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Pattern of Replication of a Colicin Factor During the Cell Cycle of *Escherichia coli*

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The ColBM, *trp*, *lac* episome was transferred to a *lacZ* derivative of *Escherichia coli* B/r, and the manner of replication of this colicinogenic factor was followed through the cell cycle. The results suggest a pattern of replication not connected with any particular stage in the cell cycle.

Bacterial plasmids are relatively small extrachromosomal molecules of deoxyribonucleic acid (DNA) that are maintained during growth of various strains of bacteria. The mechanism by which the replication of plasmids is controlled is not understood at the moment.

The replication of one plasmid, the F*lac* episome, has previously been shown to occur at a specific time in the middle of the division cycle of *Escherichia coli* B/r over a wide range of growth rates (10). For other plasmids, however, the pattern of replication appears to be different. Bazaral and Helinski (1) have presented evidence that not all ColE1 plasmids are replicated over one cycle but that the molecules to be replicated are chosen at random from a pool of plasmids. Since some plasmids are replicated twice during a cycle, they could not all be replicated at the same time. Similar results have been reported for the resistance transfer factor (RTF) plasmid DNA (9). The difference between the pattern of replication of the F*lac* episome and the ColE1 and RTF plasmids may result from the fact that there are only a small number of F*lac* episomes per cell (2), whereas there is a demonstrably larger pool of ColE1 (1) and RTF (9) plasmids in each cell.

To investigate this problem further, the pattern of replication of one additional plasmid, the colicinogenic factor ColBM, *trp*, *lac*, has been investigated by using similar methods as previously described for the study of the replication of the F*lac* episome (10).

Colicinogenic factors are genetic units of varying complexity in which the structural genes for the synthesis of one or several colicins may be associated with genes governing other properties such as repression, immunity, susceptibility to male-specific phages, etc., as well as with genes originating from the bacterial chromosome (3). Some of these colicinogenic factors carry fertility genes that make it possible to transfer them to different strains by conjugation.

One such hybrid colicinogenic factor, the ColBM, *trp*, *lac* episome carrying bacterial *trp* and *lac* genes was kindly provided by P. Fredericq. To our knowledge it is the only colicinogenic factor carrying *lac* operon genes. The origin of this episome is rather complex. It is essentially composed of colicin genes and *trp* genes derived from a strain of *E. coli* (K 260) and of *lac* genes derived from *E. coli* K-12.

The ColBM, *trp*, *lac* factor was transferred at low frequency from the K-12 strain [58LC16 *mal thi lacY* Δ (*trp-tonB*)] to a B/r strain carrying the markers *thy lacZ* (derived from strain DF obtained from David Freifelder; cf. Zeuthen and Pato [10]). Recombinants were scored on lactose minimal plates supplemented with thymine (10 μg/ml) and checked for low thymine requirement, Lac$^+$ phenotype, and the production of colicin.

The replication of the ColBM, *trp*, *lac* episome during the cell cycle of *E. coli* B/r was investigated by following the inducibility for β-galactosidase in synchronously growing cultures of *E. coli* B/r *thy lacZ*/ColBM, *trp*, *lac* obtained with the membrane selection technique of Helmstetter and Cummings (7), and in cells separated from exponentially growing cultures (according to their ages) by the membrane elution procedure of Helmstetter (4). The amount of enzyme synthesized in response to a

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short period of induction is assumed to reflect the relative number of copies of the β-galactosidase structural genes (*lacZ*) present at any stage of the cell cycle. The pattern of replication of the bacterial chromosome can be followed by pulse labeling the culture with ^{3}H-thymidine and determining the amount of radioactivity incorporated by cells of different ages, as described by Helmstetter (4) and Helmstetter et al. (6).

Inducibility for β-galactosidase in a synchronously growing culture of *E. coli* B/r *thy lacZ*/ ColBM, *trp*, *lac* increases exponentially through three division cycles (Fig. 1) in contrast to the stepwise increase observed previously for the chromosomal *lac* marker (5, 8) and for the *lac* marker carried on an F*lac* (10). In the corresponding membrane elution experiment (Fig. 2), the amount of β-galactosidase pulse-induced per cell decays exponentially with a $t_{1/2}$ equal to the generation time of the culture. In contrast, the elution curve of counts per minute per cell after ^{3}H-thymidine pulse labeling shows the characteristic steps, in agreement with a DNA synthesis rate doubling in the middle of the cycle, corresponding to an event of chromosomal initiation (4, 6, 10).

The results are interpreted to indicate that the replication of the ColBM, *trp*, *lac* episome

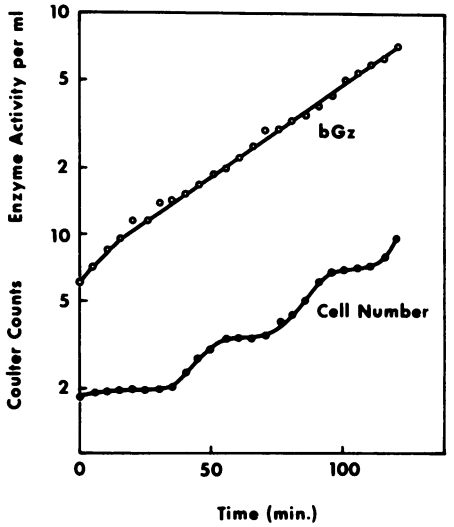

FIG. 1. *Time course of β-galactosidase inducibility in a synchronously growing population of E. coli B/r thy lacZ/ColBM, trp, lac obtained by the membrane selection technique of Helmstetter and Cummings (7). Cells were grown in a glucose minimal medium supplemented with thymine (10 μg/ml) and deoxyguanosine (50 μg/ml) at 37 C in a shaking water bath. The doubling time during exponential growth was 52 min, slightly prolonged compared to the parent strain without the colicin factor. A 100-ml amount of exponentially growing culture at an optical density of 0.300 at 450 nm was filtered onto a 0.22-μm pore size membrane filter (Millipore type GSWP), which was subsequently inverted and continuously eluted for 2 hr at 37 C. The eluate was collected in a chilled flask, filtered, and resuspended into prewarmed medium at a 10-fold concentration compared to the original eluate. Cell numbers were determined with a specially constructed Coulter counter after dilution of samples into formaldehyde-saline solution. Inducibility was determined by inducing samples from the culture with 10^{-3} M IPTG (isopropyl-β-D-thiogalactopyranoside, Mann Biochemical) for 10 min. Enzyme activity was assayed with ONPG (o-nitrophenyl-β-D-galactopyranoside, Calbiochem) as substrate.*

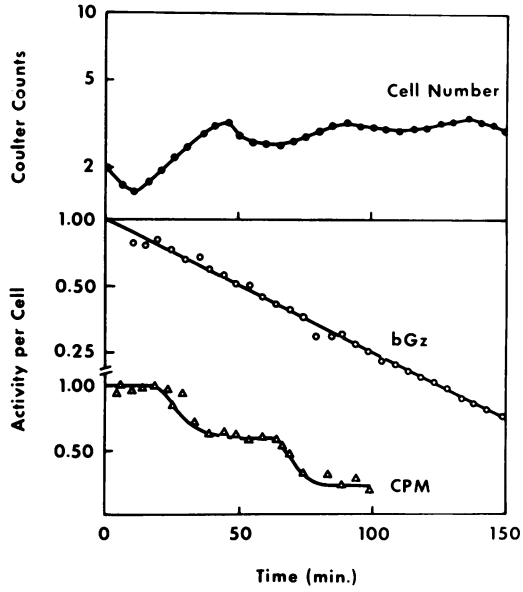

FIG. 2. *Elution patterns of cell numbers, β-galactosidase activity per cell, and radioactivity per cell for a membrane-bound glucose minimal culture of E. coli B/r thy lacZ/ColBM, trp, lac. A 100-ml amount of a glucose-grown culture as in the previous experiment was subjected to a 2-min pulse of 20 μCi of thymidine-methyl-^{3}H at a specific activity of 40 μCi/μmole, and a 3-min pulse of 10^{-3} M IPTG. Both pulses were terminated by filtration onto a membrane filter and subsequent washing with fresh medium. Immediately thereafter, elution with prewarmed medium was started at 37 C, and samples were collected consecutively. Samples for the determination of radioactivity were precipitated with cold 5% trichloroacetic acid containing 100 μg of carrier thymidine per ml. After filtration and washing, samples were counted in a toluene-based scintillation mix. Samples for the determination of β-galactosidase activity were assayed after incubation for 20 min at 37 C to allow full expression of β-galactosidase activity. For a full description of the procedures see Zeuthen and Pato (10).*

occurs essentially at random during the cell cycle of *E. coli* B/r and cannot be associated with any particular physiological event in the cell cycle as might be the case for the F*lac* episome (10). This conclusion is in good agreement with the results obtained for the ColE1 (1) and RTF (9) plasmids which indicate that these plasmids are replicated by the selection at random from a large pool of plasmids. The size of this pool is not known for the ColBM, *trp*, *lac* episome, but the kinetics of induction for β-galactosidase suggests that it is larger than for the F*lac* episome.

Thus, five of the replicons harbored by *E. coli*—the bacterial chromosome, the F*lac* episome, and the RTF, ColE1, and ColBM episomes—have been found to represent three different classes of replicons. The common and unique elements involved in their replication remain to be elucidated.

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