An approach is thus suggested to the transfer of genetic material not only into hematopoietic, but also into stromal precursor cells. The suggested method offers the possibility of marking precursor cells of the hematopoietic microenvironment for their further study.

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MUTATIONS OF GENETIC REGION FIN OF F-LIKE PLASMID pAP18-1

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Conjugative transfer of bacterial plasmids is controlled by their genetic region, under whose control a particular type of transfer inhibitor is synthesized in the cell [6, 9]. However, the genetic structure of this region has not yet been studied. It is likewise not known whether a change in the type of inhibitor synthesized under plasmid control takes place.

The aim of this investigation was to identify mutation changes in genes of the fin-region of F-like plasmid pAP18-1 (Tc, Col V), which is a carrier of the transfer inhibition system of the Fin V group [5].

EXPERIMENTAL METHOD

We used previously identified mutant pAP18-1 drd of plasmid pAP18-1, derepressed with respect to transfer functions, and also its transposon-containing derivatives pAP18-1 drd::Tn 5 and pAP18-1 drd::Tn9 [5], which cannot inhibit transfer of standard plasmid Flac (phenotype Fin^-). In conjugation crosses of the bacteria we used standard strains *E. coli* K-12 with chromosomal markers of antibiotic resistance (AP115 Nal, AP106 Str, HB101 Str), containing or not containing the test plasmids. Conjugation transfer of plasmids, sensitivity of bacteria to pilus-specific phage NS2, and ability of the

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TABLE 1. Levels of Inhibition of Transfer of Plasmid Flac by Fin$^+$-Revertants of Plasmids pAP18-1 drd::Tn 5 and pAP18-1 drd::Tn 9

Original plasmid	Fin$^+$-revertants		
	group	number of revertant in group	indices of inhibition of transfer of plasmid Flac
pAP18-1 drd :: Tn5	1	0	—
	2	9	140—527
	3	12	1266—9287
	4	1	33636—61139
	5	2	235294—453846
pAP18-1 drd :: Tn9	1	3	5,9—41
	2	3	385—987
	3	7	1625—5342
	4	4	19117—55319
	5	3	128333—866666

plasmids to inhibit the transfer function of standard derepressed plasmids were studied by methods described previously [1, 5]. Indices of inhibition of transfer of standard derepressed plasmids by the test plasmids were determined as the ratio of the frequency of transfer of the derepressed plasmid from cells of monoplasmid transconjugates to the frequency of transmission of this same plasmid from cells of diplasmid transconjugates. Treatment of the bacteria (induction of mutations in the fin region) with nitrosoguanidine was carried out by the standard method [3]. Plasmid DNA was isolated by the method of Meagher et al. [8] with minor modifications. Restriction of plasmid DNA by specific endonuclease SalGI was carried out by the standard method [7].

EXPERIMENTAL RESULTS

After treatment of *E. coli* AP106 cells containing plasmid pAP18-1 drd::Tn 5 or pAP18-1 drd::Tn 9 with nitrosoguanidine 60 clones of bacteria were selected which had lost their sensitivity to the pilus-specific phage NS2. A study of the conjugation properties of these bacteria showed that most of them also are characterized by reduced frequency of transfer of the plasmid contained in them. In particular, the frequency of transfer of the plasmid varied between 10^{-1} and 10^{-6} per donor's cell. When evaluating these data, we drew the preliminary conclusion that the changes described above in the phenotype of bacteria of the selected clones were connected with mutations of the plasmids pAP18-1 drd::Tn 5 and pAP18-I drd::Tn 9 contained in them, i.e., bacteria of selected clones contain mutant plasmids.

In the next experiments, the aim of which was to study the properties of the hypothetical mutants of the plasmids, it was found that 45 such mutants can inhibit the functions of the tra-genes of plasmid Flac, i.e., can behave like plasmids of the Fin$^+$ type. Consequently, these mutants can be regarded as Fin$^+$ revertants of Fin$^-$-plasmids pAP18-1 drd::Tn 5 and pAP18-I drd::Tn 9.

Analysis of the ability of Fin$^+$-revertants, induced by nitrosoguanidine, to inhibit the transfer functions of plasmid Flac showed that quantitative differences in the levels of their inhibitory activity exist between the revertants, and that on the basis of levels of inhibition, the mutant plasmids studied can be classified into five different groups (Table 1). Additional determination of the ability of the test Fin$^+$ plasmids to inhibit the transfer function of derepressed plasmids pAP53::Tn 5 and pAP53::Tn 9, in which sensitivity was discovered only to a transfer inhibitor of the Fin V type [4], established that 31 of 45 plasmids possess this property. Consequently, like the original repressed Fin$^+$-plasmid pAP18-1, the mutant plasmids studied can determine synthesis of a transfer inhibitor of the Fin V type.

To determine the types of transfer inhibitors in the mutant plasmids in whose genome no genetic system responsible for synthesis of the Fin V inhibitor was found, we used other F-like derepressed plasmids with a previously established type of sensitivity to transfer inhibitors. In particular, we used plasmids pAP22-2 (Fin OP, V), pAP11-2::Tn 5 (FinOP, U, V) pAP11-2::Tn 9 (FinOP, U, V, W) pAP11-2::Tn 1 (FinOP, Q, U, V, W), pAP22-2::Tn 1 (Fin U, V) [4].

By analysis of double plasmid transconjugants containing one of the mutant plasmids tested and one of the derepressed test plasmids, showed that under the control of fin-genes of three mutant plasmids (pAP18-1::Tn9-36, pAP18-1::Tn9-87, pAP18-1::Tn9-106) a transfer inhibitor of the Fin OP type is synthesized. In relation to plasmid pAP18-1::Tn 9-30 we obtained data suggesting that a transfer inhibitor of the Fin Q or Fin W type may be synthesized. Thus mutagenesis

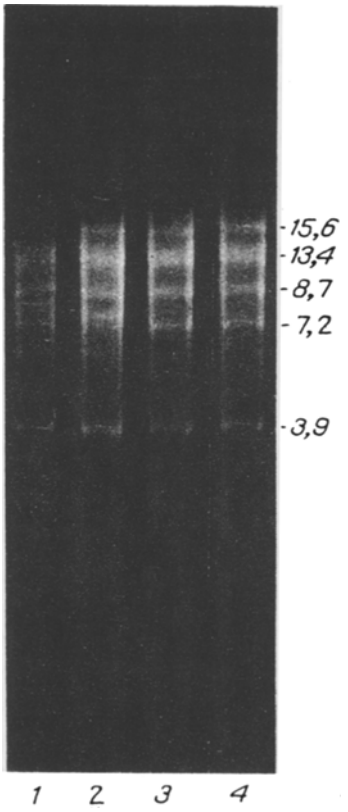

Fig. 1. Electrophoretic fractionation of restriction products of DNA of plasmid pAP18-1 and its mutant forms by restriction endonuclease SalG1. 1) pAP18-1::Tn9-30; 2) pAP18-1::Tn9-36; 3) pAP18-1::Tn9-25; 4) pAP18-1.

of plasmid pAP18-1 drd::Tn 9 is accompanied by the switch of the transfer inhibitor Fin V, synthesized under its control, into an inhibitor of the Fin OP and Fin Q or W type. However, it does not yet seem possible to identify the fin-systems of plasmid pAP18-1::Tn 9-30 more accurately with the aid of the test plasmid kit used.

To locate the fin-region in the genome of the mutant plasmids, complementation analysis of mutants of the Fin^+-group was carried out. For this purpose, plasmid cells containing one of the mutant plasmids and one of the recombinant plasmids, which we constructed previously by cloning plasmid pAP18-1 drd [2], were selected. The sensitivity of these cells to pilus-specific phage MS2 was studied. A positive complementation effect was found in the case of recombinant plasmid pAP105, selected during cloning of SalG1 fragment f5 of plasmid pAP18-1 drd, and also 22 mutant plasmids. Of the mutant plasmids, 14 were characterized by restoration of the Fin V phenotype, three by ability to determine synthesis of a transfer inhibitor of the Fin OP type, one – of Fin Q or Fin W, whereas in four types, the inhibitors controlled by them could not be determined because of the incompatibility with the test plasmids. These results suggest that genetic loci fin V, fin OP, fin Q, or fin W are located in the SalG1 fragment f5 of plasmid pAP18-1 drd, which measures 3.9 megadaltons.

In additional experiments a comparative study was made of the restriction maps of DNA of mutant plasmids (pAP18-1::Tn9-25, pAP18-1::Tn 9-36, pAP18-1::Tn 9-30), characterized by the phenotype Fin V, Fin OP, Fin Q, or Fin W (respectively), and also the original Fin V^+-plasmid pAP18-1, using endonuclease SalG1. It will be clear from Fig. 1 that mutations of the plasmids leading to reversion of the $Fin^- \rightarrow Fin^+$ phenotype were not accompanied by any changes in the specific restriction sites of their DNA.

The general conclusion can be drawn that mutations in the genome of plasmid pAP18-1 may be both forward and back mutations and may lead to changes in the system controlling genetic transfer of this plasmid, consisting of the appearance of the Fin^- phenotype or of its reversions to the Fin^+ phenotype.

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