Molecular and Cellular Biology, Feb. 1991, p. 641–654
0270-7306/91/020641-14$02.00/0

Vol. 11, No. 2

A Variant Octamer Motif in a *Xenopus* H2B Histone Gene Promoter Is Not Required for Transcription in Frog Oocytes

CRAIG HINKLEY and MICHAEL PERRY*

Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030

Received 1 August 1990/Accepted 1 November 1990

Xenopus **oocytes, arrested in G_2 before the first meiotic division, accumulate histone mRNA and protein in the absence of chromosomal DNA replication and therefore represent an attractive biological system in which to examine histone gene expression uncoupled from the cell cycle. Previous studies have shown that sequences necessary for maximal levels of transcription in oocytes are present within 200 bp at the 5′ end of the transcription initiation site for genes encoding each of the five major *Xenopus* histone classes. We have defined by site-directed mutagenesis individual regulatory sequences and characterized DNA-binding proteins required for histone H2B gene transcription in injected oocytes. The *Xenopus* H2B gene has a relatively simple promoter containing several transcriptional regulatory elements, including TFIID, CBP, and ATF/CREB binding sites, required for maximal transcription. A sequence (CTTTACAT) in the H2B promoter resembling the conserved octamer motif (ATTTGCAT), the target for cell-cycle regulation of a human H2B gene, is not required for transcription in oocytes. Nonetheless, substitution of a consensus octamer motif for the variant octamer element activates H2B transcription. Oocyte factors, presumably including the ubiquitous Oct-1 factor, specifically bind to the consensus octamer motif but not to the variant sequence. Our results demonstrate that a transcriptional regulatory element involved in lymphoid-specific expression of immunoglobulin genes and in S-phase-specific activation of mammalian H2B histone genes can activate transcription in nondividing amphibian oocytes.**

Histones are encoded by a multigene family whose expression has served as a paradigm for studies of the regulation of gene expression during the cell cycle and in early embryonic development. The fundamental role of histones in assembling DNA into nucleosomes requires that stoichiometric amounts of these structural proteins are available for the assembly of newly replicated DNA into chromatin in all proliferating cells. Toward this end, the expression of the five major histone gene classes in somatic cells is coupled to DNA replication and coordinately regulated at transcriptional and posttranscriptional levels during the cell cycle (for a review, see reference 62). Histone gene transcription rates and mRNA half-lives increase by 3- to 5-fold each at the onset of S phase, resulting in an approximately 25-fold increase in the amount of histone mRNA in the mid-S phase (4, 30). It is anticipated that a shared mechanism underlies the coordinate transcriptional regulation of histone genes; however, the molecular details of this process are unknown. Unlike cell cycle-regulated histone genes, histone variants encoded by separate genes are expressed in some species in a cell cycle-independent and cell-type-specific manner. These include testis-specific (6, 36), erythrocyte-specific (11, 63), and embryo-stage-specific (15, 39, 49) histone genes.

Analyses of histone gene transcription in injected embryos (15, 39), oocytes (9, 22–24, 29, 73), transfected cell cultures (18, 38, 72), and in vitro transcription systems (18, 26, 65, 69) have determined that sequences involved in the transcriptional regulation of individual histone genes are generally confined to promoter regions composed of multiple distinct sequence motifs. These motifs are frequently specific to a particular histone gene class (27, 54, 71). Histone-class-specific sequence elements are targets for ontogenic and cell cycle regulation of H1 (12, 39) and H2B (2, 33, 38) histone genes in several species. Proteins interacting with regulatory elements in the promoters of H1 (12, 18, 70), H2A (2, 53), H2B (1, 2, 17), H3 (15, 64), and H4 (10, 26, 69) histone genes have been described. In particular, an octanucleotide core that is part of a larger conserved H2B-specific promoter element has been implicated as the target for cell cycle regulation of a human H2B gene (38). The octamer motif is a regulatory element in the promoters of other ubiquitously expressed genes (3, 7, 46, 57), a lymphoid cell-specific regulatory element in immunoglobulin promoter and enhancer regions (16, 19, 43, 45, 72), and a DNA replication element in the adenovirus origin of replication (56). Several proteins that bind to octamer motifs have been purified, and the corresponding genes have been cloned (for a review, see reference 31). One protein, Oct-1 (NF-A1, OTF-1, NF-III), is ubiquitous (66, 68), whereas another protein, Oct-2 (NF-A2, OTF-2), is restricted to lymphoid cells (40, 66). Recent studies have detected a family of octamer binding proteins, in addition to Oct-1 and Oct-2, in adult mouse tissues and at different stages of embryonic development (51, 60, 61). The role of Oct-1 or other octamer-binding proteins in the cell cycle regulation of H2B gene transcription is unclear.

Xenopus histone genes are organized into two predominant types of tandemly repeating clusters that are distinguished by their internal organization and by the presence of genes encoding specific H1 subtypes (54, 75). Genes from these clusters are expressed in oocytes, embryos, and cultured cells (55). The synthesis and accumulation of large pools of histone mRNA and protein during oogenesis occur in the absence of chromosomal DNA replication and therefore appear to represent a mode of expression distinct from that occurring in the S phase of the cell cycle and presumably during early embryogenesis. Analysis of the expression

* Corresponding author.

of cloned *Xenopus* histone genes in cultured amphibian cells, transfected mouse cells, and frog oocytes has demonstrated that their transcription is replication dependent in somatic cells and replication independent in oocytes (52). These studies suggest that histone gene transcription undergoes a transition from constitutive expression during oogenesis to cell cycle-regulated expression at some point in embryogenesis. Because *Xenopus* oocytes are an abundant and accessible population of cells naturally arrested at a specific point in the cell cycle (namely, G_2 of the first meiotic division), we used them to examine the mechanisms by which histone gene transcription is regulated in nondividing cells.

We describe here our analysis of the transcriptional regulatory sequences required for expression of an H2B histone gene in frog oocytes. Multiple sequence motifs, including TATA, CCAAT, and ATF binding sites, were required for maximal transcription. Sequence-specific interactions were documented for oocyte-derived proteins and the ATF element. Although an octamerlike element in the H2B promoter was not functional in oocytes, substitution of this promoter element with a consensus octamer motif stimulated transcription of the H2B promoter. In accord with these results, frog oocytes were found to contain proteins capable of interacting with the consensus octamer motif but not with the variant octamer sequence. We discuss the implications of these observations with respect to cell cycle regulation of histone gene transcription.

MATERIALS AND METHODS

Plasmids. The histone genes used in these studies are derived from the *xlh3* histone gene cluster (54, 75). The wild-type H2B template, pH2B(−320), contains 320 bp of 5′-flanking sequence upstream of the start site of transcription and an in-frame synthetic *Eco*RI restriction site at position +365 that allows transcripts from injected templates to be distinguished from endogenous H2B mRNA (29). Linker-scanning mutant templates were generated by oligonucleotide site-directed mutagenesis with a single-strand M13/H2B (−320) template by published methods (59, 76). Mutagenic oligonucleotides were designed to introduce an *Asp*718 (*Kpn*I) recognition site at a different position for each mutant template. After characterization by restriction enzyme digestion and DNA sequence analysis, the 900-bp *Sal*I-*Sst*I fragment containing the H2B promoter and coding region was subcloned into pUC19 to create the pLS (pUC19 plus LS) mutants (Fig. 1). The pLS templates are numbered to indicate the boundaries of the region containing altered nucleotides; i.e., pLS(−105/−98) contains base changes between nucleotides −105 and −98.

The pH2B(−87) 5′ deletion mutant was constructed by removing a 250-bp fragment, including both the ATF and AP5 sites, from pLS(−93/−87) by digestion with *Asp*718 and religation. Synthetic oligonucleotides were introduced into the *Asp*718 site of pH2B(−87). The oligonucleotides contain sequences corresponding to the ATF (5′ GTACATGACGTCATGTAC), AP5 (5′ GTACCATGCCACAGAGAGCCTA), ATF-AP5 (5′ GTACGCTTTGACGTCATGCCACAGAGCCAG), and H2B-OCTA (5′ GTACAGCCTGCTTTACATGG) sites in the *Xenopus* H2B promoter and Hu OCTA (5′ GTACCTTATTTGCATAAGC) corresponding to the conserved octamer motif in a human H2B histone gene promoter (65). The underlined sequences denote potential factor binding sites. The relationship of each oligonucleotide to the H2B promoter is shown in Fig. 1B.

The pH2B(−33) 5′ deletion mutant was generated by removing sequences upstream of pLS(−40/−33) by digestion with *Asp*718 and religation as described above. Oligonucleotides were cloned into the *Asp*718 site to generate the pH2B (−33) oligonucleotide templates.

The pH2Bdel(−60/−33) template contains an internal deletion of sequences from nucleotides −60 to −33 (including the variant octamer element). This template was prepared by inserting the 270-bp *Sst*I-*Asp*718 fragment from the upstream region of pLS(−60/−53) into pH2B(−33). Double-stranded oligonucleotides were inserted into the unique *Asp*718 site in pH2B(−60/−33).

pUC(−155/−20), containing sequences from nucleotides −155 to −20 of the H2B promoter, was prepared by insertion of a blunt-ended 140-bp *Eco*RI-*Pvu*II fragment from pH2B(−155) into the pUC19 polylinker.

Oocyte injections and analysis of RNA. Adult female *Xenopus laevis* frogs were obtained from *Xenopus* I (Ann Arbor, Mich.). Oocyte injection and RNA extraction were done as previously described (29), except that [α-^{32}P]GTP was omitted from the injections. Extracted RNA was suspended in 10 μl of TE (10 mM Tris [pH 7.5], 1 mM EDTA) per oocyte. Specific transcription of the H2B gene was analyzed by S1 nuclease protection (5). Radiolabeled probes were prepared by phosphorylation with T4 polynucleotide kinase and [γ-^{32}P]ATP by using dephosphorylated, *Eco*RI-digested (H2A and H2B) or *Bam*HI-digested (H1A) plasmids containing the appropriate histone gene. For S1 analysis, a reaction mixture containing 10 ng of probe, 80% formamide, 100 mM KCl, 10 mM piperazine-*N*-*N*′-bis(2-ethanesulfonic acid) (pH 6.8), 1 mM EDTA, and RNA equivalent to the amount in one oocyte was incubated overnight at 50°C. After hybridization, single-strand nucleic acids were hydrolyzed by digestion with 10 U of S1 nuclease for 30 min at 37°C in 100 μl (total volume) containing 10 μl of hybridization reaction, 10 μl of 10× S1 nuclease digestion buffer (4 M NaCl, 0.4 M sodium acetate [pH 4.5], 0.1 M $ZnCl_2$), and 79 μl of H_2O. After extraction twice with phenol-chloroform (1:1), once with chloroform, and precipitation with 3 volumes of 95% ethanol, the samples were suspended in 95% formamide and fractionated by electrophoresis on a 6% acrylamide–50% urea–0.5× TBE (44.5 mM Tris [pH 8.3], 44.5 mM boric acid, 1 mM EDTA) gel. Dried gels were subjected to autoradiography, and gene-specific transcripts were quantitated by using a Bio-Rad model 620 densitometer. H2B-specific transcription was normalized to transcription from a coinjected H1A or H2A gene. As a consequence of initial studies not described in this report, we found that the site of H2B transcription initiation occurs 5 bp downstream of the previously reported position (29). The nucleotide numbering has been revised to reflect these results.

Oocyte extract preparation. Total ovarian tissue was removed from adult female frogs and either used immediately or frozen in liquid nitrogen and stored at −80°C. Approximately 4 g of tissue was homogenized with a tissue processor (Tissumizer; Tekmar, Cincinnati, Ohio) in 8 ml of homogenization buffer (20 mM Tris [pH 7.3], 20% glycerol, 50 mM NaCl, 0.2 mM EDTA, 1 μg of leupeptin [Sigma] per ml, 1 μg of pepstatin [Sigma] per ml, 5 mM 1,4-dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 M urea [Ultrapure; U.S. Biochemical Corp.]). The homogenate was centrifuged for 10 min at 4,000 × *g* under silicon oil (Sigma). The supernatant was removed and centrifuged under oil for 30 min at 24,000 × *g*. The supernatant was used directly in gel mobility shift or DNase I footprint experiments. Protein concentrations, measured by the Bradford protein assay (Bio-Rad), were typically 5 to 10 mg/ml. Identical results were obtained

A

```
        -160      -150      -140      -130      -120      -110      -100       -90       -80
                                                                              ATF    AP5       CBP
        GTTAATGGACGTTGCCGGGAAGCTTTACTTACCACCAATCGTCTGGAGAAAAGCTGCGCTTTGACGTCATGCCACAGAGCCAGCCAATGG
-105/-98                                                                    AGG ACCT
-93/-87                                                                              G TAC T
-85/-80                                                                                      GTA CT
-79/-74                                                                                            GT CCT

        -70       -60       -50       -40       -30       -20       -10        1        10
                                  OCTAMER         TATA
        GAATCAGTGTCACGGCGCAGCCTGCTTTACATGGGCAGGGTATAAAAGCAGCTGCAGCCGGAGCAGCACTTCATCGTTTGCTTTATAGA
-70/-64 GGT CCT
-60/-53           A  TA CT
-50/-44                     A G ACCT
-40/-33                               A  T CCT
-32/-25                                         AGGT CCT
-21/-14                                                   AG T C T
-9/-3                                                               AG T  C
+3/+8                                                                         A G AC
```

B

```
-110      -100       -90       -80       -70       -60       -50       -40       -30
AAAGCTGCGCTTTGACGTCATGCCACAGAGCCAGCCAATGGGAATCAGTGTCACGGCGCAGCCTGCTTTACATGGGCAGGGTATAAAAGCA

   GTACGCTTTGACGTCATGCCACAGAGCCAGGTAC  ATF/AP5        GTACAGCCTGCTTTACATGGGTAC    H2B-OCTA
      GTACATGACGTCATGTAC               ATF               GTACCTTATTTGCATAAGCGTAC  Hu-OCTA
             GTACCATGCCACAGAGCCTAGTAC  AP5
```

FIG. 1. Nucleotide sequence of the *xlh3* H2B promoter and pLS templates. (A) Continuous sequence is that of the *Xenopus xlh3* histone H2B gene promoter from nucleotides −162 to +17. Nucleotides are numbered relative to the transcription initiation site (*). Conserved sequence elements and factor binding sites are underlined. Nucleotide substitutions for each of the pLS templates are shown. (B) The sequences of synthetic double-stranded oligonucleotides used in this study are shown relative to their corresponding sites in the *Xenopus* H2B promoter. The sequence of the upper strand only is shown. Underlined bases in each oligonucleotide are identical with the corresponding nucleotides in the H2B promoter.

by using extracts prepared from enzymatically defolliculated oocytes. Extracts made with increased concentrations of NaCl (0.2 to 1.0 M) contained large quantities of proteins (presumably stored yolk proteins) that precipitated on dialysis or upon dilution to lower ionic strengths. We found that the addition of 2 M urea resulted in increased solubilization of proteins binding to the ATF site and the conserved octamer motif. Binding activity was not increased by the addition of urea to oocyte extracts previously prepared without urea.

Gel mobility shift assays. Gel mobility shift assays were performed on ice in 20- to 40-μl reaction volumes. Oocyte extract (10 μl) was preincubated for 5 min with 1 μg of poly (dI-dC) and 0.5 μg of *Alu*I-digested pUC19. Volumes were adjusted by the addition of 20 mM Tris (pH 7.3)–20% glycerol–50 mM NaCl–5 mM dithiothreitol–5 mM $MgCl_2$–1 mM $CaCl_2$. The presence of 0.25 to 1.0 M urea in the binding reactions had no discernible effect on the binding of oocyte factors to the probes used in this study. Binding reactions were initiated by the addition of 10^4 cpm of 3′ end-labeled oligonucleotides, and the mixtures were then incubated for an additional 15 min on ice. The reactions were subjected to electrophoresis on a 6% acrylamide–0.5× TBE gel, which was then dried and subjected to autoradiography.

DNase I footprinting. The probe used for DNase I protection studies was a 140-bp *Sst*I-*Bam*HI fragment from pUC (−155/−20), which contains sequences from nucleotides −155 to −20 of the H2B promoter. The 140-bp fragment was purified by electrophoresis on a 10% acrylamide–0.5× TBE gel and recovered from gel slices by elution overnight at 37°C in TE. Footprint binding reactions were the same as those for gel mobility shifts, except that 5×10^4 cpm of probe was used and the amount of nonspecific competitor was reduced to 200 ng of poly(dI-dC). Upon completion of the binding reaction, DNase I was added and samples were transferred to a room-temperature water bath for 90 s. Nuclease digestions were terminated by the addition of 60 to 80 μl of stop mix (0.4 M sodium acetate, 20 mM EDTA, 1% sodium dodecyl sulfate, 10 μg of yeast RNA per ml). DNA was extracted twice with phenol-chloroform (1:1) and once with chloroform and was recovered by precipitation with 3 volumes of 95% ethanol. DNA was suspended in 95% formamide and heated for 10 min at 65°C before electrophoresis on a 6% acrylamide–50% urea–0.5× TBE gel, which was then dried and subjected to autoradiography.

RESULTS

Multiple positive-acting regulatory sequences are required for H2B transcription in oocytes. Analysis of the expression

of injected histone genes in oocytes has shown that sequences upstream of nucleotide −105 are not required for maximal transcription of the *xlh3* H2B gene and that a transcriptional regulatory element exists between nucleotides −105 and −75 (29). Inspection of the nucleotide sequence downstream of nucleotide −105 revealed several potential binding sites for known transcription factors, including TFIID (TATA box factor), Oct-1, CBP, AP5/TEF-I, and ATF/CREB (Fig. 1A). To investigate which, if any, of these sequences were required for H2B transcription in oocytes, we used oligonucleotide-directed mutagenesis to construct a set of linker-scanning mutants (Fig. 1A) spanning the region of the H2B promoter required for maximal levels of transcription in oocytes (nucleotides −105 to +10). Mutations were designed to maximize nucleotide changes in the potential factor binding sites mentioned above.

To assess the effects of promoter-specific mutations, supercoiled templates were injected into oocytes, and the accumulation of accurately initiated transcripts was determined by S1 nuclease protection. An equal amount of a separate histone gene template (either H1A or H2A) was coinjected with each template tested to minimize variations between oocytes and sets of injections. Representative results for these injections are shown in Fig. 2. No mutant template was transcribed at a significantly higher rate than the wild-type promoter, pH2B(−320), suggesting that negative regulatory elements, or repressors, are not involved in H2B transcription in oocytes. Several of the mutant templates were transcribed much less efficiently than the wild-type H2B gene; the greatest decreases in transcription occurred when templates containing mutations in the TATA and CCAAT elements were used. Mutagenesis of either of these regulatory sequences resulted in a 5- to 10-fold decrease in the amount of accurately initiated H2B mRNA. In particular, two templates with altered CCAAT box motifs, pLS(−79/−74) and pLS(−85/−80), were transcribed at approximately 20% of the efficiency of the wild-type H2B gene. The result for pLS(−79/−74) was not surprising, since this mutation substantially altered the CCAAT sequence motif. The pLS(−85/−80) mutation does not alter the pentanucleotide core sequence of the CCAAT regulatory element; however, it does substitute pyrimidines for two purines immediately preceding the CCAAT consensus sequence. Chodosh et al. (8) found that high-affinity binding sites for two CCAAT box binding proteins, CP1 and CP2, contain two purine residues preceding the initial cytosine residue. These observations suggest that the decreased transcriptional efficiency of pLS(−85/−80) results from reduced interactions between a maternal CP1- or CP2-like factor and the CCAAT element.

Mutagenesis of the TATA box resulted in displacement of the transcription initiation site to a position about 15 nucleotides upstream of the normal site in addition to significantly reducing the amount of H2B RNA synthesis (Fig. 2, lane 9). Similar effects have been found in previous studies that have examined the function of the TATA box in the transcription of several other genes by using in vivo and in vitro approaches. These results are consistent with the general conclusion that the TATA element is required for maximal levels of transcription and is involved in fixing the site of transcription initiation for RNA polymerase II (48).

Less-pronounced effects were observed for mutations that alter the distal end of the H2B promoter from nucleotides −105 to −87. That region contains sequence motifs apparently homologous to binding sites for two previously characterized transcription factors. A palindromic sequence

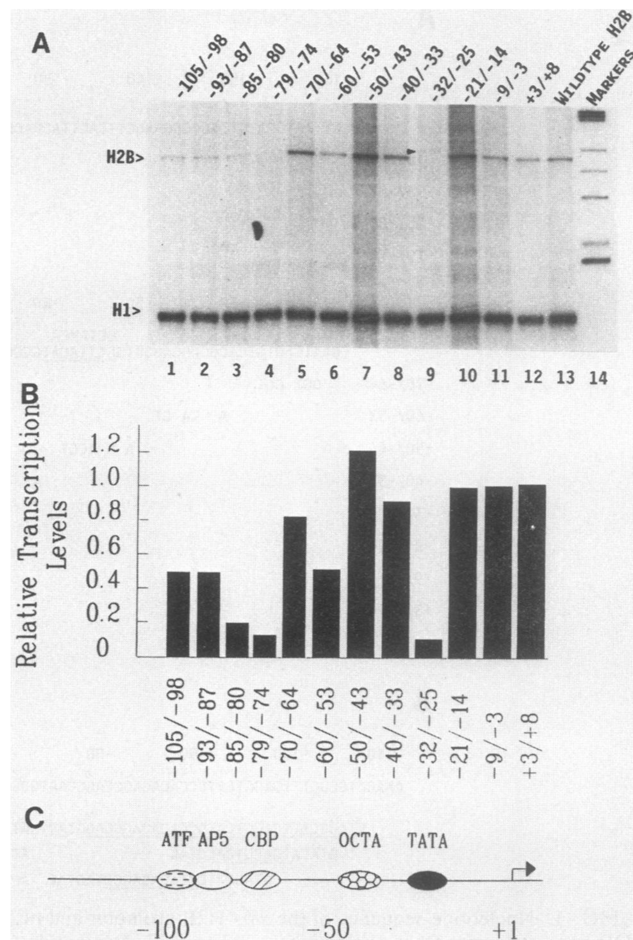

FIG. 2. Multiple promoter elements are required for *Xenopus* H2B transcription in oocytes. (A) S1 nuclease protection assay with RNA from oocytes coinjected with a wild-type H1 template and either the wild-type H2B gene (lane 13) or the pLS mutant templates (lanes 1 through 12). Arrows on the left indicate protected fragments that correspond to accurate transcription initiation of the H1 and H2B templates. A reproducibly observed protected fragment resulting from transcription initiation at a site approximately 15 bp upstream of the normal start site is denoted with an arrowhead (lane 9). (B) Relative transcription levels of the pLS templates. Transcription of the injected pLS templates was normalized to the H1 internal control and expressed relative to transcription of the wild-type H2B gene. Each bar represents the average obtained from three separate injections of each template. (C) H2B promoter region from approximately nucleotides −320 to +20. Promoter elements are shown, and the transcription start site is indicated by the arrow at +1.

(TGACGTCA) at the distal end of the 20-bp region extends from nucleotides −101 to −94 and represents a perfect match to the ATF/CREB transcription factor consensus binding site, a common *cis*-acting sequence present in promoters regulated by a variety of different agents (42, 47). A family of related proteins encoded by distinct genes interacts with ATF sites in mammalian cells (25). The pLS(−105/−98) template alters the 5′ half-site of this sequence and here resulted in a two- to fourfold decrease in the amount of accurately initiated H2B mRNA compared with that initiated by the wild-type template. Although this region is not highly conserved among vertebrate H2B genes, it is noteworthy that the corresponding region of a human cell cycle-regu-

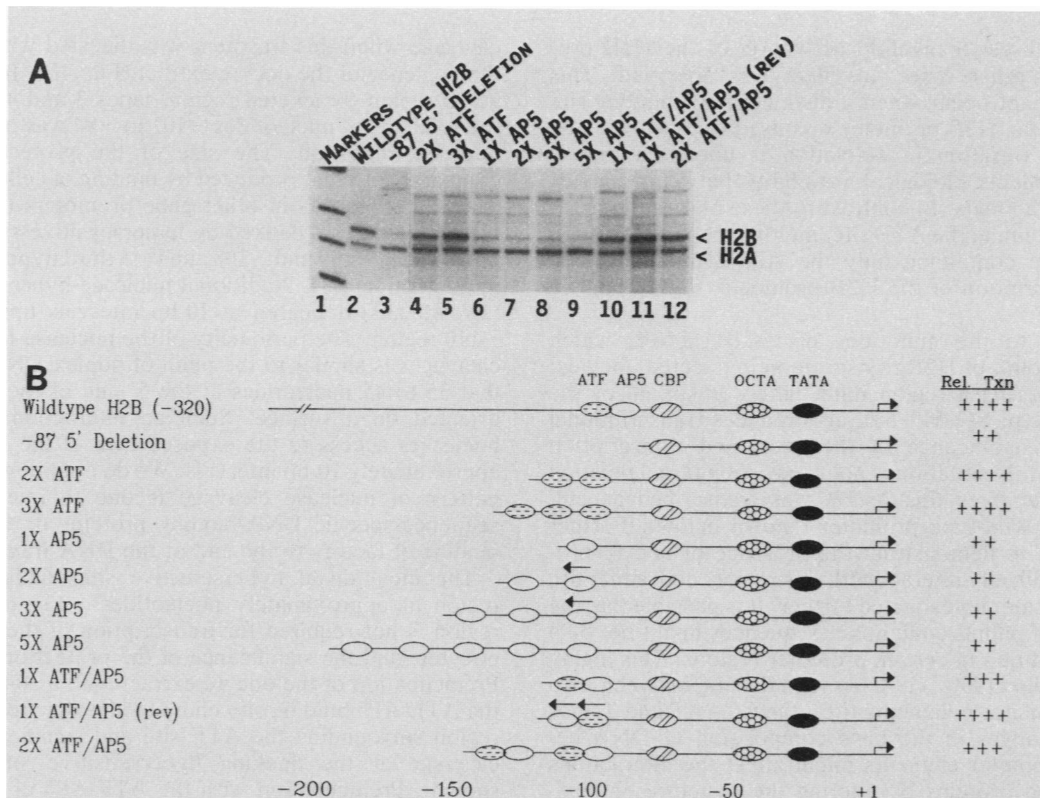

FIG. 3. ATF motif is required for maximal transcription of the H2B promoter in oocytes. (A) Results of an S1 nuclease protection assay with RNA from oocytes injected with wild-type H2B (lane 2), the nucleotide −87 5′ deletion mutant pH2B(−87) (lane 3), or the pH2B(−87) template containing ATF and/or AP5 oligonucleotides (lanes 4 through 12). H2B templates were coinjected with a *Xenopus* H2A template as an internal control. Fragments protected from S1 nuclease digestion due to accurate transcription initiation of the H2A and H2B templates are indicated. Additional bands are due to protection of the wild-type probe by readthrough transcripts from mutant templates. (B) Promoter region of the injected H2B templates. Arrows above oligonucleotide motifs show their orientation relative to the wild-type promoter. The amount of transcription of each template was determined by densitometric scanning, normalized to that of the coinjected H2A gene, and expressed relative to the wild-type H2B gene. The level of transcription is indicated to the right of each template. Each plus sign corresponds to 25% of wild-type transcription.

lated H2B gene contains a series of direct repeats that include two ATF half-sites. Mutagenesis of this region of the human H2B gene reduced transcription by about 50% in transfected cells (38) and in nuclear extracts (65). The sequence CATGCCACA at the proximal end of the 20-bp region of the *Xenopus* H2B promoter is partially complementary (8 matches out of 9 bp) to the GT-IIC motif, or core element ($\mathrm{TGTGG}^{\mathrm{AAA}}_{\mathrm{TTT}}\mathrm{G}$), of the simian virus 40 enhancer. The GT-IIC motif is required for enhancer activity in HeLa cells and in nondifferentiated and differentiated F9 embryonal carcinoma cells (50, 74) and interacts with several nuclear proteins, including TEF-I and AP5 (13, 35). For simplicity, we refer to this motif in the H2B promoter as the AP5 site. The pLS(−93/−87) template contains nucleotide substitutions in this sequence and was transcribed about two- to threefold less efficiently than the wild-type H2B promoter.

Since the distal promoter element spans a potentially complex region of the H2B promoter, we undertook to define the functional sequences precisely. Given the intimate juxtaposition of the ATF and AP5 motifs in the H2B promoter, it was of interest to determine whether both sequences were required for transcriptional activity or whether either one alone was sufficient. Therefore, oligonucleotides containing the ATF and AP5 sequence motifs were separately introduced into a 5′ deletion mutant lacking this region to test whether either motif restored transcription to wild-type levels. In addition, an oligonucleotide containing both sequence motifs was inserted at this position (Fig. 1B). The results of microinjection of these templates show that the ATF motif is a functional regulatory element in the −100 region and that the AP5 element does not participate in the activation of the H2B promoter (Fig. 3). The template lacking the ATF and AP5 binding sites, pH2B(−87), was transcribed at a reduced efficiency relative to the wild-type template (compare lanes 2 and 3). The introduction of multiple copies of the ATF motif activated transcription of the mutant H2B promoter (Fig. 3, lanes 4 and 5). Activation also occurred when the promoter contained one or two copies of the oligonucleotide consisting of both elements (Fig. 3, lanes 10 through 12). Maximal transcription was seen with a template containing one copy of the ATF-AP5 element in the opposite orientation from that found in the wild-type H2B promoter. The ATF site is 10 bp further upstream in the template with the ATF-AP5 oligonucleotide in the forward orientation compared with its location when the ATF-AP5 oligonucleotide is in the reverse orientation, suggesting that the activity of the ATF site in oocytes is sensitive to its spacing relative to another H2B promoter element, possibly the CCAAT or TATA motif.

The extent of activation conferred by the ATF site varied occasionally. In some cases, the presence of an ATF site

resulted in a three- to fivefold activation of the H2B promoter, but in other cases no effect was observed. This variability did not occur when a distinct factor binding site that activated the H2B promoter was used (see below). The origin of the variation in activation is uncertain, but it presumably reflects biological variability between oocytes from different animals. In contrast to these observations for templates containing the ATF site, multiple copies of a 20-bp oligonucleotide containing only the AP5 element did not activate transcription of the H2B promoter (Fig. 3, lanes 6 through 9).

In addition to the mutations discussed above, which reduce the amount of H2B transcription in injected oocytes, a mutation altering a region immediately upstream of the octamer motif, pLS(−60/−53), also reduces transcriptional activity. The significance of the decreased transcription resulting from this mutation is not clear. An internal deletion mutant that removes this region was transcribed as efficiently as the wild-type promoter (shown below). Further analysis failed to demonstrate the presence of oocyte proteins that specifically interact with this region; consequently, this region was not investigated further. It is possible that the introduction of palindromic linker sequences might not be a neutral substitution in certain promoter regions. This highly G+C-rich region (90% G+C) is equidistant between two functional regulatory elements (i.e., the CCAAT and TATA elements). Changes in the base composition of DNA between two promoter elements might affect the interactions between bound proteins by altering the structure or flexibility of the connecting DNA.

Proteins in oocyte extracts bind to the ATF site in the H2B promoter. To investigate whether oocytes contain factors that interact specifically with the −100 element, oocyte extracts were tested in a gel mobility shift assay with labeled oligonucleotides containing ATF, AP5, or combined ATF-AP5 binding site probes. The DNA-protein complexes assumed a simple pattern when these binding sites were incubated with increasing amounts of oocyte extract (Fig. 4A). A single complex (arrowhead) appeared when the ATF site was used as a probe (Fig. 4A, lanes 1 through 3). A complex of similar mobility and a more rapidly migrating nonspecific complex appeared when the ATF-AP5 probe was used (lanes 4 through 6). Only the nonspecific complex was seen with the AP5 site probe (lanes 7 through 9). The specificity of the observed complexes was shown by competition with unlabeled oligonucleotides. The amount of the major complex formed with the ATF probe was reduced with increasing amounts of the homologous binding site (lanes 10 through 14), whereas increasing amounts of an oligonucleotide containing the AP5 site showed no effect on this complex (lanes 15 through 19). The formation of the more slowly migrating complex observed with the ATF-AP5 probe was specifically inhibited by the ATF site (lanes 20 through 24) but not by the AP5 site (lanes 25 through 29). Formation of the rapidly migrating nonspecific complex was not inhibited by either competitor; in addition, the predominant complex formed with the AP5 probe was not specific, as evidenced by a lack of competition with excess AP5 oligonucleotide (data not shown). We conclude that the ATF consensus sequence is a binding site for an oocyte factor and that similar complexes involving specific protein-DNA interactions are formed with the ATF and ATF-AP5 binding sites.

We performed a nuclease protection analysis with a fragment containing sequences from nucleotides −155 to −22 of the *Xenopus* H2B promoter. Several regions had altered cleavage when this fragment was digested with DNase I in the presence of the oocyte extract (Fig. 4B). In particular, a 21-bp region (bracketed region, lanes 3 and 4) centered on the ATF site at nucleotides −101 to −94 was protected from nuclease digestion. The size of the protected region is comparable to that produced by binding of cellular factors to ATF/CREB motifs in other gene promoters (44). The protected region was flanked by hypersensitive sites located at nucleotides −109 and −108 and by a third hypersensitive site at nucleotide −84. Additional nuclease-hypersensitive sites (arrowheads) appeared at 10-bp intervals upstream of the −110 region. The periodicity of the nuclease-hypersensitive cleavages is similar to the pitch of duplex DNA, suggesting that 35 to 45 nucleotides at the 5′ end of the fragment are oriented on a surface. Such an interaction would allow nucleases access to the exposed face of the DNA helix at approximately 10-bp intervals. We do not know whether this pattern of nuclease cleavage is due to interactions with sequence-specific DNA-binding proteins or to nonspecific binding of factors to the end of the DNA fragment.

The most distal hypersensitive sites flank a protected region at approximately nucleotides −137 to −132. This region is not required for transcription of the H2B gene in oocytes, and the significance of the protection is uncertain. Preincubation of the oocyte extract with a 30-fold excess of the ATF-AP5 binding site eliminated protection of the 21-bp region surrounding the ATF site and significantly reduced cleavage at the flanking hypersensitive sites (data not shown). Preincubation with the ATF-AP5 or AP5 oligonucleotides did not affect protection of the −137 and −132 region or cleavage at the distal hypersensitive sites, however, suggesting that these alterations in the cleavage pattern are independent of interactions at the ATF binding site. In conjunction with results from the functional studies described above, these results show that a factor present in oocytes interacts specifically with the ATF site in the H2B distal promoter element and suggest that sequences flanking the ATF site are important for transcriptional activation.

Variant octamer motif is not required for transcription in oocytes. The conserved octamer motif is found in histone H2B genes from a variety of organisms and is necessary for S phase-specific transcription of human and rat H2B genes (33, 38). The nucleotide sequence of the *xlh3* H2B promoter contains an octamerlike sequence (CTTTACAT) that differs by 2 bp from the consensus octamer element (ATTTGCAT) whose location, about 15 bp upstream of the TATA box, is highly conserved among vertebrate H2B gene promoters (27, 71). It was intriguing, therefore, that the linker-scanning mutant that abolished the H2B octamer motif was transcribed as efficiently as the wild-type H2B gene (Fig. 2, lane 7), suggesting that this octamer motif has either very weak or no transcriptional activity in oocytes. Since the activity of weak transcriptional regulatory elements can sometimes be enhanced when the element is present in multiple copies, we sought to determine whether multiple copies of the *xlh3* H2B octamer sequence would activate H2B transcription. For this purpose, we constructed a minimal H2B promoter in which the promoter elements upstream of the TATA box were removed. Single and multiple copies of the variant H2B octamer binding site were inserted into a site adjoining the TATA element and tested for activity in injected oocytes. The template containing the TATA box alone, pH2B(−33), was transcribed at a 10-fold lower level than the wild-type template (Fig. 5, lanes 1 and 2). When single or multiple copies of oligonucleotides containing the variant octamer motif were inserted next to the TATA box, the amount of

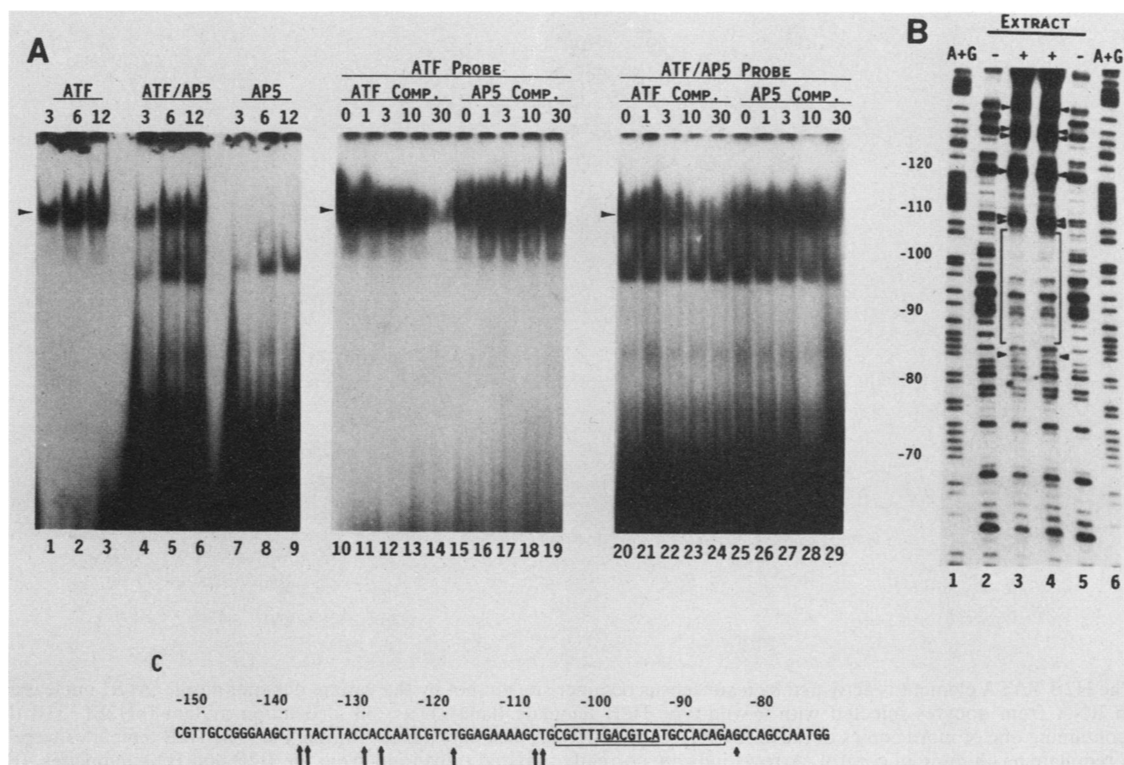

FIG. 4. ATF element is protected from nuclease digestion by oocyte proteins. (A) Double-strand oligonucleotide probes containing ATF (lanes 1 through 3), ATF-AP5 (lanes 4 through 6), or AP5 (lanes 7 through 9) binding sites used in gel mobility shift assays with either 3 μl (lanes 1, 4, and 7), 6 μl (lanes 2, 5, and 8), or 12 μl (lanes 3, 6, and 9) of oocyte extract. Specific complexes are indicated with arrows. The oocyte extract (12 μl) was incubated with 0.5 ng of the ATF oligonucleotide probe and increasing amounts of either the ATF oligonucleotide (lanes 10 through 14) or the AP5 oligonucleotide (lanes 15 through 19) competitors: no competitor (lanes 10 and 15), equimolar competitor (lanes 11 and 16); 3-fold molar excess (lanes 12 and 17), 10-fold molar excess (lanes 13 and 18), and 30-fold molar excess (lanes 14 and 19). Competitions were performed as above with the oocyte extract and the ATF-AP5 probe with increasing amounts of the ATF oligonucleotide (lanes 20 through 24) or the AP5 oligonucleotide (lanes 25 through 29) competitor: no competitor (lanes 20 and 25), equimolar competitor (lanes 21 and 26), 3-fold excess (lanes 22 and 27), 10-fold excess (lanes 23 and 28), and 30-fold molar excess (lanes 24 and 29). (B) A 140-bp 3′ end-labeled DNA fragment containing H2B promoter sequences from nucleotides −155 to −22 was used in a DNase I protection assay with oocyte extract. The promoter region is numbered relative to the transcription start site. The protected region surrounding the ATF motif is indicated by brackets, and hypersensitive sites are indicated by arrowheads. (C) Regions of altered nuclease cleavage in the *Xenopus* H2B promoter are depicted as described above. The region protected from nuclease digestion by oocyte proteins is boxed. The ATF consensus site is underlined.

transcription did not increase (lanes 3 through 5). This result agrees with the previous data and shows that the variant octamer motif does not activate the H2B promoter in injected oocytes.

The octamer motif is a functional enhancer element required for maximal transcription of small nuclear RNA genes in *Xenopus* oocytes (3, 46, 57). Since these results suggest the presence of a transcriptionally active octamer-binding factor in oocytes, we examined whether a consensus octamer element was able to activate transcription of the H2B promoter. Oligonucleotides containing a consensus octamer site were inserted in one and two copies immediately upstream of the TATA element. When a template containing a TATA box and one copy of the consensus octamer element was injected into oocytes, a stimulation of transcription was observed relative to transcription of the template containing the H2B TATA box alone (Fig. 5, lanes 6 and 7). The insertion of two consensus elements resulted in further stimulation of the basal promoter in a manner that suggested an additive, rather than synergistic or cooperative, activation. These results show that the consensus octamer element is sufficient to activate transcription from the H2B minimal promoter containing only a TATA box. We next sought to determine whether this effect was simply due to the element's close proximity to the H2B TATA box or whether it was capable of activating transcription from further upstream. We therefore introduced oligonucleotides containing each octamer motif approximately 55 bp further upstream at the 5′ end of the nucleotide −87 H2B deletion mutant. Analysis of the expression of these templates is shown in Fig. 6. The presence of one copy of the consensus octamer sequence and up to three extra copies of the variant octamer motif did not activate H2B transcription (Fig. 6, lanes 4 through 6); however, the introduction of two consensus octamer elements restored wild-type levels of transcription (lane 7). These results agree with the data from the previous experiment and show that the consensus octamer element is capable of activating the transcription of an H2B promoter in frog oocytes.

We next examined the function of the octamer element in its normal context in the H2B promoter. A template that had a 27-bp internal deletion that removed sequences from

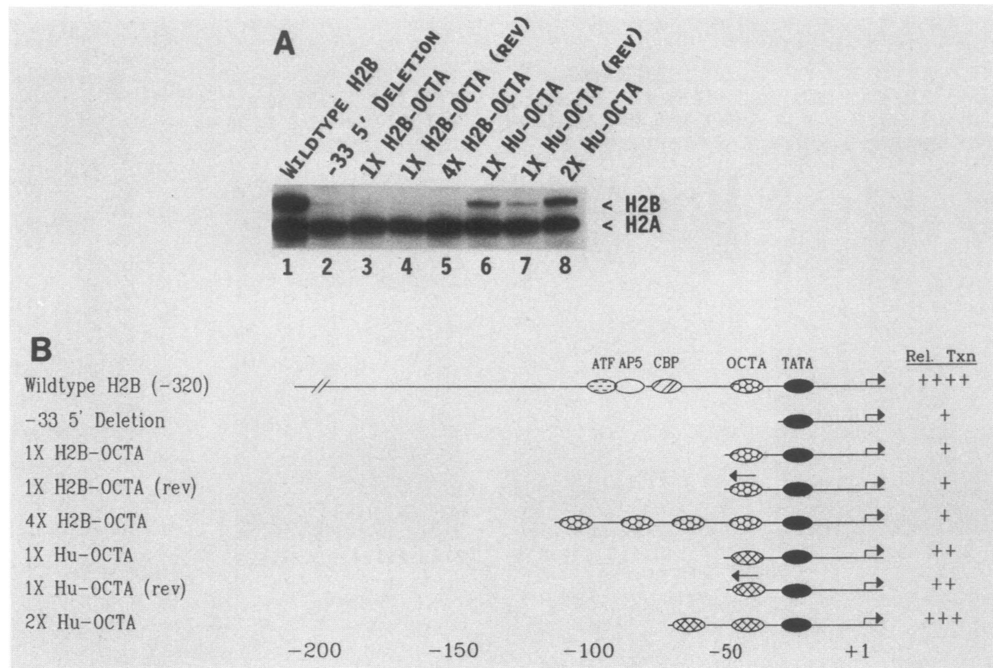

FIG. 5. The H2B TATA element is activated by a consensus octamer site but not by the variant octamer motif. (A) S1 nuclease protection analysis with RNA from oocytes injected with a wild-type H2B template (lane 1), a −33 5′ deletion mutant [pH2B(−33)] (lane 2), or pH2B(−33) containing one or more copies of the H2B-OCTA or Hu-OCTA elements (lanes 3 through 8). The H2B templates were coinjected with an H2A template as an internal control. Arrows indicate correctly initiated transcripts from the H2B and H2A templates. (B) Diagram of the templates used for injection. Ovals represent oligonucleotides present in each template. Arrows above oligonucleotide motifs show their orientation relative to the wild-type promoter. Plus signs to the right of each template indicate their transcription levels normalized to those of the H2A template and expressed relative to those of the wild-type H2B gene.

nucleotides −60 to −33, including the variant octamer motif, was engineered. Oligonucleotides containing each octamer motif were inserted into a unique restriction site at the junction of nucleotides −60 and −33, and the resulting templates were assayed by oocyte injection. The template with the internal deletion was transcribed at a level equivalent to that of the wild-type template, demonstrating that sequences between nucleotides −33 and −60 (including the octamer motif) are not required for maximal H2B transcription in oocytes (Fig. 7, lane 2). Similarly, transcription was unaltered for templates containing a single copy of either octamer motif (lanes 3, 4, 7, and 8).

Surprisingly, templates containing three copies of the variant octamer motif were transcribed at a level that was significantly lower than that of the wild-type promoter (Fig. 7, lanes 5 and 6). It is unlikely that the decreased transcription of these templates is due to a repressor that interacts with the variant motif. As shown above, mutagenesis of this sequence did not activate transcription of the H2B promoter as expected for a transcriptional silencer or negative regulatory element. Further studies (discussed below) have failed to detect proteins that specifically interact with this region. Because the oligonucleotide containing the variant octamer sequence is 20 bp long, corresponding to two complete turns of the helix, it appears unlikely that the inactivation is due to an altered stereospecific alignment of upstream elements relative to the TATA element or to the site of transcription initiation. Based on data from functional studies with oligonucleotides containing the ATF and ATF-AP5 binding sites described above, we believe that the activity of the distal promoter region is sensitive to its distance from the TATA element. Decreased transcription would result from displacement of the distal region by neutral sequences. Presumably, this effect would be offset by the insertion of activating sequences between the TATA sequence and distal promoter elements. That multiple copies of the consensus octamer element maintain high levels of H2B transcription (Fig. 7, lanes 9 and 10) is consistent with this interpretation. In conjunction with the studies described above, these results demonstrate that the ability of the octamer element to activate the H2B promoter is not significantly affected by the presence of other functional regulatory elements. It should be noted that none of the synthetic templates containing multiple ATF or octamer sites was transcribed at levels significantly higher than that observed for the wild-type promoter. The mass of DNA injected per oocyte (200 pg) in our assays is 50-fold less than the amount generally required for maximal transcription of genes transcribed by RNA polymerase II in injected oocytes. Nonetheless, it is possible that a component of the transcriptional apparatus, such as an accessory protein that mediates activation of the H2B promoter by upstream promoter elements, may be rate limiting in *Xenopus* oocytes.

An oocyte factor binds the consensus octamer sequence but not the variant octamer motif. Because the consensus octamer binding site, but not the variant element, activated the H2B promoter, we reasoned that oocytes contained factors that were able to bind specifically to the consensus, but not the variant, octamer site. To test this idea, we used the oocyte extract described above in a band shift assay with each octamer motif. Incubation of increasing amounts of oocyte extract with the labeled consensus octamer site probe resulted in the formation of two distinct complexes, an abundant complex with significantly reduced electrophoretic

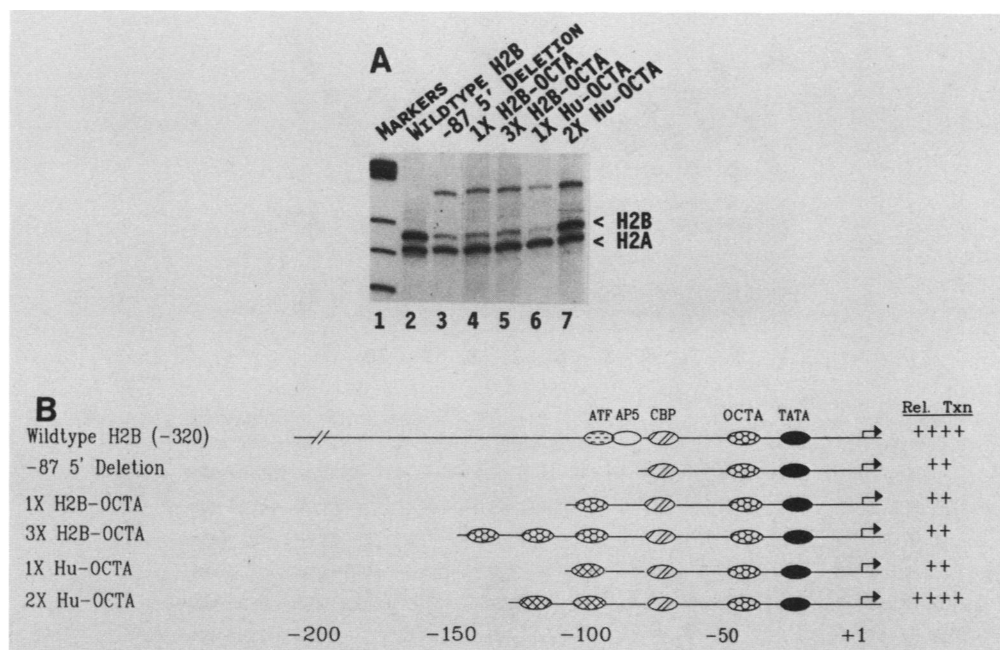

FIG. 6. Multiple copies of the consensus octamer element activate transcription of a promoter when present upstream of the CCAAT box. (A) Results of an S1 nuclease protection assay with RNA from oocytes injected with wild-type H2B (lane 2), a −87 5′ deletion mutant [pH2B(−87)] (lane 3), or the pH2B(−87) template containing the variant H2B-OCTA or consensus Hu-OCTA oligonucleotides (lanes 4 through 7). H2B templates were coinjected with a *Xenopus* H2A template as an internal control. (B) Promoter region of the injected H2B templates. The amount of transcription from each template, normalized to that of the coinjected H2A gene and expressed relative to that of the wild-type H2B gene, is indicated by plus signs.

mobility and a less intense, more rapidly migrating species (arrowheads; Fig. 8, lanes 1 through 3). The specificity of these complexes was tested in a competition assay with increasing amounts of oligonucleotide competitors. Both complexes were readily affected by competition from increasing amounts of the oligonucleotide containing the consensus octamer site (lanes 7 through 11), whereas neither complex was affected by the presence of the competitor containing the variant octamer motif (lanes 12 through 16). These data suggest that multiple complexes result from sequence-specific interactions between oocyte octamer binding factors and the consensus octamer site. The more slowly migrating complex contains maternal Oct-1, since preincubation of oocyte extracts with antisera (provided by W. Herr) prepared against human Oct-1 specifically inhibits the formation of this complex (6a). The identity of the oocyte factor(s) involved in the more rapidly migrating complex and its potential relationship to Oct-1 are uncertain.

Incubation of the oocyte extract with a labeled oligonucleotide containing the variant octamer sequence also resulted in the formation of two distinct complexes; however, much-reduced levels of the slowly migrating complex were observed (Fig. 8, lanes 4 through 6). When the extract was incubated in the presence of increasing amounts of the homologous competitor, no competition occurred (lanes 17 through 21). In contrast, the consensus octamer sequence specifically inhibited the formation of the slowly migrating complex (lanes 22 through 26). These results suggest that the slowly migrating complex resulted from low-affinity binding of an octamer-binding factor (presumably Oct-1) to the variant octamer site. The more rapidly migrating complex containing the variant octamer sequence was not specific, since neither octamer site competed with it. Because of the lack of specific, high-affinity binding to the H2B variant octamer site, we considered that the synthetic oligonucleotide might lack sufficient sequence flanking the H2B octamer site for efficient protein binding. Therefore, we isolated a 50-bp restriction fragment containing 20 bp of wild-type sequence on both ends of the octamer motif from the H2B promoter. Using this restriction fragment as a probe in a gel mobility shift assay with oocyte extracts, we were unable to detect proteins that interact specifically with the H2B octamer motif (data not shown). We conclude that frog oocytes contain Oct-1 and perhaps other octamer binding factors. At least one of these factors is capable of activating the transcription of promoters bearing a consensus octamer element. The inactivity of the variant octamer element in the wild-type *Xenopus* H2B promoter and synthetic derivatives is due to the absence of factors capable of binding with high affinity to the variant motif.

DISCUSSION

The results of this study show that at least three distinct promoter elements are involved in transcription of a *Xenopus* H2B gene in oocytes. Mutagenesis of the TATA box and the CCAAT box sequences resulted in the greatest effects on transcription. These two sequences are common regulatory elements present in the promoters of many genes transcribed by RNA polymerase II. Factors interacting with these sequences are functionally interchangeable between yeast and mammalian cells, demonstrating the highly conserved nature of these factors and supporting the suggestion that they play a fundamental role in the basic transcription machinery. A third regulatory element, an ATF site located at about

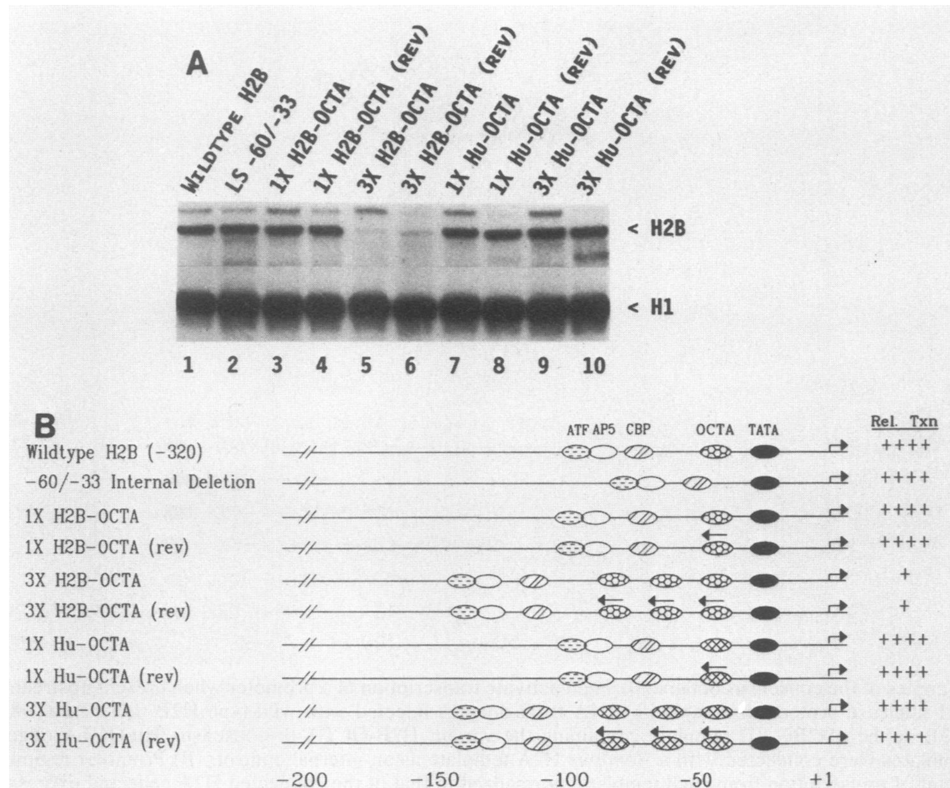

FIG. 7. Inactivation of transcription by displacement of distal promoter elements is prevented by the consensus octamer element. (A) Results of an S1 nuclease protection assay with RNA from oocytes injected with wild-type H2B (lane 1), an H2B promoter internal deletion mutant [pH2Bdel(−60/−33)] (lane 2), or the pH2Bdel(−60/−33) template containing variant H2B-OCTA or consensus Hu-OCTA oligonucleotides (lanes 3 through 10). A *Xenopus* H1 template was coinjected as an internal control. (B) Promoter region of the injected H2B templates. Arrows above oligonucleotide motifs show their orientation relative to the wild-type promoter. The amount of transcription from each template, normalized to that of the coinjected H1 gene and expressed relative to that of the wild-type H2B gene, is indicated by plus signs.

nucleotide −100, was also required for H2B transcription in oocytes.

Several lines of evidence show that the activity of the nucleotide −100 element of the *Xenopus* H2B gene is mediated by the ATF motif. First, mutations in and around the ATF consensus sequence reduced the activity of the H2B promoter. Second, introduction of multiple copies of the ATF motif activated H2B transcription, whereas no activation was seen after insertion of single or multiple copies of the AP5 element. Third, factors in oocyte extracts were found to interact specifically with a 25-bp region centered around the consensus ATF binding site. We were unable to detect binding of oocyte factors to an adjacent AP5 (GT-IIC) site, either alone or in combination with the ATF site. Sequences flanking the ATF site in the H2B promoter appear to be important for transcriptional activation. Similar observations were made for cyclic AMP-responsive promoters containing ATF sites (14). That the ATF motif is a functional element in the *Xenopus* H2B promoter is supported by previous studies of regulatory elements required for cell cycle-regulated transcription of a human H2B histone gene. LaBella et al. (38) showed that a series of short direct repeats between nucleotides −100 and −110 were essential for basal transcription and S-phase-specific activation of a human H2B gene promoter. A deletion of this region, which contains two ATF half-sites in a tandem arrangement, reduced H2B transcription equally in S-phase cells and in cells arrested in G_1.

The ATF (or CRE) motif is present in many viral and cellular promoters and apparently consists of a 5-nucleotide core (TGACG) flanked by bases that are preferred but not invariant (44). In some cases, the ATF binding site is composed of the 8-nucleotide palindromic sequence TGACGTCA containing two half-sites, as seen in the *Xenopus* H2B promoter described here. The ATF sites in several promoters are regulatory targets for cyclic AMP and the adenovirus E1A gene product. However, many other cellular promoters containing ATF sites, including the *Xenopus* H2B promoter, are not known to be regulated by either cyclic AMP or E1A. Sequences encoding a family of related cellular proteins that interact with ATF sites have been isolated (21, 25, 32). Proteins encoded by the ATF cDNA clones contain a highly conserved leucine zipper motif thought to mediate heterodimer formation (25). It is presumed that different ATF proteins bind selectively to ATF sites in various promoters, providing a basis for differential regulation of the target genes.

A highly conserved sequence, the octamer motif, is present at similar locations in the promoters of H2B genes in a variety of animal species ranging from sea urchins to humans. That the consensus octamer motif functions as a promoter element in oocytes is surprising for two reasons. First, when the octamer motif is placed in the context of an enhancer or promoter activating an mRNA-coding gene, it generally displays a lymphoid-specific activity that correlates with the expression of the B cell-specific transcription

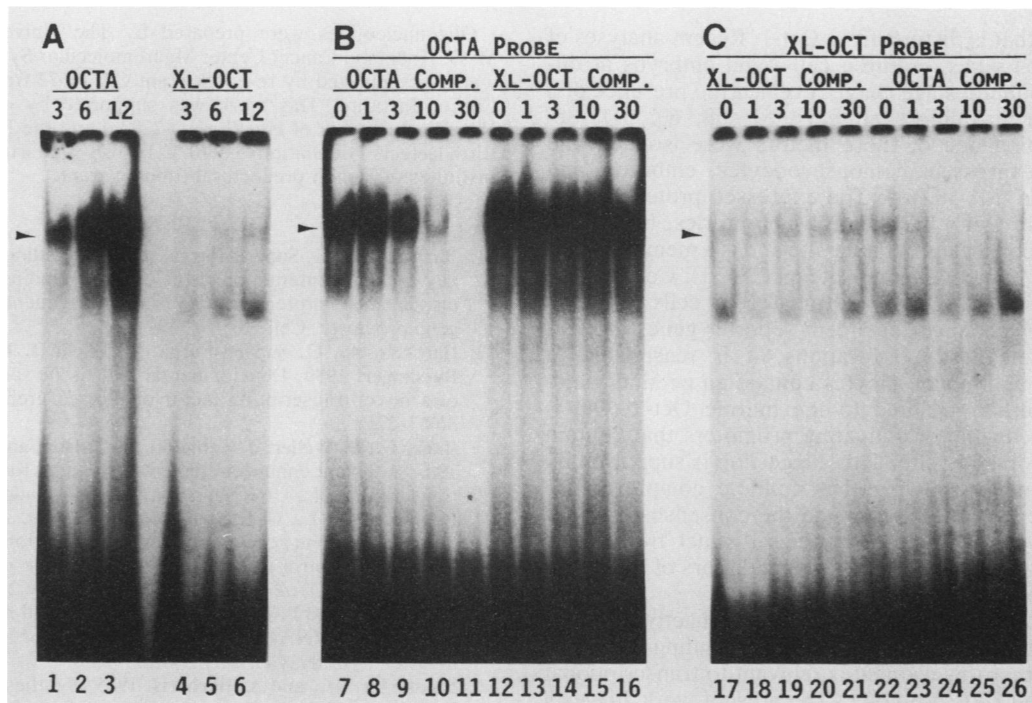

FIG. 8. Proteins in oocyte extracts bind to the consensus octamer element but not to the H2B octamer element. (A) Double-strand oligonucleotide probes containing Hu-OCTA (lanes 1 through 3) or H2B-OCTA (lanes 4 through 6) binding sites were used in gel mobility shift assays with either 3 μl (lanes 1 and 4), 6 μl (lanes 2 and 5), or 12 μl (lanes 3 and 6) of oocyte extract. Specific complexes are indicated with arrows. (B) The oocyte extract (12 μl) was incubated with the Hu-OCTA probe and increasing amounts of either the Hu-OCTA oligonucleotide (lanes 7 through 11) or H2B-OCTA oligonucleotide (lanes 12 through 16) competitors, no competitor (lanes 7 and 12), equimolar competitor (lanes 8 and 13), 3-fold molar excess (lanes 9 and 14), 10-fold molar excess (lanes 10 and 15), and 30-fold molar excess (lanes 11 and 16). (C) Competitions were performed as above with the oocyte extract (12 μl) and the H2B-OCTA probe with increasing amounts of the H2B-OCTA oligonucleotide (lanes 17 through 21) or the Hu-OCTA oligonucleotide (lanes 22 through 26) competitors, no competitor (lanes 17 and 22), equimolar competitor (lanes 18 and 23), 3-fold molar excess (lanes 19 and 24), 10-fold molar excess (lanes 20 and 25), and 30-fold molar excess (lanes 21 and 26).

factor Oct-2 (19, 43, 72). Second, studies of cell-cycle regulation of mammalian H2B genes have implicated the octamer sequence as an important regulatory element required for S-phase-specific transcription (33, 38). This regulation appears to be mediated by a ubiquitous protein, Oct-1, that binds specifically to the octamer consensus element (17). *Xenopus* oocytes are naturally arrested in G_2 of the first meiotic division; therefore, an S-phase-specific regulatory element would not be expected to function in these cells. The ability of the consensus octamer sequence to activate the transcription of synthetic promoters in oocytes suggests that the cell type specificity of this element is less stringent than previously thought.

How can the paradox of the activity of the consensus octamer motif in nonlymphoid, nondividing cells and inactivity of the variant octamer motif be explained? One possibility is that Oct-1, a weak transcriptional activator of mRNA-encoding gene promoters, might be present in oocytes at sufficient levels to allow octamer-dependent transcriptional activation. Affinity-purified Oct-1/OTF-1 stimulates transcription of octamer-dependent promoters when added to in vitro transcription systems (17, 34, 41). Amphibian oocytes accumulate many macromolecules that are used at later stages of development. Conceivably, frog oocytes accumulate sufficient amounts of Oct-1 to directly activate octamer-containing promoters. This explanation makes biological sense in view of the presumed role for this protein as a transcription factor for snRNA promoters (3, 46, 57) and in DNA replication (56).

An alternative possibility is suggested by studies showing that coexpression of the herpes simplex virus transactivator VP16, also called Vmw65 and α-TIF, confers the property of transactivation on Oct-1. VP16 induces by protein-protein interactions the formation of a multiprotein complex containing Oct-1 and at least two other cellular proteins at a degenerate octamer site termed the TAATGARAT motif (20, 37). VP16 appears to serve two distinct functions: it acts as an allosteric effector that alters the DNA-binding specificity of Oct-1, and it provides a potent transactivation function through a *GAL4*-like acidic domain. It has been suggested that the presence of a cellular VP16 analog could be responsible for the octamer-dependent cell cycle regulation of the H2B promoter (67). A similar protein, if present in amphibian oocytes, could account for the activation of promoters containing octamer sites. Thus, this model predicts that multiprotein complexes involving Oct-1 and the cellular VP16 analog should be present in oocytes. These complexes are expected to have a reduced electrophoretic mobility compared with that observed for the binding of Oct-1 alone. Although we did not observe such complexes in oocyte extracts, it is possible that they were not stable under the conditions employed.

Finally, octamer-dependent activation of the H2B promoter in frog oocytes might be mediated by an octamer

binding factor that is distinct from Oct-1. Recent analyses of various mouse tissues, cultured cells, and embryos at different developmental stages have revealed the presence of a family of octamer-binding proteins (51, 58, 60, 61). Sequences encoding one of these factors were isolated and shown to be expressed in mouse oocytes, embryos, and early stem cells (51, 58, 61). The expressed protein, designated Oct-3 and Oct-4 by different laboratories, binds specifically to the consensus octamer site and is a member of the POU family of homeodomain proteins (28, 31). Constitutive expression of Oct-3 (Oct-4) in transfected cells activates transcription of octamer-dependent reporter genes (51, 58, 61). In light of these observations, it is reasonable to anticipate that amphibian oocytes contain an octamer-binding protein, perhaps related to the murine Oct-3 (Oct-4) factor, that is capable of activating promoters that contain the consensus octamer site. This suggestion is supported by the present studies showing that multiple complexes are formed between oocyte factors and the consensus octamer site. Oocyte octamer-binding proteins distinct from Oct-1 are potential candidates for maternal mediators of transcriptional activation.

Because the variant octamer element is inactive in cells that have transcriptionally active octamer-binding factors, it is unclear whether this element is relevant to transcriptional activation of the H2B promoter. The present work suggests that the failure of the variant octamer site to activate H2B transcription in frog oocytes is due to the absence of factors capable of high-affinity binding to this sequence. We presume that the reduced affinity of the oocyte Oct-1 protein is largely due to the two nucleotide differences in the variant sequence, since one of these alterations involves a central guanosine residue whose methylation prevents binding of Oct-1 and Oct-2 to the consensus sequence (66). The sequences flanking the variant octamer motif in the H2B promoter evidently are unable to compensate for these alterations within the core motif. Since the variant octamer motif is inactive in nonreplicating oocytes, the present data are consistent with but obviously do not prove a model in which this sequence is specifically required for transcription of the *Xenopus* H2B promoter during the S phase. The relevant issue is whether the variant octamer motif activates the H2B promoter during the S phase or in a developmental stage-specific manner. It is possible that the *xlh3* H2B gene is not activated during the S phase but is expressed at a constitutive level, perhaps due to the inability of Oct-1 to bind to the variant octamer site in the H2B promoter. In this case, cell cycle-regulated H2B expression might be achieved by the selective activation of H2B genes in other histone gene clusters. Preliminary results suggest, however, that transcription of injected *xlh3* H2B genes in gastrula-stage embryos depends on the variant octamer motif and that embryos at this stage contain factors that bind specifically to the variant motif (unpublished observations). In this case, it appears that the function of the variant octamer site in embryos is mediated either by a factor distinct from Oct-1 or by a modified form of Oct-1 that is capable of binding to the variant octamer site. *Xenopus* oocytes, eggs, and embryos have provided a valuable biochemical system for illuminating fundamental mechanisms involved in cell cycle control. This system should be useful to further examine the control of genes expressed in a cell cycle-regulated manner.

ACKNOWLEDGMENTS

We thank Bill Klein and Eric Olson for their comments on the manuscript.

Oligonucleotides were prepared by The University of Texas M. D. Anderson Cancer Center Macromolecular Synthesis Facility, which is supported by research grant CA-16672 from the National Cancer Institute. This work was supported by grants from the National Institutes of Health (HD-22025) and the March of Dimes Birth Defects Foundation (5-586). C.H. was supported by a National Institutes of Health predoctoral training grant.

REFERENCES

1. **Barberis, A., G. Superti-Furga, and M. Busslinger.** 1987. Mutually exclusive interaction of the CCAAT-binding factor and of a displacement protein with overlapping sequences of a histone gene promoter. Cell **50**:347–359.
2. **Barberis, A., G. Superti-Furga, L. Vitelli, I. Kemler, and M. Busslinger.** 1989. Developmental and tissue-specific regulation of a novel transcription factor of the sea urchin. Genes Dev. **3**:663–675.
3. **Bark, C., P. Weller, J. Zabielski, L. Janson, and U. Pettersson.** 1987. A distant enhancer element is required for polymerase III transcription of a U6 RNA gene. Nature (London) **328**:356–359.
4. **Baumbach, L. L., G. S. Stein, and J. L. Stein.** 1987. Regulation of human histone gene expression: transcriptional and posttranscriptional control in the coupling of histone messenger RNA stability with DNA replication. Biochemistry **26**:6178–6187.
5. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease digested hybrids. Cell **12**:721–732.
6. **Busslinger, M., and A. Barberis.** 1985. Synthesis of sperm and late histone cDNAs of the sea urchin with a primer complementary to the conserved 3′ terminal palindrome: evidence for tissue-specific and more general histone gene variants. Proc. Natl. Acad. Sci. USA **82**:5676–5680.

6a. **Byars, D., C. Hinkley, and M. Perry.** Unpublished data.

7. **Carbon, P., S. Murgo, J.-P. Ebel, A. Krol, G. Tebb, and I. W. Mattaj.** 1987. A common octamer motif binding protein is involved in the transcription of U6 snRNA by RNA polymerase III and U2 snRNA polymerase II. Cell **51**:71–79.
8. **Chodosh, L. A., A. S. Baldwin, R. W. Carthew, and P. A. Sharp.** 1988. Human CCAAT-binding proteins have heterologous subunits. Cell **53**:11–24.
9. **Clerc, R. G., P. Bucher, K. Strub, and M. L. Birnstiel.** 1983. Transcription of a cloned *Xenopus laevis* H4 histone gene in the homologous frog oocyte system depends on an evolutionary conserved sequence motif in the −50 region. Nucleic Acids Res. **11**:8641–8657.
10. **Dailey, L., S. M. Hanly, R. G. Roeder, and N. Heintz.** 1986. Distinct transcription factors bind specifically to two regions of the human histone H4 promoter. Proc. Natl. Acad. Sci. USA **83**:7241–7245.
11. **Dalton, S., J. R. Coleman, and J. R. E. Wells.** 1986. Transcription of the histone H5 gene is not S-phase regulated. Mol. Cell. Biol. **6**:601–606.
12. **Dalton, S., and J. R. E. Wells.** 1988. A gene-specific promoter element is required for optimal expression of the histone H1 gene in S-phase. EMBO J. **7**:49–56.
13. **Davidson, I., J. H. Xiao, R. Rosales, A. Staub, and P. Chambon.** 1988. The HeLa cell protein TEF-1 binds specifically and cooperatively to two SV40 enhancer motifs of unrelated sequence. Cell **54**:931–942.
14. **Deutsch, P. J., J. P. Hoeffler, J. L. Jameson, J. C. Lin, and J. F. Habener.** 1988. Structural determinants for transcriptional activation by cAMP-responsive DNA elements. J. Biol. Chem. **263**:18466–18472.
15. **DiLiberto, M., Z.-C. Lai, H. Fei, and G. Childs.** 1989. Developmental control of promoter-specific factors responsible for the embryonic activation and inactivation of the sea urchin early histone H3 gene. Genes Dev. **3**:973–985.
16. **Dreyfus, M., N. Doyen, and F. Rougeon.** 1987. The conserved decanucleotide from the immunoglobulin heavy chain promoter induces a very high transcriptional activity in B-cells when introduced into an heterologous promoter. EMBO J. **6**:1685–1690.
17. **Fletcher, C., N. Heintz, and R. G. Roeder.** 1987. Purification and

characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2B gene. Cell 51:773–781.
18. **Gallinari, P., F. LaBella, and N. Heintz.** 1989. Characterization and purification of H1TF2, a novel CCAAT-binding protein that interacts with a histone H1 subtype-specific consensus element. Mol. Cell. Biol. 9:1566–1575.
19. **Gerster, T., P. Matthias, M. Thali, J. Jiricny, and W. Schaffner.** 1987. Cell type-specificity elements of the immunoglobulin heavy chain gene enhancer. EMBO J. 6:1323–1330.
20. **Gerster, T., and R. G. Roeder.** 1988. A herpesvirus trans-acting protein interacts with transcription factor OTF-1 and other cellular proteins. Proc. Natl. Acad. Sci. USA 85:6347–6351.
21. **Gonzales, G. A., K. K. Yamamoto, W. H. Fischer, D. Karr, P. Menzel, W. Biggs, W. W. Vale, and M. R. Montminty.** 1989. A cluster of phosphorylation sites on the cyclic-AMP regulated nuclear factor CREB predicted by its sequence. Nature (London) 337:749–752.
22. **Grosschedl, R., and M. L. Birnstiel.** 1980. Identification of regulatory sequences in the prelude sequences of an H2A histone gene by the study of specific deletion mutants *in vivo*. Proc. Natl. Acad. Sci. USA 77:1432–1436.
23. **Grosschedl, R., and M. L. Birnstiel.** 1980. Spacer DNA sequences upstream of the T-A-T-A-A-A-T-A sequence are essential for promotion of H2A histone gene transcription *in vivo*. Proc. Natl. Acad. Sci. USA 77:7102–7106.
24. **Grosschedl, R., M. Machler, U. Rohrer, and M. L. Birnstiel.** 1983. A functional component of the sea urchin H2A gene modulator contains an extended sequence homology to a viral enhancer. Nucleic Acids Res. 11:8123–8136.
25. **Hai, T., F. Liu, W. J. Coukos, and M. R. Green.** 1989. Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. Genes Dev. 3:2083–2090.
26. **Hanly, S. M., G. C. Bleecker, and N. Heintz.** 1985. Identification of promoter elements necessary for transcriptional regulation of a human histone H4 gene in vitro. Mol. Cell. Biol. 5:380–389.
27. **Harvey, R. P., A. J. Robins, and J. R. E. Wells.** 1982. Independently evolving chicken histone H2B genes: identification of a ubiquitous H2B-specific 5′ element. Nucleic Acids Res. 10: 7851–7863.
28. **He, X., M. N. Treacy, D. M. Simmons, H. A. Ingraham, L. W. Swanson, and M. G. Rosenfeld.** 1989. Expression of a large family of POU-domain regulatory genes in mammalian brain development. Nature (London) 340:35–42.
29. **Heindl, L. M., T. S. Weil, and M. Perry.** 1988. Promoter sequences required for transcription of *Xenopus laevis* histone genes in injected frog oocyte nuclei. Mol. Cell. Biol. 8:3676–3682.
30. **Heintz, N., H. L. Sive, and R. G. Roeder.** 1983. Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. Mol. Cell. Biol. 3:539–550.
31. **Herr, W., R. A. Sturm, R. G. Clerc, L. M. Corcoran, D. Baltimore, P. A. Sharp, H. A. Ingraham, M. G. Rosenfeld, M. Finney, G. Ruvkun, and H. R. Horvitz.** 1988. The POU domain: a large conserved region in the mammalian *pit*-1, *oct*-1, *oct*-2, and *Caenorhabditis elegans unc*-86 gene products. Genes Dev. 2:1513–1516.
32. **Hoeffler, J. P., T. E. Meyer, Y. Yun, J. L. Jameson, and J. F. Habener.** 1988. Cyclic AMP-responsive DNA-binding protein: structure based on a cloned placental cDNA. Science 242:1430–1433.
33. **Hwang, I., and C.-B. Chae.** 1989. S-phase-specific transcription regulatory elements are present in a replication-independent testis-specific H2B histone gene. Mol. Cell. Biol. 9:1005–1013.
34. **Johnson, D. G., L. Carayannopoulos, J. D. Capra, P. W. Tucker, and J. H. Hanke.** 1990. The ubiquitous octamer-binding protein(s) is sufficient for transcription of immunoglobulin genes. Mol. Cell. Biol. 10:982–990.
35. **Jones, N. C., P. W. J. Rigby, and E. B. Ziff.** 1988. Trans-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. Genes Dev. 2:267–281.
36. **Kim, Y.-J., I. Hwang, L. L. Tres, A. L. Kierszenbaum, and C.-B. Chae.** 1987. Molecular cloning and differential expression of somatic and testis-specific H2B histone genes during rat spermatogenesis. Dev. Biol. 124:23–34.
37. **Kristie, T. M., J. H. LeBowitz, and P. A. Sharp.** 1989. The octamer-binding proteins form multi-protein-DNA complexes with the HSV αTIF regulatory protein. EMBO J. 8:4229–4238.
38. **LaBella, F., H. L. Sive, R. G. Roeder, and N. Heintz.** 1988. Cell-cycle regulation of a human H2b gene is mediated by the H2B subtype-specific consensus element. Genes Dev. 2:32–39.
39. **Lai, Z.-C., R. Maxson, and G. Childs.** 1988. Both basal and ontogenic promoter elements affect the timing and level of expression of a sea urchin H1 gene during early embryogenesis. Genes Dev. 2:173–183.
40. **Landolfi, N. F., J. D. Capra, and P. W. Tucker.** 1986. Interaction of cell-type-specific nuclear proteins with immunoglobulin V_H promoter region sequences. Nature (London) 323:548–551.
41. **LeBowitz, J. H., T. Kobayashi, L. Staudt, D. Baltimore, and P. A. Sharp.** 1988. Octamer-binding proteins from B or HeLa cells stimulate transcription of the immunoglobulin heavy-chain promoter in vitro. Genes Dev. 2:1227–1237.
42. **Lee, K. A. W., T.-Y. Hai, L. SivaRaman, B. Thimmappaya, H. C. Hurst, N. C. Jones, and M. R. Green.** 1987. A cellular protein, activating transcription factor, activates transcription of multiple E1A-inducible adenovirus early promoters. Proc. Natl. Acad. Sci. USA 84:8355–8359.
43. **Lenardo, M., J. W. Pierce, and D. Baltimore.** 1987. Protein-binding sites in Ig gene enhancers determine transcriptional activity and inducibility. Science 236:1573–1577.
44. **Lin, Y. S., and M. R. Green.** 1988. Interaction of a common cellular transcription factor, ATF, with regulatory elements in both E1A- and cyclic AMP-inducible promoters. Proc. Natl. Acad. Sci. USA 85:3396–3400.
45. **Mason, J. O., G. T. Williams, and M. S. Neuberger.** 1985. Transcription cell type specificity is conferred by an immunoglobulin V_H gene promoter that includes a functional consensus sequence. Cell 41:479–487.
46. **Mattaj, I. W., S. Lienhard, J. Jiricny, and E. M. De Robertis.** 1985. An enhancer-like sequence within the *Xenopus* U2 gene promoter facilitates the formation of stable transcription complexes. Nature (London) 316:163–167.
47. **Montminy, M. R., and L. M. Bilezikjian.** 1987. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature (London) 328:175–178.
48. **Nakajima, N., M. Horikoshi, and R. G. Roeder.** 1988. Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA box-promoter interactions of TFIID. Mol. Cell. Biol. 8:4028–4040.
49. **Newrock, K. M., L. H. Cohen, M. B. Hendricks, R. J. Donnelly, and E. S. Weinberg.** 1978. Stage-specific mRNAs coding for subtypes of H2A and H2B histones in the sea urchin embryo. Cell 14:327–336.
50. **Nomiyama, H., C. Fromental, J. H. Xiao, and P. Chambon.** 1987. Cell-specific activity of the constituent elements of the simian virus 40 enhancer. Proc. Natl. Acad. Sci. USA 84:7881–7885.
51. **Okamoto, K., H. Okazawa, A. Okuda, M. Sakai, M. Muramatsu, and H. Hamada.** 1990. A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. Cell 60:461–472.
52. **Old, R. W., S. A. Sheikh, A. Chambers, C. A. Newton, A. Mohammed, and T. C. Aldrige.** 1985. Individual Xenopus histone genes are replication-independent in oocytes and replication-dependent in Xenopus or mouse somatic cells. Nucleic Acids Res. 13:7341–7358.
53. **Palla, F., C. Caterina, I. Albanese, L. Anello, F. Gianguzza, M. G. DiBernardo, C. Bonura, and G. Spinelli.** 1989. Cis-acting elements of the sea urchin histone H2A modulator bind transcriptional factors. Proc. Natl. Acad. Sci. USA 86:6033–6037.
54. **Perry, M., G. H. Thomsen, and R. G. Roeder.** 1985. Genomic organization and nucleotide sequence of two distinct histone gene clusters from *Xenopus laevis*. J. Mol. Biol. 185:479–499.

55. **Perry, M., G. H. Thomsen, and R. G. Roeder.** 1986. Major transitions in histone gene expression do not occur during development in *Xenopus laevis*. Dev. Biol. **116:**532–538.
56. **Pruijn, G. J. M., W. Van Driel, and P. C. Van Der Vliet.** 1986. Nuclear factor III, a novel sequence-specific DNA-binding protein from HeLa cells stimulating adenovirus DNA replication. Nature (London) **322:**656–659.
57. **Roebuck, K. A., R. J. Walker, and W. E. Stumph.** 1987. Multiple functional motifs in the chicken U1 RNA gene enhancer. Mol. Cell. Biol. **7:**4185–4193.
58. **Rosner, M. H., M. A. Vigano, K. Ozato, P. M. Timmons, F. Poirier, P. W. J. Rigby, and L. M. Staudt.** 1990. A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature (London) **345:**686–692.
59. **Schold, M., A. Colombero, A. A. Reyes, and R. B. Wallace.** 1984. Oligonucleotide-directed mutagenesis using plasmid DNA templates and two primers. DNA **3:**469–477.
60. **Scholer, H. R., A. K. Hatzopoulos, R. Balling, N. Suzuki, and P. Gruss.** 1989. A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. EMBO J. **8:**2543–2550.
61. **Scholer, H. R., S. Ruppert, N. Suzuki, K. Chowdhury, and P. Gruss.** 1990. New type of POU domain in germ line-specific protein Oct-4. Nature (London) **344:**435–439.
62. **Schumperli, D.** 1988. Multilevel regulation of replication-dependent histone genes. Trends Genet. **4:**187–191.
63. **Shannon, M. F., P. L. Wigley, and J. R. E. Wells.** 1985. Histone H5 and H1 cross-reacting material is restricted to erythroid cells in chicken. FEBS Lett. **186:**180–184.
64. **Sharma, A., T. J. Bos, A. Pekkala-Flagan, P. K. Vogt, and A. S. Lee.** 1989. Interaction of cellular factors related to the Jun oncoprotein with the promoter of a replication-dependent hamster histone H3.2 gene. Proc. Natl. Acad. Sci. USA **86:**491–495.
65. **Sive, H. L., N. Heintz, and R. G. Roeder.** 1986. Multiple sequence elements are required for maximal in vitro transcription of a human histone H2B gene. Mol. Cell. Biol. **6:**3329–3340.
66. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323:**640–643.
67. **Stern, S., M. Tanaka, and W. Herr.** 1989. The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. Nature (London) **341:**624–628.
68. **Sturm, R. D., G. Das, and W. Herr.** 1988. The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeo subdomain. Genes Dev. **2:**1582–1599.
69. **Van Wijnen, A. J., K. L. Wright, J. B. Lian, J. L. Stein, and G. S. Stein.** 1989. Human H4 histone gene transcription requires the proliferation-specific nuclear factor HiNF-D. J. Biol. Chem. **264:**15034–15042.
70. **Van Wijnen, A. J., K. L. Wright, R. F. Massung, M. Gerretsen, J. L. Stein, and G. S. Stein.** 1988. Two target sites for protein binding in the promoter region of a cell cycle regulated human H1 histone gene. Nucleic Acids Res. **16:**571–592.
71. **Wells, D., and C. McBride.** 1989. A comprehensive compilation and alignment of histones and histone genes. Nucleic Acids Res. **17**(Suppl.):r311–r346.
72. **Wirth, T., L. Staudt, and D. Baltimore.** 1987. An octamer oligonucleotide upstream of a TATA motif is sufficient for lymphoid-specific promoter activity. Nature (London) **329:**174–178.
73. **Younghusband, H. B., R. Sturm, and J. R. E. Wells.** 1986. Mutagenesis of conserved 5′ elements and transcription of a chicken H1 histone gene. Nucleic Acids Res. **14:**635–644.
74. **Zenke, M., T. Grundstrom, H. Matthes, M. Wintzerith, C. Schatz, A. Wildeman, and P. Chambon.** 1986. Multiple sequence motifs are involved in SV40 enhancer function. EMBO J. **5:**387–397.
75. **Zernik, M., N. Heintz, I. Boime, and R. G. Roeder.** 1980. Xenopus laevis histone genes: variant H1 genes are present in different clusters. Cell **22:**807–815.
76. **Zoller, M. J., and M. Smith.** 1984. Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template. DNA **3:**479–488.