METHYLATION OF *DNA-CYTOSINE INDUCED BY UV* IRRADIATION OF ESCHERICHIA COLI

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Thymine starvation and ultraviolet (UV) irradiation induce error-prone DNA repair ("SOS" repair) (Witkin 1976; Pons & Mennigmann 1979). A cytosine methylase activity is induced in Escherichia coli by thymine starvation (Tribe & Pinney 1980) and we now report experiments designed to detect methylation of cytosine after UV irradiation.

E.coli strain J6-2 dcm$^+$thy$^-$codes for a cytosine methylase and is thymine-requiring. Strain J6-2 dcm$^-$thy$^-$(S) lacks cytosine methylase activity and is sensitive (S) to thymine starvation. J6-2 dcm$^-$thy$^-$(R) similarly lacks cytosine methylase activity but is resistant (R) to thymine starvation. These strains are lysogenic for phage lambda (λ) and methylation of deoxycytidine groups in DNA was assayed by comparing the titre of induced λ on an indicator strain carrying the plasmid RN3, with the titre on a plasmidless strain. RN3 codes for an endonuclease that degrades (restricts) DNA in which specific cytosine bases are unmethylated. The ratio of the titres on the two strains gives the efficiency of plating (EOP) of the phage and the closer this is to unity, the greater is the level of cytosine methylation. Deoxyadenosine methylation in DNA was also assayed using an indicator strain harbouring plasmid R124; this codes for an endonuclease that restricts DNA in which specific adenine groups are unmethylated. Phage λ was induced from stationary phase nutrient broth-grown cultures. These were washed twice to remove λ already present and resuspended in 0.05M phosphate buffer pH 7.2. They were irradiated with a UV dose of 28 J per m^2, diluted 1 in 10 into fresh nutrient broth and incubated at 37^0. Samples were then taken, filter sterilized and the filtrates titred for phage.

Table 1. EOP of phage λ present before, and 15 min after, UV irradiation.

Strain from which phage obtained.	EOP (RN3$^+$ titre:RN3$^-$ titre)			EOP (R124$^+$titre:R124$^-$titre)		
	pre-UV	post-UV		pre-UV	post-UV	
J6-2 dcm$^+$thy$^-$	1.1×10^{-2}	9.0×10^{-1}	(82)	4.0×10^{-5}	5.3×10^{-5}	(1.3)
J6-2 dcm$^-$thy$^-$ (S)	3.3×10^{-5}	1.4×10^{-2}	(424)	3.6×10^{-5}	4.0×10^{-5}	(1.1)
J6-2 dcm$^-$thy$^-$ (R)	3.8×10^{-5}	3.0×10^{-5}	(0.8)	5.1×10^{-5}	5.2×10^{-5}	(1.0)

Figures in brackets are fold increase in EOP produced by UV irradiation.

There were highly significant increases in the plating efficiency of phage liberated 15 min after UV from strains J6-2 dcm$^+$thy$^-$and J6-2 dcm$^-$thy$^-$(S) using the RN3$^+$ indicator strain (Table 1). EOP's of phage obtained from these strains 30 and 60 min post-UV were also significantly higher than controls. No increase in EOP was detected for phage liberated 15, 30 or 60 min post-UV from the J6-2 dcm$^-$thy$^-$ (R) strain. UV irradiation therefore induces DNA-cytosine methylation in the former two strains, but not in the latter. These results exactly parallel those obtained after thymine starvation (Tribe and Pinney 1980). No increase in DNA-adenine methylation was detected in the three strains (Table 1).

The rate at which λ was liberated from each strain was found to be identical: at 90 min post-UV, phage titres had risen to 1.3×10^8, 1.6×10^8 and 2.2×10^8 for the J6-2 dcm$^+$thy$^-$, J6-2 dcm$^-$thy$^-$(S) and J6-2 dcm$^-$thy$^-$(R) strains, respectively. Since λ production is a consequence of SOS induction (Meyn et al 1977) the liberation of λ from the J6-2 dcm$^-$thy$^-$(R) strain, which does not methylate DNA-cytosine (Table 1), indicates that cytosine methylation is not a prerequisite for the induction of SOS repair, but occurs as a consequence of it.

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