MOLECULAR AND CELLULAR BIOLOGY, Aug. 1990, p. 3926–3933
0270-7306/90/083926-08$02.00/0

Vol. 10, No. 8

Transcriptional Potentiation of the Vitellogenin B1 Promoter by a Combination of Both Nucleosome Assembly and Transcription Factors: an In Vitro Dissection

BLAISE CORTHÉSY,† PHILIPPE LÉONNARD, AND WALTER WAHLI*

Institut de Biologie Animale, Université de Lausanne, CH-1015 Lausanne, Switzerland

Received 5 March 1990/Accepted 26 April 1990

The *Xenopus laevis* vitellogenin B1 promoter was assembled into nucleosomes in an oocyte extract. Subsequent RNA polymerase II-dependent transcription from these DNA templates fully reconstituted in chromatin in a HeLa nuclear extract was increased 50-fold compared with naked DNA. Remarkably, under specific conditions, production of a high level of transcripts occurred at very low DNA (1 ng/μl) and HeLa nuclear protein (1.6 μg/μl) concentrations. When partially reconstituted templates were used, transcription efficiency was intermediate between that of fully reconstituted and naked DNA. These results implicate chromatin in the process of the transcriptional activation observed. Depletion from the oocyte assembly extract of an NF-I-like factor which binds in the promoter region upstream of the TATA box (−114 to −101) or deletion from the promoter of the region interacting with this factor reduced the transcriptional efficiency of the assembled templates by a factor of 5, but transcription of these templates was still 10 times higher than that of naked DNA. Together, these results indicate that the NF-I-like factor participates in the very efficient transcriptional potentiation of the vitellogenin B1 promoter which occurs during nucleosome assembly.

The packaging of eucaryotic DNA in chromatin raises the question of whether this structural organization influences gene activity. In vivo studies have revealed that transcriptionally active genes are more accessible to nuclease digestion than their inactive counterparts (9). The pattern of the hypersensitive sites detected suggests that nucleosomes are absent from specific regions upstream of the transcription initiation site, which instead are occupied by sequence-specific binding proteins (6). This has led to the speculation that nucleosome-free promoter-proximal regions are an absolute requirement for efficient transcription in vivo. Furthermore, nucleosome assembly in vitro has been associated with transcription repression of both class III and II genes: histone H1 is implicated in the repression of chromatin-assembled templates transcribed by RNA polymerase III (13, 22, 25), while RNA polymerase II-dependent transcription from the adenovirus type 2 major late promoter is abolished when it is assembled in nucleosomes in vitro (12, 15, 26). However, electron microscopy studies of insect chromatin have revealed that many RNA polymerase II transcription units appear to display a normal nucleosome spacing near their initiation sites (17).

Here, we have chosen to analyze the effect of chromatin organization on the in vitro expression from the *Xenopus laevis* vitellogenin gene B1 promoter. In vitro transcription from the B1 promoter is apparently not significantly regulated by upstream elements in a HeLa nuclear extract (3). In contrast, in vitro analysis of the same promoter in *Xenopus* liver extracts revealed a strong control of expression by upstream negative and positive *cis*-acting sequences (3, 4). We tentatively attributed the constitutive level of expression in the HeLa nuclear extract to the use of naked DNA templates and to their ability to directly associate with abundant general HeLa transcription factors that govern efficient RNA synthesis by RNA polymerase II. Thus, it was of interest to test whether the expression of the *Xenopus* vitellogenin B1 promoter organized in nucleosomes can be modulated by specific *cis* elements in HeLa nuclear extracts. Using oocyte extracts, we first reconstituted histone-DNA complexes which mimic the physiological structure of chromatin and then compared the in vitro transcription potential of the nucleosome-assembled templates with that of naked templates. Surprisingly, very low concentrations of fully reconstituted templates were efficiently transcribed by RNA polymerase II in the HeLa cell nuclear extract, in contrast to partially reconstituted templates, which were much less active. These results reveal a role of chromatin structure in template potentiation in vitro. Moreover, unlike naked DNA, transcription from promoter deletion mutants assembled in nucleosomes indicates that the NF-I-like binding site in the vitellogenin B1 promoter actively modulates their expression. Our results represent the first evidence that transcriptional potentiation results from the combination of both nucleosome assembly and the binding of a promoter-specific transcription factor. Whether this is a particular feature of the vitellogenin B1 promoter or not will become apparent when promoters containing well-mapped *cis*-acting elements and thus lending themselves ideally to similar in vitro assays have been analyzed.

MATERIALS AND METHODS

Plasmid DNAs. Plasmid pB1(−596/+8)CAT8+ (23) carries a stretch of the *Xenopus* vitellogenin B1 promoter introduced upstream of the chloramphenicol acetyltransferase (CAT) coding sequences in the pEMBL8+ vector. The preparation of the other two 5′ deletion mutants used in this study is described elsewhere (3). To generate the 3′ constructs, we first linearized the p(−596/+8)UC-9 plasmid at position −41 in the vitellogenin sequence by *Bgl*II treatment and further digested it with Bal31. The truncated promoter (−596/Δ3′) was then reintroduced into the CAT8+ vector.

* Corresponding author.
† Present address: Howard Hughes Medical Institute Research Laboratories, Unit in Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA 94305-5425.

For structural analyses, the plasmids pB1(−596/+8)CAT8+ and pB1(−41/+8)CAT8+ were internally labeled with [γ-^{32}P]ATP at the *Eco*RI site within the CAT8+ polylinker and recircularized as described before (19).

Preparation of the oocyte extracts and the HeLa nuclear extracts. Ovaries from mature female frogs were collected in 50-ml tubes containing modified Barth saline (MBS medium [2]) lacking $CaCl_2$, torn into pieces, and digested with 0.2% collogenase (Sigma type I) in the same medium at room temperature with gentle rotary shaking. Complete dispersal of the oocytes required 4 to 6 h. The defolliculated oocytes (stages 5 and 6) were then washed twice in extraction medium (30 mM Tris hydrochloride [Tris-HCl, pH 7.9], 90 mM KCl, 10 mM sodium β-glycerophosphate, 2 mM EGTA [ethyleneglycol tetraacetic acid], 1 mM dithiothreitol [DTT]) and sorted under a microscope to remove all damaged, abnormal, or immature cells. *X. laevis* oocyte extracts were prepared as described by Glikin et al. (7). Extracts were divided into 100-μl portions, quick-frozen in liquid nitrogen, and stored at −70°C. HeLa nuclear extracts were prepared from cells grown in spinner culture by the method of Shapiro et al. (24), as modified by Corthésy et al. (3).

Chromatin assembly assays. In order to assay nucleosome assembly by plasmid supercoiling, a time course reconstitution reaction was performed in a final volume of 133 μl containing 200 ng of labeled relaxed covalently closed circular DNA, 80 μl of oocyte extracts (360 μg of protein), 30 mM Tris-HCl (pH 7.9), 90 mM KCl, 10 mM sodium β-glycerophosphate, 2 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$, and 3 mM ATP. The reaction was carried out in a 1.5-ml conical tube to obtain a high ratio of surface to volume (7). For each time point, 13.3 μl was taken, and a equivalent volume of Stop-mix (50 mM EDTA [pH 7.9], 0.67% sodium dodecyl sulfate, 3.3 mg of proteinase K per ml) was added. Samples were incubated at 37°C for 30 min, purified by phenol-chloroform extraction, electrophoresed into a 0.8% agarose gel, and autoradiographed. Spacing of nucleosomes on reconstituted templates generated by 4-h incubations was assayed by digesting assembly mixes, prepared in exact proportions to the 133-μl reaction mix described above, with 0.06 U of micrococcal nuclease per ng of DNA at 25°C in the presence of 3 mM $CaCl_2$. Portions (15 μl) were taken at the indicated times, and the digestion was quenched by addition of 2 μl of 0.5 M EDTA, pH 7.9. Samples were proteinase K treated and processed as above.

In vitro transcription. (i) Purified DNA. RNA synthesis was performed in a 50-μl final volume containing the indicated amount of supercoiled pB1(−596/+8)CAT8+ plasmid and 12 μg of HeLa nuclear extract, 7 μg of oocyte extract, or both. Final concentrations for transcription were 10% glycerol, 60 mM KCl, 33 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.9), 6 mM $MgCl_2$, 0.6 mM DTT, 0.06 mM EDTA, 5 mM creatine phosphate, 1 μl of RNasin (Promega; 40 U/μl). Then 4 mM sodium β-glycerophosphate and 0.4 mM EGTA were also added to take into account their presence in the oocyte extract buffer and thus demonstrate that these components do not affect transcription. The mixture was kept on ice for 15 min, and transcription was initiated by the addition of 0.6 μM (final concentration) of each ribonucleotide. The reactions were allowed to proceed for 45 min at 30°C, and the RNA was purified and analyzed by primer extension as described previously (4).

(ii) Nucleosome-assembled DNA. Covalently closed circular DNA (50 ng) was incubated as described above. In control experiments, the plasmids were incubated with 20 μl of extraction buffer, used for oocyte extract preparation, and complemented with 5 mM $MgCl_2$ and 3 mM ATP. After 4 h of nucleosome reconstitution at 19°C, 5 μl of HeLa nuclear extracts or bovine serum albumin (added to equal the protein concentration of the nuclear extract) diluted in a mixture of components bringing the samples back to standard transcription conditions (33 mM HEPES [pH 7.9]), 10% glycerol, 60 mM KCl, 6 mM $MgCl_2$, 0.6 mM DTT, 0.06 mM EDTA, 0.6 mM NTPs, 1 μl of RNasin, and 5 mM creatine phosphate) was added. Transcription reactions were carried out for 45 min at 30°C, and the RNA produced was processed as above. Alternatively, DNA templates were incubated prior to nucleosome assembly at 5 ng/μl in 10-μl reaction mixes (containing 3.5 μg of HeLa nuclear extracts) for 15 min at 0°C. Final concentrations for preincubation were 10% glycerol, 60 mM KCl, 33 mM HEPES (pH 7.9), 6 mM $MgCl_2$, 0.06 mM EDTA, and 0.6 mM ATP. Assembly conditions and continuation of the assay were as described above. Sensitivity of the vitellogenin B1 promoter to α-amanitin (1 μg/ml) (see Results) indicates that further purification of assembled templates to avoid possible nonspecific RNA polymerase III transcription (26) is not required.

RESULTS

Nature of the reconstituted templates. To test the effect of chromatin reconstitution on promoter activity, we used the in vitro assembly system derived from *Xenopus* oocytes as described by Glikin et al. (7) and applied previously to the study of the adenovirus type 2 major late promoter (12, 15, 26). Figure 1A shows the changes in the topology of the vitellogenin B1 promoter [pB1(−596/+8)CAT8+; 50 ng of labeled DNA] produced by increasing incubation times with the oocyte extract, in the presence of 3 mM ATP and 5 mM $MgCl_2$. The input labeled plasmid DNA contained primarily covalently closed circular molecules (relaxed form I) with some nicked circles (form II) and linear molecules (form III). It was gradually supercoiled (form I) with slow association kinetics, taking between 3 and 4 h for completion. An identical nucleosome-induced supercoiling time course was observed with all the vitellogenin promoter constructs used in this study (data not shown). The reconstitution ability of the extract with increasing amounts of two different vitellogenin 5′ deletion mutants, the pB1(−596/+8)CAT8+ and pB1(−41/+8)CAT8+ plasmids, was also examined (Fig. 1B). Up to 200 ng of DNA was efficiently supercoiled, indicating the high assembly potential of the extracts.

To address more directly the extent of nucleosome assembly, the reconstituted pB1(−596/+8)CAT8+ templates were analyzed by micrococcal nuclease digestion. Figure 1C shows a 190-base-pair (bp) periodicity (lane 4), with average DNA sizes of the monomer to the hexamer bands of 190, 380, 570, 760, 950, and 1,140 bp, respectively. These fragments in the digest indicate both efficient chromatin assembly and regular nucleosome spacing. The fraction of input plasmid organized in nucleosomal structures was estimated to be 70% by densitometric quantitation, corresponding to values obtained by others in similar assembly assays (12, 26). We conclude from the above experiments that nucleosome assembly occurred on the vitellogenin B1-*cat* gene (vit-CAT) constructs. Furthermore, the 190-bp fragments obtained by nuclease treatment, further trimmed to 146-bp core particle fragments upon extended digestion, are in good agreement with digestion products obtained from cellular chromatin (16).

Comparative analysis of the transcriptional activity of in vitro-assembled vitellogenin chromatin and naked templates in HeLa nuclear extracts. Transcriptional activity of the nucle-

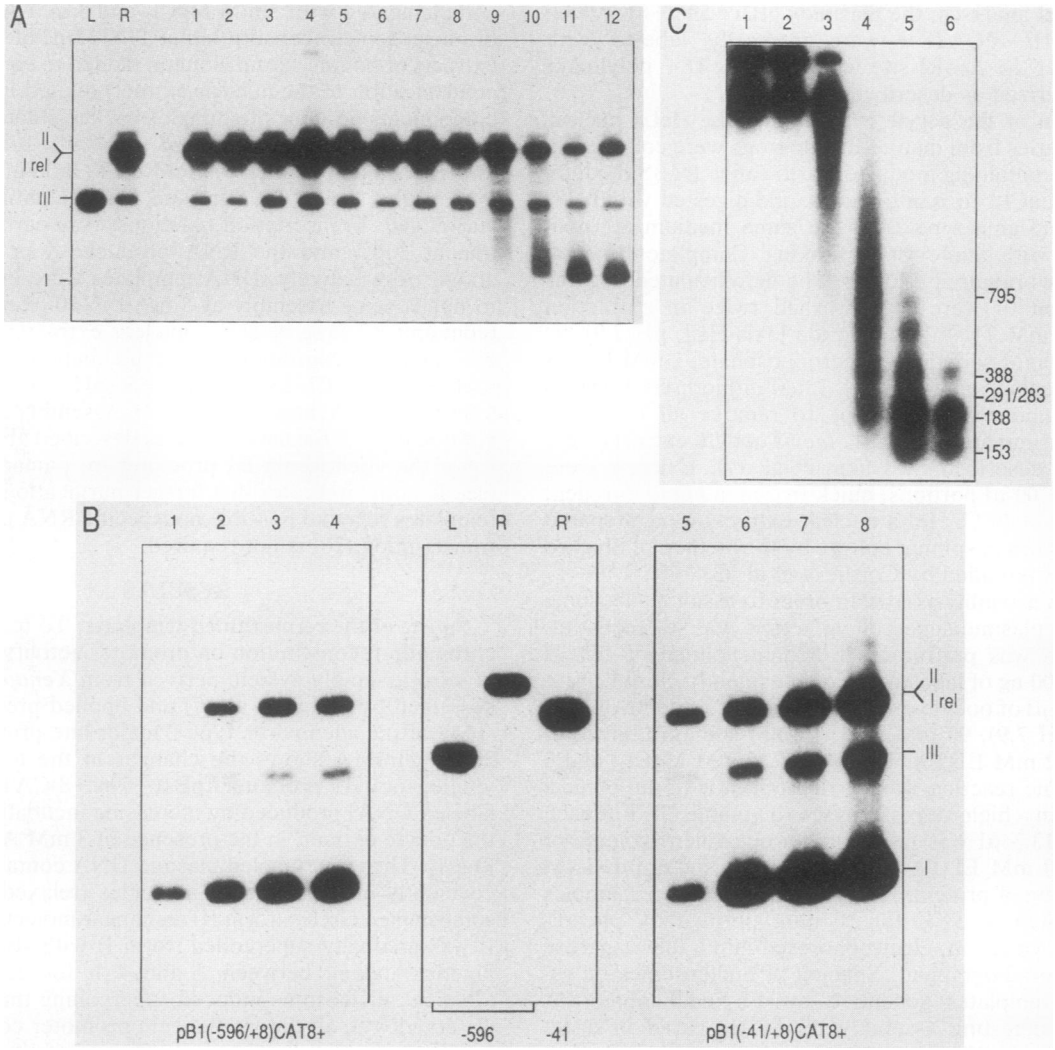

FIG. 1. Analysis of nucleosome-assembled templates. (A) Chromatin reconstitution in oocyte extracts as a function of incubation time: Samples of pB1(−596/+8)CAT8+ DNA incubated in 20-μl oocyte extracts were taken at the indicated time points and electrophoresed on a 0.8% agarose gel. Lanes: 1, 4-h incubation in 10 mM Tris-HCl (pH 7.5)–1 mM EDTA; 2, 4-h incubation in extraction buffer; 3, 0 min; 4, 3 min; 5, 6 min; 6, 10 min; 7, 15 min; 8, 25 min; 9, 40 min; 10, 1 h; 11, 2 h; 12, 4 h; L, linearized pB1(−596/+8)CAT8+ DNA; R, covalently closed circular relaxed plasmid DNA. The positions of topological forms (I, II, III, and relaxed form I [I rel]) are given on the left. (B) Chromatin reconstitution with increasing amounts of pB1(−596/+8)CAT8+ (left panel) and pB1(−41/+8)CAT8+ (right panel) DNAs. Lanes 1 to 4 and 5 to 8 contain, respectively, 50 ng, 100 ng, 150 ng, and 200 ng of covalently closed circular DNA samples incubated in 20-μl oocyte extracts for 4 h and analyzed on a 0.8% agarose gel. In the central panel, lane L contains the linearized −596 construct; lanes R and R′ correspond to covalently closed circular −596 and −41 constructs, respectively. (C) Micrococcal nuclease digestion of reconstituted chromatin. After 4 h of assembly, samples were digested with 10 U of nuclease for the times indicated and analyzed on a 1.5% agarose gel. Lanes: 1, reconstituted DNA; 2, 0 min; 3, 1 min; 4, 2 min; 5, 4 min; 6, 8 min. The positions of molecular weight markers run on the gel are shown on the right.

osome-assembled DNA was analyzed in a cell-free system and compared with that of naked DNA by using the two vitellogenin B1 promoter constructs pB1(−596/+8)CAT8+ and pB1(−41/+8)CAT8+. When naked DNA was transcribed in the HeLa nuclear extract, only a low amount of transcripts was detected (Fig. 2A, lanes 1 and 4). In contrast, when DNAs assembled into nucleosomes were used as templates, very efficient transcription occurred from the vitellogenin promoter (Fig. 2A, lanes 2 and 5). Furthermore, this transcription activity was sensitive to α-amanitin (1 μg/ml) (Fig. 2A, lanes 3 and 6), demonstrating that the transcripts indeed were RNA polymerase II products (20). Together, these results demonstrate that transcriptional activity of the vitellogenin B1 promoter is dramatically increased when organized in chromatin.

Since this promoter is the first whose transcription is potentiated rather than inhibited after nucleosome assembly, it was not possible to measure transcription from a different promoter used as an internal control under the same conditions. Thus, the validity of our results was assessed by repeating each experiment three to five times. We noticed that a similar technical difficulty was encountered by others working on the expression of assembled templates (12, 15, 26). Since our results are in contrast with those obtained with other promoters (see Introduction), we have tested, in parallel, transcription from the adenovirus type 2 major late

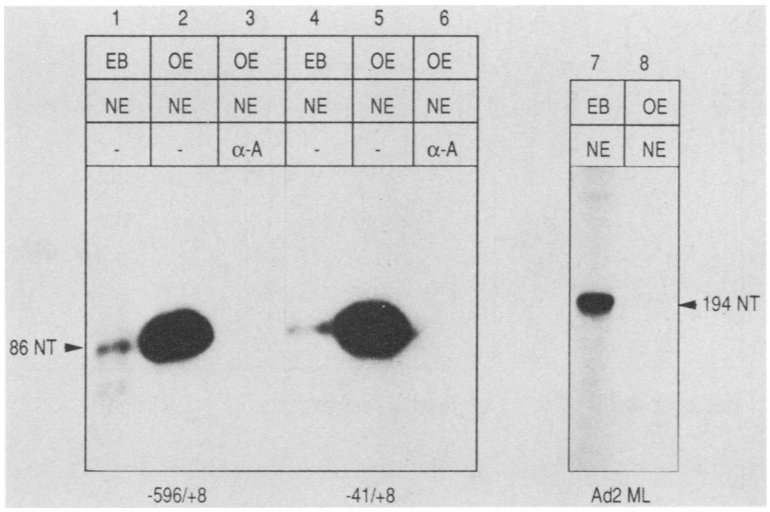

FIG. 2. In vitro transcription from optimally and partially reconstituted chromatin templates. Transcripts generated from 50 ng of pB1(−596/+8)CAT8+, pB1(−41/+8)CAT8+, and pML(C_2AT) plasmids with HeLa nuclear extracts (NE) in the absence of nucleosomes (lanes 1, 4, and 7) or after chromatin assembly (lanes 2, 5, and 8). Lanes 3 and 6 are duplicates of lanes 2 and 5 with α-amanitin (α-A; 1 μg/ml) in the test tube. EB, Oocyte extraction buffer; OE, oocyte whole-cell extract; NT, nucleotides; Ad2ML, adenovirus type 2 major late promoter.

promoter (11, 12, 15, 26) inserted in the p(C_2AT) plasmid developed by Sawadogo and Roeder (21) (G-free cassette).

In the HeLa nuclear extract used above, transcription from the naked plasmid generated the expected 194-nucleotide product (Fig. 2A, lane 7). When the same plasmid was reconstituted into nucleosomes prior to the addition of the HeLa extract, transcription was almost totally abolished (Fig. 2A, lane 8). This result is in agreement with earlier studies (11, 12, 15, 26) and therefore strongly suggests that the potentiation of vitellogenin transcription by nucleosomes is indeed a promoter-specific process.

To rule out that the potentiation observed did not depend only on components of the assembly mixture but rather truly resulted from nucleosome assembly, we first demonstrated that the oocyte extracts did not transcribe the naked vitellogenin promoter as efficiently as the HeLa extract (Fig. 3A and B). This experiment also indicates that oocyte extracts by themselves are not "poisonous" for transcription. We next demonstrated that the elevated transcription observed after reconstitution of templates was not due to a diffusible stimulatory factor from the nucleosome assembly extract, which would act independently of template organization during transcription in the HeLa cell extract. Additivity rather than synergism in the amount of the transcripts produced from naked DNA was observed when the two extracts were mixed (Fig. 3C). This indicates that the transcriptional potentiation described is not the result of the complementation of one extract by the other, but is dependent on the chromatin structure of the template. Finally, it would still be possible that a factor present in the oocyte extract acts only on reconstituted DNA independently of the extract used for transcription. This can be ruled out because in the oocyte whole-cell extract, transcription of the reconstituted template was much lower than in the HeLa nuclear extract (Fig. 3D, compare lanes 1 and 3). In addition, no transcription was observed in the absence of freshly added nuclear proteins from HeLa cells or oocytes (Fig. 3D, compare lane 2 with lanes 1 and 3). Together, these experiments suggest that template reconstitution specifically favors B1 promoter recognition by the HeLa cell transcription machinery.

To further correlate chromatin structure to transcriptional activation, we assayed transcription from templates carrying very few nucleosomes. Figure 3E shows that these templates, whose extent of reconstitution was assessed from the agarose gel (Fig. 1A, lane 9) and corresponded to less than 30%, were weakly transcribed. Comparison of lane 1 with lanes 2 to 6 in Fig. 3E clearly indicates that the partially reconstituted template supported RNA synthesis to a level intermediate between that of fully reconstituted and naked template, further reflecting a role of the chromatin organization in the potentiation process.

Chromatin assembly prevents nonspecific protein binding and favors interactions between transcription factors and promoter. We next examined whether binding of proteins of the HeLa nuclear extract to the template prior to its assembly in chromatin influences transcription. When low amounts of purified DNA (50 ng) were preincubated with HeLa nuclear extracts prior to nucleosome formation, very few vitellogenin transcripts were observed (Fig. 4A, lanes 1 and 2), corresponding to the level obtained with naked DNA. This suggests that nonspecific prebinding of HeLa proteins at low template concentration prevents promoter potentiation by specific transcription factors or blocks efficient chromatin assembly. Alternatively, HeLa nuclear extracts might contain activities that are capable of modifying the DNA template and consequently reducing the rate of transcription. The control experiments presented below demonstrate that the former interpretation is indeed the more likely.

First, the use of 150 ng of vit-CAT DNA rather than 50 ng in the in vitro transcription assay (Fig. 4B, lane 1) or addition of 1 μg of poly(dI-dC)-poly(dI-dC) to 50 ng of specific template (Fig. 4B, lane 2) allowed the recovery of transcription of both the nucleosome-assembled and the naked DNAs. This indicates that titration of nonspecific binding activities with an increased amount of vit-CAT DNA or with a synthetic copolymer is sufficient to relieve inhibition. Furthermore, when preincubation was performed with a correspondingly larger amount of the HeLa extract, thus restoring the initial ratio of DNA to protein, transcription was reduced back to a low level (Fig. 4B, lane 5). Second,

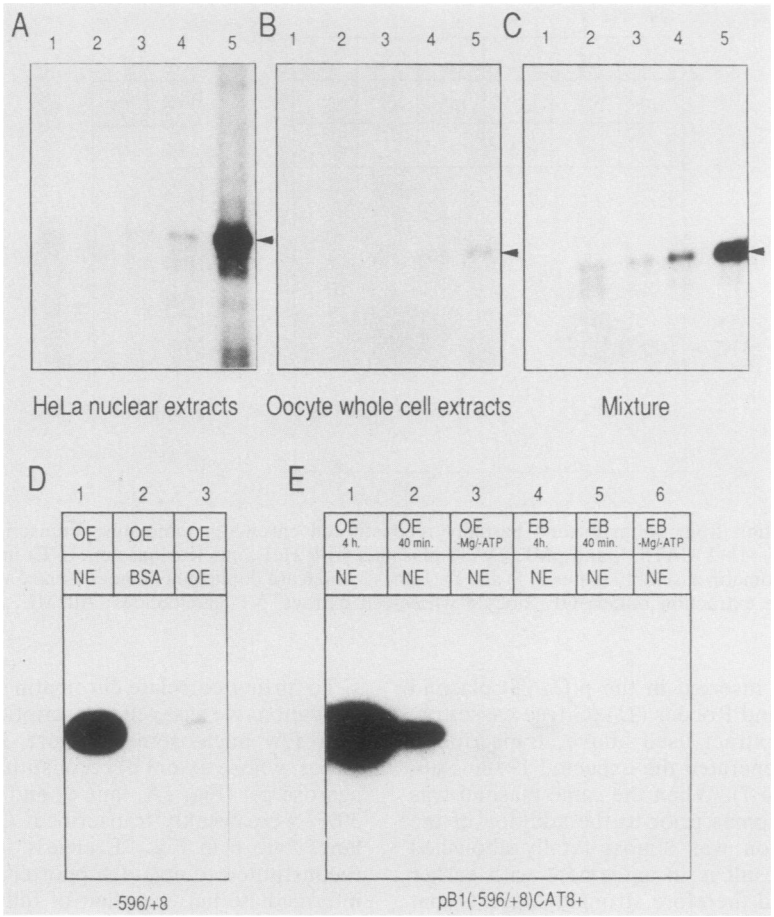

FIG. 3. Effect of DNA template concentration on transcription from the vitellogenin B1 promoter. RNA was synthesized in vitro in 50-μl reactions with either 12 μg of HeLa cell nuclear extracts (A), 7 μg of oocyte extracts (B), or a mixture of the two (C) and naked pB1(−596/+8)CAT8+ DNA in the amount indicated. Lanes: 1, 500 ng of DNA plus α-amanitin (1 μg/ml); 2, 50 ng; 3, 100 ng; 4, 200 ng; 5, 500 ng. The arrowheads indicate the position of the correctly initiated transcripts (86 nucleotides) as determined by primer extension analysis. (D) Transcription of reconstituted pB1(−596/+8)CAT8+ is dependent on the addition of factors present in the extracts. Lane 1, HeLa proteins; lane 2, bovine serum albumin (same amount of protein as in lane 1); lane 3, oocyte whole-cell extract. (E) 50 ng of pB1(−596/+8)CAT8+ reassembled after optimal incubation time (lane 1), after 40 min of incubation (lane 2), or incubated without chromatin reconstitution (lane 3) was used as a template for transcription in HeLa nuclear extracts. Naked DNA was also transcribed, with oocyte extraction buffer (EB) replacing the oocyte whole-cell extracts (OE) (lanes 4, 5, and 6).

covalently closed circular DNA preincubated with HeLa nuclear extracts was supercoiled in the oocyte extract with an efficiency indistinguishable from that of naked DNA (Fig. 4C, compare lanes 1 and 4), indicating that inhibition of transcription is not due to poor chromatin reconstitution of the preincubated template. This experiment also indicates that HeLa nuclear extracts do not degrade the template or modify its topology when assembled in nucleosomes. In addition, efficient reconstitution truly depends on oocyte components; incubation of covalently closed circular DNA for 1 h in HeLa nuclear extracts resulted in less supercoiling (lanes 2 and 3) than a 40-min incubation in oocyte extracts (see above). Together, these data suggest that nucleosome preformation prevents the association of nonspecific proteins with the template and thus facilitates the specific transcription factor-promoter interactions required for high transcription activity.

Effects of promoter upstream sequences on transcription of reconstituted templates. The standard reactions used in the aforementioned experiments (Fig. 3 and 4) contained 50 to 100 ng of vit-CAT reconstituted template. Under these conditions, both the −596 and the −41 constructs were strongly transcribed. To assess expression regulation by upstream sequences and to ensure that specific DNA-binding factors are not limiting for transactivation, we assayed transcription in the HeLa nuclear extract from a significantly lower amount (10 ng) of several reconstituted mutant templates (Fig. 5A).

The results in Fig. 5B show that the activity of the chromatin assembled pB1(−41/+8)CAT8+ and the pB1(−596/−135//−41/+8)CAT8+ templates was fivefold weaker than that of the pB1(−596/+8)CAT8+, pB1(−138/+8)CAT8+, and pB1(−596/−72//−41/+8)CAT8+ constructs (compare lanes 4 and 5 with lanes 1, 2, and 3), suggesting that prebinding of a factor(s) between positions −135 and −72 increases by about fivefold the transcriptional capacity of the reconstituted chromatin. This indicates that the 50-fold transcriptional increase reported above results from a specific combination of chromatin assembly, which by itself accounts for a 10-fold stimulation of transcription, and at least one transcription factor responsible for an additional 5-fold induction. We have previously reported

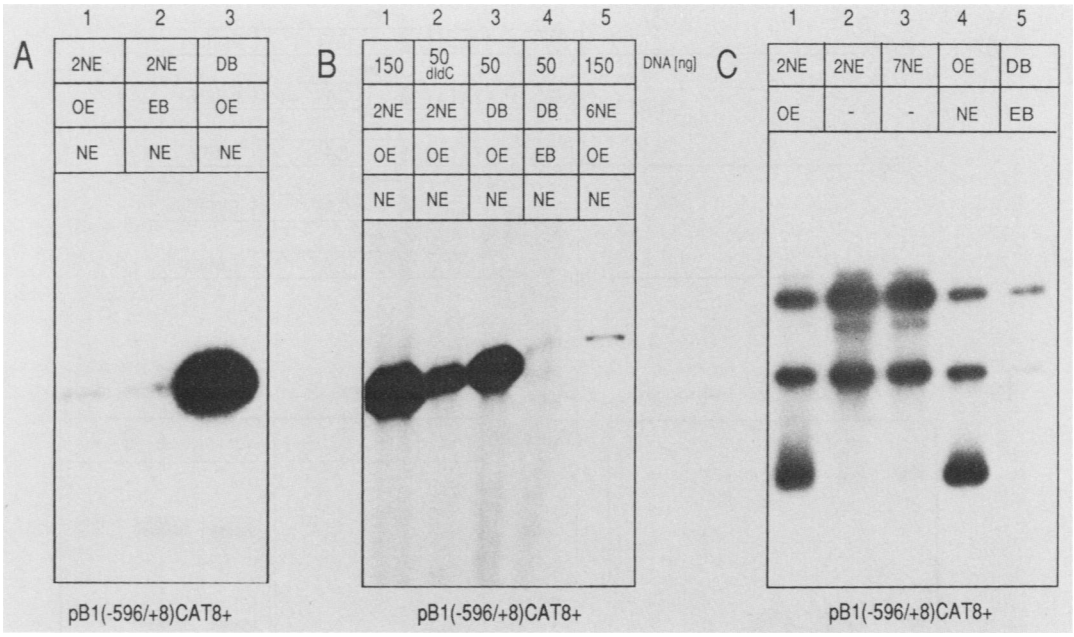

FIG. 4. Transcription from the nucleosome-assembled template preincubated with HeLa nuclear extracts. (A) 50 ng of pB1(−596/+8)CAT8+ was preincubated with either 3.5 μg (2 μl) of HeLa nuclear extracts (2NE; lanes 1 and 2) or nuclear dialysis buffer (DB) (lane 3). Material corresponding to lanes 1 and 3 was assembled into nucleosomes, while sample 2 was left unreconstituted. Transcription was initiated with 8.5 μg of fresh HeLa nuclear extract added to the mixtures. (B) The negative effect on transcription of the preincubation step is relieved by increased DNA concentrations. Lane 1, Preincubation of 150 ng of DNA with 3.5 μg of HeLa nuclear extract (2NE); lane 2, 50 ng of DNA coincubated with 1 μg of poly(dI-dC)-poly(dI-dC) prior to reconstitution; lane 3, duplicate of assay 3 in panel A; lane 4, preincubation of 50 ng of DNA in dialysis buffer left nonassembled; lane 5, preincubation of 150 ng of DNA with 10.5 μg (6 μl) of HeLa nuclear extract (6NE). (C) Lack of effect of HeLa nuclear extracts on chromatin-dependent supercoiling of DNA. Lane 1, Addition of 3.5 μg of HeLa nuclear extract (2NE) prior to chromatin reconstitution of 50 ng of DNA; lanes 2 and 3, incubation of 50 ng of DNA with 3.5 μg (2NE) and 12 μg (7NE) of HeLa nuclear extract for 4 h, respectively; lane 4, incubation of 50 ng of DNA with 20 μl of oocyte extract for 4 h; lane 5, covalently closed circular DNA incubated in dialysis buffer (DB) and oocyte extraction buffer (EB).

that purified human NF-I and a *Xenopus* liver NF-I-like activity are capable of interacting specifically with the region −114 to −101 in the vitellogenin B1 promoter (3). Such a binding activity is also present in oocyte extracts (B. Corthésy, I. Theulaz, J.-R. Cardinaux, and W. Wahli, unpublished data) and is a good candidate for inducing the fivefold transcriptional enhancement observed. Indeed, transcription experiments with reconstituted DNA (Fig. 3 and 4) indicated that potentiation of the promoter during nucleosome assembly occurred even if the preincubation step with the HeLa nuclear extract was omitted. This implies that the component(s) responsible for potentiation of the B1 promoter must be present in the oocyte extracts.

In order to test this assumption and identify this activity, we depleted oocyte extracts of the NF-I-like activity by incubation of the extract with an excess of oligonucleotide containing the NF-I binding site (Fig. 5C). Compared with the template assembled under normal conditions (lane 1), DNA molecules reconstituted with NF-I-depleted oocyte extracts were significantly less potent templates for subsequent transcription in the HeLa nuclear extract (lanes 2 and 3). This result indicates that the reconstituted DNA that had not bound the oocyte NF-I-like activity prior to assembly was significantly less active. The specificity of the effect was confirmed by the fact that the NF-I-depleted oocyte extract assembled chromatin with the same efficiency as nontreated extracts (Fig. 5C, lanes 4, 5, and 6). This last experiment again suggests that the binding of at least one specific transcription factor, the NF-I-like factor, combined with the assembly in nucleosomes of the vitellogenin B1 promoter results in a very efficient potentiation of transcription.

DISCUSSION

In this study, we have coupled nucleosome assembly and cell-free transcription systems to investigate the functional interactions of the vitellogenin B1 promoter with nucleosomal proteins and transcription factors. This approach has allowed us to demonstrate that transcription in vitro is not restricted to high template-to-protein ratios (5, 14, 24) but can also occur efficiently at very low concentrations of reconstituted templates. The possible mechanisms by which such a process takes place are discussed below in terms of DNA structure and involvement of binding sites for transcription factors, in particular the NF-I-like *cis* element in the vitellogenin B1 promoter (3).

With the aim of analyzing a possible nucleosome-mediated effect on the promoter activity, we assembled template DNA in chromatin and assayed its ability to be transcribed in vitro. Quite unexpectedly, we found that the activity of two reconstituted DNAs comprising the B1 promoter (−596/+8 and −41/+8) was dramatically increased compared with that of naked templates. These results suggest that the increased level of transcription depends on the underlying chromatin structure. Thus, we hypothesize that supercoiled DNA bearing nucleosomes is used more efficiently as a template than its naked covalently closed circular counterpart because of a topological configuration more propitious for transcription.

Our results with the B1 promoter are in contrast with transcription analyses of the reconstituted 5S RNA gene (8, 18, 25) and the adenovirus type 2 major late promoter (12, 15, 26; this study). In these latter two systems, chromatin-

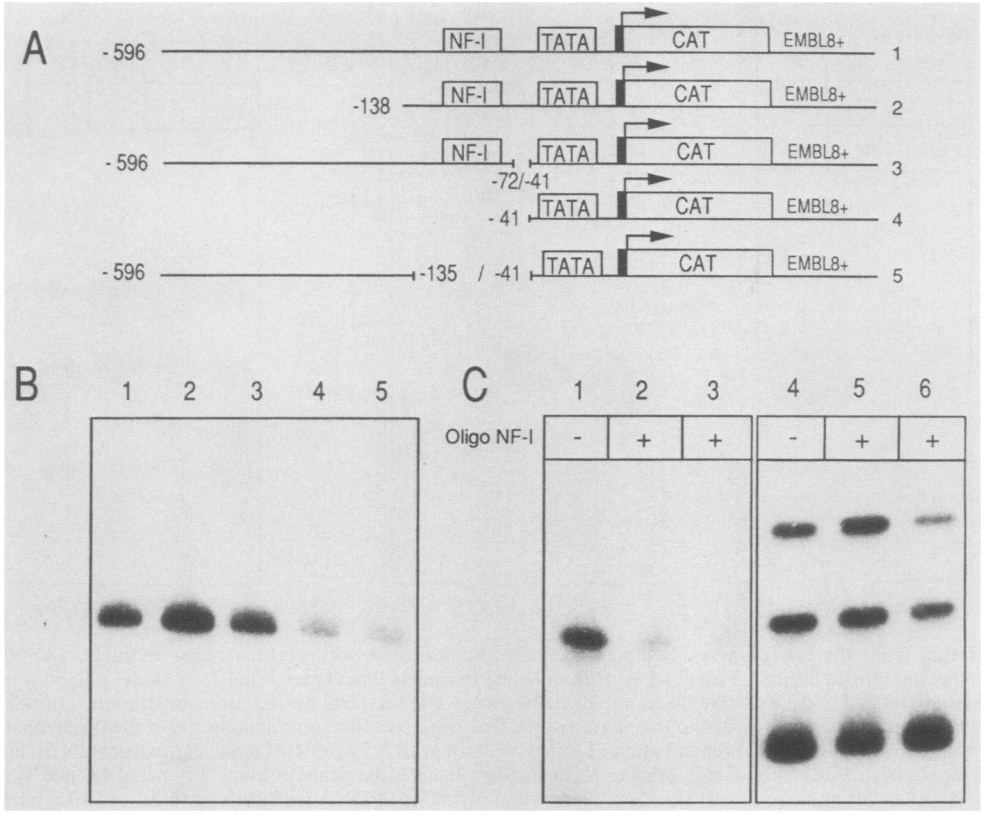

FIG. 5. Effect of upstream sequences on transcription from chromatin templates. (A) Schematic representation of vitellogenin B1 sequences present in the mutants used for transcription in panel B. Promoter elements are not drawn to scale. (B) Analysis of the ability of nucleosome-assembled deletions (10 ng) depicted in panel A to sustain transcription in vitro. Lanes: 1, pB1(−596/+8)CAT8+; 2, pB1(−138/+8)CAT8+; 3, pB1(−596/−72//−41/+8)CAT8+; 4, pB1(−41/+8)CAT8+; 5, pB1(−596/−135//−41/+8)CAT8+. (C) Potentiation is prevented by NF-I titration in the oocyte extract. Lanes: 1, transcription from 50 ng of reconstituted pB1(−596/+8)CAT8+ template; 2 and 3, as in lane 1 but with a 50-fold and 200-fold molar excess of NF-I oligonucleotide, respectively; 4, 5, and 6, reconstitution in the presence of a 0-, 50-, or 200-fold molar excess of NF-I oligonucleotide, respectively.

assembled templates were not transcribed or required preincubation with cellular factors prior to chromatin assembly to be transcriptionally active. For instance, binding of TFIID to the adenovirus major late promoter during nucleosome assembly potentiated subsequent initiation by RNA polymerase II (26), while the conjugated binding of TFIIIA and displacement of histone H1 were essential for generating active 5S chromatin templates (8, 22, 25). Thus, differences in binding sites for specific transcription factors or differences in binding site arrangements in the adenovirus and the vitellogenin promoters could account for the repression or activation observed here with the two reconstituted templates.

We have previously identified a binding site for a *Xenopus* NF-I-like and the human CTF/NF-I activities at positions −114 to −101 in the vitellogenin B1 promoter (3). We have also demonstrated that in vitro transcription from naked vitellogenin B1 templates in HeLa nuclear extracts was not modulated at this site (3). Here we show that, in contrast, the absence of the NF-I-like *cis*-acting element in the reconstituted templates partially diminished promoter activity. Therefore, we postulate that the binding of an NF-I-like activity from the oocyte combined with nucleosome assembly of the template is implicated in the potentiation seen in this study. Consistently, oligonucleotide-mediated titration of this activity in the oocyte extract leads to a decrease in the level of transcription, further arguing for a role of NF-I in the potentiation process taking place during chromatin assembly.

The presence of TFIID and other general transcription factors in the oocyte extract can account for the low rather than absent activity of the vitellogenin constructs containing the TATA box element but lacking the NF-I-binding site. In contrast, as mentioned above, the adenovirus type 2 major late promoter assembled in chromatin is not efficiently transcribed unless it has been preincubated with TFIID purified from HeLa cells (12, 15, 26). This suggests that potentiation of the adenovirus promoter cannot be achieved by the *Xenopus* TFIID present in the oocyte-derived assembly system. Alternatively, the *Xenopus* TFIID may bind more efficiently to the B1 TATA box than to that of the adenovirus major late promoter. Furthermore, the absence of possible binding sites in the latter for additional oocyte factors stabilizing the TFIID-promoter interaction could also explain the nucleosome-mediated repression of transcription.

In the vitellogenin promoter, binding of the NF-I-like activity could mediate potentiation by facilitating the formation of a stable preinitiation complex on the chromatin-assembled template. It implies that binding of the NF-I-like activity is not prevented by nucleosome assembly and that the factor is stably attached at its cognate binding site and thus represents an integral component of the activated chromatin template. This would suggest that, as essential

transcription components, NF-I and possibly TFIID may facilitate promoter recognition in chromatin by allowing access of RNA polymerase II and other factors to the transcription initiation site. They could carry out this function by maintaining the entry site for RNA polymerase II free of nucleosomes or by favorably presenting other *cis* elements which would potentiate transcription. Ultimately, the reconstitution of transcription solely from purified components (1, 10) will be required to understand how activators interact with basic transcription factors in naked DNA as well as in chromatin-assembled templates.

In conclusion, this article shows that nucleosome assembly can potentiate transcription in a template-dependent manner involving specific *cis* elements. Our results further confirm the hypothesis that potentiation of transcription and transcription initiation can be considered two uncoupled but not independent regulatory events. The appearance in several genes of tissue-specific nuclease-hypersensitive sites preceding transcription (9, 27) is also consistent with this assumption. Together, our data indicate that the use of in vitro assembly-transcription systems such as the one described in this communication will prove very useful for assaying the activity of specific DNA-binding factors that show no relevant function on naked DNA templates in vitro.

ACKNOWLEDGMENTS

We thank M. Sawadogo and R. Roeder for the generous gift of the plasmid pML(C_2AT); I. Corthésy-Theulaz, D. Feinstein, G. Krey, N. Mermod, M. Tsai, and A. Wolffe for critical reading of the manuscript and helpful comments; A.-M. Mérillat for preparation of the figures; and C. Goy for typing the manuscript.

This work was supported by research grants from the Swiss National Science Foundation and the État de Vaud.

LITERATURE CITED

1. **Buratowski, S., S. Hahn, P. A. Sharp, and L. Guarente.** 1988. Function of a yeast TATA element-binding protein in a mammalian transcription system. Nature (London) **334**:37–42.
2. **Coleman, A.** 1984. Expression of exogenous DNA in *Xenopus* oocytes, p. 49–69. *In* B. D. Hames and S. J. Higgins (ed.), Transcription and translation. IRL Press, Washington, D.C.
3. **Corthésy, B., J.-R. Cardinaux, F.-X. Claret, and W. Wahli.** 1989. A nuclear factor I-like activity and a liver-specific repressor govern estrogen-regulated in vitro transcription from the *Xenopus* vitellogenin B1 promoter. Mol. Cell. Biol. **9**:5548–5562.
4. **Corthésy, B., R. Hipskind, I. Theulaz, and W. Wahli.** 1988. Estrogen-dependent *in vitro* transcription from the vitellogenin promoter in liver nuclear extracts. Science **239**:1137–1139.
5. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
6. **Emerson, B. M., and G. Felsenfeld.** 1984. Specific factor conferring nuclease hypersensitivity at the 5′ end of the chicken adult β-globin gene. Proc. Natl. Acad. Sci. USA **81**:95–99.
7. **Glikin, G. C., I. Ruberti, and A. Worcel.** 1984. Chromatin assembly in *Xenopus* oocytes: in vitro studies. Cell **37**:33–41.
8. **Gottesfeld, J., and L. S. Bloomer.** 1982. Assembly of transcriptionally active 5S RNA gene chromatin *in vitro*. Cell **28**:781–791.
9. **Gross, D. S., and W. T. Garrard.** 1988. Nuclease-hypersensitive sites in chromatin. Annu. Rev. Biochem. **57**:159–197.
10. **Horikoshi, M., C. K. Wang, H. Fujii, J. A. Cromlish, P. A. Weil, and R. G. Roeder.** 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor, TFIID, that binds to the TATA box. Nature (London) **341**:299–303.
11. **Knezetic, J. A., G. A. Jacob, and L. S. Luse.** 1988. Assembly of RNA polymerase II preinitiation complexes before assembly of nucleosomes allows efficient initiation of transcription on nucleosomal templates. Mol. Cell. Biol. **8**:3114–3121.
12. **Knezetic, J. A., and D. S. Luse.** 1986. The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II *in vitro*. Cell **45**:95–104.
13. **Lassar, A. B., D. H. Hamer, and R. G. Roeder.** 1985. Stable transcription complex on a class III gene in a minichromosome. Mol. Cell. Biol. **5**:40–45.
14. **Manley, J. L., A. Fire, M. Samuels, and P. A. Sharp.** 1983. *In vitro* transcription: whole-cell extracts. Methods Enzymol. **101**:568–582.
15. **Matsui, T.** 1987. Transcription of adenovirus 2 major late and peptide IX genes under conditions of in vitro nucleosome assembly. Mol. Cell. Biol. **7**:1401–1408.
16. **McGhee, J. D., and G. Felsenfeld.** 1980. Nucleosome structure. Annu. Rev. Biochem. **49**:1115–1156.
17. **McKnight, S. L., and O. L. Miller, Jr.** 1977. Electron microscopic analysis of chromatin replication in the cellular blastoderm *Drosophila melanogaster* embryo. Cell **12**:795–804.
18. **Morse, H. R.** 1989. Nucleosomes inhibit both transcriptional initiation and elongation by RNA polymerase III *in vitro*. EMBO J. **8**:2343–2351.
19. **Razvi, F., G. Gargiulo, and A. Worcel.** 1983. A simple procedure for parallel sequence analysis of both strand of 5′-labeled DNA. Gene **23**:175–183.
20. **Roeder, R. G.** 1976. Eukaryotic nuclear RNA polymerases, p. 285–329. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Sawadogo, M., and R. G. Roeder.** 1985. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell **43**:165–171.
22. **Schlissel, M. S., and D. D. Brown.** 1984. The transcriptional regulation of *Xenopus* 5S RNA genes in chromatin: the role of the active stable transcription complexes and histone HI. Cell **37**:903–913.
23. **Seiler-Tuyns, A., P. Walker, E. Martinez, A.-M. Mérillat, F. Givel, and W. Wahli.** 1986. Identification of estrogen-responsive DNA sequences by transient experiments in a human breast cancer cell line. Nucleic Acids Res. **14**:8755–8770.

24 **Shapiro, D. J., P. A. Sharp, W. Wahli, and M. J. Keller.** 1988. A high-efficiency HeLa cell nuclear transcription extract. DNA **7**:47–55.

25. **Wolffe, A. P.** 1989. Dominant and specific repression of *Xenopus* oocyte 5S RNA genes and satellite DNA by histone H1. EMBO J. **8**:527–537.
26. **Workman, J. L., and R. G. Roeder.** 1987. Binding of transcription factor TFIID to the major late promoter during *in vitro* nucleosome assembly potentiates subsequent initiation by RNA polymerase II. Cell **51**:613–622.
27. **Yaniv, M., and S. Cereghini.** 1986. Structure of transcriptionally active chromatin. Crit. Rev. Biochem. **21**:1–26.