COINTEGRATE FORMATION DURING MOBILIZATION OF NONCONJUGATIVE PLASMIDS BY pAP42 PLASMID FOR GENETIC TRANSFER

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Mobilization of nonconjugative plasmids by conjugative plasmids Δ and F for transfer from donor *E. coli* cells into recipient cells of this species gives rise to transconjugants, in which the mobilizing and mobilized plasmids are not physically united, exist as independent replicons, and dissociate after mobilization into the original structures [2, 4, 5]. Meanwhile the results of a study of the mobilizing ability of F-like conjugate plasmids pAP38, pAP39, pAP41, pAP42, and pAP43 which, like plasmids Δ and F, are genetic transfer factors, suggest that plasmids pAP41, pAP42, and pAP43 are perhaps incorporated into nonconjugative plasmids mobilized by them for transfer [1].

This paper presents data showing that during mobilization physical union takes place between F-like derepressed transfer factor pAP42 and nonconjugative plasmids pACYC184 and RSF2124, as a result of which stable cointegrative structures are formed.

EXPERIMENTAL METHOD

The mobilizing ability of factor pAP42 and its variants pAP42::Tn1 and pAP42::Tn9 was determined in "three-parent" crosses in which the donors were cells of *E. coli* strain AP115 met thi lac Nal^r, containing transfer factor pPA 42, pAP42::Tn1, or pAP42::Tn9. The "intermediate recipients" were cells of *E. coli* UB1636 F^- trp his lys lac mal gal $T6^rT1^{sr}$ spc str, containing nonconjugative plasmid pMR5 ApTcCmNm or pSC101 Tc, and *E. coli* C600 thr leu thi lac rif cells, containing one of the following nonconjugative plasmids: pBR322 ApTc, pACYC184 CmTc, RSE2124 Ap ColE1 or RSF1010 Susm. The "final" recipients were *E. coli* C600 Rif^r cells. Transconjugants were selected on nutrient agar (NA) with the addition of the necessary antibiotics (25 μg/ml) or sulfonamides (2000 μg/ml). Rifampicin and nalidixic acid were added to the selective media in concentrations of 50 μg/ml. Sensitivity of the transconjugants to phage MS2 was determined by the agar layers method. To determine colinogenicity of the transconjugants, strain *E. coli* Φ was used as the indicator culture. The donor ability of the transconjugants was determined in crosses with recipient cells of *E. coli* AP 115. Spontaneous and ethidium bromide-induced (600 μg/ml) elimination of conjugative and nonconjugative plasmids from the transconjugants was studied by testing them for resistance to antibiotics and sensitivity to phage MS2. Tetracycline-resistant variants were separated from cultures of tetracycline-sensitive transconjugants by selection of colonies growing after seeding $2.0 \cdot 10^{-8}$ transconjugant cells on NA with tetracycline (25 μg/ml). To determine compatibility (incompatibility) of the plasmid cointegrates formed in the transconjugants from "three-parent" crosses with the original transfer factor, the latter was introduced into the transconjugants, which were then tested for the presence of both types of plasmids.

EXPERIMENTAL RESULTS

The experiments were started with "three-family" crosses of *E. coli* undertaken to determine the ability of plasmid pAP42 and its transposon-containing variants pAP42::Tn1 and pAP42::Tn9 to mobilize nonconjugative plasmids pMR5, pACYC184, RSF2124, pSC101, pBR322, and RSF1010 for transfer from donor cells into recipient cells, from which transconjugants containing conjugative (mobilizing) and nonconjugative (mobilized) plasmids were isolated. The results of these experiments showed that plasmid pAP42 and its variants pAP42::Tn1 or pAP42::Tn9 mobilize nonconjugative plasmids pMR5, pBR322, pACYC184, RSF2124, and RSF1010 for

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TABLE 1. Properties of Transconjugants from Crosses of Plasmid pAP42 with Nonconjugative Plasmids

Transconjugant	Phenotype	Frequency of transfer							How conjugative and nonconjugative plasmids are eliminated
		plasmid pAP42	nonconjugative plasmids (markers)						
			Ap	Tc	Km	Sm	Su	Cm	
7-83 (pAP4 : : Tn9/pMR5)	ApTcKm CmMS2^s	$1{,}8 \cdot 10^{-1}$	$1{,}7 \cdot 10^{-2}$	$1{,}9 \cdot 10^{-6}$	$1{,}2 \cdot 10^{-5}$	—	—	—	Separately
M-15 (pAP42/pBR322)	ApTc MS2^s	—	$1{,}5 \cdot 10^{-1}$	$5{,}9 \cdot 10^{-6}$	—	—	—	—	»
16—61 (pAP42 : : Tn9/pBR322)	ApTcCm MS2^s	$2{,}5 \cdot 10^{-1}$	$1{,}06 \cdot 10^{-1}$	$4{,}8 \cdot 10^{-1}$	—	—	—	—	»
5—13 (pAP42/pACYC184)	TcCm MS2^s	—	—	$4{,}0 \cdot 10^{-4}$	—	—	—	$2{,}9 \cdot 10^{-1}$	»
6-42 (pAP42 : : Tn1/pACYC184)	TcCmAp MS2^s	$2{,}35 \cdot 10^{-1}$	—	0	—	—	—	$2{,}2 \cdot 10^{-1}$	Jointly
P-2 (pAP42/RSF2124)	ApMS2^s	—	$4{,}27 \cdot 10^{-2}$	—	—	—	—	—	»
6-71 (pAP42 : : Tn9/RSF2124)	ApMS2^s	$1{,}08 \cdot 10^{-1}$	$1{,}21 \cdot 10^{-1}$	—	—	—	—	—	»
15-15 (pAP42/RSF1010)	SuSm MS2^s					$7{,}76 \cdot 10^{-5}$	$3{,}61 \cdot 10^{-5}$		Separately
7-41 (pAP42 : : Tn1/RSF1010)	SuSmAp MS2^s	$4{,}94 \cdot 10^{-1}$	—	—	—	$6{,}05 \cdot 10^{-4}$	$3{,}95 \cdot 10^{-4}$	—	»
5-53 (pAP42 : : Tn9/RSF1010)	SuSmCm MS2^s	$6{,}04 \cdot 10^{-1}$	—	—	—	$8{,}4 \cdot 10^{-4}$	$3{,}57 \cdot 10^{-4}$	—	»

transfer. As regards plasmid pSC101, no transconjugants could be isolated from crosses in which its mobilization was studied. The frequency of mobilization of plasmids pMR5, pBR322, and pACYC184 was found to differ when determined according to frequencies of transfer of different markers of these plasmids. For instance, the frequency of mobilization of these plasmids, determined from the frequency of transfer of the Tc marker, was one order of magnitude less than the frequencies of mobilization determined according to the frequency of transfer of markers Ap and Km (of plasmid pMR5), AP (of plasmid pBR322), or Cm (of plasmid pACYC184). A decrease in the frequency of mobilization was observed when determined according to the frequency of transfer (mobilization) of the Tc marker of nonconjugative plasmids in the case of other transfer factors also [1, 6].

To determine the character of the connections between the mobilizing (pAP42, pAP42::Tn1, or pAP42::Tn9) and the mobilized (one of the nonconjugative) plasmids, several transconjugants were selected from each cross, and their donor ability and the stability of the plasmids contained in them were studied. From "three-parent" crosses in which the mobilizability of plasmid pMR5 by transfer factors pAP42 and pAP42::Tn9 was determined, transconjugants 20-1 and 7-83 with the ApTcKm $MS2^S$ and ApTcKmCm $MS2^S$ phenotypes respectively were selected. Transconjugants M-15 and 16-61 with ApTc $MS2^S$ and ApTcCm $MS2^S$ phenotypes respectively were selected from crosses to determine the mobilizability of plasmid pBR322 by factors pAP42 and pAP42::Tn9. Transconjugants P-2 and 6-71 with ApColEI $MS2^S$ and ApColEICm $MS2^S$ phenotypes respectively were selected from crosses to determine mobilizability of plasmid pACYC184 by factors pAP42 and pAP42::Tn1. Finally, transconjugants 15-15, 7-41, and 5-53 with SuSm $MS2^S$, SuSmAp $MS2^S$, and SuSmCm $MS2^S$ phenotypes respectively were selected from crosses in which mobilizability of plasmid RSF1010 by factors pAP42, pAP42::Tn1 and pAP42::Tn9 was determined. The study of the donor ability of transconjugants selected from all crosses was undertaken by determining the frequency of transfer of the conjugative (mobilized) plasmids by the transconjugants in crosses with recipient cells of *E. coli* C600 Rif^r. It was assumed that equal frequencies of transfer of mobilizing and mobilized plasmids would indicate that they were fused, whereas different freuqencies would indicate the separate existence of these plasmids. The results of determination of the donor ability of the transconjugants are given in Table 1. It will be clear from Table 1 that when the donors were transconjugants 6-42 (pAP42::Tn1/pACYC184), P-2 (pAP42/PSF2124) and 6-71 (pAP42::Tn9/RSF2124), the frequencies of transfer of factor pAP42 and pAP42::Tn1, or pPA42::Tn9 and the frequencies of transfer of nonconjugative plasmids pACYC184 and RSF2124 were similar. If, however, the donors were transconjugants 7-83, M-15, 16-61, 5-13, 15-15, 7-41, and 5-53 the frequencies of transfer of the conjugative and nonconjugative plasmids were different. It must also be noted that the frequency of mobilization of nonconjugative plasmids pMR5, pBR322, and pACYC184, determined relative to the Tc marker, was considerably lower than frequencies determined relative to other markers. The results suggested that transconjugants 6-42 (pAP42::Tn1/pACYC184) P-2 (pAP42/RSF2124) and 6-71 (pAP42::Tn9/RSF2124) transmit the transfer factors contained in them fused with nonconjugative plasmids. In other words, the conjugative and nonconjugative plasmids contained in these transconjugants are united into cointegrative structures (pAP42::Tn1/pACYC184, pAP42/RSF2124 and pAP42::Tn9/RSF2124). The cointegrate pAP42::Tn1/pACYC184 present in transconjugate 6-42 does not contain the Tc marker which was present in the original nonconjugative plasmid pACYC184. This suggested that the formation of this transconjugant is probably linked with incorporation of the transfer factor into the nonconjugative plasmid in the region of the locus determining resistance to tetracycline.

It is also clear from Table 1 that transconjugants 7-83, M-15, 16-61, 5-13, 15-15, 7-41, and 5-53 transmit conjugative and nonconjugative plasmids separately. This indicates that these plasmids exist separately in these transconjugants, i.e., they are not physically connected.

To confirm the view on the character of connections between conjugative and nonconjugative plasmids in transconjugants from "three-parent" crosses, in the next experiments levels of spontaneous and ethidium bromide-induced elimination of conjugative and nonconjugative plasmids contained in the transconjugants chosen for study were determined (Table 1). Removal of transfer factor and nonconjugative plasmid from transconjugants 6-42, P-2, and 6-71 was found to take place jointly, confirming their cointegrative character, whereas from transconjugants 7-83, M-15, 16-61, 5-13, 15-15, and 5-53 these plasmids are removed independently, confirming the separate (independent) character of their existence. These results thus confirm the suggestion that plasmid cointegrates pAP42::Tn1/pACYC184, pAP42/

RSF2124, and pAP42::Tn9/RSF2124 are formed in the process of mobilization of nonconjugative plasmids for transfer by conjugative plasmids. These cointegrates were designated plasmids pRH1, pRH2, and pRH3 respectively.

Since the gene determining resistance to tetracycline is inactivated in the pRH1 plasmid cointegrate contained in transconjugant 6-42, as was mentioned above, this suggested the possibility of incorporation of the conjugative plasmid into the nonconjugative in the Tc locus. To determine the stability of the pRH1 cointegrate, in the next experiments tetracycline-sensitive transconjugant 6-42 cells containing this cointegrate were seeded on medium with tetracycline, on the assumption that the appearance of tetracycline-resistant variants would reflect dissociation of the test cointegrate at the same Tc locus. However, on seeding $2 \cdot 10^{-8}$ tetracycline-sensitive cells on a dish with NA and tetracycline, no tetracycline-resistant colonies were found, evidence of the resistance of the tested cointegrates.

In the final experiments compatibility (decompatibility) of transfer factor pAP42 with cointegrative plasmid pRH1 was determined. For this purpose transfer factor pAP42::Tn5 was introduced into transconjugant 6-42 cells containing plasmid pRH1 and the resulting secondary transconjugants were analyzed for their plasmid content. As these experiments showed, transfer factor pA42::Tn5 completely (100%) displaced the cointegrative plasmid pRH1 from the cells, evidence of their absolute incompatibility, and also that, when uniting with the nonconjugative plasmid, factor pAP42 does not lose its "individuality."

Summing up these results it can be concluded that they demonstrate the formation of plasmid cointegrates, similar in properties to typical R-plasmids, during mobilization of nonconjugative plasmids pACYC184 and RSF2124 for transfer by conjugative plasmid pAP42. Cointegrate formation has hitherto been demonstrated only in the case of conjugative and nonconjugative R-plasmids [3].

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