Mutation Research, 106 (1982) 217–224
Elsevier Biomedical Press

A96

Three mechanisms for ultraviolet radiation mutagenesis in *Escherichia coli* K-12 *uvrB5*: Specificity for the production of back and suppressor mutants

Neil J. Sargentini [1], Richard C. Bockrath [2] and Kendric C. Smith [1]

[1] *Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305, and* [2] *Department of Microbiology, Indiana University School of Medicine, Indianapolis, IN 46223 (U.S.A.)*

(Received 25 January 1982)
(Revision received 7 May 1982)
(Accepted 1 June 1982)

The ultraviolet radiation mutant frequency response (MFR) curve for *Escherichia coli* K-12 *uvrB5* (SR250) has been resolved into three components that were suggested to be the result of three independent mutagenic mechanisms (Sargentini and Smith, 1979). They are: a 'one-hit' mechanism that determines the observable linear MFR at UV radiation fluences of 0–0.5 J m^{-2}, but which operates at least up to 6 J m^{-2}; a 'two-hit' mechanism that yields a fluence-squared MFR at fluences greater than ~ 0.5 J m^{-2}; and a kinetically complex (KC) process (previously we have called this the ?-hit process) that yields a third MFR component that is observed only between 1 and 3 J m^{-2}. The theoretical nature of these three independent mutagenic mechanisms was deduced largely from the calculated resolution of three MFR components from the observed UV radiation-induced MFR.

In this study we have examined the UV radiation fluence relationship for the production of two classes of amber nonsense mutation revertants: suppressor mutants and back mutants. Our results support the existence of the proposed three mutagenic mechanisms, and associate the two-hit and KC mechanisms with suppressor mutant production, and the one-hit mechanism with back mutant production.

The *E. coli* strains used in this study are listed in Table 1. The origin of the *lacZ53*(Am) mutation has been described (Sargentini and Smith, 1979). The *leu-308*(Am) mutation (allele number provided by B.J. Bachmann, personal communication) is discussed in Bockrath and Palmer (1977), and was put into the *uvrB5 lacZ53* strain (SR250) to produce the double-amber strain (SR825), as described in Table 1. Media, preparation of cells, and UV radiation mutagenesis procedure were as before (Sargentini and Smith, 1979), with three exceptions: each mutant-selection plate was spread with 0.1 ml of cells ($\sim 1 \times 10^7$ colony-forming units per ml), the Lac^+ selection plates contained glucose at 300 μg/ml, and the Leu^+ selection plates contained L-leucine at 6 μg/ml.

Abbreviations: KC, kinetically complex; MFR, mutant frequency response.

0027-5107/82/0000–0000/$02.75

TABLE 1

STRAINS OF *E. coli* USED

Stanford Radiology No.	Genotype [a]	Source or derivation [b]
SR250	F⁻ *leuB19 metE70 thyA36 deo(C2?) lacZ53*(Am) *rha-5 rpsL151 uvrB5* λ (P1)	DY145 (Youngs and Smith, 1973)
SR719	F′ *pro*⁺ *lac*⁺ *IQ L8/ara Δ(pro lac) thi*	GM1, J.H. Miller
SR733	*leu-308*(Am) *tyr*(Oc)	WU36-10, R.C. Bockrath
SR824	F⁻ *metE70 thyA36 deo(C2?) ara lacZ53*(Am) *rha-5 rpsL151 uvrB5* λ	SR250 × P1::Tn *9*cts·SR719, select Leu⁺
SR825	F⁻ *leu-308*(Am) *metE70 thyA36 deo(C2?) lacZ53*(Am) *rha-5 rpsL151 uvrB5* λ (P1)	SR824 × P1::Tn *9*cts·SR733, select Ara⁺

[a] Genotype nomenclature is that used by Bachmann and Low (1980). All strains are derivatives of *E. coli* K-12 except SR733, which is a derivative of *E. coli* B/r.

[b] The transduction procedure using bacteriophage P1 has been described (Sargentini and Smith, 1979). Leu⁺ indicates leucine prototrophy. Ara⁺ indicates ability to grow on minimal medium containing 0.4% L-arabinose as the sole carbon source.

Lac⁺ (able to grow on lactose in place of glucose) or Leu⁺ (prototrophic for leucine) revertant colonies (generally a 200 colony sample for each UV radiation fluence tested) were picked from mutant-selection plates and isolated by transferring them onto lactose plates (for Lac⁺ revertants) or leucine-deficient plates (for Leu⁺ revertants). These isolated revertants were then analyzed to determine if they were back mutants or suppressor mutants. To analyze Lac⁺ and Leu⁺ revertants of strain SR825 (*leu*[Am] *lac*[Am]), cells were transferred onto both leucine-deficient plates and lactose plates. Isolates that could grow on both kinds of plates were classified as suppressor mutants (Witkin, 1963). Those that could only grow on one kind of plate were considered to be back mutants. To analyze Lac⁺ revertants of strain SR250, isolates were tested with a wild-type and three nonsense mutant strains (NG75, NG273, and PS205) of bacteriophage T4. This is the assay of Bockrath and Palmer (1977) except that the isolates were tested for phage-mediated cytolysis on R-plates (Miller, 1972), and incubation was for 2 days at 37°C. These protocol modifications produced more distinct lysis patterns. This assay separates revertants into back mutants and suppressor mutants [class 1 amber, class 2 amber or class 3 amber (indistinguishable from class 3 ochre) suppressor mutants].

In the experiments with strain SR250 (mutants differentiated by their sensitivity to bacteriophage T4 mutants), the spectrum of mutants induced by UV radiation was quite different from that arising spontaneously (data not shown). Back mutants were the most common class of spontaneous mutants, except in a few experiments where the class 3 amber/ochre suppressor mutants prevailed. Class 1 and class 2 amber suppressor mutants were always rare (~ 5%). After UV irradiation, only back mutants and class 2 amber suppressor mutants (*supE* mutants; i.e., tRNA recognizing UAG and specifying glutamine) were produced, but their MFR were clearly

different. The mutant frequency data for back mutants and class 2 amber suppressor mutants correlated well with the theoretical curves (Sargentini and Smith, 1979) for the one-hit and two-hit mutagenic processes, respectively, in strain SR250 (Fig. 1a). In the fluence range 1–2.5 J m^{-2}, the suppressor MFR, but not the back MFR, correlates with the KC component (Fig. 1a). Thus, we assume that the KC mechanism is involved only in the production of class 2 amber suppressor mutants.

UV radiation-induced MFR data have been plotted with logarithmic coordinates (e.g., Witkin, 1974; Sargentini and Smith, 1979) in order to gain information regarding the number of radiation 'hits' required to produce a mutation. We have plotted our Lac^+ data (strain SR250) in this fashion (Fig. 1b) and found that the back mutants yield a slope close to 1.0, while the suppressor mutants have a slope close to 2.0. These data were also linearly regressed, fitting the expression: $\log MF = k + h \log f$; where MF is the UV radiation-induced mutant frequency, k is an extrapolation constant, h is the linear regression slope (i.e., presumably the number of hits required for mutation induction), and f is the UV radiation fluence. The mean linear regression slopes for the regressed data obtained with strain SR250 are

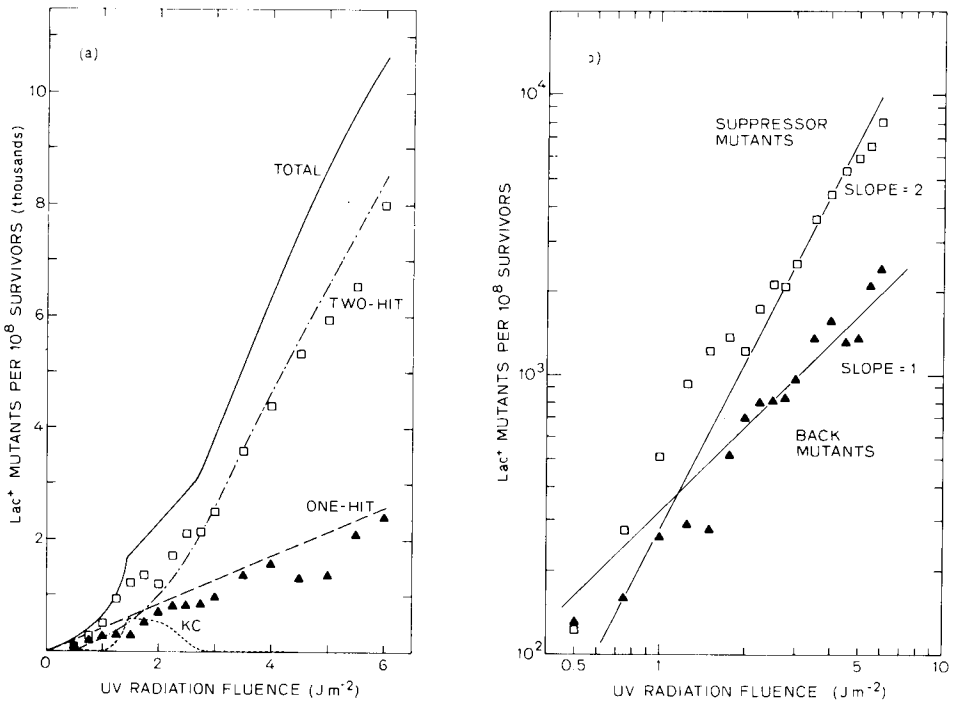

Fig. 1. UV radiation mutagenesis to Lac^+ of *E. coli* K-12 *uvrB5 lacZ53* (SR250). Lac^+ mutants were separated by bacteriophage T4 sensitivity into class 2 amber suppressor mutants (□) and back mutants (▲). These data (the means of data from 4 to 7 experiments) are superimposed over previously published curves (one-hit, two-hit, and KC) that were resolved from a total Lac^+ MFR curve for strain SR250 (Sargentini and Smith, 1979) (a), or their logarithmic values are compared to lines with slopes of 1.0 or 2.0 (b).

TABLE 2

MEAN LINEAR REGRESSION SLOPES FOR UV RADIATION-INDUCED Lac$^+$ BACK AND SUPPRESSOR MUTANTS OF *E. coli* K-12 *uvrB5 lacZ53* (SR250) [a]

Method for mean slope derivation	Back mutants		Class 2 amber suppressor mutants		Total Lac$^+$ mutants	
	2–6 J m^{-2}	All data	2–6 J m^{-2}	All data	2–6 J m^{-2}	All data
Mean of weighted slopes [b]	0.96 (53) [c]	1.15 (79)	1.77 (53)	1.45 (79)	1.46 (54)	1.49 (91)
Slope from regression of all data, all experiments	1.03 (53)	1.21 (79)	1.69 (53)	1.54 (79)	1.58 (54)	1.56 (91)

[a] UV radiation-induced mutant frequency response data for Lac$^+$ mutants (back, suppressor, and total) were converted to logarithmic values and then used to determine the *linear regression slope*.

[b] The slope value from each experiment (a total of seven) was multiplied by the number of data points (6–16 points for '2–6 J m^{-2}' and 8–19 points for 'All data') in that experiment. The products were averaged to obtain the *mean of weighted slopes*.

[c] The value in parentheses is the number of data points regressed. Negative data points (i.e., the result of induced mutant values that were less than the value for unirradiated cells) were excluded from the linear regression analysis, as were occasional widely disparate data points. In both instances, if a back or suppressor mutant point, in a given experiment, was excluded, then the corresponding suppressor or back mutant point, respectively, was also excluded. This explains the difference between the number of data points regressed in the back and suppressor mutant categories compared to the number regressed for the total mutant category.

presented in Table 2. Values derived for UV radiation fluences of 2–6 J m^{-2} are given separately because this analysis would allow us to mainly see the effect of the one-hit and two-hit mechanisms (Fig. 1a). This analysis also indicates that Lac^+ back mutants are produced by a one-hit mechanism. The suppressor mutants, however, even in the range of 2–6 J m^{-2}, have a regression slope that seems consistently less than 2.0 (i.e., ~ 1.7) (Table 2) and is suggestive of a MFR having both one-hit and two-hit components (as discussed in Sargentini and Smith, 1979).

In an attempt to clarify this situation, a second procedure was developed for analyzing back and suppressor mutants. This used strain SR825, the *lacZ53 leu-305* double-amber derivative of strain SR250. As with strain SR250, the spontaneous Lac^+ or Leu^+ revertants of strain SR825 were almost always back mutants (data not shown). The MFR for the total UV radiation-induced Lac^+ or Leu^+ mutants of strain SR825 are shown in Fig. 2a. These curves generally resemble the shape of the curve for the total MFR for Lac reversion in strain SR250, with the major difference being the lack of a low fluence, nonlinear MFR in the 0–1.5 J m^{-2} fluence range, as was seen for strain SR250 (included in Fig. 2a for comparison). The Lac^+ and Leu^+ mutants from the experiments described in Fig. 2a were separated into back or suppressor mutants, and the logarithmic values of these data were plotted (Fig. 2b, Lac^+; Fig. 2c, Leu^+). As with strain SR250, the Lac^+ back mutants of strain SR825 had a slope of 1.0 (Fig. 2b). The Leu^+ back mutants comprised only about 2% of the total UV radiation-induced Leu^+ mutants (data not shown). The suppressor MFR for strain SR825 showed a large component with a slope of 1.0 at low fluences (Figs. 2b, c), while the low fluence suppressor MFR for strain SR250 was nonlinear (Fig. 1a) and had a slope of ~ 2 (Fig. 1b). A similar change from a nonlinear low fluence MFR (approximating a two-hit phenomenon) to a linear MFR (approximating a one-hit phenomenon) has also been accomplished in *E. coli* B/r *uvrA* by inducing the cells for error-prone repair (reviewed in Witkin, 1976) either with thermo-induction of a *tif* strain (Witkin, 1974, 1975) or with a UV radiation pretreatment (Doudney, 1976). However, unlike the thermo-induced *tif* strain, strain SR825 was not significantly different from the parent strain (SR250) in its level of spontaneous mutagenesis (Lac^+) (data not shown). We presume that the KC mechanism was modified in strain SR825 (relative to strain SR250), because the 'bump' is missing in the total (Fig. 2a) and suppressor MFR curves (cf. Figs. 1b and 2b, c).

The net result from both procedures for differentiating UV radiation-induced mutants was that the production of back mutants and suppressor mutants (especially at fluences of 3–6 J m^{-2}) was associated with the respective one-hit and two-hit mutagenic mechanisms proposed earlier (Sargentini and Smith, 1979). A comparison of MFR shapes for strains SR250 and SR825, and knowledge of the effects on the MFR shape associated with the induction of error-prone repair in the experiments of Witkin (1974, 1975) and Doudney (1976) suggest to us that the KC process is inducible and is also associated with suppressor mutant production (the small bump in Figs. 1a, b and the slope = 1.0 component in Figs. 2b, c), but this point needs verification.

Even though the correlation of back and suppressor mutant production with the previously proposed theoretical MFR components (Sargentini and Smith, 1979) provides good support for the validity of those components, several questions still

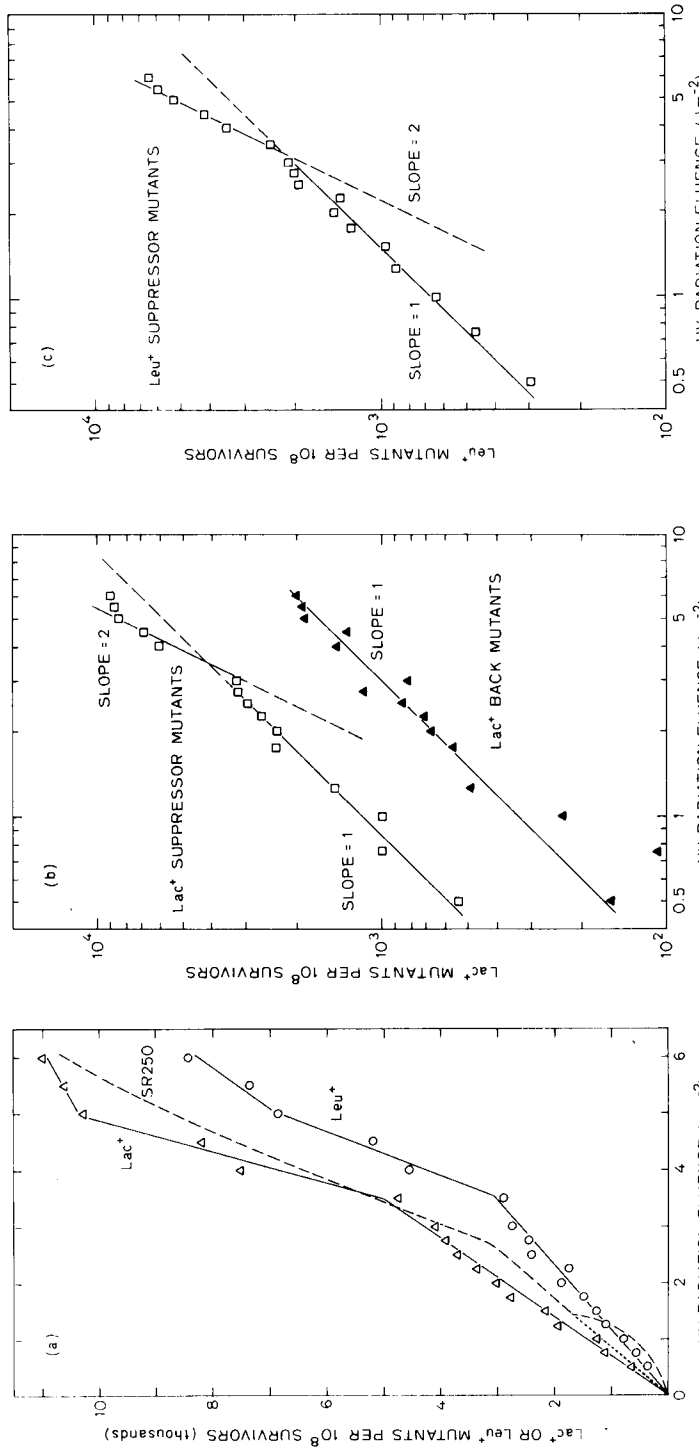

Fig. 2. UV radiation mutagenesis to Lac^+ or Leu^+ of *E. coli* K-12 *uvrB5 lacZ53 leu-308* (SR825). Data for total Lac^+ or Leu^+ revertants are the average of three experiments (a). The total Lac^+ mutant curve (— — —) for strain SR250 from Fig. 1a is included for comparison. The line (· · · · · ·) represents the effect of preinduction of mutagenic repair, as per Doudney (1976). Total Lac^+ or Leu^+ mutants from the three experiments were separated by testing the unselected phenotype (Lac^+ or Leu^+) into suppressor mutants (□) and back mutants (▲, Lac^+ only), and their logarithmic values were compared to lines with slopes of 1.0 or 2.0 (b, c; respectively).

remain. (1) Why did Bridges et al. (1967), who performed a similar study for Trp (ochre) reversion (from tryptophan auxotrophy) in *E. coli* B/r strain WP2, find that the UV radiation induction of suppressor mutants showed two-hit kinetics and back mutants showed one-hit kinetics in the *uvr*$^+$ strain, but both classes of mutants showed two-hit kinetics in the *uvrA* strain (WP2$_s$)? A possible explanation is the difference in strain background and mutation assay used. (2) What is the molecular nature of the two-hit and KC mutagenic mechanisms, and why does this latter mechanism cease to function above ~ 3 J m^{-2} in a *uvrB* strain? We suspect that our KC mechanism is the two-hit process described by Witkin (1976) where one hit induces the premutational lesion and the other hit somehow induces error-prone repair (Doudney and Young, 1962; Witkin and George, 1973; Doudney, 1975). The data to support this two-hit process (Witkin, 1976; Doudney, 1976) not only shows an inducible character, but also is consistent with this process ceasing to operate in *uvrA* cells irradiated with more than 3 J m^{-2}*. We suspect that our two-hit mechanism is the alternative two-hit process reviewed by Witkin (1976), where the two hits produce lesions in close proximity but in opposite strands of the DNA duplex. When this portion of the chromosome is replicated, overlapping daughter-strand gaps are formed and together may represent a lesion that can only be repaired by an error-prone process (Doubleday et al., 1975; Sedgwick, 1975a, b, 1976). This two-hit process would be expected to play a larger role at higher fluences (as our two-hit mechanism does), where lesions have a higher probability of being produced in close proximity to each other. Another possible explanation for our two-hit component is that it arises from a stochastic interdependence of mutation and killing, i.e., δ-effects (Eckardt and Haynes, 1977). However, the parallelness of the UV radiation survival curve for strain SR250 and the 'apparent survival' curve for the Lac$^+$ mutants of strain SR250 (calculated according to Eckardt et al., 1982) over the range of 0–6 J m^{-2} (data not shown) suggests that a true two-hit mechanistic component (and not δ-effects) is involved in our data. (3) What is the molecular nature of the one-hit process, and why does it correlate only with the induction of back mutants? It may simply result from single one-hit lesions being acted upon by a constitutive level of error-prone postreplication repair, or alternatively, it may result from base excision repair (reviewed in Lindahl, 1979). In connection with the latter possibility, a *uvr* gene-independent excision repair process for nondimer photoproducts has been reported (Youngs and Smith, 1976).

Acknowledgements

This investigation was supported by Public Health Service research grant CA-02896, and research program grant CA-10372 from the National Cancer Institute (K.C.S.), and research grant GM 21788 (R.C.B.). We wish to thank Drs. Rakesh C. Sharma and Tzu-chien V. Wang for helpful criticism, Drs. R.H. Haynes and R.G. Miller for helpful advice in the evaluation of our data, and Diane Harper, Cheryl A. Jue, and John C. Miller for skillful technical assistance.

* Note: We are discussing Doudney's fluence data after making the two-fold correction that he indicated was appropriate (Doudney, 1977).

References

Bachmann, B.J., and K.B. Low (1980) Linkage map of *Escherichia coli* K-12, edn. 6, Microbiol. Rev., 44, 1–56.

Bockrath, R.C., and J.E. Palmer (1977) Differential repair of pre-mutational UV-lesions at tRNA genes in *E. coli*, Mol. Gen. Genet., 156, 133–140.

Bridges, B.A., R.E. Dennis and R.J. Munson (1967) Differential induction and repair of ultraviolet damage leading to true reversions and external suppressor mutations of an ochre codon in *Escherichia coli* B/r WP2, Genetics, 57, 897–908.

Doubleday, O.P., B.A. Bridges and M.H.L. Green (1975) Mutagenic DNA repair in *Escherichia coli*, II. Factors affecting loss of photoreversibility of UV-induced mutations, Mol. Gen. Genet., 140, 221–230.

Doudney, C.O. (1975) The two lesion hypothesis for UV-induced mutation in relation to recovery of capacity for DNA replication, in: P. Hanawalt and R.B. Setlow (Eds.), Molecular Mechanisms for the Repair of DNA, part A, Plenum, New York, pp. 389–392.

Doudney, C.O. (1976) Complexity of the ultraviolet mutation frequency response curve in *Escherichia coli* B/r: SOS induction, one-lesion and two-lesion mutagenesis, J. Bacteriol., 128, 815–826.

Doudney, C.O. (1977) Is DNA polymerase I necessary for most UV-induced mutation from tryptophan auxotrophy in *Escherichia coli* B/r WP2? Mutation Res., 45, 147–152.

Doudney, C.O., and C.S. Young (1962) Ultraviolet light induced mutation and deoxyribonucleic acid replication in bacteria, Genetics, 47, 1125–1138.

Eckardt, F., and R.H. Haynes (1977) Kinetics of mutation induction by ultraviolet light in excision-deficient yeast, Genetics, 85, 225–247.

Eckardt, F., R.H. Haynes, B.A. Kunz and D. Averbeck (1982), Mutation Res., in press.

Lindahl, T. (1979) DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision-repair, Progr. Nucleic Acid Res. Mol. Biol., 22, 135–192.

Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sargentini, N.J., and K.C. Smith (1979) Multiple, independent components of ultraviolet radiation mutagenesis in *Escherichia coli* K-12 *uvrB5*, J. Bacteriol., 140, 436–444.

Sedgwick, S.G. (1975a) Genetic and kinetic evidence for different types of postreplication repair in *Escherichia coli* B, J. Bacteriol., 123, 154–161.

Sedgwick, S.G. (1975b) Inducible error-prone repair in *Escherichia coli*, Proc. Natl. Acad. Sci. (U.S.A.), 72, 2753–2757.

Sedgwick, S.G. (1976) Misrepair of overlapping daughter-strand gaps as a possible mechanism for UV-induced mutagenesis in *uvr* strains of *Escherichia coli*; A general model for induced mutagenesis by misrepair (SOS repair) of closely spaced DNA lesions, Mutation Res., 41, 185–200.

Witkin, E.M. (1963) One-step reversion to prototrophy in a selected group of multiauxotrophic substrains of *Escherichia coli*, Genetics, 48, 916.

Witkin, E.M. (1974) Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r; Evidence that ultraviolet mutagenesis depends upon an inducible function, Proc. Natl. Acad. Sci. (U.S.A.), 71, 1930–1934.

Witkin, E.M. (1975) Persistence and decay of thermoinducible error-prone repair activity in nonfilamentous derivatives of *tif-1 Escherichia coli* B/r; The timing of some critical events in ultraviolet mutagenesis, Mol. Gen. Genet., 142, 87–103.

Witkin, E.M. (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*, Bacteriol. Rev., 40, 869–907.

Witkin, E.M., and D.L. George (1973) Ultraviolet mutagenesis in *polA* and *uvrA polA* derivatives of *Escherichia coli* B/r; Evidence for an inducible error-prone repair system, Genetics, 73 (Suppl.), 91–108.

Youngs, D.A., and K.C. Smith (1973) Evidence for the control by *exrA* and *polA* genes of two branches of the *uvr* gene-dependent excision repair pathway in *Escherichia coli* K-12, J. Bacteriol., 116, 175–182.

Youngs, D.A., and K.C. Smith (1976) Single-strand breaks in the DNA of the *uvrA* and *uvrB* strains of *Escherichia coli* K-12 after ultraviolet irradiation, Photochem. Photobiol., 24, 533–541.