CYTOSINE METHYLATION DURING THYMINE STARVATION: EFFECT OF NICOTINAMIDE

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When bacteria are deprived of thymine they die. Pinney and Tribe (1977) have shown that a thymine-requiring (*thy*$^-$) *Escherichia coli* 1100 strain, which lacks the ability to methylate DNA cytosine bases (*dcm*$^-$) is much less sensitive to thymine deprivation than its *thy*$^-$ *dcm*$^+$ parent. However, when the *dcm*$^-$ allele was transferred into *E.coli* strain J6-2 *dcm*$^+$ *thy*$^-$ two classes of J6-2 *dcm*$^-$ *thy*$^-$ transductants were produced. One was resistant (R) to thymineless death, whilst the other was as sensitive (S) as the J6-2 *dcm*$^+$ *thy*$^-$ parent. Plasmid RN3 carries the RII restriction (Res) and modification (Mod) system coding for endonuclease EcoRII and methylase ModRII respectively. The *dcm*$^+$ chromosomal gene product recognises and methylates the same cytosine bases as the ModRII methylase and thus protects against EcoRII restriction (May and Hattman 1975). Nicotinamide (Nic) inhibits DNA methylation (Razin et al 1975) and we now report its effects on thymineless death, thymineless elimination of plasmids and on cytosine methylation in λ phage.

To test if cytosine methylation was inhibited by Nic, *E.coli* strains 1100 *dcm*$^-$, 1100 *dcm*$^-$ (RN3 Res$^+$) and 1100 *dcm*$^-$ (RN3 Res$^-$) were grown in the presence and absence of 100 mM Nic. All strains grew at the same rate in the absence of Nic. However, whilst Nic inhibited the growth of strain 1100 *dcm*$^-$ (RN3 Res$^-$), the viability of strain 1100 *dcm*$^-$ (RN3 Res$^+$) decreased to less than 10% after 3 hr exposure. In the latter strain inhibition of methylation by Nic results in DNA that is susceptible to EcoRII restriction and DNA double strand breaks therefore occur. The addition of 100 mM Nic during thymine starvation increased the survival level of strain 1100 *dcm*$^+$*thy*$^-$ after 5 hr from 0.05% to 25%, but Nic had no effect on the rate of thymineless death of strain 1100 *dcm*$^-$*thy*$^-$. Plasmid RN3 Res$^-$ is eliminated from thymine-starved *E.coli* 1100 *dcm*$^+$*thy*$^-$ but not from 1100 *dcm*$^-$*thy*$^-$ (Tribe and Pinney 1977) and it was found that Nic abolished this plasmid elimination. Thus Nic induces the *dcm*$^-$ phenotype in the 1100 *dcm*$^+$ strain in respect of both sensitivity to thymineless death and of thymineless plasmid elimination.

E.coli J6-2 is lysogenic for phage λ. DNA-cytosine methylation could therefore be estimated by measuring the efficiency of EcoRII restriction on spontaneously liberated λ. Phage from unstarved cultures and from cultures starved of thymine in the presence or absence of Nic were titred on strains 1100 *dcm*$^-$ and 1100 *dcm*$^-$ (RN3 Res$^+$). The ratio of these titres gives the efficiency of plating (EOP) of λ.

Table 1. EOP of phage λ produced before and during thymine starvation

Strain from which phage obtained	Time of thymine starvation (hr) 0	3	3(+Nic)
J6-2 *dcm*$^+$ *thy*$^-$	1.0×10^{-2}	9.0×10^{-2} (9)	7.0×10^{-3}
J6-2 *dcm*$^-$ *thy*$^-$ (S)	2.0×10^{-5}	2.8×10^{-4} (14)	1.1×10^{-5}
J6-2 *dcm*$^-$ *thy*$^-$ (R)	9.0×10^{-6}	1.8×10^{-5} (2)	1.3×10^{-5}

Figures in brackets are the fold increase in EOP produced by thymine starvation.

The increase in EOP produced by thymine starvation in J6-2 *dcm*$^+$ *thy*$^-$ and J6-2 *dcm*$^-$ *thy*$^-$ (S) shows that cytosine methylation is being induced in these strains. This methylation is abolished by the presence of Nic in the thymine starved cultures. Little increase in EOP is produced by thymine starvation of strain J6-2 *dcm*$^-$ *thy*$^-$ (R) suggesting that it lacks any methylase activity.

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