MOBILIZATION ABILITY OF GENETIC TRANSFER FACTORS pAP38, pAP39, pAP41, pAP42, AND pAP43

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F-like genetic transfer factors pAP38, pAP39, pAP41, pAP42, and pAP43 have been found in cells of *E. coli* strains isolated from man and animals [1, 2]. Since the ability of these factors to undergo mobilization for transfer of nonconjugative plasmids has not been studied, the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

The mobilization ability of transfer factors pAP38, pAP39, pAP41, pAP42, and pAP43 was determined in "three-parent" crosses in which cells of *E. coli* AP106 $trp^{-}his^{-}lac^{-}str^{+}$, containing one of these plasmids,* were used as donors. The "intermediate" recipients were *E. coli* cells carrying one of the following nonconjugative plasmids: pSF2124 (Ap), pBR322 (ApTc), pACYC184 (LmTc), pSC101 (Tc), pMR5 (ApKmTc), or SmSu. The "final" recipients were *E. coli* strains Ap 115 $met^{-}thi^{-}lac^{-}nal^{r}$ and C600 $thr^{-}leu^{-}thi^{-}lac^{-}str^{r}$. These crosses were carried out by the standard method. Transconjugants were selected on meat-peptone agar (MPA) with the addition of the necessary antibiotics (in a concentration of 25 μg/ml).

In control experiments the mobilization ability of F'lac plasmid, which has been well studied in this respect, was determined by the same method.

Inheritance of nonselective markers by the transconjugants was determined by seeding them on MPA with the addition of the corresponding agents. The donor ability of the transconjugants was studied in crosses with *E. coli* C600 rif^{r} recipient cells by the standard method.

EXPERIMENTAL RESULTS

The results of the "three-parent" crosses, in which the mobilization ability of the test genetic transfer factors and also of F'lac factor was investigated, are given in Table 1.

As Table 1 shows, all the transfer factors studied (pAP38, pAP39, pAP41, pAP42, pAP43), like the F'lac plasmid, are capable of mobilizing nonconjugative plasmids pSF2124, pMR5, and SuSm for transfer. Transfer factors pAP42 and F'lac also are capable of mobilizing nonconjugative plasmid pBR322 for transfer, whereas factors pAP41, pAP43, and F'lac can mobilize plasmid pACYC184. However, none of the transfer factors including F'lac could mobilize plasmid pSCC101 for transfer.

It will also be clear from Table 1 that during mobilization of nonconjugative plasmids controlling resistance to more than one antibiotic for transfer it was impossible to obtain

*Abbreviations used: trp) tryptophan, his) histidine, lac) lactose, leu) leucine, thr) threonine, met) methionine, thi) thiamine, nal) nalidixic acid, Ap) ampicillin, Tc) tetracycline, Km) kanamycin, Sm or str) streptomycin, Lm) levomycetin, Su) sulfanilamide, rif) rifampicin.

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TABLE 1. Mobilization Ability of Transfer Factors

Transfer factor	Mobilized plasmid	Transconjugants and their frequency (per recipient cell)				
		Ap	Km	Lm	Tc	Sm
pAP38	pSF2124	$5 \cdot 10^{-2}$				
	pBR322	—			—	
	pACYC184			—	—	
	pSC101				—	
	pMR5	$0{,}05 \cdot 10^{-5}$	—		—	
	SuSm					$0{,}03 \cdot 10^{-5}$
pAP39	pSF2124	$2{,}88 \cdot 10^{-2}$				
	pBR322	—			—	
	pACYC184			—	—	
	pSC101				—	
	pMR5	$0{,}29 \cdot 10^{-3}$	$0{,}19 \cdot 10^{-4}$		—	
	SuSm					$0{,}1 \cdot 10^{-5}$
pAP41	pSF2124	$0{,}9 \cdot 10^{-2}$				
	pBR322	—			—	
	pACYC184			$0{,}2 \cdot 10^{-5}$	—	
	pSC101					
	pMR5	$0{,}34 \cdot 10^{-4}$	$0{,}33 \cdot 10^{-5}$		—	
	SuSm					$0{,}5 \cdot 10^{-5}$
pAP42	pSF2124	$0{,}8 \cdot 10^{-2}$				
	pBR322	$0{,}14 \cdot 10^{-4}$			—	
	pACYC184			—	—	
	pSC101				—	
	pMR5	$2{,}8 \cdot 10^{-3}$	$0{,}16 \cdot 10^{-3}$		—	
	SuSm					$0{,}22 \cdot 10^{-3}$
pAP43	pSF2124	$2{,}8 \cdot 10^{-2}$				
	pBR322	—			—	
	pACYC184			$0{,}18 \cdot 10^{-5}$	—	
	pSC101				—	
	pMR5	$0{,}31 \cdot 10^{-5}$	$0{,}25 \cdot 10^{-5}$		—	
	SuSm					$0{,}64 \cdot 10^{-3}$
F'lac	pSF2124	$9 \cdot 10^{-2}$				
	pBR322	$0{,}22 \cdot 10^{-5}$			$0{,}1 \cdot 10^{-5}$	
	pACYC184			$0{,}32 \cdot 10^{-4}$	$0{,}22 \cdot 10^{-5}$	
	pSC 101				—	
	pMR5	$0{,}23 \cdot 10^{-5}$	$0{,}18 \cdot 10^{-5}$		—	
	SuSm					$0{,}3 \cdot 10^{-4}$

transconjugants of all the possible classes (during selection for resistance to each antibiotic). For example, it was impossible to identify Tc transconjugants during mobilization of nonconjugative plasmids pMR5, pBR322, and pACYC184 by all transfer factors (except F'lac), although mobilization of these plasmids in individual experiments was demonstrated by selection of transconjugants for other markers, including control experiments with F'lac factor. On the basis of these results it was postulated that mobilization of a particular nonconjugative plasmid for transfer by a conjugative plasmid may be accompanied by inactivation of some of the former's markers, evidently as a result of incorporation of the mobilizing plasmid into the mobilized plasmid in the region of these markers. To test this hypothesis nonselective markers of transconjugants obtained in each "three-parent" cross were analyzed.

By studying transconjugants from all crosses in order to detect nonselective markers it was found (Table 2) that transconjugants Ap^r (mobilizing plasmid pAP42, mobilized plasmid pBR322) and Lm^r (mobilizing plasmids pAP41 and pAP43, mobilized plasmid pACYC184) were Tc^s, i.e., sensitive to tetracycline, although these nonconjugative plasmids control resistance to tetracycline. Conversely transconjugants Ap^r and Km^r obtained in crosses during mobilization of plasmid pMR5 by all transfer factors, including F'lac, possessed all the markers.

Additional experiments also confirmed the donor ability of transconjugants identified in "three-parent" crosses and containing one or other of the test transfer factors and also one each of the nonconjugative test plasmids. *E. coli* C600 F^-rif was used as the recipient. These experiments showed that combined structures (transfer factor + nonconjugative plasmid) are transferred to the recipient cells; inactivated resistance genes of the nonconjugative plasmid, moreover, lost their own properties when they were located in new host cells.

These results are evidence that all the transfer factors studied are capable of mobilizing various nonconjugative plasmids for transfer. The mechanism of this mobilization is not clear [4], but in the present case it is probably linked with incorporation of the mobilizing

TABLE 2. Analysis of Nonselective Markers of Transconjugants

Mobilizing plasmid	Mobilized plasmid	Selective marker	Number of transconjugants (in %) containing nonselective marker				
			Ap	Tc	Km	Lm	Su
pAP42		Ap		0			
F'lac	pBR322	Tc	100				
		Ap		100			
pAP41		Lm		0			
pAP43	pACYC184	Lm		0			
		Lm		0			
F'lac		Tc				100	
pAP38		Ap		100	100		
pAP39		Ap	100	100	100		
		Km		100			
pAP41	pMR5	Km	100	100			
		Ap		100	100		
		Ap		100	100		
pAP42		Km	100	100			
F'lac		Ap		100	100		
		Km	100	100			
pAP43		Ap	100	100			
		Km	100	100			
pAP38		Sm					
pAP39		Sm					
pAP41		Sm					
pAP42		Sm					
pAP43		Sm					
F'lac	Sm Su	Sm					

plasmid into the mobilized. In particular, factor pAP42 is undoubtedly incorporated into plasmid pBR322, and factors pAP41 and pAP43 into plasmid pACYC184 into the region of the Tc gene, inactivating that gene. This hypothesis is confirmed by results showing that incorporation of cloned DNA into nonconjugative plasmids in the region of the Tc gene is accompanied by inactivation of the latter [3, 5]. It is possible that the same mechanism of mobilization applied also to factors pAP38 and pAP39. Examples of mobilization of plasmid pMR5 by various factors, demonstrated by selection of Ap or Km transconjugants, under plasmid pBR322 by factor F', demonstrated by selection of both Ap and Tc transconjugants, are evidence that there may be several sites of incorporation of the mobilizing into the mobilized plasmid.

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