Histone H1 Binding Does Not Inhibit Transcription of Nucleosomal *Xenopus laevis* Somatic 5S rRNA Templates†

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ABSTRACT: It has long been proposed that selective binding of histone H1 is, in part, responsible for the differential developmental regulation of the oocyte and somatic 5S rRNA genes in *Xenopus laevis*. In this study we show that histone H1 binds both oocyte and somatic genes equally after reconstitution into mononucleosomes or oligonucleosome arrays. Furthermore, we show that the binding of histone H1 selectively represses only oocyte gene transcription and that an RNA polymerase III transcription complex is able to initiate transcription of nucleosomal somatic templates regardless of whether histone H1 is present. These results support a model in which the differential regulation of the 5S rRNA genes is not simply due to the prevention of histone H1 binding by transcription complexes on the somatic genes, but rather to a difference in the histone H1 interaction with the somatic and oocyte genes.

Xenopus laevis produces two major types of 5S rRNA: the somatic type, which is transcribed in most cell types, and the oocyte type, which is transcribed in the early stages of oogenesis and to a lesser extent in embryogenesis (*1*). Each type of 5S rRNA is transcribed from a distinct multigene family, and considerable research has focused on explaining the differential expression of these genes. Several studies have suggested that this differential regulation is partly due to the presence of histone H1, a general repressor of transcription (*2, 3*), but the mechanism of this effect is unknown. Immediately following DNA replication, there is a competition between histones and transcription factors for DNA binding sites. In *X. laevis* somatic cells, it is believed that, as a result of this competition, the somatic genes acquire a complete transcription complex, thus preventing the formation of repressive chromatin structures on these genes. In contrast, the oocyte genes become organized into repressive chromatin structures, including histone H1, which prevent access of the transcription factor machinery (*4, 5*). The differing fate of these genes has been attributed to a faster rate of assembly of transcription complexes on the somatic gene as compared to the oocyte (*6*). As a result of this, the oocyte genes are expressed only when histone H1 is absent and TFIIIA levels are relatively high as is the case during oogenesis.

A second, not necessarily mutually exclusive, mechanism for the differential regulation of these genes is that histone H1 shows a preference for binding to the oocyte genes when compared to the somatic counterparts. This is seemingly confirmed by observations of a preferential binding of histone H1 to the oocyte gene over the somatic gene due to differences in the AT base pair content of the 5′ and 3′ flanking regions of the two 5S rRNA gene families (*7*). However, this latter study was performed with naked DNA which binds H1 in a different manner than nucleosomal DNA (*8*), and because the oocyte genes have been shown to be packaged into nucleosomes (*9*) in somatic cells, the significance of this result is limited.

The object of this study was to determine whether histone H1 preferentially interacts with nucleosomal oocyte 5S rRNA genes as compared to somatic genes. The results show that histone H1 bound equally well to both oocyte and somatic genes after nucleosome reconstitution but that this binding repressed only oocyte gene transcription. These results support a model in which the differential regulation of the 5S rRNA genes is not simply due to the prevention of histone H1 binding by transcription complexes on the somatic genes, but rather to a difference in the interaction of histone H1 with the somatic and oocyte genes.

MATERIALS AND METHODS

Protein Purification. The purification of histone H1 from HeLa cells was carried out as described previously (*10*). Nucleosome core particles with low or high levels of acetylation were obtained from HeLa cells grown in the absence or presence of sodium butyrate, respectively (*11*). Purified histone octamers were prepared from chicken erythrocytes (*12*).

Preparation of DNA and Labeling of 5S rRNA Gene Fragments. The ∼200 bp fragments of the *Xenopus laevis* 5S rRNA genes, designated Xlo(−38 → +149) and Xls(−52 → +147) (*13*), were excised from plasmids by *Hin*dIII/*Eco*RI digestion, 3′ end-labeled with Klenow and [α-^{32}P]-dATP, and purified by nondenaturing gel electrophoresis. The 720 bp oocyte and 880 bp somatic full-length 5S rRNA genes were isolated by repeatedly passing *Hin*dIII digests

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of plasmids pXlo8 and pXls11 (*14*) respectively over a Sephacryl S-1000 column (*15*).

Nucleosome Reconstitution. The octamer exchange method (*16*) was used to reconstitute mononucleosomes containing the ~200 bp radiolabeled fragments of the oocyte and somatic 5S rRNA genes. Approximately 200 fmol of labeled DNA and 3 μg of HeLa cell nucleosome cores were mixed in 25 μL of 0.8 M NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM β-mercaptoethanol, and 0.1 mM PMSF.[1] Following incubation for 30 min at 37 °C, the nucleosomes were incubated at 4 °C for 16 h. The exchange reactions were then serial diluted to 0.6 and 0.1 M NaCl by the addition of 50 mM Tris-HCl (pH 7.5) and 0.1 mM PMSF at 30-min intervals at 4 °C. Histone octamers, with or without histone H1, were reconstituted onto full-length oocyte and somatic 5S rRNA genes using a modification of a salt dialysis gradient technique (*17*). In brief, DNA, at a concentration of 100 μg/mL, was mixed with a 3−4-fold molar excess of histone octamers in 2 M NaCl and dialyzed by step gradient to 0.5 M NaCl (*15*). The reconstitution reactions were diluted 6-fold in 0.5 M NaCl, 10 mM Tris-HCl (pH 7.5) and concentrated to the original volume using a Centricon 30 microconcentrator (AMICON Inc., Beverly, MA). Following concentration, histone H1 [dissolved in 0.5 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA] was added at 1 mol of H1/mol of histone octamers and the reactions were dialyzed against 10 mM NaCl, 10 mM Tris-HCl (pH 7.5) overnight at 4 °C.

Histone H1 Electrophoretic Mobility Shift Assay. Histone H1 binding reactions were performed with approximately 0.5−1 fmol of labeled nucleosomes or free DNA in 10 μL of 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 6% glycerol, 0.1 mg/mL BSA for 20 min at room temperature. The amount of histone H1 is indicated in the figure legends. The binding reactions were electrophoresed on 0.8% agarose, 0.5× TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) gels at 3.5 V/cm at 20 °C. The gels were dried at 50 °C and autoradiographed.

Micrococcal Nuclease Digestion of Chromatosomes. Nucleosomes reconstituted on full-length 5S rRNA genes were adjusted to 1 mM $CaCl_2$ and digested with 10 units/mL of micrococcal nuclease. Digestions were stopped by adjusting the solution to 5 mM EDTA, and the DNA was deproteinized by adding SDS to 0.25% and phenol/chloroform extracting. The digestion products were ethanol precipitated and resolved on a 4% acrylamide gel (*18*).

In Vitro Transcription of Reconstituted 5S rRNA Genes. *Xenopus* oocyte nuclear extracts were prepared from *X. laevis* ovaries (*19*, *20*) using the modification of ref 21 such that the final Mg^{2+} concentration of the extracts was 2 mM. Forty nanograms of template DNA (either nucleosomal or uncomplexed DNA) was transcribed at 30 °C for 60 min with 8.2 μL of transcription extract in 10 μL of 8 mM HEPES (pH 7.5), 1.6 mM DTT, 80 nM EDTA, 40 mM KCl, 0.4 mM ATP, CTP and UTP, 1 μM [α-^{32}P] GTP, and 4% glycerol. The reactions were terminated by the addition of 90 μL of 0.3 M Tris-HCl (pH 7.4), 0.3 M sodium acetate, 0.5% SDS, 2 mM EDTA, and 3 μg/mL tRNA, followed by extraction with phenol:chloroform:isoamyl alcohol (25:24:1) and by ethanol precipitation. Precipitated transcripts were resuspended in 98% formamide, 10 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol, heated at 90 °C for 10 min, and electrophoresed at 20 V/cm on a pre-electrophoresed 8% acrylamide (19:1 acrylamide:bisacrylamide) and 8.3 M urea gel in 1× TBE. The gel was dried and autoradiographed.

Southern Blot Hybridization. Micrococcal nuclease-digested fragments, resolved on a 4% acrylamide gel, were blotted onto Zeta-Probe membrane (Bio-Rad) by applying the dry membrane to the gel and vacuum-drying at room temperature for 60 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-^{32}P] dATP end-labeled, *Hin*dIII/*Eco*RI fragment derived from plasmid pXls (*22*) which contains the entire somatic 5S rRNA coding sequence but no native untranscribed sequences.

RESULTS AND DISCUSSION

The object of this study was to make a direct comparison between the *X. laevis* somatic and oocyte 5S rRNA genes with respect to the binding of histone H1 to nucleosomal DNA. Initially, we reconstituted mononucleosomes onto ~200 bp fragments of each gene. These fragments were designated Xlo(−38 → +149) and Xls(−52 → +147) [the prefix indicates the source of the 5S rRNA gene (i.e., Xlo refers to the oocyte while Xls refers to the somatic gene) and the numbers within the brackets represent the 5′ and 3′ ends of the DNA fragments in relation to the transcription initiation site]. These fragments were selected because they include the entire 5S rRNA coding sequence, including intragenic promoter, and thus the region of DNA to which histone H1 binding would exhibit its greatest effect. When reconstituted, both fragments position nucleosomes at multiple positions (*13*). Xlo(−38 → +149) positions histone octamers with downstream boundaries of +111 and +131 with respect to the transcriptional start site, while octamers reconstituted on Xls(−52 → +147) have boundaries of +104, +134, and +144. Although at first glance these multiple translational positions would be expected to complicate analysis of the results, because the oocyte genes have been shown to contain multiple, overlapping positions in vivo (*9*), we feel that this is a better representation than a gene fragment with a strong octamer positioning signal such as that of the *Xenopus borealis* gene (*16*). An H1 titration of these DNA fragments was performed to determine whether a difference in H1 binding to naked DNA could be observed. The results (Figure 1A) suggest that the difference in H1 binding between the oocyte and somatic 5S rRNA genes was negligible. The oocyte gene fragment formed aggregates at lower H1 concentrations when compared to its somatic counterpart, but the difference was minor.

To compare the binding of histone H1 to nucleosomal oocyte and somatic 5S rRNA genes, the gene fragments were reconstituted with HeLa cell histone octamers and tested for H1 binding. The results (Figure 1B) demonstrate that histone H1 bound equally to both fragments after nucleosome

[1] Abbreviations: BSA, bovine serum albumin; bp, base pair; DTT, dithiothreitol; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.

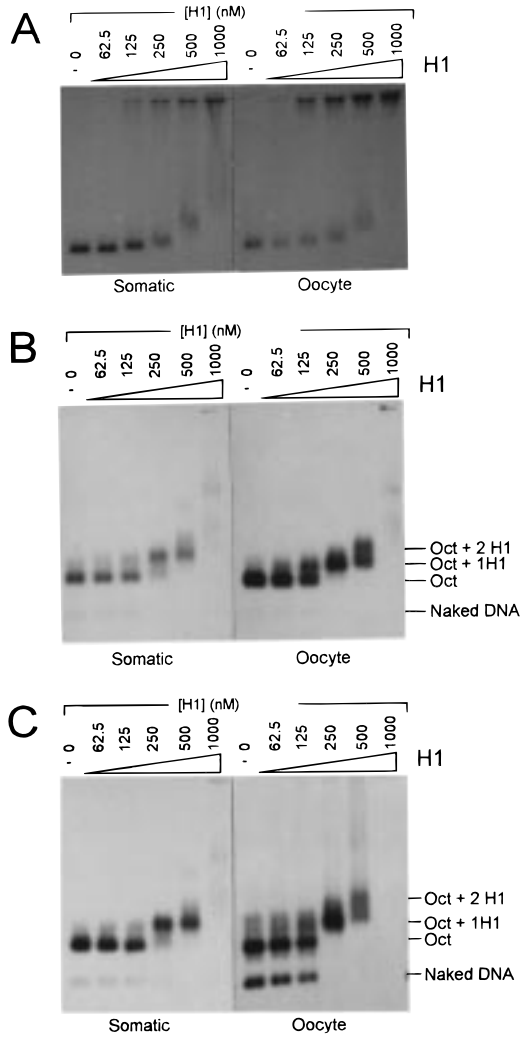

FIGURE 1: Histone H1 binds equally to the oocyte and somatic *Xenopus laevis* 5S rRNA gene fragments, Xlo(−38 → +149) and Xls(−52 → +147), after reconstitution with histone octamers. The figure shows the binding of increasing amounts of histone H1 to naked DNA (A), DNA reconstituted with control HeLa core histones (B), and DNA reconstituted with hyperacetylated HeLa core histones (C). Approximately 0.5−1 fmol of DNA was incubated with the indicated amounts of histone H1 for 20 min at room temperature and electrophoresed on 0.8% agarose gels.

reconstitution. It is interesting to note that in the case of the oocyte gene there appeared to be a secondary shift which is suggestive of more than one molecule of histone H1 interacting in a stable manner with one nucleosome. Although these results do not show an increased affinity of histone H1 for a nucleosomal oocyte gene, they do suggest that the oocyte nucleosome is capable of binding more than one molecule of histone H1.

Histone acetylation has long been linked to transcriptional activity, and studies have shown that the histone amino termini modulate the interaction of histone H1 with nucleosomes (*23*). Previously it was demonstrated, using a fragment of the *X. borealis* somatic 5S rRNA gene, that histone acetylation does not prevent the binding of histone H1 to nucleosomal DNA (*24*, *25*). This result must be interpreted with caution, however, as it has been suggested that in vivo histone H1 is not bound to this sequence (*4*, *5*). Figure 1C shows a titration similar to that in Figure 1B with the exception that the core histones were isolated from butyrate-treated HeLa cells. Once again, histone H1 did not show a preference for binding to a nucleosomal oocyte gene fragment when compared to a somatic gene fragment, but again, a secondary shift of the oocyte nucleosome was observed at higher concentrations of histone H1.

The disadvantage of using mononucleosomes for this investigation was that only the regions immediately flanking the 5S rRNA genes could be included. In the case of the oocyte gene, these regions are not as AT-rich as sequences further upstream. It has been suggested that it is the presence of extremely AT-rich sequences which is responsible for the increased binding of histone H1 to the oocyte gene (*7*), so it is possible that a preference for histone H1 binding to oocyte mononucleosomes was not detected because the fragments used excluded these sequences. To overcome this problem, the repressive effects of histone H1 on the transcription of nucleosomal templates was tested using full-length 5S rRNA gene repeats (720 bp for the oocyte gene and 880 bp for the somatic) rather than ∼200 bp fragments. This allowed more than one nucleosome to be reconstituted per DNA strand and possibly introduced internucleosome interactions. After reconstitution with histone octamers in the absence of histone H1, a micrococcal nuclease digestion of the reconstituted nucleosomal arrays was performed (Figure 2A). Both the oocyte and somatic 5S rRNA genes produced ∼146 bp nuclease-resistant fragments characteristic of packaging with histone octamers. Agarose gel electrophoresis of the reconstituted arrays (Figure 2B) demonstrates that DNA reconstituted with histone octamers alone migrated predominantly as one diffuse band although there were some slower migrating complexes (lanes 4 and 6). This indicates that the bulk of the nucleosome arrays had a similar amount of histone octamers. After addition of histone H1, the electrophoretic mobility of both the oocyte and somatic arrays was retarded, indicating that histone H1 was capable of binding to both genes.

To test the effect histone H1 has on 5S rRNA transcription, the reconstituted arrays were transcribed in *Xenopus* oocyte extracts. The results of the transcription analysis (Figure 3) demonstrate that reconstitution of an oocyte gene repeat with histone octamers repressed 5S rRNA transcription by approximately 33% (compare lanes 2 and 3). In contrast, when the somatic gene was reconstituted, there did not appear to be a significant effect on 5S rRNA transcription (compare lanes 5 and 6). When histone H1 was included in the reconstitution, residual oocyte gene transcription decreased a further 67% (compare lanes 3 and 4). Transcription of the somatic 5S rRNA gene was relatively unaffected (compare lanes 6 and 7) with only a 20% decrease in the transcripts produced. These results suggest that, in contrast to the oocyte 5S rRNA gene, RNA polymerase III was able to initiate transcription on somatic templates regardless of whether histone H1 was present.

One possible explanation why histone H1 incorporation had little effect on transcription of the somatic 5S rRNA gene is if the binding of histone H1 to this gene is atypical when compared to the bulk of the nucleosomes in vivo. To test this hypothesis, nucleosome arrays with histone H1 were digested with micrococcal nuclease. In native chromatin, histone H1 has been shown to protect an additional 20 bp's of DNA from micrococcal nuclease as compared to that of

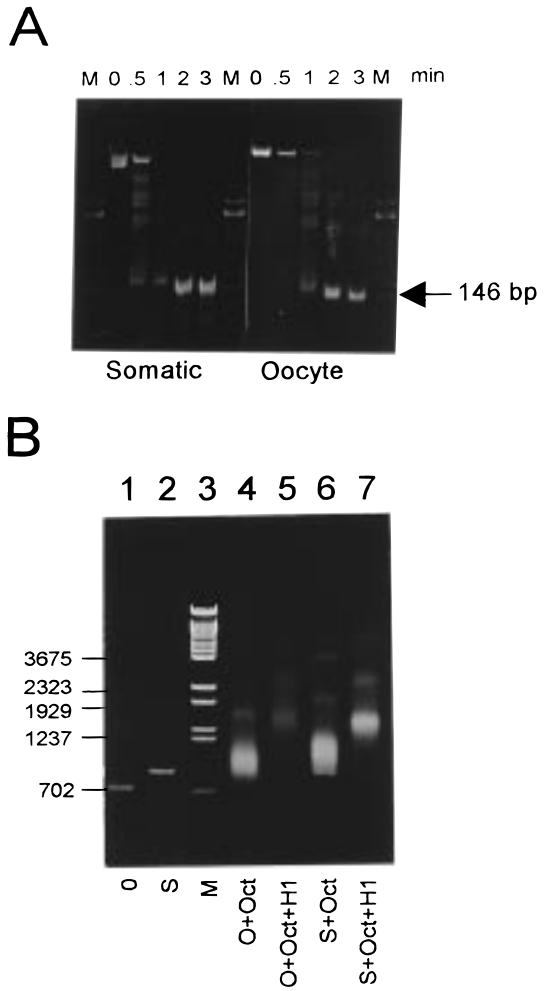

FIGURE 2: Histone H1 binds to both oocyte and somatic 5S rRNA full-length gene repeats after reconstitution with histone octamers. (A) Micrococcal nuclease digestion of histone octamers reconstituted on full-length *Xenopus laevis* oocyte and somatic genes. Digestions were carried out at a nucleosome concentration of 0.1 mg/mL (DNA weight) and an enzyme concentration of 10 units/mL for the minutes indicated on the top of each lane. The resulting DNA fragments were deproteinized and electrophoresed on a 4% nondenaturing gel: M, *Hha*I cut pB*R*322. (B) The binding of equal molar amounts of histone H1 to individual full-length 5S rRNA gene repeats after reconstitution with histone octamers as demonstrated by 1% agarose gel electrophoresis: lanes 1 and 2, uncomplexed oocyte (O) and somatic (S) full-length 5S rRNA genes; lanes 4 and 6, oocyte (O+Oct) and somatic (S+Oct) genes after reconstitution with histone octamers; lanes 5 and 7, oocyte (O+Oct+H1) and somatic (S+Oct+H1) genes after reconstitution with histone octamers and histone H1; M, *Bst*EII cut λ DNA.

the histone octamer alone (*26*). The micrococcal nuclease digestion patterns of the reconstituted oocyte and somatic 5S rRNA genes (Figure 4A) demonstrate that, in the presence of histone H1, ~165 bp's of DNA were protected from digestion. This indicates that histone H1 was bound to the reconstituted oocyte and somatic genes in a fashion similar to that found in native chromatin. It is possible that although some of the nucleosomes were capable of binding histone H1, the octamers in direct contact with the actual somatic 5S rRNA coding sequence excluded H1, thus explaining the lack of transcription repression of this gene. Although the results shown in Figure 1B,C suggest this was not the case, the possibility was tested using southern blot analysis. Nucleosome arrays reconstituted on the somatic gene repeat, with and without histone H1, were digested with micrococcal

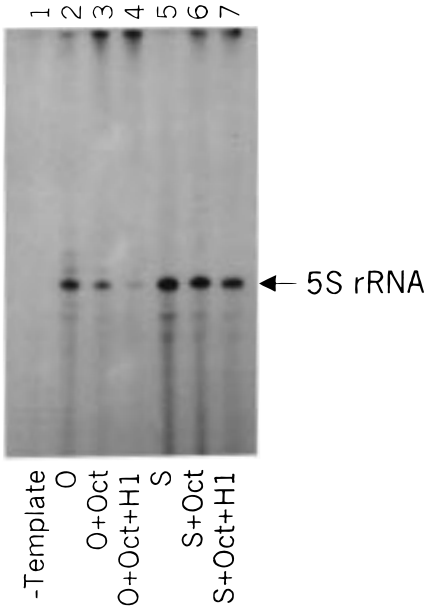

FIGURE 3: Histone H1 represses 5S rRNA transcription of oocyte but not somatic genes reconstituted with histone octamers. Approximately 40 ng of the oocyte (lanes 2−4) or somatic (lanes 5−7) 5S rRNA genes, either uncomplexed (lanes 2 and 5), reconstituted with chicken erythrocyte core histones (lanes 3 and 6), or reconstituted with chicken erythrocyte core histones and HeLa cell histone H1 (lanes 4 and 7), was transcribed in *Xenopus* oocyte extracts. Transcripts were analyzed by denaturing polyacrylamide gel electrophoresis (8% acrylamide and 8.3 M urea in 1× TBE): lane 1, transcription in the absence of template.

nuclease, blotted, and probed for the 5S rRNA coding sequence. The results (Figure 4B) demonstrate that the somatic 5S rRNA coding sequence was packaged with both histone octamers and histone H1 as indicated by hybridization of the 5S rRNA coding sequence with the ~165 bp micrococcal nuclease resistant fragment. This, together with the transcription studies (Figure 3), indicates that histone H1 binding does not inhibit transcription initiation of nucleosomal *X. laevis* somatic 5S rRNA templates.

The lack of effect of both histone octamers and histone H1 on somatic 5S rRNA transcription has been demonstrated by others (*27*). However, for the first time, this study has been able to show that histone H1 is actually bound to the somatic genes without repressing transcription. The characterization of histone H1 as a "general repressor of transcription" originated from the results of several in vitro transcription studies (*2, 28*−*30*). This was further supported by studies which showed that histone H1 inhibits transcription factor binding to nucleosomal DNA (*28, 31*). Recent lines of evidence, however, including this study, have shown that histone H1 can bind nucleosomes without affecting the assembly of the transcription machinery. For example, it has been demonstrated that the RNA polymerase I basal transcription factor, UBF, is able to displace histone H1 when binding nucleosomal DNA (*32*). Furthermore, the RNA polymerase II *trans*-acting factor, NF-1, has been shown to bind linker DNA regardless of the presence of histone H1 (*33*). These results suggest that although histone H1 can repress transcription in some instances, it is a selective repressor of transcription initiation.

The results of this study also shed new light on the basis for the differential transcription of the oocyte and somatic

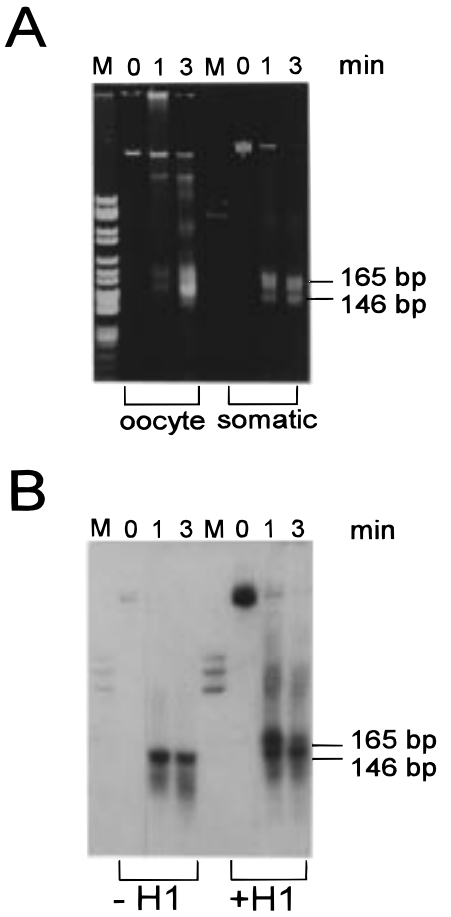

FIGURE 4: Histone H1 protects an additional ~20 bp's of DNA from micrococcal nuclease, and the somatic 5S rRNA coding sequence is in contact with both the histone octamer and the histone H1. A, Micrococcal nuclease digestion into chromatosomes of full-length *Xenopus laevis* oocyte and somatic genes. Digestions were carried out at a nucleosome concentration of 0.1 mg/mL (DNA weight) and an enzyme concentration of 10 u/mL for the minutes indicated on the top of each lane. The resulting DNA fragments were deproteinized and electrophoresed on a 4% nondenaturing gel: M, *Hha*I cut pB*R*322. B, southern blot hybridization of a similar gel as that shown in A containing micrococcal nuclease digested somatic gene repeats reconstituted without (−H1) and with (+H1) histone H1, probed with the [α-^{32}P]dATP labeled 5S rRNA coding sequence.

5S rRNA genes in somatic cells. Previous explanations relied on the concept that histone H1 prevented the formation of an RNA polymerase transcription complex. Only those genes which could form active transcription complexes, prior to the addition of histone H1, would be programmed for transcription (*4*, *5*). In this study, it was shown that histone H1 could directly interact with nucleosomes on the somatic 5S rRNA coding sequence without preventing transcription. However, this does not seem to be the case for the oocyte gene, as seen in this study and the results of others (*27*). Thus, these results support a model in which the differential regulation of the 5S rRNA genes is not due to a competition of histone H1 with the transcription machinery for DNA sites, but rather to a difference in the interaction of histone H1 with the somatic and oocyte genes. This is not entirely surprising since studies show that transcriptionally active genes are not necessarily devoid of histone H1 (*34*). It has been suggested that it is not whether histone H1 binds which determines transcriptional repression but rather how histone H1 binds (*35*).

Finally, it could be argued that we have used a heterologous histone H1 of mammalian origin for our analysis. However, we have used a somatic human H1 mixture which includes the histone H1 A variant. Somatic histone H1 A from *Xenopus* has been shown to be an in vivo repressor of oocyte 5S rRNA gene transcription (*36*). Computer sequence analysis reveals that human histone H1 A and histone H1 A from *Xenopus* exhibit a 62% sequence similarity which represents a very high score for histones of the H1 family. In addition, our results suggest that it is the DNA sequence and/or its particular interaction with the histone octamer which confers the specificity of the differential repressive H1 binding observed in the case of the 5S rRNA gene.

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