GENETICS

Interactions Between the Genetic Transfer-Inhibiting Systems finU and finV in Polyplasmid Complexes of *Escherichia coli* K-12 Cells

E. V. Grishina, V. P. Shchipkov, and A. P. Pekhov

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The inhibition of conjugative transfer of derepressed plasmids was appreciably increased in polyplasmid complexes of *Escherichia coli* K-12 cells containing two repressed conjugative plasmids with the finV system of genetic transfer regulation, in comparison with the individual effects of each of the two plasmids. Combination of two plasmids with the finU system or of plasmids with the finU and finV systems did not result in such an increase of inhibitory activity.

Key Words: *plasmid; system of genetic transfer regulation; polyplasmid complex*

Several traJ-independent genetic systems (fin-systems) regulating the conjugative transfer of plasmids are known. These systems code for the synthesis of inhibitors determining the efficacy of plasmid transfer in bacterial populations [1,3,4].

Up to the present time, bacterial cells containing only one plasmid with one fin-system of regulation have been used in studies of such systems. However, bacterial cells may carry several plasmids, each of which possesses a particular system of transfer regulation. Hence, we deemed it interesting to investigate the effects of one such system on the other under conditions of the simultaneous presence of several plasmids with fin-systems of their own in the same bacterial cell. The goal of this study was to follow up the interactions of the traJ-independent genetic systems finU and finV in experimental polyplasmid complexes.

MATERIALS AND METHODS

Reference plasmids of the repressed type (rd) JR66a and R485 from N. Willetts' collection (UK) determining the FinU and FinV inhibitors, respectively, and rd-plasmids determining the inhibitors of the same types: pAP3 (FinU), pAP7-1 (FinU), pAP18-1 (FinV), and pAP41::Tn9 (FinV) identified in our laboratory were used in the study. For testing the activity of a respective transfer inhibitor, we used a reference Flac plasmid derepressed (drd) for gene transfer functions (sensitivity to Fin OP, Q, U, V, W, and C inhibitors) and F-like drd-plasmids pAP22-2::Tn1 (Fin U, V) and pAP53::Tn5 (FinV) selected by us [2]. Cells of *Escherichia coli* strains C600 Str and AP132 Nal were hosts (donors) and recipients of the plasmids.

Conjugative transfer of plasmids was carried out using standard crossing of bacteria. The index of drd-plasmid transfer inhibition was defined as the ratio of the incidence of transfer of this plasmid from the cells of a single-plasmid donor to the incidence of its transfer from donor cells containing two or three different plasmids simultaneously.

RESULTS

As the first step of the study, we constructed different diplasmid and triplasmid complexes (plasmid

Department of Biology and General Genetics, Russian University of Peoples' Friendship, Moscow (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

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TABLE 1. Capacity of Repressed Plasmids to Inhibit the Transfer of Reference drd−Plasmid Flac in Tri− and Diplasmid Complexes of *E. coli* K−12 Cells

Composition of triplasmid complex	Composition of diplasmid complex	Type of rd-plasmid inhibitor	Index of inhibition of Flac plasmid transfer
R485		FinV	
pAP18-1		FinV	$(4.1\text{-}5.3)\times10^3$
Flac	pAP18-1	FinV	
	Flac		$(2.2\text{-}2.8)\times10^3$
	R485	FinV	
	Flac		$(7.5\text{-}7.7)\times10$
pAP3		FinU	
pAP7-1		FinU	$(0.7\text{-}0.8)\times10^2$
Flac	pAP3	FinU	
	Flac		$(0.9\text{-}1.0)\times10^2$
	pAP7-1	FinU	
	Flac		$(2.6\text{-}9.8)\times10^3$
pAP3		FinU	
pAP18-1		FinV	$(1.4\text{-}1.8)\times10$
Flac	pAP3	FinU	
	Flac		$(0.9\text{-}1.0)\times10^2$
	pAP18-1	FinV	
	Flac		$(2.2\text{-}2.8)\times10^3$
JR66a		FinU	
R485		FinV	$(1.1\text{-}2.9)\times10^4$
Flac	JR66a	FinU	$(2.3\text{-}6.7)\times10^4$
	Flac		
	R485	FinV	
	Flac		$(3.7\text{-}5.3)\times10^2$

transconjugates) of *E. coli* C600 cells (listed in Table 1) in order to assess the capacity of specific repressed plasmids (alone or in different combinations) to inhibit the transfer of Flac plasmid. Monoplasmid transconjugates of C600 cells with Flac plasmid were used for control.

Studies of the efficacy of conjugative transfer of Flac plasmid from tri- and diplasmid donor cells to recipient AP132 cells in comparison with the transfer of this plasmid from monoplasmid C600 cells showed some differences in the inhibitory activity of the examined rd-plasmids functioning in the complexes formed (Table 1).

Table 1 demonstrates that the simultaneous presence of two rd-plasmids (R485 and pAP18-1) in the cells, each of these plasmids coding for FinV inhibitor, amplifies the effect of inhibition of Flac plasmid transfer in comparison with the individual effects of each plasmid. On the other hand, we did not observe such a boosting effect in the triplasmid complex consisting of rd-plasmids determining the synthesis of FinU inhibitor (pAP3 and pAP7-1) and Flac drd-plasmid.

These results suggest that the activity of FinU inhibitor manifests itself in only one of the two rd-plasmids in this complex, probably pAP3 plasmid.

In complexes with rd-plasmids pAP3 and pAP18-1 containing different fin-systems (finU and finV, respectively) even a certain decrease of Flac transfer inhibition is possible in comparison with the inhibitory activity of the fin-system of each of these plasmids separately (Table 1).

For further study of the detected peculiarities of the above fin-systems we prepared and investigated tri-, di-, and monoplasmid transconjugates with F-like drd-plasmids pAP53::Tn5 and pAP22-2::Tn1, characterized by a narrower spectrum of sensitivity to transfer inhibitors in comparison with the reference Flac plasmid (Table 2).

Table 2 shows that the effect of inhibition of drd-plasmid transfer detected previously for Flac plasmid is enhanced for all possible combinations of rd-plasmids R485, pAP18-1, and pAP41::Tn9 coding for FinV inhibitor. It is noteworthy that this increase was the most marked when drd-plasmid pAP53::Tn5 was used as the test system; this plasmid possesses sensitivity only to FinV inhibitor, i.e., it is a monosensitive test plasmid.

Results confirming the previous conclusions drawn from studies of transconjugates with Flac plasmid were obtained for all the examined plasmid complexes containing two different rd-plasmids determin-

TABLE 2. Capacity of Repressed Plasmids to Inhibit the Transfer of drd−Plasmids pAP53::Tn5 (V) and pAP22−2::Tn1 (U/V) in Tri− and Diplasmid Complexes of *E. coli* K−12 Cells

Composition of triplasmid complex	Composition of diplasmid complex	Type of rd-plasmid inhibitor	Inhibited drd-plasmid	Index of inhibition of drd-plasmid transfer
R485		FinV	pAP53::Tn5	
pAP18-1		FinV		$(0.8\text{-}2.2)\times10^5$
AP53::Tn5	R485	FinV		
	pAP53::Tn5		pAP53::Tn5	8.3-8.5
	pAP18-1	FinV		
	pAP53::Tn5		pAP53::Tn5	$(2.1\text{-}2.7)\times10^3$
R485		FinV		
pAP41::Tn9		FinV	pAP53::Tn5	$(2.0\text{-}5.6)\times10^3$
pAP53::Tn5	R485	FinV		
	pAP53::Tn5		pAP53::Tn5	2.8-6.8
	pAP41::Tn9	FinV		
	pAP53::Tn5		pAP53::Tn5	$(0.6\text{-}1.5)\times10^2$
pAP18-1		FinV		
pAP41::Tn9		FinV	pAP22-2::Tn1	$(6.2\text{-}9.2)\times10^5$
pAP22-2::Tn1	pAP18-1	FinV		
	pAP22-2::Tn1		pAP22-2::Tn1	$(1.1\text{-}1.7)\times10^5$
	pAP41::Tn9	FinV		
	pAP22-2::Tn1		pAP22-2::Tn1	$(3.3\text{-}3.7)\times10$
pAP3		FinU		
pAP7-1		FinU	pAP22-2::Tn1	$(0.4\text{-}1.1)\times10^2$
pAP22-2::Tn1	pAP3	FinU		
	pAP22-2::Tn1		pAP22-2::Tn1	$(0.3\text{-}1.1)\times10^2$
	pAP7-1	FinU		
	pAP22-2::Tn1		pAP22-2::Tn1	3.7-8.2
pAP7-1		FinU		
pAP41::Tn9		FinV	pAP22-2::Tn1	$(5\text{-}11)\times10$
pAP22-2::Tn1	pAP7-1	FinU		
	pAP22-2::Tn1		pAP22-2::Tn1	$(2.6\text{-}3.3)\times10$
	pAP41::Tn9	FinV		
	pAP22-2::Tn1		pAP22-2::Tn1	$(2.2\text{-}9.6)\times10$

ing the synthesis of FinU or FinU and FinV inhibitors (Table 2).

These data attest, on the one hand, that the genetic systems finU and finV interact in the regulation of plasmid transfer, but, on the other, that there are essential differences in the types of interactions between Fin-products of different plasmids. For example, in plasmid complexes containing two different plasmids each of which possesses the FinV system an appreciable increase of the inhibitory effect is observed, whereas combinations of two finU or finU and finV systems does not lead to such a boost or is even associated with a decrease of the inhibitory activity. These differences may be related to the mechanisms of action of plasmid-transfer inhibitors FinU and FinV separately. However, the specific mechanisms of the effects observed are still to be investigated.

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