Estrogen Receptor (ER)-α, but Not ER-β, Mediates Regulation of the Insulin-like Growth Factor I Gene by Antiestrogens*  

Brigitte Fournier‡§, Sabine Gutzwiller†, Tanja Dittmar‡, Gabriele Matthias‡, Paul Steenberghi, and Patrick Matthias‡  

From the §Arthritis & Bone Metabolism Therapeutic Area, Novartis Pharma Research, 4002 Basel, Switzerland, ‡Department of Physiological Chemistry, University Medical Center, Utrecht 3584, The Netherlands, and ¶Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland

The importance of insulin-like growth factor I (IGF-I) on maintenance of skeletal integrity has been widely recognized. Although osteoblasts secrete some IGF-I, the liver is the primary endocrine source for IGF-I. We have studied the regulation of the human IGF-I promoter in the hepatocyte cell line Hep3B, and we have shown that the IGF-I promoter, when co-transfected in Hep3B cells together with an estrogen receptor (ER)-α expression vector, was transcriptionally regulated by raloxifene or raloxifene-like molecules but not by 17β-estradiol and 4(OH)-tamoxifen. The induction mediated by raloxifene is antagonized by 17β-estradiol and 4(OH)-tamoxifen. The induction mediated by raloxifene is antagonized by 17β-estradiol and mediated selectively by ER-α, but not by ER-β. Transfer of IGF-I promoter sequences from −733 to −65 or from −375 to −65 to a minimal Fos promoter resulted in a comparable responsiveness to raloxifene. This region contains two CAAT/enhancer-binding protein sites and an activator protein 1 site, both of which have been shown to be involved in estrogen receptor-mediated transactivation. When the CAAT/enhancer-binding protein sites were mutated in a construct bearing the sequence from −375 to −65 in front of the minimal Fos promoter, raloxifene induction was reduced, whereas mutation of the other elements did not affect induction. In addition, using chimeric proteins, we delineated the domains of ER-α that confer to ER-α transactivation abilities on the IGF-I promoter that are not exhibited by ER-β. These data shed new light on the mechanism of action of antiestrogens and might help explain, at least in part, the bone-protective effects observed for some antiestrogens in ovariectomized animals.

IGF-I1 is a pleiotropic hormone involved in several aspects of growth and development, and it plays an important role in maintaining bone mass (1). In the skeletal tissue, IGF-I originates from the circulation and from the osteoblasts, where its production is regulated by several major bone hormones, such as parathyroid hormone (2), prostaglandins (3), and estrogen (4, 5). The main endocrine source of IGF-I is the liver, where it is produced under the control of growth hormone (6). As aging progresses, a decrease in growth hormone release by the pituitary glands causes a reduction in the production of circulating IGF-I by the liver (7), and, in addition, the local skeletal IGF-I concentration declines. The dramatic decrease in circulating estrogen occurring at menopause might play a central role in the decrease of this local skeletal IGF-I production. This continuous decline in IGF-I activity with age may be a major cause of bone loss (8).

Estrogen and antiestrogen effects are mediated through the estrogen receptor (ER), a ligand-dependent transcription factor. The ER exerts its transcriptional control after interaction of receptor dimers with specific DNA sequences such as the classical estrogen-responsive elements (EREs) or other DNA sequences in the regulatory regions of target gene promoters (9). Antiestrogens can act as estrogen antagonists or partial agonists. Three distinct classes of antiestrogens have been described, ranging from pure antagonists such as ICI 182,780 to partial agonists such as tamoxifen and raloxifene, which exert tissue-selective estrogen-like effects (10). The mechanism of action of these mixed agonists/antagonists is still not completely unraveled. The elucidation of the crystal structure of ER in complex with estrogen or raloxifene (11) has provided some molecular evidence for the mechanism of antagonism of this antiestrogen. Recent data indicate that differences in tissue selectivity between raloxifene and tamoxifen are based upon differences in ligand-dependent conformation of the ligand-binding domain (12). In addition, the existence of a second recently discovered estrogen receptor, ER-β (13), could also help explain various aspects of the agonist and antagonist profile of antiestrogens in different tissues and cell contexts (14).

Raloxifene and its derivatives have recently drawn a lot of attention because they are being proposed as a first generation of specific estrogen receptor modulators (SERMs) (15) with beneficial effects on bone and the cardiovascular system and a limited effect on the uterus and acting as an antiestrogen on breast. The mechanism of action of raloxifene has previously been studied using the TGF-β3 promoter, in which a raloxifene response element (RRE) bearing no homology to the classical ERE has been described (16). In this case, the RRE appeared to be essential but not sufficient to support the raloxifene response; an additional, as yet unidentified element of the TGF-β3 promoter was also required for raloxifene induction (17). Because of the potential impact of liver IGF-I production on bone and more generally on the aging process (6, 7, 8), we studied the effects of estrogen and antiestrogens on transcription of the human IGF-I gene. The human IGF-I gene contains
two promoters, which show cell-specific expression. A preferential usage of the more proximal promoter I has been seen in the adult human liver, where it accounts for 80% of IGF-I transcripts (18). For this reason, in this study we used a piece of promoter I and tested its regulation in a liver-derived cell line, Hep3B. Estrogen regulation of the chicken IGF-I gene has already been demonstrated (19), but the effects of antiestrogens were not investigated. Here we show that the antiestrogens raloxifene, arzoxifene, and the so-called pure antiestrogen ICI 182,780 all increase IGF-I gene transcription, whereas 17β-estradiol and 4(OH)-tamoxifen, another class of antiestrogens, did not elicit any increase in IGF-I transcriptional activity. Strikingly, induction of the IGF-I promoter by these antiestrogens was mediated specifically by ER-α, but not by ER-β, although ER-β efficiently activated an ERE-tk-luc reporter in an estrogen-dependent manner. We also found that both the AF1 and AF2 domains of ER-α are responsible for specific transactivation of the IGF-I promoter by ER-α. Furthermore, we found that two C/EBP sites are necessary for this ER-α-mediated induction of the IGF-I promoter.

MATERIALS AND METHODS

Cell Cultures—The human hepatoma cell line Hep3B was obtained from the American Type Culture Collection (ATCC HB 80 64). The cells were cultivated in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 1% penicillin/streptomycin, and 1% L-Glu. During the experiments, cells were kept in phenol red-free medium consisting of 5% fetal calf serum, 1% penicillin/streptomycin, and 0.05% of estrogen receptor

The reporter (ERE)2tk-luc was a kind gift from Dr. R. Schule (Tumor Biology Center, University of Freiburg, Freiburg, Germany). The human estrogen receptor expression vector HEO and HEGO were obtained from Dr. P. Chambon's laboratory (21), and the ER-α DNA was transferred into pcDNA3 expression vector (InVitrogen, Groningen, The Netherlands). The human estrogen receptor β expression vector was made by inserting full-length human ER-β (22) into pcDNA3.

The plasmids IGF-733-CAT and IGF-375-CAT were generated by inserting the region corresponding to 733 to +55 of the IGF-I promoter I was cloned as described in Ref. 20. The reporter (ERE)tk-luc was a kind gift from Dr. R. Schule (Tumor Biology Center, University of Freiburg, Freiburg, Germany). The human estrogen receptor expression vector HEO and HEGO were obtained from Dr. P. Chambon's laboratory (21), and the ER-α DNA was transferred into pcDNA3 expression vector (InVitrogen, Groningen, The Netherlands). The human estrogen receptor β expression vector was made by inserting full-length human ER-β (22) into pcDNA3.

The plasmids IGF-733-CAT and IGF-375-CAT were generated by inserting the region corresponding to –733 to –65 (Asp-718; Psv II fragment) or the region from –375 to –65 (Xba I; Psv II fragment) in both orientations upstream of a minimal Fos promoter driving a CAT reporter (23). A C/EBP mutant IGF-375-CAT construct was generated using a Quick-change site-directed mutagenesis kit from Stratagene (La Jolla, CA). The modified oligonucleotides for the mutation of the first C/EBP site (–119, –111) were GCCCAAAAGTCTCCCTC-GAGAATCTTTGCG (upstream) and CTG GCA AAG TTA TTC TCGG (downstream) (SAL I; downstream). The oligonucleotides for the mutation of the second C/EBP site (–151, –143) were GCCAAG-GTATCATATTCCTTTGTCAGCATGG (upstream) and GCATGTTGACAAGATATGATCCTTGTC (downstream). The oligonucleotides for the mutation of the RRE site were CTCTACTCAAACTTTGGCCAGAGATCAGAGAGAAGCCAA (upstream) and TTGTTCTTCT-CTCTTTCTCTCTTGCAGATATTGAGTA (downstream). The oligonucleotides for mutation of the AP-1 site were CCCAGCAGATTTCTCCAGACTGCAGAAGTCA (upstream) and GCTGCTGTTT-TCATGGTTTGTTGCGGAAG (downstream).

To create chimeric molecules, the A/B domain, the DNA-binding domain, and the ligand-binding domain from ER-α or ER-β were polymerase chain reaction-amplified in such a way that in-frame restriction sites (Nsi I and Sal I, respectively) were introduced at the beginning and/or end of these fragments. These different fragments were cloned in pGEMT, sequenced, and used to construct chimeras by recombining them at the introduced restriction site; these chimeras have the structure A/B domain-Nsi I site-DNA-binding domain-Sal I site-ligand-binding domain. Detailed structure is available upon request.

Transient Transfection—Cells were seeded in 12-well plates at a density of 5 × 10^6 cells/cm². DNA-mediated gene transfer was performed using 2 μl of Fugene6 from Roche Molecular Biochemicals. Transfection time was 4 h, and total DNA amount was 0.5 μg consisting of the reporter plasmid (0.45 μg) and 0.05 μg of estrogen receptor α or estrogen receptor β expression vector. Cells were cultured for another 24 h in the presence of vehicle (in general, ethanol) at a final concentration of 0.01%; we used Me2SO at a final concentration of 0.01% for arzoxifene and raloxifene) or tested with hormones 17β-estradiol, 4(OH)-tamoxifen, raloxifene, or the pure antiestrogen ICI 182,780. After incubation with different hormones or antimethanes, the cells were rinsed with phosphate-buffered saline and lysed with 250 μl of the reporter lysis buffer (Promega, Madison, WI). Cell lysate was scraped out of the plates, and luminescence was measured using an automatic LB96P luminometer (Berthold, Regensdorf, Switzerland). Results were expressed in relative luminescence units/μg protein.

CAT activity was analyzed in cell extracts (prepared by three freeze-thaw cycles) using a procedure described previously (23). Samples were run for 1 h on TLC plates (Merck), and radioactivity was measured by a PhosphorImager (Molecular Dynamics). CAT activity represents the percentage of the acetylated substrate. Results are expressed as the percentage of acetylated chloramphenicol/mg protein.

RESULTS

The IGF-I Promoter Is Regulated in an ER-α-dependent Manner by Several Antiestrogens, but not by 17β-Estradiol—We compared the effects of estrogen and antiestrogens on the regulation of the human IGF-I gene. To examine the respective induction of estrogens and antiestrogens, we transfected Hep3B cells with a reporter construct containing the IGF-I promoter in front of the luciferase gene (IGF-I-luc) and co-transfected an expression vector for ER-α. We found that raloxifene, arzoxifene, and ICI 182,780, but not 4(OH)-tamoxifen, dose-dependently stimulated transcription from the IGF-I-luc reporter. By contrast, 17β-estradiol was not able to activate the IGF-I promoter (Fig. 1). However, raloxifene-induced transcription activation was partially counteracted by 17β-estradiol, confirming that the activation of transcription by raloxifene is dependent on the estrogen receptor (Fig. 2).

ER-α, but not ER-β, Mediates Antiestrogen Induction of the IGF-I Promoter—A recently identified second estrogen receptor, ER-β, has an expression pattern and functional properties that differ from those of ER-α (24). The transactivation properties of ER-β and ER-α have already been compared using reporter systems containing classical ERs and AP-1 sites (25): different ligands, such as estrogen or the antiestrogens ralox-
ifene, 4(OH)-tamoxifen, or ICI 164,384, displayed very different activity profiles, depending on both the ER subtype and the response elements. We therefore tested the effect of 4(OH)-tamoxifen, raloxifene, arzoxifene, and ICI 182,780 on transactivation of the IGF-I promoter by ER-/H9252. In this case, we could not detect activation (data not shown). However, as shown in Fig. 3, ER-/H9252 was fully functional when tested on a classical ERE reporter construct and displayed in this assay a response to estrogen that was twice as great as the one observed with ER-/H9251. In a recent study using full-length human ER-/H9252, similar results were obtained (26).

It has previously been shown that estrogen receptors α and β, which may be co-expressed in cells, are able to form functional heterodimers on DNA. We therefore compared ER-α and ER-β with their heterodimers ER-α/ER-β on an ERE-driven luciferase reporter. To do this, we performed transfections using equivalent amounts of expression vectors for ER-α and ER-β, conditions that have been shown to lead predominantly to the formation of heterodimers (27, 28). Under these experimental conditions, we found that transcription of the ERE reporter is stimulated to a level similar to that of ER-α alone (Fig. 3a). A similar experiment was done using the IGF-I promoter and confirmed that raloxifene is able to activate the IGF-I promoter through ER-α, but not through ER-β; in addition, when ER-α and ER-β expression vectors were co-transfected at equimolar levels in Hep3B cells, raloxifene induction of the IGF-I promoter was fully maintained (Fig. 3b). This may indicate that the ER domains implicated in activation of the IGF-I promoter may be different from those involved in ERE activation. It is interesting to note that ER-α and, to a lesser extent, ER-β are both expressed in Hep3B cells (data not shown).

The A/B Domain and the Ligand-binding Domain of ER-α Mediate Activation of the IGF-I Promoter—Because only ER-α was able to mediate induction of the IGF-I promoter by raloxifene, we sought to define which part of the receptor was responsible for this selective effect. To do so, we created chimeric proteins that replaced the A/B domain (bearing the transactivation function 1 (AF-1)), the DNA-binding domain, or the ligand-binding domain (bearing the activating function 2 (AF-2)) of ER-α with the corresponding region from ER-β. As shown in Fig. 4, a and b, the general pattern of activity of these chimeras was found to be qualitatively the same when
Looking at the response to the antiestrogens arzoxifene and ICI 182,780, similar but somewhat weaker results were obtained with raloxifene (data not shown). Replacement of the A/B domain of ER-α with the corresponding domain of ER-β resulted in a dramatic decrease of transactivation activity by antiestrogens. Replacement of the ligand-binding domain, which bears the AF-2 function, also led to a similar effect. In addition, exchange of both domains (βαβ) did not lead to a further impairment of activation. By contrast, the chimeric protein αβα, which has the DNA-binding domain from ER-β, displayed an activity similar to that of intact ER-α, indicating that the DNA-binding domain is not implicated in this effect. These observations suggest that both the A/B domain and the ligand-binding domain of ER-α are needed to specifically mediate antiestrogen induction of the IGF-I promoter. When tested on anERE reporter and using 17β-estradiol as an inducer (Fig. 4c), we show that only the chimeric proteins βαβ displayed an activity that is almost twice that of ER-α, as already shown with full-length ER-β (Fig. 3a). The differences in activity between ER-α and ER-β would rely not only on the A/B domain of ER-β, which displays a low homology with the A/B domain of ER-α, but also on its ligand-binding domain.

Identification of the Antiestrogen Responsive Region in the IGF-I Promoter—A response element mediating raloxifene induction has only been identified in the TGF-β3 RRE promoter, where it has been defined as a so-called RRE (16, 17). By computer analysis of the IGF-I promoter, we identified a stretch of nucleotides in the −82 to −100 region showing 90% identity to 18 of the 40 nucleotides described as RRE in the TGF-β3 promoter (Fig. 5). By analogy, we called this region IGF-I-RRE. To test whether the region encompassing the putative IGF-I RRE was responsible for the raloxifene response, we made chimeric constructs, which contained −773 to −65 (IGF-773-CAT) or −375 to −65 (IGF-375-CAT) from the IGF-I promoter sequence in front of a minimal Fos promoter. Hep3B cells were transfected with these reporter plasmids together with the ER-α expression vector, and their raloxifene response was tested. As shown in Fig. 6, raloxifene was able to activate both the longer construct (data not shown) and the shorter construct (IGF-375-CAT) from the IGF-I promoter sequence in front of a minimal Fos promoter.
compounds and the so-called pure antiestrogen ICI 182,780, but not by 4(OH)-tamoxifen and 17β-estradiol. Estrogen was able to counteract the effects of raloxifene, as is the case for the TGF-β3 promoter and the quinone reductase promoter (17, 29). This suggests a competition between these ligands for the binding domain of the estrogen receptor and confirms that these antiestrogen effects are indeed mediated by the estrogen receptor. Activation of the IGF-I promoter by antiestrogens is specifically mediated by ER-α, not by ER-β. In this case, antiestrogens act as activators of transcription. These findings illustrate that, depending on the promoter context and the ligand used, ER-α and ER-β can regulate gene activity differentially. Interestingly, and in agreement with our observations, in a recent in vivo study (30) it was shown that antiestrogens are able to increase IGF-I mRNA levels in the mouse uterus and that ER-β does not play a role in mediating this activation. Furthermore, by using chimeras between ER-α and ER-β, we found that both AF-1 and AF-2 of ER-α are required to mediate activation of the IGF-I promoter by antiestrogens.

Within the IGF-I promoter, several potential binding sites were examined for their contribution to activation by ER-α in response to antiestrogens such as raloxifene. By mutation analysis, we showed that both C/EBP sites have a major contribution to the raloxifene inducibility of the IGF-I promoter. C/EBPs are transcriptional regulators that are highly expressed in liver and have previously been shown to be involved in ER-mediated promoter activation (31–33). C/EBP-β has been shown to be necessary for ER-α mediated down-regulation of the interleukin 6 promoter and to cooperate with a nuclear factor xB site (32). Stein and Yang (31) demonstrated a direct in vitro interaction between a glutathione S-transferase-ER-α fusion protein and C/EBPβ. Furthermore, both ER-α and C/EBPα are required for maximal activation of the promoter of the very low density apolipoprotein gene promoter by estrogen (33). However, although it is known that ER-α and C/EBP can interact and form a complex in solution, the demonstration of complex formation between these two proteins has not been done by gel shift assay in any of the reports cited above. In line with this, we failed to observe the formation of a C/EBP-ER complex on the IGF-I C/EBP sites. It is worth mentioning that a similar situation has been observed for the glucocorticoid receptor, which was also shown to interact in solution with C/EBP but could not be demonstrated to do so by gel shift assay (34). In addition, using extracts from transfected cells that were treated or not treated with ER agonists or antagonists, we did not observe any alteration in the affinity of interaction between C/EBP and the corresponding C/EBP sites by hand shift analysis (data not shown). Mutation of a sequence in the IGF-I promoter that has a strong homology to the TGF-β3-RRE had no deleterious effect. Similarly, mutation of an AP-1-like site, another potentially important site for ER-mediated transcription, did not affect activation of the IGF-I promoter by ER-α.

Regulation of the IGF-I gene by estrogen has already been described in different tissues or cell types. Previous studies have shown that estradiol treatment increases IGF-I mRNA in the uterus of ovariectomized rats (35) and in osteoblastic cells (4). Here we found that 17β-estradiol had no positive effect on IGF-I transcription in Hep3B cells; on the other hand, antiestrogens such as raloxifene led to an increase in IGF-I transcription. In contrast, the chicken IGF-I promoter was found to be induced by estrogen in another hepatocyte cell line, HepG2 (19). Our observations are consistent with recent clinical studies showing that tamoxifen and 17β-estradiol decrease the circulating IGF-I levels in women (36, 37) and with the fact that the predominant source of circulating IGF-I is the liver.
Our findings might also partially explain the bone-protective effect of raloxifene or raloxifene derivatives such as arzoxifene. A recent study (38) showed that a new SERM, MDL 103,323, increases the circulating level of IGF-I and postulates that the increase in IGF-I induced by MDL 103,323 might contribute to its favorable effects on bone strength because an increase in bone density has been observed. Thus, an increase in IGF-I transcription might represent a general mode of action of antiestrogens, except for those of the tamoxifen class, which display agonistic activity in bone in vivo. Finally, a recent clinical study from Garnero et al. (39) established a clear link between a decrease in IGF-I serum concentration and an increased risk of osteoporotic fractures independent of bone mineral density, supporting the possible relevance of the IGF-I increase for the prediction of the effects of SERMs on bone metabolism.

In conclusion, in this study we propose a new mechanism of action of SERMs that positively regulate the IGF-I gene through ER-α. These findings might have useful implications for the monitoring of SERM activity in osteoporotic patients.

Acknowledgments—We thank J. Wirsching for excellent technical help and H. J. Keller for the kind gift of ER-β plasmid. We thank R. Gamse for careful reading of the manuscript and helpful discussion.

REFERENCES

1. Rosen, C. J., Donahue, L. R., and Hunter, S. J. (1994) Proc. Soc. Exp. Biol. Med. 206, 83–102
2. McCarthy, T. L., Centrella, M., and Canalis, E. (1989) Endocrinology 124, 1247–1255
3. McCarthy, T. L., Centrella, M., Raisz, L. G., and Canalis, E. (1991) Endocrinology 128, 2895–2900
4. Ernst, M., Heath, J. K., and Rodan, G. A. (1989) Endocrinology 125, 825–833
5. Kassem, M., Okazaki, R., Harris, S. A., Spelsberg, T. C., Conover, C. A., and Riggs, B. L. (1998) Calcif. Tissue Int. 62, 60–66
6. Rotwein, P. (1991) Growth Factors 3, 3–18
7. Lamberts, S. W. J., van den Beld, A. W., and Van der Lely, A.-J. (1997) Science 278, 419–424
8. Pfeilschifter, J., and Ziegler, R. (1998) Eur. J. Endocrinol. 138, 617–618
9. Katzenellenbogen, J. A., O'Malley, B. W., and Katzenellenbogen, B. S. (1996) Mol. Endocrinol. 10, 119–131
10. McDonnell, D. P., Clemm, D. L., Herrmann, T., Goldman, M. E., and Pike, J. W. (1995) Mol. Endocrinol. 9, 659–669
11. Brazowski, A. M., Pike, A. C. W., Dauter, Z., Hubbard, R. E., Bonn, T., Engstrom, O., Ohman, L., Greene, G. L., Gustafsson, J.-A., and Carlquist, M. (1997) Nature 389, 753–758
12. Grese, T. A., Sluka, J. P., Bryant, H. U., Cullinan, G. J., Glasebrook, A. L., Jones, C. D., Matsumoto, K., Falkowitz, A. D., Sato, M., Termine, J. D., Winter, M. A., Yang, N. N., and Dodge, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 14105–14110
13. Kuiper, G. G. J. M., Enmark, E., Pelto-Huikko, M., Nilsson, S., and Gustafsson, J.-A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5280–5283
14. Barkheim, T., Carlsson, B., Nilsson, Y., Enmark, E., Gustafsson, J.-A., and Nilsson, S. (1998) Mol. Pharmacol. 54, 105–112
15. Mitlak, B. H., and Cohen, F. H. (1997) Horm. Res. (Basel) 48, 155–163
16. Yang, N. N., Venugopalman, M., Hardikar, S., and Glasebrook, A. (1996) Science 273, 1222–1225
17. Yang, N. N., Venugopalman, M., Hardikar, S., and Glasebrook, A. (1997) Science 275, 1249
18. Jansen, E., Steenbergh, P. H., van Schaik, F. M. A., and Sussenbach, J. S. (1992) Biochem. Biophys. Res. Commun. 187, 1219–1226
19. Umayahara, Y., Kawamori, R., Watada, H., Imano, E., Iwama, N., Morishima, T., Yamasaki, Y., Kojimoto, Y., and Kamada, T. (1994) J. Biol. Chem. 269, 16433–16442
20. Nolten, L. A., van Schaik, F. M. A., Steenbergh, P. H., and Sussenbach, J. S. (1994) Mol. Endocrinol. 8, 1636–1645
21. Tora, L., White, J., Iren, C., Tasset, D., Webster, N., Scheer, E., and Chambon, P. (1989) Cell 59, 477–487
22. Ogawa, S., Inoue, S., Watanabe, T., Hiroi, H., Orimo, A., Hosoi, T., Ouchi, Y., and Muramatsu, M. (1998) Biochem. Biophys. Res. Commun. 245, 122–126
23. Pierce, J. W., Lenardo, M. J., and Baltimore, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1482–1486
24. Enmark, E., Pelto-Huikko, M., Grandien, K., Lagercrantz, S., Lagercrantz, J., Fried, G., Nordenstjolj, M., and Gustafsson, J.-A. (1997) J. Clin. Endocrinol. Metab. 82, 4258–4265
25. Paeche, K., Webb, P., Kuiper, G. G. J. M., Nilsson, S., Gustafsson, J.-A., Kushner, P. J., and Scanlan, T. S. (1997) Science 277, 1508–1510
26. Jones, P. S., Parrott, E., and White, I. N. H. (1999) J. Biol. Chem. 274, 32008–32014
27. Cowley, S. M., Hoare, S., Mosselman, S., and Parker, M. G. (1997) J. Biol. Chem. 272, 19858–19862
28. Pettersson, K., Grandien, K., Kuiper, G. G. J. M., and Gustafsson, J.-A. (1997) Mol. Endocrinol. 11, 1486–1496
29. Montano, M. M., and Katzenellenbogen, B. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2581–2586
30. Klotz, D. M., Curtis Hewitt, S., Korach, K. S., and Diaugustine, R. P. (2000) Endocrinology 141, 3430–3439
31. Stein, B., and Yang, M. X. (1995) Mol. Cell. Biol. 15, 4971–4979
32. Galien, R., Evans, H. F., and Garcia, T. (1996) Mol. Endocrinol. 10, 713–722
33. Calkhoven, C. F., Snippe, L., and Ab, G. (1997) Eur. J. Biochem. 249, 113–120
34. Boruk, M., Savory, J. G. A., and Hache, R. J. G. (1998) Mol. Endocrinol. 12, 1749–1763
35. Murphy, L. J., Murphy, L. C., and Friesen, H. G. (1987) Mol. Endocrinol. 120, 1882–1888
36. Deenstra, A., Robertson, C., Ballardini, B., Paggi, D., Guerrerri-Gonzaga, A., Bonanni, B., Manetti, L., Johansson, H., Barreca, A., Bettega, D., and Costa, A. (1999) Eur. J. Cancer 35, 596–600
37. Cuppari, E., Harmann, M., and Blackman, M. R. (1998) Endocrinol. Rev. 19, 20–39
38. Ammann, P., Bourrin, S., Bonjour, J.-P., Brunner, F., Meyer, J.-M., and Rizzoli, R (1999) Osteoporosis Int. 10, 369–376
39. Garnero, P., Sornay-Rendu, E., and Delmas, P. D. (2000) Lancet 355, 888–889
40. Shibonga, J. D., Dobign, H., Harden, R. M., and Turner, R. T. (1998) Endoer- nology 139, 3736–3742