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Lack of a Relation Between Deoxyribonucleic Acid Methylation and Thymineless Death in *Escherichia coli*

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When thymine-requiring bacteria are incubated in a thymineless growth medium, viability is lost (S. S. Cohen and H. D. Barner, Proc. Natl. Acad. Sci. U.S. **40**:885, 1954). This phenomenon, termed thymineless death, has been widely studied but is not understood. Recently, D. Luzzati (J. Bacteriol. **92**:1435, 1966) has shown that, accompanying thymineless death of *Escherichia coli*, there is a loss of ability to synthesize messenger ribonucleic acid (RNA) and that this is most likely due to some unknown alteration of deoxyribonucleic acid (DNA) which reduces its ability to serve as a template for RNA polymerase. One possible alteration is uncontrolled methylation. This possibility arises from the work

TABLE 1. *Viability of Escherichia coli B/r (Thy⁻) to which UV-irradiated T3 phage (10^{-5} survival) are adsorbed*

Expt	No. of cells before addition of phage	No. of cells 10 min after addition of phage[a]
I	6.9×10^7	6.7×10^7
II	1.0×10^8	1.2×10^8

[a] In both experiments, the multiplicity of adsorbed phages was about 5 after 10 min.

of M. Gold and J. Hurwitz (Cold Spring Harbor Symp. Quant. Biol. **28**:149, 1963), who showed that DNA extracted from thymine-starved *E. coli* (this DNA was prepared by me) had lost the ability to serve as a substrate for the *E. coli* DNA methylating enzymes. Although this could be a result of hypermethylation during thymine starvation, no definite conclusion about uncontrolled methylation could be drawn since trace amounts of denatured DNA (and possibly DNA altered in other ways) strongly inhibit the methylases.

M. Gold and J. Hurwitz (J. Biol. Chem. **239**: 3866, 1964) further showed that methylated DNA is a good template for the *E. coli* RNA polymerase, from which Luzzati suggested that, if uncontrolled methylation does occur, it has little to do with the loss of ability to synthesize messenger RNA and with thymineless death. I would like to report an experiment done in vivo which confirms Luzzati's suggestion.

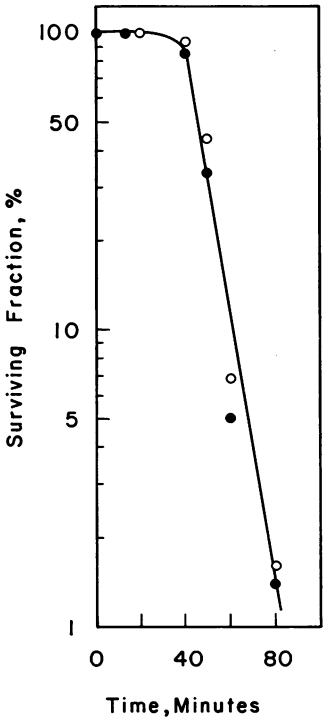

FIG. 1. *Thymineless death of Escherichia coli B/r (Thy⁻) infected with phage T3. The phage had been UV-irradiated to a survival level of 10^{-5}. Phage was added at t = 0. At t = 5, the multiplicity of infection was 5.5 (70% adsorption). (○) With phage; (●) control, without phage.*

M. Gefter et al. (J. Biol. Chem. **241**:1995, 1966) showed that, when coliphage T3 infects *E. coli*, an enzyme is synthesized which cleaves *S*-adenosylmethionine, the methyl donor in methylation reactions, thereby preventing methylation.

When cells of *E. coli* were infected at a multiplicity of 5 with T3 irradiated with ultraviolet (UV) light to a survival of 10^{-5} and then superinfected with phage T4, the T4 DNA was not methylated. Since cells infected with UV-irradiated T3 are viable (Table 1), we might ask whether such T3-infected cells can undergo thymineless death.

The system used was *E. coli* B/r (Thy^-). Thymineless death of this strain has already been described (D. Freifelder, J. Bacteriol. **90:** 1153, 1965) and is shown in Fig. 1. Phage T3 was kindly provided by M. Gefter. Cells were grown to about 2×10^8/ml, washed, collected by membrane filtration, and resuspended at 10^8/ml in iced thymineless medium. The suspension was divided into two parts: to one portion nothing was added; to the other was added a sevenfold excess of T3 (UV-irradiated to a survival of 10^{-5}). Both cultures were warmed to 37 C, and thymineless death curves were obtained as shown in Fig. 1. The two survival curves are identical. Hence, thymineless death occurs normally in the absence of methylation or at least under conditions in which hypermethylation of pre-existent DNA is certainly greatly reduced. Therefore, Luzzati's suggestion is probably correct and the nature of the lesion affecting the template activity of the DNA must be sought elsewhere.

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