HOST UMUC$^+$ GENE FUNCTION MIMICS PLASMID R46-MEDIATED PROTECTION OF *ESCHERICHIA COLI* EXPOSED TO DNA DAMAGE

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Plasmid R46 increases survival and mutagenesis in *Escherichia coli* exposed to ultraviolet light (UV), methyl methane sulphonate (MMS), bleomycin (BLM) and cis-platinum (II) diaminodichloride (PDD), which cause single-strand DNA damage. However, whilst enhancing the mutagenic activities of proflavine (PF) and mitomycin C (MTC), which bind the two strands of DNA together, the plasmid fails to protect *E. coli* against these compounds (Attfield and Pinney, 1982). R46 also restores mutability to *E.coli* strains carrying the chromosomal *umuC*$^-$ mutation, which led Walker and Dobson (1979) to suggest that UV-protecting plasmids code for an activity analogous to the host *umuC*$^+$ gene function. If this is correct, the *umuC*$^+$ gene product should mimic plasmid R46 in protecting cells against single-strand, but not double-strand DNA-damaging agents, whilst increasing mutagenesis induced by both types of agents. We report experiments designed to test this hypothesis.

Plasmid-less *E.coli* strains TK701 *his*$^-$ *umuC*$^+$ and TK702 *his*$^-$ *umuC*$^-$ were incubated at 37^0 in nutrient broth containing MMS, BLM, PDD, PF or MTC. Survival was determined by diluting samples in nutrient broth, plating on nutrient agar and incubating overnight. UV sensitivity was tested by plating cells on nutrient agar, exposing them to UV at 254 nM and incubating overnight. The *umuC*$^-$ mutant was considerably more sensitive than the *umuC*$^+$ strain to the single-strand DNA-damaging agents UV, MMS, BLM and PDD, but was no more sensitive to the double-strand binding drugs PF and MTC (Table 1).

Table 1. Effect of the *umuC* gene on survival of *E.coli* exposed to DNA damage.

Agent	UV	MMS	BLM	PDD	PF	MTC
Concentration (μg/ml)	-	1000	1	100	100	1
*% survival *umuC*$^+$	9.3	30.0	6.5	1.6	4.5	3.2
*% survival *umuC*$^-$	0.010	16.0	0.32	0.20	11.0	2.5

*After 3hr exposure to drug or a UV dose of 60 Jm^{-2}.

Mutagenesis in both strains was determined as the frequency of reversion to histidine independence per 10^8 survivors after drug or UV treatment. Table 2 shows that whereas the *umuC*$^-$ strain was refractory to mutagenesis by any of the agents, the *umuC*$^+$ strain was mutated by UV, MMS, PDD and MTC. Neither BLM nor PF was mutagenic in this test system.

Table 2. Effect of the *umuC* gene on mutagenesis of *E.coli* exposed to DNA damage.

Agent	UV	MMS	BLM	PDD	PF	MTC
Concentration (μg/ml)	-	1000	1	100	100	1
*Mutagenesis *umuC*$^+$	33.0	24.0	1.2	10.0	0.80	4.0
*Mutagenesis *umuC*$^-$	1.3	1.2	0.60	1.4	0.60	0.60

*Ratios of mutation frequencies, treated:untreated cells.

Thus, the *umuC*$^+$ gene product does mimic R46 in conferring resistance to UV, MMS, BLM and PDD, which cause single-strand DNA damage, and in not protecting against PF and MTC, which bind to both strands of DNA. Moreover the *umuC*$^+$ gene product, like R46, increases the mutagenicity of single-strand DNA-damaging agents and the double-strand crosslinker MTC. These results provide further evidence for the analogy between *umuC*$^+$ gene- and plasmid R46-mediated protection and mutagenesis.

Attfield, P.V., Pinney, R.J. (1982) Mutat.Res.in press.
Walker, G.C., Dobson, P.P. (1979) Molec.Gen.Genet. 172: 17-24.

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