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THE ATTENUATION OF ECORII RESTRICTION BY PROPAGATION OF BACTERIOPHAGE LAMBDA IN UV-IRRADIATED *E. COLI*

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Restriction endonuclease enzymes are key tools in genetic engineering. They cut DNA at specific target DNA sequences. To prevent the cell from degrading its own DNA, a restriction endonuclease always occurs in conjunction with a modification enzyme, which protects DNA by methylating the same target DNA sequences. The EcoRII restriction-modification system is carried on bacterial plasmids. Its modification enzyme methylates the internal cytosine in the target DNA sequence CC(A/T)GG. The chromosomal *dcm* gene of *Escherichia coli* K-12 also codes for a DNA cytosine methylase that methylates the same cytosine residue and thus protects against the EcoRII restriction endonuclease.

E.coli strain J6-2 *dcm*$^+$ and a *dcm*$^-$ derivative (Pinney & Tribe 1977) carried bacteriophage (phage) lambda (λ) as a prophage. The presence or absence of DNA-cytosine methylation was confirmed using the radiochemical technique of Crooks et al(1984), and by titring phage lysates from these strains on unirradiated EcoRII restriction-positive (r^+) and on EcoRII restriction-negative (r^-) strains of *E coli*. The ratio of the titres on the two strains (r^+/r^-) gives the efficiency of plating (EOP) of λ, and the lower the EOP the greater is the level of restriction. The EOP of control λ propagated on the unirradiated *dcm*$^+$ bacteria was approximately 10^{-1}; λ propagated on the unirradiated *dcm*$^-$ strain gave an EOP of 5×10^{-5}.

If phage are titred on bacteria that have previously been exposed to DNA damage, e.g. by exposure to ultraviolet light (UV), they become resistant to cleavage by restriction enzymes (restriction alleviation) (RA) (Day 1977). We investigated whether the *dcm* gene was inducible by DNA damage, which would explain the mechanism of RA for EcoRII restriction.

The *dcm*$^+$ and *dcm*$^-$ strains were exposed to UV (48 Jm^{-2}) to induce λ synthesis. UV induction produced no increase in EOP of λ from *dcm*$^+$ bacteria (Fig 1), indicating no increase in DNA-cytosine methylation following UV irradiation. Thus the *dcm* gene is not inducible. However, there were marked increases in EOP following UV induction of λ from the *dcm*$^-$ strain (Fig 1). Similar results (data not shown) were also obtained with the *E.coli* 1100 *dcm*$^+$ and *dcm*$^-$ strains of Hattmann *et al* (1973).

We have therefore discovered a novel form of RA resulting from UV treatment of bacteria from which phage are produced rather than UV treatment of bacteria on which these phage are then titred. This cannot be due to DNA-cytosine methylation since it occurs in *dcm*$^-$ strains. However, because it inhibits DNA cutting, any form of RA is a potential hindrance to genetic engineering techniques.

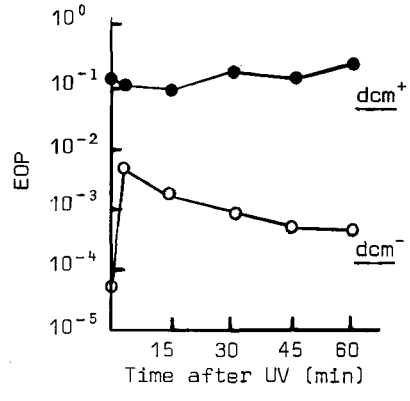

Fig.1 UV induction increases EOP of phage λ propagated on *dcm*$^-$ but not *dcm*$^+$ strains of *E.coli*

Crooks, P.A. et al (1984) J. Pharm. Pharmacol. 36: 85-89
Day, R.S. (1977) J. Virol. 21: 1249-1251
Hattman, S. et al (1973) J. Bacteriol. 115: 1103-1107
Pinney, R.J., Tribe, M.J. (1977) J. Pharm. Pharmacol. 29: 12P