Transient Versus Sustained Phosphorylation and Nuclear Accumulation of ERKs Underlie Anti- Versus Pro-apoptotic Effects of Estrogens*

Jin-Ran Chen, Lilian I. Plotkin, José Ignacio Aguirre, Li Han, Robert L. Jilka, Stavroula Kousteni, Teresita Bellido, and Stavros C. Manolagas‡

From the Division of Endocrinology & Metabolism, Center for Osteoporosis & Metabolic Bone Diseases, University of Arkansas for Medical Sciences and the Central Arkansas Veterans Health Care System, Little Rock, Arkansas 72205

Sex steroids exert anti-apoptotic effects on osteoblasts/osteocytes but exert pro-apoptotic effects on osteoclasts, in both cases requiring activation of the extracellular signal-regulated kinases (ERKs). To explain the mechanistic basis of this divergence, we searched for differences in the kinetics of phosphorylation and/or in the subcellular localization of ERKs in response to 17β-estradiol in the two cell types. In contrast to its transient effect on ERK phosphorylation in osteocytic cells (return to base line by 30 min), 17β-estradiol-induced ERK phosphorylation in osteoclasts was sustained for at least 24 h following exposure to the hormone. Conversion of sustained ERK phosphorylation to transient, by means of cholera toxin-induced activation of the adenylate cyclase/cAMP/protein kinase A pathway, abrogated the pro-apoptotic effect of 17β-estradiol on osteoclasts. Conversely, prolongation of ERK activation in osteocytes, by means of leptomycin B-induced inhibition of ERK export from the nucleus or overexpression of a green fluorescent protein-ERK2 mutant that resides permanently in the nucleus, converted the anti-apoptotic effect of 17β-estradiol to a pro-apoptotic one. These findings indicate that the kinetics of ERK phosphorylation and the length of time that phospho-ERKs are retained in the nucleus are responsible for pro-versus anti-apoptotic effects of estrogen on different cell types of bone and perhaps their many other target tissues.

Sex steroids prevent bone loss by suppressing the rate of bone turnover and maintaining a focal balance between bone formation and resorption (1–3). Suppression of bone turnover results from the attenuating effects of sex steroids on the birth rate of osteoblast and osteoclast progenitors. Maintenance of a focal balance between formation and resorption results from opposing effects on the life span of osteoblasts and osteoclasts, an anti-apoptotic effect on the former and a pro-apoptotic effect on the latter cell type (1, 4). As in osteoblasts, sex steroids exert anti-apoptotic effects on osteocytes, which are former osteoblasts entombed in the mineralized bone matrix and forming an extensive cell communication network that perceives and responds to mechanical strains by compensatory bone augmentation and reduction (5). A shortening of the life span of bone-forming cells in combination with prolongation of the life span of bone-resorbing cells represent critical pathophysiologic changes in most acquired metabolic bone diseases, including the osteoporosis that results not only from sex steroid deficiency but also from glucocorticoid excess or old age (4, 6–9). Interestingly, other agents, such as parathyroid hormone and bisphosphonates, used commonly for the treatment of metabolic bone diseases, exert their beneficial effects on bone by regulating the rate of birth of new osteoblasts or osteoclasts or their apoptosis (10–12).

Earlier work from our group has elucidated that the anti-apoptotic effect of estrogens or androgens on osteoclasts results from nongenotropic mechanisms of action of their classical receptors, causing rapid and transient phosphorylation and nuclear translocation of extracellular signal-regulated kinases (ERKs), as well as modulation of the phosphatidylinositol 3-kinase and c-Jun NH2-terminal kinase (JNK) signaling cascades (13, 14). Phosphorylation of cytoplasmic kinases and their transport to the nucleus leads in turn to the modulation of the activity of transcription factors such as Elk-1, CCAAT enhancer-binding protein β, and cAMP-response element-binding protein or c-Jun/c-Fos, which are also required for the anti-apoptotic actions of sex steroids.

In direct contrast to their anti-apoptotic effects on osteoblasts and osteocytes, estrogens and androgens exert pro-apoptotic effects on mature osteoclasts (4, 13–17). In our earlier work, 4-estren-3α,17β-diol, a synthetic ligand of the estrogen receptor (ER) or androgen receptor that reproduces the non-genotropic effects of classical sex steroids with minimal effects on classical transcription, was as potent as estradiol or 5α-dihydrotestosterone not only in protecting osteoblasts/osteocytes from apoptosis but also in promoting osteoclast apoptosis. Conversely, 1,2,5-tris(4-hydroxyphenyl)-4-propiyprazol, a compound that has potent classical transcriptional activity but minimal effects on ERK or JNK kinases, did not affect osteoclast (or osteoblast) apoptosis. These lines of evidence strongly suggest that the pro-apoptotic effect of estrogens on osteoclasts,

*This work was supported in part by National Institutes of Health Grants PO1-AG13918 (to S. C. M.), AR46823 (to R. L. J.), and KO2-AR02127 (to T. B.) and by the Department of Veterans Affairs Merit Review grant and a Research Enhancement Award Program (to S. C. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: Division of Endocrinology and Metabolism, Slot 587, University of Arkansas for Medical Sciences and the Central Arkansas Veterans Health Care System, Little Rock, Arkansas 72205

The abbreviations used are: ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; ER, estrogen receptor; estradiol, 17β-estradiol; RANKL, receptor activator of NF-κB ligand; M-CSF, macrophage colony-stimulating factor; GFP, green fluorescent protein; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; RFP, red fluorescent protein; nRFP, nuclear localized RFP; ANOVA, analysis of variance; IL, interleukin.
similar to their anti-apoptotic effect on osteoblasts, is mediated by a nongenotropic mechanism of receptor action that, as we show herein, also involves ERK activation.

In general, activation of ERK1/2 leads to cell survival, whereas activation of JNK or p38 induces apoptosis (18). Nonetheless, there is evidence that in several cell types, ERKs may also transmit pro-apoptotic signals (19–21). In addition, phosphorylated ERKs may promote their export back to the cytoplasm (30).

**EXPERIMENTAL PROCEDURES**

**Materials**—Estradiol, etoposide, cholina toxin, and leptomin B were purchased from Sigma. Recombinant mouse receptor activator of NF-κB ligand (RANKL) and recombinant human macrophage colony-stimulating factor (M-CSF) were purchased from R&D Systems (Minneapolis, MN). U0126 was purchased from Promega (Madison, WI). PD98059 was purchased from New England Biolabs (Beverly, MA). 4-Amino-5-(4-methylphenyl)-7-(4-butyl)pyrazololo(3,4-d)pyrimidine was purchased from BIOSOURCE (Camarillo, CA). Phenol red-free minimum essential medium α was purchased from Invitrogen. Bovine calf serum, charcoal-stripped serum, and fetal bovine serum were purchased from Hyclone Laboratories (Logan, UT).

**Plasmids**—Wild-type ERK2 fused to green fluorescent protein (GFP-ERK2), and the mutants GFP-ERK2 312A and 321A were provided by Dr. Roni Seger (Department of Biological Regulation, The Weizmann Institute of Sciences, Rehovot, Israel) (31). Wild-type mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) was provided by N. G. A. R. Fried (University of Colorado, Boulder, CO) (29). The SV40 large T antigen nuclear localization sequence (33) was attached to the amino terminus of the cDNA construct encoding red fluorescent protein (pDsRed1-N1, Clontech, Palo Alto, CA) to obtain an RFP targeted to the nucleus (nRFP). All the constructs used in this study have been shown to produce functional proteins.

**Cell Culture**—Osteoclasts were formed in *ex vivo* cultures of non-adherent murine bone marrow aspirates (devoid of stromal/osteoblastic cells) from 7-week-old C57Bl mice or RAW264.7 cells stimulated with 30 ng/ml M-CSF and 30 ng/ml soluble RANKL in minimum essential medium α with 10% fetal bovine serum, as described previously (9). The murine long bone-derived osteocytic cell line MLO-Y4 was provided by Dr. L. Bonewald (University of Missouri-Kansas City). Cells were cultured in phenol red-free minimum essential medium α supplemented with 2.5% fetal bovine serum, 2.5% bovine calf serum, 100 units/ml penicillin, 100 units/ml streptomycin, and 2.5% chicken embryo extract in 2.5% bovine serum. Cells/cm² on collagen type I-coated plates, as described previously (10).

**Western Blot Analysis**—The phosphorylation status of ERK1/2 in bone marrow-derived osteoclasts was determined by immunoblotting using a mouse monoclonal antibody recognizing tyrosine phosphorylated ERK1/2 or a rabbit polyclonal antibody recognizing total ERK1/2 followed by incubation with either an anti-mouse or an anti-rabbit antibody conjugated with horseradish peroxidase. Blots were developed using chemiluminescence according to the manufacturer's recommendations. Quantitation of the intensity of the bands in the autoradiograms was performed using a VersaDoc™ imaging system (BioRad).

**Quantification of Apoptotic Cells**—MLO-Y4 cells were treated with vehicle or 1 ng/ml leptomin B alone or in combination with 50 μM PD98059 for 30 min followed by a 30-min treatment with either 10⁻⁷ M estradiol. Subsequently, vehicle or 50 μM etoposide was added and cells were incubated for an additional 6 h. Apoptotic cells were quantified by trypan blue uptake, as described previously (10). The effect of wild-type or GFP-ERK2 mutants on the anti-apoptotic actions of estradiol was determined by evaluating the nuclear morphology of cells transiently transfected with the GFP-ERK2 constructs together with wild-type MEK and nRFP to follow the localization of the cell nuclei, as described previously (14). Apoptosis of osteoclasts, derived from bone marrow cells cultured with 30 ng/ml M-CSF and 30 ng/ml soluble RANKL, was quantified by assessing changes in cell morphology following staining with tartrate-resistant alkaline phosphatase and hematoxylin counterstaining.

**Transient Transfection and Subcellular Localization of ERK2—MLO-Y4 cells were transiently transfected using Lipofectamine Plus (Invitrogen) with GFP-ERK2 and wild-type MEK along with nRFP. Following transfection, cells were cultured for 48 h. Subsequently, they were serum-starved by culturing in the presence of 2% bovine serum albumin for 1 h and treated with vehicle or 10⁻⁷ M estradiol for 5 min. Cells were then fixed in neutral buffer formalin for 8 min. In the experiment showing the effect of leptomin B in the subcellular localization of GFP-ERK2, 48 h after transfection, cells were serum-starved for 40 min in the absence or in the presence of 1 ng/ml leptomin B. This was followed by the addition of 10⁻⁷ M estradiol and incubation for an additional 5 min. The percentage of cells showing nuclear accumulation of GFP-ERK2 was quantified (using a fluorescence microscope) by enumerating cells exhibiting increased GFP in the nucleus compared with the cytoplasm. At least 250 cells from fields selected by systematic random sampling were examined for each experimental condition. RAW264.7 cells (1 x 10⁶) were cultured for 2 days in the presence of 30 ng/ml RANKL and M-CSF in 10-cm cell culture dishes. Cells were then trypsinized, replated in the presence of RANKL and M-CSF, and co-transfected 24 h later with GFP-ERK2, wild-type MEK, and nRFP using Lipofectamine Plus. Twenty-four hours following transfection, cells were treated with vehicle or 10⁻⁸ M estradiol.

**Statistical Analysis**—Data were analyzed by one-way analysis of variance (ANOVA). The Student-Neuman-Keuls method was used to estimate the level of significance of differences between means.

**RESULTS**

**Induction of ERK Phosphorylation Is Required for the Pro-apoptotic Effect of Estradiol on Osteoclasts**—In cultures of mature bone marrow-derived osteoclasts, estradiol induced ERK phosphorylation within 15 min of treatment, and the magnitude of this effect increased progressively thereafter for at least 24 h (Fig. 1A). U0126, an inhibitor of MEK, at an ERK phosphorylation-inhibiting concentration (Fig. 1B), abrogated the pro-apoptotic effect of estradiol in osteoclasts (Fig. 1C), indicating that the pro-apoptotic effect of sex steroids on osteoclasts, like their anti-apoptotic effects on osteoblasts, requires ERK signaling. However, the time kinetics and pattern of sustained ERK phosphorylation for at least 24 h seen in osteoclasts is completely distinct from the transient ERK phosphorylation we have extensively documented previously in several osteoblastic and osteocytic cell lines, including MLO-Y4 cells (14) and primary osteoclasts (data not shown). In all these cell types, ERK phosphorylation in response to estradiol is detectable by 2 min, reaches a peak by 5 min, and returns to base line by 30 min.

**Sustained Versus Transient Nuclear Accumulation of ERK2 in Osteoclasts and Osteocytes**—In osteoblastic/osteocytes, estradiol or 5α-dihydrotestosterone not only induces rapid phosphorylation of ERKs but also causes rapid translocation of the phosphorylated kinase into the nucleus (13, 14). This finding, together with evidence that cell survival can depend on the duration of ERK nuclear accumulation (21, 29), prompted us to compare the kinetics of this phenomenon in osteoclasts and osteocytes (Fig. 2). To this end, osteoclasts derived from the RAW264.7 monocytic cell line as well as MLO-Y4 cells were transiently co-transfected with constructs containing GFP-ERK2, MEK, and nRFP. Subsequently, cells were exposed for different lengths of time to estradiol. Using epifluorescence microscopy, we followed the kinetics of the subcellular localization of GFP-ERK2 in response to estradiol treatment and determined the percentage of cells exhibiting accumulation of GFP-ERK2 in the nucleus. In osteoclasts, ERK2 nuclear accumulation occurred rapidly (within 2 min) and increased progressively thereafter over a 24-h period (Fig. 2A). The kinetics of ERK nuclear accumulation in response to estradiol in a single osteoclast over a 3-h time period are depicted in Fig. 2B. Prior to estradiol exposure, GFP-ERK2 exhibited a diffuse cytoplasmic distribution. However, as early as 2 min after the addition of estradiol into the cultures, the green fluorescence condensed within discrete regions corresponding to the red fluorescence-labeled nuclei. The intensity of the foci of green fluorescence increased progressively over the next 3 h, with a...
corresponding decrease in the fluorescence seen in the cytoplasm. By contrast, in the case of osteocytes, after an initial progressive increase for a 2-h period, ERK2 accumulation declined subsequently to reach base-line levels by 4 h (Fig. 2C).

Cholera Toxin Converts Sustained Phosphorylation of ERKs by Estradiol to Transient and Abrogates the Pro-apoptotic Effect of Estradiol in Osteoclasts—To explore the possibility that the opposite effects of estradiol on the fate of osteoclasts and MLO-Y4 cells resulted from the distinct temporal pattern of ERK phosphorylation, we shortened the duration of ERK activation in osteoclasts with cholera toxin, which activates the α subunit of G proteins, thus leading to constitutive activation of the adenylate cyclase/cAMP/protein kinase A pathway. Activated protein kinase A in turn suppresses ERK phosphorylation (34). The temporal pattern of ERK phosphorylation in response to estradiol was converted from sustained to transient in osteoclasts, which were pretreated with cholera toxin as ERK phosphorylation reached a zenith by 5 min, returned to base line by 30 min, and remained low for at least 2 h (Fig. 3A). Cholera toxin by itself had no significant effect on ERK phosphorylation over vehicle-treated cells. In support of our hypothesis, conversion of the temporal pattern of estradiol-induced ERK phosphorylation from sustained to transient was associated with a complete abrogation of the pro-apoptotic effect of estradiol (Fig. 3B).

Prolonging Nuclear Accumulation of Activated ERKs in MLO-Y4 Cells Converts the Anti-apoptotic Effect of Estradiol to Pro-apoptotic—Finally, we examined whether prolonging the nuclear retention of phosphorylated ERKs altered the anti-apoptotic effect of estradiol in osteocytes. In this experiment, nuclear retention of ERKs was accomplished by pre-treating MLO-Y4 cells with leptomycin B, an inhibitor of chromosomal region maintenance 1/exportin 1-mediated nuclear export of proteins (Fig. 4A). As shown in Fig. 4B, addition of leptomycin B had no effect on the survival of vehicle-treated MLO-Y4 cells. Strikingly however, estradiol stimulated apoptosis in the presence of leptomycin B. Furthermore, in leptomycin B-treated MLO-Y4 cells, estradiol could no longer prevent the pro-apoptotic effect of etoposide. Pretreatment of MLO-Y4 osteocytes with the ERK inhibitor PD98059 abolished the pro-apoptotic effect of the hormone in the presence of leptomycin B. The latter finding strongly suggested that the pro-apoptotic effect of estradiol in leptomycin B-treated cells did indeed depend on the nuclear retention of phosphorylated ERKs, or at least ERK-regulated proteins, which are retained in the nucleus in leptomycin B-treated cells.

To determine whether the subcellular localization of phosphorylated ERKs (as opposed to ERK-regulated proteins) dictates the pro- versus the anti-apoptotic effects of estradiol, we transfected MLO-Y4 cells with GFP-ERK2 or GFP-ERK2 mutants that accumulate either in the nucleus or the cytoplasm (Fig. 5A). In these mutants, substitution of residues 312–319 of ERK2 to alanines has been shown to prevent the cytosolic retention and association of ERK2 with MEK1 without affecting ERK activation, whereas substitution of residues 321–327 to alanines impairs nuclear translocation of ERK2 (31). In contrast to the anti-apoptotic effect of estradiol in cells transfected with the wild-type ERK2, in cells transfected with the nuclear-anchored ERK2, estradiol not only failed to prevent etoposide-induced apoptosis but also induced apoptosis by itself (Fig. 5B). On the other hand, in cells transfected with the ERK2 mutant that was localized in the cytoplasm, estradiol had no effect on the survival of transfected cells by itself, and it could not prevent the pro-apoptotic effect of etoposide.

**DISCUSSION**

The results of the present study demonstrate that estradiol requires ERKs to induce both anti- and pro-apoptotic signals in two distinct cell types, osteocytes and osteoclasts, respectively. The opposite outcome of ERK activation on apoptosis can be explained by differences in the duration of ERK phosphorylation as well as the duration of the accumulation of ERKs in the nucleus. Specifically, we show that one can abolish the pro-apoptotic effect of estradiol in osteoclasts and even convert the anti-apoptotic effect of the hormone in osteocytes into a pro-
apoptotic one by altering the temporal pattern of ERK activation and the time that ERKs are retained in the nucleus. Hence, the temporal pattern of ERK activation, rather than the cell context, is the critical determinant of whether ERK activation will lead to survival or apoptosis. In agreement with this conclusion, cytokines like interleukin-1 (IL-1), IL-6, tumor necrosis factor, or RANKL exert potent anti-apoptotic effects on osteoclast progenitors and mature osteoclasts (35) by inducing transient activation of ERKs (36–39). Conversely, selective c-Src inhibitors stimulate osteoclast apoptosis, both in vitro and in vivo, by inducing sustained ERK phosphorylation (21).

Converting sustained into transient ERK phosphorylation in response to estradiol in this work was accomplished by cholera toxin, an agent that stimulates cAMP-dependent activation of protein kinase A (34). Up-regulation of cAMP by calcitonin or dibutyryl cAMP itself suppresses osteoclast apoptosis (40, 41). Therefore, it is possible that the anti-apoptotic properties of cAMP are indeed due to its ability to convert sustained ERK activation into transient (in response to whatever stimuli promote osteoclast death in vivo). Future studies will be required to explore this possibility.

We and others had proposed earlier that estrogens suppress osteoclastogenesis by attenuating the production of osteoclastogenic cytokines by stromal/osteoblastic cells and that this effect results from a genotropic action of the ER (2). In support of this notion, neutralization of IL-6 with antibodies or knock-out of the IL-6 gene in mice prevents the ovariectomy-induced up-regulation of osteoclast progenitors in the marrow and the increase in osteoclast number in trabecular bone sections and also protects against the loss of bone (8, 42, 43). The suppressive effect of estrogens on IL-6 production results from protein-protein interactions between the ER and other transcription factors like NF-kB and CCAAP enhancer-binding protein β (43–45). Similar trans- or cis-interactions...
of the ER influence the synthesis of other cytokines involved in osteoclastogenesis, including tumor necrosis factor, M-CSF, and osteoprotegerin (46–48).

In view of the evidence that IL-6, tumor necrosis factor, or RANKL have potent anti-apoptotic effects on osteoclast progenitors and mature osteoclasts, the suppression of osteoclastogenesis by estrogens may be due, at least in part, to an increase of osteoclast apoptosis resulting from the inhibitory effect of estrogens on the production of these cytokines by stromal osteoblastic cells. A pro-apoptotic effect of estrogens on osteoclasts may also result from stimulation of transforming growth factor β, an agent known to induce apoptosis of osteoclasts and their hematopoietic progenitors (16). Nonetheless, the demonstration of direct pro-apoptotic effects of estrogens on osteoclasts in the present report (i.e. effects that were manifested in osteoclast cultures devoid of stromal/osteoblastic cells) argues strongly that at least part of the anti-osteoclastogenic effect of estrogens is exerted directly on osteoclasts. This contention is supported by evidence that the caspase inhibitor DEVD reverses the anti-osteoclastogenic effect of estrogens. Furthermore, it is consistent with the presence of the ER in osteoclasts (49, 50) and the observation that estrogens modulate the recruitment of myelopoietic osteoclast progenitors through a stromal cell-independent mechanism involving apoptosis (15). In any event, the fact that the pro-apoptotic effect of estrogens on osteoclasts in our studies was demonstrable in the presence of RANKL and was associated with sustained ERK activation strongly suggest that prolongation of ERK signaling by estrogens in osteoclasts must have overridden the RANKL-induced transient ERK activation signal and thereby reversed the pro-survival effects of the cytokine.

Besides ERKs (and phosphatidylinositol 3-kinase), JNK1 is also phosphorylated by RANKL and is required for the anti-apoptotic effect of the cytokine on osteoclasts (51). However, estrogens in the presence of RANKL down-regulate JNK1/c-Jun signaling, an action that mediates their anti-osteoclasto-

---

2 J.-R. Chen, L. I. Plotkin, J. I. Aguirre, L. Han, R. L. Jilka, S. Kousteni, T. Bellido, and S. C. Manolagas, unpublished observations.

---

**FIG. 3.** Cholera toxin converts sustained phosphorylation of ERKs by estradiol to transient in osteoclasts and abolishes estradiol-induced osteoclast apoptosis. Bone marrow-derived osteoclasts were treated with vehicle (veh) or 1 µg/ml cholera toxin (ChT) for 30 min prior to the addition of 10⁻⁸ M estradiol (E₂) for the indicated periods of time. ERK1/2 phosphorylation (A) and apoptosis (B) were assessed as described in Fig. 1. Bars indicate means ± S.D. of triplicate determinations. *, p < 0.05 versus vehicle by ANOVA.
Inhibition of ERK nuclear export induces MLO-Y4 apoptosis in the presence of estradiol. Cells were transiently transfected with GFP-ERK2 fusion protein along with wild-type MEK and nRFP. Transfected cells were serum-starved for 45 min in the absence or in the presence of 1 ng/ml leptomycin B and treated with vehicle or estradiol (E2) for the last 5 min. Nuclear accumulation of GFP-ERK2 is shown in the upper panels, and the expression of nRFP in the nuclei is shown in the lower panels. MLO-Y4 cells were treated with vehicle or 1 ng/ml leptomycin B alone or in combination with 50 μM PD98059 for 30 min followed by a 50-min treatment with 10^{-7} M estradiol. Subsequently, vehicle or 50 μM etoposide was added, and cells were incubated for an additional 6 h. Apoptosis was assessed by trypan blue uptake, as described under “Experimental Procedures.” Bars indicate means ± S.D. of triplicate determinations. *, p < 0.05 versus vehicle by ANOVA.

Recent work by Lean et al. (53) has suggested that estrogens suppress osteoclastogenesis by increasing thiol antioxidants in osteoclasts through an increase in glutathione and thioredoxin reductases. We have confirmed and extended these findings by demonstrating that both the pro-apoptotic effect of estrogens on osteoclasts and their ability to suppress osteoclastogenesis do indeed require glutathione. More importantly, we have determined that the regulation of glutathione and thioredoxin reductases by estrogens are the result of a nongenotropic mechanism of action mediated by cytoplasmic kinases and most likely kinase-mediated transcriptional control of the production of nonprotein thiols (54). Hence, the preponderance of existing evidence favors the conclusion that estrogens decrease the number of osteoclasts by shortening the life span of mature osteoclasts as well as by suppressing osteoclastogenesis; the latter is due, at least in part, to direct as well as indirect (cytokine-mediated) effects of the hormone on apoptosis.

In conclusion, the results of the present study strongly suggest that the kinetics of ERK phosphorylation and the length of time that phospho-ERKs are retained in the nucleus are responsible for the pro-versus anti-apoptotic effect of estrogens on bone cells, perhaps by determining the activation of a distinct set of transcription factors. Whether this is true for the numerous other cell targets of estrogen action will require future studies.

Acknowledgements—We thank Charles A. O’Brien for helpful discussions, Robyn DeWall for assistance in the writing of the manuscript, and Verenda G. Lowe, Aaron D. Warren, and Kanan Vyas for their technical assistance.

REFERENCES
1. Manolagas, S. C. (2000) Endocr. Rev. 21, 115–137
2. Manolagas, S. C., Koutsteni, S., and Jilka, R. L. (2002) Recent Prog. Horm. Res. 57, 385–409
3. Manolagas, S. C., Koutsteni, S., Chen, J.-R., Schuller, M., Plotkin, L. I., and Bellido, T. (2004) Kidney Int. Suppl. 91, S41–S49
4. Koutsteni, S., Chen, J.-R., Bellido, T., Han, L., Ali, A. A., O’Brien, C., Plotkin, L. I., Fu, Q., Mancino, A. T., Wen, Y., Vertino, A. M., Powers, C. C., Stewart, S. A., Ebert, R., Parfitt, A. M., Weinstein, R. S., Jilka, R. L., and Manolagas, S. C. (2002) Science 298, 843–846
5. Aarden, E. M., Burger, E. H., and Nijweide, P. J. (1984) J. Cell. Biochem. 25, 287–299
6. Jilka, R. L., Weinstein, R. S., Takahashi, K., Parfitt, A. M., and Manolagas, S. C. (1999) J. Clin. Invest. 102, 274–282
7. Weinstein, R. S., Jilka, R. L., Parfitt, A. M., and Manolagas, S. C. (1999) J. Clin. Invest. 102, 1732–1740
8. Jilka, R. L., Hangoc, G., Giraszale, G., Passeti, G., Williams, D. C., Abrams, J. S., Boyce, B., Brommeyer, H., and Manolagas, S. C. (1992) Science 257, 88–91
Transient Versus Sustained Phosphorylation and Nuclear Accumulation of ERKs Underlie Anti- Versus Pro-apoptotic Effects of Estrogens
Jin-Ran Chen, Lilian I. Plotkin, José Ignacio Aguirre, Li Han, Robert L. Jilka, Stavroula Kousteni, Teresita Bellido and Stavros C. Manolagas

J. Biol. Chem. 2005, 280:4632-4638.
doi: 10.1074/jbc.M411530200 originally published online November 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M411530200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 17 of which can be accessed free at http://www.jbc.org/content/280/6/4632.full.html#ref-list-1