determinants of two different fin systems controlling the synthesis of FinU and FinV transfer inhibitors. This hypothesis is confirmed by the quantitative differences between the levels of inhibition by cointegrative plasmids of the transfer of reference drd plasmids, which are sensitive simultaneously to FinU and FinV inhibitors, and the level of inhibition of the transfer of pAP18-1::Tn9 plasmids, which are sensitive to only one of these inhibitors. FinV. Differences in IPI values of reference plasmids pAP22-2::Tn1, pAP11-2::Tn5, and pAP10-2::Tn9 detected during their inhibition by cointegrative plasmids in comparison with the inhibitory activities of "parent" plasmids pAP42, pRSF2124 and pUB781 also support this hypothesis.

Our data confirm the presence of specific fin systems in the genomes of some nonconjugative plasmids, this permitting us to expect the appearance of such plasmids in the course of evolution of more intricate genetic structures, to which conjugative plasmids belong.

These results allow us to conclude that the formation of conjugative cointegrative plasmids is paralleled by the formation in their genomes of complex fin systems capable of providing synthesis of transfer inhibitors of various functional types; but how many such systems a cointegrative plasmid genome may contain whether there may be "hybrid" fin systems be among them, and how they function are still question to be answered.

REFERENCES

1. L. V. Maksimenko and A. P. Pekhov, *Byull. Eksp. Biol. Med.*, № 8, 199-201 (1992).
2. A. P. Pakhov, V. P. Shchipkov, E. V. Gubar', *et al.*, *Zh. Microbiol.*, № 12, 31-36 (1980).
3. A. P. Pekhov, *Bacterial Plasmids* [in Russian], Moscow (1986).
4. A. P. Pekhov and V. P. Shchipkov, *Molek. Genet.*, № 10, 3-13 (1986).
5. R.C. Clowes and W. Hayes, *Genetics*, Oxford (1968).
6. N. Willetts and R. Skurray, *Ann. Rev. Genet.*, **14**, 14-76 (1980).
7. N. Willetts and R. Skurray, *Cell Molec. Biol.*, **2**, 1110-1113 (1987).

Role of *E.coli* K-12 Chromosomal THR-LEU Segment in Expression of the FIN System of Inhibition of pAP53 F-Like Plasmid Transfer

N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov

UDC 579.842.11:579.252.5].08

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 9, pp. 306-307, September, 1993
Original article submitted April 1, 1993

Key Words: *plasmid; transfer inhibition system; chromosomal genes*

Genetic transfer of plasmids is determined by transfer plasmid genes (tra genes) whose functioning is controlled by a plasmid system of genetic regulation (fin system). The ability of plasmids to disseminate in natural bacterial populations depends on fin system activity [1,7]. Meanwhile, certain sites of the bacterial chromosome have an undoubted influence of the expression of tra genes [5,6]. However, the role of chromosomal genes in the expression of a particular fin system in uncnown.

We tried to elucidate the effect of chromosomal genes E cli K-12 cells on the activity of a

Deparitment of Biology and General Genetics, Russan Peoples' Friendship University, Moscow. (Presented by T. T. Beerezov, Member of the Russian Academy of Mdical Sciencers)

0007-4888/93/0009-1148$12.50

TABLE 1. Genetic Recombinants Ca pable of Stimulating pAP53 Plasmid Transfer Inhibition (Tis^+)

Host cell	Duration of conjugation hybridization, min	Selective marker	Number of Tested recombinants	Number of Tis^+ recombinations	
				absolute	%
AB1157	5	Thr−Leu	25	6	24
		Pro	25	0	0
		Thr−Leu	25	9	36
AB1157	120	Pro	25	2	8
		Arg	25	2	8
		His	25	0	0
C600	120	Thr−Leu	25	5	20

system inhibiting transfer of pAP53 F-like plasmid sensitive to type Fin V transfer inhibitor [2] and to pinpoint the localization of these genes in an annular bacterial chromosome.

MATERIALS AND METHODS

Previously indentified F-like plasmids pAP18-1 (Tc, Col V) and a derepressed (drd) Col plasmid pAP53 transposon-containing variant [3] were used in the study. *E.coli* K-2 daughter strain cells AB1157, (Thr-Leu-Pro-Arg-His-Str), C600 (thr-Leu-Rif), AP132 (Nal), and AP115 (Met-Nal) were used as plasmid hosts and *E.coli* K-12 Hfr C cells as donors of chromosomal genetic material. Conjugate hybridisation of bacteria and genetic recombinant selection were carried out according to standard methods. The index of pAP53 plasmid transfer inhibition by pAP18-1 plasmid was defined as the ratio of the incidence of drd plasmid transfer from two-plasmid transconjugate cells. The index of this ratio represents the quotient from dividing the transfer inhiibition index (TII) values for Thr^+-Leu^+ recombinant cells by the value of the initial strain cells (Thr^--Leu^-). The presence of F and F-like plasmides in the cells was judged on the basis of cll sensitivity to MS2 pilus-specific phage.

RESULTS

When investigating pAP18-1 plasmid inhibitory activity (Fin V) towardc drd plasmid pAP53::Tn5 in *E.coli* K-12 ells, we found that the level of this activity in prototrophic strains (e.g., in pAP132 strain cells) was 4-8 times higher than in some auxotrophic strains (AB1157, C600). Assuming this to be due sme features of the genetic organization of the ssaid *E.coli* K-12 auxotrophic strains obta ined as a result of repeated treatment of the initial strains with various mutagens [4], we attenpted to detect these features.

Genetic recombinations of four types (The^+-Leu^+-Pro^+-Arg^+ and His^+ not containing plasmid F in their genomes were selected in conjugation hybridization of strains Hfr C and AB1157. Such recombinants were then used as recipients to obtain single-plasmid and two-plasmid transconjugates containing the tested plasmids, which lwere investigated to assess the lvels of pAP18- plasmid inhibitory activity toward plasmid pAP53::Tn5.

The results, summarized in Table 1, indicate that some of the examined recombinants were able to increase the level of pAP18-1 plasmid ihibitory activity to a value close to the values obtained for prototrophic cells. We termed thist characteristic of the said recombinants Tis (ransfer inhibition stimulation). Table 1 demonstrates that in the majority of cases genetic recombinants selected for the closely linked chromosomal markers Thr^+-Leu^+ posessed the Tis^+ phenotype. Analysis of tshe nonselective markers of the tested recombinants howed that all Tis^+ recombinants selected for markers other than Thr^+-Leu^+ possessed the same markers as nonselective ones (that is, they had the Tis^+-Thr^+-Leu^+ phenotype).

Table 2 presents data on TII values of pAP53 plasmid in cells of the initial strain AB1157 (Thr^-

TABLE 2. Efficacy of pAP53::Th5 Plasmid Transfer Inhibition by pAP18−1 Plasmid in Thr^+−Leu^+ Relcomdinant Cells and Initial Strains of *E.coli* K−12

Host cells	Duration of conjugation hybridization, min	Plasmid pAP53::ThP5 TII in cells of initial strains	Plasmid pA53::Th5 TII in Thr^+-Leu^+ recombinants	Index of TII ratio
AB1157	5	$3.0 \cdot 10^2 - 1.8 \cdot 10^3$	$1.6 \cdot 10^3 - 4.6 \cdot 10^4$	5.3−26
AB1157	120	$1.1 \cdot 10^2 - 4.7 \cdot 10^3$	$1.2 \cdot 10^3 - 9.2 \cdot 10^4$	10.9−19.5
C600	120	$1.6 \cdot 10^4 - 4.3 \cdot 10^4$	$1.0 \cdot 10^5 - 3.6 \cdot 10^5$	6.2−8.8

-Leu⁻-Pro⁻-Arg⁻-His⁻) and in selected Thr⁺-Leu⁺ recombinants with the Tis⁺ phenotype. It is evident that the TII ratio values of such recombinants in cells of the initial strain indicate an increased lrvrl of pAP53::Tn5 plasmid transfer inhibition in Thr⁺-Leu⁺ recombinants by 5.3-26 times.

Analyzing the results, we postulated the presence of a genetic site tis on the *E.coli* K-12 cell chromosome which is necessary for normal expression of the plasmid system of conjugation transfer regukation with type Fin V inhibitor participation. It is probable that, in contrast to Hfr C strain cells, cells of the semiauxotrophic strain AB1157 are characterized by mutational changes in their genetic region tis which phenotypicawlly manifest themselves in a changed fin V system activity. It may be assumed that the this region is linked to the Thr-Leu chromosomal segment (genetic region) of the *E.coli* K-12 chromosome.

To test this hypothesis we selected and examined Thr⁺-Leu⁺ recombinants resulting from hybridization of cells from the Hfr C and C600 Rif strains. As is seen from Tables 1 and 2, the results of these experiments confirmed this region linkage to the Thr⁺-Leu⁺ segment of the *E.coli* K-12 chromosome.

Summining up the results, we may conclude that besides plasmid genes provodong functionong of the type fin V plasmid transfer genetic regulation system, a significant role in this process is played by the Thr-Leu chromosomal segment.

REFERENCES

1. A. P. Pekhov, *Bacterial Plasmids* [in Russian], Moscow (1986).
2. V. P. Shchipkov, N. I. Buyanova, G. I Myandina, and A. P. Pekhov, *Byull. Eksp. Biol. Med.*, № 8, 226-227 (1985).
3. V. P. Shchipkov, N. I. Buyanova, and A. P. Pekhov, *Antibiotiki*, № 1, 28-31 (1986).
4. B. J. Bachmann, *Bact. Rev.*, **26**, 525-557 (1972).
5. L. Beutin and M. Achtman, *J. Bact.*, **139**, 730-737 (1979).
6. N. S. Willetts and R. Surray, *Ann. Rev. Genet.*, **14**, 41-76 (1980).
7. N. S. Willetts and R. Surray, in: *Escherichia coli and Salmonells typhimurium: Cellular and Molecular Biology*, Vol. 2, Washington (1987), pp. 1110-1133.