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Beginning a Genetic Analysis of Conjugational Transfer Determined by the F Factor in *Escherichia coli* by Isolation and Characterization of Transfer-Deficient Mutants

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Eighty-four transfer-deficient mutants of F*lac* have been isolated; 27 of these bear amber mutations and 1 mutant is temperature-sensitive. All the mutants transfer between 10^{-2} and $<10^{-5}$% as well as wild-type F*lac*, all are curable by acridine orange treatment, and all are resistant to the female-specific phage ϕ_{II}. Some of the mutants are partially sensitive to female-specific phage tau. Sixty-three of the mutants are resistant to the male-specific phages f1, f2, and Qβ; 15 are resistant only to f2; and 6 are sensitive to all three male-specific phages. Most of the mutants are still poor recipients in conjugation, but four of the mutants resistant to f1, f2, and Qβ have become good recipients. Those mutants resistant to all three male-specific phages do not seem to make F-pili.

The sex factor F and derivative F-prime elements promote conjugational transfer of F and of chromosomal deoxyribonucleic acid (DNA) to recipient cells. Although the mechanism is not yet understood, the specific surface appendages termed F-pili and coded for by F seem to be involved (3). F-pili also serve as the adsorption site for male-specific phages such as f2 and Qβ [icosahedral, ribonucleic acid (RNA)-containing] which attach to the sides of the F-pili, and f1 (filamentous, single-stranded DNA-containing) which attaches to the tip (4, 5, 7). In addition, F codes for other functions which may or may not be related to donor ability, such as replication, inhibition of female-specific phage, and poor recipient ability in conjugation (here called surface exclusion; 14).

Several workers have isolated mutant F-prime or Hfr strains defective in their donor ability (10, 15). These mutants were mainly resistant to male-specific phage, and all were nonconditional except for a temperature-sensitive mutant isolated by Walker and Pittard (23).

This report describes the isolation and characterization of a large number of mutants of an F*lac* element which have lost the ability to transfer, including, in particular, a series of amber-suppressible mutants. Later papers will describe a genetic analysis of these mutants.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are described in Table 1. All strains except JC3086, JC3206, and KL98 were derived from "Su$^-$ ΔX74" (renumbered JC6589), which carries no amber or ochre suppressors but carries the *lac* deletion ΔX74 (9). Phage- and antibiotic-resistant derivatives were obtained as spontaneous mutants. JC3272 was a nitrosoguanidine (NTG)-induced double mutant from JC3051. JC3206 was derived from a different parental strain, X193 (M. Achtman, Ph.D. Thesis, Univ. of California, Berkeley, 1969).

The sources of the parental strains used to make this series of derivatives were as follows: CR63 from G. Edlin, AB2597 from P. Scotti, "Su$^-$ ΔX74" from C. Willson, X193 from S. Brenner, and KL14 and KL98 from K. B. Low.

The parental F*lac* episome was isolated by J. Scaife as a LacZ$^+$ revertant of an F*lac* element derived by F. Jacob (F_{42}) and carrying *lacI3* and *lacZ1* mutations.

Tables 2 and 3 present a list of the transfer-deficient mutants isolated in the present study together with some of their properties, to be discussed below.

Terminology. Cells carrying the parental F*lac* episome are transfer-proficient (Tra$^+$), whereas cells carrying mutant episomes are transfer-deficient (Tra$^-$). Episomal mutations leading to transfer deficiency are termed *tra-n*, where *n* is a unique number assigned to each transfer mutation. Upon genetic classification, these mutations are termed *traXn*, where *X* is a letter identifying the cistron to which *tra-n* is assigned.

TABLE 1. *Bacterial strains*[a]

Strain no.	Sex	Lac	Gal	His	Try	Str	Spc	T1	T6	Su_I	Other markers	Derivation
JC3051	F^-	−	+	−	−	R	S	S	R	−	Mal^-	$T6^R$ mutant from JC6589
JC3054	F^-	−	+	−	−	R	R	R	R	−	Mal^-	$T1^R$ Spc^R mutant from JC3051
JC3086	Hfr	+	+	+	+	S	S	S	S	−	Thy^-	Thy^- mutant from KL14 (trimethaprim selection)
JC3206	F*lac*	+/−	−	+	−	S	R	S	R	+	Ura^- Pro^-	See text
JC3272	F^-	−	−	−	−	R	S	S	R	−	Lys^-	Gal^- Lys^- mutant from JC3051
JC3273	F*lac*	+/−	−	−	−	R	S	S	R	−	Lys^-	F*lac* derivative of JC3272
JC5455	F^-	−	+	−	−	S	R	R	R	−		Mal^+ Str^S recombinant from JC3054[b]
JC5458	F^-	−	+	+	−	R	S	S	S	+		His^+ Su_I^+ transductant of JC6589[c]
JC5459	F^-	−	+	+	−	R	S	S	S	−		His^+ Su^- transductant of JC6589[c]
JC6255	F^-	−	+	+	−	S	S	S	S	+		Mal^+ Str^S recombinant from JC5458[b]
JC6256	F^-	−	+	+	−	S	S	S	S	−		Mal^+ Str^S recombinant from JC5459[b]
JC6318	F*lac*	+/−	+	−	−	R	S	S	S	−		F*lac* derivative of JC6589
JC6589	F^-	−	+	−	−	R	S	S	S	−	Mal^-	See text
JC6650	F^-	−	+	−	+	S	S	S	S	−	Su_{III}^+	Try^+ Su_{III}^+ transductant of JC6256[c]
KL98	Hfr	+	+	+	−	S	S	S	S	−		

[a] The nomenclature is that recommended by Demerec et al. (11) and Taylor and Trotter (22).
[b] The donor strain in these matings was JC3086.
[c] The donor strain for Su_I^+ was CR63, and for Su_{III}^+ AB2597.

For convenience in identifying a given mutant episome in different bacterial hosts, each was assigned a unique number of the type JCFLm. The parental episome is designated JCFL0, and all mutants in the JCFL series are derived from JCFL0. JC indicates the laboratory in which the mutant was isolated; F, that it is a mutant of F (rather than of a resistance-transfer factor, which could be designated R, or of a colicinogenic factor, which could be designated C); and L, that the parental episome carried *lac* genes. Similarly, mutants of an F*his* episome could be designated JCFHm. This system is analogous to that successfully used for numbering bacterial strains, and could, we believe, be adapted for general use in labeling plasmid mutants.

Phage strains and methods. T4 amber N82 was used to detect strains carrying an amber suppressor, and T4 ochre 427 to ensure that the strains used did not carry ochre suppressors.

T6 lysates were obtained by growing *Escherichia coli* B to 3×10^8 cells/ml in H broth and infecting with T6 at a multiplicity of 0.1. After vigorous aeration at 37 C for 4.5 hr, the lysate was treated with chloroform, stored overnight in a refrigerator, and clarified by low-speed centrifugation. Such lysates routinely contained 10^{10} to 10^{11} phage/ml.

The f1, f2, and Qβ lysates were made by adding phage at an input multiplicity of 10 to an exponential culture of JC3273 at about 5×10^7 cells/ml in L broth containing 5 mM $CaCl_2$, and shaking for 2 hr at 37 C. The lysates were clarified by low-speed centrifugation and sterilized by Millipore filtration (for f1) or with chloroform (for f2 and Qβ). The f1 and f2 lysates were stored frozen, and Qβ lysates were stored at 5 C.

A similar technique was used to obtain ϕ_{II} lysates, except that a culture of JC5455 at 5×10^8 cells/ml was used as host.

To obtain tau lysates, the $(tau)^+$ lysogen JE103 was grown to 2×10^8/ml in tryptone broth. The cells were centrifuged and resuspended in tris(hydroxymethyl)-aminomethane-Mg buffer at the original concentration and exposed to 600 ergs/mm^2 of ultraviolet light. The cells were diluted with two volumes of tryptone broth and were incubated with shaking for 5 hr at 37 C. Clearing was not observed, but after removal of cells by centrifugation such preparations contained about 10^9 plaque-forming units (PFU)/ml. The tau preparation was used immediately or after storage in a refrigerator for not more than 1 to 2 days, as the phage is unstable.

Phages f1, f2, Qβ, and ϕ_{II} were assayed by mixing 0.1 ml of a dilution of the phage lysate (in buffer) with two to three drops of a standing overnight culture (at 37 C) of the bacterial tester strain; 2.5 ml of LC top agar was added, and the mixture was poured on an L plate. Phage tau was similarly assayed except that the phage was allowed to adsorb for 15 min at 37 C before adding Trypticase (BBL) top agar and plating on Trypticase plates.

For all spot tests, two or three drops of a standing overnight culture of the bacterial tester strain were added to 2.5 ml of the appropriate top agar and poured on the appropriate agar plate. After the agar had set, small drops of appropriate dilutions of the phage to be tested were spotted on the plates.

T4 amber N82 was obtained from S. Silver; T4 ochre 427, from M. Monk; f1, f2, Qβ, and ϕ_{II}, from R.

TABLE 2. *Amber-suppressible transfer-deficient mutants*

Episome no.	JC3272 derivative strains		JC6255 derivative strains		JC6650 derivative strains		Phage sensitivities[b]			
	No.	Transfer efficiency[a]	No.	Transfer efficiency[a]	No.	Transfer efficiency[a]	f1	f2	Qβ	tau
JCFL0	JC3273	100	JC6582	100	M177	100	S	S	S	R
JCFL1	JC6611	$<5 \times 10^{-6}$	JC6608	50	M187	74	R	R	R	R
JCFL2	JC6208	$<1 \times 10^{-5}$	JC6517	92	JC6655	41	R	R	R	R
JCFL3	JC6206	$<6 \times 10^{-6}$	JC6465	1	JC6654	1	R	R	R	R
JCFL4	JC6214	$<2 \times 10^{-5}$	JC6536	70	M186	70	R	R	R	R
JCFL5[c]	JC6135	$<5 \times 10^{-5}$	JC6563	160	M182	121	R	R	R	R
JCFL6	JC6186	$<2 \times 10^{-5}$	JC6565	30	M203	52	R	R	R	R
JCFL7	JC6176	$<5 \times 10^{-5}$	JC6557	0.1	JC6653	3×10^{-3}	R	R	R	R
JCFL8[c]	JC6129	2×10^{-4}	JC6559	21	M185	57	S	R	S	R
JCFL12	JC6612	2×10^{-2}	JC6613	59	M183	17	R	R	R	R
JCFL13	JC6615	$<1 \times 10^{-6}$	JC6616	120	M184	114	R	R	R	R
JCFL14	JC6695	2×10^{-2}	JC6619	100	M181	19	S	R	S	R
JCFL15	JC6621	$<6 \times 10^{-6}$	JC6622	2.6	JC6652	0.5	R	R	R	R
JCFL16	JC6624	$<1 \times 10^{-5}$	JC6625	6	JC6656	48	R	R	R	R
JCFL17	JC6627	$<1 \times 10^{-5}$	JC6628	50	M205	111	R	R	R	R[d]
JCFL18	JC6630	$<6 \times 10^{-6}$	JC6631	80	M180	17	R	R	R	R
JCFL20	JC6645	$<1 \times 10^{-5}$	JC6646	70	M202	46	R	R	R	R
JCFL24	M201	1×10^{-1}	JC6643	160	M179	74	R	R	R	R
JCFL78	M111	$<6 \times 10^{-6}$	M78	49	M105	97	R	R	R	R
JCFL79	M110	$<6 \times 10^{-6}$	M79	58	M104	60	S[e]	S[e]	S[e]	R
JCFL80	M109	$<7 \times 10^{-6}$	M80	71	M103	7×10^{-3}	R	R	R	R
JCFL81	M108	2×10^{-4}	M81	59	M102	71	R	R	R	R[d]
JCFL82	M107	$<1 \times 10^{-5}$	M96	14	M101	10	R	R	R	R
JCFL83	M106	2×10^{-4}	M97	180	M100	93	S	R	S	R
JCFL86	M163	1×10^{-3}	M164	125	M162	67	R	R	R	R
JCFL88	M171	$<1 \times 10^{-5}$	M172	120	M170	7×10^{-3}	R	R	R	R
JCFL89	M148	3×10^{-3}	M155	140	M204	157	S	R	S	R
JCFL90	M149	1×10^{-5}	M156	35	M142	21	R	R	R	R

[a] Transfer ability was measured in a standard interrupted 40-min mating at 42 C as described in the text, with JC5455 as recipient and selection for Lac$^+$ [Spc^R] progeny. The results are expressed as a percentage of the results obtained with wild-type F*lac* derivatives of the three host strains. In absolute terms, these were approximately 100 Lac$^+$ conjugants per 100 donor cells. For 3272 derivatives the donor abilities were calculated as a ratio to the number of Lac$^+$ [Spc^R] clones present at the end of the mating, whereas the donor abilities of the other derivatives are expressed as a ratio to the number of Lac$^+$ donor cells introduced into the mating mixture.

[b] Measured by use of spot or plaque tests, or both, with the JC3272 derivative strains as indicator bacteria. R, resistance to the phage; and S, sensitivity.

[c] These mutants were isolated after EMS treatment; all other mutants were isolated after NTG treatment.

[d] Some killing (but no plaques) was observed when 10^4 to 10^6 phage were spotted on a lawn.

[e] M110 gave only faint plaques with male-specific phage.

Valentine; and tau, from E. Ohtsubo.

Media. The complex and synthetic media described by Willetts, Clark, and Low (25) were used, except that in later experiments, and in those for the determination of surface exclusion, a synthetic medium based upon M9 (1) was used.

The media used in assaying tau phage were described by Parkinson (17), except for the tryptone broth. This contained 5 g of NaCl and 10 g of tryptone (Difco) per liter of water.

The H broth used for preparing T6 lysates contained 8 g of Nutrient Broth (Difco), 5 g of Bacto peptone (Difco), and 5 g of NaCl per liter. After autoclaving, 5 ml of a 20% solution of glucose/liter was added.

Chemicals. NTG was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis., and ethyl methane sulfonate (EMS), from Eastman Organic Chemicals, Rochester, N.Y. Acridine orange was obtained from Allied Chemical Corp., New York, N.Y., and British Drug Houses, Poole, England. Later supplies were purified from divalent metal ions by NaOH precipitation and chloroform extraction, following a procedure obtained from R. B. Uretz (*personal communication*).

Spectinomycin sulfate was the generous gift of G. B. Whitfield, The Upjohn Co., Kalamazoo, Mich.

Mutagenic treatment. NTG was dissolved in 1 M citrate buffer (*p*H 5.5) at a concentration of 500 μg/ml, and was sterilized by filtration. Bacterial cultures were grown to 5×10^8 cells/ml in L broth, centrifuged, and resuspended in one-tenth volume of water. NTG solution was added to give a final concentration of 50 μg/ml, and the mixture was incubated at 37 C for 15

TABLE 3. *Nonconditional transfer-deficient mutants*

Episome no.	JC3272 derivatives		Phage sensitivities[b]				Episome no.	JC3272 derivatives		Phage sensitivities[b]			
	No.	Transfer efficiency[a]	f1	f2	Qβ	tau		No.	Transfer efficiency[a]	f1	f2	Qβ	tau
JCFL0	JC3273	100	S	S	S	R	JCFL51	JC6396	$<1 \times 10^{-5}$	R	R	R	R[d]
JCFL9	JC6209	2×10^{-3}	S	R	S	R	JCFL52	JC6399	7×10^{-5}	R	R	R	R[d]
JCFL10	JC6153	1×10^{-3}	R	R	R	S/R	JCFL53	JC6400	$<2 \times 10^{-5}$	R	R	R	S/R
JCFL25	JC6148	$<3 \times 10^{-5}$	R	R	R	R	JCFL54[c]	JC6448	$<4 \times 10^{-5}$	R	R	R	R
JCFL26[c]	JC6160	4×10^{-4}	R	R	R	R	JCFL55	JC6457	$<3 \times 10^{-5}$	R	R	R	R
JCFL27	JC6169	6×10^{-5}	R	R	R	R	JCFL56	JC6543	$<1 \times 10^{-4}$	R	R	R	R[d]
JCFL28	JC6172	1×10^{-4}	R	R	R	R	JCFL57	JC6202	$<5 \times 10^{-5}$	R	R	R	R
JCFL29	JC6174	3×10^{-5}	R	R	R	R	JCFL58	JC6213	2×10^{-2}	S	R	S	R
JCFL30	JC6184	1×10^{-4}	R	R	R	R	JCFL59	JC6292	5×10^{-4}	R	R	R	R
JCFL31	JC6185	$<3 \times 10^{-5}$	R	R	R	R	JCFL60	JC6449	1×10^{-5}	S	R	S	R
JCFL32	JC6259	2×10^{-3}	R	R	R	R	JCFL62[c]	JC6542	$<5 \times 10^{-5}$	R	R	R	S/R
JCFL33[c]	JC6386	$<4 \times 10^{-6}$	R	R	R	S/R	JCFL63	JC6180	$<2 \times 10^{-5}$	S	R	S	R
JCFL34	JC6458	$<2 \times 10^{-5}$	R	R	R	R	JCFL64	JC6397	$<3 \times 10^{-5}$	S	R	S	R
JCFL35	JC6459	$<2 \times 10^{-5}$	R	R	R	R[d]	JCFL65	JC6171	$<3 \times 10^{-5}$	S	S	S	R
JCFL36[c]	JC6130	4×10^{-3}	S	R	S	R	JCFL66[c]	JC6126	$<4 \times 10^{-5}$	R	R	R	S[f]
JCFL37	JC6201	2×10^{-4}	S	R	S	R	JCFL67[c]	JC6132	7×10^{-4}	R	R	R	R
JCFL38	JC6395	4×10^{-5}	S	R	S	R	JCFL68[c]	JC6138	$<3 \times 10^{-5}$	R	R	R	R
JCFL39[e]	JC6140	1×10^{-2}	S	R	S	R	JCFL69	JC6149	$<2 \times 10^{-5}$	R	R	R	S/R
JCFL40	JC6179	1×10^{-2}	S	S	S	R	JCFL70	JC6314	$<2 \times 10^{-5}$	R	R	R	R
JCFL41	JC6296	5×10^{-3}	S	S	S	R	JCFL71	JC6162	$<2 \times 10^{-5}$	R	R	R	R
JCFL42	JC6311	$<2 \times 10^{-4}$	S	S	S	R[d]	JCFL72	JC6450	$<2 \times 10^{-5}$	R	R	R	R[d]
JCFL43[c]	JC6123	7×10^{-3}	R	R	R	R	JCFL73	JC6194	3×10^{-4}	R	R	R	R
JCFL44[c]	JC6128	6×10^{-4}	R	R	R	R	JCFL74	JC6197	1×10^{-5}	R	R	R	R
JCFL45	JC6203	$<2 \times 10^{-4}$	R	R	R	R	JCFL75	JC6199	3×10^{-4}	R	R	R	R
JCFL46	JC6231	4×10^{-3}	R	R	R	R	JCFL76	JC6207	$<2 \times 10^{-5}$	R	R	R	R
JCFL47	JC6289	3×10^{-4}	S	R	S	R	JCFL77	JC6211	$<2 \times 10^{-5}$	R	R	R	R[d]
JCFL48	JC6323	$<5 \times 10^{-5}$	R	R	R	R	JCFL96[c]	JC6136	1×10^{-3}	R	R	R	R
JCFL49	JC6387	4×10^{-5}	R	R	R	R	JCFL98	JC6452	$<1 \times 10^{-5}$	S	S	S	R
JCFL50	JC6393	5×10^{-5}	R	R	R	R[d]	JCFL99	JC6290	$<2 \times 10^{-5}$	R	R	R	S/R

[a] See footnote *a* of Table 2.

[b] Measured by use of spot or plaque tests, or both, with the JC3272 derivative strains as indicator bacteria. R indicates resistance to phage, S indicates sensitivity, and S/R indicates that the efficiency of plating was less than 5% but more than 0.01% of that on an F^- culture. In the last case, the plaques were usually faint.

[c] These mutants were isolated after EMS treatment; all other mutants were isolated after NTG treatment.

[d] Some killing (but no plaques) was observed when 10^4 to 10^6 phage were spotted on a lawn.

[e] This mutant is temperature-sensitive; its relative transfer efficiency at 30 C was 100%.

[f] Efficiency of plating was more than 5% as great as on an F^- culture.

to 20 min. An equal volume of phosphate buffer was then added to inactivate the NTG. The suspension was centrifuged, and the cells were resuspended in L broth at 10^8 cells/ml.

For EMS mutagenesis, a culture containing 5×10^8 cells/ml was centrifuged, and the cells were resuspended in one-fifth volume of buffer. To 5 ml of such a suspension, 0.1 ml of EMS was added, and the mixture was shaken and incubated at 37 C for 20 min. The suspension was then centrifuged, and the cells were resuspended at 10^8 cells/ml in L broth.

Mating procedures. Liquid medium matings were carried out as described in the text. Earlier matings were carried out in 125-ml flasks, by mixing 0.5 ml of donor culture with 4.5 ml of recipient, but later ones were performed in tubes (18 by 150 mm) with the use of 0.6 ml donor culture with 1.4 ml recipient. The same efficiency of mating was observed with either technique. Matings were mechanically interrupted by vortexing 1-ml samples for 1 min with a Super-mixer (Labline Instruments Inc., Melrose Park, Ill.), and later with a vibratory blendor (13). For T6 interruption of matings with derivatives of the $T6^S$ strains JC6255 and JC6650 as donors, 0.5 ml of the mating mixture was added to 0.5 ml of T6 at 10^{10} to 10^{11} PFU/ml and shaken for 10 to 15 min. Samples were plated by the top-layer technique with the use of 2.5-ml volumes of top agar containing 0.1 ml of L broth to avoid "step-down" conditions (12).

Plate matings by replica plating were performed according to the procedure described by Clark and Margulies (6).

Acridine orange curing. The technique described by Bastarrachea and Willetts (2), with 25 or 50 μg of acridine orange/ml, was used, except that an inoculum of about 20 cells/ml was used. Cured Lac^- clones were detected by plating dilutions on lactose-EMB plates.

^{32}P-f2 adsorption and penetration. ^{32}P-labeled f2 phage was prepared according to Ippen (Ph.D. Thesis, Univ. of California, Berkeley, 1966).

Adsorption was measured by mixing 2 ml of L broth containing 0.001 M $CaCl_2$, 1 ml of an exponential bacterial culture containing 2×10^8 cells/ml, and 0.1 ml of labeled f2 diluted to 10^9 to 10^{10} PFU/ml (10,000 counts per min per ml). The mixture was incubated for 5 min at 0 C, and then was diluted with 5 volumes of distilled water. The diluted mixture was filtered through an 0.45-μm membrane filter (Millipore Corp.) and washed twice with 10-ml volumes of distilled water. The filter was dried and then immersed in toluene-based scintillation fluid; the radioactivity was measured in a scintillation counter.

Intracellular penetration of labeled f2 RNA into the cell was measured by R. Valentine according to the method described by Silverman and Valentine (20).

Mating pair formation. Mating pair formation was determined by the technique described by Hane (Ph.D. Thesis, Univ. of Pennsylvania, Philadelphia, 1969). Cultures were grown in previously filtered broth to 3×10^8 to 4×10^8 cells/ml. For each mating pair determination, 1 ml of the donor culture was mixed with 1 ml of the recipient culture and incubated at 42 C for 20 min. As controls, separate 2-ml volumes of the donor and recipient cultures were similarly incubated. Duplicate or triplicate samples were removed with Drummond Microcap capillary pipettes, which did not disrupt mating pairs, and were gently diluted to approximately 2×10^5 cells/ml. The number of particles per milliliter was measured with a Coulter counter.

Surface exclusion. Surface exclusion was measured by using the mutant F*lac* derivatives of JC3272 as recipients in crosses with the Hfr strain KL98, which transfers *his*$^+$ as a proximal marker, selecting His$^+$ [Lac$^+$ Str^R] recombinants. Volumes of 2.5 ml of exponential cultures of donor and recipient at 2×10^8 cells/ml were mixed and incubated at 37 C for 60 min. Care was taken to ensure that the recipient cultures contained $\geq$99% Lac$^+$ cells.

RESULTS

Mutant isolation. After treatment with EMS or NTG as described in Materials and Methods, the L-broth cultures were immediately dispensed in portions of 1 ml to 50 to 100 tubes and allowed to grow overnight. Only one mutant of a given phenotype was chosen per tube culture to avoid inclusion of identical sibling mutants. Dilutions of the cultures were spread on lactose-EMB plates and, from these, 100 to 200 Lac$^+$ clones were patched on L plates from each tube. After incubation, these were used for the plate matings shown schematically in Fig. 1.

In plate mating 1, Tra$^-$ clones were detected by their inability to transfer *lac*$^+$ to JC5458 and JC5459. Mutants carrying amber mutations would not be detected at this stage, as the mutations would be suppressed by the Su$_I^+$ suppressor in JC3206. In plate mating 2, the Lac$^+$ progeny resulting from the first plate matings were themselves tested for ability to transfer *lac*$^+$ to JC3054. Mutant episomes carrying amber mutations were transferred with high efficiency by the

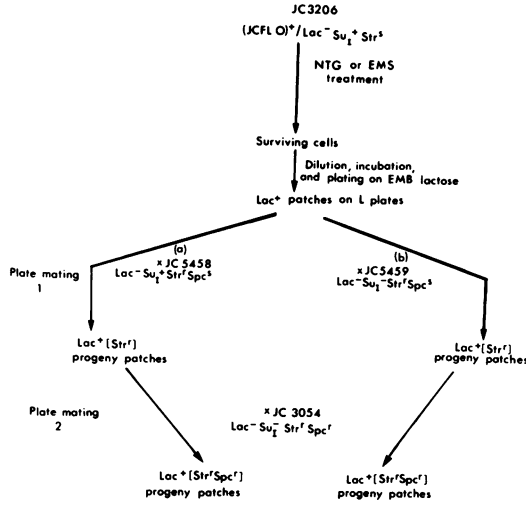

FIG. 1. *Schematic representation of mutant detection procedure.*

Lac$^+$ progeny of the Su$_I^+$ strain JC5458, and with low efficiency by the Lac$^+$ progeny of the Su$^-$ strain JC5459.

Putative Tra$^-$ clones were purified by single-colony isolation, and the mutant episomes were transferred to JC3272, a Su$^-$ strain which is also T6^R and hence useful in the series of complementation experiments we shall describe in a later publication. For most mutants, which were somewhat leaky, this was accomplished by inoculating a culture with about 10^6 cells/ml of both donor and recipient, incubating overnight, and plating to select Lac$^+$ [Str^R] clones. If this was unsuccessful, a method involving the introduction of an F*his* element (F52) into cells carrying the mutant F*lac*, to give a temporarily Tra$^+$ heterozygote was always successful. The rationale of this technique, which was used for dominance and complementation tests, will be described in more detail in a later paper (Achtman, Willetts, and Clark, *in preparation*). Episomes carrying amber-suppressible transfer mutations were, of course, transferred at almost normal frequencies from the original Su$_I^+$ JC3206 derivative. These were transferred in addition to the T6^S Str^S Su$_I^+$ strain JC6255 and the T6^S Str^S Su$_{III}^+$ strain JC6650, and the derivatives were used to measure the efficiency and pattern of suppression.

The mutant F*lac* derivatives of JC3272 were tested for their ability to transfer *lac*$^+$ to JC5455 by plate matings, selecting Lac$^+$ [Spc^R] progeny. (Note that the Su$^-$ recipient previously used, JC5459, would not be appropriate here because no contraselection would be possible; hence, a Spc^R Su$^-$ recipient JC5455 was used.) The confirmed Tra$^-$ derivatives were purified by single-

colony isolation. Since the Tra$^-$ phenotype was transferred concomitantly with the Lac$^+$ phenotype, the mutations to be discussed henceforth must all be episomally located. In several cases, the Tra$^-$ phenotype was not transferred with the Lac$^+$ phenotype. These might have been cases of chromosomal *tra* mutations or not. So many explanations of this behavior are possible that we felt it necessary to ignore these cases to characterize first the episomal mutations. All mutant F*lac* derivatives of JC3272, JC6255, and JC6650 were tested for curability by acridine orange to ensure that in all cases the episomes were autonomous rather than integrated into the chromosome.

The procedures outlined above resulted in the acquisition of 202 mutant episomes. Twenty-eight were isolated by screening 5,000 colonies surviving EMS treatment, and 155 were isolated by screening 10,000 colonies surviving NTG treatment. The latter included six mutants carrying amber mutations suppressed in Su$_I^+$ strains; since these amber mutants were particularly useful, further cultures were treated with NTG, and the surviving clones were screened specifically for amber-suppressible mutants. Nineteen were isolated from 50,000 such clones.

Transfer ability of the mutants. Transfer ability was measured in a series of quantitative crosses. As a necessary preliminary, experiments were performed to determine at what temperature and for how long matings should be conducted for maximal efficiency. A 0.5-ml amount of a culture of the wild-type (JCFL0)$^+$ donor strain JC3273 was mixed with 14.5 ml of a culture of the F$^-$ recipient JC5455 in a 250-ml flask. Samples were removed at various times and vortexed, and the number of Lac$^+$ [Str^R] donor clones and Lac$^+$ [Spc^R] progeny clones was determined. The ratio (Lac$^+$ progeny clones)/(Lac$^+$ donor cells at time of sampling) proved to be a more reliable measurement of transfer than the more usual ratio of (Lac$^+$ progeny clones)/(Lac$^+$ donor cells introduced into the mating mixture), possibly because it takes into account growth of the donor cells and also removes some sources of dilution error.

Figure 2A shows the results of two such matings, one performed at 37 C and one at 42 C. With the strains used, mating was clearly more efficient at 42 C (see also 26), and on this basis all further matings were performed at this temperature.

In addition, both curves in Fig. 2A are biphasic. The nature of the events leading to this phenomenon were further investigated by mixing 0.5 ml of a culture of a T6^S (JCFL0)$^+$ strain, JC6318, with 14.5 ml of a culture of the T6^R Spc^R F$^-$ recipient strain JC5455. At 22 min after mixing, the mating mixture was divided into two parts: one part was mixed with an equal volume of T6 lysate to kill the donor strain, and the other part was mixed with an equal volume of broth to serve as a control. The improved means of calculating the transfer efficiency discussed above was not possible for the T6-treated culture because the donor cells were killed, and Fig. 2B shows simply the number of Lac$^+$ [Spc^R] progeny cells present in samples taken from these mating mixtures at various times (corrected for the twofold dilution). In the T6-treated culture, the number of Lac$^+$ [Spc^R] progeny began to increase again 50 min after the initiation of mating, even though the original Lac$^+$ donor cells had been killed by the T6 treatment; the rate of increase was too rapid to be accounted for by growth of progeny cells. The simplest interpretation is that the Lac$^+$ progeny cells became capable of transferring F*lac* to the residual excess F$^-$ cells 50 min after the initiation of mating. Other control experiments in which a second F$^-$ strain was added to the mating mixture after killing the original (JCFL0)$^+$ donor strain with T6 confirmed that, although a few F*lac* episomes were transferred by the first recipient between 20 and 50 min after the initiation of mating, large-scale retransfer began only after 50 to 55 min. The standard length of time for quantitative matings was therefore chosen to be 40 min: this gave an efficiency of transfer of about 150 to 250% for each (F*lac*)$^+$ cell in the donor population, and was short enough to avoid errors due to retransfer.

Under these conditions, plate matings, followed in some cases by quantitative liquid matings, showed the efficiency of transfer promoted by the mutant episomes to vary from 20% to less than 10^{-5}% of that promoted by the wild-type episome (Tables 2 and 3). Our desire was to limit this first study to those mutants which we could be relatively certain had lost completely the function of the product produced by the mutant gene. The amber mutants provided a convenient scale by which to measure loss of function because they ranged in efficiencies of transfer from 10^{-1}% (one mutant) and 2×10^{-2}% (two mutants) to less than 10^{-5}% (eight mutants). Hence, we excluded from this study the 118 nonamber (i.e., absolute) mutants which promoted transfer at levels of 10^{-1}% or greater and included in our study 84 mutants consisting of all of the amber mutants (27 total) and the 57 remaining nonamber mutants. The excluded mutants may represent "leaky" mutations in which partial function remains to the mutant product or else mutations completely inactivating a partly expendable product.

Efficiency and pattern of suppression. Suppres-

sion was measured in standard 40-min crosses at 42 C, followed by T6 interruption, with the use of closely related Su_I^+ and Su_{III}^+ hosts for the episomes carrying amber mutations. Most mutants were equally good donors in either strain: JCFL1, for instance, was transferred with an efficiency of 50% from the Su_I^+ strain, and 74% from the Su_{III}^+ strain. However, some mutations were poorly suppressed by either Su_I^+ or Su_{III}^+, or by both; for example, JCFL16 was poorly suppressed in a Su_I^+ host. Presumably, poor suppression results from insertion by that suppressor of an amino acid that does not restore full activity to the mutant protein.

Phage-resistance properties. JC3273, carrying JCFL0, is sensitive to the male-specific phages f1, f2, and Qβ, and is resistant to the female-specific phages tau (10^{-6} as many plaques as on an F^- strain) and ϕ_{II} (approximately 1% as many plaques as on an F^- strain; such plaques are much smaller than those formed on the F^- strain). The mutant strains were all still resistant to ϕ_{II} phage, but a number of them had become partially sensitive to tau. The strain most sensitive to tau, JC6126 (carrying JCFL6), yielded approximately 10% as many plaques as the F^- when used as a tester strain; the least sensitive strain showed more killing but no plaques when a drop containing 10^4 to 10^6 phage was applied to the bacterial lawn. Many of the mutant strains had lost sensitivity to the male-specific phages. The qualitative results of plaque and spot tests on the mutant strains are given in Tables 2 and 3.

The mutant strains fell into three phenotypic groups on the basis of sensitivity to male-specific phages (sensitivity to tau will be further dealt with in the Discussion): (I) mutants resistant to all the male-specific phages tested, (II) mutants resistant to the RNA phage f2 but sensitive to the RNA phage Qβ and to the DNA phage f1, and (III) mutants sensitive to all three phages.

Representative mutant strains were tested quantitatively for adsorption of radioactively labeled f2 phage. The mutants thus tested included at least two members of each of the complementation groups to be described elsewhere (Achtman, Willetts, and Clark, and Willetts and Achtman, *in preparation*). These results are shown in Table 4. Mutants in phenotypic group I did not adsorb f2 phage, whereas mutants in groups II and III did.

The male-specific phage sensitivity pattern of group II mutants is the same as that of some Hfr mutants described by Silverman and Valentine (20). These authors showed that these Hfr mutants allowed unimpaired adsorption of f2, but only poor intracellular penetration of f2 RNA. One of the group II mutants described above, JC6129 carrying JCFL8, was therefore tested for penetration of ^{32}P-labeled f2 RNA at different multiplicities of infection (Fig. 3). Penetration was, indeed, impaired at all multiplicities just as it was in the case of the Hfr mutants.

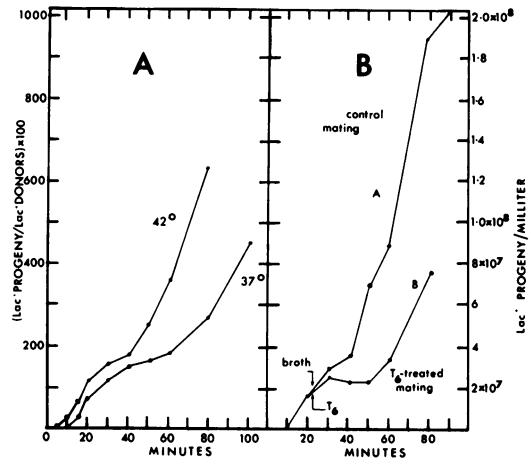

FIG. 2. *Mating kinetics.*

Mating pair formation. A mating pair formed of two (or more) cells is counted by the Coulter counter as a single particle, and the ratio (M) of the number of particles observed in the mating mixture to the average of the numbers of particles in the two separate parental cultures is therefore inversely related to the frequency of mating pair formation. M is not influenced by nonspecific aggregation. In eight separate experiments with JC3273 carrying JCFL0 as donor, M varied from 49 to 76%, with an average of 64%.

The results obtained with representative mutants are shown in Table 4. From comparison with the results obtained with JC3273, a frequency between 57 and 86% is taken to indicate a positive result, and one between 97 and 127%, a negative result (i.e., no mating pair formation). In all cases except one, strains which were sensitive to male-specific phages, and must therefore have produced F-pili (i.e., strains in groups II and III), formed mating pairs, whereas group I strains, resistant to the male-specific phages, were unable to do so. Thus, the F-pilus is necessary and may even be sufficient for mating pair formation, even in the absence of transfer as suggested previously by Walker and Pittard (23). The one exception was M110, carrying JCFL79, which also formed only faint plaques with male-specific phages, especially f1, although it adsorbed ^{32}P-f2 as well as JC3273. These results are explained by production of an abnormally low number of pili per cell (Achtman and Brinton, *unpublished experiments*).

Surface exclusion. Although the mechanism of surface exclusion, by which a cell carrying an F

TABLE 4. *Other properties of representative mutants*

Episome no.	Phage group[a]	Adsorption of ^{32}P-f2[b]	Mating pair formation[c]	Surface exclusion index[d]
JCFL0	—	100	49-76 (+)	300
JCFL1	I	0.5	108	300
JCFL2	I	0.2	102	80
JCFL4	I	ND[e]	ND	1.5
JCFL5	I	0.2	ND	ND
JCFL6	I	ND	ND	500
JCFL7	I	ND	117	130
JCFL8	II	103	86 (+)	500
JCFL10	I	−7	104	1.8
JCFL12	I	3	125	ND
JCFL13	I	0	106	300
JCFL18	I	0.2	ND	ND
JCFL25	I	0	108	170
JCFL26	I	−2	114	1.3
JCFL27	I	−1	99	250
JCFL33	I	2	100	500
JCFL35	I	−5	111	250
JCFL38	II	ND	59 (+)	170
JCFL40	III	101	70 (+)	300
JCFL41	III	107	57 (+)	ND
JCFL42	III	148	73 (+)	500
JCFL43	I	4	113	250
JCFL44	I	5	ND	ND
JCFL55	I	−3	105	90
JCFL58	II	93	ND	ND
JCFL60	II	110	80 (+)	ND
JCFL65	III	ND	ND	110
JCFL67	I	0.2	97	300
JCFL79	III	106	111	ND
JCFL81	I	0	114	ND
JCFL82	I	−5	106	300
JCFL83	II	213	79 (+)	ND
JCFL86	I	−2	107	ND
JCFL88	I	0	103	ND
JCFL90	I	0	127	2.0

[a] Male-specific phage group as defined in the text.

[b] Specific adsorption defined as (counts per minute adsorbed to mutant − counts per minute adsorbed to JC3272) × 100/(counts per minute adsorbed to JC3273). The experiment was performed as described in Materials and Methods.

[c] Mating pair formation (M) was measured as described in Materials and Methods. Values of M under 97 are considered positive.

[d] Measured as described in Materials and Methods: the surface exclusion index is defined as the ratio of the number of His$^+$ [Str^R] recombinants obtained with JC3272 to the number obtained with the strain tested, in crosses with KL98.

[e] ND means not tested.

factor acts as a poor recipient in conjugation, is not yet understood, it was considered possible that some of the transfer-deficient mutants might simultaneously have lost the ability to synthesize the surface component presumably responsible. Representative mutants were therefore tested (Table 4). Most of the mutants still manifested

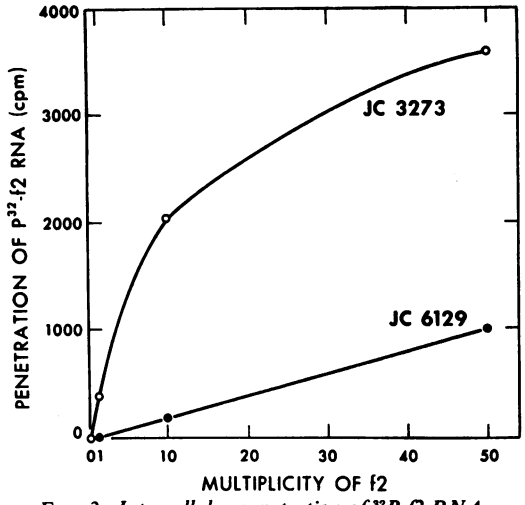

FIG. 3. *Intracellular penetration of ^{32}P-f2 RNA.*

surface exclusion, including many mutants in phenotypic group I which have lost the F-pilus. This indicates that this structure is not responsible for exclusion, in agreement with the conclusions of Curtiss et al. (8). However, four mutants, all in phenotypic group I, were as good recipients as F$^-$ strains, so that the product of the gene in which these mutations occur may be essential both for F-pilus formation and for surface exclusion (Willetts and Achtman, *in preparation*).

DISCUSSION

NTG proved to be a more effective mutagen than EMS for the isolation of transfer-deficient mutants (Table 5). Among the nonleaky mutations isolated, 6 of 54 (NTG-induced) and 2 of 13 (EMS-induced) were suppressible by an amber suppressor (the other 19 amber mutants were isolated separately without scoring for nonamber mutations). In a study of the *his* mutations induced by various mutagenic treatments (aminopurine, ultraviolet light, or X rays), Whitfield, Martin, and Ames (24) found that about 50% were nonsense mutations. The nonamber *tra* mutations have not yet been tested to see whether they include any ochre or UGA mutations, and therefore it is still possible that the proportion of nonsense mutations may be similar to that found for the *his* operon.

Approximately one-half of the mutants isolated (119 of 202, including that one carrying the amber mutant JCFL24) transferred relatively well (i.e., at least 10^{-1}% as well as wild type), in accordance with previous studies (10, 15). These transfer mutations would be considered "leaky" if we were to use as a base line for "nonleaky" the level of residual transfer shown by all but

three of the amber mutants (i.e., 10^{-2} to $<10^{-5}$% of the amount shown by wild type). The results of Ou and Anderson (16) indicate, however, that there may be two kinds of effective contacts between donor and recipient cells, one more efficient in transfer than the other. This opens up the possibility that some transfer mutants may show reduced, but not necessarily greatly reduced, transfer ability in proportion to the participation of different structures in alternative modes of conjugation.

The residual levels of activity of the amber *tra* mutants are somewhat lower than those found for amber mutants in the *lac* operon, in alkaline phosphatase, or in the penicillinase operon, which are approximately 10^{-2} to 10^{-1}% of wild type (18, 21; Sherratt, Ph.D. Thesis, Edinburgh Univ., Edinburgh, Scotland, 1969). In the latter cases, the residual level of activity has been attributed to spontaneous misreading by ribosomes, and was decreased by some *str* mutations (21). Hence, one might conclude that the *str* mutation in the donor strain used in these experiments (JC3272) caused the very low residual transfer levels. Most mutant episomes, however, showed the same very low residual transfer level in both the Str^S Su^- host (JC6256) and the Str^R Su^- host (JC3272). Another explanation is therefore needed. Possibly, spontaneous misreading of amber mutations in *tra* genes does occur with the same probability as misreading of amber mutations in other genes, but the level of transfer proteins produced does not allow an equivalent level of transfer, because several molecules of each protein may be necessary for each transfer event (i.e., many transfer proteins may be found in multimeric structures). On the other hand, suppression by Su_I^+ and Su_{III}^+ was quite efficient in most cases, even though a full complement of active proteins was presumably not produced; hence, at the present time we cannot resolve the matter of the very low residual transfer activity shown by amber *tra* mutants.

The transfer mutants fell into three groups on the basis of their response to male-specific phages. Mutants with similar phenotypes have been described by Ohtsubo et al. (15). Those mutants resistant to male-specific phages (group I) probably do not synthesize any F-pili (Achtman and Brinton, *unpublished experiments*), and this lack of F-piliation is sufficient to explain both their lack of transfer and their resistance to male-specific phages. The other mutants, resistant to f2 only (group II), or sensitive to all male-specific phages (group III), produced seemingly normal pili, in that they adsorbed radioactively labeled f2 and formed mating pairs normally. Clearly, other functions than piliation are required for transfer, as also concluded by Curtiss et al. (8) from physiological experiments. Possibly, mutants in groups II and III are deficient in enzymes necessary for the DNA metabolism associated with conjugation.

TABLE 5. *Comparison of NTG-induced and EMS-induced mutants*

Type of mutation	Phage group[a]	No. of mutants[b] obtained after treatment with	
		NTG	EMS
Nonconditional	I	31	10
	II	9	1
	III	5	0
Amber-suppressible	I	21	1
	II	3	1
	III	1	0
Temperature-sensitive	II	1	0

[a] Male-specific phage groups are explained in the text in the section in Phage resistance properties.

[b] The mutants included are those with a transfer frequency of ≤0.1% of wild type. A total of 5,000 clones surviving EMS treatment were screened, and 60,000 clones surviving NTG treatment, although the nonconditional mutants induced by NTG were obtained from only 10,000 of these.

Most of the mutants were still resistant to the female-specific phage tau, and all were resistant to the female-specific phage ϕ_{II}. There was no apparent relationship between male-specific phage sensitivity or resistance and female-specific phage sensitivity or resistance. Since one mutant is almost as sensitive to tau as the F^- but as resistant to ϕ_{II} as the wild-type $(JCFL0)^+$ strain, resistance to tau and ϕ_{II} are probably coded for by different genes.

Since all of the transfer-deficient mutants were resistant to ϕ_{II} and still curable by acridine orange, these phenomena are probably not related to conjugation. However, some group I mutants had lost the property of surface exclusion and had become good recipients in conjugation. Measurements by the Coulter counter technique, of mating pair formation between the donor strain JC3273 and a strain carrying either a surface exclusion-deficient mutant (JCFL90) or a surface exclusion-proficient group I mutant (JCFL27), showed that mating pair formation was correlated with lack of surface exclusion (Willetts and Achtman, *in preparation*). Besides demonstrating the specificity of the Coulter counter technique, this result suggested that surface exclusion operates at least partly by decreasing the formation of mating pairs.

The genetic basis for the various phenotypes of the transfer-deficient mutants described above will be reported in subsequent publications.

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