COMPETITION BETWEEN HISTONES FOR DNA AND ITS POSSIBLE ROLE IN SELF-ASSEMBLY OF EUCHROMATIN AND HETEROCHROMATIN

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During the 15 years which elapsed after the discovery of the phenomenon of competition between histones for DNA [7] only one paper has been published [1] in which this phenomenon was analyzed in systems in which only two histone fractions could compete for DNA. In both investigations cited competition between histones was used only as an experimental method to analyze the relative affinity of the different fractions for DNA.

The possibility that histones may compete for DNA in the cell has not hitherto been analyzed. To verify that such competition is possible, it is essential to know the quantitative ratio between total histone of chromatin and DNA at which competition between histones begins to arise. To assess the biological significance of this phenomenon it is important to study the selectivity in relation to individual histone fractions under conditions when all histones, allowing for their mutual association, can compete for DNA. The investigation described below was devoted to the study of these problems.

EXPERIMENTAL METHOD

Freshly frozen calf thymus tissue was cut into small pieces and homogenized for 30 sec with a knife homogenizer at 14,000 rpm in 200 ml 0.075 M NaCl + 0.025 M EDTA-Na_2, pH 8.0. After the tissue homogenate had been washed 5 times in this medium, the resulting DNP was used for isolation of DNA and for acid extraction of total histone of chromatin. The methods used for isolation were described previously [3]. DNA was depolymerized on an ultrasonic disintegrator (MSE) to a molecular weight of 1×10^6. The DNA concentration was determined by Spirin's method [4] and the protein concentration by Lowry's method [11], using for calibration solutions made up on the basis of an extinction coefficient of $E^{1\%}_{230} = 42.5$ [13]. A DNA–histone mixture was obtained in 0.15 M NaCl + 0.7 mM sodium-phosphate buffer, pH 7.0, and centrifuged for 13-16 h at 114,000g (L2-65B centrifuge, 40.3 rotor, 40,000 rpm). The residue was dispersed in 2 M NaCl (2.5 ml) and used for analysis. The composition of histones in the supernatant and in residues of the mixtures was determined by electrophoresis by Laemmli's method [9].

EXPERIMENTAL RESULTS

The results of one experiment to study the DNA and histone content in the supernatant obtained after centrifugation of total histone–DNA mixtures in medium with near-physiological ionic strength (0.15 M NaCl + 0.7 mM sodium-phosphate buffer, pH 7.0) are given in Fig. 1A. The values of histone/DNA ratios in residues of centrifuged mixtures are given in Fig. 1B. As Fig. 1B shows, when the histone/DNA ratio in the original mixture was below or equal to 1, all the histone bound with DNA and passed into the residue during centrifugation. Some of the DNA, free from protein, remained in solution.

After centrifugation of mixtures with a histone/DNA ratio equal to or higher than 1.1, all the DNA was in the residue but part of the histone remained in the supernatant, i.e., it did not form a complex with DNA because of commencing competition between the histones for binding sites on DNA. The histone–DNA ratio in nucleohistones formed in DNA–total histone mixtures depended on the quantitative proportion of the components of the mixture and it increased if competition between histones for DNA was stronger (i.e., with a higher value of the histone/DNA ratio in the mixture). However, when the histone/DNA ratio in the mixtures

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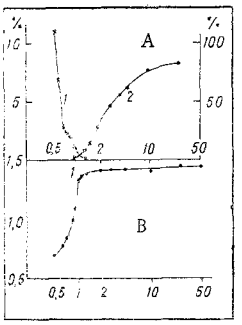

Fig. 1

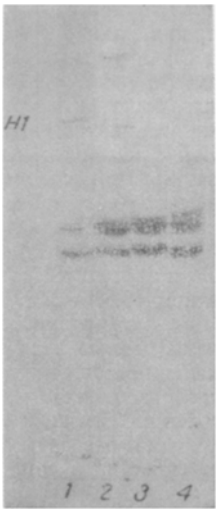

Fig. 2

Fig. 1. Content of DNA (1) and histone (2) in supernatant (A) and residue (B) of DNA–total histone mixtures in 0.15 M NaCl + 0.7 mM sodium-phosphate buffer, pH 7.0. Abscissa, histone/DNA ratios in initial mixtures (on logarithmic scale); ordinate: A) percentage fractions of DNA (left) and histone (right) remaining in supernatant of mixtures after removal of nucleohistone by centrifugation (C_{DNA} = 100 μg/ml in all initial mixtures); B) histone/DNA ratio in nucleohistone complexes formed in DNA–total histone mixtures with different proportions of components, sedimented by centrifugation.

Fig. 2. Electrophoretic analysis of histones in residues of DNA–total histone mixtures. Values of histone/DNA ratio in initial mixtures: 1) total histone (control); 2) 1.25; 3) 1.5; 4) 2.0.

exceeded a certain value, the protein content in the nucleohistones became practically independent of the quantity of histone added to the DNA, even if in a 50-fold excess over DNA. It is possible to speak of saturated DNA–histone complexes which, in different experiments, were characterized by the following values of the histone/DNA ratio: 1.46, 1.43, 1.62, 1.37, and 1.36.

Electrophoretic analysis of histones in residues of the mixtures showed a decrease in the content of histone H1 and its complete disappearance when the histone/DNA ratios in the initial mixtures were 1.25 and 1.5 respectively (Fig. 2). An increase in the histone/DNA ratio to 1.5 thus leads to complete suppression of the ability of histone H1 to bind with DNA in medium with physiological ionic strength.

Analysis of the supernatant of DNA–histone mixtures shows (Fig. 3) that competitive displacement of histone H1 from DNA is a strictly selective process. Between the remaining histone fractions competition was absent within the range of histone/DNA ratios from 1.1 to 1.4, for these histones were not found in the supernatant of the above-mentioned mixtures. Starting from a histone/DNA ratio of 1.5 or more, to begin with histone H2B appeared in the solution, followed by the remaining fractions, i.e., competition began between histones of the nucleosomal nuclei (H2A, H2B, H3, H4).

The chief result of the experiments described above is that even a minimal excess of total histone by weight relative to DNA leads to the formation of a nucleohistone poor in the H1 fraction. The possibility of self-assembly of a nucleohistone completely deprived of the H1 fraction, but containing all other histone fractions in the same proportions as in the DNA solution, also was demonstrated.

The biological importance of these facts can be interpreted in the light of the fact that numerous investigations have shown that the histone/DNA ratio in cell nuclei is much higher than 1:1 [6, 12]. Much evidence also has been obtained to show the selective distribution of histone H1 in relation to euchromatin and heterochroma-

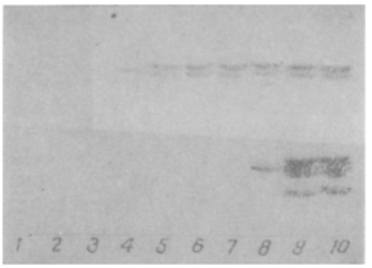

Fig. 3. Electrophoretic analysis of histones in supernatant of DNA–total histone mixtures. Values of histone/DNA ratio in initial mixtures: 1) 0.9; 2) 1.0; 3) 1.1; 4) 1.2; 5) 1.3; 6) 1.4; 7) 1.5; 8) 1.7; 9) 1.8; 10) 1.9.

tin. Numerous investigations have shown that histone H1 either is absent in euchromatin or is present in a smaller quantity than in heterochromatin [5, 10, 15].

If these results are correct, they suggest a simple mechanism of assembly of euchromatin and heterochromatin, ensuring stability of differential activity of genes in cell generations. This mechanism, which is responsible for epigenetic inheritance, is based, in the writers' opinion, on the widely accepted position, recently confirmed [2], of sequential replication of DNA of euchromatin and heterochromatin and the views on coordination between DNA and histone synthesis [5]. If the essence of this coordination of synthesis is such that during replication of euchromatin DNA an excess of histones is supplied to the nucleus relative to the synthesized DNA, a chromatin totally lacking or deficient in histone H1 will be formed. If during replication of heterochromatin DNA the quantity of histones supplied to the nucleus is equal to or less than the quantity of DNA synthesized, a chromatin containing all histone fractions in the same proportions as they were supplied to the nucleus will be formed. As a result, selective distribution of histone H1 may be achieved in relation to active and inactive genes. If absence or deficiency of histone H1 is sufficient to ensure that the corresponding genes are capable of transcription, the suggested mechanism may ensure stability of the differential state of the cells during proliferation.

Factors leading to a disturbance of coordination of DNA and histone synthesis in maternal cells may modify the differential activity of the genes in the daughter cells and, consequently, may be responsible for various forms of pathology of the cells and tissues of higher organs. In fact, a decrease or increase in the quantity of protein (proteins) synthesized by the cell, and loss or, conversely, appearance of ability to synthesize a certain protein may be essential for the cell to perform its functional load in that particular organism. In particular, in virus infection coordination between DNA and histone synthesis is disturbed [8] and the host cell begins to synthesize new types of proteins [14].

A change in the differentiated state of the cell may also take place, according to the suggested mechanism, on account of disturbances in the system responsible for the sequence of euchromatin and heterochromatin DNA replication (possibly in regions of the genome where DNA synthesis is initiated or in protein fractions responsible for initiation of DNA synthesis).

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EFFECT OF LOW TEMPERATURE ON MEMBRANE PERMEABILITY OF RED BLOOD CELLS RECONSTITUTED IN MEDIA OF VARIED IONIC COMPOSITION

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The role of physicochemical changes in the medium in the mechanisms of low temperature injury to cells, the most important manifestation of which is a disturbance of their permeability [9], has now been studied sufficiently completely [4]. Meanwhile the importance of the structural and functional state of the plasma membranes still remains largely unexplained although existing information [1, 8] suggests that it is this factor which largely determines the optimal program for freezing the cells and the choice of cryoprotective agents. For these reasons it was decided to study the effect of low temperatures on permeability of red blood cells whose membranes were modified by means of various agents. In the investigation described below the action of mineral cations (Na^+, K^+, Ca^{++}, Mg^{++}) was studied; as a result of adsorption on the membrane these cations significantly alter its permeability for water and ions and also its mechanical properties [2, 7].

EXPERIMENTAL METHOD

A convenient model with which to study this problem is reconstituted red blood cells, for the ionic composition on both sides of their plasma membrane can be varied within wide limits [6]. Reconstituted red blood cells were obtained from human donors' blood kept for 2-5 days, as described in [3], with some modifications to the composition of the lytic and reconstituting media, which are indicated in the appropriate captions to the figures. The red blood cells were quickly frozen in reconstituting medium by immersing polyethylene ampuls, each containing 0.5 ml of the sample, in liquid nitrogen. Thawing was carried out on a waterbath at 37°C. The rate of freezing was 200-400°C/min.

Membrane permeability was assessed by the ability of the reconstituted red blood cells to retain hemoglobin, K^+ and Na^+ ions, and ^{14}C-sucrose (Czechoslovakia, specific activity 340 mCi/mmole). The hemoglobin concentration was determined after centrifugation in the residue and supernatant by a spectrophotometric method [5]. The K^+ and Na^+ concentration in the residue of the reconstituted red blood cells was determined by flame photometry [5].

^{14}C-sucrose was added to the lytic solution in a dose of 1 μCi/ml. After reconstitution, sucrose not incorporated into the red cells was washed out by centrifugation in reconstituting solution under the same conditions as the hemoglobin. Radioactivity of the labeled sucrose was determined in the acid-soluble fraction of the residue and supernatant of the reconstituted red cells after freezing and thawing. The samples were counted on an SL-40 scintillation counter. ZhS-7 fluid (1 liter dioxane, 5 g PPO, 100 g naphthalene) was used as the scintillator. The specific volume of the reconstituted red blood cells was determined on a microhematocrit centrifuge (Adams Readacrit).

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