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Mechanisms of Glucocorticoid Action in Bone Cells

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Abstract Glucocorticoids play an important role in the normal regulation of bone remodeling; however continued exposure of bone to glucocorticoid excess results in osteoporosis. In vivo, glucocorticoids stimulate bone resorption and decrease bone formation, and in vitro studies have shown that while glucocorticoids stimulate osteoblastic differentiation, they have important inhibitory actions on bone formation. Glucocorticoids have many effects on osteoblast gene expression, including down-regulation of type I collagen and osteocalcin, and up-regulation of interstitial collagenase. The synthesis and activity of osteoblast growth factors can be modulated by glucocorticoids as well. For example, insulin-like growth factor I (IGF-I) is an important stimulator of osteoblast function, and expression of IGF-I is decreased by glucocorticoids. The activity of IGF I can be modified by IGF binding proteins (IGFBPs), and their synthesis is also regulated by glucocorticoids. Thus, glucocorticoid action on osteoblasts can be direct, by activating or repressing osteoblast gene expression, or indirect by altering the expression or activity of osteoblast growth factors. Further investigation of the mechanisms by which glucocorticoids modulate gene expression in bone cells will contribute to our understanding of steroid hormone biology and will provide a basis for the design of effective treatments for glucocorticoid-induced osteoporosis.

Key words: osteoblast, osteoporosis, insulin-like growth factor, collagen, matrix metalloproteinase

Bone remodeling is regulated by systemic hormones and locally produced factors acting in concert to maintain bone mass. Investigations of the actions of hormones on bone have revealed that glucocorticoids cause marked effects on bone metabolism and that continued exposure of skeletal tissue to excessive amounts of glucocorticoids results in osteoporosis. However, the exact mechanisms by which glucocorticoids act on bone are not known.

In vivo, glucocorticoids enhance bone resorption and decrease bone formation [Lukert and Raisz, 1990]. The decrease in bone formation caused by glucocorticoids could be due to direct actions of glucocorticoids on bone or due to indirect mechanisms. Glucocorticoids inhibit gonadotropin and sex steroid production, and the resulting hypogonadism could cause a decrease in bone mass [Lukert and Raisz, 1990]. Additionally, alterations in the growth hormone/insulin-like growth factor (IGF) I axis, such as a decrease in growth hormone release or action, may play a role in the inhibitory effects of glucocorticoids on bone formation in vivo [Luo and Murphy, 1989]. Increased bone resorption appears to be the result of secondary hyperparathyroidism, due to a decrease in gastrointestinal calcium absorption and an increase in the urinary excretion of calcium. Although the extent and duration of the excessive secretion of parathyroid hormone (PTH) is not known, it is likely that in conditions of glucocorticoid excess, PTH increases bone resorption as well as having other effects on bone metabolism.

GLUCOCORTICOID EFFECTS ON OSTEOCLAST AND OSTEOBLAST DIFFERENTIATION

Studies testing the effect of glucocorticoids on bone resorption in vitro have not yielded uniform conclusions due to differences in the systems, culture conditions, and length of glucocorticoid treatment used. Some researchers found that glucocorticoids inhibited PTH-stimulated bone resorption in vitro [Stern, 1969; Raisz et al., 1972]. However, more recent studies have demonstrated that glucocorticoids stimulate bone resorption in cultured calvaria [Reid et al.,

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1986; Gronowicz et al., 1990]. The effects of glucocorticoids on osteoclast recruitment/differentiation and activity have been dissociated using the model system of bone chips implanted subcutaneously into rats [Defranco et al., 1992]. It was shown that glucocorticoids inhibited the recruitment and differentiation of bone resorbing cells, but stimulated the bone resorbing activity. This may be related to the hypothesized "coupling" of osteoblastic activity to bone resorption [Rodan and Martin, 1981], since one of the effects of glucocorticoids on bone is the promotion of osteoblast differentiation.

In vitro, glucocorticoids have complex effects on osteoblast gene expression. As with the osteoclast studies, these actions are dependent on the stage of osteoblast growth and differentiation, and on the cell model and culture conditions used. Glucocorticoids can enhance selected parameters of bone formation and induce cells of the osteoblastic lineage to differentiate into mature cells expressing the osteoblastic phenotype [Bellows et al., 1987; Cheng et al., 1994; Leboy et al., 1991]. For example, when bone marrow stromal cells, the source of osteoprogenitor cells, are cultured in the presence of dexamethasone they express an osteoblast-like phenotype. In both human and rat marrow stromal cell cultures, dexamethasone inhibits cell growth while increasing alkaline phosphatase expression, which is an osteoblastic marker [Cheng et al., 1994; Leboy et al., 1991]. In rat marrow stromal cell cultures, dexamethasone induced osteopontin and osteocalcin transcripts, which are also markers of osteoblastic differentiation [Leboy et al., 1991]. In the human system, Cheng et al. [1994] found that dexamethasone augmented the ability of the marrow stromal cells to respond to two potent stimulators of osteoblast gene expression, PTH and prostaglandin E_2 (PGE_2). This effect may be partially due to the ability of glucocorticoids to increase PTH binding and receptor expression [Ureña et al., 1994]. Promotion of the osteoblastic phenotype by glucocorticoids may involve modulation of Id (inhibitor of differentiation) gene expression. Id is a ubiquitously expressed basic helix-loop-helix protein which can interact with and down-regulate the activity of several helix-loop-helix transcription factors [Benezra et al., 1990]. Expression of Id is high in early proliferating cultures of mouse osteoblast-like MC3T3 cells, but decreases significantly as the cultures mature, suggesting an inverse relationship between Id expression and differentiation [Ogata et al., 1993]. However, bone morphogenetic protein-2 (BMP-2), a member of the transforming growth factor (TGF) β superfamily, induces osteoblast differentiation; and Id expression is induced by BMP-2 and by dexamethasone in MC3T3 cells as well as in primary cultures of fetal rat calvarial osteoblasts. It is possible that BMP-2 and dexamethasone share a common pathway in the induction of Id expression [Ogata et al., 1993]. Studies to dissect the relationship between glucocorticoid or BMP-2 induction of differentiation and the expression of Id and other helix-loop-helix proteins will contribute to our understanding of the mechanisms by which mesenchymal cells take on the osteoblastic phenotype.

GLUCOCORTICOID MODULATION OF OSTEOBLAST GENE EXPRESSION

Although glucocorticoids increase osteoblast differentiation, their inhibitory actions on multiple aspects of osteoblastic function have a major impact on bone mass (Table I). Glucocorticoids have two important inhibitory actions on bone formation: 1) they inhibit cell replication, therefore depleting a cell population capable of synthesizing bone collagen, and 2) they inhibit type I collagen gene expression [Delany et al., in press, a]. These two actions are time and dose dependent, and may be responsible for the inhibition of bone formation and possibly the osteopenia secondary to glucocorticoid excess. In human and rat skin fibroblasts, glucocorticoids decrease type I procollagen transcripts by decreasing procollagen mRNA stability, but they have no effect on collagen gene transcription [Hämäläinen et al., 1985; Raghow et al., 1986]. However, in cultured rat osteoblasts, cortisol decreases transcription from the α(I) collagen gene [Delany et al., in press, a]. Thus the mechanisms by which glucocorticoids down-regulate type I procollagen transcripts are tissue specific. This would indicate differential regulation of collagen synthesis in bone and may be related to the manner in which fibroblasts and osteoblasts regulate basal levels of collagen expression. Re-

TABLE I. Effects of Glucocorticoids in Osteoblasts

1. Induce the differentiation of osteoblast precursors
2. Enhance PTH receptor expression
3. Decrease replication of preosteoblasts
4. Inhibit type I collagen and osteocalcin expression
5. Increase interstitial collagenase expression
6. Decrease tissue inhibitor of metalloproteinases

cently, Pavlin et al. [1992] reported that the 5′ region of the rat α(I) collagen promoter contains a 2.3 kb sequence required for high basal expression of chimeric α1(I) collagen promoter/CAT constructs in osseous cells, but it is not required in fibroblastic cells. These results support the notion that the mechanisms regulating collagen gene expression are tissue specific. A positive, orientation-specific cis-acting element is found in the first intron of the human α1(I) collagen gene [Liska et al., 1990], and an AP-1 (activator protein-1) sequence within this motif is required for its function. Antagonism between the AP-1 complex and the glucocorticoid receptor is well documented [König et al., 1992; Jonat et al., 1990; Kerppola et al., 1993; Schule et al., 1990; Yang-Yen et al., 1990] and may play a role in the down-regulation of α1(I) collagen gene transcription in osteoblasts. In human osteoblast cultures, treatment with dexamethasone causes a transient increase in mRNAs for Fos and Jun, two components of the AP-1 complex. C-fos was increased 10-fold and c-jun was increased 2-fold compared to the untreated control [Subramaniam et al., 1992]. The relative ratio of Fos:Jun determines whether glucocorticoids negatively or positively regulate certain genes. For example, Jun in the presence of high levels of Fos causes the proliferin promoter to be down-regulated by glucocorticoids [Diamond et al., 1990], and it is possible that a similar mechanism is functioning in the α1(I) collagen promoter. The glucocorticoid receptor also can repress transcriptional activation mediated by Spi-1/PU.1, an ets-related transcription factor which binds to the sequence GAGGAA [Gauthier et al., 1993]. Although a core glucocorticoid binding sequence is found within the promoter region of the rat α1(I) collagen gene [Lichtler et al., 1989], the DNA sequences responsible for the down-regulation of gene transcription by glucocorticoids have not yet been characterized, and this repression of gene transcription may involve a number of elements. Further investigation of the mechanisms underlying the tissue specific nature of glucocorticoid down-regulation of α1(I) collagen expression, as well as the regulation of basal collagen gene expression, will be an important focus for future studies.

Glucocorticoids also modify the expression of osteoblast specific genes, including osteocalcin. Osteocalcin expression is tightly regulated by glucocorticoids during the development of bone. Glucocorticoids prevent the stimulatory effect of 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on osteocalcin gene expression and multiple glucocorticoid receptor binding sites have been identified by in vitro footprinting of the osteocalcin promoter in both human and rat [Stromstedt et al., 1991; Heinrichs et al., 1993]. One glucocorticoid receptor binding site overlaps or is adjacent to the TATA box of the osteocalcin gene, suggesting that the glucocorticoid receptor may interact with the TATA box binding factor, TFIID, causing competition or steric hindrance [Morrison and Eisman, 1993]. The osteocalcin gene also contains several active AP-1 sites, which appear to contribute to the basal activity of the promoter. Glucocorticoid repression of basal osteocalcin promoter activity may involve interaction between the glucocorticoid receptor and components of the AP-1 complex, causing "mutual inhibition" which is independent of DNA binding, as it has been postulated for the collagenase promoter [Jonat et al., 1990; Morrison and Eisman, 1993]. Both the human and the rat osteocalcin promoter contain classic CCAAT motifs typically found in eukaryotic promoters, as well as vitamin D response elements (VDREs) in close proximity to the glucocorticoid receptor binding sites. The glucocorticoid receptor may interact with the proteins binding these DNA sequences to mediate the down-regulation of vitamin D induced transcription [Heinrichs et al., 1993]. The osteocalcin gene serves as an excellent model for studying transcriptional repression by glucocorticoids. Using the osteocalcin promoter to examine the functional interactions between the glucocorticoid receptor and other transcription factors, such as AP-1 and the vitamin D receptor, will contribute to our knowledge of what constitutes a negative GRE and the mechanisms by which glucocorticoids down-regulates gene expression [Landers and Spelsberg, 1992; Beato, 1989; Akerblom and Mellon, 1991].

Glucocorticoids have complex and unique effects on collagen degradation. In nonskeletal cell systems, glucocorticoids inhibit the synthesis of interstitial collagenase (matrix metalloproteinase 1), the primary enzyme responsible for initiating the degradation of type I collagen [Matrisian and Hogan, 1990; Jonat et al., 1990; Delany and Brinckerhoff, 1992; Brinckerhoff et al., 1986; Firestein et al., 1991]. However, in rat osteoblasts glucocorticoids increase collagenase mRNA expression and immunoreactive collagenase [Shalhoub et al., 1992; Delany et al., in press, b], and the increased expression of this enzyme may contribute to the decrease in bone

mass associated with glucocorticoid excess. Stromelysin, or matrix metalloproteinase 3, degrades noncollagenous extracellular matrix components such as laminin, fibronectin, and proteoglycans [Matrisian and Hogan, 1990]. Stromelysin expression has been documented in some cultured osteosarcoma cell lines and can be induced in cultures of normal osteoblasts by mononuclear cell conditioned medium [Rifas et al., 1994; Meikle et al., 1992]. Since collagenase and stromelysin are often coordinately regulated by cytokines and hormones [Matrisian and Hogan, 1990], it will be important to determine if glucocorticoids regulate osteoblast stromelysin expression in the same manner as collagenase. In addition to metalloproteinases, cortisol also affects the expression of the tissue inhibitors of metalloproteinases (TIMPs), a family of polypeptides which inhibit metalloproteinase activity by forming stoichiometric enzyme-inhibitor complexes [Matrisian and Hogan, 1990; Goldberg et al., 1992]. Bone is a site of TIMP-1 expression and our initial studies show that treatment with cortisol decreases osteoblast TIMP 1 mRNA expression without modifying TIMP 2 or 3 transcripts. In contrast, glucocorticoids strongly induce TIMP-3 mRNA in murine fibroblasts [Leco et al., 1994]. These results reinforce the hypothesis that skeletal cells respond to glucocorticoids in a unique fashion which is different from nonskeletal cells. Glucocorticoids may promote bone turnover by increasing collagenase expression while decreasing TIMP mRNA, but in nonskeletal cells, such as fibroblasts, glucocorticoids have a sparing effect on the extracellular matrix by decreasing collagenase and increasing TIMP production. Research aimed at dissecting the mechanisms by which glucocorticoids exert this tissue specific regulation on collagenase and TIMPs is central to our understanding of glucocorticoid action and extracellular matrix remodeling.

GLUCOCORTICOID REGULATION OF OSTEOBLAST GROWTH FACTOR SYNTHESIS AND ACTIVITY

Although glucocorticoids can directly modulate osteoblast gene expression, some of their effects may be due to modifications in the synthesis or activity of growth factors secreted by bone cells (Table II). While certain systemic growth factors are able to modulate bone cell function, growth factors synthesized by bone cells may play a more significant role in cell function than their systemic counterparts, and could act in an autocrine or paracrine fashion. Skeletal cells synthesize and respond to a number of growth factors including insulin-like growth factors (IGF) I and II, transforming growth factor beta (TGF β) 1, 2, and 3, acidic and basic fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) A and B. Skeletal cells also secrete prostaglandins, BMPs, and cytokines of the interleukin and colony stimulating factor family [Canalis et al., 1993a].

TABLE II. Regulation of Growth Factor Expression and Activity by Glucocorticoids in Osteoblasts

	Synthesis	Activation	Binding proteins	Receptors
IGF I	↓	Ø	⇵	?
IGF II	Ø	Ø	⇵	?
TGF β	Ø	↑	?	Shift
FGF 1 and 2	NT[a]	NT	NT	NT
PDGF A and B	NT	NT	NT	NT

[a]NT, not tested.

TGFβ is a mitogen produced by osteoblasts which stimulates bone matrix apposition rates [Hock et al., 1990]. In bone, it is secreted as a latent complex consisting of the 25 kd TGFβ homodimer non-covalently associated with the 75 kd remainder of the precursor proregion [Bonewald et al., 1991]. Glucocorticoid treatment stimulates osteoblasts to activate latent TGFβ, possibly by inducing the production and secretion of lysosomal proteases [Oursler et al., 1993]. However, glucocorticoids also shift the binding of $TGF\beta_1$ from signal transducing to nonsignal transducing receptors and decrease its mitogenic properties in bone cells [Centrella et al., 1991]. $TGF\beta_1$ prevents the recruitment of osteoclasts [Chenu et al., 1988], and has been reported to stimulate or inhibit bone resorption, depending on the cell system used [Pfeilschifter et al., 1988; Oreffo et al., 1990; Tashjian et al., 1985; Dieudonne et al., 1991]. The activation of TGFβ by glucocorticoids in bone cells may be relevant to the actions of the steroid on bone resorption.

Glucocorticoids inhibit the expression of IGF-I but do not modify IGF-II synthesis by osteoblasts. The actions of glucocorticoids on the IGF-I axis seem critical to their bone effects since IGF-I is among the most prevalent growth factors produced by skeletal cells and has marked stimulatory effects on bone formation. IGF-I enhances osteoblastic collagen synthesis and increases the replication of cells of the osteoblastic

lineage, likely preosteoblasts which will eventually differentiate into osteoblasts. In addition, IGF-I decreases collagenase expression and collagen degradation in calvariae [Canalis et al., 1993a,b]. Since glucocorticoids and IGF-I have opposite effects on bone cells, we postulate that the inhibitory actions of glucocorticoids on IGF-I synthesis are potentially relevant to their effects on bone formation. IGF-I expression can be decreased by glucocorticoids directly, or indirectly via the activation of TGFβ, a factor which can also inhibit IGF-I expression [Canalis et al., 1993b]. However, the kinetics of the response suggest that glucocorticoids decrease IGF-I by a direct mechanism. Down-regulation of IGF-I mRNA is apparent as early as 6 hours after glucocorticoid treatment [McCarthy et al., 1990], and the mechanisms by which this occurs are currently under study. In addition, glucocorticoids modify the binding of IGF-I to its receptor, an effect related to changes in bone cell density [Bennett et al., 1984].

Modulation of IGF binding proteins (IGFBPs) is an another important mechanism by which glucorticoids can regulate IGF-I and -II function in bone (Table III). So far, six IGFBPs, termed IGFBP-1 through -6, have been identified, and osteoblasts express transcripts for all of these binding proteins. The pattern of basal and stimulated IGFBP production varies according to the osteoblastic cell line examined [Hassager et al., 1992]. While many studies have emphasized the inhibitory effects of IGFBPs on bone cell replication and collagen synthesis, it is currently unknown if, under physiological conditions, osteoblasts are exposed to concentrations of IGFBPs that are sufficiently high to modify bone formation. Investigations conducted in nonskeletal cell systems suggest that IGFBPs play a complex role in cell growth and their actions might be different if they are associated with the cell matrix than if they are present in the culture medium [Jones et al., 1993]. It is important to note that cyclic AMP inducers are the major stimulators of IGF-I synthesis by the osteoblast, and they stimulate the synthesis of IGFBP-3, -4, and -5, a possible mechanism of controlling the exposure of bone cells to endogenous IGF-I [McCarthy et al., in press]. In an opposite fashion, glucocorticoids decrease IGF-I as well as IGFBP-3, -4, and -5 synthesis in human osteoblasts [Okazaki et al., 1994]. IGF-I is known to stabilize IGFBP-5 in human osteoblasts and the inhibition of IGF-I by glucocorticoids could destabilize this binding protein [Conover and Kieffer, 1993]. The decrease of IGFBP-5 levels by glucocorticoids could be important in the mechanism of action of glucocorticoids in bone since IGFBP-5 is the only binding protein known to enhance bone growth. Although glucocorticoids modify IGFBP synthesis in bone cells, their mechanism of action needs to be elucidated.

TABLE III. Regulation of IGFBPs by Glucocorticoids in Osteoblasts

	Human	Rat
IGFBP 1	↑	NT
2	NT[a]	↓
3	↓	↓
4	↓	↓
5	↓	↓
6	Ø	↑

NT, not tested.

While glucocorticoids have important effects on TGFβ activation and on the IGF/IGFBP axis in bone, less is known about their effects on the synthesis or activity on other skeletal growth factors. In smooth muscle cells, glucocorticoids inhibit the constitutive and thrombin-induced expression of PDGF A [Nakano et al., 1993], an effect not reported in bone cells. Similarly, the actions of glucocorticoids on the expression on FGFs and BMPs are currently not known. Since PDGFs and FGFs have important mitogenic actions in bone, changes in their synthesis or activity could result in alterations in the process of bone repair. In UMR 201 osteosarcoma cells glucocorticoids induce the expression of osteonectin (SPARC), which has been shown to bind to and prevent the action of PDGF B chains [Ng et al., 1989; Raines et al., 1992]. It is possible that similar effects occur in bone cells and that glucocorticoids may modulate the mitogenic activity of PDGF B through its actions on osteonectin synthesis.

CLINICAL AND THERAPEUTIC IMPLICATIONS

Glucocorticoid excess is a major cause of osteoporosis and little is known about the pathogenesis of this disease. The effects of glucocorticoids on bone metabolism and their mechanisms of action appear to be complex and are still poorly understood. For the prevention and treatment of glucocorticoid-induced osteoporosis, inhibitors of bone resorption and gonadal steroids have beneficial, but limited effects [Reid and Grey, 1993]. It was recently shown that calreticulin, a calcium-binding protein, interferes with cellular responses to glucocorticoids and other

steroid hormones [Burns et al., 1994; Dedhar et al., 1994]. This effect is mediated through the interaction of calreticulin with the DNA-binding domain of the steroid hormone receptors, resulting in an inhibition of the transcriptional activity of the receptors. Interestingly, calreticulin can interact with integrin α subunits, which are important components in osteoclast induced bone resorption. It is tempting to speculate that calreticulin may have important functions in the maintenance of skeletal homeostasis and regulation of osteogenesis, acting as a signal modifier between the membrane-bound integrin α receptor and nuclear steroid hormone receptors. Further investigations are needed to define the specificity and mechanisms of action of calreticulin on steroid receptors before potentially useful drugs can be designed. This goes hand-in-hand with the need to clarify the effects of glucocorticoids on bone formation and their mechanisms of action. If we assume that skeletal IGF is essential for the maintenance of bone mass, inhibition of its synthesis by glucocorticoids could be important in the development of osteoporosis. An attempt to reverse this process could be made by the systemic administration of IGF-I, possibly in combination with growth hormone. The limitations of this approach are the nonspecific effect of the two hormones in nonskeletal tissues, likely to occur after their prolonged administration. Another alternative could be reversal of the inhibition of IGF-I synthesis in bone cells, and this has been achieved in vitro with PTH. Since patients with glucocorticoid induced osteoporosis have hyperparathyroidism, this approach may seem less logical. However, the skeletal actions of continuous PTH administration, such as in hyperparathyroidism, are probably quite different from its actions after its intermittent administration. One could postulate suppressing the secretion of endogenous PTH with calcium and vitamin D and administering PTH in an intermittent fashion. Since there is no effective treatment for glucocorticoid-induced osteoporosis, it is essential to increase our understanding of the mechanism of action of glucocorticoids so that novel approaches to treatment can be devised.

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REFERENCES

Akerblom IE, Mellon PL (1991): Repression of gene expression by steroid and thyroid hormones. In: Parker MG (ed): "Nuclear Hormone Receptors: Cellular Functions, Clinical Abnormalities. San Diego: Academic Press, pp 175–198.

Beato M (1989): Gene regulation by steroid hormones. Cell 56:335–344.

Bellows CG, Aubin JE, Heersche JNM (1987): Physiological concentrations of glucocorticoids stimulate formation of bone nodules from isolated rat calvaria cells in vitro. Endocrinology 212:1985–1992.

Benezra R, Davis RL, Lockshon D, Turner DL, Weintraub H (1990): The protein Id: A negative regulator of helix-loop-helix DNA binding proteins. Cell 61:49–59.

Bennett A, Chen T, Feldman D, Hintz RL, Rosenfeld RG (1984): Characterization of insulin-like growth factor I receptors on cultured rat bone cells: Regulation of receptor concentrations by glucocorticoids. Endocrinology 115: 1577–1583.

Brinckerhoff CE, Plucinska IM, Sheldon LA, O'Connor GT (1986). Half-life of synovial cell collagenase mRNA is modulated by phorobl myristate acetate by not by *all-trans*-retinoic acid or dexamethasone. Biochemistry 25:6378–6384.

Bonewald LF, Wakefield L, Oreffo ROC, Escobedo A, Twardzik DR, Mundy GR (1991): Latent forms of transforming growth factor-β (TGFβ) derived from bone cultures: identification of a naturally occurring 100-kDa complex with similarity to recombinant latent TGFβ. Mol Endocrinol 5:741–751.

Burns K, Duggan B, Atkinson EA, Famulski KS, Nemer M, Bleackley, Michalak M (1994): Modulation of gene expression by calreticulin binding to the glucocorticoid receptor. Nature 367:476–480.

Canalis E, Pash J, Gabbitas B, Rydziel S, Varghese S (1993b): Growth factors regulate the synthesis of insulin-like growth factor-I in bone cell cultures. Endocrinology 133: 33–38.

Canalis E, Pash J, Varghese S (1993a): Skeletal growth factors. Crit Rev Eukaryot Gene Expr 3:155–166.

Centrella M, McCarthy TL, Canalis E (1991): Glucocorticoid regulation of transforming growth factor β1 activity and binding in osteoblast-enriched cultures from fetal rat bone. Mol Cell Biol 11:4490–4496.

Cheng S-L, Yang JW, Rifas L, Zhang S-F, Avioli LV (1994): Differentiation of human bone marrow osteogenic stromal cells in vitro: Induction of the osteoblast phenotype by dexamethasone. Endocrinology 134:277–286.

Chenu C, Pfeilschifter J, Mundy GR, Roodman GD (1988): Transforming growth factor B inhibits formation of osteoclast-like cells on long-term human bone marrow cultures. Proc Natl Acad Sci USA 85:5683–5687.

Conover CA, Kieffer MC (1993): Regulation and biological effect on endogenous insulin-like growth factor binding protein-5 in human osteoblastic cells. J Clin Endocrinol Metab 76:1153–1159.

Dedhar S, Rennie PS, Shago M, Leung Hagesteijn C-Y, Yang H, Filmus J, Hawley RG, Bruchovsky N, Cheng H, Matusik RJ, Giguere V (1994): Inhibition of nuclear hormone receptor activity by calreticulin. Nature 367:480–483.

Defranco DJ, Lian JB, Glowacki J (1992): Differential effects of glucocorticoid on recruitment and activity of osteoclasts induced by normal and osteocalcin-deficient bone implanted in rats. Endocrinology 131:114–121.

Delany AM, Brinckerhoff CE (1992): Post-transcriptional regulation of collagenase and stromelysin gene expression by epidermal growth factor and dexamethasone in cultured human fibroblasts. J Cell Biochem 50:400–410.

Delany AM, Gabbitas BY, Canalis E (in press, a): Transcriptional repression of the α1(I) procollagen gene by cortisol in cultured rat osteoblasts. Proceedings of the 76th Annual Meeting of the Endocrine Society (abstract).

Delany AM, Jeffrey JJ, Canalis E (in press, b): Cortisol increases interstitial collagenase expression in osteoblasts by post-transcriptional mechanisms. J Bone Miner Res Suppl (abstract).

Diamond MI, Miner JN, Yoshinaga SK, Yamamoto KR (1990): Transcription factor interactions: Selectors of positive or negative regulation from a single DNA element. Science 249:1266–1272.

Dieudonne SC, Foo P, van Zoelen EJJ, Burger EL (1991): Inhibiting and stimulating effects of $TGF\beta_1$ on osteoclastic bone resorption in fetal mouse bone organ cultures. J Bone Miner Res 6:479–487.

Firestein GS, Paine MM, Littleman BH (1991): Gene expression (collagenase, tissue inhibitor of metalloproteinases, complement, and HLA-DR) in rheumatoid synovium. Quantitative analysis and effect of intraarticular corticosteroids. Arthritis Rheum 34:1094–1105.

Gauthier J-M, Bourachot B, Doucas V, Yaniv M, Moreau-Gachelin F (1993): Functional interference between the Spi-1/PU.1 oncoprotein and steroid hormone or vitamin receptors. EMBO J 12:5089–5096.

Goldberg GI, Strongin A, Collier IE, Genrich LT, Marmer BL (1992): Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin. J Biol Chem 267:4583–4591.

Granowicz G, McCarthy MB, Raisz LG (1990): Glucocorticoids stimulate resorption in fetal rat parietal bones in vitro. J Bone Min Res 5:1223–1230.

Hämäläinen L, Oikarinen J, Kivirikko KI (1985): Synthesis and degradation of type I procollagen mRNAs in cultured human skin fibroblasts and the effect of cortisol. J Biol Chem 260:720–725.

Hassager C, Fitzpatrick LA, Spencer EM, Riggs BL, Conover CA (1992): Basal and regulated secretion of insulin-like growth factor binding proteins in osteoblast-like cells is cell line specific. J Clin Endocrinol Metab 75:228–233.

Heinrichs AAJ, Bortell R, Rahman S, Stein JL, Alnemri ES, Litwack G, Lian JB, Stein GS (1993): Identification of multiple glucocorticoid receptor binding sites in the rat osteocalcin gene promoter. Biochemistry 32:11436–11444.

Hock JM, Canalis E, Centrella M (1990): Transforming growth factor-β stimulates bone matrix apposition and bone cell replication in cultured fetal rat calvariae. Endocrinology 126:421–426.

Jonat C, Rahmsdorf HJ, Park KK, Cato ACB, Gebel S, Ponta H, Herrlich P (1990): Antitumor promotion and antiinflammation: Down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell 62:1189–1204.

Jones JI, Gockerman A, Busby WH Jr, Camacho-Hubner C, Clemmons DR (1993): Extracellular matrix contains insulin-like growth factor binding protein-5: Potentiation of the effects of IGF-I. J Cell Biol 121:679–687.

Kerppola TK, Luk D, Curran T (1993): Fos is a preferential target of glucocorticoid receptor inhibition of AP-1 activity in vitro. Mol Cell Biol 13:3782–3791.

König H, Ponta H, Rahmsdorf HJ, Herrlich P (1992): Interference between pathway-specific transcription factors: Glucocorticoids antagonize phorbol ester-induced AP-1 activity without altering AP-1 site occupation in vivo. EMBO J 11:2241–2246.

Landers JP, Spelsberg TC (1992): New concepts in steroid hormone action: Transcription factors, proto-oncogenes, and the cascade model for steroid regulation of gene expression. Crit Rev Eukaryot Gene Expr 2:19–63.

Leboy PS, Beresford JN, Devlin C, Owen ME (1991): Dexamethasone induction of osteoblast mRNAs in rat marrow stroma cell cultures. J Cell Physiol 146:370–378.

Leco KJ, Khokha R, Pavloff N, Hawkes SP, Edwards DR (1994): Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. J Biol Chem 269:9352–9360.

Lichtler A, Stover ML, Angilly J, Kream B, Rowe DW (1989): Isolation and characterization of the rat α1(I) collagen promoter. Regulation by 1,25-dihydroxyvitamin D. J Biol Chem 264:3072–3077.

Liska DJ, Stack JL, Bornstein P (1990): A highly conserved intronic sequence is involved in transcriptional regulation of the α1 (I) collagen gene. Cell Regul 1:487–498.

Lukert BP, Raisz LG (1990): Glucocorticoid-induced osteoporosis: Pathogenesis and management. Ann Intern Med 112:352–364.

Luo J, Murphy LM (1989): Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-I MRNA abundance in the intact rat. Endocrinology 125:165–171.

Matrisian LM, Hogan BLM (1990): Growth factor-regulated proteases and extracellular matrix remodeling during mammalian development. Curr Top Dev Biol 24:219–259.

McCarthy T, Casinghino S, Centrella M, Canalis E (in press): Complex pattern of insulin-like growth factor binding protein expression in primary rat osteoblast enriched cultures: Regulation by prostaglandin E_2, growth hormone, and the insulin-like growth factors. J Cell Physiol.

McCarthy TL, Centrella M, Canalis E (1990): Cortisol inhibits the synthesis of insulin-like growth factor-I in skeletal cells. Endocrinology 126:1569–1575.

Meikle MC, Bord S, Hembry RM, Compston J, Croucher PI, Reynolds JJ (1992): Human osteoblasts in culture synthesize collagenase and other matrix metalloproteinases in response to osteotropic hormones and cytokines. J Cell Sci 103:1093–1099.

Morrison N, Eisman J (1993): Role of the negative glucocorticoid regulatory element in glucocorticoid repression of the human osteocalcin promoter. J Bone Miner Res 8:969–975.

Nakano T, Raines EW, Abraham JA, Wenzel IV FG, Higashiyama S, Klagsbrun M, Ross R (1993): Glucocorticoid inhibits thrombin-induced expression of platelet-derived growth factor A-chain and heparin-binding epidermal growth factor-like growth factor in human aortic smooth muscle cells. J Biol Chem 268:22941–22947.

Ng KW, Manji SS, Young MF, Findlay DM (1989): Opposing influences of glucocorticoid and retinoic acid on transcriptional control in preosteoblasts. Endocrinology 3:2079–2085.

Ogata T, Wozney JM, Benezra R, Noda M (1993): Bone morphogenetic protein 2 transiently enhances expression of a gene, Id (inhibitor of differentiation), encoding a helix-loop-helix molecule in osteoblast-like cells. Proc Natl Acad Sci USA 90:9219–9222.

Okazaki R, Riggs BL, Conover CA (1994): Glucocorticoid regulation of insulin-like growth factor-binding protein expression in normal human osteoblast-like cells. Endocrinology 134:126–132.

Oreffo ROC, Bonewald L, Kukita A, Garrett IR, Seyedin SM (1990): Inhibitory effects of the bone-derived growth factors osteoinductive factor and transforming growth factor-β on isolated osteoclasts. Endocrinology 126:3069–3075.

Oursler MJ, Riggs BL, Spelsberg TC (1993): Glucocorticoid-induced activation of latent transforming growth factor-β by normal human osteoblast-like cells. Endocrinology 133: 2187–2196.

Pavlin D, Lichtler AC, Bedfalov A, Kream BE, Harrison JR, Thoms HF, Gronowicz GA, Clark SH, Woody CO, Rowe DW (1992): Differential utilization of regulatory domains within the α1(I) collagen promoter in osseous and fibroblastic cells. J Cell Biol 116:117–126.

Pfeilschifter JP, Seyedin S, Mundy GF (1988): Transforming growth factor beta inhibits bone resorption in fetal rat long bone cultures. J Clin Invest 82:680–690.

Raghow R, Gossage D, Kang AH (1986): Pretranslational regulation of type I collagen, fibronectin, and a 50-kilodalton noncollagenous extracellular protein by dexamethasone in rat fibroblasts. J Biol Chem 261:4677–4684.

Raines EW, Lane TF, Iruela-Arispe ML, Ross R, Sage EH (1992): The extracellular glycoprotein SPARC interacts with platelet-derived growth factor (PDGF)-AB and -BB and inhibits the binding of PDGF to its receptors. Proc Natl Acad Sci USA 89:1281–1285.

Raisz LG, Trummel CL, Wener JA, Simmons H (1972): Effect of glucocorticoids on bone resorption in tissue culture. Endocrinology 90:961–967.

Reid IR, Grey AB (1993): Corticosteroid osteoporosis. Bailliere's Clin Rheumatol 7:573–587.

Reid IR, Katz JM, Ibbertson HK, Gray DH (1986): The effects of hydrocortisone, parathyroid hormone and the bisphosphonate, APD, on bone resorption in neonatal mouse calvaria. Calcif Tissue Int 38:38–43.

Rifas L, Fausto A, Scott MJ, Avioli LV, Welgus HG (1994): Expression of metalloproteinases and tissue inhibitors of metalloproteinases in human osteoblast-like cells: differentiation is associated with repression of metalloproteinase biosynthesis. Endocrinology 134:213–221.

Rodan GA, Martin TJ (1981): Role of osteoblasts in hormonal control of bone resorption: A hypothesis. Calcif Tissue Int 33:349–351.

Schüle R, Rangarajan P, Kliewer S, Ransone LJ, Bolado J, Yang N, Verma IM, Evans RM (1990): Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. Cell 62:1217–1226.

Shalhoub V, Conlon D, Tassinari M, Quinn C, Partridge N, Stein GS, Lian JB (1992): Glucocorticoids promote development of the osteoblast phenotype by selectively modulating expression of cell growth and differentiation associated genes. J Cell Biochem 50:425–440.

Stern PH (1969): Inhibition by steroids of parathyroid hormone-induces Ca^{45} release from embryonic rat bone in vitro. J Pharmacol Exp Ther 168:211–217.

Stromstedt P-E, Poellinger L, Gustafsson J-A, Carlstedt-Duke J (1991): The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: A potential mechanism for negative regulation. Mol Cell Biol 11:3379–3383.

Subramaniam M, Colvard D, Keeting PE, Rasmussen K, Riggs BL, Spelsberg TC (1992): Glucocorticoid regulation of alkaline phosphatase, osteocalcin, and proto-oncogenes in normal human osteoblast-like cells. J Cell Biochem 50:411–424.

Tashjian Jr AH, Voelkel EF, Lazzaro M, Singer FR, Roberts AB, Derynck R, Winkler ME, Levine L (1985): α and β human transforming growth factors stimulate prostaglandin production and bone resorption in cultured mouse calvaria. Proc Natl Acad Sci USA 82:4535–4538.

Ureña P, Iida-Klein A, Kong X-F, Juppner H, Kronenberg HM, Abou-Samra A-B, Segre GV (1994): Regulation of parathyroid hormone (PTH)/PTH-related peptide receptor messenger ribonucleic acid by glucocorticoids in ROS 17/2.8 and OK cells. Endocrinology 134:451–456.

Yang-Yen HF, Chambard J-C, Sun Y-L, Smeal T, Schmidt TJ, Drouin J, Karin M (1990): Transcriptional interference between c-Jun and the glucocorticoid receptor: Mutual inhibition of DNA binding due to direct protein-protein interaction. Cell 62:1205–1215.