THE RELATIONSHIP BETWEEN DNA SYNTHESIS AND CONJUGATION IN ESCHERICHIA COLI.[1]

Noel Bouck[2] and Edward A. Adelberg

Department of Microbiology
Yale University
New Haven, Connecticut

Received March 4, 1963

In Escherichia coli the ability to act as genetic donor in conjugation is determined by the presence of the sex factor, F. In F+ cells, F is present in an autonomous state; in Hfr cells, F is firmly bound to the chromosome. The chromosome has been deduced on genetic grounds to be circular in F+ cells, and also in Hfr cells which are not physiologically in the donor state (Taylor and Adelberg, 1961). Hfr cells in the donor state, however, exhibit a break in the chromosome adjacent to the attached sex factor. The break is recognized solely by the fact that one end of the broken chromosome, designated "Origin", becomes the leading point for transfer during conjugation. The chromosomal markers are transferred in linear order, the attached sex factor penetrating the recipient last (Wollman and Jacob, 1958)[3].

Hfr donor cells thus appear to undergo "breakage" of the chromosome at the time of conjugation, in the sense that a free end (the Origin) is created. Apparent breakage might occur in one of two ways. Conjugation might activate an enzyme which hydrolyzes phosphodiester linkages in the DNA. The enzyme might be associated with the sex factor, causing the break to occur at the site of F attachment. On the other hand, it has been suggested to us by Dr. A. J. Clark that a free end might arise as a direct consequence of chromosomal replication. The latter hypothesis is supported by the work of Nagata (1962) who has shown that replication starts at the attached sex factor in Hfr cells and proceeds linearly around the circular chromosome; the Origin is the last point to be replicated. When the process is complete the two daughter chromosomes must somehow undergo ring-closure. Thus, Origin may exist as a free end only for a

---

[1]This work was supported by Grant No. AI-04377 from the National Institutes of Health.

[2]Pre-doctoral Fellow of the National Institutes of Health.

[3]For a recent review of the conjugation process, see Clark and Adelberg (1962).

brief period of time between the completion of a replication cycle and the occurrence of ring-closure.

Following this line of reasoning we propose the following model: The completion of a replication cycle has two alternative consequences. In recipient cells, or in non-conjugating donor cells, replication is shortly followed by ring closure. In conjugating donor cells, on the other hand, replication is followed by the transfer of one of the daughter chromosomes before ring closure can occur. The transfer process itself is independent of DNA synthesis.

This model leads to the following picture of conjugation. When a non-synchronous population of Hfr cells is mixed with a population of F^- cells, specific pairs are quickly formed (de Haan and Gross, 1962). At the moment a given specific pair is formed, the Hfr chromosome is already partially replicated. Replication continues after contact, and transfer is initiated at the moment that the replication process in the Hfr cell reaches the Origin. If DNA synthesis is inhibited in the male population just before mating, transfer will take place in a very few specific pairs--namely, those in which the chromosome had just completed replication at the moment of conjugation. If, on the other hand, an inhibitor of DNA synthesis is added to samples withdrawn from the mating mixture at intervals, transfer will continue in those pairs in which it had been initiated by the completion of the replication cycle during conjugation. In this type of experiment a graph of the number of recombinants against the time of addition of inhibitor should yield a curve rising linearly from the origin. The present paper reports the results of such an experiment.

Materials and Methods. Crosses were performed between Hfr strain AB259 (the Hayes Hfr) and F^- strain AB2102. Strain AB259 transfers its markers in the order O-thr-leu-pro-lac-gal-try-his----[4]. It is wild type for all markers except thi-. Strain AB2102 carries the markers thr-, leu-, pro-, lac-, T6-r, gal-, try-, his-, str-r, mal-, thi-, and mtl-.

Matings were performed by the technique of de Haan and Gross (1962) with the following modifications. Cells were grown in minimal medium (Adelberg and Burns, 1960). Exponential male cells were mixed with exponential female cells in minimal medium at a ratio of 1 male to 10 females, to give a final cell concentration of 4×10^8

[4] Symbols: O, Origin; thr, threonine; leu, leucine; pro, proline; lac, lactose; gal, galactose; try, tryptophan; his, histidine; str-r, streptomycin resistance; mal, maltose; thi, thiamine; mtl, mannitol; T6-r, resistance to phage T6.

cells/ml. After five minutes incubation of 37°C with gentle shaking, the cells were diluted 1:500 and incubated at 37°C with continued gentle shaking. Two 10 ml samples were withdrawn from this diluted suspension of pre-formed specific pairs at various time intervals thereafter. One sample was immediately agitated violently for one minute, using a Waring Blendor, and the contents plated on several selective media to obtain different classes of recombinant colonies. To the other sample was added .04 ml of phenethyl alcohol, which has been shown by Berrah and Konetzka (1962) to be a specific inhibitor of DNA synthesis. The final concentration of phenethyl alcohol (0.4 per cent) was found by preliminary experiment to stop DNA synthesis in strain AB259 while reducing the viable count by only 36 per cent.

Samples to which phenethyl alcohol had been added were shaken at 37°C for an additional 95 minutes, following which they were agitated with the Waring Blendor and plated as in the case of the untreated samples. All plates were incubated at 37°C for 48 hours, at which time the recombinant colonies were counted.

Results. The results of a typical experiment are shown in Figure 1. It can be seen that the samples which were agitated and plated immediately without phenethyl alcohol treatment (graph A) give the classical picture of the kinetics of zygote formation. Since transfer was halted abruptly at the time of sampling by the shearing apart of specific pairs, the curves for the different markers extrapolate to different places along the abscissa. On the other hand, the samples which were treated with phenethyl alcohol and incubated for an additional 95 minutes before agitation and plating (graph B) give a family of curves all of which extrapolate to the origin. These results

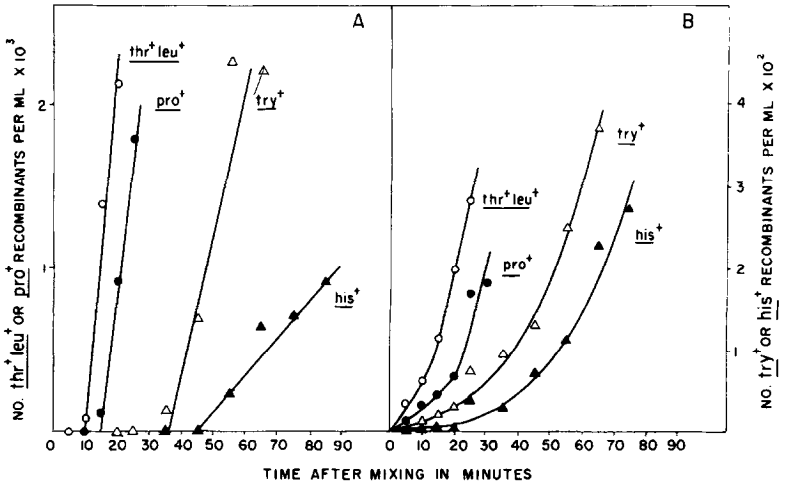

Figure 1. The effect of phenethyl alcohol on the kinetics of recombinant formation. All data corrected for the loss of viability of the recipient population due to phenethyl alcohol.

indicate that phenethyl alcohol inhibits the initiation of chromosome transfer, but does not stop transfer once it has begun. The concave shape of the curves is unanticipated and suggests that phenethyl alcohol, in addition to inhibiting the initiation of transfer, affects one or more other steps in the recombination process.

Discussion. de Haan and Gross (1962) have measured the degree of asynchrony in a population of donor cells with respect to the delayed initiation of chromosome transfer, and have suggested that "the delay may be due to the fact that transfer can only be initiated at a particular stage in the division cycle of the donor cell." Our model defines that stage as the replication of the chromosomal Origin.

The results shown in Figure I are in agreement with our model, which states that DNA synthesis in the donor bacterium is necessary for the initiation of chromosome transfer but not for the transfer process itself. Our model does not indicate which strands of DNA are transferred. Assuming the chromosome to consist of two complementary strands there should be four complete strands at the moment that transfer is initiated. If the strands are assorted according to a semi-conservative model of replication, the transferred material would consist of one new and one old strand.

References.

Adelberg, E. A., & S. N. Burns (1960) J. Bact. 79, 321.
Berrah, G., & W. Konetzka (1962) J. Bact. 83, 738.
Clark, A. J., & E. A. Adelberg (1962) Ann. Rev. Microbiol. 16, 289.
de Haan, P. G., & J. D. Gross (1962) Genet. Res. Cambridge 3, 251.
Nagata, T. (1962) Biochem. and Biophys. Res. Comm. 8, 348.
Taylor, A. L. & E. A. Adelberg (1961) Biochem. Biophys. Res. Comm. 5, 400.
Wollman, E. L. & F. Jacob (1958) Ann. Inst. Pasteur 95, 641.