COMPLEMENTARY REGIONS OF THE NUCLEAR PRECURSOR OF MESSENGER RNA

A. P. RYSKOV, G. N. YENIKOLOPOV and S. A. LIMBORSKA*
Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow 117312, USSR

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1. Introduction

It has been shown previously that after self-annealing of the nuclear precursor of messenger RNA (pre-mRNA) at high values of C_0t about 20% of the material becomes resistant to ribonuclease digestion [1]. This was explained by the existence of self-complementary regions in the molecules of pre-mRNA and by the ability of these sequences to form double-stranded complexes after annealing.

In this paper we report on the isolation and the most important properties of this fraction of RNA (self-complementary RNA or scRNA). Its possible origin is discussed.

2. Materials and methods

Pre-mRNA was isolated by the hot phenol fractionation technique from Ehrlich ascites carcinoma cells, labelled with ^{32}P for 1–1.5 hr [2]. After centrifugation in a sucrose gradient in the presence of SDS the fraction of RNA heavier than 20S was collected and additionally purified as described previously [2]. Pre-mRNA was annealed in 2 × SSC at 65°C at appropriate values of C_0t. The reaction was stopped by rapid cooling, after which the sample was digested with a mixture of pancreatic RNAase (50 μg/ml) and guanylic RNAase** (50 μnits/ml in conditions of high ionic strength (2 × SSC) at 37°C for 40 min and the content of RNAase-stable acid-precipitable material was determined.

For preparative isolation of self-complementary sequences the fraction of pre-mRNA was annealed at C_0t values of 2500–3000. The material was treated with RNAase as described above and then with pronase (100 μg/ml) at 25°C for 30 min. The sample was applied to a Sephadex G-25 column. RNA was eluted with 0.2 M sodium acetate. Those fractions which entered the exclusion volume were collected, deproteinized by 1% SDS-phenol and precipitated with cold ethanol. The material was dissolved in water, denatured by heating and reprecipitated with ethanol several times.

DNA content was measured by the Burton–Dische procedure [3].

Base composition of the [^{32}P]scRNA was determined according to Markham and Smith [4]. Thermal elution of double-stranded fragments from hydroxyapatite (Bio-Gel HTP, DNA grade; Bio-Rad Lab.) was performed in 0.06 M Na–phosphate buffer as described previously [5]. Electrophoresis of scRNA in polyacrylamide gel with formamide was done according to Staynov et al. [6]. Hybridization of denatured scRNA sequences was carried out in 2 × SSC + 0.5% SDS at 65°C with DNA gels, cross-linked by UV-irradiation [7] at RNA:DNA = 1:1500. In the DNA-driven reaction, the C_0t value was approximated by

* Present address: Institute of Oncology, Academy of Medical Sciences of the USSR. *Abbreviations:*
C_0t = concentration (mole/liter) × time(sec)
Poly A = polyadenylic acid
1 × SSC = 0.15 M NaCl + 0.15 M Na-citrate, pH 7.0
SDS = sodium dodecyl sulfate.

* Guanylic ribonuclease from Actinomyces (EC 2.7.7.26) equivalent to T1 ribonuclease was kindly provided by Dr R. I. Tatarskaya.

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assuming that the rate of hybridization with immobilized DNA is 10 times slower than that in solution [8,9]. Reassociation of denatured scRNA sequences took place at 65°C in 2 X SSC.

3. Results

Fig. 1 shows the results of the self-annealing of pre-mRNA and of the fraction of RNA, extracted from nuclei at 40°C and which consists of ribosomal RNA and its precursors [2]. One can see, that over 20% of pre-mRNA becomes RNAase-stable after self-annealing at high C_0t values. Ribosomal RNA anneals only up to 2.8% at C_0t = 1000; this could perhaps be explained by some contamination with pre-mRNA.

After treatment of annealed pre-mRNA with a mixture of RNAases the hydrolysate was chromatographed on Sephadex G-25 (fig. 2). RNAase stable material, eluting in the excluded volume was collected.

The data obtained strongly suggest that the RNAase-stable material recovered after self-annealing of pre-mRNA is really double-stranded RNA. DNA is practically absent in this preparation (< 1%); the preparation is sensitive to RNAase after denaturation and in conditions of low ionic strength; polyA content is only 0.5% as determined by adsorption on nitrocellulose filters [10] and 1.4% by the results of retention on polyU–sepharose columns [11]; scRNA has a symmetrical base composition with $(G+C)/(A+U)$ = 2.00; A = 16.7% ± 1.3%, U = 16.3% ± 2.5%; G = 30.4% ± 1.2%; C = 36.6% ± 1.5%.

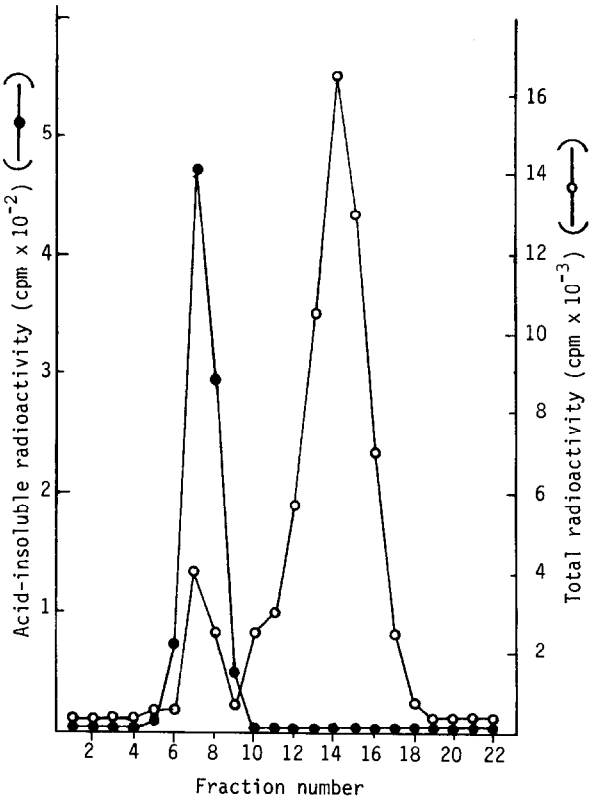

Fig. 2. The isolation of scRNA from an RNAase hydrolysate of pre-mRNA after its self-annealing at the C_0t = 2500.

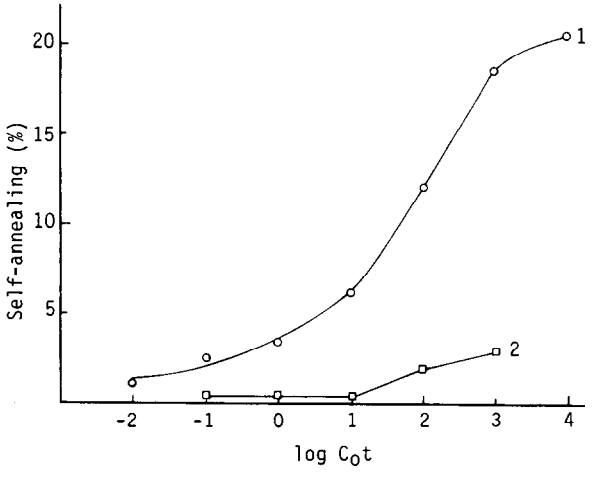

Fig. 1. The formation of double-stranded RNAase-resistant structures in the course of annealing of pre-mRNA (1) and nuclear ribosomal RNA (2).

Fig. 3 shows the thermoelution of scRNA from hydroxyapatite. This curve has the form typical of the melting profiles of doublestranded nucleic acids, with a narrow interval of melting and T_m = 79°C. After denaturation all material is eluted at 25°C as single-stranded RNA.

The molecular weight of the single strand of scRNA determined by electrophoresis in polyacrylamide gels with formamide is 15 000 (45–50 nucleotides, fig. 4).

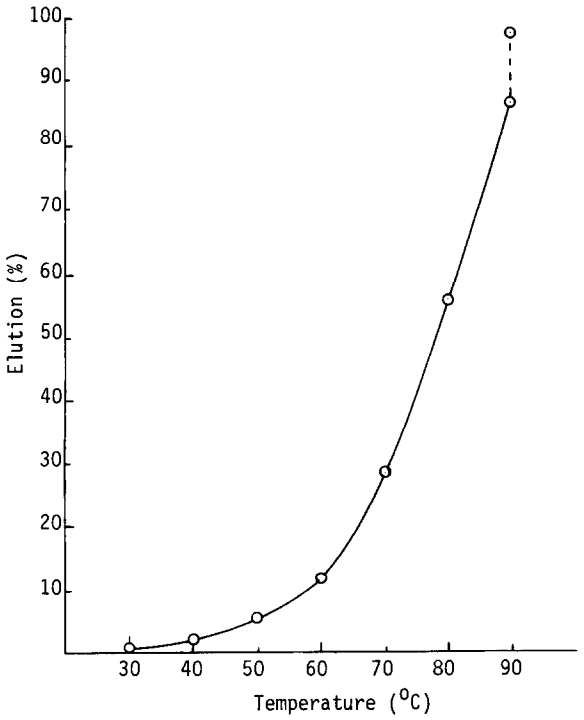

Fig. 3. Thermal elution of scRNA from hydroxyapatite. scRNA was applied to a hydroxyapatite column in 0.06 M phosphate buffer at 20° C. After heating to the desired temperature the column was washed with 10 vol of the same buffer, 5 ml fractions were collected and radioactivity was monitored by Cherenkov counting.

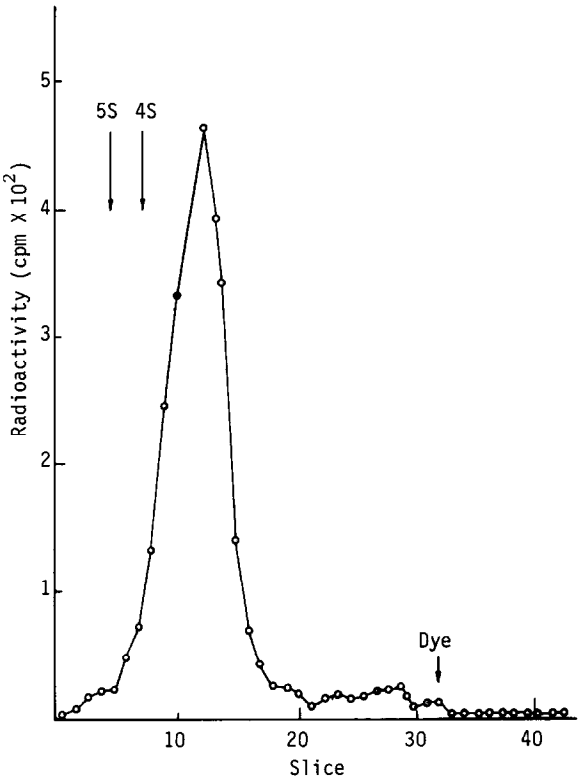

Fig. 4. Electrophoresis of denatured scRNA in polyacrylamide gel. Electrophoresis was carried out for 6 hr at 110 V in 15% gel, with formamide as solvent.

The kinetics of hybridization of denatured scRNA sequences with DNA gels are given in fig. 5. The curve presents a biphasic profile, giving $C_0t_{1/2}$ value for the first part as 2×10^{-2} and for the second part as 1.2×10^2. It can be seen that the effectiveness of the reaction reaches 30%. The most probable reason for this is that degradation of RNA takes place during prolonged incubation, as mentioned by others [12,13]. The yield of hybridization is not affected by higher DNA/RNA ratios (10 000:1) at high C_0t values ($\simeq 10^4$) showing that the hybridization reaction has in fact reached its plateau. From the values of $C_0t_{1/2}$ for both parts of the hybridization curve one can see that scRNA is transcribed only from the reiterated sites present in mouse RNA. This means that almost all the rapidly hybridizing fraction of pre-mRNA is self complementary.

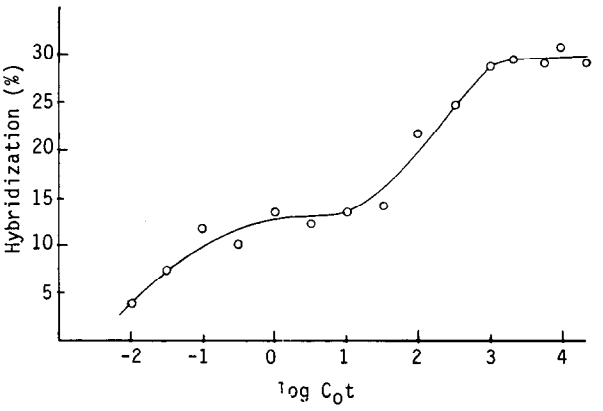

Fig. 5. Hybridization curve of denatured scRNA sequences the excess of DNA at different C_0t values.

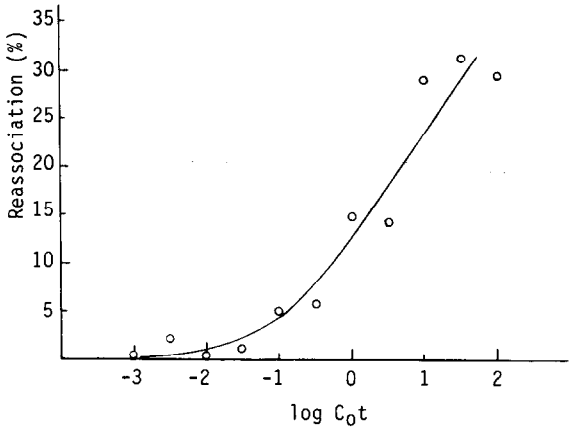

Fig. 6. Reassociation curve of denatured scRNA sequences. Denatured scRNA was incubated in 2 × SSC at 65°C for the time necessary to obtain the appropriate C_0t value. After annealing the sample was diluted with 2 × SSC and content of the total and acid-precipitable radioactive material was determined.

The reassociation curve of denaturated scRNA presents the characteristic shape obtained for homogeneous material, with $C_0t_{1/2} = 1.5$ (fig. 6). This corresponds to a complexity of about 7×10^5 base pairs [14]. Since the size of scRNA is equal to about 50 base pairs, it can be calculated that 15 000 different kinds of self-complementary sequences may be found in pre-mRNA. Unfortunately it is not known what fraction of the genome is transcribed as scRNA and thus we cannot make any calculations as to the number of such regions in the mouse genome.

Thermoelution curves of the RNA–DNA hybrid and secondary RNA–RNA renaturant are close to that of the initial double-stranded complex (scRNA) with $T_m = 80°C$ (not shown here). It must be remembered that possible divergences of reiterated sequences will eliminate the possibility of precise base-pairing [14,15].

4. Discussion

In this paper the isolation and properties of self-complementary sequences in pre-mRNA are described. One of the essential characteristics of those sequences is their high molecular weight reaching at least 15 000 for one strand. The probability of coincidental existence of such long complementary sequences in pre-mRNA is very low. There are several possible explanations for the origin of scRNA:

1) Specific symmetrical transcription of the reiterated sites of mouse nuclear DNA; 2) transcription of a special fraction of DNA–'inverted repetitions' [16, 17], separated with stretched nucleotide sequences; and 3) existence in the genome of such transcribing sequences, the functions of which do not change with their orientation with respect to the sense strand of a given transcriptional unit. In this instance, due to the transcription of the differently oriented sequences of various operons, one would find self-complementary species of RNA which is the case. At present it is difficult to choose between these possibilities.

As can be seen from the curve of hybridization with DNA and the renaturation curve (fig. 5 and 6), there are two types of self-complementary sequences in the mouse genome, differing in the degree of repetition, but in pre-mRNA they exist in equal quantities. It may be proposed that 1) transcription of both types of scRNA is proportional to their genome content, but its degradation rates are different; and 2) transcription of both types of sequences is equimolar. In this case the transcription of sequences of these two types occurs with different effectiveness. It is important to note, that self-complementary sequences as well as the haiprin-like sequences described previously [5,18,19] are transcribed from reiterated sites of the genome. It may be concluded that existence of mutually complementary sequences is a distinctive property of the rapidly-hybridizing fraction of pre-mRNA.

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