The SOS response regulates adaptive mutation

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Upon starvation some *Escherichia coli* cells undergo a transient, genome-wide hypermutation (called adaptive mutation) that is recombination-dependent and appears to be a response to a stressful environment. Adaptive mutation may reflect an inducible mechanism that generates genetic variability in times of stress. Previously, however, the regulatory components and signal transduction pathways controlling adaptive mutation were unknown. Here we show that adaptive mutation is regulated by the SOS response, a complex, graded response to DNA damage that includes induction of gene products blocking cell division and promoting mutation, recombination, and DNA repair. We find that SOS-induced levels of proteins other than RecA are needed for adaptive mutation. We report a requirement of RecF for efficient adaptive mutation and provide evidence that the role of RecF in mutation is to allow SOS induction. We also report the discovery of an SOS-controlled inhibitor of adaptive mutation, PsiB. These results indicate that adaptive mutation is a tightly regulated response, controlled both positively and negatively by the SOS system.

DNA repair | *Escherichia coli* | signal transduction | RecF | RecA

The bacterial SOS response, studied extensively in *Escherichia coli*, is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis are induced (1). SOS is the prototypic cell cycle check-point control and DNA repair system, and because of this, a detailed picture of the signal transduction pathway that regulates this response is understood. A central part of the SOS response is the de-repression of more than 20 genes under the direct and indirect transcriptional control of the LexA repressor. The LexA regulon includes recombination and repair genes *recA*, *recN*, and *ruvAB*, nucleotide excision repair genes *uvrAB* and *uvrD*, the error-prone DNA polymerase (pol) genes *dinB* (encoding pol IV) (2) and *umuDC* (encoding pol V) (3), and DNA polymerase II (4, 5) in addition to many functions not yet understood. In the absence of a functional SOS response, cells are sensitive to DNA damaging agents.

The signal transduction pathway leading to an SOS response (reviewed by ref. 6) ensues when RecA protein binds to single-stranded DNA (ssDNA), which can be created by processing of DNA damage, stalled replication, and perhaps by other means (7–9). The ssDNA acts as a signal that activates an otherwise dormant co-protease activity of RecA, which allows activated RecA (called RecA*) to facilitate the proteolytic self-cleavage of the LexA repressor, thus inducing the LexA regulon (10). Activated RecA also facilitates the cleavage of phage repressors used to maintain the quiescent, lysogenic state, and UmuD, creating UmuD′, the subunit of UmuD′C (pol V) that allows activity in trans-lesion error-prone DNA synthesis (6).

An intriguing feature of the SOS response is inducible mutation (11, 12). LexA-repressed pol V participates in most UV mutagenesis, by inserting bases across from pyrimidine dimers (3). Pol IV is required for an indirect mutation phenomenon in which undamaged phage λ DNA is mutated when added to UV-irradiated (SOS-induced) cells (13). There may be other mutagenic mechanisms induced by the SOS response.

Adaptive mutation (also called stationary-phase mutation) is a collection of phenomena in which mutations form in stressed or starving, nongrowing, or slowly growing cells, and at least some of these mutations allow growth (reviewed by refs. 14–19). It is a model for mutational escape of growth-control, such as in oncogenesis, tumor progression, and resistance to chemotherapeutic drugs (16, 20–22), and also, like SOS mutagenesis, implies that evolution can be hastened when the need arises (23).

Adaptive mutation has been studied most extensively using an assay for reversion of a *lac* +1 frameshift allele on an F′ sex plasmid in *E. coli* starved on lactose medium (24). The adaptive mutations are unlike Lac^+ mutations in growing cells in that they form during (not before) exposure to selective conditions (25), and occur via a unique molecular mechanism (reviewed by refs. 18 and 19) that requires homologous recombination proteins RecA, RecBC, and RuvABC (22, 26, 27). The adaptive mutations occur in a hypermutable subpopulation of the starved cells (28–30) during a transient period of limiting mismatch-repair activity (31) and possess a unique sequence spectrum of −1 deletions in mononucleotide repeats (32, 33) identical to that of mismatch repair defective cells (34).

As reviewed above, the cells undergoing adaptive mutation are transiently differentiated and mutable. However, the mechanism(s) by which the environment induces this differentiation, the signals from the environment, and the signal transduction pathway(s) provoking adaptive mutation are unknown. We have examined the role of the SOS response in adaptive mutation and report both positive and negative control of adaptive mutation in the Lac system by the LexA repressor. First, we report that SOS induction of the LexA regulon is required for efficient adaptive mutation. Simple overproduction of RecA, a recombination protein controlled by LexA, does not substitute. Second, we provide evidence that RecF protein is required for efficient mutation in its SOS-inducing capacity. This implies that the DNA signal provoking SOS during adaptive mutation is not a DNA double-strand break (DSB) as postulated previously (e.g., ref. 18), and implies that there are ssDNA intermediates in mutation other than at DSBs. Third, we find evidence of an SOS-controlled repressor of adaptive mutation, PsiB, a protein known to inhibit RecA* activity. The adaptive mutation response appears to occur within a narrow window in the continuum of levels of SOS induction. These results (*i*) indicate that adaptive mutation is a tightly regulated response, (*ii*) identify part of the signal transduction pathway that controls it, and (*iii*) illuminate possible DNA intermediates in that signal transduction pathway.

Abbreviations: ssDNA, single-stranded DNA; pol, polymerase; DSB, double-strand break.

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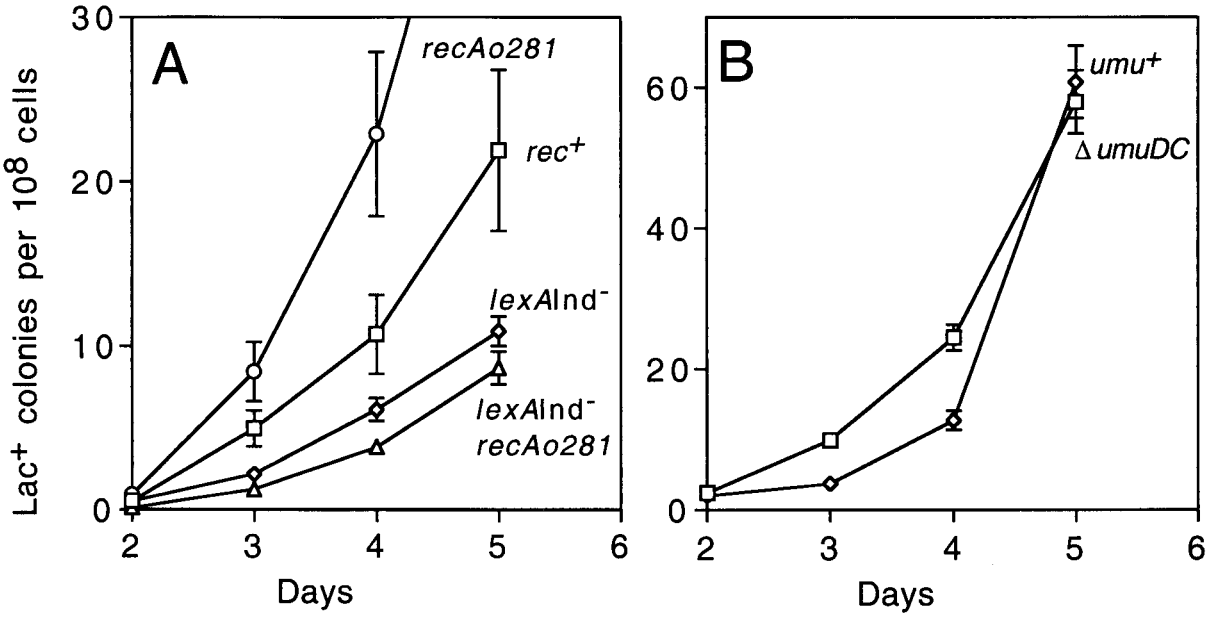


Fig. 1. (*A*) Induction of a LexA-regulated gene(s) other than or in addition to *recA* is required for efficient Lac$^+$ adaptive mutation. ○, *recAo281*; □, *rec*$^+$; ◇, *lexA3(Ind*$^-$*)*; △, *lexA3*(Ind$^-$) *recAo281*. (*B*) *umuDC* is not required for adaptive mutation. □, *Δ(umuDC)595*::*cat*; ◇, *umu*$^+$. In this and all figures, all strains shown together were tested in parallel, and the means ± SEM (error bars) of several independent cultures tested in parallel are displayed.

Materials and Methods

All new *E. coli* strains were constructed using standard P1 transduction techniques (35). The mutant alleles used were *recAo281* (36), *lexA3*(Ind$^-$) (37), *lexA51*(Def) (38), *sulA211* (*E. coli* Genetic Stock Center, New Haven, CT), *recF332*::Tn*3* (39), *dinI*::*kan* (40), *psiB*::*cat* (A. Bailone, Orsay, France), and *Δ(umuDC)595*::*cat* (41). Strains used in the mutation assay are derived from FC40 (24), which carries a deletion of the chromosomal *lac-pro* region and an F′ carrying *pro*$^+$ and a *lacI33ΩlacZ* fusion with a +1 frameshift mutation such that the cells are phenotypically Lac$^-$. Mutation assays were as described previously (27), including that cell viability measurements for all experiments reported showed no net growth or death of the frameshift-bearing cells. Some variability is seen in absolute values from experiment to experiment, but relative values between strains remained the same within a minimum of three repeats. Single representative experiments are shown (see Figs. 1–3) and the consistency of results across multiple repeats summarized (see Fig. 4).

Results

Induction of a LexA Controlled Gene(s) Other Than or in Addition to RecA Is Required for Adaptive Mutation. The *lexA3*(Ind$^-$) allele encodes a noncleavable mutant LexA protein (42, 43) containing a substitution of Gly-84 to Asp (44, 45). In *lexA3*(Ind$^-$) cells, the LexA regulon is repressed and cannot be induced. In a strain carrying *lexA3*(Ind$^-$), adaptive mutation is decreased 3- to 4-fold (Fig. 1*A*), as seen previously (24). This result indicates a requirement for induced levels of a LexA-repressed gene(s) for efficient adaptive mutation. The LexA-repressed gene(s) could be required absolutely for adaptive mutation if the basal level of expression in uninduced cells is sufficient for some adaptive mutation to occur.

recA is repressed by LexA, and is induced >10-fold during the SOS response (1). RecA is essential for adaptive mutation (22), making it a reasonable candidate for being required at induced levels. To test this hypothesis, we used a *recA* operator-constitutive allele, *recAo281*, that produces induced levels of RecA constitutively (36). In *lexA3*(Ind$^-$) *recAo281* cells, RecA is produced at levels similar to those during SOS induction (36). This allele does not restore the level of adaptive mutation in *lexA3*(Ind$^-$) cells to the level of *lexA*$^+$ cells (Figs. 1*A* and 4*A*), in contrast with data reported previously (24). The strain used by those authors was shown subsequently not to carry *lexA3*(Ind$^-$) (26, 46). This failure to restore mutation with a

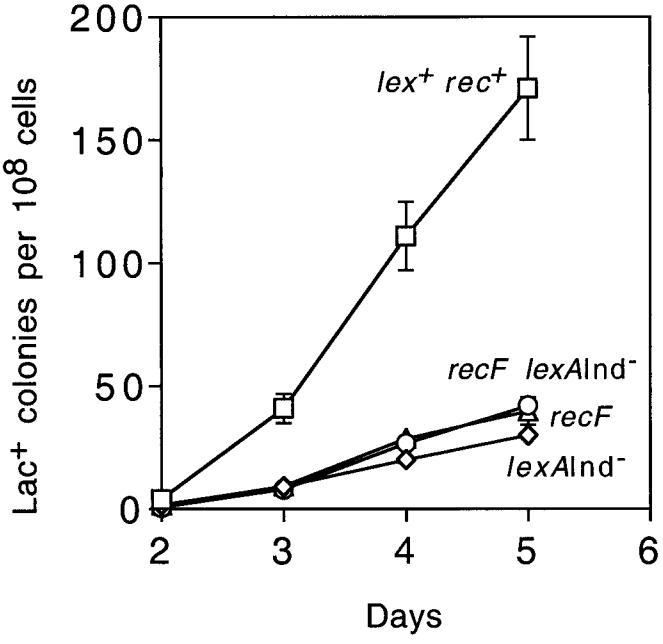
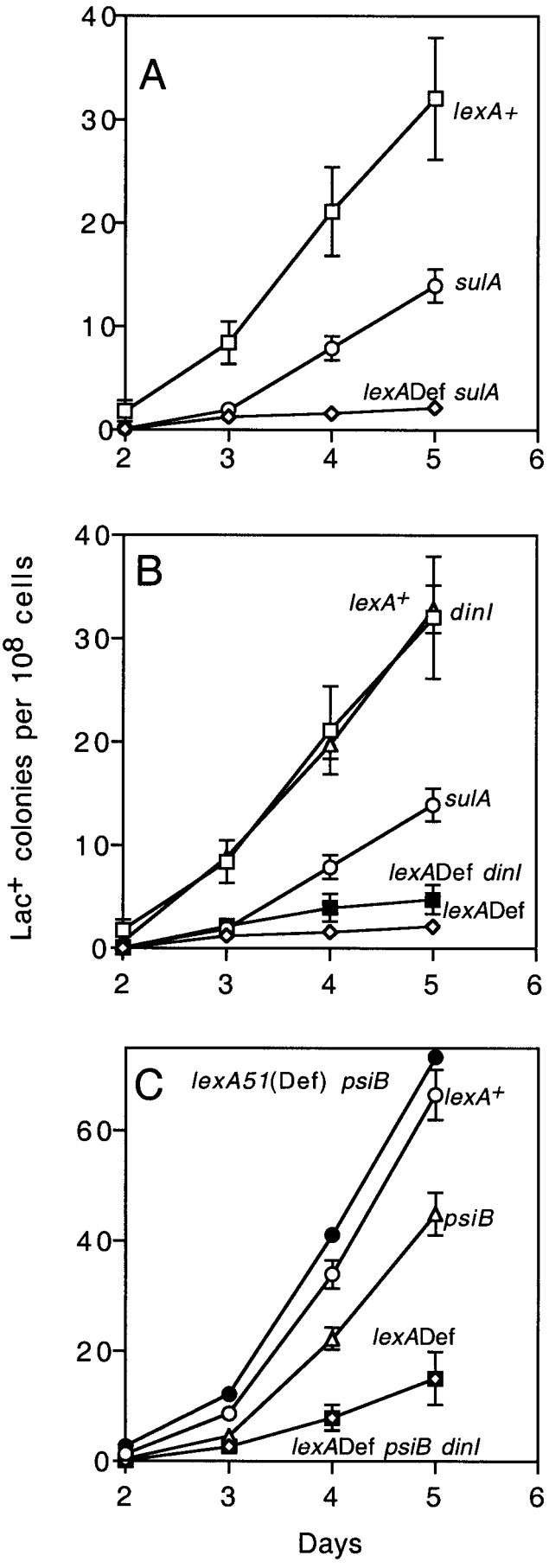


Fig. 2. RecF$^+$ promotes adaptive mutation and acts via the same pathway as LexA. □, *rec*$^+$ *lexA*$^+$; △, *recF332*::Tn*3*; ○, *recF332*::Tn*3 lexA3(Ind*$^-$*)*; ◇, *lexA3(Ind*$^-$*)*.



constitutively expressing *recA* allele indicates that *recA* is either not the LexA-repressed gene, or not the only LexA-repressed gene, required at induced levels for efficient adaptive mutation.

The LexA-repressed function required at induced levels is not the mutagenic UmuDC complex (Fig. 1*B*). This agrees with work showing that a *recA* allele unable to cleave UmuD to the mutagenically active form, UmuD′, does not affect the level of adaptive mutation (24).

RecF Is Required for Efficient Adaptive Mutation. RecF protein plays a poorly defined role(s) in recombination *in vivo* (47, 48). RecF is also required for SOS induction by some DNA damaging agents that produce single-stranded lesions (and not those that produce DSBs) (49). In a *recF* mutant, adaptive mutation is decreased 3- to 5-fold (Fig. 2 and 4*B*). To test whether RecF promotes adaptive mutation via its recombination capacity or via allowing SOS induction, a *recF lexA3*(Ind$^-$) strain was examined. *recF* is epistatic with *lexA3*(Ind$^-$) (Fig. 2), suggesting that the role of RecF in mutation is to allow SOS induction and not via recombination (alternatives discussed below).

A LexA-Controlled Inhibitor of Adaptive Mutation. Because induction of some protein(s) is required, we tested whether constitutive de-repression of the LexA-repressed genes promotes adaptive mutation. Cells lacking LexA must also carry a mutation in the *sulA* gene to be viable because SulA is a LexA-repressed protein that inhibits cell division (50). A *sulA* mutation by itself decreases adaptive mutation slightly (Figs. 3*A* and 4*C*). This could be because more cell division occurs during SOS in the absence of SulA, such that sister chromosomes have more opportunity to segregate and thus less opportunity to recombine. Sister chromosomes are a possible source of the homologous DNA used in the recombination required for adaptive mutation in this system (22). In contrast to the simplest prediction, the *lexA51*(Def) *sulA* cells show greatly decreased adaptive mutation (Fig. 3*A*). This finding indicates that constitutive de-repression of some LexA-repressed gene(s) inhibits adaptive mutation.

We tested two candidates for the LexA-repressed inhibitor(s) of adaptive mutation. DinI is a LexA-repressed protein that inhibits recombination and SOS induction by binding and altering RecA (40). Its proposed function is to help return cells to normal after an SOS response. We find that loss of *dinI* in a *lexA51*(Def) cell has little effect (Figs. 3*B* and 4*C*), indicating that DinI is not an important LexA-repressed inhibitor of adaptive mutation. However, a different anti-SOS protein encoded by the F plasmid, PsiB (51), appears to be the LexA-repressed inhibitor. In the absence of LexA, the loss of PsiB restores adaptive mutation to normal (Figs. 3*C* and 4*C*). PsiB also interacts with RecA to decrease RecA* activity (51). In addition, loss of PsiB in *lexA*$^+$ cells diminishes adaptive mutation. This finding implies that the extent of RecA* activity is crucial to adaptive mutation, indicating a tight regulatory control over adaptive mutation, as does the following result. When *psiB* and *dinI* are both removed in a *lexA51*(Def) strain, adaptive mutation is diminished greatly relative to *psiB lexA51*(Def) (Figs.

Fig. 3. LexA-repressed inhibitor(s) of Lac$^+$ adaptive mutation. (*A*) Complete de-repression of the LexA regulon inhibits mutation. The *lexA* defective strain carries *lexA51*(Def) *sulA211* (◇), *lexA*$^+$ (□), and *sulA211*(○). The *sulA* mutation, required for viability of *lexA51*(Def) strains, also depresses mutation modestly (discussed in text). (*B*) The LexA-controlled inhibitor of adaptive mutation is not DinI. Both *lexA51*(Def) strains also carry *sulA211*. □, *dinI*$^+$ *lexA*$^+$; △, *dinI*::*kan lexA*$^+$; ○, *sulA211*; ■, *lexA51*(Def) *sulA211 dinI*::*kan*; ◇, *lexA51*(Def) *sulA211*. (*C*) PsiB inhibits adaptive mutation in LexA de-repressed cells. All strains shown carry *sulA211*. Additional alleles carried are as follows: ○, *lexA*$^+$; ●, *lexA51*(Def) *psiB*::*cat*; △, *psiB*::*cat*; ◇, *lexA51*(Def); ■, *lexA51*(Def) *psiB*::*cat dinI*::*kan*. Results are discussed in the text.

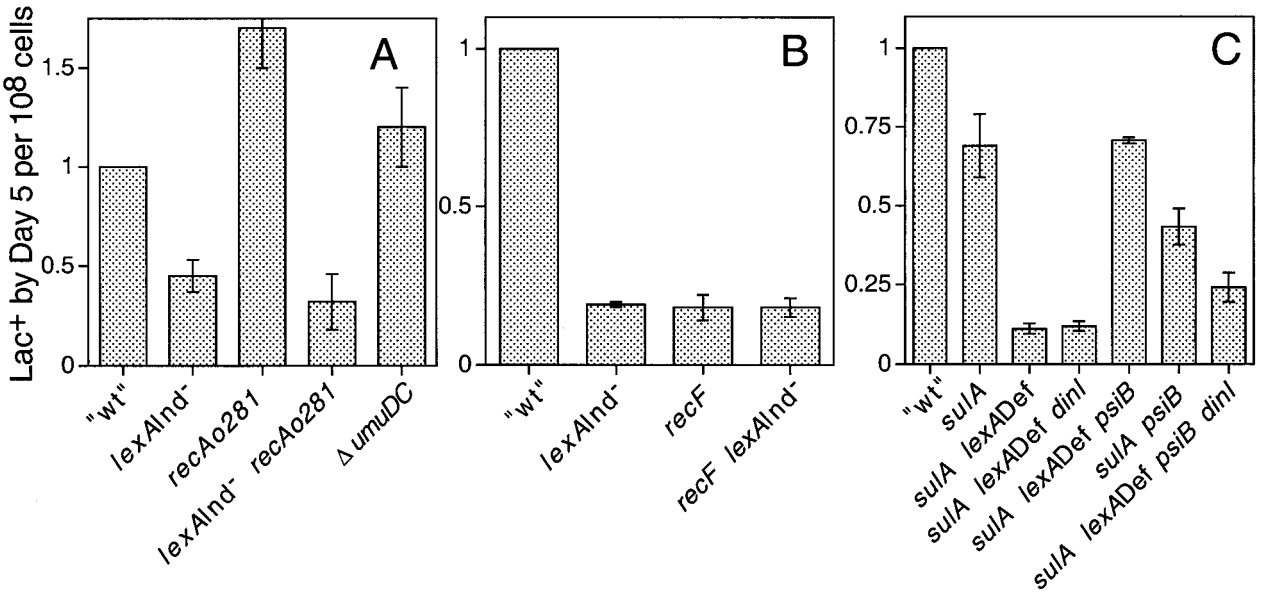


Fig. 4. Comparison of various SOS altered mutants with "wild-type" (wt) in multiple experiments. The fold-differences in accumulation of Lac^+ mutant colonies by day 5 is displayed between of each of the mutants listed and the nonmutant parental strain (wt; set equal to 1) assayed in parallel over multiple experiments. The numbers (n) of experiments averaged (error bars equal 1 SEM) are as follows: wt, $n = 4$; *lexA3*(Ind^-), $n = 4$; *recAo281*, $n = 4$; *lexA3*(Ind^-) *recAo281*, $n = 4$, Δ*umuDC*, $n = 3$ (*A*); $n = 3$ for all genotypes displayed (*B*); and $n = 3$ for all genotypes displayed except "wt" and *sulA lexAdef* for which $n = 4$ (*C*). "wt" is isogenic with all of the mutants used, and carries wild-type alleles of all *rec*, *lex*, *sul*, *din*, and *psi* genes and Δ*(pro-lac)* Rif *thi* F′ pro^+lacIq *lacI33*Ω*lacZ*.

3*C* and 4*C*). This result suggests that *psiB* is not a direct inhibitor of adaptive mutation, but modulates it by modulating the SOS response, and that adaptive mutation is very sensitive to the extent and/or duration of the SOS response, and to levels of RecA* activity. Because RecA* activity is implicated as being important even when the LexA regulon is fully derepressed [in *lexA51*(Def) cells], these results might imply that a target of the RecA* co-protease activity in addition to LexA is important in adaptive mutation (this and alternatives are discussed below).

To summarize: (*i*) PsiB appears to inhibit adaptive mutation when the LexA regulon is constitutively de-repressed in a *lexA*Def mutant; and (*ii*) two proteins that modulate RecA* activity, DinI and PsiB, affect adaptive mutation positively and negatively. These data suggest that RecA* activity is critical in adaptive mutation, that if RecA* activity is either too high or too low, mutation is decreased. These results indicate a tight control over adaptive mutation by factors modulating the SOS response, and provide evidence of SOS regulation of adaptive mutation independent of particular LexA alleles.

Discussion

The results reported indicate that adaptive mutation in the Lac system in *E. coli* is regulated by the SOS system. This identifies SOS as a signal transduction pathway controlling the transient, differentiated condition (52) of adaptive mutation, and likewise identifies adaptive mutation as a new form of SOS mutagenesis.

The LexA Regulon and Adaptive Mutation. We have shown that efficient Lac adaptive mutation requires SOS-induced levels of a LexA-repressed function(s) other than or in addition to RecA (Fig. 1). As discussed above, no real conflict exists between previously reported data (24) and ours.

Two Roles for RecA. RecA is both a signal sensor/transducer molecule for the SOS response and an important recombination protein (53). Because recombination proteins RecBCD, RuvAB, and RuvC are also required for adaptive mutation (22, 26, 27), and RuvAB and RuvC do not affect SOS induction (1), a recombinational role for RecA in adaptive mutation has been clear. The current results indicate that the SOS activation function of RecA is also required for efficient adaptive mutation. These data allow one to understand the previously perplexing finding that a special *recA* mutation conferring recombination-proficiency and SOS deficiency reduces adaptive mutation in this system (24).

DNA Intermediates in Signal Transduction. Efficient SOS induction requires either RecBC or RecF, depending on whether the DNA intermediate that triggers the SOS response is a double-strand end (RecBCD) or ssDNA not at a double-strand end (RecF) (49). RecF is partially required for adaptive mutation (Fig. 2), and the data suggest that this requirement reflects a requirement for RecF in SOS induction during adaptive mutation: (*i*) loss of RecF decreases adaptive mutation to the same (partial) extent as the LexA-uncleavable mutation (Figs. 2 and 4*B*); and (*ii*) RecF deficiency does not reduce mutation further in a strain that is already LexA uncleavable (Figs. 2 and 4*B*), as expected if the sole function of RecF in mutation is to promote LexA cleavage. The converse possibility, that LexA induction is required to produce RecF, is unlikely because RecF is not thought to be LexA regulated (1). Although not ruled out by our data, schemes in which LexA is imagined to function in a RecF-specific recombination route are more complicated, and so are not favored.

The indication that the RecF function in adaptive mutation is to promote the SOS response implies that the ssDNA signal inducing SOS during adaptive mutation is not at a double-strand end (DSE). This is surprising considering that adaptive mutation in this system absolutely requires RecBC (22), an enzyme that operates only at DNA DSEs and breaks (DSBs), and which catalyzes recombinational DSB-repair in *E. coli* by generation of ssDNA at DSEs (54, 55). One possible explanation is that the timing of SOS induction in adaptive mutation necessarily precedes DSB formation. Another is that perhaps, although DSBs form, single-strand lesions are more abundant during adaptive mutation, and so are more important SOS-inducing signals. Whichever is the case, these results allow us to infer a new DNA

intermediate in adaptive mutation: ssDNA other than single-strands exposed at double-strand ends. DSEs (22) and Holliday junctions (26, 27) are the only other DNA intermediates implicated in adaptive mutation, to date.

The ssDNA-inducing SOS during adaptive mutation could be exposed at nicked DNA at the F′ origin of transfer, stalled replication forks or chemically damaged DNA. If nicks at the F′ transfer origin are the signal, this could explain why transfer (Tra) proteins (but not actual transfer) are required for efficient adaptive mutation (56, 57), despite evidence that the F′ need not be covalently linked with the DNA undergoing mutation (28, 58, 59). A *trans* role for the F′ (also suggested by ref. 30), such as inducing *trans*-acting SOS proteins, seems sensible. Further work will be required to determine when, where, and how the ssDNA signal is generated.

Positive and Negative Control. It was surprising to find that in addition to LexA-controlled factor(s) that promote adaptive mutation, there is a LexA-repressed inhibitor, PsiB (Fig. 3). PsiB is a RecA co-protease inhibitor encoded by the F plasmid (51) and may be repressed by LexA (implied by our data, see Fig. 3*C*). The chromosomally encoded DinI protein also blocks RecA co-protease activity and recombination (40). Both of these proteins may promote a speedy return to the non-SOS state after the DNA damage that induced the response has been repaired. The *dinI* deletion had no effect on mutation in either *lexA51*(Def) or *lexA*$^+$ cells, but decreased mutation in the absence of PsiB (Figs. 3*C* and 4*C*). This finding may imply that DinI competes poorly for RecA binding in the presence of PsiB. This apparently perplexing result suggests that levels of RecA* are crucial to successful adaptive mutation. For example, adaptive mutation might be regulated temporally by the SOS response, with both early entry (in LexA-defective cells) and early exit (PsiB$^+$) or late exit (PsiB$^-$ DinI$^-$) from the SOS response being inhibitory to adaptive mutation. Alternatively, cells lacking both PsiB and DinI may simply not survive the SOS induction and hypermutation to form (Lac$^+$) colonies, as follows.

SOS and Hypermutability Are Differentiated States. Recombination-dependent adaptive mutation occurs in a hypermutable subpopulation of the stressed cells (10^{-4} to 10^{-5}) (28, 59). We suggest that SOS induction may be the event that differentiates subpopulation cells from the main population. Although no net cell death was observed during the experiments with the *dinI psiB* strain (see *Materials and Methods*), death of only the subpopulation would have been undetectable.

The discovery that the LexA regulon includes both repressor(s) and promoter(s) of adaptive mutation implies that adaptive mutation is a tightly regulated process. SOS is the first signal transduction pathway found to control adaptive mutation in this system.

Candidate Genes and Molecular Mechanism. The LexA-repressed gene(s) needed at induced levels for efficient Lac-adaptive mutation have not been identified. However, some plausible candidates are suggested by our current picture of the molecular mechanism of adaptive mutation in this system (17–19). The mutations are suggested to result from DNA polymerase errors that occur during the DNA replication (22) now known to be associated with some recombinational double-strand break-repair in *E. coli* (60). The source of the DSBs in the starving cells is not yet known. DSBs may result from stalled replication (22, 61, 62), processing of single-stranded nicks at the F′ transfer origin (63, 64), endonucleases, or chemical damage, or other (e.g., ref. 65). Mismatch repair activity is diminished transiently (28, 34, 52) in the stressed, mutating cells due to a transient limitation of MutL (31, 66). This allows the errors to be fixed as mutations. DNA pol III is implicated in the replication (60, 67, 68). Finally, the mutational process occurs in a small subpopulation of the stressed cells, in which hypermutation occurs at hotspots (not uniformly; ref. 19) throughout the bacterial genome (28–30, 59).

There are several candidate LexA-regulated genes (apart from RecA) whose induction might promote this adaptive mutation mechanism. (*i*) RuvAB recombination proteins (1, 55) are required absolutely for mutation in this system, presumably for the recombination that promotes DNA replication (26, 27). These are expressed constitutively, and may not need to be induced for full recombination (see ref. 69). (*ii*) We found that loss of the SulA cell division inhibitor protein (50, 70) reduces adaptive mutation slightly. Perhaps inhibition of cell division increases the chance of recombination between sister DNA molecules, or lack of division control results in death of some of the subpopulation, which would not be measurable in cell viability determinations. (*iii*) An attractive possibility is the LexA-repressed mutagenic DNA polymerase pol IV, encoded by *dinB* (2, 71). LexA represses three DNA polymerases. Of them, pol II (high accuracy polymerase) inhibits Lac adaptive mutation (46, 67), as if it competes with the mutagenic polymerase that makes the mutations. Pol V (UmuD′C, an error prone polymerase) has no effect (Fig. 1*B*; ref. 24), and pol IV is currently being examined. Pol IV is required for phage λ untargeted mutagenesis (13), and when overexpressed, increases spontaneous mutations (especially −1 frameshifts) up to 800-fold (72). Although DNA pol III is implicated in adaptive mutation (67, 68), the data do not rule out the possibility that another polymerase makes the mutations, or that adaptive mutations are made by both pol III and pol IV (73).

Generality. This report describes the second example of SOS mutagenesis in starving cells independent of UmuDC, both of them dependent on RecA and RecBC. In the first example, aging colonies induce SOS and mutation (74, 75). That SOS response requires cAMP, a signal molecule produced during starvation, and RecB. This is similar to recombination-dependent adaptive mutation (studied here), but the two mutation routes have some different genetic requirements (reviewed by ref. 18) and may represent closely related SOS mutagenesis mechanisms promoted by starvation. UmuDC-dependent SOS transversion mutagenesis in starving cells has also been described (76, 77). Other stationary-phase stress- or starvation-induced mutagenesis mechanisms exist in prokaryotes and eukaryotes (reviewed by refs. 17 and 18), and there are many examples in the literature of recombination-associated mutation in eukaryotes (reviewed in refs. 17, 18, 52, and 78). Components of the regulatory mechanisms of these processes have been described only for transcription-associated mutation, which involves the stringent response (amino acid starvation) (79, 80), SOS-mutagenesis in aging colonies (74, 75) and starving cells (76, 77), *phoPQ* involvement in *ebgR* mutation (81), and this report. Understanding the regulation of all of the different adaptive or stationary-phase mutation mechanisms will illuminate when, how, and whether cells adjust their mutation rates and mechanisms, thereby inducing heritable changes, and presumably increasing their options for survival.

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