A SITE OF DISCONTINUITY IN THE INTERACTION BETWEEN DNA AND HISTONES IN NUCLEOSOMES OF SEA URCHIN EMBRYO CHROMATIN.

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Summary: Digestion of chromatin DNA in nuclei of sea urchin embryos with pancreatic nuclease and with micrococcal nuclease give additional details concerning the interaction between DNA and histones. A specific site of hydrolysis appears to be located on the nucleosome in such a position as to split the DNA unit length in two equivalent fragments of about 60-70 base pairs in length. The complete digestion of chromatin DNA appears to depend on the low stability of the nucleosome containing the split DNA fragments.

Introduction

Recent reports have shown that chromatin is composed of a repeating unit containing eight histone molecules and a DNA segment 200-130 base pairs in length (1-8, 14). The assembly of the histones in chromatin has been studied by cross-linking adjacent molecules (9). Similar experiments in chromatin and in isolated nucleosomes have led to specific models of the histone octamer organization (10). Here we wish to report experiments concerning the interaction of DNA with the histones, as derived from the study of chromatin DNA hydrolysis with two nucleases. The results strongly suggest that, in sea urchin chromatin, in addition to the hydrolysis sites between one nucleosome and another, there is in the nucleosome, a specific hydrolysis site preferentially cut by nucleases, that gives rise to two DNA fragments of approximately the same length.

Materials and Methods

Preparation of sea urchin embryo nuclei. Paracentrotus lividus sea urchins were collected from the bay of Naples. The animals were opened and eggs obtained by shaking gonads in filtered sea water. Eggs were filtered through a gauze cloth, washed twice by suspending in filtered sea water and sedimenting at low speed centrifugation, suspended in a small volume of sea water and fertilized with a sperm suspension. The embryo cultures were allowed to grow at 20°C under gentle rotatory agitation at concentrations of 3000-4000 embryos per milliliter. At the blastula stage the embryos were concentrated and nuclei prepared according to Hinegardner (11). Embryos were homogenized in 2 mM $MgCl_2$ in a Dounce type B homogenizer and the homogenate was centrifuged on a discontinuous sucrose gradient.

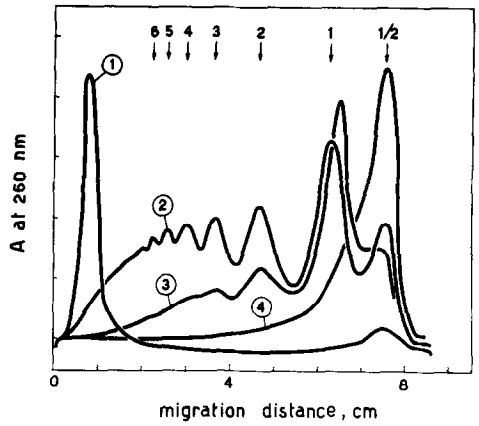

Fig. 1- Densitograms of electrophoretic separations on 2.5% polyacrylamide gel of DNA prepared from untreated blastulae nuclei (1) and from blastulae nuclei digested with 650 U/ml of micrococcal nuclease to the following percents of acid-soluble DNA: (2), 10%; (3), 30%; (4), 36%. Electrophoresis was carried out for 2 hours at 5 mA per gel at room temperature. Marker t-RNA migrates in the band labelled 1/2. The position and number of each band is indicated by the arrows.

Digestion of chromatin in sea urchin embryo nuclei. The digestion of chromatin in nuclei was carried out as previously described (5). The individual nucleases were added to the nuclear suspensions and the amounts of DNA digested were determined spectrophotometrically by measuring the acid-soluble nucleotides at different digestion times. DNA concentration of the nuclei suspensions was determined by diphenylamine reaction (12), and the contribution of nuclear RNA was estimated by orcinol reaction (13).

Preparation and analysis of the DNA fragments. The preparation and analysis of the DNA fragments were carried out as previously described (5) with minor modifications. Hydrolysis reactions were stopped by diluting the samples in 4% SDS and 4mM EDTA, final concentrations. The DNA fragments were purified by incubating samples with 50 ug/ml of RNase A for two hours and with 50 ug/ml of Proteinase K (Merck) for one hour at 30°C, after two extractions with chloroform-isoamyl alcohol (24:1, v/v).

Results and Discussion

The chromatin in nuclei of sea urchin embryos, when digested with micrococcal nuclease, is split into DNA fragments that, on acrylamide gel electrophoresis, give patterns similar to those reported for digestion of chromatin in nuclei of other origins (2-8, 14) (Fig. 1). These fragments appear to be multiples of a unit fragment of about 130 base pairs. A unit length fragment of the same size has already been reported for the digestion of sea urchin sperm chromatin (5).

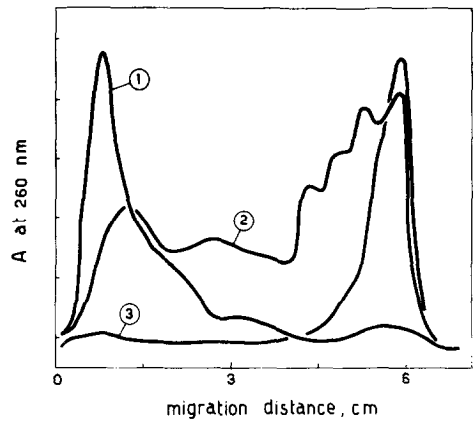

Fig. 2- Densitograms of electrophoretic separations in dissociating conditions on a 4% polyacrylamide gel in 98% formamide, of DNA prepared from untreated blastulae nuclei (1) and from blastulae nuclei digested with 23 U/ml of pancreatic nuclease, to the following percents of acid-soluble DNA: (2), 4%; (3), 9%. Electrophoresis in formamide was carried out for 6 hours at 4 mA per gel at room temperature. Marker t-RNA migrates in the band of curve (3).

As shown in Fig. 1 the size distribution of the DNA fragments changes at increasing percent of acid-soluble DNA and when about 40 % of DNA is acid-soluble the remainder is in a single band showing approximately the electrophoretic mobility of t-RNA.

When sea urchin embryo nuclei are digested with pancreatic nuclease, added at such a concentration as to digest the chromatin at approximately the same rate as micrococcal nuclease, no obvious pattern of bands multiple of a unit length is apparent. Early in the digestion process there is a discrete series of bands and the faster moving one is about 60-70 base pairs in length, similar to the faster moving band that predominates at long incubation times with micrococcal nuclease (Figs. 1 and 2). When less than 10% of the DNA is acid-soluble the remaining DNA migrates practically in a single band with approximately the electrophoretic mobility of t-RNA (Fig. 2). At longer incubation times larger and larger amounts of DNA become acid-soluble but the electrophoretic pattern of the remaining higher molecular weight fragments does not change. Such results clearly indicate that also pancreatic nuclease hydrolyses chromatin DNA in a

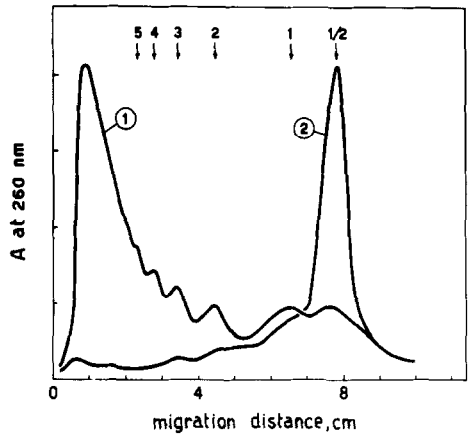

Fig. 3- Densitograms of electrophoretic separations on 2.5% polyacrylamide gels of DNA prepared from blastulae nuclei digested with micrococcal nuclease to 5% acid-soluble oligonucleotides (1), and digested additionally with 23 U/ml of pancreatic nuclease to a total of 9% acid-soluble oligonucleotides (2). Other conditions as in Fig. 1.

non-random manner cutting preferentially segments of similar lengths. A simple way to explain the apparent differences in the digestion products of chromatin DNA by the two nucleases was to assume that chromatin DNA was cut not only between one nucleosome and another, but also on each nucleosome giving rise to fragments about 50 percent the length of the unit fragment. The difference between the DNA patterns observed with the two nucleases depended essentially on the different hydrolysis rates at the two sites. Micrococcal nuclease would digest more rapidly the inter nucleosomes sites, while pancreatic nuclease would digest both sites at approximately the same rate. In order to test this hypothesis, chromatin in nuclei was digested first for 30 seconds with micrococcal nuclease, a sample was withdrawn and then pancreatic nuclease was added and the chromatin digested for an additional 30 seconds. The total acid-soluble DNA was less than 10% of the total. As shown in Fig. 3, all the DNA fragments formed after the initial hydrolysis with micrococcal nuclease, after the pulse with pancreatic nuclease, are almost quantitatively transferred under the single band with the mobility of t-RNA. This result shows that the two nucleases cut chromatin DNA at similar positions though at different rates.

In agreement with such an interpretation is the finding that calf thymus chromatin digestion products with pancreatic nuclease, spleen nuclease and micrococcal nuclease are all similar when fractionated on sucrose gradients (14). Indeed, it is unlikely that such a fractionation procedure discriminates nucleosomes with intact DNA fragments and nucleosomes with DNA fragments split into two parts. Appreciable amounts of DNA fragments shorter than 60-70 base pairs in length, have never been observed. This suggests that nucleosomes with split DNA units are unstable and dissociate. The next hydrolytic step is the complete digestion of the two unprotected DNA fragments. A detailed digestion model will be reported elsewhere.

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