhove, J. (1991) in *Methods of Protein Sequence Analysis*, ed. Gustavsson, J. H. (Birkhäuser Verlag, Basel), pp. 103–114.
18. Rosenfeld, J., Capdevielle, J., Guillemot, J. C. & Ferrara, P. (1992) *Anal. Biochem.* **203,** 173–179.
19. Karas, M. & Bahr, U. (1990) *Trends Anal. Chem.* **9,** 321–325.
20. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
21. Miller, J. H. (1972) in *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
22. Chappell, J. B. (1964) *Biochem. J.* **90,** 225–237.
23. Petit, C., Cayrol, C., Lesca, C., Kaiser, P., Thompson, C. & Defais, M. (1993) *J. Bacteriol.* **175,** 642–646.
24. Gupta, S. C. & Dekker, E. E. (1984) *J. Biol. Chem.* **259,** 10012–10019.
25. Swenson, P. A. & Schenley, R. L. (1974) *Int. J. Radiat. Biol.* **25,** 1167–1170.
26. Lewis, L. K., Harlow, G. R., Gregg-Joly, L. A. & Mount, D. W. (1994) *J. Mol. Biol.* **241,** 507–523.
27. Little, J. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1375–1379.
28. Payne, N. S. & Sancar, A. (1989) *Mutat. Res.* **218,** 207–210.
29. Lewis, L. K., Jenkins, M. E. & Mount, D. W. (1992) *J. Bacteriol.* **174,** 3377–3385.
30. Nohmi, T., Battista, J. R., Dodson, L. A. & Walker, G. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1816–1820.
31. Barbé, J., Guerrero, R. & Villaverde, A. (1983) *Mutat. Res.* **121,** 171–175.