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Con⁻ Mutants: Class of Mutants in *Escherichia coli* K-12 Lacking a Major Cell Wall Protein and Defective in Conjugation and Adsorption of a Bacteriophage

RONALD A. SKURRAY, ROBERT E. W. HANCOCK, AND PETER REEVES
Department of Microbiology, University of Adelaide, Adelaide, South Australia 5001

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We describe a new class of mutants of *Escherichia coli* K-12 defective in conjugation (Con⁻ mutants). They lack a major protein of the outer membrane and are defective as recipients with Hfr and F′ donors and as recipients for several F-like R factors and Col factors. In the case of crosses with an Hfr donor, we have shown that the Con⁻ recipient is defective in pair formation. The mutants are resistant to certain phages due to loss of receptor activity.

The mating type system of *Escherichia coli* K-12, with separate donor and recipient mating types, was first demonstrated in the early 1950s (for review see Hayes [17]). Since that time the properties of donor cells have been extensively studied both in functional and genetic terms. In particular, these studies have defined the genetic determinants for, and the properties of, the sex pilus on all donor cells (see Achtman [1], Brinton [7], and Willetts [49] for reviews).

Conjugation occurs in a series of stages, which are commonly considered to be: (i) formation of specific pairs between donor and recipient cells; (ii) a possible separate stage of conversion to effective pairs; (iii) mobilization of donor deoxyribonucleic acid (DNA) for transfer; (iv) DNA transfer to the recipient; and (v[a]) integration of transferred chromosomal DNA into the recipient chromosome or (v[b]) establishment of a transferred plasmid in the recipient (10).

The recipient cell is presumably involved at every stage, but studies so far have concentrated on stage (iv) (41, 42, 47) and, to the greatest extent, on stage (v[a]), where the discovery of Rec⁻ mutants defective in this step (9) has enabled spectacular progress (8). In contrast to our knowledge of donor properties relevant to the early stages of conjugation, very little is known of the corresponding recipient properties.

Ou (25) has shown that zinc treatment of recipient cells affects stages (i) or (ii) of pair-forming ability, and Walmsley (43) has shown that at any given time only a proportion of recipient cells are able to form pairs (stages [i] and [ii]). Monner et al. (22) have recently isolated mutants which are defective in an as yet undefined stage of conjugation, although other properties of the mutants certainly suggest that it is pair formation which is blocked.

In this paper we describe mutants (Con⁻) defective as recipients in conjugation due to a failure in pair formation, and describe other properties of these mutants, including phage resistance, the absence of two cell-wall proteins, and the specificity of the defect in pair formation for certain F-like plasmids.

MATERIALS AND METHODS

Media. Nutrient broth, nutrient agar, and minimal agar (supplemented with glucose and growth factors as required for recombinant selection) were as previously described (34, 35). Eosin-methylene blue agar (Difco-0511) was supplemented with galactose (0.5%, wt/vol) or lactose (1%, wt/vol). Lactose tetrazolium agar was made according to the formula of Achtman et al. (3). Streptomycin, where required, was added to 100 μg/ml except where noted.

Bacterial strains. The strains of *E. coli* K-12 used are listed in Table 1. Strain P400 was selected after cotransduction of the *non-9* allele (26) with *his*⁺ from strain ES368 into AB1133.

Strain P972 is a low-level thymine (1 μg/ml)-requiring mutant of strains AB261 selected on low thymine after the prior introduction of a *thyA* mutation by the method of Stacey and Simson (37). The derivatives of strain JC6256 carrying Col V2, ColVBTrp, R100-1, R136i⁻, R1-19, R386, and R538-1drd (12, 49) were kindly provided by N. Willetts.

Preparation of bacterial cultures. Bacterial cultures (usually in nutrient broth) were prepared as previously described (34, 36). All cultures were incubated at 37 C.

Antibiotic testing. Oxoid multodisks 11-14D and 30-9C were used to test sensitivity to a range of

TABLE 1. *Bacterial strains*[a]

Strain no.	Genotype	Source
AB259	HfrH/*thi rel* λ⁻	B. Bachmann, Coli Genetic Stock Center
AB261	HfrP4X/*metB*(λ)	B. Bachmann
AB1133	F⁻/*thi argE his proA thr leu mtl xyl ara galK lacY str supE* λ⁻	A. L. Taylor
D11	F⁻/*trp pro his str tsx* (λ)	D. Monner
D21	As D11 but *ampA-I*	D. Monner
D31	As D21 with 2nd *amp* mutation	D. Monner
D31m4	As D31 but φW[r]	D. Monner
ES368	F⁻/*lon non leu ade trp strA*	E. C. Siegel
JC6256	F⁻/*trp lac*	N. Willetts
JC6583	F′*lac*/*trp lac* λ⁻	N. Willetts
P400	As AB1133 but *his*⁺ *non*	
P459	As P400 but *con*	
P460	As P400 but *con*	
P461	As P400 but *con*	
P462	As P400 but *con bfe*	
P601	F′*gal*/*met* (λ)	
P729	Hfr P4X/*metB tra* (λ)	
P972	As AB261 but *thyR*[b]	
P980	HfrH/*thi rel tra* λ⁻	

[a] Abbreviations and nomenclature are essentially that of Demerec et al. (11) and Taylor and Trotter (40).

[b] The symbol *thyR* is used for a low-level thymine-requiring strain when it is not known whether the locus is *drm* or *dra* (5).

antibiotics of bacteria plated on 0.7% agar overlays. Agar plates containing ampicillin were also used, and ampicillin resistance was measured as the maximum concentration that did not reduce the viable count of bacteria plated (22).

Phage strains. The strains used have all been, or will be, described elsewhere. (R. E. W. Hancock and P. Reeves, manuscript in preparation; 36).

Phage adsorption. Two methods were used for phage adsorption. In the first, phage K3 was added to logarithmic-phase bacteria (2×10^8 cells/ml) at a multiplicity of infection of 0.5 to 1.0 in the presence of 0.001 M KCN. After 30 min at 37 C, this was diluted 100-fold, 5% (vol/vol) chloroform added, the solution was vortexed for 5 s on a Whirlimixer (Fissons Scientific Industries Ltd.) and shaken for 10 min at 37 C, and suitable dilutions were plated for infective centers. Nutrient broth substituted for bacteria in the control. This method tested only irreversible adsorption.

The second method tested both reversible and irreversible adsorption. Therefore, a negative result in the first test and a positive result in the second would imply that a phage adsorbed reversibly. Phages and bacteria were mixed for 7 min at 37 C as described above, but without the addition of cyanide. They were then centrifuged ($5,000 \times g$) and the supernatant fluid was assayed for unattached phage.

Phage resistance. Phage resistance was tested by using either a multiple syringe phage applicator (50) or by efficiency of plating tests. Full details will be described elsewhere (Hancock and Reeves, manuscript in preparation).

Mating procedures. Liquid media matings for examination of recombinant formation with Hfr, F*lac*, and F*gal* donors were done as previously described (36). Equal volumes (1 ml) of logarithmic-phase cultures of donor and recipient strains were mixed for 60 min at 37 C, mechanically agitated to separate mating pairs, diluted, and plated on selective media; recombinants with Hfr donors were scored on supplemented minimal agar, and those with F*lac* and F*gal* donors were scored on eosin-methylene blue agar. Col- and R-factor transfer was measured by the method of Finnegan and Willetts (14), with strains P400 and P460 as recipients and strain JC6256 derivatives carrying the required plasmid as donor. Essentially, logarithmic-phase cultures were mixed in a 1:10 ratio for 30 min, diluted, and then plated on selective media (nutrient agar containing streptomycin [2,000 μg/ml] and either tetracycline [16 μg/ml, used with strains R100-1, R136i⁻, and R386] or chloramphenicol [50 μg/ml; used with strains R1-19 and R538-1drd]). Transfer of Col factors was measured by plating dilutions of the mating mixture on nutrient agar plus streptomycin and, after overnight growth followed by chloroform vapor treatment of the agar surface, overlaying the plates with a colicin-sensitive indicator to detect colicin-producing colonies of strains P400 and P460.

Lethal zygosis on solid and in liquid media. Lethal zygosis tests were performed as described by Skurray and Reeves (34). Results of the solid-media lethal-zygosis plate test (for sensitivity of a streptomycin-resistant recipient strain to a streptomycin-sensitive donor strain) were expressed as sensitive, partially sensitive, or resistant. Standard conditions for lethal zygosis in liquid media (ratio 20:1) were used.

Pair formation. Logarithmic-phase bacteria (strains P972 and P400 or P460) at about 2×10^8 cells/ml were mixed in equal amounts and held at 37 C for 15 min and then chilled in an ice bath before careful dilution in ice-cold broth. Samples (0.1 ml) of a 10^{-5} dilution were transferred to 4 ml of broth standing above a prewashed membrane filter (Gelman; 47-mm diameter, 22-μm pore), and the bacteria were collected on the filter by suction. Wide-mouth pipettes were used for all dilutions to avoid disrupting mating pairs. Membranes were placed on nutrient agar plates for overnight incubation at 30 C and then transferred to lactose tetrazolium plates for about 4 h. Lac⁻ recipient and Lac⁺ donor colonies were easily distinguished, as were colonies containing many Lac⁺ and Lac⁻ sectors and derived from mating pairs. The preliminary incubation on nutrient agar was necessary, since otherwise strain P972 overgrew the Lac⁻ recipient colonies and made scoring difficult.

Sample preparation and polyacrylamide gel electrophoresis. Whole-cell envelope was prepared from cells grown in nutrient broth to logarithmic phase (7×10^8 cells/ml). The cells were harvested in

0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.8, and broken with a French pressure cell. Cell debris was removed by centrifugation (5,000 × *g*, 15 min), and $MgCl_2$ was added to 2 mM. The supernatant fluid was centrifuged (78,000 × *g*, 60 min) in a Spinco 30 rotor, and the pellet was resuspended in Tris-$MgCl_2$ as above, and centrifuged (78,000 × *g*, 60 min). The resultant pellet was suspended in deionized distilled water at a protein concentration of about 10 mg/ml (estimated by the Schacterle and Pollack method [27]) as the cell envelope preparation. Cell wall or outer membrane was prepared from the whole envelope by the method of Schnaitman (29), using Triton X-100 to solubilize the cytoplasmic membrane. The outer membrane was recovered by centrifugation (150,000 × *g*) and washing in water.

Three methods were used for solubilization of the above preparations in sodium dodecyl sulfate (SDS) before polyacrylamide gel electrophoresis. Method (i) used the room-temperature solubilization described by Neville (23). In method (ii), samples were solubilized by the complete method II of Schnaitman (30). This method included preliminary heating at 37 C, dialysis against SDS-urea, and final heating at 100 C for 5 min. Method (iii) used method II of Schnaitman (30), omitting only the final 100 C heating.

Samples of cell wall and cell envelope solubilized by method (i) were run on 11% polyacrylamide gels using a 3.2% polyacrylamide stacking gel and Tris-borate (pH 8.64) containing 1% SDS as the upper-chamber buffer and Tris-hydrochloride (pH 9.18) as the lower-running buffer (23).

Samples prepared by methods (ii) and (iii) were run on either 7.5% polyacrylamide gels using either the pH 7.2 Maizel buffer system (21) or the pH 11.4 to pH 4.1 Bragg-Hou gel system (6). These were the conditions used by Schnaitman (30–33).

Gels were stained with Coomassie brilliant blue (23, 38) and, after destaining, scanned with either a Joyce Loebel Chromoscan MkII densitometer or a Gilford gel scanner.

Gas-liquid chromatography. Lipopolysaccharide (LPS) was extracted by the phenol-chloroform-petroleum ether technique (15). The methods for preparation of alditol acetates and gas-liquid chromatography, which will be published more fully elsewhere (Hancock and Reeves, in preparation), were based on the techniques of Schmidt et al. (28) and Eriksson-Grennberg et al. (13).

RESULTS

Isolation of mutants. The mutants we describe were isolated during studies on phage resistance, and full details will be published elsewhere (Hancock and Reeves, in preparation). However, all the mutants were spontaneous and of independent origin. Those described here (strains P459, P460, P461, and P462) were all isolated as resistant to phage K3 or related phages.

Resistance to phages. The adsorption of phage K3 to strain P400 could be readily demonstrated, with 99.5% adsorbed by method (i) and 84.4% adsorbed by method (ii).

In contrast, adsorption of phage K3 to strains P459, P460, and P461 was undetectable by either method. The resistance of these strains is thus apparently due to loss of receptor such that neither reversible nor irreversible adsorption can occur.

The mutants all retained sensitivity to phages T1, T2, T3, T4, T5, T6, T7, φI, U3, W31, H, φII, φW, and φ3, and resistance to C21 and MS2.

Recombination and mating pair formation. The presence of a defect in recipient ability was first suspected when the mutants were tested for sensitivity to lethal zygosis (Table 2, Fig. 1) and found to be fully resistant. They were then tested as recipients in crosses with F*lac*, F*gal*, and Hfr strains (Table 2), and in all cases their recipient ability was reduced to 0.1 to 1% of the control level.

The ampicillin-resistant strains of Monner et al. (22) were also tested; we confirmed their observations regarding the defect in conjugation and further found them to be resistant to lethal zygosis (Table 2, Fig. 2).

When strains P400 and P460 were tested for ability to form mating pairs with an Hfr strain, as described in Materials and Methods, strain P460 was found to form pairs at only 0.5% the efficiency of strain P400 (Table 3). The absence of sectored colonies in the P400 cross after blending confirmed that we were indeed scoring mating pairs.

The specificity of the defect in recipient ability was examined by using as donors strains carrying various R or Col factors. Whereas R100-1 and R136i$^-$ transferred equally well to strains P400 and P460, R1-19, R386, R538-1drd, ColVBTrp, and ColV2 all transferred to strain P460 at 1 to 0.01% the efficiency with which they transferred to strain P400.

Sensitivity to antibiotics. The loss of a phage receptor and of conjugation ability suggests a change in surface structure, and, since such changes often correlate with changed antibiotic sensitivity, we tested the sensitivity of the Con$^-$ mutants to a series of antibiotics, as in Materials and Methods. No change in sensitivity was detected, all strains being resistant to 500 μg of sulfafurazole, 10 μg of oleandomycin, 5 U of penicillin G, 25 μg of streptomycin, 10 μg of methicillin, 10 μg of fusidic acid, 5 μg of novobiocin (but partially sensitive to 30 μg), and 5 μg of cloxacillin; they were sensitive to 50 μg of colistin methane sulfonate, 5 μg of ka-

TABLE 2. *Lethal zygosis and conjugation properties of recipient strains*

Strain no.	Relevant phenotype	Sensitivity to lethal zygosis[a]	Recipient ability[b] with		
			Hfr	*Flac*	*Fgal*
P400	Con$^+$	++	1	1	1
P459	Con$^-$	–	$<10^{-3}$	$<10^{-3}$	5×10^{-4}
P460	Con$^-$	–	$<10^{-3}$	$<10^{-3}$	$<5 \times 10^{-4}$
P461	Con$^-$	–	5×10^{-3}	$<10^{-3}$	10^{-3}
P462	Con$^-$	–	2.5×10^{-3}	$<10^{-3}$	ND[c]
D11	Amp^s	++	1	ND	ND
D21	Amp^{r}I	++	1	ND	ND
D31	Amp^{r}III	±	0.5	ND	ND
D31m4	Amp^{r}III, ϕW^r	–	4×10^{-3}	ND	ND

[a] By solid media plate test with HfrH (AB259) as described previously (34). Symbols: ++, Sensitive; ±, partially sensitive; –, resistant.

[b] Defined as the ratio of the number of recombinants obtained with the mutant strain to the number obtained with the parent (P400 or D11). With strain P400 or its Con$^-$ derivatives as recipients, Thr$^+$Leu$^+$Str^r, Lac$^+$Str^r, and Gal$^+$Str^r recombinants were selected by using the Hfr strain AB259, the *Flac* strain JC6583, and the *Fgal* strain P601, respectively; with strains D11, D21, D31, or D31m4 as recipient, Pro$^+$Str^r recombinants were selected by using the Hfr strain AB261.

[c] ND, Not done.

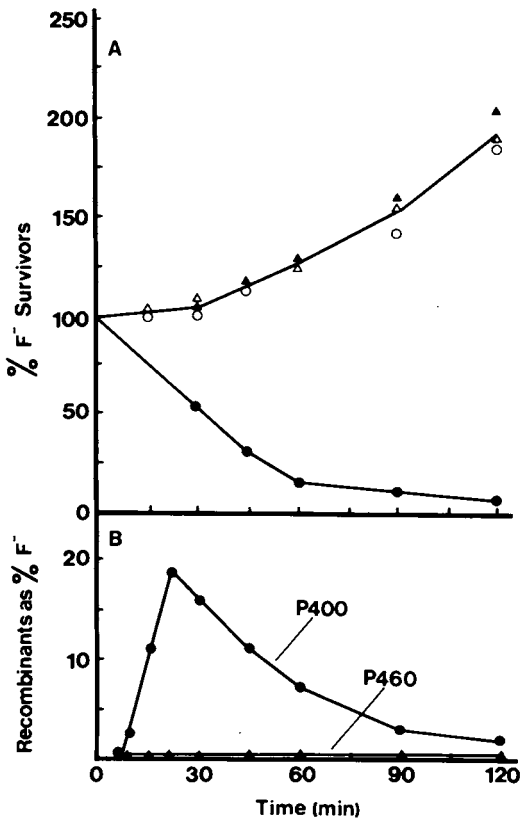

FIG. 1. *Sensitivity of strains P400 and P460 to lethal zygosis. Stationary-phase cultures of P400 (●, ○) and P460 (▲, △) were diluted and mixed with an early logarithmic-phase culture of the HfrH strain AB259 (closed symbols) or with a similar culture of the Tra$^-$Hfr strain P980 (open symbols). Samples were diluted and plated for survivors (A) and for Thr$^+$Leu$^+$Str^r recombinants (B).*

nomycin, 10 μg of neomycin, and 2 μg of ampicillin.

Other studies (13, 39; Hancock and Reeves, in preparation) have demonstrated changes in sensitivity to erythromycin, novobiocin, oleandomycin, methicillin, fucidic acid, and ampicillin to be associated with changes in cell wall structure.

The result with ampicillin is particularly interesting since strains D31 and D31m4 of Monner et al. (22) were ampicillin resistant and defective in conjugation.

Our mutant strains were therefore further tested for resistance to ampicillin by the technique of Monner et al. (22) and, if anything, were slightly supersensitive, being resistant to 0.5 or 1 μg/ml, whereas strains D31 and D31m4 are resistant to more than 100 μg/ml. Both parent strains D11 and P400 were resistant to 1.5 μg/ml.

Cell envelope chemistry. To look directly for cell envelope changes, we determined the sugar composition of the LPS from strain P400 and mutant strains P459 and P460, and also studied the envelope proteins by polyacrylamide gel electrophoresis. We could detect no variation in the sugar composition, which was heptose, glucose, and galactose, or in the molar proportions.

The polyacrylamide gel electrophoresis system we used first was the SDS system developed by Neville (23) for mammalian mem-

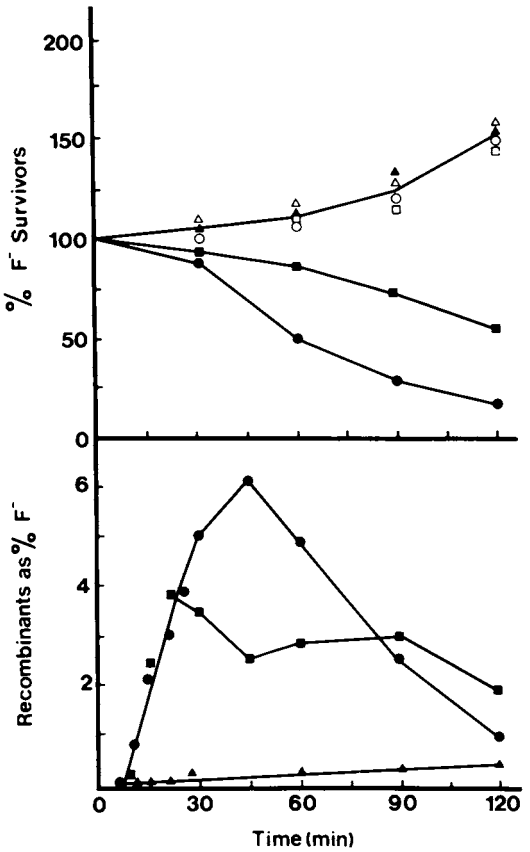

FIG. 2. *Sensitivity to lethal zygosis of ampicillin-resistant mutants of an F⁻ strain. Stationary-phase cultures of the recipient strains D11 (Amp^s; ●, ○), D31 (Amp^{r}III; ■, □), and D31m4 (Amp^{r}III φW^r; ▲, △) were diluted and mixed with an early logarithmic-phase culture of the Hfr strain AB261 (closed symbols) or with a similar culture of the Tra⁻ mutant P729 (open symbols). Samples were diluted and plated for survivors and for Pro⁺Str^r recombinants. Results with the strain D21 were similar to those with D11.*

TABLE 3. *Pair formation*

Strain		Treatment	No. of colonies[a]		
Hfr	F⁻		Lac⁺ donor	Lac⁻ recipient	Sectored
P972	P400	None	3,916	2,434	265
P972	P400	Blended	6,078	1,783	1
P972	P460	None	3,418	2,575	5

[a] Total from 48 plates, 12 from each of four experiments.

branes (method [i]), and this clearly showed that a major protein band of the whole envelope was dramatically reduced in amount in strain P460 in comparison with that of the parent strain P400 (Fig. 3A, B). The Triton X-100 treatment described by Schnaitman (29) did not solubilize the band (Fig. 3C, D), which is thus identified as an outer membrane or cell wall component. The molecular weight of the affected band was estimated at 28,000 by comparison with standard proteins. The 28,000-molecular weight band was apparently completely absent in the Triton X-100-treated preparation of the mutant, and it is possible that the small band present at this position in the envelope of the mutant was a different, Triton X-100-soluble protein that runs at the same place in the gel and is not a residual amount of the protein(s) affected by the mutations in strain P460.

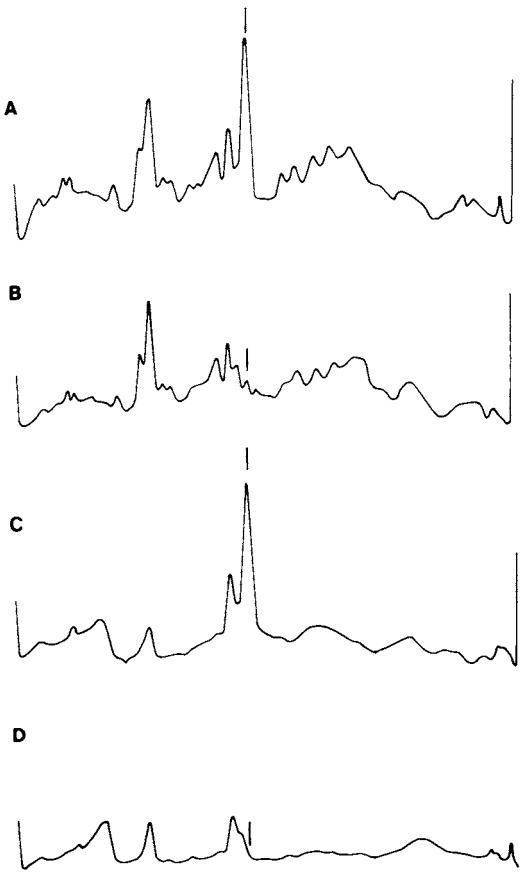

FIG. 3. *Comparison by densitometry of whole-envelope proteins of P400 (A) and P460 (B), run by using the Neville gel (23) system. (C) and (D) show profiles of outer-membrane proteins of strains P400 and P460, respectively, in the same gel system. The 28,000-molecular weight major peak is indicated by the vertical line.*

Although the Neville system gave very good resolution of bands, the pattern was different to that found by Schnaitman (29, 30), Bragg and Hou (6), or Inouye and Yee (18) using various different SDS-gel systems. To enable direct comparison with other work on *E. coli* K-12, we prepared and ran our cell wall preparations under the conditions used by Schnaitman (Fig. 4 and 5).

DISCUSSION

The mutants we describe lack a phage receptor, the ability to form recombinants with Hfr, F′, and certain R-factor donor cells, and two outer-membrane proteins.

We named the mutants "conjugation deficient" (Con$^-$) and propose the designation to cover defects affecting recipient ability in any stage from (i) through to (iv) and any defect affecting both (v[a]) and (v[b]) as defined above. Operationally, a Con$^-$ mutant is one that is defective as a recipient with both F′ and Hfr donor strains. They are thus readily distinguished from Rec$^-$ mutants (9), which are defective only with Hfr donors. Corresponding mutants in donor cells are termed Tra$^-$ regardless of the stage affected (2, 24).

All mutants described here were selected as phage resistant, and, apart from quantitative variations in their recipient abilities (Table 2), had identical phenotypes. The single exception to this was strain P462, which appeared to carry two mutations (*con* and *bfe*), although this has yet to be confirmed by genetic analysis.

One of our Con$^-$ mutants was studied further and found to be defective in pair formation (stage [i] or [ii] of conjugation).

Polyacrylamide gel electrophoresis of the proteins, of both the cell envelope and the separated outer membrane, showed that under most of the conditions used a major outer membrane band was almost completely absent in the Con$^-$ mutant strain P460. Although the Neville system (Fig. 3) gave good resolution of bands and the results show that one band is affected, the results with the Maizel and the Bragg and Hous systems are more easily interpreted, since they have been used by Schnaitman in his recent analysis of the major proteins of the outer membrane of *E. coli*.

E. coli has four major outer-membrane proteins, 1, 2, 3a, and 3b (32), of which only proteins 1, 3a, and 3b are present in strain K-12 (33). With method (iii) for sample preparation and the pH 7.2 Maizel buffer system, proteins 1 and 2 run together as peak A and proteins 3a and 3b run together as peak C (30, 32, 33). Our Con$^-$ mutant P460 had peak C very much reduced (Fig. 4B) in comparison with strain P400 (Fig. 4A). The heated samples (method [ii]) run under the same conditions showed that all the major proteins ran at peak B (Fig. 4C, D) as observed by Schnaitman (30). However, in our hands, both with strains P400 and P460, a small amount of material ran at the position of peak C, comparable in amount to that observed with unheated P460 (Fig. 4B). It seems likely that this residual amount of material in peak C (Figs. 4B, C, and D) represents a minor protein and is not a residual amount of proteins 3a or 3b; although further analysis is obviously required, we provisionally consider strain P460 to lack both proteins 3a and 3b.

Unheated preparations (method [iii]) of strains P400 and P460 also differed in the amount of peak B and possibly also peak A (Fig. 4A, B). The material in peak B is presumably due to either protein 1, 3a, or 3b running partly in this position, even when the heating step is omitted, since peak B is a product of these proteins (Fig. 4C, D). Because there is so much more peak B in P400 than in P460 (Fig. 4A, B), we conclude that it is largely derived from proteins 3a and b. It should be noted that the material in peaks B and C of P460 (Fig. 4B) is the maximum amount of proteins 3a and 3b that could be present. The reduction in one of the components of peak A in P460 is much less than the reduction in peak C (Fig. 4B) and may not be significant.

Under alkaline conditions (Bragg and Hou gels), Schnaitman (32, 33) showed that both proteins 3a and 3b run together as peak 3, which is again virtually absent in strain P460 (Fig. 5B).

Thus the results with both neutral and alkaline gels support the hypothesis that the Con$^-$ mutants described in this paper lack the two outer-membrane proteins 3a and 3b.

The Con$^-$ mutant P460 has a growth rate in nutrient broth similar to that of the parent P400, and the Con$^-$ mutants have no other obvious defects. In particular, the sugar composition and content of their LPS appears normal, and their reaction to a wide range of antibiotics is unaffected. Our observations with Con$^-$ mutants, then, enable us to conclude that proteins 3a and 3b are not essential for normal cell growth. Furthermore, since protein 2 is not present in *E. coli* K-12 (33), and a multiple-phage-resistant mutant (*tfr,tsx*) lacking protein 1 has already been described (33), it appears that none of the four major outer membrane

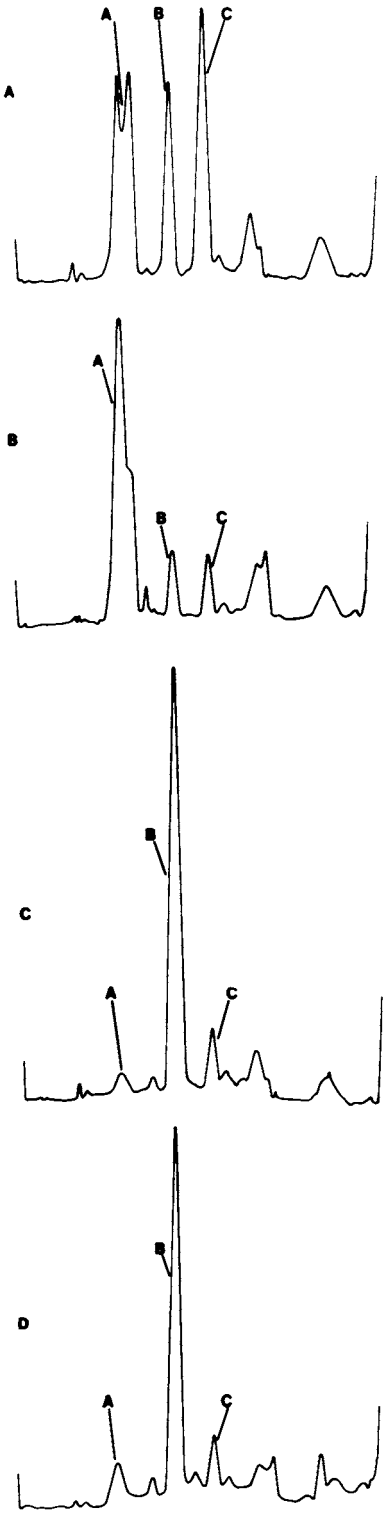

FIG. 4. *Comparison by densitometry of stained bands of outer-membrane proteins, run by using the pH 7.2 Maizel buffer system and either heating (method [i]) or not heating (method [iii]) the samples at 100 C before loading onto SDS-polyacrylamide gels. (A) and (B) show the outer-membrane proteins of strains P400 (Con$^+$) and P460 (Con$^-$), respectively, solubilized by method (iii). (C) and (D) show the outer-membrane proteins of strain P400 (Con$^+$) and P460 (Con$^-$), respectively, solubilized by method (ii). The peaks are labeled A, B, and C according to the Schnaitman nomenclature (32) as described in the text.*

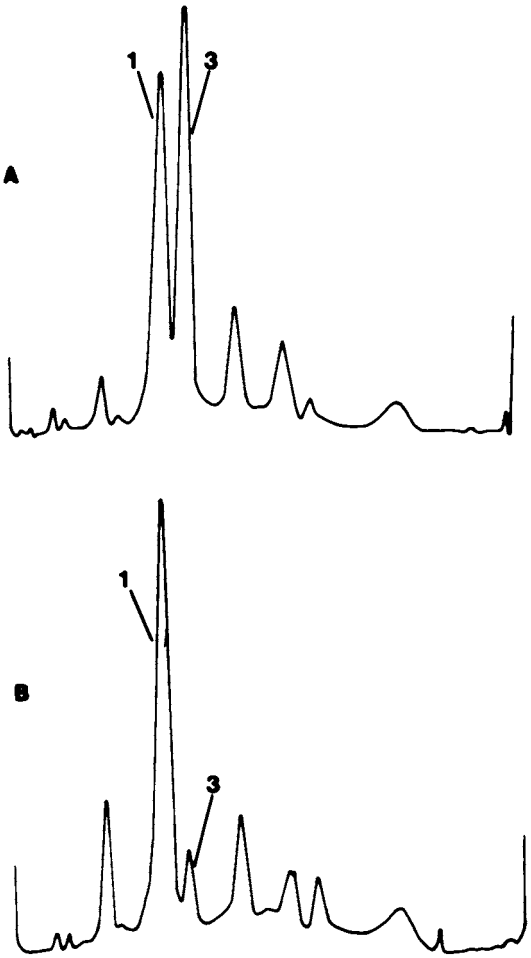

FIG. 5. *Comparison by densitometry of stained bands of the outer-membrane proteins of strains P400 (A) and P460 (B) run on the Bragg-Hou gel system after solubilization by method (ii). The peaks are labeled 1 and 3 in accordance with the Schnaitman nomenclature (32) as described in the text.*

proteins is individually essential for cell survival. It will be of interest to see whether a *tfr,tsx,con* strain lacks all the major outer membrane proteins, as one would predict, and if so, whether its membrane seems otherwise normal. The results of Ames et al. (4) and Koplow

and Goldfine (19) on heptose-deficient mutants that also lack all the major outer-membrane proteins suggest that they will at least be viable.

Two other classes of mutants have recently been observed to be defective as recipients in conjugation. The *amp*^{r}III mutants described by Monner et al. (22) have an altered LPS and are ampicillin resistant and defective in conjugation. In this study we have further shown that these mutants are resistant to lethal zygosis. Although it was suggested (22) that these mutants are defective in pair formation, the method used (16) does not distinguish the various stages of conjugation, as has been pointed out before (43). The *amp*^{r}III mutants differ from the Con$^-$ mutants not only in having altered LPS but also in being resistant to ampicillin.

The proteins of the *amp*^{r}III mutants have not been reported on as yet, and, although their overall properties have been used to advance the hypothesis that the LPS is a receptor in pair formation, it remains possible that they lack surface proteins which fulfill this role. Indeed, the work on heptose-deficient mutants (4, 19) provides a precedent for mutants defective in their LPS being also deficient in proteins.

The other class of mutants defective as recipients are certain LPS mutants of *S. typhimurium* (44) or *E. coli* E56b (08:K27:H$^-$) (45, 46). Mutations which affect the composition of the core polysaccharide of the LPS may reduce recipient ability with respect to certain R factors. However, once again we cannot exclude the possibility that the effect on conjugation is indirect and due to an effect on outer-membrane proteins, and again there is no evidence as to what stage of conjugation is blocked.

Proteins 3a and 3b are very similar (32), and a simple hypothesis to explain our observations with Con$^-$ mutants is that one or both proteins serve for the attachment of the pilus (and phage K3). The Con$^-$ mutants, missing two similar outer-membrane proteins and with an intact LPS, provide the best evidence so far for the involvement of a specific surface component of the recipient in pair formation. Nonetheless, we cannot at present exclude the possibility that the relationships between the various effects of the pleiotropic Con$^-$ mutation are indirect. Since proteins 3a and 3b are two of the three major proteins of the outer membrane in *E. coli* K-12, it is at the very least possible that they play a structural role, and their absence may affect the insertion or function of other cell wall components, including a K3 phage receptor and some component involved in pair formation.

The observation that Con$^-$ mutants are resistant to lethal zygosis further supports the hypothesis that pair formation is a prerequisite for lethal zygosis (34, 35). Until it is known which stage of conjugation is blocked in the Amp^{r}III mutants, we cannot draw any definite conclusion from the observation that they are also resistant to lethal zygosis.

It is interesting to note that the Con$^-$ mutant P460, although defective as a recipient with F′, Hfr, R1-19, R386, and R538-1drd donors, is competent as a recipient with R100-1 and R136i$^-$ donors. R100-1 is similar to the F factor of *E. coli* K-12 in that mutants in any of twelve *tra* genes (all except *traI* or *traJ*) can be complemented by R100-1 (48, 49). Presumably, the R100-1 and F-factor *tra* genes, and hence transfer systems, are very similar, although the pili encoded by R100-1 do differ from those encoded by the F factor both in their serology and the sensitivity of cells to male-specific phages.

We are continuing our studies on the specificities of the Con$^-$ mutant defect in relation to various R factors. It is clearly possible that the variation in pilus structure, detected previously by serology and phage sensitivity, determines the ability of these pili to initiate pair formation with Con$^-$ mutants.

We are also investigating the role of proteins 3a and 3b in phage K3 attachment and pair formation. However, whether or not these two closely related proteins are directly involved in pair formation, we hope that Con$^-$ mutants will be useful in analyzing the early stages of conjugation in the way that Rec$^-$ mutants have been useful in the analysis of the later stages of conjugation concerned with recombination.

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