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Escherichia coli Females Defective in Conjugation and in Adsorption of a Single-Stranded Deoxyribonucleic Acid Phage

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We predicted that, among mutants resistant to infection by single-stranded deoxyribonucleic acid viruses, there would be some also resistant to "infection" by single-stranded conjugal deoxyribonucleic acid. Approximately 5% of the *Escherichia coli* K-12 females selected for resistance to phage ST-1 were defective as recipients in conjugation. These spontaneous mutants fell into two classes. Type A accepted both plasmid and chromosomal markers at greatly reduced frequencies ($<10^{-6}$ of normal for at least one strain), formed "rough" colonies, and (unlike their parent) were nonflagellated. Type B strains accepted both chromosomal and plasmid markers at reduced frequencies (10^{-2} to 10^{-1} of normal), were temperature sensitive for growth, and showed increased susceptibility towards antibiotics and deoxycholate. Both classes of mutants also were resistant to certain female-specific viruses.

Genes may survive natural selection either by being useful to cells (e.g., the genes for β-galactosidase or ribonucleic acid polymerase) or by being infectious. Plasmids (9, 29, 37) and viruses are examples of the latter type of genes. Both code for structures that attach to cell surfaces to facilitate their infections. The protein product of the virus forms a coat which protects the viral nucleic acid in the extracellular environment. The protein product of the plasmid forms a tunnel (pilus) by means of which plasmid deoxyribonucleic acid (DNA) probably (7) passes directly from cell to cell.

Resistance of bacteria to viral infection often depends on whether viral attachment to cell receptors is prevented (27). With viruses as selective agents, numerous bacterial mutants resistant to attachment by one of more viruses have been isolated and shown to be altered in some component of the cell envelope (27, 39). One would expect that cell envelope alterations likewise would lead to bacteria which become resistant to pilus attachment, and so become resistant to infection by plasmid DNA. It seemed likely that pili and viruses would share certain attachment sites and that, therefore, it might be possible to use viruses to select for bacteria resistant to infection by plasmids, including R factors which are responsible for most clinical cases of antibiotic resistance (14). Since infecting conjugal DNA is single stranded (25, 38), infection by conjugal DNA and single-stranded viral DNA in particular might involve cell structures in common. This was tested by examining female colonies resistant to a single-stranded DNA phage for their abilities to conjugate. Approximately 5% of the females tested were found to be defective as recipients in conjugation. These mutants, which fall into two classes, are characterized.

MATERIALS AND METHODS

Bacteria and phage. Sources and properties of bacterial strains are shown in Table 1. Phage sources were as follows: ST-1, φX174, and φXtB, C. Dowell; P1, T. Jones; χ, J. Adler; T3, T7, W31, φI, and φII, K. Ippen-Ihler; T3L, M. Malany; D108, K. Mise; and Mu-1, M. Howe. φX174 DNA, ST-1 DNA, and bacterial spheroplasts were prepared by minor modifications of the procedure of Guthrie and Sinsheimer (19).

Bacterial media and growth. Unless otherwise stated, bacteria were grown on YTG, which contained (per liter); tryptone, 8 g; yeast extract (Difco), 2 g; and glucose, 2 g. For plates, 15 g of agar (Difco) per liter was used, except for motility testing (3) when 3 g/liter was used. YT is the same as YTG except that glucose is omitted. When Ca^{2+} (3 mM) is added for phage growth, YTG and YT are designated YTGC and YTC, respectively. Minimal medium contained (per liter); NH_4Cl, 1 g; $MgSO_4$, 250 mg; K_2HPO_4, 150 mg; NaCl, 1 g; carbon and energy source, 2 g; 1 M tris(hydroxymethyl)aminomethane (Tris; pH 7.3), 75 ml; and a trace of thiamine.

Chemicals. Antibiotic disks were the highest concentrations available from Difco. Trimethoprim and acridine orange were from Sigma Chemical Co. Nitrosoguanadine (NTG) was from Aldrich Chemical Co. [^{14}C]leucine (26 mCi/mmol) was from Calatomic.

TABLE 1. *Bacterial strains used*

Strain	Properties	Source
C600	F⁻ *thr leu lac* Sm^R	C. Dowell
C600-ST-1^R	ST-1^R derivative of C600	By ST-1 selection
C600/F'Lac	F'Lac derivative of C600	A330XC600
C600-Thy⁻	*thy* derivative of C600	By trimethoprim (31) selection
A330	F'Lac Sm^S	C. Dowell
A330-ST-1^R	ST-1^R derivative of A330	By ST-1 selection
AT706	HfrH *fuc* Sm^S	B. Bachmann
FR3A	F⁻ *thr leu lac* Sm^R ST-1^R type A mutant	See text
FR19B	F⁻ *thr leu lac* Sm^R ST-1^R type B mutant	See text
FR19B/F'Lac	F'Lac derivative of FR19B	A330XFR19B
FR19B-Thy⁻	*thy* deriv. of FR19B	By trimethoprim (31) selection
Hfr75	HfrH *gal bio leu* λCI_{857}	K. Shimada
J53	I-type plasmid ($Kana^R$)/*met pro* Sm^S	E. Meynell
CSH26	F⁻ *ara lac pro* Sm^S	Cold Spring Harbor
CSH26/F'Lac	F'Lac derivative of CSH26	A330XCSH26
JC12	Hfr PO12 *mtl xyl lac gal metB purF* Sm^S	A. J. Clark
KL228	Hfr PO13 *leu gal lac* Sm^S	K. B. Low

[^{14}C]thymidine (40 mCi/mmol) was from New England Nuclear Corp.

Isolation of defective conjugants using ST-1. ST-1-resistant colonies were obtained by spreading 10^8 cells of strain C600 (F⁻ Lac⁻) with 10^8 particles of phage ST-1 on YTC plates. Colonies which grew were patched directly onto lawns of 10^8 cells of an ST-1-resistant derivative of an F'Lac donor, A330. These lawns were on minimal plates that contained lactose (to select for plasmid tranfer) and streptomycin (for counter selection) and required amino acids. Plates were incubated at 43 C for 2 days, after which strains that conjugated normally showed patches of confluence growth, but some strains showed patches with few or no colonies. The latter strains were purified from YT plates upon which all strains had been patched for preservation at 30 C, and were studied further. No mutagenesis was used. An ST-1-resistant derivative of A330 was used so that excess ST-1 carried in the patch would not lyse the donor.

Zygotic induction for direct selection of defective conjugants. To select against females which mated well, Hfr75 (43), which donates early the thermally inducible prophage λCI_{857}, was mixed with λ-resistant C600 recipient cells at concentrations of 10^8 and 10^7/ml, respectively. Cells were shaken gently in YTG at 37 C for 3 h during which time most of the C600 cells were killed by zygotic induction. The mixed culture was then diluted 1/100 and shaken vigorously at 43 C for 1 h to induce the prophage, which killed the males and also the rare females in which prophage transfer had led to lysogeny rather than to zygotic induction. The culture, containing C600 cells that did not receive the prophage from Hfr75, then was gently shaken at 37 C until it was visibly turbid, at which time additional Hfr75 was added (10^8 cells/ml). The procedure was repeated 10 times, after which the culture was streaked and individual colonies were tested for their mating ability.

Conjugation experiments. Males and females were grown separately at 37 C in YTG shaking cultures, which were allowed to stand without shaking for 10 min prior to mixing. Unless stated otherwise, cells were mixed to final concentrations of 5×10^7/ml for males and 2×10^8/ml for females.

Other genetic techniques. Thymidine-requiring mutants of FR19B and C600 were isolated by growth in the presence of trimethoprim (31). Curing of F'Lac was accomplished by growing dilute cultures (10^1 to 10^2 cells/ml) for 24 h in YTG containing 25 μg of acridine orange per ml. Mutagenesis of bacteria and phage was by NTG. For bacteria (strain A330), cells were incubated for 20 min at 37 C in YTG containing 50 μg of NTG per ml. For phage (P1), cells and phage were plated in a soft-agar lawn containing 30 μg of NTG per ml, and after 12 h phage stocks were prepared by scraping the lawns.

Measurement of protein and DNA synthesis. Cultures were labeled with radioactive leucine or thymidine. At appropriate times, 25-μliter samples were removed and applied to Whatman 3MM filter squares (ca. 1 cm²). Squares were passed successively through three beakers containing cold 5% trichloroacetic acid and two beakers containing cold 95% ethanol (5 to 10 min in each beaker). They were then allowed to dry and were placed in vials containing Aquasol (New England Nuclear Corp.). Radioactivity was measured in a scintillation counter.

RESULTS

Isolation of females defective in conjugation. Colonies of the female strain C600 which were resistant to a single-stranded DNA phage were patch-tested for their ability to accept an F'Lac plasmid. The phage used was ST-1 (6), a φX174-like phage which, unlike φX174, naturally infects *E. coli* K-12 strains. Twenty of the 390 ST-1-resistant Lac⁻ colonies were unable to be converted to Lac⁺ by F'Lac at 43 C (for details, see above). These 20 strains were examined for a number of properties and found to divide clearly into two classes, type A and type B (Table 2).

Mating and growth properties of type A

TABLE 2. *Properties of type A and B mutants*

Organism	Conjugation frequency[a]	Growth at 43 C on YTG plates	Morphology on YT or YTG plates	Susceptibility to antibiotics and deoxycholate	Sensitivity to "female-specific" phage	Sensitivity to phage P1	Sensitivity to phage λ	Flagellated
Type A mutants	10^{-6}-10^{-4}	Yes	Rough	Normal	Partially resistant[b]	Sensitive	Sensitive	No
Type B mutants	10^{-2}-10^{-1}	No	Smooth	Increased	Partially-resistant[b]	Resistant	Partially resistant	Yes
C600 parent	1	Yes	Smooth	Normal	Sensitive	Sensitive	Sensitive	Yes

[a] Relative to C600. Formation of Thr$^+$ Leu$^+$ recombinants from Hfr donor AT706 and of Lac$^+$ "recombinants" from F'-Lac donor A330, at 37 C.

[b] See Table 3.

mutants. Twelve type A mutants were tested quantitively for their abilities to accept plasmid genes (F'Lac from A330) and chromosomal genes (Thr$^+$ Leu$^+$ from Hfr AT706). For each mutant, plasmid and chromosomal marker transfer occurred at less than 10^{-4} the level for the wild-type recipient C600. One type A strain, designated FR3A, was used preferentially in further study.

The nature of the very rare plasmid transfer which did occur between strains FR3A and A330 was investigated to determine whether transfer was due to: (i) mating at very low frequency between genetically homogeneous populations of FR3A and A330, (ii) A330 mutants which were capable of overcoming the FR3A barrier, or (iii) FR3A revertants. To distinguish among these alternatives, 20 colonies which had accepted F'Lac, accumulated from several A330 × FR3A crosses, were cured of their F'Lac plasmids and then tested for their ability to be reinfected by F'Lac plasmids. Nineteen clearly were C600-like revertants of FR3A in that they mated at normal frequency, grew as smooth colonies (Table 2), and were ST-1 sensitive. The remaining strain also mated at a much higher frequency than did FR3A, although somewhat below the wild type frequency. The successful A330 × FR3A matings thus involved FR3A revertants.

Further attempts to detect either an altered plasmid (analogous to a host range mutant of a "conventional" virus) or an unaltered plasmid which could overcome the FR3A block were made, including the uses of mutagenized A330 donors, abnormally high cell densities and/or the presence of ST-1 to select against revertants among the FR3A population. In every case, however, the successful maters within an FR3A population, when cured of their F'Lac plasmids, were found to mate at high rather than low frequency and, thus, were revertants. Mating of nonrevertant FR3A was undetectable and occurs at least 10^{-6} below wild-type frequency. Type A mutants grow more slowly than wild type, which contributes to the high apparent reversion rate. (In YTG, FR3A grew at 80% of the C600 rate at 30 C and 50% of the C600 rate at 43 C.)

At least two different pili types, F and I, have been distinguished immunologically and on the basis of sensitivity to different pili-specific phage (20, 30). A plasmid coding for I pili and bearing kanamycin resistance was transferred from strain J53 to FR3A at $<10^{-3}$ the frequency with which it was transferred to C600, indicating that FR3A is markedly resistant to infection mediated by I-type as well as F-type pili.

Mating and growth properties of type B mutants. Eight of the twenty ST-1-resistant colonies that did not mate when patched onto lawns of F'Lac bacteria at 43 C also were incapable of growth on YTG plates at 43 C. This initially suggested that their defect did not concern mating at all, but simply growth. All eight, however, proved also to be impaired in conjugation at temperatures nonrestrictive for growth. In crosses with both an F'Lac and an Hfr donor at 30 C (growth unimpaired), plasmid marker transfer (Lac$^+$ from A330) and chromosomal marker transfer (Thr$^+$ Leu$^+$ from AT706) were 1 to 9% of the level of each to C600. These eight strains comprise mutant type B, whose properties are summarized in Table 2. A strain at the low end of the mating range, FR19B, was used preferentially in further study.

The type B strains exhibited increased susceptibility to certain antibiotics and to deoxycholate, and showed osmotic reversibility of their temperature sensitivity, each property suggestive of strains with altered envelopes (4, 8, 15, 22, 36, 41, 50). Disks containing either erythromycin or novobiocin caused significant clear zones on FR19B lawns, although they caused no clear zones on C600 or FR3A lawns. YTG plates containing 0.6% deoxycholate supported 10^6-fold fewer FR19B colonies although C600 and FR3A colony formation was reduced

only 100-fold. Type B strains did not grow at 43 C on YTG plates but did grow at 43 C on YTG plates containing 200 mM NaCl. Their sensitivity to ultraviolet radiation was unchanged from that of wild type.

Since DNA synthesis can be impaired by certain envelope mutations (17, 22, 44) and may be involved in conjugal DNA uptake (7, 12), experiments were performed to see whether the temperature sensitivity of type B mutants specifically is caused by a defect in DNA synthesis. The results, obtained with thymidine-requiring derivatives of FR19B and C600, indicate that this is not the case, since DNA synthesis at the restrictive temperature is not affected more quickly than either protein synthesis (Fig. 1) or cell growth (Fig. 2).

The temperature sensitivity of the type B mutants was affected by several factors. Removing glucose from YTG permitted growth of FR19B at 43 C, although the addition to YTG of either cyclic adenosine monophosphate (to relieve catabolite repression) or of NaOH (to maintain pH neutrality) did not permit growth at 43 C. Also, the permissive growth temperature prior to the shift to 43 C markedly affected growth cessation at 43 C (Fig 2). These properties were not explored further. Factors that affect the temperature sensitivity of envelope mutations in unexplained ways have been reported before (e.g., yeast extract, reference 41).

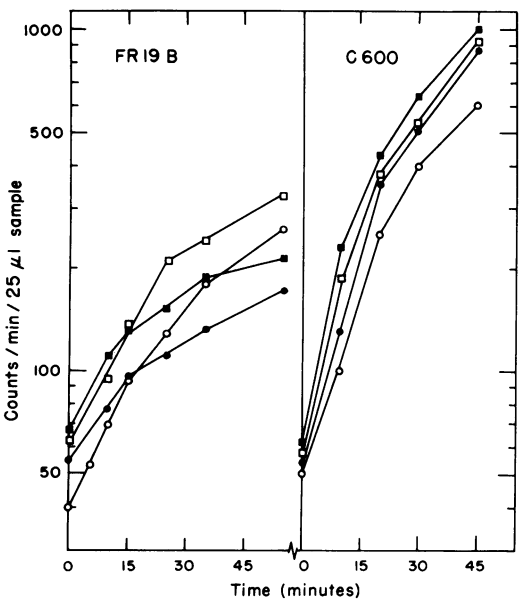

FIG. 1. *DNA and protein synthesis after a shift to 43 C. Log-phase cultures of FR19B-Thy⁻ and C600-Thy⁻ growing in YTG plus 50 µg of thymidine per ml at 37 C (2 × 10⁸ cells/ml) were inoculated either with 10⁶ counts of [¹⁴C]thymidine per ml or 10⁷ counts of [¹⁴C]leucine per ml at time zero. Each culture was then split into two, incubated at both 37 and 43 C, and sampled at intervals. Growth of the cultures was indistinguishable from those of Fig. 2. Symbols: ○, [¹⁴C]leucine incorporated at 37 C; ●, [¹⁴C]leucine incorporated at 43 C; □, [¹⁴C]thymidine incorporated at 37 C; ■, [¹⁴C]thymidine incorporated at 43 C.*

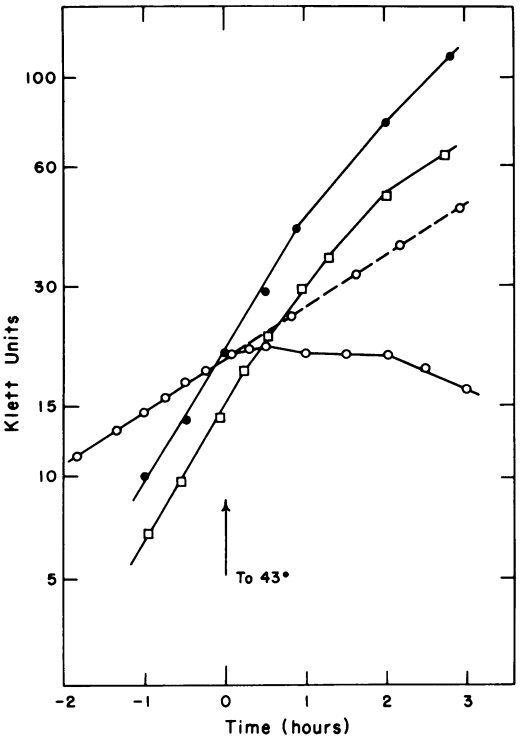

FIG. 2. *Growth after shift to 43 C. Log-phase cultures of FR19B and C600 growing in YTG at either 30 or 37 C were shifted to 43 C at time zero. Symbols: □, FR19B originally growing at 30 C; —○—, FR19B originally growing at 37 C; ●, C600, originally growing at 37 C. ---○---, FR19B which was not shifted to 43 C.*

Phage resistance of type A and type B mutants. Type A and type B mutants were obtained by screening among ST-1-resistant strains. Resistance to ST-1 in each case is due to the inability of the phage to adsorb (Fig. 3). In a separate experiment, spheroplasts of strains FR3A, FR19B, and C600 each were shown to be susceptible to infection by ST-1 and φX174 DNA.

Each mutant type was found to be resistant also to other phage. As expected, type A and type B mutants were resistant to the single-stranded DNA phage φXtB (5), a variant of φX174 capable of infecting *E. coli* K-12 strains. Type B (but not type A) mutants were resistant to P1 and partially resistant to phage lambda. Attempts to find a phage that could transduce

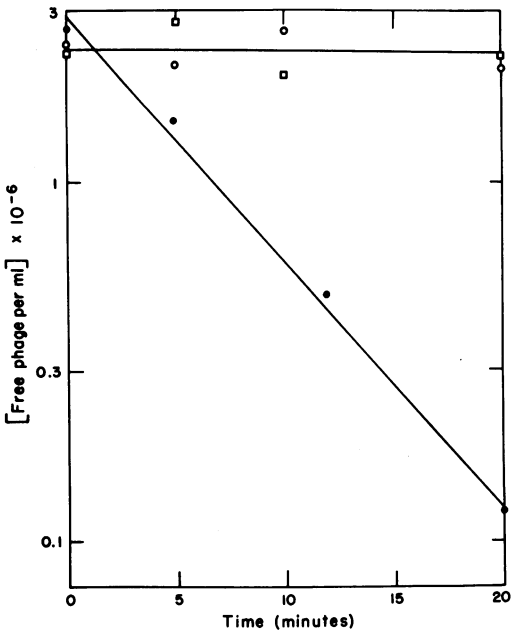

FIG. 3. *Adsorption of ST-1 to FR3A, FR19B, and C600. Log-phase bacteria were suspended at 10^8 cells/ml and incubated for 10 min at 37 C in broth containing (per liter); tryptone, 10 g; KCl, 5 g; $CaCl_2$, 10^{-2} mol; and KCN, 10^{-2} mol. ST-1 was incubated separately for 10 min under the same conditions. Bacteria and phage then were mixed. At various times, samples were diluted 100-fold into broth lacking cyanide, chloroformed, and plated for plaque-forming units. (Phage which adsorb to or penetrate cells are inactivated by the chloroform treatment.) Symbols: ●, C600; ○, FR3A; and □, FR19B.*

type B strains were unsuccessful—no host range mutants of P1 on type B strains could be found among 10^{11} mutagenized (NTG) or unmutagenized P1 infectious particles, and type B mutants were resistant to generalized transducing phage D108 (32) and Mu-1 (23).

Both type A and type B mutants showed altered sensitivity to the related phage T3, T7, and φII (13, 24), and to φI and W31 (Table 3). These phages all are "female specific" (1, 55), as can be seen by comparing their plating efficiencies on C600/F⁺ relative to C600 (Table 3). Resistance of a strain to female-specific phage may or may not be related to the mechanism that inhibits the phage from infecting male strains. With T3 and T7, for example, male resistance is due to F-factor products that block intracellular phage development (35). On the other hand, female T3-T7-resistant mutants arise as a result of envelope alterations (11, 50). To test whether the resistance of type A and type B mutants results from an envelope alteration or is more closely related to the mechanism of male resistance, T3L, a T3 variant that is not inhibited in males (Table 3), was used. Since FR3A and FR19B (but not C600/F⁺) were resistant to T3L as well as to T3 (Table 3), resistance probably is due to altered adsorption. Because of poor adsorption of many female-specific phage to *E. coli* observed here and elsewhere (33), we were unable to measure directly their adsorption to *E. coli* K-12.

Multiple phage resistance also occurred among those strains selected for ST-1-resistance which were not impaired in conjugation. Of 10 such independently arising ST-1-resistant strains of C600 picked at random, 4 were P1 resistant and 3 were T3-T7 resistant.

Either of two female-specific phage could be used in place of ST-1 to obtain mutants defective in conjugation. Fifty C600 colonies selected for resistance to T3, 50 selected for resistance to W31, 60 selected for resistance to φI, and 90 selected for resistance to φII were patch-mated with A330 by the procedure that had been used with ST-1 to isolate the type A and B mutants. Two of the $T3^R$ and four of the $W31^R$ colonies were defective maters, approximately the same proportion that had been observed with ST-1. These six strains all were ST-1 resistant. Among the φI and φII-resistant strains, >90% of which were mucoid, no defective maters were found.

Type A mutants are nonflagellated. By light microscopy, FR3A was observed to be nonmotile, although its parent C600 is motile. Nonmotility of type A strains was confirmed by their inabilities to swarm on "sloppy agar" plates (3) and by their resistance to phage χ, which requires motile flagella for infection (42). By electron microscopy, strain FR3A was observed to lack flagella, either attached to the cells or present in the supernatant. FR19B was flagellated normally, and no other

TABLE 3. *Strain sensitivity to "female-specific" phage*[a]

Phage	Sensitivity		
	C600/F⁺	FR3A	FR19B
φ1	10^{-2} (S)	10^{-2}	$<10^{-4}$
φ11	1 (S)	1 (S)	3×10^{-2}
W31	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
T7	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
T3	10^{-2} (S)	2×10^{-2}	$<10^{-4}$
T3L[b]	1	10^{-4}	$<10^{-4}$

[a] For each phage, plating efficiencies relative to C600 are shown. (S) indicates that plaques are smaller than on C600. Plates and soft agar were YTGC.

[b] A T3 variant which is not female specific. See text.

structural differences between FR3A, FR19B and C600 were apparent.

Mating properties of ST-1-resistant male strains. Two hundred ST-1-resistant colonies of an F'Lac donor (CSH26/F'Lac) were patch-mated against C600 in order to test whether any were impaired in their ability to donate the F'Lac plasmid. None were. FR19B/F'Lac was found to be unimpaired in its ability to donate the F'Lac plasmid (same frequency of transfer as from C600/F'Lac). No FR3A/F'Lac was available for testing.

Mapping of the type B defect. The mating frequency of FR19B was just sufficient to permit the lesion to be localized on the genetic map by conjugation. Preliminary crosses between FR19B and a group of 16 Hfr strains with different transfer origins, selecting for the ability of recombinants to grow on YTG at 43 C, showed the temperature-sensitive defect of FR19B to lie between the transfer origins of JC12 (same origin as AB312) at approximately 50 min and of KL228 (same origin as AB313) at approximately 74 min (28). A three-point cross was then constructed involving *xyl*, *mtl* and the temperature-sensitive defect. The results (Table 4) show the order of the three markers to be *xyl*, *mtl*, TS, which, in conjunction with the above Hfr-origin results, localizes the temperature-sensitive defect between 70 and 74 min on the standard genetic map (51). For all recombinants tested, temperature sensitivity, ST-1-resistance, and reduced mating segregated as one marker. Because of the phage resistance of type B mutants, transduction could not be used to map the lesion more precisely.

Reduced mating is not due to lethal zygosis. *E. coli* females may be killed by an excess of males in a phenomenon known as lethal zygosis (2, 47, 48). If a female mutant arose with increased sensitivity to males, its reduced survival during conjugation might appear as an impaired ability to conjugate. This was ruled out for strains FR3A and FR19B by plating for survivors (on Sm plates) after matings with normal and excess levels of (Sm^S) males. There was no significant decrease in survivors.

Zygotic induction used to directly select for strains with reduced recipient ability. Zygotic induction was used to select directly for C600 derivatives that mated poorly. Strains were selected for survival after repeated exposure to Hfr strains which donated early in conjugation the λCI_{857} prophage (see above). The C600 derivatives so obtained showed reduced ability to accept plasmid and chromosomal markers in conjugation, but also were sensitive to the male-specific phage MS2. Presumably, F^+ or F' factors produced by the HfrH strain infected C600, resulting in C600/F^+ or C600/F' strains which masked the presence of any mutant C600 F^- strains with reduced recipient ability. F'Gal strains containing the λ prophage on the plasmid (16) did not mate well enough to be useful in the selection procedure.

DISCUSSION

Plasmids may be viewed as intracellular viruses which spread by direct cell-to-cell contact, using pili as attachment organelles (7). We thought that, since the DNA transferred in conjugation is single-stranded, the attachment and/or penetration steps in infection by single-stranded viral DNA and by conjugal DNA might utilize cell components in common. If this were true, mutants defective as recipients in conjugation, which are difficult to select for directly, should appear among mutants selected for resistance to single-stranded DNA phage. This proved to be the case for 5% of the ST-1-resistant strains tested.

Most properties of the mutants defective as recipients in conjugation are typical of strains resistant to conventional viruses due to envelope alterations. Type A mutants have an altered ("rough") colony morphology and are unable to adsorb phage ST-1. Their lesion does not involve foreign DNA generally, since they accept transduced markers at normal frequency. Type B mutants have increased sensitivity to antibiotics and deoxycholate, show osmotically reversible temperature sensitivity, and also are unable to adsorb phage ST-1. The type A and B lesions do not involve intracellular single-stranded DNA metabolism generally,

TABLE 4. *Three-factor conjugation cross of xyl, mtl and the type B defect*[a]

xyl	*mtl*	Temperature sensitivity	No. obtained
D[b]	R	D	1
D	R	R	15
R	D	D	4
R	D	R	0

[a] From a cross between donor JC12 (Xyl^-, Mtl^-, non-temperature sensitive) and recipient FR19B (Xyl^+, Mtl^+, temperature-sensitive), several hundred recombinants for the distal markers Thr^+ Leu^+ were scored for *xyl* and *mtl* to find 20 strains in which a crossover between *xyl* and *mtl* occurred. Those 20 strains were scored for the type B lesion by streaking on YTG at 43 C. The results show that the temperature defect and *mtl* co-segregate 19 times, indicating the order of the markers to be *xyl*, *mtl*, type B defect.

[b] Recombinant types. D and R refer to donor and recipient phenotype, respectively.

since both types of mutants are susceptible to infection by naked ST-1 DNA and ϕX174 DNA.

Whether the defect in flagella formation of type A mutants is represented by one of the 14 known *fla* cistrons (45, 46) or is a novel one is not known. No *fla* mutants previously have been reported to be defective in conjugation. Pleiotropic mutants which share one or more properties with type B mutants have been described previously among envelope mutants selected for either colicin tolerance (4, 40), colicin resistance (22), osmotically correctable temperature sensitivity (17, 41), or temperature-sensitive DNA synthesis (8). None of these mutants has been reported to be defective as recipients in conjugation, and all differ from type B mutants in one or more additional physiological properties.

Strains severely defective as recipients in conjugation have been reported previously among lipopolysaccharide-altered strains selected for several steps of ampicillin resistance (34). These strains had either increased or decreased susceptibility to certain female-specific viruses but were not tested with single-stranded phage. Type A or B mutants do not have altered resistance to ampicillin. Other workers have noted variations, both decreases and increases, in recipient mating frequency among cell wall mutants of *E. coli* (54) and *Salmonella* (26, 52).

Type A and B mutants were resistant to certain female-specific phage, and type B mutants were resistant to P1. Since ST-1-resistant mutants which mated normally also were found commonly to be resistant to P1 and/or the female-specific phage T3 and T7, it seems that ST-1, certain female-specific phage, P1, and the F- and I-type pili either have adsorption sites in common or can be blocked from separate sites by single mutations affecting the cell envelope. We were able to isolate strains defective as recipients in conjugation also by selecting with either of two female-specific, double-stranded DNA phage in place of ST-1. These mutants arose at approximately the same frequency as when ST-1 was used to select, were all ST-1-resistant, and were similar, if not identical, to the type A mutants.

Because we looked among strains preselected for viral resistance, the search was biased in favor of mutants with envelope alterations. Alternatively, recipients with reduced mating ability could be directly selected for by intense exposure of recipients either to detrimental donor genes, or to the physically harmful effect (lethal zygosis) of mating. We used the λ prophage as the detrimental donor genes—certain dominant alleles on F factors, such as the streptomycin-sensitive allele or the *lac* i^s (super-repressor) allele under lactose-selective conditions, also might be used. The strains which we selected for in this way had reduced recipient ability because they had received F^+ or F′ factors. Skurrey and Reeves (49), using lethal zygosis for selection, also selected for an F^+ or F′ strain. By improving these methods, it should be possible to obtain a varied group of defective recipients which would be useful in defining the various recipient functions in conjugation.

Can type A mutants, unable to form flagella, form sex pili? Might an envelope alteration which prevents attachment of the distal end of a sex pilus also prevent attachment of the proximal end? These questions were not answered directly because of the inability to infect FR3A with an F′Lac plasmid. An indirect answer was obtained by screening 200 ST-1-resistant clones of an F′Lac strain for their abilities to transfer F′Lac in patch matings. These clones, which should have included several type A mutants, all were capable of transferring F′Lac, suggesting that type A mutants are not abnormal as donors. Type B mutants directly were shown to be unimpaired as donors.

The spread of antibiotic resistance by plasmids (R factors) is a problem of major clinical importance (14). For two decades, man's primary defense against antibiotic resistance has been the development of new antibiotics. As this approach becomes less effective, the inhibition of plasmid transfer could assume practical significance. Demonstrating that exposure of bacteria to certain viruses selects for bacteria which are defective in their ability to obtain plasmid genes is possibly a small step in this direction.

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