Dual Regulation of Stromelysin-3 by Fibroblast Growth Factor-2 in Murine Osteoblasts*

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Osteoblasts express stromelysin-3, a matrix metalloproteinase associated with normal remodeling processes and with stromal fibroblasts surrounding many invasive carcinomas. Fibroblast growth factors (FGFs) play an important role in skeletal development, fracture repair, and osteoblast function. The osteoblastic cell line MC3T3 was used to study the regulation of stromelysin-3 by FGF-2. Acutely, FGF-2 decreased stromelysin-3 mRNA levels, whereas prolonged treatment caused an induction of stromelysin-3 mRNA. RNA stability studies and nuclear run-off assays indicated that acute treatment with FGF-2 decreased stromelysin-3 mRNA stability but did not alter gene transcription. However, the induction of stromelysin-3 after prolonged treatment with FGF-2 resulted from increased gene transcription, with no effect on RNA stability. The stimulatory effect was protein synthesis-dependent, whereas the inhibitory effect was not. This study demonstrates dual regulation of stromelysin-3 by FGF-2: acute destabilization of stromelysin-3 mRNA, followed by induction of gene transcription. This complex regulation may be important in the function of stromelysin-3 in bone and in remodeling processes, such as wound and fracture repair.

Stromelysin-3 or matrix metalloproteinase-11 was originally cloned from stromal fibroblasts surrounding invasive breast cancer cells (1). Subsequently, it was found to be expressed by stromal cells surrounding other invasive carcinomas, including those of the lung, colon, and skin (2–4). In addition, stromelysin-3 expression is associated with normal remodeling processes, such as wound healing, involving mammary gland, and cycling endometrium (4–6). Stromelysin-3 is synthesized as an inactive proenzyme of ~60 kDa. However, it differs from most members of the matrix metalloproteinase family in that the proenzyme is processed intracellularly by a furin-like enzyme within the Golgi apparatus, and the mature, processed ~45-kDa enzyme is released from the cell (7). The mature 45-kDa form of stromelysin-3 does not appear to cleave major extracellular matrix components. To date, the only characterized substrates for the enzyme are α1 proteinase inhibitor, a serine protease inhibitor, and insulin-like growth factor (IGF)1-binding protein-1, a regulator of IGF bioavailability (8, 9). In contrast, mature stromelysin-3 forms that lack the C-terminal hemopexin-like domain have stromelysin-like activity and are able to degrade casein and some extracellular matrix components, including laminin, fibronectin, and aggrecan (10). It is possible that the role of stromelysin-3 in remodeling processes involves the activities of both the intact mature form and proteolytically cleaved form of the enzyme.

Stromelysin-3 transcripts were detected in osteogenic tissue of mouse embryos, suggesting that osteoblasts express the enzyme (11). Bone is a continuously remodeling tissue, and osteoblasts produce metalloproteinases in response to local and systemic growth factors and hormones. For example, systemic parathyroid hormone induces collagenase-3 in osteoblasts, as do local factors, including fibroblast growth factor-2 (FGF-2), interleukin 1, and tumor necrosis factor α (12–15). In non-skeletal cells, stromelysin-3 is regulated by growth factors, including platelet-derived growth factor, epidermal growth factor, and FGF-2; however, the mechanisms responsible for this induction have not been reported (1). It is possible that such growth factors regulate stromelysin-3 expression in osteoblasts.

In the skeleton, FGFs regulate development, fracture repair, and osteoblast function (16, 17). FGF-2 is a potent mitogen for cells of the osteoblastic lineage, and it represses the differentiated function of the cell (18). For example, FGF-2 represses type I collagen, osteocalcin, and alkaline phosphatase mRNA expression in osteoblasts (19, 20). FGF-2 is expressed during fracture repair, and local administration of FGF-2 stimulates the repair process, suggesting a role in matrix remodeling (16). The importance of FGFs in skeletal development is illustrated by the finding that loss or gain of function mutations in FGF receptors can lead to skeletal defects (21–23). Because stromelysin-3 is expressed in areas of active remodeling and FGFs play a role in remodeling and repair in bone, we hypothesized that stromelysin-3 is expressed by osteoblasts and that it is regulated by FGF-2 in these cells. In the present study, cultures of the nontransformed murine osteoblastic cell line MC3T3-E1 were used to characterize the mechanisms by which FGF-2 regulates stromelysin-3 expression in osteoblasts.

EXPERIMENTAL PROCEDURES

Cell Cultures—MC3T3-E1 is an osteogenic cell line derived from neonatal mouse calvaria. Early passage MC3T3 cells were cultured in a minimum essential medium (Life Technologies, Inc.) containing 20 mM HEPES and 10% fetal bovine serum (Summit Biotechnologies, Ft. Collins, CO) (24). Cells were grown to confluence, rinsed, and transferred to serum-free medium containing 0.1% bovine serum albumin (Fluka Chemical Corp., Ronkonkoma, NY) and 50 μg/ml ascorbic acid for 24 h. Cultures were then exposed to test or control medium in the absence of serum for 2–48 h. In cultures used for Western blot analysis, the fibroblast growth factor; UTR, untranslated region; DRB, 5,6-dichloro- benzimidazole riboside; kb, kilobase.
medium did not contain bovine serum albumin and was harvested after the first 24 h of incubation, and the cells were then exposed to fresh medium for a second 24-h period. In all other cultures, the medium was not renewed.

Primary cultures of mouse osteoblastic cells were isolated from parietal bones of neonatal CD-1 mice (25). This procedure was approved by the Institutional Animal Care and Use Committee of St. Francis Hospital and Medical Center. Parietal bones, dissected free of sutures, were subjected to five sequential 15-min digestions with bacterial collagenase (CLS II, Worthington Biochemical, Freehold, NJ). Cells harvested from digestions 3–5 were cultured as a pool at an initial plating density of approximately 10,000 cells/cm². These cells have been demonstrated to have osteoblastic characteristics (25, 26). Cells were cultured in an fibroblast-identified Eagle’s medium supplemented with nonessential amino acids, 20 mM HEPES, 100 µg/ml ascorbic acid, and 10% fetal bovine serum. When the cells reached confluence, approximately 1 week after plating, they were rinsed and transferred to serum-free medium for 24 h and then exposed to test or control medium for 2–48 h, without renewal of medium.

Cycloheximide, indomethacin, and 5,6-dichlorobenzimidazole riboside (DRB) (Sigma) were dissolved in absolute ethanol, and at dilutions of <1:1,000, an equal amount of ethanol was added to control cultures. FGF-2 (Austral, San Ramon, CA) was dissolved in minimum essential medium.

Northern Blot Analysis—Total cellular RNA was isolated with guanidine thiocyanate, at pH 7.0, followed by a phenol-chloroform (Sigma) extraction as described (27) or by using PURESprint RNA isolation kits (Genta Systems, Minneapolis, MN). Equal amounts of RNA (15 µg) were denatured and subjected to electrophoresis through formaldehyde-agarose gels, and the RNA was blotted onto Gene Screen Plus as directed by the manufacturer (DuPont). A 1.6-kilobase (kb) SphI/SalI fragment of the mouse stromelysin-3 cDNA (provided by P. Basset, IGBMC, Illkirch, France) and a 750-base pair rat cyclophilin cDNA (provided by P. Danielson, La Jolla, CA) were labeled with [α-32P]dCTP (3000 Ci/mmol; DuPont) by random primed second strand synthesis (Prime-A-Gene kit, Promega, Madison, WI) (1, 28). Hybridizations were carried out at 42 °C in 50% formamide, 750 mM sodium chloride, 50 mM sodium phosphate, 6 mM EDTA, 5 mM Denhardt’s solution, and 0.4% SDS (Sigma) (29). Posthybridization washes were performed at 65 °C in 150 mM sodium chloride, 15 mM sodium citrate, and 0.1% SDS. Autoradiographs were analyzed by densitometry, and stromelysin-3 mRNA levels were normalized to those of cyclophilin.

Nuclear Run-off Assay—Nuclei were isolated from confluent MC3T3 cells by Dounce homogenization in a Tris-HCl buffer containing 0.5% Nonidet P-40 (Sigma). Nascent transcripts were labeled by incubation of nuclei in a reaction buffer containing 500 µM each ATP, GTP, CTP, and UTP (800 Ci/mM, DuPont) (26). RNA was isolated by treatment with DNase I and proteinase K, followed by ethanol precipitation. Linearized plasmid DNA containing approximately 1 µg of cDNA was immobilized onto GeneScreen Plus by slot blotting according to the manufacturer’s directions (DuPont). cDNA for rat cyclophilin and 18S rRNA (American Type Culture Collection, Rockville, MD) were used as controls for loading of the radiolabeled RNA (28, 30). The plasmid vector pGEM5zf+ (Promega) was used as a control for nonspecific hybridization. Equal counts per minute of [32P]RNA from each sample were hybridized to cDNA using the same conditions as for Northern blot analysis and were visualized by autoradiography.

Western Blot Analysis—Conditioned medium samples were stored at –80 °C, the addition of 0.1% polyethylene glycol monosulfate (Tween-20, Pierce), 10 mM EDTA, 50 mM Tris-HCl, pH 8.0, and 20 µg/ml phenylmethylsulfonyl fluoride. Prior to electrophoresis, 750 µl of conditioned medium were precipitated with 3.3% trichloroacetic acid and resuspended in reducing Laemmli sample buffer containing 2% SDS. Proteins were fractionated by polyacrylamide gel electrophoresis using a 12% gel and transferred to Immobilon P membranes (Millipore, Bedford, MA). Membranes were blocked with 5% bovine serum albumin and exposed to a 1:1000 dilution of rabbit antiserum raised against recombinant human stromelysin-3 (provided by Dr. S. Weiss, Ann Arbor, MI) (9, 31). The membranes were then washed and exposed to horseradish peroxidase-conjugated goat anti-rabbit IgG antiserum, washed, and developed using a horseradish peroxidase chemiluminescent reagent (DuPont). Immunoreactive bands were visualized using Reflection x-ray film (DuPont).

Statistical Analysis—Slopes of RNA decay curves were analyzed by the method of Sokal and Rohlf (32).

### RESULTS

MC3T3 osteoblasts constitutively express a major stromelysin-3 transcript of approximately 2.2 kb, with a minor, slower mobility transcript of approximately 3.2 kb. The larger size transcript is observed primarily when the smaller size transcript is absent. FGF-2 had two distinct effects on stromelysin-3 mRNA levels. FGF-2 at 30 ng/ml decreased stromelysin-3 transcripts after 2 h of treatment, and a decrease of 62 ± 1% (mean ± S.E.; n = 6) was observed after 6 h (Fig. 1). After 24 h of treatment, FGF-2 had a modest and variable effect on stromelysin-3 transcript levels, causing only a 6 ± 1% (n = 7) decrease (Fig. 1). After 48 h of treatment, FGF-2 increased stromelysin-3 transcripts 3.5 ± 0.4-fold (n = 6). Both the stimulatory and inhibitory effects of FGF-2 on stromelysin-3 mRNA were dose-dependent, and the lowest effective dose tested was 1 ng/ml (Fig. 2).

Multiple stromelysin-3 protein species were detected by Western blot analysis of MC3T3 cell conditioned medium (Fig. 3). The mature form of stromelysin-3, lacking the propeptide, has a molecular mass of approximately 45 kDa; however, the most abundant species present in the osteoblast conditioned medium had a molecular mass of approximately 34 kDa, probably representing a proteolytic fragment (7, 8, 10). In addition, an approximately 62-kDa species was detected, which probably represents the proenzyme (8). Treatment with FGF-2 for 24 h decreased the abundance of the 34-kDa fragment by 55 ± 2% (n = 3), whereas secretion of the 62-kDa species was modestly increased or unchanged. Treatment with FGF-2 for 48 h increased the level of 34-kDa stromelysin-3 species by approximately 8.4 ± 2.2-fold (n = 3), whereas the 62-kDa form was increased by a lesser extent. When the conditioned medium samples were concentrated using chloroform/methanol precipitation and then trichloroacetic acid, a similar distribution of stromelysin-3 species was observed, suggesting that fragmentation of stromelysin-3 was not due to acidification of the samples (Ref. 33 and data not shown). These results indicate that changes in transcript levels are followed by changes in polypeptide levels.

The inhibition of stromelysin-3 transcripts by FGF-2 after 6 h of treatment was observed in the presence and absence of 2
FGF-2 at 30 ng/ml decreased stromelysin-3 mRNA levels, whereas 48 h treatment with FGF-2 increased stromelysin-3 mRNA (Fig. 8).

To confirm that the regulation of stromelysin-3 by FGF-2 was not a cell line-specific phenomenon, primary cultures of osteoblastic cells were prepared from neonatal mouse calvaria.

Fig. 3. Effect of FGF-2 at 30 ng/ml on stromelysin-3 protein levels in MC3T3 cells treated for 24 or 48 h. Conditioned medium from control or FGF-2-treated cultures was subjected to Western blot analysis using rabbit anti-human stromelysin-3 antiserum. The migration of the molecular mass standards is shown on the right. These results are representative of three cultures.

Fig. 4. Effect of FGF-2 at 30 ng/ml, in the presence or absence of indomethacin at 10 μM, on stromelysin-3 mRNA expression in MC3T3 cells treated for 6, 24, or 48 h. Total RNA from untreated cells or cells treated with FGF-2 or indomethacin (Indo) was subjected to Northern blot analysis and hybridized with a 32P-labeled stromelysin-3 cDNA (S3); the blot was stripped and hybridized with a labeled cyclophilin (cyclo) cDNA. Transcripts were visualized by autoradiography. These results are representative of three cultures.

Fig. 5. Effect of FGF-2 at 30 ng/ml, in the presence or absence of cycloheximide at 2 μg/ml, on stromelysin-3 mRNA expression in MC3T3 cells treated for 24 or 48 h. Total RNA from untreated cells or cells treated with FGF-2 or cycloheximide (CX) was subjected to Northern blot analysis and hybridized with a 32P-labeled stromelysin-3 cDNA (S3); the blot was stripped and hybridized with a labeled cyclophilin (cyclo) cDNA. Transcripts were visualized by autoradiography. These results are representative of three cultures.

DISCUSSION

Although stromelysin-3 was first found expressed by stromal fibroblasts surrounding invasive breast cancer cells, it was subsequently found to be expressed in other nonpathological remodeling processes. This study confirms that cultured osteoblasts constitutively express stromelysin-3 (10). Both MC3T3 cells and primary cultures of murine osteoblasts express a major stromelysin-3 transcript of approximately 2.2 kb and a minor transcript of approximately 3.2 kb. Presently, it is difficult to determine whether the 3.2-kb transcript represents a gene product closely related to stromelysin-3 or whether it is due to the use of alternative transcription initiation sites, splicing, or polyadenylation. Others have not detected the minor transcript in human fibroblastic cells or in NIH 3T3 cells, suggesting that the 3.2-kb stromelysin-3 transcript may be cell type-specific (36, 37). Furthermore, cultured murine osteoclasts can express gelatinases and membrane type matrix metalloproteinase-1; however, preliminary studies indicate that they do not express stromelysin-3 (38, 39). This also suggests cell type-specific expression of stromelysin-3 in bone.

FGFs are important regulators of osteoblast gene expression.

μg/ml cycloheximide (Fig. 4) (26). In contrast, the recovery of stromelysin-3 mRNA levels in cells treated with FGF-2 for 24 h was prevented by cycloheximide (Fig. 4A). In cells treated with cycloheximide for 48 h, stromelysin-3 transcripts were no longer detectable (Fig. 4B). These data suggest that the stimulatory effect of FGF-2 on stromelysin-3 mRNA expression is dependent on a new protein synthesis. Because FGF-2 stimulates prostaglandin production, and prostaglandin E2 increases stromelysin-3 mRNA levels, the cyclooxygenase inhibitor indomethacin was used to determine whether prostaglandin synthesis played a role in the stimulatory effect of FGF-2 on stromelysin-3 (34). Indomethacin had no effect on the stimulation of stromelysin-3 by FGF-2, suggesting a prostaglandin-independent mechanism (Fig. 5).

To determine whether FGF-2 modified the stability of stromelysin-3 mRNA in osteoblasts, the RNA polymerase II-specific inhibitor DRB was used to arrest transcription, and the decay of stromelysin-3 mRNA was monitored by Northern blot analysis (25, 35). In a first set of experiments, serum-deprived cultures were exposed to control medium or to 30 ng/ml FGF-2 for 1 h and then treated with 72 μM DRB for up to 5 h. In transcriptionally arrested osteoblasts, the half-life of stromelysin-3 mRNA was approximately 2.5 h, but in the presence of FGF-2, the stability of the transcript decreased by one-half, to approximately 1.25 h (Fig. 6, left panel). In a second set of experiments, serum-deprived cultures were exposed to control medium or to 30 ng/ml bFGF for 24 h and then treated with 72 μM DRB for up to 5 h. In these cultures, the half-life of stromelysin-3 mRNA was approximately 2.5 h, and the stability of the transcripts was not altered by treatment with FGF-2 (Fig. 6, right panel). These data indicate that destabilization of stromelysin-3 mRNA is an acute effect of FGF-2 and that this effect is no longer evident after 24 h of treatment with the growth factor. To determine whether there was a transcriptional component to the regulation of stromelysin-3 by FGF-2, nuclear run-off assays were performed. FGF-2 did not alter the rate of stromelysin-3 gene transcription after 2 (not shown) or 6 h of treatment. However, after 24 or 48 h, FGF-2 increased stromelysin-3 gene transcription 1.8–3-fold (Fig. 7). These data suggest that the acute FGF-2-mediated decrease in stromelysin-3 mRNA is due to a decrease in metalloproteinase transcript stability, and the increase in stromelysin-3 mRNA observed after 48 h of treatment is due to an increase in gene transcription.

To confirm that the regulation of stromelysin-3 by FGF-2 was not a cell line-specific phenomenon, primary cultures of osteoblastic cells were prepared from neonatal mouse calvaria.

A. M. Delany, unpublished data.
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They play a role in skeletal development and fracture repair, events associated with extracellular matrix remodeling (16, 17). Our data reveal a complex regulation of stromelysin-3 by FGF-2 in osteoblasts. Acutely, FGF-2 decreases stromelysin-3 mRNA and protein expression by decreasing the stability of the transcript. Nuclear run-off data failed to demonstrate a transcriptional component to this down-regulation, indicating that the reduction in stromelysin-3 RNA stability alone accounts for the decrease in steady-state mRNA levels. Down-regulation of stromelysin-3 mRNA by FGF-2 is independent of protein synthesis, suggesting a direct effect of the growth factor. FGF receptors are ligand-activated tyrosine kinases (40). It is possible that changes in protein phosphorylation resulting from the activation of the FGF receptor alters the activity of stromelysin-3 RNA-binding proteins and causes a decrease in RNA stability. It is suggested that the activity of several RNA-binding proteins is regulated by changes in protein phosphorylation. In particular, protein kinase C pathways are implicated in regulating the activity of ferritin and RNA-binding proteins associated with urokinase-type plasminogen activator, urokinase receptor, and ribonucleotide reductase component R2 transcripts (41–43). Such RNA-binding proteins could be nucleases or proteins that protect the transcript from degradation.

Frequently, sequences contained in the 3′-untranslated region (UTR) of the mRNA are associated with the regulation of transcript stability. For example, AU-rich elements within the collagenase-1 3′-UTR play a role in destabilizing the collagenase mRNA (44). Like collagenase-1, collagenase-3 and stromelysin-1 can be regulated by posttranscriptional mechanisms, and the 3′-UTRs of these transcripts contain AU-rich sequences (29, 45). AU-rich motifs are important in the destabilization of short-lived cytokine and proto-oncogene mRNAs and are implicated in increasing stability of the transcripts in response to cytokines (46). In transcriptionally arrested osteoblasts, collagenase-3 mRNA has a longer half-life than stromelysin-3 mRNA (29). However, the stromelysin-3 3′-UTR does not contain classical AU-rich motifs seen in the 3′-UTR of collagenase-1 or collagenase-3 (1). Therefore, it is likely that the mechanisms regulating the stability of stromelysin-3 mRNA differ from those regulating collagenase-3 or other metalloproteinase mRNAs containing AU-rich motifs.

Nuclear run-off assays indicate that FGF-2 stimulates stromelysin-3 gene transcription after 24 and 48 h of treatment. The delayed induction of stromelysin-3 mRNA by FGF-2 and the ability of cycloheximide to block the induction suggest that the phenomenon is a secondary effect of the growth factor. It is possible that the transcriptional increase is mediated by a newly synthesized, FGF-2-regulated protein. For example, one transcription factor known to be induced by FGF is c-Jun (47). To date, only the human stromelysin-3 promoter has been cloned and characterized. The proximal promoter lacks a functional AP-1 (Fos/Jun binding) site; therefore, c-Jun may not have direct action on the stromelysin-3 promoter (36, 48). The lack of a functional AP-1 site and the presence of a DR1 type retinoic acid responsive element are some of the characteristics that make the human stromelysin-3 promoter different from other matrix metalloproteinase genes (36, 48). Similar to collagenase-1 and stromelysin-1, the stromelysin-3 promoter contains a PEA-3 site, which could potentially bind members of the c-Ets family of transcription factors. In contrast to these metalloproteinases, this site does not appear to be functional because a promoter fragment containing the PEA3 motif is not transactivated by c-Ets-1 (48). Identification of the DNA elements necessary for transcriptional induction by FGF-2 could provide insight into the regulation of stromelysin-3 in development and wound or fracture repair.
Western blot analysis of osteoblast conditioned medium showed little intact, mature stromelysin-3, whereas the most abundant form was a 34-kDa species. This 34-kDa species may lack the hemopexin-like C-terminal domain (49). Stromelysin-3 peptides lacking the hemopexin-like domain have been shown to have stromelysin-like activities (10). Recently, a 35-kDa stromelysin-3 species was purified from FGF-2 treated tumor/stroma cocultures (50). This 35-kDa stromelysin-3 is cleaved at the N-terminal end and lacks a portion of the catalytic domain, suggesting that it lacks the metalloproteinase activity associated with the intact enzyme. The generation of the 35-kDa stromelysin-3 requires the presence of FGF-2 and is thought to involve a matrix metalloproteinase-dependent mechanism (50). Thus, the 34-kDa stromelysin-3 found in cultured osteoblasts may be truncated at the N terminus, although this peptide is observed in the presence and absence of exogenous FGF-2. However, endogenous FGFs may be present because osteoblasts can secrete FGFs and store them in their matrix (51).

The function of stromelysin-3 in bone is not yet defined. Stromelysin-1 and -2 are not constitutively expressed by normal osteoblasts; in contrast, stromelysin-3 is constitutively expressed (15). Because forms of stromelysin-3 that lack the hemopexin-like domain have activities similar to that of other stromelysins, cleaved stromelysin-3 may provide stromelysin-like activity to the bone environment (10). Through its ability to degrade serine protease inhibitors, stromelysin-3 may potentiate the matrix degradation cascade in remodeling, because serine proteinases can activate other matrix metalloproteinases, such as collagenases and stromelysins 1 and 2 (52). Indeed, hormonal regulation of stromelysin-3 appears to be important in normal remodeling processes. For example, stromelysin-3 is induced by thyroid hormone in the metamorphosing intestine of the frog Xenopus laevis. Its expression peaks at the metamorphic climax, in concert with endogenous thyroid hormone levels (53). Similarly, stromelysin-3 synthesis is repressed by progesterone in endometrial cells. In cycling human endometrium, stromelysin-3 expression is high in the proliferative and menstrual phases (when tissue growth and remodeling occurs and progesterone levels are low) (6).

In addition, stromelysin-3 can affect growth factor activity through its degradation of IGF-binding protein-1, a protein that limits IGF I bioavailability (9). IGF I has important anabolic effects on bone. It stimulates cell proliferation and collagen synthesis while decreasing the expression of collagenase-3 (54). It is probable that stromelysin-3, like collagenase and stromelysin, can degrade IGF-binding proteins that are important in bone (55, 56). Other proteins found abundantly in bone, such as noncollagenous extracellular matrix components, may also be target substrates for stromelysin-3. Breast cancer cells preferentially metastasize to bone, and it is suggested that stromelysin-3 contributes to the survival of tumor cells outside their compartment of origin (37). It is possible that the ability of breast cancer cells to flourish in the bone environment is due, in part, to the constitutive expression of stromelysin-3 by osteoblasts.

In conclusion, we find dual regulation of stromelysin-3 by FGF-2 in osteoblasts. The FGF-2-mediated decrease in metalloproteinase mRNA levels is due to transcriptional inactivation, whereas the subsequent increase in mRNA levels is due to transcriptional induction. Further characterization of stromelysin-3 substrates and the regulation of its expression by growth factors and cytokines will clarify the function of this enzyme in bone and in pathological states.

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REFERENCES

1. Basset, P., Belloq, J. P., Wolf, C., Stoll, I., Hutin, P., Limacher, J. M., Podhajzer, O. L., Chenard, M. P., Rio, M. C., and Chambon, P. (1990) Nature 348, 699–704
2. Anderson, I. C., Sugarbaker, D. J., Ganju, R. K., Tsarwhas, D. G., Richards, W. G., Sunday, M., Kobzik, L., and Shipp, M. A. (1995) Cancer Res. 55, 4120–4126
3. Porte, H., Chastre, E., Prevot, S., Nordlinger, B., Empeureur, S., Basset, P., Chenard, M. P., and Chambon, P. (1995) Int. J. Cancer 60, 1543–1545
4. Lefevbre, O., Wolf, C., Limacher, J. M., Hutin, P., Wendling, C., LeMeur, M., Maes, J. P., and Rio, M. C. (1992) J. Invest. Dermatol. 99, 870–872
5. Lefevbre, O., Wolf, C., Lefevbre, O., and Rio, M. C. (1995) Development 121, 947–955
6. Podhajcer, O. L., Chenard, M. P., Rio, M. C., and Chambon, P. (1996) Endocrinology 135, 774–781
7. Yamaguchi, T. P., and Rossant, J. (1995) Cell 81, 485–491
8. Chambon, P., Centrella, M., and McCarthy, T. (1988) J. Clin. Invest. 81, 1572–1577
9. Hurley, M. M., Abreu, C., Harrison, J. R., Lichtler, A. C., Raisz, L. G., Kream, B. E. (1993) J. Biol. Chem. 268, 5888–5893
10. Rodan, S. B., Wesolowska, G., Yoon, K., and Rodan, G. A. (1989) J. Biol. Chem. 264, 19994–19994
11. Jabs, E. W., Li, X., Scott, A. F., Meyers, G., Chen, W., Eccles, M., Mao, J., Welgus, H. G., and Kahn, A. J. (1987) J. Biol. Chem. 262, 26607–26612
12. Partridge, N. C., Jeffrey, J. J., Ehlich, L. S., Teitelbaum, S. L., Fliszar, C., and Basset, P. (1995) J. Biol. Chem. 270, 25526–25712
13. Murphy, G., Segain, J.-P., Mohler, J. C., and Hurley, M. M. (1995) J. Biol. Chem. 270, 25952–25959
14. Podhajcer, O. L., Chenard, M. P., Rio, M. C., and Chambon, P. (1995) J. Biol. Chem. 270, 25952–25712
15. Murphy, G., Segain, J.-P., O'Shea, M., Cockett, M., Ioannou, C., Lefevbre, O., Chenard, M. P., and Basset, P. (1995) J. Biol. Chem. 270, 25954–25955
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43. Heise, T., Nath, A., Jungermann, K., and Christ, B. (1997) J. Biol. Chem. 272, 20222–20229
44. Vincenti, M. P., Coon, C. I., Lee, O., and Brinckerhoff, C. E. (1994) Nucleic Acids Res. 22, 4818–4827
45. Delany, A. M., and Brinckerhoff, C. E. (1994) J. Cell. Biochem. 50, 400–410
46. Greenberg, M. E., and Belasco, J. G. (1993) Control of Messenger RNA Stability, pp. 161–215, Academic Press, Inc., New York
47. Okazaki, R., Ikeda, K., Sakamoto, A., Nakano, T., Morimoto, K., Urakawa, K., Ogata, E., and Matsumoto, T. (1992) J. Bone Miner. Res. 7, 1149–1155
48. Anglard, P., Melet, T., Guerin, E., Thomas, G., and Basset, P. (1995) J. Biol. Chem. 270, 20337–20344
49. Noel, A., Santavicea, M., Stoll, I., L’Hoir, C., Staub, A., Murphy, G., Rio, M.-C., and Basset, P. (1995) J. Biol. Chem. 270, 22866–22872
50. Mari, B. P., Anderson, I. C., Mari, S. E., Ning, Y. Y., Lutz, Y., Kohzik, L., and Shipp, M. A. (1998) J. Biol. Chem. 273, 618–626
51. Globus, R. K., Plouet, J., and Gospodarowicz, D. (1989) Endocrinology 124, 1539–1547
52. Matrisian, L. M., and Hogan, B. L. M. (1990) Curr. Top. Dev. Biol. 24, 219–259
53. Patterson, D., Hayes, W. P., and Shi, Y.-B. (1995) Dev. Biol. 167, 252–262
54. Delany, A. M., and Canalis, E. (1997) in Growth Factors and Cytokines in Health and Disease (LeRoith, D., and Bondy, C., eds) JAI Press, Greenwich, CT
55. Fowlkes, J. L., Suzuki, K., Nagase, H., and Thrailkill, K. M. (1994) Endocrinology 135, 2810–2813
56. Thrailkill, K. M., Quarles, L. D., Nagase, H., Suzuki, K., Serra, D. L., and Fowlkes, J. L. (1995) Endocrinology 136, 3527–3533