Communication

Transcriptionally Active Xenopus laevis Somatic 5 S Ribosomal RNA Genes Are Packaged with Hyperacetylated Histone H4, Whereas Transcriptionally Silent Oocyte Genes Are Not*

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The relationship between histone acetylation and transcription of the Xenopus laevis oocyte and somatic 5 S ribosomal RNA genes was investigated. Chromatin fragments from a X. laevis kidney cell line were immunoprecipitated with an antibody specific for hyperacetylated histone H4. The DNA from the hyperacetylated chromatin was probed with both oocyte- and somatic gene-specific sequences, and the results showed that the upstream, nontranscribed region of the transcriptionally active somatic genes is packaged with acetylated histone H4. In contrast, the corresponding region of the transcriptionally silent oocyte genes is packaged with hypoacetylated histone H4 in this cell line. Further study also showed that this region of the oocyte genes was less sensitive to digestion with the enzyme, micrococcal nuclease. Together these results suggest that, as described for both RNA polymerase I and II transcribed genes, transcriptionally silent 28 S rRNA genes in rat tissue culture cells are packaged with nonacetylated histones (7). This latter result is despite the fact that the same study showed these silent genes exhibited increased sensitivity to micrococcal nuclease, another hallmark of transcriptionally poised genes.

Xenopus laevis produces two major types of 5 S rRNA: the somatic type, which is transcribed in most cells, and the oocyte type, which is produced only during early oogenesis, early embryogenesis, and in certain tissue culture cell lines (8, 9). Each 5 S rRNA type is transcribed from a distinct multigene family, and considerable research has focused on explaining the differential expression of these genes in somatic cells. Recently, it has been reported that histone acetylation enhances RNA polymerase III transcription of dinucleosomal 5 S rRNA gene templates (10), suggesting a possible role for histone acetylation in 5 S rRNA transcription. It was also demonstrated that in a X. laevis kidney cell line transcribing low levels of oocyte 5 S rRNA, a small portion of oocyte genes were packaged with hyperacetylated histones (11). Although this seemingly indicates a correlation between histone acetylation and 5 S rRNA transcription, it is not known whether the active oocyte genes are the subset packaged with acetylated histones in this cell line. In this study we sought to examine the relationship between histone acetylation and transcription of the X. laevis oocyte and somatic 5 S rRNA genes by determining whether these gene repeats are packaged with hyperacetylated histone H4 in somatic cells in which the genes are differentially regulated.

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The acetylation of lysine residues within the amino-terminal "tails" of core histones is known to be associated with transcriptionally active genes, although the basis of this relationship is not well understood (for reviews see Refs. 1–4). The use of antibodies, specific for hyperacetylated histones, to map core histone acetylation of active and inactive genes has suggested that acetylation is not a consequence but rather a precondition of transcription. It has been demonstrated in chicken embryonic erythrocytes, for example, that the β globin genes are packaged with acetylated histones prior to, during, and after activation during development (5). Furthermore, the platelet-derived growth factor B chain gene is also found packaged with hyperacetylated histones prior to gene induction in a human hematopoietic stem cell line (6). In contrast to this apparent constitutive presence of acetylated histones on "poised" RNA polymerase II transcribed genes, transcriptionally silent 28 S rRNA genes in rat tissue culture cells are packaged with nonacetylated histones (7). This latter result is despite the fact that the same study showed these silent genes exhibited increased sensitivity to micrococcal nuclease, another hallmark of transcriptionally poised genes.

EXPERIMENTAL PROCEDURES

Cell Culture and Northern Blot Analysis—X. laevis K2 cells were as generous gift of Paul Labhart (Scripps Research Institute, La Jolla, CA). Cells were cultured at room temperature in 0.5 × Leibovitz L-15 medium (Life Technologies, Inc.) supplemented with 10 mM HEPES, pH 7.5, 1 × PSN antibiotic (Life Technologies, Inc.), 2 mM l-glutamine, and 10% fetal calf serum. Total cellular RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform extraction technique (12) and electrophoresed for 16 h at 10 V/cm on a pre-electrophoresed (at 25 V/cm for 4 h), 12.5% acrylamide (29:1 acrylamide:bis-acrylamide) and 2.5 μg/ml gel in 1 × TBE (90 mM Tris borate, 1 mM EDTA). Following electrophoresis, the RNA was blotted onto a Zeta-Probe membrane (Bio-Rad) by applying the membrane dry to the gel, and vacuum drying at room temperature for 90 min (the gel detached once the membrane was rehydrated in 2 × SSC (0.3 M NaCl, 30 mM trisodium citrate, pH 7)). The membrane was alkaline fixed, blocked, and hybridized, as per manufacturer’s instructions to a 180 bp, [α-32P]dATP end-labeled, Hin-dIII/EcoRI fragment from plasmid pXS1 (10) that contains a copy of the X. laevis oocyte 5 S rRNA gene coding sequence. In vitro transcribed X. laevis oocyte and somatic 5 S rRNAs were prepared by T7 RNA polymerase (New England Biolabs, Beverly, MA) transcription of DraI-digested plasmids pXS1 and pXS2, respectively (13), as per the manufacturer’s instructions.

Western Blot Analysis—A polyclonal antibody specific for acetylated histone H4 was prepared as described previously (14). Western blot analysis (15) was performed with histones isolated from HeLa cells grown in the presence of sodium butyrate (16).

1 The abbreviations used are: rRNA, ribosomal RNA; bp, base pair; PAGE, polyacrylamide gel electrophoresis; PIPES, 1,4-piperazinediethanesulfonic acid.
Control Immunoprecipitation—200 ng of HeLa mononucleosome cores either nonacetylated (fraction E from nonbutyrate-treated HeLa cells) (16) or hyperacetylated (fraction B from butyrate-treated HeLa cells) (16) were 5' end-labeled with \([\alpha{^32P}]dATP\) and polynucleotide kinase (17). The labeled cores were diluted with a 1000 molar excess of unlabeled chicken erythrocyte mononucleosome cores (17) to produce a solution with a final mononucleosome concentration of 1 ng/ml. The samples were treated with 1% formaldehyde at room temperature for 20 min, dialyzed to remove the formaldehyde and immunoprecipitated as described in the following section.

Chromatin Isolation, Immunoprecipitation, and Dot Blot Analysis—Chromatin fragments were isolated from X. laevis tissue culture cells that had been fixed with 1% formaldehyde for 20 min at room temperature. The cells were harvested by centrifugation at 2000 × g and resuspended sequentially to a concentration of approximately 5.5 × 10⁷ cells/ml in ice-cold phosphate-buffered saline, buffer I (0.25% Triton X-100, 10 mM EDTA, 0.5 mM EGTA, 10 mM Tris-HCl, pH 7.5), and buffer II (200 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5). The final pellet was resuspended in a one-half volume of buffer III (1% SDS, 10 mM EDTA, 0.5 mM EGTA, 10 mM Tris-HCl, pH 7.5) and sonicated 10 times for 10 s each. Immunoprecipitation of hyperacetylated nucleosomes was performed by diluting the sonicated chromatin in buffer IV (1% Triton X-100, 150 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl, pH 7.5) to a final DNA concentration of 100 ng/μl, mixing 100 μg with 7.5 μl of undiluted polyserum and rotating at 4 °C for 1 h. Immunocomplexes were precipitated with protein A-Sepharose CL 4B beads, and the DNA was deproteinized as described by others (18). The DNA was dot blotted onto Zeta-Probe membranes, blocked, and hybridized as per manufacturer's instructions to total X. laevis DNA labeled with \([\alpha{^32P}]dATP\) by random priming (Promega Corp., Madison, WI) at 30°C in 10 mM Pipes, pH 6.8, 50 mM NaCl, 5 mM MgCl₂, 1 mM CaCl₂, 0.1 mM phenylmethylsulfonyl fluoride and digested with 4 units/μg micrococcal nuclease at 37 °C for various timed intervals. The digests were terminated, and the nucleic acids were lysed by adding the solution to 10 mM EDTA and 0.5% SDS. The DNA was deproteinized by digestion with 0.2 mg/ml proteinase K for 3 h at 37 °C followed by extraction with phenol/chloroform/isoamyl alcohol (25:24:1). The samples were ethanol precipitated, electrophoresed on a 1% agarose gel in 1 × TAE (20), Southern blotted, and probed with the ~360-bp [α-32P]dATP end-labeled, HindIII/EcoRI fragment derived from plasmid pXlo8 or a 459-bp [α-32P]dATP end-labeled, HindIII/EcoRI fragment derived from plasmid pXlsl1 (19). The latter two probes contain the 5' nontranscribed spacer of the X. laevis oocyte and somatic 5 S rRNA genes, respectively.

Chromatin Isolation, Micrococcal Nuclease Digestion, and Southern Blot Analysis—Nuclei isolated from X. laevis tissue culture cells (16) were suspended in 10 mM Pipes, pH 6.8, 50 mM NaCl, 5 mM MgCl₂, 1 mM CaCl₂, 0.1 mM phenylmethylsulfonyl fluoride and digested with 4 units/μg micrococcal nuclease at 37 °C for various timed intervals. The digests were terminated, and the nucleic acids were lysed by adding the solution to 10 mM EDTA and 0.5% SDS. The DNA was deproteinized by digestion with 0.2 mg/ml proteinase K for 3 h at 37 °C followed by extraction with phenol/chloroform/isoamyl alcohol (25:24:1). The samples were ethanol precipitated, electrophoresed on a 1% agarose gel in 1 × TAE (20), Southern blotted, and probed with the ~360-bp [α-32P]dATP end-labeled, HindIII/EcoRI fragment derived from plasmid pXlo8.

RESULTS AND DISCUSSION

This study examined the relationship between histone acetylation and transcription of the oocyte and somatic 5 S rRNA genes in X. laevis by determining whether these genes are packaged with hyperacetylated histone H4 in somatic cells in which the genes are differentially expressed. Previously it has been reported that certain Xenopus tissue culture cell lines express low levels of oocyte 5 S rRNA (8), and thus it was necessary to determine whether the oocyte genes were truly repressed in the somatic cell line used in this investigation. To this end, total cellular RNA was isolated from tissue culture cells, resolved by partially denaturing PAGE, blotted, and probed with a sequence specific for the oocyte 5 S rRNA gene. Because of a six-nucleotide difference between the oocyte and somatic 5 S rRNAs, the two molecules migrate differently in this partially denaturing gel system. This can be seen by comparing lanes 1 and 2 in Fig. 1, which show that the T7 polymerase-transcribed somatic 5 S rRNA migrated faster than its oocyte counterpart. Lane 3 of Fig. 1 shows the results of Northern blot analysis of the total cellular RNA from the Xenopus tissue culture cell line. The results show only one band, the migration of which closely resembled that of the somatic 5 S rRNA (Fig. 1, lane 1). No oocyte-like transcripts were observed. The lowest limit of detection of this analysis was ~0.5 pg of 5 S rRNA. This, taken together with the fact that there are 50 times more oocyte than somatic genes, suggests that the oocyte genes are transcribed at a rate at least 5000-fold less than that of the somatic in this cell line. It must be noted that the T7 transcribed somatic 5 S rRNA migrated slightly slower than that isolated from the tissue culture cells. The T7 transcribed 5 S rRNAs were 121 nucleotides in length because of run-off transcription of a DraI-digested plasmid, whereas the native 5 S rRNAs are only 120 nucleotides. This extra nucleotide has been shown not to participate in the secondary structure of 5 S rRNA. The conclusion of these results is that in the Xenopus tissue culture cell line used in this study, the oocyte genes are transcriptionally silent.

In this investigation, an antibody specific for hyperacetylated histone H4 was used to immunoprecipitate chromatin fragments. This antibody, prepared as described previously (14), was raised against the first 20 amino acids of tetraacetylated histone H4 from Tetrahymena, which differs significantly from that of Xenopus. To determine whether this antibody shows the same specificity for hyperacetylated Xenopus histone H4, a Western blot analysis was performed using histones from butyrate-treated HeLa cells (X. laevis histone H4 shares the identical first 20 amino acids with human H4). By comparing lane 1 of Fig. 2A, which shows Coomassie-stained histones, with the immunodetected histones in lane 2, it can be seen that the antibody used in this study strongly reacted with the di- to tetra-acetylated H4 but did not recognize nonacetylated H4. Thus this antibody would be expected to only immunoprecipitate nucleosomes packaged with hyperacetylated H4. To verify this, a control immunoprecipitation was performed. The starting samples for these control immunoprecipitations consisted of unlabeled, nonacetylated nucleosome cores (chicken erythrocyte) containing a 0.1% fraction of either radiolabeled acetylated or radiolabeled hypoacetylated HeLa cell nucleosome core particles. These samples were used to demonstrate that the antibody used in this study could selectively fractionate the hyperacetylated from the nonacetylated nucleosome cores as seen in Fig. 2B.

Chromatin fragments isolated from the X. laevis kidney cell line were immunoprecipitated with an antibody recognizing hyperacetylated histone H4. The resulting nucleosomal DNA was deproteinized and probed by dot blot analysis for the presence of the oocyte or somatic 5 S rRNA gene repeats (Fig. 3). Equal amounts of both the bound and unbound DNA fragments (aH4) were loaded as is demonstrated by hybridization.
and unbound fractions were blotted, if the oocyte or somatic 5 S rRNA genes were packaged with acetylated histone H4, there would be an enrichment for these sequences in the immunoprecipitate, and a more intense signal for the bound fraction than that for the unbound. The results show that in the case of the somatic genes, there was an ~5-fold enrichment for this sequence in the bound fraction, suggesting that the upstream nontranscribed sequence is packaged with hyperacetylated histones. In the case of the oocyte genes, although there was some signal, there was not an enrichment for the oocyte 5 S rRNA genes in the bound fraction. The fact that the signal for the bound is less intense than that of the unbound suggests that the upstream, nontranscribed sequence of the oocyte genes were selectively not precipitated, indicating that this region of the oocyte 5 S rRNA genes was not packaged with hyperacetylated histone H4. Assuming that the acetylation state of the spacer nucleosomes reflects that of any nucleosomes on the 5 S rRNA coding sequence, these results indicate that the transcriptionally active somatic 5 S rRNA genes are packaged with acetylated histones, whereas the transcriptionally silent oocyte genes in X. laevis are not. It should be noted that because the 5 S rRNA transcription complex spans a region greater than the 120 bp 5 S rRNA coding region (21, 22), it would be unexpected for this complex to form without displacing any nucleosomes located on this sequence. This is supported by DNase I footprint analysis of 5 S rRNA genes containing transcription complexes in vivo (23, 24). Thus, it is unlikely that the transcriptionally active somatic 5 S rRNA genes used for this study have nucleosomes on the actual coding sequence.

Because the above results demonstrate that the transcriptionally silent oocyte 5 S rRNA genes were not packaged with hyperacetylated histone H4, this would seemingly suggest the presence of nonacetylated histones on these genes. To verify the presence of nucleosomes on these genes, tissue culture cell chromatin was digested with micrococcal nuclease and Southern blotted. If the upstream, nontranscribed sequence of the oocyte gene had a low abundance of nucleosomes, this region of DNA would be sensitive to micrococcal nuclease digestion. Fig. 4 shows that this is not the case because digestion of the oocyte gene with micrococcal nuclease resulted in micrococcal nuclease-resistant fragments characteristic of the presence of nucleosomes on these genes, which has been shown by others (11,
Histone Acetylation of Oocyte and Somatic 5 S rRNA Loci

23, 24). These results also show that the oocyte genes in this cell line were less sensitive to micrococcal digestion than the bulk of the chromatin because more time was required to produce the same extent of digestion (compare the third and fourth lanes of the ethidium bromide-stained gel in Fig. 4A with those of the Southern blot in Fig. 4B).

Previous studies suggest that histone acetylation is one of the hallmarks of poised or potentially active genes (5, 6). Another well defined property of these regions is a general sensitivity to nucleases such as DNase I, DNase II, and micrococcal nuclease. In X. laevis somatic cells, the transcriptionally silent oocyte 5 S rRNA genes cannot be considered transcriptionally poised using these classifications because they are packaged with hypoacetylated histones and are less sensitive to micrococcal nuclease digestion than bulk chromatin. These results, taken together with those of others demonstrating the presence of acetylated histones on oocyte genes in cells in which oocyte 5 S rRNA is synthesized at low levels (11), suggest a link between histone acetylation and 5 S rRNA transcription. This is further supported by the fact that the transcriptionally active somatic 5 S rRNA genes are packaged with hyperacetylated histone H4.

The repression of oocyte 5 S rRNA transcription in somatic cells is reportedly because of the presence of histone H1 and reduced levels of transcription factor IIIA (25–27). The lack of histone acetylation in transcriptionally silent oocyte 5 S rRNA chromatin could then be explained by a blockage of a histone acetyltransferase activity by histone H1 or a requirement for the RNA polymerase III transcriptional machinery for the recruitment of this activity.

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