Adaptive Reversion of a Frameshift Mutation in *Escherichia coli*

John Cairns*[,1] and Patricia L. Foster[†]

**The Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115, and*
[†]Department of Environmental Health, Boston University School of Public Health, Boston University School of Medicine, Boston, Massachusetts 02118

Manuscript received October 9, 1990
Accepted April 23, 1991

ABSTRACT

Mutation rates are generally thought not to be influenced by selective forces. This doctrine rests on the results of certain classical studies of the mutations that make bacteria resistant to phages and antibiotics. We have studied a strain of *Escherichia coli* which constitutively expresses a *lacI-lacZ* fusion containing a frameshift mutation that renders it Lac$^-$. Reversion to Lac$^+$ is a rare event during exponential growth but occurs in stationary cultures when lactose is the only source of energy. No revertants accumulate in the absence of lactose, or in the presence of lactose if there is another, unfulfilled requirement for growth. The mechanism for such mutation in stationary phase is not known, but it requires some function of RecA which is apparently not required for mutation during exponential growth.

RECENT experiments have shown that certain spontaneous mutations in *Escherichia coli* seem to occur at a higher frequency when they are beneficial (SHAPIRO 1984; CAIRNS, OVERBAUGH and MILLER 1988; HALL 1988, 1990). Although there have been rather few studies of the influence of circumstances upon the production of spontaneous mutants, it is a tenet of neo-Darwinism that the frequency of mutational events should be independent of their immediate utility. For that reason, the new experiments are controversial and are unlikely to gain wide acceptance until the molecular biology of adaptive mutation is understood. The investigation of mechanism might be easier if there were an example of a spontaneous mutation where the stimulatory effect of usefulness had been quantitated. It should then be possible to find out if the process that produces adaptive mutations under conditions of selection requires a special set of gene products that are not necessary for the random, nonadaptive mutations that occur during normal growth.

Frameshift mutations in *E. coli lacZ* have been found to undergo reversion at high frequency when lactose is the sole source of energy (BRUCE LEVIN, personal communication; JEFFREY MILLER, personal communication). We have studied one such case and show here that the majority of the Lac$^+$ revertants are arising after the cells have been exposed to lactose. These adaptive revertants do not accumulate in the absence of lactose nor do they accumulate in the presence of lactose if there is some other, unfulfilled requirement for growth. Certain mutations in recA and lexA greatly reduce the rate of adaptive reversion under conditions of selection but seem to have no effect on the rate of nonadaptive reversion during growth. This suggests that the two classes of revertant arise by different mechanisms.

MATERIALS AND METHODS

Bacterial strains: All the strains used in this study were derivatives of P90C [F$^-$ *ara* Δ(*lacproB*)$_{XIII}$ *thi*] (COULONDRE and MILLER 1977) or a spontaneous rifamycin-resistant (Rif^R) isolate of P90C. Into this background we transferred F′ episomes carrying the *lac-pro* region with mutant alleles of *lacZ* but with *lacY*$^+$ *lacA*$^+$ *proB*$^+$. FC29 is Rif^S and carries an F′ Δ(*lacI lacZ*) (obtained from J. BECKWITH); this strain cannot revert to Lac$^+$. FC40 is Rif^R and bears an F′ with a *lacI*Ω *lacZ* fusion that eliminates the coding sequence for the last four residues of *lacI*, all of *lacP* and *lacO*, and the first 23 residues of *lacZ* (MÜLLER-HILL and KANIA 1974); transcription is initiated constitutively from the *lacI*^{q} promoter but the construct is Lac$^-$ because of a polar +1 frameshift changing CCC to CCCC at the 320th codon of *lacI* (CALOS and MILLER 1981). FC82 is Rif^S and carries the same episome as FC40 but has been made *trpE9777* by P1vir transduction; the *trpE9777* allele has a +1 frameshift creating a run of six A:T base pairs (BRONSON and YANOFSKY 1974). Alleles of *recA* were transduced into these backgrounds via linkage to *srl*::Tn*10* or *srl*$^+$; *lexA3* was transduced via linkage to *mal*::Tn*10*. The transposons were removed by subsequent transduction to *srl*$^+$ or *mal*$^+$. Genetic techniques were as described by MILLER (1972).

Media: The bacteria were routinely cultivated in roller tubes at 37° in M9 minimal medium (MILLER 1972) containing 20 μg/ml thiamine, 0.001% gelatin and 0.1–0.4% glycerol or glucose and supplemented where necessary with tryptophan. The frequency of Lac$^+$ revertants was usually determined on M9 plates containing 0.1–0.4% lactose, either by spreading or by overlaying in 2.5 ml M9 agar containing lactose. The frequency of Trp$^+$ revertants was determined on plates containing 0.2–0.4% glucose. The

[1] Present address: Clinical Trial Service Unit, The Harkness Building, Radcliffe Infirmary, Oxford OX2 6HE, England.

change, over time, in viable counts on spread plates was determined by removing small circular plugs, vortexing these in M9 and assaying the suspended bacteria on 1% peptone or LB plates containing 0.1 mg/ml rifamycin when necessary.

RESULTS

The timing of appearance of Lac$^+$ revertants: The experiments presented here were conducted with a strain that constitutively transcribes a fusion of *lacI* and *lacZ* containing a frameshift mutation (MÜLLER-HILL and KANIA 1974; CALOS and MILLER 1981). This strain (FC40) was chosen because under conditions of selection it has a high rate of reversion to Lac$^+$. When a loopful of a saturated liquid culture is spread on a lactose-M9 plate, Lac$^+$ revertants continue to be produced for a period of a week or two, until the plate is covered with colonies. Our first task was to exclude various trivial explanations for this effect.

Like many frameshift mutations in *lacZ* (ATKINS, ELSEVIERS and GORINI 1972) the +1 frameshift in FC40, although slightly leaky, does not allow cultures to synthesize enough β-galactosidase to undergo any measurable increase in optical density in M9-lactose (data not shown). There are, however, contaminating sources of energy in agar, and M9-lactose plates will support a certain amount of growth of Lac$^-$ cells. For this reason, the final number of Lac$^+$ colonies present a week after plating 10^6–10^9 FC40 cells is almost independent of the number of cells plated. To limit the amount of growth that can occur on plates, we routinely added to each aliquot of FC40 about 10^9 scavenger cells (FC29) bearing a deletion of *lacIZ*, which compete for the contaminating source of energy (SMITH and SADLER 1971; ALBERTINI *et al.* 1982). Figure 1 shows that, when this is done, the number of Lac$^+$ colonies arising each day is proportional to the number of FC40 cells that were plated. Because the proportionality is maintained even when only one or two Lac$^+$ colonies are formed per plate, the continued appearance of colonies with time cannot be attributed to local growth of Lac$^-$ cells in the neighborhood of the first colonies to be formed (cross-feeding).

Because FC40 is Rif^R, we could measure the amount of growth of FC40 in the presence of the Rif^S scavenger cells by punching out small plugs from between the Lac$^+$ colonies and assaying them for viable count on plates containing rifamycin. Figure 2 shows that in a typical experiment, the population of FC40 cells undergoes less than one doubling in the first five days after plating on M9-lactose. During this period, however, the number of Lac$^+$ colonies is rising approximately linearly from near zero to more than 40 per plate. This suggests that the revertants arise after plating as the result of some process that is time-dependent but not growth-dependent.

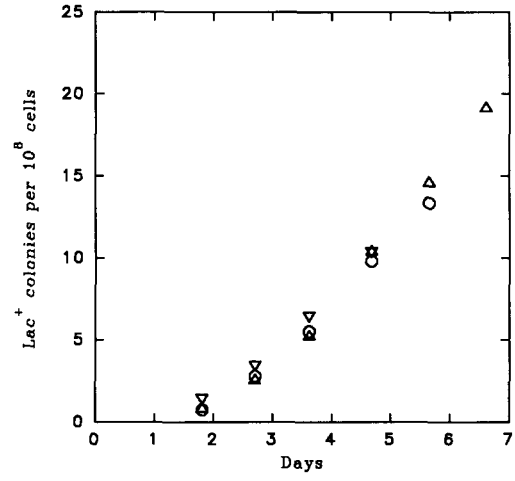

FIGURE 1.—The relation between the number of FC40 cells plated and the accumulation of Lac$^+$ colonies on lactose-M9 plates. Three cultures of FC40 were grown to saturation (average viable count 3.8×10^9/ml) in M9–0.4% glycerol from an inoculum of about 10^4 cells. Aliquots of 0.400 ml (1.5×10^9 cells) (triangles), 0.080 ml (3.0×10^8 cells) (circles) and 0.016 ml (6×10^7 cells) (inverted triangles) were mixed with 3×10^9 scavenger cells and plated in 2.5 ml top agar onto lactose-M9 plates. The colony counts are the average for 6, 12 and 18 plates, respectively.

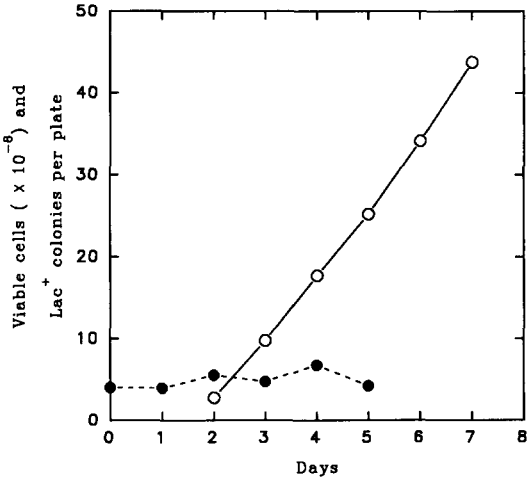

FIGURE 2.—The accumulation of Lac$^+$ colonies and the viable count of FC40 after plating on lactose-M9 plates. Six cultures of FC40 were grown to saturation in M9–0.2% glycerol. Aliquots of 0.075 ml, containing 3×10^8 FC40 cells, were mixed with 0.025 ml containing 1×10^9 scavenger cells and spread on M9-lactose plates. On each day, a small circular sample was removed from one of each set of six plates (avoiding any visible Lac$^+$ colonies) and was vortexed with 1 ml M9; the viable titer of FC40 in these suspensions was assayed on rifamycin-peptone plates (filled circles). The Lac$^+$ colony counts (open circles) are the averages for 23 plates.

If these revertants do indeed arise after plating, the numbers found among different cultures should form a Poisson distribution (LURIA and DELBRÜCK 1943). We therefore measured the distribution of revertants among 120 saturated cultures of FC40, plated with scavengers and counted each day. The cultures produced an average of less than one Lac$^+$ revertant colony by day two, but the number rose to about 20 by day 7. Two cultures were plainly exceptional; one

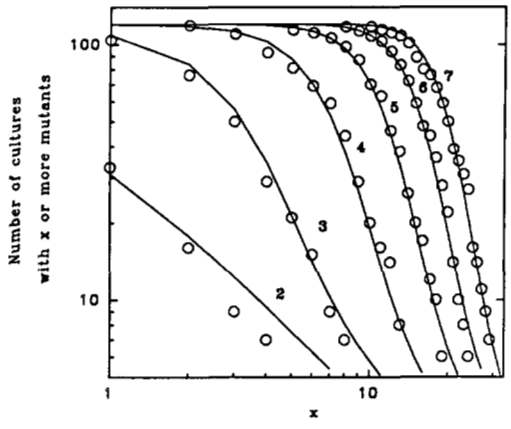

FIGURE 3.—The distribution of Lac$^+$ revertants among different cultures of FC40. A culture of FC40 in M9-glycerol was diluted 10^5-fold to give 120 0.5-ml cultures in M9–0.015% glycerol. These were grown to saturation (1.8×10^8 viable cells per culture). Each then received 9×10^8 washed scavenger cells and was plated with top agar onto M9-lactose. The number of Lac$^+$ colonies on each plate was counted each day, starting at 42 hr (when the first colonies are just visible). The figure shows the observed number of plates with x or more colonies (points) compared to the expected number (lines) for the various values of m (mutations per culture during growth) and μ (mutations per culture after plating) that are given in Table 1.

produced 25 colonies by day 2, and the other about 60 between days 5 and 7. This tells us two things. First, the presence of these "jackpots" means that some of the revertants were the result of mutations that occurred during the prior growth of the cultures; if 120 cultures included two jackpots containing at least 25 more mutants than the average culture, the mean number of mutational events during prior growth of the cultures was roughly $(2 \times 25)/120$ or 0.4 per culture (LURIA 1951). Second, the two jackpots differed in the speed with which they produced colonies, meaning that individual Lac$^+$ revertants of FC40 can differ in their rate of growth on lactose. To get a better measure of this, we checked 104 independently arising revertants that produced colonies on day 5 or 6. Seventy of these produced visible colonies on lactose plates within 48 hr but the remaining 34, although growing normally on glycerol, were slow to produce colonies on lactose.

It is convenient to describe distributions of numbers of mutants in a fluctuation test as the relation between the logarithm of the proportion of cultures with x or more mutants and log$x$ (LURIA 1951; CAIRNS, OVERBAUGH and MILLER 1988). Figure 3 shows that the distributions of revertants in this experiment after day two were much narrower than would be expected if all the mutations had occurred before the cultures were plated. To find some combination of a Luria-Delbrück distribution (with a mean of m mutations per culture before plating) (LEA and COULSON 1949) and a Poisson distribution (with a mean of μ mutations per culture after plating) that would fit each of the

TABLE 1

The distribution of Lac$^+$ revertants of FC40

Day	m	μ	χ^2	d.f.	P
2	0.30	0.00	4.48	3	0.2
3	0.35	2.00	5.84	5	0.4
4	0.40	5.50	13.16	8	0.1
5	0.45	9.50	7.94	9	0.6
6	0.50	13.50	8.76	11	0.2
7	0.50	17.50	18.53	12	0.1

Comparison of the observed and expected values shown in Figure 3. The values for m and μ were chosen by trial and error to minimize the value of χ^2, with the sole restriction that the initial value of μ on day 2 should be zero. To calculate χ^2, the counts were divided into groups so that the expected number in each group (number of cultures containing between x and $x+r$ mutants) was never less than about 5. Because an arbitrary value of m was chosen for the first set of counts, and arbitrary values of m and μ for all subsequent sets, the number of degrees of freedom (d.f.) was respectively 2 and 3 less than the number of groups in each set. The final value of m was 0.5 for populations of 1.8×10^8 cells, which corresponds to a mutation rate of 1.4×10^{-9} per cell per generation. The final value of μ was 4 per day, which corresponds to a mutation rate of 0.9×10^{-9} per cell per hr.

observed distributions, we made the simplifying assumption that the counts on day 2 (at 42 hr, when the first few colonies were just visible) comprised a Luria-Delbrück distribution, but later counts could be a mixture of Luria-Delbrück and Poisson (CAIRNS, OVERBAUGH and MILLER 1988). A crude procedure of trial and error led to a set of values for m and μ which gave expected distributions that were not significantly different from observed (Table 1).

The results of this curve-fitting can be summarized as follows. Before plating, the cultures appear to have experienced an average of 0.5 mutational events (representing a mutation rate of 1.4×10^{-9} per cell division); because m increased slowly from 0.3 to 0.5, it seems that about half of these pre-plating mutants were able to form colonies within 42 hr and the rest grew rather more slowly. After plating, the cultures averaged about four mutational events per day (representing a mutation rate of 0.9×10^{-9} per cell per hr); again, because μ increased slowly from two per day to four per day, it seems that about half of these post-plating mutants could form colonies in 2 days, and the rest grew more slowly. In other words, the way the distribution of colony numbers changed with time suggests that more than 95% of the colonies appearing in the first week after plating were the result of mutations that occurred after the cells had been put out onto lactose-minimal plates.

Conditions required for the formation of Lac$^+$ revertants: The first requirement is the presence of lactose. When FC40 is plated on plain M9 agar without an energy source, no Lac$^+$ revertants arise until the plates are overlaid with agar containing lactose (Figure 4).

FC40 is constitutive for transcription of the *lacI-*

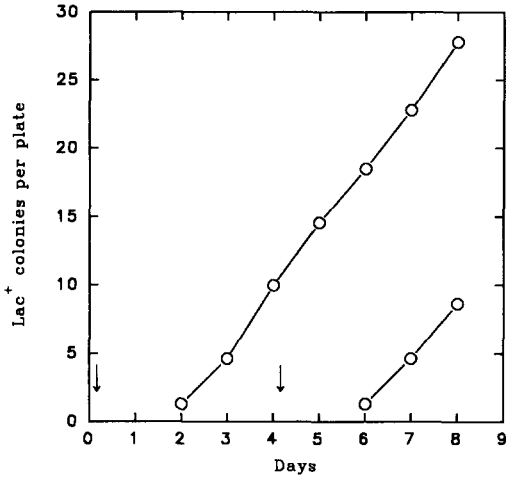

FIGURE 4.—The effect of delaying the addition of lactose upon the production of Lac$^+$ revertants by FC40. Six cultures of FC40 were grown to saturation in M9–0.2% glycerol. Aliquots of 0.1 ml, containing 2×10^8 cells, were mixed with 3×10^9 scavenger cells and plated with 2.5 ml top agar on M9 plates lacking any energy source. Each plate was then overlaid with a further 2.5 ml top agar. Finally, the plates were overlaid with 2.5 ml top agar containing 1% lactose, half of the plates immediately and half after 4 days of incubation. Each point is the average for 18 plates. The arrows mark the times of addition of lactose.

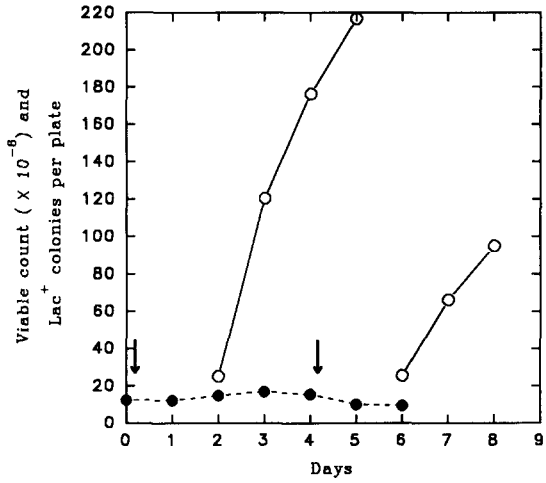

FIGURE 5.—The effect of delaying the addition of tryptophan upon the production of Lac$^+$ revertants by a Lac$^-$ Trp$^-$ strain (FC82) after plating on lactose. Six 20 ml cultures of FC82 were grown to saturation in M9 containing 0.4% glycerol and 3 μg/ml tryptophan, and then centrifuged and suspended in 0.25 ml M9. Aliquots of 0.05 ml were then spread onto one lactose plate and plated with 2.5 ml top agar onto three lactose plates. The spread plates were assayed for viable counts in the same way as for Figure 3 (closed circles). The top-agar plates were overlaid with a further 2.5 ml and finally overlaid with top agar containing 0.1 mg/ml tryptophan, either immediately or on day 4; the counts of Lac$^+$ colonies (open circles) are the averages for six plates. The arrows mark the times of addition of tryptophan.

lacZ fusion (MÜLLER-HILL and KANIA 1974) and so this effect of lactose is not because it is inducing transcription. But lactose could be acting as a nonspecific mutagen, either due to a direct effect of the lactose (or of some contaminant in the lactose) or because the frameshift in FC40 is leaky and therefore the presence of lactose allows the cells to undergo a low level of metabolism which might be mutagenic. To investigate these possibilities, we have looked at the reversion of the frameshift in a tryptophan-requiring strain (FC82). The Trp$^-$ mutation in this Trp$^-$ Lac$^-$ strain undergoes spontaneous reversion at about one-tenth the rate of reversion of the Lac$^-$ mutation, but most of the Trp$^+$ revertants, like the Lac$^+$ revertants, arise after the cells are put onto plates lacking tryptophan (data not shown). The presence of the additional mutation allowed us to show that lactose is not acting as a mutagen. No Lac$^+$ revertants accumulated until the plates were overlaid with top agar containing tryptophan (Figure 5). Thus the presence of lactose does not result in the accumulation of viable Lac$^+$ revertants under conditions where the mutation to Lac$^+$ is not, on its own, sufficient to allow the cell to start multiplying. (We also intended to study the effect of lactose on the accumulation of Trp$^+$ revertants by overlaying with glucose either on day 0 or on day 4, but this half of the experiment was not informative because the bacteria lost their ability to produce Trp$^+$ revertants when they had been deprived of tryptophan for several days.)

The results, up to this point, may be summarized as follows. During normal growth, FC40 and FC82 undergo a low rate of spontaneous reversion to Lac$^+$, presumably due to errors in replication. Revertants also accumulate when the bacteria are deprived of an energy source, but only under conditions where the mutation allows the cell to resume multiplication. It is as if cells in stationary phase are continually producing changes in sequence that are preserved if the cell starts multiplying but otherwise are discarded (perhaps by destruction or repair of the mutated sequence, or by death of the cell).

The idea that mutation in stationary phase is a distinct process would be strengthened if we could show that it required some gene products that are not required for mutation during normal growth. We have not yet succeeded in identifying such genes simply by a direct screen of a mutagenized stock of FC40, but we have found that some known gene functions are required.

Reversion rates in strains with mutations in *recA* and *lexA*: Various alleles of *recA* and *lexA* were moved into FC40 by transduction, and we measured their effect on the rate of appearance of Lac$^+$ colonies on lactose-M9 plates. (We avoided using a *recA(Def)* mutant in these experiments because of the poor viability of such strains.) Given the complex interactions between RecA, LexA and the many other genes of the SOS pathway (WALKER 1984), several combinations of alleles had to be tested in order to determine which

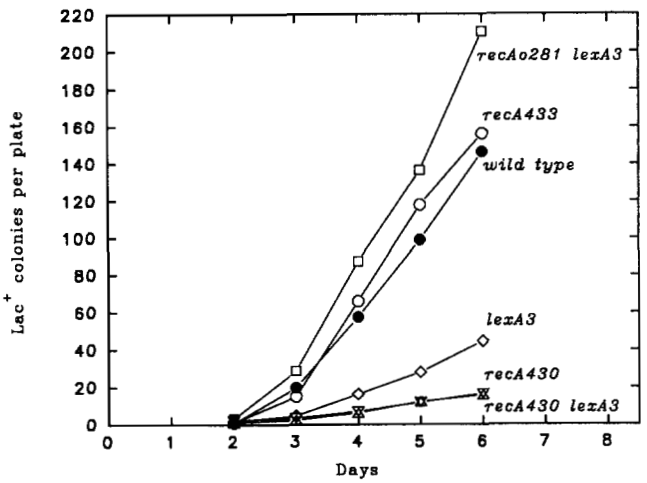

FIGURE 6.—The effect of various alleles of *recA* and *lexA* on the accumulation of Lac$^+$ colonies on lactose-M9 plates. Four or five independent cultures of each strain were grown to saturation in M9–0.1% glycerol, and a 0.1-ml aliquot of each culture was mixed with 3×10^9 scavenger cells and spread onto a plate. Plugs, removed from these plates every other day, showed that the viable counts of each strain varied by less than a factor of two over the course of the experiment.

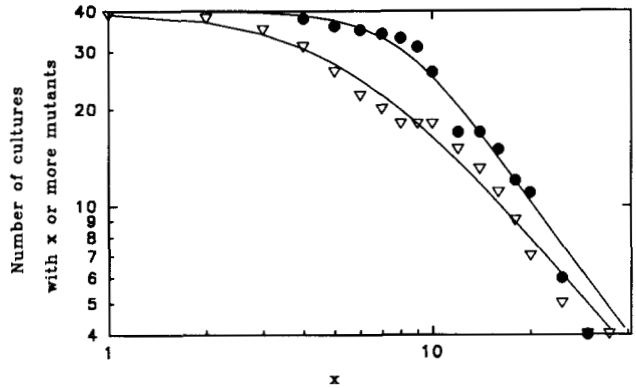

FIGURE 7.—The distribution of Lac$^+$ revertants among 40 cultures of FC40 (*recA*$^+$) and 40 cultures of FC203 (*recA430*). A culture of each strain was diluted 10^4-fold to give 40 1-ml cultures in M9–0.1% glycerol. These were grown to saturation and each then received an additional 10^9 scavenger cells and was plated with top agar onto M9-lactose. The number of Lac$^+$ colonies was counted at 47 hr. The observed distribution of FC203 revertants (downward triangles) is matched against the curve expected if the cultures experienced an average of 2.8 mutations before plating and 1 mutation afterward. The observed distribution for FC40 (closed circles) is matched against the curve expected if the cultures experienced an average of 2.8 mutations before plating and 5 afterward.

was the important function. The main results are shown in Figures 6 and 7.

A mutant allele of *lexA* (*lexA3*), which produces a LexA protein that is resistant to RecA-mediated cleavage (LITTLE *et al.* 1980), conferred about a 3-fold reduction in reversion rate; this suggested that there is a requirement for at least one of the many genes controlled directly or indirectly by LexA, such as *recA*, *umuD* and *umuC*. But it is not *umuD* or *umuC* and the pathway for SOS-mutagenesis that is involved, because the reversion rate was unaffected by a mutant *umuC*$^-$ allele (data not shown) or by mutant alleles of *recA*, such as *recA433* (Figure 6), that are deficient in processing UmuD to its active form but are not deficient in processing LexA (ENNIS, OSSANNA and MOUNT 1989). To show that the reversion rate depends on the expression of *recA* itself, rather than any of the other genes repressed by LexA, we showed that the *lexA3* allele has no effect when *recA* carries an operator-constitutive mutation (*recAo281*) (VOLKERT, MARGOSSIAN and CLARK 1981).

One allele of *recA*, *recA430*, that is deficient in processing both LexA and UmuD (ENNIS *et al.* 1985; NOHMI *et al.* 1988; SHINAGAWA *et al.* 1988) showed a 5–10-fold reduction in Lac$^+$ reversion rate (Figure 6). Although the reversion rate of the *recA430* strain was not further lowered by the *lexA3* allele, the effect of the *recA430* mutation is probably not due simply to the poor ability of RecA430 to process LexA; since the *lexA3* mutation, on its own, did not confer as great a decline in reversion rate as the *recA430* mutation, it seems likely that RecA430 is deficient in some other RecA-function that is required for the production of Lac$^+$ revertants of FC40.

These results indicate that most of the Lac$^+$ revertants of FC40, that arise after plating on lactose, arise by a process that depends on certain functions of RecA. In contrast, the rate of reversion during prior growth, before the application of selection, is not markedly affected by the presence of the *recA430* allele rather than wild type *recA*. A fluctuation test showed that in each case two cultures out of 40 contained a jackpot of more than 100 mutants, and the right-hand end of the distribution of mutant numbers, counted at 47 hours, was roughly the same for the two strains (Figure 7).

DISCUSSION

In their natural habitat, organisms like *E. coli* live under extreme nutritional constraint (OZAWA and FRETER 1964) and can divide only once or twice a day (MATIN *et al.* 1989). But *E. coli* is able to accumulate mutations even when it is not dividing (RYAN, OKADA and NAGATA 1963) or replicating its DNA (RYAN, NAKADA and SCHNEIDER 1961), and this is presumably its main source of genetic variation in nature. The process of mutation in stationary phase has the unusual property that it tends to produce whatever adaptive changes are needed to allow the resumption of growth, rather than random mutations that confer no benefit (SHAPIRO 1984; CAIRNS, OVERBAUGH and MILLER 1988; HALL 1988, 1990). To determine how this is accomplished, it would be helpful to have an example where the adaptive mutations arising in a culture in stationary phase can be distinguished from the random changes that arose earlier in the culture's history when the cells were still multiplying.

In this paper, we describe the spontaneous reversion of a frameshift in a constitutively expressed *lacI-lacZ* fusion. During exponential growth the rate of reversion is about 10^{-9} per cell generation (Table 1); most of the revertants are fully restored to the wild type growth rate on lactose and produce visible colonies on lactose plates in about 40 hr; their distribution among independent cultures is roughly the Luria-Delbrück distribution that would be expected if the mutations had occurred randomly throughout the history of each culture (Figures 3 and 7). After plating on lactose, revertant colonies continue to appear at a rate of about 10^{-9} per cell per hour (Table 1); the cells in these late-arising colonies grow on lactose at the same rate as the other revertants; the distribution of late arising colonies among independent cultures is close to the Poisson distribution that would be expected if these mutations are occurring on the plates (Figure 3). The late mutations that arise after plating cannot be attributed to growth on the plates (Figure 2) or to cross-feeding from the first colonies to be formed (Figure 1). Interestingly, they do not occur in the absence of lactose (Figure 4) or in its presence when there is some additional growth requirement (Figure 5), suggesting that mutations in stationary phase are preserved only if they quickly lead to the resumption of growth. The late mutations differ from those that occur during exponential growth because they have an almost absolute requirement for at least one of the many functions of *recA*. A mutation in *recA*, *recA430*, that affects both the expression and function of RecA shows normal Lac^+ reversion rates during growth (Figure 7), but its subsequent rate of reversion on lactose plates is greatly reduced (Figure 6) even though the cells are surviving perfectly well. A decrease in spontaneous mutation rates in the presence of certain alleles of *recA* has been reported for other systems (KONDO *et al.* 1970; SARGENTINI and SMITH 1981; ALBERTINI *et al.* 1982; WHORISKEY, SCHOFIELD and MILLER 1991) although there was no thought at that time that some of the spontaneous mutations might be arising after the onset of selection.

Adaptive mutations could be acquired in stationary phase in many different ways (CAIRNS, OVERBAUGH and MILLER 1988) but perhaps the most conservative explanation is the following (STAHL 1988; BOE 1990). The enzymes for DNA repair seem capable of resolving almost any form of damage and correcting any copying error provided that they have time to take the process to completion. Therefore, as long as a bacterium refrains from duplicating its DNA, it may be protected against spontaneous mutation. So the only spontaneous sequence changes that become irreversibly fixed in a stationary-phase population may be those that happen to be uncorrected in cells when they resume growth, and these changes will necessarily include any mutation that allowed the cell to start growing. Of course, the moment a cell is successful and resumes growth it may have no way of singling out for preservation the particular change in sequence that brought success, which could explain why mutants arising under selective conditions show a somewhat increased frequency of additional mutations in genes unrelated to the selective pressure (HALL 1990). So far we have not found a way to test this general model. Defects in certain repair pathways, such as *mutHLS*-dependent mismatch repair (BOE 1990) and *ada-ogt*-dependent alkylation repair (REBECK and SAMSON 1991), are known to increase the rate of mutation in stationary phase, but this does not mean that these are the systems that are normally eradicating the unsuccessful sequence changes. To show that these repair functions are preventing nonadaptive mutations, we would have to show that Lac^+ revertants accumulate in the absence of lactose when these pathways are defective. So far our experiments along those lines have been indecisive. There is, however, less room for doubt when a defect in some DNA-processing system is found to decrease the rate of mutation. Our results with *lexA3*, which lowers the expression of *recA*, and with *recA430*, which is known to be deficient in several functions of *recA*, suggest that RecA plays (directly or indirectly) some role in the production or fixation of adaptive sequence changes, or perhaps in the creation or termination of a special state of high mutability.

We thank J. BECKWITH, E. EISENSTADT, D. ENNIS, J. MILLER, D. MOUNT, M. VOLKERT, G. WALKER and C. YANOFSKY for strains, and M. PATTERSON and S. FRANKLIN for technical assistance. The work was supported by grants from the National Science Foundation and the Rita Allen Foundation. We are grateful to L. SAMSON for giving J. C. space in her laboratory.

LITERATURE CITED

ALBERTINI, A. M., M. HOFER, M. P. CALOS and J. H. MILLER, 1982 On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions. Cell **29:** 319–328.

ATKINS, J. F., D. ELSEVIERS and L. GORINI, 1972 Low activity of β-galactosidase in frameshift mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **69:** 1192–1195.

BOE, L., 1990 Mechanism for induction of adaptive mutations in *Escherichia coli*. Mol. Microbiol. **4:** 597–601.

BRONSON, M. J., and C. YANOFSKY, 1974 Characterization of mutations in the tryptophan operon of *Escherichia coli* by RNA nucleotide sequencing. J. Mol. Biol. **88:** 913–916.

CAIRNS, J., J. OVERBAUGH and S. MILLER, 1988 The origin of mutants. Nature **335:** 142–145.

CALOS, M. P., and J. H. MILLER, 1981 Genetic and sequence analysis of frameshift mutations induced by ICR-191. J. Mol. Biol. **153:** 39–66.

COULONDRE, C., and J. H. MILLER, 1977 Genetic studies of the lac repressor. IV. Mutagenic specificity in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **117:** 577–606.

ENNIS, D. G., B. FISHER, S. EDMISTON and D. W. MOUNT, 1985 Dual role for *Escherichia coli* RecA protein in SOS

mutagenesis. Proc. Natl. Acad. Sci. USA **82:** 3325–3329.

ENNIS, D. G., N. OSSANNA and D. W. MOUNT, 1989 Genetic separation of *Escherichia coli recA* functions for SOS mutagenesis and repressor cleavage. J. Bacteriol. **171:** 2533–2541.

HALL, B. G., 1988 Adaptive evolution that requires multiple spontaneous mutations. I. Mutations involving an insertion sequence. Genetics **120:** 887–897.

HALL, B. G., 1990 Spontaneous point mutations that occur more often when advantageous than when neutral. Genetics **126:** 5–16.

KONDO, S., H. ICHIKAWA, K. IWO and T. KATO, 1970 Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different repair capacities. Genetics **66:** 187–217.

LEA, D. E., and C. A. COULSON, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

LITTLE, J. W., S. H. EDMISTON, L. Z. PACELLI and D. W. MOUNT, 1980 Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. USA **77:** 3225–3229.

LURIA, S. E., 1951 The frequency distribution of spontaneous bacteriophage mutants as evidence for the exponential rate of phage production. Cold Spring Harbor Symp. Quant. Biol. **16:** 463–470.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MATIN, A., E. A. AUGER, P. H. BLUM and J. E. SCHULTZ, 1989 Genetic basis of starvation survival in nondifferentiating bacteria. Annu. Rev. Microbiol. **43:** 293–316.

MILLER, J. H., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MÜLLER-HILL, B., and J. KANIA, 1974 *Lac* repressor can be fused to β-galactosidase. Nature **249:** 561–562.

NOHMI, T., J. R. BATTISTA, L. A. DODSON and G. C. WALKER, 1988 RecA mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. Proc. Natl. Acad. Sci. USA **85:** 1816–1820.

OZAWA, A., and R. FRETER, 1964 Ecological mechanism controlling growth of *Escherichia coli* in continuous flow cultures and in the mouse intestine. J. Infect. Dis. **114:** 235–242.

REBECK, G. W., and L. SAMSON, 1991 Increased spontaneous mutation and alkylation sensitivity of *Escherichia coli* strains lacking the *ogt* O^6-methylguanine DNA repair methyltransferase. J. Bacteriol. **173:** 2068–2076.

RYAN, F. J., D. NAKADA and M. J. SCHNEIDER, 1961 Is DNA replication a necessary condition for spontaneous mutation? Z. Vererbungsl. **92:** 38–41.

RYAN, F. J., T. OKADA and T. NAGATA, 1963 Spontaneous mutation in spheroplasts of *Escherichia coli.* J. Gen. Microbiol. **30:** 193–199.

SARGENTINI, N. J., and K. C. SMITH, 1981 Much of spontaneous mutagenesis in *Escherichia coli* is due to error-prone DNA repair: Implications for spontaneous carcinogenesis. Carcinogenesis **2:** 863–872.

SHAPIRO, J. A., 1984 Observation on the formation of clones containing *araB-lacZ* cistron fusions. Mol. Gen. Genet. **194:** 79–90.

SHINAGAWA, H., H. IWASAKI, T. KATO and A. NAKATA, 1988 RecA protein dependent cleavage of UmuD protein and SOS mutagenesis. Proc. Natl. Acad. Sci. USA **85:** 1806–1810.

SMITH, T. F., and J. R. SADLER, 1971 The nature of lactose operator constitutive mutations. J. Mol. Biol. **59:** 273–305.

STAHL, F. W., 1988 A unicorn in the garden. Nature **335:** 112–113.

VOLKERT, M. R., L. J. MARGOSSIAN and A. J. CLARK, 1981 Evidence the *rnmB* is the operator of the *Escherichia coli recA* gene. Proc. Natl. Acad. Sci. USA **78:** 1786–1790.

WALKER, G. C., 1984 Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli.* Microbiol. Rev. **48:** 60–93.

WHORISKEY, S. K., M. A. SCHOFIELD and J. H. MILLER, 1991 Isolation and characterization of *Escherichia coli* mutants with altered rates of deletion formation. Genetics **127:** 21–30.

Communicating editor: J. W. DRAKE