MOLECULAR SIZE OF PLASMID R18 AND ITS SUBSTITUTED VARIANTS IN *Escherichia coli* CELLS

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Some R plasmids belonging to the Inc P1 incompatibility group not only have a wide range of hosts, but also have the ability to mobilize chromosomal genes with the formation of R-substituted variants [3, 6, 7, 12]. By using this feature of RP1 plasmids it is possible to correct deficiencies in certain strains of *Escherichia coli* and also to use R factors as instruments for mapping some regions of the *Pseudomonas aeruginosa* chromosome [11].

The present investigation is a direct continuation of research into the role of R plasmids of the Inc P1 group in the transfer of chromosomal genes during intergeneric crosses [3, 6, 7]. An attempt was made in it to discover differences in molecular size of R18 plasmid and its substituted variants, using methods of electron microscopy followed by statistical analysis of measurements of the outline lengths of the molecules.

EXPERIMENTAL METHOD

The following strains of *E. coli* were used: AB1157, obtained from Professor A. P. Pekhov (Patrice Lumumba Peoples' Friendship University, Moscow); AB2463, obtained from the Museum of the Institute of General Genetics, Academy of Sciences of the USSR; CSH57, from the collection of the Laboratory at Cold Spring Harbor (USA). The above strains with plasmid R18 (AB1157 and AB2463) and R18Arg (AB2463-80 and CSH57-74:7) were obtained in our department by T. S. Minina by methods described previously [6]. Plasmid DNA was isolated as in [8]. The molecular weight of the plasmid DNA was determined from the outline length of the molecules according to Lang [13]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

To determine the molecular size of the R-substituted plasmids two strains of *E. coli* were chosen which, together with plasmid R18, had received ability to grow on media without arginine. The first strain, AB2463-80, was isolated after crossing AB2463 with *P. aeruginosa* 1822 (R18) [6]. The second strain, CSH57-74:7, was obtained by infection of strain CSH57 with phage P1, propagated on transconjugant AB1157-74 which, during crossing of strain AB1157 with *P. aeruginosa* 1822 (R18), together with plasmid R18 received ability to grow on media without arginine [6]. Control strains AB2463 and AB1157 with plasmid R18 were grown under the same standard conditions (Lederberg's medium containing 0.5% glucose, 0.1% each of casaminic acids and yeast extract, and 50 μg/ml of kanamycin).

Electron-microscopic study of preparations of plasmid DNA obtained from the above-mentioned strains showed that in some cases these preparations were heterogeneous and consisted of assortments of molecules of different sizes. Subsequent statistical analysis revealed a principal and additional groups of molecules of plasmid DNA for the strains studied.

Analysis of preparations of plasmid DNA obtained from the control strains revealed that the principal group of molecules in both cases corresponded to the ordinary molecular weight

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TABLE 1. Physical Dimensions of Plasmid R18Arg Molecules

Source of plasmid DNA	Size of plasmid molecules (mol. wt. in megadaltons)		
AB2463-80	1,3±0,1 (14)	33,1±1,5 (5)	42,2±2,7 (30)
E. coli CSH57-74:7	2.0±0.0 (3)	41,6±3,3 (14)	45,4±5,8 (45)

Legend. Number of molecules found in preparation of plasmid DNA and belonging to the given group is shown in parentheses.

of plasmid R18. For instance, the mean molecular size of this plasmid from strain AB1157 was 39.4 ± 1.9 megadaltons, and that from its recA mutant AB2463 was 40.6 ± 1.0 megadaltons.

A similar investigation of samples of plasmid DNA from strains of *E. coli* which, together with plasmid R18, received ability to grow on media without arginine, revealed an increase in molecular weight in the principal group of molecules. The molecular weight of the plasmid DNA from strain AB2463-80 was 42.2 ± 2.7 megadaltons, and that from CSH57-74:7 was 45.4 ± 5.8 megadaltons. The observed increase in molecular weight of the plasmid isolated from these clones can be explained by incorporation of genetic material of the original host of this plasmid (*Bacillus pyrocyaneus*) in the composition of plasmid R18, in good agreement with genetic data obtained previously [6]. In the work just cited it is stated that the R18Arg structure in some strains, for example in CSH57-74:7, is unstable, and its disintegration leads to the formation of true plasmid R18. The present results also support these conclusions.

The results of statistical analysis of the molecular size of the two substituted plasmids (Table 1, single values not amenable to grouping are excluded) showed that in both cases the plasmid DNA was heterogeneous. Besides the principal groups there were also additional groups and, what is particularly important, in one strain a sufficiently large number of small replicons with a molecular weight of 1.3 megadaltons was found. Incorporation of chromosomal genetic material into plasmid R18 evidently leads to destabilization of its structure and the subsequent formation of smaller plasmids.

The dispersion of molecular size of R18Arg thus revealed is nothing unusual. A similar situation is found, for example, in the case of other substituted R plasmids [11, 12], biodegradation plasmids [16], the hybrid structure of R-Ent [11], and so on. It is difficult at present to analyze the reasons for this instability of the "component" plasmids, because the mechanism of transfer of chromosomal markers by R plasmids or of mobilization of nonconjugative plasmids [5] has not yet been explained. Nevertheless it can be postulated that plasmids in general are labile structures, constantly recombining with one another (and with chromosomes), thereby generating families of plasmids differing in molecular weight and other properties [15, 17]; mechanisms of both homologous and integrative recombination may be involved in this process [1, 2]. Such a possibility is indirectly confirmed by data on the polyplasmid nature of wild-type strains of various species of bacteria [4, 14]. Under laboratory conditions this is not encountered so frequently as yet, possibly because most of the work is done with artificially bred (relatively stable) plasmids. It must also be remembered that the stability of plasmids may depend on the genetic features of the host cells of the plasmids [9].

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