R PLASMIDS MEDIATE PROTECTION AND SENSITIVITY TO BLEOMYCIN

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Some R plasmids increase survival and mutagenesis in bacteria exposed to ultraviolet (UV) light, ionising radiation or chemical agents (Lehrbach et al 1977). Bleomycin (BLM) is an anti-cancer drug that binds to DNA causing strand-scission and release of free bases and oligonucleotide fragments (Takeshita et al 1978). We report experiments designed to study the effects of R plasmids on the survival of Escherichia coli exposed to BLM.

The activity of R46, a UV-protecting plasmid which is present in the cell as a single copy per chromosome, was compared with two plasmids that do not confer UV protection: R1, which is also a single copy plasmid and R6K, which in exponential phase cells is present at over 10 copies per host chromosome. The sensitivities to BLM of R^- and R^+ strains of E.coli AB1157 were determined in nutrient broth. Plasmid R1 did not alter host sensitivity to BLM. Plasmid R6K showed little effect on bacterial sensitivity to lethal concentrations of BLM, but it did produce a significant sensitizing effect on cells grown in sub-lethal concentrations. In 0.1μg ml^{-1} BLM, the growth rate of the R^- strain was approximately twice that of the R^+ strain. This suggested that repeated sub-culturing of the $R6K^+$ strain in BLM could increase the proportion of R^- cells in the population and thus effectively "eliminate" the plasmid. Therefore strain AB1157 (R6K) was subcultured daily into fresh nutrient broth containing 0.1μg ml^{-1} BLM and the proportion of R^- cells assessed by replica plating. No elimination of R6K was observed up to the twentieth subculture. However, the proportion of R^- cells then increased over the next 5 days to a maximum level of 82%. Plasmids R1 and R46 were not eliminated by similar treatment. The time lag before R^- cells were observed followed by their rapid increase in number over the next 5 days, indicated that BLM-mediated elimination may not be due solely to selective inhibition of R^+ cells in the population, but that the R6K copy number could have been reduced by the treatment to the extent that R^- cells arose after 20 days. Since R6K confers resistance to ampicillin, plasmid copy number could be estimated by measuring β-lactamase levels of the BLM-treated cultures. Using the hydroxylamine method (Dale & Smith 1971), β-lactamase levels were shown to fall linearly throughout the treatment, suggesting that BLM had indeed reduced plasmid copy number.

Plasmid R46 protected its host at all concentrations of BLM tested. UV protection by this plasmid is independent of host-mediated DNA excision repair, but is dependent upon a functional host recA gene (Tweats et al 1976). R46 was therefore transferred into E.coli strains deficient in excision or recombination repair functions, in order to investigate plasmid-mediated protection against BLM. R46-mediated protection was observed in recombination-deficient recB$^-$ and recC$^-$ strains but not in recA$^-$ strains. Protection occurred in excision repair-deficient mutants that lack endonuclease or ligase activity, but not in a strain lacking DNA polymerase I activity. R46 protection against both UV light and BLM therefore seems to involve recA gene-plasmid interactions. However, BLM protection may also be dependent upon DNA polymerase I activity as shown by lack of R46 protection in the polymerase I-deficient mutant.

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