GENETIC ORGANIZATION OF THE TRANSFER REGION OF F-LIKE PLASMID pAP18-1

N. I. Buyanova, V. P. Shchipkov, K. S. Krivskaya, and A. P. Pekhov

UDC 579.252.5:579.842.11

KEY WORDS: F-like plasmid; transfer system; tra-genes; fin locus.

Conjugation transfer of F plasmid and certain F-like plasmids is connected with the presence of a tra region in their genome, consisting of tra genes, under the control of which plasmid-specific pili and other phenotypic manifestations of the transfer system of bacterial cells containing these plasmids are formed [2, 6, 7, 10, 11]. However, the organization of genetic control of transfer of other F-like plasmids, notably plasmids found in bacteria of natural populations, has not yet been studied.

The aim of this investigation was to identify genes of the tra region and to determine its possible location in the genome of a derepressed (drd) mutant of F-like plasmid pAP18-1 (Tc, Col*V*), found previously in cells of a strain of *Escherichia coli* isolated from a sick calf [3]. The problem was solved by identifying Tra$^-$-mutants and molecular cloning of plasmid pAP18-ldrd.

EXPERIMENTAL METHOD

We used plasmid pAP18-ldrd and its genetically marked derivatives pAP18-ldrd::Tn5 and pAP18-ldrd::Tn9, obtained by insertion of transposons Tn5 and Tn9, respectively, into the plasmid gene [3]. The hosts of the plasmids were cells of *E. coli* strains AP106 (Str), HB101 (Str), and AP115 (Nal), derivatives of *E. coli* strain K12. Bacteria containing the plasmids were treated with N-methyl-N′-nitro-N-nitrosoguanidine by the standard method [1]. The plasmids were transferred in standard bacterial crosses. Ability of the bacteria to synthesize plasmid-specific pili was determined on the basis of their sensitivity to pilus-specific phage MS2. Plasmid DNA was isolated by the method of Meagher et al. [9], with minor modifications. Molecular cloning of SalI-restriction fragments of DNA of plasmid pAP18-ldrd in the composition of vector plasmid pBR325 followed the usual methods [8].

EXPERIMENTAL RESULTS

The investigation began with treatment of populations of *E. coli* AP115 cells containing plasmid pAP18-ldrd::Tn5 or pAP18-ldrd::Tn9 with nitrosoguanidine and searchin in these populations for bacterial mutants which had lost their sensitivity to pilus-specific phage MS2.

As a result of these experiments 11 clones of bacteria with resistance to phage MS2 were selected. Since the resistance of the bacteria to this phage also was maintained during subsequent conjugation transmission of plasmids from cells of the mutant clones into cells of various recipient strains of *E. coli*, it was concluded that the changes observed in the phenotype of the bacteria are connected with mutation changes in the plasmids contained in them.

The subsequent study of the conjugation properties of the selected mutants showed that most of them are characterized not only by loss of phage-sensitivity, but also by a decrease in the frequency of plasmid transfer. Meanwhile, the mutant plasmids, identified as pAP18-1::Tn5-1 and pAP18-1::Tn5-5, were characterized by a transfer frequency comparable with that of the corresponding drd-plasmid pAP18-ldrd::Tn5.

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. 110, No. 9, pp. 298-301, September, 1990. Original article submitted March 16, 1990.

 0007-4888/90/0009-1250$12.50 ©1991 Plenum Publishing Corporation

TABLE 1. Properties of F-Like drd-Plasmids pAP18-ldrd::Tn5, pAP18-ldrd::Tn9, and Their Mutants in Cells of *E. coli* AP106

Plasmid	Ability to determine MS2-sensitivity of cells	Frequency of transfer of plasmid into AP115 cells (per 100 donors)	Ability to inhibit tra-function of drd-plasmids	
			Flac	pAP53::Tn5 pAP53::Tn9
pAP18-155Tn5-1	—	13—15	—	—
pAP18-1::Tn9-2	—	$(1,8—2,0)\cdot10^{-4}$	—	—
pAP18-1::Tn9-3	—	1,5—2,0	—	—
pAP18-1::Tn5-4	—	1,3—2,0	—	—
pAP18-1::Tn5-5	—	12—20	—	—
pAP18-1::Tn5-6	—	$(3,1—9,0)\cdot10^{-2}$	—	—
pAP18-1::Tn5-7	—	0,14—0,16	+	—
pAP18-1::Tn5-8	—	2,3—4,0	+	—
pAP18-1::Tn5-9	—	$(6,8—7,1)\cdot10^{-2}$	+	—
pAP18-1::Tn5-10	—	$(1,1—1,4)\cdot10^{-2}$	+	+
pAP18-1::Tn5-11	—	$(1,4—1,5)\cdot10^{-2}$	+	+
pAP18-1drd::Tn5	+	15—18	—	—
pAP18-1drd::Tn9	+	36—42	—	—

The list of selected mutants and their characteristics (compared with the corresponding drd-plasmids are given in Table 1.

The further study of the 11 plasmid mutants showed that six of them, like the original drd-plasmids, cannot inhibit the function of the tra-genes of plasmid Flac, i.e., they belong to the Fin$^-$-plasmid category, whereas the other five mutants possessed this ability, i.e., they were plasmids of the Fin$^+$ type (Table 1).

When analyzing these data we suggested that the inability of the mutant plasmids of the Fin$^-$-group (pAP18-1::Tn5-1, pAP18-1::Tn9-2, pAP18-1::Tn9-3, pAP18-1::Tn5-4, pAP18-1::Tn5-5, pAP18-1::Tn5-6) to perform the function of pilus formation is linked with mutation changes in the tra genes of these plasmids, controlling that function. As regards plasmids of the Fin$^+$-group (pAP18-1::Tn5-7-pAP18-1::Tn5-11), loss of their ability to control the function of pilus formation can be explained, in our view, by a reverse mutation in the Fin-locus of the original drd-plasmid pAP18-ldrd::Tn5, i.e., by conversion of the drd-plasmid into an rd-plasmid. The ability of the Fin$^+$-plasmids pAP18-1-1::Tn5-10 and pAP18-1::Tn5-11 to inhibit the transfer function of drd-plasmid pAP53::Tn9 (Table 1), which is sensitive only to a transfer inhibitor of the FinV type [4], is evidence that these plasmids acquired the ability to determine synthesis of a transfer inhibitor of the FinV type. Incidentally, the original Fin$^+$-plasmid of self-repressed type pAP18-lral also possesses the same ability [3].

Considering data on partial compatibility of plasmids pAP18-ldrd::Tn5 and pAP18-ldrd::Tn9 (5), we undertook a complementation analysis of the Tra$^-$-mutants of these plasmids, i.e., mutants of the Fin$^-$-group. For this purpose we obtained two-plasmid transconjugants, i.e., cells containing two different mutant plasmids at the same time, and we tested their sensitivity to phage MS2. The results are given in Table 2, which shows that the mutant plasmids tested can be classified within the limits of no fewer than three different complementation groups, presumptive evidence of the existence of at least three different tra-regions in the genome of plasmid pAP18-1, which can be conventionally described as tra 1, tra 2, and tra 3. The study of the Tra$^-$-mutants in complementation tests with drd-plasmids Flac and pAP42::Tn1 showed that mutations of all three presumptive tra regions are complemented by tra genes of plasmid Flac, whereas in the case of plasmid pAP42::Tn1 complementation of only one of them was observed (tra 2). These results suggest the existence of similarity and differences in the allelic specificity of the tra genes of these plasmids.

To determine the localization of the identified genes of the tra regions of plasmid pAP18-ldrd in the genome, molecular cloning of SalI-fragments of DNA in the composition of the vector plasmid pBR325 (ApCmTc) was carried out. After transformation of *E. coli* HB101 cells by ligated mixtures containing one or other SalI-fragment of DNA of plasmid pAP18-ldrd and restricted DNA of the vector plasmid, from the surface of the selective medium on which transformants resistant to ampicillin (Ap^r) appeared at 37°C, variants which had lost their resistance to tetracycline (Tc^s) were selected. Since in the process of restriction of plasmid pBR325 by endonuclease SalI and subsequent incorporation of the cloned fragment into it, the tetracycline resistance gene of this plasmid was inactivated, we regarded transformants with the Ap^{r}Cm^{r}Tc^s phenotype as the most probable carriers of recombinant plasmids (vector plasmid + SalI-fragment of the cloned plasmid).

TABLE 2. Complementation Analysis of Tra⁻-Mutants of Plasmids pAP18-ldrd::Tn5 and pAP18-ldrd::Tn9

Tra⁻-mutants of plasmid pAP18-ldrd::Tn9	Tra⁻-mutants of plasmid pAP18-ldrd::Tn5			
	pAP18-1::Tn5-1 (tra 1)	pAP18-1::Tn5-4 (tra 1)	pAP18-1::Tn5-5 (tra 1)	pAP18-1::Tn5-6 (tra 2)
pAP18-1::Tn9-2 (tra 2)	+	+	+	−
pAP18-1::Tn9-3 (tra 3)	+	+	+	+

Legend. +(−) Ability (inability) of mutant plasmids to restore by complementation the function of pilus formation in cells of two-plasmid transconjugants to the level characteristic of the original drd-plasmid.

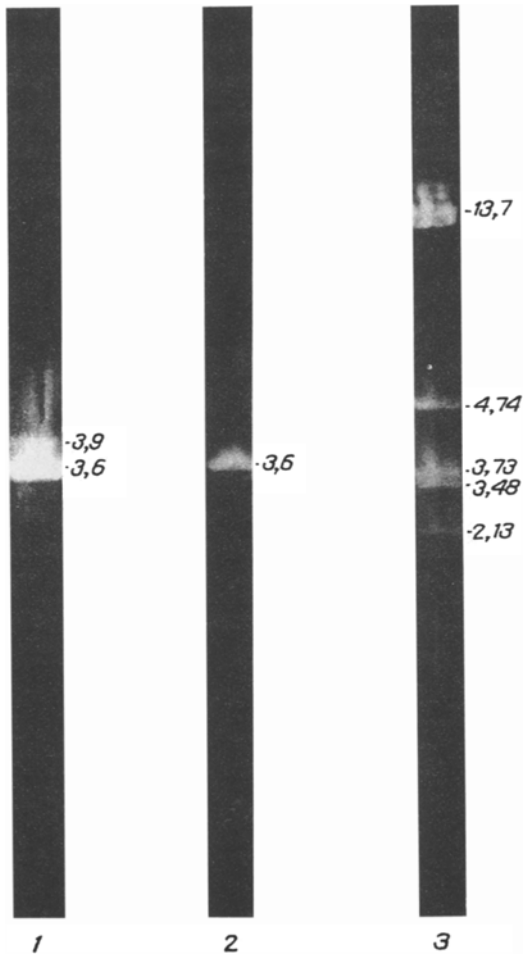

Fig. 1. Electrophoretic fractionation of restriction products of plasmid pAP105 and pBR325 by SalI enzyme. 1) pAP105, SalI, 2) pBR325, SalI, 3) λ, EcoRI.

As a result of subsequent complementation analysis of the recombinant and tra-mutant plasmids, a positive complementation effect was found in the case of the recombinant plasmid selected after conujugation of the SalI-fragment f5 of plasmid pAP18-ldrd, which we called pAP105, and the mutant plasmid pAP18-1::Tn9-2 (tra 2). Subsequent restriction analysis of the DNA of plasmid pAP105 using endonuclease SalI showed that this plasmid in fact consists of vector plasmid pBR325 and the SalI-fragment f5 (3.9 megadaltons) of plasmid pAP18-ldrd (Fig. 1). It can be concluded from the results of this complementation analysis that the tra 2 region of plasmid pAP18-ldrd, which is related to the control of synthesis of plasmid-specific pili of F-type, is located in the SalI-fragment f5 of this plasmid.

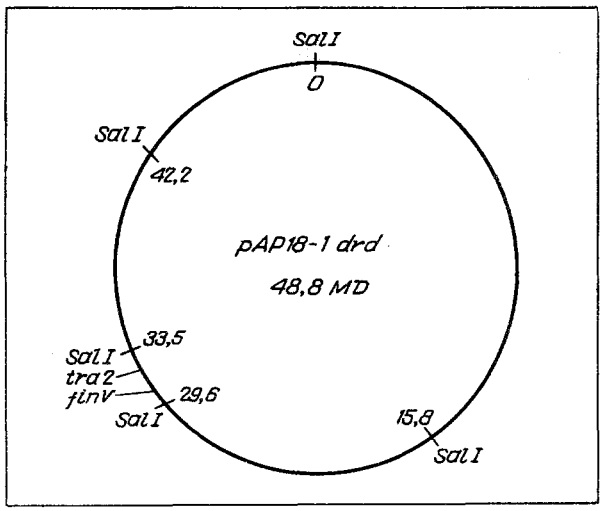

Fig. 2. Location of genes of tra region and restriction map of plasmid pAP18-ldrd (location of genes in the SalI-fragment f5 is conventional).

The study of two-plasmid transconjugants containing plasmid pAP105 and the Fin$^+$-plasmid pAP18-1::Tn5-10 or pAP18-1::Tn5-11 revealed restoration of the pilus-forming function (sensitive to phage MS2) to the level characteristic of the drd-plasmid. These results can be explained by the transdominant character of the mutation arising under the influence of nitrosoguanidine in the FinV locus of the wild-type plasmid pAP18-lrd, and leading to the formation of the test plasmid pAP18-ldrd [3]. The results also show that the FinV locus, like the tra 2 region, is located in the SalI-fragment f5 of the test plasmid. The probable localization of these genetic structures in the SalI-fragment f5 of the restriction map of plasmid pAP18-ldrd, constructed on the basis of the previous map of this plasmid [5], is given in Fig. 2.

It can be concluded from the generalization of these results that the genome of plasmid pAP18-1 contains at least three regions determining synthesis of F-like pili. One of these regions (tra 2), and also the FinV genetic locus, involved in regulation of tra functions, are located in the SalI-fragment f5 of this plasmid.

LITERATURE CITED

1. D. Miller, Experiments in Molecular Genetics [Russian translation], Moscow (1976), pp. 116-117.
2. A. P. Pekhov, Plasmids of Bacteria [in Russian], Moscow (1986).
3. V. P. Shchipkov, N. I. Buyanova, and A. P. Pekhov, Antibiotiki, No. 1, 28 (1986).
4. V. P. Shchipkov, N. I. Buyanova, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 10, 459 (1986).
5. V. P. Shchipkov, N. I. Buyanova, E. V. Shcherbakova, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 2, 191 (1988).
6. B. S. Hansen, P. A. Manning, and M. Achtman, J. Bact., **150**, 89 (1982).
7. M. A. Kehoe and T. J. Foster, Molec. Gen. Genet., **176**, 113 (1979).
8. T. Maniatis, E. F. Fritsch, and J. Sambrook, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, New York (1982).
9. R. B. Meagher, R. C. Tait, M. Betlach, et al., Cell, **10**, 521 (1977).
10. N. S. Willetts and R. Skurray, Ann. Rev. Genet., **14**, 41 (1980).
11. N. S. Willetts and R. Skurray, *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology, Vol. 2, Washington (1987), pp. 1110-1133.