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Georgiev G P, Samarina O P, Lerman M I, Smirnov M N & Severtzov A N.

Biosynthesis of messenger and ribosomal ribonucleic acids in the nucleolo-chromosomal apparatus of animal cells. *Nature* 200:1291-4, 1963.

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Pure nuclear RNAs with DNA-like and ribosomal RNA-like base composition (dRNA and rRNA, respectively) were isolated by the hot-phenol fractionation procedure and characterized in several ways. In particular, dRNA was found to be heterogeneous in size. The 45S and 35S components were detected in the rRNA fraction. The base composition of total RNA in each fraction coincided with that of newly formed RNA. Also, a method of selective inhibition of rRNA synthesis was proposed for visualization of newly formed dRNA in both the nuclei and the cytoplasm. [The SC¹® indicates that this paper has been cited in over 260 publications.]

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In the spring of 1961, in the Department of Biochemistry headed by I. Zbarsky at the Severtsov Institute of Animal Morphology, I studied with V. Mantieva the properties of nuclear RNA. As a source for its isolation, we used "phenolic nuclei," i.e., a material collected between the 0.14 M NaCl and phenol, pH 6, layers after the treatment of Ehrlich carcinoma cells with the above mixture in the cold. This material contained nuclei retaining their original shape. Its extraction with 0.14 M NaCl-phenol, pH 6, at 65°C solubilized the bulk of nuclear RNA that contained 28S and 18S peaks and a heterogeneous material when subjected to analytical ultracentrifugation. The base composition of nuclear RNA differed from that of ribosomal RNA. In particular, nuclear RNA contained more A+U. We hypothesized that this depended on the presence of a novel class of RNA with a DNA-like base composition in addition to ribosomal RNA.

Therefore, we tried to further fractionate nuclear RNA and immediately succeeded in its separation into two components: one with an rRNA-like base composition and another with a DNA-like base composition. We reported our results at the International Biochemical Congress in Moscow and soon after published

them.^{1,2} A few months later, we developed an improved method for isolation of pure rRNA and dRNA based on successive extraction of phenolic nuclei with 0.14 M NaCl-phenol, pH 6, at a stepwise elevated temperature (40, 55, and 65°C).³ In this way, nuclear dRNA (referred to as hnRNA in 1965 by other authors) was first discovered and obtained in milligram quantities with a more than 90 percent purity.

Further work, which I started together with two other postdocs, O. Samarina and M. Lerman, and a student, M. Smirnov, was to characterize dRNA. Just in the middle of the project, I was offered a job by V. Engelhardt to head a department at his Institute of Radiation and Physical-Chemical Biology (now the Institute of Molecular Biology). So, the research was finished there. We found that the base composition of both total and newly formed material in the dRNA fraction was DNA-like. dRNA was found to be heterogeneous in size as followed from the data of sucrose gradient ultracentrifugation. In addition, we demonstrated the presence of newly formed dRNA in the cytoplasm using selective inhibition of rRNA synthesis with low doses of actinomycin D. Further improvements of nuclear RNA fractionation and the properties of the RNA fractions obtained are described in reference 4.

Our research was mainly handicapped by the absence of a Spinco-L ultracentrifuge. From time to time, we could put one tube into the SW-25 rotors belonging to other institutes!

The paper describing these results was sent to *Nature* and soon we received a proof. Unfortunately, the proof had reached us too late and therefore the paper was full of errors. Even in the affiliation, instead of the Severtsov Institute of Animal Morphology, Severtsov was made one of the authors. Thus, due to the courtesy of *Nature*, I've had a unique chance to collaborate with a man who had died before I was born.

I would like to believe that frequent citation of the paper reflects the fact of dRNA (hnRNA) discovery and isolation in our experiments. However, it should be pointed out that the first description of this was given in earlier papers.¹⁻³ Another reason for citation was the method of using a low actinomycin D dose to visualize dRNA biosynthesis.

1. Georgiev G P. Ribonucleic acid of the nucleolo-chromosomal apparatus. *Biochimiya* 26:1095-107, 1961.

2. Georgiev G P & Mantieva V L. On the existence of AU-type RNA in the nucleolo-chromosomal apparatus. *Vopr. Med. Khimii* 8:93-4, 1962.

3. ———. The isolation of DNA-like RNA and ribosomal RNA from the nucleolo-chromosomal apparatus of mammalian cells. *Biochim. Biophys. Acta* 61:153-4, 1962. (Cited 225 times.)

4. Samarina O P, Mantieva V L, Ryskov A P & Georgiev G P. The hot phenol extraction of RNA, particularly nuclear dRNA. (Reid E, ed.) *Methodological developments in biochemistry. Volume 2. Preparative techniques.* London: Langman, 1973. p. 131-43.