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ARTICLES

Downregulation of Histone H4 Gene Transcription During Postnatal Development in Transgenic Mice and at the Onset of Differentiation in Transgenically Derived Calvarial Osteoblast Cultures

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Abstract In vivo regulation of cell cycle dependent human histone gene expression was examined in transgenic mice using a fusion construct containing 6.5 kB of a human H4 promoter linked to the chloramphenicol acetyltransferase (CAT) reporter gene. Transcriptional control of histone gene expression, as a function of proliferative activity, was determined. We established the relationship between DNA replication dependent H4 mRNA levels (Northern blot analysis) and H4 promoter activity (CAT assay) during postnatal development in a broad spectrum of tissues. In most tissues sampled in adult animals, the cellular representation of H4 gene transcripts declined in parallel with promoter activity. This result is consistent with transcriptional control of H4 gene expression at the cessation of proliferation. Interestingly, while H4 mRNA was detectable at very low levels post-proliferatively in brain, promoter activity persisted in adult brain, where most of the cells are terminally differentiated. This dissociation between histone gene promoter activity and histone mRNA accumulation points to the possibility of post-transcriptional regulation of histone gene expression in brain. Cultures of osteoblasts were prepared from calvaria of transgenic mice carrying the H4 promoter/CAT reporter construct. In contrast to the brain, in these bone-derived cells, we established by immunohistochemistry that the transition to the quiescent, differentiated state is associated with a transcriptionally mediated downregulation of histone gene expression at the single cell level.

Key words: CAT assays, histone gene expression, H4 promoter activity, proliferating osteoblasts, transcriptional regulation

The execution of multiple cell-specific gene regulatory programs during cell growth and differentiation in vertebrates necessitates precise spatial and temporal constraints on gene expression. This results in the selective expression of unique subsets of genes associated with tissue-specific phenotypes. One strategy to addressing the extent to which transcriptional and post-transcriptional events are operative during in vivo development is to use the incorporation of chimeric gene constructs into the vertebrate germline [Jaenisch, 1988]. Expression of cell cycle controlled histone genes is tightly coupled with DNA replication. Cellular representation of histone mRNAs transcribed from the five multicopy histone gene classes (H1, H2A, H2B, H3, and H4) and biosynthesis of the encoded proteins coordinately parallels the extent of DNA replication. This has been established in a broad spectrum of mammalian cell culture systems [Stein et al., 1984; Osley, 1991]. Because of the relationship between histone gene expression and cell division, understanding histone gene regulation is instrumental in assessing proliferation-specific gene regulation throughout the lifespan of vertebrates.

The coupling of histone gene expression with DNA replication and cell proliferation is both transcriptionally and post-transcriptionally mediated [Stein et al., 1992; Marzluff and Pandey, 1988; Osley, 1991] and includes a number of cellular processes that regulate and maintain cellular histone H4 mRNA content. Transcrip-

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tional regulation of histone genes plays a key role in the modulation of histone mRNA levels during the cell cycle, and contributes to cessation of histone gene expression at the onset of differentiation [Stein et al., 1992]. Post-transcriptional mechanisms for regulated cellular histone H4 content include 3′ end processing events resulting in the production of mature cytosolic histone mRNAs from nuclear precursor RNAs [Stauber and Schumperli, 1988; Vasserot et al., 1989; Harris et al., 1991; Eckner et al., 1991], targeted subcellular localization [Zambetti et al., 1987; Zambetti et al., 1991], and cytosolic mRNA destabilization by a 3′ exonucleolytic degradation pathway [Peltz and Ross, 1987; Morris et al., 1991]. Transcription of multiple histone genes involves a limited number of shared cis-acting elements [Sierra et al., 1983; Kroeger et al., 1987; LaBella et al., 1988, 1989; Ramsey-Ewing et al., 1992; Dalton and Wells, 1988a; Hwang and Chae, 1989] that function as protein/DNA interaction sites [Pauli et al., 1987] for both ubiquitous and proliferation-specific transcription factors [Fletcher et al., 1987; Dailey et al., 1988; Gallinari et al., 1989; Dalton and Wells, 1988b; Mitchell and Tjian, 1989; Lee et al., 1991; Tung et al., 1989; Sharma et al., 1989; van Wijnen et al., 1988, 1989, 1991b–d]. In addition, coordination of histone gene expression in mammals is controlled post-transcriptionally via a unique histone mRNA-specific stem-loop structure that is involved in 3′ end processing and turnover [Marzluff and Pandey, 1988]. Thus, the construction of chimeric genes containing histone gene promoter sequences in which the histone mRNA coding region and 3′ flanking sequences are substituted by a reporter gene, selectively eliminates the histone-specific post-transcriptional component of control. Studying the expression pattern of the introduced histone promoter/reporter gene fusion construct in conjunction with endogenous histone gene expression during development in transgenic mice provides the basis for assessing the importance of transcriptional control in the intact animal.

Gene regulatory 5′ flanking sequences are primary determinants for establishing accurate expression of exogenous genes in transgenic mice, albeit that chromosomal location of the integrated gene may influence transcriptional patterns [Wilson et al., 1990]. Recently, we have provided evidence that the initial 6.5 kB of flanking region (designated F3 promoter) of the human histone H4 gene FO108 confers downregulation of reporter gene expression during hepatic development in several lines of transgenic mice, coincident with the onset of in vivo quiescence and differentiation in liver [van Wijnen et al., 1991a]. The F3 promoter contains a complex array of protein/DNA interaction sites [Stein et al., 1992] positioned in close proximity of the mRNA initiation site [Pauli et al., 1987; van Wijnen et al., 1989, 1991d], as well as distally located cis-acting elements [Helms et al., 1987; Kroeger et al., 1987; van der Houven van Oordt et al., 1992]. This promoter also includes a series of nuclease hypersensitive regions [Chrysogelos et al., 1985; Moreno et al., 1986] and a putative nuclear matrix attachment site [Dworetzky et al., 1992]. Thus, inclusion of the entire histone gene control region ensures that the F3 promoter contains sufficient regulatory information and conformational determinants to minimize ectopic expression of the reporter gene.

Previously, we have shown for three different reporter genes driven by the F3 promoter that expression in several tissues is developmentally regulated and reflects the level of cell proliferation [van Wijnen et al., 1991a]. However, the low level of reporter gene expression prohibited quantitative assessment of tissue-related histone gene promoter activity during postnatal development. Recently, it has been shown that the inclusion of introns increases levels of reporter gene expression [Choi et al., 1991; Palmiter et al., 1991]. In this study, we have utilized transgenic strains with an enhanced reporter gene construct (F3-SiCAT) [Choi et al., 1991], containing a generic intron (Si) and the chloramphenicol acetyl transferase (CAT) mRNA coding sequence directed by the histone H4 promoter (F3), to analyze the role of histone gene transcriptional regulation during postnatal development in multiple tissues by assaying CAT activity in whole tissue preparations. Moreover, we have determined transcriptional activity at the single cell level in primary cultures of osteoblasts from calvaria of transgenic mice undergoing development of the bone-cell phenotype by in situ antibody binding. Our results clearly suggest that the human histone H4 promoter directs a proliferation-specific pattern of reporter gene expression both in the intact animal, during natural postnatal development, as well as in vitro during proliferation and differentiation of cultured primary osteoblasts derived from transgenic mice. These results indicate

that transcriptional control is a principal mechanism operative in the developmental regulation of histone gene expression at both the cell and tissue levels.

MATERIALS AND METHODS

Generation of Transgenic Mice

The H4 promoter F3-SiCAT chimeric reporter gene construct is composed of the initial 6.5 kB of histone promoter, 5′ untranslated leader sequences containing a generic intron, CAT coding sequences, and the SV40 late polyadenylation signal (Fig. 1a) [Choi, 1990; Choi et al., 1991]. This construct was introduced into the mouse germline [Gordon and Ruddle, 1983; Hogan et al., 1986] after linearization by restriction enzyme cleavage and microinjection into eggs from superovulating FVB/N mice [Choi, 1990]. Fertilized eggs were transferred into oviducts of pseudopregnant FVB/N mice. The strains of F3-SiCAT containing mice that resulted from this procedure (F3-SiCAT 1, F3-SiCAT 7, and F3-SiCAT 9) were constructed in collaboration with Drs. Theodore Choi and Rudolf Jaenisch (Whitehead Institute, Massachusetts Institute of Technology, Cambridge) [Choi et al., 1991; van Wijnen et al., 1991a]. The resulting mice were initially mated with FVB/N background mice and were screened for the presence of the transgene by Southern blot analysis. Positive mice representing the heterozygous founder class offspring (P) were subjected to crossbreeding (Fig. 1b) to select for animals containing the transgene. Presence of the transgene was established in the F4 generation in two out of three strains (Fig. 1c: Southern blot analysis of putative F3-SiCAT homozygotes).

Preparation and Analysis of Mouse Genomic DNA

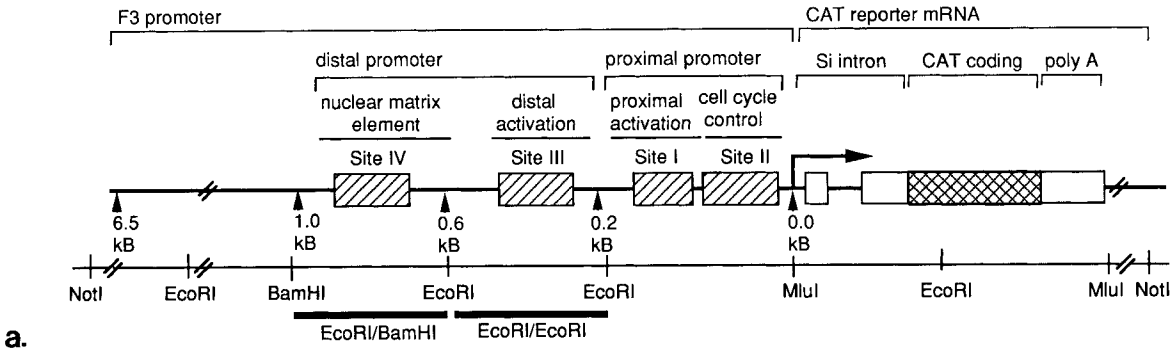
DNA isolated from 1 cm tailtips of 4-week-old mice was digested with EcoRI, electrophoretically separated in 1% agarose gels, and analyzed by Southern blotting after alkaline capillary transfer according to standard procedures [Ausubel et al., 1987; Sambrook et al., 1989]. Unique 5′ flanking DNA sequences (390 bp EcoRI/EcoRI or 475 bp EcoRI/EcoRI fragment of pFO002) [Kroeger et al., 1987] of the human F3 promoter were used as a probe. Prehybridization and hybridization was performed in 7% SDS, 0.5 M NaH_2PO_4, pH 7.4, and 1 mM EDTA at 65°C. After two consecutive washes (30 min at 65°C) with 5% SDS, 0.04 M NaH_2PO_4, pH 7.2, and 1 mM EDTA, the membrane was analyzed using a BetaScope 603 (Betagen Corp., Waltham, MA) or by autoradiography and laser densitometry (LKB 2400 UltroScan XL) (Pharmacia LKB Biotechnology, Piscataway, NJ).

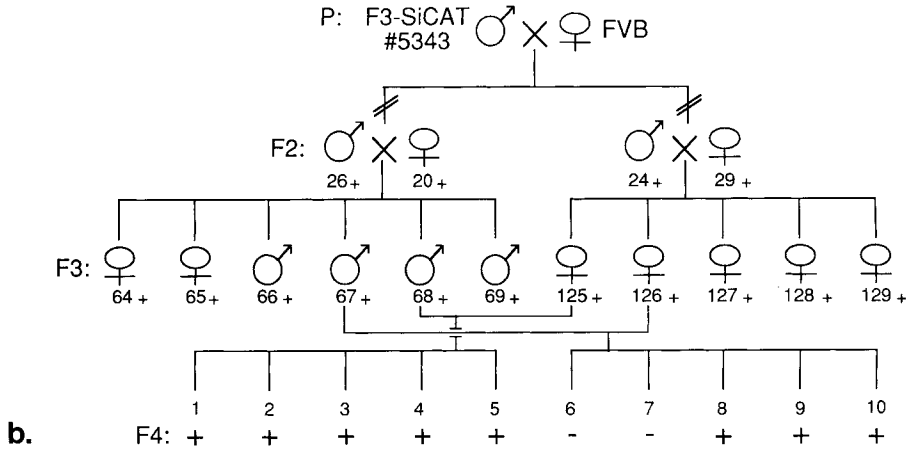
RNA Preparation and Northern Blot Hybridization Analysis

RNA samples were isolated from the same tissues that were used for CAT assays (see below), permitting a direct comparison of cellular histone mRNA levels and histone gene transcription rates. Total cellular RNA was prepared as described previously [Shalhoub et al., 1991]. Frozen samples were disrupted in 5 M guanidine thiocyanate, 2% sarcosyl, and 72 mM mercaptoethanol using a Brinkman polytron at 20,000 rpm and centrifuged for 15 min at 12,000*g* using a Beckman J2-21 centrifuge using a JA-20 rotor (12,000 rpm). The supernatant was purified by centrifugation over a cesium-chloride cushion for >18 h at 126,444*g* in a Beckman L5-65 centrifuge using a SW 41 rotor (36,000 rpm). Translucent pellets were recovered and total cellular RNA quantitated by 260 nm absorbance. Aliquots (12 μg) were electrophoretically fractionated in 1% agarose gels containing 6.6% formaldehyde. The intactness of the RNA preparations was monitored by representation of ribosomal RNA (28S and 18S) after ethidium bromide staining. For Northern blot analysis, RNA was transferred to Zetaprobe nylon (capillary transfer; >24 h) and attached to the membrane by UV crosslinking. Prehybridization and hybridization were performed overnight in 50% formamide, 5× SSC (20× SSC is 0.3 M sodium chloride, 0.3 M sodium citrate, 10× Denhardt's solution (100× Denhardt's solution is 2% Ficoll, 2% polyvinylpyrrolidone, 50 mM sodium phosphate, pH 6.5, 1% SDS, 250 μg/ml *Escherichia coli* DNA) for 24 h at 43°C. Blots were washed three times with 2× SSC/0.1% SDS, twice with 1× SSC/0.1% SDS, and once with 0.1× SSC/0.1% SDS at 65°C. Quantitation was performed by analysis of blots on a BetaScope 603 (Betagen Corp.) or by exposure to preflashed X-ray films (XAR-5; Eastman Kodak Co., Rochester, NY) using a Cronex Lightning Plus followed by scanning laser densitometry (LKB 2400 UltroScan XL).

CAT Assays

The same tissues used for RNA analysis were quickly isolated and rapidly frozen in liquid ni-





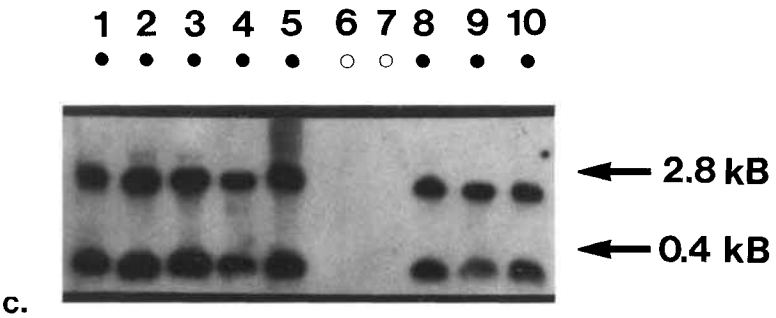


Fig. 1. **a:** Diagram of the F3-SiCAT chimeric histone gene promoter/reporter gene construct. The 6.5 kB promoter region (F3) of the H4 gene FO108 is depicted in detail. Indicated are a putative nuclear matrix attachment site and a series of cis-acting elements and trans-acting factors involved in transcriptional regulation of this gene (reviewed in Stein et al., 1992). The intron containing fragment (Si), the CAT coding region (CAT), and the polyadenylation signal (poly A) containing fragment. Not indicated are nuclease hypersensitive regions in both proximal and distal regions of the promoter (Chrysogelos et al., 1985; Moreno et al., 1986). Also depicted are restriction enzyme sites (EcoRI, BamHI, NotI, MluI) and two promoter fragments (390 bp EcoRI-EcoRI, 475 bp EcoRI-BamI) that were used as probes for Southern blot analysis. **b:** Strain breeding records documenting generation of mice containing at least one copy of the transgene based on Southern analysis. **c:** Southern blot analysis of genomic DNA. DNA was isolated from mouse tail segments, digested with EcoRI, and probed with two different fragments of the human histone H4 promoter (F3) to test mice for the presence of the transgene (black dot); e.g., lanes 6 and 7 contain DNA from mice that do not contain the transgene. The numbers above the lanes refer to mice from the F4 offspring shown in Figure 5b.

trogen. Frozen specimens were disrupted at 20,000 rpm in 1 ml of 0.25 M Tris (pH 7.8) using a polytron (IKA-works, Cincinnati, OH). After incubation for 15 min at 65°C to inactivate endogenous acetylases, reaction cocktails containing 80 μg of total protein (Bradford assay: Bio-Rad, Richmond, CA) were incubated at 37°C for 1.5 h. After extraction with ethylacetate, samples were spotted onto thin layer chromatography (TLC) plates and chromatographed for 1.5 h in 30% methanol and 70% chloroform. Plates were quantitated using a BetaScope 603. The levels of CAT activity in tissues were normalized to percent of arbitrary units detected with spleen preparations. For example, if 80 μg of total protein from spleen was incubated, we found that approximately 30–32% of the chloramphenicol became acetylated, and we have set this level of CAT activity to the equivalent of 100 arbitrary spleen units.

Immunohistochemical Labeling

Cells containing CAT protein were stained using unconjugated rabbit anti-CAT antibody (5Prime-3Prime, Inc., Boulder, CO). Cultured osteoblasts derived from transgenic mice were fixed in 4% paraformaldehyde for 2 h on ice, rinsed with Tris-saline (NaCl = 0.9%, pH 7.4), and incubated for 1 h in normal goat serum (ABC kit, Vector Laboratories, Burlingame, CA). Sections were washed in Tris-saline and incubated (overnight at 4°C) with anti-CAT antibody (dilution in Tris-saline = 1:20,000 to 1:10,000). The cells were again washed and serially incubated with biotinylated goat anti-rabbit antibody (2 h) and avidin-biotin complex to which horseradish peroxidase was coupled (2 h, Vector Laboratories). The samples were stained with diaminobenzidine and 1/1000th hydrogen peroxide for 15 min. Tissues were dehydrated (consecutive treatments for 10 min with, respectively, 70%, 95%, and 100% ethanol) and slides dipped into xylene before mounting coverslips. Immunolabeling was less or not detectable above background when cells did not contain specific antigens (nontransgenic mice were used as control) or when the primary or secondary antibodies were omitted. The same procedure using higher antibody concentrations (e.g., 1:100) produced the same results. Cells were harvested at days 1–7 and day 9.

RESULTS AND DISCUSSION

Downregulation of Endogenous Histone Gene Expression in Multiple Tissues During Postnatal Development

To establish the extent to which histone genes are expressed during postnatal development, we examined the cellular levels of histone H4 mRNA in multiple tissues at several development stages throughout most of the lifespan (up to 1.5 years). The level of H4 mRNAs as detected by Northern blot analysis (Fig. 2) represents transcripts from several members of the H4 multicopy gene family [Lichtler et al., 1982]. We have previously shown by S1 protection analysis that these H4 gene transcripts are regulated in parallel with the DNA replication dependent mouse H4 gene we have designated H4-AST [van Wijnen et al., 1991a], which is transcribed in a cell cycle controlled manner [Seiler-Tuyns and Paterson, 1987]. Based on the well-documented coupling of cell cycle regulated histone H4 gene expression, DNA replication, and cell proliferation, this result suggests that H4 mRNA levels are indicative of the level of cell proliferative activity.

H4 gene transcripts in spleen derived from mice ranging from newborn (day 1) to 1.5 years are present in consistently higher abundance than in liver, kidney, and brain (Fig. 2a). We observed that although the absolute level of total H4 mRNA decreases throughout the lifespan of the animal, the ratio between the different tissues did not show a significant difference. Therefore, we normalized the data relative to the H4 mRNA level in spleen of newborn mice (expressed as arbitrary spleen units) (Fig. 2b). There is a 95% decrease in H4 mRNA levels in spleen of adult (1.5 years) compared to newborn (day 1) mice. In contrast, the abundance of H4 mRNAs in other tissues, including liver and kidney, was low in young mice and declined to barely detectable levels during later stages of cell and tissue development. For example, kidney H4 mRNA levels were consistently higher (or the same) than those found in liver, and declined from 35% to undetectable levels compared to spleen H4 mRNA. H4 mRNA was also present in brain, although levels were more variable during the early stages of postnatal development, and ultimately decreased to below the level of detection in older animals (see Fig. 2b). The low levels of histone mRNA in adult liver, kidney, and brain which mostly contain

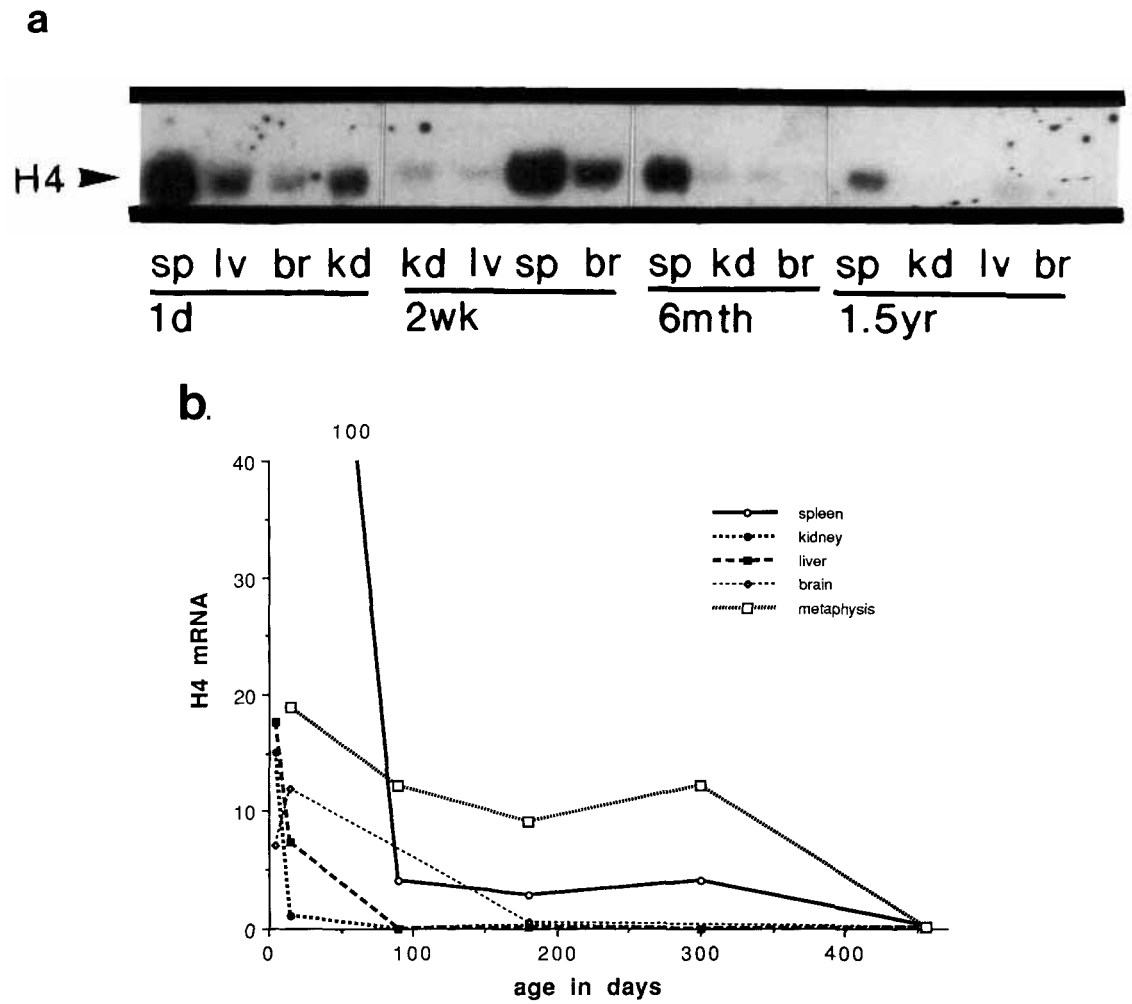


Fig. 2. **a:** Northern blot analysis of RNA samples from several mouse tissues isolated at four representative ages reflecting the murine lifespan. In each case, the hybridization signals were obtained using 10 μg total cellular RNA from liver (lv), kidney (kd), spleen (sp), and brain (br) from mice at the indicated ages (1 day, 2 weeks, 6 months, and 1.5 years). The same tissues were also analyzed for CAT activity (see Fig. 3). **b:** Quantitation of mRNA levels during postnatal development. H4 mRNA levels observed with all samples were normalized relative to the representation of H4 mRNA in spleen on day 14. The results presented in the graph show the downregulation of H4 gene expression in spleen, kidney, liver, brain, and the metaphyseal region of the femur as a function of age.

quiescent and differentiated cells reflect limited cell proliferative activity in these tissues (e.g., related to replacement of parenchymal and endothelial cells or stromal and glial cell proliferation). However, the high levels of H4 mRNA in spleen are consistent with active lymphocyte proliferation, particularly during the early period of the murine lifespan.

Hence, this result shows that endogenous H4 gene expression decreases postnatally in several tissues. This decline reflects the decreasing representation of heterogeneous populations of proliferating cells in maturing tissues occurring concomitant with completion of the principal growth period associated with tissue development. However, many cells retain cell proliferative potential to accommodate tissue repair and cell replacement.

Decreased H4 Histone Gene Promoter Activity Reflects Transcriptional Downregulation of Reporter Gene Expression During Postnatal Development

To assess the level at which downregulation of histone gene expression occurs during progression from birth to senescence, we monitored H4 promoter driven CAT activity (Fig. 3a) in three different transgenic mouse strains (SiCAT 1, SiCAT 7, and SiCAT 9) using the sensitive F3-SiCAT reporter gene construct. The presence of

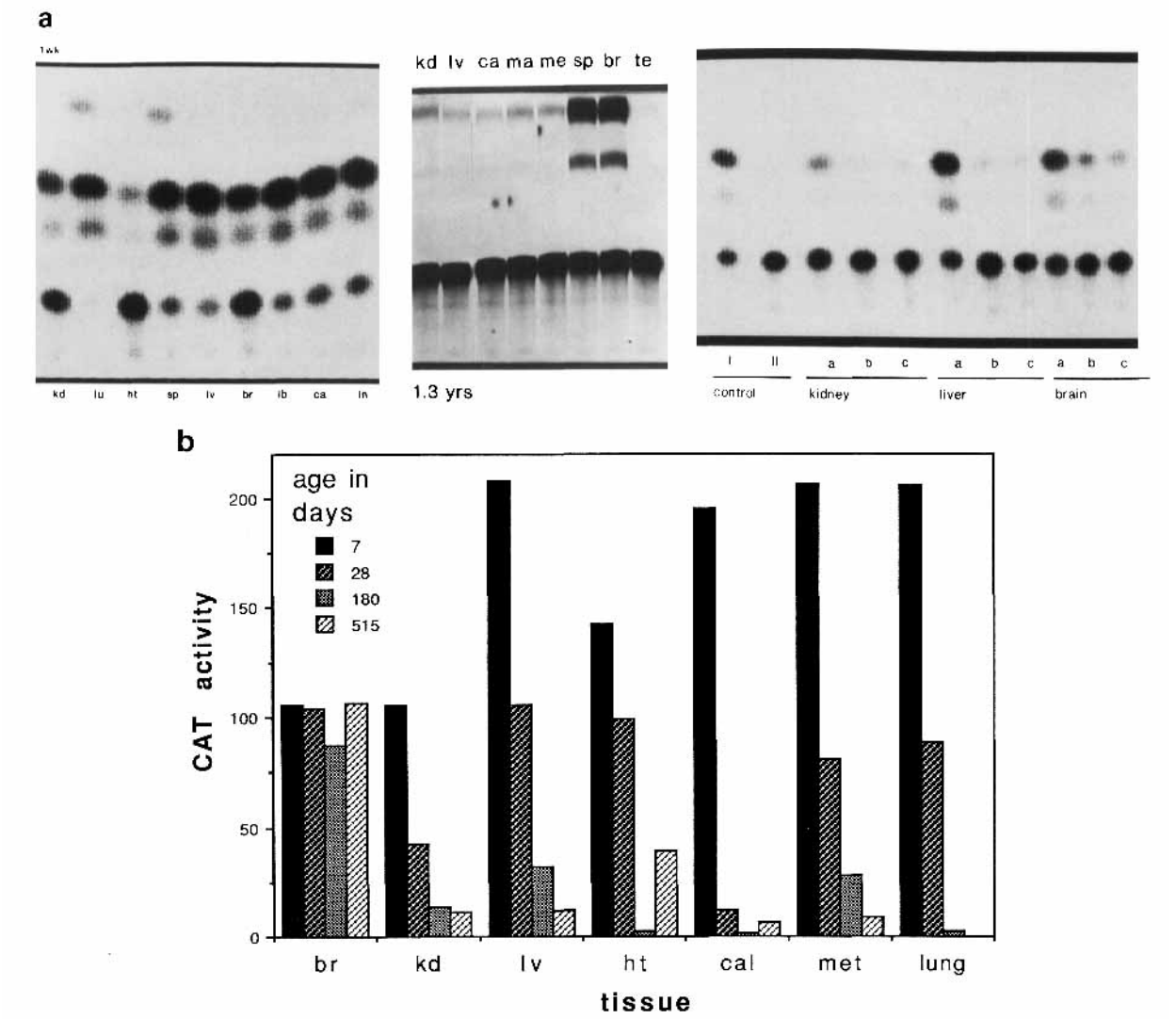


Fig. 3. Downregulation of H4 gene transcription as determined by CAT reporter gene expression during postnatal development. CAT assays were performed with the same tissues used for analysis of H4 gene expression (see Fig. 2). Multiple CAT assays were carried out using a panel of tissues isolated at different stages of the murine lifespan (ht = heart; lv = liver; br = brain; lu = lung; kd = kidney; sp = spleen; lb = limb; ca = calvaria; in = intestine; ma = mandible; me = metaphyseal region of the femur). **a:** Shown in the **left panel** and **middle panel** are CAT assays reflecting histone gene promoter activity in these tissues at 1 week and 1.3 years; the **right panel** shows an example of a CAT assay in which CAT activity was assayed simultaneously in three tissues at three developmental stages (a, day 14; b, day 180; c, day 365; control lanes: I = 0.05 units CAT enzyme, II = no protein). Note the dramatic age-dependent decline in kidney and liver to barely detectable levels. In contrast, CAT activity in brain persists, exhibiting only a slight apparent decrease which was not consistently observed (see a, middle panel, and the quantitation of multiple CAT assays in b). **b:** Shown are the mean values of representative timepoints (7, 28, 180, 515 days) indicating an age-dependent downregulation of H4 promoter activity in all tissues, with the exception of brain. kd = kidney; lv = liver; br = brain; ht = heart; cal = calvaria; met = metaphysis.

the generic Si intron results in a 300-fold increase in reporter gene expression in transgenic mice [Choi et al., 1991]. For example, whereas CAT reporter gene expression in adult liver was barely detectable in previous studies using a construct without the Si intron (designated F3-CAT), the higher levels of F3-SiCAT expression allowed quantitative determination of CAT activity in adult tissues. We observed that while the overall level of reporter gene expression in each transgenic strain (SiCAT 1, SiCAT 7, and SiCAT 9) showed significant differences, the relationship between levels of expression in each of the tissues examined remained constant (data not shown; see also Choi et al., 1991). Presumably, this is related to the site of chromosomal

integration in each strain. To facilitate analysis and to eliminate strain variation, we have quantitated data obtained using mice from the SiCAT 7 strain (Fig. 3b).

Because the SiCAT 7 transgenic mice expressed the introduced F3-SiCAT fusion gene at relatively constant levels throughout development in spleen, we normalized the levels of CAT activity in other tissues to those detected in spleen preparations. Endogenous CAT activity was not observed in any of the tissues examined, based on absence of chloramphenicol acetylation in samples from background mice lacking the transgene, nor were significant CAT inhibitory effects observed, as shown by incubating purified CAT enzyme with tissue homogenates [Choi, 1990; unpublished observations]. Because CAT activity is not influenced by these endogenous tissue-specific parameters, enzymatic activity reflecting reporter gene expression provides a direct measure for histone H4 gene transcription.

The results obtained with adult mice (180 days) (Fig. 3) show consistent tissue-related differences in the levels of CAT activity from different organs (metaphyseal region of bone > brain > spleen > > liver > kidney > lung > heart > muscle). Interestingly, we observed a reduction in relative levels of CAT activity as a function of age in all tissues, with the exception of brain (Fig. 3). This decline in promoter activity occurs in conjunction with an age-dependent decrease in H4 mRNA levels in the same animals (Fig. 2). Taken together, these observations indicate that histone gene expression during postnatal development in this in vivo model is regulated at least in part at the transcriptional level. In contrast, the difference between H4 mRNA levels and relative levels of CAT activity in brain is consistent with post-transcriptional regulation of histone genes in brain, where transcription continues in the absence of mRNA accumulation. Histone gene transcription, as reflected by CAT activity, was easily measurable in diverse brain regions including cortical, basal ganglia, and cerebellar tissues. The possibility arises that differences exist in the relative contribution of transcriptional and post-transcriptional mechanisms mediating histone gene regulation in specific brain compartments.

Transcriptional Regulation of Histone Gene Expression in Bone

Bone growth and extracellular matrix mineralization represents an important physiological process during postnatal development. Historically, the composition of bone has precluded nucleic acid isolation restricting analysis of gene expression in this tissue. However, recently a procedure has been developed to isolate mRNA from calcified bone [Shalhoub et al., 1991] which enables us to study expression of cell growth and osteoblast-related genes during bone formation and remodeling. Initially, we assessed the level of H4 gene expression in mandible and the metaphyseal region of the femur. Results obtained by Northern blot analysis (Fig. 4) show that the level of H4 mRNA isolated from the metaphysis of the femur is similar to that from spleen, and higher than that from mandible, which like the kidney and brain contains low levels of H4 mRNA. The relative level of proliferative activity in the femoral metaphysis and mandible is reflected by the respective levels of H4 mRNA.

When H4 mRNA levels were examined in the metaphyseal region of the femur as a function of age, we observed a decline during the lifespan of the animal to levels that were undetectable at 1.3 years (Fig. 2b). The decline in H4 mRNA abundance was paralleled by a similar decrease in H4 promoter activity (Fig. 3) in the femoral metaphysis. This coupled relationship indicates that transcriptional control is a key component of the observed age-dependent changes in histone gene expression. However, reduction in histone gene transcription as a function of age is more precipitous in calvaria than the metaphysis of the femur (Fig. 3). These results are consistent with the requirement for persistence of proliferative activity in long bone to support bone remodeling, which is minimal in calvaria.

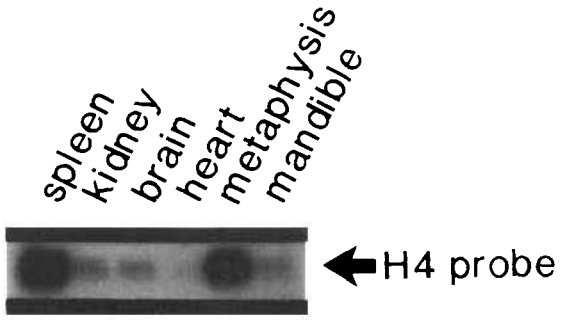


Fig. 4. Northern blot analysis of total cellular RNA isolated from two bone tissues (as indicated) of adult mice (6 months) and hybridized with a DNA probe containing H4 coding sequences. The strong signal observed with RNA isolated from the metaphyseal region of the femur (metaphysis) reflects proliferative activity in the growth plate, whereas mandible represents a skeletal tissue containing primarily quiescent or differentiated cells. The other tissues are shown for reference.

To further pursue the relationship between histone gene promoter activity and proliferation during development of the bone cell phenotype, we prepared primary cultures of osteoblasts [Aronow et al., 1990] from calvaria of SiCAT transgenic mice. When placed in culture, these cells actively proliferate while establishing a type I collagen extracellular matrix. Following completion of proliferative activity, a series of genes are sequentially expressed, leading to development of a tissue-like organization similar to calvarial bone [Owen et al., 1990]. Reporter gene expression in these osteoblast cultures was detected immunohistochemically using a CAT protein antibody, allowing assessment of histone gene transcription at the single cell level.

As shown in Figure 5, actively proliferating osteoblasts exhibit specific staining by the CAT antibody, which was particularly evident in, but not restricted to, the proximity of the nucleus. When cells were progressing through the initial proliferative period (e.g., days 1, 2, and 5) of the osteoblast developmental sequence, most cells were stained positively with the CAT antibody. When the cultures reached confluency (e.g., days 6, 7, and 9) and proliferation ceased, immunohistochemical labeling for CAT was notably absent. The apparent half-life of the CAT protein in osteoblasts reflected by antibody detection is less than 24 h. These results directly indicate at the single cell level that histone genes are transcriptionally downregulated at the completion of proliferative activity during bone cell differentiation.

In a broader context, our results suggest that transcriptional control of histone H4 genes supports selective expression during postnatal development in a series of tissues. We have established that transcriptional downregulation of a cell cycle expressed gene (histone H4) accompanies differentiation in vivo, and that regulatory elements supporting such developmental and proliferation-related control reside within the initial 6.5 kB of the histone H4 gene promoter. However, in cells of the central nervous system (brain) H4 histone gene transcription persists in the absence of H4 histone mRNA accumulation. In these cells a tissue-specific mechanism may be operative that balances constitutive H4 promoter activity with post-transcriptional rate-limiting steps determining histone mRNA levels. This will permit us to further define the

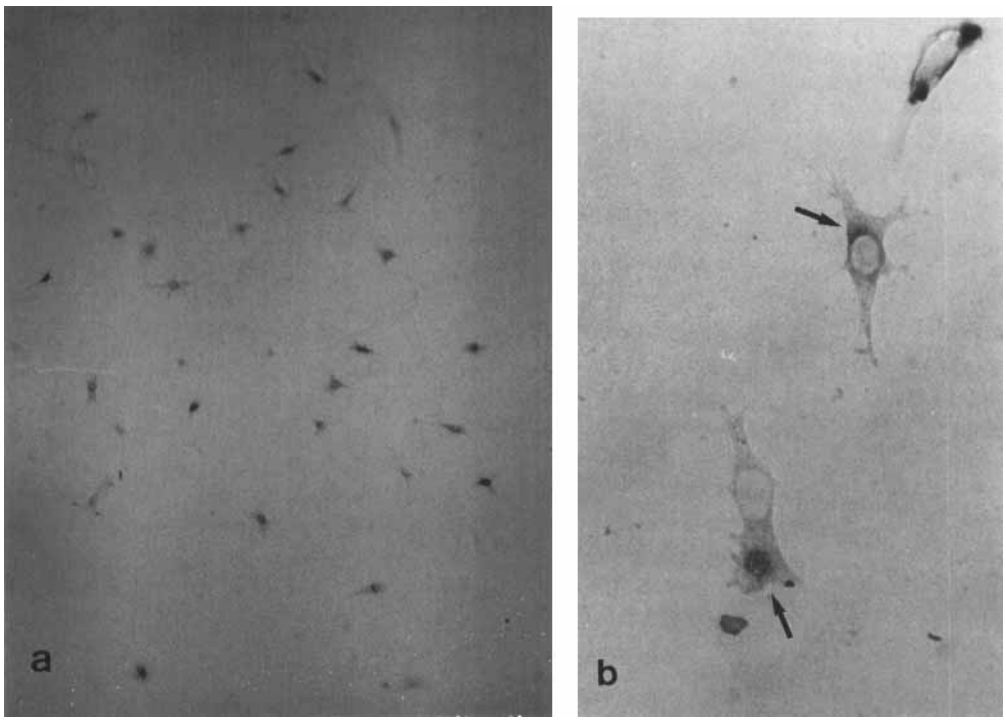


Fig. 5. Immunohistochemical staining using a CAT antibody of cultured osteoblasts derived from transgenic mice. **a:** Low magnification field of proliferating osteoblasts on day 2 where all cells are proliferating and stain positively with the CAT antibody indicating H4 gene transcription. **b:** Higher magnification micrograph showing two proliferating osteoblasts in a day 2 culture with arrows indicating regions of the cell where intense CAT antibody staining is observed. When cells have ceased to proliferate and are within a type I collagen extracellular matrix no antibody staining occurs.

relationship between transcriptional components of cell growth control and expression of tissue-specific genes post-proliferatively in response to physiological mediators of proliferation and differentiation in intact animals.

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