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Evidence for polarity of chromosome replication in F⁻ strains of *Escherichia coli*

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1. INTRODUCTION

Cairns has shown that the replication of the circular chromosome of *E. coli* is sequential. This work did not indicate whether the origin of replication is the same in all cells (Cairns, 1963). In *Bacillus subtilis* there is excellent evidence both for sequential replication and for a fixed origin of replication (Yoshikawa & Sueoka, 1963). Nagata (1963) attempted to determine whether the replication of the *E. coli* chromosome was also initiated at the same point in all cells. He used the variation in the pool size of prophage as a measure of gene duplication in synchronously dividing cells and concluded that the two Hfr strains investigated have polarized chromosome replication from fixed origins but that this is not the case for an F⁻ strain. We decided to reinvestigate the question of a fixed origin of replication of the F⁻ chromosome by measuring variation in the induced rate of enzyme synthesis at intervals throughout the cell cycle.

In synchronously dividing cultures of both *B. subtilis* (Masters, Kuempel & Pardee, 1964) and *E. coli* Hfr CS 101 (Kuempel, Masters & Pardee, 1965) the initial rates of synthesis of enzymes after induction have been found to be nearly constant during most of the cell cycle and then to increase sharply to a new value at characteristic times. This has been interpreted as a reflexion of the synchronous replication of the structural genes. This interpretation is based on the fact that the amount of a particular enzyme produced by a cell is determined in part by the number of copies of the corresponding structural gene which the cell carries (Jacob, Schaeffer & Wollman, 1960; Garen & Garen, 1963; Pittard & Ramakrishnan 1964; Donachie, 1964).

Our experiments show periodic changes in enzyme inducibility during synchronous growth of F⁻ strains and also support the interpretation that these are the result of synchronous gene replication in the cells. They therefore indicate that there is a fixed origin of replication in the *E. coli* chromosome in the absence of an integrated F factor. Evidence will also be presented that an F′ episome replicates once per cell cycle at a fixed time.

2. MATERIALS AND METHODS

Strains. E. coli K12 58–161 $St^R Met^- T_1^s T_6^R$ F⁻ was obtained from Dr E. Meynell and *E. coli* 15T⁻ from Dr K. Fisher. *E. coli* B/r was given by Dr S. Glover. The F′*Lac* donor was F′Lac^+/$Met^- Lac^+ St^S$ from the stock collection of Dr R. Clowes.

Media. All cultures were grown on M9 salts (Nagata, 1963) together with a carbon source and any required supplements. Glycerol was used as a carbon source in all experiments except that involving 15T⁻, which was grown with glucose to ensure a single period of nuclear replication per cell cycle (Lark & Bird, 1965).

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Cell counts. Cell numbers were estimated by viable counts on nutrient medium or by particle counts in formalin-fixed samples using a Coulter Counter with a 30 μ orifice.

Synchronization procedure. The sucrose gradient technique of Mitchison and Vincent (Mitchison & Vincent, 1965) was used.

Enzyme inductions. Samples were taken from synchronous cultures at intervals and added to inducer. The induction mixtures were shaken at 37°C. for 10 min. and the induction terminated by adding 50 μg./ml. chloramphenicol or, in the case of β-galactosidase, 40 μg./ml. CETAB (cetyl trimethyl ammonium bromide).

Inducer concentrations were, for β-galactosidase 10^{-3} M TMG (thiomethyl β-D-galactopyranoside), for tryptophanase 1 mg./ml. L-tryptophan and for D-serine deaminase 150 μg./ml. D-serine.

Enzyme assays. Standard procedures were used. These are given in the following references: β-galactosidase (Abbo & Pardee, 1960), D-serine deaminase (Pardee & Prestidge, 1955) and tryptophanase (Pardee & Prestidge, 1961).

3. RESULTS

(i) *Inducibility of β-galactosidase and tryptophanase in* 58–161 F^-

This K12 strain is the same as the F^- strain used in Nagata's experiments (Nagata, 1963). Figure 1 shows the results of an experiment in which the inducibility (enzyme

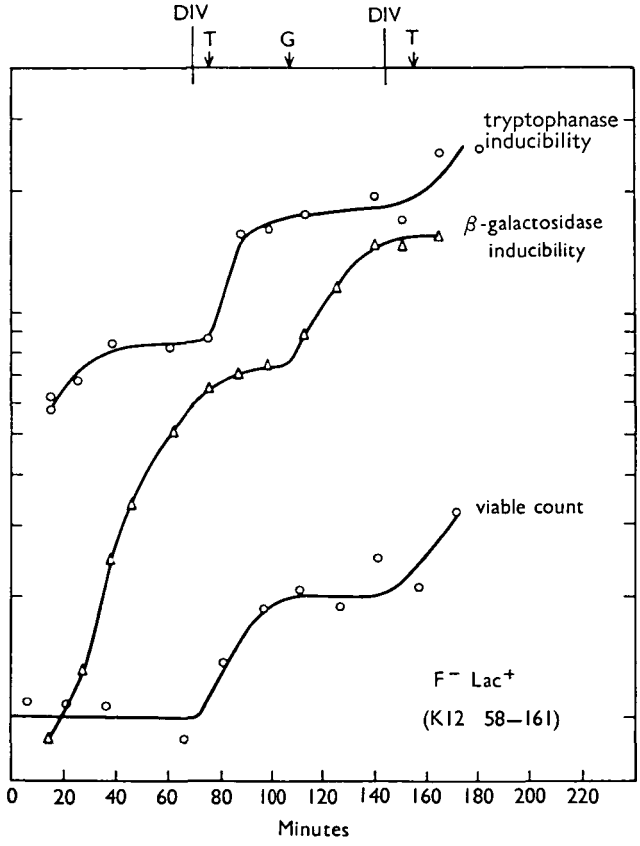

Fig. 1. Inducibility of β-galactosidase and tryptophanase during synchronous growth of *E. coli* K12 58–161 F^-. The estimated times of increase in inducibility (G, T) and cell number (DIV) are drawn at the top.

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synthesis in a 10-min. period following addition of inducer) of β-galactosidase and tryptophanase was followed throughout the first two synchronous cell divisions.

The very low level of inducibility of β-galactosidase following reinoculation is found in most experiments and probably results from a metabolic disturbance caused by the synchronization procedure. The inducibility of this enzyme is extremely sensitive to alterations in growth rate and other conditions that affect the general catabolite level. For this reason, a stepwise increase in inducibility is frequently difficult to detect during the first cell cycle. However, a sharp step is almost always found to occur in the middle of the second cycle. In contrast the inducibility of tryptophanase (which is much less disturbed by the synchronization procedures) characteristically increases at about the time of cell division.

The changes in inducibility of these two enzymes are therefore not triggered by the same event. In this connexion it is relevant to note that the chromosomal loci corresponding to these enzymes (*Lac* and *Ind*) are 30% of the genome distant from one another (Taylor & Thoman, 1964).

(ii) *Inducibility of β-galactosidase and tryptophanase in F'*Lac$^+$/Lac$^+$ 58–161

The 58–161 F$^-$ strain used in the previous experiments was infected with F'*Lac*$^+$. Genetic tests confirmed its genotype to be F'*Lac*$^+$/*Lac*$^+$ 58–161. It was thus possible to test the effect of a double set of *Lac*$^+$ genes on the inducibility of β-galactosidase. It was hoped that, as in the case of F$^+$ in *Proteus* (Rownd, 1965), the F' episome would replicate at a fixed time in the cell cycle and that this would be different from the time of replication

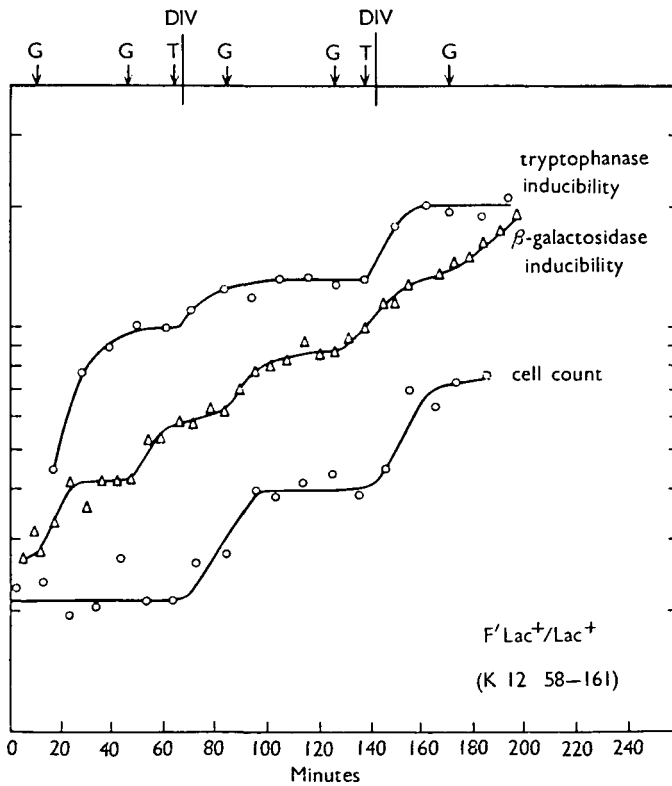

Fig. 2. Inducibility of β-galactosidase and tryptophanase during synchronous growth of F'*Lac*$^+$/*Lac*$^+$ 58–161.

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of the chromosomal *Lac* locus. If the steps reflect gene duplication, two stepwise increases in inducibility of β-galactosidase would be expected in each cell cycle.

Figure 2 shows the inducibility of β-galactosidase and tryptophanase during synchronous growth of F′*Lac*$^+$/*Lac*$^+$ 58–161. Two steps per cell cycle in the inducibility of β-galactosidase can be distinguished. This is always found with this strain. However, as in the parent strain, there is only a single step in tryptophanase inducibility in each cell cycle. This is as expected, since the *Ind* locus is not carried on the episome.

(iii) *Relationship between steps in inducibility and DNA replication*

If the sharp periodic increases in inducibility of enzymes do reflect periodic gene replication, then they should be absent in the absence of DNA replication. To test this we examined the effect of thymine starvation on the inducibility of D-serine deaminase during synchronous growth of *E. coli* 15T$^-$.

An exponentially growing asynchronous population of 15T$^-$ was collected and fractionated on a sucrose gradient. (Thymine was present in the gradient.) The top fraction of cells (the smallest cells) was then collected and washed free of thymine with warm medium.

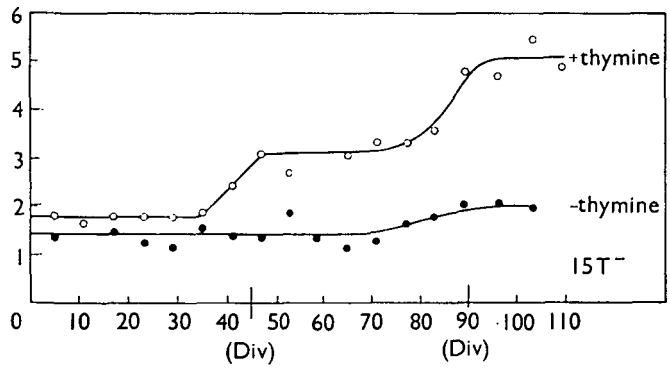

Fig. 3. Inducibility of D-serine deaminase in *E. coli* 15T$^-$ in the presence and absence of thymine. See text for experimental procedure. The estimated times of cell division in the thymine-supplemented culture are shown on the abscissa.

Equal portions of the washed cells were inoculated into medium with and without thymine. The cell counts in the thymine-supplemented culture rose in steps, while in the thymine-starved culture the counts rose immediately after inoculation and thereafter remained constant. The optical densities of the two cultures increased at the same exponential rate (doubling time, 45 min.) for about 65 min., when the density of the thymine-starved culture ceased to increase.

Figure 3 shows the inducibility of D-serine deaminase in the two cultures together with the approximate times of division in the thymine supplemented culture. It is clear that the regular stepwise increase in inducibility is dependent on the presence of thymine. In the thymine-starved culture inducibility remained at its initial level during the period when its mass (as judged by optical density) increased threefold. (A small rise in inducibility always follows the cessation of growth in the thymineless cells but this is probably an effect of change in growth rate itself.)

Such experiments show that sharp periodic changes in inducibility are dependent on continuing gene replication. Inducibility of D-serine deaminase seems to be related to the amount of DNA rather than to the total cell mass. This provides good support for the basic assumption that gene duplications are responsible for the observed stepwise increases in inducibility in synchronous cultures.

(iv) *The timing of inducibility changes*

The estimated times of initiation of periods of increasing inducibility have been collected together from all experiments so far performed. These are given in Fig. 4. Data obtained from *E. coli* B/r are also included. Since generation times vary from strain to strain, the initiation times are expressed as fractions of the corresponding cell cycle. It is clear that the inducibility changes in β-galactosidase and tryptophanase are clustered around particular times in the cell cycle. Moreover thay appear to take place at the same relative times in both K12 F⁻ and B/r F⁻ strains of *E. coli*.

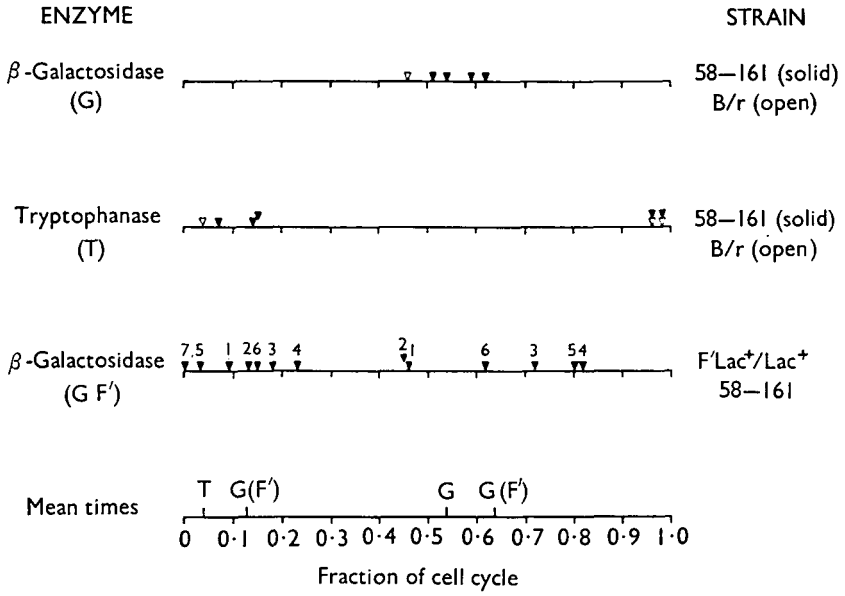

Fig. 4. Collected times of inducibility steps in β-galactosidase and tryptophanase in 58–161 F⁻, F′*Lac*/58–161 and B/r. The times are expressed as fractions of the cell cycle in which they occur. To calculate mean times, tryptophanase times which came immediately before cell division were assigned negative values and it was assumed that the times for β-galactosidase in F′*Lac*/58–161 fell into two different groups. In line 3, the different numbers refer to different cell cycles. Matching pairs of numbers indicate that these changes in inducibility occurred in the same cell cycle.

In F′*Lac*/58–161 there are evidently two periods during which inducibility changes in β-galactosidase occur. The first and second steps in each individual cycle always fall into the first and second groups (see Fig. 4). Since the second group overlaps the time of inducibility change in the haploid F⁻ strains, it appears probable that the first group results from the replication of the F′ episome. Thus it would seem that the F′ factor replicates soon after the beginning of cell separation.

SUMMARY

The inducibility of three enzymes (β-galactosidase, tryptophanase and D-serine deaminase) has been measured at various times during the cell cycles of three strains of *Escherichia coli* (K12 58–161 F⁻, B/r F⁻ and 15T⁻). In each strain sharp increases in inducibility of these enzymes occurred at characteristic periods in each cell cycle. Such increases depend on DNA replication and therefore probably reflect synchronized gene

replication. It is inferred that chromosome replication in these F$^-$ strains is sequential from a fixed origin.

Infection with F′*Lac*$^+$ results in an extra period of increase in inducibility of β-galactosidase in each cell cycle. It is concluded that the F′ episome replicates once in each cell cycle at a time soon after cell separation.

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