Individual anti-H1\(^0\) monoclonal antibodies were screened in an immunolocalization assay to isolate clones able to recognize H1\(^0\) in a differentiation-dependent manner using a murine erythroleukemia cell line. Two clones were selected, one recognizing H1\(^0\) only in differentiating cells (clone 27 antibody), and the other recognizing the protein constitutively (clone 34 antibody). Both antibodies recognized a restricted region of the protein located at the N-terminal part of the globular domain. Amino acids 24–30, essential for the recognition of the protein by the clone 27 antibody, are extremely conserved in all known H1\(^0\)-like proteins from sea urchin to human. Within these residues, proline 26, responsible for a bend in this region, plays a particularly important role in the epitope recognition. The region involved in the protein recognition by clone 34 antibody is larger and encompasses amino acids 20–30. However, proline 26 does not play an essential role in the structure of this epitope. Detailed analysis of the differential recognition of H1\(^0\) in chromatin during cell differentiation and proliferation suggests that the modification of chromatin structure as well as that of H1\(^0\) conformation can account for this effect. Indeed, in vitro study of H1\(^0\)-four-way junction DNA interaction showed that the N-terminal tail domain of the protein can influence the recognition of H1\(^0\) by these antibodies when the protein interacts with DNA. The two monoclonal antibodies described here therefore seem to be valuable tools for investigating fine modulations in chromatin structure and the concomitant changes occurring in the conformation of the protein.

Linker histone is an abundant basic protein present in almost all euukaryotes. The protein is involved in the formation of higher order structure in the chromatin and the maintenance of the overall chromatin compaction. In general, linker histone has a tripartite structure: a central globular domain flanked by N- and C-terminal tail domains. The globular domain binds the linker DNA and interacts with the nucleosome where DNA enters and exits the nucleosome. Unlike core histones, linker histones diverge significantly in sequence and structure.

Numerous developmentally regulated variants of linker histone have been defined. These variants can be subdivided in three major groups in vertebrates as a function of their expression during development and cell differentiation. First, an embryonic form of linker histone is present during the oogenesis and the early development in amphibians. Replication-dependent types are present in all tissues during the life of the organism, and finally the differentiation-specific group accumulates in differentiating cells. Some members of this later group are tissue- and species-specific, like histones H5 and H1t. Others, like histone H1\(^0\), are widely expressed in many tissues and in almost all vertebrates.

Previously, we have shown that there is a tight correlation between the type of linker histone expressed and the proliferative capacities of cells during early Xenopus development. Histone H1\(^0\) appears relatively late and concomitant with a dramatic decrease in the cell proliferation during the tail bud–tailpole transition period. Therefore, crucial periods in development can be characterized by a transition in the linker-histone variants within chromatin. Nothing is known concerning the role of these variants in specific organization of the chromatin structure. Immunolocalization of these proteins using specific polyclonal and monoclonal antibodies provided interesting information concerning the distribution of a given linker histone variant in the nucleus. However, chromatin organization is extremely dynamic and is subject to permanent remodeling. One of the most striking examples of this phenomenon is early embryonic development. Indeed, transition periods have been defined during development that are characterized by the modification of both chromatin constituents and the proliferative capacities of cells. Moreover, later during development and in adult tissues, chromatin remodeling continues as adult type linker histones accumulate in cells. It is therefore of great importance to understand the nature of these remodeling processes and to evaluate their role in the expression of specific genetic programs.

The aim of this work was the identification of monoclonal antibodies raised against histone H1\(^0\), showing specific abilities in recognizing this protein in chromatin. Individual anti-H1\(^0\) monoclonal antibodies were screened in an immunolocalization assay to isolate clones able to recognize H1\(^0\) in a differentiation-dependent manner. Antibodies characterized in this work appeared to be probes that are useful for monitoring chromatin structure modifications occurring concomitantly with regulatory events such as the onset of a differentiation program and the arrest of cell proliferation.

**MATERIALS AND METHODS**

**Cell Culture**—Murine erythroleukemia (MEL)\(^1\) cells from clone G9, a subclone of F4N0, were maintained in culture in minimum essential medium (Life Technologies, Inc.) containing 10% fetal calf serum (14). To induce differentiation, MEL cells in exponential growth were treated with hexamethylen-bis-acetamide (Sigma) as described previously.

Clone 6 cells, a rat embryonic fibroblast cell line transformed by ras, were maintained in RPMI 1640 (Boehringer) supplemented with 5% fetal calf serum and glutamin 4 mM and grown in a humidified atmosphere.

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Claude Gorka, Marie-Paule Brocard, Sandrine Curtet, and Saadi Khochbin‡

From the Laboratoire de Biologie Moléculaire du Cycle Cellulaire, INSERM U309, Institut Albert Bonniot, Faculté de Médecine, Domaine de la Merci, 38706 La Tronche Cedex, France

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\(^\dagger\) To whom correspondence should be addressed. Tel.: 33-4-76-54-95-83; Fax: 33-4-76-54-95-95; E-mail: khochbin@ujf-grenoble.fr.

\(^1\) The abbreviations used are: MEL, murine erythroleukemia; AA, amino acid(s).
atmosphere of 95% air, 5% CO₂, normally at 37 °C or shifted to 32 °C to induce the cell growth arrest (15).

**Purification of Nuclei and Oligonucleosomes**—Nuclei were extracted from untreated or hexamethylene-bis-acetamide-treated MEL cells. Cells were collected and washed with phosphate-buffered saline. After centrifugation at 300 x g for 5 min, nuclei were resuspended in the same buffer lacking EDTA, EGTA, and Triton (buffer D).

To obtain chromatin, nuclei were digested with micrococcal nuclease (Boehringer, 5 units/10⁸ nuclei) for 7 min at 37 °C. Oligonucleosomes were fractionated on linear sucrose gradients in 1 mM phosphate buffer, pH 7.4, 150 mM NaCl, 0.2 mM EDTA.

**Preparation of Digested and Reconstituent Histone H1**—Mouse full-length histone H1 (clone 34) cDNA (16) or cDNAs corresponding to mutated H1 were cloned in pET expression vector (Novagen). All mutations and deletions were produced according to the method described in Refs. 17 and 18. Polymerase chain reaction products were digested with NdeI and XhoI and cloned, and their sequences were verified.

The expression of the recombinant H1 was induced by 1 mM isopropanol-1-thio-β-galactopyranoside, and the protein was extracted according to the standard protocol by 5% perchloric acid. Cytochrome c oxidase cleavage of histone H1 was performed as described by Dousson et al. (19).

**Antibodies**—Anti-H1 antibodies were monoclonal antibodies produced in our laboratory as described previously (19). Antibodies used in this work were from clones 27E8E10 (clone 27 antibody) and 34B10H4 (clone 34 antibody). For immunostaining, hybridoma supernatant was used, and for gel shift assays, Igs were purified as follows. Ascites were collected in a list mode. To determine the mean specific H1 fluorescence per cell we used ProCyt®, a computer program developed in our laboratory (available on request) (24).

**RESULTS**

**Screening of Monoclonal Antibodies for H1 Recognition in Chromatin**—Previously, we have reported the preparation of monoclonal antibodies raised against histone H1 (19). In this work we undertook a screening of these antibodies for their ability to recognize the protein in chromatin. The first screening was performed using immunodetection of the protein in undifferentiated MEL cells. This work allowed us to define two clones: one (34B10H4) able to recognize H1 in chromatin and the second one (27E8E10) not able to bind the protein in this environment (both antibodies recognizing H1 in cellular or nuclear extracts with high specificity; data not shown). For simplicity, in the text we will refer to 34B10H4 and 27E8E10, as clone 34 and clone 27 antibodies, respectively. Preliminary mapping based on the recognition of peptides obtained by partial cleavage of H1 by cytochrome c oxidase cleavage of histone H1. A series of experiments were planned to map precisely the epitopes recognized by these two antibodies and to elucidate the basis for the differential recognition of the protein in chromatin.

**Precise Epitope Mapping**—Mouse H1 cDNAs encoding the wild type protein or proteins bearing mutations affecting the N-terminal tail and the globular domain were cloned into prokaryotic expression vectors to obtain purified recombinant proteins (Fig. 1B). The wild type recombinant protein was efficiently recognized by both antibodies, and moreover, the complete deletion of the N-terminal tail domain did not affect protein recognition (Fig. 1C, S20). However, the removal of 18 AA from the N-terminal part of the globular domain completely abolished the binding of these antibodies (Fig. 1C, S39). A portion of the protein covering the AA 20–38 region therefore plays an essential role in the recognition of the protein by both antibodies (Fig. 1C, S20).

A more detailed analysis of the region recognized by these antibodies (AA 20–38 region) was performed. The comparison of the sequence covering this portion of H1 from vertebrate and H16 from sea urchin (the adult type histone H1 in sea urchin, see Ref. 25) showed that in H16, the region homologous...
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Fig. 1. Region of H1° involved in the recognition of the protein by monoclonal antibodies (clone 27E8E10 and 34B10H4 antibodies). A, partial cyanogen bromide cleavage of H1°. Peptides were resolved on 15% SDS-polyacrylamide gel (stain) and transferred to a membrane, and an immunodetection was carried out using the two antibodies (right panel). B, schematic representation of the bacterially expressed H1° bearing deletions and mutations (50 N-terminal AA are considered). The lines represent the N-terminal tail domains, and the boxes represent the globular domains. The two arrowheads in the Pro-Val mutant show the region where the mutation is placed. The gap in the 824–30 mutant represents the region deleted. C, the various mutants were analyzed on 15% SDS-polyacrylamide gel (stain) and transferred to a membrane, and an immunodetection was carried out using the two antibodies and revealed by ECL system (27E8E10 and 34B10H4). WT means wild type mouse H1°, and 810, 815, 820, 838, 841, and 844 stand for proteins having 10, 15, 20, 38, 41, and 44 N-terminal AA deleted respectively. Pro-Val (B) and P-V (C) mean a mutation converting proline 26 into valine, and 824–30 indicates a protein having a deletion covering the 24–30 AA region.

Fig. 2. Involvement of the AA 20–30 region in the recognition of the protein by the two antibodies. A, the sequences of the AA 21–39 region of H1° from human (34), mouse (16), rat (35), and Xenopus (36) were compared with the sequence of the corresponding region of the sea urchin H1° (25). The identical AA are shaded. B, total H1 extracted from Xenopus and sea urchin tissues as well as from MEL cells were resolved on 15% SDS-polyacrylamide (stain) and transferred into a membrane, and an immunodetection was carried out with the two antibodies (34B10H4 and 27E8E10, respectively). C, the scheme summarizes data presented in Fig. 1 and in A and B of this figure. The AA 20–30 and AA 24–30 regions appear to be essential for the recognition of the protein by the clone 34 antibody and the clone 27 antibody, respectively. N stands for N-terminal tail domain, and G stands for the globular domain.

Recognition of the Target Epitope by Clone 34 and 27 Antibodies—The high affinity binding of H1 to four-way junction DNA (23, 28) allows the study of the specific aspects of H1-DNA interaction with the target DNA. The interaction of H1° with four-way junction DNA is shown in Fig. 3A. The interaction is specific, as shown by the fact that only clone 27E8E10 and 34B10H4 antibodies recognize the protein in Xenopus and mouse, whereas both antibodies are able to recognize the protein in sea urchin H1°. Considering the crystal structure of H5 (26), it is obvious that the proline 26 is involved in the formation of a bend in the unstructured part of the N-terminal region before the helix I. Moreover, this proline is one of the most conserved amino acids in all known H1°s (not shown, see also Ref. 27). It was therefore important to study the influence of this residue on the recognition of the protein by these antibodies.

Using site-directed mutagenesis, we changed this proline into a valine that is supposed to destroy this bend. Interestingly, this mutation abolished almost completely the recognition of the protein by the clone 27 antibody, whereas the recognition of the protein by the clone 34 antibody is not affected (Fig. 1C, P-V).

These experiments therefore allowed establishment of a precise map of the motifs recognized by these antibodies. AA 20–30 play an important role in the recognition of the protein by clone 34 antibody, whereas the AA 24–30 are sufficient for recognition by the clone 27 antibody (Fig. 2C).

Interaction of H1° with Four-way Junction DNA and the
interactions (29). We took advantage of this model to determine how the defined target epitopes are recognized by our antibodies when H10 interacts with DNA. Conditions for a complete shift of the labeled four-way junction DNA upon the addition of recombinant H10 (wild type or mutated) were determined, excesses of the clone 27 and 34 antibodies were added to the mixture, and the shift was examined. Upon the addition of the clone 34 antibody, three different cases were observed: 1) a fraction of DNA-H10 complex interacts with the antibody and is super-shifted (Fig. 3A, WT panels, lanes +H10+Ab); 2) a fraction of DNA-H10 complex is not recognized by the antibody; and 3) a fraction is dissociated as indicated by the release of free DNA.

To discover whether the N-terminal tail of the protein can...
influence the recognition and the dissociation of the H1<sup>0</sup>-fourway junction DNA complex by these antibodies, we used H1<sup>0</sup> mutant lacking the 10 and 20 AA from the N-terminal end of the protein. The removal of the N-terminal 10 or 20 AA facilitated the dissociation of the H1<sup>0</sup>-DNA complex by the clone 34 antibody without significantly increasing the amount of super-shifted materials (Fig. 3A, 34B10H4, panels −10 and −20, lanes +H1<sup>0</sup>+Ab). Conversely, these mutations affected the formation of the ternary complex by the clone 27 antibody. Indeed, a decrease in the amount of the super-shifted material is observed (Fig. 3A, 27E8E10, panels −10 and −20, lanes +H1<sup>0</sup>+Ab). Moreover, the dissociation of the H1<sup>0</sup>-DNA complex by this antibody is also less efficient. As a control, we show that when the proline-valine mutant is used, clone 27 antibody is not able to supershift the complex nor to dissociate it, whereas clone 34 antibody (which is able to recognize this protein) supershifts and dissociates the complex (Fig. 3B, panel Pro-Val, lanes +H1<sup>0</sup>+Ab34 and +H1<sup>0</sup>+Ab27). The 824−30 mutant is able to interact with the DNA, but the addition of the described antibodies does not affect the H1<sup>0</sup>-DNA complex. The use of these mutants showed also that the supershifted materials observed upon the addition of antibodies is highly dependent on the nature of H1<sup>0</sup> and is not due to the association of DNA with the antibody or some other components present in the reaction. These observations suggest that the shortening of the N-terminal part of the protein, nonessential for the recognition of the free protein, renders the dissociation of the complex by the clone 34 antibody more efficient, although it does not significantly affect that mediated by the clone 27 antibody.

**Differential Recognition of H1<sup>0</sup> within Chromatin during the Induced Differentiation of MEL Cells**—MEL cells are virus-transformed erythroid precursors able to undergo a differentiation program under the action of a large variety of chemical inducers (30). We used this differentiation model to monitor H1<sup>0</sup> recognition by our antibodies during cell differentiation. Uninduced MEL cells or cells treated with the inducer (4 mM hexamethylene-bis-acetamide) for 6, 8, 16, 24, 32, and 48 h were fixed, and the immunofluorescence was monitored by flow cytometry after immunostaining with clone 27 and 34 antibodies. In uninduced MEL cells, whereas H1<sup>0</sup> is efficiently recognized by the clone 34 antibody, the protein is not recognized by clone 27 antibody (Fig. 4A, 0 h). In these cells, clone 27 antibody-related immunofluorescence corresponds to the background fluorescence, which is observed when anti-H1<sup>0</sup> antibody is omitted (not shown).

Clone 34 antibody-related immunofluorescence intensity changes between 0 and 6 h after induction (Fig. 4A, 6 h; a broader distribution of the immunofluorescence is observed). An accumulation of the protein during this period (31) can contribute to this increase in immunofluorescence intensity. However, despite this accumulation of H1<sup>0</sup>, clone 27 antibody is not able to detect the protein in chromatin after 6 h of induction (compare 0 and 6 h, Fig. 4A). An increase in clone 27 antibody-related immunofluorescence is visible after 8 h of induction characterized by a broader distribution of cells along the immunofluorescence axis. The onset of the differentiation program as judged by the initiation of α-globin mRNA accumulation is also observed after 8 h of induction (Fig. 4B). Another increase in the immunofluorescence intensity is observed between 32 and 48 h of induction, essentially visible for clone 27 antibody.

To know if the differential recognition of H1<sup>0</sup> by these antibodies described above is indicative of a modification of chromatin structure (a change of accessibility), we fixed nuclei isolated from uninduced MEL cells after incubation in a buffer containing increasing concentrations of NaCl and performed immunodetection of H1<sup>0</sup> as above. When nuclei were fixed after a treatment with 200 mM NaCl, a clear increase in the clone 34 antibody-related immunofluorescence is observed compared with nuclei fixed at 100 mM NaCl. Clone 27 antibody immunoreactivity did not change significantly in such conditions (Fig. 4C, 200 mM NaCl, note that the clone 34 and 27 antibody-related immunofluorescence was recorded at the basal level to better visualize the increase of the immunofluorescence intensity after the salt treatment). When the nuclei were prepared in the presence of 300 mM NaCl, the recognition of the protein by both antibodies is enhanced. These data indicate that the recognition of H1<sup>0</sup> in chromatin by clone 34 antibody is more sensitive to chromatin structure modification than that of the clone 27 antibody.

It would be interesting to know whether the differential recognition of the protein by these antibodies can also be observed on fractionated chromatin. Nuclei from both uninduced cells and cells induced for 48 h were digested by micrococcal nuclease, and chromatin fragments were fractionated on a sucrose gradient (Fig. 5A). A comparable amount of chromatin from each fraction was loaded on a filter in duplicate using a dot blot apparatus (Fig. 5B). One blot was incubated with clone 34 antibody, and the other was incubated with clone 27 antibody, and recognition of H1<sup>0</sup> was monitored by the ECL system.
Histone H1\(^0\) Accumulation and Chromatin Remodeling

**Fig. 6.** Cell cycle-dependent modulation of the chromatin structure during the induced arrest of cell proliferation. Clone 6 cells expressing thermosensitive P53 were shifted to 32 °C for the indicated times (0, 8, 16, and 24 h), and the control cells were maintained at 37 °C during this period. A, flow cytometric analysis of the immunodetection of H1\(^0\) was carried out as described in the legend of Fig. 4, except in this case cells are doubly stained for H1\(^0\) by indirect immunofluorescence and for DNA with the DNA-specific dye Hoechst 33258. This method (dual analysis of cells by cytofluorimetry; dot plot representations shown in the left panel for each temperature) allows monitoring of H1\(^0\) immunoreactivity as a function of the position of cells in the cell cycle. For each temperature, the modification of the cell cycle parameters as a function of time is visualized in histograms shown on the right side. These histograms represent the number of cells present at different positions in the cell cycle (DNA fluorescence). B and C, the induced arrest of cell proliferation is not associated with an increase in the amount of H1\(^0\) encoding mRNA and protein. RNA prepared from cells maintained at 37 °C or shifted to 32 °C was analyzed according to the Northern blot procedure. The blot was probed with a H1\(^0\), H4, or 28 S rRNA probe (B). Total H1 was extracted from proliferating cells (0 h) or after the shift of temperature (8, 16, and 24 h). Proteins were analyzed on SDS-15% polyacrylamide gels stained with Coomassie Blue and by Western blotting.

As a control, different amounts of purified H1\(^0\) were also loaded on each filter (Fig. 5B, purified H1\(^0\) panel). Fig. 5B shows that as expected the clone 27 antibody did not recognize the protein in chromatin of uninduced cells (27E8E10, 0 h lane), whereas the clone 34 antibody recognized the protein efficiently (34B10H4, 0 h lane). The purified protein was recognized with an equal efficiency by both antibodies (purified protein panel). Interestingly, 48 h after the induction of cell differentiation, clone 27 antibody was able to recognize H1\(^0\) in the chromatin (27E8E10, 48 h lane). Signals corresponding to the recognition of the protein by clone 34 antibody is also more intense for this chromatin (34B10H4, compare the 48 h lane with the 0 h lane). The same blots were then washed, and the immunodetection of histone H1 was performed using polyclonal anti-H1 antibodies. Fig. 5B (anti-H1 panel) shows that histone H1 was recognized efficiently in each fraction and proved that a comparable amount of chromatin was loaded on the two blots.

**Modification of the H1\(^0\) Recognition during the P53-mediated Arrest of Cell Proliferation—**Clone 6 cells are rat embryonic fibroblasts transformed by ras and a thermosensitive P53 mutant. At 37 °C, P53 is in a mutated conformation that is responsible for the appearance of a transformed phenotype. At 32 °C, P53 exhibits the property of the wild type protein and triggers an arrest of cell proliferation (15). We used this system to monitor H1\(^0\) accessibility during this process. A flow cytometric analysis of H1\(^0\) immunolabeling using clone 34 antibody was performed (Fig. 6A). H1\(^0\) was detected by indirect immunofluorescence (y axis) and DNA by Hoechst fluorescence (x axis). DNA fluorescence reflects the position of cells in the cell cycle (G1 cells are around channel 60, G2 cells are found around channel 120, and S phase cells are in between). A general increase of H1\(^0\) immunofluorescence is observed during the cell cycle indicating the normal doubling of cell constituents when cells accomplish DNA replication and enter the G2/M phases of the cell cycle (Fig. 6A, panel 37 °C). However, 8 h after the transfer of cells at 32 °C, a clear increase in the H1\(^0\) immunofluorescence intensity was observed, specifically visible in the G2/M cell populations (32 °C panel, dot plot representation, compare 0 and 8 h). It is precisely in this phase of the cell cycle that the first accumulation of cells is observed (Fig. 6A, note the accumulation of cells in the G2/M phase of the cell cycle, 32 °C panel, 8 h histogram). After 16 h at 32 °C, these cells enter the G0 phase and stop proliferating. An increase in the H1\(^0\) immunofluorescence is visible in these arrested cells (panel 32 °C, lane 16 h). After 24 h at 32 °C, almost all cells are in the G0/G1 phase of the cell cycle, and a clear increase of the H1\(^0\) related immunofluorescence intensity is observed in these cells compared with the control cells kept at 37 °C (panel 32 °C, lane 24 h). Clone 27 antibody did not recognize H1\(^0\) either in proliferating cells or in arrested cells (data not shown).

RNA was also prepared from these cells and used to obtain a Northern blot. The hybridization of the blot with a H1\(^0\) probe
showed that no significant variation in mRNA content can be observed during this process and that the hybridization with a H4 probe confirmed the kinetics of cell arrest at 32 °C observed by cytofluorimetric analysis (Fig. 6B).

The steady state level of H10 in proliferating cells (37 °C) or cells kept at 32 °C for different times did not show a significant variation (Fig. 6C), confirming the Northern blot data. The increase of the immunofluorescence observed is therefore essentially due to a modification of the immunoreactivity of H10 toward the clone 34 antibody during the arrest of cell proliferation.

Differential Pattern of H10 Immunolocalization by Clone 27 and Clone 34 Antibodies—To know if the differential recognition of H10 by these antibodies correlates also with a specific pattern of immunodetection, we performed a microscopic analysis of immunolabeled nuclei. *Xenopus* embryos were first used in this experiment to examine the situation in an *in vivo* context. H10 accumulates relatively late during the *Xenopus* development, and the first detectable accumulation of the protein is tissue-specific, observed in the nervous tissue, somites, and the cement gland. Later during development, at tadpole stage, the accumulation of the protein was observed in many different tissues (10). The analysis of the immunolabeled cells observed on a section of the cement gland (for example, Fig. 7A, arrowhead) shows that nuclei are immunolabeled with both antibodies and that, moreover, foci could be observed in clone 27 antibody-immunolabeled nuclei (Fig. 7B, bottom left panel) in contrast to a relatively homogenous labeling for nuclei labeled by the clone 34 antibody. Nuclei of cells forming the neighboring tissue were negative for H10 detection (Fig. 7B, compare the anti-H10 column with the Hoechst column). The same pattern of immunofluorescence was observed when we examined the pattern of clone 27 and 34 antibody immunolabeling in nuclei of differentiated MEL (data not shown). These observations suggest that the recognition of H10 by clone 27 antibody occurs only on restricted regions that could be sites of specific chromatin remodeling.

**DISCUSSION**

In this work we have precisely mapped a region of histone H10 located at the entry of the globular domain and involved in the recognition of the protein by two monoclonal antibodies. One of them, clone 34 antibody, recognizes the protein dependent on the AA 20–30 region. Recognition by the second antibody (clone 27 antibody) has been shown to be dependent on only 7 AA (AA 24–30) within this region. Moreover, an essential role of the proline 26 has been illustrated by site-directed mutagenesis. Indeed, the replacement of this proline by a valine completely abolished the recognition of the protein. These data suggest that the proline-mediated structure is important for the recognition of the protein by this antibody. Interestingly, this antibody does not recognize H10 within the chromatin of undifferentiated MEL cells. However, after the commitment in the differentiation program, H10 becomes recognizable. The AA 20–30-dependent recognition of the protein by clone 34 antibody is efficient in uninduced as well as in differentiated MEL cells.

Two explanations can be proposed for the differential recognition of H10 by the clone 27 antibody during cell differentiation. First, modification of the chromatin structure in differentiated cells can render H10 accessible to this antibody. However, clone 34 antibody, for which the recognition of H10 is also highly dependent on the AA 24–30 region, binds the protein in uninduced cells as well as in differentiated cells. Therefore, a simple modification of the accessibility of the 24–30 region cannot satisfactorily account for the differentiation-dependent reactivity of H10 toward the clone 27 antibody. The proline-valine replacement experiment suggests that recognition by clone 27 antibody could be dependent on a structure in the AA 24–30 region. Therefore, a modification of the structure of the N-terminal domain of H10 occurring during the induced differentiation of MEL cells could be a reason for the observed differential recognition by the clone 27 antibody.

The importance of the N-terminal tail of H10 in the recognition of the protein by our antibodies is also suggested by analyzing their ability to recognize H10-four-way junction DNA complex in *vitro*. At least two different kinds of H10-DNA complexes have been found. One is able to interact with the antibody and is supershifted in a gel retardation assay. In the rest of the population of H10-DNA complexes, the addition of the antibody creates a competition between the antibody and DNA for interaction with H10. This competition is accompanied

![Image](322x297 to 549x729)

**FIG. 7. Immunostaining of H10 by the clone 27 and 34 antibodies in *Xenopus* embryos.** A, *Xenopus* embryos were taken at the stage 36 of development and fixed, and cryosections were obtained. Two successive sections were subjected to immunostaining with the two anti-H10 antibodies and counterstained with Hoechst, which allows the detection of all nuclei (nuclei are shown from one section at low magnification). The white arrowhead shows the cement gland that is also shown at high magnification in B. B, consecutive 10-µm sections obtained from stage 36 embryos were used for the immunodetection of H10 by the clone 34 and 27 antibodies (left panels) and counterstained with the DNA-specific fluorochrome Hoechst 33258 (right panels).
with a release of DNA from the complex. The observed displacement of the DNA is enhanced when the N-terminal tail is shortened. The removal of 10 AA or the whole N-terminal tail domain (−20) facilitated greatly the displacement of DNA by the clone 34 antibody. This facilitated displacement could be due to a decrease in the strength of DNA-protein interaction or to a better recognition of the protein by the antibody. The first possibility is unlikely because the removal of 10 AA, although it dramatically increases the dissociation of the H10-DNA complex, does not eliminate any of the 58 lysine/arginine residues present in the protein or any of the conserved residues showed to be involved in the interaction of H1 with DNA (29). Moreover, the shortening of the N-terminal tail domain does not affect the clone 27 antibody-dependent dissociation of the complex. However, it influences the formation of the ternary complex.

These data suggest that the N-terminal tail domain of histone H10, which is nonessential for the recognition of the free protein by our antibodies, can influence the protein recognition when it interacts with DNA and strengthens the possibility of a differential recognition of the protein in chromatin due to a modification of the N-terminal tail conformation during critical stages of cell life.

A different pattern of the immunolabeling is also observed when we compared clone 34 and clone 27 antibody immunostained nuclei. Clone 27 antibody is able to reveal foci of immunoreactivity within the nuclei. Under the same conditions clone 34 antibody shows a more homogenous nuclear labeling. The appearance of foci after the immunolabeling by clone 27 antibody is observed in different Xenopus tissues, as well as in cells in culture (not shown). Because in MEL cells, labeling by clone 27 antibody is differentiation-dependent, one can assume that these foci of H10 immunolabeling correspond to sites of specific chromatin remodeling, rendering the N-terminal part of H10 recognizable by the clone 27 antibody.

The salt treatment experiment (Fig. 4C) showed that the recognition of H10 by clone 34 antibody is more sensitive to modification of chromatin structure than that of clone 27 antibody. Clone 34 antibody shows an enhanced H10 recognition after the P53-mediated arrest of cell proliferation, whereas in these cells (cycling or arrested), H10 is not recognized by clone 27 antibody. These observations suggest that chromatin remodeling events of a different nature are associated with cell arrest and differentiation.

Several reports described the use of monoclonal and polyclonal antibodies raised against different parts of histone H1, H5, and H10, as well as the use of immobilized proteases to investigate the accessibility of the linker histones in chromatin. The majority of these works showed a lower accessibility of the globular domain compared with the N- and C-terminal tail domains of linker histone in the chromatin (32, 33). Therefore, recognition of H1s by antibodies raised against the globular domain of the protein are expected to be much more sensitive to different chromatin remodeling events than that of antibodies raised against tail domains. The work presented here shows that modifications of the chromatin structure occur at precise periods during different important cellular events, such as the commitment of cells in a particular differentiation program or the arrest of cell proliferation. The clone 27 antibody can immuno-label H10 in MEL cells precisely at the onset of β-globin gene expression during the induced differentiation. This observation shows that a modification of the chromatin structure occurs concomitant with the reinitiation of erythropoiesis.

The clone 34 antibody allowed us to show a modification of the chromatin structure occurring during a restricted period of the cell cycle before the arrest of cell proliferation. The described antibodies therefore seem to be valuable tools for monitoring precise timing of chromatin remodeling associated with different regulatory events controlling the cell fate and enable investigation of these modifications in more detail.

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