The Formation of a Dissociable Plasmid Cointegrate from the Flac Factor and the Resident Plasmid of Salmonella typhimurium LT2

BY V. RODRIGUEZ LEMOINE AND R. J. ROWBURY

Department of Botany and Microbiology, University College London, London WC1E 6BT

(Received 16 January 1976; revised 24 March 1976)

SUMMARY

The Flac factor showed unstable maintenance in Salmonella typhimurium dnaC MP10LT2. The properties of a more stable lac+ derivative (SD-I) are described. SD-I was ts and carried the fi+ property and the ability to transfer the lac+ character. It contained a large plasmid of molecular weight about $1.29 \times 10^6$ daltons. The properties of SD-I and its derivatives suggested that the large plasmid was a cointegrate of Flac and the MP10LT2 plasmid. Lac+ transfer was efficient from SD-I to M799 MP10LT2 and one lac+ exconjugant contained the intact cointegrate. The cointegrate was not successfully transferred to strains lacking MP10LT2. It dissociated into apparently unaltered Flac and MP10LT2 plasmids, but the deletion of small parts of one or both plasmids during cointegrate formation could not be ruled out. Cointegrate dissociation was more marked in M799 than in SD-I especially during growth in glucose-Casamino acids minimal medium. In the presence of R1dra5g, the cointegrate (like the MP10LT2 plasmid) was stably maintained in the dnaC strain; maintenance of Flac was, however, unstable. It seems likely that replication of the cointegrate was controlled by the MP10LT2 plasmid constituent.

INTRODUCTION

During previous work on plasmid replication in dnaC and dnaC+ strains of Salmonella typhimurium, it was observed (Rodriguez Lemoine & Rowbury, 1975b) that whereas Flac was stably maintained in the dnaC+ strains at all the temperatures tested, maintenance of Flac was aberrant at permissive temperatures in the temperature-sensitive dnaC mutant. After overnight growth in broth at 32 °C, 90% of organisms were lac-. Unstable maintenance was dependent on the presence of the resident plasmid as well as on the dnaC lesion. Stably lac+ derivatives can be isolated from this strain and we describe the properties of one such derivative.

The resident plasmid MP10LT2 (previously called the LT2 plasmid) is a large stringently controlled plasmid of molecular weight about $60 \times 10^6$ daltons (Spratt, 1972; Spratt, Rowbury & Meynell, 1973; Smith et al., 1973). The plasmid, which is apparently non-transferable, is stably maintained, but cured strains (which lack the fi+ property conferred by it) have been isolated and studied (Smith et al., 1973; Spratt et al., 1973). Macrina & Balbinder (1972) isolated a large plasmid (FlacS) from a FtsI lac derivative of S. typhi-murium. FlacS showed certain properties characteristic of MP10LT2 (Macrina & Balbinder, 1973; Rodriguez Lemoine & Rowbury, 1975a). Nevertheless, the failure of FlacS DNA to hybridize with that of the MP10LT2 plasmid led Macrina, Balbinder & Bassel (1973) to conclude that FlacS might not be a hybrid of FtsI lac and MP10LT2. In this paper, we describe
the formation of a large plasmid in *S. typhimurium* **I1G** dnaC *Flac* **MPIoLT2**. This plasmid is apparently a cointegrate of *Flac* and the **MPIoLT2** plasmid, since it conferred properties characteristic of both and dissociated into apparently unaltered *Flac* and **MPIoLT2** plasmids. Transfer of the cointegrate of *Flac* and the **MPIoLT2** plasmid was studied, since it provided a possible means of transferring **MPIoLT2**. Maintenance of the cointegrate was also examined in the presence of another plasmid to ascertain which component governed replication.

**METHODS**

**Bacteria.** *Salmonella typhimurium* strains **I1G**, **I1G MPIoLT2**, **I1G MPIoLT2 Flac**, **I1G FlacS**, **PG154 MPIoLT2 Flac**, **M827** and **M799 MPIoLT2 Flac** have been described previously (Spratt *et al.*, 1973; Rodriguez Lemoine & Rowbury, 1975b). Strain M799 FlacS was derived from strain M799 MPIoLT2 by transfer of FlacS. *Escherichia coli* strain C600 is a K12 derivative and requires threonine, leucine and thiamin for growth.

**Culture media.** The minimal medium (MM) used was that of Davis & Mingioli (1950). Citrate was omitted and glucose or lactose (0.2%) was added as energy source. Required amino acids were added at 20 µg ml⁻¹ (L form), thiamin at 1 µg ml⁻¹ and Difco vitamin-free Casamino acids at 0.1%. Oxoid broth no. 2 was used as a rich liquid medium. MM and broth were solidified where necessary with 2% (w/v) Difco Bacto agar. To detect lac+ and lac− colonies, lactose–deoxycholate (DOC) agar (Meynell & Meynell, 1970) was used.

**Viable counts.** The number of viable cells in cultures was measured by plating samples (suitably diluted in 0.065 M-phosphate buffer pH 7.4) on nutrient agar or lactose–DOC agar, and counting colonies after 2 days incubation at 25 °C or 30°C.

**Tests for sensitivity to MS2 phage.** Two methods were used: (i) 0.1 ml portions of cultures grown overnight in broth were spread on nutrient agar plates and drops of MS2 phage [0.02 ml; 10¹⁰ plaque-forming units (p.f.u.)/ml] were transferred on to marked areas of the plates; (ii) 0.1 ml portions of cultures grown overnight in broth were added to 2.5 ml soft agar (nutrient broth containing 0.8% Difco Bacto agar and 0.8% NaCl) and poured on to nutrient agar plates. After setting, drops of MS2 phage (about 0.02 ml; 10¹⁰ p.f.u./ml) were transferred on to marked areas of the plates. After incubation overnight at 30 °C or 37 °C, MS2 sensitive strains showed clear areas where the phage had been placed.

**Transfer of lac+ character by conjugation.** Donor strains were grown to late-exponential phase in broth without shaking at the appropriate temperature. Recipient strains were grown to exponential phase in broth with shaking. Recipient cells (0.1 ml of a suitable dilution) were spread on lactose minimal agar plates containing the growth requirements of the recipient. Drops of donor culture [appropriately diluted in 0.85% (w/v) NaCl] were then transferred on to marked areas of the plates. *Lac*+ exconjugants were picked after 72 h at 30 °C or 48 h at 37 °C.

**Analysis of plasmid DNA.** The growth, labelling and lysis of cells, separation of plasmid DNA on alkaline sucrose gradients, and analysis of gradients were all performed as described previously (Spratt *et al.*, 1973; Rodriguez Lemoine & Rowbury, 1975a). The molecular weights of plasmids were estimated from their sedimentation behaviour on alkaline sucrose gradients (Clayton & Vinograd, 1967).

**Experiments with R*trdtrd*.** This R factor was transferred from *Escherichia coli* 153 as described previously (Rodriguez Lemoine & Rowbury, 1975a). R*+* organisms were selected by plating on minimal agar supplemented with the growth requirements of the recipient and with kanamycin (25 µg ml⁻¹) and chloramphenicol (25 µg ml⁻¹). Where strains carrying
A dissociable plasmid cointegrate

Fig. 1. Analysis of the plasmid content of the stably lac+ derivative (sd-1) by alkaline sucrose gradient centrifugation. Strains 11G MP107173 Flac and sd-1 were labelled with [3H]thymidine in lactose–Casamino acids MM. Lysis of the organisms, sedimentation of gradients, gradient analysis and counting procedures were as described in Methods. In all figures, fraction 1 corresponds to the bottom of the gradient tube. (a) sd-1; (b) strain 11G MP107173 Flac.

Fig. 2. Sedimentation on an alkaline sucrose gradient of the sd-1 plasmid and FlacS. sd-1 was grown and labelled with [3H]thymidine as described in Fig. 1. Strain 11G FlacS was grown and labelled as described in Fig. 1, but using [14C]thymidine (4 µCi ml⁻¹) instead of [3H]thymidine. The two suspensions were then mixed and lysed together. Lysis, sedimentation and all further analytical procedures were as described in Methods. (a) 14C counts, i.e. plasmid content of strain 11G FlacS; (b) 3H counts, i.e. plasmid content of sd-1.

R1drd19 were tested for the number of R+ organisms, plating was on nutrient agar with the same concentrations of kanamycin and chloramphenicol.

RESULTS

A large plasmid in a derivative of Salmonella typhimurium 11G

When S. typhimurium 11G dnaC MP107173 Flac (parent) was plated on lactose–deoxycholate agar at 25 °C, many of the organisms formed white colonies with red centres, i.e. lac+ maintenance was unstable. About 0.5% of the organisms, however, formed red stably lac+ colonies. One of these stably lac+ derivatives (sd-1) was purified and studied.

sd-1 was, like its parent, ts and was able to transfer the lac+ character to strain M799 MP107173. sd-1 was, moreover, resistant to MS2 phage and therefore carried characteristics of both Flac (lac+ character and transfer ability) and the MP107173 plasmid (fi+ character).

The plasmid DNA content of sd-1 was compared with that of its parent by running extracts on alkaline sucrose gradients. The extract prepared from the parental culture showed the two peaks characteristic of the covalently closed circular (CCC) forms of Flac (about 1.65% of the total cellular DNA as CCC plasmid) and the MP107173 plasmid (about 1.9% of the total cellular DNA as CCC plasmid). However, extracts of sd-1 cultures contained substantial amounts (about 3.6% of the total cellular DNA as CCC plasmid) of a
large plasmid (Fig. 1), as well as smaller amounts of plasmids which behaved like Flac (about 0.35% of the total cellular DNA) and the MP10LT2 plasmid (about 0.36% of the total cellular DNA).

The large SD-1 plasmid was larger (Fig. 2) than the hybrid FlacS factor (molecular weight about 109 x 10^6 daltons; Macrina et al., 1973) and its molecular weight was estimated to be 128 x 10^6 to 130 x 10^6 daltons. It seemed likely that the SD-1 plasmid was a coinegrate of Flac and the MP10LT2 plasmid, since SD-1 had properties characteristic of both. The molecular weight estimated was somewhat less than that expected for a coinegrate made by fusion of the complete genomes of Flac (molecular weight about 80 x 10^6 daltons) and the MP10LT2 plasmid (molecular weight about 60 x 10^6 daltons). Some DNA might therefore have been deleted in the fusion.

The small amounts of apparently normal Flac and MP10LT2 plasmids present in SD-1 extracts might have arisen by dissociation of a coinegrate. Dissociation would result in the presence of two types of derivative in SD-1 cultures. Organisms carrying the two constituent plasmids would arise directly; lac+ maintenance should be unstable in them. From these, lac- organisms carrying only the MP10LT2 plasmid should arise. When broth-grown cultures of SD-1 were plated on lactose-deoxycholate agar at 25 °C, 5 to 6% of the organisms formed white colonies with red centres, i.e. maintenance of the lac+ character was unstable in them (Table 1). Organisms from one of these colonies contained plasmids which behaved like

![Fig. 3](image-url)

Fig. 3. Analysis of the plasmid content of a lac- segregant of SD-1 by alkaline sucrose gradient centrifugation. Organisms of a lac- segregant from SD-1 were labelled with [3H]thymidine in glucose-Casamino acids MM. Strain PG154 MP10LT2 Flac was labelled with [14C]thymidine in lactose-Casamino acids MM. Cell suspensions were then mixed and lysed together. All subsequent procedures were as described in Methods. (a) 14C counts, i.e. plasmid content of strain PG154 MP10LT2 Flac; (b) 3H counts, i.e. plasmid content of the lac- segregant from SD-1.

![Fig. 4](image-url)

Fig. 4. Analysis of the plasmid content of a lac+ exconjugant from a cross of SD-1 x M799 MP10LT2 by alkaline sucrose gradient centrifugation. The exconjugant was grown in lactose-Casamino acids MM and labelled with [3H]thymidine as described in Fig. 1. Sedimentation, analysis of gradients and counting of samples were also performed as in Fig. 1.
A dissociable plasmid cointegrate

Fig. 5. Comparison of the derived Flac with the parental Flac by sedimentation on an alkaline sucrose gradient. Organisms of strain m827 carrying the derived Flac (see Table 1) and strain PG154 MP10L72 Flac were labelled with [3H] and [14C]thymidine respectively in lactose-Casamino acids MM. Organisms were lysed together and treated subsequently as in Fig. 2. (a) 14C counts, i.e. plasmid content of PG154 MP10L72 Flac; (b) 3H counts, i.e. plasmid content of m827 derived Flac.

Flac and the MP10L72 plasmid on alkaline sucrose gradients, but the large plasmid was absent. A further 2 to 3% of the organisms from a SD-I culture formed white (lac-) colonies on lactose-deoxycholate agar. All three of these tested (e.g. Fig. 3) contained only a plasmid which behaved like MP10L72.

Transfer properties of SD-I

As stated above, SD-I transferred the lac+ character when incubated with strains such as M799 MP10L72. However, 5 to 6% of the organisms in a SD-I culture (those showing unstable lac+ maintenance) probably carried Flac and these might have been responsible for all the lac+ transfer. Quantitative measurements rule this out. Transfer of the lac+ character to M799 MP10L72 was somewhat more efficient from SD-I (2.9 x 10^-4 lac+ exconjugants per donor organism) than from 11G MP10L72 Flac (2.4 x 10^-4 lac+ exconjugants per donor organism). It follows that organisms carrying the cointegrate were responsible for most of the lac+ transfer.

Extracts from one lac+ exconjugant from the cross of SD-I x M799 MP10L72 contained the plasmid cointegrate (Fig. 4). Dissociation of the cointegrate was more extensive in strain M799 since substantial amounts of plasmids which behaved like Flac and MP10L72 were also present (Fig. 4). Growth of the M799 derivative in glucose medium rather than lactose led to even further dissociation (about 1% of total DNA as CCC form of the SD-I plasmid in glucose-Casamino acids MM, but about 3% in lactose-Casamino acids MM).

Attempts to transfer the cointegrate into strains lacking MP10L72 were unsuccessful. Very few lac+ exconjugants arose when SD-I was crossed with m827 and these probably received only Flac since 45/45 were MS2 sensitive (i.e. lacked the fi+ property characteristic of MP10L72 and the cointegrate) and extracts from one contained only a single plasmid which sedimented like Flac on alkaline sucrose gradients. Similarly, after a cross of M799 containing the cointegrate with 11G, the three lac+ exconjugants tested were MS2 sensitive and one of them contained a single plasmid which behaved like Flac on alkaline sucrose gradients. When E. coli C600 was used as recipient in crosses with SD-I or the M799 derivative carrying the cointegrate, no lac+ exconjugants were isolated.

Properties of the plasmids deriving from dissociation of the SD-I plasmid

The large plasmid of SD-I evidently arose by fusion of Flac and MP10L72. Its molecular weight, however, appeared to be somewhat less than the sum of the molecular weights of
Flac was transferred into the 11G derivatives from m827 Flac. The derived Flac originated in an unstable lac+ derivative of SD-1. From there it was transferred to m827 and thence to the appropriate 11G derivatives. The strain 11G derivative carrying the derived MP10LT2 plasmid was a lac- segregant from SD-1. Organisms were taken from slopes of supplemented minimal agar plus lactose and grown overnight in broth at 30 °C. Results are expressed as percentages.

<table>
<thead>
<tr>
<th>Strain</th>
<th>lac− cells</th>
<th>Unstably lac+ cells</th>
<th>Stably lac+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>11G Flac</td>
<td>0.6</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>11G derived Flac</td>
<td>2.0</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>SD-1</td>
<td>2.3</td>
<td>6.6</td>
<td>91.1</td>
</tr>
<tr>
<td>11G MP10LT2 Flac</td>
<td>80.0</td>
<td>19.7</td>
<td>0.3</td>
</tr>
<tr>
<td>11G MP10LT2 derived Flac</td>
<td>71.4</td>
<td>28.4</td>
<td>0.2</td>
</tr>
<tr>
<td>11G Flac derived MP10LT2</td>
<td>78.0</td>
<td>21.7</td>
<td>0.3</td>
</tr>
<tr>
<td>11G derived MP10LT2 derived Flac</td>
<td>80.1</td>
<td>19.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

NT, not tested.

Control of cointegrate replication; replication in the presence of R1drd19

Unstable maintenance of Flac in the dnaC strain was not only caused by the MP10LT2 plasmid. Flac maintenance was also unstable (Rodriguez Lemoine & Rowbury, 1976) in the presence of R factors like R1drd19 (61% of organisms were lac− after overnight growth of S. typhimurium dnaC Flac R1drd19 in broth at 30 °C; 100% were R+), whereas the MP10LT2 plasmid was stably maintained with R1drd19 (51/51 organisms tested were...
A dissociable plasmid cointegrate

MP10_LT2+ R+ after overnight growth of *S. typhimurium* *dnaC* MP10_LT2 R1_drd19 in broth at 30 °C. When R1_drd19 was introduced into SD-I, the strain remained stably *lac*+ (2.5% of organisms were *lac*− after overnight growth of SD-I in broth at 30 °C; 2.7% were *lac*− after overnight growth of SD-I R1_drd19 in broth at 30 °C). It follows that the cointegrate behaved as though its replication could be under the control of the LT2 plasmid constituent.

**DISCUSSION**

*Flac* was not stably maintained in the presence of the MP10_LT2 plasmid in *S. typhimurium* strain 11G, but stably *lac*+ derivatives could arise in a number of ways (Rodriguez Lemoine & Rowbury, 1975b). The stably *lac*+ character of SD-I clearly derived from the presence of a single plasmid cointegrate; single plasmids can be stably maintained in strain 11G (Rodriguez Lemoine & Rowbury, 1975b; unpublished observations). The following observations support the conclusion that the cointegrate was formed by fusion of *Flac* and the MP10_LT2 plasmid: (i) the presence of the cointegrate in SD-I was associated with substantially reduced amounts of *Flac* and the MP10_LT2 plasmid (Fig. 1); (ii) some organisms in SD-I cultures carried both *Flac* and the MP10_LT2 plasmid and the cointegrate was absent from them.

The presence of *Flac* and MP10_LT2 in extracts from SD-I cultures (Fig. 1) suggested that the cointegrate was dissociable. The isolation of a derivative containing both *Flac* and MP10_LT2 from SD-I reinforced this view. The extent of dissociation was apparently increased in strain M799, especially when organisms were grown with glucose rather than lactose.

The *Flac* and MP10_LT2 plasmids which derived from the cointegrate were not significantly altered compared to the parental plasmids with respect to molecular weight or other properties tested. This supports the view that the cointegrate contains essentially the complete genomes of the two constituents. In contrast, there was a marked discrepancy between the observed molecular weight of the cointegrate and that expected for a hybrid produced by fusion of the complete genomes of *Flac* and MP10_LT2. It may be therefore that the cointegrate lacks a small part of one or both constituents. Studies of the cointegrate by restriction endonuclease "fingerprinting" or by electron microscopy will be necessary to resolve this problem. If the SD-I plasmid does carry a deletion then this might be responsible for the failure to transfer the cointegrate to strains lacking MP10_LT2.

Transferable cointegrates which have a tendency to dissociate may be useful for transferring otherwise non-transferable plasmids. The MP10_LT2 plasmid has not so far been transferred as a separate unit but transfer of the SD-I cointegrate (Fig. 4) may eventually permit establishment of the MP10_LT2 plasmid in strains lacking it.

Where plasmid hybrids are formed by the fusion of parts or the whole of two constituent plasmids then the hybrid may potentially have two distinct replication systems. Some such hybrids can apparently utilize either replication system, e.g. the replication of the hybrid pSC134 is governed by the ColE1 constituent in the presence of chloramphenicol but by the pSC101 constituent in *polA* mutants (Timmis, Cabello & Cohen, 1974). The SD-I cointegrate described here clearly contains the replication system of both constituents, since both plasmids derived from the cointegrate were able to replicate in the absence of other plasmids. In the presence of R1_drd19, replication of the cointegrate appeared to be under the control of the MP10_LT2 plasmid constituent in strain 11G since the cointegrate, like this plasmid, was stably maintained. If the cointegrate is under the control of the MP10_LT2 plasmid constituent in SD-I itself then we would expect very stable maintenance since the MP10_LT2
plasmid and FlacS are very rarely lost from strain 11G MP10LT2 or strain 11G FlacS (Rodriguez Lemoine & Rowbury, 1975b). So far we have found no lac- segregants of SD-1 which have lost the complete cointegrate; all lac- segregants tested carried the MP10LT2 plasmid and therefore arose by dissociation of the cointegrate rather than by its loss. We are further examining the replication of the cointegrate to ascertain whether the F factor constituent can govern replication under suitable conditions.

This work was supported by a grant (to R.J.R.) from the Central Research Fund of the University of London and by a Fellowship (to V.R.L.) from the Consejo de Desarrollo, Científico y Humanístico, Universidad Central de Venezuela.

REFERENCES


