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Fertility Factors in *Pseudomonas putida*: Selection and Properties of High-Frequency Transfer and Chromosomal Donors

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The octane plasmid (OCT) in *Pseudomonas putida* strains has been shown to be transferred at low frequency. However, bacteria which had newly received this plasmid showed a transient increase in donor ability. Using Octane$^+$ *P. putida* as the donor, the transfer of most chromosomal markers was shown to be independent of OCT transfer, whereas the mobilization of the octanoate catabolism genes (octanoic and acetate) was dependent on OCT plasmid transfer. The presence of a fertility factor termed FPo has been postulated to explain these results. Strains carrying only this fertility factor have been obtained from strains carrying both OCT and FPo plasmids. Strains in which the OCT plasmid was transferred at high frequencies have also been isolated, and chromosome mobilization by OCT and FPo has been compared. A different gradient of transmission by OCT and FPo has been observed. It has also been shown that chromosome transfer by OCT was dependent on the bacterial recombination system, whereas the chromosome transfer by FPo was unaffected by the presence of a *rec* mutation in the donor strain.

The genes responsible for the oxidation of octane have been shown to be located on an extrachromosomal element in *Pseudomonas putida* ATCC 17633 (strain PpG6), formerly termed *Pseudomonas oleovorans* (2). The transfer of octane plasmid (OCT) to other *Pseudomonas* species has been described previously (7, 8). Earlier studies in our laboratory by M. Shaham (unpublished results) revealed that some strains cured of the OCT structural genes retained chromosome donor ability, suggesting the presence of a plasmid aggregate capable of carrying a fertility factor on a second element. It has also been shown by Chakrabarty and Friello (5, 6) that the fertility factor accompanying the OCT plasmid could be separated from the structural genes and mobilized the chromosome independently of the octane$^+$ (Oct$^+$) phenotype, and so the octane plasmid could be considered as a plasmid aggregate of OCT structural genes and a fertility factor. We have used the abbreviation FPo for this element (FP stands for *Pseudomonas* fertility), whereas Chakrabarty (5) has used the term K. It has not been made clear whether the octane plasmid carried a fertility factor independent of the separate FPo factor.

This paper analyzes several characteristics of the low-frequency transfer of OCT FPo which behaves like a repressed plasmid aggregate. We used the high plasmid donor ability of the camphor-octane (CAM-OCT) fused plasmid (8) to isolate strains carrying the OCT structural genes in a condition that allows their transfer, as well as that of some chromosomal loci at high frequency. We also obtained strains carrying only FPo with a high plasmid donor ability. The chromosome mobilization by these high-frequency-transfer OCT and FPo plasmids was compared. The involvment of the bacterial recombination system in the mobilization of chromosomal genes by OCT and FPo was also studied.

MATERIALS AND METHODS

Bacterial strains. The derivation and the genotypic and phenotypic properties of the strains utilized are shown in Table 1, together with references to their origin.

Media. Luria medium (18) was routinely used as the complete medium, and phosphate ammonium salts medium (7) or Vogel-Bonner medium (26) was used as the minimal medium. Phosphate ammonium salts medium was used for experiments requiring specific carbon sources.

Genetic experiments. Genetic analysis was accomplished by transferring the requisite genes, both

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TABLE 1. *Pseudomonas strains used*

PpG strain no.	Genotype[a]	Phenotype[b]	Derivation or source[c]	Reference
1	wild type/CAM	Cam$^+$	Camphor enrichment	22
572	wild type/ΔCAM	Cam$^-$	1, PC	22
273	*trpB615*/CAM	Trp$^-$ Cam$^+$	1, PC	22
277	*trpB615*/ΔCAM	Trp$^-$ Cam$^-$	273, mtc	8
6	wild type/OCT FPo	Oct$^+$	Octane enrichment	2
969	*his-901*/OCT FPo	His$^-$ Oct$^+$	6, NTG	8
970	*trpB615*/CAM-OCT	Trp$^-$ Cam$^+$ Oct$^+$	969 × 273, C aux	8
972	*trpB615*/OCT FPo	Trp$^-$ Oct$^+$	969 × 277, C aux	8
1330	*met-cys-601*	Met$^-$ Cys$^-$	572, NTG	
1334	*his-621*	His$^-$	572, NTG	
1335	*thr-611*	Thr$^-$	572, NTG	
1339	*arg-621*	Arg$^-$	572, NTG	
1340	*arg-615*	Arg$^-$	572, NTG	
1343	*met-616*	Met$^-$	572, NTG	
1344	*met-616 ilv-600*	Met$^-$ Ilv$^-$	1343, NTG	
1365	*ilv-600*	Ilv$^-$	1344, T	
1386	*ace-603*	Ace$^-$ Suc$^+$	572, NTG	
1360	*trpB615 pur-631*	Trp$^-$ Ade$^-$	277, NTG, PC	
1361	*pur-631*	Ade$^-$	1360, T	
1276	*met-616*/CAM	Met$^-$ Cam$^+$	273 × 1343, C aux	
1756	*met-616*/CAM-OCT	Met$^-$ Cam$^+$ Oct$^+$	970 × 1343, C aux	
1723	*met-616*/FPo	Met$^-$ Oct$^-$	972 × 1344, C aux	
1755	*met-616*/OCT FPo	Met$^-$ Oct$^+$	972 × 1343, C aux	
1720	*trpB615*/FPo	Trp$^-$ Oct$^-$	1755 × 1360, C aux	
1750	*str-601*	Str^r	572, S	
1372	*str-601 ace-601*	Str^r Ace$^-$ Suc$^+$	1750, NTG	
1377	*str-601 ace-602*	Str^r Ace$^-$ Suc$^+$	1750, NTG	
1387	*str-601 ace-604*	Str^r Ace$^-$ Suc$^+$	1750, NTG	
1390	*str-601 oic-601*	Str^r Oic$^-$ Ace$^+$	1750, NTG	
1737	*str-601 ool-603*	Str^r Ool$^-$ Oal$^+$	1750, NTG, PC	
1605	*hft-601*	MMS^s	572, NTG	
1701	*hft-601*/OCT(ΔCAM)	MMS^s Oct$^+$	970 × 1605, C aux	
1707	*hft-601 trp-616*/OCT(ΔCAM)	MMS^s Trp$^-$ Oct$^+$	1701, PC	
1713	*hft-601 trp-616*/ΔOCT(ΔCAM)	MMS^s Trp$^-$	1707, mtc	
1711	*hft-601 trp-616*/CAM-OCT	MMS^s Trp$^-$ Cam$^+$ Oct$^+$	1756 × 1713, C aux	
1712	*hft-601 trp-616*/CAM	MMS^s Trp$^-$ Cam$^+$	1276 × 1713, C aux	
1714	*hft-601 met-616*/OCT(ΔCAM)	Met$^-$ Oct$^+$	1707 × 1343, C aux	
1400	*trpB615 rec-604*	MMS^s Trp$^-$	277, NTG	
1405	*trpB615 rec-604 ace-623*	MMS^s Trp$^-$ Ace$^-$ Suc$^+$	1400, PC	
1404	*trpB615 rec-604*/OCT FPo	MMS^s Trp$^-$ Oct$^+$	1755 × 1400, C aux	
1715	*trpB615 rec-604*/OCT(ΔCAM)	MMS^s Trp$^-$ Oct$^+$	1714 × 1400, C aux	

[a] Δ, Plasmid deleted; *hft*, high frequency transfer on the chromosome.

[b] Ool, Octanol; Oal, octanal; Oic, octanoic; Ace, acetate.

[c] PC, Enrichment in spontaneous mutants by using the killing action of penicillin plus cycloserine; mtc, plasmid curing with mitomycin C; C aux, counterselection of exconjugants employing auxotrophic requirement; T, transduction; S, spontaneous. Other symbols in the table are according to Demerec et al. (11) and Taylor and Trotter (25).

chromosomal and plasmid borne, to the cell background of *P. putida* pG1 by conjugation or transduction.

Phage Pf16 was used as previously described (13). Phage lysates were routinely prepared on Luria agar with a soft agar overlay (1) and a phage infection multiplicity of 10^{-3}. The transduction procedure was essentially that described by Wheelis and Ornston (27). A low infection multiplicity was used (0.2), and the phage were killed by adsorption with an anti-pf16 serum (K [first-order velocity constant] = 50 min^{-1}). Routine transduction frequencies were of the order of magnitude of 10^{-6} per recipient cell.

Conjugation was performed in broth cultures incubated for 3 h with stirring at 30°C (about 10^9 bacteria per ml). Donor and recipient cells were mixed in equal numbers, or the donor-to-recipient ratio was reduced if necessary. Aliquots were plated immediately on appropriate media, using counter-selection for the parent donor by auxotrophic or resistance (streptomycin) markers. Large numbers of individual donor strains were scored by the replica plating method of Miller (18).

Mutagenesis and plasmid curing. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was used at a dose of 50 μg per ml in 100 mM citrate buffer (pH 6), with

incubation for 30 min at room temperature. The rate of mortality in these conditions was approximately 10% of the initial population. When necessary, enrichment by the D-cycloserine method of Ornston et al. (21) was used. Recombination-deficient strains (Rec^-) were selected after mutagenesis as methylmethane sulfonate (MMS)-sensitive strains. The final ratio of MMS was the highest concentration permitting normal growth of the wild-type strain, 3×10^{-4} (vol/vol). Rec^- recipients were identified in standard transduction experiments by the inability of auxotrophic strains to yield prototrophic recombinants.

Mitomycin C was used by the method of Rheinwald et al. (22) for plasmid curing at a concentration of 10^2 to 10^3 cells per ml to prevent reinfection of the cured cells. Wild-type strains were cured with 5 or 10 μg of mitomycin per ml; Rec^- strains were cured with 1 to 4 μg per ml. Mitomycin C sensitivity is strain related.

RESULTS

OCT versus CAM plasmid transfer. The OCT plasmid carried by strain PpG972 was found to be transferred at a frequency of about 10^{-4} per donor, as contrasted with the CAM or the CAM-OCT fused plasmid whose transmission frequencies were approximately 10^{-1} (Table 2). Also in contrast to the $Camphor^+$ (Cam^+) strains where 100% of the cells behaved as CAM donors, fewer than 1% of Oct^+ cells were capable of transferring the OCT plasmid. When an OCT^+ donor was crossed with any cured recipient strain, the newly formed exconjugants were found to transfer the OCT plasmid at a frequency as high as 10^{-2} per donor as contrasted with 10^{-4} for parental strain PpG972. This property of high transfer frequency was unstable, however, and was lost when the strain was subcultured for several generations (Table 2). A relationship between curability and plasmid donor capacity was also observed; i.e., all of the good OCT donors were found to be curable and became Oct^- with mitomycin C treatment, whereas the strains which transferred OCT with a low frequency could not be cured of OCT and remained Oct^+ after treatment with mitomycin C.

Crosses with Oct^+ strains of *P. putida* as donors. It has been previously shown that, when Oct^+ strains are used as donors, the transfer of most chromosomal prototrophic markers is independent of OCT plasmid transfer (5). We also observed that a very low percentage of prototrophic recombinants carried the OCT plasmid. However, when the same donor strains were mated with recipient strains defective in the chromosomal genes of octanoic acid catabolism, octanoic (*oic*) or acetate (*ace*), a high percentage of the cells selected for Oic^+ or Ace^+ inherited the OCT plasmid (Table 3). A gradient in the inheritance percentage of the unselected

TABLE 2. *Plasmid transfer of CAM, CAM-OCT, and OCT with strain 572 (wild type/ΔCAM) as the recipient*[a]

Donor[b]		Plasmid transfer frequency (−log)	% Donor cells capable of transferring plasmid	Mitomycin C curable[c]
PpG strain no.	Genotype			
273	*trpB615*/CAM	1	100	+
970	*trpB615*/CAM-OCT	1	100	+
972	*trpB615*/OCT FPo	4	<1	−
1755	*met-616*/OCT FPo			
	First clone	2	70	+
	After 20 generations	5	<1	−

[a] The following exconjugants were selected for: Cam^+ (273), Cam^+ Oct^+ (970), Oct^+ (others). Plasmid transfer of single colonies was tested by replica mating.

[b] Strain 972 is a strain which has contained OCT plasmid for many generations. Strain 1755 is an exconjugant derived from the cross 972 × 1343 (Met^-), which transfers OCT at a high frequency when it has just received OCT and at a low rate after several generations.

[c] All of the cells could be cured with adequate concentrations of mitomycin C (between 5 and 10 μg per ml). No curing was obtained with strain 972 and strain 1755 which had contained OCT for several generations.

TABLE 3. *Plasmid and chromosomal locus cotransfer with strain 972 (trpB615/OCT FPo) as donor*

Recipient[a]		Selected[b]	Nonselected/selected[c] (%)
PpG strain no.	Genotype		
1390	*oic-601*[d]	Oic^+	94
1372	*ace-601*[d]	Ace^+	84
1386	*ace-603*	Ace^+	72
1330	*met-cys-601*	wild type	40
1343	*met-616*	wild type	23
1365	*ilv-600*	wild type	9
1361	*pur-631*	wild type	5
1335	*thr-611*	wild type	2
1340	*arg-615*	wild type	0
1344	*met-616 ilv-600*	Met^+/Ilv^+	43/10[e]

[a] Mutants 1372 and 1386 are given as examples of acetate mutants; 13 more Ace^- recipient strains were used, and the percentage of Ace^+ Oct^+ recombinants was between 35 and 85%. The parental strain of 1344 was 1343. Strain 1365 was obtained from 1344 by transduction of the Met^+ character.

[b] Each selected marker was transferred with the same frequency, which varied from 10^{-7} to 10^{-6}.

[c] Selected, chromosomal prototrophy; unselected, Oct^+ (OCT plasmid).

[d] Str^r (*str-601*) was present but was not scored.

[e] The first number (43) applies to the transfer of Met; the second (10) applies to that of Ilv.

marker, Oct$^+$, was observed, which suggested a linkage between *oct*, *oic*, and *ace* genes, as well as *met-cys* and *met* genes. To test the variability in the cotransfer of OCT and prototrophic markers, cotransfers to single and double mutants were compared. The percentage of inheritance of the unselected marker in individual and double mutants was found to be similar (Table 3).

It has already been said that there is evidence that strains carrying the OCT plasmid contain another fertility factor as well. The results presented so far could be explained if the OCT plasmid mobilized the chromosome somewhere in the vicinity of the *oic* or *ace* genes, giving rise to an oriented transfer of the chromosome in the order *oic*, *ace*, *met-cys*, *met*, while FPo mobilized the chromosome in such a way as to transfer *arg*, *pur*, etc. with high frequency and *oic* and *ace* with low frequency. It would follow that, when OCT had a high plasmid donor ability, mobilization by that plasmid could contribute relatively more to select recombinants in the octanoic region than when it was transferred with low frequency, which was confirmed by results shown in Table 4.

We attempted to test this hypothesis by separating FPo from the aggregate FPo OCT, as well as by obtaining a derivative of the OCT plasmid which was transferred at a high frequency. We then studied chromosome mobilization in these two kinds of donor strains, which had a high plasmid donor ability.

Isolation of FPo from strains carrying OCT and FPo plasmids. Oct$^+$ strains, presumed to carry both OCT and FPo plasmids, were mated with double mutants. The recipients inherited the OCT plasmid with low frequency when the selection was made for one marker. Most of the Oct$^-$ exconjugants were found to be donors of chromosomal markers. The strains PpG1720 and 1723 thus isolated (Table 1) were used in further conjugation experiments. It is possible that the fertility factor present in these strains is identical to the factor K described by Chakrabarty (5). However, until this is demonstrated, we will refer to this factor as FPo. When not associated with OCT, FPo transferred chromosomal markers with high frequency. This property was stable and was not lost when FPo had been present for many generations. Furthermore, each colony of a donor strain expressed its donor properties in replica mating.

Isolation of an OCT plasmid which is transferred at high frequency. For the isolation of such a plasmid, use was made of the high rate of transfer of the fused CAM-OCT plasmid. Normally when a recipient strain was infected with the CAM-OCT plasmid, 100% of the exconjugants were Cam$^+$ Oct$^+$. However, in some crosses where MMS-sensitive recipient strains were mated with a donor carrying the CAM-OCT plasmid, about 5% of the exconjugants were Cam$^-$ Oct$^+$. The OCT plasmid in strains thus obtained was autotransmissible with high frequency (10^{-1}). This characteristic was a property of the plasmid which was retained on transfer into any recipient strain. These strains were also easily cured by mitomycin C, and cured strains lost their ability to transfer chromosomal markers. Such plasmids were termed OCT(ΔCAM). One of these OCT(ΔCAM)-carrying strains, PpG1707 (Table 1), was used in further conjugation experiments.

TABLE 4. *Dependence of OCT and Ace cotransfer upon the mobility of OCT plasmid*

Donor[a]		% Of donor cells which transferred OCT plasmid	% Of OCT cotransfer[b]		
PpG strain no.	Genotype		Trp$^+$	Ade$^+$	Ace$^+$
1755	*met-616*/OCT FPo	6	1	0	15
1755 A	*met-616*/OCT FPo	42	0	2	98

[a] Donor strain 1755 was an exconjugant derived from the cross 972 *trpB615*/OCT FPo × 1343 *met-616* and transferred OCT plasmid with a low frequency. Strain 1755 A was a single colony derived from 1755, which transferred OCT plasmid with high frequency.

[b] Recipient strain 1362 Trp$^-$ Ade$^-$ Ace$^-$ was ultimately derived from strain 277 *trpB615*. No linkage was observed between the three markers. Selected, chromosomal prototrophy; unselected, Oct$^+$ (OCT plasmid).

Mobilization of the chromosome by OCT-(ΔCAM) and FPo plasmids. Because of the results shown in Table 3, it was expected that there would be a high transfer frequency of some chromosomal markers by an OCT-depressed plasmid. Table 5 shows such a frequency for the *oic*, *ace*, *met-cys*, and *met* genes. By contrast, FPo produced frequent prototrophs with *arg* genes but a low transfer frequency of the *oic*, *ace*, *met-cys*, and *met* genes. The fused CAM-OCT plasmid produced a gradient of transfer similar to that of OCT(ΔCAM), whereas CAM-bearing strains demonstrated no such gradient.

Role of the bacterial recombination system in chromosome mobilization by OCT-(ΔCAM) and FPo. Rec$^-$ recipient strains were obtained by selecting mutants sensitive to MMS and considered to be Rec$^-$ because of their very low transduction frequency. The strains used in the experiments described below may be considered analogous in their phenotypic properties to

TABLE 5. *Fertility factor transfer polarity*

Recipient		Exconjugants/donor with the following donors (Trp^-) (−log)[a]			
PpG strain no.	Genotype	1707 OCT(ΔCAM)	1711 CAM-OCT	1712 CAM	1720 FPo
1737	*ool-603*[b]	1[c]	1[c]		8
1390	*oic-601*[b]	3	3	6	6
1372	*ace-601*[b]	2	3	6	6
1377	*ace-602*[b]	4	4		6
1386	*ace-603*	3	4		
1330	*met-cys-601*	4	4	6	6
1343	*met-616*	5	5		6
1335	*thr-611*	7			5
1340	*arg-615*	7		7	2
1339	*arg-621*	6		8	4
1361	*pur-631*	7			5
1334	*his-621*	5	5		2

[a] Strains 1707, 1711, and 1712 were *hft-601 trp-616*; strain 1720 was *trpB615*.

[b] Str (*str-601*) was present but was not scored.

[c] Gene *ool* was borne on plasmid and chromosome; Ool^+ selected measured conjugation frequency. Other numbers measured the transfer frequency of chromosomal markers. All of the exconjugants inherited the corresponding plasmid.

the *recA* mutation described for *Escherichia coli*, because their transduction frequencies were decreased more than 10^3-fold.

Chromosomal transfer by donors carrying OCT(ΔCAM) or FPo into Rec^- recipient strains. A *rec* mutation in the recipient decreased the transfer of chromosomal markers in crosses with both FPo and OCT(ΔCAM) donors (Table 6). The decrease in recombination frequency was more severe with OCT(ΔCAM) (10^{-4}) than it was with FPo (10^{-2}) and was not a consequence of entry exclusion because OCT(ΔCAM) was transferred with the same frequency in Rec^+ and Rec^- recipient strains.

Transfer of extrachromosomal elements by Rec^- donor strains containing OCT-(ΔCAM) or OCT FPo plasmids. Results presented in Table 7 show that a Rec^- donor bearing an OCT(ΔCAM) plasmid gave a much lower transfer frequency of the octanoic marker than did the corresponding Rec^+ donor strain. By contrast, the arginine marker, which was transferred efficiently by a Rec^+ OCT FPo donor, showed no reduction in transfer with a Rec^- donor containing the same plasmids.

TABLE 6. *Crosses of donor strains carrying OCT(ΔCAM) or FPo plasmid with Rec^- recipient strains*[a]

Donor		Selected maker[b]	Recombination frequency (−log)	
PpG strain no.	Genotype		Rec^+ recipient	Rec^- recipient
1714	*met-616*/OCT(ΔCAM)	Ace	4	>8
1723	*met-616*/FPo	Trp	4	6

[a] The recipient strain was PpG1405 *trpB615 ace-623 rec-604*. Rec^+ recipients were MMS^r revertants of Rec^- strains.

[b] The data cited above are given with the marker which was transferred with high frequency in a Rec^+ recipient strain by each plasmid.

DISCUSSION

The OCT plasmid has a low plasmid donor ability, because a low percentage of colonies derived from the same donor strain is capable of transferring the plasmid, although bacteria which have newly received OCT pass it on at much higher frequency for a few serial subcultures. Many plasmids with such properties have been described and appear to be fertility repressed (9, 14, 15, 17, 20, 28). By contrast, CAM and fused CAM-OCT plasmids have the properties of derepressed plasmids in their wild-type form; i.e., they express their fertility in all of the cells containing them, just like the classical F-factor of *E. coli*. However, further experiments (physical isolation of FPo, OCT, and the other plasmids and determination of genetic homologies among the plasmids as well as the chromosome) will be necessary to elucidate whether OCT has its own fertility factor or whether it is a Tra^- plasmid mobilized by FPo.

To interpret chromosome mobilization by OCT FPo plasmid, we studied the donor properties (plasmid donor ability and chromosome mobilization) of strains containing only one of these plasmids (OCT or FPo), each of them being transferred at high frequency. We obtained an OCT plasmid which was transferred

TABLE 7. *Chromosomal transfer by Rec⁻ and Rec⁺ donors carrying OCT(ΔCAM) and OCT FPo plasmids*

Donor[a]		Recipient		Selected marker	Recombination frequency (−log)
PpG strain no.	Genotype	Strain no.	Genotype		
1715	*trpB615 rec-604*/OCT(ΔCAM)	1390	*oic-601*[b]	*oic*	7
	trpB615/OCT(ΔCAM)	1390	*oic-601*[b]	*oic*	3
1404	*trpB615 rec-604*/OCT FPo	1340	*arg-615*	*arg*	4
	trpB615/OCT FPo	1340	*arg-615*	*arg*	4

[a] Rec⁺ donor strains were MMS[r] revertants of Rec⁻ strains. In each cross, the recipient strain was mutated in a gene which was transferred with high frequency by the Rec⁺ donor strain.
[b] Str[r] (*str-601*) was present but was not scored.

at high frequency by segregation of CAM from the fused CAM-OCT plasmid. This plasmid, OCT(ΔCAM), can be considered to be fertility derepressed. It is interesting to note, although difficult to explain, that such segregation could be obtained by the transfer of CAM-OCT into MMS-sensitive strains alone. One explanation for the derepression of OCT plasmid in OCT(ΔCAM) is the retention of the CAM fertility factor (FPc) and the segregation of CAM structural genes in some of these exconjugants when the recipient strains were MMS sensitive. In any case, the absence of CAM structural genes in OCT(ΔCAM) is indicated by the fact that revertants to the Cam⁺ Oct⁺ phenotype have not been observed and that the Cam⁺ phenotype was not restored when the plasmid was transferred back to a MMS-resistant strain, although the derepression was retained.

The strains containing OCT(ΔCAM) transferred chromosomal markers of octonoate catabolism at very high frequencies. A reproducible pattern for the gradient of transfer frequencies for these and other markers (i.e., *oic* > *ace* > *met-cys* > *arg*) suggests that OCT(ΔCAM) may mobilize the chromosome from a fixed point of origin. Previous studies (3, 7, 12) have shown a functional homology (gene redundancy for the octanol gene) between the plasmid and the *P. putida* chromosome. It is likely that OCT(ΔCAM) behaves like an F-prime factor in *E. coli*, where homologous regions on the plasmid and chromosome provide a definite point of attachment during transfer (4, 16, 24). Our investigations with *rec* mutants further support the view that, as with an F-prime factor, the integration of OCT(ΔCAM) is required for chromosomal mobilization by this plasmid (16, 19, 28).

The FPo factor, when not associated with OCT, mobilized some chromosomal markers at high frequency, and each colony derived from a given donor culture could transfer chromosomal markers. Therefore, the FPo plasmid and OCT(ΔCAM) appear to have similar plasmid donor ability, although chromosomal mobilization by FPo produced a different marker gradient. Another difference from OCT(ΔCAM) was the independence of chromosomal transfer by an FPo plasmid donor with respect to the bacterial recombination system, implying that FPo may have its own recombination system, like several other known plasmids (10, 19, 23). The difference between the recombination systems of OCT(ΔCAM) and FPo may be tentatively explained by an argument similar to that already proposed by Moody and Hayes (19), who suggested that a long period of association between plasmid and chromosome of the bacterial host may allow for an evolutionary process to develop, allowing the plasmid [in our case the CAM plasmid from which OCT(ΔCAM) is derived] to become dependent on the host recombination system. On the contrary, the genome of a plasmid recently introduced from a different strain (as in the case of FPo) may have little analogy with the genome of the new host, implying that chromosome transfer by FPo will have to take place by a mechanism independent of the bacterial *rec* system.

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