# POLYPHOSPHATE GROUPS AT THE 5′-ENDS OF NUCLEAR dRNA FRACTIONS

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## 1. Introduction

It has been shown previously that newly-formed nuclear dRNA (RNA with DNA like base composition) has a significantly higher molecular weight than cytoplasmic mRNA [1–6], and it was suggested that the short chains of dRNA are formed by cleavage of long precursor chains and the destruction of several sequences (RNA processing) [2, 3]. The independent synthesis of short RNA chains could not, however, be excluded. To investigate this problem we have studied the 5′-ends of the newly formed nuclear dRNA isolated from animal cells. It is known that RNA synthesized in a cell-free system [7] as well as a number of viral RNAs [8, 9] and a nascent 5 S RNA of HeLa cells [10] possess a nucleoside triphosphate residue at the 5′-end. In mature RNAs $\beta$ and $\gamma$-phosphates at the 5′-end are absent. Thus, the presence of the 5′-triphosphate end indicates that this RNA is a newly formed one.

In this paper it is shown that only heavy dRNAs ($\geqslant$ 35 S) contain 5′-triphosphate ends. In the light dRNA fraction the 5′-end contains only one phosphate group. These data indicate that only giant RNA is the primary product of dRNA synthesis. The shorter chains are probably formed from heavy dRNA as a result of processing.

## 2. Materials and methods

dRNA was isolated from 20 rat livers 3 hr after injection of $Na_2H^{32}PO_4$ (1 mCi per animal) by the hot phenol fractionation technique [11, 12]. The RNA obtained in the 55–85° temperature range (dRNA) was re-precipitated by ethanol, treated by DNAase, mixed with sodium dodecyl sulphate (SDS) and layered on the top of a sucrose density gradient containing 0.25% SDS. After ultracentrifugation the heavy ($\geqslant$ 35 S) and light ($<$ 30 S) fractions were collected, purified by Sephadex G-75 gel filtration, precipitated by 5% trichloroacetic acid (TCA) and hydrolyzed in 0.5 N KOH for 24 hr at 37°. A mixture of non-labeled oligonucleotides was added (RNA digested by pancreatic RNAase) to the neutralized hydrolysates and then the material was separated on a DEAE-Sephadex column in 7 M urea [8]. The elution with a salt gradient gave a number of peaks with an increasing number of nucleotides in the chain. The material of the peaks III (trinucleotides) and IV–VI (tetra to hexanucleotides) was collected, desalted, and re-hydrolyzed with 0.5 N KOH under the same conditions to destry all surving labeled oligonucleotides. The chromatography was repeated. At this step of purification peak III contains exclusively labeled nucleoside diphosphates (pXp) and peaks IV and V-labeled nucleoside tetraphosphates (pppXp) or triphosphates (ppXp). The radioactivity was measured in a toluene scintillator liquid [8] on an Intertechnique SL-40 counter.

## 3. Results and discussion

The sedimentation pattern of total nuclear dRNA obtained by hot phenol fractionation at 85° is presented in fig. 1. One can see that the peak of radioactivity is localized in the 30–50 S zone, a significant part of material sedimenting to an even heavier zone (60–120 S). Thus the hot phenol treatment does not lead to a significant dRNA degradation.


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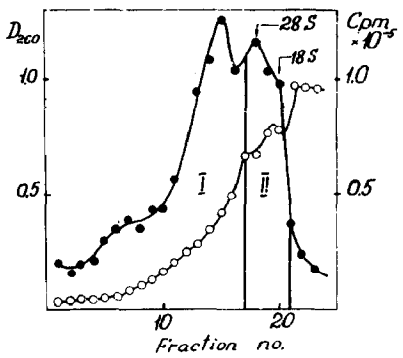


Fig. 1. Sedimentation pattern of dRNA in a sucrose density gradient. Experimental conditions: 5–20% sucrose gradient in 0.01 M tris-0.05 M NaCl -0.005 M EDTA -0.25% SDS, pH 7.5; ultracentrifugation was performed in the SW-25.2 bucket rotor of the Spinco L2 ultracentrifuge at 22,000 rpm and 21° for 6 hr. Ribosomal RNA was centrifuged in a separate tube as a marker.
o——o absorbance (in the last fractions high absorbance depends on the presence of degraded DNA).
•——• radioactivity
The heavy (I) and light (II) fractions have been taken for the analysis of phosphorylated 5′-ends.

The 5′-ends of the heavy and light RNA fractions have been studied. It is known that chromatography on DEAE-Sephadex in the presence of 7 M urea separates the compounds according to their negative charge [13]. The compounds of the pXp type move together with trinucleotides and compounds of the ppXp and pppXp type - with tetra and pentanucleotides. It should be pointed out that repeated alkaline treatment is necessary. Otherwise some surviving labeled oligonucleotides interfere with the nucleoside polyphosphates.

Table 1
Tha distribution of $^{32}P$ radioactivity between nucleoside mono-, di-, and tetraphosphates in the alkaline hydrolysates of nuclear dRNA fractions.

| RNA Fractions | Radioactivity | | | | |
|---|---|---|---|---|---|
| | Xp | pXp | | pppXp + ppXp | |
| | cpm | cpm | per cent of total | cpm | per cent of total |
| Heavy dRNA | 1,440,000 | 187 | 0.013 | 590 | 0.041 |
| Light dRNA | 600,000 | 600 | 0.1 | 25 | 0.004 |

Both pXp and pppXp were found in dRNA after alkaline hydrolysis. Alkaline phosphatase treatment of this material removes at least 95% of $^{32}P$ in the form of orthophosphate. This fact excludes the possibility of a contamination of the III–V peaks with resistant oligonucleotides.

In table 1 the distribution of the mono- and triphosphate 5′-ends among dRNA fractions of different molecular weights is presented. Polyphosphate ends may be found exclusively in the heavy zone of the sucrose density gradient. On the other hand the number of monophosphorylated ends in the light fraction is much higher than in the heavy one.

It should be pointed out that total number of phosphorylated 5′-ends in the light fraction is at least three times higher than in the heavy fraction. This correlates with the difference in molecular weights.

The data presented in this paper show that only heavy dRNAs with a molecular weight $> 2 \times 10^6$ contain 5′-triphosphate ends which may be considered as the markers of newly formed chains. One can conclude that almost all dRNA is synthesized in the form of very long chains from which the shorter chains are formed by means of cleavage and partial destruction of giant precursors. These shorter chains of nuclear dRNA seem to be precursors of cytoplasmic mRNA [2, 3]. 5′-Monophosphate ends which are present in all fractions of nuclear dRNA are formed during the processing as a result of splitting of $\beta,\gamma$-phosphates or of exo- and endo-nuclease action.

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