

## INTERRELATIONSHIPS BETWEEN UV-PROTECTION, MUTAGENESIS AND THYMINELESS ELIMINATION IN N-GROUP R PLASMIDS

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The N-group R plasmid R46 mediates at least three important gene functions. It protects against UV irradiation, it increases mutation frequency and it is eliminated during thymine starvation. All three effects are inducible and *recA*-dependent and may therefore be mediated by the same gene product. To test this we have taken advantage of the results of Birks & Pinney (1975), which suggest that only N-group R factors lacking the *EcoRII* (*hspII*) restriction specificity are eliminated by thymine starvation. The N-group plasmid RN3 both restricts and modifies and we have used a series of its restriction (r) and modification (m) mutants (Schlagman & Hattman, 1974) to test whether the restrictionless (r<sup>-</sup>) state is a prerequisite for elimination, UV protection and mutagenesis.

Thymine starvation and determination of r<sup>-</sup> clones was performed as described by Birks & Pinney (1975). UV protection was examined as described by Tweats *et al.* (1976). UV-induced mutagenesis was determined according to Mortelmans & Stocker (1976). No elimination (<0.5%) of the wild-type RN3r<sup>+</sup>m<sup>+</sup> plasmid was observed after three hours thymine starvation, whereas the r<sup>-</sup>m<sup>+</sup> derivative eliminated at a frequency of 11%. However, an RN3r<sup>-</sup>m<sup>+</sup> plasmid was not eliminated from either amber suppressor or non-suppressor hosts. This result was unexpected since R46, which is genotypically r<sup>-</sup>m<sup>-</sup>, is eliminated (Birks & Pinney, 1975). Two r<sup>-</sup>m<sup>+</sup> derivatives (pMT1 and pMT2) of RN3 were therefore constructed in this laboratory and a second mutation was introduced into their modification genes, giving r<sup>-</sup>m<sup>-</sup> plasmids pMT3 and pMT4 respectively. All pMT derivatives of RN3 were found to eliminate at frequencies greater than 10%. Both RN3r<sup>+</sup>m<sup>+</sup> and its r<sup>-</sup> derivatives increased survival after UV irradiation, and UV-induced frequencies of reversion to lysine or arginine independence in *E. coli* strain 343/112 *lys*<sup>-</sup> *arg*<sup>-</sup> were increased at least tenfold by the presence of RN3r<sup>+</sup>m<sup>+</sup>, or its r<sup>-</sup>m<sup>+</sup> mutant.

The *E. coli* K12 *mec*<sup>+</sup> chromosomal gene product recognises and methylates cytosine bases in the same nucleotide sequence as the RN3 *hspII* modification methylase (May & Hattman, 1975). We therefore compared thymineless elimination and UV protection in *E. coli* strain 1100 *mec*<sup>+</sup> and in its *mec*<sup>-</sup> derivative. No elimination (<0.5%) of R46 or RN3r<sup>+</sup>m<sup>+</sup> was observed from a *mec*<sup>-</sup> *thy*<sup>-</sup> host, compared with frequencies of 16% and 11% respectively from the *mec*<sup>+</sup> *thy*<sup>-</sup> parent strain. The R<sup>-</sup> *mec*<sup>+</sup> and R<sup>-</sup> *mec*<sup>-</sup> strains showed similar sensitivities to UV irradiation, and both R46 and RN3r<sup>+</sup>m<sup>+</sup> gave similar protective effects in the wild type *mec*<sup>+</sup> strain. However, protection was increased 100 fold in the *mec*<sup>-</sup> strain. For example, strains 1100 *mec*<sup>+</sup> and 1100 *mec*<sup>-</sup> showed about a 0.005% survival level after exposure to a UV dose of 10.4x10<sup>-5</sup> J/mm<sup>2</sup>. The presence of RN3 increased this to 0.15% in strain 1100 *mec*<sup>+</sup> and to 15.6% in strain 1100 *mec*<sup>-</sup>.

These data confirm the results of Birks & Pinney (1975): the *EcoRII* restriction specificity must be absent if N-group plasmids are to be eliminated by thymine starvation. Since RN3r<sup>+</sup>m<sup>+</sup> confers UV resistance and raises the frequency of UV-induced mutation it would appear that these latter two effects are not directly due to the gene function responsible for thymineless elimination. However, there remains an interrelationship between elimination and UV protection in that the former is abolished in a *mec*<sup>-</sup> host, whereas the latter is greatly enhanced.

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