

MOBILIZATION OF NONCONJUGATIVE PLASMID

pAP57 Hly FOR TRANSFER BY VARIOUS CONJUGATIVE
PLASMIDS

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Nonconjugative plasmids are incapable of independent genetic self-transfer from some bacterial cells to others. They can be mobilized for transfer by conjugative plasmids [2, 3, 8, 11]; this mobilization, moreover, is controlled by genes of the nonconjugative plasmids [5-7]. However, it is not known whether there is any connection between ability of a nonconjugative plasmid to be mobilized and the belonging of the mobilizing (conjugative) plasmid to one particular incompatibility group, and also between ability to undergo mobilization and the character of the pili determined by the mobilizing plasmid. It is also not clear how important is the range of transmissiveness of the mobilizing plasmid in mobilization, what is the role of the bacterial *rec A* gene, and what is the effect of transposed genetic elements on mobilization.

The aim of this investigation was to study ability of nonconjugative plasmid pAP57 Hly, identified in *E. coli* cells [1] and controlling β -hemolysin synthesis by bacteria of this species, to be mobilized for transfer.

EXPERIMENTAL METHOD

Ability of plasmid pAP57 immobilized for transfer was studied in "three-parent" crosses in which the donors were *E. coli* AP115 met thi lac or J53 pro met cells, containing one of the conjugative plasmids, *E. coli* C600 thr leu thi lac rif or AB2463 *rec A* cells, containing the nonconjugative plasmid to be tested, were intermediate recipients, and *E. coli* pAP115 nal^r cells were the final recipients. Conjugation mixtures from these crosses were seeded on nutrient agar (NA) with the addition of essential antibiotics (25 μ g/ml), nalidixic acid (50 μ g/ml), and erythrocytes (1%). The frequency of combined or separate transfer of mobilizing and mobilized plasmids from transconjugants into *E. coli* C600 rif^s recipient cells was determined in crosses by the standard technique. Plasmid elimination, spontaneous and induced by ethidium bromide (100 μ g/ml), was determined by the change in hemolytic activity and sensitivity of the bacteria to the corresponding antibiotics. Donor-specific phage MS2 was used in the phage titer elevation test (PTET).

EXPERIMENTAL RESULTS

To study the possibility of mobilization of nonconjugative plasmid pAP57 by conjugated plasmids, F-like conjugative plasmids pAP39 (inc FI/FIV), R386 (inc FI), R1 (inc FII), R124 (inc FIV), pAP38 (inc FVII), pAP42 (inc FIX), pAP43 (inc FVIII) and pAP41 (inc FI/FIV), and also plasmids R16, R40a, R711b, R27, R144, R621a, R391, R387, R466b, N3, RP4, Rts1, R905, S-a and R6K, which are reference plasmids for groups inc B, C, D, H, Ia, I γ , J, K, M, N, P, T, W, and X (respectively), were used as mobilizing plasmids in three-parent crosses. The results of these experiments are given in Table 1.

It will be clear from Table 1 that only 11 of the 23 conjugative plasmids tested (pAP41, R711b, R386, R27, R621a, R391, R387, RP4, Rts1, R905 and S-a) possessed the ability to mobilize the nonconjugative plasmid for transfer; mobilization was carried out most effectively, moreover, by F-like plasmid pAP41, and also by plasmids R27, R387, and S-a, belonging to incompatibility groups H, K, and W (respectively), and determining different pili [4]. These plasmids, as we know, are characterized by a narrow range of transmissiveness. It is also clear from Table 1 that plasmids R711, R621a, R391, and RP4, belonging to incompatibility groups D, I γ , J, and P, had the least mobilizing ability. Plasmids of D, I γ , and J groups also are known to have a narrow range of transmissiveness, but plasmids of the P group are the most transmissive. In particular, plasmid RP4

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TABLE 1. Mobilization of Nonconjugative Plasmid pAP57 for Transfer by Conjugative Plasmids

Mobilizing plasmid	Incompatibility group	Frequency of mobilization	Property of transconjugants		
			transmission of plasmids to recipient cells	spontaneous elimination of plasmids	induced elimination of plasmids
pAP38	FVII	—	Separate	Separate	Separate
pAP39	FI/FIV	—			
pAP41	FI/FIV	$1 \cdot 10^{-1}$			
pAP42	FIX	—			
pAP43	FVIII	—	Separate	Separate	Separate
R16	B	—			
R40a	G	—			
R711b	D	$0.9 \cdot 10^{-4}$			
R386	FI	$4.0 \cdot 10^{-3}$			
R1	FII	—			
R124	FIV	—			
R27	H	$0.9 \cdot 10^{-1}$			
R144	I	—			
R621a	I	$0.1 \cdot 10^{-4}$			
R391	J	$1.4 \cdot 10^{-4}$			
R387	K	$0.1 \cdot 10^{-4}$			
R446b	M	—			
N3	N	—			
RP4	P	$0.3 \cdot 10^{-4}$			
Rts1	T	$3.1 \cdot 10^{-3}$			
R905	V	$0.54 \cdot 10^{-2}$			
S-a	W	$0.42 \cdot 10^{-2}$			
R6K	X	—			

can overcome not only interspecific, but also intergeneric barriers in the transfer process. The results show that the particular incompatibility group to which the conjugative plasmid belongs, the character of the pili determined by the conjugative plasmid, and its mobilizing ability are properties which are not interconnected. Moreover, data on the insufficient mobilizing efficiency of plasmid RP4 do not confirm the conclusion according to which it is suggested that conjugative plasmids with a wide range of transmissiveness also possess greater mobilizing activity.

To study whether physical mobilizing and mobilized plasmids are connected in transconjugants bred from "three-parent" crosses, one transconjugant was selected from each of the 11 crosses named above and they were studied as donor cells in further crosses. The aim of these crosses was to discover whether the mobilizing and mobilized plasmids are transmitted separately or together. It was postulated that separate transmission will indicate the separate existence of the two plasmids, whereas combined transmission indicates cointegration of the plasmids. Analysis of the results of transfer of the two plasmids showed that the highest frequency of transmission is possessed by the mobilizing plasmid, i.e., the two plasmids are transmitted separately.

For the same purpose the level of spontaneous and ethidium bromide-induced elimination of plasmids from the transconjugants was studied. These experiments showed that removal of conjugative and nonconjugating plasmids from the transconjugants also took place at different frequencies, i.e., separately (Table 1).

The results of transfer and elimination of plasmids from transconjugants, taken together, signify that the mobilized and mobilizing plasmids do not form cointegrates that exist in a separate state independently of one another.

To discover whether homology exists between the mobilized and mobilizing plasmids, parallel "three-parent" crosses were carried out in which the donors were *E. coli* AP115 cells containing plasmid pAP41, and *E. coli* AB2463 rec A cells, containing nonconjugative plasmids pAP57, served as recipients. These experiments showed that the frequency of mobilization of the nonconjugative plasmid chosen for study in Rec⁻ cells is the same as in the "three-parent" crosses described above, in which Rec⁺ cells were the intermediate recipients. These results signify that mobilization is unconnected with homology of the mobilizing and mobilized plasmids.

In the final experiments the effect of transposons Tn1 and Tn9 on mobilization was investigated. For this purpose *E. coli* containing conjugative plasmids pAP41, pAP41: :Tn1, or pAP41: :Tn9 were used as donors in three-parent crosses, and *E. coli* AP 2463 cells containing nonconjugative plasmid pAP57 were used as intermediate recipient. As these experiments showed, plasmid pAP41 and pAP41: :Tn1 mobilized plasmid pAP57 for transfer with a frequency of the order of $1 \cdot 10^{-1}$, whereas plasmid pAP41: :Tn9, although capable of mobilizing, did so with a much lower frequency. The subsequent study of the donor properties of transconjugants bred in these experiments and their ability (spontaneously induced) to eliminate the plasmids contained in them showed that the latter do not form cointegrates and exist in a separate state.

To sum up the results of these experiments it can be concluded that mobilization of nonconjugative plasmid pAP57 for transfer is dependent neither on the incompatibility group to which the mobilizing plasmid belongs, nor on the character of the pili determined by it, nor on the range of its transmissiveness. These results also exclude covalent binding of the mobilizing and mobilized plasmids as a result of their homology, with subsequent formation of cointegrative structures, as one of the mechanisms of mobilization. The mechanism of mobilization of this plasmid requires further study.

LITERATURE CITED

1. O. B. Gigani and A. P. Pekhov, in: Abstracts of Proceedings of the 6th Working Conference on the "Plasmid" Program [in Russian], Moscow (1981), p. 41.
2. O. B. Gigani and A. P. Pekhov, *Byull. Éksp. Biol. Med.*, No. 5, 82 (1982).
3. F. Bolivar, R. L. Rodriguez, P. J. Greem, et al., *Gene*, 2, 9 (1977).
4. D. E. Bradley, *Plasmid*, 4, 155 (1980).
5. G. Dougan, J. H. Crosa, and S. Falkow, *J. Infect. Dis.*, 37, 676 (1978).
6. G. Dougan, M. Saul, G. Warren, et al., *Mol. Gen. Genet.*, 158, 325 (1978).
7. G. Dougan and D. J. Sherratt, *Mol. Gen. Genet.*, 151, 151 (1977).
8. K. G. Hardy, *Bact. Rev.*, 39, 464 (1975).
9. G. Warren and D. Sherratt, *Mol. Gen. Genet.*, 151, 197 (1977).
10. N. Willetts and C. Crowther, *Genet. Res. (Cambridge)*, 37, 311 (1981).
11. I. G. Young and M. I. Poulos, *Gene*, 4, 175 (1978).