# THE COMPARISON OF MELTING PROPERTIES OF THE 28 S NUCLEAR MESSENGER-LIKE RNA, 28 S RIBOSOMAL RNA AND 9 S GLOBIN mRNA FROM PIGEON ERYTHROID CELLS

K. G. GASARYAN, A. I. NICKOLAEV and V. Z. TARANTUL
*I. V. Kurchatov Institute of Atomic Energy, Moscow, USSR*

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## 1. Introduction

Secondary and tertiary structures of an RNA molecule most probably determine specificity of its relations with other substances and its functions. Since pioneering works by Doty [1] and Spirin [2] the conformation of RNA has not been a subject of careful investigation except tRNA. Just recently it has been possible to reveal with the help of electron microscopy a number of characteristic structural components in 28 S rRNA [3] and in some mRNAs [4].

The high resolution ultraviolet spectrophotometry now provides many possibilities of studying RNA conformation by detailed thermal denaturation. Few attempts have been made to use this approach to study rRNA [5] and polysomal mRNA [6,7] molecules, and no work of this kind has been done with nuclear messenger-like RNAs.

A discrete class of metabolically stable nuclear messenger-like RNA with sedimentation coefficients about 28 S has been described [8]. This RNA (designated as 28 S snRNA) constitutes about 75% of the nuclear 28 S RNA in pigeon bone marrow erythroblasts, 13–15% of the total RNA present in these cells. Hybridization-competition experiments revealed no sequence homology between 28 S snRNA and 28 S rRNA, in spite of their conspicuous size-similarity. At the same time 28 S snRNA exhibits a principal sequence resemblance to heterogeneous nuclear RNA, differing from the latter by its strikingly high metabolic stability. We suggested that 28 S snRNA represented nuclear stores of unprocessed (or partially processed) precursors for messengers RNAs. Another possibility that this RNA plays some regulatory functions within the nucleus was also discussed [8].

Here we present the thermal denaturation characteristics of the 28 S snRNA and two known RNA species: 28 S rRNA and polysomal 9 S globin mRNA. The results show that RNAs display distinct melting properties which are considered to reflect the peculiarities of the secondary and tertiary structures of their molecules.

## 2. Materials and methods

Bone marrow cells (erythroblasts) and immature cells of peripheral blood (reticulocytes) were obtained from a pigeon rendered anaemic by phenylhydrazine [9]. 28 S snRNA and 28 S rRNA were prepared as described [8]. 9 S mRNA was obtained from reticulocyte polysomes by chromatography on poly(U)–Sepharose (Pharmacia) [10]. Thermal denaturation curves were obtained on a Beckman Acta M VI spectrophotometer. Continuous electric heating provided the linear increase of temperature up to 90°C at 0.4°C/min. In a typical experiment the quartz cells were charged with 100–150 μg RNA in 2.5 ml solution containing 0.5 M Na-phosphate buffer (pH 7.2), 0.05% sodium dodecylsulphate, 0.5 mM EDTA and different concentrations of NaCl. The solutions and glassware were pretreated with 0.1% diethylpyrocarbonate followed by heating at 95°C for 2 h to remove the reagent.



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## 3. Results and discussion

The melting curves of the RNAs were found to be reproducible enough in the range 25–90°C, which justified calculation of $\Delta A_{260}/\Delta T$ at 1°C intervals. In fig.1 the slope differential is plotted versus temperature. The differences between RNAs are evident: a smooth differential melting curve of 28 S snRNA and discrete melting pattern of 9 S mRNA and especially of 28 S rRNA. This points to the existence in the 28 S rRNA molecules of discrete classes of helical regions the denaturation of which results in the separate peaks on the differential plot. Three peaks on the differential plot of 28 S rRNA in the high-temperature region most probably are created by the denaturation of GC-rich hairpins, probably those long (200–300 base pairs) double and triple hairpins of extremely high GC content which have been revealed recently in pigeon 28 S rRNA by electron microscopy [3]. In contrast the denaturation of 9 S mRNA reveals several discrete transitions in the low-temperature region of the curve suggesting that the molecules of this RNA contain at least three thermolabile helices. The differential melting curve of 9 S mRNA resembles that of ovalbumin mRNA [7] and differs from the differential curve of rabbit globin mRNA [6].

Figure 2 presents the dependence of hyperchromicity ($h_{260}$) of RNAs evaluated from the difference in absorbance at 90°C and 25°C ($h_{260}$ = 100% ($A_{90°C}$–$A_{25°C}$)/$A_{25°C}$) on ionic strength in the 0.01–0.5 M

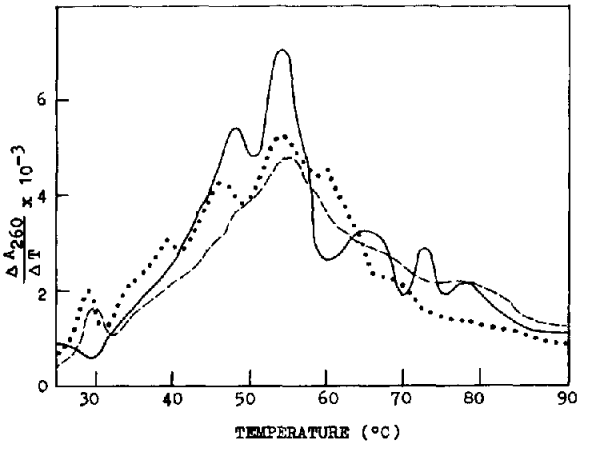


Fig.1. Differential melting curves of RNAs in 0.05 M NaCl. (——) 28 S rRNA; (– – –) 28 S snRNA; (· · · ·) 9 S mRNA.

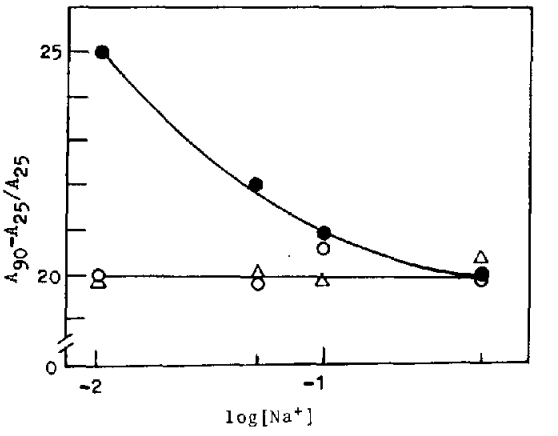


Fig.2. Dependence of the hyperchromicity of RNAs on the NaCl concentration. (●–●) 28 S rRNA; (○–○) snRNA; (△–△) 9 S mRNA.

NaCl range. The $h_{260}$ value appeared to be rather constant in this range for 9 S mRNA and 28 S snRNA (i.e. for the messenger-like RNAs), but shows significant ionic strength dependence for 28 S rRNA. The decrease of $h_{260}$ value observed for 28 S rRNA points to the existence in this RNA of secondary structures enriched in GC nucleotides, which are stabilized at increased salt concentrations in such a degree that they remain undenatured at 90°C. These structures may be identified with the above helices the denaturation of which resulted in the peaks on the differential curve in fig.1. The data of fig.2 show that at least 20% of the hyperchromicity of 28 S rRNA is most probably caused by these hairpins. It should be mentioned that the hairpins visualized in this RNA by electron microscopy include about 20% of its sequences [3].

Experimental and theoretical evidence indicates that melting temperature both for double-stranded and single-stranded polynucleotides is a linear function of the logarithm of ionic strength [5,6]. The value $dT_m$/dlog[NaCl] may vary depending on conformation, in particular on compactness of the molecules as the compactness must shield internal structures from cation influence.

The dependence of the RNA melting temperature ($T_m$) on log[NaCl] is given in fig.3. All three RNAs show an increased $T_m$ with addition of NaCl indicated by a slope $dT_m$/dlog[NaCl] which is different for each

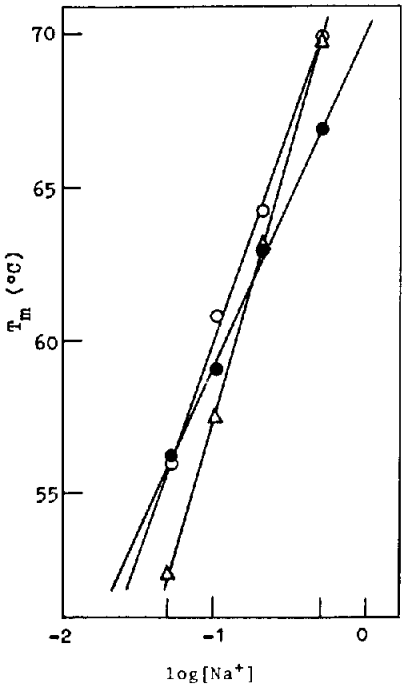


Fig.3. Dependence of the mean melting temperature of RNAs on the logarithm of NaCl concentration. (•—•) 28 S snRNA; (○—○) 28 S rRNA; (△—△) 9 S mRNA.

type of RNA. This dependence is sharper for 9 S mRNA and 28 S rRNA than for 28 S snRNA. We assume that a reason for this difference may consist in the extent of compactization of RNAs, i.e., 28 S snRNA becomes more compact, 28 S rRNA and 9 S mRNA molecules remaining relatively open structures with the increase of ionic strength.

In conclusion, in 28 S rRNA most of the double-stranded structures are relatively thermolabile denaturing at $t \leqslant T_m$. About 20% of the paired bases are highly enriched in guanine and cytosine and are involved at least in three large hairpins. It is suggested that these hairpins restrict compactization of 28 S rRNA molecule. Such an open tertiary structure stabilized by the hairpins might be significant as a factor determining the conformation of large ribosomal subunit.

28 S nuclear messenger-like RNA differs distinctly from the 28 S rRNA not only in its primary structure [8] but also in the thermal denaturation characteristics, i.e., in the secondary and tertiary structures. No discretely denaturing structures were revealed in this RNA by thermal denaturation. This may be a consequence either of inhomogeneity of this RNA class or actual absence of separately melting helices. The latter interpretation suggests that this RNA consists of highly flexible chains of about 4500 nucleotides long containing only short double-stranded structures, and/or scattered complementary sequences. The secondary structure formed in this RNA does not prevent the compactization of the molecules. Such a property might be essential in view of the suggestion that 28 S snRNA, which exists in the nuclei in a form of ribonucleoprotein tightly associated with chromatin (unpublished data), represents a storage form of unprocessed stable messenger RNA precursors. The ability to form compact structures may be required to provide the packing of these RNP particles in chromatin. As has been repeatedly demonstrated by Bernhard's group [11,12] chromatin may contain a lot of such metabolically stable RNP granules.

9 S mRNA contains at least three thermolabile hairpins each melting separately at $t < T_m$. Its tertiary structure seems to be relatively open even at high ionic strength, the compactization of the molecule being supposedly restricted by its short length.

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