Distribution of histone variants in the sea urchin chromatin fractions obtained by selective micrococcal nuclease digestion

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Abstract

Chromatin fractions differing in their transcriptional activity were isolated by selective micrococcal nuclease digestion of nuclei from sea urchin embryos (Strongylocentrotus droebachiensis) at the gastrula and pluteus stage. The electrophoretic analysis of the chromatin proteins at the gastrula stage showed that a soluble, transcriptionally active fraction of chromatin was enriched with early variants of histones H1 and H2A. The early and late variants of histone H2A at the pluteus stage were distributed randomly between chromatin fractions. However, the content of both variants of histone H1 was essentially decreased in the soluble transcriptionally active fraction of chromatin.

Introduction

It has been reported that the structure of transcribing chromatin is less compact than that of inactive chromatin (1, 2). This may be explained by the presence of specific histone variants and the absence of histone H1. During the early embryogenesis of sea urchins, three distinct variants of histone H1, H2A and H2B are synthesized: CS-variants before the 16 cell stage, then α-variants up to the blastula stage and, finally, from the late blastula stage -β,γ,σ-variants (3). Simpson reported that these variants of histone have demonstrable effects on nucleosome structure (4). Changes in nucleosome conformation may lead to alterations in chromatin structure at other levels. Therefore, it can be assumed that histone variants could play a role in the control of gene expression. In this study we used the micrococcal nuclease treatment of sea urchin chromatin at the gastrula and pluteus stages to determine the distribution of histone variants between chromatin fractions differing in transcriptional activity. The data showed that at the gastrula stage, when primary cell differentiation occurs along with the changes in the activity and structure of chromatin, the early α-variants of histone H1 and H2A are preferentially associated with the transcriptionally active chromatin fraction.

Materials and methods

Embryos of the sea urchin Strongylocentrotus droebachiensis were prepared as described previously (5). Embryos at the pluteus stage were collected by centrifugation, washed several times with membrane-filtered sea water and incubated (3 × 10⁴ embryos/ml) with gentle stirring for 120 min at 7-8 °C in the presence of ¹⁴C-protein hydrolyzate (0.4 MBq/ml). To label DNA, the embryos at the gastrula stage were incubated with [³H]thymidine (0.8 MBq/ml) for several hours. Penicillin (100 U/ml) and streptomycin (50 U/ml) were added to the incubation medium. After incubation, the embryos were washed three times with membrane-filtered ice-cold sea water by centrifugation and dissociated into cells (5).

The cells were resuspended and homogenized in
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...buffer containing 0.15 M sucrose, 0.06 M KCl, 0.015 M NaCl, 0.05 M LiCl, 0.05 M Na2S2O5, 1 mM phenylmethylsulphonylfluoride (PMSF), 0.5–1% Triton X-100, 0.01 M Tris, pH 6.8. After homogenization, 0.4 volume of the solution containing 0.5 M sucrose, 0.06 M KCl, 0.015 M NaCl, 0.05 M LiCl, 0.01 M Tris, pH 6.8 was added. Nuclei were collected by spinning at 600 g for 10 min and resuspended in nuclease digestion buffer containing 0.25 M sucrose, 0.1 mM PMSF, 0.01 M Tris, pH 7, washed twice by centrifugation at 600 g for 10 min and finally resuspended at a concentration of 2–3 mg/ml. Nuclear hydrolysis was performed as described by Pospelov et al. (6) until 8–10% of the DNA was acid-soluble (under these conditions 20% of chromatin is in soluble form). Soluble (S) and pellet (P) chromatin fractions were separated by centrifugation at 10,000 g for 10 min.

Total histone was extracted from chromatin with 0.4 N H2SO4 according to Panyim and Chalkley (7). Histones H1, H2A, H2B were selectively extracted from chromatin using an aqueous two-phase system containing 7% Dextran T500, Mw = 460 · 103, Pharmacia Fine Chemicals, Sweden, 5% polyethylene glycol 6000 (Carbowax 6000), M = 6 · 103, Union Carbide, USA, 0.9 M NaCl, 0.01 M sodium phosphate, pH 7 (8).

Histone electrophoresis was performed in a polyacrylamide slab gel (15%) containing 0.9 M acetic acid, 8 M urea and 6 mM Triton X-100 (9). The gels were stained with Serva Blue G, FRG. The gels were scanned with the microphotometer M4Q III, FRG. The peak area of the densitograms was a linear function of the amount of the protein up to 10 µg per electrophoretic band. Therefore for quantitative measurements we applied 50–60 µg of total histone per gel. Baselines for the stained gels were set by adjusting the slit width on the spectrophotometer until blank regions of the gel showed zero adsorbance. Relative areas were then measured by cutting out appropriate regions of the scans and weighing them. Determinations were repeated three times and averaged. DNA and RNA were obtained as described by Vorob'ev and Kosjuk (10). The transcriptional activity of S and P chromatin fractions was judged by hybridization of [3H]labeled DNA with poly(A)-RNA. Poly(A)-RNA was prepared on a cellulose-oligo (dT) column (11). [3H]DNA/RNA hybridization reactions were carried out in 0.12 M phosphate buffer, pH 6.8, 1 mM EDTA, 0.25% SDS, as described by Levy and Dixon (12). Samples containing 2 µg [3H]DNA and 1 mg RNA were sealed in capillaries, heated to 100 °C for 3 min to denature the nucleic acids and then quickly equilibrated to 60 °C and incubated for various periods of time. At the end of each incubation period, samples were diluted with buffer, containing 0.003 M ZnCl2, 0.05 M NaCl, 0.03 M CH3COONa, pH 4.5, nuclease S1 (1000 units/ml) and incubated for 1 h at 45 °C. After incubation tRNA was added (100 µ/ml) and samples were precipitated in ice-cold 14% trichloracetic acid, filtered onto glass fibre filters and counted.

Results and discussion

It is known that in the early embryogenesis of sea urchin at the blastula-prism stages, one-third of the genome is transcriptionally active (13, 14). To obtain the putative fraction of transcribing chromatin, nuclei were hydrolyzed so that 8–10% of the DNA was acid-soluble.

The results of the hybridization of [3H]DNA from S and P fractions with poly(A)-containing RNA isolated from gastrula embryos are depicted in Figure 1. Hybridization curves show that a solu-

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**Fig. 1.** Hybridization of gastrula [3H]DNA with gastrula poly(A)-RNA. [3H]DNA was isolated from total chromatin (K) and chromatin fractions obtained by micrococcal nuclease digestion: soluble fraction (S), pellet (P) (see Methods).