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Detection of a Proliferation Specific Gene During Development of the Osteoblast Phenotype by mRNA Differential Display

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Abstract Fetal rat calvarial-derived osteoblasts in vitro (ROB) reinitiate a developmental program from growth to differentiation concomitant with production of a bone tissue-like organized extracellular matrix. To identify novel genes which may mediate this sequence, we isolated total RNA from three stages of the cellular differentiation process (proliferation, extracellular matrix maturation, and mineralization), for screening gene expression by the differential mRNA display technique. Of 15 differentially displayed bands that were analyzed by Northern blot analysis, one prominent 310 nucleotide band was confirmed to be proliferation-stage specific. Northern blot analysis showed a 600–650 nt transcript which was highly expressed in proliferating cells and decreased to trace levels after confluency and throughout the differentiation process. We have designated this transcript PROM-1 (for proliferating cell marker). A full length PROM-1 cDNA of 607 bp was obtained by 5′ RACE. A short open reading frame encoded a putative 37 amino acid peptide with no significant similarity to known sequences. Expression of PROM-1 in the ROS 17/2.8 osteosarcoma cell line was several fold greater than in normal diploid cells and was not downregulated when ROS 17/2.8 cells reached confluency. The relationship of PROM-1 expression to cell growth was also observed in diploid fetal rat lung fibroblasts. Hydroxyurea treatment of proliferating osteoblasts blocked PROM-1 expression; however, its expression was not cell cycle regulated. Upregulation of PROM-1 in response to TGF-β paralleled the stimulatory effects on growth as quantitated by histone gene expression. In conclusion, PROM-1 represents a small cytoplasmic polyA containing RNA whose expression is restricted to the exponential growth period of normal diploid cells; the gene appears to be deregulated in tumor derived cell lines. J. Cell. Biochem. 64:106–116.

Key words: osteoblasts; proliferation; growth control; differential display; differentiation

The underlying mechanism involved in regulation of cellular proliferation and differentiation is a fundamental question of developmental biology and numerous physiologic and pathologic events including cancer. In the case of bone, like other tissues, cell growth and specialization must be maintained and stringently regulated throughout the life of the organism to support tissue remodeling. We have examined molecular mechanisms contributing to growth and differentiation using an in vitro model in which osteoblasts derived from 21-day-old fetal rat calvaria undergo active cellular proliferation, matrix maturation, and matrix mineralization in concert with differentiation of the osteoblast [Owen et al., 1990; Aronow et al., 1990; Pockwinse et al., 1992]. This developmental program is reflected by changes in the levels of subsets of osteoblast expressed genes from the proliferative to differentiated stages [Lian et al., 1992; Stein et al., 1990]. For example, genes characteristic of the proliferation period include cell growth regulated genes, such as histone H4, fos and jun, as well as genes mediating formation of the extracellular matrix, such as type I collagen and fibronectin. In the post-proliferative period, genes are maximally expressed that promote maturation and competency for mineralization, including alkaline phosphatase and matrix gla protein. With the onset of mineralization in the final stages of

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osteoblast differentiation, osteocalcin and osteopontin are expressed at maximal levels.

We have examined regulatory mechanisms mediating the progression of osteoblast differentiation by examining factors associated with stage specific expression of phenotypic genes. It is apparent that events which occur in the proliferation period are critical for determining commitment to further progression of the bone cell lineage. In proliferating osteoblasts, expression of subsets of gene family members involved in osteoblast differentiation is observed. Several of the bone morphogenic proteins are expressed early and downregulated while others remain at detectable levels [Harris et al., 1994a]. Similarly, cfos and cjun are downregulated at the end of the growth period followed by upregulation of related family members, fra-2 and jun-D [McCabe et al., 1995]. TGF-β must also be downregulated at the end of the growth period for further differentiation of the cells to mature osteoblasts. Indeed, addition of TGF-β and extension of the proliferation period blocks further maturation to a committed cell lineage [Breen et al., 1994; Harris et al., 1994b]. It has even been observed that modifications in expression of factors regulating the cell cycle clock occur at the onset of differentiation. For example, cyclin E becomes upregulated in differentiated osteoblasts in a mineralized matrix but not in post-proliferative cultures where mineralization has been blocked by TGF-β [Smith et al., 1995]. The requirements for stringent regulation of genes in the proliferation period provide compelling evidence for the importance of defining differentially expressed genes in proliferating osteoblasts. Identification of such genes will provide us with further insight into mechanisms mediating cell proliferation and differentiation [Stein and Lian, 1993; Stein and Lian, 1995; Stein et al., 1996].

Several methods have been introduced to distinguish the uniquely expressed genes of different phenotypes. These include subtractive hybridization of mRNA [Lee et al., 1991], RNA finger printing [McClelland et al., 1994], and mRNA differential display [Liang and Pardee, 1992]. The differential display technique has several advantages over other methods: it allows direct comparison of multiple and different samples; requires only a very small amount of sample; and, is a much more comprehensive screening method allowing a selection of candidate clones in a short period. In this study, we selected mRNA from three different stages of osteoblast differentiation and screened gene expression by mRNA differential display. We have isolated a cDNA corresponding to a transcript encoding a 37 amino acid peptide designated PROM-1, which shows proliferation stage specific expression in diploid cells and deregulation in tumor derived cells. Interestingly, PROM-1 is not restricted to osteoblasts but is present in proliferating cells derived from several tissues.

MATERIALS AND METHODS

Cell Culture

Osteoblasts (ROB) were prepared from calvaria of 21 day fetal rats. Following removal of dura and periosteum from the parietal bone, cells were collected from calvaria by three sequential digestions of 20, 40, and 90 min at 37°C in 2 mg/ml collagenase P (Boehringer Mannheim Biochemicals, Indianapolis, IN)/ 0.25% trypsin (Gibco/BRL, Grand Island, NY). Cells from digests one and two were discarded and cells from the third digest were grown in minimal essential medium (MEM: Gibco) supplemented with 10% fetal calf serum (FCS) at a density of 6.5×10^5 cells/100 mm dish (Corning, Corning, NY). Osteoblast mineralization was enhanced by the addition of 50 mg/ml ascorbic acid and 10 mM β-glycerophosphate in medium. For differential display, RNA was prepared from cells harvested on day 2, approximately 70% subconfluent culture, when cells were actively proliferating; day 12, when the cells have reached monolayer confluency but have not multilayered to form visible nodules; and day 21, representing a highly mineralized culture. RNAs were isolated from each of these cell populations for confirmation of expression of the differentially displayed product by Northern blot analysis. RNAs were also isolated from cells harvested at additional points during the osteoblast differentiation time course, as specified in the figure legends.

Osteoblast-like rat osteosarcoma cells, ROS 17/2.8 [Rodan et al., 1983], MC3T3-E1 cells [Sudo et al., 1983], and primary cultured fetal rat lung fibroblasts (FRL) were cultured and harvested to obtain RNA for Northern blot analysis. $1,25(OH)_2D_3$ was kindly provided by Dr. M. Uskokovic, Hoffman-La Roche Inc. TGF-β was purchased from R & D Systems, (Minneapolis, MN).

RNA Isolation and Purification

Cells were scraped with PBS, pelleted by centrifugation at 800 × *g* for 5 min at 4°C and stored at −70°C until used. Total cellular RNA was extracted with TriZol (Gibco/BRL, Grand Island, NY) [Chomczynski and Sacchi, 1987] according to the manufacturer's instructions. Cytoplasmic RNA was extracted as follows. The pellet of harvested cells was resuspended in a lysis buffer (50 mM, pH 8.0, Tris Cl containing 100 mM NaCl, 5 mM $MgCl_2$ and 0.5% Nonidet p-40), then incubated for 5 min on ice. Nuclei and cell debris were removed by centrifugation at 15,000 × *g* for 2 min at 4°C. RNA was extracted with TriZol from the supernatent containing cytoplasmic extract. Extracted RNA (100 µg) was incubated for 30 min at 37°C with 20 units of RNasin (Promega, Madison, WI) and 20 units of RNase free DNase I (Promega, Madison, WI) in 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$. After sequential extraction with phenol/chloroform and chloroform, the RNA was ethanol precipitated in the presence of 0.3 M sodium acetate and redissolved in diethyl pyrocarbonate-treated water. RNA concentration was determined by absorption at 260/280 nm wave length, and the integrity of RNA was assessed by the 28S/18S rRNA ratio after electrophoresis. The RNA was aliquoted and stored at −70°C until used.

Differential Display

Differential mRNA display analysis was carried out according to the original method [Liang and Pardee, 1992; Liang et al., 1993] with minor modifications. Total RNA (300 ng) was reverse transcribed in a 30 ul reaction mixture with 300 units of superscript MMLV reverse transcriptase (Gibco/BRL, Grand Island, NY) and 100 units of RNasin in the presence of 2.5 mM of one of the degenerate primers (T_{11}VA, T_{11}VC, T_{11}VG, and T_{11}VT; V denotes A, C, or G) and 20 mM dNTP for 60 min at 42°C. Control reactions were performed in the absence of reverse transcriptase. Two µl of the reverse transcription product was amplified with the GeneAmp kit (Perkin-Elmer Cetus, Foster City, CA) in the presence of 2.5 mM T_{11}VG as a 3′ primer, 0.5 mM HY1 (AGACACCATG) as a 5′ primer with dNTP concentration at 2 µM in a final 20 µl reaction mixture. The buffer, $MgCl_2$ and Taq polymerase concentrations were as suggested by the manufacturer. PCR parameters were 94°C for 1 min, 40°C for 1 min, and 72°C for 1 min with 40 cycles, and a final 5 min elongation at 72°C.

Amplified cDNAs were then separated on a 6% denaturating gel. The gel was blotted on Whatman 3 MM paper and dried. The dried gel was exposed for 24–48 h to BioMax film (Kodak, Rochester, NY) with orientation by radioactive marker. The cDNA bands of interest were excised and eluted with 100 µl of TE. The eluted cDNA was ethanol precipitated in the presence of 0.3 M sodium acetate and 2 µl of 10 mg/ml glycogen as a carrier. One half of the recovered cDNA was reamplified under the same PCR conditions as the first PCR except that the dNTP concentrations were increased to 20 µM without adding isotope. Ten of 50 µl of the PCR product were electrophoresed in an agarose gel with a molecular mass ladder (Gibco/BRL, Grand Island, NY) as a standard to estimate molecular weight and concentration of cDNA in the PCR product. The remaining samples were stored at −20°C for screening and cloning. The PCR product was cloned into the pCR II vector using the TA cloning system (Invitrogen, San Diego, CA). The cloned cDNA insert was sequenced with Sequenase Version 2.0 (USB, Cleveland, OH). The nucleotide sequences obtained were compared with known sequences by searching GenBank and EMBL databases (March, 1996) with the Fasta program (Genetic Computer Group, Madison, WI).

Screening by Northern Blot Analysis

RNA samples used for differential display were monitored with respect to representation of 18S and 28S ribosomal RNA as internal standards and the intactness of the RNA was ascertained by electrophoretic fractionation on 5.5% formaldehyde, 1% agarose gels, and ethidium bromide staining. For screening, 20 µg of total cellular RNA were fractionated as described above and Northern transfer performed using Zetaprobe membrane (Bio-Rad, Melville, NY). RNA was cross-linked to filters by UV irradiation for 1 min and stored until use. DNA probes, either PCR product or cloned cDNA, of PROM-1, human histone H4, pF0002 [Pauli et al., 1989], rat osteocalcin, pOC 3.4 [Lian et al., 1989], rat alkaline phosphatase, pRAP54 [Noda et al., 1987], and human ribosomal LS6 [Wilson et al., 1978] were labelled with a α-^{32}P-dCTP (3,000 Ci/mmol; NEN, Boston, MA) by random primer technique [Fein-

berg and Vogelstein, 1983]. The blot was prehybridized in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.1% SDS and 100 µg/ml salmon sperm DNA at 42°C for 3 h. For hybridization, 10^6 cpm/ml of heat denatured radioactive DNA probe was added and incubated at 42°C overnight. Following hybridization, the blot was washed three times in 2 × SSC/0.1% SDS at room temperature for 15 min each, twice in 0.1 × SSC/0.1% SDS at room temperature for 20 min each. Blots were exposed to Kodak XAR film at −70°C with intensifying screens.

5′ RACE (Rapid Amplification of cDNA End)

To generate the 5′ end of the PROM-1 RNA, 3 different gene specific primers (GSP1, 2 and 3) were designed from the unique sequence of original clone. 5′ RACE [Frohman et al., 1988] was performed with a 5′ RACE kit (Gibco/BRL, Grand Island, NY) according to the manufacturer's instructions. GSP1 was used for reverse transcription. GSP2 was used for the 3′ primer of the first PCR reaction, and GSP3 was used as the 3′-primer of the consecutive nested PCR (see Fig. 2). The final PCR product was cloned into the pCR II vector and sequenced. A full length clone was constructed by joining the original differential display clone to the 5′ RACE product at the Hinf I site.

RESULTS

The representation of differentially expressed mRNAs in cultured fetal rat calvarial cells at three stages of osteoblast differentiation was analyzed by using combinations of ten arbitrary 5′ decamers and four T_{11}VN 3′ primers. Each primer combination displayed 40–60 bands between 150–500 bp range. Figure 1A shows the clones exhibiting differential expression of mRNA between log phase growth (lane P), cultures with cells at both monolayer confluency and with proliferating cells forming nodules (lane C), and the mature post-proliferative osteoblasts in a mineralized matrix (lane M). The arrow indicates the selected band, which was highly expressed in proliferating cells, and then decreased to a basal level in confluent and mineralized cultures. The band was excised, eluted, amplified, and used as a probe for Northern blot analysis (Fig. 1B) which demonstrated a 600–650 nt transcript and corresponded to the expression pattern observed from the differential display. The same source of RNA used for differential display was examined for expression of histone H4 (H4), alkaline phosphatase (ALP), and osteocalcin (OC) mRNAs as markers of growth and differentiation of the osteoblast cells (Fig. 1C).

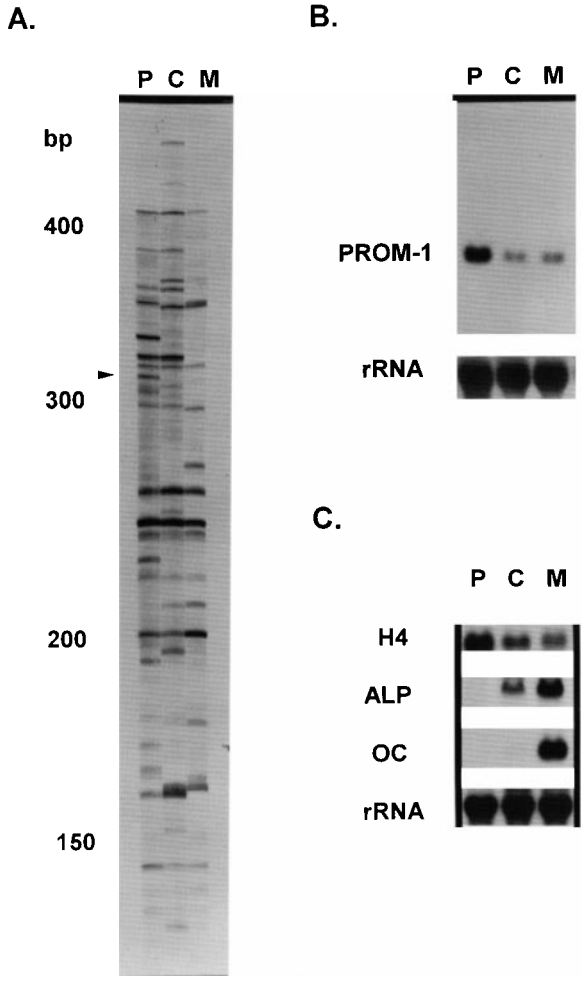

Fig. 1. Differential display of total cellular RNA from rat osteoblasts during in vitro differentiation. **A:** Total cellular RNAs of three different time points of osteoblast differentiation was reverse-transcribed with T_{11}VG as primer. Reverse transcription products were amplified with HY1 (ACACACCATG) and T_{11}VG under the conditions described in Materials and Methods. The amplified product of interest is indicated by an arrow. The sizes of bands were calculated from co-migrating known sequences following a sequencing reaction. **B:** Expression of PROM-1 RNA was screened by Northern blot analysis using the same source of RNA as was used for differential display. Reamplification product of the differentially displayed band was used as a probe for Northern hybridization. The 28S ribosomal RNA indicated that the same amount of RNA was loaded at each lane. P, Proliferating cells at day 2; C, confluent cells at day 12; M, mineralization period at day 21. **C:** The 10 µg of total cellular RNA used for differential display was screened for alkaline phosphatase (ALP), histone H4 (H4), and osteocalcin (OC) gene expression to assess the extent of ROB cell differentiation. Consistency of RNA loading is reflected by hybridization to 28S ribosomal RNA.

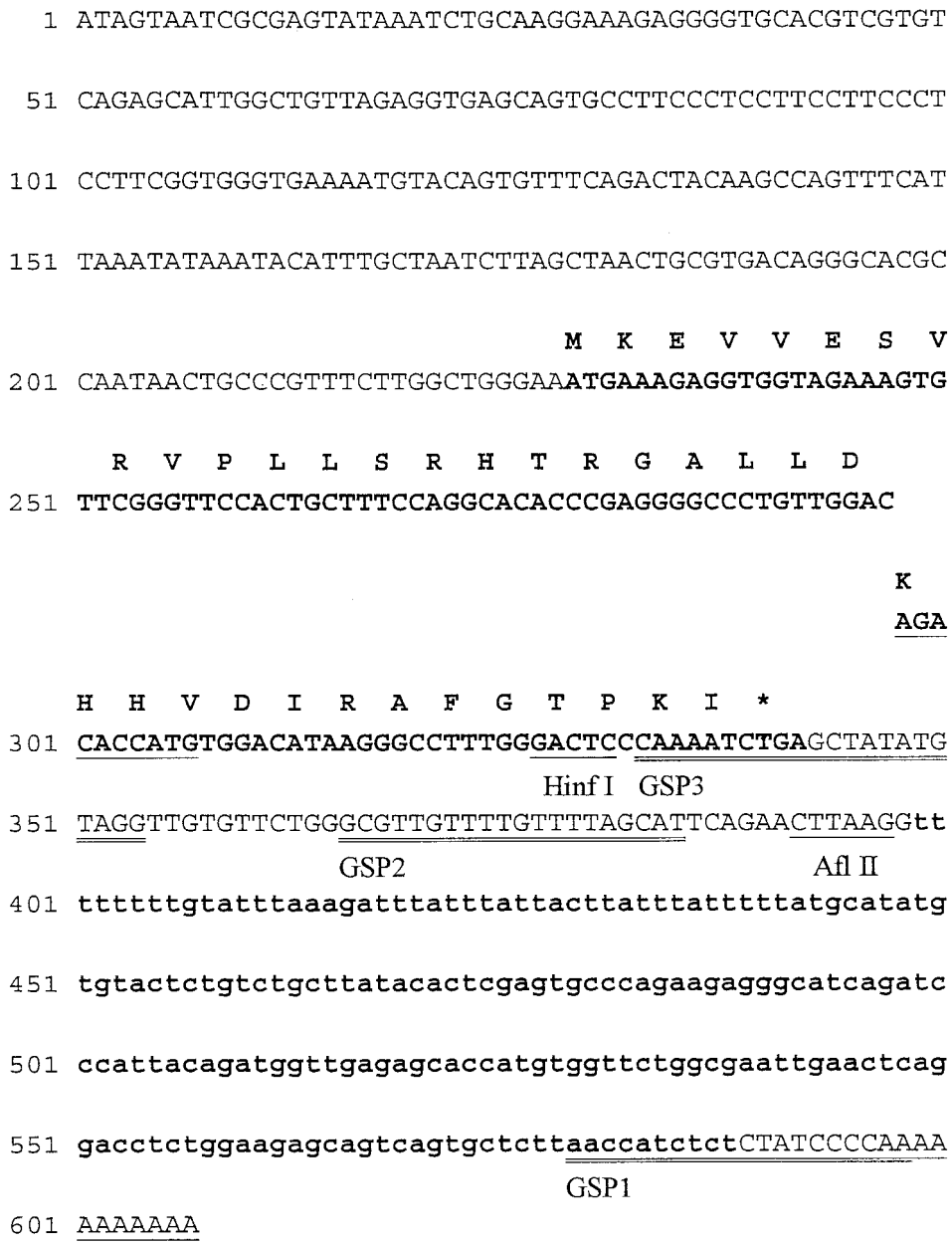

Fig. 2. Nucleotide sequence of PROM-1. Reamplified fragment was cloned into pCR II vector with the TA cloning system. The insert was sequenced with T7 and SP6 promoter primers. The restriction site Afl II was used to separate a unique sequence and repetitive sequence. The repetitive sequences are marked by bold face small letters. The primers used for differential display are underlined. The primers used for 5′ RACE are double underlined and marked as gene specific primer (GSP) 1, 2, and 3. The upper part of the sequence represents 5′ RACE product. The original clone and the 5′ RACE product were joined by ligation at the Hinf I restriction site. A putative open reading frame is indicated by bold face capital letters; the translated amino acid sequence is shown above.

The nucleotide sequence of the cloned DNA fragment is presented in Figure 2. The clone has sequences corresponding to the 3′- and 5′-primers at each end and is 310 bp length. Sequence comparison revealed that the 3′ end of the clone is highly homologous (75%) to the B2 repetitive sequence of mouse. However, the 5′ end showed no homology to any previously reported sequences. To obtain a full length transcript, 5′ RACE was performed as described in Materials and Methods. Three different gene specific primers, as indicated by the double underlined sequences, were designed from unique sequences for nested PCR analysis (Fig.

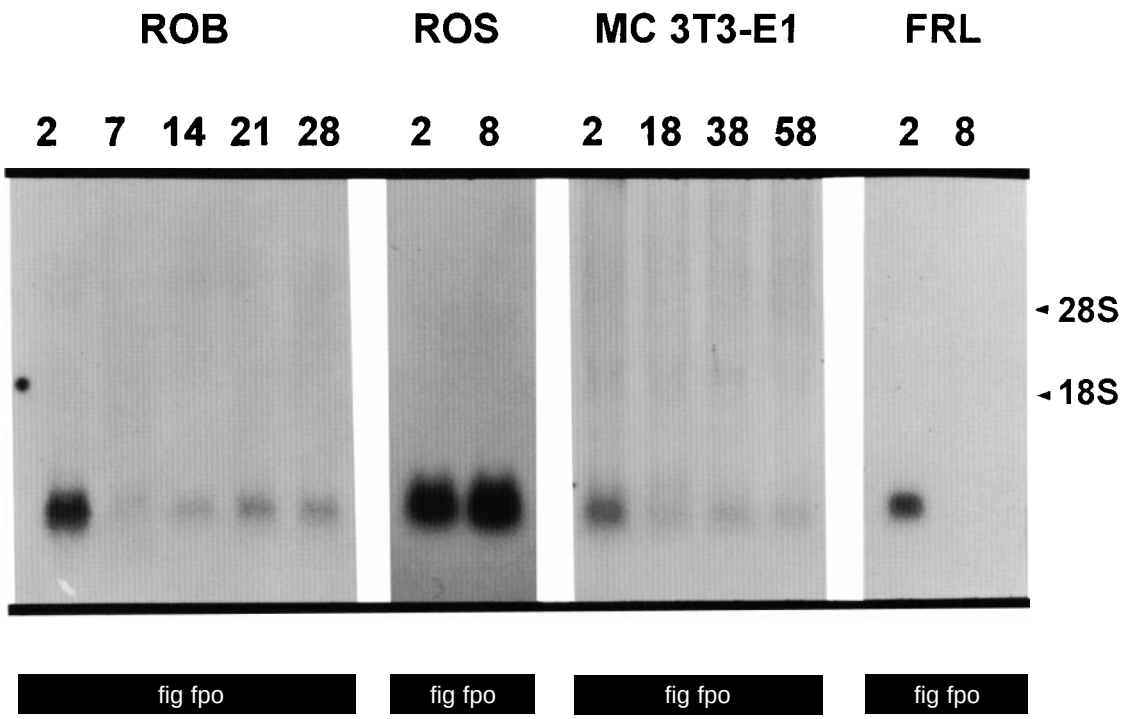

Fig. 3. Expression of PROM-1 in rat osteoblasts (ROB), ROS 17/2.8, MC3T3-E1 and fetal rat lung (FRL) fibroblasts is related to cell growth. RNA blots containing 10 µg of total cellular RNA for each lane were hybridized with the PROM-1 full length cDNA as a probe. Numbers indicate the day of culture from which RNA was purified. Consistency of RNA loading is reflected by hybridization to 28S ribosomal RNA.

2). From this approach, we obtained a 607 bp cDNA sequence which corresponded to the RNA size observed by Northern blot analysis. To confirm that the 5′ RACE product was the 5′ part of the original clone, an additional 5′ primer was designed from the 5′ RACE product sequence. The PCR reaction of this primer and one of the gene specific primers from the unique sequence domain had a product of the expected size (data not shown). The sequence of the 5′ RACE product revealed no significant homology with any known sequences in the databases at either the nucleotide or amino acid levels.

To better define expression of this clone in relation to the growth period of osteoblast phenotype development, the representation of full length transcripts was analyzed at different times during proliferation and differentiation of ROB cells by Northern blot analysis (Figure 3). Maximal levels of expression were observed in actively proliferating cells (day 2). During the late proliferation phase, at confluency (day 7) and thereafter, only trace levels were detected. Notably, a slight increase was observed in late stage mineralized cultures, at which time apoptosis and some compensatory proliferation occur [Lynch et al., 1994]. Mouse MC3T3-E1 cell cultures exhibited almost the same expression pattern, but the signal was lower. In contrast, the transcript was abundantly expressed in the ROS 17/2.8 osteosarcoma cells, and high levels of expression were sustained post-proliferatively. These same profiles of expression were observed with cytoplasmic and total cell RNA preparations. The constitutive expression of genes related to cell growth control and differentiation has been well documented in transformed and tumor derived cells [reviewed in Stein et al., 1996].

We examined expression of this clone in other normal diploid cells. In contrast to osteoblasts which multilayer, fetal rat lung (FRL) fibroblasts exhibit very strict contact inhibition of monolayer growth at confluency. In these cultures, the transcript was expressed at high levels in proliferating cells, but was absent from confluent cultures. Based on these observations of restricted expression to proliferating normal diploid cells and deregulated expression in tumor cells, we are designating the gene transcript we identified by differential display PROM-1 (proliferating cell marker).

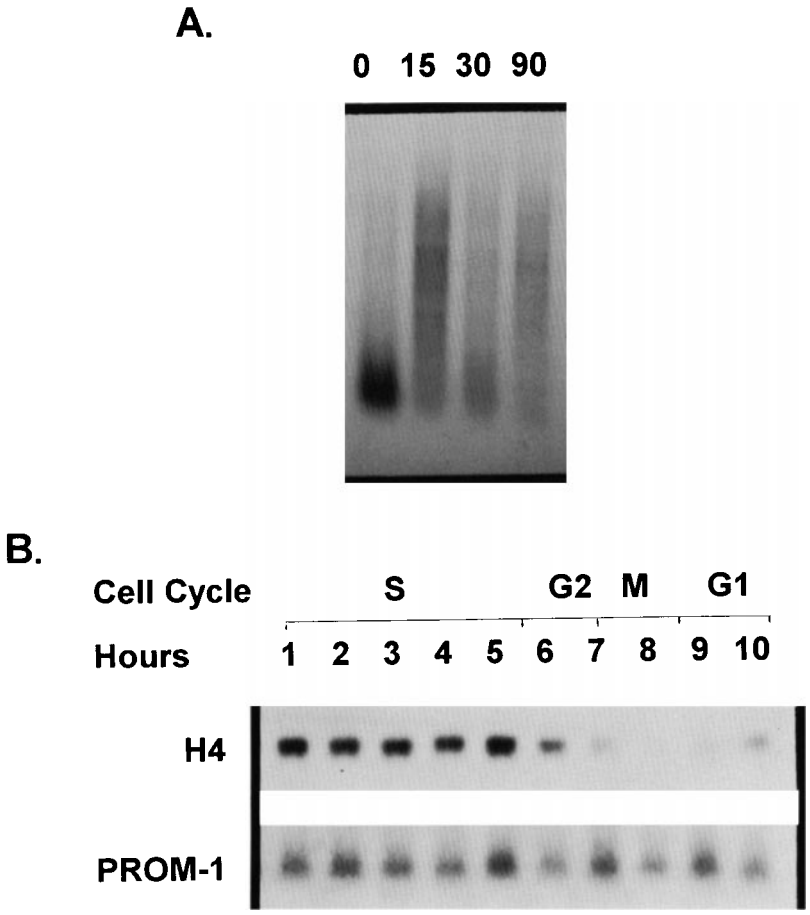

Fig. 4. **A:** Hydroxyurea treatment of proliferating ROB cells downregulates PROM-1 RNA levels. ROB cells were plated at a density of 3×10^5 cells/100 mm dish. After 48 h of seeding, cultures were treated with 1 mM of hydroxyurea for indicated times and the PROM-1 gene expression was monitored by Northern blot analysis. **B:** PROM-1 RNA levels do not show cell cycle related changes in synchronized MC3T3-E1 mouse osteoblast-like cell line. Actively proliferating cells were exposed to 2 mM thymidine twice to obtain a cell synchrony at the G1/S phase boundary. After release from the double thymidine block, cells were harvested at each h up to 10 h for RNA preparation. Cell synchrony was monitored by Northern blot analysis of histone H4 signal expression. Time points 1–5 h represents S phase, 6–8 h represents G2/M phase, and 9–10 h represents the G1 phase.

To further explore the relationship of PROM-1 expression to cell proliferation, DNA synthesis was blocked by hydroxyurea treatment of ROB cells. We examined cells harvested after 0, 15, 30, and 90 min after addition of 1 mM hydroxyurea [Kockx et al., 1994]. Hydroxyurea treatment resulted in rapidly decreased levels of PROM-1 mRNA, within 15 min (Fig. 4A). However, when PROM-1 expression was examined at different stages of the cell cycle in both ROB and MC3T3-E1 cells synchronized by a double thymidine block, the expression levels were identical (Fig. 4B). Therefore, PROM-1 expression reflects a function that is related to cell growth but is not dependent on or related to progression through the cell cycle.

To ascertain regulation of PROM-1 expression by modulators of osteoblast growth and differentiation, the effects of TGF-β and the hormone $1,25(OH)_2D_3$ were examined in ROB cells (Fig. 5). $1,25(OH)_2D_3$ had no influence on PROM-1 expression in either proliferating (day 3) or differentiated cells (day 11 and 19), while other genes (e.g., osteocalcin) were responsive (Fig. 5B). TGF-β treatment had little or no effect on PROM-1 mRNA levels during the proliferation stage. In contrast, TGF-β upregulated PROM-1 more than three fold in postconfluent differentiated osteoblasts (day 11 and 19). However, the level to which PROM-1 was induced by TGF-β in differentiated osteoblasts was lower than the endogenous level observed during the proliferation period. Responses of other genes regulated by TGF-β are also shown (Fig. 5B). Growth is stimulated, as demonstrated by the increase in histone H4, and osteo-

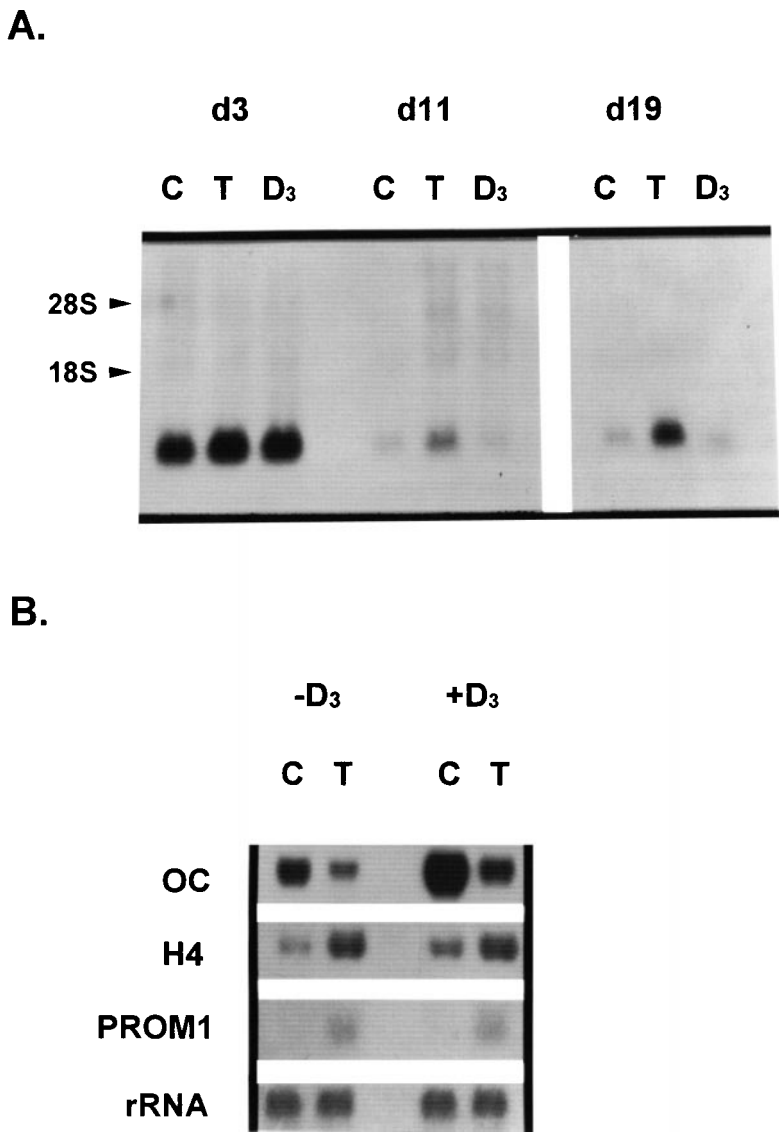

Fig. 5. Effect of TGF-β and/or vitamin D treatment on PROM-1 gene expression. The cells were seeded and maintained as described in Materials and Methods. **A:** Cells were harvested at day 3, day 11, and day 19 for RNA purification. At 48 h before harvest, medium was replaced by Hams F12 containing 2% charcoal-treated FCS for 24 h and then incubated for another 24 h with 10^{-8} M $1,25(OH)_2D_3$(D3), or 2.5 ng/ml of TGF-β (T) or vehicle alone (C). **B:** Cells were treated with 10^{-8} M $1,25(OH)_2D_3$ and/or 2.5 ng/ml of TGF-β as described above. PROM-1 gene expression on day 19 was compared with osteoblast growth and differentiation markers, histone H4 (H4), and osteocalcin (OC). Consistency of RNA loading is reflected by hybridization to 28S ribosomal RNA.

calcin is downregulated. These comparisons suggest that PROM-1 is not regulated directly by TGF-β but that the modest changes in PROM-1 expression reflect the parallel modification in proliferative activity in the cultures in response to the growth factor.

DISCUSSION

Differential mRNA display [Liang and Pardee, 1992] provides an effective method for isolation of genes showing selective expression in a variety of biological systems [Liang et al., 1992; Nishio et al., 1994; Utans et al., 1994; Zimmermann and Schultz, 1994; Donohue et al., 1995; Swisshelm et al., 1995]. After technical modifications [Liang et al., 1993; Bauer et al., 1993; Callard et al., 1994; Reeves et al., 1995; Donohue et al., 1995], this technique has become a sensitive and comprehensive approach for identification of genes which are differentially expressed, including genes expressed at low levels.

In this study, by differential display PCR coupled with 5′ RACE, a cDNA of 607 bp in length was cloned and Northern blot analysis identified a cytoplasmic polyA+ RNA of corresponding size. Sequence analysis revealed that the 3′ 175 bp of the cDNA has −75% homology with B2 repetitive sequences of the mouse, which is homologous to the human Alu repetitive sequence. According to a computerized search of known mRNA sequences, approximately 5% contain this repetitive sequence, usually in 3′ untranslated region (UTR). Occasionally (15%) B2 repetitive sequences are present in the 5′ UTR and very rarely are observed in the coding region [Yugul et al., 1995]. It should be noted that the RNA sample used for this experiment was DNase I treated; therefore, it is unlikely that the clone was derived from a DNA contaminant. Additionally, the cytoplasmic RNA preparation yielded a hybridization signal with the clone. Novel sequences are present in the 5′ 60% of the clone, which are not homologous with any known gene at either the nucleotide or amino acid level precluding an indication of functional activity. The putative amino acid sequence of the cDNA encodes for a short peptide with 37 amino acids.

As shown by differential display and Northern blot analysis, this gene was expressed in a proliferation-related manner. In normal diploid rat osteoblast cultures, maximal expression was observed during the peak of the proliferation period. Trace levels of expression occur in the late proliferation period when post-confluent proliferation supports focal formation of nodules. However, the gene was completely suppressed after confluency in cell lines exhibiting monolayer contact growth inhibition (e.g., FRL cells). In contrast, PROM-1 expression was retained in confluent post-proliferative osteosarcoma cell cultures. This result suggests that PROM-1 gene expression is stringently controlled in normal cells, but is deregulated in tumor cells. Interestingly, PROM-1 mRNA levels were rapidly downregulated by hydroxyurea inhibition of DNA synthesis; but, PROM-1 is not cell cycle regulated.

A relationship of PROM-1 to cell proliferation is supported by the influence of TGF-β on PROM-1 mRNA levels. TGF-β1 is one of the most abundant growth factors in the bone extracellular matrix. When bone resorption occurs, TGF-β1 is released and serves as a potent regulator of the surrounding bone and osteoprogenitor cells. TGF-β1 has been shown to stimulate osteoblast growth and to function as a differentiation activator in vivo [Centrella et al., 1994]. However, in cultured rat osteoblasts, TGF-β acts as a suppressor of genes related to bone cell differentiation, while retaining a growth stimulatory effect [Breen et al., 1994; Harris et al., 1994b; Staal et al., 1996]. TGF-β treatment increased cell growth and suppressed osteocalcin gene expression in post-proliferative differentiated cells. PROM-1 expression was upregulated by TGF-β treatment, paralleling the increase in histone H4 expression.

Consistent with these observations, expression of RNAs containing B2 repetitive sequences, as with PROM-1, have previously been associated with cell growth. For example, such B2 containing genes are upregulated following serum-induced cell growth [Edwards et al., 1985] and decrease expression during induced differentiation of murine erythroleukemia cells [Khochbin et al., 1991]. Most interestingly, tumor cells contain more of this class of RNAs [Karamerov et al., 1982] and a subset of these gene transcripts are predominantly expressed in tumor cells [Kohnoe et al., 1987]. More recently, Clawson et al. [1996] reported that RNAs containing B2 repetitive sequences are highly compartmentalized in a subset of hepatocytes within the initiation foci following hepatocarcinogen exposure. These authors suggested that the level of B2 transcript in the cytoplasm may be functionally linked to control of hepatocyte proliferation.

In summary, we have isolated a novel cDNA expressed selectively in proliferating cells, designated PROM-1. The gene is highly expressed in osteosarcoma cells, suggesting deregulation in tumor cells. However, functional interrelationships between PROM-1 expression and competency for proliferation, cell cycle progression, or parameters of tissue-specific gene expression remain to be experimentally established.

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