ABSENCE OF PLASMID-MEDIATED DNA POLYMERASE ACTIVITY FROM UV-INDUCED STRAINS OF *ESCHERICHIA COLI*

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Many R plasmids confer ultraviolet (UV) light resistance and increase UV-induced mutagenesis in host bacteria (Pinney, 1978). DNA polymerases are believed to play an important role in DNA repair and mutagenesis (Witkin, 1976). MacPhee (1974) using plasmid R205 in a *Salmonella typhimurium polA*$^-$ strain, which is deficient in DNA polymerase I, and Lehrbach et al (1977) with plasmid pMG2 in *Pseudomonas aeruginosa polA*$^-$ have demonstrated that DNA polymerase activity is associated with these repair and mutator plasmids. Experiments were therefore undertaken to look for a similar activity in *Escherichia coli* strains containing the known UV mutator plasmids R205, R46 and its derivative pKM101.

Plasmids were transferred by conjugation into *E.coli* JG138 *polA1*$^-$ and into its *polA*$^+$ parent strain MM300. Assays for polymerase I activity were performed on crude extracts prepared by ultrasonic disintegration of cells in 0.1M Tris, 0.01M $MgSO_4$ buffer (pH 7.4). Sonicated calf thymus DNA was used as template for polymerisation, and DNA synthesis was measured by the incorporation of tritium-labelled deoxythymidine triphosphate into acid insoluble material.

DNA polymerisation occurred in reaction mixtures containing extracts of the R$^-$ *polA*$^+$ strain, but the presence of R plasmids in this strain did not increase its polymerase activity. No activity could be demonstrated in R$^+$ or R$^-$ *polA1*$^-$ strains. Experiments were repeated with ATP added to the reaction mixtures since DNA polymerases II and III require this cofactor. Once again no plasmid-associated DNA synthesis occurred in the *polA1*$^-$ strains. Polymersiation was, however, reduced in the *polA1*$^+$ strain suggesting that ATP-dependent nuclease(s) might be degrading the synthesised DNA. N-ethyl maleimide(NEM), which inhibits the ATP-dependent exonuclease V (Strike and Emmerson, 1974), was therefore added to the system. The ATP-induced reduction of *polA*$^+$-dependent DNA synthesis was inhibited by NEM, but again no activity could be demonstrated in the R$^+$ or R$^-$ *polA1*$^-$ strains.

Post-UV protein synthesis is required for plasmid-determined UV protection (Tweats et al, 1976) and a similar requirement was shown for UV-induced mutagenesis. DNA polymerisation experiments were therefore repeated using cells that had been exposed to UV and then allowed to grow for 40 min before disruption. Using such UV-induced preparations it was still not possible to detect any plasmid-associated polymerase activity even in the presence of ATP and NEM.

The results presented here and those of Kronish and Walker (1979) show that plasmid-associated DNA polymerase activity is not evident in all *polA*$^-$ strains. This suggests that the plasmid itself does not code for a polymerase but that a plasmid gene-product may interact with a host polymerase and change its characteristics.

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