ON THE OCCURENCE OF HISTONE ACETYLTRANSFERASES IN SEA URCHIN EGGS AND SPERM

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1. Introduction

The primary structure of histones F3 and F2a1 has been highly conserved during evolution [1–6]. Interestingly, these histones display different degrees of microheterogeneity in different organisms and cell types which results from acetylation, methylation and phosphorylation of specific lysyl and seryl residues. The function of the amino acid side chain modifications within histones is presently unknown. In sea urchin sperm, however, no such microheterogeneity of any of the five histones could be observed [7]. This observation has been discussed to indicate a possible involvement of histone modification, especially of acetylation, in the activation of the genetic material since during early embryo-genesis of sea urchins histones become extensivley acetylated [8,9].

We have been wondering whether in the genetically inactive sea urchin sperm the enzymes responsible for histone acetylation are absent or only prevented from functioning.We also ask whether histone acetyltransferases are present in sea urchin eggs or whether they possibly become induced and newly synthesized after fertilization. It was found that in sea urchin eggs two histone acetyltransferases A and B are present whereas in sea urchin sperm no histone acetyltransferase activity could be observed.

2. Methods

Sea urchins (*Arbacia lixula*) were collected at the Mediterranean coast and used the same day or kept at 4°C for not more than 12 hr. Ripe animals were induced to spawn into different beakers with filtered sea water by injecting 1 ml of 0.5 M KCl into the coelomic cavity. The suspensions of eggs or sperm were passed through a double layer of cheese-cloth to remove coarse particles, centrifuged for 10 min at 2500 *g* and then washed three times with filtered sea water. The last sediment was frozen immediately at −20°C. For the following experiments only those batches of eggs were used which gave better than 90% fertilization after addition of freshly diluted sperm.

The extraction procedure for histone acetyltransferases and the estimation of enzyme activity were essentially the same as described for rat thymus nuclei [10].

1×10^8 frozen sea urchin eggs from about 40 *Arbacia lixula* females were homogenized in 10 vol of buffer A (0.075 M Tris–Cl, pH 7.9, 5 mM 2-mercaptoethanol, 0.25 mM EDTA, 1 mM $MgCl_2$) containing 1 M $(NH_4)_2SO_4$ at full speed with an MSE-homogenizer for 30 sec. Solubilization of the enzyme proteins was acheived by sonicating (Branson sonifier, 20 kHz, 125 Watt) the crude homogenate in 60 ml aliquots for 120 sec followed by stirring at 4°C for 1 hr. Crystalline $(NH_4)_2SO_4$ was slowly added to a final concentration of 3.5 M. After stirring for 2 hr in the cold the extract was centrifuged for 20 min at 23 000 *g*. The resulting protein pellet, containing pigment granules, was suspended in 20 vol of buffer B (0.01 M Tris–Cl, pH 7.9, 5 mM 2-mercaptoethanol, 0.25 mM EDTA, 0.01 M NH_4Cl), solubilized by aid of a Douncehomogenizer and dialyzed against 20 vol of the same buffer. After removal of precipitated proteins by centrifugation at 23 000 *g* for 10 min, the protein solution was applied to DEAE-cellulose equilibrated with medium B. Adsorbed protein was eluted with a linear gradient of 0.01–0.35 M NH_4Cl. Proteins

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were similarly extracted and fractionated from 2.5×10^{11} sperm cells obtained from 15 male sea urchins.

Histone acetyltransferase activity was measured by the incorporation of [^{14}C]acetate from 1-[^{14}C]acetyl coenzyme A (NEN Corp., spec. act. 56.6 mCi/mM) into hot TCA precipitable acceptor histones. The incubation mixture contained in a final vol of 0.25 ml: 15 μmol Tris–Cl, pH 8.0, 25 μmol KCl, 1.8 μmol NH_4Cl, 0.045 μmol EDTA, 0.9 μmol 2-mercapto-ethanol, 0.0145 μCi 1-[^{14}C]acetyl coenzyme A, 20 μg total histone from calf thymus and 100 μl of the enzyme fraction. The reaction was started by the addition of acetyl CoA. Incubation was performed for 15 min at 37°C and terminated by precipitating 0.2 ml of the incubation mixture on filter paper discs (Schleicher and Schuell, 2043 b) in ice cold 25% TCA. The filter papers were processed as described [10].

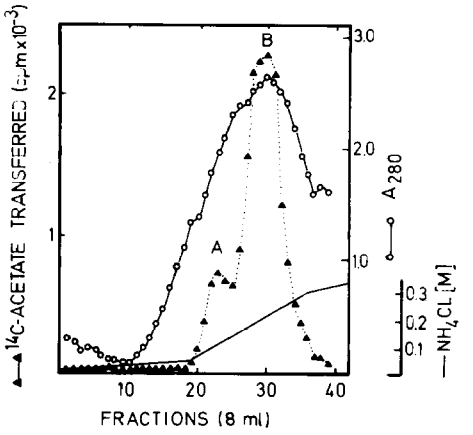

Fig.1. DEAE-cellulose chromatography of histone acetyltransferases from sea urchin eggs. Proteins were extracted from 1×10^8 eggs as described in Methods and separated on a 2.5 × 8 cm column of DEAE-cellulose equilibrated with medium B using a linear 0.01–0.35 M NH_4Cl gradient. Enzyme activity was measured with 100 μl of the fractions collected.

3. Results and discussions

Our previous studies showed that multiple forms of histone specific acetyltransferases are present in a variety of mammalian tissues [10,11]. We used the same isolation methods to search for these enzymes in eggs and sperm of the sea urchin *Arbacia lixula*. After separating the proteins from the bulk of nucleic acids by 3.5 M ammonium sulfate precipitation they were passed over DEAE-cellulose. Proteins were eluted from the ion exchanger with a linear 0.01–0.35 M ammonium chloride gradient and tested for acetyltransferase activity using calf thymus histones and [^{14}C]acetyl-coenzyme A as acetate acceptor and donor, respectively [10,11].

In fig.1 the DEAE-cellulose chromatography of sea urchin egg protein is shown. As is the case for mammalian histone acetyltransferases two enzyme fractions, designated as A and B, were isolated from sea urchin eggs. The enzymes eluted at ammonium chloride concentrations of 0.12 M and 0.2 M. Chromatographically, the two histone acetyltransferases A and B from sea urchin eggs and rat liver tissues have similar elution properties on hydroxyapatite [10].

When proteins extracted from sea urchin sperm were examined for histone acetyltransferases no enzyme activity, neither in the crude protein extract nor after separation of the sperm proteins on DEAE-cellulose, was observed. It could be argued that for sperm acetyltransferases homologous histones are needed as the substrate. We consider this possibility as unlikely since; 1) from our experience the acetyltransferases from different species and organs, although specific for histones, are equally active with homologous and heterologous histones as substrate; 2) histones F3 and F2a1, the main substrates for the modifying enzymes, are highly stable during evolution and the two histones derived from sea urchin sperm and calf thymus reveal no difference in electrophoretic mobility [7] suggesting very similar primary structures; 3) calf thymus histones did serve as substrate for the acetyltransferases from sea urchin eggs. The absence of histone acetyltransferase from sea urchin sperm is consistent with the finding that sperm histones are not acetylated [7]. Since these enzymes are present in sea urchin eggs it is tempting to speculate that egg histones are subject to acetylation and/or that the enzymes are used after fertilization to modify the histones of the very compact sperm chromatin. Recently it was demonstrated that during oogenesis in *Xenopus laevis* histones are newly synthesized, acetylated and stored in the egg nucleus [12]. Although histone messenger RNA has been demonstrated in sea urchin eggs [13], synthesis of

histones has not been observed. Whatever the function of histone acetyltransferases in sea urchin eggs might be, the eggs are obviously furnished with the enzymes needed to acetylate histones during early cleavages.

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