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Post-UV Survival and Mutagenesis in DNA Repair-proficient and -deficient Strains of *Escherichia coli* K-12 Grown in 5-Azacytidine to Inhibit DNA Cytosine Methylation: Evidence for Mutagenic Excision Repair

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Abstract—Inhibition of cytosine methylation by growth in 5-azacytidine (5-azaC), did not affect the sensitivities to DNA damage induced by exposure to ultraviolet light (UV) of *Escherichia coli* K-12 strains AB1157 *dcm*$^+$, which is fully DNA repair-proficient, LR68 (a *dcm* derivative of AB1157), JC3890 *dcm*$^+$ *uvrB*, deficient in error-free excision repair, TK702 *dcm*$^+$ *umuC*, deficient in error-prone repair, or TK501 *dcm*$^+$ *uvrB umuC*, which lacks both excision repair and error-prone repair. However, growth in 5-azaC increased the post-UV survival of strains AB2463 *recA*(Def), AB2470 *recB* and AB2494 *lexA*(Ind$^-$), which are deficient in the induction or expression of recombination repair or error-prone repair of DNA. Spontaneous mutation frequencies were increased in strains LR68, AB2463, AB2470 and AB2494 by growth in 5-azaC, but remained unaltered in strains AB1157, JC3890, TK702 or TK501. Growth in 5-azaC significantly increased UV-induced mutation frequencies in strains AB2463 and AB2470, significantly reduced UV-induced mutation in strain JC3890, but had little effect on UV-induced mutation in the other strains. The results suggest that 5-azaC may induce a normally error-free DNA repair pathway to become error-prone and therefore genotoxic.

The control of many DNA-dependent processes is determined by sequence-specific methylation of DNA bases. For example, DNA-methylation is believed to regulate gene expression in eukaryote cells (Cedar 1988), and to protect or sensitize prokaryotic cells to DNA-restriction endonucleases (Marinus 1987). Inhibitors of DNA methylation are therefore under investigation as potential drug candidates, and we have described simple in-vitro (Crooks et al 1984) and in-vivo (Radnedge & Pinney 1992) bacterial screening tests to assess their activity and specificity.

Three *Escherichia coli* K-12 chromosomal genes encode DNA methylases. The *hsdM* gene product methylates the second adenine in 5′-AAC(6N)GG-3′ sequences and thus protects against DNA strand cleavage by the *Eco*K restriction endonuclease (Marinus 1987). Dam protein methylates the adenine group in 5′-GATC-3′ sequences, enabling strand recognition by mismatch repair enzymes (Wagner & Meselson 1976; Glickman et al 1978), and the *dcm* gene product methylates the 5 position of the internal cytosine in the sequence 5′-CC(A/T)GG-3′ (May & Hattman 1975a,b), which is also the recognition sequence of the *Eco*RII restriction and modification system (Schlagman & Hattman 1974). Dcm-methylated DNA is therefore protected against *Eco*RII restriction, but no other function has yet been demonstrated for the Dcm methylase. Strains carrying the *dcm-6* allele are unable to perform very short patch (VSP) DNA repair (Lieb 1987; Zell & Fritz 1987), which corrects T.G mispairs induced by deamination of 5-MeC in 5′-CMeC(A/T)GG-3′ sequences. However, VSP repair is restored to *dcm-6* mutants by a plasmid carrying the cloned *dcm*$^+$ allele, but not by a plasmid encoding the *Eco*RII methylase, and it would therefore appear that the *dcm* gene product itself it not involved in VSP repair (Lieb & Bhagwat 1988), but that VSP repair is determined by a second, partially overlapping sequence that may be transcribed from the same promoter as *dcm* (Bhagwat et al 1988).

To investigate whether cytosine methylation has a role in DNA repair pathways other than VSP, we initially attempted to transduce a *dcm* allele into various DNA repair-deficient strains of *E. coli*, but this was unsuccessful (Radnedge 1988). We now report an approach to this problem that utilizes 5-azacytidine (5-azaC) to inhibit DNA-cytosine methylation. 5-azaC inhibits bacterial cytosine methylation irreversibly (Friedman 1981) by noncompetitive inhibition of DNA methyl transferases (Santi et al 1983, 1984). It is capable of inactivating the *dcm* methylase without inactivating DNA-adenine methylation (Friedman 1979) and can also inhibit methylation of DNA without significantly inhibiting protein synthesis. 5-azaC-grown cells were therefore used to determine the effects of inhibition of cytosine methylation on post-UV survival and mutagenesis of strains deficient in recombination repair (*recA*, *recB*), SOS error-prone repair (*recA*, *lexA* and *umuC*) and excision repair (*uvrB*).

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Materials and Methods

Bacterial strains

The bacterial strains used are listed in Table 1. They are all derivatives of *Escherichia coli* K-12. The *recA13* allele

Table 1. Strains of *Escherichia coli* K-12 used.

Strain	Genotype	Source or reference
AB1157	*thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 hisG4 argE3 rpsL31 tsx-33 supE44 dcm*$^+$ λ^- F^-	Howard-Flanders et al (1964)
LR68	as AB1157, but *dcm his*$^+$	Radnedge (1988)
AB2463	as AB1157, but *recA13* (Def)	Howard-Flanders & Theriot (1966)
AB2470	as AB1157, but *recB21*	Howard-Flanders (1968)
AB2494	as AB1157, but *leu*$^+$ *arg*$^+$ *metB1 lexA3*(Ind$^-$)	Howard-Flanders (1968)
JC3890	as AB1157, but *DE301* (deletion *bio-chlA* giving *uvrB*)	Howard-Flanders (1968)
TK702	*thi-1 proA2 hisG4 lacY1 galK2 xyl-5 mtl-1 supE44 umuC36*	Kato & Shinoura (1977)
TK501	as AB1157, but *DE301 umuC36*	Kato & Shinoura (1977)

present in strain AB2463 makes the strain completely defective in all RecA functions (Walker 1984). Strain AB2494 *lexA3* codes for a LexA protein that is not cleaved on SOS induction (Walker 1984): SOS-inducible functions are therefore absent in this strain. The *recB21* allele abolishes recombination and repair activities due to the RecBCD nuclease in strain AB2470 (Clark 1973). SOS error-prone DNA repair is absent from strain TK702 *umuC36* (Kato & Shinoura 1977), and the *DE301* deletion eliminates UvrB activity, and therefore excision repair from strains JC3890 and TK501 (Howard-Flanders 1968).

Growth of cells in 5-azaC

Fully-supplemented DM medium (4·5 mL) (Davis & Mingioli 1950) was inoculated with a loopful of cells, obtained by sweeping through the region of semi-confluent growth on a fresh fully-supplemented DM plate-culture of the organism, and vortexed thoroughly. A sample (0·5 mL) of the resulting uniform suspension was inoculated into $4 \times 4{\cdot}5$ mL quantities of fully-supplemented DM medium, and incubated overnight at 37°C with shaking. Each resulting 5 mL culture was then added to 95 mL fully-supplemented DM medium in a 250 mL conical flask at 37°C. The viable counts of these suspensions were determined initially and again after 3 h incubation at 37°C on an orbital shaker, by diluting in DM salts solution and plating on fully-supplemented DM agar. Sufficient 5-azaC solution was then added to give final concentrations of 0, 0·1, 1·0 or 10·0 μg mL^{-1}, and cultures grown for a further 3 h. Cells were then harvested, washed twice in DM salts solution and the viable counts of the washed 5-azaC-grown suspensions determined.

Post-UV survival

Washed cultures, grown in the presence or absence of 5-azaC, were serially diluted in DM salts solution and 0·02 mL of each dilution placed on segmented nutrient agar plates to give three replicates for each dilution for each UV dose. Immediately the inocula had soaked into the agar, the plates were irradiated under a Hanovia model 12 low pressure mercury lamp (Hanovia Lamps Ltd, Slough, Bucks, UK), which emitted light at 254 nm. The dose rate was 1 J m^{-2} s^{-1} as determined with a Blak-Ray UV meter model J-225 (Ultraviolet Products Inc., San Gabriel, CA, USA). Counts performed on unexposed plates gave initial viability levels. UV irradiation of exposed plates and all manipulations subsequent to it were performed in a darkened laboratory to prevent photoreactivation. Plates were incubated at 37°C for 24 h or until all the surviving colonies had grown through.

Spontaneous and UV-induced mutagenesis

Volumes (0·1 mL) of undiluted, washed cultures were spread on DM plates fully-supplemented for all growth requirements except histidine, which was present in a trace concentration of 0·05 mM to allow a few rounds of cell division. Spontaneous mutation frequencies were determined from the number of His$^+$ revertant colonies that grew on unirradiated plates. UV-induced mutation frequencies were obtained using plates exposed to the dose of UV that produced 50% survivors in the particular strain being tested. Control counts and determination of post-UV survival levels were performed on fully-supplemented DM medium including histidine. Plates were incubated at 37°C for 2 days. Spontaneous mutation frequencies are expressed as revertants per cell plated. The frequency of UV-induced mutation is expressed as His$^+$ revertants per survivor.

Results

The effect of 5-azaC on growth and post-UV survival of DNA repair-deficient strains of E. coli

Addition of 0·1 μg mL^{-1} 5-azaC to fully-supplemented DM medium caused only a slight decrease in growth rate of *E. coli* AB1157 *dcm*$^+$ (Table 2), whereas 1·0 μg mL^{-1} significantly reduced the rate of cell division and 10 μg mL^{-1} 5-azaC virtually abolished it. Higher concentrations were lethal (results not shown). 5-azaC at 0·1, 1·0 and 10·0 μg mL^{-1} were therefore considered to be suitable concentrations for the demonstration of the effect of 5-azaC on the UV sensitivities of the various strains.

Viable counts carried out on cultures after 3 h incubation in 5-azaC are given in Table 2. The initial counts of strain AB1157 *dcm*$^+$ and its *dcm* derivative LR68, were comparable, whereas after 3 h incubation in fully-supplemented DM containing 10 μg mL^{-1} 5-azaC, the viable count of LR68 was fivefold that of the *dcm*$^+$ parent (Table 3). However, the post-UV survival levels of both strains were not affected by growth in DM containing 5-azaC (Fig. 1).

The growth rates of JC3890 *uvrB*, TK702 *umuC* and TK501 *uvrB umuC* decreased with increasing concentrations of 5-azaC, but cell division occurred in all concentrations as high as 10 μg mL^{-1}. The UV sensitivities of these three

Table 2. Viable counts mL^{-1} of *E. coli* cultures grown for 3 h in DM medium containing 5-azaC.

Strain	Initial count	Concn 5-azaC ($\mu g\ mL^{-1}$) 0	0·1	1·0	10·0
AB1157 *dcm*$^+$	$5{\cdot}9\times10^6$	$7{\cdot}1\times10^8$	$6{\cdot}0\times10^8$	$8{\cdot}0\times10^7$	$1{\cdot}2\times10^7$
LR68 *dcm*	$4{\cdot}2\times10^6$	$5{\cdot}7\times10^8$	$2{\cdot}7\times10^8$	$1{\cdot}3\times10^8$	$6{\cdot}1\times10^7$
JC3890 *dcm*$^+$ *uvrB*	$2{\cdot}8\times10^5$	$1{\cdot}3\times10^7$	$1{\cdot}3\times10^7$	$5{\cdot}4\times10^6$	$4{\cdot}4\times10^6$
TK702 *dcm*$^+$ *umuC36*	$6{\cdot}4\times10^6$	$3{\cdot}8\times10^8$	$3{\cdot}8\times10^8$	$6{\cdot}5\times10^7$	$1{\cdot}6\times10^7$
TK501 *dcm*$^+$ *umuC36 uvrB*	$2{\cdot}7\times10^5$	$5{\cdot}0\times10^6$	$5{\cdot}0\times10^6$	$2{\cdot}7\times10^6$	$1{\cdot}9\times10^7$
AB2463 *dcm*$^+$ *recA13*(Def)	$1{\cdot}3\times10^6$	$2{\cdot}6\times10^7$	$2{\cdot}6\times10^7$	$3{\cdot}0\times10^5$	$2{\cdot}8\times10^5$
AB2470 *dcm*$^+$ *recB21*	$1{\cdot}5\times10^6$	$6{\cdot}4\times10^6$	$6{\cdot}4\times10^6$	$1{\cdot}9\times10^5$	$1{\cdot}0\times10^5$
AB2494 *dcm*$^+$ *lexA3*(Ind$^-$)	$4{\cdot}3\times10^6$	$2{\cdot}9\times10^8$	$1{\cdot}5\times10^7$	$1{\cdot}7\times10^6$	$1{\cdot}3\times10^6$

strains were not affected by growth in minimal medium containing 0·1, 1·0 or 10·0 $\mu g\ mL^{-1}$ 5-azaC (Fig. 1).

E. coli strains AB2463 *recA*(Def), AB2470 *recB* and AB2494 *lexA*(Ind$^-$) were more sensitive to 5-azaC than the DNA repair-proficient strain AB1157, confirming the results of Bhagwat & Roberts (1987). Viable counts after 3 h incubation in minimal medium showed 0·1 $\mu g\ mL^{-1}$ 5-azaC to be sub-inhibitory, but 1·0 and 10·0 $\mu g\ mL^{-1}$ to be lethal to all three strains (Table 2). In addition to their 5-azaC sensitivity, all three strains showed enhanced UV resistance after growth in minimal medium containing 5-azaC. Post-UV survival of strain AB2463 *recA*(Def) increased over tenfold when cells were grown in 0·1, 1·0 or 10·0 $\mu g\ mL^{-1}$ 5-azaC (Fig. 2). Thus a significant increase in UV resistance was produced in this strain by growth in the sub-inhibitory concentration of 0·1 $\mu g\ mL^{-1}$. *E. coli* AB2470 *recB* also showed enhanced post-UV survival when incubated in minimal medium containing the lethal (Table 2) 5-azaC concentrations of 1·0 and 10·0 $\mu g\ mL^{-1}$, but not when grown in the sub-inhibitory concentration of 0·1 $\mu g\ mL^{-1}$ (Fig. 2). The enhancement of post-UV survival of strain AB2494 *lexA*(Ind$^-$) increased with increasing concentration of 5-azaC (Fig. 2): there was about a tenfold increase in survival after a UV dose of 20 J m^{-2} when grown in the sub-inhibitory concentration of 0·1 $\mu g\ mL^{-1}$, whereas cells were more than one hundred times more resistant after exposure to the lethal (Table 2) concentration of 10 $\mu g\ mL^{-1}$ (Fig. 2).

Strains carrying mutations that affect recombination or recombination repair, such as AB2463 *recA*(Def) and AB2470 *recB*, or the control of SOS repair, such as AB2463 *recA*(Def) and AB2494 *lexA*(Ind$^-$) were therefore made more resistant to UV by growth in 5-azaC. However, the DNA repair-proficient strains AB1157 *dcm*$^+$ and LR68 *dcm* and the remaining DNA repair-deficient strains tested, JC3890 *uvrB*, TK702 *umuC* and TK501 *uvrB umuC*, were not affected. It is possible that enhancement of post-UV survival produced by growth in 5-azaC resulted from heightened DNA repair activity induced either by the drug itself or by the result of its interaction with DNA. If such induced DNA repair activity is error-free, the UV-induced mutation frequencies would not increase. Conversely, if repair is error-prone then UV-induced mutation frequencies would be expected to rise. The effect of pre-growth in the presence of 5-azaC on UV-induced mutation was therefore investigated.

Spontaneous and UV-induced mutagenesis of strains grown in 5-azaC

Spontaneous reversion frequencies of cells grown in 0 and 10 $\mu g\ mL^{-1}$ 5-azaC are shown in columns 1 and 2, respectively, of Table 3, and UV-induced reversion frequencies are shown in columns 3 and 4, respectively, of Table 3.

The spontaneous reversion frequency of strain LR68 *dcm* was much lower than that of the AB1157 *dcm*$^+$ parent when both strains were grown in the absence of 5-azaC (column 1, Table 3). However, growth in 10 $\mu g\ mL^{-1}$ 5-azaC increased the spontaneous mutation in strain LR68 *dcm* over 40-fold to become comparable with that of AB1157 *dcm*$^+$ grown in the presence or absence of 5-azaC (column 2, Table 3).

Strains AB2463 *recA*(Def), AB2470 *recB* and AB2494 *lexA*(Ind$^-$), which showed enhanced post-UV survival after incubation for 3 h in minimal medium containing 10 $\mu g\ mL^{-1}$ 5-azaC (Fig. 2), also exhibited enhanced spontaneous rever-

Table 3. Effect of growth in 10 $\mu g\ mL^{-1}$ 5-azaC on spontaneous and UV-induced mutation frequencies.

Strain	His$^+$ revertants per 10^8 viable cells[a] 1	2	3	4	Fold increase in mutation frequency produced by growth in 5-azaC Spontaneous	UV-induced
AB1157 *dcm*$^+$	5·3	6·1	656·0	1717·4	+1·2	+2·6
LR68 *dcm*	0·2	8·2	111·0	511·0	+41·0	+4·6
AB2463 *dcm*$^+$ *recA13*(Def)	1·8	15·0	4·8	55·8	+8·3	+11·4
AB2470 *dcm*$^+$ *recB21*	20·8	331·5	39·7	655·7	+15·9	+16·5
AB2494 *dcm*$^+$ *lexA3*(Ind$^-$)	0·1	7·2	6·2	5·0	+72·2	−1·2
JC3890 *dcm*$^+$ *uvrB*	0·5	0·4	113·0	8·0	−1·3	−14·1
TK702 *dcm*$^+$ *umuC36*	0·1	0·1	0·1	0·2	1·0	+2·0
TK501 *dcm*$^+$ *umuC36 uvrB*	0·3	0·7	1·2	3·9	+2·3	+3·3

[a] 1, Spontaneous reversion frequency; 2, spontaneous reversion frequency of 5-azaC-grown cells; 3, UV-induced reversion frequency of control cells; 4, UV-induced reversion frequency of 5-azaC-grown cells.

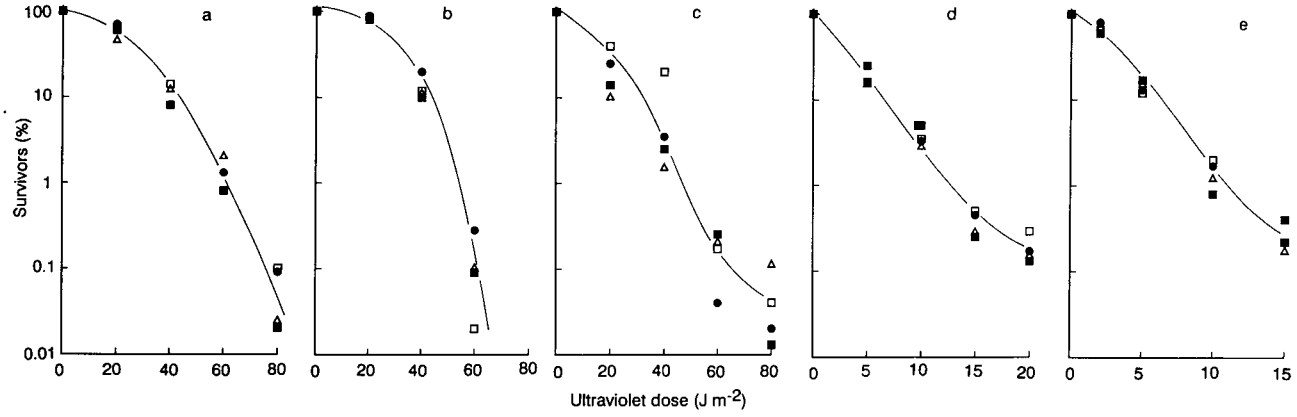

FIG. 1. *E. coli* strains in which growth in 5-azaC did not significantly affect sensitivity to UV irradiation: a, AB1157 *dcm*$^+$; b, LR68 *dcm*; c, TK702 *umuC*; d, JC3890 *uvrB*; e, TK501 *uvrB umuC*. Concentrations of 5-azaC (μg mL^{-1}), 0 (■), 0·1 (△), 1·0 (●), 10·0 (□).

sion frequencies after growth in the compound (Table 3). However, mutation frequencies in strains JC3890 *uvrB*, TK702 *umuC* and TK501 *uvrB umuC*, which did not show significantly enhanced post-UV survival when grown in the presence of 5-azaC (Fig. 1), were not significantly increased by growth in 5-azaC (Table 3). There was thus a positive correlation between enhanced post-UV survival of bacteria grown in minimal medium containing 5-azaC and enhanced spontaneous reversion frequency of cells grown under the same conditions.

The UV-induced reversion frequencies of 5-azaC-grown cells of *E. coli* AB1157 *dcm*$^+$ and its *dcm* derivative LR68 were 2·6 and 4·6 times higher, respectively, than the values obtained with control cells grown in 5-azaC-free medium (Table 3). However, the effect of growth in 5-azaC on UV-induced mutagenesis was greatest in strains where 5-azaC also increased post-UV survival. Table 3 shows that UV-induced mutagenesis of strains AB2463 *recA*(Def) and AB2470 *recB* was increased 11·4- and 16·5-fold, respectively, by growth in 5-azaC. This also correlated with the effect of the compound on the spontaneous reversion frequencies of these two strains (Table 3). However, the increase in spontaneous mutagenesis produced by 5-azaC in strain AB2494 *lexA*(Ind$^-$) was not reflected by a similar increase in UV-induced mutagenesis (Table 3). The excision repair-deficient strain JC3890 *uvrB* was the only strain that showed a significant decrease in UV-induced mutation frequency when grown in 10 μg mL^{-1} 5-azaC. This reduction was abolished when the *umuC* allele was also present, as in strain TK501 *uvrB umuC* (Table 3). UV-induced reversion in strain TK702 *umuC* was not significantly affected by growth in 5-azaC.

Discussion

Our initial aim was to determine the effect of the Dcm$^-$ phenotype on post-UV survival and mutagenesis of DNA repair-deficient derivatives of *E. coli* K-12. However, attempts at co-transducing the defective *dcm* allele with the adjacent *his*$^+$ operon into *dcm*$^+$ *his* DNA repair-deficient strains were unsuccessful (Radnedge 1988).

We therefore grew *dcm*$^+$ strains in 5-azaC to mimic the effect of the Dcm$^-$ phenotype. The validity of this approach is proved by the observations that not only is the susceptibility of phage λ_{vir} to *Eco*RII restriction increased by addition

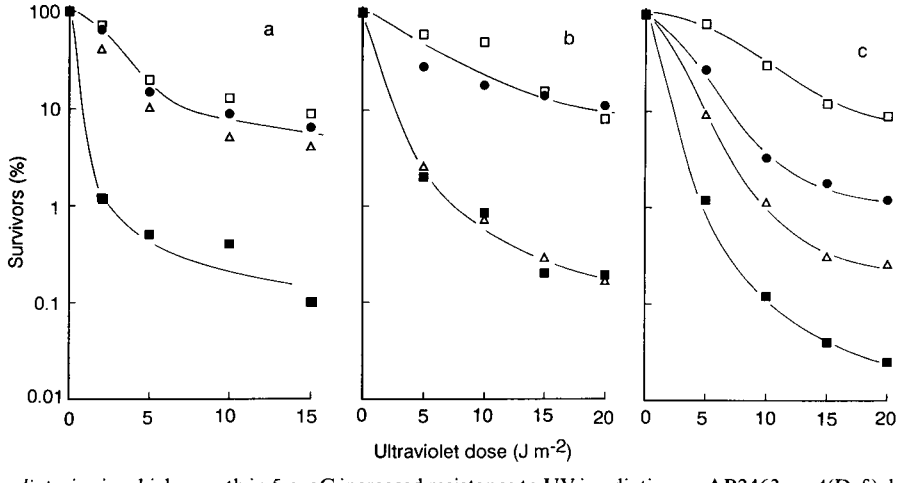

FIG. 2. *E. coli* strains in which growth in 5-azaC increased resistance to UV irradiation: a, AB2463 *recA*(Def); b, AB2470 *recB*; c, AB2494 *lexA*(Ind$^-$). Concentrations of 5-azaC (μg mL^{-1}), 0 (■), 0·1 (△), 1·0 (●), 10·0 (□).

of 5-azaC to the medium when the phage is propagated on a Dcm$^+$ host (Radnedge & Pinney 1992), but also LR68, the *dcm* derivative of AB1157, was more resistant to 5-azaC than the *dcm*$^+$ parent. The latter observation is analogous to the 5-azaC resistance of *E. coli* B strains, which do not carry the *dcm* locus (Friedman 1982). Sensitivity to 5-azaC is probably due to the presence of lethal DNA cross-links formed by the Dcm methylase between the 6-positions of 5-azaC residues on opposite DNA strands (Santi et al 1983; Friedman 1986). These cross-links are absent from the methylase-deficient strains, which are therefore more resistant to 5-azaC.

5-azaC, 10 μg mL^{-1}, was bactericidal to the DNA repair-deficient strains AB2463 *recA*(Def), AB2470 *recB* and AB2494 *lexA*(Ind$^-$). In experiments using AB2463 in complex medium, Barbe et al (1986) found that the viable cell count was reduced to 10% of the original after a 60 min exposure to 20 μg mL^{-1} 5-azaC. Results presented in Table 2 show that exposure of strain AB2463 to 10 μg mL^{-1} 5-azaC in minimal medium reduced viability to 21·5% of the initial count after 3 h. Barbe et al (1986) also demonstrated that *E. coli lexA*(Ind$^-$) and *recA*(Def) mutants were killed after 60 min exposure to 20 μg mL^{-1} 5-azaC in complex medium and Bhagwat & Roberts (1987) showed that *E. coli recA* (Def) and *lexA*(Ind$^-$) mutants are extremely sensitive to 20 μg mL^{-1} 5-azaC. However, our results (Table 2) show that concentrations of 5-azaC as high as 10 μg mL^{-1} are subinhibitory to strains AB1157 *dcm*$^+$, LR68 *dcm*, JC3890 *uvrB*, TK702 *umuC*, and TK501 *uvrB umuC*, at least when grown in a fully-supplemented basal-salts medium such as DM.

Growth in 5-azaC increased the post-UV survival of strains AB2463 *recA*(Def), AB2470 *recB*, and AB2494 *lexA*(Ind$^-$) (Fig. 2). No strain tested showed decreased post-UV survival after growth in 5-azaC. The enhancement of post-UV survival by 5-azaC could be due to the induction of a DNA repair pathway, to the enhancement of an already functioning repair system or simply to the slowing down of DNA replication by 5-azaC, which would increase the time available for DNA repair to be effected. The only repair mechanism known to be error-prone and therefore mutagenic is SOS repair, which is not induced in undamaged cells (Walker 1984). Post-UV protein synthesis is required for its expression, which is why cells were plated on medium fully-supplemented for all growth requirements except histidine, which was present in trace amounts to allow a few rounds of replication. However, since 5-azaC increased post-UV survival in *E. coli* strains AB2463 *recA*(Def) (deficient in SOS and recombination repair), AB2470 *recB* (deficient in recombination repair) and AB2494 (deficient in SOS induction), it would appear that increased post-UV survival cannot be due to the induction of SOS repair or recombination (daughter strand gap) repair.

5-azaC has previously been shown to be mutagenic in *E. coli* (Fucik et al 1965) and *Salmonella typhimurium* (Podger 1983). The effect of 5-azaC on spontaneous and UV-induced mutagenesis in the different DNA repair-deficient *E. coli* strains was therefore determined to show whether any induced DNA repair was mutagenic or error-free, once again plating on medium that allowed protein synthesis to ensure full induction of all repair processes. The spontaneous reversion frequency of the *dcm* mutant LR68 was one twenty-sixth of that of the *dcm*$^+$ parent (column 1, Table 3). Since LR68 contains only unmethylated cytosine bases, spontaneous deamination of these will yield uracil residues (Coulondre et al 1978), which are efficiently excised by uracil-DNA glycosylase encoded by the *ung* gene (Lindahl 1974). However, 5-meC bases present in the DNA of the wild-type AB1157 *dcm*$^+$ strain will be deaminated to thymine, and specific removal of thymine residues from T.G mispairs (in sequences other than that recognized by VSP repair) is less efficient than removal of uracil groups from U.G mispairs because in the former case an unnatural base is not being removed (Ehrlich et al 1990). Thus, the frequency of G.C-A.T transition mutations will be higher in wild-type strains than in *dcm* derivatives.

5-azaC is incorporated into DNA and converted to 5-azauridine (5-azaU) (Doskocil & Sorm 1971). 5-azaU does not inhibit the uracil-DNA glycosylase of *Bacillus subtilis* (Chao & Duker 1984) but it is not clear whether 5-azaU itself is excised by uracil-DNA glycosylase. If it is excised, then the spontaneous mutation frequency of 5-azaC-grown cells would be expected to be lower than in cells grown in 5-azaC-free medium; their mutation frequencies would be comparable to *dcm* mutants. However, if 5-azaU is not removed by uracil-DNA glycosylase then mutation frequencies should be comparable with those of the *dcm*$^+$ parent. Since the mutation frequencies of 5-azaC-grown *dcm*$^+$ and *dcm* strains are similar (Table 3) then excision of 5-azaU by uracil-DNA glycosylase appears unlikely.

Strains in which growth in 5-azaC increased UV resistance (Fig. 2) also exhibited 5-azaC-enhanced increases in spontaneous reversion frequencies (Table 3). UV-induced mutation was also elevated in two of these three strains. However, it is unlikely that 5-azaC induces mutations via the SOS error-prone repair pathway since it does not induce *recA::lacZ* or *umuDC::lacZ* fusions (Barbe et al 1986), but does increase 9-aminoacridine mutagenesis in a *recA* background (Podger & Grigg 1986), UV-induced mutation in strain AB2463 *recA*-(Def) (Table 3) and post-UV survival in strain AB2494 *lexA*(Ind$^-$) (Fig. 2), all of which are deficient in the induction or expression of the SOS pathway (Walker 1984). Similarly, 5-azaC-mediated increases in UV-resistance and spontaneous and UV-induced mutation frequencies cannot be due to recombination repair since they are apparent in strains AB2463 *recA*(Def) and AB2470 *recB* (Fig. 2, Table 3). The fourteenfold decrease in UV-induced mutation produced by 5-azaC in strain JC3890 *uvrB* (Table 3) implicates excision repair as the mutagenic DNA repair pathway. However, excision repair has been considered to be error-free and therefore non-mutagenic (Hanawalt et al 1979). A study of other mutants involved in excision repair would enable a more precise definition of the site of interaction of 5-azaC with this repair pathway to be made. Exposure of cells, post-UV, to conditions in which excision repair was allowed to procede in the absence of SOS repair, for example by suspending cells post-UV in buffer before plating, might also confirm the involvement of excision, rather than SOS repair in mutagenesis.

A mechanism for error-prone excision repair is difficult to postulate on the basis of these results, although it could be argued that the most likely stage for the introduction of errors would be during DNA base incorporation. Until now,

5-azaC has been considered to be mutagenic in mammalian systems because of aberrant gene activation resulting from hypomethylation of DNA (Cedar 1988). The findings presented here indicate that the induction of an error-prone pathway, which is normally error-free, may also contribute to the mutagenicity of 5-azaC and, by analogy, other agents that inhibit DNA methylation.

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