Loss and Repair of Conjugal Fertility and Infectivity of the Resistance Factor and Sex Factor in *Escherichia coli*

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ABSTRACT

HIROTA, YUKINORI (University of Osaka, Osaka, Japan), TOSHIO FUJII, AND YUKINOBU NISHIMURA. Loss and repair of conjugal fertility and infectivity of the resistance factor and sex factor in *Escherichia coli*. *J. Bacteriol.* **91**:1298-1304, 1966.—The drug-resistance factor, R, and the sex factor, F, have homologous traits, including contagious transmission, mediation of sexuality of the host cell, and autonomous replication in their host bacteria. Cooperation between F and R factors was found with a mutant R factor, which is nontransmissible in F⁻ bacteria, becoming transmissible when introduced into bacteria carrying F. Conversely, the chromosome of a sterile male strain carrying the mutant sex factor, F^r, becomes transmissible when an R factor is introduced into the cell. The genetic determinants of R factors have been analyzed by isolation of mutant R factors, by sexual conjugation of the host bacteria, and by transduction of R factors with phage P1kc. The fertility determinant of the R factor, *m*, is inseparable from the determinant for its infectivity, but can be separated from the loci for autonomous replication of the R factor. R and F thus carry genetic determinants governing the same functions.

Conjugally transmissible elements of bacteria, such as the sex factor, F, the genetic determinant of maleness (3, 17, 22), bacteriocinogenic factors conferring on bacteria the ability to produce antibiotic substances of protein nature called bacteriocines (9, 11, 14), and the contagious resistance factor, R, which confers on enteric bacteria resistance to several antibiotics (1, 29), are known to have common properties.

They may exist in the autonomous state and replicate independently of the bacterial chromosome (4, 7). Host bacteria carrying them may conjugate with bacteria having no such element, to which the factors are transferred with high efficiency (1, 10, 17, 22, 29), and chromosomal characters are transferred with low efficiency (25, 30, 32).

It seemed of interest to analyze the striking parallelism known to exist between the wide variety of contagious agents of natural origin. Accordingly, genetic studies were carried out with mutants of the drug resistance factor and of the sex factor.

The introduction of the resistance factor, R, into an F⁻ strain of *Escherichia coli* allows the

F⁻R⁺ strain to mate with F⁻R⁻ strains, and this phenomenon has been named R mating. In variants of the resistance factor, a coincidence was found in the loss of two functions, namely, conjugal fertility and infectivity of the resistance factor itself (32).

The present report is concerned with: loss or acquisition of conjugal fertility and infectivity by a forward or a back mutation of the R factor, extranuclear transmission of wild and mutant R factors, repair of the defects of R factors by F factors, and the repair by R factors of a mutational defect of F which affects chromosome transfer.

Preliminary accounts of this work have been published previously (12, 13, 28).

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial cultures used in this work were mutants of *E. coli* K-12. The principal strains used are listed in Table 1.

Male-specific bacteriophages used were: phage f1 (27, 33), provided by N. D. Zinder, The Rockefeller Institute, New York, N.Y.; phage MS2 (6), provided by E. T. Bolton, Carnegie Institution of Washington, Washington, D.C.; phage M12 (19), provided by

P. H. Hofschneider of Max-Planck-Institut, Munich, Germany. All of them lyse male strains of *E. coli* K-12, specifically.

Phage τ is a female-specific bacteriophage which forms plaques only on *E. coli* K-12 F⁻ strains (15).

Genetic markers Nutritional requirements (- indicates dependence; +, independence) are shown by the following symbols: *met*, methionine; *thr*, threonine; *leu*, leucine; *thy*, thymine; *his*, histidine; *try*, tryptophan; *B₁*, thiamine; and *ade*, adenine

Fermentation markers (- indicates nonfermentation; +, fermentation) include: *ara*, arabinose; *gal*, galactose; *lac*, lactose; *mtl*, mannitol; and *xyl*, xylose. The different suffixes indicate different isolations.

Sexuality of bacteria is represented as follows: F⁺, male carrying the contagious fertility factor, F; F⁻, female lacking this factor; Hfr, mutant male showing a high frequency of recombination; and F^r, sterile male carrying a defective mutant of F factor which is incapable of donating the host chromosome. R, or R factor, is a term for the infectious drug-resistance factors.

The symbol *m* refers to a gene controlling conjugal infectivity and fertility associated with the R factor.

Transduction of R factors by phage P1kc, mating of bacteria, disinfection of F factor or R factor by acridine treatment, and infection with R factors. The procedures reported previously were employed in the present study (18, 26, 32).

Media. Scoring of sugar fermentation and drug resistance was carried out on eosin-methylene blue-sugar-agar (21) with or without addition of one of the drugs, such as 20 μ g/ml of dihydrostreptomycin, 25 μ g/ml of chloramphenicol, 25 μ g/ml of tetracycline, or 200 μ g/ml of a sulfanilamide derivate, sodium sulfisomidine (Dainihon seiyaku). Eosin-methylene blue-sugar-agar (18), a synthetic medium, was used for selection of prototrophic recombinant colonies.

RESULTS

Loss of conjugal fertility and infectivity associated with the resistance factor. Experiments to elucidate the relationships among conjugal infectivity (R infection), conjugal fertility associated

with the R factor (R mating), and the autonomous state of the R factor, were carried out.

Ninety-nine mutant R factors having lost infectivity and 30 mutant R factors incapable of R mating were isolated independently by irradiation with ultraviolet light or were found among transductants with P1kc (32). Indirect selection by the replica plating method was used for their isolation (23).

The mutants which were incapable of R infection were tested for their R mating activity. All of them were inactive (Table 2).

Mutants isolated for their inability to effect R mating were also tested for their ability to effect R infection. All except two were inactive (Table 3).

Removal of the R factor from the exceptional mutants, by acridine treatment, and reintroduction of the original R factor or F factor by mixed culture resulted in decreased frequency of mating capacity. Introduction of the R factor of the exceptional mutants into other F⁻ strains resulted

TABLE 2. Mutants of R factor with lowered infectivity

Frequency*		No. of mutants
R mating	R infection	
<10 ⁻⁸	<10 ⁻⁸	77
<10 ⁻⁸	10 ⁻⁸	4
<10 ⁻⁸	10 ⁻⁷	7
<10 ⁻⁸	10 ⁻⁶	3
10 ⁻⁸	10 ⁻⁸	1
10 ⁻⁸	10 ⁻⁷	7
10 ⁻⁵	10 ⁻²	Wild type

* Frequency of R mating or R infection is expressed as the number of recombinant colonies (*mer*⁺ *mal*⁺) or R⁺ colonies (*chl*⁺ *mal*⁻) formed per donor bacterium used.

TABLE 3. Mutant strains which lost R mating activity

Frequency		No. of mutants
R mating	R infection	
<10 ⁻⁸	<10 ⁻⁸	22
<10 ⁻⁸	10 ⁻⁸	1
10 ⁻⁸	10 ⁻⁷	1
10 ⁻⁸	5 × 10 ⁻⁷	1
10 ⁻⁸	10 ⁻⁶	1
10 ⁻⁸	10 ⁻⁵	2
10 ⁻⁸	10 ⁻²	2*
10 ⁻⁵	10 ⁻²	Wild type

* Exceptional mutant strains which carry the mutation on their host genome and not on the R factor.

TABLE 1. Strains of *Escherichia coli* K-12 used

Strain	Genotype*
W6.....	F ⁺ <i>met</i> ⁻
W1895.....	Hfr ₁ (Cavalli) <i>met</i> ⁻
W4354.....	F ⁻ <i>met</i> ⁻
JE1940.....	F ⁻ <i>lac</i> ⁻ <i>ara</i> ₂ ⁻ <i>mtl</i> ⁻ <i>xyl</i> ⁻ <i>thr</i> ⁻ <i>leu</i> ⁻ <i>thy</i> ₁₅ ⁻ <i>ade</i> ⁻ <i>his</i> ⁻ <i>try</i> ⁻ <i>gal</i> ₆ ⁻
W2252.....	Hfr ₁ (Cavalli) <i>met</i> ⁻
JE1031.....	Hfr ₂ (Hayes) <i>met</i> ⁻ <i>B</i> ₁ ⁻
JE1936.....	F ^r <i>met</i> ⁻ <i>B</i> ₁ ⁻ R ₁₀₀₋₁ ⁺

* Only relevant markers are shown.

in as high a frequency of R mating and R infection as with the original R factor.

It was thus proved that the site of mutation of the exceptional mutant strains is not on the R factor, but on the host chromosome. A detailed study of the site of mutation of the exceptional mutants will be reported elsewhere.

Certain combinations of nontransmissible mutant R factors of independent origin became transmissible when harbored in the same host cell, and such combinations also had R mating activity (Table 4).

Table 5 shows a remarkable coincidence of the reacquisition of the two functions of R by reverse mutation.

Location of wild and mutant resistance factors in host bacteria, and the mode of inheritance of R factors. Nontransmissible mutant R factors, m^- , as well as wild-type R factors, can be removed with acridine (32). This suggests that wild and mutant R factors are in an extranuclear state, replicating independently from the host chromosome. This possibility was tested further by analysis of linkage of R with chromosomal

markers in the sexual conjugation system of *E. coli* K-12. The results (Table 6) can be summarized as follows. Resistance factors, both mutant and the wild type, showed no specific linkage with any of the chromosomal markers used in these crosses; the recombinant progenies of different genotypes received the resistance factor at equal frequencies. Noninfectious mutants of the resistance factor were transmitted from Hfr to F^- cells with rather high frequency, independently of chromosomal markers. The frequency differed depending upon the R factors used. This remarkable fact will be further analyzed in the following section. Certain fractions of the progeny bacteria from crosses of $Hfr R^- \times F^- R^+$ lost their R factor, independently of the chromosomal markers of the recombinants.

The fact that resistance factors, both wild and nontransmissible mutants, exist in an extra-chromosomal state shows that the capacity for autonomous replication of R may be separated from the other functions, conjugal fertility and infectivity.

Repair of conjugal infectivity and fertility of defective R factors by the sex factor. An experiment was carried out to confirm the finding that noninfectious mutants of the R factor were transmitted from Hfr to F^- cells with rather high frequency. Nontransmissible R mutants were transduced into methionine-requiring mutants of F^+ (W6), Hfr (W1895), and F^- (W4354) by phage P1kc. R factor infectivity and R infection were restored in bacteria having the F factor. This repair was not observed when F^- was used for the host (Table 7).

Thus, conjugal infection of the R factor is repaired by the F factor. The state of the F factor, either chromosomal or extrachromosomal, seems to have no effect on the majority of mutant R factors (except for three isolates).

Of 15 mutant R factors of independent origin, 12 were transmitted from Hfr R^+ or F^+R^+ to F^-R^- recipients. The two mutants were transmitted at a lower frequency in sexual crosses than were other m^- mutants, even though they were in an Hfr host (Table 7, lines 3 and 4). One mutant R factor was most infectious when it was in an F^+ host (Table 7, line 2).

The m^- mutant R factors transferred from Hfr R^+ strains to F^-R^- recipients were all nontransmissible, since the F factor of an Hfr is transferred to recipients with chromosomal markers at very low frequency (about 10^{-3} /Hfr cell). The m^- R mutants transferred from F^+R^+ to F^-R^- were transmissible, since the easy contagious transmission of F factors from F^+ to F^- cells results in transfer of both factors, F and R, into F^-R^- cells. Strains which received the R

TABLE 4. Cooperation of nontransmissible mutant R factors of independent origin on R infection and R mating

R infection	R mating	Combination of mutant R factors*	
+	+	R_{100-27}	R_{100-33}
		R_{100-27}	R_{100-42}
		R_{100-70}	R_{100-33}
		R_{100-70}	R_{100-42}
		R_{100-70}	R_{100-43}
		R_{100-70}	R_{100-46}
		R_{100-70}	R_{100-53}
		R_{100-70}	R_{100-54}
		R_{100-71}	R_{100-42}
		R_{100-71}	R_{100-47}
—	—	R_{100-70}	R_{100-52}
		R_{100-71}	R_{100-43}
		R_{100-71}	R_{100-45}
		R_{100-71}	R_{100-46}

* Suffixes of R factor signify independent origin of mutations.

TABLE 5. Reverse mutations of m^- R factors

R factor	No. of R factors tested	Rate of R mating	Rate of R infection
Original.....	1	10^{-5}	10^{-2}
m^- mutant.....	1	10^{-8}	10^{-6}
m^+ reverse mutant....	5	10^{-5}	10^{-2}

TABLE 6. Frequency of unselected male markers transferred to recombinants in various crosses

♂	cross	♀	Lac	L	Ara	T	M	Mtl	Xyl	Thv	Ade	His	Try	Gal	R	Total no. of recombinants
W1895×JE1940	JE1347 (W1895R ⁺ ₁₀₀₋₂₇)×JE1940	* R ⁺ %	404 (89%)	355 (78%)	368 (81%)	419 (92%)	0 (0%)	127 (28%)	124 (27%)	202 (44%)	145 (32%)	190 (42%)	288 (63%)	454 (100%)		454 (100%)
			290 (71%)	311 (77%)	340 (84%)	380 (94%)	0 (0%)	54 (14%)	59 (15%)	147 (36%)	144 (35%)	295 (73%)	228 (56%)	406 (100%)	338 (83%)	406 (100%)
			240 (83%)	261 (84%)	284 (84%)	288 (76%)		42 (78%)	48 (81%)	124 (84%)	120 (83%)	250 (85%)	192 (84%)	338 (83%)		338 (83%)
			416 (87%)	364 (76%)	369 (77%)	423 (88%)	0 (0%)	27 (6%)	32 (7%)	98 (21%)	108 (23%)	103 (22%)	207 (43%)	478 (100%)	10 (2%)	478 (100%)
W1895×JE2075 (JE1940R ⁺ ₁₀₀₋₂₇)	JE1348 (W1895R ⁺ ₁₀₀₋₇₀)×JE1940	* R ⁺ %	391 (91%)	292 (68%)	329 (76%)	348 (89%)	0 (0%)	75 (17%)	67 (16%)	121 (28%)	147 (34%)	140 (32%)	244 (56%)	432 (100%)	175 (41%)	432 (100%)
			164 (42%)	129 (44%)	144 (44%)	152 (40%)		38 (51%)	37 (55%)	57 (46%)	60 (41%)	63 (45%)	106 (43%)	175 (41%)		175 (41%)
			334 (82%)	282 (69%)	317 (78%)	325 (79%)	0 (0%)	86 (21%)	84 (21%)	175 (40%)	321 (78%)	213 (52%)	257 (63%)	409 (100%)	42 (10%)	409 (100%)
			38 (11%)	32 (11%)	37 (12%)	39 (12%)		17 (20%)	16 (19%)	32 (18%)	38 (12%)	30 (14%)	36 (14%)	42 (10%)		42 (10%)
W1895×JE1942 (JE1940R ⁺ ₁₀₀₋₃₁)	JE1349 (W1895R ⁺ ₁₀₀₋₃₁)×JE1940	* R ⁺ %	402 (86%)	379 (78%)	416 (85%)	470 (97%)	0 (0%)	59 (12%)	66 (14%)	168 (34%)	281 (58%)	350 (72%)	394 (81%)	487 (100%)	129 (26%)	487 (100%)
			114 (28%)	107 (28%)	115 (28%)	127 (27%)		30 (51%)	32 (48%)	59 (35%)	82 (29%)	92 (26%)	101 (26%)	129 (26%)		129 (26%)
			357 (71%)	297 (59%)	319 (64%)	456 (91%)	0 (0%)	138 (28%)	145 (29%)	229 (46%)	376 (75%)	281 (56%)	315 (63%)	501 (100%)	104 (21%)	501 (100%)
			89 (25%)	77 (26%)	85 (27%)	94 (21%)		51 (37%)	52 (36%)	70 (31%)	85 (23%)	90 (32%)	96 (30%)	104 (21%)		104 (21%)

W1895: Hfr, Lac⁺ L⁺ Ara⁺ T⁺ M⁺ Mtl⁺ Xyl⁺ Thy⁺ Ade⁺ His⁺ Try⁺ Gal⁺.
JE1940: F⁻ Lac⁻ L⁻ Ara₂⁻ T⁻ M⁻ Mtl⁻ Xyl⁻ Thy₁₅⁻ Ade⁻ His⁻ Try⁻ Gal⁻.
* Overnight cultures of donor and recipient bacteria were mixed (10⁷ donors, 10⁸ recipients) and incubated for 2 hr at 37 C without shaking. The mating mixture was diluted appropriately and plated on eosin-methylene blue-galactose-agar supplemented with tryptophan (80 µg/ml), histidine (50 µg/ml), thymine (20 µg/ml), threonine (50 µg/ml), adenine (20 µg/ml), and leucine (50 µg/ml). After 2 days of incubation at 37 C, gal⁺ met⁺ recombinant colonies were transferred to master plates, and then replica-plated to test their unselected markers.

TABLE 7. Frequency of infection of mutant R factors carried in Hfr, F⁺, and F⁻ strains

Frequency of infection of R in			No. of mutant R tested
Hfr	F ⁺	F ⁻	
10 ⁻³	10 ⁻²	<10 ⁻⁷	12
10 ⁻⁶	10 ⁻³	<10 ⁻⁷	1
<10 ⁻⁷	10 ⁻⁵	<10 ⁻⁷	1
<10 ⁻⁷	<10 ⁻⁷	<10 ⁻⁷	1
10 ⁻²	10 ⁻²	10 ⁻²	Wild type

factor but not the F factor were not infectious. When an F factor of an F⁺R⁺ strain was cured with acridine orange, the remaining R factor became noninfectious. For example, an experiment gave F⁻R⁺ 862 (96.2%), F⁺R⁻ 0, F⁻R⁻ 4 (0.4%), and F⁺R⁺ 30 (3.4%) when it was treated with 20 µg/ml of acridine orange (18), where no F⁻R⁺ was found in untreated control experiments.

These results suggest that the repair of conjugal infection and conjugal fertility occurs without the formation of a stable complex of F and R factors. In other words, it suggests the presence of a hypothetical, diffusible substance essential for synthesis of a common structure for both infectious transmissibility and conjugal fertility.

An analogous phenomenon has been reported by Harada et al. (16), who explained the repaired transmission by postulating the formation of a hybrid of F and R factors. Our results suggest another mechanism for the cooperation of F and R factors.

Defective males, F^r mutants, and the repair of male activity by the resistance factor. An Hfr cell which is unable to donate its chromosome to an F⁺ recipient has been known as an F refractory mutant, F^r, or as a sterile male. Such a cell cannot be infected with F from another donor, which distinguishes it from an F⁻ strain (24). This indicates the presence of a marker in the genome of F controlling immunity of the sterile male strains to superinfection by a second F factor.

Thirty-nine F^r mutants were isolated from W2252, a Cavalli-type Hfr, and 15 mutants from JE1031, a Hayes-type Hfr, by replica plating. Their fertility, or frequency of chromosome donation from male to F⁻ female through conjugation, was about 10³ to 10⁵ times below that of the original Hfr males.

Mutants derived from Hfr Cavalli inhibit growth of the female-specific phage τ (15). The original strain of Hfr Hayes used was sensitive to the phage; however, the Hfr and the infertile mutants were immune to superinfection by the F

factor. Sensitivity of those mutants to male-specific phages, such as f1, MS2, or M12, was tested. The majority of the F^r mutants isolated were resistant to the male-specific phages.

The fertility and sensitivity of F^r mutant strains to male-specific phages were recovered when an m⁻ mutant R factor was introduced into the defective males.

Oriented transfer of the recovered male

FIG. 1. Solid lines and dotted lines represent the increase in number of recombinants from the cross of JE1031 (Hfr H R⁻) and of JE1936 (F⁺R₁₀₀₋₁⁺) used as donor, respectively. A 0.5-ml portion of an overnight culture of parents in brain-heart infusion (BHI) was inoculated into 5 ml of fresh BHI and shaken for 2 hr at 37 C. Young cultures of donor (1 ml) and recipient (10 ml) were mixed in a flask and incubated at 37 C. Samples of the mating mixture were taken at intervals, diluted 100 times with ice-cold Davis minimal medium (DM) minus glucose, and blended in a homogenizer. They were plated on various selective agar plates with 10 ml of soft agar. R infection was selected on DM agar plus glucose and 25 µg of chloramphenicol per ml; Ara⁺, Lac⁺, and Gal⁺ selection was on DM agar supplemented with 100 µg of streptomycin per ml and arabinose, lactose, or galactose, respectively.

chromosome, F^+R^+ , was the same as that of the original Hfr from which the sterile males were derived. A typical timing experiment of the mating process of the recovered male and of the original Hfr strain are shown in Fig. 1. Curing of the R factor of F^+R^+ strains always results in loss of the mating activity.

DISCUSSION

The results may be summarized as follows. Conjugal fertility and infectivity associated with the resistance factor R were inseparable by mutation or by transduction with P1kc. This suggests that a locus controlling a common mechanism, such as a specific surface structure, is involved in the transfer of the host chromosome and in the infective transmission of the R factor itself. This view is supported by the discovery of pili which are produced only by cells harboring the R factor; the presence of pili in R^+F^- bacteria and their absence in bacteria harboring the m^- mutant R factor has been observed (Nishimura et al., *in preparation*).

The wild and m^- mutant R factors used were found to be in an extrachromosomal state. In other words, two functions, conjugal fertility and infectivity of the R factor, are controlled by a locus m , and are separable by mutation or transduction from the function of autonomous replication of the R factor.

Extranuclear inheritance of the wild R factor in *Salmonella* was reported by Dubnau and Stocker (8), except in a case where the R factor carried a part of the genome of the transducing phage P22. In our experiments, wild-type and mutant R factors behaved as extranuclear factors, in good agreement with the findings of Dubnau and Stocker. The term episome (20) may therefore not be suitable for R factors until the integrated state of an R factor is clearly shown.

The m^- mutant R factors regain the functions of conjugal fertility and infectivity when two m^- mutant R factors of independent origin or when one wild and one m^- mutant R factor are present together in the same cell. Furthermore, F factors, whether wild-type F, F' , or the integrated F of an Hfr strain, may restore the infective transmission of the m^- mutant R factors.

Sterile males carrying the defective sex factor, F^+ , recovered their chromosome-donating ability when a wild-type R factor or a mutant R factor defective for infection was introduced; a certain fraction of F^+ strains, however, did not recover their fertility. This suggests that both the R factor and the F factor possess a gene controlling the formation of F pili (2).

The restoration of the F pili of sterile males by the introduction of an R factor has been con-

firmed by electron microscopy (Nishimura et al., *in preparation*).

Phenomena similar to those reported here have been known to be associated with colicinogenic factors. Noninfectious col factors such as col E2 or col K, or a poorly transmissible factor such as col E1, acquire high transferability by introduction of a transmissible col factor (col I or col B) as well as by introduction of an F factor (31).

The analogous effect that infection of an F^+ mutant with a colicinogenic factor such as col I causes recovery of its activity of chromosome donation has been reported (5).

The results summarized above suggest that the transmissible R factor carries a gene controlling conjugal infection of the R factor: m , which confers on the host bacteria the ability to form pili or some other common structure for conjugal infectivity and fertility, and it directs the formation of an F-specific surface substance essential for F mating. Determinants controlling the autonomous replication of R factor may be separated from m .

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