JOURNAL OF BACTERIOLOGY, Nov., 1965
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Vol. 90, No. 5
Printed in U.S.A.

Interaction Between Colicinogenic Factor V and the Integrated F Factor in an Hfr Strain of *Escherichia coli*

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Received for publication 30 June 1965

ABSTRACT

KAHN, PHYLLIS L. (Princeton University, Princeton, N.J.), AND DONALD R. HELINSKI. Interaction between colicinogenic factor V and the integrated F factor in an Hfr strain of *Escherichia coli*. J. Bacteriol. **90**:1276–1282. 1965.—The production of colicin V by strains of *Escherichia coli* is determined by a colicinogenic factor, *colV*. The *colV* factor possesses a genetic determinant of fertility, F_v. $V^+F_v^+$ cells are characteristically susceptible to a male-specific phage, μ, and able to transfer the *colV* factor and chromosomal markers to recipient cells. The present work describes an interaction of the *colV* factor with the chromosome of the Hfr strain, HfrH. A *colV*-containing HfrH strain, designated $HfrHV^+F_v^+(1)$, was isolated and shown to be insensitive to phage μ and impaired in its fertility properties. Loss of the *colV* factor by this strain, either spontaneous or induced by acridine orange, resulted in a further 10^3- or 10^4-fold loss in fertility. This additional loss of fertility was restored by reinfection of these strains with the *colV* factor. The *colV* interaction with the HfrH chromosome also can result in defects in the fertility properties of the *colV* factor. Altered *colV* factors were found in recombinants isolated from a cross between the $HfrHV^+F_v^+(1)$ strain and F^- recipients. It is postulated that in the $HfrHV^+F_v^+(1)$ strain an interaction of the *colV* episome with the integrated F region of the chromosome occurs, with a resulting modification of the fertility properties of the HfrH strain. This interaction can also result in a defect in certain properties of the *colV* factor.

The ability of a bacterium to produce colicin V is determined by an episomal element, designated colicinogenic factor V (*colV*). In a previous communication (Kahn and Helinski, 1964), the *colV* factor was shown to possess a genetic determinant of fertility (F_v) in addition to the genetic determinant for colicin V production. The properties of a bacterium associated with the presence of the F_v fertility determinant are quite similar to those determined by the ordinary F factor of *Escherichia coli* (Kahn and Helinski, 1964). The promotion of conjugation and chromosomal recombination by the F and the F_v fertility determinants are qualitatively identical. In addition, the presence of either genetic determinant renders an *E. coli* cell sensitive to the male-specific bacteriophage, μ.

Fredericq (1963) demonstrated the ability of the *colV* factor to become integrated into the bacterial chromosome and to acquire a linkage with certain chromosomal genes. In the present study, further evidence for the interaction of *colV* with the chromosome of a bacterium is presented. When a high-frequency recombining strain, HfrH, is infected with *colV*, two distinct types of colicinogenic HfrH strains are obtained. The properties of one of these strains is best explained by an interaction of the *colV* factor with the fertility region of the HfrH chromosome. This episome-episome interaction results in an interference of the fertility properties of the HfrH strain and causes defects in the fertility properties of colicinogenic factor V.

MATERIALS AND METHODS

Organisms. The strains used in this study are described in Table 1.

Media and culture methods. The medium and most of the methods have been described previously (Kahn and Helinski, 1964). For this investigation, colonies were tested for the production of colicin V by replica plating. Individual colonies were grown on a master plate overnight, or at least until heavy growth was evident. To a very dry nutrient broth plate, 4 ml of soft agar containing approximately 5×10^6 cells of a strain

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TABLE 1. *List of strains**

Strain	Colicin produced	Auxotrophic characteristics	Resistance markers
C600	—	$thr^-leu^-thi^-lac^-$	—
C600/E_1VAz	—	$thr^-leu^-thi^-lac^-$	Az, E_1, V
C600$V^+F_v^+$	V	$thr^-leu^-thi^-lac^-$	Az, E_1, V
C600 S^r/E_1V	—	$thr^-leu^-thi^-lac^-$	S, E_1, V
YS40	—	his^-pro^-	S
YS40/E_1V	—	his^-pro^-	S, E_1, V
YS40$V^+F_v^+$	V	his^-pro^-	S, E_1, V
YS57	—	$his^-pro^-try^-$	T_1, S, V
HfrH/E_1V	—	thi^-	Az, E_1, V
HfrH$V^+F_v^+$(h)†	V	thi^-	Az, E_1, V
HfrH$V^+F_v^+$ (l)†	V	thi^-	Az, E_1, V
HfrH$V^-F_v^-$ (l)†	—	thi^-	Az, T6, E_1, V
K94	V	—	—

* The following abbreviations are used: *his*, histidine; *try*, tryptophan; *pro*, proline; *thr*, threonine; *leu*, leucine; *thi*, thiamine; *lac*, lactose; S, streptomycin; Az, sodium azide; E_1, colicin E_1; V, colicin V; *colV*, colicinogenic factor V; μ^r, resistant to bacteriophage μ. The source of most of these strains was indicated previously (Kahn and Helinski, 1964). YS57 was kindly supplied by C. Yanofsky.

† The properties of this strain are described in the text.

sensitive to colicin V were added. The master plate was then replicated onto this seeded plate. Upon incubation, noncolicinogenic colonies appeared as a heavy growth on a turbid background, whereas the colicinogenic colonies were surrounded by a clear zone.

Recombination. Except for the following modifications, the recombination experiments were carried out as described earlier (Kahn and Helinski, 1964). Mating cells were mixed in a ratio of 10 females to 1 male, and conjugation was interrupted by chilling portions of the culture and subjecting them to violent agitation with a Vortex mixer for 1 min. Crosses were stopped after 2 hr, except where a different time is specifically indicated. In certain experiments the recombinants were picked, grown overnight, and then tested for susceptibility to a male-specific phage, μ (Dettori, Maccacaro, and Piccinin, 1961). When necessary, these cultures were tested for recombination by the method previously described for testing a large number of cultures (Kahn and Helinski, 1964). When it was desired to test strains to a very low level of recombination, overnight cultures of the donor and recipient strains were diluted 20-fold and mixed at a 1:1 ratio in nutrient broth. After 4 hr of growth this mixture was centrifuged, and was concentrated 10-fold in saline; 0.1 or 0.2 ml of the concentrated suspension was plated. Recombinants at a level of 1 in 10^9 cells could be detected by this method.

Transfer of colicinogenic factors. Conditions for the transfer of colicinogenic factors were those described by Kahn and Helinski (1964).

RESULTS

Fertility properties of the HfrH and HfrH$V^+F_v^+$ strains. An interaction between the *colV* factor, which possesses genetic determinants for colicin V production (V^+) and fertility (F_v^+), and the HfrH chromosome was suggested by the effect of the *colV* factor on the recombination rate of the HfrH strain. The presence of the *colV* factor reduced the recombining ability of a certain colicinogenic isolate of HfrH, designated HfrH$V^+F_v^+$(l), by approximately 80% for the *TL* marker (Table 2). Other colicinogenic isolates of HfrH, designated HfrH$V^+F_v^+$(h), appeared to be unaffected by the presence of the *colV* factor. An interaction between the *colV* factor and the chromosome of the HfrH strain was also indicated by the difference in transfer of the *colV* factor from HfrH$V^+F_v^+$(l) and HfrH$V^+F_v^+$(h). *ColV* is transferred approximately 3% as efficiently from the HfrH$V^+F_v^+$(l) strain as from the HfrHV^+-F_v^+(h) strain. Although precise data on the frequency of occurrence of the two types of HfrH$V^+F_v^+$ strains have not yet been obtained, it appears that, after infection of HfrH with the *colV* factor, the occurrence of the HfrH$V^+F_v^+$(h) type is more probable.

The presence of the *colV* factor in the HfrHV^+-F_v^+(l) strain also was found to alter the susceptibility of this HfrH strain to the male-specific phage, μ. Under the conditions employed, phage μ failed to form plaques on the HfrH$V^+F_v^+$(l) strain (Table 3). The μ^r property of this strain was also indicated when its susceptibility to phage μ was examined by the patch test under conditions previously described (Kahn and Helinski, 1964).

Acridine orange treatment of the HfrH$V^+F_v^+$

TABLE 2. *Fertility of the HfrH and HfrHV$^+$F$_v^+$ strains**

Strain	Method of obtaining HfrHV$^-$F$_v^-$	No. of TL^+ recombinants per 10^4 donors
HfrH	—	366
HfrHV$^+$F$_v^+$(h)	—	264
HfrHV$^+$F$_v^+$(l)	—	60
HfrHV$^-$F$_v^-$(h)	Acridine orange treatment†	413
HfrHV$^-$F$_v^-$(l)	Spontaneous segregant	0.011
HfrHV$^-$F$_v^-$(l)	Acridine orange treatment†	0.148

* Each strain was crossed with C600 S^r/E$_1$V, selecting TL^+ from the donor and S^r from the F$^-$ recipient. The values represent the average recombination frequency of 4 to 10 single-colony isolates of each strain listed. The crosses were performed as described in the text.

† Acridine orange treatment of the HfrHV$^+$F$_v^+$ (l) strain was carried out as described by Kahn and Helinski (1964).

TABLE 3. *Ability of phage μ to form plaques on HfrH strains**

Strain	Per cent of plaque-forming units
HfrH	100
HfrHV$^+$F$_v^+$(h)	79
HfrHV$^+$F$_v^+$(l)	0
HfrHV$^-$F$_v^-$(h)	86
HfrHV$^-$F$_v^-$(l)	0

* The number of plaque-forming units produced with each of the above strains was determined by mixing 2×10^7 cells of each strain with 150 plaque-forming units of phage μ (grown on the HfrH strain) in 2.5 ml of soft agar and adding the mixture to a nutrient agar plate. After incubation at 37 C overnight, the plaques were counted.

strains. To test whether the presence of *colV* in the HfrH strain in certain cases induces a permanent alteration of the fertility properties of this strain, the *colV* factor was removed by treatment with acridine orange. HfrHV$^+$F$_v^+$ strains which have lost the *colV* factor have been designated HfrHV$^-$F$_v^-$. Removal of the *cclV* factor had no effect on the Hfr recombining property in the HfrHV$^+$F$_v^+$(h) strain (Table 2). However, the removal of *colV* from the HfrHV$^+$F$_v^+$(l) strain resulted in a striking decrease in fertility. This was also observed upon spontaneous loss of the *colV* factor from the HfrHV$^+$F$_v^+$(l) strain. In both cases the recombining ability of the HfrHV$^+$-F$_v^+$(l) strain was reduced by a factor of approximately 10^3 to 10^4. Growth in acridine orange per se, when *colV* was not removed, had no effect on the fertility of the HfrHV$^+$F$_v^+$(l) strain. Removal of the *colV* factor also did not result in a restoration of the phage μ susceptibility of the parent HfrH strain (Table 3).

Restoration of fertility to the sterilized HfrHV$^-$-F$_v^-$(l) strain. An attempt was made to restore the fertility of the HfrH clones that had been sterilized by loss of the *colV* factor. Table 4 shows the result of infecting the HfrHV$^-$F$_v^-$(l) strain with *colV* and *colE$_1$* from different donor strains. The transfer of the *colV* factor to the sterilized HfrH strain invariably resulted in a considerable restoration of the lost fertility. The degree of restoration of fertility depended upon the source of the *colV* factor. Restoration of phage μ sensitivity also depended upon the source of the *colV* factor. The restored HfrHV$^+$-F$_v^+$(l) strains transfer their chromosome with the same orientation as is exhibited by the parent HfrH strain. Colicinogenic factor E$_1$ did not restore fertility. Also, growth of the HfrHV$^-$-F$_v^-$(l) strain in the presence of an F$^+$ donor strain did not result in a restoration of fertility. In this case, however, the actual transfer of the F factor to HfrHV$^-$F$_v^-$(l) could not be established.

Appearance of V-colicinogenic strains resistant to phage μ. Previously, it had been shown (Kahn and Helinski, 1964) that the genetic determinant of colicin V production is closely associated with a genetic determinant of fertility (F$_v$). In addition to other evidence, it was observed that, regardless of the combination of donor and recipient strains employed, the V$^+$ and F$_v^+$ characteristics were always transferred simultaneously to recipient cells. This was not found to be the case when colicin V-producing recombinants from a cross with HfrHV$^+$F$_v^+$(l) as donor were tested for their susceptibility to phage μ (Table 5). When TL^+, *his*$^+$, *pro*$^+$, and *try*$^+$ recombinants from crosses between HfrHV$^+$F$_v^+$(l) and certain F$^-$ recipient strains were isolated and examined, a large percentage of these recombinants appeared to receive the ability to produce colicin V and not the susceptibility to phage μ. These recombinants have been designated V$^+\mu^r$. The dissociation of the property of phage μ susceptibility from the *colV* factor showed the following characteristics. (i) It occurs to an appreciable extent only when HfrHV$^+$F$_v^+$(l) is used as the donor. It was not observed at all in crosses where YS40V$^+$F$_v^+$ or C600 V$^+$F$_v^+$ served as donors, and was found only infrequently with HfrHV$^+$F$_v^+$(h) as the donor. (ii) Even with the HfrHV$^+$F$_v^+$(l) strain as donor, V$^+\mu^r$ recipients were observed only amongst re-

TABLE 4. *Fertility of HfrHV⁻Fv⁻ (l) after infection with colV and colE₁**

Cross			Fertility of HfrHV⁻Fv⁻ (l) infected with a *col* factor†	
Source of *col* factor	*Col* factor transferred	Recipient of *col* factor	*TL* recombinants	No. of single colonies averaged
—	—	HfrH	342	6
—	—	$HfrHV^+F_v^+$ (l)	49	9
—	—	$HfrHV^-F_v^-$ (l)	0.0024	10
K94	*colV*	$HfrHV^-F_v^-$ (l)	60	4
$HfrHV^+F_v^+$ (l)	*colV*	$HfrHV^-F_v^-$ (l)	29	7
$HfrHV^+F_v^+$ (h)	*colV*	$HfrHV^-F_v^-$ (l)	13	5
$C600E_1^+V^+$	*colV*	$HfrHV^-F_v^-$ (l)	7	3
$C600E_1^+V^+$	*colE*$_1$	$HfrHV^-F_v^-$ (l)	0.0041	1
$HfrHE_1^+$	*colE*$_1$	$HfrHV^-F_v^-$ (l)	0.0071	7
$C600E_1^+F^+$	*colE*$_1$	$HfrHV^-F_v^-$ (l)	0.0037	6

* The transfer of colicinogenic factors E_1 and V to $HfrHV^-F_v^-$ (l) was carried out as described by Kahn and Helinski (1964). The donor and recipient strains were crossed for 2 hr. When an HfrH strain was the colicinogenic donor, T6 was used to counterselect the donor strain. In the other crosses, sodium azide was employed to counterselect the donor.

† After infection with *colV* or *colE*$_1$, the colicinogenic $HfrHV^-F_v^-$ (l) strain was purified and crossed with the F^- strain C600 S^r/E_1V at 37 C for 2 hr as described in the text. The recombination values are given as the number of *TL* recombinants per 10^4 colicinogenic $HfrHV^-F_v^-$ (l) donor cells. The number of independent single-colony isolates of the $HfrHV^-F_v^-$ (l) strain having received *colV*, or *colE*$_1$, and that were tested for fertility, is indicated in the last column.

TABLE 5. *Marker and strain dependence for the production of $V^+\mu^r$ strains**

Donor	Recipient	Recombinant selected	No. of V^+ recipient clones examined	Per cent of V^+ clones that are $V^+\mu^r$
$C600V^+F_v^+$	$YS40/E_1V$	pro^+S^r	96	0
$YS40V^+F_v^+$	$C600S^r/E_1V$	pro^+TL^+	100	0
$HfrHV^+F_v^+$ (h)	$YS40/E_1V$	pro^+S^r	156	0.6
$HfrHV^+F_v^+$ (h)	$C600S^r/E_1V$	TL^+S^r	70	2.9
$HfrHV^+F_v^+$ (l)	$C600S^r/E_1V$	TL^+S^r	24	96.0
$HfrHV^+F_v^+$ (l)	$YS40/E_1V$	pro^+S^r	64	43.7
$HfrHV^+F_v^+$ (l)	$YS40/E_1V$	$pro^+his^+S^r$	33	48.4
$HfrHV^+F_v^+$ (l)	$YS40/E_1V$	$pro^-his^+S^r$	38	13.0
$HfrHV^+F_v^+$ (l)	$YS40/E_1V$	None†	97	0
$HfrHV^+F_v^+$ (l)	YS57	$pro^+try^+S^r$	35	42.9
$HfrHV^+F_v^+$ (l)	YS57	$pro^-try^+S^r$	38	7.9

* The strains were crossed for 2 hr as described in the text.

† $YS40/E_1V$ receiving *colV* without chromosomal genes from the donor strain $HfrHV^+F_v^+$ (l).

combinants. Those recipients acquiring *colV* by transfer with no apparent recombination were $V^+\mu^s$. (iii) A clear marker dependence is visible, the appearance of $V^+\mu^r$ strains correlating with the integration of an early marker such as *TL* or *pro*. When selection is made for a later marker such as *his* or *try*, the percentage of $V^+\mu^r$ among the recipients is greatly decreased except where the *his* or *try* recombinants are also *pro* recombinants. (iv) The appearance of the $V^+\mu^r$ strains was independent of the duration of the mating (between 20 and 120 min). (v) None of the recombinants examined exhibited $V^-\mu^s$ characteristics. The recombinant was always $V^+\mu^s$ or $V^+\mu^r$.

Characterization of the $V^+\mu^r$ strains. When the $V^+\mu^r$ strains were mated with noncolicinogenic F^- strains, they appeared to fall into several classes on the basis of their ability to transfer the altered *colV* factor and to recombine (Table 6). In general, the $V^+\mu^r$ strains were inferior to the

usual $V^+F_v^+$ strain with regard to fertility properties; many were totally unable to act as genetic donors in crosses. These sterile V^+ strains form class A in Table 6. In class B are found $V^+\mu^r$ clones unable to transfer the *colV* factor autonomously at a high level (<0.2%) but able to recombine at a low level. When the recombinants were picked and tested, most were found to produce colicin V, and all of the colicinogenic recombinants retained the parental resistance to phage μ. The $V^+\mu^r$ strains in class C are capable of both recombining with F^- strains and transferring the *colV* factor, but these functions are carried out at a diminished rate. With some of the class C strains, the $V^+\mu^r$ state is retained upon transfer of the damaged *colV* factor; in other strains in this category, the $V^+\mu^s$ property was restored upon transfer of the damaged *colV* factor to a suitable recipient. The strains whose *colV* factor exhibited this change in its characteristics showed no detectable inhomogeneity of the $V^+\mu^r$ property. It should be noted that $V^+\mu^r$ strains with an ability to promote recombination equivalent to that of a $V^+F_v^+$ strain were not isolated; $V^+\mu^s$ strains that had lost the ability to recombine were not obtained.

The $V^+\mu^r$ strains in each of the three categories were isolated at approximately the same frequency. Furthermore, the particular type of $V^+\mu^r$ strain appeared to be independent of the type of recombinant selected from the cross between the $HfrHV^+F_v^+(l)$ strain and an F^- recipient.

Reconstruction of a $V^+F_v^+$ strain. When the unaltered *colV* factor is transferred to an F^+ recipient, a definite incompatibility between the $V^+F_v^+$ and F^+ characters in the recipient is observed (Kahn and Helinski, 1964). An attempt was made to determine whether the $V^+\mu^r$ recombinants had lost their incompatibility with the F factor. In almost every case tested, the $V^+\mu^r$ strains appeared to be unable to coexist stably with the normal F factor of *E. coli*, as judged by their inability to become susceptible to phage μ after mating with an F^+ strain. In two $V^+\mu^r$ strains, however, a slight sensitivity to phage μ was observed. When these strains were plated out, they were found to segregate $V^+\mu^r$ and F^+ cells at a high frequency, with some clones retaining the $V^+\mu^s$ characteristics. After three such subcultures, two strains were picked which exhibited a stable $V^+\mu^s$ characteristic. Further tests showed that the properties of the stable $V^+\mu^s$ strains were identical to those of a typical $V^+F_v^+$ strain. These strains always transferred the ability to produce colicin V concurrently with the genetic determinant of fertility; they promoted genetic recombination to the level of a $V^+F_v^+$ strain; and, upon curing with acridine orange, the abilities to produce colicin V and to elicit a sensitive response to phage μ were always removed together. Subcultures of the same $V^+\mu^r$ strains that had not been mated with an F^+ strain did not yield any $V^+\mu^s$ segregants or show any instability of the *colV* factor.

TABLE 6. *Properties of $V^+\mu^r$ strains*

Type	Promotion of recombination	Autonomous transfer of *colV*
A	–	–*
B	+†	–*
C	+†	+

* No evidence of transferability when tested at a level that would detect transfer to 0.2% of the recipient cells.

† Recombination rate of approximately 1:10 to 1:100 of normal *colV* promoted recombination.

DISCUSSION

The data clearly indicate an effect of the *colV* factor on the integrated fertility factor in the HfrH strain. The various manifestations of this are perhaps most explicable by an interaction between *colV* and the region of the chromosome of HfrH harboring the fertility factor. As summarized in Table 7, two distinct types of $HfrHV^+F_v^+$ strains appear. In the $HfrHV^+F_v^+(h)$ strain, the *colV* factor appears to exist as an autonomous cytoplasmic element. The recombination frequency of this strain is quite similar to that of the parent noncolicinogenic HfrH strain; the transfer of *colV* from this HfrH strain is high; and colicinogenic recombinants from crosses where $HfrHV^+F_v^+(h)$ is the donor also possess the F_v^+ properties typically associated with the

TABLE 7. *Summary of properties of the $HfrHV^+F_v^+$ strains*

Strain	Recombining ability	Transfer of *colV* factor	Susceptibility to phage μ	Concurrent loss of fertility and *colV* factor	Alteration of *colV* factor
$HfrHV^+F_v$ (h)	High	High (100%)	+	–	Low
$HfrHV^+F_v^+$ (l)	Low	Low (3%)	–	+	High

ability to produce colicin V (Kahn and Helinski, 1964). In addition, when HfrHV$^+$F$_v^+$(h) is cured of its *colV* factor by growth in a medium containing acridine orange, its Hfr fertility is undisturbed. This was expected, since Hirota (1960) showed that acridine orange has no effect on an F factor integrated into the chromosome as in an Hfr strain. The only known peculiarity of this strain is its ability to retain the *colV* factor stably. Scaife and Gross (1962) could not isolate F$^+$ or Hfr strains also harboring an autonomous *F-lac* factor. In addition, an incompatibility between the F factor and the *colV* factor has been observed (Kahn and Helinski, 1964).

The properties of the second type of colicinogenic HfrH strain, HfrHV$^+$F$_v^+$(l), are significantly different. Its recombination rate is reduced to one-seventh that of the parent HfrH strain, and its ability to transfer *colV* is also lower than that of most other V$^+$ strains. It is also less susceptible to phage μ that is HfrH. Most significant, however, is the concurrent loss of Hfr fertility with loss of the *colV* factor either by acridine orange curing or spontaneous segregation. One explanation for this is that the *colV* factor is in some way associated with the integrated F factor, and its removal results in the concurrent removal of some part of the HfrH fertility factor. The association of the *colV* factor with the F factor site might also interfere with the synthesis of the specific surface structure necessary for mating, and this may account for the lower recombination rate and the poorer μ susceptibility observed with the HfrHV$^+$F$_v^+$(l) strain. Nagel de Zwaig and Anton (1965) similarly reported a reduction of recombining ability of an Hfr strain as a result of the presence of a *colV-colI* factor. In addition, the episomic element, the RTF factor, has been shown to interfere with the fertility properties of an Hfr strain (Watanabe and Fukasawa, 1962; Watanabe, Fukasawa, and Takano, 1962).

It is also significant that those cells which have lost their colicinogenicity retain a low level of fertility. Fertility can be restored to its original level by reinfection with the *colV* factor. The sterilized HfrHV$^-$F$_v^-$ strain behaves as if it has a sex factor affinity locus. Richter (1957) reported a similar situation with certain F$^-$ recombinants of an Hfr cross. When these recombinants were subsequently infected with the F factor, they became Hfr strains identical to the original parental Hfr strain. Adelberg and Burns (1960) similarly reported the isolation of F$^-$ strains with a sex factor affinity locus. These F$^-$ strains could be obtained by acridine orange treatment of the Hfr strain P4X-1, and could be converted to high-frequency donors upon reinfection with either the F$_1$ or F$_2$ episome.

The appearance of V$^+\mu^r$ strains among the recombinants of an HfrHV$^+$F$_v^+$ (l) cross may also be an effect of this postulated interaction between *colV* and the HfrH chromosomal fertility site. The V$^+\mu^r$ strains appear to an appreciable extent only from the HfrHV$^+$F$_v^+$(l) strain and largely when an early marker from this strain has been integrated into the recipient. They do not appear at all in a cross where a low-frequency recombining colicinogenic strain is used as donor (Table 4 and Kahn and Helinski, 1964), or when transfer of *colV* occurs without corresponding recombination of a chromosal marker. Their appearance is strongly correlated with integration of a marker close to the origin of the Hfr and is independent of the time of mating. It has also been noted that, of the altered *colV* containing recombinants, only V$^+\mu^r$ strains are obtained. V$^-\mu^s$ strains were not observed. There are two possible reasons for this: (i) there is a definite order to the transfer, with the genetic determinant for the production of colicin V transferred first and the determinant for the μ-receptor site last, or (ii) the genetic region necessary to maintain the *colV* factor in a clone is very close to the episomal locus responsible for colicin V production. Thus, although the V$^-\mu^s$ episome can be formed, it is highly probable that it can not maintain itself and is diluted out of the culture with growth. Experiments are currently underway to investigate the nature of the lesion of a V$^+\mu^r$ strain. As indicated previously, the μ^r characteristic is not maintained upon transfer from certain V$^+\mu^r$ strains in the class C category. Thus, it is possible that in certain cases the loss of the property of susceptibility to phage μ by *colV* factors is due to a reparable damage rather than to a deletion of genetic material.

Whatever the nature of the damage to the V$^+\mu^r$ strains, all those tested still retain incompatibility to the F factor of *E. coli*. They also appear to retain their susceptibility to curing of these factors by acridine orange. Both of these properties are absent in a colicinogenic factor devoid of any F properties such as the *colE$_1$* factor (Kahn and Helinski, 1964). According to the "replicon" hypothesis of Jacob, Brenner, and Cuzin (1963), the incompatibility between related episomes may be explained by a competition for a limited number of specific attachment sites. Attachment to such a site is also a prerequisite for replication. Thus, a *colV* factor sufficiently damaged to have lost its incompatibility with the F factor might also be unable to replicate and maintain itself in the clone.

When an attempt was made to introduce F into two V$^+\mu^r$ strains, it appeared that a stable combination of the V$^+$ characteristic of the altered

colV factor and the μ^s characteristic of the F factor occurred and was dependent on a recombination between the two. The resultant $V^+\mu^s$ strain appeared in all respects identical to the usual $V^+F_v{}^+$ strains (Kahn and Helinski, 1964), and the two characteristics appeared to be determined by one episome with respect to their transfer and removal by acridine orange. The possibility does exist, however, that the temporary possession of an F factor by these two $V^+\mu^r$ strains, which are usually unable to transfer *colV*, enabled the transfer of the *colV* factor to take place, and that, once out of the original $V^+\mu^r$ cell, the defective *colV* factor was repaired, making the recipient $V^+\mu^s$. Harada et al. (1964) isolated a recombinant of a drug-resistance factor and an *F-lac* factor. A similar type of recombination event may be responsible for the restoration of fertility to the $V^+\mu^r$ strain after infection with the F factor.

The data on the properties of the $HfrHV^+F_v{}^+$ (1) strain and the appearance of the $V^+\mu^r$ recombinants suggest some interaction between the *colV* factor and the integrated F factor of this strain. Possibly, a crossover similar to that postulated by Scaife and Gross (1963) and Pittard and Adelberg (1964) is occurring. This could result in the addition of the genes determining the production of colicin V to the origin of the chromosome, while the F_v genes remain with the integrated F factor. Upon integration into the recipient chromosome of a marker close to the origin, the deoxyribonucleic acid responsible for the $V^+\mu^r$ characteristic becomes an autonomous genetic element.

The data presented here also emphasize the possibility of an interaction between a colicinogenic factor and a specific region of a bacterial chromosome. It is notable that this interaction can be strong enough to interfere with the maintenance of the integrated F factor on the chromosome. The most likely explanation for this interaction is a chemical homology between the genetic determinant of fertility in the *colV* factor and the region of the HfrH chromosome possessing the integrated F factor.

Acknowledgments

This investigation was supported by Public Health Service research grant AI-05210-01 from the National Institute of Allergy and Infectious Diseases. Phyllis L. Kahn held a Post-doctoral Research Fellowship from the National Institute of Allergy and Infectious Diseases.

We thank Judith Hardy and Doris White for technical assistance.

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