COINTEGRATION BETWEEN F-LIKE CONJUGATIVE PLASMIDS

E. V. Grishina and A. P. Pekhov

UDC 579.842.11:579.252.5].083.1

KEY WORDS: conjugative plasmids; inhibition of conjugative functions; cointegration.

Transfer of plasmids of repressed type (rd) is regulated by inhibitor proteins, synthesized under the control of intrinsic genes (fin-systems), whereas transfer of derepressed (drd) plasmids is regulated by the action of inhibitors synthesized under the control of inhibitory rd-plasmids, located in the same cell. Several genetic fin systems and types of inhibitors synthesized under their control have now been identified and the directions of action of the inhibitors have been determined [1]. However, the nature of the physical connections between inhibiting and inhibited plasmids when located in the same bacterial cell is a completely unstudied problem.

Since previous investigations showed that F-like plasmid pAP22-1::Tn1 can inhibit the conjugative functions of F-like plasmid PAP18-1 (TcColV)drd, the aim of the present investigation was to study physical relations between plasmids when located in *E. coli* cells.

EXPERIMENTAL METHOD

Strains of *E. coli* C600 Str, and also AP106 Str, AP132 Nal, and AP115 Nal, which are derivatives of strain K-12 and contain plasmids pAP22-1::Tn1 and pAP18-1 (TcColV)drd, were used in the experiments. Genetic markers of the test plasmids (resistance to antibiotics, colicinogenicity, sensitivity to donor-specific phages) were detected and conjugation transmission of plasmids and their elimination from the bacterial cells were carried out by standard methods [2, 3]. Plasmid DNA was isolated by centrifugation in a CsCl gradient [5]. DNA was recorded by the use of EcoRI enzyme. Restriction fragments were "dispersed" by horizontal slab electrophoresis in 0.8% agarose gel and their size determined by the use of plasmid DNA of phage λ as the reference DNA [4].

EXPERIMENTAL RESULTS

The work began with insertion of plasmids pAP22-1::Tn1 and pAP18-1 drd into *E. coli* C600 cells, followed by selection of transconjugants containing both these plasmids. A study of selective recombinants with the aim of revealing the inhibitory capacity of plasmid pAP22-1::Tn1 showed that this plasmid does in fact inhibit the transfer function of plasmid pAP18-1 drd; inhibitory capacity; moreover, is manifested against both pilus formation, determined by plasmid pAP18-1 drd, and also the actual transfer of plasmid pAP18-1 drd.

To discover the character of the connection between inhibiting and inhibited plasmids in diplasmid transconjugants *E. coli* C600 (pAP22-1::Tn1/pAP18-1 drd), these transconjugants were later crossed with AP115 recipient cells and the mixtures were seeded on media so that transmission of plasmids pAP22-1::Tn1 and pAP18-1 drd could be recorded separately. When doing these experiments, we assumed that "confluent" transmission of the two plasmids would indicate their cointegration (combination), whereas separate transfer would be evidence that inhibiting and inhibited plasmids exist in the cells in a separate state. The results of these experiments are given in Table 1.

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 294-295, September, 1991. Original article submitted February 3, 1991.

 0007-4888/91/0009-1312$12.50

TABLE 1. Genetic Transfer of Plasmids from Diplasmid Donors to *E. coli* C600 and *E. coli* AP115

Clones of plasmid donor cells	Plasmid	Selective marker	Frequency of transfer per donor cell	Analysis of nonselective markers of transconjugants (for 100 clones studied)		
				number of clones containing markers		
				Tc	Col	Ap
1	pAP18-1 drd	Tc	$3{,}0 \cdot 10^{-1}$		100	80
	pAP22-1::Tn1	Ap	$3{,}0 \cdot 10^{-1}$	100	100	
2	pAP18-1 drd	Tc	$7{,}2 \cdot 10^{-2}$		100	76
	pAP22-1::Tn1	Ap	$6{,}0 \cdot 10^{-2}$	97	97	
3	pAP18-1 drd	Tc	$4{,}5 \cdot 10^{-2}$		100	72
	pAP22-1::Tn1	Ap	$8{,}3 \cdot 10^{-2}$	95	95	
	pAP18-1 drd	Tc	$2{,}6 \cdot 10^{-1}$			
	pAP22-1::Tn1	Ap	$1{,}5 \cdot 10^{-4}$			

TABLE 2. Masses (in M_D) of EcoRI-Restriction Fragments of Plasmid DNA

Plasmid	Restriction fragments and their molecular mass															Total mass
	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10	f11	f12	f13	f14	f15	
pAP22—1:Tn1	17,7	10,6	5,1	3,9	2,7	2,4	1,7									44,1
pAP22-1: :Tn1+ +pAP18—1 drd	19,4	17,7	9,1	5,8	5,3	3,7	3,7	3,1	2,7	2,5	2,3	0,9	0,9	0,5	0,5	78,1
pAP18-1 drd	19,4	9,1	5,2	3,5	3,5	3,1	2,5	0,9	0,9	0,5	0,5					49,1

Table 1 shows that the donor cells of each of the transconjugant clones transmit both plasmids to *E. coli* C600 cells with equal frequencies, and, moreover, close to the frequency of transmission of plasmid pAP18-1 drd, used as the control. On the other hand, the results of analysis of nonselective markers of the transconjugants, given in Table 1, show that inheritance of genes of drug resistance and colicinogenicity takes place jointly in 72-100% of cases, despite the use of mutually excluding media for selection of the transconjugants. The results of these experiments thus suggested that transmission of inhibiting and inhibited plasmids from donor cells to recipient cells is "united" as a result of their combination (cointegration).

To obtain further proof of the physical connection of the inhibiting and inhibited plasmids we studied elimination of these plasmids from transconjugant *E. coli* C600 cells, induced by acridine orange (75 and 100 μg/ml), ethidium bromide (200 and 300 μg/ml), and sodium dodecylsulfate (10% solution). As these experiments showed, plasmids pAP22-1::Tn1 and pAP18-1 drd were insensitive to the eliminating action of the eliminating substances used. However, elimination of plasmids pAP22-1::Tn1 and pAP18-1 drd was not found even after treatment of bacteria containing these plasmids separately, one at a time, i.e., monoplasmid cells, with these eliminating substances. In control experiments, however, in which elimination of plasmid Flac by means of ethidium bromide (300 μg/ml) was studied, elimination of this plasmid amounted to 11.7%. Thus, the results of these experiments neither confirm nor disprove the hypothesis regarding "united" (cointegrative) existence of inhibiting and inhibited plasmids.

The hypothesis regarding cointegration of inhibiting and inhibited plasmids was confirmed in experiments to study isolation of plasmid DNA from cells in which plasmids pAP22-1::Tn1 and pAP18-1 drd were kept together and separately, followed by EcoRI restriction analysis. The results of these experiments are given in Table 2.

It will be clear from Table 2 that plasmid pAP22-1::Tn1 contains seven EcoRI fragments in its genome with a total molecular mass of 44.1 M_D, plasmid pAP18-1 drd contains 11 fragments with a total molecular mass of 49.1 M_D, and the hypothetical plasmid cointegrative structure (pAP22-1::Tn1 + pAP18-1 drd) consists of 15 EcoRI-fragments with molecular mass of 78.1 M_D.

The general conclusion can be drawn from these results that inhibition by conjugative plasmid pAP22-1::Tn1 of the repressed type of transfer function of conjugative plasmid pAP18-1 drd is accompanied by the formation of a cointegrative plasmid structure on the basis of these two plasmids. Data on cointegration of two conjugative plasmids are important not only for an understanding of the mechanisms of genetic regulation of plasmid transfer, but also to explain the evolution of conjugative plasmids possessing several Tra-operons.

LITERATURE CITED

1. A. P. Pekhov and V. P. Shchipkov, Molec. Genet., No. 10, 3 (1986).
2. A. P. Pekhov, V. P. Shchipkov, E. V. Gubar', et al., Zh. Mikrobiol., No. 12, 31 (1980).
3. V. P. Shchipkov, Byull. Éksp. Biol. Med., No. 1, 70 (1982).
4. D. L. Daniels, J. R. De Wet, and F. R. Blattner, J. Virol., **33**, 390 (1980).
5. R. B. Meagher, R. C. Tait, M. Betlach, and H. W. Boyer, Cell, **10**, 521 (1977).