Chromatin structure of histone genes in sea urchin sperms and embryos

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ABSTRACT

The nucleosomal organization of active and repressed α subtype histone genes has been investigated by micrococcal nuclease digestion of *P. lividus* sperm, 32-64 cell embryo and mesenchyme blastula nuclei, followed by hybridization with ³²P-labeled specific DNA probes. In sperms, fully repressed histone genes are regularly folded in nucleosomes, and exhibit a greater resistance to micrococcal nuclease cleavage than bulk chromatin. In contrast, both coding and spacer α subtype histone DNA sequences acquire an altered conformation in nuclei from early cleavage stage embryos, i.e., when these genes are maximally expressed. Switching off of the α subtype histone genes, in mesenchyme blastulae, restores the typical nucleosomal organization on this chromatin region. As probed by hybridization to *D. melanogaster* actin cDNA, actin genes retain a regular nucleosomal structure in all the investigated stages.

INTRODUCTION

Several lines of evidence suggest that gene expression requires local modifications of the chromatin structure. However, no conclusive evidence has yet been obtained on whether all the transcribed regions retain a typical nucleosomal organization. Contrasting evidence has in fact been reported by E.M. analysis of active transcriptional units and by micrococcal nuclease digestion experiments, followed by hybridization with specific probes (for reviews, see 1-3). Differences in the results obtained by these two experimental approaches may arise from artefacts and technical problems in preparing the samples to be analyzed, but may also reflect, at least in part, real differences in the active chromatin structure, depending on the rate of transcription and the particular gene and cell type chosen for the analysis.

The increased sensitivity of active genes to DNase I provides in any event clear evidence for an altered conformation of the transcribed chromatin regions. Several distinctive features of active chromatin have been recently described (1-3); it contains HMG-associated nucleosomes, highly acetylated histones, and the DNA itself is often undermethylated, at least in higher eu-

karyotes (4). Another difference in the nucleosomal organization of chromatin regions in the active vs. the non-transcribed state could be the way in which histone octamers are positioned along the DNA filament. By nucleosome mapping on specific genes, both random and non-random location have been described with respect to the underlying nucleotide sequences (2,5,6). In particular, nucleosomes appear to be preferentially located in specific positions on the histone gene cluster of *Drosophila*, at least in the spacer regions (7). Analysis of *X. laevis* tRNA genes shows that nucleosomes are in one regular, predominant phase in erythrocyte chromatin, where these genes are repressed, while they appear to be either randomly arranged or in many different phases in liver and kidney cells, actively synthesizing tRNA (8).

Sea urchin histone genes provide an excellent model system to study, in order to correlate gene activity with modifications of chromatin structure. It is well known that sea urchin chromatin undergoes significant structural changes during development. Both the average nucleosomal repeat length and the degree of sensitivity to nuclease digestion have been reported to vary in the course of embryogenesis (9). These findings, which reveal modifications in the overall chromatin organization, have been related to changes in the rate of transcription and cell division and to the presence of different stage - specific histone variants in chromatin (10). Nothing as yet is however known about the organization of specific genes in the sea urchin genome. At least two sets of histone genes are expressed in sea urchin embryos, i.e., the subtype α actively transcribed up to early blastula and the genes coding for the β , γ and δ histone variants, which are expressed at early and late stages (11-14). A wealth of information on the sequence organization has been accumulated on the highly reiterated α subtype histone genes, whereas very little is known on the organization of the subtypes expressed at later stages. In all sea urchin species, the α subtype genes are tandemly repeated and the coding sequences, which are present with identical orientation along the same DNA strand, are intermingled with spacer DNA regions of similar length (15, 16).

We have analyzed the nucleosomal organization of these genes by micrococcal nuclease digestion of chromatin from 32-64 cell stage embryos and mesenchyme blastulae and sperms, in order to determine whether differences could be detected in the structural features of this chromatin region in its active and repressed state.

MATERIALS AND METHODS

Nucleosomal DNA preparation. Eggs of *P. lividus* were fertilized and cultured

at a concentration of 10.000/ml in Millipore-filtered sea water containing antibiotics (17). Embryos were collected at the 32 to 64 cell stage and at the mesenchyme blastulae stage and nuclei were isolated as described (18), with some modifications. All operations were performed at 4°C. Embryos were pelleted and washed once in buffer I (0.1 M Na phosphate, pH 6.0 - 0.1 M LiCl - 0.15 mM spermine - 0.5 mM spermidine - 1 mM EDTA⁺ - 1 mM EGTA⁺ - 0.5 mM PMSF⁺) containing 24 mM EDTA. Embryos were then resuspended in buffer I and homogenized. The crude nuclear pellet was washed 2-3 times in buffer I containing 0.1 % NP40 and further purified by centrifugation through a 1.0 M sucrose cushion in the same buffer, at 15.000 g for 15 min. Sperm nuclei were isolated as described by Spadafora et al. (19). Purified nuclear pellets from sperms, 32 to 64 cell stage embryos and mesenchyme blastulae were washed once in RSB (10 mM NaCl - 5 mM MgCl₂ - 10 mM TRIS-HCl, pH 7.4) and resuspended in RSB supplemented with 1 mM CaCl₂. Micrococcal nuclease (Worthington) digestion was performed at 37°C as described in the legends to figures. At the end of incubation, EDTA was added to a final concentration of 10 mM. Nuclei were centrifuged at 500 g for 10 min, to produce a first supernatant and a nuclear pellet, which was lysed by resuspension in 0.2 mM EDTA, pH 7.0. The lysate was made 5 mM EDTA, pH 7.0, and briefly centrifuged (500 g for 2 min) to give a second supernatant and a nuclear residue. The extent of chromatin digestion was estimated by measurement of absorption at 260 nm of the acid-soluble material in the first supernatant. A factor of 1.68 was used to correct for hyperchromicity (20). For the experiments to be described, DNA was extracted from the second supernatant. DNA extracted from the total nuclear lysate gave identical results. Before DNA extraction, the samples were incubated with 100 µg/ml preboiled pancreatic RNase (Sigma), at 37°C for 30 min, then NaCl and SDS⁺ were added to a final concentration of 0.4 M and 1 % respectively and the samples were treated with Proteinase K (Merck) for 2 h. DNA was phenol-chloroform extracted and ethanol precipitated. DNA pellets were resuspended in TE buffer (10 mM TRIS, pH 8.0 - 1 mM EDTA) and kept at 4°C. Digestion of DNA with restriction endonucleases was carried out under the conditions suggested by the supplier.

Blot hybridization. Nucleosomal DNA fragments (10-20 µg/well) were fractionated by electrophoresis on 1.5 % agarose slab gel using a TRIS - borate - EDTA buffer system (21) and transferred to nitrocellulose sheets according to Southern (22). Blots were probed with nick-translated (23) histone DNA fragments which were isolated from PH70 DNA by restriction enzyme digestion and electroelution from the agarose gel. Nitrocellulose filters, after DNA transfer, were preincubated for 4 h at 66°C in 3 X SSC (1 X SSC is 0.15 M NaCl - 0.015 M Na

citrate) - 10 X Denhardt's solution (24), then hybridized for 18 h at 66°C in a mix having the same composition of the preincubation solution and containing 100 µg/ml denatured salmon sperm DNA and $1-2 \times 10^6$ cpm/ml of denatured fragment. After hybridization, the filters were extensively washed in 2 X SSC at 66°C and 0.1 X SSC at 45°C, dried and autoradiographed with Fuji RX film and Kodak fast tungstate intensifying screen.

RESULTS

Chromatin organization of histone DNA in active and repressed state.

The transcription of the α subtype histone genes is developmentally regulated. These genes are expressed up to the stage of early blastula and then are shut off (16). Since the chromatin organization undergoes significant modifications during sea urchin embryogenesis (9), we have asked whether similar stage - specific structural rearrangements occur also at the level of particular genes. One way of answering this question is to determine whether or not nucleosomes are on phase on histone DNA and whether the same phasing is held after fertilization and during the course of embryogenesis.

To this end, a preliminary experiment was performed by the indirect end labeling technique (25, 26), in order to see whether, after micrococcal nuclease and restriction enzyme digestion, histone DNA bands could be detected by hybridization with a histone DNA fragment.

Fig. 1 shows the restriction map of a fragment of pPH70 clone which bears P.lividus α subtype histone genes (11). PH70 contains seven genes, more than one repeat, but lacks one of the expected H4 genes (G.Spinelli and J.Telford, unpublished data). Only the restriction enzyme sites generating the DNA fragments utilized in the present work are indicated.

Purified nuclei of P.lividus sperms and embryos at the 32-64 cell stage

Fig. 1: Restriction map of P.lividus histone DNA. pPH70 DNA was digested with the indicated restriction enzymes and the following probes were electroeluted from the agarose gel and nick-translated: (a) 2.1 kb HpaI - HindIII; (b) 0.8 kb SalI - SalI; (c) 0.95 kb EcoRI - EcoRI; (d) 1.1 kb HindIII - HpaI.

were incubated with micrococcal nuclease. The release into the post-nuclear supernatant of the acid-soluble material was on the order of 8-10 %. Nucleosomal DNA was extracted and digested with HpaI restriction enzyme. The DNA fragments obtained were separated according to size by agarose gel electrophoresis, blotted onto nitrocellulose filter and hybridized with a nick-translated 2.1 kb HpaI-HindIII fragment (probe a in Fig.1). The autoradiograph of Fig. 2 shows a very interesting and unexpected feature. While nucleosomal DNA of sperms (lane E) shows the characteristic oligomeric pattern, and HpaI restriction enzyme digestion of this DNA generates several sharp bands (lane A), neither discrete DNA fragments nor a nucleosomal pattern are revealed, even after prolonged exposure, by hybridization of the labeled probe with blots of embryos nucleosomal DNA digested (lane C) or not digested (lane D) with HpaI. By ethidium bromide staining, on the contrary, both embryo DNA samples presented the expected ladder of multimer bands (not shown). Lane B of Fig. 2 represents 32-64 cells stage embryo nuclear DNA which, although slightly degraded by the endogenous nuclease, exhibits after digestion with HpaI a hybridization band of 7 kb, which corresponds to the size of the *P.lividus* histone DNA repeating unit.

Although this first experiment could not allow to draw any conclusion with

Fig. 2: Southern blot probed with nick translated HpaI-HindIII fragment. Sperm nuclei (20 O.D.₂₆₀/ml) and 32-64 cell embryo nuclei (14 O.D.₂₆₀/ml) were digested with 20 U/ml micrococcal nuclease for 20 min and with 50 U/ml micrococcal nuclease for 10 min respectively. The release of acid-soluble material was on the order of 8 - 10 % of the starting O.D.₂₆₀. 10 µg of sperm nucleosomal DNA and about 2 µg of embryo nucleosomal DNA, undigested or digested with HpaI, were analyzed on 1.5 % agarose gel, blotted onto nitrocellulose filter and hybridized to 2.1 kb nick-translated HpaI-HindIII DNA fragment (probe a in Fig. 1). Lane A: sperm nucleosomal DNA digested with HpaI. Lane B: 32-64 cell embryo nuclear DNA digested with HpaI. Lane C: 32-64 cell embryo nucleosomal DNA digested with HpaI. Lane D: 32-64 cell embryo undigested nucleosomal DNA. Lane E: sperm undigested nucleosomal DNA.

regard to the nucleosome phasing problem, even in sperm chromatin, because of the lack of adequate controls (a more careful investigation on this point is still in progress), nevertheless it did clearly indicate that a typical nucleosomal array is present on the α subtype histone genes region in sperms. Furthermore, it provided a stimulating, even though very preliminary indication that the chromatin organization of histone genes could be drastically modified in cells actively transcribing them. Since however in this particular experiment the amount of embryo DNA loaded on the gel was much lower than that of sperm DNA and contained fewer high molecular weight oligomers, the interpretation of the results was rather ambiguous. The low intensity of hybridization and the absence of a typical nucleosomal pattern or discrete DNA bands in lanes C and D of Fig. 2 could be attributed in fact to the higher extent of chromatin digestion by micrococcal nuclease. We were then prompted to perform the following experiments in order to determine whether nucleosomal DNA fragments could be obtained from the histone gene chromatin region, and to separately analyze the organization of coding and spacer regions within the cluster.

Chromatin organization of coding and spacer histone DNA sequences.

In order to better investigate chromatin histone genes organization in the active state, we incubated 32-64 cell embryo purified nuclei with different a-

Fig. 3: Ethidium bromide staining pattern of nucleosomal DNA. Nucleosomal DNA of sperms, 32-64 cells and mesenchyme blastula embryos was fractionated on 1.5 % agarose gel. Lane A: DNA from sperm nuclei (20 O.D.₂₆₀/ml) incubated with 20 U/ml micrococcal nuclease for 20 min. 8% acid-soluble material released. Lane B: DNA from undigested 32-64 cell embryo nuclei. Lane C-F: DNA from 32-64 cell embryo nuclei (12.5 O.D.₂₆₀/ml) incubated with 2 U/ml, 5 U/ml, 15 U/ml and 30 U/ml micrococcal nuclease respectively, for 10 min. The amount of acid-soluble material released was: (C) 0.6 %; (D) 0.7 %; (E) 1.38 %; (F) 2.84 %. Lane G: mesenchyme blastula embryo nuclei (5 O.D.₂₆₀/ml) incubated with 10 U/ml micrococcal nuclease for 5 min. 6% acid-soluble material released. 10 μ g DNA were analyzed in each lane, except for lane E, in which 20 μ g were used. Size markers (pBR 322 cut by HpaII) are indicated in basepairs.

mounts of micrococcal nuclease (from 2 to 30 U/ml) to the point when from 0.6% to 2.84% of the starting O.D.₂₆₀ were released into the post-nuclear supernatant. The DNA extracted from these samples, and from non-incubated nuclei, was analyzed on agarose gel in parallel with nucleosomal DNA from sperm and mesenchyme blastulae cells. The ethidium bromide staining pattern is shown in Fig. 3. One slot (E) was intentionally overloaded with 32-64 cell stage embryos nucleosomal DNA. On the basis of the migration distance of marker DNA fragments of known length, the repeat size was calculated to be 232.5 ± 3.5 for sperm chromatin, 198 ± 2.5 at the 32-64 cell stage and 200 ± 5 for mesenchyme blastula nucleosomal DNA.

As can be seen in lanes C and D, a complete nucleosomal pattern is already present after very mild micrococcal nuclease digestion of 32-64 cell stage embryo nuclei, although most of the material is in the high molecular weight region. The multimer to small oligomer ratio is inverted after more extensive digestion (lane F). Sperms and mesenchyme blastulae nucleosomal DNA are shown in lanes A and G respectively. Despite the fact that chromatin from these cells was digested to a similar extent (6-8 % acid-solubility), there is a striking difference in the multimers to monomer ratio. We feel that this must be somehow correlated to the different higher order structure and degree of

Fig. 4: Autoradiographic pattern of nucleosomal DNA probed with whole pH70. The DNA samples analyzed on the agarose gel shown in Fig. 3 were transferred to a nitrocellulose filter and hybridized to nick-translated whole pH70. Lanes A - G: same as in Fig.3

condensation of chromatin in nuclei from sperms and embryos at various stages. This question will however be discussed in greater detail elsewhere.

Aliquots of the same sample preparations were analyzed on several agarose gels identical to that whose ethidium bromide staining pattern is shown in Fig. 3 or to that shown in Fig. 7a. The DNA was then transferred by Southern blotting (22) onto nitrocellulose filters, which were each hybridized to one of the following nick-translated probes (Fig. 1): whole pPH70; Sal I - Sal I H3 spacer sequences; EcoRI - EcoRI fragments containing H 4 coding sequences; HindIII - HpaI fragment. The results obtained by hybridization with the first three probes are illustrated in Fig. 4, 5 and 6 respectively. Analogous results were obtained with the HindIII - HpaI fragment (not shown). Sperms and blastulae nucleosomal DNA gave rise, after hybridization with all probes, to an oligomeric pattern which closely resembles the ethidium bromide staining, except for the weaker intensity of the dimer respect to the higher oligomer bands in the hybridization patterns of sperm DNA. Thus, the fully repressed histone genes in sperm cells appear to be more resistant than bulk chromatin to micrococcal nuclease digestion. Hybridization of 32-64 cell stage embryo nucleosomal DNA with the same histone DNA probes generates, on the contrary, a smeared pattern superimposed on which a few broad bands are only barely visible. They are mainly in correspondence of the trimer - tetramer mesenchyme

Fig. 5: Autoradiographic pattern of nucleosomal DNA probed with spacer DNA. Aliquots of the same sperm, 32 - 64 cell embryos and mesenchyme blastulae nucleosomal DNA samples shown in Fig. 3 were analyzed on 1.5 % agarose gel, transferred to a nitrocellulose filter and hybridized to the 0.8 kb SalI - SalI DNA fragment (probe b in Fig. 1) containing H3 spacer sequences.

Lanes A - G: same as in Fig. 3

blastulae nucleosomes in the lanes containing mildly digested nucleosomal DNA (Fig. 4, lane C), while very faint traces of monomers and dimers appear in more extensively digested DNA samples (Fig. 4, lane D). This can better be seen after hybridization with whole pH70 or the EcoRI - EcoRI fragment. With all probes, however, most of the hybridization appears to occur as a continuous smear, rather than localized in discrete bands, even in very mildly digested nucleosomal DNA samples. Lane B represents the hybridization of high Mr DNA, extracted from 32-64 cell embryo undigested nuclei.

The results of these hybridization experiments carried out with different probes corresponding to either coding or spacer histone DNA sequences reinforce our initial observation that the whole α subtype histone gene cluster chromatin, in its maximum active state, undergoes dramatic structural modifications.

Do all structural genes present an altered chromatin structure in 32-64 cell stage embryos?

The experiments described in the previous section clearly indicate that the α subtype histone gene cluster chromatin presents an altered conformation at the early embryo stage. As shown in Fig. 3, however, this is not a general feature of 32-64 cells stage embryo bulk chromatin. By ethidium bromide

Fig. 6: Autoradiographic pattern of nucleosomal DNA hybridized to H4 DNA. Sperm, 32-64 cell embryos and mesenchyme blastulae nucleosomal DNA aliquots from the same sample preparations utilized in the previous experiments were analyzed on 1.5 % agarose gel, transferred to nitrocellulose filter and hybridized to nick-translated 0.95 EcoRI - EcoRI DNA fragment (probe c in Fig.1) containing H4 coding sequences. Lanes A - E: same as in Fig.7

staining, a typical nucleosomal pattern is in fact clearly evident.

In order to determine the type of organization and the sensitivity to micrococcal nuclease of structural genes which are silent or expressed at low rate in the early cleavage embryos, we studied the chromatin structure of actin coding sequences. The amount of actin mRNA in sea urchin embryos increases in the course of development, and it is very low before hatching (27). The actin genes family in sea urchin consists of several non-reiterated members (28), all of which cross-hybridize very efficiently with the *Drosophila* actin cDNA (unpublished observation and 29). Therefore actin cDNA cloned in pACY 184 plasmid (a kind gift of V.Pirrotta) was nick-translated and hybridized to a Southern blot of sperms and embryos nucleosomal DNA. Fig. 7a shows the ethidium bromide staining pattern of the agarose gel, onto which were

Fig. 7: Chromatin structure of the actin genes. (a) Ethidium bromide staining. (b) Autoradiograph of Southern blot probed with nick-translated *D.melanogaster* actin cDNA. Lane A: sperm nucleosomal DNA digested to 8 % acid-solubility. Lane B: DNA from undigested 32-64 cell embryo nuclei. Lane C: 32-64 cell embryo nucleosomal DNA digested to 0.7 % acid-solubility. Lane D: 32-64 cell embryo nucleosomal DNA digested to 2.84 % acid-solubility. Lane E: mesenchyme blastula nucleosomal DNA digested to 6 % acid-solubility. 20 μ g DNA were analyzed in each lane.

loaded aliquots of the same DNA samples used in the previous experiments. Fig. 7b shows the autoradiograph of the filter. By hybridization with the actin probe, a typical ladder of DNA bands is clearly evident not only in correspondence to sperm (lane A) and mesenchyme blastulae (lane E) DNA, but also in lanes C and D, which contain 32-64 cell stage nucleosomal DNA, digested to 0.7 % and 2.84 % acid-solubility respectively. Comparison of the ethidium bromide staining (Fig. 7a, lanes C and D) and hybridization pattern (Fig. 7b, lanes C and D) further suggests that the sensitivity to digestion of the actin genes chromatin region is very similar to the sensitivity of bulk chromatin.

DISCUSSION

The experiments described in this paper were undertaken to study the chromatin structure of the α subtype sea urchin histone genes in their active and inactive state.

The fine structural analysis of transcriptionally active genes, carried out on a number of different organisms, has provided consistent evidence for an altered conformation of the active chromatin regions (for recent reviews on this subject, see 1-3). Most of the information today available has come from DNase I digestion experiments, which have revealed several distinctive features common to many eukaryotic genes in their active state. The use of micrococcal nuclease to probe the structure of active genes has led instead to different results depending on the particular gene analyzed. In some cases, after micrococcal nuclease digestion, chromatin regions which are actively transcribed exhibit a typical nucleosomal pattern (30,31), even though their DNA may be more sensitive to enzymatic degradation. However, the Drosophila hsp 70 gene and the 5'-flanking regions of the chicken ovalbumin gene have been reported to lose, upon activation, the nucleosomal organization (32-34).

As shown in this paper, the sea urchin α subtype histone genes chromatin also undergoes profound modifications in cells which actively express them. As revealed by the autoradiographs shown in Fig. 4, 5 and 6, α subtype histone genes are clearly organized in nucleosomes in chromatin from sperm cells, where they are fully repressed, and from mesenchyme blastula embryos, in which transcription of these genes has already been shut off. Bulk 32-64 cell stage embryo chromatin is organized in nucleosomes (Fig. 3), with a repeat unit size of 198 ± 2.5 . However, the nucleosomal pattern appears totally blurred in the autoradiographs when DNA isolated from early stage embryo nuclei mildly digested by micrococcal nuclease (0.6 % to 2.84 % acid-solubility) is hybridized to nick-translated probes containing histone genes sequences. Some bands can

indeed be detected above the background. This is more clearly evident when DNA fragments containing H4 coding sequences or the whole pPH70 are used as a probe (Fig. 6 and 4). They are however too faint and broad to establish the size of the corresponding DNA fragments.

The main conclusion to be drawn from the present data is that the periodic organization of chromatin, as revealed by micrococcal nuclease digestion, is disrupted in the histone genes region during transcription. The very faint bands visible above the smeared background and the monomers and dimers present in traces in the hybridization pattern (Fig. 4) may be taken as an indication that not all the 400 tandemly repeated copies of the α subtype histone gene cluster are simultaneously active or transcribed at the same high rate. A small percent of the copies, or/and histone genes orphans, recently discovered (35), may then retain a typical periodic chromatin structure, giving rise to DNA fragments of discrete size after micrococcal nuclease digestion. The alternative possibility that these bands may be generated by cross-hybridization of the probes with other sets of histone genes, expressed at later developmental stages, appears more unlikely, also in view of the low copy number recently found for the late subtype histone genes (personal communication),

Whatever the special features of histone genes chromatin in the active state may be, they clearly characterize both the coding and the spacer regions of the cluster. Analysis of the same regions in mesenchyme blastulae nuclei shows that inactivation of the α subtype histone genes restores the typical nucleosomal structure. At the present, we have no data to determine when the structural modifications of the histone genes chromatin are first established. Preliminary experiments, however, indicate that this chromatin region has the typical nucleosomal organization in unfertilized egg nuclei.

The appearance of a hybridization pattern which extends from the high to the low molecular weight region of the gel when 32-64 cell stage embryo nucleosomal DNA is probed with labeled histone DNA fragments suggests that actively transcribed histone genes are still bound by proteins and thus protected from micrococcal nuclease digestion, even though they do not exhibit the periodic arrangement typical of bulk chromatin. Naked DNA of all embryonic stages, if incubated with micrococcal nuclease in very mild conditions (0.03 U/ μ g DNA at 10°C) is in fact rapidly digested to a size of less than 500 bp (not shown). Visual comparison of the ethidium bromide staining and autoradiographic patterns of early stage embryos chromatin shows instead that bulk and histone DNA sequences are cleaved to roughly give the same average size distri-

bution by micrococcal nuclease. Although the slightly lower than expected overall intensity of the autoradiographic pattern, when compared to the ethidium bromide staining of the 32-64 cell embryos nucleosomal DNA may suggest that the histone genes are digested away more rapidly than bulk chromatin, more quantitative data would be required to support this observation.

In contrast, comparison of the staining and autoradiographic pattern of sperm nucleosomal DNA reveals a greater than average resistance of the α subtype histone genes to micrococcal nuclease. Even within a highly condensed chromatin such as that of sperm cells may thus exist regions characterized by different degrees of compaction. No significant difference can instead be detected in the average repeat size between histone genes and bulk chromatin in these cells.

Upon inactivation, in mesenchyme blastula embryos, both the overall chromatin structure and the sensitivity to micrococcal nuclease of the α subtype histone genes region become indistinguishable from that of bulk chromatin, in contrast with what found in DNase I digestion experiments. By DNase I digestion in fact, at least some features typical of active chromatin regions appear to be retained by globin and ovalbumin genes even after their transcription has been shut off (1, 36, 37).

The results described in this paper indicate quite clearly that the organization of the histone gene chromatin is severely affected by the transcriptional process. However, nothing can be said from the present data either in favor or against the presence of histone octamers on active genes. Several alternative hypotheses can be formulated to interpret our data. The possibility of a complete removal of the histone octamers from the DNA strands during transcription cannot be excluded, but is not supported by any direct evidence. In our opinion, the blurring of the periodic pattern in the active histone gene chromatin could be explained equally well by the two following hypotheses: 1) because of the presence of highly modified histone molecules or some specific non-histone protein, the internal structure of the nucleosomes is so "relaxed" that micrococcal nuclease is not prevented from digesting the core DNA. If in fact the enzyme could attack equally well linker and core DNA, most probably only a smeared pattern, similar to that we have observed, would be detectable after hybridization with specific probes; 2) as a consequence of the frequent RNA polymerase passing along the histone genes, the nucleosome cores could slide along the DNA filament, so that the size of the linker DNA would be totally randomized. If this were the case, again no discrete DNA fragments or oligomeric pattern could be expected to be generated by micrococcal

nuclease digestion. Similar hypotheses have been suggested to explain the results obtained in analyzing the chromatin structure of the *Drosophila* hsp 70 gene (32) and the 5'-flanking regions of the chicken ovalbumin gene (34) in their active state. Those results are to a large extent similar to the ones we have reported for the α subtype sea urchin histone genes, although with some differences.

One last point which clearly emerges from the experiments described in this paper, and which deserves some attention, is that the conformational changes observed in the intensely transcribed α subtype histone genes chromatin are not detected in the actin genes, which are either silent or transcribed at a much lower rate (27). The same 32-64 cell stage embryo nucleosomal DNA which gives a smeared hybridization pattern when probed with histone DNA sequences, generates in fact a typical nucleosomal pattern when hybridized to actin cDNA.

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Abbreviations: EDTA, Ethylenediamine tetra acetic acid. EGTA, Ethylene glycol-bis-(2-amino ethyl ether)-N,N'-tetra acetic acid. PMSF, Phenylmethyl sulphonyl fluoride. SDS, Sodium dodecyl sulfate. bp, base pair. kbp, kilobase pair. hsp, heat shock protein.

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