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Interaction between Denatured DNA and RNA of B. Stearothermophilus Involving only One Half of Total DNA

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Biofisica. — *Interaction between Denatured* DNA *and* RNA *of* B. Stearothermophilus *Involving only One Half of Total* DNA. Nota di MARIO AGENO (*), ELISABETTA DORE (*), CLARA FRONTALI (*), MARIA ARCA (**), LAURA FRONTALI (**) e GIORGIO TECCE (**), presentata (***) dal Corrisp. M. AGENO.

During the last year a series of experiments was performed in our laboratories to try to ascertain if only one strand of DNA is copied *in vivo* by messenger RNA in bacteria, as is found in phages [1–3] and as is indicated also for *E.coli* DNA [4]. The esperimental design was to determine accurately the fraction of denatured DNA not involved in hybrid formation with *m*-RNA, by means of the analysis of bands formed in CsCl density gradient in the analytical ultracentrifuge. In the course of these experiments it was found that a peculiar type of DNA–RNA hybrid molecule is formed when solutions of denatured DNA and ribosomal RNA are mixed at room temperature. This work reports results which indicate that exactly one half of the total DNA is involved in the formation of this kind of hybrid (which we will call pseudo-hybrid to distinguish it from hybrids formed after prolonged annealing at 60°), and describes some of its properties. While this paper was in preparation, a paper by Opara-Kubinska, Kubinsky and Szibalsky [5] appeared which reports results very similar to ours for the interaction of DNA and RNA from several bacilli.

The materials used in this work are DNA and RNA from various sources. Among others from *B.stearothermophilus* whose nucleic acids were already studied by our group [6–9]. Extraction was performed according to the procedures of Marmur [10] and Monier et al. [11]. Extracted RNA was fractionated on a MAK column [12], and the material of the peaks containing ribosomal and messenger RNA was used in the experiments, after reconcentration and dialysis against SSC (0.15 M NaCl; 0.015 M Na-citrate). This procedure reduces the sedimentation coefficient of RNA from 19 and 29 S to 4–5 S. Fig. 1 shows the profile obtained in CsCl density gradient *a*) for 45 μg RNA and *b*) for 2 μg denatured DNA alone. Note that denatured DNA gives an asymmetrical band [6]. Fig. 1 *c* shows the profile given in the same conditions by a mixture containing 2 μg denatured DNA and 35 μg RNA. Near the band of denatured DNA a heavier band has appeared, having approximately the same area, but a larger width, than the first one. The interpretation of this band as due to a new kind of DNA–RNA interaction

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was tested at first by enzyme treatment. While DNAase completely destroys both bands, the effect of RNAase is not so clearcut. Fig. 2 shows the shape of the bands given by an RNA–DNA mixture *a*) before and *b*) after RNAase treatment; in these graphs the continuous background due to RNA fragments was subtracted, and the resulting curve decomposed into two gaussian curves [13]. In Table I results of this analysis are reported for two such experi-

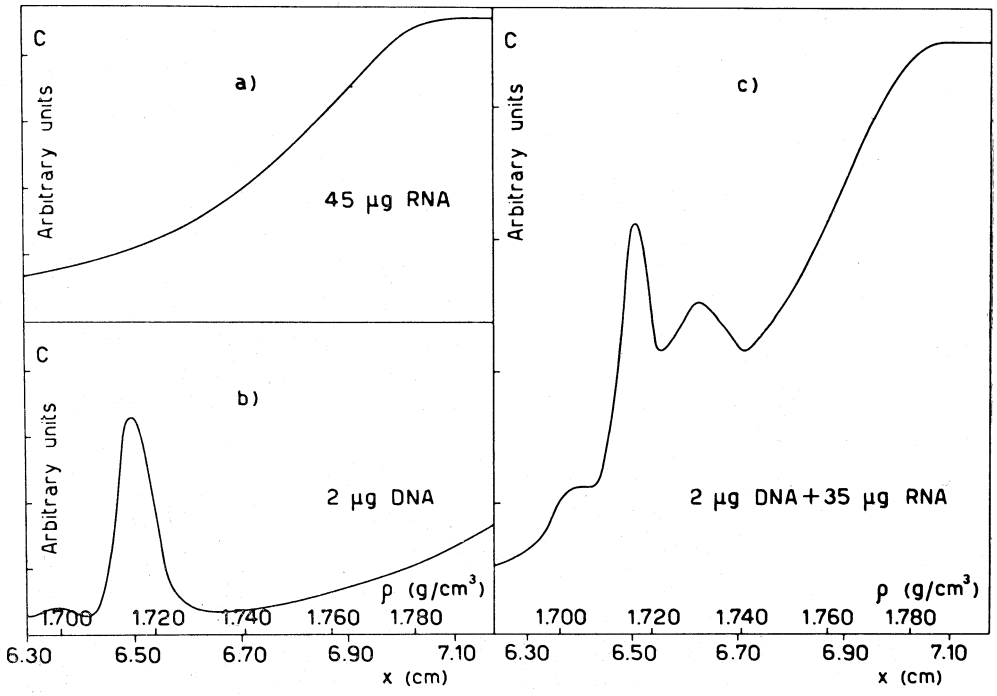

Fig. 1. – Densitometer tracings of U.V. photographs taken at the analytical ultracentrifuge in CsCl density gradient after 24 hours at 44770 rpm. Amounts of nucleic acids in the centrifuge cell are indicated. The minor band appearing on the left side of denatured DNA may be due to a small amount of non denatured or renatured DNA.

ments. It is seen that RNAase treatment, while leaving completely unaffected the denatured DNA band, reduces the density and the width of the heavier band. The ratio between the areas of the two gaussians is in every case very near to 1.

All these results are consistent [14] with the hypothesis that the heavier band is due to a hybrid molecule formed by only one half of the strands in the denatured DNA solution. The small density difference between the two bands, together with the fact that the ratio between the areas is equal to 1, suggests that the pseudo-hybrid, far from being a complete double strand, is given by a certain number of small RNA fragments bound to a DNA strand in such a way as to involve only a minor fraction, say a few per cent, of the whole genome. This model is supported by the fact that RNAase treatment, while affecting the density difference, leaves unaltered the areas

of the two gaussians, thus suggesting that a measurable density difference may be given by the attachment (or detachment) of pieces not significantly contributing to the measured absorption. It is believable that RNAase digests the free ends of RNA fragments attached to a DNA strand by some short nucleotide sequence. This would explain why RNAase treatment also reduces the dispersity of the heavier band.

TABLE I.

Denatured DNA	RNA	RNAase	$\bar{x}_2-\bar{x}_1$ mm	$\Delta\rho \cdot 10^3$ g/cm^3	σ_1 mm	σ_2 mm	σ_2/σ_1	A_2/A_1
2 μg	35 μg	no	0.87	9.2	0.23	0.34	1.47	0.95
2 μg	35 μg	+	0.50	5.5	0.22	0.23	1.11	0.90
2 μg	35 μg	+	0.48	4.6	0.20	0.25	1.23	1.00

$\bar{x}_2-\bar{x}_1$ is the center to center distance of the two gaussians expressed in mm in the centrifuge cell; $\Delta\rho$ is the density difference.

σ_1 and σ_2 are respectively the standard deviations of the denatured DNA and heavier band; A_2/A_1 is the ratio between their areas.

TABLE II.

DNA μg	RNA μg	RNA s	$\bar{x}_2-\bar{x}_1$ mm	$\Delta\rho \cdot 10^3$ g/cm^3	σ_2/σ_1	A_2/A_1
2	22	26	0.75	8.7	2	1.08
2	22	6.5	1.05	12.5	1.9	1.02
2	35	5.3	0.96	11.4	1.75	0.99
2	35	3.7	0.87	10.7	1.47	0.95

s is the sedimentation coefficient in Svedberg; for the other symbols see Table I.

These findings prompted us to investigate the effect of the degradation of RNA on the formation of the heavy band. In order to study this point we made use of high molecular weight RNA separated from soluble RNA by means of repeated precipitations [15]. Such RNA preparations contain practically no slower fragments when observed in sedimentation velocity experiments. The analysis in two gaussian components of different experiments performed with degraded RNA of known sedimentation coefficient, yields the data reported in Table II. It is seen that the ratio of the width

of the second band to the first decreases when reducing the size of RNA molecules. This could be explained by the fact that the pseudo-hybrid formed with short RNA fragments is more homogeneous with respect to density than the pseudo-hybrid formed with a relatively small number of longer molecules. Again the ratio between the areas is in every case equal to 1.

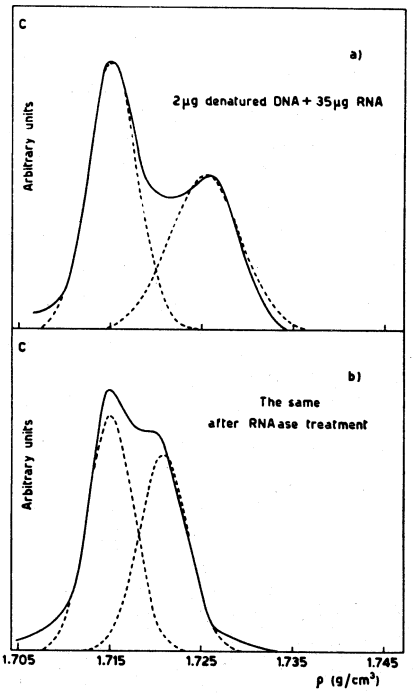

Fig. 2. – Decomposition in gaussian components of the curves obtained from densitometer tracings:

a) for a mixture of 2 µg denatured DNA and 35 µg RNA; *b*) for the same mixture after 30 min. treatment with RNAase (1 µg/ml) at 37° C.

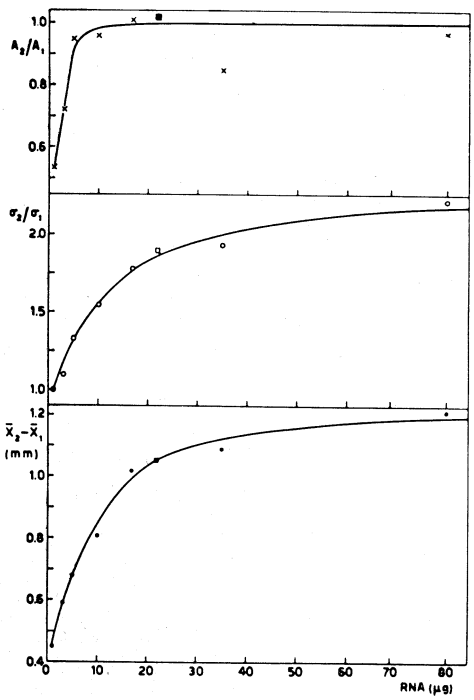

Fig. 3.

a) Center to center distance; *b*) ratio between standard deviations and *c*) ratio between areas of the two gaussian components, versus RNA amount (in µg) in a mixture containing 2 µg denatured DNA.

The following data concern the more fundamental problem of determining which is the maximum fraction of denatured DNA involved in the formation of the pseudo-hybrid.

A series of experiments was conducted on the same RNA preparation, by varying the ratio of RNA to DNA amount in the mixture. The results of the decomposition into two gaussian curves are summarized in Table III and in fig. 3. By increasing the amount of RNA, a saturation value is reached for the distance between the centers of the gaussians, and for the ratios of their widhts and areas. Most important is the fact that already for an RNA/DNA ratio equal to 2 the limit of 1 is reached for the ratio between the areas.

This strongly suggests the hypothesis that only one of the two DNA strands is able to interact with RNA. This hypothesis is supported by the following observations:

a) the first band (that of denatured DNA) retains the same width throughout Table III, indicating that the DNA fraction interacting with RNA has the same molecular weight and the same overall base composition as the bulk of DNA;

b) for very low amounts of RNA, the standard deviation of the two gaussians is exactly the same, indicating that the two fractions of DNA thus separated have the same molecular weight.

TABLE III.

DNA μg	RNA μg	$\bar{x}_2 - \bar{x}_1$ mm	$\Delta\rho \cdot 10^3$ g/cm^3	σ_2 mm	σ_1 mm	σ_2/σ_1	A_2/A_1
2	1	0.45	5.3	0.28	0.28	1.00	0.50
2	3	0.59	7.2	0.28	0.31	1.11	0.70
2	4	0.68	8.2	0.28	0.37	1.33	0.95
2	10	0.81	9.8	0.28	0.43	1.55	0.96
2	17	1.02	12.2	0.28	0.50	1.78	1.10
2	35	1.12	13.5	0.27	0.51	1.94	0.85
2	80	1.21	14.6	0.28	0.62	2.22	0.97

For the symbols see Table I.

Of course, an unequivocal confirmation of this hypothesis can only be reached through preparative separation of the two bands and demonstration of the inability to renature of DNA strands from either component. Preliminary results in this sense were obtained by passing the mixture on a MAK column-modified according to Sueoka and Cheng [16]. Two peaks are recovered, the first of which does not renature even after prolonged annealing while the second one renatures only partially. These results will be reported elsewhere.

We finally would like to recall attention to a few other observations:

a) the observed asymmetry in the band of denatured DNA is significantly reduced after treatment RNAase; this suggests that it is due to some RNA contamination in DNA solutions;

b) the formation of this kind of hybrid molecule is not limited to *B.stearothermophilus* since a similar phenomenon was observed with nucleic acids from *B.megatherium*. Furthermore it is not specific, since the formation

of the heavy band was observed by mixing RNA from *E.coli* and DNA from *B.stearothermophilus*. No band was observed in the reciprocal mixture (RNA from *B.stearothermophilus* and DNA from *E.coli*) and with RNA and DNA from *E.coli*;

c) soluble RNA from *B.stearothermophilus* does not form pseudo-hybrids with denatured DNA.

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RIASSUNTO. — (Interazione tra DNA e RNA di *B.stearothermophilus* interessante solo la metà del DNA totale).

Si descrive un'esperienza che dimostra che, in un batterio, una sola delle due eliche del DNA è capace di formare un complesso con RNA ribosomico. Si utilizza a questo scopo un nuovo tipo di molecola ibrida che si forma a temperatura ambiente tra una ben determinata delle due eliche del DNA e frammenti di RNA ribosomico. Vengono studiate le proprietà del complesso e l'elica del DNA non impegnata in esso viene isolata per via cromatografica, e si verifica che essa non è in grado di rinaturare con se stessa.