GENETICS

Specificity of *E. coli* K-12 Chromosomal Segment Regulating the Expression of Systems Inhibiting Flac Plasmid Transfer

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 11, pp. 559-561, November, 1997
Original article submitted December 2, 1996

Specificity of *E. coli* K-12 chromosomal Thr-Leu-segment (genetic locus tis) regulating the expression of systems inhibiting Flac plasmid transfer is revealed. The findings point to a complex (polygenous) structure of this locus.

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

Chromosome segments of bacterial host cells participate in the regulation of F plasmid and some other F-like plasmid transfer along with plasmid genetic systems (fin-systems) [1-4]. Previously, we revealed the role of *E. coli* K-12 Thr-Leu chromosome segment in the expression of fin V, an F-like plasmid pAP53 system [2]. Possible effect of chromosome locus, denoted as locus tis, on the function of other known systems regulating plasmid transfer is still unclear. In this study we compared the specificity of locus tis toward fin systems OP, Q, U, V, and W, capable of inhibiting conjugative transfer of Flac plasmid [5,6].

MATERIALS AND METHODS

Reference derepressed (drd) Flac plasmid and reference repressed (rd) plasmids R100 (fin OP), TP108 (fin Q), JR66a (fin U), R485 (fin V), and R455 (fin W) from N. Willetts' collection (UK) were used. Plasmid hosts were *E. coli* K-12 cells C600 (Lac, Thr, Leu, and Rif) and AP132 (Lac and Nal). *E. coli* strain Hfr C were donors of the tis chromosome locus.

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Conjugation crossing-over of bacteria and selection of genetic recombinants and plasmid transconjugates were carried out routinely. For assessing the capacity of Fin^+ plasmids to inhibit the functions of Flac plasmid transfer genes (Tra-function), the transfer inhibition index (TII) was calculated as the ratio of the rate of Flac plasmid transfer from single-plasmid donor cells to the rate of the same plasmid transfer from diplasmid transconjugate cells. Functional activity of "sex" piles whose production is regulated by the Flac plasmid tra genes was assessed from the sensitivity of relevant bacterial cells to pile-specific MS2 phage.

RESULTS

Conjugation crossing over of Hfr C (Tis^+) cells with C600 (Tis^-) recipient strain cells was performed to obtain genetic recombinants containing tis locus. The resultant Thr^+Leu^+ recombinants were used as bacterial hosts containing drd Flac plasmid and one reference rd plasmid with a known fin type. The corresponding single-plasmid transconjugates (recombinant cells containing Flac plasmid alone) were used as control.

Flac plasmid TII for each reference Fin^+ plasmid was estimated from the results of subsequent cros-

0007-4888/97/0011-1116$18.00

TABLE 1. Expression of Plasmid fin Systems in *E. coli* K-12 Cells with Tis$^+$ and Tis$^-$ Phenotype

Host cell Tis phenotype	Plasmid content of host cell	fin system type of rd plasmid	Flac TII	Tis$^+$ effect value
C600 Tis$^+$	R100+Flac	OP	232-430	7.5-22.5
	TP108+Flac	Q	10-3200	33-3440
	JR66a+Flac	U	1600-31660	10.7-107
	R485+Flac	V	385-406	8.0-10.0
	R455+Flac	W	203-200000	10.0-5263
C600 Tis$^-$	R100+Flac	OP	10-57.6	
	TP108+Flac	Q	0.3-0.93	
	JR66a+Flac	U	149-295	
	R485+Flac	V	35-48	
	R455+Flac	W	20-38	

sings of the resultant diplasmid and single-plasmid transconjugates with the recipient strain AP132 cells. At least 50 plasmid transconjugates were investigated for identifying the Tis$^+$/Tis$^-$ phenotype of the resultant recombinants in all cases.

The Thr$^+$Leu$^+$ recombinants obtained in our experiments are characterized by different TII values, permitting us to divide them into two phenotypic groups (Tis$^+$ and Tis$^-$, Table 1). For characterization of Tis$^+$ effect, its value was determined in each case, i.e., the ratio of Flac TII value derived in study of C600 Tis$^+$ cells to similar value for C600 Tis$^-$ cells.

Table 1 shows that the value of Tis$^+$ effect varies from 7.5 to 5263 and differs appreciably for individual fin systems. The incidence of Tis$^+$ effect in Thr$^+$Leu$^+$ recombinants varied in different fin systems. For fin systems Q and W, Tis$^+$ effect was detected in 72 and 68% of all examined Thr$^+$Leu$^+$ recombinants, respectively. For fin OP, fin U, and fin V, these values varied from 20 to 29%.

A special series of experiments was carried out to assess the specificity of Tis$^+$ effect of individual Thr$^+$Leu$^+$ recombinants, initially detected for R455 rd plasmid (fin W) toward other plasmids carrying other fin systems. The results permit us to identify 3 phenotypical classes of such recombinants. In the first case, Tis$^+$ effect was observed only toward fin W system but was completely absent for rd-plasmids with fin systems OP, Q, and V. In the second case, this effect was observed for fin W and fin OP systems.

Group 3 Thr$^+$Leu$^+$ recombinants exerted Tis$^+$ effect toward fin W group, but fin U system was completely unable to inhibit Flac plasmid transfer, i.e., Flac plasmid TII in this case was even lower than for Tis$^-$ cells. The opposite effects of recombinants of this group toward two different fin systems may be due to the genetic structure of tis locus.

The results indicate a complex (polygenous) structure of identified chromosome tis locus of *E. coli* K-12 cells. The detected differences in the specificity of Tis$^+$ effect in different groups of recombinants may be explained by the genetic structure of this locus, which formed as a result of its rearrangement. Further studies are needed to verify this conclusion.

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