REPLICATION OF DNA DURING F'Lac TRANSFER

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F'Lac is a DNA-containing (Silver, 1963) episomal element which can be transferred from a male donor strain of the bacterium, E. coli, to a female recipient by conjugation. The mechanism by which this transfer occurs is not known. Suggested models for transfer are of three types: those for which DNA synthesis is unnecessary, those for which DNA synthesis accompanies transfer, and those for which DNA synthesis precedes transfer. In most investigations, the nature of the DNA transferred under normal conditions of DNA synthesis has been examined. Herman and Forro (1963) have shown that if radioactively labeled males are mated with females in non-radioactive medium, the transferred labeled DNA behaves as a single conserved unit during subsequent replication of the recipient cell, suggesting that the DNA is replicated in the non-radioactive medium prior to or during transfer. Ptashne (1965) mated heavy-density-labeled males with light females in a light medium and showed that all transferred DNA was of a hybrid density, and reached a conclusion similar to that of Herman and Forro. An alternative explanation for both of these results is that replication occurs immediately after transfer, an idea proposed by Stouthamer, de Haan, and Bulten (1963). Gross and Caro (1965) studied chromosomal transfer in Hfr males using quantitative autoradiography and concluded that the DNA is replicated during transfer; substantial evidence was given for lack of replication in the female.

The nature of the transferred DNA can be studied in another way. If a thymine-requiring male transfers F'Lac in a medium containing

5-bromouracil deoxyriboside (BUDR) instead of thymine, the presence of BUDR in the transferred DNA can be detected by virtue of its sensitization to the effects of short or long wavelength ultraviolet irradiation (Greer, 1960; Stahl, et al., 1961). DNA transferred without replication would be resolved as a fraction of F'Lac recipients relatively resistant to the irradiation. In the present paper, these experiments are described and it is shown that, in agreement with the results of others quoted above, the transferred DNA has been replicated prior to or during transfer.

MATERIALS AND METHODS

Several strains of E. coli K-12 were used which have been designated DF18 (♂) and DF30 (♀). The history of these strains is given in Table I.

TABLE I.

Designation	Genotype*	Origin
200P	♂ F'Lac/Lac-T-L-Bl-$Sm^{S}T6^{S}$	F. Jacob, Pasteur Inst.
CR34	♀ Lac-T-L-Bl-Met-$Sm^{S}T6^{S}$Thy-	M. Meselson, Harvard Univ.
DF3	Same as CR34, but Sm^{R}	Ultraviolet mutation of CR34
DF30	Same as DF3, but Pur-	UV treatment of DF3
DF6	Same as CR34 but $T6^{R}$	Spontaneous mutant
DF18	♂ F'Lac+/Lac-T-L-Bl-$Sm^{S}T6^{R}$Thy-	200P x DF6

*Abbreviations: Lac, ability to ferment lactose; F'Lac, male episomal particle carrying Lac gene; T, threonine; L, Leucine; Bl, Vitamin Bl; Met, methionine; Thy, thymine; Pur, purine; Sm^{R}, streptomycin resistance; $T6^{R}$, phage T6 resistance.

The growth medium was tris-glucose-0.1% casamino acids supplemented with 5 μg/ml thymine and 20 μg/ml hypoxanthine (H) which satisfies the purine requirement of DF30. All media transfers were by membrane filtration.

Lactose fermentation was assayed on MacConkey agar plates (Difco) containing 100 μg/ml streptomycin.

The cross was performed as follows: DF18 and DF30 were grown with aeration to 2 x 10^8/ml. DF18 was transferred to iced thymineless, hypoxanthineless medium. The DF30 females were transferred to thymine-containing, hypoxanthineless medium, incubated at 37°C for 20 minutes with shaking and transferred again to iced thymineless, hypoxanthineless medium. (Following this incubation period, DF30 will not incorporate C^{14}-thymine into DNA unless hypoxanthine or adenine is added.) The DF18 males were then added at a ratio of 1-2 males/10 females and 2 μg/ml BUDR and 1 μg/ml thymine was added. (In such a BUDR+Thy medium, cells will grow indefinitely without loss of viability.) The mating mixture was very gently shaken for 30 minutes at 37°C, diluted 100-fold into iced buffer and shaken on a Vortex mixer for 1 minute to separate the mating pairs. The cells were then irradiated and plated. In a cross with thymine alone, ca. 50-100% of the DF18 males transfer F'Lac in 30 minutes. With the BUDR added, this is reduced to about 1-5%. In order to determine survival levels after irradiation with some accuracy, up to 10^5 cells are plated. The resolution of MacConkey agar is such that one red Lac+ colony is easily detected against a background of 10^4-10^5 pink Lac-colonies.

At all times, cells containing BUDR were protected from inactivating wavelengths of visible light.

Ultraviolet irradiation was accomplished with a 15-watt General Electric germicidal lamp. For visible light irradiation, the cells were iced and irradiated with an unfiltered General Electric 150-watt reflector spot lamp at a distance of 7 inches. For both irradiations, the cells were suspended in 0.01 M phosphate, pH 7.5, 0.001 M $MgSO_4$, 0.0001 M $CaCl_2$.

RESULTS AND DISCUSSION

In a control experiment, DF18 and hypoxanthine-starved DF30 were

mated in the presence of 1 μg/ml thymine for 30 minutes, diluted 100-fold and vortexed. Aliquots of the diluted suspension were irradiated with ultraviolet or visible light and plated on MacConkey agar. Inactivation of Lac+ and Lac- cells by ultraviolet proceeded at the same rate. There was little or no inactivation of either type by the visible light.

In a second experiment, the cells were mated in the BUDR-containing medium, diluted, vortexed, and irradiated. Figure 1 shows the result of ultraviolet irradiation. The Lac+ colonies are inactivated at a considerably greater rate than the Lac- colonies (which are inactivated at the same rate as those obtained from the thymine mating experiment). This

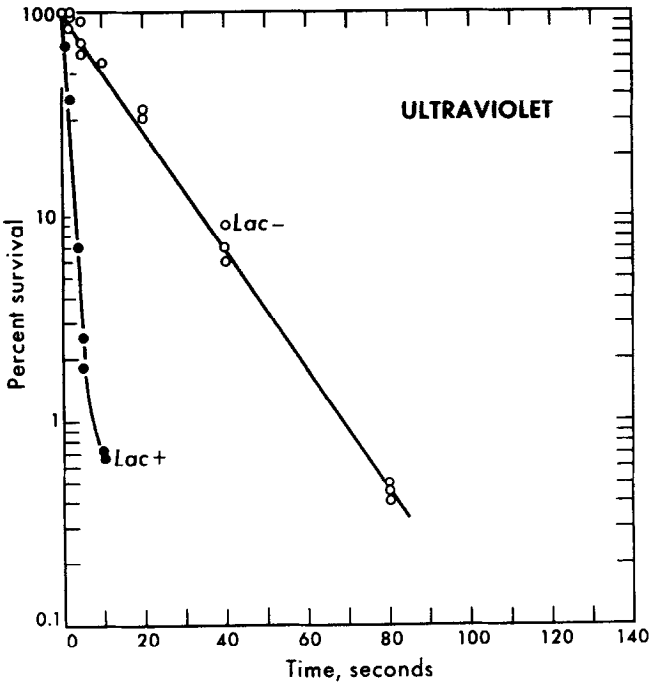

Figure 1-- Inactivation by ultraviolet light of viability and the ability to ferment lactose in DF30 females which have received a BUDR-labeled F'Lac from DF18 males by bacterial conjugation. The curve labeled Lac- represents loss of viability of Lac- colonies. The curve labeled Lac+ represents loss of the Lac character. These latter cells presumably are not killed but rather are converted to Lac-.

is presumably a reflection of the sensitizing effect of the BUDR contained in the transferred DNA (Greer, 1960). The Lac+ inactivation curve remains exponential for about two decades, implying that at least 99% of the transferred episomes have nearly the same BUDR content, i.e., there are few, if any, which contain only thymine.

The result of the irradiation with visible light is shown in Figure 2. The Lac- cells are not inactivated indicating that they have incorporated little, if any, BUDR. This should be compared with the rapid inactivation of DF30 grown for 3 generations in the BUDR medium. However, cells which have received the F-Lac are sensitive to visible

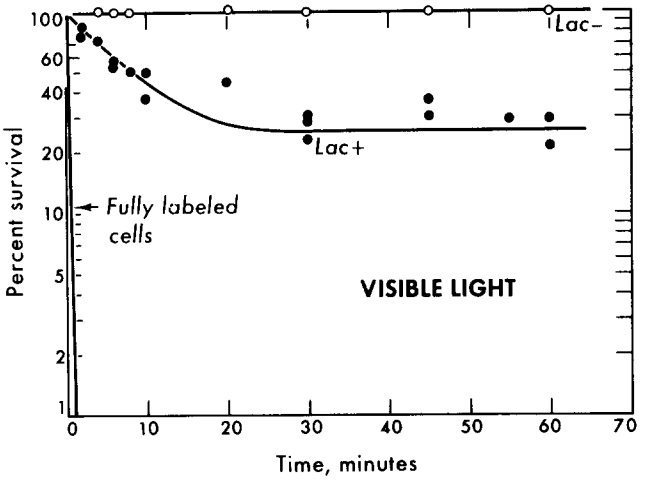

Figure 2-- Inactivation by visible light of viability and the ability to ferment lactose in DF30 females which have received a BUDR-labeled F'Lac from DF18 males by bacterial conjugation. The curve labeled Lac- represents the viability of Lac- colonies. The curve labeled Lac+ represents loss of the Lac character. These latter cells presumably are not killed but rather are converted to Lac-. The curve marked "fully labeled cells" represents loss of viability of cells whose chromosomes are extensively labeled in both strands with BUDR.

light (that is, the Lac+ character is sensitive). Because of the low frequency of transfer in the BUDR medium, it is not possible to tell if the cell itself is killed or if only the F'Lac is inactivated, although the latter is the more likely.

The visible light inactivation differs from the UV case in that there is a resistant fraction of about 30%. It is possible that this represents transfer of F'Lac containing no BUDR but this seems to be ruled out by the UV result. Fox and Meselson (1963) have shown that only 50% of a population of phage λ which contains BUDR in only one strand of its DNA can be inactivated by visible light, from which they concluded that, for reasons still to be determined, damage in one of the two complementary strands is non-lethal. It is likely that a similar effect accounts for the resistant fraction encountered in the visible light experiment, although the inactivation is greater than 50%. Presumably, this is a result of a small fraction of episomes which have replicated in the BUDR medium prior to the transfer process and therefore contain BUDR in both strands.

It might be asked why there is no resistant fraction in the UV experiment. This result agrees with other results for bifilarly labeled DNA (Green, 1960) and is explained on the basis of BUDR inhibiting the systems which repair UV-irradiated DNA (Howard-Flanders, et al., 1962). This inhibition is presumably independent of the location of the BUDR.

The above experiments may be interpreted as follows: When male cells containing F'Lac are mated with female cells, the transferred F'Lac is replicated either prior to or during the transfer process. Some particles may replicate twice prior to transfer or once prior to and once during transfer. The percentage transferred without replication is probably less than 1%. Whether this is true of mating in thymine without BUDR and if this is also the case for Hfr transfer cannot be stated.

These results are consistent with those of Herman and Forro, Ptashne, and Gross and Caro and with the replicon theory of Jacob, Brenner, and Cuzin (1963).

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