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Interaction between denatured DNA and ribosomal RNA: properties of pseudohybrids isolated on nitrocellulose filters

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Biologia molecolare. — *Interaction between denatured* DNA *and ribosomal* RNA: *properties of pseudohybrids isolated on nitrocellulose filters.* Nota di MARIA ARCÀ, LAURA FRONTALI e GIORGIO TECCE, presentata (*) dal Corrisp. M. AGENO.

RIASSUNTO. — L'interazione che si verifica a temperatura ambiente tra RNA ribosomiale e DNA denaturato dei bacilli è stata studiata col metodo della filtrazione su membrane di nitrocellulosa. Sono state così determinate le condizioni per la formazione del complesso o pseudoibrido, la sua resistenza alla RNAasi e le condizioni che ne provocano la dissociazione.

La quantità di pseudoibrido formato in presenza di eccesso di RNA ha consentito di stabilire quale percentuale dell'elica trascrivente del DNA può interagire con lo RNA.

Le caratteristiche di questo tipo di ibrido vengono brevemente discusse anche in relazione al suo possibile significato fisiologico.

The occurrence of a new type of interaction taking place at room temperature between ribosomal RNA and denatured DNA has been recently extensively investigated by means of CsCl density gradient centrifugation [1, 2, 3]. The name of "pseudohybrids" has been proposed to distinguish real specific DNA–RNA hybrids from these complexes, which are formed at room temperature between denatured DNA from several microorganisms and homologous, as well as heterologous RNA.

It has been previously demonstrated that only one DNA strand is involved in the formation of these complexes [1–3], and recent experiments performed with DNA from phages having strands of different buoyant density have shown that only the physiologically active strand partecipates in the formation of pseudohybrids [4].

A similar interaction takes place between one strand of denatured DNA and polyguanylic acid [3, 5, 6], while both strands interact with poly–U.

To investigate further the nature and the characteristics of these complexes, we have undertaken a study of the formation of pseudohybrids by means of the nitrocellulose membrane technique [7].

In most of this work a heterologous mixture was used containing [^{14}C]–RNA from *E. coli* and denatured DNA from *B. stearothermophilus*. A uracil requiring strain of *E. coli* was grown in a minimal medium [8] containing 4 μg/ml [^{14}C] uracil (specific activity 150 μC/mg); after two doublings a hundred-fold excess cold uracil was added and after 0.8 doublings growth was stopped by pouring the culture on crushed ice. Cells were collected by centrifugation and RNA extracted as previously reported [9]. The specific activity of the RNA was 5000 counts/min/μg.

(*) Nella seduta dell'8 aprile 1967.

DNA was prepared from *B. stearothermophilus* and from *E. coli* as previously reported [9]. Native DNA preparations were denatured by heating for 10′ at 100° solutions containing less than 20 μg/ml DNA in 1/10 SSC (SSC = standard saline citrate = 0,15 M NaCl, 0.015 M Na citrate).

Mixtures containing various quantities of denatured DNA from *B. stearothermophilus*, and of [^{14}C] RNA from *E. coli* in 1 ml 2×SSC were kept at 25° for 5 hours, unless otherwise stated. After a twentyfold dilution with 2 × SSC the samples were filtered through nitrocellulose membrane filters (Membranefilter MF 30) and the filters were washed with 100 ml of 2 × SSC. Membranes were then placed on alluminium planchets and counted in a Nuclear Chicago gas flow counter. The radioactivity resistent to RNAase was evaluated as reported by Spiegelman and Gillespie [10] by immersing the filters for 60′ at room temperature in 5 ml of 2 × SSC containing 20 μg/ml pancreatic RNAase (Worthington heated at 90° for 10′). The filter was then washed again with 100 ml of 2 × SSC and counted. Alternatively the treatment with RNAase was performed by adding RNAase to the sample before the filtration.

The results obtained with these two procedures were substantially similar. The proportion of RNAase resistent radioactivity was about 15 % of the radioactivity initially retained on the filter. The results of a typical experiment are reported in Table I.

TABLE I.

Radioactivity retained on nitrocellulose filters from mixtures of denatured DNA *and ribosomal* RNA *incubated at room temperature.*

COMPOSITION OF THE MIXTURE	Counts/min	Counts/min after RNAase treatment
40 μg DNA from *B. st.* + 30 μg [^{14}C] RNA from *E.coli* in 1 ml	3050	450
30 μg [^{14}C]RNA from *E. coli* in 1 ml	60	5
40 μg DNA from *E. coli* + 30 μg [^{14}C]RNA from *E. coli* in 1 ml	364	160
40 μg DNA from *B. st.* + 30 μg [^{14}C]RNA from *E. coli*; filter washed with boiling water	50	

The formation of pseudohybrids is not immediate and the rate of the reaction depends on the DNA and RNA concentration. In the conditions usually adopted in the course of this work the reaction was complete after 5 hours.

In the homologous mixture containing [^{14}C] RNA and DNA from *E. coli* the radioactivity retained on the filter after incubation at room temperature

was only slightly higher than that retained after the filtration of samples containing only [^{14}C] RNA.

This is consistent with the results of preceding experiments by analytical ultracentrifugation which had failed to demonstrate the formation of detectable quantities of pseudohybrids between RNA and DNA from *E. coli* at room temperature.

The data reported in fig. 1 on the dependence on temperature of the radioactivity retained on filters show that the formation of pseudohybrids is maximal at 28° and decreases rapidly at higher temperatures.

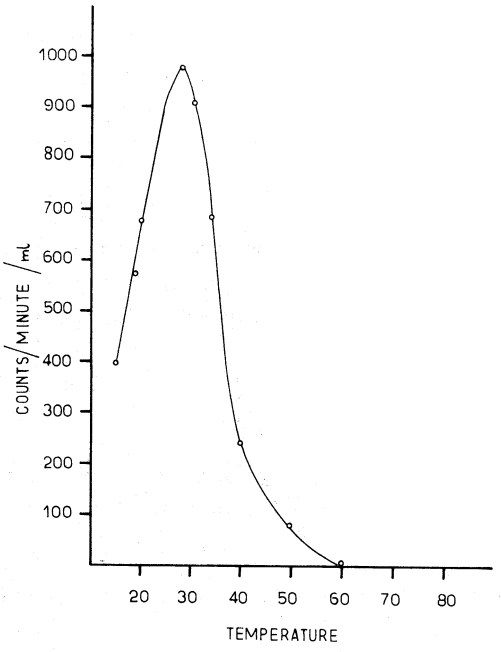

Fig. 1. – Radioactivity retained on nitrocellulose filters as a function of temperature: mixture containing 40 μg/ml denatured DNA from *B. stearothermophilus* and 20 μg/ml [^{14}C] RNA from *E. coli*.

TCA precipitable radioactivity was not found to decrease after incubation at various temperatures.

The heat stability of the complex formed at this temperature was investigated by incubating for 10′ at various temperatures the pseudohybrid preformed at 28°. Results are reported in fig. 2.

To investigate what part of the physiologically active DNA strand is capable of interacting in this way with ribosomal RNA, we have studied the formation of pseudohybrids as a function of RNA concentration. As can be seen from fig. 3 a saturation plateau is reached for a ratio retained RNA single DNA strand of 0.084. If RNAase resistent radioactivity is taken as a measure of the length of base paired segments in pseudohybrids, we can say that 1.1 % of one DNA strand can interact in this way with ribosomal RNA.

This rules out the possibility that this kind of interaction takes place at the sites for ribosomal RNA on bacterial DNA: in fact these sites have been demonstrated to cover 0.3 % of the single DNA strand [11].

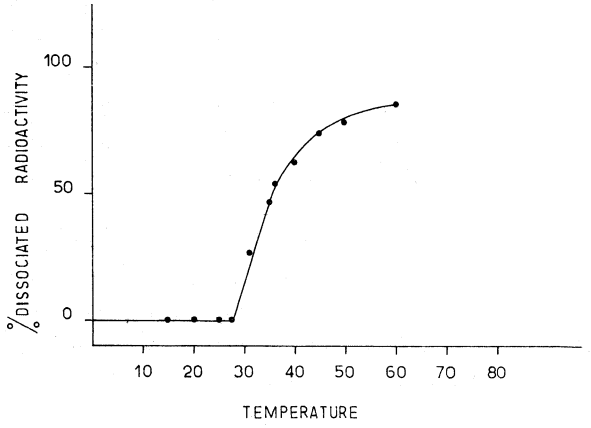

Fig. 2. – Dissociation curve of the complex between denatured DNA from *B. stearothermophilus* and [^{14}C] RNA from *E. coli*.

Assay conditions: a solution containing 40 µg/ml denatured DNA from *B.st.* and 30 µg/ml [^{14}C] RNA in 2 SSC was incubated for 5 hours at 28°. After a twenty-fold dilution aliquots were taken, incubated for 10' at various temperatures, chilled in an ice bath and filtered.

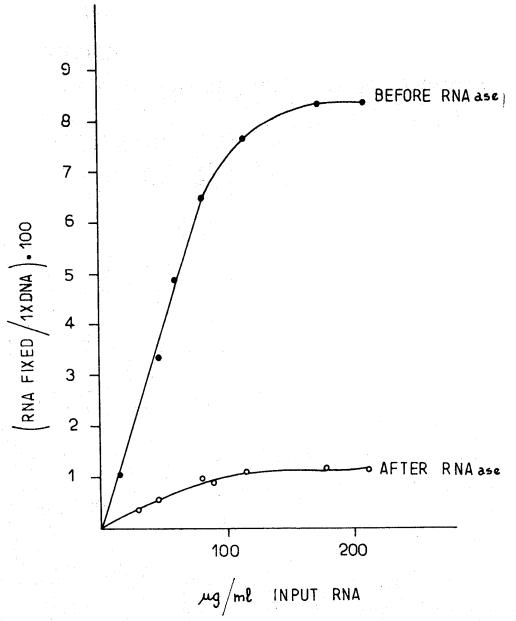

Fig. 3. – RNA retained on nitrocellulose filters per DNA single strand as a function of the quantity of input RNA.

The dissociation curve, which has a T_m of 35° supports the idea that in pseudohybrids base paired regions are formed with the participation of short nucleotide sequences of ribosomal RNA and of the physiologically active

DNA strand. Unpaired RNA tails are digested by RNAase and the saturation curve after RNAse treatment shows that the paired sequences do not exced 1.1 % of the DNA strand.

Several questions remain to be answered: first of all whether or not the interaction between denatured DNA and ribosomal RNA is quite the same as has been observed with synthetic polynucleotides. If this is the case, then the interaction should take place between G–rich sequences of ribosomal RNA and C–rich sequences of the transcribed DNA strand. However it is not yet clear why G–rich regions on the other strand of DNA fail to interact with either ribosomal RNA or poly C.

As for the possibility of a physiological meaning of this kind of hybrid, two different hypotheses have been put forward. Since evidence has recently been found indicating that ribosomes are involved in the DNA dependent synthesis of RNA [12, 13], it is conceivable that the interaction between ribosomal RNA and determinate sites on DNA might guide ribosomes to the regions on the chromosome where transcription initiates.

Another hypothesis has been put forward by Szybalski [5, 6], namely that pyrimidine rich clusters might have a peculiar secondary structure which could result in a high affinity for DNA dependent RNA polymerase; therefore these clusters might correspond to initiation points of RNA transcription. In this case interaction of DNA with ribosomal RNA should be purely accidental.

The possible relationship between the above characteristics of pseudo-hybrids and these hypotheses are now under investigation.

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