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Deoxyribonucleic Acid Replication and Cell Division in *Escherichia coli* at 33 C

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At 33 C (60-min generation time) the time required to replicate the chromosome is C = 60 min. The time between the end of a round of replication and cell division is D = 20 min, as at 37 C. Nalidixic acid and a temperature shift in a *dnaB* mutant give identical results for the determination of the end of a round of replication.

The life cycle of *Escherichia coli* B/r growing at 37 C has been described in detail by Helmstetter et al. (4). Irrespective of the generation time (if it is <60 min), it takes a constant time C = 40 min to replicate the chromosome. After the end of a round of chromosome replication, a constant time D = 20 min elapses until the cell divides.

Since many investigators use temperature-sensitive mutants in the investigation of the *E. coli* life cycle, it is desirable to extend the Helmstetter model to cells growing at temperatures commonly used with these mutants.

E. coli WM-301 (F^-, *leu*, *pro*, *lac*, *try*, *his*, *arg*, *thy*, *str*, *met*, *dra* or *drm*, hsp^{K12}) is a B/r strain except for the hsp^{K12} marker and a derivative of strain HB-50 from H. Boyer. *E. coli* WM-331 is a derivative of WM-301 carrying a temperature-sensitive mutation *dnaB* (B/r7 of Mikolajczyk and Schuster [9]).

Cells were grown in glucose-minimal medium (3) and synchronized by a modification of the membrane selection technique of Helmstetter and Cummings (5, 8). Synchronous growth was followed with a Coulter Counter combined with a Nuclear Data multichannel analyzer.

The rate of deoxyribonucleic acid (DNA) replication was determined with 4-min pulses of ^{3}H-thymidine (2.5 μCi/ml; 20-30 Ci/mmole).

Cell division becomes insensitive to the inhibition of DNA replication when the chromosome is fully replicated. This effect has been used to determine the end of a round of DNA replication by Clark (2), who inhibited DNA synthesis with nalidixic acid, and by Helmstetter and Pierucci (6), who inhibited replication by thymine starvation.

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Figure 1A and B compares the effects of inhibition of DNA synthesis by nalidixic acid and by a shift to the nonpermissive temperature in strain WM-331.

To a synchronized culture growing in glucose-minimal medium at 33 C, nalidixic acid (10 μg/ml) was added at different times during the life cycle, and the residual cell division was determined (Fig. 1A). In a parallel culture, samples were shifted to 42 C at different cell age, and cell division occurring at 42 C was measured (Fig. 1B). The cell number reached after inhibition of DNA synthesis was plotted against the time of addition of nalidixic acid or the time of the temperature shift, respectively. This procedure gives the potential of cells to divide after blocking replication, i.e., the proportion of cells that is beyond the end of the replication cycle.

In both experiments, the end of a round of replication for the average cell was at 40 min, cell division occurred at 60 min, (both taken from the inflection points of the curves). Inflection points were determined as the mid-point between two plateaus.

Inhibition of replication by nalidixic acid and by a temperature shift in a *dnaB* mutant give identical results in the determination of the end of a round of DNA replication, thus both techniques seem to give a valid measurement for this step in the life cycle.

Cell division, the end of a round of DNA replication, and initiation were compared in cultures growing synchronously at 33 and at 37 C (Fig. 2A and B). The end of a round of replication was determined as described for Fig. 1A. The rate of DNA replication increased in steps, giving about a factor 2 increase in rate. This, together with the time observed for the

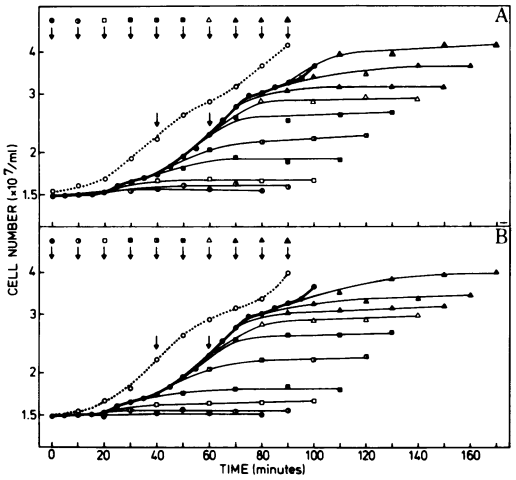

FIG. 1. *End of a round of DNA replication in E. coli WM-331 growing at 33 C. A, Nalidixic acid (10 μg/ml) was added to a synchronized culture at different cell age (arrows). Cell number was measured in the different samples. The maximal cell number reached in the presence of the drug reflects the number of cells that reached the end of a round of replication, and was plotted versus the time of addition of the inhibitor (···O···). Control without nalidixic acid (——●——). B, Samples of a synchronized culture were shifted to 42 C at different cell age (arrows). Cell number was determined and plotted as in A. The inflection points of the curves are indicated by arrows.*

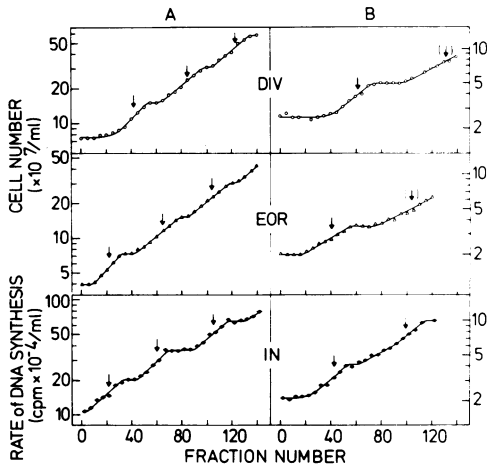

FIG. 2. *Cell division, end of a round of DNA replication, and initiation of replication in E. coli WM-301 at (A) 33 C and (B) 37 C. End of a round of DNA replication (EOR) was determined as in Fig. 1A. Arrows indicate the inflection points of the curves. (↓) were set at 1.5 times the level of the preceding plateau. Synchronization was always poorer at 33 C as compared to 37 C.*

end of a round of replication, indicates that no dead time without replication occurred. The time of initiation is taken from the inflection points of the curves.

As shown in Fig. 2A for the culture growing at 37 C with 40-min generation time, successive steps of initiation occurred in 40-min intervals. The time between the end of a round of replication and division was 20 min. Thus times C and D were as predicted by the Helmstetter model (4).

At 33 C (Fig. 2B) generation time was 60 min. As in Fig. 2A, initiation and the end of a round of DNA replication occurred at the same time. The intervals between successive steps of initiation, however, were 60 min. The time between the end of a round of replication and cell division was 20 min.

The increase of generation time from 40 to 60 min when changing temperature from 37 to 33 C thus is reflected in the time required to replicate the chromosome, C being 60 min at 33 C. The time between the end of a round of replication and cell division, however, remained constant, D = 20 min.

This time D is mainly determined by the requirement to form polar caps (7). Obviously this process is much less affected by a change in temperature than DNA replication.

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