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Molecular basis of the activation of basal histone $H1^0$ gene expression

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ABSTRACT

Histone $H1^0$ is encoded by a gene that is expressed only in cells committed to differentiation. We have previously cloned the *Xenopus laevis* $H1^0$ gene and studied elements involved in the regulation of its expression in transfected *Xenopus laevis* A6 cells, and in microinjected embryos. In this work, in order to understand the basis of the action of these elements, we used an A6 cell nuclear extract and showed that the $H1^0$ promoter is able to direct efficient *in vitro* transcription, which is highly dependent on a functional TATA box. However, in contrast to what we observed *in vivo*, in transfected A6 cells, the *in vitro* transcription was independent of major regulatory elements, defined *in vivo*. We then used this *in vitro* system to reconstitute $H1^0$ gene regulation. The creation of a repressive environment by the addition of purified histone H1 to the *in vitro* transcription system allowed us to obtain transcription dependent on the integrity of the regulatory elements. Investigating the basis of this regulation we found that protein-DNA interaction on the proximal promoter region was dependent on the integrity of proximal elements, and moreover the distal regulatory element, the UCE, was able to modulate this interaction. We conclude that the role of these regulatory elements is to maintain the basal TATA-dependent transcription of $H1^0$ under repressive condition: i.e., H1-mediated repression of transcription, or chromatin assembly in general.

INTRODUCTION

Vertebrate somatic tissues express three major classes of histone H1. Firstly, an embryonic type, described as yet only in amphibians, is expressed specifically during early development (1–5). Secondly, the common histone H1 proteins, a group of closely-related polypeptides that appear during development at the same time as zygotic expression initiates (3–7); they remain present in almost all tissues throughout the life of the animal. Finally, adult type-H1 histones accumulate in cells reaching a terminal stage of differentiation. Two members of this family have been characterized : $H1^0$, which is expressed in the majority of differentiated somatic tissues in vertebrates (8–10), and histone H5 which is expressed during maturation of avian erythrocytes (11).

Commitment of cells to differentiation is accompanied by activation of $H1^0$ gene expression (12–13). Cis-acting elements involved in $H1^0$ gene expression have been described for *Xenopus laevis* (10), mouse (14–16), and human (17). These elements are highly conserved in these three species, indicating that an evolutionarily conserved mechanism may be involved in the regulation of $H1^0$ gene expression.

We have identified in the*Xenopus laevis* $H1^0$ gene promoter, several cis-acting elements involved in the regulation of the gene expression (10). Two of these elements are located in the proximal promoter region at −40 and −103 (see figure 1), and show respectively 100% homology with two elements involved in the regulation of two other histone-encoding genes: i) an H1-box (18), located approximately at −100 bp with respect to the initiation site of the histone H1 gene and ii) an H4-box involved in the expression of the histone H4 gene. This latter element is conserved in all histone H4 genes (19), and it has also been found at the same position in the histone H5 promoter (called in this case UPE), (20). The particular feature of the $H1^0$ proximal promoter is that these boxes are found together at the same relative positions with respect to the initiation sites in *Xenopus laevis* and mouse as well as in the human histone $H1^0$ promoters (figure 1). In addition, we have described an upstream conserved element (UCE), located at −435 bp from the initiation site in *Xenopus laevis*. This element is highly conserved between human, mouse and Xenopus (19 identical bases out of 20) (see figure 1) ; it interacts with nuclear factors in A6 cell nuclear extract and it is involved in the enhancement of basal $H1^0$ gene expression (10).

In the present work, using an *in vitro* system consisting of A6 cell nuclear extract, we could reproduce a basal, TATA-dependent transcription which was, in contrast to the *in vivo* situation, dependent neither on the integrity of the UCE nor on the integrity of the proximal H1 and H4 boxes. Nevertheless, this *in vitro* system allowed us to show that these elements were involved in a mechanism which allows the basal TATA-dependent

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transcription to occur under repressive conditions. We discuss the possibility that these elements are involved in the activation of $H1^0$ gene expression during development by relieving chromatin-mediated repression of basal transcription during cell differentiation.

MATERIALS AND METHODS

Preparation of CAT constructs containing mutations of the histone $H1^0$ promoter

The preparation of all constructs used in this study has been described previously (10). All mutagenesis made use of the PCR-based overlap extension method (21,22).

In vitro transcription under permissive conditions

500 ng of supercoiled vector were incubated in 80 μg of an A6 nuclear extract for 30 min at room temperature using the conditions previously described for *in vitro* transcription with this extract (23). Briefly, the standard in vitro transcription assay was done in 25 μl final volume of a buffer consisting of 25 mM Hepes−KOH, pH 7.6, 12.5 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 1 mM spermidine, 1 mM PMSF, 10 μg/ml leupeptin, 10% glycerol, and 0.5 mM each of 4-ribonucleotide 5′-triphosphate and 10 U RNase inhibitor (Promega). Nucleic acids were then extracted and transcribed RNA was reverse-transcribed using an end-labelled primer with a sequence complementary to the first 27 bases of the CAT gene (23). Briefly, transcripts were annealed with 0.05 pmol of 5′-^{32}P- labeled primer (2×10^5 cpm) in 10 μl of 20 mM Tris−HCl, pH 8.0, 0.2 mM EDTA, 0.25 M KCl at 65°C for 5 min and at 55°C for 30 min, before cooling to room temperature. The annealed primer was elongated using 2 U of avian myeloblastosis virus reverse transcriptase (Promega) in 30 μl of 20 mM Tris−HCl, pH 8.0, 80 mM KCl, 8 mM $MgCl_2$, 80 μg/ml actinomycin D, 10 mM DTT, 0.4 mM each of 4-deoxyribonucleotide 5′-triphosphate, and 10 U of RNase inhibitor at 42°C for 1 hour. The reverse transcription product was then analyzed on a 6% polyacrylamide sequencing gel.

In vitro transcription under repressive conditions

500 ng of supercoiled vectors were incubated in 80 μg of an A6 nuclear extract in a volume of 21 μl on ice for 30 min. 4 μl of a solution containing the indicated amounts of purified histone H1 were added to the mixture and the incubation was carried out for another 30 min at room temperature. After reverse transcription, the products were analyzed as described above.

DNase I digestion/primer extension analysis

500 ng of different supercoiled vectors were incubated in 80 μg of an A6 nuclear extract for 30 min on ice in a final volume of 11.5 μl of a mixture containing the other components necessary for *in vitro* transcription (23), minus NTPs. 500 ng of purified histone H1 were then added (except for the negative control), and incubation was carried out for another 30 min at room temperature. 1 μl of a DNase I solution (250 ng/ml), was added for 1 min at room temperature and the digestion was stopped by the addition of 200 μl of a solution of 20 mM DTA, 0.2 M NaCl and 1% SDS. After extraction, DNA was precipitated and 20 ng of the digested vector were used for a primer extension analysis using the same end-labelled CAT primer as the one used in the *in vitro* transcription, according to the previously published protocol (24).

Analysis of the proximal promoter region by restriction enzyme digestion

A6 cell extract. 500 ng of a supercoiled plasmid containing a CAT gene under the control of wild type, or mutant, $H1^0$ promoter were incubated in 80 μg of an A6 nuclear extract, in a final volume of 25 μl, as described above. The incubation was carried out for 30 min on ice followed by 15 min at room temperature. 10 units of the each restriction enzyme were added and the incubation was continued for another 30 min at room temperature. After phenol/chloroform and chloroform extraction, DNA was precipitated, digested with XhoI enzyme (which cuts at −313), and run on a 2% agarose gel. After denaturation and transfer to a membrane, the blot was hybridized to a mixture of ^{32}P-labelled DNA fragments corresponding to the proximal promoter region and the vector sequence.

Embryo extract. Embryonic extracts were obtained as described (25) and the experiment was carried out as above except that the incubation media contained, in addition, 2mM Tris−HCl pH7.6, and 0.02% Triton X100. The DTT and EDTA concentrations were 3mM and 1mM, respectively.

RESULTS

Reconstitution of the regulation of $H1^0$ *in vitro*

The CAT gene was fused to the $H1^0$ promoter, in which different regulatory elements had been destroyed by sequence replacement (see figure 1). These vectors were used to direct an *in vitro* transcription, in an A6 nuclear extract. A ^{32}P end labelled primer complementary to the initiation codon of the CAT gene was used to perform primer extension, on RNA transcribed

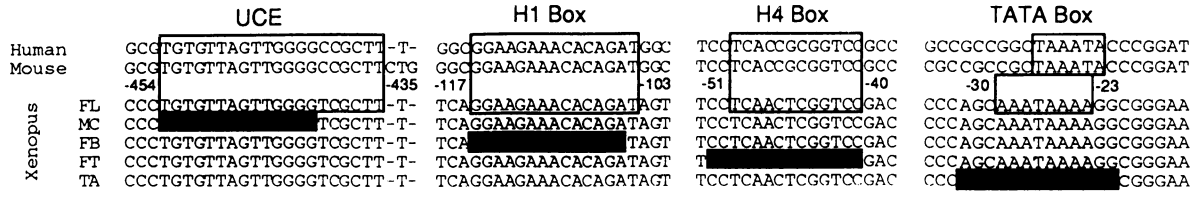

Figure 1. Distal and proximal regulatory elements involved in the regulation of $H1^0$ gene expression. Homology between human, mouse and *Xenopus laevis* sequences is shown at the level of indicated elements. FL designates wild type sequence and MC, FB, FT and TA indicate different vectors bearing mutations at the level of UCE, H1 box, H4 box and the TATA box region respectively (black boxes). Sequences covered by black boxes were replaced by unrelated sequences described previously (10).

in vitro. Analysis of the extension product showed that transcription was strictly dependent on the integrity of the TATA box (construct TA, fig. 2). However, in contrast to what we had observed previously in transfected A6 cells (10), efficient basal transcription was not affected by the destruction of regulatory elements; neither the proximal H4- and H1 boxes nor the distal UCE (fig. 2, construct FT, FB and MC, receptively).

We then tried to reconstitute *in vitro*, the regulation of $H1^0$ gene expression. Recent work on the mechanism of the activation of transcription by transcription factor Gal4-VP-16, showed that this factor can activate basal transcription *in vitro*, through an antirepression mechanism, by relieving the repression of transcription due to the interaction of histone H1 with naked- or chromatin-reconstituted vector (24, 27).

Since in the *in vitro* transcription assay, efficient basal transcription was not dependent on upstream elements, we asked the question whether these elements were involved in an antirepression mechanism rather than in a true activation (24, 26, 27). Should this latter hypothesis be true, the destruction of these elements would have no effect on the efficiency of transcription in the absence of a repressive environment: i.e., chromatin assembly or simply the addition of histone H1 to the extract.

We then performed *in vitro* transcription in the presence of increasing amounts of histone H1, added after incubation of the vector in the extract for 30 min on ice, concomitant with the transfer of tubes to room temperature, and initiation of transcription, according to the protocol described by Croston *et al.*, (24). In every case the addition of histone H1 repressed transcription (fig. 3). However, the repression was more efficient when vector mutated in the regulatory elements, UCE, H1- and H4 box, was used as template (fig. 3, construct MC, FB and FT respectively). This experience allowed us to reconstitute the control of basal transcription by the upstream regulatory elements. For instance, in the presence of 500 ng H1, transcription was essentially observed from the wild-type promoter (FL construct, fig. 3). This observation prompted us to investigate the basis of the action of these different regulatory elements.

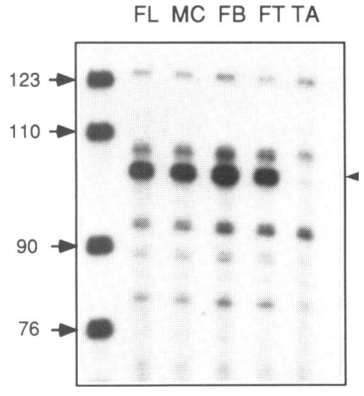

Figure 2. *In vitro* transcription using wild type or mutated $H1^0$ promoter-CAT vector. 500 ng of the indicated supercoiled vector were incubated in 80 μg of an A6 nuclear extract for 30 min at room temperature. Nucleic acids were then extracted and transcribed RNA was reverse-transcribed using an end-labelled primer with a sequence complementary to the first 27 bases of the CAT gene. The reverse transcription product was then analyzed on a 6% polyacrylamide sequencing gel. The arrow on the right indicates the position of the correctly initiated transcript. Numbers to the left indicate positions of marker. The nature of each mutation is indicated in fig. 1.

Cis-acting elements-dependent protein–DNA interaction in the $H1^0$ gene proximal promoter region

It has been shown that the addition of histone H1 to the extract, besides the creation of a repressive environment, allows the detection of structural features induced by the binding of transcription factor (24). We thus monitored the structure of the $H1^0$ gene proximal promoter region, under normal *in vitro* transcription, or repressive conditions, by DNase I digestion/primer extension analysis. This technique which is an indirect analysis of DNase I-digested fragments, allows the analysis of protein–DNA interaction on a supercoiled plasmid, under effective conditions of *in vitro* transcription (28). The result of such an analysis is shown in figure 4A, and figure 4B represents a quantitative estimation of these data.

In the presence of histone H1 in the extract, a DNase I hypersensitive site was observed on the $H1^0$ proximal promoter region, located between the TATA-box and the H4-box of the wild type promoter (FL construct, fig. 4A and B, +H1 panel). The presence of this site was strictly dependent on the integrity of the H4-box, since it disappeared when this box was mutated (fig. 4A and B, +H1 panel, FT construct).

The mutation in UCE considerably enhanced the accessibility of this site to DNase I (fig. 4B, +H1 panel, MC construct), indicating the capacity of UCE, a distal regulatory element, to modulate protein–DNA interaction in the proximal promoter region under repressive conditions. The data indicate that factors interacting with UCE somehow direct an increased protection of the H4 box-dependent hypersensitive site.

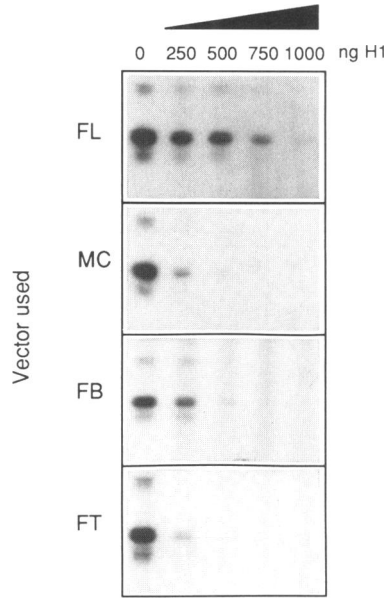

Figure 3. *In vitro* transcription under repressive conditions. 500 ng of the indicated supercoiled vectors were incubated in 80 μg of an A6 nuclear extract on ice for 30 min. A solution containing the indicated amounts of purified histone H1 were added to the mixture and the incubation was carried out for another 30 min at room temperature. After reverse transcription, the products were analyzed as in figure 2. The nature of each mutation is indicated in fig. 1.

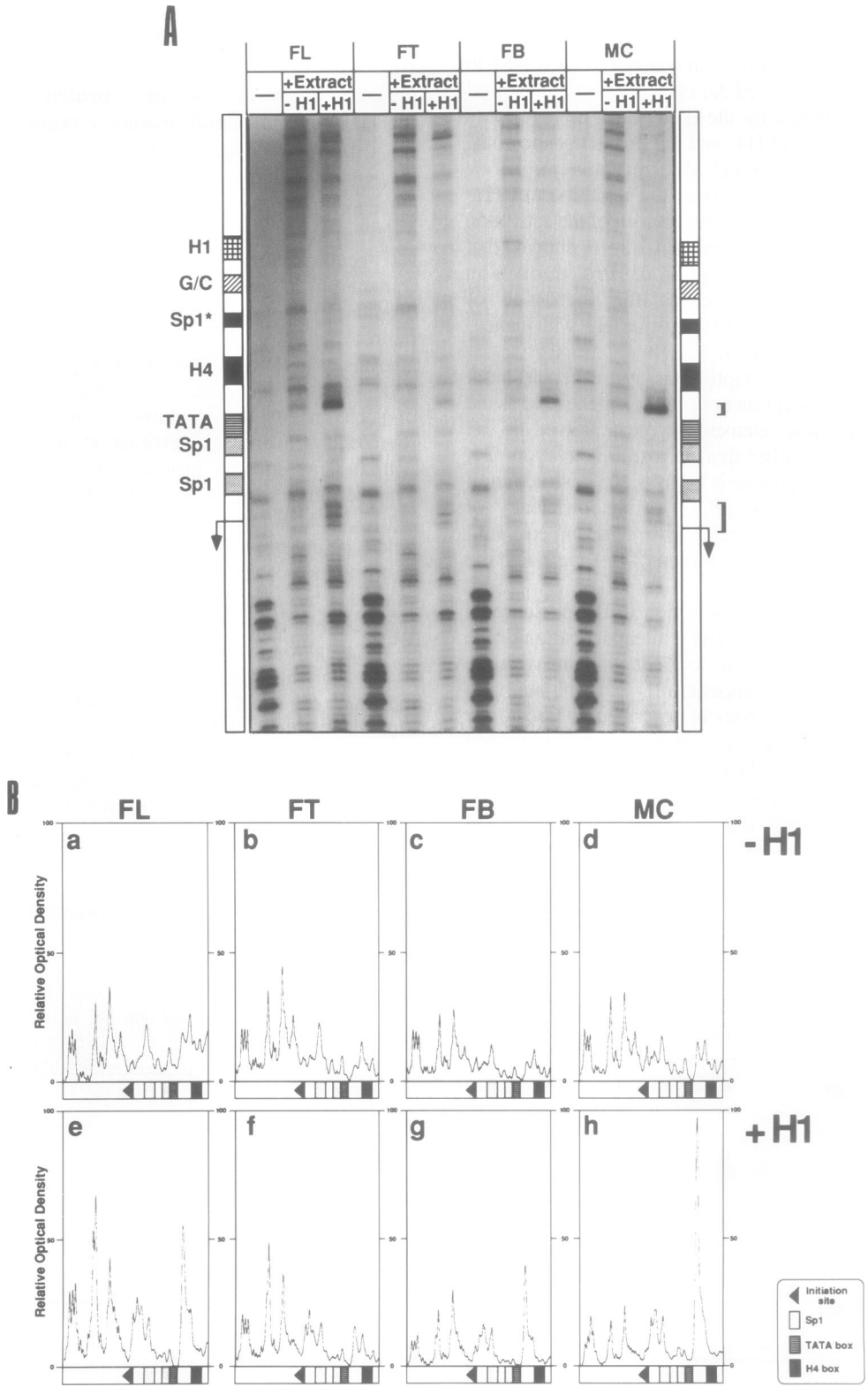

Figure 4. Analysis of the $H1^0$ proximal promoter region under repressive conditions by DNase I digestion / primer extension. (**A**) 500 ng of different supercoiled vectors were incubated in 80 μg of an A6 nuclear extract for 30 min on ice. A mixture containing other components necessary for *in vitro* transcription (23) minus NTPs, plus or minus 500 ng of purified histone H1, were then added and incubation was carried out for another 30 min at room temperature. DNase I digestion was carried out for 1 min at room temperature. After extraction, DNA was precipitated and a portion of digested vector was used for a primer extension analysis using the same end-labelled CAT primer as above. The position of the major putative binding sites for transcription factors are indicated on the left and on the right of the figure. The arrow indicates the position of the start site. Positions of important DNase I sensitive sites are indicated on the right by brackets. Mutant designation is as in figure 1. −H1 indicates the absence of H1 and +H1 the presence of H1. (−) indicates vector digested in the absence of extract and H1. (**B**) Densitometric analysis of primer extension product is shown. The position of each putative binding site for transcription factor and the initiation site are indicated.

The destruction of H1-box did not greatly affect the accessibility of this particular site (fig. 4B, +H1 panel, FB construct).

In addition to the H4 box-dependent DNaseI hypersensitive site, other structural motifs are influenced by different mutations. For instance, a region immediately downstream of the initiation site showed a clear modification of its accessibility to DNase I, qualitatively and quantitatively, specially when the H1 box and UCE were mutated (fig. 4B, +H1 panel, FB and MC constructs).

In the absence of histone H1, the DNase I digestion/primer extension analysis did not show any significant modulation of the structure of this region (fig. 4B, −H1 panel).

Restriction enzyme digestion analysis of the proximal promoter region

DNase I digestion/ primer extension analysis showed that UCE can influence the hypersensitivity of a site located downstream, between TATA- and the H4 box. this hypersensitive site appears only in the presence of H1, probably because of an efficient exclusion of histone H1 from this region, due to the assembly of factors on the H4 box. In the absence of histone H1 no particular hypersensitive site dependent on regulatory elements can be observed. However we wished to know if the upstream UCE can modulate protein−DNA interaction downstream, on the proximal promoter region, in the absence of H1, under normal conditions of *in vitro* transcription.

The strategy we chose was to assay the accessibility of three target sequences present at + 30, −62 and −124, to their respective restriction enzymes, XbaI, PstI and DraIII in an A6 nuclear extract under *in vitro* transcription conditions (see fig. 5A). In order to perform the experiment, the supercoiled plasmid was incubated in the extract for 30 min on ice. Tubes were then transferred and maintained at room temperature for 15 min before the addition of the appropriate restriction enzyme. The incubation with enzymes was carried out for another 30 minutes, before the extraction of DNA. The extracted plasmid is then digested to completion with XhoI restriction enzyme cutting at 313 bases upstream of the initiation site, between UCE and the H1 box. Therefore the amount of insert liberated after XhoI digestion is proportional to the extent of XbaI, PstI and DraIII cuts at their respective sites in the extract. In other words the amount of insert is proportional to the accessibility of restriction enzymes to their target sequence.

Destruction of UCE (construct MC) located between −435 and −455, considerably increased the accessibility of the Pst I enzyme to its target sequence (figure 5B), located between the H1- and the H4-boxes (see figure 5A). Interestingly this site is very close to the previously observed DNase I hypersensitive site (about 30 bp, upstream). In contrast, the destruction of UCE had little or no effect on the accessibility of XbaI and DraIII to their respective sites compared to that observed when wild type promoter was used (figure 5B, in XbaI and DraIII panels, compare FL and MC constructs). This shows that the structural feature induced by UCE affects a specific portion of the proximal promoter region.

The destruction of either the H1-box or the H4-box, or both, did not change the sensitivity of the PstI site, compared to that of the wild type promoter (fig.5B, PstI panel, compare FB, FT and FTB, with FL construct). These mutations did not affect the cutting by XbaI or DraIII neither. However, it must be noted that when the H1-box was mutated, the accessibility of DraIII to its target sequence was slightly increased, probably because

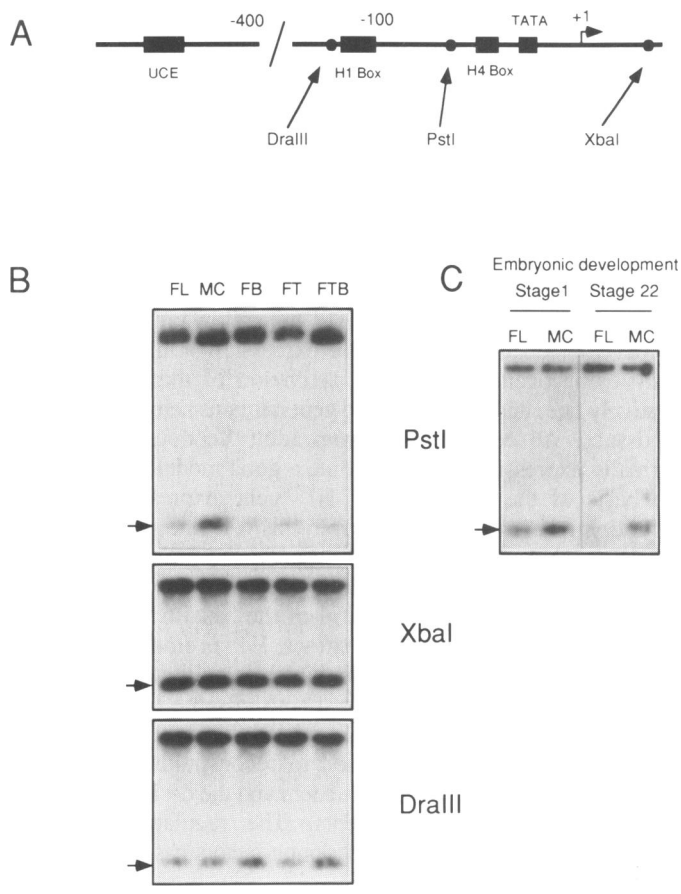

Figure 5. Analysis of the $H1^0$ proximal promoter region under permissive conditions by restriction enzyme digestion. Analysis in A6 extract; 500 ng of a supercoiled plasmid containing a CAT gene under the control of wild type or mutant $H1^0$ promoter were incubated in an A6 nuclear extract. The incubation was carried out for 30 min on ice and for 15 min at room temperature. 10 units of the indicated restriction enzyme were added and the incubation was continued for another 30 min at room temperature. DNA was precipitated, digested with XhoI enzyme which cuts at −313, and run on a 2% agarose gel. After denaturation and transfer to a membrane, the blot was hybridized to a mixture of ^{32}P-labeled DNA fragments corresponding to proximal promoter region and vector sequence. The amount of the generated restriction fragment reflects the accessibility of PstI, XbaI and DraIII enzymes to their respective target sequences in the extract. (**A**) Shows the structure of the $H1^0$ promoter region and the positions of the restriction sites investigated. (**B**) Southern blot of indicated vectors digested as described above. The restriction enzyme utilized is indicated on the right of each panel ; the nature of each mutation is shown in figure 1. FTB is a vector which carries both FB and FT mutations. Arrows on the left indicate the positions of the generated restriction fragments and in each case the upper band represents the linearized vector. Analysis in embryo extract; (**C**) the experiment was carried out as above and the effect of Pst I digestion is shown for each of the extracts.

of the proximity of this sequence to the H1-box (vector FB and FTB in figure 5B, DraIII panel).

To confirm the specificity of this phenomenon, we prepared extracts from *Xenopus laevis* embryos that were non-competent (stage 1) or competent (stage 22) for $H1^0$ gene expression (10), and added PstI restriction enzyme to each sample. Figure 5C shows that, after incubation of plasmids containing wild type promoter (FL construct) and UCE-mutated promoter (MC construct), with stage 1 embryo extract, similar levels of PstI digestion were observed for both constructs. In contrast, at stage 22, the PstI site became hypersensitive to PstI enzyme only when

UCE was mutated, while it was protected in a wild type vector. In other words, in cells competent for $H1^0$ expression, UCE can influence protein–DNA interaction in the proximal promoter region, which results in a protection of the PstI site.

DISCUSSION

An important question concerning the regulation of $H1^0$ gene expression is to know how this gene is activated in a variety of independent differentiation pathways.

The cloning of the Xenopus $H1^0$ gene allowed us to consider the developmental regulation of the expression of this gene during early embryogenesis. The full activation of this gene occurs relatively late, and thereafter, the gene remains active in a variety of tissues. A6 cells derived from adult Xenopus kidney and normally expressing $H1^0$, constitute a good model to investigate the basis of the regulation of $H1^0$ gene expression. Recent definition of a nuclear extract from this cell line, able to direct efficient RNA polymerase II-dependent transcription (23), prompted us to use this system to investigate the role of different regulatory elements in the control of the basal transcription of $H1^0$ gene. We find that in this extract, $H1^0$ promoter can direct an efficient specific transcription which is only dependent on a functional TATA box. In contrast, in the same line, the basal expression of $H1^0$ is tightly regulated by several upstream elements, *in vivo*. We then tried to reconstitute *in vitro*, the regulation of gene expression to understand the basis of the action of different regulatory elements. The regulation of basal transcription, controlled by transcription factors has been recently reconstituted *in vitro* (24, 26, 27, 29), and *in vivo*, in microinjected Xenopus oocytes (30). It appears that the factor-dependent activation of transcription is largely dependent on the repressive action of the chromatin. Indeed, transcription factors predominantly acted to relieve the chromatin-mediated repression.

Chromatin assembly drastically represses $H1^0$ gene expression *in vitro* (Khochbin, unpublished data) and *in vivo* in microinjected oocytes (30). Interestingly, in microinjected oocytes, in the absence of chromatin assembly an efficient TATA-dependent $H1^0$ gene expression is observed (31). These observations confirm the repressive role of chromatin on $H1^0$ gene expression. We then wished to know if factors interacting with important regulatory elements controlling $H1^0$ gene expression could activate the basal transcription through opposition to the repressive action of the chromatin. Unfortunately systems allowing chromatin assembly *in vitro*, in an extract, or *in vivo*, use oocytes, eggs or early embryos, where endogenous $H1^0$ gene is not expressed, and therefore, they lack the necessary regulatory factors. A6 nuclear extract prepared from $H1^0$-expressing cells, contains factors interacting specifically with the main regulatory elements of $H1^0$ promoter (10). However, conditions for efficient chromatin assembly have not yet been defined in such extracts. Therefore in order to perform our analysis we took advantage of data published by Croston *et al.* (24), showing that the addition of purified histone H1 to the extract, at the time of transcription initiation, is sufficient to reproduce results obtained when vector assembled in chromatin was used. This approach allowed us to reconstitute *in vitro*, regulatory element-dependent, $H1^0$ gene expression. Indeed, transcription from the wild type promoter was more resistant to the repressive action of histone H1 than that observed from mutated promoters. The experimental protocol for this experiment is such that histone H1 is added after the incubation of the template with the extract. Therefore we can assume that the interaction of different factors with their target sequences in the wild type promoter prevent the nucleation of histone H1 assembly on the initiation site region. This property is dependent on the integrity of proximal and distal elements: UCE, and the H1- and H4 boxes, appear to participate together in a mechanism which fails to function if only one of the components is destroyed. consistent with this conclusion, we showed previously that, in transfected A6 cells, efficient expression from $H1^0$ promoter was dependent on the integrity of these three elements. In a UCE-depleted promoter, a residual TATA-dependent expression of CAT protein was observed and the destruction of either the H1-box or H4-box, or both, had no effect on this expression. In contrast these mutations greatly affected the enhanced $H1^0$ gene expression when a UCE-containing promoter is used. These observations lead us to suppose that UCE cooperates with elements located at the proximal promoter region to induce a high level of $H1^0$ expression (10).

The analysis of the proximal promoter region by DNase I/ primer extension, revealed modulation of DNase I accessibility on the proximal promoter region. This modulation was dependent on the integrity of the regulatory elements and was observable only in the presence of histone H1 in the extract. A DNase I hypersensitive site was observed between the TATA- and the H4 box; the presence of this site was strictly dependent on the integrity of the H4 box. This behaviour resembles that observed by Croston *et al.* (24), where a hypersensitive DNase I site was revealed in the adenovirus E4 gene promoter, only in the presence of histone H1, and was dependent on the binding of the transcription factor, GAL4-VP16, on its targets located up to 1.2 Kb upstream of the initiation site.

In the $H1^0$ proximal promoter, the presence of a DNase I hypersensitive site, appeared to be also dependent on the integrity of the UCE located approximately 400 bp upstream. This observation confirms our previous conclusion suggesting possible interaction of the UCE with the proximal promoter region (10). Our data suggest that factors binding to the UCE are able, somehow, to reduce the exposure of the H4 box-dependent hypersensitive site. We could not show a relationship between this hypersensitive site and the H1 box,. but the destruction of the H1 box itself also modulates the accessibility of the initiation site region to DNase I.

Restriction enzyme analysis in non repressive conditions, allowed us to show that the interaction of protein in the extract with the proximal promoter region is also directed by the regulatory elements studied, and could be revealed in the absence of histone H1. This approach allowed us to confirm the influence of the UCE on protein–DNA interaction in a restricted region of the proximal promoter region, observed previously by DNase I/primer extension analysis. It also confirmed a developmentally regulated interaction of factors with $H1^0$ promoter, observed before (31), during early Xenopus embryogenesis. The observation of these types of interaction dependent on regulatory elements in the extract, when these same elements do not participate in regulating the efficiency of basal transcription (transcription in the absence of H1), suggest that their function is other than a direct stimulation of the basal level of transcription.

Based on the data presented in this work, we propose that elements involved in the enhancement of basal $H1^0$ gene expression function predominantly through an antirepressive

mechanism. However, one should be aware of the fact that the repressive conditions obtained by H1, mimics only the chromatin-mediated repression of transcription and does not reflect the *in vivo* situation. *In vivo*, these elements could accomplish their function by modifying the chromatin structure of the $H1^0$ promoter. Indeed there exists good evidence showing that $H1^0$ gene expression is highly sensitive to chromatin structure. Hyperacetylation of chromatin through the inhibition of cellular deacetylase efficiently induces $H1^0$ gene expression. This phenomenon, first shown in HeLa cells treated with butyrate (32), a known inhibitor of histone deacetylases, has been observed in other mammalian cells in culture (33). Previously we showed that the Xenopus $H1^0$ gene was also highly inducible upon inhibition of histone deacetylation during early development (10, 31). The inducibility of $H1^0$ upon butyrate treatment first appeared after gastrulation (10), concomitant with the appearance of hyperacetylated histones (4). This butyrate-induced over-expression of $H1^0$ occurs before the full activation of $H1^0$ gene expression, which normally takes place later during development. This observation suggests that the modification of chromatin structure due to chromatin acetylation, before the full activation of the gene can create a situation which mimics what happens later under the influence of the appropriate transcription factors. In order to confirm these hypotheses we are currently investigating the state of chromatin structure of the $H1^0$ promoter region in non-$H1^0$ expressing embryonic cells and in cells where $H1^0$ is fully activated.

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REFERENCES

1. Smith, R.C., Dworkin-Rastl, E. and Dworkin, M.B. (1988) *Genes Devl.* 2, 1284–1295.
2. Ohsumi , K. and Katagiri, C. (1991) *Dev. Biol.* 147, 110–120.
3. Hock, R., Moorman, A., Fisher, D. and Scheer, U. (1993) *Dev. Biol.* 158, 510–522.
4. Dimitrov, S., Almouzni, G., Dasso, M. & Wolffe, A.P. (1993) *Dev. Biol.* 160, 214–227.
5. Dworkin-Rastl, E., Kandolf, H. and smith, R.C. (1994) *Dev. Biol.* 161, 4225–439.
6. Wolffe, A.P. (1989) *EMBO J.* 8, 527–537.
7. Clarke, H.S., Oblin, C. and Bustin, M. (1992) *Development* 115, 791–799.
8. Gjerset, R., Gorka, C., Hasthorpe, S., Lawrence, J.J. and Eisen, H. (1982) *Proc.Natl.Acad.Sci.USA.* 79, 2333–2337.
9. Moorman, A.F.M., De boer, P.A.J., Charles, R. (1987) *Differentiation* 35, 100–107.
10. Khochbin, S. and Wolffe, A.P. (1993) *Gene (Amst)*128, 173–180.
11. Affolter, M. , Cote, J., Renaud, J. and Ruiz-Carrillo, A. (1987) *Mol.Cell.Biol.* 7, 3663–3672.
12. Rousseau, D., Khochbin, S., Gorka, C. and Lawrence, J.J. (1991) *J.Mol.Biol.* 217, 85–92.
13. Rousseau, D., Khochbin, S., Gorka, C. and J.J. Lawrence. (1992) *Eur. J. Biochem.* 208, 775–779.
14. Breuer, B., Fischer, J. & Alonso, A. (1989) *Gene (Amst)* 81, 307–314.
15. Breuer,B., Steuer, B. and Alonso, A. (1993) *Nucleic Acids Res.* 21, 927–934.
16. Steuer, B., Breuer, B. and Alonso, A. (1992) *Biochem. Biophys. Res. Commun.* 188, 1153–1160.
17. Bouterfa, H.L., Triebe, S.M. and Doenecke, D.R. (1993) *Eur. J. Biochem.* 217, 353–360.
18. Dalton, S. and Wells, J.R.E. (1988) *EMBO J.* 7, 49–56.
19. Van Wijnen, A.J., Van Den Ent, F.M.I., Lian, J.B., Stein, J.L. and Stein, G.S. (1992) *Mol. Cell Biol.* 12. 3273–3287.
20. Rousseau, S., Renaud, J. and Ruiz-Carrillo, A. (1989) *Nucleic Acids Res.* 17, 7495–7511.
21. Higuchi, R., Krummel, B., and Saiki, R.K. (1988) *Nucleic Acids Res.* 16, 7351–7356.
22. Ho, N., Hunt, H.D., Horton, R.M., Pullen, J.K. and Pease, L.R. (1989) *Gene* 77, 51–59
23. Toyoda, T and Wolffe, A.P. (1992) *Anal. Biochem.* 203, 340–347.
24. Croston, G.E., Laybourn, P.J., Paranjape, S.M. and Kadonaga, J.T. (1992). *Gene Devl.* 6, 2270–2281.
25. Hinkley, C. and Perry, M. (1992) *Mol.Cell Biol.* 12, 4400–4411.
26. Laybourn, P.J. and Kadonaga, J.T. (1991) *Science* 254, 238–345.
27. Laybourn, P.J. and Kadonaga, J.T. (1992) *Science* 257, 1682–1685.
28. Gralla, J.D. (1985) *Proc.Natl.Acad.Sci.USA.* 82, 3078–3081.
29. Kamakaka, R.T., bluger, M. and Kadonaga, J.T. (1993) *Gene Devl.* 7, 1779–1795.
30. Almouzni, G. and Wolffe, A.P. (1993) *Gene Devl.* 7, 2033–2047.
31. Almouzni, G. Khochbin, S., Dimitrov, S. and Wolffe, A.P. (1994) *Dev. Biol.* In Press
32. Kress, H. Tönjes, R. & Doenecke, D.R. (1986) *Nucleic Acids Res.* 14, 7189–7197.
33. Brocard, M.P., Rousseau, D., Lawrence, J.J. & Khochbin, S. (1994) *Eur. J. Biochem.* 221, 421–425.