Expression of a Mutation Affecting F Incompatibility in the Integrated but Not the Autonomous State of F

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Previously we have described a mutant Hfr strain in which incompatibility between the integrated F factor and an autonomous F-prime (F') factor was abolished. The mutation (inc) was located in the integrated F factor. F-prime factors isolated from the mutant Hfr strain have the same incompatibility behavior as those isolated from normal Hfr strains. Reintegration of these F' factors into the chromosome restores the Inc- phenotype characteristic of the mutant Hfr. The inc mutation thus affects incompatibility between integrated F and autonomous F(Fi-Fa, incompatibility) but not incompatibility between two autonomous F factors (Fa-Fa, incompatibility). The implications of this finding for the mechanism of plasmid incompatibility are discussed.

Plasmid incompatibility has been demonstrated in Escherichia coli between an integrated F factor and an autonomous F factor (Fi-Fa incompatibility) and between two autonomous F-prime factors (Fa-Fa incompatibility; for review, see reference 4). Previously we have described a mutant of an integrated F factor in which Fi-Fa incompatibility was impaired (3). However, F' factors isolated from this Inc- Hfr strain were incompatible with other F' factors and with wild-type integrated F factors. It was not shown whether the normal incompatibility behavior of these F' factors was due to a second reverse mutation from inc to inc+ or whether it reflected a difference in the expression of incompatibility between the integrated and the autonomous state of the F factor. In the present paper we report results that support the second possibility. We have isolated Hfr strains in which F' factors isolated from the Inc- Hfr strain had become reintegrated into the chromosome, and these Hfr strains are Inc−, like the original Inc- Hfr strain. We conclude that the inc mutation is expressed phenotypically in an integrated F factor but not in an autonomous F factor.

MATERIALS AND METHODS

Media and materials used in matings have been described previously (3, 5). Preliminary screening of donor and recipient ability of strains was performed by cross-streaking donor and recipient cultures on appropriate selective media. Results of the streak matings were usually confirmed by standard broth matings. Transductions were performed by the method of Glansdorff (6). The Rec phenotype was tested by replica plating or spotting cultures onto freshly poured plates of neopeptone agar containing between 3 and 6 parts of methyl methane sulfonate per 10,000 parts of medium and incubating overnight at 37 C. Rec- cells do not grow in the presence of methyl methane sulfonate, whereas Rec+ cells grow in a nearly normal fashion. Experiments involving thermosensitive mutations used a restrictive temperature of 42 C and a permissive temperature of 30 C. Bacterial strains used are described in Table 1; a map of relevant gene locations, Hfr sex factors, and F' factors is shown in Fig. 1. F' factors were isolated from the Inc- Hfr by mating the Hfr with a recA female strain and selecting for inheritance of markers near the integrated F factor (3).

RESULTS

Integration of F' factors in a seg-2, recA strain. In the seg-2 mutant described by Hatha-way and Bergquist (7), F' factors cannot replicate at restrictive temperatures (42 C) but replicate normally at 30 C. This provides a means for selection of Hfr strains from F' strains at the nonpermissive temperature.

F' factors E1, E2, E3, E4, E5, and E6 derived from the Inc- Hfr strain MA1091 were transferred to the seg-2 strain PB1010 thy lys by conjugation from their KL250 derivatives. In the seg-2 background the replication of these plasmids was temperature sensitive. To select for integration of presumptive Inc- F's, E1 to E6 derivatives of PB1010 thy lys were streaked onto minimal medium selecting for Thy+ Lys+ at 42 C. A small number of colonies grew out after 2 to 3 days. These presumptive Hfr colonies were tested for ability to transfer his+ to MA124 (recA+) and for ability to transfer lys+...
to RG274R (recA\textsuperscript{+}). A large number of the thermoresistant Thy\textsuperscript{+}, Lys\textsuperscript{+} colonies proved to be Hfr\textquoteright}s, which transfer his\textsuperscript{+} at high frequency to the Rec\textsuperscript{+} recipient and lys\textsuperscript{+} at very low frequency to the Rec\textsuperscript{−} recipient. The low-frequency transfer of lys\textsuperscript{+} to a Rec\textsuperscript{+} recipient is presumably due to F\textquotesingle;s present in some cells. All of these Hfr\textquoteright}s formed by integrating the E series of plasmids also transferred lys\textsuperscript{+} and his\textsuperscript{+} to RG274 (recA\textsuperscript{+}) at high rates but transferred serA\textsuperscript{+} to RG274 at very low rates, suggesting that the plasmids are integrated at or very near the integration site of their parental Hfr, MA1091.
It was necessary to introduce the recA allele into these Hfr strains, since incompatibility is best tested in a recA background to avoid recombination between the F’ and the chromosome. To obtain Rec^- derivatives of the Hfr strains derived by integrating the E plasmids, they were mated in the F^- phenocopy state with Hfr KL16-99 selecting for Pro^+ in the recipients while counter-selecting the donor cells with streptomycin. Colonies were purified and tested for their Rec phenotype with methyl methane sulfonate. Rec^- clones showing Hfr properties and an Ara^-,Leu^- phenotype were saved for further testing.

Testing strains having E plasmids integrated in a recA^- seg-2 genetic background for their Inc phenotype. Eighty-five Rec^- derivatives having integrated E plasmids were tested as follows: F^- phenocopies were cross-streaked with exponentially growing cultures of both KLF1/AB2463 and KLF4/AB2463 with selection for Leu^+ progeny. Both KLF1 and KLF4 carry the seg^- allele. In seg^+/seg-2 diploids of PB1010, replication of the F’ is normal at 42°C. Since the seg^- allele is dominant, then an inability of F’ factors to exist stably in our derived Hfr strains would be due to the inc^+ gene and not to the seg^-2 gene. Leu^- colonies were picked and streaked on MacConkey arabinose agar. Eight-one of the Rec^- derivatives gave rise to stable Leu^-Ara^- derivatives, which could subsequently donate the KLF1 or KLF4 plasmid at high rate to KL250 Gal^+ Spe^- (Table 2). Spontaneously cured Ara^- derivatives were still Hfr and were good recipients in matings with KLF1/AB2463 and with KLF4/AB2463. Therefore, these 81 Rec^- Hfr’s are Inc^- . The remaining four derivatives gave rise to much lower numbers of Leu^- colonies when mated in F^- phenocopy with KLF1/AB2463 and with KLF4/AB2463. The few Leu^- colonies arising in the matings were highly unstable for Ara^- when checked on MacConkey arabinose agar. These derivatives are Inc^+ [for example, strain (E4)311 in Table 3]. The inc^- allele in these strains was probably inherited from KL16-99 when the strains were mated to obtain Rec^- derivatives. This reflects the location of the inc^+ allele on that portion of F transferred early by Hfr KL16-99 in conjugation, in agreement with published mapping data (12).

The Inc phenotypes were confirmed for representative Rec^- derivatives in quantitative mating experiments. One Rec^-Inc^- Hfr derivative of each F’ factor was used as a recipient (in F^- phenocopy) in matings with KLF1/AB2463 and with KLF4/AB2463. Matings were performed in neopeptone broth at 37°C for 1 h at a donor-recipient ratio of 1:10 and were plated on selective medium to determine numbers of recipients becoming Leu^+. An Inc^- derivative was also tested as an F’ recipient as were two Hfr strains, MA1077 (Inc^+) and MA1091 (Inc^-), of known Inc phenotype (3). An F’ seg^-2 strain was used as a control recipient. The data are summarized in Table 3. They support the conclusion that, although F’ factors derived from an Inc^- Hfr are phenotypically Inc^+, they regain the Inc^- phenotype when reintegrated into the chromosome.

Since a thermosensitive seg^-2 mutant was used to integrate the E plasmids, it was of interest to see if seg^-2 was still present and phenotypically functional in the Hfr’s derived

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**Table 2. Screening Rec^- derivatives harboring integrated E episomes for Inc phenotype**

<table>
<thead>
<tr>
<th>Episome integrated in Hfr strain</th>
<th>No. of Rec^- Hfr derivatives giving stable Leu^- Ara^- progeny in matings with KLF1 and KLF4/AB2463 tested^c</th>
<th>No. of stable Ara^- derivatives that transfer an autonomous F’leu/no. tested^b</th>
</tr>
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<tbody>
<tr>
<td>E1</td>
<td>30/32</td>
<td>30/30</td>
</tr>
<tr>
<td>E2</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>E3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>E4</td>
<td>9/10</td>
<td>9/9</td>
</tr>
<tr>
<td>E5</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>E6</td>
<td>25/26</td>
<td>25/25</td>
</tr>
</tbody>
</table>

^a Several Leu^- progeny from each mating were streaked onto MacConkey arabinose agar to check for a stable Ara^- phenotype.

^b Recipient in cross-streak matings was KL250 Gal^- Spe^-.

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*FIG. 1. Map of the E. coli chromosome showing approximate locations of some relevant genes, Hfr sex factors, and F’ factors.*
by integrating the E plasmids. F'8 was introduced by conjugation from CGSC2605 into a few Inc-Rec- derivatives that had retained the gal- allele of PB1010. Segregation of F'8 was checked by streaking Gal+ derivatives on MacConkey agar with galactose as carbon source and incubating the plates at 42 and 30°C. In all strains tested, the Gal+ phenotype was thermostable, indicating that the Seg- phenotype is still present in our Hfr derivatives. At 30°C there was no segregation of F'gal from the Inc-Hfr's.

Isolation and testing of F'ser integrated in MA176. Besides the F-primes carrying early markers, counterclockwise from the Inc-Hfr sex factor in strain MA1091, we also isolated several F-primes carrying the late serA+ (3) (see Fig. 1). Like the other F-primes derived from the Inc-Hfr, they are also phenotypically Inc+. These were also tested to determine whether the mutant inc gene is carried in an unexpressed state. In this case, however, integration was obtained in a strain not containing the seg-2 mutation. The F'ser was introduced into strain MA176 by mating with F'ser/MA219 and selecting for Ser+ in the recipient. A culture of F'ser/MA176 was plated to give approximately 300 colonies/plate and incubated overnight at 37°C. The next day, this master plate was replica-plated onto three neopeptone plates, which were incubated at 37°C. After 4 to 5 h, the neopeptone plates were replica plated onto lawns of suitable F- strains to test for F'ser transfer and for chromosomal transfer. In both cases spectinomycin was used for counterselection. A few colonies that showed chromosomal transfer but no F' transfer were picked, grown to exponential phase of growth, and rechecked for donor ability by cross-streak matings with the same recipients on selective minimal medium. Two Hfr strains that showed good transfer of his+ to the Rec+ recipient but low transfer of serA+ to the Rec- recipient were isolated. One of these, (F'ser-I) MA176 was used as a recipient in a mating with the recA Hfr strain MA1046 to obtain Rec- derivatives.

Seven clones that were phenotypically Hfr, Thr-, Leu-, Gal-, Lac-, Str+, Rec- were isolated. All seven cultures were tested as recipients, in the F'-phenocopy state, of Thr+ Leu- from KLF1/AB2463. Six strains were good donors and good recipients (Inc-). A seventh strain was a good Hfr donor but a poor recipient for KLF1, probably having inherited inc+ from MA1046 when the Rec- derivative was obtained.

One of the Inc-Rec- derivatives was further tested by mating it (in F'-phenocopy) with the F'gal (F'8) donor CGSC2605. A large number of stable Gal+ progeny were obtained. When 50 of the Gal+ progeny were tested for their ability to donate F'gal to a Rec- Gal- recipient strain (H19 Arg- Rec-) and to donate the chromosomal markers lys+, trp+, and pro+ to a Rec+ recipient (x478 Spe+), it was found that 49 out of the 50 Gal+ derivatives were both F'gal and chromosome donors.

This confirms the finding that, when an Hfr strain is obtained by integrating the F'ser, the Inc- phenotype is restored. The fact that the integration of F'ser into MA176 gives the same

<table>
<thead>
<tr>
<th>Recipient strain</th>
<th>Putative Inc phenotype</th>
<th>Leu+ progeny/ml of mating mixture × 10^3 in matings with:</th>
<th>% Leu+ progeny able to transfer chromosomal and F' genetic markers*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KLF1/AB2463</td>
<td>KLF4/AB2463</td>
</tr>
<tr>
<td><strong>(E1) 132</strong></td>
<td>-</td>
<td>190</td>
<td>77</td>
</tr>
<tr>
<td><strong>(E2) 231</strong></td>
<td>-</td>
<td>5,700</td>
<td>97</td>
</tr>
<tr>
<td><strong>(E3) 112</strong></td>
<td>-</td>
<td>3,300</td>
<td>420</td>
</tr>
<tr>
<td><strong>(E4) 111</strong></td>
<td>-</td>
<td>1,700</td>
<td>74</td>
</tr>
<tr>
<td><strong>(E4) 311</strong></td>
<td>+</td>
<td>0.35</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>(E5) 2211</strong></td>
<td>-</td>
<td>830</td>
<td>NT</td>
</tr>
<tr>
<td><strong>PB1010 thy rec</strong></td>
<td>F-</td>
<td>56,000</td>
<td>67,000</td>
</tr>
<tr>
<td><strong>MA1077</strong></td>
<td>+</td>
<td>1.7</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>MA1091</strong></td>
<td>-</td>
<td>6,600</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Leu+ transfer to KLF250Gal+Spc was evidence of F' transfer; His+ transfer to MA124 was evidence of chromosomal transfer. Twenty single colonies from each strain were tested as donors.

a F-primes E1 to E5 are all integrated into a PB1010 derivative background and carry the markers leu recA seg2 ara str.

b Colonies were found that acted as either chromosomal or F' donors, but not both.
d NT, Not tested.
results as those obtained by integrating the series of E episomes into a seg-2 strain eliminates the possibility that the IncC phenotype results from the presence of the seg-2 mutation.

In conclusion, the inc mutation originally characterized by DeVries and Maas (3) is expressed only by integrated F; derived F′ factors have an IncA phenotype. When these F′ factors are reintegrated into the chromosome, the IncA phenotype of the parental Hfr is restored.

DISCUSSION

The results presented lead to the conclusion that the inc mutation affects F-R-F′ incompatibility but not F-R-F′ incompatibility. Moreover, the effect is produced when the mutant F factor is in the integrated state but not when it is in the autonomous state. A difference between F-R-F′ incompatibility and F-R-F′ incompatibility has also been observed with an R factor, R386, which belongs to the same incompatibility group (FI) as the F factor. It was shown by Dennison (2) and by us (DeVries and Maas, unpublished data) that this R factor segregates with higher frequency when present with an F′ factor than when present in an Hfr strain. It should be noted that, in this case, the integrated F factor is IncC′ and the diminished F-R-F′ incompatibility is due to greater stability of R386 in Hfr strains than in F′ strains.

In regard to the mechanism underlying incompatibility, our results suggest that the mechanism of F-R-F′ incompatibility may be different from that of F-R-F′ incompatibility. However, this suggestion must be viewed with caution, because with plasmids belonging to the same incompatibility group one finds large differences in the degree of incompatibility, as determined by the rate of loss of a plasmid in a clone that originally harbored an incompatible pair. This variation is illustrated in a recent paper by Uhlin and Nordstrom (11), who have shown that the degree of incompatibility between an incompatible pair of R factors can be altered as a result of mutation in one of the R factors. The genetic background of the host is also important in determining the degree of incompatibility. San Blas et al. (8) have described a chromosomal mutation that abolishes incompatibility between a specific pair of F′ factors but does not affect incompatibility with other F′ factors. We have found (Santos and Maas, unpublished data) that incompatibility between R386 and an F′lac factor is much greater in one host strain than in another. Viewed against this information, especially the observed differences in stability of R386 in Hfr and F′ strains, our results with the IncA mutant may be interpreted as being due to a quantitative difference in the expression of the incompatibility function between an integrated F factor and an autonomous F factor.

The genes for F-R-F′ incompatibility and F-R-F′ incompatibility have been found to be located within a 9-kilobase segment of F comprising about 10% of the total F factor. This was shown by demonstrating that a hybrid plasmid, mini-F′ lac, is incompatible with both autonomous F′ factors and integrated F (DeVries and Maas, unpublished data). The hybrid plasmid was isolated by Lovett and Helinski (Helinski, personal communication), and its F coordinates were determined by Guyer, Figurski, and Davidson (Guyer, personal communication) and by us (Palchaudhuri and Maas, unpublished data) to be about 40–49 F. This finding is at least not inconsistent with the idea that the same genes determine F-R-F′ incompatibility and F-R-F′ incompatibility and thus does not argue against the possibility of a common mechanism for both kinds of incompatibility. It should be noted that incompatibility between another mini-F′ and F′lac has been reported (10).

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LITERATURE CITED


