SHORT COMMUNICATION

Transposition of Tn951 (Tnlac) and Cointegrate Formation are Thermosensitive Processes

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The frequency of transposition of Tnlac to pGC200, an IncFII R plasmid, increased during the storage of the host strain. This result is explained by the fact that the transpositional event is temperature-dependent: it occurred readily when the host strain was grown at 30 °C but it was nearly undetectable when the host strain was grown and kept at 37 °C. Fusions between two different plasmids carrying Tnlac with pGC200 were also thermosensitive, suggesting a relation between cointegrate formation and transposition. Lactose did not influence the frequency of transposition of Tnlac.

INTRODUCTION

Plasmids conferring a lactose-positive phenotype have now been discovered in a wide variety of Enterobacteriaceae, including Salmonella (Falkow & Baron, 1962; Synenki et al., 1973; Le Minor et al., 1974) and Yersinia (Cornelis et al., 1976). Although it is difficult so far to estimate how common these plasmids are and how often they actually disturb the routine detection of the pathogens, it is obvious that extrachromosomal lac genes are of great medical concern.

Tn951 is a transposon of 16.6 kilobases (kb) encoding lactose fermentation. It was originally discovered by its ability to transpose from pGC1, a lactose plasmid that originated in Yersinia enterocolitica, to RP1, a well-known resistance plasmid that belongs to incompatibility group P1 (Cornelis et al., 1978, 1979). The lactose operon from Tn951 is homologous to the E. coli lac operon but the homology is limited to the three lac genes Y, Z and I (Cornelis et al., 1978).

This paper presents data indicating that transposition of Tn951 is a temperature-sensitive process: it occurs readily when the host strain is grown at 30 °C but only poorly, if at all, when the strain is grown at 37 or 40 °C.

METHODS

Bacteria. Escherichia coli K12 strains: JC3272 his, lys, trp, lacΔX74, strA (Achtman et al., 1971); JC6310, a recA derivative of JC3272; C600 thr, leu, thi, lac. Yersinia enterocolitica strain W22708 belongs to serotype 9; it is a restriction-deficient mutant (Cornelis & Colson, 1975).

Plasmids. These were: pGC1, a conjugative (Tra+) plasmid of 50 kb encoding lactose fermentation (Cornelis et al., 1976); RP1, a plasmid of 57 kb that belongs to incompatibility group P1 and confers resistance to ampicillin (Ap'), kanamycin (Km') and tetracycline (Tc') (Grinsted et al., 1972); pGC9115, an RP1::Tn951 derivative which is a transfer defective mutant of pGC9114 (Cornelis et al., 1978); pGC530, an RP1::Tn951 derivative in which insertion of Tn951 inactivated the tetracycline resistance gene of RP1 (Cornelis et al., 1979); pGC200, a transfer derepressed plasmid of group FII conferring resistance to kanamycin (Km'), chloramphenicol (Cm'), streptomycin (Sr') and sulfonamides (Su') [described as RY2dvd2 by
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Cornelis & Colson (1975); pSC101, a plasmid of 9.1 kb conferring resistance to tetracycline (Tcr) (Cohen & Chang, 1973). Plasmid pGC604 is described in this paper.

Genetic transfer and selective media were as described by Cornelis et al. (1976).

Isolation of plasmid DNA and electron microscopy were done according to the methods outlined by Cornelis et al. (1978).

RESULTS

Effect of strain storage on the transposition frequency

Experiments with a strain containing both pGC1 and RP1 suggested that ageing of the strain might have an effect on the transposition frequency (results not shown). The effect of ageing of the strain on the transposition frequency was therefore examined in detail in a system where transposition could easily be distinguished from fusion. A derivative of E. coli JC6310 containing pGC9115 and pGC200 was constructed at 37 °C and stored on Dorset egg medium at 4 °C. At regular intervals, an inoculum of this strain was taken from the Dorset egg medium, grown in Tryptic soy broth at 37 °C and crossed for 3 h with E. coli C600. Transconjugant plasmids were selected on minimal medium containing threonine, leucine, thiamin and lactose as sole carbon source and about 50 colonies were tested for tetracycline resistance in order to discriminate between transposition and fusion or mobilization. The transfer of pGC200 was monitored by selection on minimal medium containing threonine, leucine, thiamin, glucose and chloramphenicol. The frequency of transposition increased during the storage from less than $1.4 \times 10^{-6}$ after 3 d to $9 \times 10^{-8}$ after 13 d and $8 \times 10^{-4}$ after 3 months.

Effect of temperature on the transposition frequency

To examine the effect of growth temperature, derivatives of JC6310 containing both pGC9115 and pGC200 were constructed and grown at 30, 37 and 40 °C. They were finally mated at 37 °C with E. coli C600. As shown in Table 1, the number of transconjugants containing recombinant molecules was markedly reduced at 40 and 37 °C, compared with 30 °C. This reduction applied both to pGC200::Tn951 transposition products and to the

<table>
<thead>
<tr>
<th>Source of transposon</th>
<th>Growth temp. (°C)</th>
<th>In E. coli JC6310</th>
<th>In Y. enterocolitica W22708</th>
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<tbody>
<tr>
<td></td>
<td>Fusion</td>
<td>Transposition</td>
<td>Fusion</td>
</tr>
<tr>
<td>pGC9115</td>
<td>30</td>
<td>$2.0 \times 10^{-4}$</td>
<td>$1.0 \times 10^{-4}$</td>
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<tr>
<td></td>
<td>37</td>
<td>$2.2 \times 10^{-7}$</td>
<td>$&lt; 0.7 \times 10^{-7}$</td>
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<tr>
<td></td>
<td>40</td>
<td>$2.1 \times 10^{-7}$</td>
<td>$&lt; 2.1 \times 10^{-7}$</td>
</tr>
<tr>
<td>pGC604</td>
<td>30</td>
<td>$5.7 \times 10^{-5}$</td>
<td>$1.2 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>$6.0 \times 10^{-7}$</td>
<td>$&lt; 2.0 \times 10^{-7}$</td>
</tr>
</tbody>
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NT, Not tested.
Fig. 1. DNA heteroduplex molecules constructed between pGC602 and pGC604. Since Tn951 (16.6 kb) is larger than pSC101 (9.1 kb), rehybridization is more likely to occur between the transposon moieties than between the vehicle moieties. A total of 17 heteroduplex molecules were observed. In 14 molecules rehybridization took place between the Tn951 parts. Eight of these molecules had the same structure as the molecule shown in (a). In two molecules, double-stranded (DS) Tn951 DNA was circularized, presumably owing to its inverted repeats, and pSC101 DNA emerged as two single-stranded (SS) loops, inserted, of course, at the same point of the transposon (b). Four molecules were of the same type as in (b) but the two pSC101 loops were underwound (c). Finally, three molecules were observed in which rehybridization took place between the pSC101 moieties (d); Tn951 DNA emerged as two single-stranded loops inserted about 4.3 kb apart. The average lengths measured in these molecules were 9.0 ± 0.5 kb for pSC101 and 16.3 ± 0.7 kb for Tn951.

presumed pGC9115::pGC200 cointegrates. In order to rule out an effect of growth rate rather than of temperature, a similar experiment was conducted in Y. enterocolitica, an organism that grows faster at 30 °C than at 37 °C. As shown in Table 1, there was again a marked difference between the results at 30 and 37 °C, suggesting that it is the temperature that is important rather than the growth rate.

In order to establish that the transposition of TnIac is a temperature-sensitive process, it seemed desirable to observe the effect of temperature on transposition of TnIac from another source. Tn951 was therefore transposed to pSC101. The strategy used to construct this plasmid was very straightforward: plasmid pGC530 was introduced into E. coli C600-(pSC101) at 30 °C. Total plasmid DNA was extracted and used to transform E. coli JC6310. Among 800 Lac<sup>+</sup>, tetracycline-resistant clones, four were kanamycin-sensitive. Restriction analysis (results not shown), as well as electron microscopic examination of heteroduplex molecules (Fig. 1), confirmed that these four clones (designated pGC601 to pGC604) were indeed pSC101::Tn951 derivatives.

Plasmid pGC604 was chosen as a source of TnIac and pGC200 was again used as a
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Plasmid pGC200 was transferred into JC6310(pGC604) at 37 and 30 °C and the strains were mated with E. coli C600. Transposition was detected by selecting for Kmr Cmr Smr Sur Lac+ exconjugants and screening for those which were Te+. Transposition could only be detected after growth of the host at 30 °C. However, the frequency of transposition in this system appears to be much lower than that observed when RP1::Tn951 was used as a source of the transposon (Table 1).

Possible cointegrates, selected as Kmr Cmr Smr Sur Tcr Lac+, also appeared much more frequently at 30 than at 37 °C (Table 1). Twenty exconjugants selected at 30 °C were subsequently crossed at 37 °C with E. coli JC3272 and selection was made for kanamycin resistance. Five of these 20 cotransferred the Lac+ character at frequencies ranging from 0.5 to 0.95 while the remaining 15 cotransferred both markers at a frequency of more than 10^-4. Since the frequency of cotransfer in the original double was only in the range of 10^-5, one can assume that the Kmr Cmr Smr Sur Tcr Lac+ exconjugants resulted from some kind of recA-independent, transient cointegration. These results thus indicate that transient cointegration between pGC200 and pGC604 is a thermosensitive process.

Effect of induction of the lactose operon on the transposition frequency

The lac genes from Tn951 are homologous to the E. coli lac operon (Cornelis et al., 1978) and inducible (unpublished results). It seemed interesting to investigate whether the derepression of the lac operon could result in an increase of the transposition frequency. Clones of JC3272(pGC200)(pGC9115) were therefore constructed at 37 and 30 °C, grown on minimal medium containing either glucose or lactose as sole carbon source and transposition was monitored as described above. The transposition frequency at 30 °C was in the range of 10^-4, irrespective of the carbon source. At 37 °C the frequency was always lower than 4.7 x 10^-6. Thus, the derepression of the lac operon had no influence on the transposition frequency of Tn951.

DISCUSSION

The frequency of transposition of Tn951 (Tnlac) in E. coli can vary up to a 1000-fold according to the growth temperature of the host strain. This influence of temperature can also be observed when transposition takes place in a strain of Y. enterocolitica. Since the latter strain grows faster at 30 °C than at 37 °C, it is clear that the influence of temperature reflects the thermosensitivity of the system rather than an effect of the DNA replication rate. This thermosensitivity of the transposition process is not unique. The frequency of transposition of Tn3 (TnA) is reduced at 45 °C compared with 32 °C (Kretschmer & Cohen, 1977). However, our experiments showed not only that the transposition of Tn951 is a thermosensitive process but also that recA-independent, transient cointegration is temperature-dependent. This common thermosensitivity clearly supports the idea that the two events are related, as recently proposed by the models of Shapiro (1979) or Grindley & Sherratt (1978). In particular, according to Shapiro, a cointegrate structure with two directly repeated copies of the transposon is an obligatory intermediate in the transposition event between two circular molecules. The molecular analysis of the Tn951-generated cointegrates will determine whether these structures carry Tn951 as directly repeated copies.

Finally, it is striking that Tn951 was originally discovered on pGC1, a transfer-thermosensitive conjugative plasmid. Moreover, pGC1 itself was initially isolated from a strain of Y. enterocolitica, a bacterial species known to grow faster at 30 °C than at 37 °C. This suggests that Tn951 might have originated in a saprophytic environment bacterium. Since the lac genes from Tn951 are homologous to the E. coli lac operon, one might extrapolate that the lac operon from E. coli itself derives from a saprophytic environment bacterium.
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REFERENCES


