AN ATTEMPT TO LOCALIZE 5S RNA CISTRONS IN ESCHERICHIA COLI

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Summary : Three different loci for 5S RNA cistrons are localized.

It has been shown that DNA contains sequences complementary to ribosomal RNA. In Escherichia coli the existence of cistrons for both 16S and 23S RNA was inferred from hybridisation experiments (1, 2, 3).

5S RNA described as a component of the 50S subunits of ribosomes (4) is an original piece of RNA different from either 16S and 23S RNA or from tRNA. The existence of DNA sequences complementary to 5S RNA has been demonstrated (5).

BROWNLEE et al. (6) have studied the nucleotide sequence of 5S RNA in two different strains of Escherichia coli: K_{12} CA 265 and MRE 600. In each, they described two major species differing by a single base substitution, at position 13 for the MRE 600 strain and at position 12 for the K_{12} strain. Furthermore one of us (B. JARRY, unpublished experiments) detected in K_{12} strains a single base substitution at position 91 or 92.

Analysis of the T_1 RNase degradation products of the whole 5S RNA of the two strains is given in table I. Deduced sequences in each case are shown in fig. 1.

In the MRE 600 strain, the hexanucleotide CCUUAG is detected among the T_1 RNase degradation products with a molarity close to 0.5 while trinucleotides CCG and UAG which occur elsewhere in the molecule are found with molarities higher than 3. CCG and CCUUAG are degradation products from the sequence types I and III respectively (6). The trinucleotide CAG is detected with a molarity of 0.10-0.20 and comes from species II (fig. 1) (our unpublished results).

In the K_{12} strains, the trinucleotide CAG is detected among the T_1 RNase degradation products, with a molarity of 0.5 and is interpreted as specific of species II. The trinucleotides CCG and UAG

Type	Sequence	Relative amount in K 12	Relative amount in MRE 600
	11 12 13 14 15 16		
I	C—C—G—U—A—G	0.50	0.30-0.50
II	C—A—G—U—A—G	0.50	0.10-0.20
III	C—C—U—U—A—G	—	0.40-0.50
	87 88 89 90 91 92 93 94 95 96		
A	U—C—U—C—(U C)—C—A—U—G	0.10-0.20	—
B	U—C—U—C—C—C—C—A—U—G	0.80-0.90	1.00

Figure I
Nucleotides sequences in 5S RNA

are found with molarities higher than 3. However the decanucleotide U_4C_4AG, which is not detected in MRE 600 5S RNA T_1 RNase digests, exists at a molarity of 0.10-0.20. This decanucleotide corresponds to a C-U substitution either at position 91 or at position 92 (fig.1) (our unpublished results). The 5S molecules which have U at position 91 or 92 are designed as having the A sequence. The other molecules which have C at position 91 or 92 have the B sequence. We don't known yet wether the type A sequence is associated with type I or type II sequences. The hexanucleotide CCUUAG cannot be detected and is therefore specific for MRE 600 5S RNA.

We took advantage of these clear cut differences between strains K_{12} and MRE 600 to map the cistron(s) specific for type III sequence or for type A sequence.

Methods

The strains we used are described in table II.

An Hfr derivative of MRE 600 was constructed, after curing of a colicinogenic factor, by infecting with F'_{ts74} lac a derivative of MRE 600 *his*$^-$ *try*$^-$ *lac*$^-$ *strA*^{R} and screening for lactose$^+$ at high temperature. This Hfr injects its chromosome clockwise (fig. 2).

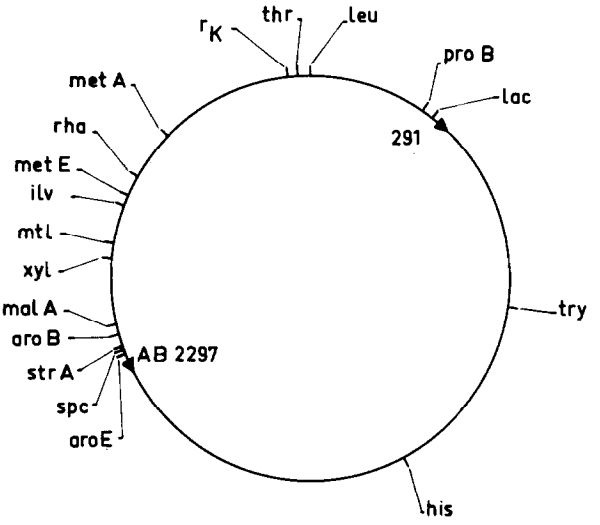

FIGURE II

Genetic map of E. coli

F^-K_{12} strains were made r_k^- to avoid the restriction between the two strains. Details on the building of the strains will be given elsewhere (B. JARRY and R. ROSSET, manuscript in preparation).

MRE 600 Hfr were mated at 30° in L medium (7) with F^-K_{12} strains. P_1 transducing phage was grown at 30° on MRE 600 and its derivatives and transduction was done according to LENNOX (8).

High specific activity ^{32}P-labelled 5S RNA after phenol extraction (4) and purification by polyacrylamide gel electrophoresis was analyzed after digestion with T_1 RNase by the fingerprinting technique of SANGER et al. (9).

Results

1) Transfer of 5S RNA cistrons from MRE 600 to K_{12}

We first checked the possibility to transfer genetic markers from MRE 600 to K_{12} ; a mating between 291 and K 921 rha$^-$ gives recombinants which received markers from 291 (table 3).

5S RNA of both parents and recombinants were analyzed by fingerprinting the T_1 RNase digest. The molarities of the differents degradation products were calculated as described in the legend of table I. The molarities of the trinucleotide CAG, the hexanucleotide CCUUAG and of the decanucleotide U_4C_4AG are given in table III.

TABLE 1

End-products of T_1 RNase digestion of 5S RNA

Sequence	MRE 600 wt Found+	MRE 600 wt Calc. from the sequence	K 12 wt Found++	K 12 wt Calc. from the sequence
G	9.80	11	7.40	11
CG	4.96	5	5.00	5
AG	2.10	2	2.70	2
UG	4.20	4	4.10	4
CCG	3.80	3.30-3.50	3.30	3.50
AAG	1.10	1	1.10	1
CCAG	0.92	1	0.91	1
AAACG	0.86	1	0.83	1
CCUG	0.94	1	0.98	1
UAG	3.80	3.30-3.50	4.00	4.00
AUG	1.02	1	0.97	1
AACUG	0.83	1	0.66	1
AACUCAG	0.61	1	0.79	1
ACCCCUAG	0.75	1	0.81	1
UCCCACCUG	0.82	1	0.76	1
UCUCCCCAUG	0.76	1	0.61	0.80-0.90
CCUUAG	0.42	0.5	not detected	0
pUG	0.84	1	—	1
CAU_{OH}	—	1	0.57	1
CAG	0.17	0.10-0.20	0.50	0.50
C_4U_1AG	not detected		0.15	0.10-0.20

Yields were obtained by measuring the radioactivity in the area of the paper containing the nucleotide and the results are expressed relative to :

$$\frac{(AAG) + (CCAG) + (CCUG) + (AUG)}{14} = 1.00$$

(+) They are unbiased averages for four experiments
(++) These results are the average of three experiments

The oligonucleotide U_4C_4AG is present in the 5S RNA of recombinants which received the lac-metA region of the Hfr chromosome to the same extent as in the 5S RNA of the parental female (class 1 and 2 recombinants). But it disappears when distant markers are transfered (class 3 recombinants) suggesting that the cistron (or cistrons) specific for type A sequence of K_{12} strains has been replaced by a cistron of type B sequence of MRE 600 strain ; a preliminary conclusion is that this cistron (or cistrons) is located between metA and strA.

The nucleotide CCUUAG appears in recombinants which received the marker strA but not his (class 4 recombinants). The 5S RNA pattern of such a recombinant, is the pattern of MRE 600. Therefore

TABLE 2

Name	Strain	Genotype
MRE 600	MRE 600	Wt *xyl*$^-$ col MRE 600
291	MRE 600	Hfr MRE 600 *his*$^-$ *try*$^-$ *str*Ar *xyl*$^-$
K 921 rha$^-$	K 12	F$^-$ *thr*$^-$ *leu*$^-$ *met*A$^-$ *rha*$^-$ r_k^- m_k^-
M 4-1	K 12	F$^-$ *thr*$^-$ *ilva*$_{105}$ *his*$_4$ *met*E$_{46}$ *try*$_3$ *pro*$_2$ *mal*A$_1$ *mtl*$_1$ *gal*$_2$ *lac*$_4$ r_k^- m_k^- *str*A$_8$ $T_1T_6^R$ *spcr*
T 121	K 12	F$^-$ *thr*$^-$ *leu*$^-$ *met*A$^-$ *aro*B$^-$ *str*Ar r_k^- m_k^-
T 1211	K 12	F$^-$ *thr*$^-$ *leu*$^-$ *met*A$^-$ *aro*B$^-$ *mal*A$^-$ *str*Ar r_k^- m_k^-
T 94	K 12	F$^-$ *thr*$^-$ *leu*$^-$ *met*A$^-$ *aro*E$_{24}$ *str*Ar r_k^- m_k^-
AB 2297	K 12	Hfr *ilva*D$_{1321}$ *pur*C$_1^-$ *xyl*H$_1$ su$_{16}^+$

Bacterial strains

TABLE 3

Mating 291 X K 921 rha$^-$

Class	Markers transferred	Number of recombinants analyzed	CAG	CCUUAG	U_4C_4AG
1	*Thr leu*	2	0.40±0.10	n.d.(+)	0.15±0.05
2	*Thr leu metA*	3	0.33±0.10	n.d.	0.12±0.05
3	*Thr leu metA rha*	1	0.22±0.10	n.d.	n.d.
4	*Thr leu metA rha xyl Sm*	1	0.15±0.05	0.40±0.10	n.d.
	291		0.15±0.05	0.40±0.10	n.d.
	K 921 rha$^-$		0.50±0.10	n.d.	0.15±0.05

291 (10^8 cells/ml) and K 921 rha$^-$ (5 x 10^8 cells/ml) were allowed to mate in L medium for 45 min at 37°. Cells were washed in minimum and plated on minimum glucose plates supplemented with methionine and biotine. Thr$^+$ Leu$^+$ recombinants were selected, purified on same plates and screened for unselected markers. Only recombinants classes the 5S RNA of which was analyzed are described in the table. Thr$^+$ Leu$^+$ markers were transferred with a frequency of 10^{-4}. 5S RNA was isolated and analyzed as described in methods ; molaritie are calculated as in the legend of table 1. Only the molarities of specific sequencies described are shown in this table.

(+) n.d. = not detected

TABLE 4

Mating 291 X M4-1

Class	Markers transferred	Number of recombinants analyzed	CAG	CUUAG	U_4C_4AG
5	pro metE	1	0.22±0.10	n;d.	n.d.
6	pro metE Ilva mtl	1	0.50±0.10	n.d.	n.d.
7	pro metE Ilva mtl xyl	1	0.40±0.10	n.d.	n.d.
8	pro metE Ilva mtl xyl mglA	1	0.50±0.10	n.d.	n.d.
		2	0.25±0.10	0.15±0.05	n.d.
	M 4-1		0.40±0.10	n.d.	0.10±0.05
	291		0.15±0.05	0.40±0.10	n.d.

Mating was done under the same conditions as for table 3. Selection of metE$^+$ recombinants was done on minimum plates complemented with isoleucine, valine, histidine, tryptophane and spectinomycin (ROSSET and GORINI, 1969). Selected recombinants were purified and screened for unselected markers. Only classes the 5S RNA of which was analyzed are given. 5S RNA was analyzed as in table 3.

the cistrons specific for type III sequence are transferred later than the rha marker ; at the same time the type II sequence disappears (suggesting that the two types of cistrons are alleles).

2) Analysis of the region strA-metA

To locate the map position of 5S RNA cistrons more precisely the poly-auxotroph K_{12} female M 4-1 was mated with 291 and the 5S RNA of class 5 to 8 recombinants was analyzed.

None of the recombinants which received metE have the type A sequence : the cistron(s) specific for the type A sequence in K_{12} strains is localized before metE and therefore between metE and metA.

None of the recombinants of class 5 to 7 have 5S RNA molecules with the type III sequence ; out of the 3 recombinants analyzed which received malA (class 8 recombinants), 2 received at least one cistron specific for the type III sequence : a preliminary conclusion was that a 5S RNA cistron is localized between spc and malA loci.

3) Analysis of the region aroE-malA

By P_1 transduction we transferred from MRE 600 to a K_{12} derivative the regions aroE-aroB (in two steps) and aroB-malA

TABLE 5

Transduction from MRE 600 to K_{12} derivatives

Recipient	Region transferred	CAG	CCUUAG	U_4C_4AG
T_{94}	aroE-strA	0.45±0.05	not detected	0.15±0.05
T_{121}	aroB-strA	0.50±0.05	not detected	0.15±0.05
T_{1211}	aroB-malA	0.54±0.05	0.17±0.05	0.10±0.05

P_1 lysates were grown on MRE 600. Selection was performed for aro^+ transductants on minimum plates supplemented with glucose, threonine, leucine and methionine. Transductants were purified on the same plates and screened for unselected marker. Two transductants of each class were analyzed for their 5S RNA sequences as in table 3.

(table 5). Only transductants which received both markers aroB-malA have 5S RNA with the oligonucleotide CCUUAG present with a molarity of 0.15 ± 0.05 : at least one of the cistrons specific for type III sequence is localized between markers aroB and malA.

4) Region his-aroE

No recombinant of class 8 has a 5S RNA pattern like the parent 291, suggesting that a cistron(s) is localized between markers spc and his. A class 4 recombinant (which has the same pattern as the parent 291) was made $malA^-$ (λ^R) and crossed with the Hfr AB 2297 which transfers anticlockwise aroE as a very early marker, selecting $strA^+$ $malA^+$recombinants. The type III sequence was still detected in these recombinants with a molarity of 0.15. We must conclude that at least one cistron in MRE 600 is actually present in a third region between his and aroE markers.

Discussion and conclusion

We have found some minor forms of 5S RNA always with the same molarity 0.15 ± 0.05 : the type A sequence in K_{12}, the type I sequence in K_{12} (figure 1). If we assume that every 5S RNA cistron is expressed at the same level, these results suggest that each of these minor forms are the transcription products of one cistron and that there are 5-10 cistrons for 5S RNA. Results from hybridization experiments agree reasonnably well with our determination (5, 10).

Using intraspecific crosses between two strains which

differ by bases substitution in 5S RNA, we have been able so far to determine 3 loci for 5S RNA cistrons : one between metE and metA for a sequence specific of K_{12} and two, between aroB and malA and between his and aroE, for a sequence specific of MRE 600. It is clear that this method enables us to map only those cistrons for which nucleotide sequence differences are observed in the two parental strains used.

The first hybridization studies (1) suggested that 16S and 23S RNA cistrons are clustered and more recently PURDOM et al. (11) concluded that 16S and 23S cistrons occur in tandems and that these tandems are scattered on the chromosome ; PACE and PACE (10) proposed that 16S, 23S and 5S cistrons, present at the same level per genome, could be part of a transcription unit with only one promotor site. The localization by ATWOOD (12) of 16S and 23S cistrons, by SYPHERD (13) of some 23S cistrons could fit with our mapping for the cistron specific for type A sequence ; the existence of some loci close to strA for 16S and 23S RNA cistrons (14, 15) could agree with our data for type III sequence. On the other hand KAPLAN (personnal communication) has found most of the 16S and 23S cistrons between ilv and rib markers where we found so far no 5S RNA cistron ; but we cannot exclude the possibility that the cistrons we do not detect are localized in this region. On the other hand a different situation has already been described in Xenopus laevis (16) where 5S RNA cistrons are not intermingled in 18S RNA and 28S RNA cistrons.

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