

THE CHARACTERISTICS OF THE NUCLEAR NUCLEOPROTEINS OF SOME EXPERIMENTAL TUMORS AND OF CHICK EMBRYOS

I. B. Zbarskii and L. P. Ermolaeva

Group on the Biochemistry of Cell Structure (Head-Prof. I. B. Zbarskii),
A. N. Severtsev Institute of Animal Morphology (Dir.-Corresponding Member
AN SSSR Prof. G. K. Khrushchov) Academy of Sciences of the USSR, Moscow
(Presented by Active Member AMN SSSR S. E. Severin)
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According to investigations carried out in our laboratory, the cell nuclei of tumor tissues actually differ in protein and nuclein composition from the nuclei of normal cells [3-7, 12, 13]. These differences are not found in nuclei isolated from the cells of embryonic tissues [4, 6, 7, 12]. A number of authors have mentioned the difference in deoxyribonucleoprotein complexes (DNP) of tumors [11, 12], which are evidently due to the relatively high content of nonhistone, tryptophan-containing proteins.

The characteristics of the tumor nuclear nucleoproteins have been demonstrated by immunological methods [10, 11]. Also, in tumors some differences are observed in the properties of histones [15].

These investigations indicate that the nuclear nucleoproteins of tumors differ from the normal, which may be very significant for the nature of tumor growth. It should be remarked, however, that the above results were obtained by different methods which were not always reliable. The composition of the nuclear nucleoproteins depends to a great extent on the method of their isolation [17], and the preparation of DNP in the native state is of no little difficulty [2, 14].

The investigations of Vendrely and co-workers show the homogeneity in DNP composition of different tissues, but a great difference is found in this depending on the presence of histones or protamines in the composition of DNP [19, 22].

Considering a number of deficiencies in method in the earlier experiments, chiefly connected with the method of obtaining the isolated nuclei [21], which could result in a change in the DNP composition, we studied the nuclear nucleotides of some tissues, isolating the nuclei and DNP preparations exceedingly carefully in an attempt to preserve the original composition. In the study mentioned [9] we succeeded in showing that DNP samples from different organs of the rat and chick scarcely differ from each other in many respects, but have a different composition from the DNP of sturgeon sperm, which con-

tains protamines. The composition of the DNP which we obtained differed, however, from that in the investigations of the above mentioned authors [19, 20].

In the present work we have studied the DNP obtained from isolated cell nuclei of some primary tumors and from chick embryos.

METHOD

We studied DNP obtained from isolated cell nuclei of whole chick embryos on the seventh and eleventh day of incubation, the liver of 19-day chick embryos, the cells of Ehrlich ascitic tumor of mice (on the seventh day after transfer) and of Walker rat carcinosarcoma 256. The cell nuclei were separated by the two-step sucrose-glycerophosphate method [1]. The nuclei from the tumors contained a pyroninophilic residue, evidently of cytoplasmic origin. Therefore the nuclei from the Ehrlich ascitic tumor cells were partially separated by the method of osmotic shock [18].

According to the method which we have described [8, 9] the globulin fraction is fully removed from the isolated nuclei, and then the DNP is extracted and precipitated by dilution with water [9].

Analysis of the resulting DNP preparations was carried out in the same way as in the work mentioned [9].

RESULTS

The composition of the DNP is given in Table 1 in percentage of the DNP preparation under conditions of 16% nitrogen content. The nitrogen content was determined on the air-dried preparation.

The content of diamino acids in the DNP preparation is given in Table 2.

The results which we obtained showed that DNP from chick embryos in practice does not differ from the earlier-studied DNP from 11-day chick embryos, liver of 19-day embryos and adult chickens, and DNP of rat organs [9]. Like the DNP mentioned, the nitrogen/phosphorus ratio is here 4.0-4.8, the DNA content does not exceed 40%, the

TABLE 1. Composition of DNP Separated from Isolated Nuclei (in Percent of DNP Containing 16% Nitrogen)

Source of preparation	Nitrogen	Phosphorus	N/P	DNA	Arginine	DNA Arginine	No. moles arginine per atom DNA phosphorus
7 day whole chick embryo	14.0	3.1	4.5	39.4	7.8	5.05	0.38
11 day whole chick embryo	13.6	3.4	4.0	40.2	7.4	5.45	0.35
Liver of 19 day chick embryo	14.0	2.9	4.8	38.5	7.3	5.26	0.36
Walker carcinoma 256	12.0	3.2	3.8	46.3	7.2	6.44	0.30
Ehrlich ascitic tumor: sucrose-glycerophosphate method	11.5	4.5	2.6	46.5	6.3	7.39	0.26
	14.4	3.6	4.0	41.1	7.0	5.88	0.33
osmotic shock method	11.3	4.5	2.5	52.0	9.8	5.30	0.36
	11.0	4.2	2.6	51.7	10.2	5.06	0.38

TABLE 2. Content of Diamino Acids in DNP Obtained from Isolated Nuclei

Source of preparation	No. g amino acid per 100 g DNP contg. 16% N			No. molecules amino acid per atom DNA phosphorus			
	Arginine	Lysine	Histidine	Arginine	Lysine	Histidine	Total diamino acids
7 day whole chick embryo	7.8	8.0	1.0	0.378	0.462	0.055	0.895
11 day whole chick embryo	7.4	7.3	2.0	0.352	0.414	0.107	0.873
Liver of 19 day chick embryo	7.3	8.3	1.3	0.362	0.490	0.072	0.924
Walker carcinoma 256	7.2	8.2	1.3	0.297	0.403	0.060	0.760
Ehrlich ascitic tumor: sucrose-glycerophosphate method	6.3	7.6	1.2	0.258	0.372	0.054	0.684
	7.0	8.5	1.2	0.325	0.470	0.062	0.857
osmotic shock method	9.8	11.7	1.4	0.360	0.513	0.058	0.931
	10.2	13.1	1.0	0.376	0.576	0.041	0.993

DNA/ arginine ratio is 5.0-5.5, and there is about 0.35 mole arginine per atom of DNA phosphorus.

DNP obtained from tumor nuclei was less constant in composition. The first three preparations, the DNP obtained from Walker carcinoma, the Ehrlich ascite tumor cells obtained using sucrose - glycerophosphate and that from the ascitic tumor obtained by the osmotic shock method, were close in composition to "normal" and "embryonal" DNP. They differed in having a higher DNA content and somewhat less arginine. Correspondingly, the nitrogen/phosphorus ratio was somewhat lower, and the DNA/arginine somewhat higher. Per atom of phosphorus, there was a somewhat smaller number of moles of arginine. These differences were small and the small number of DNP preparations and the considerable differences between them did not create assurance of the significance of these differences. The differences between the preparations might depend on the fact that they were ob-

tained by one method from different tumors and by different methods from one tumor. Nevertheless, this result shows the necessity for a more careful comparison of the composition of tumor and normal DNP.

On the other hand, the last two preparations, obtained from the nuclei of Ehrlich ascitic tumor cells isolated by the method of osmotic shock, actually differed in high content of DNA, arginine, and lysine, low nitrogen/ phosphorus ratio and large number of total diamino acids per atom of phosphorus of DNA. It should be noted that in obtaining these two preparations the DNP solution had a relatively low viscosity, and when diluted with water precipitated only a small amount of DNP. This fact leads us to assume that the last two preparations have undergone a partial splitting, probably because of breakdown of the nonhistone protein. The assumption certainly requires testing, but there are a number of indications in the literature of considerable changes in DNP composi-

tion with modification of the method of its separation [17] and also with its reprecipitation by dilution with water [12].

Thus, in the present study on the materials of DNP of chicken embryos we have confirmed the conclusions of our previous work [9] both as to the homogeneity of DNP of different tissues and the fact that original DNP has per atom of DNA phosphorus 0.8-0.9 mole of diamino acids. This value agrees with the data of Davison and Butler [16] and considerably exceeds the value of 0.65 mole of diamino acids given by Vendrely [20]. Though the results of our work for tumor DNP do not show a difference in its composition, yet they indicate with great probability the possibility of such differences. Our results also confirm the difficulty of separating DNP in the original state and the existence of differences in DNP composition depending on the method of obtaining the cell nuclei. This fact is evidently the reason for the variations in literature data on the composition of DNP and the peculiarities of tumor DNP.

The explanation of the individuality of composition of tumor DNP can be very important for the pathogenesis of malignant tumors. The evidence for such differences requires surmounting a number of methodological difficulties.

SUMMARY

Deoxyribonucleoprotein (DNP) obtained from isolated cellular nuclei of chick embryos as well as the DNP of the liver of adult hens and rats contained 40% of DNA (the ratio DNA/ arginine being 5.0-5.5 with 0.8-0.9 moles of diamino acids per atom of DNA phosphorus). Tumor DNP's differed in having a higher content of DNA and a lower percentage of arginine. In two samples with low viscosity and probably degraded, the DNA and diamino acid content was particularly high, which stresses the dependence of the composition of the DNP on the conditions under which it is obtained.

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