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Transient alterations of the chromatin structure of sea urchin early histone genes during embryogenesis

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ABSTRACT

We describe features of the chromatin structure of the early histone gene family of Strongylocentrotus purpuratus during development. Before and after the histone genes are transcriptionally active, chromatin structure is quite similar with well-defined spaced nucleosomes and no major 5'-flanking sites hypersensitive to nucleases. During the period when the genes are active, marked changes in chromatin structure occur. Micrococcal nuclease digestion generates monomer nucleosomes and only trace amounts of higher multimers. Regions hypersensitive to an endogenous nuclease and DNAase I appear in the 5'-flanking regions of genes for H2A, H2B and H3. Each region consists of four sites spanning a DNA length of 200-250 base pairs. In each case, one major cutting site is near the TATA box; the bulk of the sensitive region is in the nontranscribed spacer. Other sites, in 3'-flanking regions of the genes, are sensitive to nucleases only when the histone genes are no longer transcribed.

INTRODUCTION

The early histone genes of sea urchins provide a fine example of developmentally regulated transcriptional expression (for review see 1). This tandemly repeated gene family is present in about 400 copies per haploid genome. Early in development, at about the 16-cell stage, these histone genes begin to be transcribed. Transcription rates increase during development and at the 100-200 cell stage a large portion of newly synthesized mRNA is derived from the histone genes (2). At the time of hatching, blastula stage, the early histone gene set becomes quiescent and another family of "late" histone genes are transcribed to produce the nuclear proteins for the rest of sea urchin larval life. The early histone gene family has been cloned from several species of sea urchin. In each case, the repeated element is 6-7 kbp in length and contains one gene for each of the five histones, H1, H2A, H2B, H3 and H4. All genes are transcribed in the same direction.

The mechanism of transcriptional control of this gene family is not known. In the urchin Psammechinus miliaris, Grosschedl and Birnstiel (3)

demonstrated that a sequence 5' to the H2A gene was necessary for active transcription when the cloned gene was injected into Xenopus oocytes. The sequence lay ca. 200 bp upstream from the translation start site and, interestingly, was functional in either orientation relative to the structural gene. This sequence, called a "modulator", thus bears strong relationships to subsequently described "enhancer" sequences in viruses and in other cellular genes (for review see 4).

Certain features of the chromatin structure of transcriptionally competent genes have recently been elucidated in several systems (for review see 5). Active genes are generally more readily digested by several different nucleases than inactive genes. Sites which are hypersensitive to nucleases, especially to DNAase I, have been shown to be characteristically present in active chromatin; most often such sites are near or at the 5' end of the coding sequence. In most cases, nucleosomes are present on both active and inactive genes. These features of chromatin architecture are common to two classes of genes in higher eukaryotic cells. The first, exemplified by globin (6), consist of genes which are present in terminally differentiated cells and active only in these specific cell types. The second, with heat shock genes (7) as an example, consists of genes which are active at all times in cells albeit at markedly different levels of transcription depending upon physiological conditions. The yeast HIS3 gene similarly is maintained in a transcriptionally competent, nuclease sensitive strucure whether transcription is at a basal level or increased several-fold by amino acid starvation (8). For these cases, it appears that once a gene has acquired the chromatin structure characteristic of the transcriptionally active state, that structure persists even though active transcription diminishes or ceases. The relationship of these chromatin structure alterations to control of transcription thus remains unclear.

For other genes, changes in chromatin structure have been correlated more directly with transcription. The acid phosphatase gene of S. cerevisiae, PHO5, is active when cells are grown in the absence of phosphate and repressed when phosphate is present in the medium. Changes in nucleosome positioning and hypersensitive sites in 5' flanking regions occur as a function of the transcriptional activity of the PHO5 gene (9). While not a reversible change in chromatin organization, the mating type loci have different chromatin structures dependent on whether they are active, when at the MAT locus, or inactive, at the HMLα or HMRa locus (10). The 5S rRNA genes of Tetrahymena thermophila also show altered patterns of nuclease sen-

sitivity as a function of both absolute (macro- vs. micronuclei) and relative (fed vs. starved cells) transcriptional levels (11).

As a gene family in cells which are continuing to divide, the early histone gene set of sea urchins might be expected to have changes in chromatin structure which correlate with transcriptional activity in a fashion more like the yeast PHO5 gene rather than like the housekeeping or terminally differentiated genes described above. Indeed, during the course of our studies, Bryan, Olah and Birnstiel (12) described changes in the chromatin structure of the early histone genes of P. miliaris during embryogenesis. During the period when the gene is active, nucleases preferentially cut the chromatin DNA in regions centered at the TATA box upstream from each of the five histone genes. Preferential cutting disappeared when the genes were repressed at blastula stage.

We have investigated the chromatin organization of the early histone genes in S. purpuratus during development. While monomer nucleosomes are present on active genes, we can not demonstrate a nucleosome ladder, suggesting that this highly active gene family has highly irregular or infrequent nucleosomes. Well defined ladders are present when the genes are inactive. Upstream from the genes for H2A, H2B and H3, we find nuclease sensitive regions which are developmentally regulated; they are absent in sperm, present in 100-200 cell embryos, and absent at hatching blastula and gastrula stages. Mapped at reasonably high resolution, these regions are not centered at the TATA box, although they occur in close proximity to this genetic element. Each region consists of several nuclease sensitive sites spanning a length of about 200 bp. Another set of sensitive sites are present when the gene family has been rerepressed; these sites are located in the spacer regions near the 3' ends of two of the histone genes.

MATERIALS AND METHODS

S. purpuratus were obtained from Pacific Biomarine and maintained in artificial sea water (Instant Ocean). Spawning was induced by intracoelomic injection of 0.55 M KCl. Eggs were collected in sea water and washed several times by settling while sperm was collected "dry". Embryos were grown in artificial sea water at 16°C at a density of 10^7/liter. When embryos were to be harvested prior to hatching, hardening of the fertilization membrane was prevented by growth in the presence of 1 mM 3-aminotriazole; the membrane was removed by forcing the embryos through 52 μm nylon mesh.

Sperm nuclei were prepared as described (13). Embryo nuclei were pre-

pared by washing embryos twice in calcium/magnesium-free sea water containing 2 mM EDTA and once in Hewish and Burgoyne (14) buffer containing 0.34 M sucrose and 2 mM EGTA. Cells were broken by Dounce homogenization and debris removed by filtration through 20 µm nylon mesh. Nuclei were pelletted at 4°C for 10 min at 10000 xg and washed once in Hewish/Burgoyne buffer containing 1.5 M sucrose prior to digestion. In some instances, nuclei were washed with NaCl-containing solutions of various concentrations prior to digestion, as indicated.

Nuclease digestions were carried out at 37°C for one min using indicated concentrations of DNAase I or micrococcal nuclease (Worthington). Reactions were terminated by addition of EDTA and sodium dodecyl sulfate to concentrations of 12.5 mM and 0.5%, respectively. Digested nuclei were incubated at 65°C for one min and DNA was purified using proteinase K digestion, phenol-chloroform extractions, ethanol precipitation and reprecipitation from 2.5 M ammonium acetate. Secondary restriction was carried out under conditions specified by enzyme suppliers. Restriction endonucleases were obtained from New England Biolabs, Bethesda Research Labs, or Boehringer-Mannheim.

Plasmids pCO2A, pBR313 containing the Hind III 6.54 kbp S. purpuratus histone gene repeat (15), or pSP17', the 1.9 kbp EcoR I S. purpuratus histone gene segment containing H2A and H3 genes originally in pSP17 (16) recloned in pBR322, were grown, amplified and prepared using conventional methods. Figure 1 shows a partial restriction map of the region studied based on published sequences (17) and unpublished sequence data (L. Kedes, personal communication). Fragments to be used as probes were excised from the plasmids, purified twice by agarose gel electrophoresis and nick-translated to specific activities of $5x10^8$-10^9 cpm/µg.

DNA fragments were separated by electrophoresis on submerged 1.0 - 1.6% agarose gels in 89 mM Tris, 89 mM boric acid, 2 mM EDTA and transferred to nitrocellulose filters (18). After baking, filters were prehybridized at 65°-70°C in 6xSSC and 4xDenhardt's solution for one hour. Hybridization was carried out for 18 hours at 70°C in 6xSSC, 4xDenhardt's, 0.1% sodium dodecyl sulfate, 1 mM EDTA, 0.25 mg/ml tRNA and radioactively labeled probe. Filters were washed three times for 30 min each with 0.1% sodium dodecyl sulfate, 0.1xSSC at 70°C and dried. Autoradiography was carried out using Kodak XR5 film and a DuPont Cronex intensifying screen at -70°C.

RESULTS

The tandemly repeated early histone gene set of S. purpuratus is a classic developmentally regulated gene family. Active transcription of these

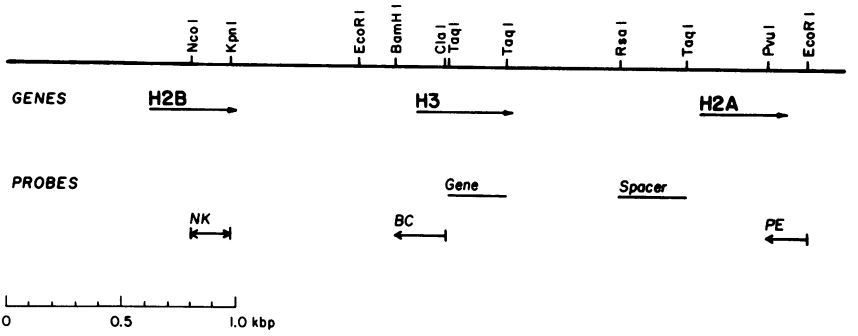

Figure 1 A partial map of the early histone gene repeat of S. purpuratus. The positions and direction of transcription of the genes for histones H2B, H3 and H4 are indicated. Probes used for micrococcal nuclease digests are the 1.95 kbp EcoR I fragment which contains genes for H3 and H2A together with flanking and intervening spacer segments, a Taq I fragment which derives entirely from the H3 gene and a Taq I - Rsa I fragment which contains only spacer sequences 5' to the H2A gene. Indirect end label probes for endogenous nuclease and DNAase I digests were an Nco I - Kpn I fragment, used to map in both directions, a BamH I - Cla I fragment used to map from the Cla I site and an EcoR I - Pvu I fragment used to map from the EcoR I site.

genes begins shortly after fertilization; at morula, the time of maximal activity, histone mRNA comprises about one-third of the newly synthesized message in the developing embryo. At the time of hatching, early blastula stage, this histone gene set is rerepressed and another, less abundant and not tandemly organized, set of histone genes is activated and provides the basic proteins of chromatin during the remainder of sea urchin larval life.

Figure 1 shows a map of a portion of the early histone gene repeat of S. purpuratus (15-17), detailing the positions and direction of transcription of genes for H2A, H2B and H3, locations of a selected set of restriction endonuclease sites and the strategy used for mapping the chromatin organization of these genes during sea urchin development. We describe (i) the nucleosomal organization of these genes before, during and after transcription and (ii) the location of sites which are highly sensitive to digestion by either an endogenous endonuclease or DNAase I using indirect end labeling (7,19) with the probes indicated in the figure.

Nuclei were isolated from different developmental stages during S. purpuratus embryogenesis and digested with micrococcal nuclease. DNA was purified and electrophoresed on agarose gels. At all stages, well defined nucleosome ladders were present for bulk chromatin, as judged by ethidium bromide staining of the gels (Figure 2). Approximate nucleosome repeat lengths for the

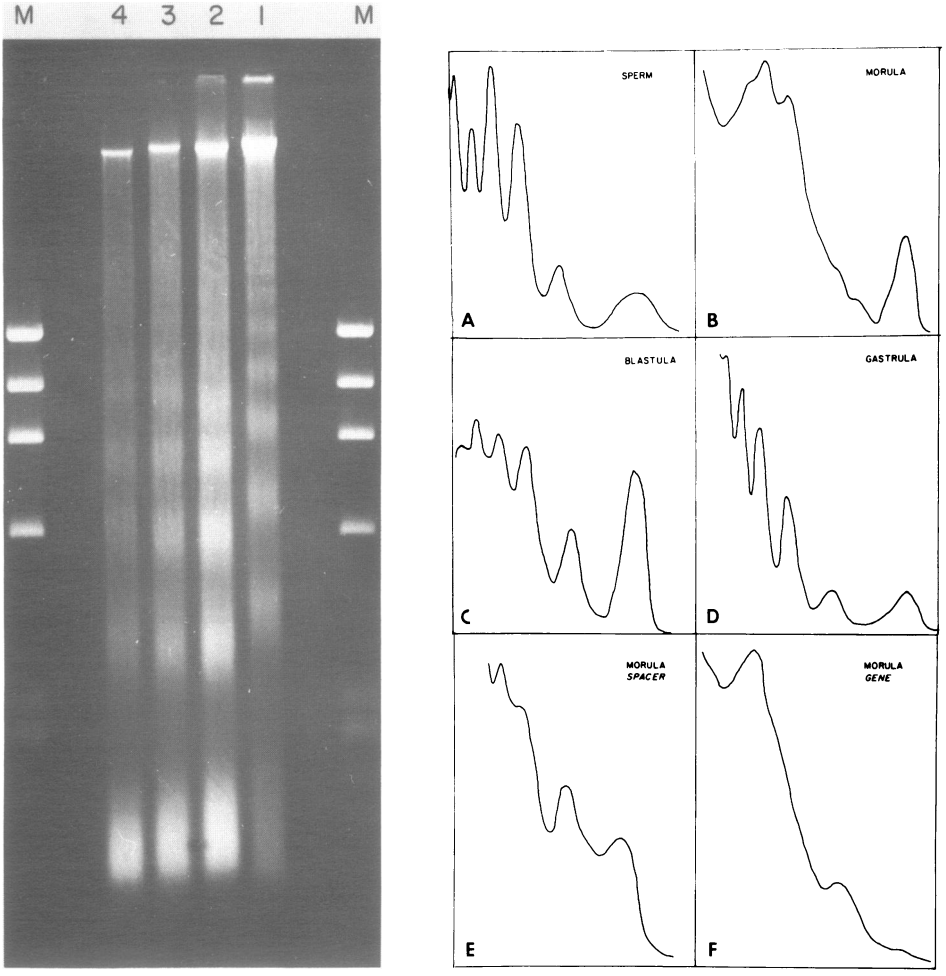

Figure 2 Micrococcal nuclease digests of nuclei from various developmental stages during S. purpuratus embryogenesis. Nuclei were digested with micrococcal nuclease (500 U/ml for lane 1 and 250 U/ml for lanes 2-4 for 1 min), DNA purified and electrophoresed on agarose gels. The left figure is an ethidium bromide stained gel of digests from nuclei of sperm (lane 1), morula (lane 2), blastula (lane 3) and gastrula (lane 4). The lanes M contain a Hae III digest of ϕX174-RF DNA. For the right figure, DNA was transferred to nitrocellulose filters and probed with the 1.95 kbp EcoR I fragment (A-D) or the spacer (E) or gene (F) specific probes detailed in the legend to Figure 1. Stages used were (A) sperm, (B), (E) and (F) morula, (C) blastula and (D) gastrula. Autoradiograms were scanned with an E-C Corporation densitometer. The ordinant is linear with optical density.

several stages were sperm, 245 bp; morula, 210 bp; blastula, 225 bp; and gastrula, 220 bp.

The separated DNA fragments were transferred to nitrocellulose and hybridized with probes from the histone gene segment to ascertain the nucleosomal organization of the early genes during activation of transcription and subsequent rerepression. Figure 2 shows the results of a typical experiment. Sections A-D trace the digestion patterns of nuclei from sperm, where the gene family is inactive, through morula, a stage of high activity, to blastula and gastrula when the genes are no longer transcribed. The blots were probed with the nick translated EcoR I fragment containing the genes for H2A and H3 (Figure 1).

The sperm nuclei contain a well defined micrococcal nuclease ladder of nucleosomes, indicative of an organized structure typical of bulk chromatin at any stage of development. The repeat length for the ladder is about 250 bp. In contrast to this organized pattern, digestion of nuclei from stages where the gene set is active leads to a highly heterogeneous set of DNA fragments containing histone genes. A mononucleosome peak is present; at longer fragment lengths only a broad smear with minor fine structure is seen (Figure 2B). The fine structure may relate to nuclease sensitive regions within the repeat. This finding suggests that few nucleosomes are present on the histone gene family when transcriptionally active and that those which are present are spaced widely and highly irregularly. We asked whether this situation obtained for only the structural gene segments or for the whole tandemly repeated segment by hybridizing similar blots with DNA fragments derived from a structural gene (Figure 2F) or from a spacer region (Figure 2E). The results are essentially the same for both cases; a mononucleosome peak appears below a highly heterogeneous array of fragment sizes in the micrococcal nuclease digest. The spacer probe appears to detect a fraction of sequences which are present in dinucleosomes, although this is only a trace of the degree of organization present for stages when the gene family is repressed. Digestion to greater or lesser extents did not qualitatively affect the patterns of hybridization.

At blastula, the early histone gene family is rerepressed. Concurrent with this alteration in transcriptional activity, a defined micrococcal nuclease digestion pattern reappears (Figure 2C). The spacing of the bands is shorter than that observed in sperm, 220 bp, and the degree of resolution of the various bands is less than that present in the earlier repressed stage. As development proceeds to gastrula, the defined micrococcal nuclease digest-

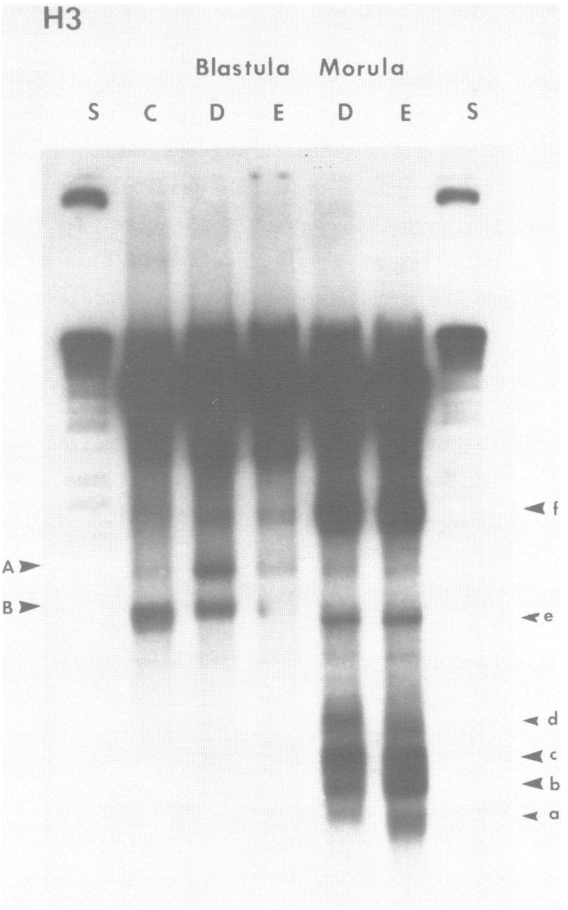

Figure 3 Nuclease hypersensitive sites in the 5'-flanking region of the H3 histone gene. Nuclei from blastula or morula stage were digested with the endogenous endonuclease (E) or DNAase I (D), 50 U/ml for 1 min, as indicated. Lane C is a sample of S. purpuratus DNA digested with DNAase I, 4 U/ml for 1 min. Lanes S are partial Sau 3A digests of pCO2A. After digestion, samples were purified, secondarily restricted with Nco I, subjected to agarose gel electrophoresis and transfer to nitrocellulose filters, and probed with the Nco I - Kpn I fragment shown in Figure 1. In this and Figures 4 and 5, lower case letters adjacent to arrowheads refer to sites which are preferentially cut when the histone genes are transcriptionally active; upper case letters codify sites which are cut when the genes have been rerepressed.

ion pattern persists and becomes even more clear cut (Figure 2D). Indeed, the resolution of individual bands at gastrula is similar to that for sperm. We suggest that rerepression of the early histone gene set which occurs at blastula leads to reestablishment of a defined pattern of spaced nucleosomes.

During further divisions, the spacing and overall organization of the nucleosomes is further refined to lead to the digestion pattern at gastrula (c.f. Figure 2C and D).

We attempted to define the positions of nucleosomes on the histone genes at various developmental stages using indirect end labeling methodology. Unfortunately, the presence of a number of sequence selective cutting sites for the nuclease in protein free DNA precludes any meaningful interpretation of such experiments.

Figures 3-6 show the results of indirect end labeling experiments addressing the presence of nuclease sensitive sites during sea urchin development. In each case, data are shown which reflect the presence of an endogenous endonuclease in the nuclei which is active in the presence of both calcium and magnesium. Also shown are data derived from digestion with DNAase I in the presence of magnesium and EGTA.

At morula stage, both nucleases cut at four reasonably narrow regions to the 5' side of the H3 gene (Figure 3, bands a-d). The positions of the two central, strongest sites (b and c) are nearly identical for the endogenous endonuclease and DNAase I. The shorter and longer fragments (a and d) are somewhat larger for DNAase I than for the endogenous enzyme. These sites are not preferentially cut by DNAase I in sperm nuclei, prior to the activation of transcription (data not shown), and, similarly, are not preferentially susceptible in protein free DNA (Figure 3). Strikingly, in nuclei from blastula, a stage when the early histone gene family is rerepressed, the sensitive sites near the 5' end of the H3 gene are absent (Figure 3). Figure 6 summarizes the locations of the sensitive sites for the three genes studied. Band d arises from cutting at or near the translation initiation codon. The two major fragments, b and c, are due to cutting about 20 and 90 bp 5' to the TATA box while the shortest fragment, a, maps to a cutting site about 160 bp upstream from this element.

The H3 gene region per se does not, for the most part, contain any hypersensitive sites for nucleases. There is one sensitive site near the 3' end of the coding sequence, about 25 bp from the translation termination codon (band e), which is developmentally regulated in a fashion similar to the sites in the 5'-flanking segments. This site is weakly cut in protein free DNA. In contrast to this regulated pattern, there are two sites in the 3'-flanking spacer region of the H3 gene which are less accessible to nuclease when the gene is active, but are sensitive when the gene is repressed in blastula nuclei (Figure 3, bands A and B). These sites are positioned 30 and

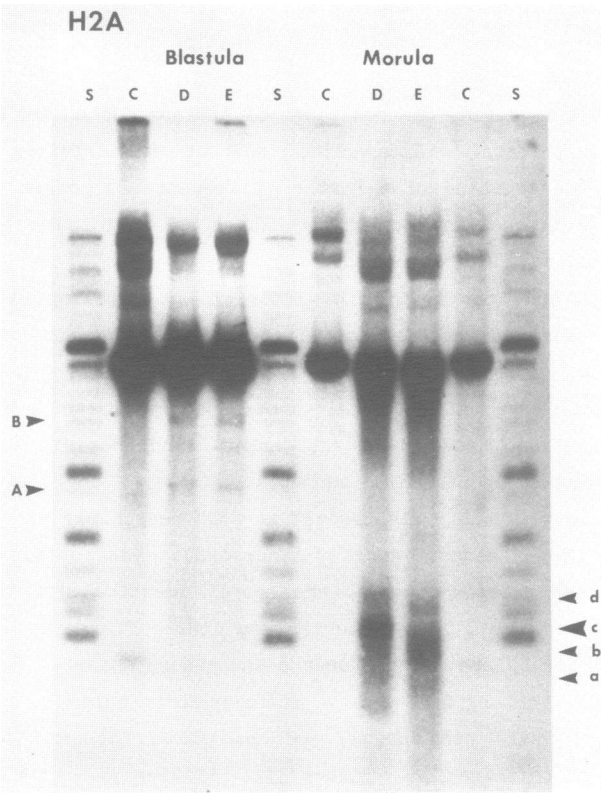

Figure 4 Nuclease hypersensitive sites in the 5'-flanking region of the H2A histone gene. Lane identifiers are as in the legend to Figure 3. Secondary restriction was with EcoR I and the blots were probed with the EcoR I - Pvu I fragment shown in Figure 1. The lower case labeled arrowheads indicate the positions of the sites cut by DNAase I; in this case, there are different sites within a common region cut by this exogenous enzyme and the endogenous nuclease.

210 bp to the 3' side of the termination codon. One of these sites is also cut strongly in naked DNA. Finally in the map shown in Figure 3 there is another set of sites which are sensitive in the active gene and not so in the repressed gene (band f). These result from cutting 5' to the neighboring H2A gene; they are mapped with higher precision in experiments shown in Figure 4.

Similar to the case for H3, sites in the 5'-flanking region of the gene for histone H2A are sensitive to both the endogenous nuclease and DNAase I when the gene is active, at morula, and not when the gene is rerepressed at blastula (Figure 4). Again these sites are not, in general, sensitive to the exogenous nuclease in naked DNA. The pattern for the sites near H2A differs

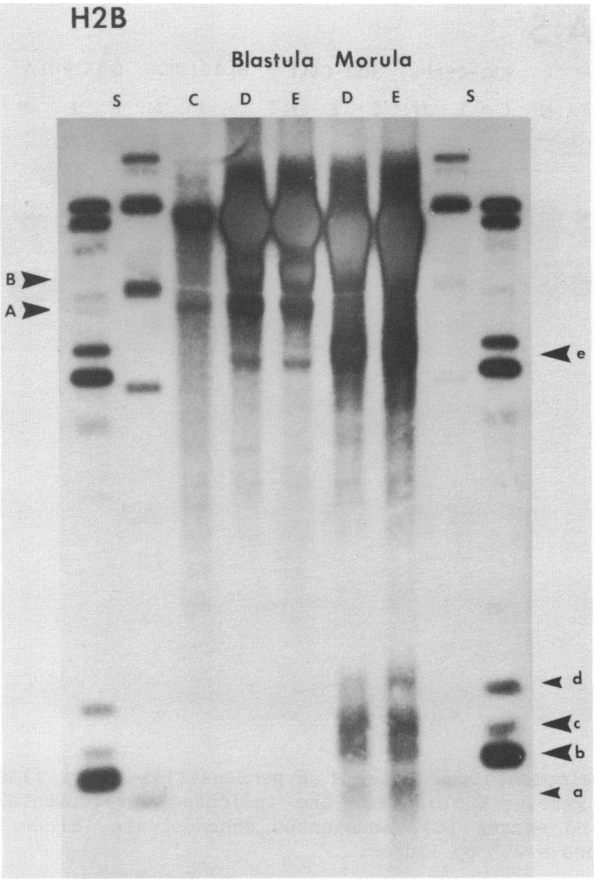

Figure 5 Nuclease hypersensitive sites in the 5'-flanking region of the H2B histone gene. Lane identifiers are as in the legend to Figure 3, except that two standard (S) lanes are shown; the innermost of these is a partial Hae III, and the outermost a partial Sau 3A digest of plasmid pCO2A. Secondary restriction was with Kpn I and the blots were probed with the Kpn I - Nco I fragment shown in Figure 1.

from that for H3; in the case of H2A, digestion appears to involve a region of sensitivity flanked by two (endogenous nuclease) or three (DNAase I) less intensively cut bands. The positions of the major bands differ for the two enzymes although there is some overlap.

Both enzymes cut chromatin DNA weakly in the region of the translation start site (band a). The major band for the endogenous nuclease spans from about 15 bp to the right of the transcription start site to 70 bp to the left of this site; it is centered at about the TATA box. The 5'-most site for the

Figure 6 Developmental profile of hypersensitive sites flanking the H2A early histone gene. Nuclei from the indicated developmental stages were digested with no enzyme (C), endogenous endonuclease (E) or DNAase I (N). The standards are a 255 bp ladder.

endogenous enzyme is about 130 bp upstream from the cap site. DNAase I also digests chromatin near the cap site (band b), at about the middle of the endogenous endonuclease major band. The major region for DNAase I cutting extends from -50 to -110 with a peak of intensity at -85 (band c). The 5'-most DNAase I site maps to about 150 bp 5' to the cap site (band d). Again, the striking loss of the hypersensitive sites when the gene is rerepressed at blastula occurs for the region to the 5' side of the H2A gene also. Bands A and B observed for the inactive gene and absent for the active gene (Figure 4) are the sites mapped in the spacer 3' to H3 in Figure 3.

Figure 5 presents data mapping cutting sites for the two enzymes in the 5'-flanking region of the gene for histone H2B. The two enzymes detect similar structural features in this area, a broad region containing two apparent major cutting sites (bands b and c) is flanked by weaker cutting sites (bands

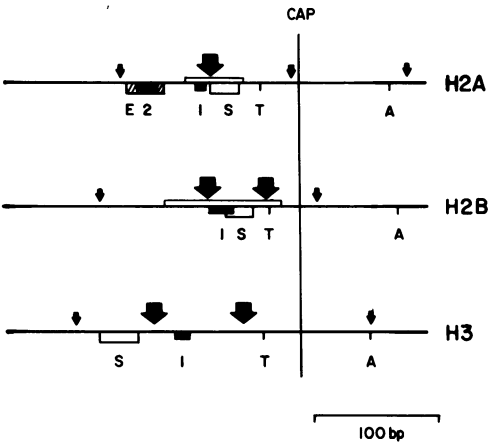

Figure 7 Schematic representation of cutting sites in the 5'-flanking regions of the early H2A, H2B and H3 genes which are hypersensitive to DNAase I when the genes are transcriptionally active. The three genes are aligned based on the start site for transcription (CAP). Also shown are the positions of the TATA box (T) and the translation initiation codon (A). Cutting sites mapped in Figures 3-5 are indicated as the arrows above each linear gene representation. Below each line are shown features of the DNA sequence which are discussed in the text: (solid boxes 1 and 2), conserved upstream elements found in 5'-flanking regions of histone genes from several species; (open box S) an unusual repeated sequence for each of the three genes; and (cross hatched box E) the position of an element in the sea urchin P. miliaris which functions as a modulator (or enhancer-like) element.

a and d). Band a maps just to the 3' side of the cap site. Band b derives from cutting at the TATA box; the region including bands b and c spans from -15 to - 110 relative to the cap site. The most distal band, d, arises from cutting at about -160. Again there is a striking developmental regulation of the hypersensitive sites.

Higher in the gel, two other sets of developmentally regulated sites are present. Band e is present in the chromatin from cells where the gene is active and largely missing at blastula stage. This site maps to the 5'-flanking region of the gene for histone H4. Two sites present in blastula nuclei and absent in the active gene chromatin (bands A and B) are located in the spacer to the 3' side of the gene for histone H1.

Figure 6 shows a full developmental profile for hypersensitive sites to the 5' side of the H2A gene, using both the endogenous endonuclease and exogenous DNAase I. As shown before, the sites are present on the active gene chromatin, at the 100- and 200-cell stages. The sites are absent prior to transcription of the genes, in sperm nuclei. When transcription is rerepres-

sed, at blastula, the sites are no longer present; they remain absent at gastrula and further in development.

Certain other experiments are relevant to these results. The endogenous endonuclease is present at blastula and morula, as shown by the presence of blastula and morula specific cutting in the absence of added DNAase I. Further, proteins eluted from nuclei by salt washes at the two stages include the nuclease (data not shown). We feel that a positive effector leads to the appearance of hypersensitive sites on the active gene; this contention is based on the lack of similar sites in protein-free DNA and by observations that the sensitive sites are not detected after nuclei are washed with 0.35 M NaCl-containing buffers (data not shown). The salt wash data are not unambiguous since the nuclei are highly aggregated after such treatment and an artefactual lack of sensitivity can not be precluded.

DISCUSSION

Nucleosome Organization

In contrast to a number of genes whose nucleosomal organization persists irrespective of the transcriptional state of the gene, the early histone genes of sea urchins appear to not be tightly packaged with histones when active. Thus, essentially no discrete species of nucleoprotein larger than a mononucleosome is observed when the genes are being transcribed. The observation is true for both structural gene segments and for nontranscribed spacer regions. It should be noted that this gene family is highly active during early stages of development; perhaps the persistence of more or less regular nucleosome ladders for less vigorously transcribed genes is a reflection of the density of RNA polymerase molecules relative to histone octomers. If dissociation of histones from DNA is a prerequisite for transcription of a segment of chromatin, the highly active histone genes may lack regions devoid of polymerase sufficiently long to accomodate a dinucleosome. Similar conclusions regarding the absence of histones on actively transcribed genes have recently been made by others for Tetrahymena 5S rRNA genes (11) and Drosophila hsp 70 heat shock genes (20).

Before and after the histone genes are transcribed, well defined nucleosome ladders are present. We note that the resolution of the individual nucleosome oligomer bands at blastula, just after the histone genes are inactivated, is not as high as at gastrula, one or two cell divisions later. At gastrula, the resolution of the ladder is equivalent to that seen for sperm. It would appear that the remodeling of chromatin structure of the repressed

genes is a continuing process even after transcription ceases. Maturation of the organization of the chromatin requires a longer time than the simple reestablishment of a multinucleosome structure. We have not been able to determine whether phasing exists on the histone genes and, if so, whether establishment of phasing occurs during this maturation.

Nuclease sensitive regions

The results presented here clearly corroborate features of those obtained by Bryan, Olah and Birnstiel (12) concerning the chromatin architecture of the early sea urchin histone genes in another species. Nuclease sensitive regions are absent in 5' flanking regions of the individual histone genes prior to activation of transcription. Such regions appear when the genes are actively transcribed and, nearly uniquely, disappear when the gene is again transcriptionally dormant later in development. Noted in the Introduction are the observations of others that in terminally differentiated cells, nuclease sensitive sites are present once a gene is competent for transcription and persist even in the absence of transcription. Whether remodeling to a state in which nuclease sensitive regions are absent is characteristic of genes which are active only early in development remains to be determined. Of interest would be the chromatin structure of a gene which is activated early in the developmental lineage of a committed cell and then inactivated prior to terminal differentiation. A related question of interest is whether replication is required for remodelling from a state with hypersensitive sites to one without.

While confirming the general observation of developmentally associated sensitive regions in the sea urchin histone gene set, our observations differ somewhat in detail from those previously reported (12). Specifically, the higher resolution afforded by mapping from restriction sites nearer the regions of interest has allowed us to define each sensitive region as consisting of several sensitive sites. The positions of these sites are shown diagramatically in Figure 7. For each of the three genes, the 3'-most site hypersensitive to nucleases occurs within the transcription unit. For H2A and H3 it is at about the position of the translation initiation codon.

The major hypersensitive area for all three genes consists of a broad segment of chromatin DNA (or two bands for H3) with less sensitive flanking bands, positioned at or to the left of the TATA box/cap area of the gene. In each case, the hypersensitive region spans about 200 bp of DNA. Of interest, the developmentally regulated nuclease sensitive region upstream from the Sgs4 glue protein locus in Drosophila similarly consists of three sites over

a 150 bp span (21), although this region is more distal to the gene than others discussed here. Several nuclease sensitive sites over a 120-180 bp region are characteristically present near the 5' end of the yeast TRP1 (22) and URA3 (Thoma, F., unpublished observations) genes. Whether these sets of cutting sites in putative control regions reflect a common architecture for chromatin DNA remains to be determined.

Might these sites reflect the binding of RNA polymerase II during transcription? We feel this to be unlikely given the static occurrence of the hypersensitive sites and the dynamic nature of the transcription process. Further, digestion patterns and the actual positions of the sensitive regions differ for the three genes (Figure 7), leading us to favor the interpretation that the sensitive regions result from interaction of positive regulatory elements with upstream control regions. Given the lack of sequence or structure homology in the three nuclease sensitive regions (see below), it is hard to envision their interacting with a common regulatory element, even though the footprinted DNA structure detected by nuclease digestion is somewhat similar.

In contrast to the general situation where nuclease sensitive sites are thought to be characteristic of active genes, we have found several locations on the histone gene set where sensitive sites are present on inactive genes and absent during transcription. Bryan, Olah and Birnstiel (12) also located a site present in protein-free DNA which was digested when genes were inactive and inaccessible in the active state. The significance of these sites remains unknown.

Regardless of the features leading to the presence of the hypersensitive sites on the transcriptionally active genes, the most striking feature of their occurrence is regulation during development. For most other genes it appears that, once established, a nuclease sensitive site persists in a particular gene region, regardless of the actual state of transcription of the gene. Such sites thereby mark a transcriptionally competent state. It is clear that in the case of this histone gene family which is activated and then rerepressed, the transcriptionally competent state can be reversed to the earlier, stably repressed, structure.

Features of the DNA of sensitive regions

Examination of the three sensitive region DNA sequences both for direct homology and for structural homology based on purine-pyrimidine pairs (23) has revealed no obvious features which would suggest interactions with a common regulatory element. Each of the three regions has an unusual tandemly

repeated element; these are shown in Figure 7 where the positions of the sequences are indicated by the open boxes labeled "S". In the sensitive region near the H2B gene is an internally symmetric heptanucleotide which is perfectly repeated eight bp later.

5' GCATACGGACCGCAGCATACG 3'

Within the H2A sensitive region a pentanucleotide is tandemly repeated about an octanucleotide inverted repeat.

5' GTCACAATGCCCCCCGACGGTCAC 3'

Finally, in the H3 region, there is a undecanucleotide nearly perfectly repeated with a twenty bp spacing.

5' TATGTGCAAAGTCAATAAAATGTGTCGAAAG 3'

This nucleotide sequence is highly related to the consensus SV-40 enhancer core sequence (4):

H3(a)	5' TaTGTGcAAAG 3'
H3(b)	5' TGTGTcGAAAG 3'
SV-40	5' TGTGTGGAAAG 3'.

The spacing of the two enhancer-like sequences is such that both will present on the same face of the DNA double helix, making their occurrence of even greater interest. Note that this is also true for the repeated pentanucleotide sequences in the H2A sensitive region.

Based on sequence comparison of histone genes from Xenopus, sea urchin, chicken and human, Perry, Thomsen and Roeder (24) have described a set of "upstream elements" which have been rather well conserved in the 5'-flanking region of several histone genes. They have suggested that these DNA regions might be important in regulation of transcription of the histone genes. The positions of two such elements near the H2A gene and one each for H2B and H3 are shown in Figure 7 as the numbered solid boxes. In each case, the position of the upstream element lies within the region which is hypersensitive to DNAase I in transcriptionally active genes. Thus, the chromatin structure of these upstream elements changes in a fashion which correlates with the transcriptional capacity of the histone genes. While not demonstrating a cause and effect relationship of these sequences, transcriptional regulation and the detected alterations in chromatin organization, the current results are not inconsistent with this interpretation.

Grosschedl and Birnstiel (3) showed that deletion of a modulator sequence, located 184-524 bp 5' to the translation initiation site of the H2A gene decreased transcription of the gene when injected into oocytes 15-20 fold. The sequence was functional in either orientation, thus meeting, at least

partially, criteria subsequently established for enhancer elements. Further studies refined the sequences required to augment transcription as being between positions -111 and -165, relative to the transcription start site (25). If a similar transcriptional modulatory element occurs in the same position relative to the ATG in S. purpuratus, it is between the left and central nuclease sensitive sites upstream from the H2A gene.

In summary, we have described reversible alterations in the chromatin structure of the early histone genes of the sea urchin S. purpuratus. The developmentally programmed changes correlate with transcriptional capacity of the gene set. We suggest that this highly active gene family is deficient in histones during transcription and that redeposition of histones following repression is succeeded by further remodelling of chromatin structure during later cell divisions. We have defined three nuclease sensitive regions and show that each consists of several sites spanning about 200 bp. Each region contains a conserved element common to the 5'-flanking sequences of histone genes from several species. Within one of these regions there is a tandemly repeated sequence with strong homology to the SV-40 consensus core enhancer. A second region includes a section of the gene demonstrated to function in P. miliaris as an upstream modulatory element.

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