Suppressible Base Substitution Mutations Induced by Angelicin (Isopsoralen) in the *Escherichia coli lacI* Gene: Implications for the Mechanism of SOS Mutagenesis

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Angelicin- plus near-UV-induced mutations were *umuC* dependent in *Escherichia coli* K-12. Angelicin, a monofunctional psoralen derivative, is believed to damage DNA almost exclusively at pyrimidine bases. To broaden our knowledge about the mutagenic specificity of SOS-dependent mutagens, we determined the mutational specificity of 233 suppressible *lacI* mutations induced by angelicin. More than 90% of the nonsense mutations arose via transversion substitutions. The three most frequently mutated sites were at A · T base pairs and accounted for more than one-third of all induced nonsense mutations. The two hottest sites were at the only occurrences of the 5'-TATA-3' tetranucleotide in *lacI*, a sequence expected to be a preferred binding site for a psoralen. Both A · T-to-T · A and A · T-to-C · G transversions were well induced by angelicin treatment, but the frequency of each transversion depended on the particular site. We also detected significant induction of transversion mutations at G · C sites. The induction of transversions by an SOS-dependent mutagen that generates lesions at pyrimidines supports the idea that DNA lesions influence the selection of bases that are incorporated via the process of SOS repair.

The mutagenicity of DNA lesions that block DNA synthesis in *Escherichia coli* depends largely on inducible functions provided by the *umuDC* operon and the *recA* gene (21, 29, 43). Although the biochemical functions of the UmuDC and RecA products in SOS mutagenesis (43) are unknown, one important feature of the process involving these proteins has been deduced: mutations generated via SOS mutagenesis occur at sites of DNA damage (9, 14, 28). Replication bypass of lesions on a template strand is the most straightforward explanation for the targeting of mutations to sites of DNA damage (16).

The base substitution specificities of several different SOS-dependent mutagens were determined by employing the *E. coli lacI* nonsense system (27, 28). All but two of the mutagens analyzed by this system, UV and neocarzinostatin, were agents which damage DNA preferentially at purines (summarized in reference 9). To broaden the base of knowledge about base substitution mutagenesis via the SOS response, we decided to examine the mutagenic specificity of an additional SOS-dependent mutagen that preferentially damages DNA at pyrimidines. Here we present the spectrum of *lacI* nonsense mutations induced by angelicin. Angelicin is a monofunctional psoralen that engages in specific photochemical reactions with pyrimidine bases in DNA after irradiation with light in the near-UV range (2, 6). The major products of reactions between psoralens and DNA are adducts linked by a cyclobutane ring to the 5-6 position of pyrimidines; these adducts block DNA replication in vitro (31, 32). We show that mutation induction by angelicin treatment is *umuC* dependent in *E. coli* and evaluate the distribution of 233 angelicin-induced *lacI* nonsense mutations. Our analysis leads us to suggest that angelicin adducts to pyrimidines are mutagenic lesions that induce transversions at both A · T and G · C base pairs via a replication bypass mechanism.

MATERIALS AND METHODS

Bacterial strains. *E. coli* EE270 was constructed by transducing the *uvrB5* allele (1, 18) linked to a Tn10 insertion in *chlA* into *E. coli* GM1 (3). The resulting strain was excision repair defective and carried an F' *pro*⁺ *lac*⁺ episome in a $\Delta(lac\ pro)\ thi$ chromosomal background. *E. coli* TK603 and TK610 have been described previously (19); they are an isogenic pair of *uvrA* strains differing only at the *umuC* locus. TK603 is wild type for *umuC*, while TK610 bears the *umuC36* allele. The additional genetic markers for these strains are as follows: *thr-1*, *leu-6*, *proA2*, *his-4*, *ilv-325*, *thi-1*, *lacY1*, *galK2*, *ara-14*, *xyl-14*, *mtl-1*, *tsx-33*, *strA31*, *supE44*.

Media. The preparation of selective media and indicator plates has been described by Coulondre and Miller (3). The minimal medium for culturing bacteria before mutagenesis contained E salts (42), 0.2% glucose, and 5 μ g of thiamine per ml. Broth plates were LP (26).

Mutagenesis. Angelicin (the generous gift of Madhu Pathak) was stored with dessicant at 4°C and dissolved to a concentration of 1 mg/ml of 95% ethanol as needed. Angelicin treatment of TK603, TK610, and EE270 was identical except for the indicated differences. Bacteria were grown in minimal medium to a concentration of 2×10^8 cells per ml (or to a Klett reading of 60 [green filter] for the TK603 and TK610 strains), washed once by centrifugation, suspended at the initial cell density in 10 mM MgSO₄ (or single-strength E salts for the TK603 and TK610 strains), and starved for 1 h at 37°C before mutagen treatment. Treatment consisted of incubating the starved cells with angelicin at a concentration of 10 μ g/ml for 45 min (1 h for the TK603 and TK610 strains) at room temperature, followed by irradiating 1.5-ml samples distributed into glass petri dishes (60 by 15 mm) placed 15.5 cm below two 40-W BL fluorescent near-UV lamps for 50 s (or 30, 60, and 90 s for the TK603 and TK610 strains). The intensity of the lamps

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TABLE 1. Mutability by angelicin is *umuC* dependent

UVA dose (s)	Viable cells/ml		His ⁺ revertants/ml	
	Umu ⁺	Umu ⁻	Umu ⁺	Umu ⁻
0	8.5×10^8	1.9×10^9	200	50
30	5.0×10^8	6.3×10^8	1,350	20
60	1.2×10^8	2.6×10^8	1,950	20
90	5.2×10^7	7.0×10^7	940	60

as measured with an International Light germicidal photometer with a WB 320 filter was estimated to be 67 J/m² per s. Irradiated cells were diluted and plated directly on broth plates to determine survival levels. Samples (0.1 ml) of irradiated EE270 cells were inoculated into 150 tubes containing 5 ml of minimal medium and grown out at 37°C overnight. Samples from each overnight culture were spread on plates containing phenyl-β-D-galactoside as the sole carbon source to select for Lac constitutives (3). Nonsense mutations were identified as previously described, by screening for suppressible mutations with suppressors Su2, Su3, SuB, and SuC (3). One amber and one ochre mutant from each culture were ultimately chosen and identified by comparing their suppression patterns and map positions to the known *lacI* nonsense alleles (3). To estimate induction of His⁺ revertants in the treated TK603 and TK610 strains, we spread 0.1-ml samples on minimal plates containing all nutritional requirements in excess except for histidine which was present at 1 μg/ml. These plates were incubated at 37°C for 3 days at which time the number of His⁺ revertants was counted.

RESULTS

SOS dependence of angelicin-induced mutations. To determine whether angelicin-induced mutations were SOS dependent, we monitored the reversion of the *his-4* ochre allele in Umu⁺ and Umu⁻ strains. The results are summarized in Table 1. Angelicin treatment led to a 100-fold increase in His⁺ revertants in the Umu⁺ strain. The *umuC36* mutation, however, which eliminates UV mutability (19), completely eliminated the induction of His⁺ revertants by angelicin treatment. Thus, we conclude that angelicin-induced reversion of *his-4* is SOS dependent. As reversion of the *his-4* ochre allele can occur via several different base substitution events (20) it seems reasonable to infer that, in general, base substitution mutations induced by angelicin are going to be SOS dependent. These results are in accord with the observation that pMK101 was required for the efficient induction by angelicin of *hisG46* revertants in *Salmonella typhimurium* (23).

Induction of *lacI* mutants by angelicin. Irradiating *E. coli* EE270 with near-UV in the presence of 10 μg of angelicin per ml resulted in a survival level of approximately 5% (4.8×10^{-2}). The incidence of Lac constitutive mutants among the outgrown population of surviving cells was 4.8×10^{-5} or 4.4-fold greater than in controls (untreated bacteria or bacteria treated with either angelicin only or near-UV only). Of the induced Lac constitutives (5,500 screened), 6.4% were suppressible by amber suppressors and 7.6% were suppressible by ochre suppressors, whereas only 4% of 200 spontaneous Lac constitutives were suppressible by amber or ochre suppressors. Thus, the incidence of nonsense mutants induced by angelicin plus near-UV was 15-fold over background. As fewer than 70 sites in the entire *lacI* gene are potential targets, the high proportion of *lacI* nonsense mu-

tations among all induced Lac constitutives suggests that base substitution mutations account for a large fraction of angelicin-induced mutations.

We assigned 233 independent occurrences of angelicin-induced *lacI* nonsense mutations (125 ochre and 108 amber) to 1 of the 69 sites at which amber and ochre mutations arise by single base pair substitutions (27). The data describing this collection are summarized in Tables 2 to 4, in Fig. 1, and in the following sections.

Summary of angelicin-induced mutations by class of mutation. Angelicin induced all the base substitutions which lead to *lacI* nonsense mutations (Table 2; nonsense mutations are not generated by A · T-to-G · C transitions). Transversion mutations accounted for 94% of all the nonsense mutants and were detected at 73% of the available sites (37 of 51), whereas the G · C-to-A · T transition was detected at less than half of the 26 available sites. Approximately 40% of the mutations arose via G · C-to-T · A or A · T-to-T · A transversions. Transversions were detected at 89% (24 of 27) of the potential G · C sites and at 54% (13 of 24) of the potential A · T sites. Ignoring the contribution of the major hotspots, the difference in the degree of saturation between the potential G · C and A · T transversion sites appears to be significant (with Yates correction, $\chi^2 = 6.05$; $P < 0.02$).

We conclude that transversions at both A · T and G · C sites are the major base substitution mutations induced by angelicin treatment. The small number of transition mutations in our collection are probably not spontaneous in origin since they differ from the spontaneous spectrum (3).

Site specificity and frequency distribution of angelicin-induced mutations. Table 3 summarizes the data by site of occurrence, and Fig. 1 displays the same data arranged by frequency of occurrence. Clearly, angelicin-induced mutations were not randomly distributed among the monitorable sites. The three most frequently mutated sites were at A · T base pairs and accounted for 5% of all Lac constitutives induced by angelicin. The two hottest sites (O2, monitoring the A · T-to-T · A transversion, and O33/A30, monitoring either the A · T-to-T · A or A · T-to-C · G transversion) were at the only two occurrences of the 5'-TATA-3' tetranucleotide sequence in *lacI*. Some G · C residues were also hotspots: nonsense mutations at four G · C sites were recovered at frequencies comparable to those of three of the well-mutated A · T sites and higher than those of nine other A · T sites (Fig. 1).

No mutations were detected at 28 sites representing examples of all monitorable base substitution mutations. Assuming that, hotspots aside, the frequency distribution fits a Poisson distribution, we would have expected not to recover mutations at nine sites in a sample of this size (see reference

TABLE 2. Summary of base substitution events in the *lacI* gene generated by angelicin plus near-UV

Substitution	No. of monitorable sites	No. of sites found	Total no. of occurrences	% of analyzed mutations
G · C to A · T	26	12	15	6
G · C to T · A	24	22	93	40
A · T to T · A	19	10	91	39
A · T to C · G	5	3	31	13
G · C to C · G	3	2	3	1
Total events	77	49	233	
Amber	37	29	108	
Ochre	40	20	125	

14 for method of analysis). The difference between observed and expected is significant ($P < 0.01$ by chi-square test) and provides further evidence for the idea that mutations are targeted to sites of DNA damage (9, 14, 28). Half of the cold sites were potential G · C-to-A · T transitions. Since angelicin nearly saturated the G · C sites at which transver-

TABLE 3. Distribution of *lacI* nonsense mutations induced by angelicin plus near-UV^a

Base substitutions	Amber site	No. of independent occurrences	Ochre site	No. of independent occurrences
G · C to A · T	A5	1	O9	0
	A6 ^b	1	O10	0
	A9	1	O11	0
	A15 ^b	2	O13	0
	A16	0	O17	0
	A19	1	O21	0
	A21	1	O24	0
	A23	1	O27	0
	A24	1	O28	0
	A26	1	O29	1
	A31	1	O34	0
	A33	3	O35	0
	A34 ^b	0		
	A35	0		
G · C to T · A	A2	8	O3	0
	A7	3	O6	2
	A10	10	O7	1
	A12	2	O8	1
	A13	4	O14	3
	A17	1	O15	2
	A20	3	O19	8
	A25	2	O20	5
	A27	4	O25	3
	A28	6	O26	5
			O30	16
			O31	1
			O32	5
			O36	0
A · T to T · A	A11	2	O1	1
	A18	11	O2	35
	A32	0	O4	0
	X9	0	O5	0
	A36	4	O12	0
			O16	2
			O18	5
			O22	19
			O23	3
			O33	7
			Y3	0
			Y4	0
			Y6	0
			Y8	0
A · T to C · G	A3	4		
	A4	0		
	A14	3		
	A22	0		
	A30	24		
G · C to C · G	A1	1		
	A8	2		
	A29	0		

^a Sites at which nonsense mutations are detected are identified by their amber (A) or ochre (O) alleles (3).

^b Sites containing 5-methylcytosines (CCAGG). These are spontaneous *lacI* hotspots.

FIG. 1. Frequency distribution of 233 angelicin-induced amber and ochre mutations in the *lacI* gene of *Escherichia coli*. The histograms show the number of sites at which *k* mutations were found (14).

sions are monitorable (Tables 2 and 3), the scarcity of transition mutations at G · C sites probably reflects the mutagenic specificity of angelicin lesions.

Mutagenic specificity of angelicin-induced mutations. Nonsense mutations at the eight tyrosine codons in *lacI* can arise via two different transversions (Table 4): at TAC codons via either G · C-to-T · A or G · C-to-C · G transversions and at TAT codons via either A · T-to-T · A or A · T-to-C · G transversions. An analysis of mutations at these sites revealed whether there was preferential induction of one transversion over another.

One of the three TAC targets was well mutated by angelicin treatment (*tyr-273*; Table 4). All 16 mutations at *tyr-273* arose via the G · C-to-T · A transversion. The other two TAC targets were too poorly mutated to reveal any specificity. We found no obvious feature of primary sequence surrounding the different TAC targets which correlated with their mutability.

Two of the five TAT codons, *tyr-126* and *tyr-282*, were well mutated (Table 4). *tyr-282* has one of the two occurrences of the 5'-TAT-3' tetranucleotide in *lacI*, and here we found a strong preference (24 to 7) for the A · T-to-C · G transversion over the A · T-to-T · A transversion (Table 4). Among the eight mutations collected at *tyr-126*, however, there was no obvious preference for one transversion over the other.

DISCUSSION

Previous investigations on mutagenic specificity of psoralens. The studies of Drake and McGuire (8) on psoralen, Piette et al. (30) on 4'-hydroxymethyl-4,5',8-trimethylpsoralen, Levin and Ames (23) on angelicin, and Yatagai and Glickman (44) on 8-methoxypsoralen (8-MOP) showed that psoralens can induce transversion mutations. Piette et al. (30) and Levin and Ames (23) examined mutations at a small number of sites in their respective systems and concluded that psoralens specifically generated A · T-to-C · G transversions. Yatagai and Glickman (44) determined by sequence analysis the base changes which generated 131 *lacI* mutants induced by 8-MOP. They found that 8-MOP, a bifunctional psoralen, induced frameshift as well as base substitution mutations. The A · T-to-T · A substitution was the transversion most frequently induced by 8-MOP, but the site or sequence specificity of transversion mutations was not described (44). Saffran and Cantor (35, 36)

observed the induction of transition mutations in the *tetA* locus of plasmid pBR322 after cross-linking of DNA by a psoralen derivative carrying sulphhydryl arms to direct it to mercurated nucleotides in DNA. This result is quite different from those obtained by other investigators and ourselves and may be peculiar to the system used by these authors.

We monitored mutation induction by angelicin treatment at approximately 70 different sites in the *lacI* gene of *E. coli* and found evidence for the specific induction of several types of transversion mutations. Our results, therefore, extend our understanding of the mutational specificity of psoralens and underline the importance and value of monitoring mutations at many sites to gather information about mutagenic specificity.

Spectrum of *lacI* nonsense mutations induced by angelicin. The two most frequently mutated sites in the *lacI* gene were at the only occurrences at the 5'-TATA-3' tetranucleotide, a sequence which is expected to be a preferred binding site for planar molecules such as psoralens (5, 38, 40). Thus, for these sites there appears to be a good correspondence between psoralen chemistry and biology.

Angelicin treatment induced both types of transversion mutations at A · T sites, but the frequencies of each type varied widely from site to site. At the hottest spot, O2 (*leu-6*), we detected 35 independent occurrences of the A · T-to-T · A transversion. At the second hottest spot, O33/A24 (*tyr-282*), A · T-to-C · G transversions dominated over A · T-to-T · A transversions by 24 to 7. O2 and O33/A24 alleles are generated at different sites within the 5'-TATA-3' sequence: O2 arises via a change in the first base pair, whereas O33/A24 arise via changes in the second base pair. As only one base substitution was monitorable at the O2 site, we do not know whether the specificity difference between O2 and O33/A24 is more apparent than real.

Angelicin treatment also induced transversion mutations at G · C sites but, in contrast to A · T sites, these were predominantly of one type. Thus, at O30/A29 (*tyr-273*) we found that the G · C-to-T · A transversion dominated over G · C-to-C · G transversions by 16 to 0. Three other sites at which the G · C-to-T · A transversion can be monitored were also well induced (A2, A10, and O19). Of course, we cannot rule out the possibility that had there been additional sites to monitor the G · C-to-C · G event we might have found an instance of this event being strongly induced. The paucity of G · C-to-A · T transitions in our collection (which were monitorable at 26 different sites) implies, however, that psoralen damage to G · C sites preferentially induces transversion mutations.

Identity of mutagenic lesions induced by angelicin. The major chemical damage to DNA after near-UV irradiation of DNA in the presence of psoralens results from adduct formation at the 5-6 position of pyrimidines with a preference for thymine over cytosine (reviewed in reference 2). Piette and Hearst (32) investigated the sites of termination of DNA synthesis in vitro using single-stranded DNA templates modified with 4'-hydroxymethyl-4,5',8-trimethyl-psoralen. They found that every termination site occurred at the base preceding each thymine residue and some cytosine residues. Studies with 8-MOP revealed a strong preference for adduct formation with thymidine on the 3' side of a 5'-TpA site (40). Other investigations suggested that alternating pyrimidine-purine sequences were preferred DNA-binding sites for planar molecules including psoralens (5, 38). Rigorous structural analyses of psoralen-DNA adducts have been published for only some bifunctional psoralens (reviewed in reference 2), but recent studies by Dall'Acqua et

TABLE 4. Angelicin-induced mutations at the TAC and TAT tyrosine residues in the *lacI* gene

Sites ^a	Coding position (and normal codons)	Independent occurrences of mutant codons	
		TAG	TAA
A1, O3	Tyr-7 (TAC)	1	0
A8, O8	Tyr-47 (TAC)	2	1
A29, O30	Tyr-273 (TAC)	0	16
A3, O4	Tyr-12/4/0 (TAT)		
A4, O5	Tyr-17/0/0 (TAT)		
A14, O18	Tyr-126/3/5 (TAT)		
A22, O23	Tyr-204/0/3 (TAT)		
A30, O33	Tyr-282/24/7 (TAT)		

^a Both amber and ochre mutations can be generated at these sites, allowing both transversions to be monitored.

al. (6) suggested that angelicin follows the rules governing monoadduct formation by bifunctional psoralens.

Minor psoralen DNA lesions can be induced by psoralen treatment via the generation of active oxygen species (2, 4), and de Mol et al. (7) concluded that singlet oxygen effects were responsible for a substantial fraction of the mutagenicity of 8-MOP. It may be possible that some of the less frequently observed *lacI* mutations (e.g., the transitions) arose as a consequence of minor oxidative damage to DNA. The observation that mutational hotspots correspond to preferred psoralen binding sites makes it seem reasonable to assume, however, that at least the well-induced mutagenic events were induced by the major psoralen photoadducts to pyrimidines.

Significance of transversion induction by psoralens. A transversion mutation arises when a purine replaces a pyrimidine or a pyrimidine replaces a purine. Thus, a straightforward, if tautologous, explanation for the induction of transversions by psoralens is that replication bypass of pyrimidine-psoralen adducts is accompanied by preferential incorporation of dTMP or dCMP. Had purines been incorporated preferentially opposite damaged pyrimidines, transition mutations at G · C sites would have been a prominent feature of the angelicin-induced *lacI* spectrum. Other interpretations invoking more complicated rules for base incorporation opposite psoralen-damaged pyrimidines cannot, of course, be excluded. The preferential incorporation of pyrimidines opposite psoralen lesions would appear to be a property associated with the psoralen lesion as two other pyrimidine-damaging treatments, UV and neocarzinostatin, preferentially induce transition mutations (3, 12, 33).

The rules governing SOS-dependent base incorporation opposite lesions are unknown. DNA polymerases have a tendency to incorporate dAMP opposite apurinic sites in vitro (22, 24, 39). Preferential dAMP incorporation accounts for induction of transversion mutations by apurinic sites and transition mutations by apyrimidinic sites in SOS-induced bacteria (10-13, 15, 22, 24, 33, 34, 37; P. L. Foster and E. F. Davis, Proc. Natl. Acad. Sci. USA, in press). The preference for dAMP, however, is not absolute. For example, Kunkel (22) showed that dTMP is the next most likely nucleotide to be incorporated at an apurinic site and that the magnitude of the preference for dAMP over dTMP varied among the apurinic sites.

A specific base-pairing mechanism resembling the model for transversion mutations proposed by Topal and Fresco (41) was suggested by Piette et al. (30) to explain the induction of A · T-to-C · G transversions by psoralen

adducts to thymine. Piette et al. (30) proposed that a psoralen-modified thymine in the template strand rotates to the *syn* conformation and subsequently pairs with dCMP. Although the model may be extended to explain the induction of G · C-to-T · A transversions by psoralen adducts to C, it does not, however, seem able to account for the induction of A · T-to-T · A transversions by angelicin. Biochemical studies with psoralen-modified DNA may shed light on the mechanisms of transversion induction.

The results described here lead us to imagine that bypass replication of a DNA lesion via SOS repair can, in principle, incorporate any of the four common deoxynucleoside monophosphates: the specific deoxynucleoside monophosphate which is incorporated is selected by rules that are sensitive to the chemical features of a particular lesion and its surrounding bases. SOS mutagenesis may, therefore, share features in common with SOS-independent mutagenesis induced by base analogs (17) or simple alkylating agents (25).

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LITERATURE CITED

- Backendorf, C., H. Spaink, A. P. Barbeiro, and P. van de Putte. 1986. Structure of the *uvrB* gene of *Escherichia coli*. Homology with other DNA repair enzymes and characterization of the *uvrB5* mutation. *Nucleic Acids Res.* **14**:2877–2890.
- Cimino, G. D., H. B. Gamper, S. T. Isaacs, and J. E. Hearst. 1985. Psoralens as photoactive probes of nucleic acid structure and functions: organic chemistry, photochemistry, and biochemistry. *Annu. Rev. Biochem.* **54**:1151–1193.
- Coulondre, C., and J. H. Miller. 1977. Genetic studies of the *lac* repressor. IV. Mutagenic specificity in the *lacI* gene of *Escherichia coli*. *J. Mol. Biol.* **117**:577–606.
- Dall'Acqua, F. 1985. Furocoumarin chemistry and its main biological implications. *Curr. Probl. Dermatol.* **15**:137–163.
- Dall'Acqua, F., M. Terbojevich, S. Marciani, D. Vedaldi, and M. Recher. 1978. Investigations on the dark interaction between furocoumarins and DNA. *Chem. Biol. Interact.* **21**:103–115.
- Dall'Acqua, F., D. Vedaldi, S. Caffieri, A. Guiotto, F. Bordin, and G. Rodighiero. 1984. Chemical basis of the photosensitizing activity of angelicins. *Natl. Cancer Inst. Monogr.* **66**:55–60.
- de Mol, N. J., G. M. Beijersbergen van Henegouwen, G. R. Mohn, B. W. Glickman, and P. M. van Kleef. 1981. On the involvement of singlet oxygen in mutation induction by 8-methoxypsoralen and UVA irradiation in *Escherichia coli* K-12. *Mutat. Res.* **82**:23–30.
- Drake, J. W., and J. McGuire. 1967. Properties of *r* mutants of bacteriophage T4 photodynamically induced in the presence of thiopyronin and psoralen. *J. Virol.* **1**:260–267.
- Eisenstadt, E. 1987. Analysis of mutagenesis, p. 1016–1033. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
- Eisenstadt, E., A. J. Warren, J. Porter, D. Atkins, and J. H. Miller. 1982. Carcinogenic epoxides of benzo[a]pyrene and cyclopenta[cd]pyrene induce base substitutions via specific transversions. *Proc. Natl. Acad. Sci. USA* **79**:1945–1949.
- Eisenstadt, E., M. Wolf, and I. H. Goldberg. 1980. Mutagenesis by neocarzinostatin in *Escherichia coli* and *Salmonella typhimurium*: requirement for *umuDC* or plasmid pKM101. *J. Bacteriol.* **144**:656–660.
- Foster, P. L., and E. Eisenstadt. 1983. Distribution and specificity of mutations induced by neocarzinostatin in the *lacI* gene of *Escherichia coli*. *J. Bacteriol.* **153**:379–383.
- Foster, P. L., and E. Eisenstadt. 1985. Induction of transversion mutations in *Escherichia coli* by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine is SOS dependent. *J. Bacteriol.* **163**:213–220.
- Foster, P. L., E. Eisenstadt, and J. Cairns. 1982. Random components in mutagenesis. *Nature (London)* **299**:365–367.
- Foster, P. L., E. Eisenstadt, and J. H. Miller. 1983. Base substitution mutations induced by metabolically activated aflatoxin B₁. *Proc. Natl. Acad. Sci. USA* **80**:2695–2698.
- Friedberg, E. C. 1985. DNA repair. W. H. Freeman and Company, New York.
- Goodman, M. F., R. L. Hopkins, R. Lasken, and D. N. Mhaskar. 1985. The biochemical basis of 5-bromouracil- and 2-aminopurine-induced mutagenesis. *Basic Life Sci.* **31**:409–423.
- Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics* **53**:1119–1136.
- Kato, T., and Y. Shinoura. 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Mol. Gen. Genet.* **156**:121–131.
- Kato, T., Y. Shinoura, A. Templin, and A. J. Clark. 1980. Analysis of ultraviolet-light-induced suppressor mutations in the strain of *Escherichia coli* K-12: an implication for molecular mechanisms of UV mutagenesis. *Mol. Gen. Genet.* **180**:283–291.
- Kitagawa, Y., E. Akaboshi, H. Shinagawa, T. Horii, H. Ogawa, and T. Kato. 1985. Structural analysis of the *umu* operon required for inducible mutagenesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **82**:4336–4340.
- Kunkel, T. A. 1984. Mutational specificity of depurination. *Proc. Natl. Acad. Sci. USA* **81**:1494–1498.
- Levin, D. E., and B. N. Ames. 1986. Classifying mutagens as to their specificity in causing the six possible transitions and transversions: a simple analysis using the *Salmonella* mutagenicity assay. *Environ. Mutagen.* **8**:9–28.
- Loeb, L. A. 1985. Apurinic sites as mutagenic intermediates. *Cell* **40**:483–484.
- Loechler, E. L., C. L. Green, and J. M. Essigman. 1984. *In vivo* mutagenesis by *O*⁶-methylguanine built into a unique site in a viral genome. *Proc. Natl. Acad. Sci. USA* **81**:6271–6275.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Miller, J. H. 1978. The *lacI* gene: its role in *lac* operon control and its use as a genetic system, p. 31–88. In J. H. Miller and W. S. Reznikoff (ed.), *The operon*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Miller, J. H. 1982. Carcinogens induce targeted mutations. *Cell* **31**:5–7.
- Perry, K. L., S. J. Elledge, B. B. Mitchell, L. Marsh, and G. C. Walker. 1985. *umuDC* and *mucAB* operons whose products are required for UV light- and chemical-induced mutagenesis: UmuD, MucA, and LexA proteins share homology. *Proc. Natl. Acad. Sci. USA* **82**:4331–4335.
- Piette, J., D. D.-Deburgh, and H. Gamper. 1985. Mutagenesis of the *lac* promoter region in M13 mp10 phage DNA by 4'-hydroxymethyl-4,5',8-trimethylpsoralen. *Proc. Natl. Acad. Sci. USA* **82**:7355–7359.
- Piette, J., and J. Hearst. 1983. Termination sites of the *in vitro* nick-translated reaction on DNA that had photoreacted with psoralen. *Proc. Natl. Acad. Sci. USA* **80**:5540–5544.
- Piette, J., and J. Hearst. 1985. Sites of termination of *in vitro* DNA synthesis on psoralen phototreated single-stranded templates. *Int. J. Radiat. Biol.* **48**:381–388.
- Povirk, L. F., and I. H. Goldberg. 1985. Endonuclease resistant apyrimidinic sites formed by neocarzinostatin at cytosine residues in DNA: evidence for a possible role in mutagenesis. *Proc. Natl. Acad. Sci. USA* **82**:3182–3186.
- Povirk, L. F., and I. H. Goldberg. 1986. Base substitution

- mutations induced in the *cI* gene of lambda phage by neocarzinostatin chromophore: correlation with depyrimidination hotspots at the sequence AGC. *Nucleic Acids Res.* **14**: 1417-1426.
35. Saffran, W. A., and C. R. Cantor. 1984. Mutagenic SOS repair of site-specific psoralen damage in plasmid pBR322. *J. Mol. Biol.* **178**:595-609.
36. Saffran, W. A., and C. R. Cantor. 1984. The complete pattern of mutagenesis arising from the repair of site-specific psoralen crosslinks: analysis by oligonucleotide hybridization. *Nucleic Acids Res.* **12**:9237-9248.
37. Schaaper, R. M., and L. A. Loeb. 1981. Depurination causes mutations in SOS-induced cells. *Proc. Natl. Acad. Sci. USA* **78**:1773-1777.
38. Sobell, H. M., T. D. Sakore, S. C. Jain, A. Banerjee, K. K. Bhandary, B. S. Reddy, and E. D. Lozansky. 1983. β -Kinked DNA—a structure that gives rise to drug intercalation and DNA breathing—and its wider significance in determining the premelting and melting behavior of DNA. *Cold Spring Harbor Symp. Quant. Biol.* **47**:293-314.
39. Strauss, B., S. Rabkin, D. Sagher, and P. Moore. 1982. The role of DNA polymerase in base substitution mutagenesis on noninstructional templates. *Biochemie* **64**:829-838.
40. Tessman, J. W., S. T. Isaacs, and J. E. Hearst. 1985. Photochemistry of the furan-side 8-methoxypsoralen-thymidine monoadduct inside the DNA helix. Conversion to diadduct and to pyrone-side monoadduct. *Biochemistry* **24**:1669-1676.
41. Topal, M. D., and J. R. Fresco. 1976. Complementary base pairing and the origin of substitution mutations. *Nature (London)* **263**:285-289.
42. Vogel, H. Y., and D. M. Bonner. 1956. Acetylornithase of *E. coli*: partial purification and some properties. *J. Biol. Chem.* **218**:97-106.
43. Walker, G. C. 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol. Rev.* **48**:60-93.
44. Yatagai, F., and B. W. Glickman. 1986. Mutagenesis by 8-methoxypsoralen plus near-UV treatment: analysis of specificity in the *lacI* gene of *Escherichia coli*. *Mutat. Res.* **163**:209-224.