Journal of Cellular Biochemistry 57:62–70 (1995)

Temporal Variation of c-Fos Proto-Oncogene Expression During Osteoblast Differentiation and Osteogenesis in Developing Rat Bone

M. Machwate, A. Jullienne, M. Moukhtar, and P.J. Marie

INSERM Unité 349, Cell and Molecular Biology of Bone and Cartilage, Lariboisiere Hospital, 75010 Paris, France

Abstract To delineate the implication of c-fos protooncogenic in the osteogenie process, we have investigated the temporal pattern of c-fos mRNA expression in fetal and neonatal rat bone during intramembranous and endochondral bone formation. Northern blot analysis of mRNA extracted from calvaria and femur showed that expression of c-fos, Histone H4, and osteocalcin mRNAs followed a temporal sequence during bone development. The levels of histone H4 mRNA, a marker of cell proliferation, were high at early stages of fetal development of calvaria and femur, and decreased until birth. In both the postnatal calvaria and femur, c-fos mRNA levels increased transiently at birth and preceded a rise in osteocalcin transcripts, a marker of the mature osteoblast phenotype. The immunohistochemical analysis showed that c-Fos protein was expressed in osteoprogenitor cells in the perichondrium and periosteum, and not in mature osteoblasts which expressed markers of differentiated osteoblasts such as type-I collagen, bone sialoprotein, and osteocalcin. Thus, the transient c-fos proto-oncogene expression during the postnatal life that precedes the osteocalcin expression may be involved in the transition from the precursor state to mature osteoblasts. These results suggest that c-fos proto-oncogene may play an important role in osteogenesis during rat postnatal life.

Key words: c-fos, calvaria, femur, osteogenesis, proliferation, differentiation, osteoblast, mRNA

Bone formation during development is a complex process which differs with the type of bone considered, the site of bone formation, and the stage of development. Endosteal bone development involves a sequence of events characterized initially by the formation of cartilage, the recruitment of osteoblasts, and the deposition of an extracellular bone matrix, whereas the intramembranous bone formation involves the direct deposition of a bone matrix. The molecular basis of osteoblast recruitment and differentiation in the processes of endosteal and periosteal osteogenesis is not fully understood. In vitro, the progression of osteogenesis is characterized by the sequential expression of genes related to the development of the osteoblast phenotype [Owen et al., 1990a; Turksen and Aubin, 1991; Poliard et al., 1993]. The proliferation of osteoblast precursors is associated with the transient expression of genes associated with cell growth [Owen et al., 1990a; Stein and Lian, 1993]. Differentiating osteoblasts express alkaline phosphatase, type-I collagen, and osteopontin genes, whereas the expression of osteocalcin and bone sialoprotein appears to be restricted to more mature osteoblastic cells [Bianco et al., 1991; Turksen and Aubin, 1991; Rodan and Noda, 1991]. In vivo, changes in extracellular matrix genes have been described during skeletal growth in rats [Yoon et al., 1987; Shalhoub et al., 1991]. However it is not known whether the inverse relationship between osteoblast proliferation and differentiation described in vitro [Owen et al., 1990a; Stein and Lian, 1993] also occurs in vivo during the early fetal and postnatal normal bone development.

There are several lines of evidence suggesting that the c-fos proto-oncogene may play an important role in the control of bone cell proliferation and differentiation [Müller, 1986; Verma and Graham, 1987; Curran, 1988]. The c-fos protooncogene is the cellular homologue of the v-fos oncogene present in murine osteosarcoma viruses [Finkel et al., 1966; Curran et al., 1982]. C-fos is an immediate early gene involved in the proliferative response to growth factors in a variety of cells [Greenberg and Ziff, 1984], including osteoblastic cells [Merriman et al., 1990;

Received May 3, 1994; accepted May 24, 1994.

Address reprint requests to Dr. P.J. Marie, INSERM Unité 349, 6 rue Guy-Patin, 75010 Paris, France.

Okazaki et al., 1992; Matsumoto et al., 1993]. The c-fos gene product (c-Fos protein) is a nuclear protein able to interact with members of the Jun family, forming the AP-1 transcription factor [Chiu et al., 1988; Curran and Franza, 1988]. The presence of functional AP-1 binding sites in the promoter of genes expressed by osteoblasts [Schüle et al., 1990; Angel and Karin, 1991], and the induction of c-fos expression by calciotropic hormones [Lian et al., 1991; Candeliere et al., 1991; Lee et al., 1994] suggest that c-fos may be implicated in the regulation of the osteoblast function. High c-fos mRNA [Dony and Gruss, 1987; McMahon et al., 1990] and protein levels [Caubet and Bernaudin, 1988; DeTogni et al., 1988] were found in areas of active osteoblast proliferation and differentiation in developing bone in rodents, and in fetal human bone [Sandberg et al., 1988a,b], suggesting that c-fos may have an important role in the development of osteogenesis. This is supported by the observation that overexpression of c-fos in transgenic mice induces new bone formation [Ruther et al., 1987, 1989; Wang et al., 1991]. Other studies have shown that deregulated fos expression interferes with osteogenic differentiation [Goralczyk et al., 1990], and that osteoblasts are target cells for transformation in c-fos transgenic mice [Grigoriadis et al., 1993].

To delineate the role of c-fos in normal osteoblast proliferation and differentiation during intramembranous and long bone osteogenesis, we have investigated the temporal pattern of c-fos mRNA expression in fetal and neonatal developing rat bone. The results indicate that c-fos expression during normal postnatal rat bone osteogenesis is followed by the induction of genes characterizing osteoblastic cells differentiation.

MATERIALS AND METHODS

Bone Samples

Femurs and calvarias were obtained from Sprague-Dawley fetal rats at 17, 19, and 21 days of age, from newborn rats, and from 1, 3, and 6 days old neonatal rats. The bones were removed and carefully dissected to remove mesenchymal connective tissues and to preserve the periosteum. For histology and immunohistochemistry, the bones were immediately fixed with 2% paraformaldehyde for 1 h, treated with 4% sucrose for 1 h, embedded in Tissue-Tek (Miles Inc., Elkhart, IN) and stored at −80°C until sectioning using a cryostat (Frigocut, Reichert-Jung, Heidelberg, Germany). The sections were collected on poly-L-lysine coated slides and stored at −80°C until they were stained using Mayer's hematoxylin, or used for immunohistochemistry. For RNA preparation, the calvaria and femurs were microdissected under sterile conditions. The femurs were separated into two portions consisting of the epiphyseal plate and the metaphysis plus the diaphysis. Calvaria samples and the different femur fractions obtained at the same site and from the same time point obtained from an average of 35 rats were pooled separately and stored at −80°C. Four series of similar independent experiments were performed.

RNA Isolation and Northern Blot Analysis

Total RNA was extracted from each pool of tissue using the guanidinium thiocyanate-phenol-chloroform method [Chomczynski and Sacchi, 1987]. Briefly, samples were homogeneized in a denaturing solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, 0.1 M β-mercaptoethanol, and 1 mM EDTA. RNA was extracted with water saturated phenol and purified by isopropanol precipitations. Aliquots containing 16 μg of total RNA were fractionated by gel electrophoresis on 1% agarose gels as described [McMaster and Carmichael, 1977], blotted onto GeneScreen transfer membranes (New England Nuclear, Boston, MA), and hybridized under stringent conditions. After hybridization, the filters were washed and the bound probe was detected by autoradiography at −80°C using X-ray films (Amersham, Arlington Heights, IL) and intensifying screens. The filters were re-probed for 18S signal to take in account the variations in RNA loading. The autoradiograms obtained were scanned using a densitometer (Transidyne 2500, General Corp., Ann Arbor, MI) and the integrated area under the curve for each signal was standardized against the signal for 18S rRNA. Three series of similar independent experiments were performed.

DNA Probes

The following DNA probes were used: a PstI/PvuII v-fos (FBJ-MSV) cDNA fragment of 1 kbp (Clontech, Palo Alto, CA), a Xenopus Boralis histone H4 genomic Hind III-XbeI fragment of 594 bp, a rat osteocalcin cDNA EcoRI insert of 520 bp [Yoon et al., 1988], and a 1975 bp mouse SalI/EcoRI 18S cDNA fragment [Raynal et al., 1984]. The (^{32}P)-dCTP-labelled cDNA probes

(specific activity: 2–4; 10^8 cpm/μg) were prepared by nick-translation (Boehringer, Mannheim, Germany) or random-priming (Promega, Madison, WI) methods using the manufacturer procedures.

Immunohistochemistry

Affinity-purified anti-Fos immunoglobulin (Oncogene Science, Uniondale, NY) used at 20 μg/ml was employed to detect the Fos protein on 30 μm thick sections by indirect immunofluorescence. The sections were first incubated with 2% BSA to remove the non-specific binding sites, permeabilized with 0.1% Triton X-100 for 30 *min at* 4°C, and washed three times with PBS for 10 min. This step was repeated at the end of subsequent treatments. The sections were treated with 0.1% H_2O_2 for 30 min to inactivate endogenous peroxidases and were exposed to the primary antibody overnight at 4°C. The sections were then treated with the second antibody (Amersham) coupled to horseradish peroxidase. To verify the specificity of the immunolabeling, control sections were treated with the same concentration of the anti-Fos antibody which has been pre-incubated with a 10-fold excess of Fos peptide (Oncogene Science).

Collagen and noncollagenous proteins were detected by immunochemistry as described previously [Machwate et al., 1993]. Fixed sections were washed in PBS with 0.01% Triton X-100, exposed to 0.1% H_2O_2 for 30 min, treated with 2% BSA for 30 min, washed in PBS/0.01% Triton X-100, and exposed to one of the following antiserum diluted 1/200: anti-rat osteocalcin raised in the rabbit [Modrowski et al., 1992], anti-human C-telopeptide (synthetic) of α1(I)-collagen (LF-67) crossing to the rat protein, or anti-bone sialoprotein (BSP) (LF-87) crossing to rat [Fisher et al., 1983; Bianco et al., 1991]. Control sections were treated with nonimmune serum instead of the primary antibody and diluted at the same dilution. After exposure for 2 h at room temperature, the sections were washed three times for 10 min in PBS/Triton-X 100, and exposed to a second donkey antibody linked to horseradish peroxidase (Boehringer) for 1 h. After washing in PBS, the specific antigen-antibody binding was revealed by exposing the cells to 1 mg/ml diaminobenzidin in 10 mM Tris buffer (pH 7.6) and 3 μl/ml of 3.0% H_2O_2. The immunohistochemical staining was visualized by light microscopy.

RESULTS

Expression of c-fos Proto-Oncogene During Fetal and Neonatal Osteogenesis

The development of the rat calvaria is characterized by the progressive formation of a bone matrix in fetal life and by a marked increase in bone formation during the postnatal life. Northern blot analysis (Fig. 1) and integration of signals after correction for RNA loading (Fig. 2) showed a temporal variation in the expression of c-fos, histone H4, and osteocalcin genes in the developing calvaria. C-fos mRNA levels progressively increased from 17 to 21 days of fetal life, rose abruptly at one day after birth and fell to undetectable levels at 3 and 6 days of postnatal life (Figs. 1, 2A). The levels of histone H4 mRNA, which are indicative of cell proliferation, were high at 17 days of fetal life, decreased until birth, and increased slightly in the postnatal life (Figs. 1, 2B). Osteocalcin mRNA levels, a marker of the late stage of osteoblast maturation, were low in the fetal life (Fig. 1) and increased progressively after birth (Fig. 1, 2C). These data indi-

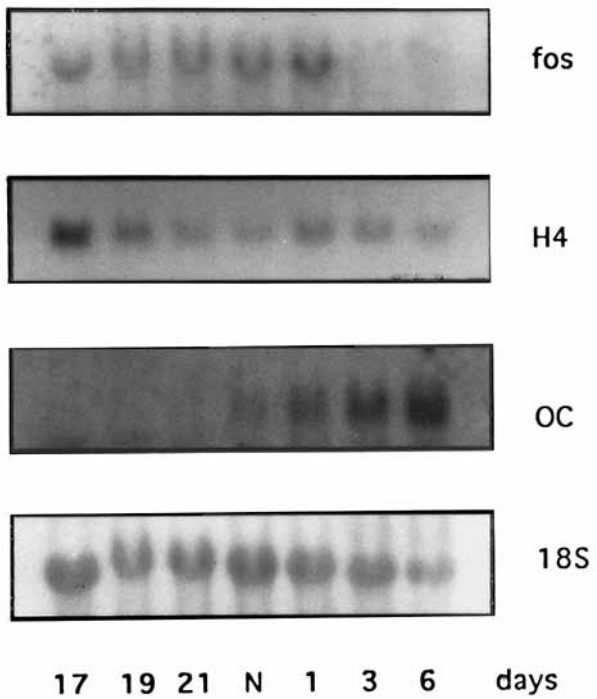

Fig. 1. Northern blot analysis showing the evolution of expression of *c-fos, histone H4, and osteocalcin (OC) compared to* 18S during fetal and neonatal development of the rat calvaria. The RNA was isolated from pooled calvarias obtained at 17, 19, and 21 days of fetal life, in newborn rats (N), and at 1, 3, and 6 days of postnatal life, and 16 μg of RNA were analysed for expression of the different genes. The blot was reprobed with the different probes. This is representative of four separate experiments.

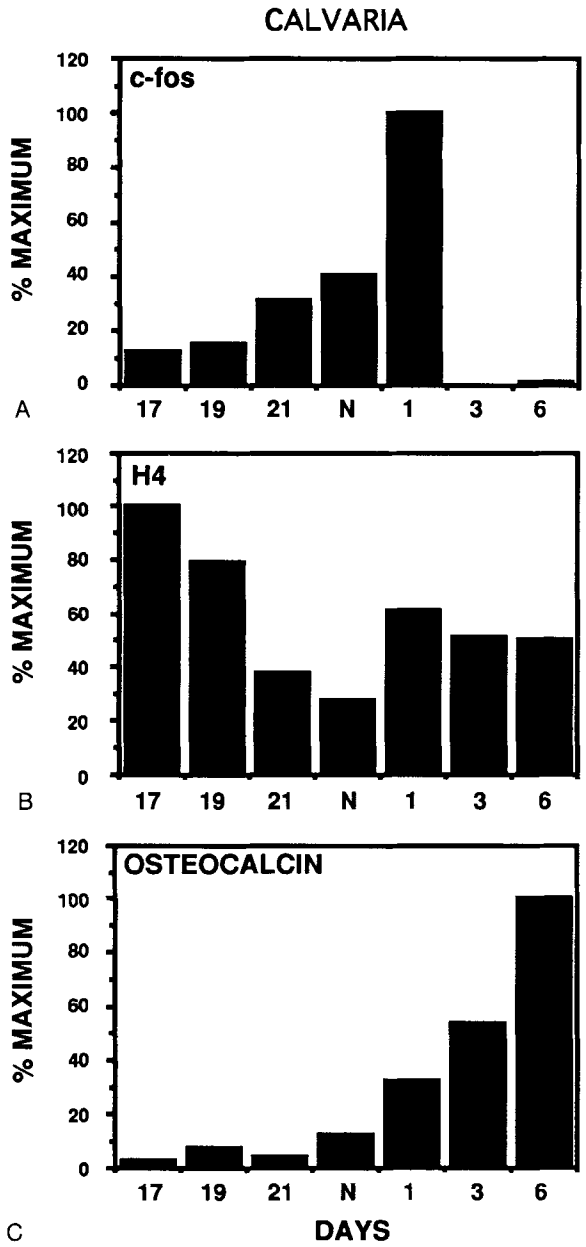

Fig. 2. Temporal pattern of expression of c-fos (A), histone H4 (B), and osteocalcin (C) in fetal and neonatal rat calvaria. The mRNA values were determined by northern blot analysis of total RNA obtained at 17, 19, and 21 days of fetal life, in newborn rats (N), and at 1, 3, and 6 days of postnatal life, and are expressed as a percentage of maximum expression calculated from the densitometric units normalized to 18S ribosomal RNA. Note the peak of c-fos expression occurring at 1 day after birth which was followed by the elevation of osteocalcin mRNA levels in the postnatal calvaria.

cate that the transient increase in the expression of c-fos mRNA in the neonatal calvaria is followed by a rise in the expression of osteocalcin, a marker of differentiated osteoblasts.

The development of the femur is characterized by a rapid bone growth from 17 to 21 days

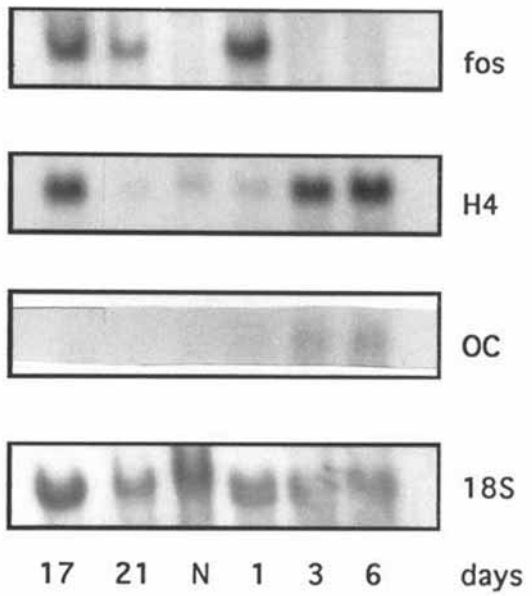

Fig. 3. Northern blot analysis showing the change in the expression of c-fos, histone H4, and osteocalcin (OC) compared to 18S during the development of the femur in the fetal and neonatal rat. The RNA was isolated from pooled femurs obtained at 17 and 21 days of fetal life, in newborn rats (N), and at 1, 3, and 6 days of postnatal life, and 16 μg of RNA were analyzed for the expression of different genes. The blot was reprobed with the different probes. The signal for osteocalcin was low even on over exposed blots. This is representative of four separate experiments.

and by a marked increase in bone formation and elongation after birth. The temporal sequence of gene expression was evaluated by northern blot analysis (Fig. 3) after integration and correction for the variations in RNA loading (Fig. 4). We found that c-fos mRNA levels were elevated at 17 days of fetal life and decreased until birth. At 1 day of life, c-fos mRNA increased transiently and declined thereafter at 3 and 6 days of postnatal life (Figs. 3, 4A). Histone H4 mRNA levels were high at 17 days of fetal life, decreased until birth and increased again at 3 and 6 days after (Figs. 3, 4B). Osteocalcin mRNA levels in femur were low in fetal life. The levels were higher after birth and increased at 1–6 days of postnatal life (Figs. 3, 4C). The comparative analysis of gene expression in calvaria and femur indicates that the transient rise in c-fos expression at birth was followed by a rise in osteocalcin expression during postnatal osteogenesis.

Expression of the c-Fos Protein in the Developing Bone

The cellular localization of the c-fos product during osteogenesis was evaluated by immuno-

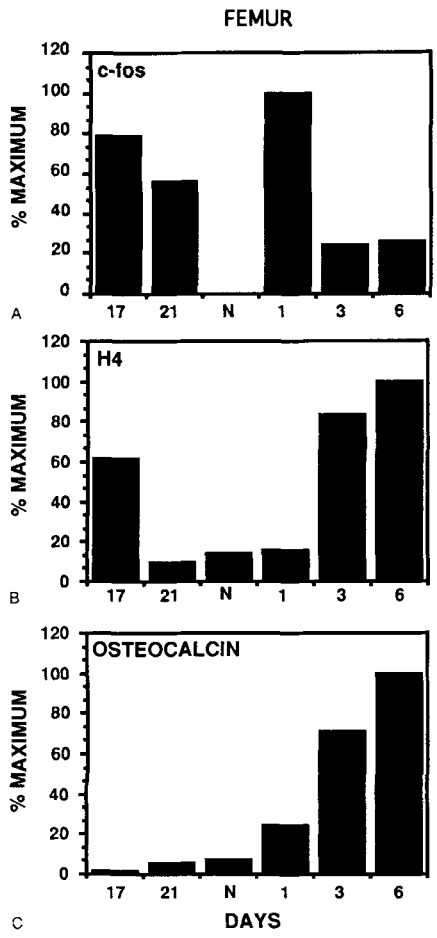

Fig. 4. Temporal pattern of expression of c-fos (A), histone H4 (B), and osteocalcin (C) in fetal and neonatal femur during development. The mRNA values were determined by northern blot analysis of total RNA obtained at 17 and 21 days of fetal life, in newborn rats (N), and at 1, 3, and 6 days of postnatal life, and are expressed as a percentage of maximum expression calculated from the densitometric units normalized to 18S ribosomal RNA. Note the peak of c-fos expression at one day after birth which was followed by the progressive increase in osteocalcin mRNA levels in the postnatal femur.

histochemistry on frozen bone sections. At 17 days of fetal life, a c-Fos signal was mainly located in the bone marrow cells (Fig. 5A). A specific signal for the c-Fos protein was found in the perichondrium and periosteum at all the subsequent stages of femur development. The specificity of immunostaining was checked out by using parallel sections incubated with a neutralized first antibody. The control sections showed no detectable staining (Fig. 5F). At 19 (Fig. 5B, G) and 21 (Fig. 5C, H) days of fetal life, a strong specific signal for c-Fos protein was found in perichondrial cells. A specific signal for c-Fos protein was also found in both the perichondrium and periosteum at 3 (Fig. 5D, I) and 6 (Fig. 5E) days of postnatal life. On the other hand, no signal for the c-Fos protein was found in cells covering the surface of the bone matrix (Figs. 5D, E and I) which were characterized as mature osteoblasts (see below). The c-Fos immunostaining in periosteal cells was found to decrease in the postnatal life (Table I). A specific staining for the c-Fos protein was also found in few cells of the hypertrophic cartilage in the zone adjacent to the metaphyseal bone (Fig. 5J). This staining occured until one day of life and declined thereafter (Table I), suggesting that c-fos proto-oncogene may be involved in endochondral bone formation.

Similar results were found in calvaria (not shown) in which a specific signal for the c-Fos protein was found in the periosteal cells, whereas the more mature osteoblastic cells located along the bone surface were not labelled (Table I). As in the femur, c-Fos expression in these cells decreased after 1 day of age (Table I).

Localization of Osteoblast Maturation Proteins During Osteogenesis

To determine the maturity state of cells expressing the c-Fos protein, we have studied the expression of three markers of osteoblast differentiation during osteogenesis. Figure 6 shows the immunostaining for collagen type-I, bone sialoprotein and osteocalcin in calvaria, and femur at 3 days of age. Similar results were found at earlier stages (data not shown). Only osteoblastic cells localized along the bone matrix in the periosteal and endosteal sites of the calvaria and femur were found to express type-I collagen, osteocalcin and bone sialoprotein, markers of the late stage of osteoblast differentiation (Fig. 6, Table II). These cells were not found to express the c-Fos protein (Fig. 5D,I,E, Table I). These data indicate that, during calvarial and femur development, c-Fos protein is expressed mainly in osteoblast precursors, but not in the mature osteoblasts.

DISCUSSION

A functional relationship between cell proliferation and differentiation is believed to be a fundamental concept of development. During in vitro osteogenesis by rat calvaria cells, the proliferating osteoblastic cells express cell cycle (Histone H4) and cell growth (fos, myc) regulated genes. Following the downregulation of the proliferative activity, several genes of the osteoblast phenotype are expressed in vitro [Owen et al., 1990a; Lian et al., 1991; Stein et al., 1993].

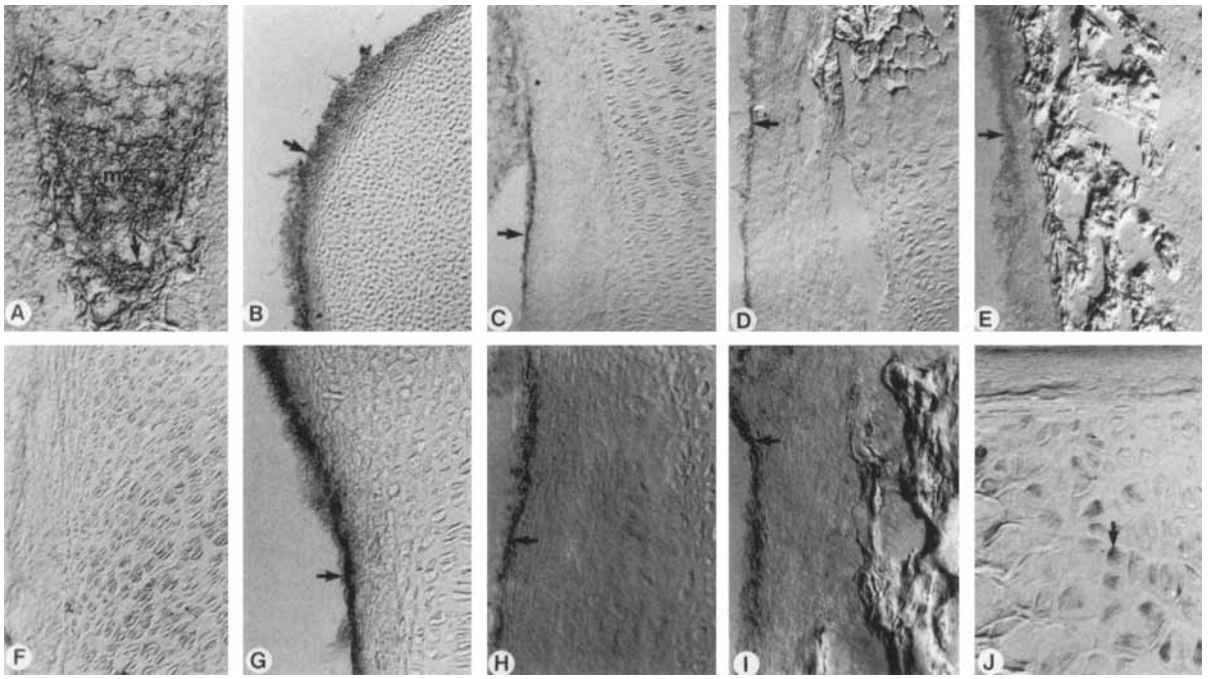

Fig. 5. Localization of c-Fos protein in the developing femur. Frozen sections of femurs at 17 (**A, F**), 19 (**B, G**), 21 (**C, H**) days of fetal life, and at 3 (**D, I**) and 6 (**E, J**) days of postnatal life were processed for immunohistochemistry and incubated with a specific c-Fos antibody (A–E, G–J), or with c-Fos antibody pre-incubated with a 10-fold excess of Fos peptide as controls (F). A specific signal for c-Fos protein was present in the marrow (A) and in pre-osteoblastic cells located in the perichondrium (B–D, G, H) and periosteum (D, E, I) (arrows). Only the outer layer of cells was stained (arrows), whereas the more mature cells covering the surface of the matrix were not stained (D, E, and I). c-Fos expression was also detected in some chondrocytes of the hypertrophic zone of the cartilaginous growth plate after birth (J). A–E, ×125; F–J, ×250.

TABLE I. Spatial and Temporal Expression of Fos Protein During Rat Calvaria and Femur Development*

Developmental stage (days pre- or postnatal)	Calvaria			Femur		
	Precursor cells	Mature osteoblasts	Cartilaginous cells	Perichondrial/ periosteal cells	Mature osteoblasts	Chondrocytes
19	++	−	−	++	−	++
0	++	−	−	++	−	++
1	++	−	+a	++	−	++
3	+	−	+a	+	−	+

*++, A strong and specific immunostaining for the Fos protein using a specific antibody; +, a weak immunostaining for the Fos protein; +a, immunostaining of a fraction of the cartilaginous cells closed to the bone matrix; and −, absence of immunostaining for the protein.

These in vitro studies indicate that the osteoblast proliferation and differentiation activities involve the expression of distinct genes. In addition there is some evidence that the c-fos proto-oncogene expression suppresses the osteoblastic phenotype and may be involved in osteoblastic cell proliferation in vitro [Lian et al., 1991]. In the present in vivo study, we have found that there is a temporal variation in histone H4 and osteocalcin mRNA levels during early osteogenesis in the calvaria and the femur. The decreased histone mRNA levels, used as a marker of cell proliferation, during the late stages of fetal life preceded the increase of the osteocalcin mRNA levels in both the calvaria and femur. This is consistent with the pattern of gene expression observed during in vitro osteogenesis indicating that there is a reciprocal relationship between the decline in proliferation and the initiation of expression of the osteoblast phenotype [Lian et al., 1991; Stein et al., 1993]. Interestingly, we found that the expression of c-fos

TABLE II. Expression of Osteoblast Differentiation Proteins in 3 Days Postnatal Rat Calvaria and Femur*

	Periosteal/ perichondrial precursor cells	Mature endosteal and periosteal osteoblasts	Cartilaginous cells
Calvaria			
Collagen type I	−	++	−
Osteocalcin	−	++	−
BSP	−	++	−
Femur			
Collagen type I	+	++	−
Osteocalcin	−	++	−
BSP	−	+	−

*++, A strong and specific immunostaining; +, a weak immunostaining; and −, absence of immunostaining for the corresponding protein.

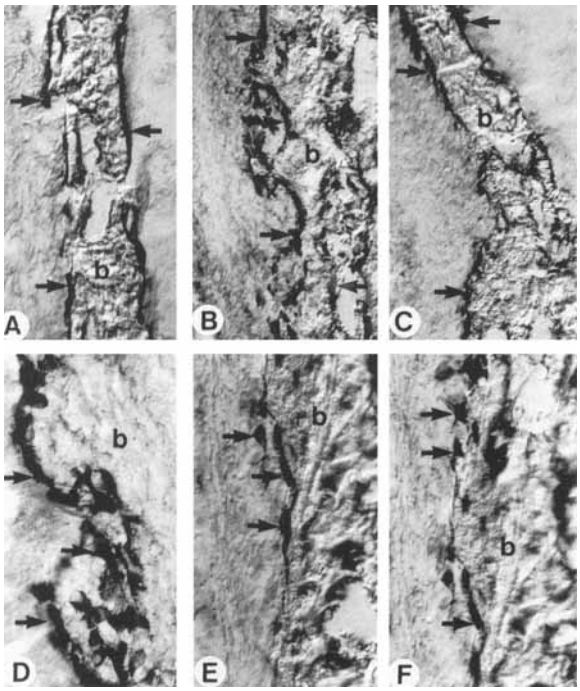

Fig. 6. Localization of osteoblast differentiation proteins in the calvaria and femur. Frozen sections of calvaria (**A–C**) and femur (**D–F**) at 3 days of age were immunostained for osteocalcin (A, D), collagen type-I (B, E), and bone sialoprotein (C, F). Only mature osteoblasts lining the bone matrix expressed collagen type-I, osteocalcin and bone sialoprotein, markers of the differentiated osteoblasts, whereas the layers of pre-osteoblastic cells located at distance of the bone did not express these proteins. Similar results were obtained at other time-point during development.

proto-oncogene during the late stages of fetal life differs in the calvaria and femur. This may be explained by the presence of different cell types in the two bones. Femur extract contains mRNAs that belong to heterogeneous cell populations, including hematopoietic cells that are known to express the c-fos proto-oncogene in vivo [Müller et al., 1984]. Thus, the expression of this proto-oncogene in the femur during the last stages of fetal life may be related to the growth of marrow cells. Unlike the femur, the calvaria extract contains mRNAs that mainly belong to cells of the osteoblast lineage. The histone H4 mRNA expression in the calvaria therefore predominantly denotes the osteoblastic cell proliferation activity. We found that, unlike in the in vitro osteogenesis model [Owen et al., 1990a], the high levels of histone transcripts in the calvaria during the prenatal life were not paralleled with an increased c-fos proto-oncogene expression. One possible explanation is that c-fos expression was higher at earlier stages of fetal calvaria development. Alternatively, the growth factors that are involved in the control of osteoblastic cell proliferation during the fetal life in the calvaria may not require c-fos proto-oncogene expression for their effect. This is in accordance with the fact that osteogenesis in transgenic mice lacking c-fos is normal during the fetal and the first days of postnatal lives [Johnson et al., 1992; Wang et al., 1992]. The osteopetrosis and the decreased osteoblast recruitment described in these mice only appear one to 2 weeks after birth. These findings taken together suggest that c-fos proto-oncogene expression is more likely involved in postnatal than in prenatal osteogenesis in rodents.

Both in calvaria and femur, the growth rate and osteogenesis are markedly stimulated during the postnatal life. The increase in osteocalcin transcript levels during this early postnatal period denotes an increased maturation of bone forming cells. We found that this increased osteocalcin expression was preceded by a transient expression of c-fos proto-oncogene. This postnatal transient expression of c-fos could not be attributed to mechanical stress that may occur during newborn delivery or tissue dissection. Indeed, we found that the c-fos expression peaked at 1 day of postnatal life, whereas the transient expression induced by mechanical stress is a more rapid phenomenon [Verrier et al., 1986; Closs et al., 1990]. The transient expression of c-fos proto-oncogene found in rat calvaria and femur was also reported in new-

born mice [Kasik et al., 1987; Grigoriadis et al., 1993], suggesting a role of this proto-oncogene in postnatal osteogenesis.

The immunohistochemical analysis showed that the c-Fos protein is only present in precursor cells but not in mature osteoblasts. These results are consistent with previous in vivo studies showing by in situ hybridization [Dony and Gruss, 1987; Sandberg et al., 1988a; McMahon et al., 1990] and immunohistochemistry [Caubet and Bernardin, 1988] that c-fos is expressed in the perichondrial growth region of fetal bone [Sandberg et al., 1988b]. Thus, the transient c-fos proto-oncogene expression during the postnatal life that preceded the osteocalcin expression may be involved in the transition from the precursor state to mature osteoblasts. These results are consistent with in vitro findings showing that the decline in c-fos proto-oncogene expression precedes the expression of osteoblast specific genes [Owen et al., 1990b; Lian et al., 1991]. Several genes of the osteoblast phenotype, including osteocalcin, are known to be regulated by Fos proteins. The osteocalcin promoter contains consensus sequences that bind the Fos/Jun complex [Demay et al., 1990; Ozono et al., 1990]. The binding of the Fos/Jun complex to these sequences suppresses the basal and induced osteocalcin gene expression, whereas the down-regulation of Fos/Jun complex in more mature osteoblasts allows the transcription of this gene [Owen et al., 1990b; Schüle et al., 1990; Lian and Stein, 1992].

The causative factors responsible for the transient change in c-fos mRNA levels at birth are unknown. The change in c-fos expression may be related to the induction by systemic or local growth factors that remain to be determined. Interestingly, a recent study showed that the subcutaneous administration of PTH (1–84) induced a sequential c-fos mRNA expression in rat long bone cells [Lee et al., 1994]. The present data indicating that the expression of c-fos proto-oncogene is followed by induction of osteogenic differentiation in vivo, suggest a physiological role of c-Fos during normal postnatal rat bone development.

ACKNOWLEDGMENTS

The authors wish to thank Dr. L.W. Fisher, Bone Research Branch, NIDR, NIH, Bethesda, MD, for the generous gift of antibodies against bone proteins, and Dr. M. Noda (Japan) for the osteocalcin probe.

REFERENCES

Angel P, Karin M (1991): The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1072:129–157.

Bianco P, Fisher FL, Young MF, Termine JD, Gehron Rohey P (1991): Expression of bone sialoprotein (BSP) in human developing skeletal and non-skeletal tissues as revealed by immunostaining and in situ hybridization. Calcified Tissue International 49:421–426.

Candeliere GA, Prud'homme J, St Arnaud R (1991): Differential stimulation of fos and jun family members by calcitriol in osteoblastic cells. Mol. Endocrinol. 5:1780–1788.

Caubet JF, Bernaudin JF (1988): Expression of the c-fos protooncogene in bone, cartilage and tooth forming tissues during mouse development. Biol Cell 64:101–104.

Chiu R, Boyle WJ, Meek J, Smeal T, Hunter T, Karin M (1988): The c-fos protein interacts with c-jun/AP-1 to stimulate transcription of AP-1-responsive genes. Cell 54:541–552.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Closs EI, Murray BA, Schmidt J, Schon A, Erfle V, Strauss PG (1990): C-fos expression precedes osteogenic differentiation of cartilage cells in vitro. J Cell Biol 111:1313–1323.

Curran T (1988): The fos oncogene. In: Reddy EP, Skalka AM, Curran T (eds): The Oncogene Handbook. Amsterdam, New York, Oxford: Elsevier Science Publishers, pp. 307–326.

Curran T, Franza BR (1988): Fos and Jun: The AP-1 connection. Cell 55:395–397.

Curran T, Peters G, Van Beveran C, Teich NM, Verma IM (1982): The FBJ murine osteosarcoma virus: Identification and molecular cloning of biologically active proviral. DNA J Virol 44:674–682.

Demay MB, Gerardi JM, Deluca HF, Kronenberg HM (1990): DNA sequences in the rat osteocalcin gene that bind the 1,25-dihydroxyvitamin D_3 receptor and confer responsiveness to 1,25-dihydroxyvitamin D_3 Proc Natl Sci USA 87:369–373.

DeTogni P, Niman H, Raymond V, Sawchenko P, Verma IM (1988): Detection of fos protein during osteogenesis by monoclonal antibodies. Mol Cell Biol 8:2251–2256.

Dony C, Gruss P (1987): Proto-oncogene c-fos expression in growth regions of fetal bone and mesodermal web tissue. Nature 328:711–714.

Finkel JP, Biskis BO, Jinkins P (1966): Virus induction of osteosarcoma in mice. Science 151:698–701.

Fisher LW, Whitson SW, Avioli LV, Termine JD (1983): Matrix sialoprotein of developing bone. J Biol Chem 258: 12723–12727.

Goralcxzyk R, Closs EI, Ruther U, Wagner EF, Strauss PG, Erfle V, Schmidt J (1990): Characterization of fos-induced osteogenic tumours and tumour-derived murine cell lines. Differentiation 44:122–131.

Greenberg ME, Ziff EB (1984): Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature 311:433–437.

Grigoriadis AE, Schellander K, Wang ZQ, Wagner EF (1993): Osteoblasts are target cells for transformation in c-fos transgenic mice. J Cell Biol 122:685–701.

Johnson RS, Spiegelman BM, Papaioannou V (1992): Pleiotropic effects of a null mutation in the c-fos proto-oncogene. Cell 71:577–586.

Kasik JW, Wan Y-JY, Ozato K (1987): A burst of c-fos gene expression in the mouse occurs at birth. Mol Cell Biol 7:3349–3352.

Lee K, Deeds JD, Chiba S, Un-No M, Bond AT, Segre GV (1994): Parathyroid hormone induces sequential c-fos expression in bone cells in vivo: in situ localization of its receptor and c-fos messenger ribonucleic acids. Endocrinology 134:441–450.

Lian JB, Stein GS (1992): Transcriptional control of vitamin D-regulated proteins. J Cell Biochem 49:37–45.

Lian JB, Stein GS, Bortell R, Owen TA (1991): Phenotype suppression: A postulated molecular mechanism for mediating the relationship of proliferation and differentiation by fos/jun interactions at AP-1 sites in steroid responsive promoters elements of tissue-specific genes. J Cell Biochem 45:9–14.

Machwate M, Zerath E, Holy X, Hott M, Modrowski D, Malouvier A, Marie PJ (1993): Skeletal unloading in rat decreases proliferation of rat and bone marrow-derived osteoblastic cells. Am J Physiol (Endocrinol Metabol 27) 264:E790–E799.

Matsumoto A, Okitsu M, Deyama Y, Yoshimura Y, Suzuki K, Hisada Y, Uematsu T (1993): c-fos m-RNA expression after treatment with fetal bovine serum and epidermal growth factor for osteoblastic MC3T3-E1 cells cultured in a low environment. Biochem Biophys Res Comm 193:77–87.

McMahon AP, Champion JE, McMahon JA, Sukhtame VP (1990): Developmental expression of the putative transcription factor Egr-1 suggests that Egr-1 and c-fos are coregulated in some tissues. Development 108:281–287.

McMaster GK, Carmichael GG (1977): Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc Natl Acad Sci USA 74:4835–4840.

Merriman HL, LaTour D, Likhart TA, Mohan S, Baylink DI, Strong DD (1990): Insulin-like growth factor-I and insulin-like growth factor-II induce c-fos in mouse osteoblastic cells. Calcif Tissue Int 46:258–262.

Modrowski D, Miravet I, del Pozo E (1992): Dynamics of circulating osteocalcin in rats during growth and under experimental conditions. Horm Metab Res 24:474–477.

Müller R (1986): Cellular and viral fos genes: Structure, regulation of expression and biological properties of their encoded products. Biochem Biophys Acta 823:207–225.

Müller R, Müller D, Guilbert L (1984): Differential expression of c-fos in hematopoietic cells: Correlation with differentiation of monomyelocytic cells in vitro. 3:1887–1890.

Okasaki R, Ideda K, Sakamoto A, Nakano T, Morinoto K, Kikuchi T, Urakawa K, Ogata E, Matsumoto T (1992): Transcriptional activation of c-fos and c-jun protooncogenes by serum growth factors in osteoblast-like MC3T3-E1 cells. J Bone Miner Res 7:1149–1155.

Owen TA, Aronow M, Shalhoub V, Barone LM, Wilming L, Tassinari MS, Kennedy MB, Pockwinse S, Lian JB, Stein GS (1990a): Progressive development of the rat osteoblast phenotype in vitro: Reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of bone extracellular matrix. J Cell Physiol 143:420–430.

Owen TA, Bortell K, Yocum SA, Smock SL, Zhang M, Abate C, Shalhoub V, Aronin N, Wright K, Van Wijnen AJ, Stein JL, Curran T, Lian JB, Stein GS (1990b): Coordinate occupancy of AP-1 sites in the vitamin D-responsive and CCAAT box elements by Fos-Jun in the osteocalcin gene: model for phenotype suppression of transcription. Proc Natl Acad Sci USA 8:9990–9994.

Ozono K, Liao J, Kerner SA, Scott RA, Pike JW (1990): The vitamin D-responsive element in the human osteocalcin gene. J Biol Chem 265:21881–21888.

Poliard A, Lamblin D, Marie PJ, Buc-Caron MH, Kellermann O (1993): Commitment of the teratocarcinoma-derived mesodermal clone-C1 towards terminal osteogenic differentiation. J Cell Sci 106:503–512.

Raynal F, Michot B, Bachellerie JP (1984): Complete nucleotide sequence of mouse 18S rRNA gene: Comparison with other available homologs. FEBS Lett 167:263–268.

Rodan GA, Noda M (1991): Gene expression in osteoblastic cells. Euk Gene Expression 1:85–98.

Ruther U, Garber C, Komitowski D, Müller R, Wagner EF (1987): Degranulated c-fos expression interferes with normal bone development in transgenic mice. Nature 325:412–416.

Ruther U, Komitowski D, Schubert FR, Wagner EF (1989): C-fos expression induces bone tumors in transgenic mice. Oncogene 4:861–865.

Sandberg M, Autio-Harmainen H, Vuorio E (1988a): Localization of the expression of types I, III and IV collagen, TGF-β1 and c-fos genes in developing human calvarial bones. Dev Biol 130:324–334.

Sandberg M, Vuorlo T, Hirvonen H, Alitalo K, Vuorio E (1988b): Enhanced expression of TGF-β and c-fos mRNAs in the growth plates of developing human long bones. Development 102:461–470.

Schüle R, Umesono K, Mangelsdorf DJ, Bolado J, Pike JW, Evans RM (1990): Jun-Fos and receptors for vitamins A and D recognize a common response element in the human osteocalcin gene. Cell 61:497–504.

Shaloub V, Jackson ME, Lian JB, Stein GS, Marks SC (1991): Gene expression during skeletal development in three osteopetrotic rat mutations: Evidence for osteoblast abnormalities. J Biol Chem 266:9847–9856.

Stein GS, Lian JB (1993): Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. Endocrinol Rev 14:424–442.

Turksen K, Aubin JE (1991): Positive and negative immunoselection for enrichment of two classes of osteoprogenitor cells. J Cell Biol 114:373–384.

Verma IM, Graham WR (1987): The fos oncogene. Adv Cancer Res 49:29–52.

Verrier B, Müller RD, Bravo R, Müller R (1986): Wounding a fibroblast monolayer results in the rapid induction of the c-fos proto-oncogene. EMBO J 5:913–917.

Wang Z, Ovit QC, Grigoriadis AE, Mohle-Steinlein U, Ruther U, Wagner EF (1992): Bone and haematopoietic defects in mice lacking c-fos. Nature 360:741–745.

Wang ZQ, Grigoriadis AE, Mohle-Steinlein U, Wagner EF (1991): A novel target cell for c-fos-induced oncogenesis: development of chondrogenic tumors in embryonic stem cell chimeras. EMBO J 10:2437–2450.

Yoon K, Buenaga RF, Rodan GA (1987): Tissue specificity and developmental expression of rat osteopontin Biochem Biophys Res Commun 148:1129–1132.

Yoon K, Ruthedge SJC, Buenaga RF, Rodan GA (1988): Characterization of the rat osteocalcin gene: Stimulation of promoter activity by 1,25-dihydroxyvitamin D3. Biochemistry 27:8521–8526.