Genetic Determinant in *Escherichia coli* Affecting Thymineless Death and Ultraviolet Sensitivity

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We have infected *Escherichia coli* strain B3 with F' factors carrying different lengths of the K-12 chromosome. When the F' factor carries the tsx to purE segment, the resulting hybrid merodiploid shows increased sensitivity to thymineless death, although leaky deoxyribonucleic acid synthesis is unaffected, as well as increased sensitivity to ultraviolet irradiation. The dominant or partially dominant character of the effect indicates that it is not a product of allelic differences between *E. coli* K and B at either *ras* or *lon*.

Thymine starvation of *Escherichia coli* results in loss of viability (1). Although the mechanism of thymineless death is unknown, the existence of synergism between thymine starvation and ultraviolet irradiation (3) suggests that the same functional unit (presumably the bacterial chromosome) is the site of both kinds of lethal damage. This report concerns evidence for a gene that jointly affects sensitivity to thymine starvation and ultraviolet irradiation.

The first indication of a genetic region affecting thymineless death was obtained from experiments on the regulation of alkaline phosphatase (EC 3.1.3.1) synthesis. Under standard conditions, the synthesis of phosphatase is repressed in media containing excess phosphate (6). Transfer of F'13, an episome derived from *E. coli* K-12 (5), to *E. coli* B3 (a thymine-requiring mutant of *E. coli* B) results in a novel regulatory property: the hybrid merodiploid shows derepressed synthesis of alkaline phosphatase in excess phosphate following thymine starvation at 30°C, whereas the parental B3 strain does not show this behavior (4). We have observed an additional difference between the two strains: the merodiploid shows a greater rate of mortality during thymine starvation at 30°C (Fig. 2a). This difference is not attributable to a disparity in residual deoxyribonucleic acid (DNA) synthesis. Both strains are fairly leaky at 30°C, but the extent, 28% of the thymine-sufficient rate as determined by 14C-adenine incorporation into DNA (8), is the same.

This difference in survival would be explained if the F'13 episome carries a genetic determinant affecting the severity of thymineless death. F'13 carries a segment of the K-12 chromosome spanning *lac* clockwise through *pur E* (9). It is possible, therefore, that the determinant involved is a K-12 allele of a gene in this region. To test this possibility, we constructed merodiploid derivatives of B3 with F' factors carrying different lengths of this region of the K-12 chromosome (Fig. 1).

Figure 2b shows the survival of these hybrid merodiploids during thymine starvation. The merodiploids with the two shorter F' factors die at virtually the same rate as the parental B3 haploid strain. This shows that neither merodiploidy

![Figure 1](http://jb.asm.org/)

**Fig. 1.** F' factors used in this study. The upper line represents the *lac*-purE region of the chromosome of *E. coli*. The lower three lines represent the portions of this region carried by the three F' factors: 18 (in our stock collection) is *Flac* which carries *lac* but not *proC* (obtained from W. Fangman); 98 extends to *tsx* but not *purE* (obtained from E. Singer); 306 is ORF-8 of Berg and Curtiss and extends slightly past *purE* (obtained from C. Berg). (In each of these F' factors, F is integrated between *lac* and *proB*.) Merodiploid derivatives were constructed as described earlier (4).
per se nor the presence of an autonomous F episome affects the severity of thymineless death. The longest F' factor, in contrast, produces an increased rate of killing, just as F'13 does. It must therefore carry a determinant of the rate of thymineless death which is not present on either of the shorter F' factors and which, therefore, lies in the tsx-pur E region of the map.

Furthermore, as shown in Fig. 3, there are parallel differences in sensitivity to ultraviolet irradiation among these merodiploids. Thus, there is a gene in the same region, carried on the K-12 chromosome, that governs ultraviolet sensitivity. The simplest interpretation, especially in view of the dominance of enhanced sensitivity to

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**Fig. 2.** Thymineless death of haploid and merodiploid strains. Exponential cultures of the indicated strains, growing at 30°C in tris(hydroxymethyl)aminomethane glucose minimal medium (4) supplemented with 20 μg of thymine per ml, were centrifuged, washed two times, and suspended in minimal medium lacking thymine. They were cultivated at 30°C at a cell density of about 10^6/ml, with samples spread at intervals on nutrient agar plates to assay for viability. (a) B3L1 (○) and B3L1/F'13 (●), (b) B3L1 (○), B3L1/F'18 (○), B3L1/F'98 (▲), and B3L1/F'306 (△).

**Fig. 3.** Ultraviolet inactivation of haploid and merodiploid strains. Exponential cultures of the indicated strains were spread on nutrient agar plates and irradiated with a Hanovia germicidal lamp at approximately 20 ergs per mm^2 per sec. B3L1 (○), B3L1/F'98 (△), B3L1/F'306 (□).
both treatments, is that a single locus is responsible for both phenotypic effects. One explanation of the results is that there is an allelic difference between races K and B of *E. coli* for a gene in the *tsx-purE* interval and that the K-12 allele is dominant for increased sensitivity to both kinds of DNA damage. Alternatively, the differential lethality may be a consequence of gene dosage for some genetic element in this region. We might add that the different merodiploids exhibit similar sensitivities to nalidixic acid, a compound that can induce lysogenic bacteria (2). This indicates that a defective prophage is not involved.

Two genes which affect ultraviolet sensitivity, *lon* and *ras*, have previously been found to map in the *tsx-pur E* interval. However, in contrast to the effect described here, resistance is dominant to sensitivity for both loci (9, 10).

The relationship between thymine starvation and the regulation of alkaline phosphatase synthesis will be discussed in a separate report. However, it should be noted that there is a correlation between the extent of mortality produced by thymine starvation and the extent of phosphatase derepression. For conditions of mild killing, as originally reported (4), there is no derepression; greater rates of killing do produce derepression in K and B strains of *E. coli* (A. Wilkins, Ph.D. thesis, University of Washington, Seattle, 1969).

**LITERATURE CITED**