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The Cellular Protooncogenes c-*fos* and *egr-1* Are Regulated by Prostacyclin in Rodent Osteoblasts and Fibroblasts

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ABSTRACT

PGs are local regulators of various cellular functions. They exert their effects via specific PG receptor subtypes. Induction of c-*fos* gene expression has been described for arachidonic acid and its metabolite PGE_2. We demonstrate that another very short half-lifed prostanoid metabolite, namely prostacyclin (PGI_2), is a regulator of immediate-early genes. PGI_2 transiently induced the growth-associated immediate-early genes c-*fos* and *egr-1* in osteoblastic as well as fibroblastic cell lines. Furthermore, we showed that PGI_2 dose dependently stimulated new DNA synthesis in the osteoblastic cell line MC3T3-E1. Although PGI_2 is known to be a potent inducer of cyclooxygenases, we showed that this pathway is not necessary for protooncogene induction by PGI_2. Our data indicate a direct effect of PGI_2 on immediate-early gene expression, which does not depend on the synthesis of other prostanoids.

Intracellular signal transduction mechanisms were studied with the protein kinase inhibitor H-7, a potent inhibitor of PGI_2-induced c-*fos* expression. Experiments with phorbol esters revealed that protein kinase C activity is not obligatory for the effect of PGI_2 on c-*fos* expression. We conclude from these results that PGI_2, a rapidly inactivated prostanoid, has a major impact on cellular oncogene expression and growth in mesenchymally derived cells. (*Endocrinology* **137:** 4536–4541, 1996)

PGs ARE IMPORTANT local regulators of many cellular functions (1, 2). Because of their multiple biological actions, their application in medical treatment is wide-spread (3), although their exact mode of action remains to be clarified.

By activation of phospholipase A_2 (4), arachidonic acid, the prostanoid precursor, is released from membrane phospholipids and is further metabolized by the specific key enzymes, cyclooxygenases 1 and 2 (5). PGs exert their various biological activities via specific nuclear (6, 7) or G protein-coupled transmembrane receptors (8) inducing intracellular signal transduction cascades, including 1) Ca^{2+} signaling, 2) inositol trisphosphate production, 3) cAMP accumulation, and 4) activation of protein kinases.

Much information exists about the role of the prostanoid metabolites in bone formation and bone resorption and about the expression of specific receptors in bone cells (9, 10). In the osteoblast-like cell line MC3T3-E1, receptors have been described for PGE_2 (11, 12) and proposed for $PGF_{2\alpha}$ as well as PGE_1, which may be the same as the prostacyclin (PGI_2) receptor (13, 14).

PGE_2 has been shown to stimulate DNA synthesis and collagen production in bone organ cultures (15) as well as in isolated primary osteoblasts *in vitro* (16). Under most culture conditions, PGE_2 is a potent mitogen for bone cells. In addition, a large number of *in vivo* studies indicate that PGE_2 and PGE_1 can stimulate bone formation (for a review, see Ref. 9). Likewise, $PGF_{2\alpha}$ induces mitogenesis in MC3T3-E1 cells and increases protein kinase C (PKC) activity (17).

In contrast to other PGs, the short half-lifed PGI_2 is not well studied in bone cells. It was shown to be produced in bone organ cultures (18, 19). Furthermore, PGI_2 has been suggested as a mediator of stress, with a preferential role in adaptive bone remodeling (20). Recently, we have shown that application of hydrostatic pressure to MC3T3-E1 cells resulted in a rapid increase in c-*fos* messenger RNA (mRNA) expression. This effect could be inhibited by indomethacin, indicating PGs as mediators. Radioimmunological analysis revealed that PGI_2 is a likely candidate in mediating the effect of hydrostatic compressive stress on bone cells by regulating the level of c-*fos* mRNA (21).

PGI_2 is rapidly converted (half-life, 2–12 min) into its stable, but biologically inactive, metabolite 6-keto-$PGF_{1\alpha}$. This rapid inactivation makes PGI_2 a perfectly regulated system that allows activation of receptor proteins and pointed signaling within a short period of time only.

Cellular immediate-early genes such as c-*fos* and *egr-1* are regulated by the concerted action of multiple signals. c-*fos* transgenic mice were shown to develop osteosarcoma, indicating the central role of this regulatory protein in skeletal growth and development (22, 23). Egr-1, a nuclear Zn^{2+} finger protein and transcriptional regulator, was shown to be coregulated with c-*fos* in the developing mouse (24). Both immediate-early transcription factors serve as nuclear couplers of early cytoplasmic events to long term alterations in gene expression (25, 26).

In this paper we show that the short half-lifed PGI_2 is a potent stimulator of protooncogene expression in rodent osteoblastic and fibroblastic cell lines.

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Materials and Methods

Cell culture

The cell lines used were of osteoblastic and fibroblastic origin: the mouse osteoblast-like cell line MC3T3-E1 (donated by Dr. Kumegawa, Meikai University, Sakado, Japan), the rat osteosarcoma-derived cell line UMR-106 (kindly provided by H. J. Leis, University of Graz, Graz, Austria), and the mouse fibroblastic cell line NIH-3T3. All cell lines were seeded in tissue culture dishes (Corning, Corning, NY) at a density of about 75,000 cells/cm^2 and cultured in αMEM (Sebak, Suben, Austria) supplemented with 5% FCS (Sebak) and 30 μg/ml gentamicin. The cells were kept in humidified air under 5% CO_2 at 37 C. All cell lines were subcultured twice a week before reaching confluence. For induction experiments, the medium was changed to αMEM supplemented with 0.1% BSA 4 h before the addition of the inducers. PGI_2 (Cayman Chemical Co., Ann Arbor, MI), PGE_2 (Upjohn, Puurs, Belgium), iloprost (Ilomedin) a stable PGI_2 analog (Schering, Vienna, Austria), or arachidonic acid (Sigma Chemical Co., St. Louis, MO) were added at the concentrations indicated. Cycloheximide (Sigma) at a final concentration of 10 μg/ml and actinomycin D (Serva, Heidelberg, Germany) at 2 μg/ml were used as translational and transcriptional inhibitors, respectively. Indomethacin (1 μM; Sharp and Dohme, Munich, Germany) and the protein kinase inhibitor H-7 (20 μM; Calbiochem, San Diego, CA) were used for signal transduction studies. All inhibitors were added 20 min before the addition of PGI_2. Phorbol ester [1 μM; phorbol 12-myristate 13-acetate (PMA); Sigma] was used as a PKC activator and to down-regulate PKC activity by long term treatment.

Northern blotting

Cytoplasmic RNA was isolated (27), and its total amount was estimated spectrophotometrically. Ten micrograms of total RNA were applied to each lane of a 1% agarose gel containing 2.2 M formaldehyde (28). After electrophoresis, the RNAs were transferred to a nylon filter (GeneScreen, New England Nuclear Corp., Boston, MA). Hybridization was performed for 10–16 h in a solution consisting of 10% dextran sulfate, 500 μg/ml sheared salmon sperm DNA, 1 M NaCl, and 1% SDS. As hybridization probes we used a rat *egr-1* complementary DNA (cDNA) *BglII* fragment and a mouse c-*fos* cDNA (29). To ensure even loading, we hybridized the same Northern blots using the entire rat glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA (GAPDH cDNA was kindly provided by Dr. Busslinger, Institute of Molecular Pathology, Vienna, Austria). Probe labeling was performed by random primed labeling of the excised insert using a commercially available kit (Boehringer Mannheim, Mannheim, Germany) with [α-^{32}P]deoxy-CTP (3000 Ci/mmol; New England Nuclear). After stringency washes, the filters were evaluated in an Instant Imager (Packard Instrument Co., Meriden, CT). The bands were quantitated densitometrically, normalized to GAPDH, and expressed as a percentage of the control value. Data are presented as the mean ± SE of three or more separate experiments. Statistical significance was analyzed using ANOVA (*post-hoc* test, Fisher's protected least significant difference test).

[^{3}H]Thymidine incorporation experiments

Cells were seeded at a density of 20,000 cells/cm^2 in 24-multiwell plates and grown in αMEM supplemented with 5% FCS to near confluency. The medium was changed to serum-free αMEM, and the culture was continued for an additional 24 h. Treatments were performed by adding PGI_2, PGE_2 or arachidonic acid in αMEM for 24 h at the concentrations indicated. [*Methyl*-^{3}H]thymidine (70–90 Ci/mmol; New England Nuclear) in αMEM was added to give a final concentration of 3 μCi/ml for the last 5 h of incubation. The cell layers were washed three times with PBS, and incorporated [^{3}H]thymidine was precipitated by adding 10% TCA. Thereafter, the acid-precipitable material was dissolved in 250 μl 0.1 N NaOH at 60 C and transferred to TopCount microplates (Packard Instrument Co.). Liquid scintillation cocktail, Microscint-20 (Packard), was added, and incorporated [^{3}H]thymidine was counted in a TopCounter (Packard). Statistical significance was analyzed using ANOVA (*post-hoc* test, Fisher's protected least significant difference test).

Preparation of nuclear extracts

MC3T3-E1 cells were grown to confluency in αMEM supplemented with 5% FCS. Afterward, the cells were starved in serum-free culture medium for 24–48 h. Nuclear extracts were prepared from untreated cells or cells treated with iloprost (Ilomedin) for 30 or 50 min. In brief, the cells were scraped in ice-cold PBS, collected by centrifugation, and resuspended on ice in 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, and 10 mM KCl. The cells were homogenized in a glass Dounce homogenizer (Kontes Co., Vineland, NJ), and nuclei were collected by centrifugation at 3,000 × *g* for 20 min. Nuclei were extracted in a high salt extraction buffer [20 mM HEPES (pH 7.9), 25% glycerol, 1.2 M KCl, and 0.2 mM EDTA] for 30 min and pelleted at 25,000 × *g* for 30 min. Supernatants were dialyzed against extraction buffer substituted with 0.1 M KCl. The dialyzed proteins were stored frozen at −25 C.

Western immunoblotting

After estimation of the protein contents with bicinchoninic acid/copper (II) sulfate (Sigma), 20 μg protein were applied per lane, and electrophoresis was performed under denaturing conditions on 10% polyacrylamide-SDS gels according to the method of Laemmli (30). After semidry transfer to nitrocellulose membranes (Immobilon-P, Millipore Intertech, Bedford, MA), the blots were probed with a c-Fos antibody (sc-52, Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:100 dilution and detected by a secondary antibody against rabbit IgG coupled to alkaline phosphatase (Boehringer). For estimation of the mol wt, a color marker (Sigma) was used.

Results

PGI_2 induced the mRNA synthesis of the immediate-early genes c-fos and egr-1 in both osteoblastic and fibroblastic cell lines

The time course of c-*fos* mRNA induction in MC3T3-E1 cells treated with 2×10^{-6} M PGI_2 is shown in Fig. 1A. The increase in c-*fos* mRNA was detectable after 10 min and reached a prominent peak after 25 min. Thereafter, the amount of c-*fos* mRNA declined rapidly to low levels at 60

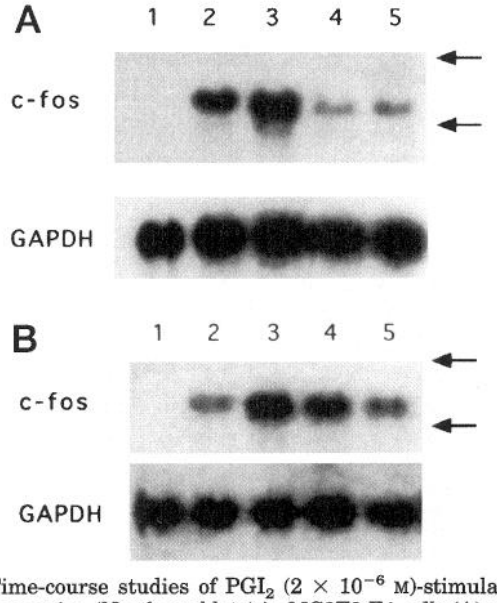

FIG. 1. Time-course studies of PGI_2 (2×10^{-6} M)-stimulated c-*fos* mRNA expression (Northern blots) in MC3T3-E1 cells (A) and NIH-3T3 cells (B). Total RNA was isolated at various time points of induction. Ten micrograms were applied to each lane. Lane 1, Control; lane 2, PGI_2, 10 min; lane 3, PGI_2, 25 min; lane 4, PGI_2, 40 min; lane 5, PGI_2, 60 min. As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers.

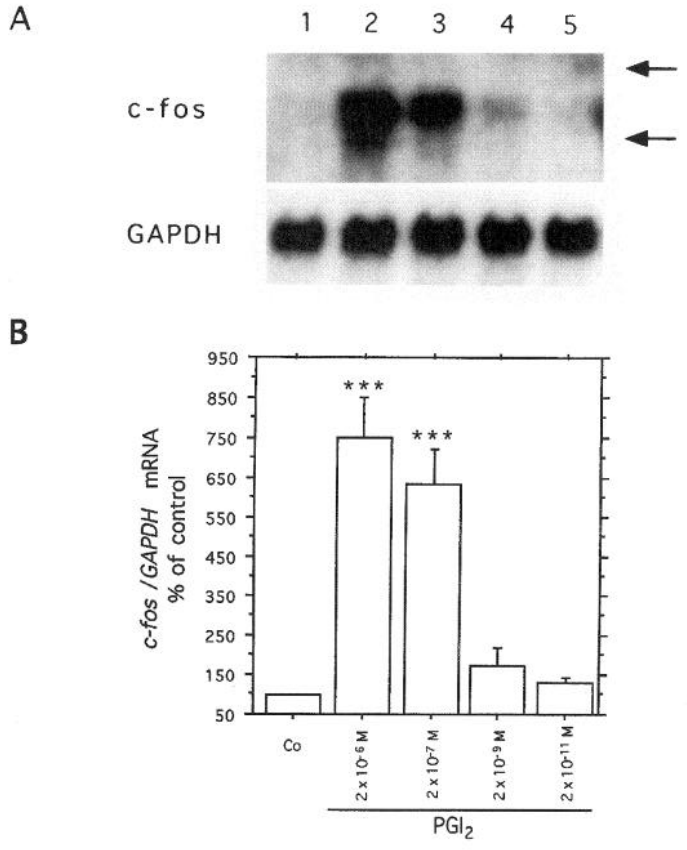

FIG. 2. Dose dependence of PGI$_2$-stimulated c-*fos* mRNA expression in UMR-106 cells. A, Northern blot (20 μg total RNA; 25-min PGI$_2$ treatment). Lane 1, Control; lane 2, 2×10^{-6} M PGI$_2$; lane 3, 2×10^{-7} M PGI$_2$; lane 4, 2×10^{-9} M PGI$_2$; lane 5, 2×10^{-11} M PGI$_2$. As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers. B, Densitometric analysis of the Northern blots shown in A after normalization to GAPDH. Data are given as a percentage of the control value (mean ± SE; n = 4). ***, $P < 0.001$ (treated *vs.* control).

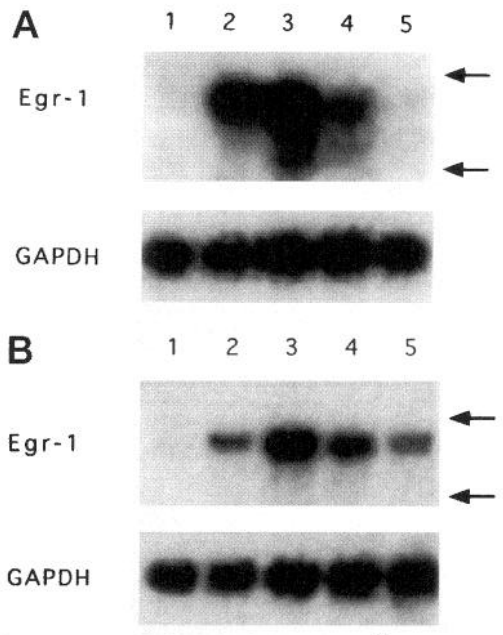

FIG. 3. Time-course studies of PGI$_2$ (2×10^{-6} M)-stimulated *egr-1* mRNA expression (Northern blots) in MC3T3-E1 cells (A) and NIH-3T3 cells (B). Total RNA was isolated at various time points of induction; 10 μg were applied to each lane. Lane 1, Control; lane 2, PGI$_2$, 10 min; lane 3, PGI$_2$, 25 min; lane 4, PGI$_2$, 40 min; lane 5, PGI$_2$, 60 min. As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers.

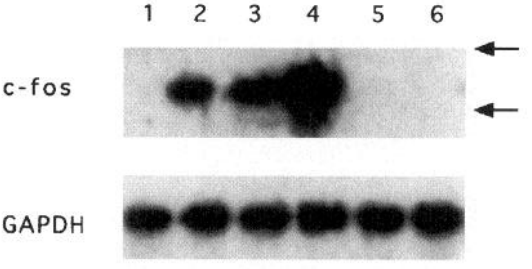

FIG. 4. PGI$_2$ (2×10^{-6} M; 30 min)-induced c-*fos* mRNA expression does not depend on *de novo* protein synthesis. MC3T3-E1 cells were preincubated for 10 min with 10 μg/ml cycloheximide (CHX) or 2 μg/ml actinomycin D (Act D) as translational and transcriptional inhibitors, respectively. Lane 1, Control; lane 2, PGI$_2$; lane 3, CHX; lane 4, PGI$_2$ plus CHX; lane 5, Act D; lane 6, PGI$_2$ plus Act D. As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers.

min. The dose-response relationship was tested in MC3T3-E1 cells and the osteosarcoma cell line UMR-106. c-*fos* mRNA levels were detectable after 25 min of treatment with PGI$_2$ at concentrations as low as 2×10^{-11} and 2×10^{-9} M, but were markedly increased at higher concentrations (2×10^{-7} and 2×10^{-6} M). c-*fos* mRNA was undetectable in untreated controls (Fig. 2). There was no difference in the dose response between UMR-106 cells and MC3T3-E1 cells. A comparable effect of PGI$_2$ on c-*fos* mRNA expression was observed in NIH-3T3 mouse fibroblasts (Fig. 1B), indicating that this effect is not a specific feature of osteoblasts.

As it is known that in skeletal regions undergoing substantial bone formation, *egr-1* and c-*fos* genes are coregulated (24), we were interested in the effect of PGI$_2$ treatment on *egr-1* expression. Indeed, the time course of *egr-1* mRNA induction paralleled that described above for c-*fos* mRNA (Fig. 3, A and B). MC3T3-E1 (Fig. 3A) as well as NIH-3T3 cells (Fig. 3B) responded rapidly to PGI$_2$ treatment with an increase in *egr-1* mRNA levels. This response was transient, with a maximum after 25 min and a decline thereafter.

Translational and transcriptional regulation of PGI$_2$-induced c-*fos* mRNA expression was studied with cycloheximide (CHX) and actinomycin D as translational and transcriptional inhibitors, respectively. In MC3T3-E1 cells, the addition of CHX (10 μg/ml) resulted in increased levels of c-*fos* mRNA (Fig. 4). However, treatment with PGI$_2$ additionally increased the amount of c-*fos* mRNA. In the presence of CHX, the effect of PGI$_2$ on c-*fos* mRNA expression was more pronounced, indicating the lack of feedback regulation. These data suggest that *de novo* protein synthesis is not needed for the induction of c-*fos* mRNA by PGI$_2$. In contrast, actinomycin D (2 μg/ml), an inhibitor of RNA polymerase II, completely blocked the c-*fos* mRNA response. Consequently, actinomycin D was also a potent inhibitor of c-*fos*

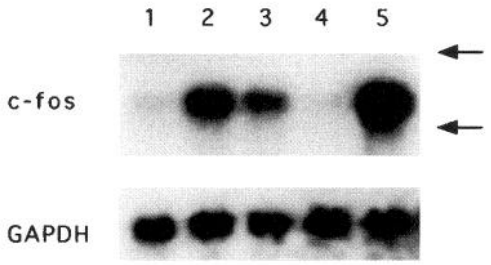

FIG. 5. Comparison of the effects of 30-min treatment with PGI_2, arachidonic acid, or PGE_2 at equimolar concentrations (2×10^{-6} M) on c-*fos* mRNA expression. Lane 1, Control; lane 2, PGI_2; lane 3, arachidonic acid; lane 4, arachidonic acid plus indomethacin (1 μM); lane 5, PGE_2. As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers.

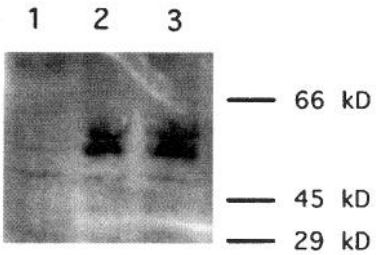

FIG. 6. Western immunoblot. Fos protein in nuclear extracts of MC3T3-E1 cells cultured for 24 h in serum-free αMEM and treated with or without 2×10^{-6} M iloprost. Lane 1, Control; lane 2, iloprost, 30 min; lane 3, iloprost, 50 min. Indicated are the positions of protein markers (kilodaltons).

mRNA expression in UMR-106 and NIH-3T3 cells (data not shown).

Recently, it was reported that arachidonic acid and PGE_2 have the capacity to increase c-*fos* mRNA expression in Swiss 3T3 fibroblasts (31). Therefore, we compared the effects of PGI_2 in MC3T3-E1 cells with those of PGE_2 or arachidonic acid (both at 2×10^{-6} M). As shown in Fig. 5, all three compounds induced c-*fos* mRNA expression after 30 min. As expected, the effect of arachidonic acid was markedly inhibited by indomethacin (1 μM). The response to PGE_2 treatment was significantly more pronounced than that to PGI_2 or arachidonic acid (Fig. 5). Indomethacin had no influence on the effect of the prostanoids on oncogene expression after 30 min (see Fig. 7).

To show that c-*fos* induction at the mRNA level results in an increase in Fos protein, Western immunoblotting was performed. Two major proteins of the c-Fos family were detected after 30 and 50 min of PGI_2 (iloprost) treatment (Fig. 6).

The signal transduction pathways involved in the effect of PGI_2 were tested using protein kinase inhibitors. Interestingly, the addition of H-7 (20 μM), an inhibitor of PKC, and cyclic nucleotide-dependent protein kinases inhibited the effect of PGI_2 on c-*fos* mRNA expression in MC3T3-E1 as well as NIH3T3 cells (Fig. 7). In another series of experiments (Fig. 8), c-*fos* expression after treatment of MC3T3-E1 cells with phorbol ester (PMA) for 10, 20, and 30 min and 24 h was studied. As a short term effect (10–30 min), stimulation of c-*fos* mRNA expression was found. In contrast, after 24 h, c-*fos* mRNA levels returned to the basal level, indicating the down-regulation of PKC activity, as described in other osteoblast cultures (32). However, c-*fos* expression at this time was inducible by PGI_2, indicating the involvement of alternate signal transduction pathways. As expected, additional PMA treatment was not able to stimulate c-*fos* mRNA after 24 h of PKC down-regulation (data not shown).

PGE_2 is known to stimulate the proliferation of osteoblasts (16). In agreement with this finding, treatment of MC3T3-E1 cells with 2×10^{-5} M PGE_2 for 24 h significantly increased [^{3}H]thymidine incorporation (Fig. 9). [^{3}H]Thymidine incorporation was also significantly increased to the same extent by 2×10^{-5} M PGI_2 (Fig. 9). This effect of PGI_2 on proliferation was dose dependent in the concentration range between 2×10^{-7} and 2×10^{-5} M (Fig. 9). Arachidonic acid (2×10^{-5} M),

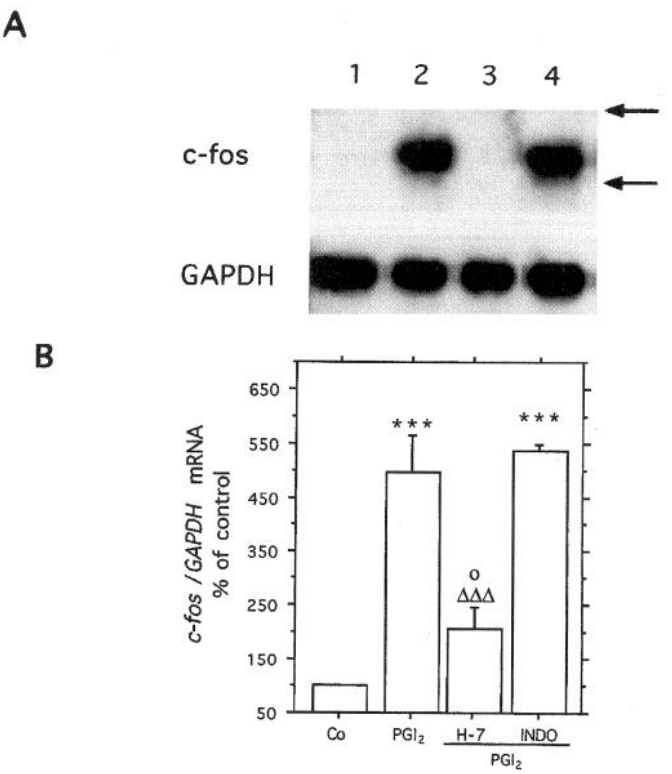

FIG. 7. Influence of the protein kinase inhibitor H-7 and indomethacin on PGI_2 (2×10^{-6} M)-induced c-*fos* expression in MC3T3-E1 cells. A, Northern blot (10 μg total RNA; 30 min). Lane 1, Control; lane 2, PGI_2; lane 3, PGI_2 plus H-7 (20 μM); lane 4, PGI_2 plus indomethacin (INDO; 1 μM). As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers. B, Densitometric analysis of the Northern blots shown in A after normalization to GAPDH. Data are given as a percentage of the control value (mean ± SE; n = 3–4). ***, $P < 0.001$ (treated *vs.* control); ΔΔΔ, $P < 0.001$ (H-7 *vs.* PGI_2); O, P = NS (*vs.* control).

the precursor of prostanoid metabolites, had no effect on DNA synthesis over 24 h.

Discussion

The identification of protooncogene activators and the characterization of signal transduction mechanisms are fundamental for the understanding of cell regulation. We now present convincing data that PGI_2 acts as a potent protoon-

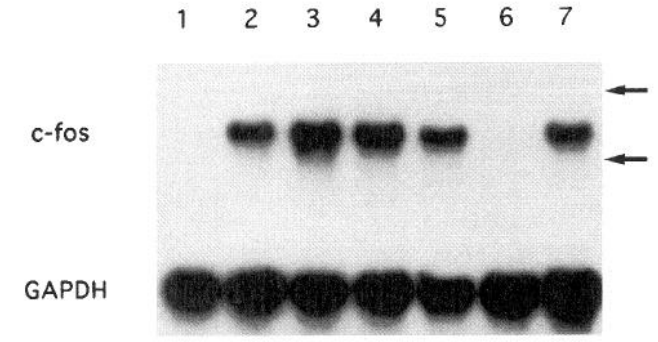

FIG. 8. Effect of phorbol ester (PMA; 1 μM) on c-*fos* mRNA expression in MC3T3-E1 cells. PKC activity is not obligatory for PGI_2 (30 min) induced c-*fos* mRNA expression. The results of a Northern blot (20 μg total RNA) are shown. Lane 1, Control; lane 2, PMA, 10 min; lane 3, PMA, 20 min; lane 4, PMA, 30 min; lane 5, PGI_2, 30 min; lane 6, PMA, 24 h (down-regulation of PKC activity); lane 7, PMA, 24 h (down-regulation of PKC activity), plus PGI_2, 30 min. As a control for RNA loading, hybridizations using a GAPDH probe were performed. *Arrows* indicate the 28S/18S ribosomal RNA markers.

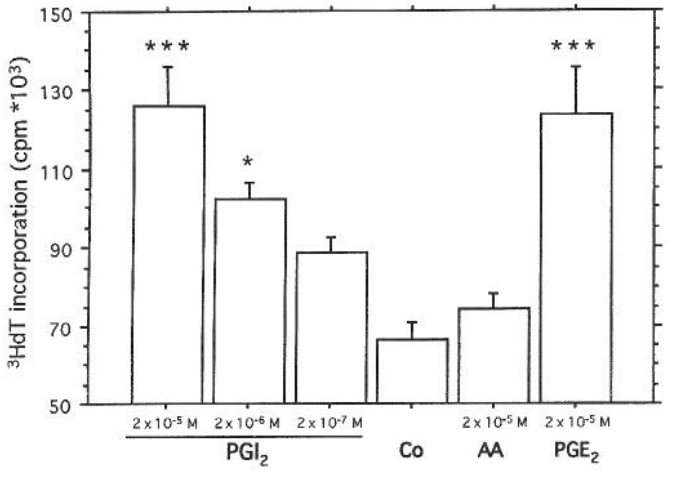

FIG. 9. Effects of PGI_2, PGE_2, and arachidonic acid on new DNA synthesis in MC3T3-E1 cells. Twenty-four h after treatment, [^{3}H]thymidine incorporation into MC3T3-E1 cells was measured (counts per min). Values are the mean ± SE (n = 4–10). *, $P < 0.05$; ***, $P < 0.001$ (treated *vs.* control).

cogene activator. Hence, to the best of our knowledge no investigation has been performed to elucidate the effect of PGI_2 on oncogene expression. We show for the first time that PGI_2 regulates c-*fos* and *egr-1* gene expression in rodent osteoblastic as well as fibroblastic cell lines within 30 min.

The cellular protooncogene c-*fos* and the immediate-early gene *egr-1* are involved in developmental processes (22, 26). c-*fos* and *egr-1* show overlapping patterns of expression at ossification sites and in mesenchymally derived cells in the developing mouse (24). In contrast to our results with PGI_2, it was shown that another prostanoid, PGE_2, has the capacity to induce only c-*fos* mRNA in UMR 106–01 osteoblast-like cells, yet did not stimulate *egr-1* mRNA (33).

To investigate the signal transduction pathways involved, we had to show, first, that the induction of c-*fos* expression by PGI_2 does not depend on *de novo* protein synthesis and, second, as expected, that it depends on new transcription. c-*fos* encodes for an unstable mRNA whose cytoplasmic lifespan is increased when translation is blocked by protein synthesis inhibitors (34, 35), leading to the so-called superinduction phenomenon. More recently, the absolute requirement of translation for c-*fos* mRNA degradation was demonstrated (36). However, some inhibitors, such as CHX, act positively as nuclear signaling agonists and induce c-*fos* on their own (37).

Various PGs are known to induce cyclooxygenase isoenzymes, resulting in stimulation of the synthesis of PGH metabolites. The PGI_2 analog iloprost has the capacity to induce cyclooxygenase activity in MC3T3-E1 cells (14). From this finding, one could speculate that PGI_2-stimulated cyclooxygenase activity results in increased synthesis of other PG metabolites, which, in turn, are responsible for the stimulation of c-*fos* expression. We clearly can exclude such an indirect mechanism 1) because of the immediate c-*fos* mRNA accumulation by 10 min, which is less than the time necessary for cyclooxygenase activation (1 h) (14), 2) because treatment with the cyclooxygenase inhibitor indomethacin did not alter the c-*fos* response, and 3) because the experiments with CHX indicate that *de novo* protein synthesis is not obligatory.

The PGI_2 analog iloprost induces cAMP production in MC3T3-E1 cells, whereas inositol trisphosphate production was reported not to be significantly stimulated (14). In contrast, in Chinese hamster ovary cells, iloprost increased cAMP as well as the generation of inositol phosphates, indicating multiple signaling pathways (38). For arachidonic acid (31, 39)- and PGE_2 (40)-induced c-*fos* induction, cAMP-dependent as well as cAMP-independent mechanisms have been suggested. Treatment with H-7, an unspecific inhibitor of protein kinases, resulted in complete inhibition of the response. An additional series of experiments with phorbol ester (PMA) showed that PKC activity is not a necessary prerequisite for PGI_2-mediated c-*fos* expression. The response to PGI_2 was also present after 24-h down-regulation of PKC by PMA. As a control at this time point, additional PMA treatment did not result in c-*fos* mRNA expression. Inhibition of adenylate cyclase with SQ22536 resulted in significant, but incomplete, inhibition of the effect of PGI_2 effect on c-*fos* expression (data not shown). We conclude from these data that among other protein kinases, cAMP-dependent kinases play a role in PGI_2-induced signal transduction to immediate-early gene expression.

The immediate-early transcription factors couple short term responses to long term alterations in gene expression. The induction of immediate-early genes is closely related to proliferation and/or differentiation (25). In our hands, PGI_2 exhibited mitogenic activities on MC3T3-E1 cells, as shown by [^{3}H]thymidine incorporation. Its effect on proliferation after 24 h was as potent as that of PGE_2.

In conclusion, we show that PGI_2 is an inducer of immediate-early genes in osteoblasts and other cells of mesenchymal origin and by this mechanism might influence the proliferation and differentiation of these cells.

Acknowledgments

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