Comparisons of F Factors and R Factors: Existence of Independent Regulation Groups in F Factors

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The similarity of sex pili mediated by F factors and R(f1+H) factors and the ability of R(f1+) factors to control by repression the functioning of pilus genes encoded by the F factor suggested that F factors and R(f1+) factors are closely related. Further comparisons of the episomal properties of F factors and R(f1+) factors, however, indicated many differences. F factors contain information for a restriction system for phages φII and T7. Cells containing R factors are sensitive to these phages. Furthermore, R(f1+) factors do not repress the F factor φII restriction system in cells containing both an R(f1+) factor and an F factor. R factors and F factors are heteroimmune epimemes. In addition, an R(f1+) factor in cells containing both an R factor and an F factor does not fully repress the expression of F-factor immunity to an incoming second F factor. R-factor and F-factor replication systems are not identical. Wild-type F-factor replication genes will complement the mutant Ft,141ac+ replication genes in cells containing two F factors. The Ft,141ac+ episome is retained when these cells are grown at 42°C; however, cells containing an R(f1+) factor and Ft,141ac+ lose the Ft,141ac+ when grown at 42°C, at the same rate as cells containing only the Ft,141ac+. The replication system of the R(f1+) factor will not complement the mutant Ft,141ac+ replication system.

Transmissible extrachromosomal elements in the Enterobacteriaceae can exhibit a number of different properties, including fertility (18), autonomous replication (12), restriction of certain bacteriophages (6), immunity or exclusion of other extrachromosomal elements (21, 23), and the synthesis of sex pili (3). In addition, portions of the bacterial chromosome (19) or genes determining antibiotic resistance (21) may be associated with these elements.

Fertility, or the transfer of deoxyribonucleic acid (DNA) from donor cell to recipient cell, is a complex function which requires the synthesis of specific sex pili as well as a specific DNA transfer apparatus (3, 18). Drug-resistance factors (R factors) have been placed into two categories according to the type of sex-specific pili they produce and according to their effect on the fertility of cells containing both F factors and R factors (14, 23). The majority of cells in cultures of strains harboring wild-type F+ factors and substituted F' factors (such as F'lac+) produce F-type pili and show a high degree of fertility (3). However, in most cells of a culture of a strain carrying a wild-type R(f1+) factor, sex pilus synthesis is repressed and fertility is much lower. In contrast, in most cells of a culture to which an R(f1+) factor has been recently transferred, phenotypic derepression of fertility occurs and sex pilus of the F type are produced (16, 23). When such a wild-type R(f1+) factor is present in a cell containing an F factor, F pilus synthesis is greatly decreased and the ability of the cell to transfer the F factor is reduced (23). From this observation, and the existence of fertility-depressed mutants of the R(f1+) factors which normally synthesize F pilus in most of their host cells (16) and which no longer decrease the fertility of F factors in the same cell, it has been suggested that pilus synthesis is normally under repressive control in R(f1+) cells (17).

The second category of extrachromosomal elements, exemplified by R(f1+) factors, produce a new class of sex pilus, the I pili (14). R(f1+) elements have no effect upon the expression of fertility and sex pilus production of F factors present in the same cell (14, 23). The existence of fertility-depressed mutants of R(f1+) factors which give rise to I pili synthesis in most cells and demonstrate a high level of fertility suggests that R(f1+) extrachromosomal elements are also under repressive control, although this control is different from that of R(f1+) elements (16, 23).
The similarity in sex pilus production and the ability of $R(f^+)$ factors to control by repression the expression of pilus genes encoded by the $F$ factor suggested that $F$ factors and $R(f^+)$ factors are closely related. It was, therefore, of interest to consider the relationship between other known functions of $R$ and $F$ factors, such as phage restriction, immunity, and replication.

Most $F$ factor-containing strains restrict the multiplication of phages $\phi II$ (7) and $T7$ (6), whereas isogenic $F^-$ derivatives lacking only the $F$ sex factor are fully sensitive to these phages. By contrast, strains containing $R$ factors are sensitive to $T7$ (23) and the phage W31 (24), another $F$ factor-restricted phage. We have found that strains containing $R$ factors are also $\phi II$-sensitive. The sensitivity of strains containing $R$ factors to $T7$ and $\phi II$ may result simply from the absence of the gene(s) for the phage restriction system or, alternatively, the phage restriction system may be repressed. In either case, it is of interest to determine whether $R(f^+)$ repression of $F$ functions extends to the $\phi II$-$T7$ restriction system.

$R(f^+)$-, $R(f^-)$-, and $F$-containing strains exhibit a property of immunity or exclusion (18, 21, 23); i.e., each type of element prevents the establishment of a second episode of a homologous type in a cell. Strains containing both $R(f^+)$ and $R(f^-)$ factors exhibit little or no immunity to the establishment of an $F$ factor (23), suggesting that $R$ factors and $F$ factors have different immunity systems. However, it is not known whether the presence of $R(f^+)$ in a cell containing both an $F$ factor and an $R$ factor affects the immunity of the $F$ factor towards the establishment of a second $F$ factor.

$F$ factors are autonomously replicating, extrachromosomal elements, each of which is an independent replicon. This system has been best defined by a series of mutant $F^{lac^-}$ episomes which are temperature-sensitive in replication. At low temperature (34°C), host cells retain their resident $F^{lac}$, but at high temperature (42°C) the $F^{lac}$ is lost and the cells become $F^-$ (12). This temperature-sensitive mutation is known to be within the genome of the episome, because episome replication remains temperature-sensitive when the episome is transferred to other host strains (12). $R$ factors must also have their own replication system, but it is not known whether the $R$-factor replication system is the same as that of $F$ factors. However, if one accepts the hypothesis of Jacob and Monod (13) for sex factor immunity, then the coexistence of $F$ factors and $R$ factors in the same cell implies that these episomes have different replication systems.

MATERIALS AND METHODS

Strains, episomes, and phages. The strains and episomes used in these experiments are listed in Table 1. All episomes studied were transferred into strain TM-1 by selecting for $LAC^+$ (or $MEL^+$), $SPC^+$ in the case of $F$ factors, or drug resistance in the case of $NR.1$ and $NR.3$ (originally carried by CSH-2). In a second series of crosses, $F$ factors (originally carried by TM-2) were introduced into TM-1 derivatives carrying $NR.1$ or $NR.3$ by selecting for $LAC^+$ (or $MEL^+$), drug-resistant recipients.

Phages used in these experiments were R17, a ribonucleic acid $F$-specific phage (15) obtained from J. Watson; fd, a DNA $F$-specific phage (24) obtained from T. August; $\phi II$, an $F$-restricted phage (7) obtained from B. Low; and $T7$, an $F$-restricted phage (6) obtained from W. C. Summers.

Media and growth conditions. All strains were grown in ML broth which contained, per liter: tryptone, 10 g; yeast extract, 5 g; NaCl, 5 g; and KH$_2$PO$_4$, 2 g. R plates and R soft agar (11) were used for plaquing phage. Minimal plates contained M63 medium (4) plus vitamins (0.1%) and 50 mg of lactose (0.5%). TZL plates contained 25.6 g of Difco Antibiotic Medium No. 2 and 50 mg of 2,3,5-triphenyltetrazolium chloride and 10 g of lactose per liter.

Antibiotics used to verify the presence of $R$ factors were streptomycin, tetracycline, and chloramphenicol, all of which were obtained from commercial sources, and spectinomycin, which was a gift from The Upjohn Co. The concentration of each antibiotic used in both liquid and solid media was 20 μg/ml.

Single-step growth curves of $\phi II$ in cells containing $R$ and $F$ factors. A culture was grown to 3 × 10⁶ cells/ml in ML broth. KCN was added to a final concentration of 0.01 M, and incubation was continued for 2 min. Phage $\phi II$ was added at a multiplicity of 0.1 and incubation at 37°C was continued for 5 min. The $\phi II$-infected culture was then diluted by 10⁶ and 10⁷ in ML broth. Incubation at 37°C was continued, and samples of the infected culture were taken at time intervals and plated on TM-1 to determine the $\phi II$ titer.

Mating conditions. In experiments designed to measure the immunity of strains containing both $R$ factors and $F$ factors, TM-1/(F'W4680 + NR.1) and TM-1/(F'W4680 + NR.3) were used as recipients. To construct these strains, F'W4680 (F$^{lac^-}_{exo}$), which is $MEL^+$, was transferred to TM-1/NR.1 and TM-1/NR.3 by selecting for $MEL^+$, antibiotic-resistant cells.

To determine the immunity of $F$ factor- and $R$ factor-containing cells, episome F'M15 (F$^{lac^-}_{exo}$) was transferred to TM-1/(F'W4680 + NR.1) and to TM-1/(F'W4680 + NR.3) by mixing in flasks approximately 5 × 10⁷ cells of TM-2/F'M15 with approximately 5 × 10⁷ cells of TM-1/(F'W4680 + NR.1) or TM-1/(F'W4680 + NR.3) in a volume of 1 ml. After incubation at 37°C for 60 min, the mating mixture was plated to select for LAC⁺, SPC⁺ cells. Deletion M15 lacks the $\alpha$ segment of the z gene of the lac operon (2, 20), and deletion W4680 lacks the $\beta$ segment of the z gene (5, 20). However, cells containing both the M15 mutation and the W4680 mutation
are LAC+ because of intracistronic complementation (20). The retention of the R factor was insured by including the appropriate antibiotics in the plating medium. Retention of the F factor originally present was insured by selecting for LAC+ cells.

Where noted, recipient strains were grown with aeration at 37 C for 24 hr (to stationary phase) prior to the mating. Such a procedure produces cells which behave phenotypically like F- cells, i.e., F- phenotypes (10).

The ability of episome-containing cells to serve as recipients is measured by the number of LAC-, SPCR or MEL, SPCR (antibiotic-resistant if applicable) cells per input TM-2/F'M15. Temperature inactivation of F\textsubscript{lac} The procedure used was that described by Cuzin and Jacob (9). Cells containing F\textsubscript{nu1}lac\textsuperscript{+} were grown to stationary phase in ML broth at 34 C. At time zero, portions of the culture were diluted into fresh medium and incubated at 42 C. Samples were removed at intervals and plated in duplicate on TZL plates in the presence or absence of antibiotics. One set of TZL plates with antibiotics and one set without antibiotics were incubated at 42 C to determine the fraction of cells containing integrated F\textsubscript{lac} (LAC+ at 42 C). The remaining TZL plates (with and without antibiotics) were incubated at 34 C to determine the fraction of LAC+ cells remaining in the culture after growth for various times at 42 C.

The temperature inactivation of F\textsubscript{lac} is plotted as the log N/N\textsubscript{0} against the number of generations, where N equals the fraction of LAC+ cells in each sample after incubation at 42 C, minus the fraction of cells containing integrated F\textsubscript{lac} (LAC+ at 42 C) in this sample. N\textsubscript{0} equals the fraction of LAC+ cells in the sample taken at time zero, minus the fraction of cells containing integrated F\textsubscript{lac} in the zero-time sample.

**RESULTS**

To test the interaction of F factors and R factors, it was first essential to introduce these elements into an isogenic background. Therefore, all of the episomes to be studied were transferred into strain TM-1 as outlined in Materials and Methods. To verify the existence of autonomous F\textsuperscript{r} factors in strains containing both R factors and F factors, the rate of segregation of LAC+ cells was determined. In every case, the loss of the F\textsuperscript{r}lac episomes occurred at the same frequency, regardless of the presence or absence of R factors of either type in the same cell.

**Phage sensitivity.** The result of plating F-specific and F-restricted phages on cells containing F factors and R factors is shown in Table 2. Strains TM-1, TM-1/F\textsuperscript{lac}, TM-1/NR.1, and TM-1/NR.3 were used as the controls. A number of observations can be made.

First, strains containing only F factors are sensitive to phages R17 and fd. However, when NR.1 is present in the same cell, these cells are resistant to the F-specific phages. R(fii\textsuperscript{+}) repressive control of F plus appears to be functioning.

Second, cells containing NR.3 alone are insensitive to R17 and fd, but the presence of NR.3 in F factor-containing cells does not diminish the sensitivity of these cells towards the F-specific phages.

Third, TM-1 and derivatives containing R factors alone are fully sensitive to phages 81I and T7. All F factor-containing cells used in our experiments are resistant to these phages. If an R factor (fii\textsuperscript{+} or fii\textsuperscript{-}) and an F factor are present...
TABLE 2. Response of episome-containing strains to F-specific and F-restricted bacteriophages*

<table>
<thead>
<tr>
<th>Series</th>
<th>Episomes present</th>
<th>Efficiency of plating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R17</td>
</tr>
<tr>
<td>Controls</td>
<td>None</td>
<td>&lt;5 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>F'lac⁺</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>NR.1</td>
<td>&lt;5 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>NR.3</td>
<td>&lt;5 × 10⁻³</td>
</tr>
<tr>
<td>F'lac⁺</td>
<td>F'lac⁺ + NR.1</td>
<td>&lt;4 × 10⁻³</td>
</tr>
<tr>
<td></td>
<td>F'lac⁺ + NR.3</td>
<td>0.79</td>
</tr>
<tr>
<td>F'13</td>
<td>F'13</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>F'13 + NR.1</td>
<td>&lt;2 × 10⁻²</td>
</tr>
<tr>
<td>E5014</td>
<td>E5014</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>E5014 + NR.1</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
<td></td>
<td>E5014 + NR.3</td>
<td>1.70</td>
</tr>
</tbody>
</table>

* In each case, strain TM-1 served as the episome-carrying cell. Standards for φII and T7 were obtained with TM-1; for R17 and fd, with TM-1/F'lac⁺. Each F'lac⁺-containing cell segregated LAC⁻ derivatives at characteristic frequency regardless of the presence of R factors.

* Pinpoint plaques.

in the same cell, the cell remains resistant to φII and T7. Neither R factor prevents restriction of φII and T7 by the three F factors tested.

To confirm the results of the plating tests, single-step growth experiments were performed with φII in F⁻, R⁻, and episome-containing cells (Fig. 1). The burst size in cells containing F factors and in cells containing both the R(φI⁺ or φI⁻) factor and the F factor is reduced to less than 5% of that obtained with F⁻, R⁻ cells (60 plaque-forming units/cell). In a separate experiment, not presented here, cells containing NR.1 gave identical burst sizes as compared with TM-1. The φII burst size in TM-1/NR.3 was reduced from that obtained in the R⁻ F⁻ alone, but was still 10 times greater than that obtained in NR.3 + F factor-containing cells.

**Immunity of cells containing both R and F factors.** The establishment in a cell of a second episode of a homologous type is prevented by the immunity system of the first episode. The immunity of F factor-containing cells, in the presence or absence of R factors in the same cell, was determined by testing the ability of these cells containing both R and F factors to accept a second F factor. Strain TM-2/F'M15 was used as the donor, and derivatives of TM-1 containing F'W4680 and NR.1, or F'W4680 and NR.3, were used as recipients. Selection in each cross was for LAC⁺ (by complementation), antibiotic-resistant cells. Recipients used as controls were TM-1, TM-1/F'W4680, TM-1/NR.1, and TM-1/NR.3. Selection in these crosses was for MEL⁺, SPC⁺, LAC⁺, SPC⁺; MEL⁺, SPC⁺; TC⁺; CM⁺; and MEL⁺, SPC⁺, TC⁺ cells, respectively. Retention of the resident R factor in the recipients was ensured by including the antibiotics in the plating medium. Retention of the F'W4680 originally present was ensured by selecting for LAC⁺ cells. [It should be recalled that deletion M15 lacks the φ segment of the z gene of the lac operon (2, 20), and deletion W4680 lacks the β segment of the z gene (5, 20). Therefore, cells containing both the M15 mutation and the W4680 mutation are LAC⁺ because of intracistronic complementation (20).] The LAC⁺ cells selected may be either true F'W4680 + F'M15 cells or recombinant F'lac⁺ cells (10).

These crosses were performed in two ways: recipients were grown for 24 hr (to stationary phase) prior to the mating (column 3, Table 3) or fresh cultures of recipients in log-phase growth were used in the matings (column 4, Table 3). Both sets of results show that the cells containing R(φI⁺) alone accept the F'M15 episome to approximately the same extent as the F⁻, R⁻ control, but the presence of R(φI⁻) alone leads to a 90% decrease in acceptance of F'M15.

F'W4680-containing cells grown to stationary phase prior to mating demonstrate a high degree of immunity or exclusion of the second F factor. The degree of F'M15 exclusion from (F'W4680 + NR.1) recipients in stationary phase is essentially the same as that observed for the cells containing only F'W4680. Therefore, full repression of the F'W4680 exclusion of F'M15 has not occurred in these cells. If full repression of F'W4680 immunity had occurred, one would predict the ratio of recipients per input donor to be equivalent to that observed when TM-1/NR.1 was used as a recipient.

It should be noted that, in these matings, the acceptance of F'M15 by the strain containing the two episomes was the same, whether or not the recipient was grown to stationary phase.
However, the acceptance of F'M15 by cells containing F'W4680 alone or by the cells containing (F'W4680 + NR.3) could not be measured until the recipients were grown to stationary phase. When grown to stationary phase, these recipients accepted F'M15 to approximately the same extent as cells containing NR.1 and F'W4680.

**R-factor replication.** To explore relationships between the R-factor replication systems and that of F factors, a complementation experiment was attempted. It has been reported that in rare cells containing two different F factors, one of which is F'w (temperature-sensitive) and the other wild type for replication, the temperature-sensitive episome is retained upon cultivation at high temperature (8). Presumably the defect of the mutant episome at high temperature can be complemented by the wild-type episome, thus allowing replication of the F'w episome. If R-factor replication systems are the same as, or similar to, the F-factor system, then the addition of an R factor to an F'tlac+-containing cell should allow the retention of the F'tlac+ at high temperature.

Cells containing F'tlac+ and either NR.1 or NR.3 were constructed by use of methods described above. The phage sensitivities of the resultant F't8 + R strains are identical to those of the F'tlac+ + R factor strains reported in Table 2. In addition, at 34 C, cells containing both the R factor and F'tlac+ segregate LAC- cells at a frequency of 5 \times 10^{-3}, indicating that the F'tlac+ episome is autonomous in these strains.

The sensitivity of F'tlac+ replication to incubation at high temperature (42 C) in cells containing the F't8 + R strains are identical to those of the F'tlac+ + R factor strains reported in Table 2. In addition, at 34 C, cells containing both the R factor and F'tlac+ segregate LAC- cells at a frequency of 5 \times 10^{-3}, indicating that the F'tlac+ episome is autonomous in these strains.

The sensitivity of F'tlac+ replication to incubation at high temperature (42 C) in cells containing the F'tlac+ alone or together with NR.1 or NR.3 was determined by the method of Cuzin and Jacob (9). F'tlac+ -containing cells were grown to stationary phase at 34 C. At time zero, portions of the culture were diluted into fresh medium and incubated at 42 C. At intervals, samples were removed and plated at 34 C to determine the fraction of LAC+ cells in the population and for total cell counts.

To obtain a temperature inactivation curve, the logarithm of the fraction of LAC+ cells remaining in the culture at any time, divided by the fraction of LAC+ cells at time zero, was plotted as a function of the number of generations obtained at 42 C. As can be seen in Fig. 2, the rate of loss of F'tlac+ from the R factor-containing cells was as great as or greater than the rate of loss of F'tlac+ when present alone.

The following controls were performed: (i) cultures grown at 34 C showed no decrease in the fraction of LAC+ cells; (ii) the total cell count at each sample time was the same when plated in the presence or absence of drugs, indicating that no significant segregation of the R factors had occurred after growth at 34 or 42 C; (iii) samples plated at 34 C in the presence of drugs exhibited the same fraction of LAC+ cells as did samples plated in the absence of drugs, indicating that the presence of drugs in the plates did not influence the fraction of LAC+ cells.

We can conclude that the decrease in the fraction of LAC+ cells after growth at 42 C cannot be due to the plating conditions, nor to the absence of the R factors in the cells. Neither the R(\(fi^+\)) nor the R(\(fi^-\)) factor is able to repair the replication defect of F'tlac+ at 42 C. Therefore, the replication system of the F factor is different from that of either of the two R factors tested.

**DISCUSSION**

F factors and R(\(fi^+\)) factors mediate the synthesis of the same type of sex pilus, the F pilus (14), and this synthesis appears to be under the control of the same repressor (17). However, this repression does not appear to extend to other F-factor functions such as the phage restriction system(s) or F factor-mediated immunity.

F factor-containing strains restrict the multiplication of phages \(\phi II\) and T7, whereas R factor-containing strains are sensitive to these phages. However, F factor-mediated restriction of \(\phi II\)
Factor-containing strains.

The mechanism responsible for immunity is unclear. Jacob et al. (12, 13) suggested that immunity might result from competition for membrane attachment sites, and the lack of immunity between nonhomologous episomes merely reflects a difference in the membrane attachment sites. Novick (18) proposed that immunity is also a property of the exterior of the male cell and that the resident episome is responsible for a surface property which prevents entry of the superinfecting episomal DNA.

R(\(f^{+}\))-mediated immunity cannot be identical to F-mediated immunity. In addition, R(\(f^{+}\)) immunity is not repressed, because R(\(h^{+}\))-containing cells are immune to the establishment of a second R(\(f^{+}\))-factor. We have shown that R factors do not greatly decrease F-mediated immunity. If immunity is due to competition for unique membrane sites, R factor repression should not extend to F-factor immunity because F-factor membrane attachment would be a host-mediated function. If some F-factor product such as an exterior membrane component is responsible for immunity, then R(\(f^{+}\)) repression does not extend to this F-factor function. However, our results do not allow a choice between models for sex factor immunity.

Our results suggest that the R(\(f^{+}\)) factor in cells containing R(\(f^{+}\)) + F factors represses some F-factor function, allowing the cells to accept a second F factor at low levels. This might result from the repression of external sex pilus formation or of some other unknown property. Growth to stationary phase of nonrepressed F\(^{+}\) cells has the same effect of slightly reducing the immunity of F\(^{+}\) cells to homologous episomes. We have shown, however, that absence of pili on the surface of cells containing R(\(f^{+}\)) and F factors [via R(\(f^{+}\)) repression] is not sufficient to reduce fully the immunity of male cells.

The fact that R(\(f^{+}\)) factors probably do not have a \(\phi II\) or T7 restriction system(s) and the fact that the R(\(f^{+}\)) factors do not have the same type of immunity system as that of F factors suggest that R factors and F factors are indeed quite different. Our comparison of the replication

<table>
<thead>
<tr>
<th>Table 3. F'M15 acceptance of episome-containing strains</th>
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<tbody>
<tr>
<td><strong>Recipient</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>TM-1</td>
</tr>
<tr>
<td>TM-1/F'W4680</td>
</tr>
<tr>
<td>TM-1/NR.1</td>
</tr>
<tr>
<td>TM-1/NR.3</td>
</tr>
<tr>
<td>TM-1/(F'W4680 + NR.1)</td>
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<tr>
<td>TM-1/(F'W4680 + NR.3)</td>
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\(^{a}\) The donor was TM-2/FM15.

\(^{b}\) Recipients were grown to stationary phase prior to mating.

\(^{c}\) Freshly grown recipients were used.

Fig. 2. Temperature inactivation of F\(_{\text{R}}\)lac\(^{+}\) in R factor-containing strains.

and T7 operates even if R factors are present in the same cell. Thus, the R factor-mediated repression of F-factor pilus formation does not extend to the \(\phi II\) and T7 restriction system(s). It is, therefore, likely that a mechanism for restriction of these phages is lacking in the two R factors tested.

R(\(f^{+}\))-, R(\(f^{-}\))- , and F factor-containing strains exhibit the property of immunity or exclusion (21, 23); i.e., the establishment in a cell of a second episome of a homologous type is prevented. Strains containing R(\(f^{+}\)) and R(\(f^{-}\)) factors exhibit little or no immunity to the establishment of an F factor (22); therefore, R factors and F factors have different immunity systems.

The mechanism responsible for immunity is unclear. Jacob et al. (12, 13) suggested that immunity might result from competition for membrane attachment sites, and the lack of immunity between nonhomologous episomes

Goldstein and Malamy (1975) established that factors have the property of immunity, or exclusion, from infection. They showed that the establishment of the second episome of a homologous type is prevented. Strains containing R(\(f^{+}\)) and R(\(f^{-}\)) factors exhibit little or no immunity to the establishment of an F factor (22); therefore, R factors and F factors have different immunity systems.

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systems of these two extrachromosomal elements indicates a further difference. Cells containing R factors wild type in replication and F' \text{lac}^+ factors which are temperature-sensitive in replication (F_{\text{ts14lac}^+}) lose the F_{\text{ts14lac}^+} at high temperature at the same rate or at a greater rate than cells containing only the F_{\text{ts14lac}^+}. R factors are unable to repair the replication defects in the F_{\text{ts14lac}^+}, whereas other F factors are known to have this ability. Thus, R-factor replication is not identical to that of the F factor. This result is expected if the hypothesis of Jacob et al. (12, 13) for sex factor immunity is correct. According to this hypothesis, the coexistence of F factors and R factors in the same cell implies different membrane attachment sites and, therefore, different replication systems.

Our comparison of R factors and F factors suggests that F factors and NR.1 are similar only in fertility and the control of fertility. Furthermore, our results provide evidence for the existence of at least three separately controlled units in substituted F factors: (i) a structural gene(s) involved in pilus formation which can be regulated by the R(fi+)-repressor; (ii) other unique F-factor functions (immunity, \PhiII and T7 restriction, and autonomous replication) which are either not regulated, absent, or different in R factor-containing cells; (iii) incorporated chromosomal genes (one or more, depending on the F') which are subject to normal induction and repression characteristic of the corresponding chromosomal genes (e.g., lac genes are induced by isopropyl-\beta-D-thiogalactoside).

Our results indicate that NR.3, the R(fi-) factor, may have a generalized restriction system in addition to the normal epimural immunity. Several lines of evidence suggest this conclusion. First, NR.3-containing strains accept F factors at a lower frequency (10%). This phenomenon has been previously observed by Watanabe (23). However, (NR.3 + F)-containing cells appear to accept a second F at essentially the same frequency as F-containing cells. But the sensitivity of the experiments concerning the immunity of cells containing both an R factor and an F factor is probably not great enough to allow accurate detection of differences less than an order of magnitude between the immunity of (NR.3 + F)-containing cells and F factor-containing cells. Second, NR.3-containing strains plate \PhiII at approximately F-, R- strain efficiency (Table 2), but the burst size of \PhiII in these strains is about half that obtained in female strains or in NR.1-containing strains. Finally, the rate of inactivation of F_{\text{ts14lac}^+} at 42 C is the same in strains containing only F_{\text{ts14lac}^+} and in strains containing (NR.1 + F_{\text{ts14lac}^+}), whereas the rate of inactivation at 42 C of the F_{\text{ts14lac}^+} is greater in (F_{\text{ts14lac}^+} + NR.3)-containing cells (Fig. 2).

It is not immediately obvious why the presence of a generalized restriction system should lead to a more rapid elimination of the F_{\text{ts14lac}^+} episome when NR.3-containing cells are incubated at 42 C. NR.3 does not increase the segregation frequency of F_{\text{ts14lac}^+} at 34 C.

NR.3 would seem to contain a generalized restriction system acting on incoming DNA or nonreplicating DNA. Enhanced immunity to incoming F DNA might arise from the NR.3 immunity system, or it may be a result of the presence of an additional restriction system determined by this episome. It is not known whether this restriction system is the same as the \lambda and T1 restriction system defined by Watanabe et al. (23).

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LITERATURE CITED


