A Cold Sensitive dnaA Mutant of E. coli which Overinitiates Chromosome Replication at Low Temperature

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Summary. A heat resistant mutant of E. coli dnaAts46 was isolated, which grows normally only at temperatures above 39°. After a temperature shift from 42° to 32° the mutant overproduces DNA relative to protein. This is due to overinitiation of rounds of chromosome replication at low temperature, as indicated by hybridization and other experiments. The mutation is cotransduced by Pl with ilv and could not be separated from dnaAts46 by transduction.

Introduction

The temperature sensitive replication-initiation mutant dnaAts46 was isolated by Kohiyama (1968) and studied extensively by Hirota et al. (1970). A dnaAts46 mutant placed at 42° continues DNA synthesis until all previously initiated rounds of replication are completed but is unable to initiate new rounds. We have isolated temperature resistant “revertants” of dnaAts46. Some of these were not true revertants and carried suppressor mutations with interesting new properties. One such suppressor mutation, studied here, will be called dnaAcos because it renders its carrier cell cold sensitive. The mutation cotransduces with ilv and could not be separated from dnaAts46 by Pl transduction. It seems to be very closely linked to dnaAts46 and may represent an intragenic suppressor mutation since it also affects the initiation of chromosome replication. Unlike dnaAts46, however, dnaAcos is not blocked in the initiation of DNA replication but, instead, produces an excess of DNA, relative to cell mass, at 32°. This increased rate of DNA replication is due to an excess of initiation.

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Materials and Methods

The bacterial strains used in this study are listed and described in Table 1.

The growth media used were: Tryptone (Difco Tryptone 10 g, NaCl 5 g per liter), LB (Difco Tryptone 10 g, Yeast extract 5 g, NaCl 10 g/l) or M9 glucose minimal medium supplemented with thiamine (0.5 µg/ml), thymine (2 µg/ml) and 20 µg/ml of required amino acids.

14C-labeled φ80imm DNA was prepared according to Louarn et al. (1977); 3H-labeled Mu-1 DNA was a gift of A. Bruschi.

Hybridizations were carried out on Sartorius membrane filters (MF50) in the presence of 2 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate) and 50% formamide as described by Kourilsky et al. (1971). The principle and the techniques of the hybridization experiments were basically those of Louarn et al. (1974). However, in order to simplify the procedure we loaded the filters with crude lysates rather than purified bacterial DNA. Cells were concentrated by filtration on Millipore filters, washed, and resuspended in TSE-2. (TSE-2 is 0.5 M NaCl, 0.05 M Tris pH 7, 0.05 M EDTA). The cells were lysed by adding an equal volume of 1 M NaOH and the suspension was boiled for 10 min in a water bath. The suspension was cooled and neutralized with HCl; 1 M Tris-buffer pH 7 was added to a final concentration of 0.1 M. An equal volume of 10 × SSC was added and variable aliquots of this solution loaded on filters presoaked in 6 × SSC. The filters were washed twice with 6 × SSC, dried, and heated for 2 h at 80° under vacuum before their use for hybridization.

The method was tested using a culture of strain LC531 (ile::Mu-1 (φ80imm ind )) in which the chromosomes had previously been aligned by amino acid starvation for 3 h in order to obtain a Mu/φ80 ratio of one (Bird et al., 1972). The hybridization mixture contained 0.5 µg of 3H-labeled Mu-1 DNA and 0.5 µg of 14C-labeled φ80imm DNA. The hybridizations were carried out in a volume of 0.5 ml at 42° for 5 days.

The results with the aligned test culture showed that at cell concentrations from 10⁸ to 8 × 10⁶ lysed cells per filter the amount of labeled DNA hybridized was proportional to the load on the filter and the 3H/14C ratio measured remained constant around 1. At higher cell concentrations on the filters, the efficiency of hybridization and the 3H/14C ratio decreased. The decrease in efficiency is undoubtedly due to the fact that, at high cell concentration, the Mu-1 and φ80 sequences in the hybridization mixture are no longer in large excess relative to their homologs on the filter. The decrease in 3H/14C ratios may be attributed in part...
Table 1. Bacterial strains and their origins

<table>
<thead>
<tr>
<th>Strain Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>CBO129</td>
<td>F- derivative of W1485 thy-leu-thi (Caro and Berg, 1969)</td>
</tr>
<tr>
<td>LC256</td>
<td>spontaneous ile mutant of CBO129 isolated by penicillin screening (Y. Nishimura)</td>
</tr>
<tr>
<td>CRT46</td>
<td>dnaAts46 ile mutant of CR34 (Hirota et al., 1970)</td>
</tr>
<tr>
<td>LC252</td>
<td>CRT46 ile transductant by P1 from LC607 ile (Y. Nishimura)</td>
</tr>
<tr>
<td>LC257</td>
<td>dnaAts46 ile transductant of LC256 from LC252 (Y. Nishimura)</td>
</tr>
<tr>
<td>LC343</td>
<td>leu thy (proB lac), dnaAts4 cycI (J.-M. Louarn)</td>
</tr>
<tr>
<td>LC384</td>
<td>spontaneous temperature resistant, cold sensitive mutant of LC257 (this paper)</td>
</tr>
<tr>
<td>LC576</td>
<td>LC257 (rbs::Mu-1); phage Mu-1 is inserted near the origin of replication in gene rbs (J.-M. Louarn)</td>
</tr>
<tr>
<td>LC577</td>
<td>LC257 (bgl::Mu-1); phage Mu-1 is inserted near the origin of replication, in the opposite direction relative to LC576 (J.-M. Louarn)</td>
</tr>
<tr>
<td>LC674</td>
<td>LC576 (rbs::Mu-1) (80imnmind) dnaAcos, a spontaneous cold sensitive mutant of LC576, lysogenized with phage 80imnmind (this paper)</td>
</tr>
<tr>
<td>LC675</td>
<td>LC577 (bgl::Mu-1) (80imnmind) dnaAcos, a spontaneous cold sensitive mutant of LC577, lysogenized with phage 80imnmind (this paper)</td>
</tr>
</tbody>
</table>

Table 1 shows bacterial strains and their origins. To increased absorption of 3H beta particles by the accumulated cell mass on the filter, Control experiments did show that a large fraction of cell proteins remain bound to the filter under the conditions of loading. It is important, for these reasons, not to overload the filters.

In the hybridization experiments filters were loaded with no more than 4 x 10^6 cells. This amount was decreased when the DNA/mass ratio of the cells increased due to experimental treatment of the culture. Four filters were made for each point; two of them were hybridized against the phage mixture, one against E. coli DNA, and one against doubly labeled 14C-3H DNA. The 14C E. coli DNA hybridized to one filter permitted us to estimate the amount of DNA loaded on the filters and to see that it moved within the safe range of concentration. The doubly labeled E. coli DNA was hybridized to one filter to control the efficiency of the 3H counts relative to the 14C counts which was identical for all samples.

P1-transductions were performed with P1LA, a clear plaque mutant of P1kc (Caro and Berg, 1971). Temperature sensitivity of the transductants was verified by spotting a dilution of the colony tested. This is because dnaAcos has sufficient residual growth at low temperature to give a positive reading when a high concentration of cells is stabbed on the test plate.

Optical density of E. coli cultures, as an estimate of total cell mass, was measured at 450 nm on a Hitachi Model 101 spectrophotometer.

Results

A. The Genotype of LC384 dnaAcos

The strain LC384 was isolated as a spontaneous temperature resistant derivative of LC257, a strain of E. coli K12 W1485 carrying the mutation dnaAts46. LC384 will be shown here to be sensitive to a temperature of 32°, to still carry the dnaAts mutation, and to be affected in its DNA replication properties at 32°. For reasons which are explained below we have called such spontaneous mutations dnaAcos. In one typical isolation of dnaAcos mutants we spread 10⁸ cells of LC257 dnaAts46 on Tryptone plates and incubated overnight at 42°; 36 normal sized colonies were found; in addition, about 200 smaller colonies appeared after prolonged incubation. We picked the normal sized colonies, streaked them out on Tryptone plates at 42°, and tested subclones of each for growth at different temperatures. Two of the 36 colonies tested were cold-sensitive, did not grow at 37° and below, and behaved like LC384 dnaAcos with respect to their DNA-metabolism. The genetic location of the mutations was not, however, determined.

LC384 dnaAcos can give rise to cold resistant variants forming colonies at 32°. In one experiment, 10⁷ cells were spread on a Tryptone plate and incubated at 32°. Overnight growth resulted in a pale confluent background due to residual replication of dnaAcos at 32° (see later). From this, 44 colonies of normal size emerged. These were purified by restreaking and tested for temperature sensitivity. 14 did not grow at 42°. For two of these temperature sensitive colonies, DNA-synthesis was studied before and after a temperature shift to 42°. They behaved exactly like LC257 dnaAts46: after a shift to 42°, prelabeled cells continued incorporating 3H-thymine for about 45 min and then stopped at a level representing 1.5 times the value measured at the time of the shift. Among the 24 colonies which grew at 42° as well as at 32°, four different classes were distinguished on the basis of colony size, morphology, and the plaque size of phage λ obtained on them. Only one of these classes, comprising 6 clones, resembled wild type CBO129, the parent of LC257 dnaAts46. Thus, dnaAcos can revert in one step to either the dnaAts46 or the wild type phenotypes with respect to temperature sensitivity but it seems likely that at least some such reversions are due to the suppression of dnaAcos by secondary mutations. The reversion to the dnaAts phenotype as well as the effects of the mutation on DNA replication (see later) lead us to believe that the dnaAts46 allele is still present and is suppressed by the dnaAcos mutation.

We attempted to localize dnaAcos on the genetic map of E. coli by P1 transduction. The results, shown in Table 2, can be summarized and interpreted as follows: P1 grown on LC384 dnaAcos and used to transduce LC257 dnaAts46, with selection for growth at 42°, yielded only 32° sensitive transductants (line 1). The reverse transduction from LC257 dnaAts46 to LC384 dnaAcos, followed by selection at 32°, produced only 42° sensitive colonies (line 2). Thus, a dnaAts46 allele could not be transduced out of LC384 separately from dnaAcos, nor could