To what can this "delay" of the hyperchromic effect be attributed? The optical density of the nucleus is known to be determined by the state of the secondary structure of DNA which, in turn, in chromatin depends on the character of its bond with the surrounding proteins. The absence of an increase in the absorption of DNA in the composition of the modified chromatin of trisomic cells up to 80°C was evidently due to the fact that this temperature is too low to rupture the bonds between the DNA chains and to convert it into the "coiled" state. These data confirm our idea of the high degree of condensation of the aneuploid genome.

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GENETIC FEATURES OF F-LIKE PLASMID pAP10-2 CONTROLLING SYNTHESIS OF THERMOSTABLE ENTEROTOXIN BY *E. coli* CELLS

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During an investigation of the plasmid complex discovered by the writers previously [1] in cells of conditionally pathogenic strain *E. coli* AP42-1 (serogroup 0101), isolated from a diseased calf, plasmid pAP10-2 with a molecular weight of 65 megadaltons, controlling synthesis of a thermostable enterotoxin (Ent-plasmid) was identified.

The object of this investigation was to study the genetic features of this Ent-plasmid after transmission into cells of plasmid-free strains of *E. coli* K-12.

EXPERIMENTAL METHOD

The ability of bacteria to produce thermostable enterotoxin was determined by intraperitoneal injection of 0.1 ml of cell-free culture fluid into newborn suckling mice [7]. The mice were kept for 4 h at 30°C and then killed, and their small intestine was weighed. The results of the experiments were expressed as a gravimetric index (ratio of weight of intestine to weight of remainder of animal). An isogeneic plasmid-free strain of *E. coli* K-12 was used as the control.

Other genetic markers were studied and the test bacteria conjugated by standard methods [1]. The sensitivity of the bacteria to donor-specific phage MS2 was determined by the agar layers method [8] or by tests to determine the increase in titers of this phage [3]. To eliminate individual plasmids the bacteria were grown in the presence of ethidium bromide [6].

Genetic marking [11] of the test plasmid was carried out with transposon Tn9, carrying determinants of resistance of the bacteria to chloramphenicol, and a component of plasmid RP1 with a temperature-sensitive replication system (a strain of bacteria containing this plasmid

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was obtained from G. B. Smirnov, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR).

To determine the ability of marked plasmid pAP10-2 to inhibit the fertility functions of plasmid F^1-lac^+, double plasmid transconjugants were obtained and then tested for sensitivity to phage MS2 and effectiveness of transmission of plasmid F^1-lac^+ into cells of plasmid-free strains of *E. coli* K-12.

Experiments to study compatibility (incompatibility) of the marked plasmid pAP10-2 followed the usual scheme [5], using reference plasmids of seven known incompatibility groups of F-like plasmids [2, 4, 9].

EXPERIMENTAL RESULTS

To obtain a clone of bacteria carrying one Ent-plasmid, cells of a previously bred [1] transconjugant strain of *E. coli* K-12 (14R525), carrying plasmids pAP10-2 (Ent) and pAP10-1 (Ap*, Km, Lm, Sm, Su), were grown in nutrient broth (NB) containing 300 μg/ml ethidium bromide. During subsequent clonal analysis of this bacterial population a clone which had lost the markers of drug resistance (i.e., plasmid pAP10-1), but which preserved plasmid pAP10-2 (Ent), was selected. The presence of this plasmid was judged from the ability of bacteria containing it to induce a distinct increase in size of the small intestine of the newborn mice (gravimetric index 0.085; corresponding values for the control plasmid-free strain 14R525 were 0.04-0.06). A further study of these bacteria also showed that they promote reproduction of donor-specific phages of the F-group. On this basis it was concluded that plasmid pAP10-2 is an F-like plasmid.

Since the Ent-plasmid studied possessed no convenient genetic markers for a more detailed investigation, attempts were made to mark it with transposon Tn9. As a result a marked variant of this plasmid, designated pAP10-2::Tn9, was obtained.

Investigation of the conjugative properties of plasmid pAP10-2::Tn9 showed that it can be transmitted to cells of various plasmid-free strains of *E. coli* K-12 with a frequency of the order of $4.7 \cdot 10^{-4}$ to $3.6 \cdot 10^{-5}$ (to one donor cell in the conjugation mixture). However, when this plasmid was introduced into cells of strain AP132, carrying plasmid F^1-lac^+, a considerable decrease in transmission frequency was observed ($1.4 \cdot 10^{-5}$). This frequency was only 1/34 of the frequency of transmission of the test plasmid into cells of plasmid-free strain AP132, reflecting the well-marked surface exclusion of this plasmid by the resistant plasmid F^1-lac^+.

A further study of the double plasmid transconjugants thus obtained, in order to determine the ability of plasmid pAP10-2::Tn9 to inhibit the fertility functions of plasmid F^1-lac^+ revealed the extremely unstable character of their inheritance of the lac^+ marker. However, analysis of individual transconjugants which preserved both plasmids showed that the test Ent-plasmid possesses this property, i.e., it belongs to the category of Fi^+-plasmids.

The high degree of segregation of the lac^+ marker in the double plasmid transconjugant suggested that plasmid pAP10-2::Tn9 probably belongs to the same incompatibility group as plasmid F^1-lac^+, i.e., to group FI. To test this hypothesis further, compatibility of plasmid pAP10-2::Tn9 with plasmids R386, R1-19, ColB-K98, R124, F_0lac, Hly-P212, and pAP38, the reference plasmids of incompatibility groups FI, FII, FIII, FIV, FV, FVI, and FVII, respectively, was studied. From each cross (forward and backward) 20 plasmid transconjugants were analyzed and the results showed that in 82.5-90% of cases the test plasmid coexisted with a reference plasmid of group FI (Table 1), in 92.5-100% of cases with a plasmid of group FIII, in 97.5-100% of cases with a plasmid of group FVI, and in 100% of cases with plasmids of group FII, FIV, FV, and FVII.

To clarify still more the question of the incompatibility group to which plasmid pAP10-2::Tn9 belongs, a clonal test was performed. For this purpose, 20 colonies obtained by seeding separate clones of double transconjugants after subculture in NB were tested in each case for the presence of markers of both plasmids (introduced and resident). Stable coexistence of the test plasmid with reference plasmids of groups FII, FIII, FIV, FVI, and FVII was discovered in 100% of cases and with plasmid of group FV in 95% of cases. Meanwhile, clonal analysis of transconjugants carrying reference plasmid of group FI (R386) showed loss of this

*The recommendations of Novick et al. [10], are used in this paper to designate plasmids and their genetic markers.

TABLE 1. Results of Study of Compatibility of Plasmid pAP10-2::Tn9 with Reference Plasmid R386, Belonging to F^i Incompatibility Group

Plasmid		Selective marker	Frequency of transmission of marker	Analysis of plasmid transconjugant bred			Results of clonal test for plasmid compatibility			
				percent of colonies whose cells contain			no. of transconjugant clones	percent of daughter colonies whose cells contain		
introduced	(resident)			introduced plasmid	resident plasmid	both plasmids		introduced plasmid	resident plasmid	both plasmids
pAP10-2::Tn9	(R386)	Lm	$0{,}6 \cdot 10^{-3}$	100	82,5	82,5	1	100	0	0
							2	100	25	25
							3	100	15	15
							4	100	15	15
							5	100	10	10
pAP10-2::Tn9	—	Lm	$3{,}6 \cdot 10^{-3}$	100						
R386	(pAP10-2::Tn9)	Tc	$1{,}6 \cdot 10^{-4}$	95	90	90	1	10	100	10
							2	85	100	85
							3	80	100	80
							4	15	100	15
							5	95	100	95
R386	—	Tc	$1{,}6 \cdot 10^{-2}$	85						

plasmid. The effectiveness of loss of this plasmid differed for different clones tested and varied between 0 and 95% (Table 1).

An additional investigation of individual clones of double plasmid transconjugants in crosses with suitable recipient strains of *E. coli* K-12 revealed in all cases the independent (nonlinked) character of transmission of each of the two coexisting plasmids.

It can be concluded from these results that plasmid pAP10-2 is a conjugative F-like plasmid, belonging to the FI incompatibility group.

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