Induction of Filament Formation and Thymineless Death in Escherichia coli K-12

M. GHERARDI and N. SICARD

Laboratoire de Génétique Faculté des Sciences de Toulouse, Toulouse, France

Received for publication 23 January 1970

The study of isogenic strains of Escherichia coli K-12 carrying mutations which control filament formation after ultraviolet irradiation showed that there is not necessarily a relationship between filament formation and thymineless death.

Ultraviolet irradiation and thymine deficiency induce certain identical effects on nucleic acid synthesis in Escherichia coli (2, 8). For example, both treatments can lead to episome induction (11, 12, 15) and also to some filament formation (7). Since Walker and Pardee have shown for their strains that ultraviolet (UV) irradiation inhibits septum formation as a result of alterations in deoxyribonucleic acid (DNA) metabolism, the question arises whether thymineless death and inhibition of septum formation are not two independent consequences of an alteration of DNA in the absence of thymine (16). On the other hand, it has been suggested that thymineless death may be the result of inadequate cell wall synthesis (3). Indeed, a difference in survival after thymine starvation has been observed between strains, bearing a fit+, or a fit- mutation (3, 13). These strains tested, however, had a different genetic origin and therefore could be nonisogenic. In fact, isogenic strains have been used by Cummings and Mondale, who found that a strain of E. coli B sensitive to UV irradiation and able to form extensive filaments, is not more sensitive to thymine starvation than the parent strain (5). This work would have been conclusive if the strains had not contained any inducible episome. Since a sort of defective phage has been reported to be present in E. coli B (4) and it is known that prophage induction adds to the lethal effect of thymine deprivation (15), only strains which present the base line effect alone should therefore be used.

We have performed a comparative study of the sensitivity to thymine starvation of isogenic strains of E. coli K-12 (thy- arg- tht-) bearing different mutations leading to filament formation. After 4 hr of thymine starvation, the survival of this strain taken at an exponential phase of growth is 10%. If the prophage λ or colicinogenic factors are introduced in this strain, the sensitivity to thymineless death increases considerably as a function of the induction rate (14, 15). Although one cannot exclude completely the possibility that the strain is carrying an unknown prophage, these results represent the highest survival ever observed for a strain undergoing thymineless death.

The method used to introduce the lon- marker into the parental strain was the one described by Adler et al. (1). Strain 3 300 M 6 (F+) was crossed with our strain cured of the F+ factor by acridine orange and bearing a lac- factor selected after treatment with nitrosoguanidine. The lon- gene is linked to the lac marker. Lac+ mucoidal recombinants were isolated and, after purification, were tested for lon- phenotypic expression. One clone was chosen for further experimentation. Other mutants were induced by UV irradiation of the parental strain. All the mucoidal mutants were tested for their filament-forming ability after growth in nutrient broth after a low dose of UV light. Two mutants were used in the experiments presented in this report. No mapping was done for these mutants.

For thymine deprivation experiments, the strains were grown at 37° C in glucose-phosphate minimal medium, supplemented with thymine (30 μg/ml), arginine (50 μg/ml), and thiamine (1 μg/ml). When the cultures reached 5 × 10^7 to 7 × 10^8 cells per ml, the bacteria were collected by centrifugation, washed, and resuspended in the same medium, but without thymine. Samples were taken every 30 min to measure the viability and to make microscopic observations. For UV irradiation, stationary-phase cultures were washed and resuspended in minimal medium without glucose to a final concentration of 10^7 cells/ml. The bacterial suspensions were exposed to UV light under constant shaking. The irradiation was performed with a 15-w germicidal lamp (G.E.) at a dose rate of 15 ergs per mm² per sec.
The bacteria were plated immediately on complete medium and incubated in the dark to avoid photoreactivation.

The UV sensitivity of the lon recombinant differs very little from the parental strain sensitivities (Fig. 1a) and does not vary when the bacteria are plated on yeast extract tryptone or minimal media (9). Nevertheless, the bacteria did form filaments after further growth in complete medium after irradiation. UV sensitivity, filament formation, and mucoidy are pleiotropic properties of the lon gene (7). The absence of increase in UV sensitivity of the strain might result from a suppressor. The viability in the absence of thymine is the same for both the two strains (Fig. 1b). After 3 hr of starvation, the lon strain forms short filaments, whereas, in the parental strain, there is only an increase in the size of the cells which become rod shaped. The two mutants obtained after UV irradiation show similar results (Fig. 2a and b). Although one of the two mutants produces very extensive filaments after UV irradiation, the survival in the absence of thymine is the same as for the parent strain. During thymine starvation the cells of the two cultures elongate and form filaments which are at least 10 times longer than the size of the control bacteria.

This fact suggests that the filament ability, characterized by the formation of long filaments after treatment of the cells with low doses of UV light, does not influence the cellular response to thymineless death. The difference in viability between the fit and fit strains of E. coli B observed by Bazill (3) may be due to the nonidentity of the genetic background of the two strains used for comparison. It appears that in addition to the unnecessary relationship between sensitivity to UV and thymineless death in E. coli (6), filament formation and thymineless death are not always associated. Thymine starvation induces both filament formation and prophage induction. These two processes can be related (17) in some cases (10). Thus it seems possible that filament formation like prophage induction could be only a consequence of the primary effect caused by thymine starvation.

![Fig. 1. Effects of ultraviolet irradiation (a) and thymine starvation (b) on viability of Escherichia coli strains K-12 lon+ and lon-. Symbols: ∇, strain 3 300 M 6 (lon+); +, strain thy-arg-thi-; O, strain thy-arg-thi- lon-. Bacteria plated on minimal medium or yeast-extract tryptone medium (a).](image1)

![Fig. 2. Effect of ultraviolet irradiation (a) and thymine starvation (b) on viability of two filament-forming mutants of Escherichia coli K-12 thy- arg-thi-. Symbols: +, parent strain; O, mutant forming filaments after UV irradiation; ∇, mutant forming extensive filaments after UV irradiation.](image2)

We are very grateful to H. I. Adler for the supply of the lon- bacterial strain.

**LITERATURE CITED**

5. Cummings, D. J., and L. Mondale. 1967. Thymineless death in...


