fin K, fin L, fin M, AND fin N GENETIC PLASMID TRANSFER REGULATORY SYSTEMS

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Several plasmids, irrespective of their ability to undertake conjugative transfer, of the incompatibility group to which they belong, and of other properties in their genome, contain genetic fin-systems, which determine inhibition (regulation) of conjugative transfer of F plasmid. Previously six of these fin-systems have been discovered, belonging both to individual conjugative plasmids with repressed transfer function (rd-plasmids) and nonconjugative plasmids [4, 9, 10]. It was subsequently shown that these fin-systems regulate conjugative activity of F-like transfer factors also. Moreover, certain F-like transfer factors with derepressed transfer function (drd-plasmids) have been found to be able themselves to inhibit transfer of F factor [2, 4, 5]. Accordingly the problem has arisen of the specificity of the inhibitory action of fin-systems as applied to F factor and to other F-like factors.

The aim of this investigation was to find fin-systems acting on transfer of different plasmids, and to study details of their expression, including dependence on the strain of the host cells and the order of entry of the plasmids into the cells during conjugation.

EXPERIMENTAL METHOD

Previously identified F-like (pBlCmGs, pAP27Ap, CmTcSmSu, pAP35CmTcSuSm) and non-F-like (pK4ApKm, pK5ApCmTcSuSm) plasmids, R-plasmids of the rd-type, F-like rd transfer factors pAp10-2:Tn9, pAp19-1::Tn9, pAP20::Tn9, pAP22-1::Tn1, and also several other F-like transfer factors of drd type, were used (Table 2). *E. coli* of strains C600Rif, JM83Str, AP115Nal and serotyped strains AP15-2Str (antigen O-106) and AP58-2Str (antigen O-147) were used in conjugative crosses of bacteria. Conjugative crosses, determination of the sensitivity of the bacteria to pilus-specific phage, and the rising phage titer test (RPTT) were carried out by standard methods [7, 8]. Indices of inhibition (II) of the frequency of plasmid transfer, the phage seeding efficiency, and the rising phage titer index (RPTI) in cultures of diplasmid cells were determined as the ratio between the corresponding values for monoplasmid and diplasmid cell cultures of the same strain.

EXPERIMENTAL RESULTS

The ability of rd-plasmids (pAP10-2::Tn9, pAP19-1::Tn9, pAP20::Tn9, pAP22-1::Tn1, pAP27, pAP35, pB1, pK4, and pK5) to inhibit the transfer function of F factor was first studied, with determination of II of the frequency of transfer of factor Flac in the presence of rd-plasmid during crossing of bacteria of the C600 and AP115 strains, and the ability of diplasmid C600 cells to synthesize pili, adsorbing donor-specific phage f2, also was investigated.

These experiments showed that F-like plasmids (pAP10-2::Tn9, pAP35, pAB1) and non-F-like plasmids (pK4 and pK5) inhibited the transfer function of factor Flac, preventing pilus formation by diplasmid cells (except pK5)

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TABLE 1. Fin-Effects of Transfer-Repressed Plasmids on Pilus-Formation Function and Transfer of F-Like Factors

Transfer factor	Sensitivity to standard fin-systems [10]	Fin-effects due to rd-plasmid			
		pAP22—1::Tn1	pAP20::Tn9	pAP19—1::Tn9	pAP27
pAP10—2::Tn9	OP, Q, U, V	+	N	N	N
pAP11—2::Tn1	OP, Q, U, V	N	+	+	N
pAP11—2::Tn5	OP, U, V	—	+/—	—	+
pAP11—2::Tn9	OP, U, V	—	N	N	N
pAP18—1	V, W	+	+	—	N
pAP18—1::Tn5	V	+	+	+/—	—
pAP18—1::Tn9	W, V	—	+	—	N
pAP19—1::Tn1	OP, Q, U, V, W	N	+/—	+/—	N
pAP19—1::Tn9	OP, U, V	+	N	N	N
pAP22—2::Tn1	V, U	N	—	—	N
pAP22—2::Tn5	V	+	+	—	—
pAP38::Tn9	V	—	N	N	N
pAP39::Tn9::Tn5	N	—	—	—	+/—
pAP41::Tn1721	N	—	+	+	N
pAP41::Tn9::Tn1741	OP, U, V	—	+	+	N
pAP42::Tn1	U, V	N	+/—	—	N
pAP42::Tn5Fin$^+$	U, V, W	+	—	—	+/—
pAP42::Tn5Fin$^-$	OP, Q, U, V, W	—	—	—	+/—
pAP42::Tn9	V, OP, U	+	N	N	N
pAP53::Tn5	V	+	+	+	+/—
pAP53::Tn9	V	—	N	N	N

Legend. +) Fin-effect on plasmid transfer and pilus formation; +/—) Fin-effect on plasmid transfer; —) no Fin-effect; N) not tested.

and reducing the frequency of its transfer during conjugation by 92.9-1040.0, 25.2-684.2; 28.2-136.8; 38.3-51.7, and 15.3-136.8 times respectively. Conversely, as the experiments showed, F-like plasmids pAP19-1::Tn9, pAP20::Tn9, pAP22-1::Tn1, and pAP27 had no inhibitory action on any transfer functions of the factor (II of the frequency of transfer of plasmid Flac from diplasmid cells $\leq 1.1 \pm 0.4$).

The same experiments showed that plasmids pAP27, pAP35, and pK4 are compatible with factor Flac, whereas plasmids pAP10-2::Tn9, pAP20::Tn9, pAP19-1::Tn9, and pB1 partially displaced factor Flac from diplasmid cells (in 38, 33, 20, and 7% of cases respectively). Plasmid pK5 itself was partially (8%) displaced by factor Flac. With plasmids pAP22-1::Tn1 and Flac, separate elimination was observed (7 and 10% respectively).

In further investigations a search was made for F-like drd transfer factors of plasmids sensitive to fin-systems inactive relative to Flac: pAP27, pAP19-1.:Tn9, pAP20::Tn9, and pAP22-1::Tnl.

The results of these experiments are given in Table 1, which show that rd-plasmids containing fin-systems inactive against factor Flac in their genome inhibited the transfer functions of various F-like factors; the Fin-effects, moreover, differed in the range of sensitive transfer factors, in the inhibitable transfer functions, and in the degree of inhibition. Comparison of the sensitivity of F-like transfer factors to systems regulating transfer, coded by plasmids pAP22-1::Tnl, pAP20::Tn9, pAP19-1::Tn9, and pAP27, and to standard systems [10] leads to the conclusion that they are not identical.

The use of several alternative transfer factors, differing from each other in the presence of a particular transposon in the genome, in this investigation made it possible subsequently to demonstrate the effect of transposon insertion on the sensitivity of the transfer system of the test plasmids to fertility inhibitors. Loss of sensitivity to the fin-system of plasmid pAP22-1::Tnl was observed in a deletion mutant of factor pAP42::Tn5, characterized by loss of Fin activity relative to F factor (Fin-variant) and by modification of the spectrum of sensitivity to standard systems of transfer function regulation [2, 3].

Since the fin-system of plasmid pAP27 acts on both transfer functions (pilus formation and transfer proper) of only one, and, moreover, a compatible plasmid pAP11-2::Tn5), the expression of the fin-system of precisely this plasmid was next studied, using a series of clones of diplasmid cells. Clonal analysis showed that the peak level of expression of the fin-system of plasmid pAP27 was expressed as a decrease in the frequency of transfer of factor pAP11-2::Tn5 by 22.7 times, and by absence of phage-sensitivity of diplasmid C600 cells, containing both plasmids. Reduction of RPTI by 15.4 ± 0.3 times was observed under these circumstances. However, the level of expression of

TABLE 2. Effect of Host Strain and Order of Entry of Plasmids into Cell on Level of Fin-Effect Due to Plasmid pAP27

Host strain	Introduced plasmid	Resident plasmid	Surface exclusion index	Fin-effect of plasmid pAP27	
				pilus formation (II of RPTI)	transfer (II of tr. frequency)
JM83	pAP27	pAP11—2::Tn5	3,4	+(5,0)	+(3,9)
JM83	pAP11—2::Tn5	pAP27	0,9	$-(2{,}3\times10^{-4})$	+(6,9)
AP15—2	pAP27	pAP11—2::Tn5	1,8	+(3,5)	+(25,0)
AP15—2	pAP11—2::Tn5	pAP27	0,037	+(155,5)	+(9,0)
AP58—2	pAP27	pAP11—2::Tn5	3,7	+(80,0)	—(0,2)
AP58—2	pAP11—2::Tn5	pAP27	0,0031	$-(3{,}6\times10^{-3})$	—(0,5)

the fin-system of plasmid pAP27 was clonally heterogeneous, for clones for which the Fin-effect was either minimal or absent were found in populations of diplasmid cells.

Thus in relation to factor pAP11-2::Tn5 the minimal effect of inhibition was determined by a decrease in its transfer frequency by 2.7 times, while phage sensitivity of the diplasmid clones was preserved, although the phage seeding efficiency fell by 13.6 ± 0.3 times. II of the transfer frequency of F-like factors lay within the range from 7.4 to 20.5 for pAP42::Tn5 fin^+, from 6.3 to 6.8 for pAP42::Tn5 fin^-, from 1.0 to 14.5 for pAP39::Tn9::Tn5, and from 4.2 to 15.7 for pAP53::Tn5.

To study the effect of the host cell and the order of entry of plasmids into the cell on expression of the fin-system of plasmid pAP27, as the test plasmid we used factor pAP11-2::Tn5. Inhibition of the transfer functions of the test plasmid by plasmid pAP27, as the test plasmid we used factor pAP11-2::Tn5. Inhibition of transfer functions of the test plasmid by plasmid pAP27 was determined in cells of serotyped strains AP15-2 and AP58-2. In all cases the recipient consisted of cells of plasmid-free strain AP115. *E. coli* JM83 cells were used as the control.

The results of these experiments showed (Table 2) that plasmid pAP27, present in cells of the serotype strains, facilitated introduction of the test plasmid into them and increased the frequency of its transfer. With the opposite order of introduction of the plasmid pair in the cell it was found that factor pAP11-2::Tn5 either did not affect the transfer frequency of plasmid pAP27 or depressed it, but not significantly. In all cases compatibility of the tested pair of plasmids was observed.

Expression of the fin-system of plasmid pAP27 in host cells of serotyped strains AP15-2 and AP58-2 differed. Inhibition of transfer of the test plasmid was observed only in cells of strain AP15-2, and not of strain AP58-2. The Fin-effect with respect to pilus formation was resistant also in AP15-2 cells only. The Fin-effect with respect to pilus formation was discovered in cells of strains JM83 and AP58-2 only with a certain order of entry of the plasmids into the bacterial cell (the former pAP11-2::Tn5, the latter pAP27).

It is important to note that, depending on the strain of the host cells carrying the plasmid pair pAP27-pAP11-2::Tn5, the plasmid composition of transconjugants formed in crosses with plasmid-free AP115 cells also changed. In particular, combined transfer of markers of plasmid pAP27 and factor pAP11-2::Tn5 was not found in control crosses in which the donors were cells of strain JM83. However, 50% and 96% of clones of transconjugants were characterized by the simultaneous presence of markers of both plasmids, when cells of serotyped strains AP58-2 and AP15-2 respectively served as donors.

It can be concluded from the discussion of these results that among F-like plasmids there are some which contain in their genome fin-systems that are inactive against factor Flac, but which inhibit transfer functions of certain F-like factors; these plasmids, moreover, are characterized by qualitative differences in the realization of their Fin-effects. These fin-systems are found in rd-plasmids pAP19-1::Tn9, pAP20::Tn9, pAP22-1::Tnl, and pAP27. We have called these systems fin K, fin L, fin M, and fin N respectively.

The data thus obtained about the fin N system indicate that expression of these systems depends on the host cells and on the order of entry of the Fin-active and test plasmids into the cell.

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IONIC REGULATION OF RECEPTION OF ^{35}S-*tert*-BUTYLBICYCLOPHOSPHOROTHIONATE BY BRAIN MEMBRANES OF INBRED MICE DIFFERING IN EMOTIONAL STRESS RESPONSE

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Binding of ^{35}S-*tert*-butylbicyclophosphorothionate (^{35}S-TBPS) reflects the function of the effector component of the supramolecular GABA-benzodiazepine receptor complex (BDRC), a Cl^--ionophore [13]. The study of reception of this ligand by brain membranes of C57BL/6 (B6) and BALB/c (C) mice, which differ in their response to emotional stress in the open field (OF) test and to administration of benzodiazepine tranquilizers [5] has shown that its binding is characterized by different degrees of dependence on the Cl^- ion concentration in the medium for animals of the inbred lines used [2]. Interlinear differences in ionic regulation of ^{3}H-diazepam reception [1, 2], discovered previously, data on the coupling of reception in benzodiazepine and Cl^--ionophore regions of BDRC [4], and also the views of other workers on the existence of a binding site for cations in BDRC [11], were taken into consideration.

In the investigation described below, to continue the study of the mechanisms of formation of hereditary differences in receptor activity of BDRC, the effect of various salts on binding of ^{35}S-TBPS by brain membranes of B6 and C mice was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery, Russian Academy of Medical Sciences. The mice were kept under animal house conditions at the Research Laboratory of Pharmacogenetics, Institute of Pharmacology, Russian Academy of Medical Sciences, for at least 2 weeks before the experiment began, on a standard diet, with 10 mice to a cage, and with 12 h daylight alternating

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