SHORT COMMUNICATIONS

Instability of the Flac+ Factor in a dnaC Mutant of Salmonella typhimurium

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INTRODUCTION

The dnaC gene of Escherichia coli maps at 89 min on the genetic map and its product is involved in the initiation of chromosome replication (Gross, 1972). It also plays a role in plasmid replication since, for example, the Flac+ factor fails to replicate when the dnaC gene is inactivated (e.g. at restrictive temperature in a temperature-sensitive dnaC mutant; Zeuthen & Pato, 1971). Salmonella typhimurium strain 11G is a temperature-sensitive strain which forms colonies at up to about 35 °C but not at higher temperatures. It is mutated in the dnaC gene or its equivalent since the lesion, like dnaC of E. coli, maps close to the leu genes and the strain is altered in the initiation of chromosome replication at 38 °C (Spratt & Rowbury, 1971). Strain 11G also fails to replicate the resident LT2 plasmid and the Flac+ factor at 38 °C (Spratt, 1972).

Thus in both E. coli and S. typhimurium, inactivation of the dnaC gene product prevents both initiation of chromosome replication and the replication of plasmids. Here we show that under certain conditions the Flac+ factor, but not the LT2 plasmid or the hybrid Flac+S, shows an instability in strain 11G at permissive temperature which is apparently related to the presence of the mutated dnaC gene.

METHODS

Bacteria. Salmonella typhimurium strains 11G LT2+, M827 LT2−, PG154 LT2+, PG154 LT2+ Flac+ and M799 LT2+ have been described previously (Spratt & Rowbury, 1971; Spratt, Rowbury & Meynell, 1973). The isolation of strain 11G LT2− Flac+S by introduction of the Flac+S factor into 11G LT2+ has also been described (Rodriguez Lemoine & Rowbury, 1975). Strain 11G LT2− was isolated from a rare white lac− colony produced on deoxycholate-lactose agar (see below) by 11G LT2− Flac+S. Strain 11G LT2+ ts+ was isolated by plating 10⁸ cells of 11G LT2+ on nutrient agar and picking one of the large revertant colonies which grew at 37 °C.

Culture media. The minimal medium (MM) used was that of Davis & Mingioli (1950) lacking citrate and with glucose or lactose (0·2 %) as energy source. Required amino acids were added at 20 μg/ml (L form). Oxoid nutrient broth No. 2 was used throughout as a rich liquid medium. Both MM and broth were solidified where necessary with 2 % Difco Bacto agar.

To detect lac+ and lac− colonies, lactose–deoxycholate (DOC) agar (Meynell & Meynell, 1970) was used.

Measurement of total cell number and viable count. The total number of cells in cultures was measured on samples diluted in Isoton diluent (Coulter Electronics Ltd, Dunstable, Bedfordshire) with a Coulter model ZB1 electronic particle counter (30 μm orifice, aperture current 1, amplification 2, lower threshold 2·5). The number of viable cells in cultures was
measured by plating samples (suitably diluted in 0.065 M-phosphate buffer pH 7.4) on lactose–DOC agar and counting colonies after 2 days' incubation at 25 °C.

**Tests for sensitivity of strains to MS2 phage.** In some experiments Flac+ strains were tested for MS2 sensitivity. Two methods were used: (i) portions (0.1 ml) of overnight broth-grown cultures were spread on to nutrient agar plates and drops of MS2 phage (about 0.02 ml; 10^10 p.f.u./ml) looped on to areas of the plates; (ii) portions (0.1 ml) of overnight broth-grown cultures were added to 2.5 ml soft agar (nutrient broth containing 0.8% Difco Bacto agar and 0.8% NaCl). The soft agars were poured on to nutrient agar plates and, after setting, drops of MS2 phage (about 0.02 ml; 10^10 p.f.u./ml) were looped on to areas of the plates. In both types of test MS2-sensitive strains showed clear areas where the phage drops had been placed.

**Transfer of Flac+ by conjugation.** Flac+ donor strains were grown to late-exponential phase in broth without shaking at the appropriate temperature. Recipient strains were grown with shaking in broth and used in exponential phase. Recipient cells (0.1 ml of a suitable dilution) were spread on lactose minimal agar plates containing the requirements of the recipient and drops of donor culture (tenfold diluted in 0.85% w/v NaCl) were looped on to areas of the plates. Exconjugants were picked after 72 h at 30 °C (strain 11G and derivatives) or 48 h at 37 °C (other recipients).

**Separation of plasmid DNA.** The growth and labelling of cells, lysis, separation of plasmid on alkaline sucrose gradients and analysis of gradients were all performed as described previously (Spratt et al. 1973; Rodriguez Lemoine & Rowbury, 1975).

**RESULTS AND DISCUSSION**

To test for stability of Flac+ we introduced it into strain 11G LT2+ (this is the normal 11G strain carrying the resident LT2 plasmid) and its parent, strain PG154 LT2+ ts+, and into another dnaC+ strain, M799 LT2+. Organisms from all three Flac+ strains were then grown overnight in broth at 25 °C and tested for lac- cells by plating on lactose-deoxycholate agar at 25 °C. The Flac+ plasmid was stable in PG154 LT2+ and in M799 LT2+ (< 1% lac- cells) but highly unstable in 11G LT2+ (about 39% lac- cells). There were also virtually no lac- cells present after overnight growth of PG154 LT2+ Flac+ and M799 LT2+ Flac+ at 37 °C (see Table 1). Strain 11G LT2+ Flac+ will not grow at 37 °C but after overnight growth at 32 °C almost all organisms had lost the Flac+ (about 91% lac- cells). Thus not only was the Flac+ unstable in the dnaC mutant at permissive temperature but the tendency to segregate lac- cells increased with increasing temperature.

The temperature-dependent loss of Flac+ shown in liquid medium by strain 11G LT2+ Flac+ also occurred on solid medium. Organisms plated on lactose–DOC agar at 25 °C formed red (lac+) colonies with thin white (lac-) areas encircling them. Colonies formed at 32 °C were white with small red centres. Under these conditions colonies containing substantial numbers of lac- cells arose from initially Flac+ cells. It seems likely therefore that many of the lac- cells present after overnight growth of strain 11G LT2+ Flac+ in broth at 32 °C (Table 1) were formed in the same way, i.e. from the Flac+ cells present in the initial inoculum and not predominantly from the few lac- cells initially present.

Of the lac+ cells remaining after overnight growth of strain 11G LT2+ Flac+ at 25 or 32 °C, 99% still showed unstable maintenance of Flac+. When restreaked on lactose–DOC agar at 25 °C they produced red (lac+) colonies with white (lac-) areas around them.

It is assumed above that replication of Flac+ was aberrant in strain 11G LT2+ Flac+ at 32 °C and that lac- cells arose from Flac+ ones. This interpretation is supported by the
Table 1. Effect of temperature on Flac⁺ stability and viability of strains of S. typhimurium

The Flac⁺ derivatives were prepared as described in Methods. The donor strains were (i) PG154 LT²⁺ Flac⁺ for transfer of Flac⁺ to M799 LT²⁺ and M827 LT²⁻ and (ii) M827 LT²⁻ Flac⁺ for transfer of Flac⁻ to the IIG derivatives. The Flac⁺ derivatives were taken from slopes of minimal agar and lactose and grown overnight in broth at the stated temperature. Strain IIG LT²⁺ was taken from a nutrient agar slope and grown in broth at the stated temperature. For viable counts and counts of lac⁺ and lac⁻ organisms, suitable dilutions from the initial inocula and/or the overnight cultures were plated on lactose-DOC agar and the plates incubated at 25 °C. Total cell counts were made with the Coulter counter as described in Methods.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage of lac⁻ cells</th>
<th>Percentage of viable cells after overnight growth at stated temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In initial inoculum 25 °C</td>
<td>32 °C</td>
</tr>
<tr>
<td>IIG LT²⁺ Flac⁺</td>
<td>5.8</td>
<td>38.8</td>
</tr>
<tr>
<td>IIG LT²⁻ Flac⁺</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>IIG LT²⁻ Flac⁻</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>PG154 LT²⁺ Flac⁺</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>M799 LT²⁺ Flac⁻</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>IIG LT²⁻ Flac⁻+S</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>IIG LT²⁻ Flac⁻ ts⁺</td>
<td>1.3</td>
<td>1.7</td>
</tr>
</tbody>
</table>

N.T., not tested.

behaviour of the strain on solid medium. Another possibility however is that after growth at 32 °C, Flac⁺ organisms showed plating sensitivity and were unable to form colonies on solid medium. To test this, organisms were grown overnight at 32 °C and total cell counts (made with the Coulter counter) were compared with viable counts on lactose-DOC agar. For strain IIG LT²⁺ Flac⁺, 73% of organisms were viable after overnight growth at 32 °C (Table 1). Loss of viability, however, was apparently unrelated to either the presence or the instability of Flac⁺, since strain IIG LT²⁺ (which does not carry Flac⁺) and strain IIG LT²⁻ Flac⁺ (which showed stable maintenance of Flac⁺; Table 1) both had a reduced viability (after overnight growth at 32 °C) similar to that of strain IIG LT²⁺ Flac⁺ (Table 1).

Not all plasmids show instability in strain IIG. The resident LT2 plasmid was rarely lost during growth at 25 or 30 °C (data not shown) and the hybrid Flac⁺S factor, which appears to contain parts of the LT2 plasmid and can replace it (Rodriguez Lemoine & Rowbury, 1975), was maintained stably in IIG at both 25 and 32 °C (Table 1). Instability of Flac⁺ appears from the above to be associated with the dnaC lesion. To throw further light on this relationship we have tested the stability of Flac⁺ in a ts⁺ revertant of strain IIG LT²⁺. Table 1 shows that one such ts⁺ revertant showed almost stable maintenance of Flac⁺. The percentage of lac⁻ cells after growth at 37 °C was somewhat higher than for PG154 LT²⁺ Flac⁺, but although a large colony revertant was picked, this strain still showed slower growth at 37 °C than the parent and therefore may still have a dnaC product with slightly reduced activity at 37 °C. The greater stability of Flac⁺ in this revertant strain was not due to loss of the LT2 plasmid (see below), since examination of its DNA on alkaline sucrose gradients showed that both Flac⁺ and the LT2 plasmid were present. Thus all the above suggests that instability of Flac⁺ is related to inactivation of the dnaC product.

As stated above, the LT2 plasmid and Flac⁺S are stable in strain IIG at 25 and 32 °C. The LT2 plasmid apparently remains stable at 32 °C in the presence of Flac⁺, since after over-
night growth of IIG LT2+ Flac+ at 32°C all 11 of the lac+ clones tested were resistant to MS2 phage (i.e. had the fi+ property shown to be characteristic of LT2+ strains by Smith et al. 1973). Also, as stated above, 99% of these lac+ clones showed active segregation of lac- cells; such segregation is indicative of the continued presence of the LT2 plasmid with Flac+. In the absence of the LT2 plasmid, Flac+ is stable (Table I, strain IIG LT2- Flac+).

The instability of Flac+ in IIG LT2+ might indicate some major difference in replication mechanism. Another possibility is that strain IIG can stably maintain only a single plasmid even at permissive temperature; where two normally compatible plasmids are present then the resident LT2 (or its derivative Flac+S) is replicated normally while replication of the second is aberrant. To test this possibility we have introduced Flac+ into a strain of IIG which carries no autonomous LT2 plasmid (it is probably cured, since it lacks the fi+ property). The resultant strain (IIG LT2- Flac+, Table I) showed stable maintenance of Flac+ at both 25 and 32°C. It appears, therefore, that strain IIG can maintain a single plasmid (LT2 or Flac+S or Flac+) at permissive temperature but that when Flac+ is present in addition to the LT2 plasmid then the replication of the latter is favoured and Flac+ tends to be lost.

The reason for the unstable behaviour of Flac+ in strain IIG is at present conjectural. The dnaC gene product probably plays an enzymic role in the initiation process for both chromosome and plasmid, and when the level of active dnaC product is limited then there is competition for it and the ‘foreign’ Flac+ plasmid evidently fails to compete effectively for it. We are at present testing whether other plasmids show unstable behaviour in IIG LT2+.

Although Flac+ was unstable in IIG LT2+, 0.5% of the ts lac+ clones were stably lac+. These still carried the fi+ property characteristic of the LT2 plasmid. In one strain the presence of both Flac+ and the LT2 plasmid was confirmed by examining its DNA on alkaline sucrose gradients. The nature of the change in this strain is at present under investigation. Stable lac+ behaviour of IIG derivatives would also be expected if the LT2 plasmid were lost or if Flac+ recombined with the LT2 plasmid, forming a hybrid. Such a hybrid plasmid would probably confer fi+ properties. Recombination of this kind would be analogous to that involved in the formation of Flac+S from FtsI14h+ and the LT2 plasmid (Macrina & Balbinder, 1972). Studies of further stable lac+ derivatives of IIG are in progress to ascertain whether any of them contain hybrid plasmids or lack the LT2 plasmid.

The instability of Flac+ in strain IIG LT2+ may offer a method of easily isolating dnaC mutants. If dnaC mutants in general (or all initiation mutants) show poor ability to maintain Flac+ when the LT2 plasmid is present, then after mutagenesis of LT2+ Flac+ strains it may be possible to detect temperature-sensitive dnaC mutants (or initiation mutants in general) as those which show unstable Flac+ maintenance at 25°C (i.e. red colonies with white sectors or areas on lactose-DOC agar) associated with failure to grow at 37°C. This is at present under investigation.

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REFERENCES


