DOUBLE-STRANDED STRUCTURES IN THE POLY(A)-CONTAINING CYTOPLASMIC RNA

Effect of cortisone

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1. Introduction

Cortisone induces preferential activation of transcription of repetitive DNA sequences in rat liver nuclei [1–3]. A part of the transcripts of the activated repetitive sequences is transferred to the cytoplasm, according to competitive hybridization data [4].

To get further insight into the nature of the cortisone-induced RNA, the population of the cytoplasmic poly(A)-containing RNA has been studied. We report here the detection, isolation and some important properties of the double-stranded (ds) regions isolated from poly(A)-containing cytoplasmic RNA. It has been shown that the content of ds-regions is increased after cortisone injection.

2. Materials and methods

Male Wistar rats (100–120 g) were used. Cortisone-acetate in 1 ml 0.14 M NaCl (5 mg/100 g body wt) was injected intraperitoneally 3 h before the animals were sacrified. Control rats received 1 ml 0.14 M NaCl 40 min before slaughter [^{14}C]orotic acid (300 μCi/100 g body wt) was administered to both control and cortisone-treated rats. All animals had been fasting for 24 h.

Nuclear, cytoplasmic and polysomal RNA was isolated and purified as in [5].

Poly(A)-containing RNA (poly(A)$^+$-RNA) was isolated from high-molecular weight cytoplasmic or polysomal RNA with poly(U)–Sepharose chromatography [6].

Poly(A)$^+$-RNA was annealed in 2 × SSC at 65°C at appropriate values of C_0t. The reaction was stopped by rapid cooling and samples were treated with a mixture of pancreatic RNase (50 μl/ml), T_1 RNase (50 units/ml) and T_2 RNase (2 units/ml) in 2 × SSC at 37°C for 40 min. The RNase-resistant material was collected on membrane filters (0.45 μm, HAWP, USA) after precipitation with cold 5% trichloroacetic acid. All measurements were carried out on triplicate samples.

For preparative isolation of RNase-resistant ds-regions the poly(A)$^+$-RNA was treated with the mixture of RNases as above and then with pronase (100 μg/ml) at 25°C for 30 min. The material was deproteinized by 1% SDS–phenol and reprecipitated several times with cold ethanol. For renaturation experiments the material was purified on a Sephadex G-50 column.

Electrophoresis of RNase-resistant ds-RNA in 10% polyacrylamide gel containing 0.2% SDS was done at room temperature [7].

Melting temperature of the ds-RNA was determined as follows. Material was dissolved in 2 × SSC and incubated at appropriate temperature for 7 min. After rapid cooling, the samples were digested with RNase, and content of acid-precipitable material determined.

Reassociation of denatured ds-RNA was done at 65°C in 2 × SSC. Thereafter the samples were also treated with RNases.

Isolation of chromatin and in vitro RNA synthesis with RNA-polymerase from *Escherichia coli* were done as in [1].

DNA content was measured by the Burton-Dische procedure [8].

Specific radioactivity: nuclear RNA ~5 × 10^3 cpm/μg; cytoplasmic RNA, 5 × 10^2 cpm/μg; poly(A)-containing RNA, 2 × 10^3 cpm/μg.

Reagents: [^{3}H]orotic acid, 1 Ci/mmol (Amersham,

England); DNase, electrophoretically pure, RNase-free (Worthington, USA); RNAse A, T_1, T_2 and pronase (Calbiochem, USA); poly(U)–Sepharose (Pharmacia, Sweden); pyronin (Merck, FRG); [^{3}H]UTP, 46 Ci/mmol (Amersham, England); unlabelled nucleosidetriphosphates (Boehringer, FRG).

3. Results and discussion

The cytoplasmic poly(A)$^+$-RNA from cortisone-stimulated hepatocytes displays higher resistance to treatment with the mixture of RNases than control poly(A)$^+$-cytoplasmic RNA (fig.1). The same holds true for the denatured (100°C, 10 min) cytoplasmic poly(A)$^+$-RNA, isolated from normal and cortisone-treated rats (2.9% and 5.5%, respectively). Hence, in poly(A)$^+$-RNA a part of the RNase-resistant nucleotide sequences is able to restore their secondary structure immediately after denaturation. It may be suggested that these structures are self-complementary nucleotide sequences, located at close range, forming double-stranded 'hairpin-like' structures. The other part of the RNAse-resistant structures are probably intramolecular RNA duplexes formed by reassociation of complementary sequences arranged at a longer distance within one RNA molecule or intermolecular duplexes formed by reassociation of complementary sequences of different RNA molecules. The latter suggestion is favoured by the data on the kinetics of reassociation (fig.2). As can be seen from this figure, the effectiveness of the formation of the RNase-resistant structures on self-annealing of poly(A)$^+$-RNA depends on $C_0^{RNA} \cdot t$ values. One can see that the degree of self-annealing is also higher for the RNA from the livers of hormone-treated animals.

Unlike cytoplasmic RNA no difference in the resistance to RNases has been observed between nuclear RNA isolated from control and cortisone-treated rats (3.7% and 3.9%, respectively). Either the nuclear RNA from control and hormone-stimulated liver cells did not differ in their ability to form RNase-resistant structures after self-annealing (fig.3).

The observed differences in the effect of cortisone upon nuclear and cytoplasmic RNA with respect to the content of RNase-resistant regions, may be accounted for by the rapid transfer of the cortisone-induced RNA molecules, containing such structures, from nucleus to cytoplasm. Such transport might be needed for a rapid adaptive response of the cell to the hormonal stimulus. This suggestion is favoured by the data obtained for the RNA synthesized in the cell-free

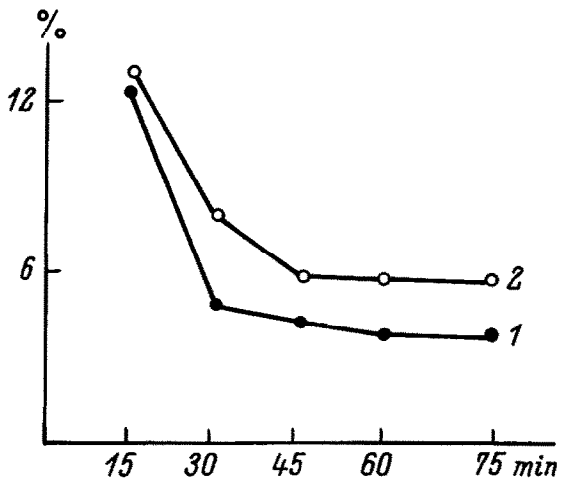

Fig.1. Digestion by (T_1 + T_2 + A) RNases of cytoplasmic poly(A)$^+$-RNA molecules isolated from livers of control and cortisone-treated rats. [^{3}H]Orotic acid-labelled poly(A$^+$)-RNA were prepared as described and samples containing ~10 000 cpm were dissolved in 0.35 M NaCl and digested at 37°C with (A + T_1 + T_2) RNases for the indicated times. Radioactivity remaining acid-precipitable during the digestion is plotted. (1) Control RNA; (2) RNA isolated after cortisone treatment.

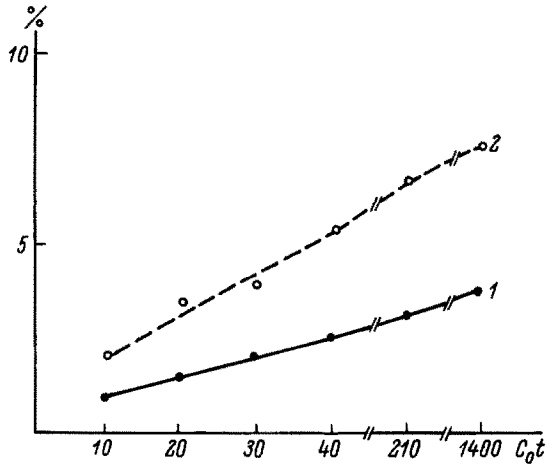

Fig.2. The formation of RNase-resistant structures in the course of annealing of poly(A)$^+$ cytoplasmic [^{3}H]RNA. (1) Control RNA; (2) RNA isolated after cortisone injection. Abscissa: $C_0^{RNA} \cdot t$ (C_0, initial RNA concentration in mol nucleotides/litre; t, time in seconds).

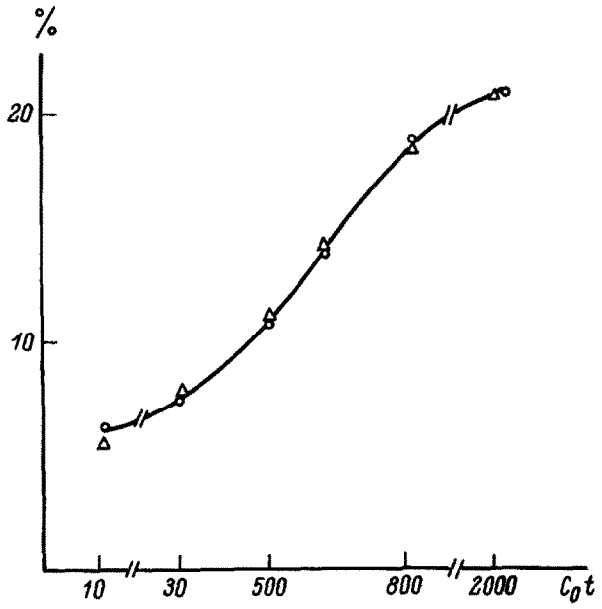

Fig.3. The self-annealing of nuclear [^{3}H]RNA, isolated from control (○) and cortisone-treated rats (△).

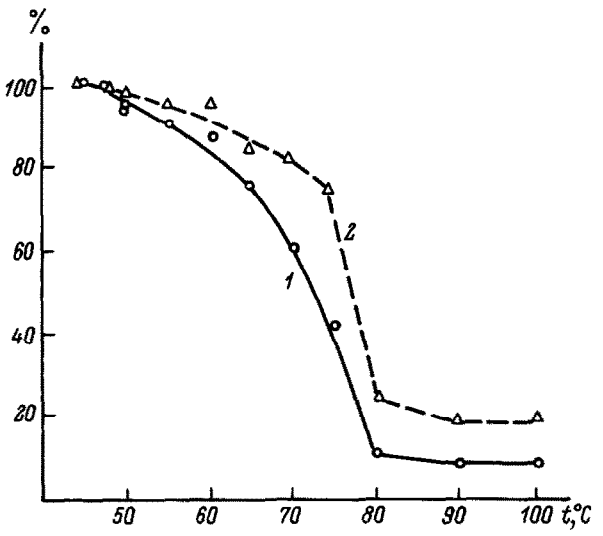

Fig.5. Melting curves of native-c-ds-RNA. (1) control c-ds-RNA; (2) c-ds-RNA from cortisone-treated rats.

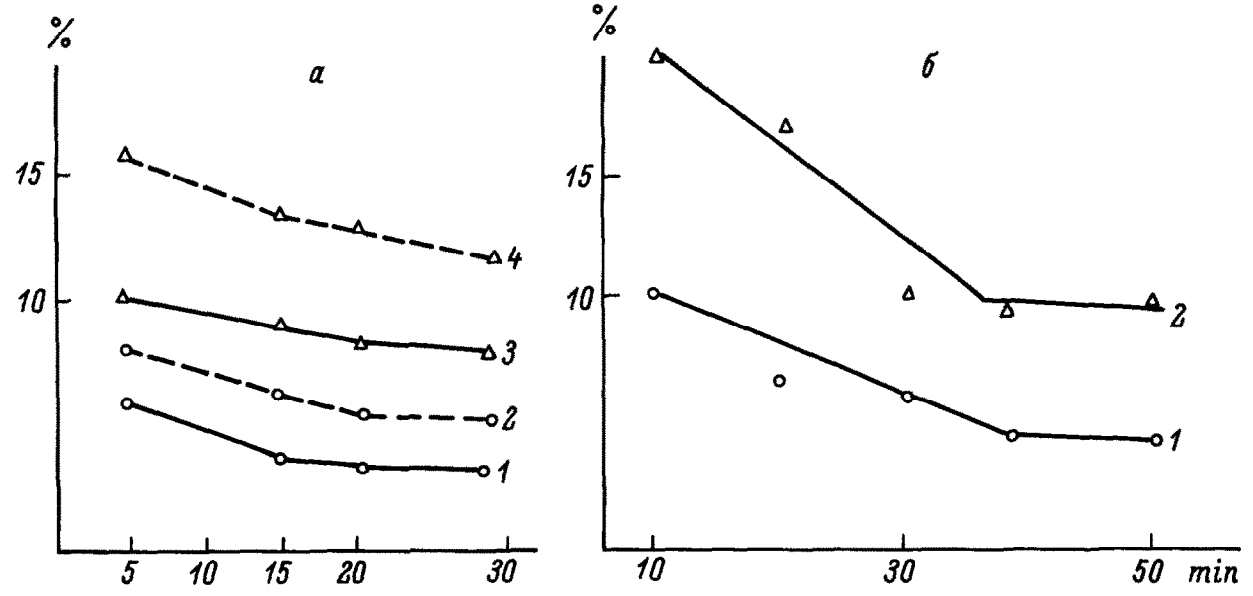

Fig.4. Digestion by (T_1 + T_2 + A) RNases of the [^{3}H]RNA, synthesized in vitro on chromatin templates. (a): (1,2) RNA synthesized on control chromatin; (3,4) RNA synthesized on chromatin from cortisone-activated hepatocytes; (1,3) denatured RNA samples; (2,4) native RNA samples. Abscissa: RNase concentration: RNase A (μg/ml). The ratio of concentration of RNase A (μg/ml) to RNase T_1 (units/ml) = 10:1. Ordinate: Resistance to RNases (%). Time of hydrolysis, 40 min. (b): (1) native RNA transcribed from control chromatin; (2) native RNA synthesized on chromatin from cortisone-stimulated hepatocytes.

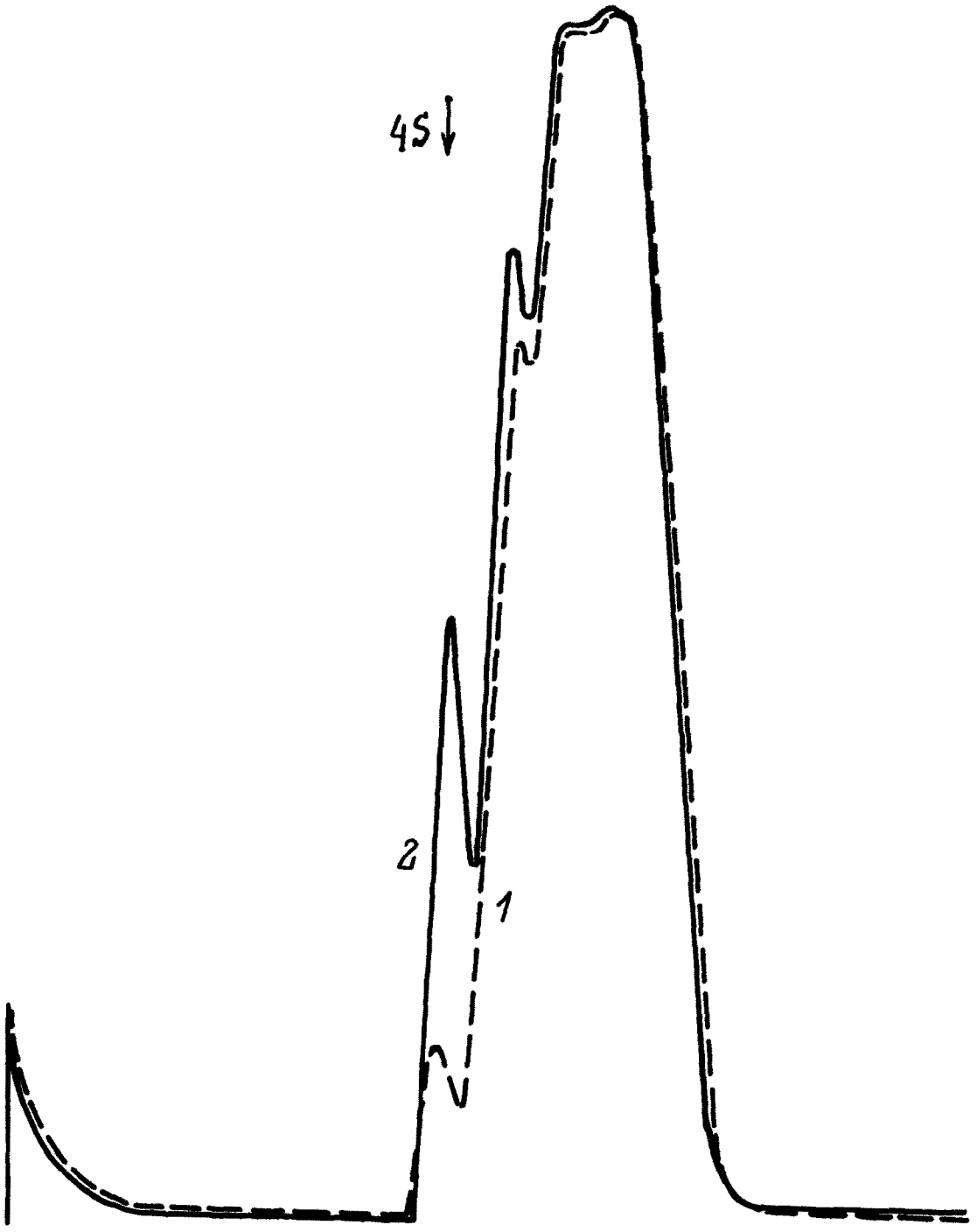

Fig.6. Electrophoresis of native c-ds-RNA in polyacrylamide gel. Electrophoresis was done in 10% gel for 2.5 h at 100 V. (1) Control ds-RNA; (2) ds-RNA isolated after cortisone treatment.

system. The RNA transcribed in vitro from chromatin templates isolated from cortisone-stimulated hepatocytes has been shown to contain a higher amount of the RNase-resistant structures (fig.4a,b).

To study the nature of the RNase-resistant structures, they were isolated as in section 2. DNA is practically absent in these samples (<1%), poly(A)-content is ~1% as determined by retention of denatured material on poly(U)-Sepharose columns.

After denaturation (100°C, 10 min) the isolated ds-RNA shows a 90% sensitivity to RNases, i.e., it becomes single-stranded. The melting profiles of RNase-resistant RNA structures are typical of ds nucleic acids. They have narrow melting intervals, and T_m = 72°C for RNA from control rats and T_m = 76°C for RNA isolated from cortisone-stimulated animals (fig.5).

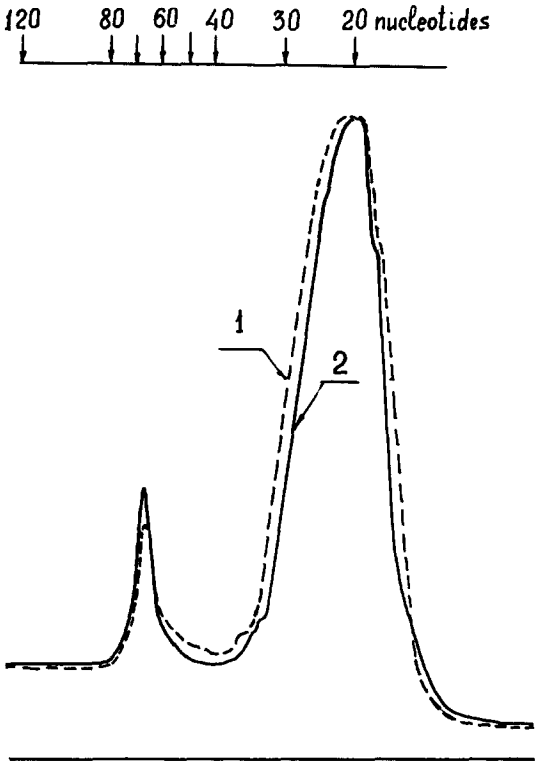

Fig.7. Electrophoresis of denatured c-ds-RNA in polyacrylamide gel. Electrophoresis was done for 2.5 h at 150 V in 5% gel. 7 M urea was used as a solvent. Buffer: 0.02 M Tris–borate, 0.002 M EDTA, pH 8.3. (1) Control ds-RNA; (2) ds-RNA isolated after cortisone treatment. Fragments of chromatin digested with DNase I [17] were used as markers.

All the above data suggest that the isolated RNase-stable material is really ds RNA (c-ds-RNA: cytoplasmic double-stranded RNA).

Fig.6 shows the electrophoresis of the isolated native c-ds-RNA on polyacrylamide gels. One can see the discrete bands of 3 size classes. The pattern of the distribution of the material from control and corti- proportions being unknown, we can calculate only sone-stimulated hepatocytes is similar, but not identical. The c-ds-RNA from the livers of cortisone-treated rats contains the higher proportion of high-molecular weight component.

The size of the denatured single-stranded c-ds-RNA determined by polyacrylamide gel electrophoresis in denaturing conditions (7 M urea) is 10–50 nucleotides (fig.7).

The reassociation curve of the denatured c-ds-RNA is presented in fig.8. Addition of a 1000-fold excess of the poly(U) did not change the effectiveness of the reassociation. Therefore poly(U)–poly(A) complexes do not take part in the formation of the ds-structures in poly(A)$^+$-RNA. It is seen from fig.6 that reassociation occurs with $C_0t_{1/2} = 1.02 \times 10^3$. This corresponds to a complexity of ~450 base pairs [9]. Since there are fractions of c-ds-RNA of several size classes, their

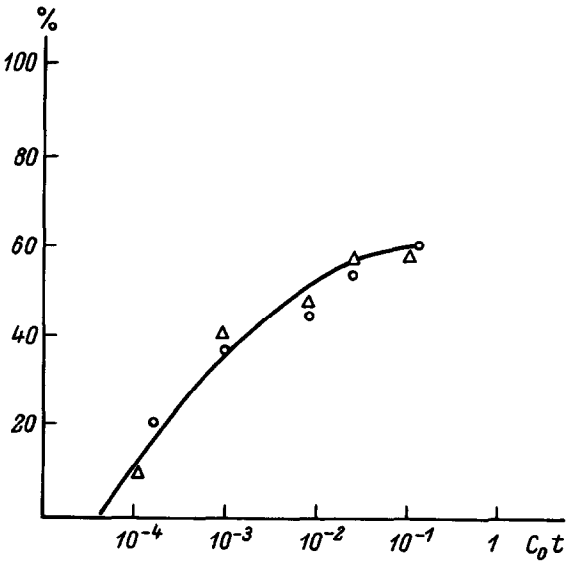

Fig.8. Reassociation curve of denatured c-ds-RNA. Denatured ds-RNA was incubated in 2 × SSC at 65°C for the time necessary to obtain appropriate C_0t values. After annealing the sample was diluted with 2 × SSC, treated with RNases and content of total acid-precipitable radioactive material was determined.

that the number of different kinds of c-ds-sequences in cytoplasmic poly(A)$^+$-RNA is $\leqslant 40$ and $\geqslant 9$.

Here the isolation and properties of ds-regions in poly(A)-containing cytoplasmic RNA are described. Similar structures were studied earlier in heterogeneous nuclear RNA [10,11]. However, ds-structures in the poly(A)-containing cytoplasmic RNA have lower molecular weights than the ds-structures in nuclear RNA [10,11]. They are probably different structures.

The data from physico–chemical studies of individual mRNA suggest the existence of a high proportion of secondary structure in molecules of mRNA, but the regions with ordered secondary structure have not been isolated. A common conclusion is that mRNA contains only short ds-regions of ~4–5 nucleotide pairs [12–16].

Cortisone has been shown to increase the content of the ds-regions within poly(A)-containing cytoplasmic RNA. This fact favours the regulatory role for the ds-RNA. The secondary and tertiary structure of the RNA molecules possibly determine the specificity of their interaction with other molecules, for example with the protein translating factors or proteins of RNP-particles, changing the effectiveness of the translation or transfer of RNA from nucleus to cytoplasm. It may be assumed that the lifetime of the RNA molecules is also changed. All these alterations may be needed for adaptive response of the cell to hormonal stimuli.

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