MOLECULAR-GENETIC STUDY OF F-LIKE PLASMID pAP18-1 MUTATIONS FOR INCOMPATIBILITY AND TRANSFER REGULATION INDUCED BY NITROSOGUANIDINE AND TRANSPOSONS Tn5 AND Tn9

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In a previous study [2] using nitrosoguanidine, the writers induced a mutant of F-like plasmid pAP18-1 (Tc, CoIV), derepressed (drd) with respect to functions of tra-genes, which was found to be a representative of the incompatibility group inc FVII. On subsequent incorporation of transposons Tn5 and Tn9 into the structure of plasmid pAP18-1 drd changes were observed in the system regulating genetic transfer of this plasmid [3]. The aim of this investigation was to discover the connection between mutations of plasmid pAP18-1 induced by nitrosoguanidine and transposons and changes in the restriction map of this plasmid under the influence of these factors.

EXPERIMENTAL METHOD

Standard strains of *E. coli* K-12 with chromosomal markers of resistance to antibiotics (AP1-6 Str, AP115 Nal, AP132 Nal, C600 Str), containing or not containing reference and test plasmids, were used. Determination of compatibility (incompatibility) of the plasmids was carried out in standard conjugation crosses, using reference plasmids of ten different incompatibility (inc) groups of F-like plasmids [2]. Plasmid DNA was isolated by the method in [5], with certain modifications, using centrifugation in a CsCl density gradient. Restriction of plasmid DNA was effected by endonucleases EcoR1 and Sal1, which were added to the incubation mixture separately (single restrictions) or in pairs. Restriction fragments were fractionated in 0.65% agarose gel by horizontal slab electrophoresis. The dimensions of the restriction fragments of DNA of the test plasmid was determined by the use of restriction fragments of phage λ DNA as molecular weight standards [4]. A physical map of plasmid pAP18-1 and its derepressed variants (pAP18-1 drd, pAP18-1 drd :: **Tn5**, PAP18-1 drd :: Tn9) was drawn by means of the algorithm described previously [1].

EXPERIMENTAL RESULTS

To obtain data on compatibility (incompatibility) of plasmid pAP18-1 and its derepressed transposon-containing variants pAP18-1 drd :: Tn5 and pAP18-1 drd :: Tn9 with reference plasmids of ten incompatibility groups, diplasmid transconjugants containing one of the test and one of the reference plasmids were obtained and investigated. The results of these investigations showed that the original plasmid pAP18-1 is compatible with plasmids of all ten inc-groups of F-like plasmids now known. Parameters of compatibility of this plasmid (percentage of clones and subclones of diplasmid transconjugants, preserving both plasmids) varied from 92 to 100%. This suggests that the F-like plasmid pAP18-1 does not belong to any of the ten incompatibility groups mentioned above, and it can probably be regarded as a representative of a new incompatibility group inc FXI.

The results of determination of compatibility (incompatibility) of plasmids pAP18 drd :: Tn5 and pAP18-1 drd :: Tn9 showed that these plasmids are compatible with reference plasmids of all inc-groups except plasmids pAP38 :: Tn1, a representative of the inc FVII group. Consequently, unlike the original (repressed) plasmid pAP18-1 (inc FXI) its derepressed variants pAP18-1 drd, pAP18-1 drd :: Tn5, and pAP18-1 drd :: Tn9 are representatives of the

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TABLE 1. Results of Studies of Compatibility (Incompatibility) of Plasmid pAP18-1 and Its Derepressed Variants with Reference Plasmid of Incompatibility Group FVII

Plasmid		Selective marker	Analysis of plasmid transconjugants	Clones of transconjugants	Results of clonal test for compatibility of plasmid	
introduced	resident		per cent of clones whose cells contain both plasmids		per cent of daughter colonies whose cells contain both plasmids	compatibility index, per cent
pAP18-1	pAP38::Tn1	Tc	17,5	1 2 3 4 5	85 85 100 90 10	92
pAP38::Tn1	pAP18-1	Ap	80	1 2 3 4 5	90 85 95 100 100	94
pAP18-1 drd	pAP38::Tn1	Tc	80	1 2 3 4 5	15 20 0 20 5	12
pAP38::Tn1	pAP18-1 drd	Ap	100	1 2 3 4 5	5 20 5 30 10	14
pAP18-1 drd::Tn5	pAP38::Tn1	Km	37,5	1 2 3 4 5	80 85 95 45 90	79
pAP38::Tn1	pAP18-1 drd::Tn5	Ap	72,5	1 2 3 4 5	20 35 25 45 25	38
pAP18-1 drd::Tn9	pAP38::Tn1	Lm	45	1 2 3 4 5	30 70 60 40 45	49
pAP38::Tn1	pAP18-1 drd::Tn9	Ap	47,5	1 2 3 4 5	35 35 25 30 60	49

Legend. During primary analysis of the diplasmid transconjugants 40 clones of each specimen were investigated. In each clonal test, 20 colonies from each clone of transconjugants (after passage) were studied.

TABLE 2. Molecular Weights (in megadaltons) of Restriction Fragments of DNA of Plasmid pAP18-1 and Its Derepressed Variants

Plasmid	Restriction enzyme	Restriction fragments and their weight												
		f1	f2	f3	f4	f5	f6	f7	f8	f9	f10	f11	f12	f13
pAP18-1	EcoR1	18,3	8,3	6,2	5,0	3,6	3,0	2,4	1,1	0,9				
	Sal1	15,6	13,4	8,7	7,2	3,9								
	EcoR1 + Sal1	11,7	8,3	5,2	3,9	3,6	3,3	3,0	2,7	2,4	1,7	1,1	1,0	0,9
pAP18-1 drd	EcoR1	18,3	10,2	5,4	3,6	3,6	3,0	2,4	1,3	1,0				
	Sal1	15,8	13,8	8,7	6,6	3,9								
	EcoR1 + Sal1	11,7	10,2	3,9	3,6	3,6	3,0	3,0	2,7	2,4	1,3	1,3	1,1	1,0
pAP18-1 drd::Tn5	EcoR1	18,3	10,2	5,4	5,3	3,6	3,6	3,0	2,4	1,0				
	Sal1	13,8	11,5	8,7	8,3	6,6	3,9							
pAP18-1 drd::Tn9	EcoR1	18,3	10,2	5,4	3,6	3,6	2,6	2,4	2,0	1,3	1,0			
	Sal1	17,4	13,8	8,7	6,6	3,9								

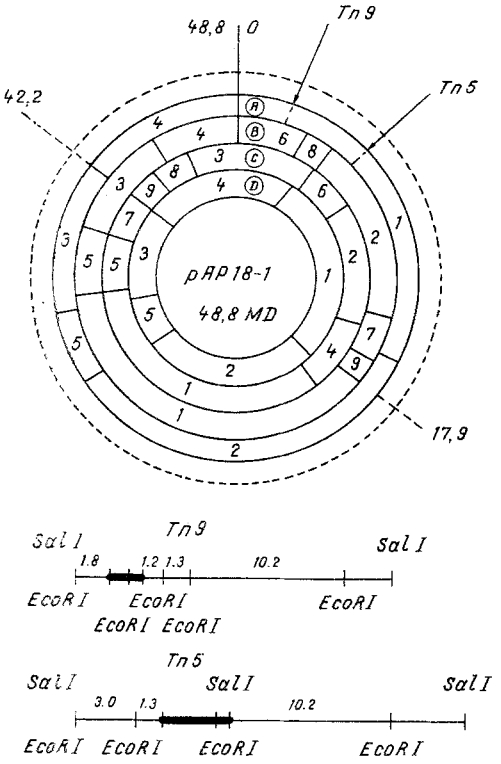

Fig. 1. Restrictions maps of plasmid pAP18-1 and its derepressed variants. A) restriction of pAP18-1 drd by enzyme SalI; B) restriction of pAP18-1 drd by enzyme EcoR1; C) restriction of pAP18-1 by enzyme EcoR1; D) restriction of pAP18-1 by enzyme SalI. Arrows indicate sites of insertions of transposons. Sizes of corresponding DNA fragments are given in Table 2.

inc FVII incompatibility group. Meanwhile, it will be clear from Table 1 that, unlike plasmid pAP18-1 drd, with a high degree of incompatibility with plasmid pAP38 :: Tn1 (compatibility index 12-14%) its transposon-containing variants are characterized by partial incompatibility with this plasmid (compatibility index 38-79%).

It can be concluded from these data that treatment of bacteria containing plasmid pAP18-1 with nitrosoguanidine was accompanied not only by mutations of the genetic system for inhibition of transfer of this plasmid, but also by mutations of genetic structures controlling incompatibility (change from inc FXI to inc FVII groups). Incorporation of transposons Tn5 and Tn9 into the structure of plasmid pAP18-1 drd did not change the incompatibility group (inc FVII) but led to a fall in the level of incompatibility.

To determine the possible connection of changes in the genetic characteristics of the plasmids studied with changes in their molecular structure, restriction analysis was carried out of DNA of plasmids pAP18-1, pAP18-1 drd, pAP18-1 drd :: Tn5 and pAP18-1 drd :: Tn9. It will be clear from Table 2 that the molecular weights of plasmids pAP18-1 and pAP18-1 drd are 48.8 megadaltons (MD) in each case. Incorporation of transposons Tn5 and Tn9 led to an increase in the molecular weight of the plasmid by 4.0 and 1.6 MD, respectively. It will also be clear from Table 2 that the mutant plasmid pAP18-1 drd, induced by the mutagen, is characterized by restructuring of some fragments by EcoR1- and SalI-restriction. Definite structural changes also are observed after incorporation of transposons into the structure of plasmid pAP18-1 drd. It can be tentatively suggested that incorporation of transposon Tn5 into EcoR1-fragment f8 (1.3 MD) leads to the appearance of a new f4 fragment (5.3 MD), whereas its incorporation into SalI-fragment f1 (15.8 MD) led to the formation of two new fragments: f2 (11.5 MD) and f4 (8.3 Md). In the case of transposon Tn9, it was probably incorporated into EcoR1-fragment f6 (3.0 MD) with the formation of two new fragments: f6 (2.6 MD) and f8 (2.0 MD), whereas its incorporation into SalI-fragment f1 (15.8 MD) led only to an increase in size of this fragment (17.4 MD).

Analysis of the restriction maps of these plasmids, drawn on the basis of results of single and paired restrictions (Fig. 1) showed that treatment of the original (repressed) plasmid pAP18-1 with nitrosoguanidine was accompanied by changes in the arrangements of the restriction endonuclease sites only in part of the molecule. A region 24.3 MD long, constituting about half of the plasmid genome and having relative coordinates of 17.9-42.2 remained unchanged under these circumstances. In the other half of the molecule nitrosoguanidine induced changes in the arrangement of the recognition sites of the restriction endonucleases, mainly in two regions of the genome with relative coordinates of 42.2-4.3 and 12.9-17.9. In this case the total number of sites was unchanged.

Thus under the influence of nitrosoguanidine and transposons Tn5 and Tn9 substantial structural changes are possible in the molecular organization of plasmid pAP18-1 and they are accompanied by changes in its incompatibility and regulation of the functions of tra-genes.

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