Host Cell Variations Resulting from F Plasmid-Controlled Replication of the Escherichia coli Chromosome

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Cell size and DNA concentration were measured in Escherichia coli K-12 ET64. This strain carries a dnaA(Ts) mutation that has been suppressed by the insertion of the F plasmid into the chromosome. ET64 can grow in a balanced steady state of exponential growth at the restrictive temperature for its dnaA allele (39°C), in which chromosome replication is controlled by the F plasmid, and at the permissive temperature (30°C), in which chromosome replication is controlled by dnaA-oriC. When cells grown at the indicated temperatures were compared, it was observed that at 39°C, the cell mass increased and the amount of cellular DNA decreased slightly; therefore, the DNA concentration was strongly reduced. These changes can neither be explained by the reduction of the generation time (which is only 10-15%) nor from observed changes in the replication time and in the time between DNA synthesis termination and cell division. Variations were mainly due to the increase in cell mass per origin of replication, at initiation, in cells grown at 39°C. Control of chromosome replication by the F plasmid appears to be the reason for the increase in the initiation mass. Other possible causes, such as the modification of growth temperature, the generation time, or both, were discarded. These observations suggest that at one growth rate, the F plasmid replicates at a particular cell mass to F particle number ratio, and that this ratio is higher than the cell mass to oriC ratio at the initiation of chromosome replication. This fact might be significant to coordinate the replication of two different replicons in the same cell.

Replication of the chromosome of Escherichia coli is regulated at the step of initiation. Replication is normally initiated at a fixed origin, oriC (2), and proceeds bidirectionally to the terminus region of the chromosome. Dependence of initiation at oriC on dnaA product is absolute (8, 22). However, the dnaA mutation can be suppressed by the insertion of a plasmid into the bacterial chromosome (11, 13, 21). There is evidence that such suppressed strains initiate replication at the plasmid site (3, 11, 13), which is in apparent contradiction with the failure of initiation at the plasmid site when integrated in dnaA+ strains. A model for the control of replication that explains these facts has been proposed by Pritchard et al. (17, 19). This model states that replicons are controlled by their own negatively acting gene products. Initiation of replication raises the inhibitor concentration, reducing the probability of further initiation; growth of the host cell between two rounds of replication progressively reduces inhibitor concentration, therefore increasing the probability of initiation. A mechanism of this type has been demonstrated to control the replication of several bacterial plasmids, e.g., ColE1 and R1 (10, 12, 18). Pritchard’s (17) model also proposes that in plasmids able to produce integrative suppression, their repressor should be diluted further than the chromosomal repressor. Consequently, these plasmids, when integrated in dnaA+ strains, are passively replicated as part of the chromosome, with initiation at the plasmid site being cancelled. In contrast, strains lacking the dnaA product can carry out initiation at the plasmid site but at a higher cell mass. To test this hypothesis, we measured the cell mass to chromosome origin ratio at initiation of replication, the so-called initiation mass (Mi) (7, 16) in a dnaA E. coli strain which was suppressed by the insertion of the F plasmid into the chromosome. Mi values are compared in this strain under conditions in which replication is controlled by oriC-dnaA or it is under the control of the integrated F replicon.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used were all derivatives of E. coli K-12. Strains ET64 thr leu thi lacY dnaA46(Ts) thyA deoC intF, ET65 thr leu thi lacY dnaA46(Ts) thyA deoC, and A4 thr leu thi lacY thyA deoC are thymine auxotrophs derived, respectively, from ET1 (21), A3 (21), and A3+ (14) by trimethoprim selection (20). A4F* is an F+ derivative of A4. Maintenance of the dnaA mutation and of the Hfr characteristic was tested in ET64 for its ability to transfer the dnaA thermosensitive allele. Matings were done with strain X36 as receptor as described previously (21).

Cells were grown at 30°C or 39°C in M9 minimal medium (1) supplemented with 20 μg of thymine per ml, 10 μg of vitamin B1 per ml, and 40 μg of the required amino acids per ml. Unless otherwise indicated, 0.4% glucose was used as the carbon source. Media were solidified with 1.5% agar for plating purposes.

Determination of cell number, cell mass, and DNA. Particle number was determined with a Coulter Counter (model ZB1; Coulter Electronics, Inc., Hialeah, Fla.). Viable cells were measured, after an appropriate culture dilution was plated on minimal agar, by counting the number of resulting colonies. Cell mass was determined as the optical density at 450 nm (OD450) with a Guilford 300-N spectrophotometer. Cell DNA was estimated in cultures that had been labeled by growth with 14C thymine at a final specific activity of 0.04 μCi/μg (Radiochemical Centre, Amersham, Bucks, United Kingdom). Samples (1 ml) were added to 1 ml of 10% trichloroacetic acid and kept on ice for at least 1 h. The precipitates were collected on Whatman GF/F filters, washed with hot water, air dried, and then placed in vials with toluene-based scintillation liquid. Radioactivity (in counts per minute) was measured in a Beckman LS7500 scintillation counter.

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Inhibition of macromolecular synthesis. Protein synthesis was inhibited by the addition of chloramphenicol (200 μg/ml). DNA synthesis was inhibited by thymine starvation. Culture samples were collected on nitrocellulose membrane filters (pore size, 0.45 μm; Millipore Corp., Bedord, Mass.), and cells were washed twice with the same volume of thymine-free growth medium and then suspended in the same medium.

Calculation of the replication time and the time between DNA synthesis termination and cell division. The replication time (C) was calculated as reported by Bremer and Churchward (4) with the equation

\[ G_0/G_t = C(\ln 2)^{2D/t} \left( 1 - 2^{-C/2t} \right) \]

where \( G_0/G_t \) is the ratio between the final amount of DNA in cultures to which chloramphenicol has been added and the DNA content of the same cultures before this treatment, \( t \) is the replication time, and \( t_d \) is the time of delay in the cessation of replication initiation after protein synthesis inhibition. The average \( t_d \) of the cultures was calculated from the kinetics of DNA content in cultures to which chloramphenicol was added, by using the \( 1/\gamma \) curve as described by Bremer and Churchward (4).

If it is assumed that there is an exponential distribution of cell ages (15), the time between DNA synthesis termination and cell division (D) is given by

\[ D = \tau \ln(1 + n)/\ln 2 \]

where \( n \) is the fraction of cells that divides in the absence of DNA synthesis. For this purpose we used thymine starvation, and \( n \) was indirectly determined (to avoid possible errors because of the presence of residual thymine or cell losses during washing) from the relationship (A. Puyet and J. L. Canovas, Bacterial Morphogenesis, abstract, p. 46, 1984).

\[ G_0/G_x = 1 + n; G_0/G_x \text{ is the ratio between DNA per cell in the cultures before and after thymine starvation.} \]

Calculation of \( D_i \) and average cell DNA content in genome equivalents. Previous authors (for a review, see reference 9) have shown that when a culture is in balanced growth and if an exponential cell age distribution is assumed (15), the average cell mass (\( \tilde{M} \)) and the average cell DNA content in genome equivalents (\( \tilde{G} \)) can be described as functions of \( C \), \( D_i \), and \( \tau \):

\[ \tilde{M} = \bar{M} \left[ 2^{C + D_i/\tau} \right] \]

\[ \tilde{G} = \tau 2^{D_i/\tau} \left( 2^{C/\tau} - 1 \right)/C(\ln 2) \]

Absolute \( \bar{G} \) values can be obtained with equation 1. \( \bar{M} \) can be calculated in relative units as a function of \( C \) and DNA concentration (DNA/M = cpm/OD_{450}) with the equation resulting from dividing equation 2 by equation 1:

\[ \text{DNA/M} = K (1 - 2^{-C/2\tau}) \bar{M} \ln 2 \]

where \( K \) is the conversion factor between radioactivity incorporated into DNA and \( \tilde{G} \).

\[ \text{RESULTS} \]

Growth and cell parameters of \( E. coli \) ET64. Strain ET64 is a stable \( dnaA46(Ts) \) mutant that was suppressed by the insertion of the F plasmid into the chromosome. This strain, as well as others used in this study, can be maintained in a balanced steady state of exponential growth, at least for all measured quantities. This fact is particularly relevant in ET64 due to its abnormal control of DNA replication. Strain ET64 does show balanced growth at both the permissive temperature for its \( dnaA \) allele (30°C) and the restrictive temperature used in this work (39°C; this is the minimal restrictive temperature for the \( dnaA6 \) protein, as shown by the analysis of DNA synthesis in strain ET65). Optical absorbance, particle number, viable cell number, and DNA content all increased at the same rate after 20 generations of exponential growth.

Comparison of cells grown in the same medium, at 30 and 39°C (Table 1), showed striking changes in cell composition, although the generation time was only slightly modified. At 39°C the average cell mass (OD_{450} per number of particles) significantly increased and the cell DNA content (cpm per number of particles) decreased somewhat; then the DNA concentration (cpm/OD_{450}) was strongly reduced.

We measured the values of \( C \), \( D_i \), and \( \tau \) in ET64 to learn the reasons for the observed changes in cell composition. The time \( C \) was calculated from the incremental increase in DNA of the cultures after the addition of chloramphenicol, taking into account the time of delay in the cessation of replication initiation after protein synthesis inhibition (4). Time \( D_i \) was determined from the variation in cell DNA content after thymine starvation (Puyet and Canovas, abstract). An example of the data provided by these treatments is illustrated in Fig. 1. The length of \( C \) and \( \tau \) and the corresponding \( G \) values (as calculated with equation 2) in ET64 cells grown at 30 and 39°C are shown in Table 1. Our measurements of \( C \) and \( D_i \) appear to be correct because the ratio of calculated \( G \) at both temperatures is practically equal to the ratio of measured radioactivity incorporated into DNA. Therefore, the cell mass increase observed in populations grown at 39°C might be derived from an increase in \( M \) because, according to equation 1, it is not produced by measured changes in \( C \), \( D \), and \( \tau \).

Effect of F plasmid-controlled replication on \( M_i \). As indicated above, a possible method of calculating \( M \) is provided by equation 1; \( M \) values obtained by this method are shown in Table 2. Apparently, \( M_i \) is much higher in cells grown at 39°C. \( M_i \) can also be calculated in relative units with equation 3. Results obtained from two independent experiments are reported in Table 2. They show a striking increase of \( M \) in ET64 cells grown at 39°C, confirming \( M \) calculations with equations. This increase is not due to changes in \( \tau \) (5), or growth temperature, or both. Higher changes of \( \tau \) in ET64 grown at 39°C, or in an isogenic strain A4 \( (dnaA4 (dnaF)) \) grown at 39°C, induce much less of an increase in \( M \) (Table 3). It can also be seen in Table 3 that a growth temperature change from 30 to 39°C produced a slight reduction of \( M_i \) in A4 cells. It was also tested whether the F plasmid affects \( M \) in normal oriC-dnaA-controlled replication. Comparison
FIG. 1. The kinetics of cell DNA content after macromolecular synthesis inhibition in strain ET64 grown at 30°C. Protein synthesis was inhibited by the addition of 200 μg of chloramphenicol per ml (A): the V curve values (see equation 8 and Fig. 3 in reference 4) corresponding to this experiment, are represented in the inset. DNA synthesis was inhibited by thymine starvation (B). Macromolecular synthesis inhibition was started at the time indicated by the arrows.

of $\bar{M}_i$ in A4 and A4F + cells in two sets of experiments run at different generation times showed no change in this parameter (Table 3). We conclude that F plasmid-controlled replication results in an increase of cell mass at initiation and, consequently, a higher average cell mass.

**DISCUSSION**

Cells of strain ET64 with F plasmid-controlled replication show a higher mass and a reduced DNA concentration if compared with cells with oriC-dnaA-controlled replication. These changes cannot be explained from observed variations in $C$, $D$, and $\tau$. Time $C$ is larger than normal in ET64 cells but this does not appear to be due to the dnaA mutation or to the modification in the replication control. Reported changes in cell size and DNA concentration are mainly originated by an increase of the $\bar{M}_i$ in cells with F plasmid-controlled replication. This suggests that at one growth rate, the F plasmid replicates at a particular cell mass: F particle number ratio, $\bar{M}_i(F)$, which is higher than the $\bar{M}_i$ of oriC.

**TABLE 2. Changes of initiation mass in ET64 cells**

<table>
<thead>
<tr>
<th>Exp</th>
<th>Growth temp (°C)</th>
<th>$\tau$ (min)</th>
<th>$\bar{M}_i$</th>
<th>Relative units</th>
<th>$10^{-9}$ × OD600</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>88</td>
<td>1.00</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>75</td>
<td>1.95</td>
<td>2.62</td>
<td></td>
</tr>
</tbody>
</table>

- Calculated with equation 3, giving to the conversion factor $K$ the value that makes $\bar{M}_i = 1$ to cells grown at 30°C in each experiment.

- Calculated with equation 1.

**TABLE 3. Effect of growth temperature, growth rate, and F plasmid presence on cell initiation mass**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source (0.4%)</th>
<th>Growth temp (°C)</th>
<th>$\tau$ (min)</th>
<th>$\bar{M}_i$ (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>Glycerol</td>
<td>39</td>
<td>86</td>
<td>1.00</td>
</tr>
<tr>
<td>A4</td>
<td>Glucose</td>
<td>39</td>
<td>54</td>
<td>1.10</td>
</tr>
<tr>
<td>ET64</td>
<td>Glucose</td>
<td>30</td>
<td>83</td>
<td>1.00</td>
</tr>
<tr>
<td>ET64</td>
<td>Glucose + 50 μg of Casamino Acids per ml</td>
<td>30</td>
<td>53</td>
<td>1.31</td>
</tr>
</tbody>
</table>

- Each pair of data corresponds to an independent experiment.

One of the statements of the model for the control of replication that has been proposed by Pritchard et al. (17, 19) is that $\bar{M}_i(F)$ should be higher than $\bar{M}_i$; thus, initiation at the F plasmid site and replication of free F plasmid are cancelled in dnaA + Hfr strains. A prediction from this model is that the actual $\bar{M}_i$ of cells with F plasmid-controlled chromosome replication should be higher than that of the isogenic cells with oriC-dnaA-controlled replication. This prediction appears to be fulfilled by the results described in this study.

Another prediction from Pritchard's model is that to ensure F plasmid maintenance at all growth conditions, the $\bar{M}_i(F)/\bar{M}_i$ ratio cannot be constant because $\bar{M}_i(F)$ must be lower that the division mass, $\bar{M}_d = \bar{M}_i^{2/3} + D\tau_i$, and the $(C + D)/\tau$ ratio is progressively reduced at slow growth rates in E. coli cells (9). Reported variations of the F to oriC ratio with growth rate (6, 17) are consistent with changes in the $\bar{M}_i(F)/\bar{M}_i$ ratio. The observed differences between $\bar{M}_i(F)$ and $\bar{M}_i$ in ET64 are higher than might be expected if it is considered that the $(C + D)/\tau$ ratio is less than 1 in most E. coli strains at $\tau$ longer than 60 min. This is not the case in ET64; therefore, the high $\bar{M}_i(F)/\bar{M}_i$ ratio resulting from our data cannot create problems for F plasmid maintenance.

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**LITERATURE CITED**