Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11736–11740, December 1995
Genetics

cAMP-dependent SOS induction and mutagenesis in resting bacterial populations

(starvation/catabolic repression/DNA repair/inducible genetic instability/evolution)

FRANÇOIS TADDEI[†], IVAN MATIC[‡], AND MIROSLAV RADMAN

Laboratoire de Mutagénèse, Institut Jacques Monod, 2, place Jussieu, 75251, Paris, France

Communicated by Evelyn M. Witkin, Rutgers University, Piscataway, NJ, August 17, 1995

ABSTRACT The inducible SOS system increases the survival of bacteria exposed to DNA-damaging agents by increasing the capacity of error-free and error-prone DNA repair systems. The inducible mutator effect is expected to contribute to the adaptation of bacterial populations to these adverse life conditions by increasing their genetic variability. The evolutionary impact of the SOS system would be even greater if it was also induced under conditions common in nature, such as in resting bacterial populations. The results presented here show that SOS induction and mutagenesis do occur in bacteria in aging colonies on agar plates. The observed SOS induction and mutagenesis are controlled by the LexA repressor and are RecA- and cAMP-dependent.

Nature rarely provides the conditions that will allow bacteria to grow exponentially, and when it does, the nutrients are quickly exhausted, leading to starvation conditions. Numerous adaptive physiological changes have been observed under these conditions (for a review, see ref. 1). Cells starving in the presence of nutrients they cannot use exhibit increased mutation frequencies in the gene under selective pressure (2–7). This still controversial finding suggests that cellular DNA metabolism is different under starvation vs. exponential growth conditions. Therefore, results obtained under the latter conditions cannot directly apply to the former but may serve as a framework for further studies. Under exponential growth conditions, a major cellular response to perturbation of DNA metabolism is the induction of the SOS system. Besides DNA repair (8, 9), recombination (10, 11), and the fidelity of replication (9, 12–14), the *Escherichia coli* SOS response affects cell division (15), transposon mobility (16), and horizontal gene transfer (17). DNA-damaging agents, such as radiation and chemicals, produce an SOS-inducing signal generally thought to be single-stranded DNA (8, 9). The binding of RecA protein to single-stranded DNA confers on RecA a co-protease activity (the symbol for activated RecA is RecA*), which stimulates the cleavage of the CI repressor of λ and the LexA repressor of numerous SOS genes, including the *recA* gene itself (8). We sought to determine if resting bacterial populations can induce their SOS functions in the absence of exogenous sources of DNA lesions. Increased genetic variability of resting bacterial populations can increase their fitness (18). Mutagenesis is one of the SOS functions that could contribute to adaptation; therefore, we have measured mutation frequencies under these conditions.

MATERIALS AND METHODS

Detection of SOS Induction on Agar Plates. *E. coli* strains used were derived from MT1 [*ilv his rpsL* nalidixic acid-resistant (λ *c*I$^+$ *cro*$^+$-*gal*$^+$)] and MT5, a *c*I(Ind$^-$) derivative of MT1 (19). Derivatives carrying the following alleles were constructed by P1-mediated transduction (20): *recA430 srl300*::Tn*10* (from D. Touati, Paris), *lexA1 malB*::Tn*9* (from GC2281; R. D'Ari collection, Paris), *recAo98 srl300*::Tn*10* (from C. Herman collection, Paris), *recB268*::Tn*10* (21), *mutS215*::Tn*10* (from G. Walker's collection; Cambridge, MA), and *katF*::Tn*10* (*katF* = *rpoS* mutation was provided by D. Touati). Bacteria were incubated at 37°C on MacConkey agar plates containing 1% (wt/vol) galactose. On these plates, the Gal$^+$ phenotype is detectable as a red color of colonies due to the change of pH caused by galactose metabolism.

Detection of SOS Induction in Liquid Media. The strains used to measure *recA* gene expression are derivatives of S90C (Δ*pro-lac ara thi strA*) (22), which carries a functional *recA* copy and is lysogenized by a λ carrying a *recA*::*lacZ* coding for a fusion protein (23). Derivatives carrying the following alleles were constructed by P1-mediated transduction: *lexA1*(Ind$^-$) *malB*::Tn*9* (from GC2281; R. D'Ari collection) and Δ*cya ilv*::Tn*5* (from GC2880; R. D'Ari collection). β-Galactosidase units were calculated according to Miller (20).

Detection of Mutagenesis in Resting Bacterial Populations. For the mutagenesis assay, NR3835 Δ(*pro-lac*) *ara thi trpE9777*; F′(*pro-lac*) and its *recA56* derivative (NR8002) were provided by R. Schaaper (Research Triangle Park, NC). The *lexA1*(Ind$^-$) *malB*::Tn*9* and Δ*cya* strains were constructed by P1 transduction. On day 0, ≈10^3 cells from an overnight liquid culture were spotted on a nitrocellulose filter (NC 45 from Schleicher & Schuell) on fresh 869 plates (NaCl at 5 g/liter, Bacto tryptone at 10 g/liter, yeast extract at 5 g/liter, and agar at 15 g/liter). On days 1 and 7, cells were resuspended in 1 ml of 10 mM $MgSO_4$ and were incubated 1 hr at 37°C in liquid 869 to allow for expression of the rifampicin resistance, as is usually done after mutagenic treatments (24). Cells were plated on either 869 containing rifampicin (100 μg/ml) or 869. Colonies were counted 2 days after plating to allow slow-growing mutants to appear. Frequency of mutation was computed from at least 20 independent cultures as described (25).

RESULTS

SOS Induction in Aging Colonies. To detect the SOS response in resting cells, we employed a reporter system involving an epigenetic switch as a consequence of λ CI repressor cleavage by the activated RecA protein (see Fig. 1 for description and ref. 19 for sensitivity of this assay to DNA-damaging treatments). In this system, SOS induction is revealed as a white to red colony color change on indicator plates after 2 days of incubation (Fig. 2). The color change does not occur if the strain carries either a *recA430* (RecA defective in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ROSE, resting organisms in a structured environment; RecA*, activated RecA.

[†]On leave of absence from: Ecole Nationale du Génie Rural des Eaux et des Forêts 19, avenue du Maine, 75015, Paris, France.
[‡]On leave of absence from: Laboratory of Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, University of Zagreb, 41000 Zagreb, Croatia.

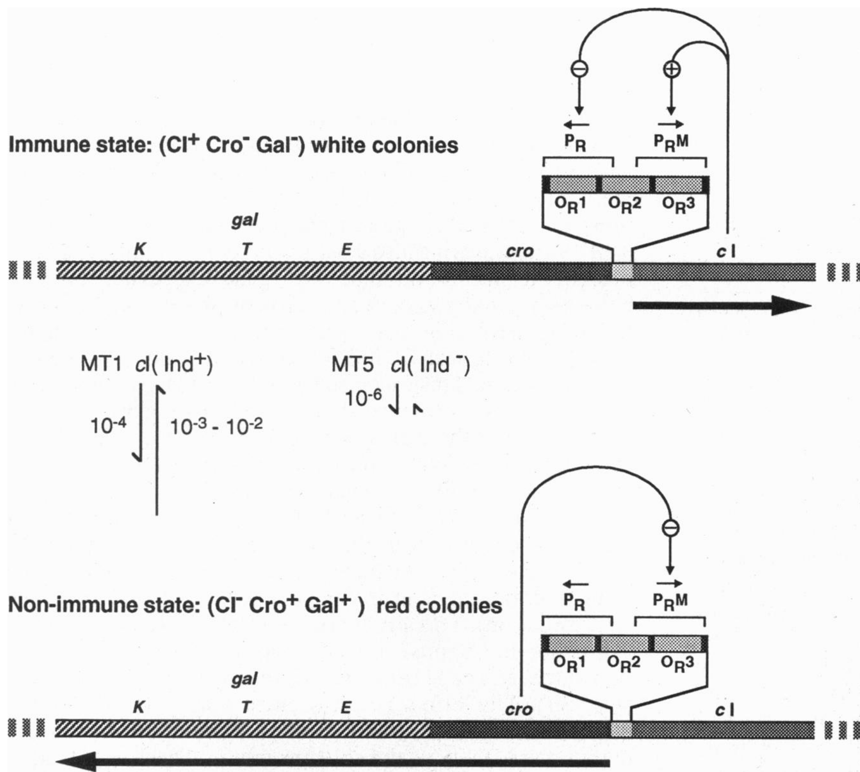

FIG. 1. The SOS-inducible epigenetic switch. This figure shows the principle of the SOS-inducible epigenetic switch in *E. coli*, which, because it is heritable (i.e., transmitted to the clonal descent of individual cell), allows detection and analysis of clones issued from progenitor cells that have undergone SOS induction even transiently (19). This "memory" effect is based on the reciprocal repression of the phage λ *c*I and *cro* genes (26), which have been fused with the promoterless *gal* operon transcribed from the λ P_R promoter and thus set under the negative control of the CI repressor (27). Inactivation of the CI repressor, either permanently by mutation or transiently by its RecA-assisted proteolytic inactivation during SOS induction, results in stable, heritable expression of the *cro* and *gal* genes. An isogenic MT5 strain with a *c*I(Ind$^-$) mutation (which prevents cleavage of CI repressor by activated RecA) detects, by color change, only the rare mutations in the *c*I gene. In MT1, the two epigenetic states switch spontaneously with the indicated frequencies.

λ CI repressor cleavage) or a *c*I(Ind$^-$) (repressor resistant to cleavage) mutation, indicating that it is due to bona fide SOS induction (Fig. 2 and Table 1). Red papillae appearing on these colonies are caused by mutations in the *c*I(Ind$^-$) gene. The

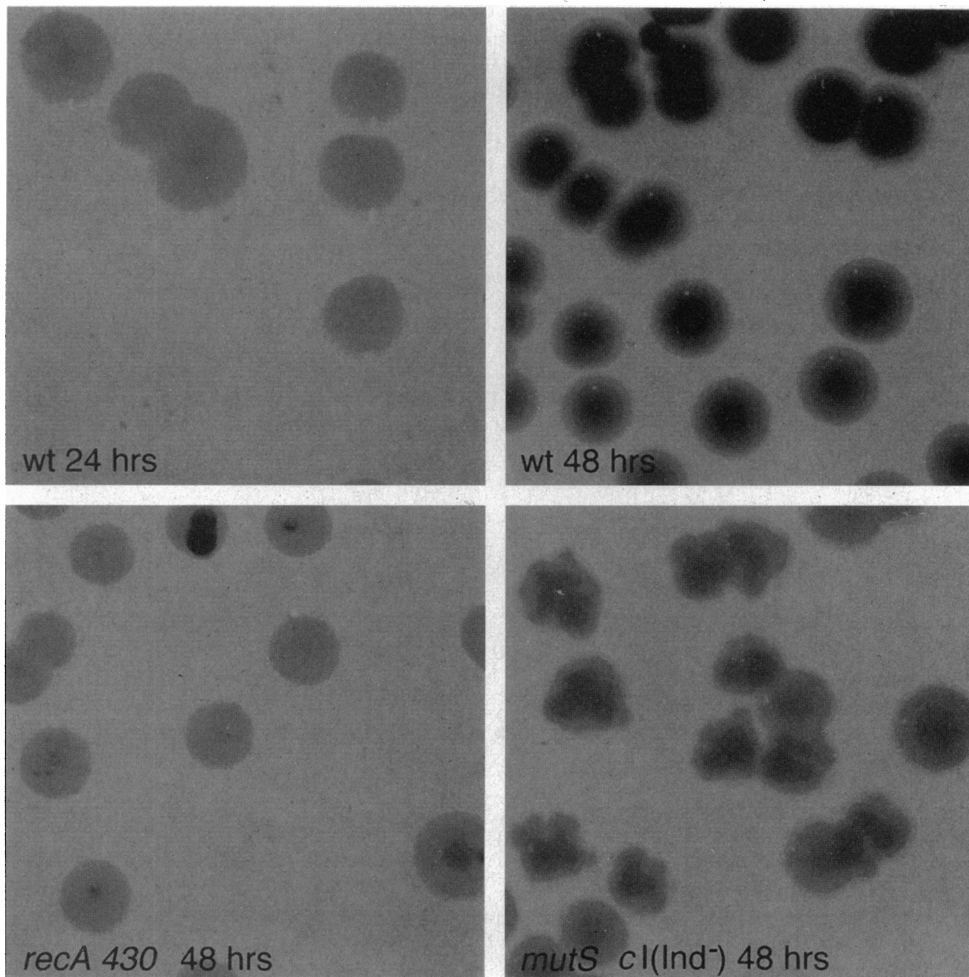

FIG. 2. Bacteria in aging colonies induce the SOS response. Colonies were photographed after 24 or 48 hr of incubation at 37°C. The different strains are indistinguishable after 24 hr (MT1 is shown), although some *c*I(Ind$^-$) *mutS*$^-$ colonies have papillae. The red (dark on the picture) papillae on the *c*I-(Ind$^-$) *mutS*$^-$ and *recA430* colonies are due to mutations inactivating *c*I [*c*I(Ind$^-$) colonies have a phenotype identical to *recA430* colonies]. The Gal$^+$ phenotype of MT1 colonies, which have turned red (dark) by day 2, is due to an epigenetic switch (white segregants upon streaking) (19). The SOS induction of cells within colonies is independent of the MacConkey medium because it has been observed on plates containing only peptone (20 g/ml), tryptone (5 g/ml), NaCl (5 g/ml), agar (15 g/liter), and water with neutral red added on day 1 or 2. wt, wild type.

Table 1. Genetics of SOS induction in colonies

Strain	Time of color change, hr
MT1 cI+	48
MT5 cI(Ind−)	48*
MT1 *recA430*	48*
MT1 *lexA1*(Ind−)	60
MT1 *lexA1*(Ind−) *recAo98*	36
MT1 *recB*	72
MT1 *rpoS*	48

Bacteria were plated (time 0) at 37°C on MacConkey agar plates containing 1% (wt/vol) galactose. Colony phenotype (white to red color change) was monitored from day 1 to day 3.
*Red papillae on *recA430* and cI(Ind−) white colonies represent mutations inactivating the CI repressor. The strain carrying a *c*I-(Ind−) mutation forms colonies with a phenotype identical to the *recA430* colonies (data not shown).

frequency of this SOS-independent genetic switch is elevated about 100-fold by the introduction of a *mutS*− mutator mutation (data not shown). In the *c*I(Ind−) *mutS*− strain, the mutation rate to Gal+ approaches the rate of the epigenetic switch observed in *c*I(Ind+) cells exponentially growing, which is due to spontaneous SOS induction under these conditions. The morphology of *mutS*− *c*I(Ind−) and *mutS*+ *c*I(Ind+) colonies is very different (see Fig. 2); the former are covered with red papillae giving a spotted texture, whereas the latter show concentric red rings giving a smooth surface. These differences in colony morphology imply that the SOS-dependent epigenetic switch in aging colonies occurs at a much higher frequency than in exponentially growing bacteria.

The starvation-specific transcriptional σ factor (28), encoded by the *rpoS* gene, is not required for the SOS induction in aging colonies (Table 1). In a *lexA1*(Ind−) strain (noncleavable SOS repressor), the time necessary for the color change was increased, whereas it was reduced in a *lexA1*(Ind−) *recAo98* (*recA* operator constitutive) double mutant, which overproduces the RecA protein (Table 1). These results suggest that it is the concentration of RecA protein rather than the concentration of RecA-activating substrate that is the limiting factor determining the efficiency of repressor cleavage. The color change is also delayed when the cells carry a *recB*− mutation (Table 1). SOS induction by double-stranded DNA breaks is known to depend on the RecBC helicase (10), which unwinds double-stranded DNA from the ends, thus providing single-stranded DNA for RecA protein binding (a prerequisite for both co-protease and recombinase activities). Therefore, the induction of SOS in aging colonies could be mediated by the presence of double-stranded DNA ends.

SOS Induction in Resting Bacterial Populations is cAMP-Dependent. Because the concentration of RecA protein is a key factor in this SOS induction, the relationship between cell metabolism and this pathway of induction in resting populations was studied by measuring β-galactosidase activity in a *recA*+ strain carrying also a *recA::lacZ* gene coding for a fusion protein. It was found that *recA* gene expression increases between the early exponential growth phase (day 0) and the following days (Fig. 3*A*). This effect is not observed in a *lexA1*(Ind−) derivative, indicating that it is due to a genuine SOS induction. This increased β-galactosidase activity is not observed in the presence of 1% (wt/vol) glucose (Fig. 3*C*), suggesting that the expression of the *recA* gene is sensitive to catabolic repression, known to be mediated by low cAMP concentration (29). However, this result is obscured by the apparent instability of the *recA::lacZ* protein fusion under starvation conditions in the presence of excess glucose. The possible effect of cAMP on SOS induction was therefore tested using a strain deficient in the synthesis of cAMP (carrying a *cya*− mutation). This strain does not show increased *recA* gene expression in response to starvation (Fig. 3*A*), and the induction of the *recA* gene can be recovered by the addition of cAMP (Fig. 3*B*). This effect is not seen in a *lexA1*(Ind−) mutant, demonstrating that cAMP controls SOS induction and not merely the activity of the *recA* promoter. These results are in contrast to the absence of a cAMP effect on SOS induction after UV irradiation (30). The cAMP dependence of SOS induction in resting bacterial populations without exogenous DNA-damaging treatments provides evidence of an interaction between the cAMP-controlled and the LexA-controlled regulons, one controlling cell metabolism, the other genetic stability. This interaction might bring under new light previous results describing an effect of both SOS and cAMP on the regulation of some genes (30, 31).

Timing of this cAMP-dependent SOS induction between day 0 and day 1 is consistent with the increase in RecA protein levels obtained using an immunoassay (32) and suggests that the SOS induction occurs after the end of the exponential growth phase when cells enter the stationary phase.

Mutagenesis in Resting Bacterial Populations. To study genetic variability in resting bacterial populations in the absence of direct selective pressure on the target gene, the

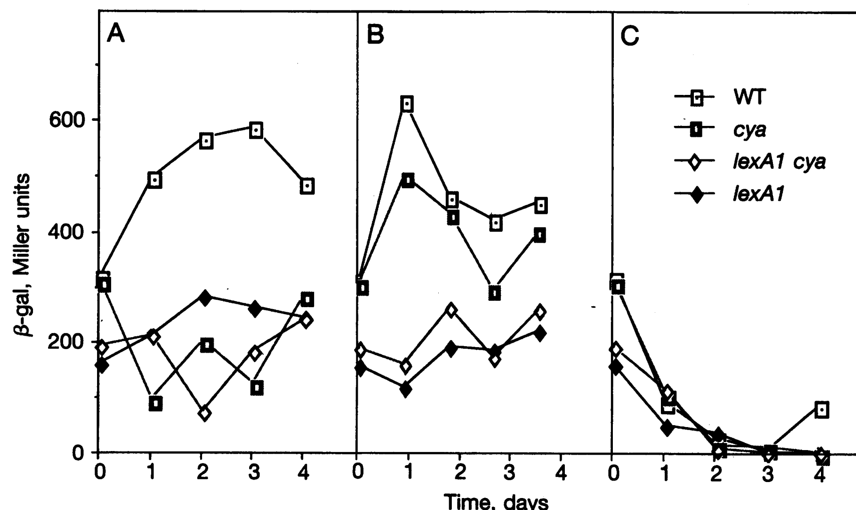

FIG. 3. The starvation-induced SOS response is controlled by cAMP levels. Cells were diluted (day 0) in rich liquid 869 medium (Luria–Bertani with 5 g of NaCl per liter) (*A*) with either cAMP (10 mM) (*B*) or 1% (wt/vol) glucose (*C*). Data presented are the average of three independent experiments. WT, wild type.

frequency of bacteria resistant to rifampicin was measured among 1- and 7-day-old colonies. Fig. 4 shows a 9-fold increase in mutation frequency between days 1 and 7. The use of a lethal selection, such as resistance to rifampicin, precludes the appearance of postplating mutations (because those rifampicin-resistant mutant genes occurring on the plates would have to be transcribed by the rifampicin-sensitive RNA polymerase in the presence of rifampicin). Thus, most likely, all rifampicin-resistant mutants arise before plating on the selective medium. Due to the lag in the phenotypic expression of the rifampicin resistance, cells from resuspended colonies were incubated in rich medium. This increase in the frequency of rifampicin resistance was not observed in the absence of this preincubation. A preincubation of 1 hr was sufficient; further incubation did not affect the mutation frequency (data not shown). To test if the preincubation was mutagenic or just required for expression of the rifampicin resistance, we employed a nonlethal selection system [selection of *lacI*$^-$ mutation, using a noninducing analog of lactose (phenyl β-D-galactopyranoside)] in which the mutant phenotype can be expressed directly on the selective plate (33). With this assay, we have observed an increase in frequency of mutation between days 1 and 7 independent of the preincubation, showing that it is not mutagenic *per se* (data not shown). Thus, the *lacI*$^-$, and probably the rifampicin-resistant, mutants must have arisen in aging colonies on filters.

On the filter, the fastest growth period is between days 0 and 1 when cell number per plate increased 10^6-fold, as compared with only a 10-fold increase between days 1 and 7. The initial population (10^3 cells) was too small to contain any mutant. On average, the frequency of rifampicin-resistant mutants increased about 10-fold between days 1 and 7 (Fig. 4). Assuming that this increase is due to errors in DNA synthesis, the ratio of mutation rates can be calculated from these figures by using a simple derivation of Drake's formulas (34),

$$m_7/m_1 = [\log(N_1/N_0)/\log(N_7/N_1)]\cdot[(f_7 - f_1)/f_1],$$

where N_0 is the number of cells initially seeded (10^3 cells), N_1 and N_7 are the numbers of cells on days 1 and 7, f_1 and f_7 are the frequencies of mutants on days 1 and 7, and m_1 (m_7) is the mutation rate between days 0 and 1 (days 1 and 7). To explain the increase in frequency of mutations between days 1 and 7, two hypotheses are possible: cellular turnover in the colony or increased mutation rate. If the increase in frequency of mutants is due only to bacterial turnover within the colony (without any increase in mutation rate), it would require 160 generations between days 1 and 7, which would imply a growth rate superior to the one between days 0 and 1. Alternatively, in the absence of DNA turnover, considering that these mutations arose during the DNA synthesis that happened while cell number increased from 10^9 to 10^{10}, the mutation rate would have increased about 50-fold. Given these numbers and the requirement for active SOS system (see Fig. 4), the increased mutation rate hypothesis seems more likely than the turnover one, but as these two hypotheses are not mutually exclusive a combination of both might be a better description of the reality.

If one assumes that the mutations arose in actively dividing cells, they should be able to express their rifampicin-resistant RNA polymerase; under such a hypothesis, it is difficult to explain the requirement for preincubation to observe the increase in rifampicin-resistant mutants. On the contrary, one can argue that the mutations occurred predominantly in the last round(s) of DNA synthesis in cells that were too starved to express the mutant phenotype. In that case, an even larger increase in mutation rate is predicted. In conclusion, it appears that the bulk of the mutations arose in nonactively dividing/resting bacteria in the structured environment of the large colonies before they met the selective agent.

To study the molecular mechanisms of this mutagenesis in resting organisms in a structured environment (ROSE) and its link with the cAMP-dependent SOS response, the *recA*, *lexA*(Ind$^-$), and *cya* strains were used. All of these strains showed a 1.5-fold increase in the mutation frequency between days 1 and 7 (Fig. 4). Whereas the level of ROSE mutagenesis is significantly different from the exponential mutagenesis in the wild-type strain ($P < 0.0001$; *t* test), the differences in the *cya*, *recA*, and *lexA*(Ind$^-$) mutant backgrounds were not statistically significant ($0.07 > P > 0.05$; *t* test). Therefore, ROSE mutagenesis is both cAMP- and SOS-dependent.

DISCUSSION

The results presented here show a cAMP-dependent SOS induction and mutagenesis in resting bacterial populations. The role for cAMP in SOS induction and mutagenesis in ROSE might also be linked with a long-standing paradox: why should starving cells spend energy to produce cAMP that will be excreted [starving cells dramatically increase their intra- and extracellular cAMP levels (35); 99.9% of the produced cAMP is excreted independently of the growth rate, repre-

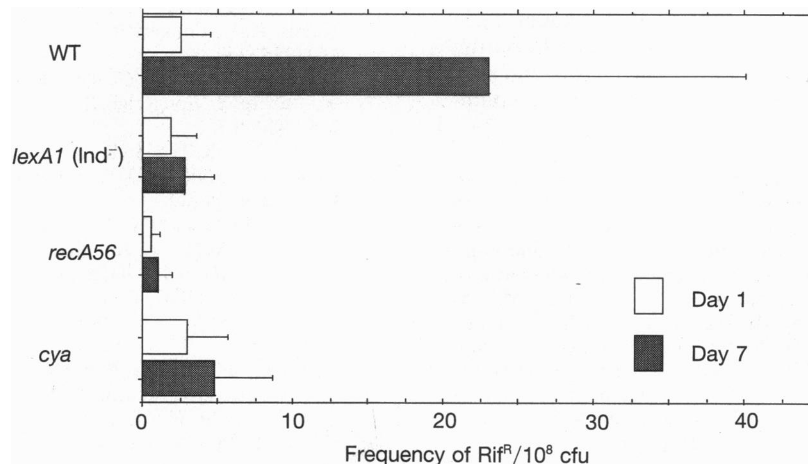

FIG. 4. SOS and cAMP-dependent mutagenesis to rifampicin resistance in aging colonies. Cells were resuspended on days 1 (open bars) and 7 (shaded bars). Cells were given a 1-hr incubation time at 37°C in 869 to allow for the expression of rifampicin resistance. The total number of colony-forming units (cfu) increased from 10^9 on day 1 to 10^{10} on day 7. Data presented are the average values of 24–49 independent cultures. Error bars represent the standard deviation. Rif^R, rifampicin resistant; WT, wild type.

senting as much as 9% of cellular energy (36)]? The function of this increased extracellular cAMP is as yet unknown. One possibility (that, to our knowledge, has been ignored) is that cAMP could serve a cell-to-cell signaling function within a bacterial colony. The extracellular cAMP concentration in a bacterial colony would be predicted to reach much higher levels than in liquid culture where cAMP is diluted in a much larger volume. It remains to be established if this putative signaling role of cAMP is responsible for the SOS induction and mutagenesis in ROSE or if cAMP acts intracellularly.

There is a striking similarity between RecA*-mediated CI cleavage in resting bacterial populations (this work) and "adaptive mutagenesis" (2, 37). Mutations such as *lexA*(Ind$^-$), *recB*, and *recA430* decrease both CI cleavage and adaptive mutagenesis, whereas constitutive expression of the *recA* gene enhances both phenomena. These results were interpreted as a requirement for the RecABC recombination activities for adaptive mutations (37). Here, an alternative is proposed—namely, that both phenomena could depend on high levels of RecA*. In adaptive mutagenesis, RecA* is required at least to induce the *recA* gene expression and possibly to inactivate or activate some other function. However, the recombination and the co-protease hypotheses in adaptive mutagenesis are not mutually exclusive (but see ref. 38). The putative role of SOS in adaptive mutagenesis is reinforced by the similarity between the spectrum of mutations observed under adaptive mutation and under SOS induction in the absence of lesions (as in a *recA441* strain, which is constitutively ON for SOS at 42°C). In both cases, the relative number of frameshifts at repetitive tracks of mononucleotides is enhanced (39–41).

Given the similarities between adaptive mutagenesis and the cAMP-dependent SOS induction observed in resting bacterial populations, it becomes interesting to test whether adaptive mutagenesis is also cAMP-dependent. The reported negative correlation between catabolite repression by glucose and mutagenesis to valine resistance on plates (42) may be related to the same pathway of cAMP-dependent and SOS-dependent mutagenesis.

Given the broad spectrum of SOS functions, SOS induction in resting bacterial populations could have many consequences for these cells other than the observed enhanced mutagenesis. In nature, bacteria oscillate between feast and famine. Many of the changes induced by famine [such as increased resistance to stresses (43), prophage induction (44), filamentation (44), point mutations (2), and genetic rearrangements (6, 18, 45)] are the same changes that occur when SOS is induced (8–13, 16, 44, 46–48). Therefore, some of the effects of famine may be due to the induction of the SOS response, and some of the SOS phenotypes that have no obvious utility under feast lifestyle [e.g., colicine production (31), restriction alleviation (49), stable DNA replication (50), and transposon mobility (16)] may prove to have adaptive value under famine conditions.

This paper is dedicated to the memory of Harrison Echols. We wish to thank our colleagues and friends and members of our laboratory and the R. D'Ari laboratory for their interest and advice during the course of this work and for their help in the writing of this manuscript. We thank P. Lechat for technical help, D. Touati for providing strains, and S. M. Rosenberg for comments on the manuscript. I.M. was supported by the Agence National de Recherche sur le SIDA and by Centre National de la Recherche Scientifique. This work was supported by grants from Agence National de la Recherche sur le SIDA, Association pour la Recherche contre le Cancer, Fondation pour la Recherche Médicale, Ligue Nationale Française contre le Cancer, and Groupement de Recherches et d'Etudes sur les Génomes.

1. Kolter, R. (1993) *Annu. Rev. Microbiol.* **47**, 855–874.
2. Cairns, J. & Foster, P. L. (1991) *Genetics* **128**, 695–701.
3. Foster, P. L. (1993) *Annu. Rev. Microbiol.* **47**, 467–504.
4. Lenski, R. E. & Mittler, J. E. (1993) *Science* **259**, 188–194.
5. Cairns, J., Overbaugh, J. & Miller, S. (1988) *Nature (London)* **335**, 142–145.
6. Shapiro, J. (1984) *Mol. Gen. Genet.* **194**, 79–90.
7. Hall, B. (1988) *Genetics* **120**, 887–897.
8. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (Am. Soc. for Microbiol., Washington, DC).
9. Livneh, Z., Cohen-Fix, O., Skaliter, R. & Elizur, T. (1993) *CRC Crit. Rev. Biochem. Mol. Biol.* **28**, 465–513.
10. Oishi, M. (1988) in *The Recombination of Genetic Material*, ed. Low, K. L. (Academic, San Diego), pp. 445–491.
11. Dimpfl, J. & Echols, H. (1989) *Genetics* **123**, 255–260.
12. Caillet-Fauquet, P., Maenhaut-Michel, G. & Radman, M. (1984) *EMBO J.* **3**, 707–712.
13. Tessman, I. & Kennedy, M. A. (1994) *Genetics* **136**, 439–448.
14. Escarceller, M., Hicks, J., Gudmundsson, G., Trump, G., Touati, D., Lovett, S., Foster, P. L., McEntee, K. & Goodman, M. F. (1994) *J. Bacteriol.* **176**, 6221–6228.
15. Huisman, O. & d'Ari, R. (1981) *Nature (London)* **290**, 797–799.
16. Kuan, C.-T. & Tessman, I. (1992) *J. Bacteriol.* **174**, 6872–6877.
17. Matic, I., Rayssiguier, C. & Radman, M. (1995) *Cell* **80**, 507–515.
18. Sonti, R. V. & Roth, J. R. (1989) *Genetics* **123**, 19–28.
19. Toman, Z., Dambly-Chaudière, C., Tenenbaum, L. & Radman, M. (1985) *J. Mol. Biol.* **186**, 97–105.
20. Miller, J. H. (1992) *A Short Course in Bacterial Genetics: A Laboratory Manual for Escherichia coli and Related Bacteria* (Cold Spring Harbor Lab. Press, Plainview, NY).
21. Lloyd, R. G., Buckman, C. & Benson, F. E. (1987) *J. Gen. Microbiol.* **133**, 2531–2538.
22. Miller, J. H. (1977) *J. Mol. Biol.* **109**, 275–301.
23. Weinstock, G. M. & McEntee, K. (1981) *J. Biol. Chem.* **256**, 10883–10888.
24. Caillet-Fauquet, P. & Maenhaut-Michel, G. (1988) *Mol. Gen. Genet.* **213**, 491–498.
25. Halliday, J. A. & Glickman, B. W. (1991) *Mutat. Res.* **250**, 55–71.
26. Eisen, H., Brachet, P., Silva, L. P. d. & Jacob, F. (1970) *Proc. Natl. Acad. Sci. USA* **66**, 855–862.
27. Dambly-Chaudière, C., Gottesman, M., Debouck, C. & Adhya, S. (1983) *J. Mol. Appl. Genet.* **2**, 45–56.
28. Lange, R. & Hengge-Aronis, R. (1991) *Mol. Microbiol.* **5**, 49–59.
29. Botsford, J. L. & Harman, J. G. (1992) *Microbiol. Rev.* **56**, 100–122.
30. Barbé, J., Gibert, I. & Guerrero, R. (1987) *Can. J. Microbiol.* **33**, 704–708.
31. Ebina, Y., Takahara, Y., Kishi, F. & Nakazawa, A. (1983) *J. Biol. Chem.* **258**, 13258–13261.
32. Karu, A. E. & Belk, E. D. (1982) *Mol. Gen. Genet.* **185**, 275–282.
33. Schmeissner, U., Ganem, D. & Miller, J. (1977) *J. Mol. Biol.* **109**, 3003–3026.
34. Drake, J. W. (1970) *The Molecular Basis of Mutation* (Holden-Day, San Francisco).
35. Buettner, M., Spitz, E. & Rickenberg, H. (1973) *J. Bacteriol.* **14**, 1068–1073.
36. Matin, A. & Matin, M. (1982) *J. Bacteriol.* **149**, 801–807.
37. Harris, R. S., Longerich, S. & Rosenberg, S. M. (1994) *Science* **264**, 258–260.
38. Rosenberg, S. (1994) *Genome* **37**, 893–899.
39. Rosenberg, S., Longerich, S., Gee, P. & Harris, R. (1994) *Science* **265**, 405–407.
40. Foster, P. L. & Trimarchi, J. M. (1994) *Science* **265**, 407–409.
41. Yatagai, F., Halliday, J. A. & Glickman, B. W. (1991) *Mol. Gen. Genet.* **230**, 84–89.
42. McPhee, D. G. (1993) *Mutat. Res.* **285**, 109–116.
43. Matin, A., Auger, E. A., Blum, P. H. & Schutltz, J. E. (1989) *Annu. Rev. Microbiol.* **43**, 293–316.
44. Witkin, E. M. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 1275–1279.
45. Naas, T., Blot, M., Fitch, W. M. & Arber, W. (1994) *Genetics* **136**, 1–10.
46. 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.
47. Witkin, E. M. (1976) *Bacteriol. Rev.* **40**, 869–907.
48. Echols, H. (1981) *Cell* **25**, 1–2.
49. Hiom, K., Thomas, S. M. & Sedgwick, S. G. (1991) *Biochimie* **73**, 399–405.
50. Kogoma, T., Torrey, T. A. & Connaughton, M. J. (1979) *Mol. Gen. Genet.* **176**, 1–9.