CLONING REGION OF GENETIC TRANSFER TRA AND FIN GENE OF CONJUGATED PLASMID pAP17-1 IN *Escherichia coli* K-12 CELLS

D. E. Kulumbetova, V. P. Shchipkov, and A. P. Pekhov

UDC 579.842.11:579.252.5:579.257/.083.1

KEY WORDS: plasmid, tra region; fin gene; transfer regulation system

Analysis of conjugation transfer of F plasmid and of various F-like plasmids in *E. coli* K-12 cells points to the existence of six genetic systems (fin-systems) for the regulation of this transfer, contained in the genomes of these plasmids, and also of individual plasmids which are not F-like [4, 5, 8, 12, 13]. The latter include conjugated plasma pAP17-1, determining synthesis of β-hemolysin [3], and also an inhibitor of the F plasmid, classified as an inhibitor of the Fin U type [1].

To study the molecular localization of the tra region and fin gene on plasmid pAP17-1, experiments were carried out on the molecular cloning of these genetic structures. The results are described below.

EXPERIMENTAL METHOD

Strain *E. coli* AP106, containing the Hly-plasmid pAP17-1::Tn9, and *E. coli* strain HB101, containing the vector plasmid pBR322 (Ap, Tc) were used. The recipients were *E. coli* strains AP132 Nal, C600 Rif, and HB101 Str.

Plasmid DNAs were isolated by centrifugation in a CsCl density gradient [6, 11]. Restriction of the plasmid DNA was carried out with endonucleases EcoRI and HindIII by the standard method [10]. The restriction fragments of DNA were fractionated in 0.65% agarose gel by horizontal slab electrophoresis. To determine the dimensions of these fragments, data on molecular mass of restriction fragments of phage λ DNA were used as standards [7]. HindIII fragments of DNA of plasmid pAP17-1::Tn9 were used for cloning. Plasmid pBR322 served as the genetic vector. Ligation [10] and transformation of *E. coli* HB101 were carried out [9]. The conjugative properties of the resulting recombinant plasmids and their ability to inhibit the transfer (tra) function of standard derepressed (dfd) plasmids Flac and pAP11-2::Tn5 were investigated by the methods described previously [2].

EXPERIMENTAL RESULTS

Restriction analysis of DNA of plasmid pAP17-1::Tn9 using restriction endonucleases EcoRI and HindIII showed that it is split by these enzymes into 10 and 11 fragments respectively (Fig. 1).

The largest HindIII fragments of DNA of the test plasmid (f1, f2, f3, f4, f5, f6) and also DNA of vector plasmid pBR322 (Ap, Tc) were isolated for the cloning experiments. After transformation of *E. coli* HB101 by ligating mixtures (the fragment of DNA of the test plasmid plus restricted DNA of the vector plasmid) variants which had lost their resistance to tetracyclene (TCS) were selected from the surface of the selective medium on which transformants resistant to ampicillin (Ap^r) grew. Since in the process of restriction by endonuclease HindIII and subsequent ligation, inactivation of the Tc gene of the vector plasmid took place, we also regarded transformants (Ap^r, Tc^s) as clones of bacteria carrying the recombinant plasmids.

To determine whether the recombinant plasmids possess the tra region arising from plasmid pAP17-1, in the next experiments the possibility of conjugation transfer of the Ap^r marker by transformants into the cells of suitable recipient Strains was determined. The possibility of inhibition of these transformants of the tra functions of plasmid Flac, sensitive to all known six transfer inhibitors [8, 12], and also of plasmid pAP11-2::Tn5, sensitive to transfer inhibitors of types Fin OP, U, and V [4] also was determined.

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 192-194, August, 1990. Original article submitted April 10, 1989.

0007-4888/90/0008-1103$12.50

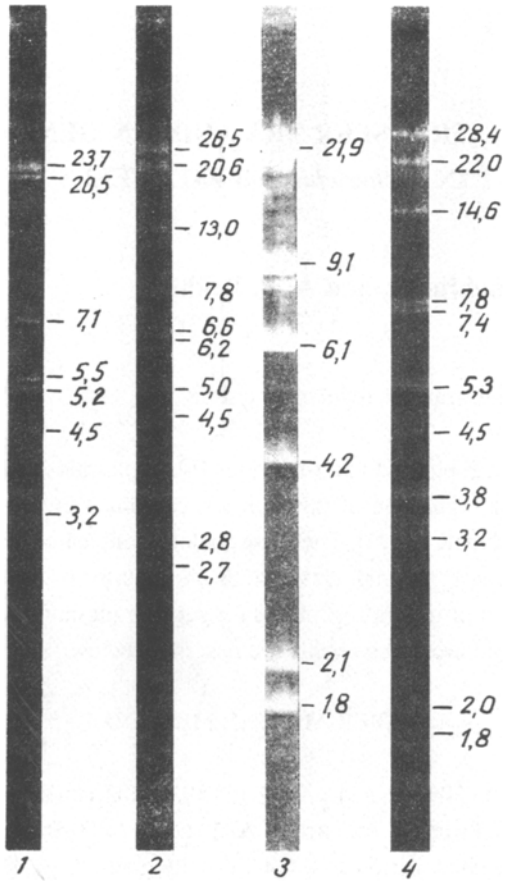

Fig. 1. Electrophoresis of restriction products of DNA of plasmid pAP17-1::Tn9. 1) λ, EcoRI (control), 2) pAP17-1:: Tn9, EcoRI, 3) λ, HindIII, 4) pAP17-1::Tn9, HindIII. Numbers indicate molecular masses of fragments (in thousands of base pairs).

TABLE 1. Conjugation and Inhibitory Properties of Plasmid pDK4

Plasmid content of HB101 cells (donors)	Frequency of transfer of plasmid into C600 Rif cells (recipients)	Frequency of transfer of standard plasmid pAP11-2:: Tn 5	Index of inhibition of transfer of plasmid pAP11-2::Tn 5
pDK4	$0{,}7 \cdot 10^{-5}$		
	$2{,}1 \cdot 10^{-5}$	—	—
pAP17-1::Tn 9	$1{,}0 \cdot 10^{-2}$		
	$1{,}2 \cdot 10^{-2}$		
pBR 322	0	—	—
pAP11-2::Tn 5	—	$5{,}3 \cdot 10^{-4}$—$2{,}0 \cdot 10^{-3}$	—
pDK4, pAP11-2: :Tn 5	—	$1{,}0 \cdot 10^{-6}$—$1{,}0 \cdot 10^{-5}$	138*
pBR 322, pAP11-2: :Tn 5	—	$0{,}3 \cdot 10^{-4}$—$2{,}5 \cdot 10^{-4}$	1,2*

Legend. Asterisk denotes mean values of six standard conjugation crosses.

As a result of the investigation a clone of transformant bacteria containing recombinant plasmid, described as pDK4, was selected; it was assumed that it was formed as a result of ligation of fragment f3 (14.6 kbp) of plasmid pAP17-1::Tn9. The bacteria of this clone were characterized by ability to transfer the plasmid in conjugation crosses with recipient bacteria of the C600 Rif strain (Table 1). Inhibition of the tra functions of plasmid Flac also took place in them (index of inhibition 20).

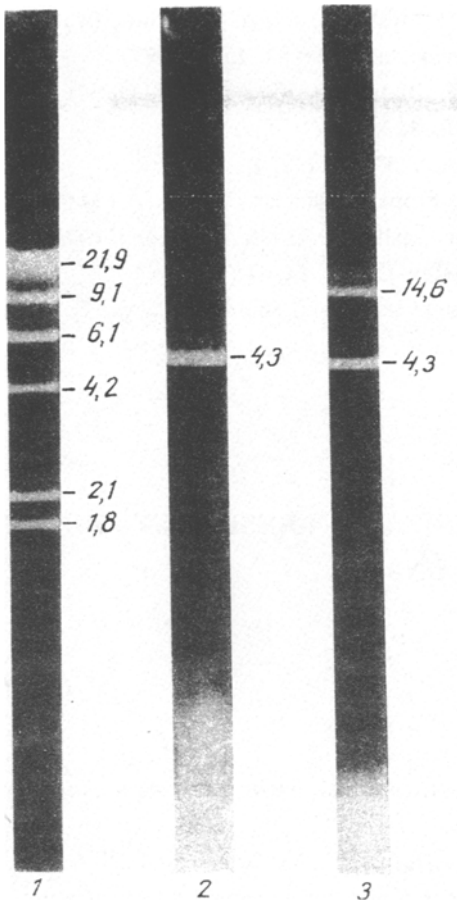

Fig. 2. Electrophoresis of restriction products of DNA of plasmid pDK4. 1) λ, HindIII, 2) pBR322, HindIII, 3) pDK4, HindIII. Remainder of legend as in Fig. 1.

It will be clear from Table 1 that unlike the nonconjugative vector plasmid pBR322, plasmid pDK4 is transferred into recipient cells, but at a lower frequency than the original conjugative plasmid pAP17-1::Tn9. This suggested that the genetic region of plasmid pAP17-1::Tn89 responsible for transfer, i.e., the tra region, is a component of the recombinant plasmid. It is also clear from Table 1 that plasmid pDK4 inhibits the tra functions of plasmid pAP11-2::Tn5, suggesting that it also contains the fin gene controlling synthesis of the transfer inhibitor of Fin U type.

To confirm that recombinant plasmid pDK4 contained the HindIII fragment f3 of the original plasmid pAP17-1::Tn9, DNA of the recombinant plasmid was isolated from HB101 cells and subjected to restriction analysis with the aid of restriction endonuclease HindIII. The results of this analysis show that plasmid pDK4 does in fact consist of the vector plasmid pBR322 and the Hind fragment f3 of the original plasmid pAP17-1::Tn9 (see Figs. 1 and 2).

It can be concluded from these results that the tra region of plasmid pAP17-1 is located in its HindIII fragment f3. This fragment also contains the fin gene determining synthesis of an inhibitor of genetic transfer of the Fin U type. Thus in plasmid pAP17-1, which is not F-like, the fin gene, just as in plasmid F, either is located in the tra region or lies next to it, so that this plasmid resembles the F-like plasmids.

LITERATURE CITED

1. D. E. Kulumbetova and V. P. Shchipkov, Molecular Biology and Genetics of Plasmids [in Russian], Moscow (1986), pp. 31-32.
2. D. E. Kulumbetova, V. P. Shchipkov, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 8, 214 (1988).
3. A. P. Pekhov, V. P. Shchipkov, T. Aran, and T. Ando, Zh. Mikrobiol., No. 9, 45 (1979).
4. A. P. Pekhov and V. P. Shchipkov, Mol. Genet., No. 10, 3 (1986).

5. V. P. Shchipkov, N. I. Buyanova, G. I. Myandina, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 8, 226 (1985).
6. H. C. Birnboimand and J. Doly, Nucleic Acids Res., **7**, 1513 (1979).
7. D. L. Daniels, Y. R. Dewet, and F. R. Blattner, J. Virol., **33**, 390 (1980).
8. M. Gasson and N. S. Willetts, J. Bact., **122**, 518 (1975).
9. M. Mandel and A. Higa, J. Molec. Biol., **53**, 159 (1970).
10. T. Maniatis, E. F. Ffitsch, and J. Sambrook, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor (1982).
11. R. B. Meagher, R. C. Tait, M. Betlach, and H. B. Boyer, Cell, **10**, 521 (1977).
12. N. S. Willetts and R. Skurray, Ann. Rev. Genet., **14**, 41 (1980).
13. N. S. Willetts and R. Skurray, *Escherichia coli* and *Salmonella typhi:* Cell and Molecular Biology, Vol. 2, Philadelphia (1987), pp. 1110-1133.

HETEROGENEITY OF DEPROTEINIZATION-RESISTANT PROTEINS FOR FIRMNESS OF BINDING TO DNA

N. I. Sjakste and T. G. Sjakste

UDC 616-006.6-008.939.6:616.6-008.93:577.113

KEY WORDS: Proteins firmly bound with DNA; nuclear matrix; NPC chromatography; DNA–protein interactions

Heterogeneity of proteins of the nuclear matrix for firmness of DNA–protein interaction has been investigated for several years. Two groups of proteins have been found: firmly bound, for dissociation of which from DNA a high temperature is required, and weakly bound, dissociating in a solution of salt and urea. Firmly bound proteins are evidently located in the replicative complex, whereas the weakly bound type are evidently involved in transcription [1, 3, 5]. The polypeptide composition of firmly and weakly bound proteins has been shown to be very similar and to be characterized by the presence of components with mol. wt. of 54-68 kD [6]. Polypeptides with this molecular weight and entering into the composition of the nuclear matrix preserve their binding with DNA after deproteinizing procedures: stronger – treatment with proteinase K and phenol [9], or milder – treatment with sarcolysin combined with high ionic strength [13]. It has been shown that a certain proportion of proteins resistant to deproteinization are noncovalently bound to DNA, whereas some are covalently bound, by a phosphodiester bond [7, 11]. Data on the functional role of the firmly bound proteins are rather contradictory. By some workers they have been ascribed the role of constant "fixer" of DNA with the nuclear matrix, and a role in both replication and transcription [10, 12], whereas others postulate a transient association with the nuclear matrix, depending on the type of differentiation [13]. Their binding predominantly with satellite DNA [12] or with unique sequences [13] has been reported. Thus, on the one hand, firmly bound proteins similar in polypeptide composition are liberated by procedures of differing strength [7, 10, 11], but on the other hand the nucleoprotein complexes of the nuclear matrix, similar in their polypeptide spectrum with one another and with the firmly bound proteins, differ in stability and in their functional role [1, 3, 5, 6]. It has consequently been suggested that firmly bound proteins may also be heterogeneous for the strength of the DNA–protein bond. The aim of this investigation was to study the strength of DNA–protein interactions in complexes of DNA with firmly bound proteins by methods of nucleoprotein-celite chromatography (NPC) and elution from nitrocellulose.

Laboratory of Cancer Cell Chemistry, Latvian Research Institute of Experimental and Clinical Medicine, Ministry of Health of the Latvian SSR, Riga. (Presented by Academician of the Academy of Medical Sciences of the USSR I. B. Zbarskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 194-196, August, 1990. Original article submitted April 5, 1989.

 0007-4888/90/0008-1106$12.50