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Ultraviolet Mutagenesis and Inducible DNA Repair in *Escherichia coli*

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INTRODUCTION

Mutagens change the genetic script by promoting errors in replication or in repair of deoxyribonucleic acid (DNA). Ultraviolet (UV) light owes its powerful mutagenic effect in *Escherichia coli* primarily to misrepair of radiation damage to the bacterial chromosome (26, 125, 230). Recent developments indicate that the error-prone repair activity responsible for UV mutagenesis in *E. coli* and some of its phages is repressed in undamaged wild-type cells, but is expressed, as one of a metabolically diverse but coordinately regulated group of inducible functions, in response to UV radiation or other agents that damage DNA or interrupt its replication (53, 77, 174, 240). This article summarizes current evidence for the inducibility of mutagenic DNA repair and explores some of its implications. Other aspects of UV mutagenesis in microorganisms have recently been reviewed (3, 62, 66, 67, 130).

Enzymatic Repair of UV Damage in *E. coli*

Enzymatic repair mechanisms operating in bacteria which minimize the damaging effects of UV radiation are described in detail elsewhere (88, 91, 93, 102). The brief summary given here is intended only to provide sufficient background for discussion of UV mutagenesis.

UV radiation produces a variety of photoproducts in DNA (169, 197, 202), among which the intrastrand cyclobutane-type dimer of adjacent pyrimidines (8) has been identified as a major cause of lethal, mutagenic and tumorogenic effects in a wide spectrum of organisms (92, 95, 216, 225). Wild-type strains of *E. coli* effectively neutralize the potentially lethal effects of as many as a thousand pyrimidine dimers by utilizing one or more of three types of enzymatic DNA repair, which are represented in Fig. 1. Photoreactivation (120) is accomplished by "photoreactivating enzyme" (PRE), which binds pyrimidine dimers in the dark but requires "photoreactivating light" (PRL) in the range of wavelengths mainly between 310 and 400 nm to "split" or monomerize them in situ (183, 184). Since PRE acts specifically on pyrimidine dimers (193), photoreversibility by this enzyme indicates that a UV-initiated biological phenomenon requires the more or less persistent presence of at least one pyrimidine dimer.

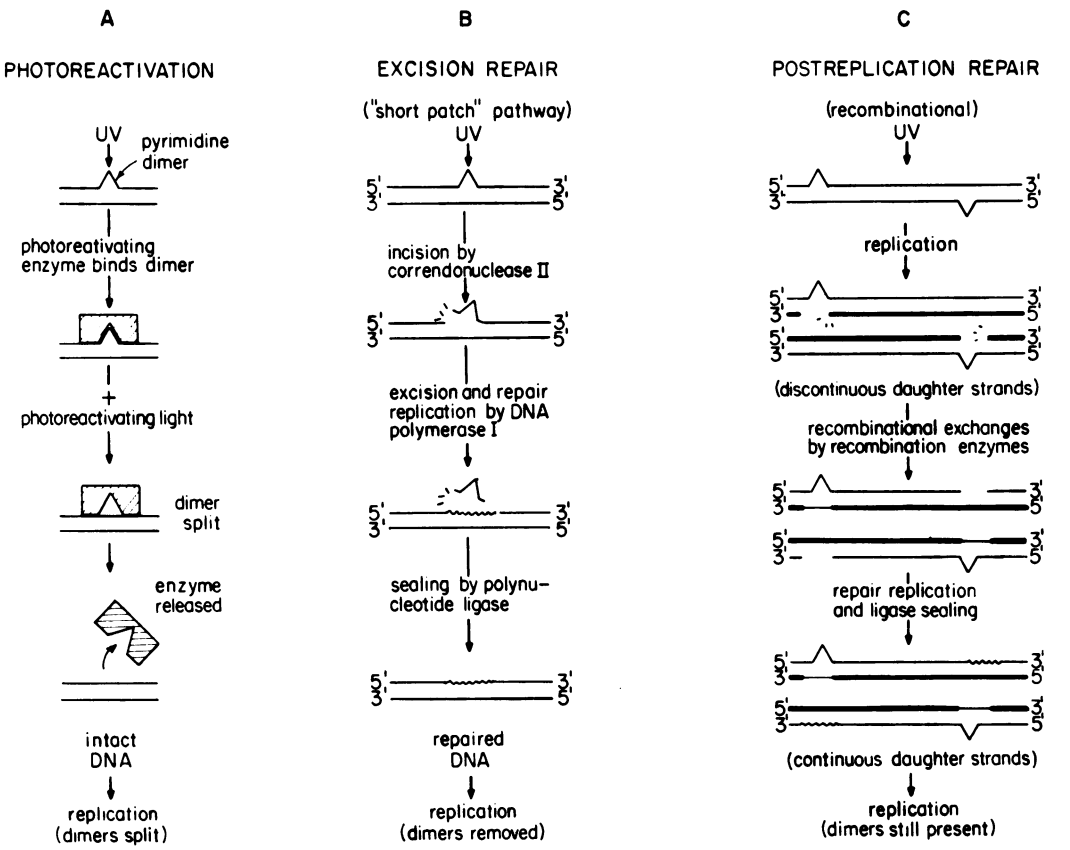

FIG. 1. *Major pathways of enzymatic repair of damage to DNA caused by UV radiation in E. coli. All three of these repair mechanisms are error-proof in principle. Symbols:* ⌒ *or* ⌣, *pyrimidine dimer or other noninstructive UV photoproduct; heavy lines, daughter strands produced in first postirradiation DNA replication; light lines, parental (UV-irradiated) strands of DNA; wavy lines, DNA polymerized during repair replication. Adapted from Hanawalt (91). See text for additional references.*

In the dark, pyrimidine dimers are not chemically reversed, but may be removed from the DNA by a multienzymatic mechanism, excision repair (20, 195). The distortion of the double helix caused by a pyrimidine dimer (or by some other kinds of DNA lesions) is recognized by correndonuclease II, the product of the *uvrA* and *uvrB* genes of *E. coli* (22), which makes a single-strand nick, or incision, on the 5′ side of the dimer. An exonucleolytic excision releases the oligonucleotide bearing the pyrimidine dimer plus some bases on either side of it, and the "excision gap" is patched by repair replication (170), the intact region of the strand opposite the gap serving as template for accurate replacement of the missing nucleotides. In wild-type *E. coli,* the excision and resynthesis steps are probably effected simultaneously by DNA polymerase I (50, 78, 119), although efficient excision requires additional gene products, including those of *uvrC* (103), *uvrE* (200, 201, 213) and *mfd* (73, 74) genes. The final step is sealing of the sugar-phosphate linkage by polynucleotide ligase. Alternate pathways of excision repair, utilizing DNA polymerase III (244) or DNA polymerase II (144), have been demonstrated in mutants lacking activity of DNA polymerase I. In addition to the "short-patch" type of excision repair shown in Fig. 1B, a "long-patch" pathway has been identified which requires the products of the *recA*$^+$ and *lexA*$^+$ gene products, among others, and occurs only in growth-supporting media (49, 245, 248).

Pyrimidine dimers, that are neither photoenzymatically split nor removed from the DNA by excision repair (as in excision-deficient Uvr$^-$ mutants kept in the dark) block the continuous progress of the DNA replication complex, but do not prevent subsequent reinitiation of DNA synthesis at a point beyond the dimer (185). When pyrimidine dimers or other noninstructive lesions are present in the template strand, daughter strands are detected initially as segments of relatively low molecular weight, their

continuity interrupted by gaps about 1,000 nucleotides long (114) which correspond approximately in number and position to the dimers in the parental strand (7, 105). These "daughter-strand gaps" are secondary UV lesions, caused by the replication of DNA containing primary UV photoproducts that prevent base pairing. The third major type of enzymatic DNA repair, postreplication repair (Fig. 1C), operates to connect the daughter-strand segments, thereby endowing them with the continuity required for further replication (185). The formation and joining of daughter-strand segments synthesized on UV-irradiated templates should not be confused with the discontinuous synthesis and much more rapid joining of Okazaki fragments at normal DNA replication forks (128). Although first demonstrated in Uvr$^-$ strains, postreplication repair occurs also in Uvr$^+$ strains capable of excising pyrimidine dimers efficiently (192, 203), implying that ability to perform excision repair does not prevent some noninstructive UV lesions from passing through a replication fork.

In excision-deficient (Uvr$^-$) strains that are otherwise normal, the major mechanism of postreplication repair is recombinational. After UV irradiation followed by DNA replication and postreplication repair, specifically labeled parental DNA is found covalently inserted into the daughter strands, the number of insertions corresponding approximately to the number of daughter-strand gaps initially produced (186). The precise mechanism of recombinational exchange operating to rejoin daughter strands is not known, but it must be such that some pyrimidine dimers originally present in the parental strands are exchanged into daughter strands during postreplication repair (71). Figure 1C shows a plausible but hypothetical scheme for recombinational postreplication repair.

E. coli is capable of performing postreplication repair via a number of distinct pathways (72, 182, 189, 190, 192, 209, 247), all of which, however, require the *recA*$^+$ genotype. In *recA*$^-$ mutants, no postreplication joining of daughter strands occurs (203), and the UV sensitivity of *uvrA*$^-$ *recA*$^-$ double mutants, which can perform neither excision repair nor postreplication repair, is such that a single pyrimidine dimer per strand of DNA kills (103). The requirement for the *recA*$^+$ gene product, which is necessary for any type of genetic recombination in *E. coli* (42), does not necessarily imply that all pathways of postreplication repair are recombinational. The phenotype of *recA*$^-$ mutants is highly pleiotropic, and these mutants lack many wild-type functions in addition to recombination ability (Table 1). The specific nature of some pathways of postreplication repair will be considered below, as they may relate to UV mutagenesis.

Error-Proof and Error-Prone Pathways of DNA Repair

The possibility that UV-induced mutations originate as errors in the repair of radiation damage (223) has been substantiated by the finding that UV mutability is eliminated by mutations in either the *recA* (124, 151, 229) or *lexA* (159, 227) genes of *E. coli*, which also cause extreme UV sensitivity and abolish some pathways of DNA repair. Although they are UV-nonmutable, *recA*$^-$ and *lexA*$^-$ strains are spontaneously mutable and are also mutable by agents believed to cause only replication errors (125, 241). Since *recA*$^-$ and *lexA*$^-$ mutants perform some kinds of DNA repair efficiently (e.g., the "short-patch" pathway of excision repair, photoreactivation) after UV irradiation, yet produce no UV-induced mutations, the enzymatic repair occurring in these strains must be accurate, whereas one or more of the pathways of DNA repair requiring both the *recA*$^+$ and *lexA*$^+$ gene products are probably error-prone and responsible for UV mutability (26, 230).

Enzymatic photoreversal of pyrimidine dimers is accurate in principle and unlikely to cause changes in the base sequence of photorepaired regions of the DNA. In fact, exposure to PRL after UV irradiation prevents most of the mutagenic effect, as well as most of the lethal effect, caused by the same UV treatment in "dark" controls (121, 167). There is no reason to believe that monomerization of pyrimidine dimers by PRE is mutagenic, per se, although the visible light used to activate PRE may exert mutagenic effects of its own (218, Fig. 5).

The "short-patch" pathway of excision repair, at least as it occurs in *recA*$^-$ and *lexA*$^-$ strains, must be accurate, since it does not contribute to a detectable level of UV-induced mutation. The low frequency of mutations which has been ascribed to errors in the repair of excision gaps (30, 164, 165) may be associated with "long-patch" excision repair, which, like UV mutagenesis, requires the *recA*$^+$ *lexA*$^+$ genotype. When Uvr$^+$ and Uvr$^-$ strains are exposed to the same low fluence of UV radiation, causing neither detectable killing nor detectable mutagenesis in the excision-proficient Uvr$^+$ strain, the Uvr$^-$ strain, unable to excise UV photoproducts, not only shows drastically reduced survival but also produces a high frequency of UV-induced mutations among the survivors (32, 98, 225). The UV hypermutability of Uvr$^-$ strains demonstrates that unexcised UV photoproducts are much more mutagenic than the same photoproducts removed from the DNA by excision,

TABLE 1. *Pleiotropic effects of recA⁻ and lexA⁻ mutations in Escherichia coli as interpreted by the SOS hypothesis*

Phenotype	*recA*⁺ *lexA*⁺	*recA*⁻	*lexA*⁻	Possible inducible *recA*⁺ *lexA*⁺-dependent (SOS) function(s)	Sources (also see text)
UV sensitivity	+	+++	++	SOS repair activity; exonuclease V inhibitor	43, 103, 106, 215
X ray sensitivity	+	+++	+++	Growth medium-dependent single-strand break repair activity; exonuclease V inhibitor	103, 246
UV inducibility of λ prophage	+	−	±	Lytic phage growth	35, 57, 97, 153
DNA degradation after UV irradiation	+	+++	+++	Exonuclease V inhibitor	106, 143, 171
Mutagenic W-reactivation of λ phage	+	−	−	SOS repair activity	53, 77, 151, 167a, 209a, 220
UV mutagenesis (bacterial)	+	−	−	SOS repair activity	124, 151, 159, 227, 229
Aberrant reinitiation of DNA replication at chromosomal origin after arrest	+	−	?	Reinitiation protein(s)	9, 172, 243
Delayed cell division after UV irradiation (filamentous growth when extreme)	+ (*lon*⁺) +++(*lon*⁻)	−	−	Septum inhibitor	57, 111, 226
Synthesis of protein X after treatment with UV radiation, nalidixic acid, etc.	+	−	−	Protein X (function not known)	88a, 89, 90, 112, 113
Respiration shutoff after UV irradiation	+	−	−	Respiration control protein	208
Chloramphenicol-sensitive postreplication repair pathway	+	−	−	SOS repair	189, 247
"Long-patch" excision repair pathway	+	−	−	SOS repair	49, 248
Thermal inducibility of SOS functions in *tif-1* mutant strains	+	−	−	All the above	40
Recombination ability	+	−	+	None	43, 156

and implies that postreplication repair (at least as it occurs in Uvr⁻ strains) is far more error-prone than excision repair. In Uvr⁻ strains, in which postreplication repair is the only type of dark repair of UV damage known to occur, it is probable that UV mutagenesis is due primarily to errors introduced into the DNA in the course of joining discontinuous daughter strands (26, 230).

The existence of two distinct pathways of postreplication repair, one error-proof and *lexA*⁺ independent, the other error-prone and *lexA*⁺ dependent, was postulated (227) to account for the properties of certain derivatives of UV-sensitive, UV-nonmutable *lexA*⁻ strains. These derivatives had been selected for increased UV resistance, and although some were nearly as UV resistant as *lexA*⁺ strains, they were still nonmutable by UV radiation. The UV-resistant, UV-nonmutable strains were assumed to have acquired improved ability to perform an error-proof type of postreplication repair, enabling them to tolerate nearly as many unexcised pyrimidine dimers as *lexA*⁺ strains without undergoing UV mutagenesis. The ability of similar UV-resistant, UV-nonmutable strains to perform postreplication repair much more efficiently than their *lexA*⁻ parents has been confirmed (215). Biochemical evidence for a pathway of postreplication repair requiring the *recA*⁺ and *lexA*⁺ gene products and associated with UV mutagenesis has recently been obtained (189).

Two hypotheses concerning the nature of error-proof and error-prone pathways of postreplication repair have been considered. Witkin (227) initially proposed that recombinational repair is accurate, and that the *lexA*⁺-dependent mechanism of daughter-strand gap-filling is an error-prone DNA polymerase activity capable of inserting nucleotides opposite pyrimidine dimers or other noncoding UV photoproducts without template instruction. A similar mechanism was suggested by Bridges et al. (27). The *lexA*⁺ gene was envisaged (227) either as conferring template independence upon a normal DNA polymerase or as itself coding an error-prone DNA polymerase such as terminal deoxynucleotidyl transferase (TDT), an enzyme capable of random "end-addition" of nucleotides to single-strand ends of DNA (15). In either case, gaps opposite pyrimidine dimers could be filled by repair replication with a high probability of error. Failure to find TDT activity in *E. coli*, and the later demonstration that DNA polymerases I, II, and III (the only known constitutive DNA polymerases) are strictly dependent upon template instruction (7, 128; M. Radman, personal communication), tended to discourage this hypothesis, at least until the requirement for an inducible function was recognized.

A second hypothesis (27, 125, 228) proposed that UV mutagenesis may be due to errors in a *lexA*⁺-dependent error-prone pathway of recombinational repair. Three experimental observations made this interpretation seem reasonable. One was the dependence of both recombination ability and UV mutagenesis on the *recA*⁺ gene product (43, 124, 151, 229) and the apparently commensurate reduction of both by *recB*⁻ and *recC*⁻ mutations (232). Another was the reduction of the yield of recombinants to as little as 30% of normal when *lexA*⁻ mutants were used as recipients in conjugation (230), an observation later confirmed but ascribed to effects other than reduced ability to perform genetic recombination (159). Most important was the approximate equality observed between the number of daughter-strand gaps repaired and the number of recombinational exchanges detected after postreplication repair (186). This relation implied that either all or nearly all postreplication repair is associated with recombination, although it did not rule out the possibility that a small fraction of the daughter-strand gaps is repaired by a nonrecombinational mechanism.

Evidence against recombinational postreplication repair as the error-prone gap-filling mechanism, however, began to accumulate in 1971, when Bridges and Mottershead (30) showed that the UV mutability of chemostat-grown *E. coli* is not enhanced by chromosomal multiplicity. In *recB* and *recC* mutants, which show reduced ability to perform genetic recombination (42, 68) and postreplication repair (247), yields of UV-induced mutations per survivor at a given UV fluence were either the same as those in wild type (99; T. Kato, R. Rothman, and A. J. Clark, personal communication) or were somewhat lower (232), depending upon the strain used and on the mutation scored. Even when reduced yields of UV-induced mutations were obtained, neither Witkin (232) nor Hill and Nestmann (99) considered the reduction to be conclusive evidence that the probability of error *per mutagenic lesion repaired* was affected by absence of exonuclease V, now known to be the product of the *recB* and *recC* genes (211). The demonstration that *E. coli* can utilize a second pathway of genetic recombination requiring the *recF*⁺ but not the *recB*⁺ *recC*⁺ genotype (101) left open the possibility that UV mutagenesis might be associated with the *recF*⁺-dependent pathway. Recent experiments (T. Kato, R. Rothman, and A. J. Clark, personal communication) have shown

that UV mutagenesis occurs in *recA*$^+$ *recB*$^-$ *recF*$^-$ strains, which are unable to perform either of the two pathways of genetic recombination. This important result suggests that the *recA*$^+$ gene product is required for UV mutagenesis for some reason other than its role in promoting genetic recombination and that neither of these pathways of recombinational postreplication repair is very error-prone. However, these experiments do not rule out the possibility that residual recombinational repair, either due to leakiness of the *rec* mutations or independent of both *recB* and *recF* gene products, may be at least partly responsible for bacterial UV mutagenesis. Recent work has demonstrated that error-prone DNA repair activity, whatever its mechanism, is not a constitutive property of undamaged wild-type *E. coli*, but is induced in response to UV radiation and other mutagenic treatments. A full description of the experimental evidence which has led to this conclusion will follow an account of the "SOS" hypothesis, an hypothesis that has stimulated much of the work to be discussed in this article and that continues to provide a unifying theoretical framework for an impressively diverse yet interconnected array of biological and biochemical investigations.

The "SOS" Hypothesis: the Regulatory Role of DNA Damage

Defais et al. (53) proposed that UV mutagenesis in *E. coli* might depend upon an inducible function which, like λ prophage, is repressed in healthy wild-type cells but is expressed in response to UV irradiation. One basis for this suggestion was the dependence of UV mutagenesis upon *recA*$^+$ and *lexA*$^+$ gene products, which are also required for prophage induction by UV radiation, and for the pleiotropic expression of other UV-inducible functions now recognized to be numerous (Table 1). The hypothesis that the *recA*$^+$ and *lexA*$^+$ gene products jointly control a coordinately regulated group of inducible functions, including an error-prone DNA repair activity, was further developed by Radman as the "SOS" hypothesis (174, 175). The designation "SOS" (the international distress signal) implies that damage to DNA (or stalled DNA replication) initiates a regulatory signal that causes the simultaneous derepression of various functions, all of which presumably promote the survival of the cell or of its phages. I shall refer to the group of inducible functions believed to belong to this regulatory unit as "SOS functions." "SOS repair" is defined here as *recA*$^+$ *lexA*$^+$-dependent error-prone DNA repair activity induced in *E. coli* by UV radiation or other agents having in common the ability to damage DNA or interrupt its synthesis.

The SOS hypothesis incorporates and integrates many observations and ideas that go back as far as a quarter of a century to the demonstration by Lwoff et al. (134) that UV radiation initiates mass induction of prophage in lysogenic bacteria. Other treatments, sharing with UV radiation the ability to arrest DNA replication, were also found to cause lysogenic induction, including exposure to X rays (129), starvation for thymine (147, 199a), incubation with mitomycin C (167c), and temperature elevation in certain mutants unable to synthesize DNA at high temperatures (154, 166, 188).

Another early observation contributing to the SOS hypothesis was Weigle's finding (220) that exposure of the host population of *E. coli* cells to a low fluence of UV radiation prior to infection substantially increases the survival of UV-irradiated λ phage, and is required for a high level of phage UV mutagenesis. This "UV-reactivation" and the mutagenesis that accompanies it require the *recA*$^+$ (151) and *lexA*$^+$ (53) genotype in the host. Defais et al. suggested that UV irradiation of the bacteria induces the functions responsible for UV reactivation and UV mutagenesis of the irradiated phage, via an induction pathway at least partially common to other *recA*$^+$ *lexA*$^+$-dependent phenomena, such as prophage induction and bacterial UV mutagenesis. Radman (174, 175) proposed that a single inducible error-prone repair replication system (SOS repair) may be responsible for both the mutagenic reactivation of UV-irradiated phage and for error-prone repair of bacterial DNA. I shall use the term "SOS repair" to denote inducible error-prone repair activity acting either on phage DNA or on bacterial DNA without implying that only one such activity is induced in *E. coli*, although there is at present no reason to invoke more than one. Since UV radiation is only one of many host treatments capable of stimulating mutagenic reactivation of UV-irradiated phage, the term "UV-reactivation" is misleading. Following Radman's suggestion (174), the terms "W-reactivation" and "W-mutagenesis" are used in Table 1 and elsewhere to refer to the Weigle effect.

Another facet of the SOS hypothesis was anticipated in an interpretation of the parallels observed between the conditions that promote or prevent prophage induction in *E. coli* lysogens and those that promote or prevent filamentous growth in *lon*$^-$ strains such as *E. coli* B (226). Witkin proposed that certain bacterial functions, including the synthesis of a septum-inhibiting protein, may be governed by repres-

sors similar enough to λ repressor to respond to the same inducer, an inducer produced only when DNA replication is interrupted. The subsequent report (122) that *E. coli* K-12 strain T44 not only induces prophage spontaneously at elevated temperatures but also grows in long filaments at 42°C, without requiring any of the usual inducing treatments, further indicated coordinate regulation of these two functions.

Table 1 lists some of the extremely pleiotropic effects of *recA*$^-$ and *lexA*$^-$ mutations, only one of which (loss of ability to perform genetic recombination) is caused by *recA*$^-$ but not by *lexA*$^-$ mutations, and for this and other reasons is not considered to be an inducible SOS function. Table 1 also shows that the *tif-1* mutation, which is responsible for the thermal inducibility of prophage and filamentous growth in strain T44 (39), causes constitutive expression of other (perhaps of all) SOS functions at elevated temperature, without requiring any other inducing treatment. When incubated at 42°C, *tif-1* mutants show no abnormality of DNA structure or replication (39), but their ability to express SOS functions at this temperature requires the *recA*$^+$ *lexA*$^+$ genotype (40). The *tif-1* mutation maps in or very near the *recA* locus (39), and may affect the same protein that is altered by *recA*$^-$ mutations. Two other mutations that are tightly linked to the *recA* locus and may map within it are *lexB*$^-$ (11) and *zab*$^-$ (40). Both *lexB*$^-$ and *zab*$^-$ mutations cause a phenotype like that of *lexA*$^-$ mutations: noninducibility of SOS functions without loss of recombination ability. The *recA* locus maps at 58 min on the recently recalibrated linkage map of *E. coli* K-12 (4), and the *lexA* locus maps at 90 min.

Evidence that SOS functions are actually derepressed in response to UV radiation and other inducing agents (or in *tif*$^-$ mutants at 42°C) is indirect in all cases except λ prophage induction, in which inactivation (198) and proteolytic cleavage (180) of λ repressor have been demonstrated after such treatments. A requirement for new protein synthesis after the inducing treatment has been shown for the expression of most of the SOS functions, and is obvious in the case of protein X.

EVIDENCE FOR THE INDUCIBILITY OF ERROR-PRONE REPAIR ("SOS REPAIR") ACTIVITY

SOS Repair of Bacteriophage DNA

The increased survival of UV-irradiated phage that occurs when the host is also irradiated before infection (W-reactivation) is accompanied by a high level of phage UV mutagenesis (220). A comparable degree of reactivation and mutagenesis of UV-irradiated λ phage occurs in unirradiated *tif*$^-$ hosts incubated before infection at 42°C for about 40 min (39), but not in *recA*$^-$ or *lexA*$^-$ derivatives of the *tif*$^-$ strain (40). When wild-type strains are UV-irradiated and incubated in growth-supporting medium for 30 min before infection, W-reactivation and W-mutagenesis of UV-irradiated λ are maximal, but neither occurs if chloramphenicol is present during the preinfection incubation (52). Like other *recA*$^+$ *lexA*$^+$-dependent functions, W-reactivation of λ requires protein synthesis after the inducing treatment for its expression.

Further evidence for common regulation of W-reactivation and prophage induction is that both can be induced "indirectly." "Indirect" induction of prophage occurs when an unirradiated F$^-$ ("female") lysogen is mated with a UV-irradiated F$^+$ or F′ ("male") donor strain (18, 19, 56). The UV-damaged DNA of the F episome (an *E. coli* sex factor), transferred by conjugation into a recipient lysogen whose own DNA is undamaged, triggers prophage induction. Indirect induction of prophage is also promoted by transfer of other UV-irradiated replicons, such as colicin I factor (152, 153) or P1 bacteriophage (181). Indirect induction of W-reactivation and W-mutagenesis has been demonstrated by George et al. (77), who mated an unirradiated F$^-$ host with UV-irradiated donors (F′ or Hfr) before infection with UV-irradiated λ phage. Mutagenic reactivation of the phage occurred as efficiently as if the host itself had been irradiated, whether the DNA transferred during conjugation was that of a UV-irradiated sex factor or a fragment of UV-irradiated Hfr chromosome, and even if the DNA transferred was restricted in the recipient. In spite of some puzzling differences (e.g., prophage is indirectly inducible only by transfer of a complete UV-irradiated replicon and not by a fragment of Hfr DNA), the indirect inducibility of both prophage and W-reactivation points to a common induction pathway. Indirect induction of another SOS function (filamentous growth) has also been observed (152; J. Donch, personal communication).

W-reactivation (SOS repair) of UV-irradiated λ phage occurs in both Uvr$^+$ and Uvr$^-$ strains, and is independent of ability to perform excision repair (176). However, the range of UV fluences in which both W-reactivation and λ prophage induction can be expressed is about 10 times lower in Uvr$^-$ strains (53, 155), as shown in Fig. 2. The photoreversibility of prophage induction (115), W-reactivation (220), and other SOS functions indicates that pyrimidine dimer is necessary for their induction. Dimers that

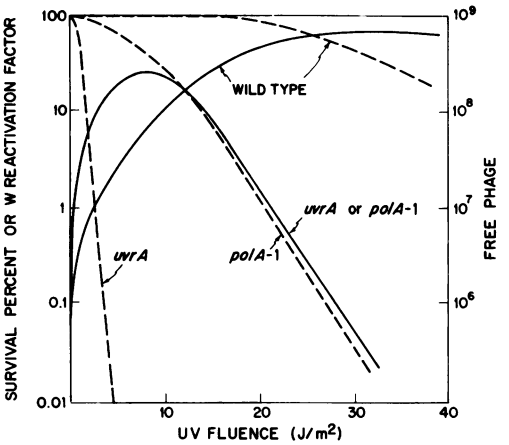

FIG. 2. *Relation between survival and induction of two UV-inducible functions in polA-1, uvrA, and wild-type strains of E. coli. Dashed lines, survival percentage. Solid lines, composite curves for induction of λ prophage (refer to "free phage") and for W-reactivation of UV-irradiated λ (refer to "W-reactivation factor") (53, 70, 155, 240).*

are repaired rapidly by the excision repair system, however, evidently fail to generate an effective SOS induction signal.

Blanco and Devoret (10) and Devoret et al. (55) have ruled out recombinational repair as the mechanism of W-reactivation, and have concluded that it expresses the activity of a previously unrecognized type of dark repair, involving neither excision of DNA damage nor recombinational exchanges, which is either induced or activated in the host by W-reactivating treatments. The occurrence of W-reactivation in single-stranded phages (167a, 209a) implies a nonrecombinational mechanism, barring multiple infection, since only one copy of the phage genome is present even after a replication. Experiments with the single-stranded phage φX174 (M. Radman, P. Caillet-Fauquet, M. Defais, and G. Villani, personal communication) have demonstrated that W-reactivation is accomplished by an inducible DNA replication mechanism, capable of polymerizing DNA past pyrimidine dimers in the template strand. These crucial studies will be described in detail in connection with the mechanism of SOS repair.

SOS Repair of Bacterial DNA

The proposal that bacterial UV mutagenesis and that of UV-irradiated λ phage undergoing W-reactivation may depend upon the same inducible error-prone repair activity (174, 175) was initially based only upon their common requirement for the *recA*$^+$ *lexA*$^+$ genotype. Evidence for inducibility of a function necessary for bacterial UV mutagenesis has come from two kinds of studies: those in which mutant strains exhibiting anomalous induction of prophage and other SOS functions were examined for corresponding anomalies of UV mutability predicted by the SOS hypothesis, and those in which an inducible pathway of postreplication repair has been demonstrated biochemically and linked to UV mutability.

Evidence from studies of repair-deficient mutants. (i) *polA-1* mutants. Mutants of *E. coli* carrying a *polA-1* mutation are deficient in the DNA polymerizing activity of DNA polymerase I (54, 87). The repair replication usually performed by this enzyme can be taken over by DNA polymerase III (192, 209, 244, 247) and to some extent by DNA polymerase II (144). Although *polA-1* mutants excise pyrimidine dimers more or less normally (21), they are slow to complete the patching and sealing of excision gaps (117, 168). The UV sensitivity of *polA-1* mutants is relatively slight (4 to 5 times wild type) compared with that of Uvr$^-$ strains unable to excise pyrimidine dimers (10 to 20 times wild type). Nevertheless, as Fig. 2 shows, excision-proficient *polA-1* strains express at least two UV-inducible SOS functions (prophage induction and W-reactivation) in the same low range of UV fluences as Uvr$^-$ strains. Filamentous growth, another SOS function, is also induced by UV in the same low range of UV fluences in excision-proficient *polA-1* mutants (J. Donch, personal communication). Since *polA-1* strains are slow to complete the repair replication step of excision repair, it appears that a persistently open excision gap is equivalent to an unexcised pyrimidine dimer as a means of initiating an effective SOS induction signal.

Witkin and George (240) suggested that both the UV sensitivity and the UV mutability of *polA-1* strains can be explained by the anomalously low range of UV fluences in which these strains express inducible SOS functions, assuming that all SOS functions are expressed in the same fluence range. The UV survival curve of a Uvr$^+$ *polA-1* strain is superimposable upon the falling portion of induction versus fluence curves for prophage and W-reactivation, as shown in Fig. 2. If SOS repair or any other inducible SOS function is necessary for survival at higher UV fluences, the survival of UV-irradiated *polA-1* populations may be limited by their ability to induce the vital function(s). Survivors, at any UV fluence, would then consist only of the fraction of the *polA-1* population in which SOS functions are expressed. Since an inducible inhibitor of exonuclease V is one of

the SOS functions shown in Table 1, the immediate cause of death in *polA-1* strains may well be double-strand breaks in DNA, produced at lower UV fluences than in wild-type strains (16, 17). However, the increased probability of producing a lethal break may be traced to a more primary cause: the inability of *polA-1* cells to express SOS functions in the same range of UV fluences as wild-type strains.

The UV mutability of *polA-1* strains (231) and of other strains now known to be deficient in the polymerizing activity of DNA polymerase I (125, 127) is the same as that of the wild type, when the frequency of induced mutations per survivor is compared at the same UV fluence. This means that a *polA-1* survivor and a Pol^+ survivor, exposed to the same UV fluence, have the same probability of undergoing mutagenesis, although the surviving fraction is much smaller in the *polA-1* population. Although other interpretations are possible (231), a *polA-1* survivor in which SOS functions are expressed may be no different from an SOS-induced Pol^+ survivor exposed to the same UV fluence in those parameters that determine the probability of undergoing UV mutagenesis. The relevant parameters are: (i) the amount of premutational UV damage per survivor, (ii) the probability that such damage will be repaired by an error-prone mechanism, and (iii) the probability of error per lesion repaired by the error-prone repair system. Unless the surviving fraction in *polA-1* populations is selected by UV radiation as the least damaged fraction, *polA-1* and Pol^+ survivors exposed to the same UV fluence should be alike in these respects. They would be alike, for instance, if successful SOS induction in a *polA-1* survivor depends upon metabolic factors unrelated to the amount of DNA damage a particular cell may have sustained. Cells that start transcription late, for example, may have the best chance of transcribing information for SOS functions from fully repaired templates and surviving.

Since the induction of SOS functions is more effective in *polA-1* strains that in the wild type at UV fluences below about 5 J/m^2 (Fig. 2), Witkin and George postulated that the DNA polymerase I-deficient mutant should be more UV mutable at these very low UV fluences, if UV mutagenesis depends upon inducible SOS repair. Significantly elevated UV mutability at sublethal UV fluences was demonstrated in *polA-1* strains, as were other properties predicted by the SOS hypothesis (235, 240).

(ii) *tif-1* mutants. Studies of *tif-1* mutants, in which SOS functions are thermally inducible without insult to DNA, have provided the strongest evidence that an inducible SOS function is required for bacterial UV mutagenesis (234, 238). Induction of the postulated SOS repair activity by incubation at 42°C should enhance the UV mutability of a tif^- mutant, when compared with a largely uninduced population containing the same amount of potentially mutagenic UV damage. UV damage is obviously necessary in an assay for enhanced UV mutagenesis, and therefore the tif^- bacteria and tif^+ controls must be UV irradiated either before or after the incubation at 42°C. Since UV radiation itself is an efficient inducer of SOS functions, thermally induced SOS repair activity should be expected to enhance UV mutagenesis only at extremely low fluences, well below those required for mass UV induction of prophage or W-reactivation (Fig. 2). In Uvr^- strains, UV fluences below about 0.5 J/m^2 induce prophage or W-reactivation in fewer than 1% of the irradiated population, yet about five to six pyrimidine dimers per genome are produced in *E. coli* per 0.1 J/m^2. Assuming that all SOS functions are induced at a given UV fluence to the same extent, exposure of tif^- bacteria to UV fluences below about 0.5 J/m^2 (Uvr^-) or 5 J/m^2 (Uvr^+) should introduce some potentially mutagenic UV photoproducts into the DNA of almost every cell, while inducing SOS functions (including the postulated SOS repair activity) in an extremely small fraction of the population.

A 10-fold enhancement of the yield of UV-induced Trp^+ mutations was obtained in a tryptophane-requiring $uvrA^-$ *tif-1* derivative of *E. coli* B/r (strain $WP44_s$) when UV irradiation with about 0.1 J/m^2 was followed by a 45-min incubation at 42°C, compared with the yield obtained from similarly treated tif^+ controls or from tif^- cells maintained after UV irradiation at 30°C (234). The magnitude of the enhancement was later increased to a maximum of about 50-fold at the lowest UV fluence used (238). Typical results are shown in Fig. 3. The amount of enhancement of UV mutagenesis provided by thermal post-treatment in the tif^- strain diminishes as the UV fluence increases, and no significant enhancement is observed at UV fluences above about 3 J/m^2. UV induction of λ lysogens and of W-reactivation in Uvr^- strains approaches 100% at about the same fluence (Fig. 2). Once the UV treatment itself is sufficient to induce SOS functions in every cell, thermal post-treatment should no longer enhance the yield of UV-induced mutations. These results are in good agreement with the expectation that postirradiation incubation at 42°C (which should induce SOS functions in every tif^- cell) should enhance UV mutagenesis at UV fluences too low to cause mass UV-induc-

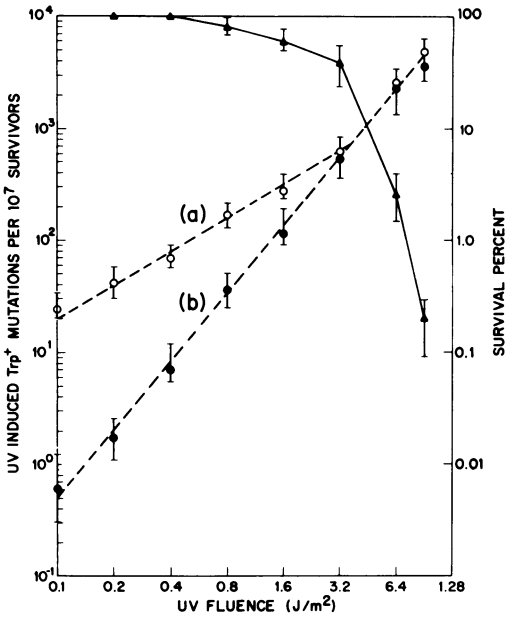

FIG. 3. *Effect of postirradiation temperature elevation on the UV mutability of strains WP2$_s$ (tif$^+$) and WP44$_s$-NF (tif-1 Sfi$^-$). Symbols;* ▲, *survival percentage (average for both strains, whether or not thermal post-treatment was given);* ●, *frequency of UV-induced Trp$^+$ mutations (average of data for tif$^+$ strain, whether or not thermal post-treatment was given, and for tif-1 strain not given thermal post-treatment);* ○, *frequency of UV-induced Trp$^+$ mutations (average of data for tif-1 strain given thermal post-treatment). (a) - - -, theoretical "one-hit" curve (slope = 1); (b) - - -, theoretical "two-hit" curve (slope = 2). Thermal post-treatment consisted of incubation for 60 min at 42°C immediately after UV irradiation and plating on 5% SEM agar. All plates were incubated at 30°C for 2 days (survival) or 3 days (mutations) starting either immediately after thermal post-treatment (if given) or immediately after plating, if thermal post-treatment was not given. Points are averages of data from four experiments. Trp$^+$ mutation frequencies shown have been corrected for spontaneous mutations by subtracting frequencies obtained from similarly treated unirradiated controls. Titers of log cultures used were 1 × 10^8 to 3 × 10^8 cells per ml. For detailed description of strains, media and methods used, see references 234 and 238.*

tion, by permitting error-prone repair of UV lesions that could otherwise not cause mutations. In uninduced cells, such lesions presumably either remain unrepaired or are repaired by error-proof mechanisms. The significance of the slopes of the curves in Fig. 3 will be considered later.

Not only was UV mutability enhanced in the *tif*$^-$ mutant by thermal post-treatment, but other predictions generated by the SOS hypothesis were fulfilled as well. Thermal enhancement of UV mutability was abolished by the presence of chloramphenicol during the heat treatment, indicating a requirement for new protein synthesis after the inducing treatment. Additives that had been shown to promote or prevent thermal induction of prophage in *tif*$^-$ lysogens (80, 123) had parallel effects on thermal enhancement of UV mutability: the enhancement was abolished by pantoyl lactone or by a mixture of cytidine and guanosine and was promoted even at 30°C by adenine. Although the basis of these effects is not understood, they provide strong evidence for common regulation of prophage and an inducible SOS function required for bacterial UV mutagenesis.

(iii) *dnaB*^{ts} mutants. Temperature-sensitive *dnaB* mutants of *E. coli*, which stop synthesizing DNA instantly at 42°C (219), also induce prophage at elevated temperatures when lysogenic (166, 188). The SOS hypothesis predicts that all SOS functions should be thermally inducible in *dnaB*^{ts} strains, including SOS repair. Enhancement of UV mutagenesis at very low fluences, virtually identical with that described for *tif*$^-$ mutants, has been demonstrated in *dnaB-43* derivatives of *E. coli* B/r (237). Although *tif*$^-$ and *dnaB*^{ts} strains are very similar in their expression of SOS functions at 42°C, the reasons for their thermal inducibility are quite different. In *dnaB*^{ts} mutants, induction is triggered by interrupted DNA replication, whereas heat treatment of *tif*$^-$ mutants induces SOS functions without damaging the DNA or arresting its synthesis.

(iv) Other mutant strains. The hyperinducibility of *polA-1* strains for SOS functions at very low UV fluences suggests that persistently open gaps in DNA may generate effective SOS signals, whereas gaps that are rapidly closed do not. Significantly elevated UV mutability at extremely low fluences (possibly indicative of hyperinducibility for SOS repair activity) has been observed in *mfd* mutants, which close excision gaps very slowly (73) and are also somewhat hyperinducible at low UV fluences for prophage and W-reactivation (74). Elevated UV mutability in the extremely low UV fluence range has been found as well in *recF* mutants (E. Witkin, unpublished observation), which are deficient in postreplication repair (72), and in *UVrA polA-1* mutants (235), in which postreplication repair may be slow. All of these observations support the hypothesis that an inducible function is required for bacterial UV mutability, its induction coordinated with that of prophage and other SOS functions. In addi-

tion, the hyperinducibility of strains in which gaps in DNA are unusually persistent implicates such gaps as possible sources of the SOS induction signal.

Evidence from studies of postreplication repair. A correlation between UV mutagenesis in *E. coli* and an inducible *lexA*$^+$-dependent pathway of postreplication repair has been demonstrated by Sedgwick (189), who showed that chloramphenicol, added just before UV irradiation of strain $WP2_s$ (a *uvrA*$^-$ *trp*$^-$ derivative of B/r), prevents both the irreversible "fixation" of UV-induced Trp$^+$ mutations against enzymatic photoreversal and the occurrence of a small fraction of postreplication repair. Inhibition of protein synthesis after UV irradiation thus causes a small number of daughter-strand gaps to remain open, and also blocks the occurrence of UV mutagenesis. Chloramphenicol does not prevent the completion of daughter-strand joining, however, in irradiated bacteria which have been allowed a 20-min period of growth before addition of the antibiotic, or in *uvrA*$^-$ *tif*$^-$ bacteria which have been incubated for 70 min at 42°C before UV irradiation. No chloramphenicol-sensitive pathway of postreplication repair was detected in UV-nonmutable *uvrA*$^-$ *lexA*$^-$ bacteria. These observations establish that UV mutagenesis is associated with the occurrence of a minor *lexA*$^+$-dependent pathway of postreplication repair that is UV inducible, or requires a UV-inducible component. Sedgwick's study also shows that the major pathway of postreplication repair in *Uvr*$^-$ *E. coli*, which is known to be recombinational (186), is constitutive, *lexA*$^+$-independent and error-free, at least in the presence of chloramphenicol. The existence of a *lexA*$^+$-dependent chloramphenicol-sensitive pathway of postreplication repair has also been demonstrated by Youngs and Smith (247).

MANIFESTATIONS OF SOS REPAIR AND THEIR SIGNIFICANCE

Mutation Frequency Response to Increasing Fluence of UV Radiation

At low fluences of UV radiation, UV-induced mutations in bacteria usually increase as the square of the UV fluence, as in the lower curve in Fig. 3. This "fluence-squared" (F^2) relation implies that two independent photon absorptions (UV "hits") are required to induce a mutation. The nature of the two "hits" has been a source of speculation for nearly two decades (25, 33, 61, 64, 148, 224, 240, 249).

Since most UV-induced mutations are photoreversible, it is generally assumed that at least one of the UV photoproducts required for mutagenesis is a pyrimidine dimer and that at least one must be located in the gene in which the particular mutation scored occurs. Two hypotheses have been argued most recently. One is the "two-lesion" hypothesis, according to which a UV-induced mutation requires the interaction of two closely spaced UV photoproducts near the site of the mutation (25, 64, 148). The other is the "one lesion + SOS induction" hypothesis (61, 240), which proposes that only one UV photoproduct is a premutational lesion in the gene scored, whereas the other is an SOS-inducing "hit," required for induction of SOS repair activity. The *tif*$^-$ mutant, in which SOS functions are thermally inducible, seemed an ideal instrument for determining which of the two hypotheses is correct. The "one-lesion + SOS induction" hypothesis predicts that *tif*$^-$ bacteria, UV-irradiated and then given a thermal post-treatment, will not require an SOS-inducing "hit," but will show a linear increase of mutation frequency as the UV fluence increases, indicative of the accumulation of premutational UV photoproducts caused by the absorption of single photons. The frequency of thermally enhanced yields of UV-induced Trp$^+$ mutations rises with an unmistakably linear slope, whereas controls show the typical F^2 response, in both *tif*$^-$ (233, 234) and *dnaB*^{ts} (237) strains, as can be seen for a *tif*$^-$ strain in Fig. 3. This result seemed to provide unambiguous support for the idea that UV mutagenesis requires an SOS-inducing "hit" plus a premutational photoproduct, the latter remaining cryptic in uninduced cells. However, if all UV mutagenesis involves SOS repair of damage caused by single UV photoproducts, mutation frequencies in normal strains should begin to rise linearly with increasing UV fluence once the level of UV radiation required for mass SOS induction is reached. The mass-induction fluence for both λ prophage induction and W-reactivation is approached at about 3 J/m^2 in Uvr$^-$ strains (Fig. 2), the same level at which thermal post-treatments no longer enhance UV mutagenesis in *tif*$^-$ (Fig. 3) and *dnaB*^{ts} (237) populations. However, as Fig. 3 shows, the yield of UV-induced mutations continues to rise approximately as the *square* of the UV fluence, in both *tif*$^-$ and *tif*$^+$ strains, thermally post-treated or not, up to UV fluences as high as 9 J/m^2. Bridges et al. (33) have also reported mutation frequency response curves that continue their F^2 slope well above the mass-induction fluence level. The data shown in Fig. 3 seem to support the "one-lesion + SOS induction" hypothesis at low UV fluences and the "two-lesion" hypothesis at higher UV fluences. Both

Witkin (238) and Doudney (63) have recently concluded that some UV-induced mutations in *E. coli* are caused by single UV photoproducts, whereas others are caused by the interaction of two closely spaced UV lesions, and that neither hypothesis is wholly correct nor wholly incorrect.

Witkin (238) has distinguished between single premutational UV lesions that require error-prone SOS repair but are not, in themselves, sufficient signals for the induction of SOS repair activity ("SOS-mutable, SOS-noninducing" lesions), and UV lesions generated by the interaction of two UV photoproducts, which are not only targets for SOS repair activity but are also effective inducers of SOS repair ("SOS-mutable, SOS-inducing" lesions). The distinction between these two types of premutational lesions was based on the kinetics of thermal enhancement of UV mutagenesis at 42°C and the kinetics of decay at 30°C of susceptibility to such enhancement in *tif*$^-$ strains. It was concluded that in uninduced cells SOS-mutable, SOS-noninducing lesions are cryptic, but contribute to overt UV mutagenesis if SOS activity is induced by some other means, such as temperature elevation in *tif*$^-$ mutants or exposure to UV fluences high enough for mass UV induction of SOS repair. Unless such cryptic premutational lesions are present in UV-irradiated but uninduced cells, thermal post-treatments could not enhance UV mutagenesis in *tif*$^-$ or *dnaB*^{ts} strains. Unless the cryptic lesions are due to single UV photoproducts, the thermally enhanced yield should not rise linearly with increasing UV fluence. However, unless one assumes that some UV-induced mutations are caused by two UV photoproducts, the F^2 accumulation of induced mutations at fluences far above the mass-induction level (33, 63; Fig. 3) would be hard to explain. Variation in the proportions of the two kinds of premutational lesions might be expected in different genes, or even in the same gene under different conditions, and could account for the complexity and variability characteristic of mutation frequency response curves (63).

The UV Lesion Responsible for Induction of SOS Functions

The composite induction curves for prophage induction and W-reactivation shown in Fig. 2 show a steep rise in the low UV fluence range similar to the increase in frequency of UV-induced mutations with the square of the UV fluence in the same range. When measured carefully, prophage induction can be seen to increase strictly as the square of the UV fluence in both Uvr$^+$ (141) and Uvr$^-$ (E. Witkin, unpublished data) *E. coli* lysogens, in striking contrast to the linear increase of induced lysogens with increasing exposure to X rays (140). If all SOS functions are assumed to respond to a common induction signal, UV radiation clearly requires two photon absorptions to generate an effective induction signal.

Sedgwick (189, 190a) has suggested that a target for SOS repair (and, by implication, an SOS-inducing lesion) may be a daughter-strand gap that partially overlaps another daughter-strand gap in the sister DNA molecule. Such a pair of overlapping daughter-strand gaps should be refractory to any type of recombinational repair, in principle, and could not be patched by the repair replication activity of the known constitutive DNA polymerases, because of the pyrimidine dimer or other noncoding UV photoproduct located opposite each of the gaps. This type of structure (Fig. 4A) would be formed when two pyrimidine dimers on opposite strands of the parent molecule are close enough so that the daughter-strand gaps produced by them overlap, either as they are originally formed or after some DNA degradation. In Uvr$^-$ strains, such gap structures could account entirely for the F^2 relation between expression of SOS functions and UV fluence in the low fluence range. Mass SOS induction, on this basis, should occur at the fluence at which every irradiated cell, on the average, will contain one pair of overlapping daughter-strand gaps after DNA replication.

In Uvr$^+$ strains, unexcised UV photoproducts could also generate overlapping daughter-strand gaps that would be refractory to constitutive repair systems, as in Uvr$^-$ strains. However, Uvr$^+$ strains could, in addition, form the kind of gap structure shown in Fig. 4B, which would also require the production of two excisable UV photoproducts close to each other on opposite strands of the irradiated DNA molecule. Excision of one of the lesions, perhaps with some DNA degradation enlarging the excision gap, could place the second lesion opposite the excision gap, thus preventing patching by DNA polymerase I. Since correndonuclease II requires a double-stranded substrate for incision near a pyrimidine dimer, the second lesion would remain unexcised at least until the first excision gap is somehow filled and sealed. Such structures, if produced in a part of the DNA replicated before UV irradiation, might be repairable by recombination, but should be refractory to any known type of constitutive repair if produced in an unreplicated part of the chromosome. Bresler (23) has suggested that

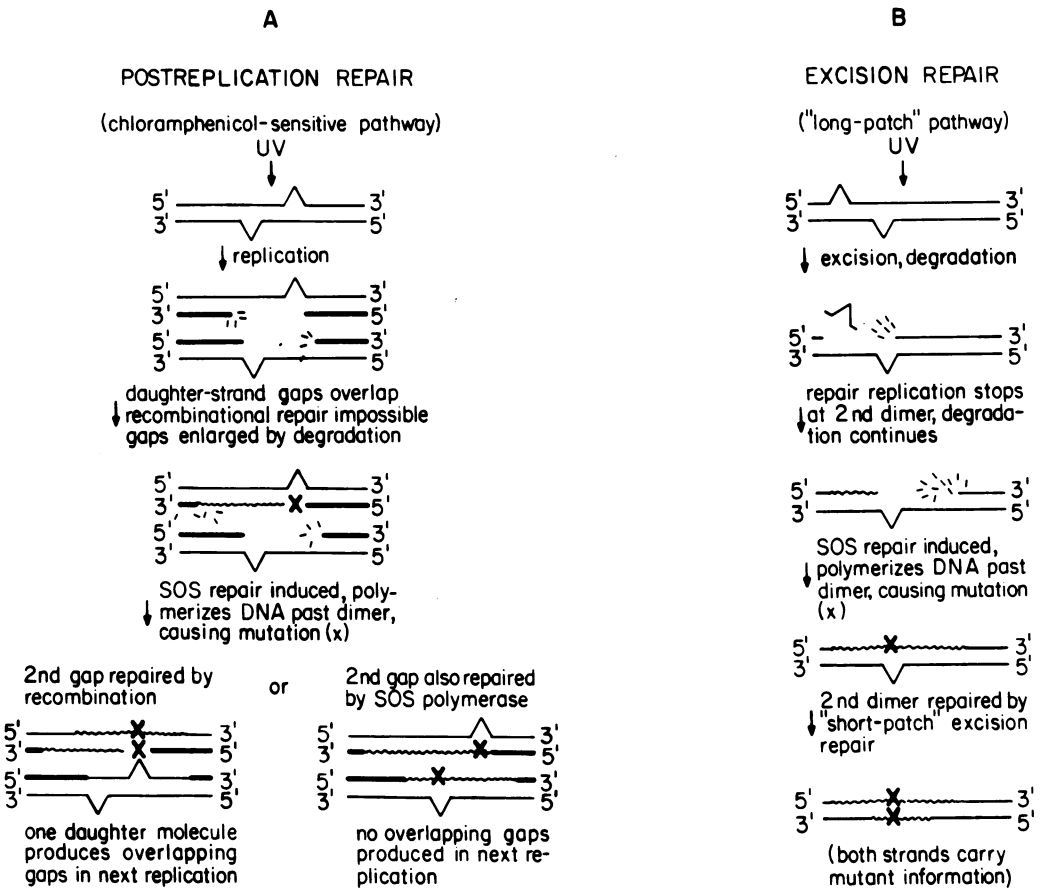

FIG. 4. *Possible pathways of error-prone repair (SOS repair) of damage to DNA caused by UV radiation in E. coli. Symbols: —^— or —v—, pyrimidine dimer or other noninstructive UV photoproduct; heavy lines, daughter strands produced during first postirradiation DNA replication; light lines, parental (UV-irradiated) strands of DNA; wavy lines, DNA polymerized during repair replication; X = mutation ("wrong" base sequence). SOS repair activity is assumed to permit replication past pyrimidine dimers or other noninstructive UV photoproducts with a high probability of error (see section of text under heading, Mechanism of SOS Repair).*

this type of structure could cause UV mutagenesis by promoting replication errors during *recA*$^+$-dependent excision gap resynthesis, but he does not believe that error-prone repair activity is inducible. Polymerization past a pyrimidine dimer, with or without errors, is not within the capacity of any of the three known constitutive *E. coli* DNA polymerases (7, 128; P. Caillet-Fauquet, M. Defais, and M. Radman, personal communication).

Excision gaps opposite a dimer (Fig. 4B) or overlapping daughter-strand gaps (Fig. 4A) would seem to confront *E. coli* with types of damage not repairable by constitutive repair systems, and therefore lethal in strains not able to induce a type of repair activity capable of closing such gaps. Unless and until such activity was induced, these otherwise nonrepairable gaps would tend to persist. Several strains in which repair defects cause unusually slow closure of gaps in DNA (*polA-1*, *recF*, *mfd*) have been found to be hyperinducible for at least some SOS functions (see above). It is a reasonable possibility that an SOS signal may be initiated by either of the types of gaps shown in Fig. 4, although there is no direct evidence for this idea. It should be emphasized that the probability that two UV photoproducts would generate such a gap structure would depend not only upon the distance between them, but also on the extent of DNA degradation occurring after UV irradiation.

Several otherwise puzzling features of UV mutagenesis can be explained if it is assumed that the two kinds of gaps shown in Fig. 4 are the primary targets for SOS repair activity in *E. coli*. Although all UV-induced mutations examined in Uvr$^-$ strains become irreversibly "fixed" against initially antimutagenic post-treatments such as PRL (164) or caffeine (239) coincidentally with DNA replication, some UV-induced mutations in Uvr$^+$ strains lose their photoreversibility as dimer excision is completed, well before DNA replication, indicating that some mutations in Uvr$^+$ strains originate as rare errors in the repair of excision gaps before DNA replication (30, 164, 165). The kind of excision gap shown in Fig. 4B could provide a site requiring *prereplication* SOS repair in Uvr$^+$ strains. Since induction of SOS repair requires considerable time (see below), gaps requiring this repair should persist as targets for exonuclease V degradation and could become considerably enlarged by the time the inducible repair system is available. This could account for the "long patch" character of the *recA*$^+$ *lexA*$^+$-dependent (i.e., inducible) pathway of excision repair, which may well be the same SOS repair activity that also repairs overlapping daughter-strand gaps. As Bresler (23) has pointed out, subsequent excision of the second UV photoproduct, once the mutagenic event has closed the first excision gap, would lead to a "pure clone" of the mutant type, at least in cells containing only one surviving copy of the genome.

Sedgwick's overlapping daughter-strand gap hypothesis (190a) could also explain why detectable UV-induced mutations are produced primarily and probably exclusively in the first postirradiation cell division (60, 230, 238), although pyrimidine dimers persist and are exchanged into daughter strands in Uvr$^-$ strains (71). Closely spaced UV photoproducts that produce overlapping daughter-strand gaps in the first postirradiation cell division would either fail to do so at all in subsequent replications, or would do so with a reduced probability, depending upon the mechanism of SOS repair (e.g., if both gaps are repaired by error-prone polymerization, the photoproducts would remain in the parental strands and no overlapping daughter-strand gaps would be produced after the first replication). Even if mutagenic gap structures are generated after the first DNA replication, the more rapid proliferation of DNA molecules not containing such damage might prevent the expression of potential second-round mutations, since most methods used to detect induced mutants limit postirradiation growth in one way or another (3).

Kinetics of Induction and Decay of SOS Repair Activity

The kinetics of induction and decay of inducible error-prone repair activity have been determined in two very different systems with remarkably similar results.

Witkin (238) investigated thermally induced SOS repair activity in *tif*$^-$ B/r derivatives that had been rendered viable at 42°C by selection for a secondary mutation (Sfi$^-$) that suppresses thermally inducible filamentous growth without altering other thermally inducible *tif*-mediated functions (75). Kinetics of induction of SOS repair activity were assayed in both Uvr$^+$ and Uvr$^-$ *tif*$^-$ Sfi$^-$ derivatives by determining the degree of enhancement of UV mutability obtained, after exposure to a low fluence of UV radiation, with increasing periods of incubation at 42°C (0 to 30 h) before completing incubation at 30°C. No significant thermal enhancement was obtained if the incubation at 42°C was shorter than 30 min. Optimal enhancement was obtained with incubations at 42°C for 45 min after UV irradiation or longer. The ability to enhance UV mutagenesis induced by a 60-min incubation at 42°C was found to decay at 30°C (the noninducing temperature) to about half its optimal level in 30 min, and was no longer detectable after 90 min at 30°C. Kinetics of induction at 42°C and of decay at 30°C of thermally induced ability to enhance UV mutability (i.e., of thermally induced SOS repair ability) were virtually identical in Uvr$^+$ and Uvr$^-$ *tif*$^-$ Sfi$^-$ strains. The time required for induction of SOS repair is consistent with the observation (69) that no error-prone gap-filling occurs during the first 20 min after UV irradiation.

Defais et al. (52) determined the kinetics of induction and decay of UV-inducible capacity to W-reactivate UV-irradiated λ phage. Irradiated λ were reactivated maximally when infection was delayed for 30 min after UV irradiation of the host cells. The UV-induced capacity for W-reactivation decayed with a half-life of 30 min once the optimal level was achieved. Although there are some differences, not surprising in view of the radically different inducing treatments and assay systems used, the two sets of kinetics obtained by Witkin and by Defais et al. are similar enough to encourage the hypothesis that the same inducible error-prone repair activity effects bacterial UV mutagenesis and W-reactivation of UV-irradiated λ.

Time of Action of SOS Repair in UV Mutagenesis

Two recent studies have attempted to determine just when UV-induced mutations are es-

tablished by the action of error-prone repair. Doubleday et al. (60) have used the kinetics of loss of photoreversibility (LOP) as a measure of irreversible commitment to UV-induced mutation caused by pyrimidine dimers in *E. coli* $WP2_s$ (*uvrA*$^-$ *trp*$^-$). Irradiated bacteria, incubated for two generations in each of three different media before plating on medium selective for Trp$^+$ mutants, produced the same number of induced mutations at the same UV fluence, but lost photoreversibility of the induced mutations at very different rates. In rich medium, photoreversibility was lost before the population had completed one cell division; in minimal medium plus tryptophane, some photoreversibility of the UV-induced Trp$^+$ mutations was still present after an average of two cell divisions; and in rich medium plus pantoyl lactone, LOP kinetics were extremely slow. UV fluence had no effect on LOP kinetics, but only on the final yield of induced mutations. The authors considered their results compatible with either of two interpretations: (i) that error-prone repair may occur after one or more rounds of DNA replication in which daughter-strand gaps are repaired by an error-free mechanism, or (ii) that error-prone repair occurs only in the first round of postreplication repair at daughter-strand gaps, which are refractory to error-free postreplication repair, perhaps because they form part of an overlapping gap structure such as that proposed by Sedgwick as the target for SOS repair activity. In that case, the premutational gaps would remain open until the error-prone system is induced and/or completes its action. According to the latter interpretation, the different rates of LOP reflect medium effects on the rate of induction and/or on the rate of action of the error-prone repair system. Overall population increase, then, need not reflect the specifically delayed division of potential mutants, and all detectable UV mutagenesis may actually occur only during the first round of postreplication repair. This study also provided clear evidence, based on segregational analysis, that most or all UV mutagenesis is "terminal," occurring only once at a given site within a clone.

Witkin (238) used *tif*$^-$ Sfi$^-$ strains (both Uvr$^-$ and Uvr$^+$) to analyze the timing of thermally induced error-prone repair activity, confirming the conclusion of Doubleday et al. that UV mutagenesis is "terminal." These experiments also showed that thermally enhanced yields of UV-induced Trp$^+$ mutations are produced primarily (possibly exclusively) before the completion of the first round of postirradiation cell division, when SOS activity is present (at 42°C). In populations consisting almost entirely of cells not containing SOS repair activity (because they were exposed to an extremely low fluence of UV radiation and were incubated afterwards at 30°C), the capacity to produce an optimally enhanced yield of UV-induced Trp$^+$ mutations in response to a delayed thermal post-treatment was fully maintained at 30°C for at least 2 to 3 h.

Photoreversibility of the enhanced yield of UV-induced mutations, rapidly lost when incubation after UV irradiation is at 42°C, was also fully maintained for at least 2 h at 30°C. It was concluded that sites for SOS repair activity are stable in uninduced cells. If such sites are daughter-strand gaps, they must be refractory to constitutive error-proof recombinational repair, remaining open and unrepaired for at least 2 to 3 h in the absence of SOS repair activity. Since the thermally enhanced yields of UV-induced mutations increase linearly with UV fluence, however, the premutational sites must be gaps produced by *single* UV photoproducts, which, in spite of being refractory to constitutive postreplication repair, are unable to generate an SOS repair-inducing signal. These are the "SOS-mutable, SOS-noninducing" lesions discussed above in connection with mutation frequency response curves.

If the target of SOS repair activity is a gap in DNA, the "SOS-mutable, SOS-noninducing" lesion responsible for thermally enhanced yields of Trp$^+$ mutations must be a single daughter-strand gap, not overlapping another in the sister molecule of DNA. In principle, nonoverlapping gaps should be susceptible to constitutive recombinational repair, which Sedgwick has found to occur efficiently at 30°C in the same *tif*$^-$ Sfi$^-$ strain (189). The refractoriness of this particular group of lesions to constitutive postreplication repair may be related to unique properties of transfer ribonucleic acid-coding genes, in which most of the Trp$^+$ mutations induced by UV in these strains (primarily ochre suppressors) probably occur. UV-induced suppressor mutations are uniquely refractory to excision repair when incubation after UV irradiation is in rich medium, a phenomenon related to "mutation frequency decline" (12, 230, 242). The same specific features that make some transfer ribonucleic acid-coding genes less susceptible to excision repair than other genes under certain conditions may also interfere with recombinational postreplication repair. For instance, possible "cloverleafing" of the nontranscribed strand during transcription could place the premutational UV photoproduct in a configuration inaccessible to repair enzymes. Another possibility is that UV photoproducts causing this type of suppressor muta-

tion have an unusual primary structure, as indicated by the action spectrum for their induction, which is quite different from the action spectra for the induction of other mutations or for the production of pyrimidine dimers (194).

Cryptic Premutational Lesions Susceptible to SOS Repair

The existence of SOS-mutable lesions incapable of inducing error-prone repair activity has some important implications. Although such lesions are cryptic in uninduced cells, they are capable of undergoing mutagenesis in cells that also receive an effective induction signal. A case in point is the fluorescent light used in my laboratory as a source of PRL. Visible light is known to be mutagenic (218), but the exposures required to demonstrate this are usually much longer than the times used in photoreversal experiments. However, as Fig. 5 shows, the same exposure to PRL is far more mutagenic in *tif*$^-$ cells given a 60-min incubation at 42°C immediately after illumination than in controls incubated at 30°C. This implies that PRL may produce SOS-mutable, SOS-noninducing lesions in DNA which are cryptic in uninduced cells, but contribute to overt mutagenesis in SOS-induced cells. Recognition that some sources of visible light may produce such lesions can provide an explanation for some otherwise enigmatic effects in experiments such as those (33, 148) in which UV irradiation is followed by exposure to PRL and then by a second UV irradiation. In both studies, pretreatment with UV + PRL enhanced the mutagenic effect of the second UV exposure and this effect was attributed to nonphotoreversible UV photoproducts, still present in the DNA after the PRL treatment. Actually, SOS-mutable, SOS-noninducing lesions produced by the PRL treatment itself may have caused or contributed to the enhanced mutagenesis. SOS repair activity, induced by the second UV exposure, could have potentiated the otherwise cryptic mutagenic effect of the PRL.

The production by PRL of SOS-mutable, SOS-noninducing photoproducts in DNA requires caution in the interpretation of photoreversal experiments, especially those determining kinetics of LOP. When PRL is given immediately after UV irradiation, dimers are split before SOS repair activity can be induced, and potentially mutagenic DNA lesions produced by the PRL treatment, which do not trigger SOS induction, remain cryptic. However, when the PRL treatment is delayed for about an hour after UV irradiation, UV-induced SOS repair activity is fully expressed by that time, and PRL treatment could contribute significantly to the mutations scored as nonphotoreversible UV-induced mutations. A suitable control would be equivalent PRL treatment of a heated but unirradiated *tif*$^-$ population, which would permit enumeration of the PRL-induced contribution to the "nonphotoreversible" fraction of mutations. Various sources of PRL may differ in the efficiency with which they produce SOS-mutable, SOS-noninducing photoproducts. The mutagenic effect in heated *tif*$^-$ cells of the PRL source used in my laboratory was reduced to about 50% by a filter that eliminated wave lengths below 350 nm.

Heated *tif*$^-$ populations may be useful in screening tests for environmental mutagens, some of which, like fluorescent light, may be

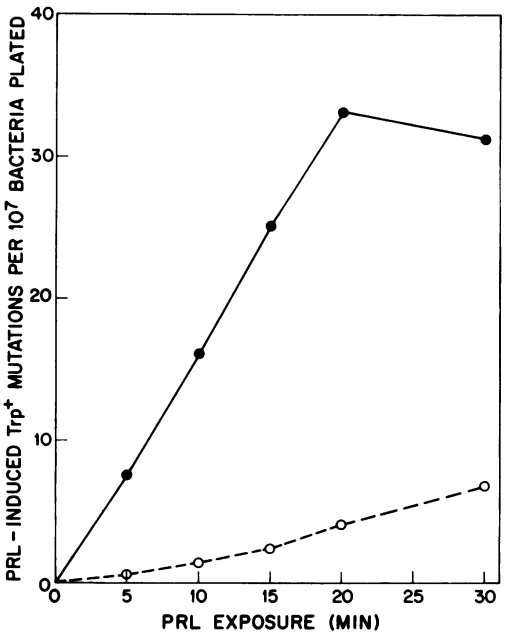

FIG. 5. *Mutagenic effect of visible light (PRL) in strain WP44$_s$-NF (tif-1 Sfi$^-$). Aliquots (1 × 10^7 to 3 × 10^7 cells) from log culture were plated on 5% SEM agar, exposed to PRL 0 to 30 min, and then incubated at 42°C for 60 min (●) or not (○). All plates were incubated for 3 days at 30°C starting either immediately after post-treatment (if given) or immediately after illumination (if no thermal post-treatment was given). Source of PRL was a pair of Westinghouse "cool white" fluorescent bulbs. Trp$^+$ mutation frequencies shown have been corrected for spontaneous mutations by subtracting frequencies obtained in similarly treated "dark" controls. Survival (monitored by plate-wash assays) did not depart significantly from 100% under any of the conditions shown. (For detailed description of strain, methods, and media used, see references 234, 238, and 240.) No significant effect of thermal post-treatment was obtained in similar experiments using the tif$^+$ strain WP2$_s$ (not shown).*

far more mutagenic in combination with an efficient SOS-inducing agent that could be suspected from their nonmutagenic or weakly mutagenic response in the usual test systems.

It should be emphasized that photoreversibility of UV-induced mutations gives no information about the nature of the premutational photoproduct. Pyrimidine dimers may be required for the induction of SOS repair activity, but once induced, the error-prone repair system may cause mutations during repair of nondimer damage as well. In Uvr^+ strains, the splitting of pyrimidine dimers may also reduce UV mutagenesis indirectly by promoting subsequent excision of nondimer UV photoproducts that would otherwise compete poorly with dimers for excision enzymes.

Mutator Effect of SOS Repair on Undamaged DNA

An unexpected byproduct of studies of *tif-1* derivatives of B/r was that incubation of untreated control populations under SOS-inducing conditions (i.e., at 42°C for 45 min or longer) resulted in a significant increase in spontaneous mutability, estimated as about 60 times the normal rate of mutation from Trp^- to Trp^+, indicating that the *tif-1* mutation is a mutator at 42°C (233, 234, 238). Mutator activity has been described also in K-12 *tif-1* strains (75). The elevated spontaneous mutability in unirradiated controls does not obscure thermal enhancement of UV mutability, since thermal post-treatments required to demonstrate such enhancement are brief (45 to 60 min), and the heated populations are small enough (2×10^7 to 3×10^7 cells per plate), so that the actual number of mutant colonies on heated but unirradiated control plates is small compared with the number on platings of UV-irradiated populations, and is subtracted before plotting UV-induced yields as in Fig. 3. Prolonged incubation at 42°C of unirradiated *tif-1* cells, however, reveals the mutator effect much more dramatically.

When SOS repair activity is induced by UV radiation, the mutations caused by the radiation should include some that are not due to error-prone repair of UV damage, but to the mutator activity of the SOS repair system in undamaged portions of the DNA. The magnitude of the mutator effect in thermally post-treated tif^- populations (238) is such that the fraction of UV-induced mutations caused by the SOS mutator activity does not exceed 10% under the conditions usually used, at least with respect to the Trp^- to Trp^+ mutations scored. This maximum estimate includes mutations that may be caused by SOS mutator activity during repair replication steps of the otherwise error-proof repair pathways (e.g., Fig. 1B and C) when these pathways operate in SOS-induced cells.

A mutator effect of thermally induced SOS repair activity has also been reported in a lig^{ts} mutant in which DNA ligase is inactive at high temperatures (157). Since prophage is thermoinducible in lig^{ts} lysogens (81), SOS repair activity should be expressed at 42°C as well. However, the data in the paper cited are not sufficient to establish that bacterial mutability is increased by heat treatment of this mutant. The increase reported is an apparent elevation of the frequency of mutants among survivors of more or less lethal heat treatments. Under conditions promoting filamentous growth, as heat treatment of lig^{ts} is known to do (72a), seemingly large mutagenic effects can often be traced to a methodological artifact (3, 227) which, if recognized, can be satisfactorily controlled (see Appendix).

If SOS repair activity is mutagenic in cells containing no structural damage in their DNA, one might expect that all agents capable of inducing prophage and other SOS functions should be at least somewhat mutagenic. In fact, a high correlation exists in *E. coli* between the mutagenicity of various treatments and their ability to induce prophage (127). SOS-inducing agents should be divisible into two distinct classes with respect to their mutagenic potency: potent mutagens, like UV radiation, which not only induce SOS repair activity but also produce numerous sites in the DNA requiring its error-prone action, and weak mutagens, which induce SOS repair activity by arresting DNA replication but do not otherwise damage the genetic material. SOS-inducing agents of the latter type should exert only the relatively weak mutagenic effect seen in $dnaB^{ts}$ unirradiated populations incubated at 42°C for 1 h, and then returned to the permissive temperature (237). Thymine starvation, nalidixic acid inhibition, and temperature elevation in lig^{ts} mutants should act as similarly weak mutagens, since these treatments arrest DNA replication but probably do not introduce any structural damage requiring SOS repair activity, except perhaps at the replication fork(s). Since SOS activity decays with a half-life of about 30 min, the mutator effect should operate exclusively or at least primarily during the first round of DNA replication after termination of these inhibitory treatments. Valid demonstrations of thymineless mutagenesis (those in which an absolute increase in the number of mutant colonies is obtained after starvation treatments causing little or no thymineless

death) usually show the expected relatively weak effect (e.g., references 29, 116). Extremely high mutation yields, calculated from data obtained after long periods of starvation which cause much thymineless death, have been reported, but in these cases the frequency of mutants in thymine-starved populations may have been grossly overestimated (see Appendix). Sublethal starvation for thymine in strain $WP2_s$ *thyA*$^-$, a derivative of *E. coli* B/r, causes a weak but genuine mutagenic effect, which is absent in its *lexA*$^-$ and *recA*$^-$ derivatives, and enhances UV mutability at low fluences as effectively as does thermal post-treatment of *tif*$^-$ or *dnaB*^{ts} strains (E. Witkin, manuscript in preparation). These observations confirm the conclusion (29) that UV radiation and thymine starvation share a common pathway of mutagenesis. Similar weak mutagenesis and strong enhancement of low-fluence UV mutagenesis have been observed after sublethal treatment with nalidixic acid (E. Witkin, unpublished data). Both thymine starvation and nalidixic acid treatment, as well as ionizing radiation, which is also virtually nonmutagenic in *lexA*$^-$ strains (29), appear to be effective inducers of SOS repair activity. The unusual kind of thymineless mutagenesis described by Bresler et al. (24), however, which operates only under special conditions and is independent of *lexA*$^+$ and *recA*$^+$ gene products, is clearly not due to inducible SOS repair activity.

Mutations have been produced in untreated phage by irradiating the host with UV radiation before infection (109, 115a) or by pretreating the host with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (126). These observations have been interpreted by Ichikawa-Ryo and Kondo (109) as expression of an inducible misreplication system in the host that elevates the yield of mutations occurring during the replication of untreated phage. These authors, however, doubt that the induced misrepair system is the same one that is responsible for UV mutagenesis of either bacterial or phage DNA, since UV irradiation of a *lexA*$^-$ mutant caused some mutagenesis of the untreated phage, although not as much as that observed in an irradiated *lexA*$^+$ host. It seems possible (as suggested by M. Volkert) that a low level of SOS repair activity may be expressed in some *lexA*$^-$ strains, too low to be readily detectable in assays for UV mutagenesis or W-reactivation, but detectable in the much more sensitive assay system provided by unirradiated phage. If so, the same SOS repair system induced in *E. coli* could be responsible for bacterial UV mutagenesis, for mutagenic W-reactivation and for the mutator effect on undamaged bacterial DNA and on untreated phage infecting mutagen-treated hosts.

PLASMIDS AND SOS REPAIR

In *Salmonella typhimurium*, certain plasmids, including colIb and the drug-resistance factor R-Utrecht, have been found to enhance UV resistance and increase UV mutagenesis in wild-type cells (65, 107, 136). These effects have generally been interpreted as indicative of plasmid-borne genes that determine or enhance error-prone repair of UV-damaged hosts. Expression of this effect by the plasmid R-Utrecht depends upon the presence in *Salmonella* of a gene product that appears to be similar to the *recA*$^+$ product of *E. coli* (137). In the *Salmonella* tester strains developed by Ames et al. (2), some R factors enhance the mutagenic effect of many carcinogens in *recA*$^+$ but not in *recA*$^-$ hosts (146). In *recA*$^-$ strains, these carcinogens are nonmutagenic with or without the R factor. An R factor introduced into *E. coli* K-12 enhances UV resistance and spontaneous mutability in wild-type hosts, but not in *recA*$^-$ or *lexA*$^-$ mutants (G. Walker, personal communication). It is possible that some plasmids carry genes specifying a unique SOS repair activity, responsive to host regulation, or genes that amplify the expression of the host's own inducible repair system.

MECHANISM OF SOS REPAIR

Although current evidence is consistent with the possibility that a single SOS repair activity is responsible for bacterial and phage UV mutagenesis, there is no proof that only one type of error-prone DNA repair is induced by UV radiation in *E. coli*. The possible mechanism(s) of SOS repair of phage and bacterial DNA will therefore be discussed separately.

Mechanism of SOS Repair in Bacteriophages

Devoret et al. (55) have reviewed the evidence that mutagenic W-reactivation of bacteriophage λ is independent of both excision repair and recombinational repair and that it depends upon a novel error-prone repair activity induced or activated in the host cell by UV radiation and other SOS-inducing treatments. The requirement for new protein synthesis after the inducing treatment (52, 167a) suggests induction rather than activation of this error-prone repair activity, or of one or more of its components.

Proof that at least one type of UV-inducible SOS repair is an error-prone DNA polymerizing activity has come recently from work done

by Radman and his collaborators with the single-stranded DNA phage ϕX174 and with synthetic template-primers (M. Radman, personal communication, data summarized in M. Radman, P. Caillet-Fauquet, M. Defais, and G. Villani, 1976, p. 537–545. *In* Screening tests in chemical carcinogenesis. R. Montesano, H. Bartsch, and L. Tomatis [ed.], IARC Publications, no. 12, Lyon). When UV-irradiated ϕX174 infects untreated *E. coli*, the phage DNA remains largely unreplicated (i.e., single stranded), and the amount of phage DNA synthesis that does occur is consistent with the interpretation that replication is arrested by the first pyrimidine dimer encountered in the template (7). It appears, therefore, that none of the constitutive DNA polymerases can copy UV-irradiated DNA past pyrimidine dimers. However, if the host is exposed before infection to UV radiation, under optimal SOS-inducing conditions, much of the single-stranded ϕX174 DNA is converted to the covalently closed double-stranded replicative form, and the extent of the UV stimulation of phage DNA synthesis is correlated with the extent of W-reactivation. When primed ϕX174 DNA is used as an in vitro template with Kornberg's clear extracts of *E. coli* (128), DNA synthesis is largely prevented by prior UV irradiation of the phage DNA. The extent of the inhibition and its photoreversibility by PRE indicate that pyrimidine dimers are blocks to this in vitro DNA synthesis. The reason for blockage by dimers of DNA synthesis by DNA polymerase I appears to be the "proofreading" 3'-5'-exonuclease activity of this enzyme, which increases two to three orders of magnitude, relative to the polymerizing activity, when the ϕX174 DNA template is exposed to a UV fluence of 500 J/m^2 (G. Villani and M. Radman, personal communication). Because of this result, and because they have failed to identify a new induced DNA polymerase, Villani and Radman have suggested that an inducible inhibitor of the proofreading activity of one or more constitutive DNA polymerases may be the mutagenic effector. In the absence of this inhibitor, nucleotides inserted opposite pyrimidine dimers or other noninstructive lesions would be promptly removed. In its presence, the stable insertion of one or more "wrong" nucleotides would permit replication to continue, albeit with a high probability of mutation. There is considerable evidence (66, 128) that spontaneous mutation rates are grossly affected by changes in the relative efficiency of polymerizing and proofreading activities in DNA polymerases. Thus, an induced inhibitor of proofreading activity would be expected to cause transient mutator activity during replication of undamaged DNA, as well as to promote mutagenesis at the site of any noninstructive lesion.

In experiments using a synthetic template-primer, poly(dT)-oligo(dA), Villani and Radman (personal communication) have developed an assay for inducible SOS repair activity. When the template-primer is UV-irradiated, crude extracts from "SOS$^-$" cultures (*tif-1* cells incubated at 32°C) fail to promote DNA synthesis. When crude extracts from "SOS" cultures (*tif-1* cells incubated at 42°C for 45 min in medium containing adenine) are used, DNA synthesis occurs on the UV-irradiated templates, and massive misincorporation (uptake of dGTP and dCTP) is observed. Some misincorporation occurs when the "SOS" crude extract is used even with unirradiated template-primer, corresponding to the mutator effect of SOS activity in undamaged *E. coli* cells. The in vitro misincorporation promoted by crude extracts from heated *tif-1* mutants is *recA*$^+$ dependent, as is SOS repair activity in vivo. The in vitro assay provides a basis for possible purification of the protein(s) responsible for SOS repair activity.

Mechanism of SOS Repair in Bacteria

Since SOS-inducing treatments induce an error-prone DNA-polymerizing activity that is capable of promoting replication past pyrimidine in ϕX174 DNA and in synthetic template-primer, the simplest assumption is that the same inducible activity is responsible for error-prone repair of UV-damaged bacterial DNA as well. The same activity (or another activity of the same kind) could effect error-prone repair synthesis to fill gaps such as those shown in Fig. 4. However, other possibilities (236), including a minor inducible pathway of recombinational repair, have not been ruled out. Bridges et al. (31) have concluded that DNA polymerase III is involved in bacterial UV mutagenesis, and that its ability to effect error-prone repair may depend upon an inducible factor. The experiments upon which this conclusion was based were done with a *polC*^{ts} derivative of *E. coli* WP2$_s$, in which DNA polymerase III is temperature sensitive. UV-induced mutations in this strain lose their photoreversibility progressively at the permissive temperature (34°C), but not when the irradiated bacteria are transferred to the restrictive temperature (42°C) after an initial postirradiation incubation for 15 min at 34°C. Bridges et al. equate the LOP with the occurrence of error-prone postreplication repair. Their expectation, if error-prone gap-filling does not require active

DNA polymerase III, was that some LOP would occur at the restrictive temperature, reflecting error-prone repair of gaps formed during the 15 min between UV irradiation and temperature elevation. Since LOP stopped abruptly as soon as the temperature was raised to 42°C, they concluded that error-prone gap-filling requires active DNA polymerase III, and proposed that its ability to insert bases opposite pyrimidine dimers in the template may depend upon an inducible factor. These results are suggestive, and, if substantiated by further evidence, may implicate DNA polymerase III, as modified by an inducible factor (e.g., a proofreading inhibitor), as the agent of SOS repair of bacterial DNA.

Analysis of the molecular basis of UV mutagenesis in *E. coli* and some of its phages reveals that base transitions, primarily from guanine-cytosine (GC) to adenine-thymine (AT) (3, 40a, 167b), are efficiently induced. In one study of UV mutagenesis in a single-stranded phage, the mutations induced were predominantly C to T transitions (101a). If polymerization past noninstructive UV photoproducts is the primary mechanism of UV mutagenesis in *E. coli*, the limited information available at the molecular level suggests that photoproducts involving C (not necessarily pyrimidine dimers) may be the most common premutational lesions, at least in wild-type strains in which most pyrimidine dimers are excised. Even if such lesions are C-containing dimers, requiring two nucleotide insertions by SOS activity without template instruction, most would produce mutations meeting the operational definition of single-base transitions, owing to the degeneracy of the genetic code, the probability that either or both of the uninstructed insertions will be correct, and the probability that only one of two amino acids that might be affected will matter.

IMPLICATIONS OF SOS REPAIR FOR CARCINOGENESIS

It is not known whether DNA damage initiates the induction of an SOS-like cluster of functions, including an error-prone repair activity, in eukaryotic cells. In bacteria, most of the agents that are effective SOS inducers are also known to be carcinogenic in mammals. This is true not only for such long-known mutagen-carcinogens as ionizing radiations, UV light, and alkylating agents. Aflatoxin B_1, a potent carcinogen, induces λ prophage and is mutagenic for λ in *E. coli* K-12 (82). The carcinogen 4-nitroquinoline-1-oxide is UV mimetic in *E. coli*, producing a type of DNA damage that is biologically equivalent to pyrimidine dimer (110). In the *Salmonella* test system (2), most mammalian carcinogens are mutagenic, and much of this mutagenesis requires the product of a *Salmonella* equivalent of the *E. coli recA*$^+$ gene (146). The mutagenicity of many carcinogens in bacteria may therefore depend upon the induction of SOS repair activity. This, of course, does not imply that inducible mutagenic DNA polymerase activity is involved in mammalian carcinogenesis. Speculation to this effect may be useful, however, if it stimulates a deliberate search for induction of similar activity in normal mammalian cells after treatment with carcinogens.

Mutagenic DNA polymerases, which promote misincorporation of "wrong" bases in vitro in assays using synthetic template-primers, have been found in human cancer cells (204). The enzyme TDT, which polymerizes DNA by random "end-addition" without template instruction (15), has also been detected in human leukemic cells (48, 145, 187, 205). TDT is normally found only in thymus, where it may act as a somatic mutator in generating diversity of antigenic responsiveness in T cells (5). The presence of TDT or other error-prone DNA polymerase does not imply a causative role for these enzymes in carcinogenesis, although errors in DNA replication have been proposed as possibly important factors in initiation of malignant transformation (e.g., references 133, 163, 187). The response of bacteria to carcinogens suggests that mammalian cells, as well, may react to DNA-damaging agents by inducing mutagenic DNA polymerase activity as a transient repair function.

Mammalian cells are capable of repairing photochemical damage to their DNA (46), as well as DNA damage caused by chemical carcinogens and mutagens (177). UV radiation produces pyrimidine dimers in the DNA of mammalian cells (212), and most mammalian cells, including normal human cells, are capable of excising them (178). A human hereditary disease, xeroderma pigmentosum (XP), is associated with various deficiencies in DNA repair capacity (44, 45, 47). The clinical symptoms of XP include extreme sensitivity to UV radiation and susceptibility to development of multiple skin carcinomas, usually the cause of early death. In one type of XP, caused by an autosomal recessive mutation, ability to perform the first step of excision repair (incision) is absent (196), as in Uvr$^-$ mutants of *E. coli*. Skin fibroblast cultures taken from this type of XP patient are not only much more sensitive to UV radiation than normal skin fibroblasts, but they are also UV hypermutable at low UV fluences (139) as are Uvr$^-$ mutants of *E. coli*.

There is no direct evidence that the cancer proneness of XP patients is a consequence of their inability to excise pyrimidine dimers. However, since unexcised dimers cause hyperinducibility for SOS functions in *E. coli*, it seems possible that the induction of an error-prone repair activity in response to unexcised UV damage may account for the hypermutability of XP cells, and may also contribute to their high rate of carcinogenesis. Pyrimidine dimers are known to be causative agents of neoplastic transformation in fish (95). Carcinogens induce prophage in *E. coli* (156a), as well as mutagenic DNA polymerase activity, and Uvr$^-$ mutants are hyperinducible for both functions. If comparable SOS-type activities are induced by carcinogens in mammalian cells, the induction of latent virus and of error-prone DNA polymerase activity should occur simultaneously, and either or both could contribute to carcinogenesis.

There are some indications that UV and X radiation induce repair functions in mammalian cells. UV- or X-irradiated human viruses are reactivated (as in W-reactivation of bacteriophage) when the mammalian host cells are also exposed to X or UV radiation (13, 14, 96, 135). It is not known whether this radiation-induced reactivation is accompanied by viral mutagenesis. A UV-inducible enhancement of postreplicative repair, inducible also by *N*-acetoxy-acetylaminofluorene, has recently been demonstrated in Chinese hamster cells (51a).

In some mammalian cell lines, including normal human cells, the DNA made shortly after UV irradiation is produced in segments of low molecular weight, which are elongated and joined with further incubation to produce strands of normal molecular weight (131). The process involved in this postreplication repair appears to be primarily a form of repair replication, since recombinational exchanges have not been detected. The DNA synthesized on UV-irradiated templates at long times after irradiation (e.g., 20 h) is produced in strands of normal molecular weight, even when the UV photoproducts causing the initial discontinuities are still present in the template strands (36, 132, 149). Although other explanations are possible, the long time required to develop the capacity to synthesize DNA continuously on damaged templates could indicate induction of a type of repair activity (possibly error prone) that is repressed in undamaged mammalian cells.

REGULATION OF SOS REPAIR AND OTHER UV-INDUCIBLE FUNCTIONS

The functions activated by DNA damage constitute a regulatory unit that is unusual, perhaps unique, in *E. coli*. Most bacterial control systems regulate blocks of genes, either linked (operons) or unlinked (regulons), all of which code products active in the same metabolic pathway, or in closely related pathways (6, 179). Regulatory molecules tend to be substrates, end products, or other metabolites bearing some direct biochemical relation to the pathway itself. SOS functions, at least conceptually, are more like the metabolically diverse but teleonomically (i.e., adaptively) related groups of functions that are simultaneously activated during eukaryotic development and differentiation (34, 100). The regulation of SOS functions is the answer from *E. coli* to a typically eukaryotic question: how to turn on a group of genes simultaneously when their coordinate expression is beneficial under particular conditions, although their products may act in unrelated pathways. An understanding of SOS regulation may therefore be of interest in considering the evolution of eukaryotic control systems.

Goldthwait and Jacob (80) proposed that λ repressor is inactivated, in lysogens treated with prophage-inducing agents, by a precursor of DNA synthesis (possibly a derivative of adenine), which accumulates when DNA replication is interrupted. Hertman and Luria (97), who found that *recA*$^-$ mutations prevent UV induction of λ prophage, suggested that extensive DNA degradation (a characteristic of *recA*$^-$ mutants) produces breakdown products that antagonize the ability of a DNA precursor-inducer to inactivate the phage repressor. Tomizawa and Ogawa (210) concluded that the production of λ inducer is a complex biochemical pathway, requiring postirradiation protein synthesis.

To explain the similar requirements for induction of prophage in lysogenic strains and of filamentous growth of *E. coli* B after UV irradiation, Witkin (226) proposed that a bacterial gene coding a septum-inhibiting protein is governed by a repressor similar enough to λ repressor to respond to the same inducer, and that the common inducer is synthesized when DNA replication is interrupted. As additional bacterial functions were identified as belonging to the same regulatory system, and as their common requirement for the *recA*$^+$ *lexA*$^+$ genotype was recognized, this model was further developed (215, 233, 235, 237, 240) to assume that all SOS functions have evolved repressors similar enough to respond to the same inducer, and that the *recA*$^+$ and *lexA*$^+$ products are necessary for their induction and/or expression. The *tif-1* mutation was believed to cause constitutive synthesis at 42°C of an intermediate in the

induction pathway, thereby bypassing the requirement for interruption of DNA synthesis as the initiating signal.

λ repressor, which binds specifically to λ DNA (173), is reported to bind selectively to nicked DNA, an observation that has led to the hypothesis that SOS functions are induced by agents that introduce single-strand nicks into DNA, thereby providing sites for competitive binding of SOS repressors (206). This model does not account for thermal inducibility of SOS functions in *tif*⁻ mutants, in which DNA is intact at the inducing temperature, or for the failure of SOS induction in *recA*⁻ and *lexA*⁻ mutants when their DNA is nicked. Selective binding of SOS repressors to nicked DNA may enhance induction by "soaking up" excess repressor molecules, but it seems inadequate to account for the primary derepression of SOS functions.

The first comprehensive model of SOS regulation, specifying the roles of *recA* and *lexA* gene products, was proposed by Gudas and Pardee (89), based largely upon their study of the induction of protein X (112, 113) in various strains. As recently elaborated by Gudas (88a), this model assumes that the *lexA*⁺ gene product is a repressor of protein X and possibly of other SOS functions, which may be linked in a single operon or scattered throughout the chromosome. Inactivation of the *lexA*-coded repressor results, directly or indirectly, in the expression of all SOS functions. In wild-type strains, SOS-inducing conditions initiate induction by causing the accumulation of breakdown products of DNA degradation, one of which interacts with the *lexA*⁺-coded repressor so as to sensitize it to inactivation by an antirepressor (possibly a protease) coded by the *recA* gene. The possibility that the *recA* gene may code a protease was proposed earlier by Roberts and Roberts (180), who found that λ repressor is proteolytically cleaved following prophage-inducing treatments. According to the Gudas-Pardee model, the *tif-1* mutation is interpreted either as a temperature-sensitive operator-constitutive mutation at the binding site of the *lexA*-coded repressor or as a mutation affecting the activity of the *recA* gene product. Gudas (88a) considers the latter possibility more probable, and it is consistent with the tight linkage between *tif-1* and *recA* mutations (40). The *recA* product, as altered by a *tif-1* mutation, would presumably function as an antirepressor at 42°C, inactivating the *lexA*⁺ product without requiring the participation of a metabolite of DNA degradation.

A valuable aspect of this model is the proposal that a DNA breakdown product may act as an inducer in SOS regulation, a possibility that is supported by the failure of nalidixic acid to induce protein X synthesis in *recB* or *recC* mutants lacking exonuclease V activity, although bleomycin, an antibiotic that promotes DNA degradation directly, induces protein X in these strains (89). The properties of the *dam-3* mutant (142), which lacks an adenine methylase and degrades its DNA chronically, are also consistent with this idea, since *dam-3* strains express at least some SOS functions constitutively. However, there is no simple correlation between DNA degradation and thermoinducibility of prophage in different *dna*ᵗˢ mutants (188).

The Gudas-Pardee model is consistent with the dominance of *lexA*⁻ mutations (159) in merodiploids with the wild-type allele. It also accounts satisfactorily for the synthesis of protein X in a number of different mutant strains. However, the assumption that the *lexA*⁺ gene product functions as a repressor of SOS functions, or that it controls a product required for the induction of these functions, is not consistent with some other facts. A major problem is λ prophage, which codes its own repressor, yet requires the *lexA*⁺ genotype for induction by SOS-inducing treatments. Gudas (88a) explains this by proposing that λ induction requires the participation of a protein or small metabolite synthesized via the *lexA*⁺-controlled SOS induction pathway or after its expression. However, in *tif-1* λ lysogens, the inactivation of λ repressor begins without a lag as soon as the temperature is raised to 42°C and proceeds without requiring new protein synthesis (221). Thus, in *tif-1* strains all proteins required for thermal inactivation of λ repressor appear to have been constitutively synthesized before the temperature elevation. Nevertheless, the *lexA*⁺ gene product is strictly required for thermal induction of λ prophage in *tif-1* strains, and this requirement is for the actual inactivation of λ repressor rather than for any subsequent step leading to cell lysis. This has been demonstrated by showing rapid lysis of *tif-1 lexA*⁻ cells lysogenized with a λ mutant (λ*c*I857) which codes a temperature-sensitive repressor, whereas the same strain lysogenized with wild-type λ does not induce its prophage at 42°C (E. Witkin, unpublished observation). Many *lexA*⁻ mutations severely depress and delay UV induction of λ prophage, and this effect, too, is exerted at the level of λ repressor inactivation (59). The Gudas-Pardee model, as well as another model (162) in which the *lexA*⁺ product is assigned the role of repressor of some SOS functions, must assume that this gene product acts in an

entirely different capacity to influence λ prophage induction. Although this is not impossible, it seems simpler to assume that the *lexA*$^+$ gene product participates in the regulation of all the functions it controls in the same way.

Another set of facts that must be explained by any model for SOS regulation is the diversity of patterns of expression of different SOS functions caused by mutations that probably map within the *lexA* locus, such as *tsl*$^-$ (158, 160) and *rnm*$^-$ (215). For example, in *tsl*$^-$ mutants, filamentous growth (160) and synthesis of protein X (89) are expressed constitutively at 42°C, but λ prophage is not thermally inducible, and UV mutability (i.e., SOS repair activity) is greatly reduced compared to the wild type (158). In *rnm*$^-$ mutants, no UV mutability is detectable, but exonuclease V activity after UV irradiation is controlled to a nearly normal extent (215), and synthesis of protein X is noninducible (D. Spencer, personal communication). In *rnm*$^-$ mutants, synthesis of the inhibitor of exonuclease V is constitutive (M. Volkert, personal communication). Models assuming that the *lexA* gene codes the common repressor of SOS functions could account for such "split" phenotypes only by assuming numerous nonidentical operator sites that respond differentially to an altered repressor. The assumption that all SOS functions belong to a single operon (89) cannot account for them in any obvious way.

A different type of model is based on Clark's suggestion (41) that the *lexA* gene codes a regulator of the *recA* gene, an attempt to account for the extensive copleiotropic effects of mutations in these two genes. Mount (personal communication) has developed a model in which the quantitative level of the *recA*$^+$ gene product (assumed to act as an antirepressor that destroys SOS repressors under SOS-inducing conditions) is one of the critical factors determining whether or not a strain can express SOS functions normally. The *lexA*$^+$ gene product is assumed to act as a regulator of *recA*$^+$ synthesis, resulting in a level of *recA*$^+$ product that permits normal SOS induction in wild-type strains. Mutations in the *lexA* gene that result in a reduced level of *recA*$^+$ product cause noninducibility of all SOS functions (the *LexA*$^-$ phenotype). Certain other mutations in the *lexA* gene (*spr*$^-$) are assumed to increase the synthesis of the *recA*$^+$ product to an abnormally high level, and thereby cause constitutive expression of SOS functions even at 30°C, if other requirements for induction are provided by a *tif-1 sfi*$^-$ genotype. Any model in which the *lexA* product regulates synthesis of the *recA* product, and in which the quantitative level of *recA* product is a critical factor in SOS induction, can explain all-or-none noninducibility or all-or-none constitutive expression of SOS functions. Such models, however, cannot easily explain the "split" phenotypes of *tsl*$^-$ or *rnm*$^-$ mutants (described above), or the quite different pattern of expression of SOS functions seen in *lex-113* mutants (58). For any one *lexA* allele, there can be only one level of *recA*$^+$ product produced under its control, whether that level be normal, abnormally high or abnormally low. Yet *tsl*$^-$ and *rnm*$^-$ mutants are noninducible for some SOS functions, whereas they express others normally or are constitutive for still others under the same conditions. In *lex-113* mutants, the phenotype is like *lexA*$^-$ in all respects, except that filamentous growth is expressed constitutively. One would have to make some complicated assumptions to explain how various *lexA* alleles (which can only either raise or lower the amount of *recA* product synthesized according to this model) can produce such a variety of patterns of "split" phenotypes with respect to SOS functions. It should be emphasized, however, that *tsl*$^-$, *rnm*$^-$, and *lex-113* mutations have not been definitively mapped within the *lexA* gene, although the linkage data (especially for *tsl*$^-$ and *rnm*$^-$) are consistent with this possibility.

Figure 6 illustrates another way of interpreting SOS regulation. The essential features of the model are as follows. (i) Each SOS function expresses the activity of a separate operon, controlled by its own repressor. (ii) SOS repressors are similar enough to bind the *lexA*$^+$ gene product when bound to their respective operator sites, but are not identical. (iii) In undamaged wild-type cells, the *lexA*$^+$ product is bound to all SOS repressors, thereby preventing their inactivation by constitutive antirepressors. In Fig. 6, the antirepressors are shown as cellular proteases, on the assumption that proteolytic cleavage is the primary mechanism of SOS repressor inactivation. In that case, the *lexA*$^+$ product may serve to block protease-sensitive sites on the SOS repressors, or it may act more positively as a protease inhibitor. Whatever the primary mechanism of derepression, the role of the *lexA*$^+$ gene product is protection of SOS repressors against inactivation in the absence of an SOS induction signal. (iv) The *recA-tif* complex (so designated because the *tif-1* mutation maps in the *recA* region and may affect the same protein) is inactive in the absence of an SOS signal, in the sense that it is unable to remove the *lexA*$^+$ product from its binding with SOS repressors. In the inactive state, the

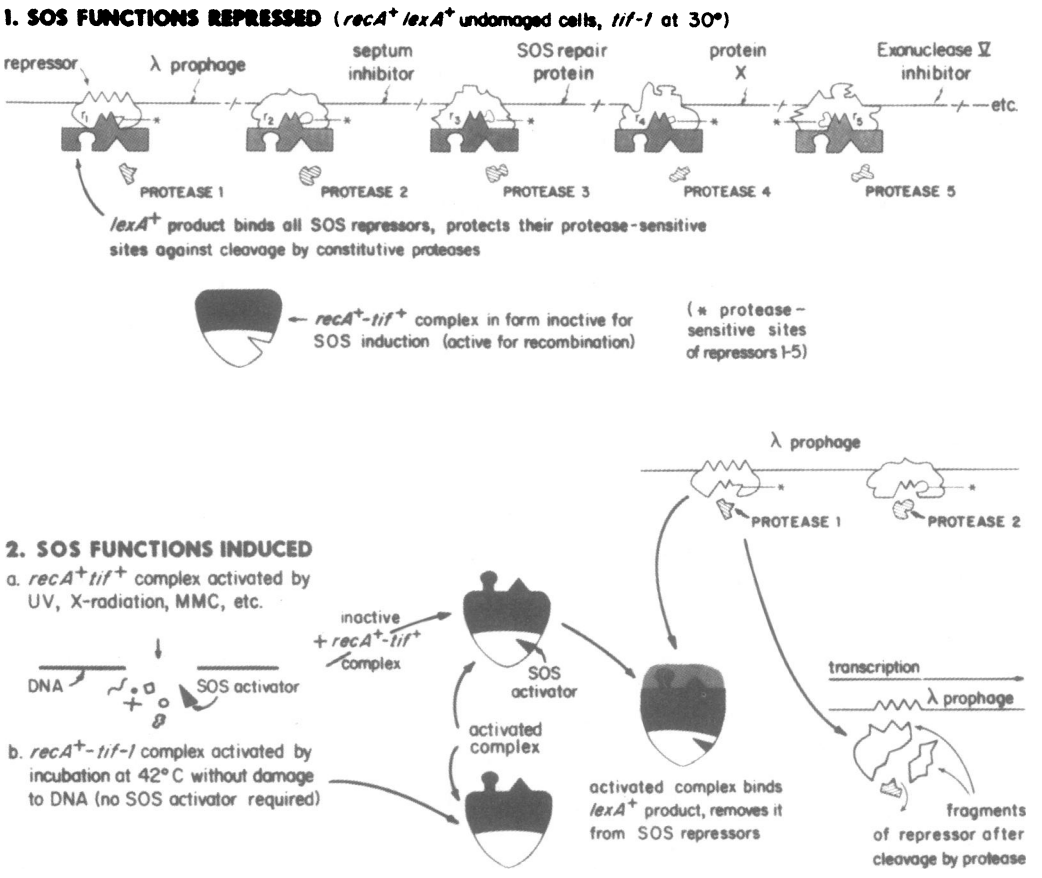

FIG. 6. *Model to account for the regulation of SOS functions in E. coli (see text for explanation and alternative versions).*

recA-tif complex may be free, as shown in Fig. 6, or bound to the *lexA*$^+$ product. In either case, activation of this complex, enabling it to remove the *lexA*$^+$ product from SOS repressors, occurs in *tif*$^+$ strains only when an SOS activator is produced as a consequence of arrested DNA replication. The activator may be a precursor of DNA replication (80) or a breakdown product of DNA degradation (89), or (as suggested by H. Ginsberg) activation may be accomplished under some conditions by a precursor and under other conditions by a breakdown product. In the *tif-1* mutant, activation of the *recA-tif* complex occurs when the temperature is raised to 42°C without requiring an activator, or at lower temperatures when adenine is present (80, 123, 234). Activation of the complex permits its binding to the *lexA*$^+$ product unless it is already so bound before activation. In either case, the binding of activated *recA-tif* complex to the *lexA*$^+$ product results in the release of the *lexA*$^+$ product from SOS repressors, thus exposing their protease-sensitive sites to proteolytic cleavage (or permitting their inactivation by some other constitutive antirepressor).

The model in Fig. 6 explains currently known facts satisfactorily and generates a number of testable predictions. Like any model offered when knowledge is incomplete, it may require drastic revision or rejection as additional facts become known, and it is proposed here as a working hypothesis. The dominance of *lexA*$^-$ mutations in merodiploids with *lexA*$^+$, regardless of which allele is located on the F′ factor (159), is readily explained. Mutations causing the noninducible phenotype for all SOS functions (the LexA$^-$ phenotype), according to this model, are those eliminating the ability of the *lexA*$^+$ product to bind the activated *recA-tif* complex or to be released from SOS repressors when bound to the activated complex. In merodiploids, any *lexA*$^+$ product bound to an SOS repressor would be

removed under SOS-inducing conditions, and could be replaced by a *lexA*$^-$ product, which would remain irreversibly bound to that repressor. If the inactivation of SOS repressors and/or the expression of SOS functions is slow compared with the rate of binding of SOS repressors by the *lexA*$^-$ product, all SOS repressors would ultimately become saturated with the irreversibly bound product in a merodiploid.

Mutations that map in the *recA* region but have a Lex$^-$ phenotype (noninducibility for SOS functions but normal recombination ability), such as *zab*$^-$ (40) or *lexB*$^-$ (11), may be *recA* alleles affecting a part of the *recA* gene product not essential for genetic recombination. Both of these mutations are recessive with respect to UV sensitivity in merodiploids with the wild-type allele (J. George, personal communication). This is consistent with the model in Fig. 6, since the noninducible phenotype in these mutants is ascribed to changes in the *rec-tif* complex, such as to prevent either its activation or its interaction with the *lexA*$^+$ product. Presence of the normal allele should restore inducibility in the merodiploid. The *tif-1* mutation, on the other hand, should be dominant, or at least partly dominant. Although most *recA*$^-$ mutations would be expected to be recessive, partial dominance of some *recA* alleles would be consistent with the model, e.g., if the mutation caused unactivated *recA* product to bind irreversibly to the *lexA*$^+$ product.

This model can explain the variety of "split" phenotypes for SOS functions caused by mutations that are tightly linked to *lexA*$^-$ mutations, and that may affect the same protein. These include *tsl*$^-$, *rnm*$^-$, and possibly *lex-113* (see above). Additional examples of "split" phenotypes in derivatives of *lexA*$^-$ strains selected for increased UV resistance have been reported (28, 191). A single mutation in the *lexA* gene could cause a variety of different patterns of expression of SOS functions, according to the model in Fig. 6. If the mutation permits normal binding of the altered *lexA* product to a particular SOS repressor, but prevents its response (when so bound) to an activated *recA-tif* complex, the mutant will be noninducible for that SOS function. If the same altered *lexA* product is unable to bind one or more other SOS repressors, the mutant will express the corresponding function(s) constitutively, since the repressor(s) will be exposed continuously to inactivation by antirepressor (e.g., cleavage by protease). The mutant may exhibit normal inducibility of still other SOS functions, if the altered *lexA* product binds normally to some SOS repressors and responds normally to activated *recA-tif* complex when so bound. Assuming nonidentical repressors, changes at different sites within the *lexA* gene could thus cause a multiplicity of different patterns of constitutivity, inducibility, and noninducibility of the various SOS functions.

A prediction generated by the model in Fig. 6 is that *lexA* mutations that cause "split" phenotypes for SOS functions should cause a corresponding pattern of "split" dominance, such that any function that is noninducible in the mutant should be dominant in merodiploids with *lexA*$^+$, whereas any function that is expressed constitutively in the mutant should be recessive. Insofar as the dominance/recessiveness of individual components of "split" phenotypes has been determined, the results agree with this prediction. In *tsl*$^-$ mutants, low UV mutability (i.e., poor inducibility of SOS repair activity) is dominant, but constitutive filamentous growth at 42°C is recessive in merodiploids with the wild-type allele (158). In the *lex-113* mutant (which is closely linked to *lexA* but not unambiguously located within the *lexA* locus) the constitutive filamentous growth of the mutant is recessive in merodiploids with the wild-type allele (J. Donch, personal communication), but its UV sensitivity and UV nonmutability (i.e., noninducibility of SOS repair activity) are dominant (86). The constitutive filamentous growth of the *lex-113* mutant is suppressed by a *lexA*$^-$ mutation (59), an observation that will add further support to the model if these mutations prove to be allelic.

Another prediction based on this model is that the phenotype of a deletion mutant lacking the *lexA* gene should be constitutive for all SOS functions, whereas the phenotype of a *recA* deletion mutant should be noninducible for all SOS functions, assuming that such mutants are viable. If recessiveness is interpreted as indicative of loss of function of the type one might expect of a deletion mutant, the recessiveness of noninducible *zab*$^-$ and *lexB*$^-$ mutations indicates that the positive action of a functional *recA* allele is necessary for SOS induction, whereas the recessiveness of the constitutive filamentous growth in *tsl*$^-$ (and possibly in *lex-113*) mutants indicates that the positive action of a functional *lexA* allele prevents induction.

The model in Fig. 6 can also explain the unique phenotype of the double mutant *tsl*$^-$ *recA*$^-$ (108, 158a, 161, 162), which is different with respect to some SOS functions from either of the single mutants. The double mutant is unlike any other *recA*$^-$ strain in its UV resist-

ance and ability to perform some error-free postreplication repair (in Uvr$^+$ strains) (158a, 161, 162), and in its constitutive synthesis of protein X (88a) and filamentous growth at 42°C (161, 162). The *tsl*$^-$ *recA*$^-$ strain differs from the *tsl*$^-$ *recA*$^+$ mutant in its inability to control DNA degradation after UV irradiation (i.e., its failure to induce the inhibitor of exonuclease V) and in its inability to produce any UV-induced mutations (158, 158a). The phenotype of the double mutant suggests an interaction between the *recA* and *lexA* gene products such that mutations in each can affect the role of the other in SOS induction. An interaction of this type is expected in the model in Fig. 6. Devoret (personal communication) has proposed a model in which the *recA* and *lexA* gene products form a complex which acts as a "vise" or clamp, detecting SOS repressors after SOS-inducing treatments and positioning them so as to permit their inactivation by an antirepressor. Such a model (if one assumes different repressors for SOS functions) could also account for "split" phenotypes and for unique phenotypes in double mutants, but could not easily account for the apparently invariable dominance of *lexA*$^-$ alleles, unless it is assumed that complete inactivation of the *lexA* product is lethal.

A final aspect of the model in Fig. 6 is that it assigns the primary repressor inactivation to proteases coded by genes outside the *recA* and *lexA* loci. The proteases are shown as different for each SOS repressor. This degree of diversity is not a necessary feature of the model, although more than one antirepressor must be postulated. If there were only one SOS antirepressor, there should be a third locus (the locus coding the antirepressor) in which mutations could eliminate inducibility of all SOS functions. Since no such mutations have been found unlinked to *recA* and *lexA*, it is assumed that more than one protease is required to inactivate all SOS repressors.

A variation of this model, which fits the facts equally well, could operate if the *lexA*$^+$ product functions actively as a protease inhibitor, rather than as a passive protector of protease-sensitive sites on SOS repressors. In that case, the *lexA*$^+$ product need not be assumed to interact with SOS repressors, but could be bound instead to the antirepressor proteases in the uninduced state, and its inactivation by activated *recA-tif* complex could result in the release of proteases and in the cleavage of SOS repressors. "Split" phenotypes exhibited by *tsl*$^-$, *rnm*$^-$ and other mutants believed to alter the *lexA* product would then be due to specificity of interaction of the altered gene product with different proteases. Although not necessarily requiring that every SOS repressor be susceptible to cleavage by a different protease, this version of the model would require that more than one protease have the capacity to cleave SOS suppressors.

A puzzling set of observations concerns the need for new protein synthesis after various SOS-inducing treatments in order to inactivate λ repressor in various lysogenic strains. The presence of chloramphenicol during incubation at 42°C does not prevent repressor inactivation in *tif-1* (221) or *dnaB*^{ts} (166) lysogens, nor does chloramphenicol, added immediately after gamma irradiation, prevent repressor inactivation in wild-type lysogens (221). After treatment with MMC or UV radiation, however, λ repressor is not inactivated in the presence of chloramphenicol (198, 210), indicating that new protein synthesis is needed to accomplish derepression of prophage when these inducers are used. These results imply that different SOS-inducing treatments are not alike in the manner in which they generate an effective SOS-inducing signal. Clarification of this question may be crucial for evaluation of SOS regulation models.

PROTEASES AND THE EXPRESSION OF SOS FUNCTIONS

The initial basis for considering protease activity as a possible primary SOS repressor-inactivating event was the proteolytic cleavage of λ repressor that occurs when λ prophage is induced, although it is not known whether this cleavage is the cause or the consequence of repressor inactivation (180). The specificity of proteases and their importance in both bacterial and mammalian cell economy are receiving increasing attention (79, 79a, 150, 178a, 207).

A strong reason for invoking protease activity as a major factor in modulating the expression of SOS functions, as well as in their induction, is the possibility that the *lon* gene, mutations in which cause extreme filamentous growth in response to SOS-inducing treatments (1, 104), may code a protease or a product that regulates protease activity. The *lon* gene is apparently identical with the gene *degT* (199). Mutations in *lon*/*degT* greatly increase the stability of specific polypeptide fragments that would otherwise be rapidly degraded, as well as the stability of some other proteins (79, 79a). Mutations designated *degR* have been reported to alter the stability of different polypeptides, suggesting a unique pattern of specificity (2a), although it is apparently not yet certain whether *degT* and *degR* are in fact distinct loci (79a). The products of the *deg* gene(s) have not been identified, but they could be proteases,

protease inhibitors, or products that otherwise regulate selectively the rate of degradation of various proteins. The pleiotropy of *lon/degT* mutations includes (among others) effects on the rate of transcription of the *gal* operon (138), on the synthesis of capsular polysaccharides (37), and on the capacity to be lysogenized by λ phage (217). All of these effects could be the consequence of altered stability of a variety of metabolically unrelated gene products due to a *lon/degT* mutation affecting (directly or indirectly) the specificity and activity of a particular protease or protease inhibitor. Sfi⁻ mutations (suppressors of filamentous growth), which eliminate the filamentous growth expressed at 42°C by *tif-1* mutants (75, 238), may also affect the structure or activity of proteases or protease inhibitors, imposing a new pattern of stability upon numerous proteins. Independent Sfi mutations which suppress the filamentous growth of the *lon*⁻ wild-type strain *E. coli* B may or may not also alter sensitivity to penicillin, and at least one such mutation (responsible for the nonfilamentous phenotype of strain B/r) radically changes the kinetics of cell division and death in the stationary phase of growth (222).

A change in the half-life of most proteins in *E. coli* may not cause a strikingly obvious change in the phenotype, as long as the protein does not become so unstable that it is essentially unable to perform its function. Altered stability of the proteins synthesized in response to an SOS induction signal, however, could drastically alter the expression of at least some SOS functions. SOS operons are transcribed only during a limited period of time while SOS-inducing conditons prevail, and rapid degradation of their products would, a priori, be expected. This expectation is consistent with the 30-min half-life of SOS repair activity (52, 238) and with the existence of a class of unstable proteins in bacteria with half-lives of 20 to 60 min (79a). When UV radiation is the inducing agent, transcription of these operons is possible only until DNA repair is completed and normal DNA replication resumes. The length of the filaments produced after exposure of *E. coli* B (a *lon* mutant) to UV radiation is strictly proportional to the amount of protein synthesis that is allowed to occur before DNA repair is completed, an observation that led to the hypothesis that the duration of the delay in septation is quantitatively related to the amount of a septum-inhibiting protein synthesized during the limited period of derepression (226). George et al. (75) have pointed out that *lon* mutations could cause extreme filamentous growth in response to SOS-inducing agents either by stabilizing the postulated septum inhibitor (if *lon/degT* codes a protease) or by increasing the rate of its synthesis. Mutations affecting protease activity and/or specificity could mimic regulatory mutations by affecting the stability of regulatory proteins. The apparent regulatory role of the *lon* product in the galactose operon (138) may exemplify the difficulty of distinguishing between mutations directly affecting regulatory protein structure and those affecting their metabolism (6).

The limited and often very brief period during which SOS operons are derepressed makes the expression of SOS functions particularly susceptible to mutations that increase or decrease rates of transcription, rates of translation, stability of mRNA or stability of proteins. To the extent that such mutations act pleiotropically on specific spectra of substrates, they may amplify or diminish the expression of different SOS functions in a variety of patterns. Mutations at any of these levels, perhaps exemplified by *lon* and *sfi* mutations, may not only "fine-tune" the expression of SOS functions but could also represent a means of generating asexually, via a single mutation, a rich diversity of phenotypic variation. Their importance in evolution should not be underestimated.

CONCLUSIONS

UV-induced mutations in *E. coli* and some of its phages are caused primarily by inaccurate repair of UV-damaged DNA. Most of the UV damage produced in the DNA of surviving bacteria is repaired by relatively "error-proof" mechanisms (e.g., photoreactivation, "short-patch" excision repair, the major pathways of recombinational postreplication repair), which do not contribute substantially to UV mutagenesis. Some kinds of DNA damage (probably single-strand gaps not repairable by any constitutive accurate mechanism) are targets for the activity of inducible "error-prone" repair activity ("SOS" repair), which is entirely responsible for UV mutagenesis in *E. coli* and in λ bacteriophage. SOS repair may participate in two minor DNA repair pathways: a "long-patch" excision repair and chloramphenicol-sensitive postreplication repair.

SOS repair activity is repressed in undamaged wild-type cells, but is induced in response to UV radiation and other agents (including many mutagens and carcinogens) that damage DNA or interrupt its replication. Such treatments generate a regulatory signal (the "SOS" signal), which initiates a complex induction process culminating in the derepression of a group of metabolically diverse but coordinately regulated functions ("SOS" functions), all of

which presumably promote the survival of the damaged cell or that of its phages. Included among SOS functions, in addition to error-prone DNA repair activity, are prophage induction, cell division delay (sometimes leading to filamentous growth), inhibition of the DNA-degrading activity of exonuclease V and "aberrant" reinitiation of DNA synthesis at the chromosomal origin. Expression of these and other inducible SOS functions requires the *recA*$^+$ and *lexA*$^+$ gene products and new protein synthesis during or after the inducing treatment. The nature of the SOS induction signal is not yet understood, nor is the mode of regulation of SOS functions.

At least one kind of SOS repair activity induced in *E. coli* has been identified as a DNA-polymerizing activity that is distinguishable from the activities of the constitutive DNA polymerases I, II, and III by its ability to polymerize DNA past pyrimidine dimers in the template strand, with a high probability of error. UV mutagenesis in *E. coli* and λ bacteriophage may be due primarily to the insertion of "wrong" bases by this UV-inducible SOS repair activity as it replicates DNA past noninstructive UV photoproducts, which are not necessarily pyrimidine dimers. The inducible component of this SOS repair system need not be a new DNA polymerase, but may be a factor that inhibits the 3'-5'-exonuclease "proofreading" activity of one or more of the constitutive DNA polymerases, or one that relaxes their template dependence in some other way. It is possible that more than one type of SOS repair activity is induced in *E. coli*. In that case, some or all of the UV mutagenesis occurring in bacterial and in λ phage DNA could be due to error-prone repair by a minor inducible pathway of recombinational repair, although there is no evidence that such a system is induced.

Anomalous induction of SOS repair activity in undamaged cells containing intact DNA capable of replication results in a mutator effect, i.e., greatly increased generalized spontaneous mutation rates. SOS repair activity in crude extracts of UV-irradiated *E. coli* also promotes misincorporation of bases during replication of unirradiated synthetic template-primer DNA. Both in vivo and in vitro, however, the presence of UV photoproducts in the DNA greatly increases the mutagenic effect of SOS repair activity.

The kinetics of induction and decay of SOS repair activity are similar, whether the error-prone activity is assayed by its capacity to enhance bacterial UV mutagenesis or to promote λ UV mutagenesis in UV-irradiated hosts. In both types of assay, the half-life of SOS repair activity is about 30 min. These observations are consistent with the possibility that the same inducible error-prone DNA polymerase activity is responsible for both bacterial and phage UV mutagenesis.

The induction of at least some SOS functions by UV radiation requires the absorption of two photons. UV-induced mutations, however, may be caused either by single UV photoproducts or by the cooperative interaction of two UV photoproducts. All or nearly all detectable UV-induced mutations occur before the second postirradiation DNA replication. In wild-type strains, some UV-induced mutations occur before DNA replication via SOS repair of gaps resulting from excision of UV photoproducts; others occur during or after the first postirradiation DNA replication via SOS repair of damage (probably gaps in the daughter strands) caused by unexcised UV photoproducts passing through a replication fork.

There is some evidence that mutagens and carcinogens induce DNA repair activities in mammalian cells. It is not known whether these activities are error-prone or whether they are induced in coordination with other SOS-like functions in resonse to DNA damage. If so, at least two such functions (induction of latent virus and of error-prone DNA repair activity) could contribute to carcinogenesis.

APPENDIX: THE CELL DENSITY ARTIFACT

Many studies of bacterial mutagenesis are flawed by an artifact that has not been generally recognized. The problem stems from the common assumption that percentage of survival, after termination of a more or less lethal treatment, is independent of the cell density at which the bacteria are subsequently plated.

The "cell density artifact" can be illustrated by describing a protocol widely used to measure the mutagenic effect of UV radiation in an auxotrophic (e.g., Trp$^-$) strain, scoring mutations to prototrophy (Trp$^+$). This kind of system, when used properly (3), allows accurate calculation of the frequency of induced mutations per survivor. A key feature of the auxotrophy to prototrophy system is the use of the same medium for assaying auxotrophic survival and for selecting prototrophic mutants. The medium used (SEM) is a minimal agar partially enriched with nutrient broth (1 to 5%, vol/vol). When a highly diluted portion of a Trp$^-$ culture (100 to 300 cells) is plated on SEM, the tryptophane in the supplement allows this small number of cells to undergo many cell divisions, so that each cell forms a small but visible colony before its growth is arrested by

exhaustion of the medium for the required growth factor. When a vary large population of Trp^- cells (10^7 to 10^8) is plated on the same medium, the required amino acid is used up after relatively few divisions, and the auxotrophic population forms a confluent but barely visible lawn. Any Trp^+ mutants either present in the inoculum or arising during the divisions on the plate can continue to grow to form large colonies. This system was originally developed by Demerec and Cahn (54a).

The use of SEM medium in the auxotrophy to prototrophy system automatically satisfies three major requirements of sound methodology for bacterial mutagenesis. (i) Survival and mutation are scored on the same medium, thus eliminating any medium effect on survival as a possible source of error. (ii) The mutagen-treated population undergoes a few cell divisions before stringent selection for the prototrophic phenotype begins, permitting phenotypic expression of any newly induced mutations. This requirement is not met when mutagen-treated auxotrophs are plated directly on minimal agar, since the phenotype of potential mutants remains auxotrophic until new information in the DNA can be transferred to the gene product. (iii) The treated population is allowed to undergo the divisions required for phenotypic expression while immobilized on solid medium, thus preserving the essential 1:1 relation between the number of mutant colonies counted (above the number produced by unirradiated controls) and the number of mutations induced. Allowing phenotypic expression to occur during a period of liquid cultivation long enough to guarantee complete expression permits division of induced mutants before plating, and makes accurate determination of mutation frequency impossible.

Before UV irradiation and after each increment of radiation exposure, both diluted and undiluted aliquots of the Trp^- cell suspension are plated on SEM agar. The diluted samples are used to calculate percent survival for each UV fluence. The undiluted samples are used to enumerate Trp^+ colonies after 2 to 3 days of incubation. The frequency of UV-induced mutations is then calculated, after subtracting the number of Trp^+ colonies on plates seeded with unirradiated controls. (The number of Trp^+ colonies on control plates depends primarily upon the *final* size of the plated population, and therefore is constant over a wide range of inoculum sizes, at a given level of nutrient broth enrichment.) Any excess of Trp^+ colonies over the number on control plates represents the number of Trp^+ mutations induced by UV radiation in the surviving fraction of the plated population. It is usually taken for granted that percentage of survival, as indicated by diluted platings, is the same as percentage of survival in the undiluted populations plated to select Trp^+ mutants. The assumption that percentage of survival is independent of cell density on the postirradiation plating medium has been shown to be invalid for UV-treated populations of the *lon*$^-$ strain B, but is valid for one of its nonfilamentous derivatives, strain B10/r (227), and is also valid for strain B/r over a wide range of cell densities (E. Witkin, unpublished observation).

Under some conditions of postirradiation cultivation, strain B, like *lon*$^-$ mutants of K-12, forms long filamentous cells and is consequently UV sensitive (104, 222). Under different postirradiation conditions, however, these bacteria divide normally and are UV resistant (1b, 1c, 2a, 226). Survival after UV irradiation, or after treatment with other SOS-inducing agents, is not irreversibly determined in *lon*$^-$ strains at the time the treatment is terminated, but can be influenced subsequently over several orders of magnitude by manipulating the composition of the medium, the temperature of incubation, or the cell density. Filamentous growth itself appears to be lethal unless cell division is resumed before a certain "critical length" is reached (117a), and is the cause of the UV sensitivity of *E. coli* B and of *lon*$^-$ mutants of K-12 to most SOS-inducing agents. High cell density is one of the factors (among others) that can prevent extreme (i.e., lethal) filamentous growth by promoting early resumption of normal cell division and radiation resistance in *lon*$^-$ strains, a phenomenon described as "neighbor restoration" (1, 53a) or "crowding recovery" (227). Thus, the "cell density artifact" applies to any strain treated with an agent that causes extensive filamentous growth under the conditions used to determine percentage of survival, but not under the conditions used to select mutants. Under these conditions, gross overestimation of the frequency of induced mutations per survivor results from extrapolating percentage of survival from diluted to undiluted platings. The actual size of the viable population from which the observed number of induced mutations is arising may be 100 times larger than estimated, if filamentous growth kills 99% of the cells plated at low density, but kills none of the cells plated at high density.

The cell density artifact due to filamentous growth can sometimes apply to *lon*$^+$ strains, such as K-12, 15TAU, and others that are as UV-resistant as strain B/r. *E. coli* K-12 wild type does not form long filaments after UV irradiation, and the cell density artifact proba-

bly does not affect studies of UV mutagenesis in this or other *lon*$^+$ strains. However, *lon*$^+$ UV-resistant strains are capable of producing long filamentous cells, and may well be subject to the cell density artifact, after relatively prolonged starvation for thymine (243a), or after incubation for 1 h or more at 42°C in *tif-1* (39, 122), *dnaB*^{ts} (99a), or *lig*^{ts} (72a) derivatives. Thus, *lon*$^+$ K-12 and similar strains appear to be capable of undergoing "filamentous death" after some SOS-inducing treatments. In contrast, strain B/r does not form filaments when its *dnaB*^{ts} or *tif-1* derivatives are incubated at 42°C, and seems to be immune to the cell density artifact under any condition examined (E. Witkin, unpublished observations).

Microscopic observation of treated bacteria, incubated for several hours at relatively high and low cell densities after an SOS-inducing treatment, can often indicate whether the cell density artifact is likely to operate. The problem can be avoided entirely by the use of a mutation scored without selection, such as mutations from Lac$^+$ to Lac$^-$ detected on indicator medium, since survival and mutations are scored on the same plates at a single cell density. This kind of system is slow and expensive, however, since large numbers of plates are required to obtain accurate mutation frequency data. The use of selected mutants (auxotrophy to prototrophy, streptomycin sensitivity to resistance) can provide reliable mutation frequency data in strains not subject to filamentous growth after the treatment used. Even in strains capable of undergoing lethal filamentous growth at low cell density after a particular treatment, accurate induced mutation frequencies can be obtained by limiting the treatment to levels still permitting 100% survival as determined by plating dilutions, or by plate-wash assays (227, 234) of cells harvested from densely populated plates after an incubation long enough to allow DNA replication but not cell division. Many SOS-inducing treatments are demonstrably mutagenic even in this high survival range, at least in strains exhibiting a "shoulder" in their survival curves. Induced mutations scored in the 100% survival range of treatment can be assumed to have arisen from the whole plated population (frequency per survivor = frequency per bacterium plated). Thus, in *lon*$^+$ K-12 and similar strains, mutagenesis by treatments such as thymine starvation or by temperature elevation in *dnaB*^{ts}, *lig*^{ts} or *tif-1* derivatives is likely to be highly overestimated, if indeed it is demonstrated at all, unless treatment is limited to levels that are sublethal in survival assays.

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ADDENDUM IN PROOF

S. Meyn, T. Rossman, and W. Troll (Proc. Natl. Acad. Sci U.S.A., in press) have shown that *tif-1*-mediated thermal inactivation of λ repressor is prevented by a protease inhibitor, antipain, which also blocks the induction and/or expression of SOS repair activity and filamentous growth. Antipain does not promote uncontrolled DNA degradation after UV irradiation of wild-type cells, however, and therefore does not seem to interfere with induction of the inhibitor of exonuclease V. These observations are consistent with the model of SOS regulation in Fig. 6, but do not support models in which a single protease is the antirepressor for all SOS functions.

Studies of merodiploids *tif-1*/*tif*$^+$ and *tif-1*/*recA* (J. George, personal communication) indicate that *tif-1* and *tif*$^+$ are codominant and that *tif-1* and *recA* mutations probably affect the same gene product. These observations support the version of the SOS regulation model in Fig. 6 in which the wild-type *rec-tif* complex (probably a single protein) is bound to the *lexA*$^+$ product in the uninduced state (see text). Complete dominance of the *tif-1* allele would be expected if the *recA-tif* complex were free, as shown in Fig. 6.

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