Genetic Evidence that Control of F Replication Is Negative

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Summary. We have taken advantage of two situations in which the incompatibility barrier between F plasmids is overcome to show that wild-type genes controlling F copy number (cop⁺) are dominant in trans over mutant genes. The simplest interpretation of our findings is that the cop mutations have inactivated a repressor gene that controls F replication. Since the cop mutations all map in a region that others have shown by sequence analysis to theoretically encode four proteins, a strong possibility exists that one of these proteins is the repressor.

Introduction

Mini-F plasmids have been constructed from wild-type F and found to have the same copy number as their full size parent (Lovett and Helinski 1976; Timmis et al. 1975). This indicates that mini-F has the same control of replication as the native plasmid; and this control appears to be negative (Tsutsui and Matsubara 1981). This latter conclusion is based on the following observations made with mini-F:ColEl cointegrates. When replication was driven by the ColEl moiety, the copy number of the cointegrate was several fold higher than the normal copy number of mini-F. If the ColEl-directed replication was suddenly prevented by inactivating the polA gene product, then the cointegrate replication ceased and did not resume for several generations. The observed lag was just that needed to dilute the cointegrate concentration to the normal copy number of the mini-F plasmid. This behavior was interpreted as reflecting dilution of a repressor of F replication that was maintained in proportion to the elevated mini-F concentration.

Prior to the observations on the behavior of mini-F:ColEl cointegrates, Manis and Kline (1978b) observed that copy mutants of mini-F also lost one of their incompatibility functions and speculated that the loss reflected inactivation of a repressor controlling copy number. More recently, Seelke et al. (1982) mapped the cop mutations isolated by Manis and Kline to somewhere within a 530 base pair region bounded by the coordinates 45.35 and 45.88 kb. In the present work we tested the ability of wild-type mini-F to repress expression of these cop mutations. In order to do this we had to overcome the strong incompatibility barrier that would prevent the formation of stable heteroplasmid cells with mini-F plasmids. This was accomplished in two different ways. Both ways showed that the cop⁺ allele was dominant to the cop allele which is consistent with negative control.

Materials and Methods

Bacterial Strains and Plasmids

The bacteria used in this study are CSH50 ara A (lac pro) thi rpsL (Miller 1972) and BK210 which has the same genotype as CSH50 but is additionally recA (Miller et al. 1978). The plasmids used in this work have been described before: pML31 (mini-F Km⁺) (Lovett and Helinski 1976) and several derivatives (Manis and Kline 1978; Seelke et al. 1982) are pictured in Fig. 1. The plasmid mutations tested for recessiveness to the cop⁺ allele were induced by nitrosoguanidine (cop44 and cop50, Manis and Kline 1978b) or ethyl methane sulfonate (cop211, 213 and 214, Seelke et al. 1982). pBK50 was formed by cutting pSC101 (Cohen and Chang 1977) with EcoRI and recombining it with the EcoRI fragment taken from pML31 (Fig. 1) (Manis and Kline 1978b). Thus, pBK50 determines kanamycin and tetracycline resistance; when used to clone BamHI fragments, the tet gene is inactivated.

In Vivo Formation of Cointegrate Structures Between Mini-F Ap⁺ and -F Km⁺ Plasmids

Cointegration between F plasmids, one of which is pML31 (Fig. 1), is rare unless the latter is deleted for some function that maps in the 40.8 to 43.1 kb region (Manis and Kline 1978a and unpublished observation). To isolate the cointegrates used in the present study, we introduced mini-F singly into CSH50 by transformation (Lederberg and Cohen 1974). Usually, the cop⁺ kam⁺ plasmid pML31 or pBK261 was introduced first, then a second plasmid, a mini-F blacap⁺ A (40.3 to 43.1 kb) mutant, was added to make the heteroplasmid. The heteroplasmid transformants were allowed to grow in AB3 broth (Difco) for 10 to 20 generations at 37 ° C. During this period the incompatibility functions destroy the heteroplasmid state in all but a few cells. These cells were selected by plating small samples of the broth culture on AB3 agar containing 25 μg ampicillin/ml and 40 μg kanamycin/ml. Our expectation and only finding was that colonies formed from initially unselected heteroplasmid cells would contain recombinant plasmids.
DM represents Tn3 insertion into pMF21 at the 46.19 kb coordinate; plasmid used for these studies. Gene symbols are: pM£4 from pMF45, pML31, pMF21, pMF45 and 46 all have the same restriction site in F at 49.1 kb: pBK261 was made in vitro between the most distal of pMF45 containing, the Tn3 transposon. The genesis of the derivatives of pML31 is as follows: pMF21 represents a deletion made in vitro for the F sequences between the 40.8 and 43.1 kb BamHI sites; pMF45 represents Tn3 insertion into pMF21 at the 46.19 kb coordinate; pMF46 represents a deletion made in vitro for the EcoRI fragment of pMF45 containing, the kan + gene; and pBK261 represents a deletion made in vitro between the most distal BsrEI site in Tn3 and the same restriction site in F at 49.1 kb: pBK261 was made from pMF45, pML31, pMF21, pMF45 and 46 all have the same copy number of 1–2/chromosome equivalent. The copy number of pBK261 is about twice that of pML31 (Seelke et al. 1982).

This conclusion was established and the general nature of the recombinational event was deduced by restriction analysis of the plasmid DNA with EcoRI endonuclease. The parental plasmids have a total of three EcoRI restriction sites and, therefore, a true recombinant formed by a single crossover event should also have three sites. The total mass of the resultant cointegrate is equal to that of the sum of the parental plasmids unless a deletion has occurred. By contrast, if a transpositional event was responsible for the formation of the stable Ap′Km′ colony, then the plasmid DNA should have a parental restriction pattern with the additional mass of a transposon unless the Tn3(Ap) or Tn903(Km) element had moved into the chromosome of the host cell. In no instance did we detect any evidence for a transpositional event although such events undoubtedly occurred.

Due to the presence of a deletion and a transposon in the F sequences of the copy mutants, it was possible to deduce from the molecular weights of the restriction fragments whether the recombinational event took place to the right or to the left of the Tn3 insertion site which maps at coordinate 46.19 kb. The logic and the expected fragment masses are presented schematically in Fig. 5. Representative examples of both situations are shown in Figs. 3 and 4.

Assay of Plasmid Concentration

Rapid estimates of copy numbers per unit mass were done by extracting plasmid DNA as described by Birnboim and Doly (1979) from 0.5 ml of overnight cultures containing a fixed amount of cell mass. Then about 20 μl of extracted DNA from each culture was subject to electrophoresis under conditions that have been described previously (Manis and Kline 1978b). This method is excellent when large differences between the Cop + and Cop − phenotypes are evident. To quantitatively verify the results of the gel electrophoresis assays, the specific activity of plasmid-determined β-lactamase was measured by using a chromogenic penicillin, nitrocefin, as a substrate (Manis and Kline 1978b). Restriction enzymes were employed according to manufacturer's instructions.

Results

Complementation Between F′ Lac + and pBK50: FCop + Cointegrates

Complementation assays between F plasmids are not easily done unless the incompatibility barrier between them is avoided. This was accomplished by forming cointegrate plasmids of two different types. The first type consisted of a cointegrate between an F compatible replicon, pSC101-Km′ (pBK50), and a mini-F replicon containing sequences 43.1 to 46.2 kb. The 43.1 to 46.2 kb region contains sufficient information to form a mini-F plasmid (Seelke et al. 1982). Manis and Kline (1978b) and Seelke (unpublished) found that the copy number for these cointegrates can be significantly greater than that of pBK50. Some representative results are given in Table 1. The data suggests that the F cop sequences are controlling replication of the cointegrates. Both sets of authors also found that cointegrates made from the F copy mutants lost the ability to express the incB + and incC + genes that are contained within the cloned F sequences (Fig. 1; Manis and Kline 1978b; Seelke et al. 1982).

This last observation led us to examine cointegrate copy numbers in the presence and absence of an F′ lac plasmid. This can be done in a semiquantitative way by comparing the intensity of the DNA represented by the cointegrate plasmid in lysates made from the appropriate cells as shown in Fig. 2. These results clearly show that the concentration of the three different cointegrates tested is significantly lowered when F′ lac is also present in the host cell. This result indicates repression of the F′directed replication of the cointegrate by the F′ lac plasmid.

Complementation Between FCop + and FCop − Plasmids

The second type of cointegrate formed to avoid the F′ incompatibility barrier was between two mini-F plasmids.

Table 1. Copy numbers of mini-F and mini-F:pBK50 cointegrates

<table>
<thead>
<tr>
<th>Parent Plasmid</th>
<th>Copy Number</th>
<th>Cointegrate Plasmid</th>
<th>Copy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMF46</td>
<td>Wild-type</td>
<td>2</td>
<td>pBK57</td>
</tr>
<tr>
<td>pBK65</td>
<td>cop44</td>
<td>18</td>
<td>pBK69</td>
</tr>
<tr>
<td>pBK213</td>
<td>cop213</td>
<td>56</td>
<td>pBK222</td>
</tr>
<tr>
<td>pBK214</td>
<td>cop214</td>
<td>26</td>
<td>pBK224</td>
</tr>
<tr>
<td>pBK50</td>
<td>Wild-type</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

* All cointegrates were constructed in the same way. The parental plasmid was digested with BamHI and the fragment containing the 43.1 to 46.2 kb F sequences was purified and ligated into the BamHI site in the tet gene of pBK50. The table is written to indicate that pBK57 has the F sequences from pMF46, etc.

b Copy numbers were determined by dye-CsCl analysis; all values are from prior publications (Manis and Kline 1978b; Seelke et al. 1982) except for pBK222 and pBK224.