cAMP-dependent SOS induction and mutagenesis in resting bacterial populations

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

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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 [ibv his rpsL nalidixic acid-resistant (λ cI + cI' -gal+)] and MT5, a cI(Ind-') derivative of MT1 (19). Derivatives carrying the following alleles were constructed by P1-mediated transduction (20): recA430 srl300::Tn10 (from D. Touati, Paris), lexA1 malB::Tn9 (from GC2281; R. D'Ari collection, Paris), recA098 srl300::Tn10 (from C. Herman collection, Paris), recB268::Tn10 (21), mutS215::Tn10 (from G. Walker's collection; Cambridge, MA), and katF::Tn10 (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 (Δpro-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::Tn9 (from GC2281; R. D'Ari collection) and Δcyu ilv::Tn5 (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 Δpro-lac ara thi trpE9777; F'(pro-lac) and its recA456 derivative (NR8002) were provided by R. Schaaper (Research Triangle Park, NC). The lexA1(Ind-') malB::Tn9 and Δcyu strains were constructed by P1 transduction. On day 0, ~10^5 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 MgSO4 and were incubated 1 hr at 37°C in liquid 869 to allow expression of the rifampicin resistance, as is usually done after mutagenic treatments (24). Plates 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

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


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Immune state: (CI⁺ Cro⁻ Gal⁺) white colonies

Non-immune state: (CI⁻ Cro⁺ Gal⁺) red colonies

\( \lambda \) CI repressor cleavage) or a cI(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 cI(Ind⁻) gene. The

![Diagram showing the principle of the SOS-inducible epigenetic switch in E. coli](image)

**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 inheritable (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 \( \lambda \) cI and cro genes (26), which have been fused with the promoterless gal operon transcribed from the \( \lambda PR \) 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, inheritable expression of the cro and gal genes. An isogenic MT5 strain with a cI(Ind-), which prevents cleavage of CI repressor by activated RecA) detects, by color change, only the rare mutations in the cI gene. In MT1, the two epigenetic states switch spontaneously with the indicated frequencies.

![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 cI(Ind⁻) mutS⁻ colonies have papillae. The red (dark on the picture) papillae on the cI(Ind⁻) mutS⁻ and recA430 colonies are due to mutations inactivating cI [cI(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.](image)

**Fig. 2.**
Table 1. Genetics of SOS induction in colonies

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time of color change, hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1 cI*</td>
<td>48</td>
</tr>
<tr>
<td>MT5 cI(Ind-)</td>
<td>48*</td>
</tr>
<tr>
<td>MT1 recA430</td>
<td>48*</td>
</tr>
<tr>
<td>MT1 lexA1(Ind-)</td>
<td>60</td>
</tr>
<tr>
<td>MT1 lexA1(Ind-) recA98</td>
<td>36</td>
</tr>
<tr>
<td>MT1 recB</td>
<td>72</td>
</tr>
<tr>
<td>MT1 rpoS</td>
<td>48</td>
</tr>
</tbody>
</table>

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 cI(Ind-) mutation forms colonies with a phenotype identical to the recA430 colonies (data not shown).

The frequency of this SOS-independent genetic switch is elevated about 100-fold by the introduction of a mutS- mutant mutation (data not shown). In the cI(Ind-) mutS- strain, the mutation rate to Gal+ approaches the rate of the epigenetic switch observed in cI(Ind+) cells exponentially growing, which is due to spontaneous SOS induction under these conditions. The morphology of mutS- cI(Ind-) and mutS+ cI(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-) recA98 (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. 3A). 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. 3C), 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. 3A), and the induction of the recA gene can be recovered by the addition of cAMP (Fig. 3B). 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...
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- mutation, 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 106-fold, as compared with only a 10-fold increase between days 1 and 7. The initial population (103 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),

\[ m_2/m_1 = \frac{\log(N_1/N_0)/\log(N_7/N_1)}{(f_7 - f_1)/f_1}, \]

where \( N_0 \) is the number of cells initially seeded (103 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 \( m_1 \) (\( m_2 \)) 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 \( 10^0 \) 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 intracellular cAMP levels (35); 99.9% of the produced cAMP is excreted independently of the growth rate, repre-

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**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. RifR, rifampicin resistant; WT, wild type.
sensing 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 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., colicin production (31), restriction alleviation (49), stable DNA replication (50), and transposon mobility (16)] may prove to have adaptive value under famine conditions.

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