CHROMOSOME TRANSFER AND THE DNA REPLICATION CYCLE IN ESCHERICHIA COLI*

Joanne Roeser and Walter A. Konetzka

Department of Bacteriology

Indiana University, Bloomington, Indiana

Received May 21, 1964

Phenethyl alcohol (PEA) has been employed as an inhibitor of DNA synthesis in order to elucidate the relationship between the synthesis of DNA in Hfr strains of Escherichia coli and the transfer of chromosomal markers during conjugation (Bouck and Adelberg, 1963; and Jacob, Brenner, and Cuzin, 1963).

Treick and Konetzka (1964) have shown recently that PEA inhibits the initiation of new cycles of DNA replication in E. coli but permits the completion of those cycles initiated before addition of PEA to the medium. Therefore, cycles of DNA replication can be controlled by the addition, removal, and readdition of PEA to a culture at specific time intervals.

The relationship of the DNA replication cycle to the ability of the Hfr to transfer chromosomal markers constitutes the subject of this investigation.

Materials and Methods. Hfr strain AB259 and F⁻ strain AB2102 were obtained from Dr. E. A. Adelberg. The order of transfer of markers by strain AB259 is O-thr-leu-pro-lac-gal-try-his----. It carries the marker thi and is str-s. Strain AB2102 carries the markers thr⁻, leu⁻, pro⁻, lac⁻, T6-r, gal⁻, try⁻, his⁻, str-r, mal⁻, thi⁻, and mtl⁻.

* This investigation was supported in part by a Public Health Service training grant (No. 503) from the Institute of General Medical Sciences, Public Health Service and in part by a PHS research grant AI 05630-01CB from the National Institute of Allergy and Infectious Diseases, Public Health Service.

Cells were grown in a medium containing: KH_2PO_4, 13.6 g; $(NH_4)_2SO_4$, 2.0 g; $CaCl_2.5H_2O$, 0.01 g; $FeSO_4.7H_2O$, 0.5 mg; $MgSO_4.7H_2O$, 0.2 g; glucose, 2.0 g; thiamine, 10.0 mg; casein hydrolysate, 2.0 g; distilled water, one liter. For C^{14}-thymidine labeling of the Hfr strain, unlabeled thymidine at 10 μg/ml, thymidine-2-C^{14} at 0.01 μc/ml, and deoxyadenosine (Boyce and Setlow, 1962) at 250 μg/ml, final concentrations were included. Phenethyl alcohol was used at a final concentration of 0.31%.

AB259 and AB2102 were inoculated from nutrient agar slants into C^{14}-thymidine-containing medium and into unlabeled medium respectively and incubated for 16 hr at 37C. Both cultures were diluted into fresh medium. The Hfr culture was incubated until a cell concentration of 3.8×10^8 cells/ml was attained. PEA was added and the culture was incubated for an additional 2 hr. Two F^- cultures were incubated so that a cell concentration of 4×10^8 cells/ml was reached at the appropriate times for mating.

The Hfr culture was then filtered through an HA Millipore filter, washed once with medium without PEA, and resuspended in medium without PEA. After 20 min incubation, PEA was again added to the culture.

Matings were carried out by diluting a sample of the Hfr culture 1:10 into a sample of the F^- culture and incubating the mixture at 37C with gentle shaking. After 10 min or 20 min, in respective matings, the mixture was diluted 1:100 into medium with or without PEA. Samples were taken at intervals, diluted in 0.85% KCl, agitated vigorously to separate the pairs, and plated for *thr*$^+$ *leu*$^+$ *str-r* and *try*$^+$ *str-r* recombinants. Plates were incubated at 37C for 36 hr.

Thymidine incorporation into strain AB259 was measured by taking samples at intervals, extracting for 30 min in cold 5% trichloroacetic acid, and filtering through GS Millipore filters. The filters were glued to planchets for determining the radioactivity in a Nuclear-Chicago gas flow counter with "Micromil" window.

Results. The two hour period of pretreatment of the Hfr with PEA has permitted DNA replication in all of the bacteria to reach the point where PEA

prevents any further DNA synthesis. From zero time on, no DNA synthesis takes place so long as the cells remain in PEA (Figure 1).

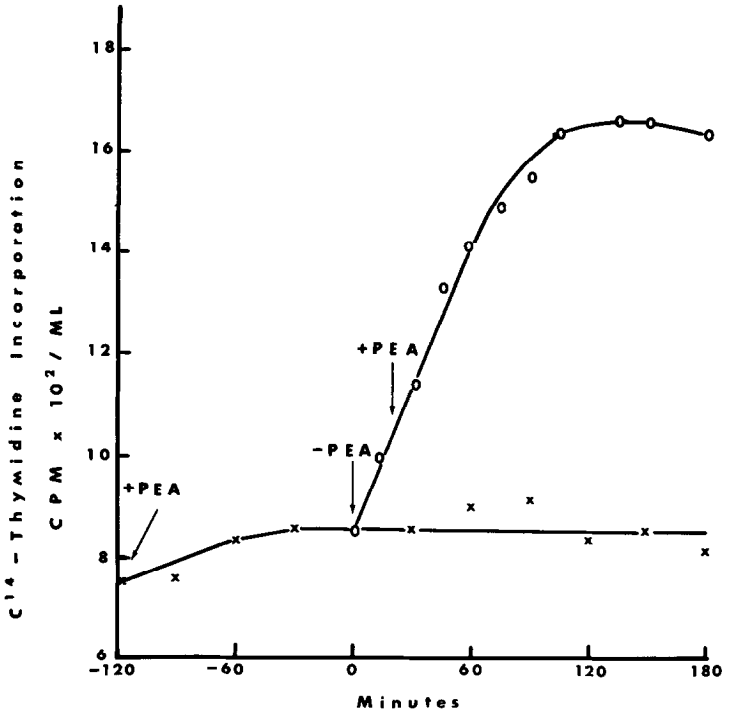

Fig. 1. Control of DNA replication in Hfr AB259 by phenethyl alcohol. Bacteria in presence of PEA throughout the experiment, X —— X. Bacteria in presence of PEA for 2 hr, PEA removed for 20 min, and PEA added again, 0 —— 0.

At zero time, the cells are filtered and resuspended in medium without PEA. The inhibition is released and a new cycle of DNA replication is initiated. Twenty minutes' incubation before readdition of PEA to the culture allows a doubling of the DNA as measured by C^{14}-thymidine incorporation (Figure 1). The continued DNA synthesis after 20 min represents the completion of the cycle of replication initiated during the 20 min period in the absence of PEA (Treick and Konetzka, 1964).

The control of DNA synthesis in Hfr cells by PEA allows an analysis of the role of DNA replication in the transfer of the Hfr chromosome during conjugation. Samples are removed at zero and forty min from the Hfr culture undergoing the PEA-controlled replication cycle shown in Figure 1 and are

mated with F⁻ cells. The kinetics of transfer of the chromosomal markers after mating are shown in Figure 2.

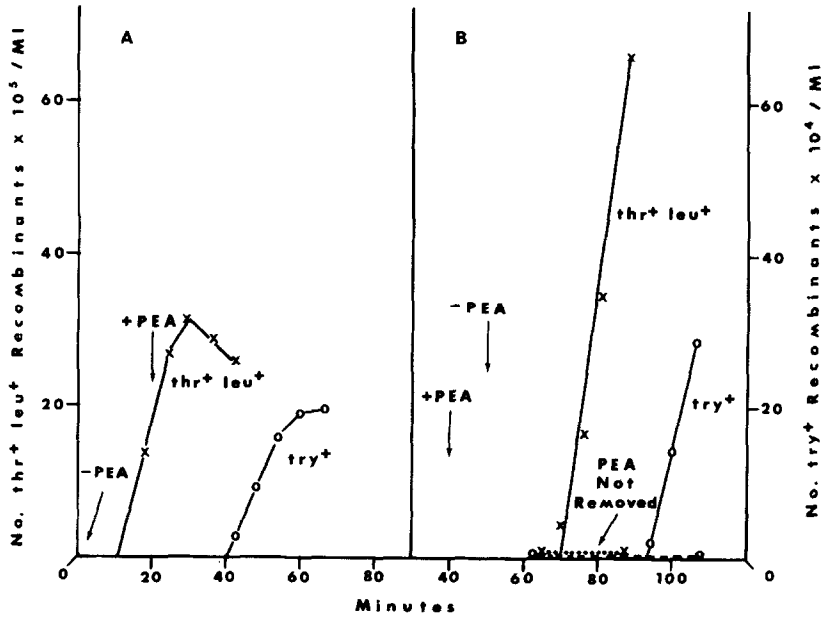

Fig. 2. Chromosome transfer during the DNA replication cycle. A. Kinetics of transfer when pair formation is concomitant with initiation of new cycles of DNA replication. PEA is removed from PEA-inhibited Hfr cells at time of mating and is added to the mating mixture 20 min later. B. Kinetics of transfer when pair formation is prior to initiation of new cycles of DNA synthesis. Mating of PEA-inhibited Hfr cells with F⁻ occurs in presence of PEA and the inhibitor is removed after 10 min. Control--mating occurs in presence of PEA and PEA is not removed. Thr+ leu+, X ···· X; try+, O ---- O.

Hfr cells mated at zero time in the absence of PEA are capable of transferring chromosomal markers as shown by the data in part A of Figure 2. The number of cells transferring markers is limited because the cells are diluted into medium containing PEA at 20 min. However, the transfer of the Try+ marker is not prevented by PEA although this marker does not begin to enter the F⁻ cells until 20 min after the readdition of PEA to the culture.

PEA-inhibited Hfr cells mated with the F⁻ strain in the presence of PEA can transfer their markers only when the inhibition is released by diluting into medium without PEA at 50 min as shown by the curves in part B of Figure 2. Thus, pair formation occurs in the 10 min period in the presence of PEA.

However, no transfer of markers is detected when the pairs remain in PEA throughout the entire period of sampling despite the fact that residual DNA synthesis is proceeding in the Hfr cells due to the release of the inhibition for 20 min. These findings show that a new cycle of DNA replication commencing after pair formation, and not DNA synthesis per se, is necessary for chromosome transfer.

Discussion. Bouck and Adelberg (1963) proposed that transfer could take place only when the closed circular chromosome opened at the completion of each cycle of DNA replication. According to this model, DNA synthesis occurring after pair formation but before the initiation of transfer permits the completion of replication cycles so that chromosomes reach the open state. Subsequent transfer is independent of DNA synthesis.

The replicon model of Jacob and Brenner (1963) states that the Hfr chromosome and the integrated sex factor are replicated by the bacterial replication system. Jacob, Brenner, and Cuzin (1963) suggested that chromosome transfer required concomitant replication of the Hfr chromosome so that one of the replicas passed into the F^- cells as it was formed.

Our results confirm the suggestion that chromosome transfer begins at a particular stage of the DNA replication cycle, namely, the beginning of a new cycle of replication. Transfer can occur only under conditions permitting the initiation of new cycles of DNA replication. The synthesis of DNA per se is not a sufficient condition for chromosome transfer. DNA synthesis which constitutes the completion of replication cycles previously initiated does not permit transfer to proceed.

In a normally growing culture, the Hfr cells will be at various stages in their replication cycles at the time of mixing with F^- cells. These cycles will be completed, and as new cycles begin, transfer will be initiated. Consequently, DNA replication is an integral part of the process of chromosome transfer.

The results obtained in this investigation are compatible with the repli-

con model and with the data of Jacob, Brenner, and Cuzin (1963) showing that DNA transferred from the Hfr to F^- cells is primarily DNA which is replicated after mixing the strains. The direction of replication of the chromosome during transfer is thus the same as the direction of transfer, proceeding from the Origin to the sex factor.

Acknowledgement: The authors wish to thank Dr. E. A. Adelberg for supplying the strains of bacteria employed in this investigation and Sally Dorwin for technical assistance.

REFERENCES

Bouck, N. and Adelberg, E.A. 1963 Biochem. Biophys. Res. Comm. 11: 24.
Boyce, R.P. and Setlow, R.B. 1962 Biochem. Biophys. Acta 61: 618.
Jacob, F. and Brenner, S. 1963 Comp. rend. acad. sci. 256: 298.
Jacob, F., Brenner, S. and Cuzin, F. 1963 Cold Spring Harb. Symp. Quant. Biol. 28: 329.
Treick, R.W., and Konetzka, W.A. 1964 Bact. Proc. 97.