THE INDUCTION OF A CHROMOSOMAL ABERRATION BY BROMOURACIL INCORPORATION IN ESCHERICHIA COLI K 12[1]

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Bromouracil (BU) and bromodeoxyuridine (BUDR) have been shown to be mutagenic by Benzer and Freese (1958), lethal by Hitchings, Falco, and Sherwood (1945), or completely innocuous by Kit, Dubbs, Piekarski, and Hsu (1963) depending on the conditions and organisms used in the test. Transforming DNA containing BU is still genetically functional as demonstrated by Ephrati-Elizur, and Zamenhof (1959) and by Szybalski, Opara-Kubinska, Lorkiewicz, Ephrati-Elizur, and Zamenhof (1960); however, Gimlin, Farquharson, and Leach (1963) report that many of those cells which are not transformed are killed by the exposure to BU-containing DNA. We wish to present evidence of a chromosomal aberration in E. coli K 12 induced by BU incorporation.

First, studies were made to determine the effect of BU incorporation on the fertility of males (Hfr) and females (F^-). To obtain BU incorporation stationary cultures of our G4 Hfr strain (Identical in chromosomal transmission to the Hays Hfr) were diluted into minimal medium containing 20 μg per ml of histidine and 5 μg per ml of thymine (T). After two hours incubation at 37° with shaking 20 μg per ml of BU was added and the incubation continued for another hour prior to mating. Females

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requiring leucine (leu) or proline (pro) were incubated under similar conditions substituting 20 μg per ml of leu or of pro for the histidine as required. Under these conditions radioactivity from BU-2-^{14}C was incorporated and microscopic observations revealed swelling and other distortions of the males. For broth matings the males and females were mixed in the proportion of 1:10 and incubated without agitation for 60 minutes. Appropriate dilutions of the mixtures were plated on minimal media suitable for scoring the recombinants. If observations were made at 20-24 hours, there was a reduction in the fertility for $\underline{leu}^+$ transfer with BU treatment while $\underline{pro}^+$ recombinants from both normal and BU males developed at the same time. Additional incubation for 12-15 hours allowed expression of the $\underline{leu}^+$ recombinants which suggests that BU interferes with recombination of the $\underline{leu}^+$ marker. There was only a slight reduction in fertility of males by BU incorporation if an extended incubation was used. When females were grown in the presence of BU for one hour by the above procedure, there was no change in their fertility.

The effect of BU incorporation on chromosomal mapping by entrance time was determined by the membrane filter mating technique of Matney and Achenbach (1962). Mating on membrane filters prevents the separation of conjugal pairs as occurs in broth matings thus allowing more accurate determination of low frequency recombinations such as terminal markers. After being established in the logarithmic growth phase and labeled with BU by the regime described above, the males and females were mixed in the proportion of 1:10 and impinged on membrane filters then washed with saline. The membranes were transferred to warm, minimal plates lacking glucose for storage until the requisite number of membranes has been prepared. No transfer of the chromosome occurred since energy is required and there is no difference in entrance times obtained when cold, minimal plates with glucose are used. The membranes were rapidly transferred to warm, minimal plates containing glucose, and timing began. At the desired times membranes were removed and placed into cold saline which

stopped chromosomal transmission. The bacteria were removed from the membranes by shaking and the conjugal pairs separated by 30 seconds of blending with a Servall omni-mixer. Appropriate dilutions were made and plated on minimal media selective for the desired recombinant. Figure 1 shows the determination of the entrance times for leu$^+$ and pro$^+$ in both normal and BU treated males. The frequency of recombination is decreased in the BU treated males; however, a reduction of the entrance time for pro$^+$ in BU treated males is apparant. Table I summarizes several experiments with both normal G4 Hfr's and a thymine-requiring mutant (G4-3) isolated by the aminopterin procedure of Okada, Yangisawa, and Ryan (1961). Normally the distance between leu$^+$ and pro$^+$ corresponds to ten minutes of mating time; however, this is consistantly reduced by growth in the presence of BU or by UV exposure.

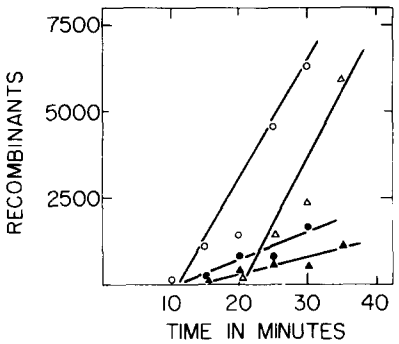

Figure 1. Determination of the entrance times for leu and pro. G4 grown in the presence of thymine as described in the text were mixed with the appropriate females and the mixture scored for leu ○ and pro △ recombinants. G4 grown in the presence of BU were mixed with appropriate females and the mixture scored for leu ● and pro ▲ recombinants. Recombinants were counted at 48 hours which is sufficient time for full expression.

Matney and Goldschmidt (1962) tested 26 clones derived from a population which had been irradiated with UV light to reduce viability to 10^{-5} and all gave evidence of the same chromosomal aberration - an entrance time of pro$^+$ of about 13 minutes. It is of interest that after broth

TABLE I

Effect of Bromouracil Treatment on Entrance Times

Male	Treatment	Leu^+	Pro^+	Leu-Pro Distance
		Minutes		
G4	None	12.0	21.5	9.5
G4	Bromouracil	11.5	14.5	3.0
G4-3	None	11.0	21.5	10.5
G4-3	Bromouracil	11.2	15.0	3.8

cultures of these isolates had remained at room temperature for several days they regained the normal chromosomal entry pattern. The transient nature of the aberration under these conditions suggests the formation of a chromosomal loop, 6.5 minutes long, that is reopened when the nuclear apparatus enters the stationary phase. However, an alternative explanation is that there is either a transposition or deletion of the 6.5 minute chromosome segment through breakage and reformation in regions containing a high concentration of AT pairs.

The effectiveness of BU and UV in inducing this chromosomal aberration suggests that the leu-pro region may contain AT rich DNA and since either deletions or transpositions may result, AT rich regions may mark the end of specific messages or functional units of the chromosome such as operons.

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