POLARITY AND SYNCHRONY IN THE REPLICATION OF DNA MOLECULES OF BACTERIA*

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The investigation of different organisms by cytological and genetic methods has led to the concept that the chromosome replicates with a polarity from one or more fixed points (Taylor, 1959; Gall, 1959; Lissouba and Rizet, 1960). Maaløe (1961) proposed that a similar polarity might characterize the replication of bacterial chromosomes which may be single molecules of DNA with no inserted linkers. The possibility of molecular synchrony which may reveal such polarity in the replication of macromolecules is being examined by observing the time course of the duplication of prophage in populations of lysogenic bacteria engaged in synchronous growth.

Two strains of E. coli K 12 have been investigated; one is Z260 which is F^- and presumed to have a closed chromosome; the other is Hfr H in which the F factor occupies a definite site on the chromosome which is transferred last during conjugation. Although there may be a possibility that the Hfr

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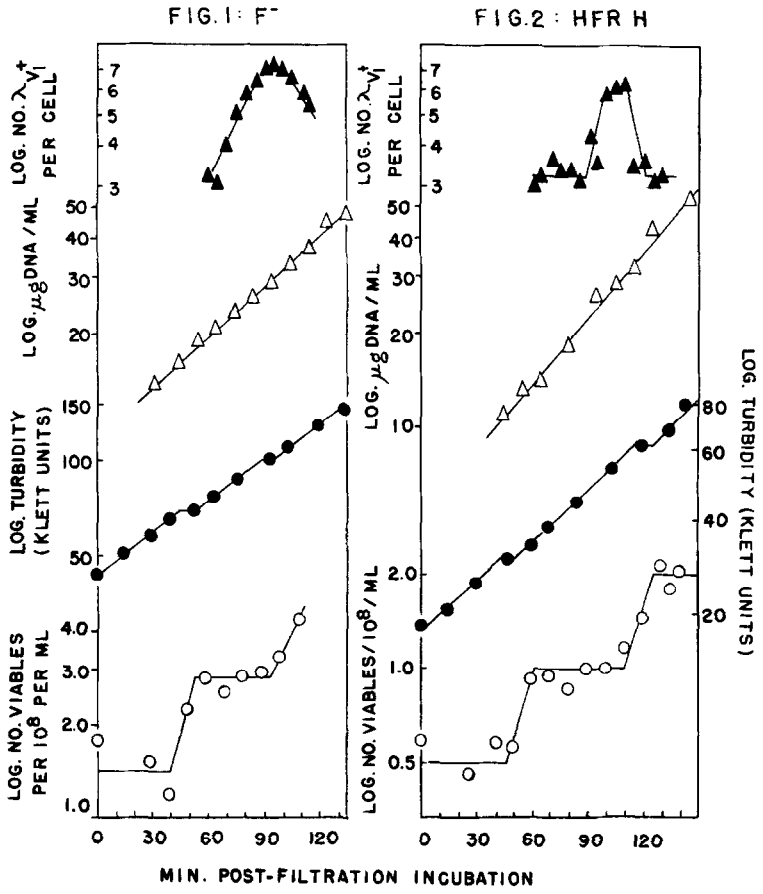
chromosome is closed when it is an F^- phenocopy (Taylor and Adelberg, 1961), it is known to be open at least at conjugation acting as Hfr. Both strains are derivatives of a single F^+M^- strain (58-161), are lysogenic for phage λ $v_1^+h^+$ and can be induced to form phage with nearly complete efficiency. The number of prophage per cell (pool size) was measured by superinfection with a mutant phage, λ v_1h, immediately upon induction. The resulting lysates were assayed for turbid (v_1^+) and clear (v_1) plaque-forming λ . The ratio of the two types is a function of both multiplicity of superinfection and pool size of prophage at the moment of induction-superinfection. The last parameter can be calculated by knowing the first two (Jacob and Wollman, 1953; Bertani, 1954); as a modification the relation was formulated taking the Poisson distribution of super-infecting phages into account. Before induction and superinfection the bacteria were synchronized by fractional filtration (Maruyama and Yanagita, 1956), the smaller cells being employed.

Fig. 1 and 2 show that both the F^- and the Hfr bacteria responded by a good synchrony in division. The increase in DNA (measured with diphenylamine reaction) and in optical density was almost exactly exponential (cf. Abbo and Pardee, 1960). On the other hand, the number of prophage increased with a pattern that differed for the F^- and Hfr strains. The continuous increase in the number of prophage per F^- bacterium, reduced only by cell division (Fig. 1), is not inconsistent with the hypothesis that the replication of DNA is polarized and synchronous but that it may begin at any point along the



continuous chromosome. Of course the alternative interpretations would be that there is no molecular synchrony, or that replication takes place from different sites in random directions. But these become unlikely when one considers the result obtained with the HfrH bacteria (Fig. 2), where the abrupt increase of the number of prophages was observed, indicating molecular synchrony and speaking for a polarized replication of DNA beginning at the posterior end of the chromosome. The number of prophages per cell begins to increase when DNA has finished some 50% of its replication and is completed when some 80% of the DNA is replicated, whereas the position of λ on the HfrH linkage map is ca. 80% from the posterior end.

These facts are consistent with the observation of Strelzoff (1962) that mutations in E. coli strain 15 were induced uniformly by 5-bromouracil in the course of a synchronous growth induced by thymine starvation. The failure to observe an abrupt increase of mutation could have been due to the fact that strain 15 is F^- and has a closed chromosome. Such experiments are being repeated with strains of Hfr where attempts will be made to deliver the mutagen in pulses.

Prophage experiments are also underway with other strains of Hfr which have λ in a different position on the chromosome, and with a single strain of Hfr which is lysogenic for two different prophages located far apart.

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