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Abstract

Euchromatin specimen prepared by the usual method formed large clumps and had various shapes under electron microscopy. A method of separation of the euchromatin specimen into chromatin fractions having relatively homogeneous form was examined and partial characterization of these fractions was carried out. The heavy euchromatin fraction was a large network of thin fibrils (about 100 A in diameter) and various thick fibers. The intermediate euchromatin fraction consisted of relatively homogeneous networks of thick knobby fibers (about 250 A in diameter). The light euchromatin fraction had metworks of thick fibers. These chromatin fractions were quantitatively prepared from sonicated nuclei of mouse ascites sarcoma cells. Twenty-one or twenty-two bands of non-histone proteins besides histones were detected in these chromatin fractions by SDS-polyacrylamide gel electrophoresis. There were significant differences in the electrophoretic patterns of non-histone proteins among these chromatin fractions.

KEYWORDS: euchromatin, SDS-polyacrylamide gel electrophoresis, electron microscopy

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FRACTIONATION AND CHARACTERIZATION OF EUCHROMATIN ISOLATED FROM MOUSE ASCITES SARCOMA CELLS

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Abstract. Euchromatin specimen prepared by the usual method formed large clumps and had various shapes under electron microscopy. A method of separation of the euchromatin specimen into chromatin fractions having relatively homogeneous form was examined and partial characterization of these fractions was carried out. The heavy euchromatin fraction was a large network of thin fibrils (about 100 A in diameter) and various thick fibers. The intermediate euchromatin fraction consisted of relatively homogeneous networks of thick knobby fibers (about 250 A in diameter). The light euchromatin fraction had networks of thick fibers. These chromatin fractions were quantitatively prepared from sonicated nuclei of mouse ascites sarcoma cells. Twenty-one or twenty-two bands of non-histone proteins besides histones were detected in these chromatin fractions by SDS-polyacrylamide gel electrophoresis. There were significant differences in the electrophoretic patterns of non-histone proteins among these chromatin fractions.

Key words : euchromatin, SDS-polyacrylamide gel electrophoresis, electron microscopy

Many reports (1-4) on the interrelation between DNA and histones have accumulated since the discovery of "nucleosomes" considered to be the basic structure of chromatin (5). There are numerous non-histone proteins in chromatin, however, there is very little evidence on the role of non-histone proteins in the structure and function of chromatin.

Euchromatin specimens prepared by the usual method formed very large clumps and had various shapes under electron microscopy (6). It is useful, therefore, to examine the preparation of homogeneous chromatin fractions for the study of chromatin.

The present paper reports on the fractionation of an euchromatin preparation isolated from mouse ascites sarcoma cells and on the partial characterization of these chromatin fractions.

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MATERIALS AND METHODS

Isolation of nuclei. SR-C_3H/He ascites sarcoma cells were harvested from tumor-bearing mice at one week after transplantation of the tumor cells (about 5×10^6 cells per animal) into their peritoneal cavities. The nuclei of the tumor cells were prepared by the Ca-sucrose method (7). The tumor cells were suspended in three volumes of 0.32 M sucrose-1.5 mM $CaCl_2$ and homogenized by a Chaikoff homogenizer. The homogenate was filtered through four layers of gauze, centrifuged at 1,000×g for 10 min and the supernatant discarded. The pellet was washed once by the same procedure, resuspended in 1.62 M sucrose-1.5 mM $CaCl_2$ and centrifuged for 10 min at 5,000×g. The pellet was resuspended in 2 2 M sucrose-1.5 mM $CaCl_2$ and centrifuged at 12,000×g for 20 min. The nuclear pellet was then washed four times with 0.01 M Tris-HCl (pH 7 1)-1.5 mM $CaCl_2$ by centrifugation at 650×g for 5 min to remove nuclear membranes, nuclear ribosomes and nuclear free proteins from the nuclei (8, 9) before sonication. The purity of the nuclear preparation was monitored by observations in the light microscope and/or electron microscope.

Fractionation of euchromatin. Chromatin specimen was prepared from the nuclear pellet described above by the method of Yasumineh and Yunis (9) with slight modifications. The nuclear pellet was suspended in 0.25 M sucrose to yield a concentration of 1.0 OD_{425nm} unit/ml and allowed to swell while being bubbled with nitrogen for 10 min at 4°C. The nuclei were broken by sonication at 0.16 A and 20,000 cycles/sec for several min in a Branson type sonifier (Kaijo Denki Co., Tokyo) in an ice bath. Nuclear breakage was monitored at 20 sec interval using a phase contrast microscopy. Heterochromatin and nucleoli fraction was precipitated from the sonicate by centrifugation at 3,500×g for 20 min. Euchromatin in the supernatant was fractionated by a differential centrifugation. The heavy euchromatin fraction was precipitated from the supernatant by centrifuging at 10,000×g for 20 min and the intermediate euchromatin fraction was precipitated by centrifuging the supernatant at 30,000×g for 30 min. The light euchromatin fraction was precipitated from the final supernatant by centrifugation at 75,000×g for 60 min.

Chemical analysis. DNA and RNA in each chromatin fraction was extracted by the method of Schneider (10) then estimated by UV absorption, the method of Burton for DNA (11) or of Brown for DNA (12). Histones in each chromatin fraction were extracted by final concentration of 0.2 M sulfaric acid. Proteins were estimated by the method of Lowry *et al* (13).

SDS-polyacrylamide gel electrophoresis. An appropriate amount of each chromatin fraction with (or without) the treatment of final concentration of 0.2 N sulfaric acid or of the extract (histones) was mixed with 1.5 volume of 0.17 M Tris-HCl buffer (pH 7.9) containing 8.3 M urea and 3 3 M NaCl and 0.025 volume of 1 M $LaCl_3$ and kept for 30 min in an ice bath. The mixture was centrifuged at 10,000×g for 30 min and the supernatant was dialyzed and condensed by using a collogion bag system. To 0.2 ml of the supernatant, 0.06 ml of 10% SDS solution containing 10% 2-mercaptoethanol and 0.02% Brom Phenol Blue (pH 7.5) and one drop of glycerin were added. This mixture was heated in a boiling

water bath for one min and 0.04 ml of the mixture was charged on a SDS-polyacrylamide column (0.5×8.5 cm). Electrophoresis was carried out at 5 mA/tube for several h and then stained with Coomassie Brilliant Blue.

Electron microscopy. Each chromatin fraction was diluted in about 10 volumes of distilled water and adsorbed to grids coated with carbon film. The surface was rinsed with distilled water, and floated on 0.1 mM uranyl acetate. After 30 sec the excess solution was removed. The grids were examined in a Hitachi 11-C type electron microscope.

RESULTS

The heavy chromatin fraction isolated from mouse ascites sarcoma cells appeared as networks of fibers of various thickness under electron microscopy. The diameter of main thin fibrils was about 100 A (Fig. 1). The intermediate chromatin fraction contained mainly networks of knobby fibers (about 250 A in diameter, Fig. 2).

The light chromatin fraction contained networks of thick fibers whose diameter of basic fibers was about 250 A (Fig. 3). The yield ratio of heavy, to intermediate and to light chromatin fraction was almost equal.

The ratios of histones, non-histone proteins and RNA to DNA in each chromatin fraction were determined (Table 1). The ratio of histones and of non-histone proteins to DNA in these chromatin fractions varied from 1.29 to 1.51 and from 1.38 to 1.95, respectively. The ratio of RNA to DNA in these fractions was about two times higher in comparison with the ratio in the euchromatin fraction isolated from calf thymus (8).

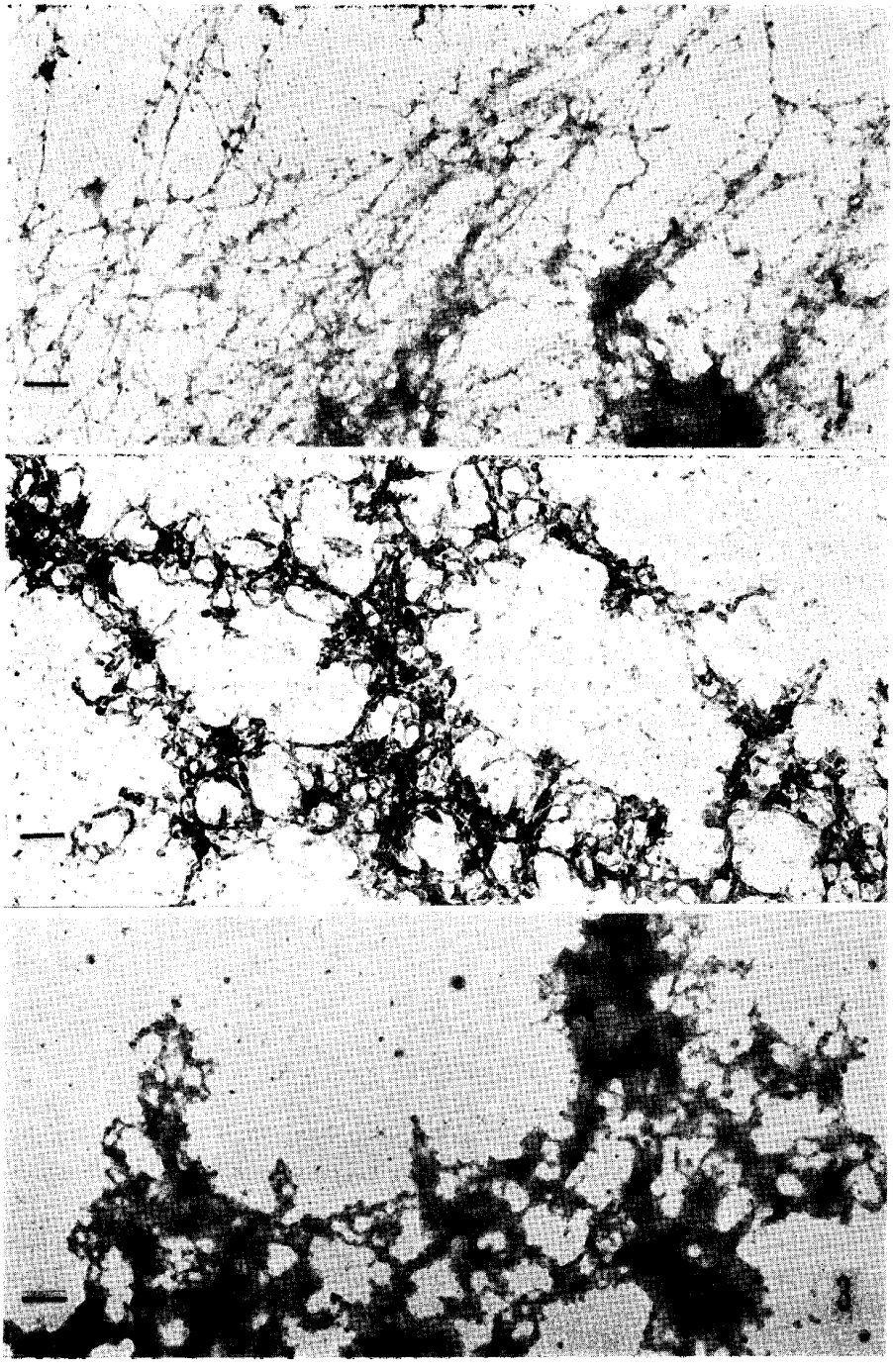
TABLE 1. CHEMICAL ANALYSIS OF CHROMATIN FRACTIONS

Chromatin fraction	DNA	Histones	Non-histone proteins	RNA
Heavy	1.00	1.51	1.95	0.310
Intermediate	1.00	1.50	1.60	0.302
Light	1.00	1.29	1.38	0.377

(Values expressed as mg based on 1.0 mg DNA)

The components of proteins in each chromatin fraction were analyzed by SDS-polyacrylamide gel electrophoresis. Four bands of histones were detected in each chromatin fraction (Fig. 4),

Twenty-one or twenty-two bands of non-histone proteins besides histones were detected in each chromatin fraction (Fig. 5). The pattern of several bands of non-histone proteins varied significantly in each chromatin fraction.



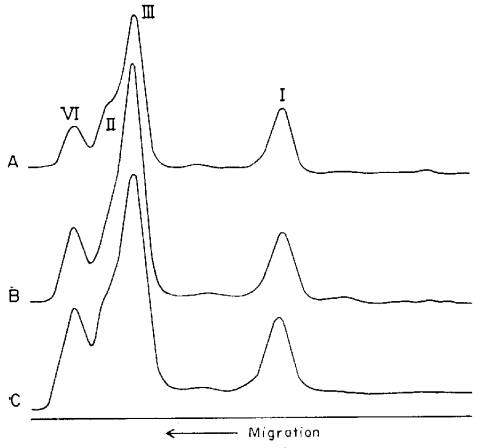


Fig. 4. Densitometer tracing of SDS-polyacrylamide gel electrophoresis patterns of total histones in heavy chromatin (A), intermediate chromatin (B) and light chromatin (C).

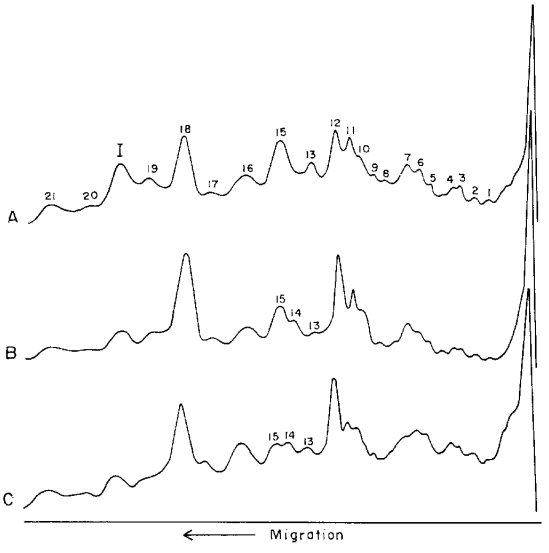


Fig. 5. Densitometer tracing of SDS-polyacrylamide gel electrophoresis patterns of total non-histone proteins in heavy chromatin (A), intermediate chromatin (B) and light chromatin (C). Mark I indicates histone I which coelectrophoresed with samples.

Fig. 1. Electron micrograph of heavy chromatin fraction isolated from SR-C_3H/He cells. Bar indicates 200 nm (×30,000).

Fig. 2. Electron micrograph of intermediate chromatin fraction isolated from SR-C_3H/He cells. Bar indicates 200 nm (×30,000).

Fig. 3. Electron micrograph of light chromatin fraction isolated from SR-C_3H/He cells. Bar indicates 200 nm (×30,000).

DISCUSSION

The diameter usually obtained for a portion of a chromosome fiber in eukaryotes by the surface spreading method is 200 to 300 A (14, 15). Ris (16) suggested that the 250 A fiber is the result of side-by side pairing of two 100 A thick fibers. On the other hand, DuPraw (14) reported that there are two types in human chromosomal fibers, *i. e.*, thinner fibril (type A), about 100 A in diameter, and thicker fiber (type B), about 250 A in diameter, and the specific gravity of the former is higher than that of the later.

In our preparation the heavy chromatin fraction consisted mainly large area of type A fibrils while the intermediate and light fractions consisted mainly of type B fibers.

Although the thickness in the solenoidal model which was proposed by Finch *et al.* (4), is of same order of magnitude as that of type B fibers, the role of non-histone proteins in the supercoiling of the nucleofilaments to the solenoid was not described. DuPraw (14) suggested that the occurrence of non-histone proteins which link DNA and are specific to type A fibril.

The cause of the high ratio of RNA to DNA in these chromatin preparations has not yet been elucidated. Some nuclear ribosomes may have been a factor. However, chromatin preparations from highly deviated tumor cells generally contain a larger amount of RNA than those from normal tissues of adult animals (17).

There were significant differences between the gel electrophoretic patterns of non-histone proteins of the three chromatin fractions. Band 15 of non-histone proteins was higher and the band 14 was lower in the heavy chromatin fraction (Fig. 5).

It is necessary to investigate further the role of non-histone proteins in the strcture and function of chromatin.

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