PLASMID R46 PROTECTS *ESCHERICHIA COLI* AGAINST SINGLE-STRAND, BUT NOT DOUBLE-STRAND DNA DAMAGE

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Plasmid R46 increases survival of bacteria exposed to DNA-damaging agents such as ultraviolet light (UV) and the anticancer drug bleomycin (BLM) (Attfield and Pinney, 1980) by an unknown mechanism. Both UV and BLM damage one strand of the DNA molecule. We have now investigated the effect of R46 on survival of bacteria exposed to the monofunctional alkylating agent methyl methanesulphonate (MMS), nitrofurazone (NFZ), which produces single-strand breaks in DNA, proflavine (PF) and adriamycin (ADM), both of which intercalate between adjacent base pairs in the DNA helix, and mitomycin-C (MTC) and cis-platinum-II-diaminodichloride (PDD). MTC crosslinks bases on opposite strands of the DNA molecule (i.e. interstrand linkage), whereas PDD functions by crosslinking bases in close proximity on one strand of the molecule (i.e. intrastrand linkage).

The effect of plasmid R46 on the sensitivity of *Escherichia coli* strain AB1157 to these DNA damaging agents was assessed by incubating cells at 37° in nutrient broth containing sub-lethal or lethal concentrations of the drugs. At hourly intervals samples were diluted in nutrient broth, plated onto nutrient agar and incubated overnight at 37° to test for survival. The plasmid had little effect on

Table 1. Effect of R46 on survival of *E.coli* AB1157 exposed to DNA damage

Drug	NFZ	MMS	PDD	MTC	ADM	PF
Concentration (μg/ml)	10	1,000	50	1	250	50
*% survival $R46^+$	12	8.0	100	2.0	16	7.1
*% survival $R46^-$	3.0	2.5	2.5	1.7	15	6.4

* After exposure to the drug for 3 h .

survival of strain AB1157 when exposed to lethal concentrations of ADM, PF or MTC (Table 1). Furthermore, R46 produced no effect on the growth rate of cells incubated with sub-lethal concentrations of these drugs. However, in contrast the plasmid markedly increased the survival of *E.coli* strain AB1157 following exposure to a lethal concentration of PDD, and it also protected against NFZ and MMS (Table 1). Cells harbouring R46 also grew more rapidly than plasmid-less cells in sub-lethal concentrations of PDD, NFZ or MMS.

Plasmid-mediated increase in bacterial survival after exposure to DNA damaging agents such as UV and MMS is thought to result from interaction of plasmid gene product(s) with host error-prone DNA repair (Walker 1977). In the search for the mechanism of plasmid-mediated repair it is therefore significant that the protective effect of plasmid R46 functions against single-strand DNA damage produced by drugs such as BLM and NFZ, which cause strand scission, and PDD, which introduces intrastrand crosslinks into DNA, whereas the protective effect is not apparent in the case of double-strand DNA damage mediated by drugs such as ADM and PF, which intercalate into DNA, and MTC, which forms interstrand crosslinks.

Attfield, P.V., Pinney, R.J. (1980) J. Pharm.,Pharmacol. 32: 31P
Walker, G.C. (1977) Molec. Gen. Genet. 152: 93-103

0022-3573/81/120035P-01$02.50/0