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Divergent Regulation of the *Osteopontin* Promoter by the Estrogen Receptor-Related Receptors Is Isoform- and Cell Context Dependent

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ABSTRACT

We sought to determine whether the estrogen receptor-related receptor gamma (mEsrrg) regulated the *Osteopontin* (*Opn*) promoter through the same AP1/CAAT box element that we have previously described for mEsrra. In HeLa cells mEsrrg used an additional site present in the 5′UTR, while in ROS17/2.8 cells the AP1/CAAT site was not used, but a completely novel site surrounding the transcription start site was used. We also find that in ROS17/2.8 cells mEsrra repressed, while mEsrrg activated the *Opn* promoter. None of the sites identified conform to established Esrr response elements (ERREs). Additionally, the two reported mEsrrg protein isoforms showed differences in their activation potential. Mutations in the activation function 2 (AF2) of mEsrra, predicted to abolish activation, surprisingly turned mEsrra into a better activator. In contrast, similar AF2 mutations in Esrrg2 abolished its ability to activate the *Opn* promoter. Mutation of the DNA binding domain of mEsrra/g2 abolished transcriptional activity in HeLa and ROS17/2.8 cells. Our data indicate, first, that the two Esrr isoforms regulate *Opn* in a cell context-dependent manner. Second, they suggest that although the DNA binding domains of mEsrra and mEsrrg are 93% identical and required for regulation, the receptors bind to distinct *Opn* promoter elements, suggesting that the two isoforms may co-regulate *Opn*, and perhaps other genes, without competing for the same site in the promoter. Finally, the results suggest that each isoform interacts differently with co-activators and co-repressors, as highlighted by the AF2 mutation that turns mEsrra into a better activator but abolishes activity of Esrrg2. J. Cell. Biochem. 114: 2356–2362, 2013.

KEY WORDS: Esrr; NR3B; GENE REGULATION; ERRE; OSTEOPONTIN; OSTEOBLAST

Nuclear receptors (NRs) are arranged from the N- to the C-terminus into five functional domains: the A/B domain contains the ligand-independent activation function 1 (AF1), a highly conserved DNA binding domain (DBD, domain C) composed of two zinc fingers with essential cysteines coordinating the zinc ions, a short linker region (domain D) followed by the ligand binding domain (E or hormone binding domain (HBD)), containing the ligand-dependent AF2 function. Binding of ligand induces a conformational change in the HBD that repositions helix 12 and displaces co-repressors, allowing for co-activators to bind leading to maximal transcriptional activation [Bain et al., 2007].

Bone homeostasis is regulated by several hormones, for example estrogen, loss of which is thought to be the primary cause of bone loss in postmenopausal osteoporosis [Riggs et al., 2002; Pacifici, 2008]. Estrogen binds to two receptors, estrogen receptor alpha (Esra, NR3A1) and Esrb (NR3A2) [Nuclear Receptors Nomenclature Committee, 1999], which can form homo- or heterodimers that regulate target genes synergistically or antagonistically depending on the promoter context [Hall and McDonnell, 1999]. A novel family of NRs was discovered when the DBD of Esra was used as a probe to screen expression libraries [Giguere et al., 1988]. The estrogen receptor-related receptor (Esrr) family is composed of three genes, Esrra (NR3B1), Esrrb (NR3B2), and Esrrg (NR3B3), which share a high degree of similarity to the Esrs with at least 68% identity in their DBD [Giguere, 2002]. However, the Esrr proteins are unable to bind estrogen due to a small ligand binding pocket and are still considered orphan receptors as no natural ligand has been found, although several synthetic compounds can inhibit the constitutive basal activity of the Esrrs, that is, act as inverse agonists [Giguere, 2002; Horard and Vanacker, 2003; Hyatt et al., 2007]. The crystal structures

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of the unliganded HDB of Esrra and Esrrg have been solved with helix 12 occupying the activated position explaining their constitutive activity, while crystal structures with inverse agonist bound show helix 12 adopting a conformation reminiscent of unliganded NRs [Greschik et al., 2002; Kallen et al., 2004; Wang et al., 2006; Kallen et al., 2007]. The Esr and Esrr proteins can physically bind to each other [Yang et al., 1996] and Esra can transcriptionally regulate Esrra [Shigeta et al., 1997; Bonnelye et al., 2002]. The Esrs bind to estrogen response elements (ERE) as either hetero- or homodimers to an inverted repeat separated by three nucleotides (IR3) with the consensus AGGTCAnnnTGACCT, while the Esrrs bind to ERREs (TCAAGGTCA) either as monomers or dimers [Giguere, 2002; Barry et al., 2006]. The close similarity in binding sites helps to explain why these two families of NRs can regulate many of the same genes [Vanacker et al., 1999; Kraus et al., 2002]. Esrrg has been shown to have a very broad binding specificity, being able to bind both inverted and direct repeat sequences with various spacing [Razzaque et al., 2004]. Two protein isoforms for Esrrg have been described differing by 29 amino acids at the N-terminus [Hong et al., 1999; Susens et al., 2000]. The additional amino acids in Esrrg2 contain a phosphorylation dependant sumoylation site, which affects the transcriptional activity of the protein [Tremblay et al., 2008; Hentschke et al., 2009]. Esrrg has recently also been shown to be able to regulate the Esrra promoter, adding another layer of complexity [Zhang and Teng, 2007].

Our lab has shown that Esrra regulates bone homeostasis in in vitro models of both cartilage and bone differentiation [reviewed in Bonnelye and Aubin, 2013]. Overexpression of Esrra leads to an increase in cartilage and bone formation, while antisense knockdown of Esrra leads to a reduction in bone formation [Bonnelye et al., 2001; Bonnelye and Aubin, 2005; Bonnelye et al., 2007]. Intriguingly, the Esrrs are more highly expressed in bone compared to the Esrs [Bonnelye and Aubin, 2002], suggesting that perhaps some of the effects of estrogen on bone might be mediated by the Esrrs and help to explain the relatively mild phenotype observed in Esr knockout mice [Couse and Korach, 1999]. In our analysis of genes that are regulated by Esrra in either the cartilage or bone differentiation models, we found that the chondrocyte master regulator *Sox9* is positively regulated as well as the gene for *Osteopontin* (*Opn*) [Bonnelye et al., 2001, 2007].

Opn is a secreted phosphoprotein found in bone matrix where it plays multiple roles, for example serving as a mineralization inhibitor [Boskey et al., 1993; Hunter et al., 1994] and a positive regulator of osteoclast activity [Tanabe et al., 2011]. Opn has previously been shown to be regulated by Esrra either positively via an ERRE (S1) [Vanacker et al., 1998; Bonnelye et al., 2001] or negatively by interfering with another orphan receptor Nurr1 bound to the same S1 ERRE in osteoblastic cell lines [Lammi et al., 2004]. Esrrg was also shown to negatively regulate *Opn* transcription by interfering with Nurr1 [Lammi et al., 2004]. Suppression of several bone specific genes by Esrrg can also occur by its ability to repress the activity of Runx2 [Jeong et al., 2009]. We recently demonstrated that Esrra represses *Opn* via a non-canonical site that is a composite inverted CAAT/AP1 site in osteoblastic cells [Zirngibl et al., 2008]. Having identified this non-canonical site for Esrra, we wanted to determine whether Esrrg similarly regulates *Opn*.

MATERIALS AND METHODS

PLASMIDS

The mEsrra and m*Opn* promoter constructs have been described previously [Zirngibl et al., 2008]. The m*Esrrg2* open reading frame, coding the for the N-terminally longer 458 amino acid Esrrg2 protein, was amplified using RT-PCR from mouse muscle cDNA and cloned into pcDIN to make pcDINmEsrrg2. Point mutations and deletions of mEsrrg2 were made using standard PCR approaches with pcDINmEsrrg2 as the starting plasmid and oligonucleotides that incorporated restriction sites for cloning and mutation identification [Ausubel et al., 1987]. pcDINmEsrrg1 was constructed by cloning the *Xmn*I–*Hin*dIII fragment into pcDIN. Oligonucleotide sequences used in making the plasmids are available upon request. All plasmids were sequence verified.

CELL CULTURE AND TRANSIENT TRANSFECTION

Cell lines and transfections were done as previously described [Zirngibl et al., 2008]. Briefly, ROS17/2.8 cells were grown in αMEM with 10% fetal bovine serum (FBS) and HeLa cells were grown in DMEM high glucose (Sigma) with 10% FBS in a humidified 5% CO_2 atmosphere at 37°C. Cells were plated the day prior to transfecting them with the indicated plasmids using Lipofectamine 2000 (Invitrogen) or PolyJet (FroggaBio). Roughly 48 h after transfection, luciferase activity was measured using the dual luciferase kit (Promega) and activity was corrected for transfection efficiency using the pRLtk plasmid (Promega).

RESULTS

mEsrrg1/2 AND mEsrra DIFFERENTIALLY REGULATE THE *OPN* PROMOTER

To determine whether mouse Esrrg (mEsrrg) regulates the m*Opn* promoter similarly to mEsrra, we tested a series of promoter constructs we made previously; these included the ERREs S1 to S6 identified by Vanacker et al. [1998] and the novel composite CAAT/AP1 site at −56 to −48 bp that we had identified as the element for mEsrra [Zirngibl et al., 2008] (Fig. 1). mEsrrg activated the m*Opn* promoter in HeLa cells five to 25-fold depending on the promoter context and the activation is independent of the previously identified ERREs (Fig. 1B). Additionally we found that the longer Esrrg2 isoform was a better activator than the smaller Esrrg1 on all the promoter constructs tested in HeLa cells. Consistent with several reports on other promoters [Lu et al., 2001; Zhang et al., 2006], mEsrrg is a much better activator than mEsrra, as mEsrra activated the m*Opn* promoter only twofold under the same conditions (Fig. 1B). Whereas mEsrra did not activate the −24 to +78 bp m*Opn* promoter fragment (Fig. 1B), mEsrrg1/2 did, suggesting that mEsrrg1/2 uses a completely novel site independent of or in addition to the CAAT/AP1 site. The very low basal activity of the −24 to +78 bp m*Opn* promoter fragment is most likely due to the fact that the CAAT and TATA box have been removed. Notably, when we tested the same m*Opn* promoter constructs in the osteoblastic ROS17/2.8 cell line, we found that mEsrrg2 activated the promoter 2- to 15-fold depending on the promoter composition, while mEsrra repressed the same promoter

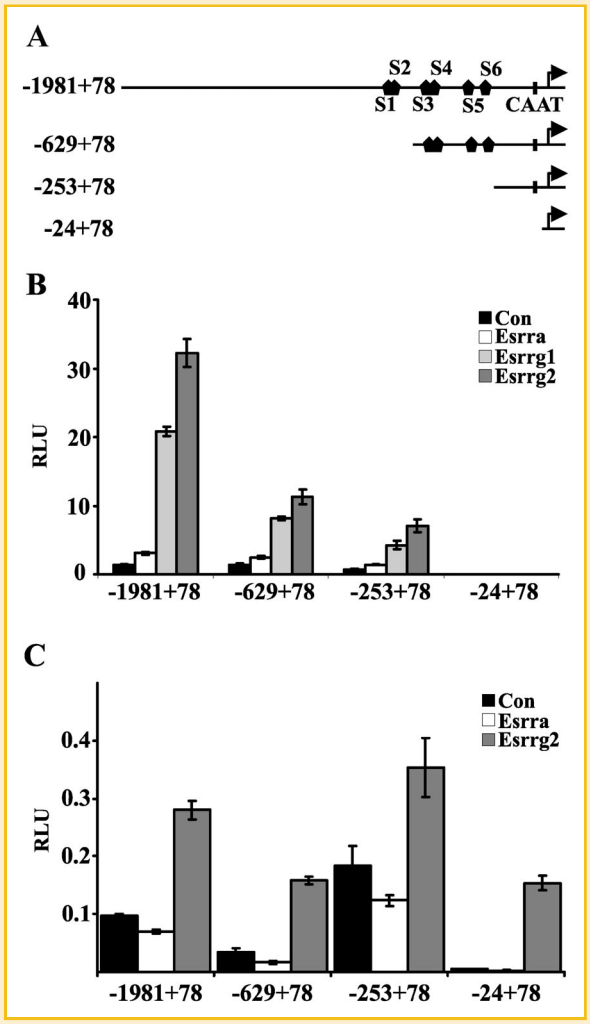

Fig. 1. Esrra and Esrrg regulate m*Opn* divergently. A: Schematic of the m*Opn* promoter constructs used and the location of the previously defined Esrr binding sites S1 to S6 [Vanacker et al., 1998] as well as the composite CAAT/AP1 site [Zirngibl et al., 2008]. B: Activation of m*Opn* expression in HeLa cells points to divergent use of ERREs. HeLa cells (70,000 cells/well) were transfected with 100 ng of m*Opn* promoter construct and 300 ng of mEsrr expression plasmids. C: Opposing regulation and differential use of ERRE in ROS17/2.8 cells by mEsrra and mEsrrg2. ROS17/2.8 cells (20,000 cells/well) were transfected with 50 ng of m*Opn* promoter construct and 400 ng of mEsrr expression plasmid. Transfection efficiency was normalized to internal control and is expressed as relative light units (RLU). The mean ± standard deviation from triplicate wells from a representative experiment is shown.

fragments as we had demonstrated previously [Fig. 1C; Zirngibl et al., 2008]. In contrast to mEsrra, which lacked activity on the −24 to +78 bp promoter, mEsrrg2 caused a robust 15-fold activation (Fig. 1C), again suggesting that mEsrrg2 uses a site distinct from mEsrra.

mEsrrg TRANSACTIVATES IN A CELL CONTEXT-DEPENDENT MANNER AND VIA NON-CANONICAL ERREs

The previous analysis suggested that mEsrrg uses a novel ERRE present in the −24 to +78 bp region of the promoter, either alone or together with the previously identified site used by mEsrra. To test these possibilities, we transfected HeLa cells with the −56 to +9 bp construct (Fig. 2A, wt) together with mEsrrg1/2, which resulted in robust two- to threefold activation of the promoter (Fig. 2B). Manual inspection of the −56 to +9 bp sequence revealed a low homology ERRE1 (5/9 bases) that we mutated (Fig. 2A, m1), which did not abolish activation by mEsrrg1/2 in HeLa cells, pointing to a different site being used. No other ERRE-like sequences are present in this minimal promoter fragment and so adenine scanning mutants were made to identify the site used by mEsrrg (Fig. 2A). While the basal activity of the 6A1 and 6A2 mutants was reduced, Esrrg1/2 were able to activate these reporters between 25% and 70% above basal levels, while the 6A3 and 6A4 mutants showed a two- to threefold induction similar to the wt promoter fragment (Fig. 2B). This suggests that mEsrrg1/2 can work through the composite CAAT/AP1 site in HeLa cells that we had identified previously for mEsrra and is also consistent with there being another site that Esrrg can use.

We similarly tested the same m*Opn* promoter fragments in ROS17/2.8 cells and found that mEsrrg2 activated the wt and m1 constructs twofold, even though the basal activity of the m1 fragment increased (Fig. 2C). To our surprise, all of our adenine scanning mutants were activated by mEsrrg2, indicating that in ROS17/2.8 cells mEsrrg2 uses a novel site altogether (Fig. 2C). As we had observed in our analysis of mEsrra regulation of m*Opn* in ROS17/2.8 cells and in contrast to what we observed in HeLa cells, the basal activity of the 6A1 and 6A2 mutants was lower, suggesting that the CAAT box is important in osteoblastic cells.

THE AF2 AND DNA BINDING DOMAINS ARE REQUIRED FOR PROPER mEsrrg2 FUNCTION

To determine which functional domains are required for the observed activation/repression, we first made deletions in both mEsrra and mEsrrg2 that remove helix 12 (mAF2) and should render them inactive (Fig. 3A). While both wild-type receptors were capable of activating the m*Opn* promoter in HeLa cells (Fig. 3B), mEsrra (mAF2) was a better activator than its wild-type (wt) counterpart, while the similar mutation in mEsrrg2 (mAF2) lead to loss of activity. A larger deletion that effectively removes both helix 11 and 12 of mEsrra (ΔC392, Fig. 3A) led to residual activity that varied between that of wild type and background levels depending on the size of the promoter fragment (Fig. 3B, and data not shown). mEsrrg2 lacking helix 11 and 12 (Δ430–456, Fig. 3A) failed to activate the m*Opn* promoter and, in some promoter contexts, functioned as a dominant negative molecule (Fig. 3B, and data not shown). In ROS17/2.8 cells, both wt and mutant forms of mEsrra repressed the m*Opn* promoter (Fig. 3C), indicating that the repression in this cell type is not determined by an intact helix 11 and 12. On the other hand, mEsrrg2 activated the m*Opn* promoter in ROS17/2.8, and both carboxy terminal deletions resulted in markedly reduced promoter activity, indicating a potential dominant negative role (Fig. 3C).

To determine the role of DNA binding for the promoter activation by mEsrra and mEsrrg2, we mutated the conserved cysteine in the first zinc finger, which had previously been shown to abolish DNA binding for mEsrrg [Huppunen et al., 2004]. Neither mEsrraC99G nor mEsrrg2C148G activated the m*Opn* promoter in HeLa (Fig. 3D,E) or

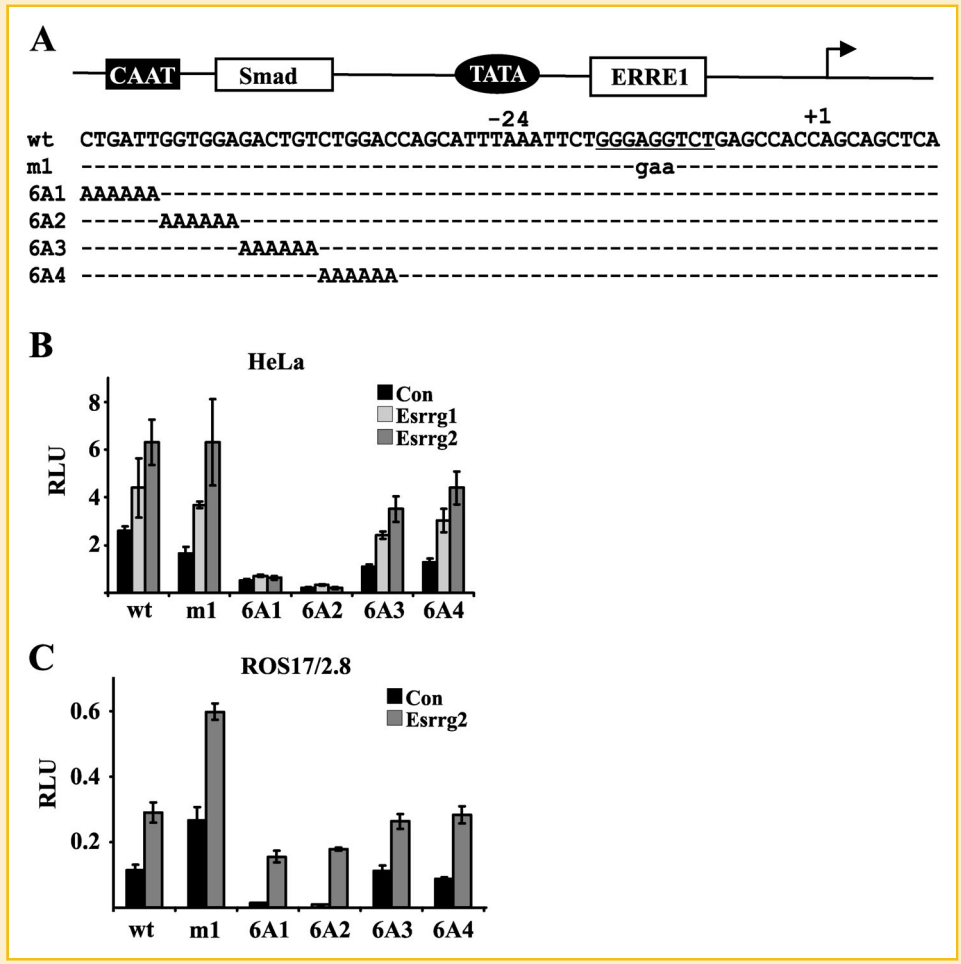

Fig. 2. Defining the non-canonical ERRE used by mEsrrg2. A: Schematic of the mutations used in the proximal m*Opn* promoter to identify the sites used. B: In HeLa cells mEsrrg uses the CAAT/AP1 site and an additional site in m*Opn* to activate transcription. HeLa cells (70,000 cells/well) were transfected with 100 ng of m*Opn* promoter construct and 300 ng of mEsrr expression plasmid. C: mEsrrg2 uses a novel site between −32 and +9 bp to activate expression in ROS17/2.8 cells. ROS17/2.8 cells (20,000 cells/well) were transfected with 50 ng of m*Opn* promoter construct and 400 ng of mEsrr expression plasmid. Transfection efficiency was normalized to internal control and is expressed as relative light units (RLU). The mean ± standard deviation from triplicate wells from a representative experiment is shown.

ROS17/2.8 cells (data not shown) indicating that a functional DBD is required for the activities that we observe.

DISCUSSION

We describe the differential use of non-canonical response elements by the highly related mEsrra and mEsrrg on the m*Opn* promoter. Whereas mEsrra regulates m*Opn* through a composite CAAT/AP1 site in both HeLa and ROS17/2.8 cells, mEsrrg regulates the m*Opn* promoter via the CAAT/AP1 plus an additional site in m*Opn* in HeLa cells (Fig. 2B) and a completely distinct site in ROS17/2.8 cells (Fig. 2C). These data are interesting because the DBD of mEsrra and mEsrrg are identical at 61/66 (92%) amino acid positions (with only 2/5 non-conserved amino acid changes) and we predicted that these two NRs would regulate m*Opn* through the same site. This suggests that promoter composition plays an important part in determining binding of NRs and not just the presence or absence of a binding site.

It is possible that the regulation of m*Opn* by the Esrrs is more influenced by other factors bound to the m*Opn* promoter as binding of the Esrrs is through non-canonical sites. The repression by Esrra or the activation by mEsrrg2 of the m*Opn* promoter does not appear to be mediated by Runx2, as the Runx2 binding site at −130 bp [Sato et al., 1998] is removed in the −56 to +9 bp promoters that we used [Fig. 2; Zirngibl et al., 2008]. We also observed that the activity of mEsrrg2 was higher than that of mEsrrg1 which was unexpected as the sumoylated form of Esrrg2 has been shown to act as a repressor of transcription [Tremblay et al., 2008; Hentschke et al., 2009]. However, in these reported studies the activity was never directly compared between Esrrg1 and Esrrg2. There could be a difference in the cofactors that are bound by the two different Esrrg isoforms or the fact that fact that the Esrrs bind to the m*Opn* promoter in a non-canonical ERRE as we have demonstrated. It is also possible that a non-sumoylated form of Esrrg2 binds to a non-canonical site. Further studies need to be done to resolve the differential activity observed between Esrrg1/2.

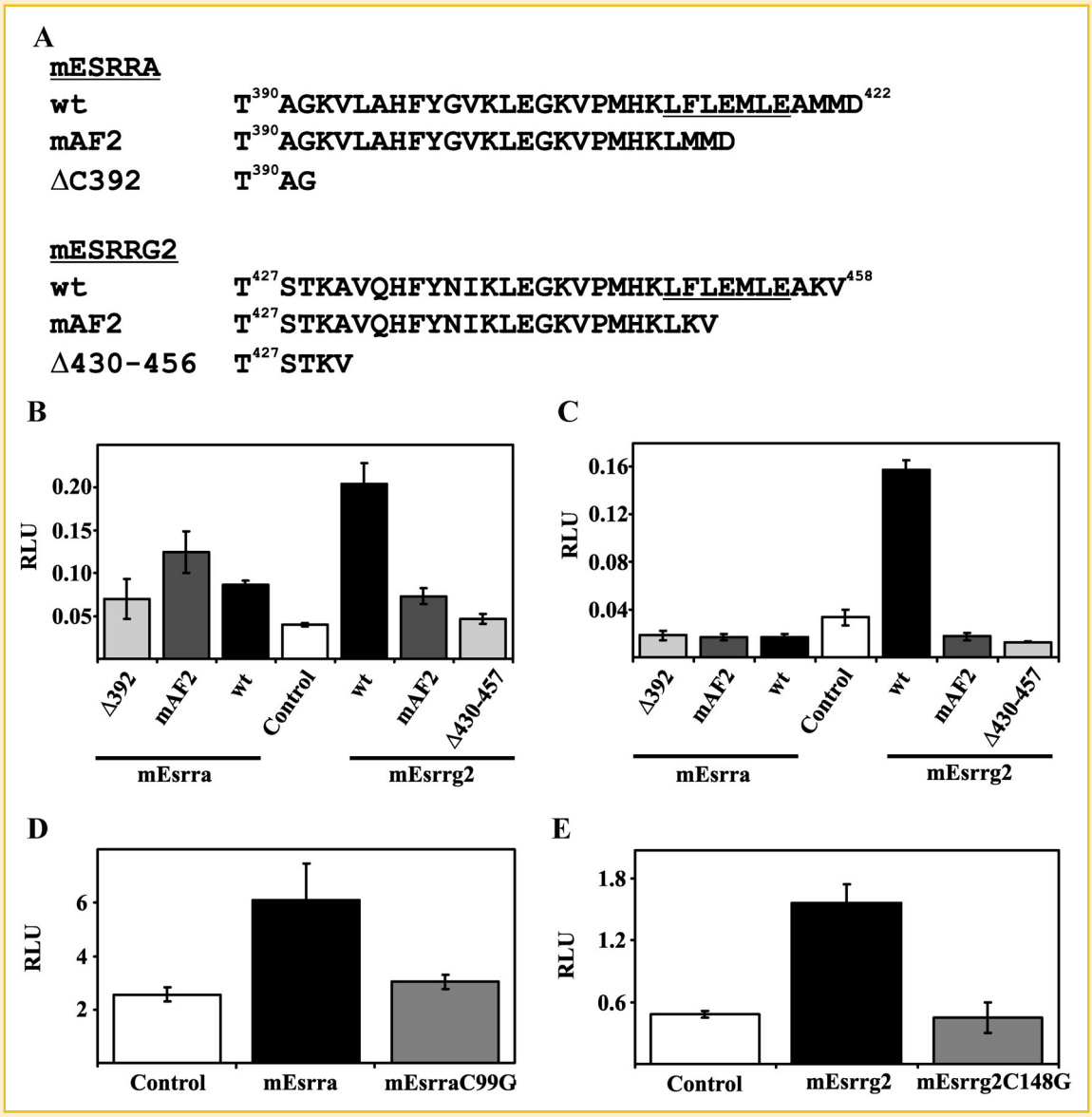

Fig. 3. A: Sequence of the carboxy terminal end of mEsrra and mEsrrg2 and indicated deletions used to test the AF2 requirement. Helix 12, which forms part of the AF2 binding surface, is underlined. B: Removal of the AF2 domain in mEsrra and mEsrrg2 reveals opposing functional consequences. HeLa cells (40,000 cells/well) (B) or ROS17/2.8 cells (20,000 cells/well) (C) were transfected with 50 ng of the −629 to +78 bp m*Opn* promoter and 400 ng of mEsrr expression plasmid. DNA binding is required for the regulation of the m*Opn* promoter by mEsrra (D) and mEsrrg2 (E) in HeLa cells. HeLa cells (40,000 cells/well) were transfected with 200 ng of the −253 to +78 bp m*Opn* promoter and 250 ng of expression plasmid in (D) or 150 ng of the −253 to +78 bp m*Opn* promoter and 150 ng of expression plasmid in (E). Transfection efficiency was normalized to internal control and is expressed as relative light units (RLU). The mean ± standard deviation from triplicate wells from a representative experiment is shown.

The second finding is that the Esrrs regulate the same promoter in a cell context-dependant manner, with Esrra repressing and Esrrg2 activating the m*Opn* promoter in ROS17/2.8 cells. At present we do not know whether these two opposing activities can compete with each other as we have not done titration experiments using both Esrrs in the same cell line. This competition, at least in ROS17/2.8 cells, would not involve interference for binding to the same site, but rather recruitment of cofactors to the promoter. That the two Esrrs appear to use different cofactors or at least bind them in a different manner is supported by our mutations involving the AF2 region. While removal of the AF2 region in Esrra turns it into a better activator, similar mutations in Esrrg2 severely reduce or even turn it into a repressor (Fig. 3B,C). We, as well as others, found that Esrrg2 is a much better activator when similar amounts of plasmid are transfected (Fig. 1B), again arguing for differential use of cofactors. We do not believe that our results are due to squelching of cofactors, as we have done titration experiments which show similar results (data not shown). This also means that by just looking at RNA expression levels of the two Esrrs one cannot easily determine what will be the transcriptional outcome. We have demonstrated that Esrra RNA is expressed at high

levels in bone, at much higher levels than the Esrs [Bonnelye and Aubin, 2002], while Esrrg is expressed at comparable levels to the Esrs (data not shown). Another possibility that needs to be considered is that Esrra and Esrrg can physically interact and repress each others activity [Huppunen and Aarnisalo, 2004] and Esrrg can regulate Esrra transcription [Zhang and Teng, 2007]. It is not yet known whether a reciprocal regulatory loop exists for Esrrg. In any case, this complex auto-regulatory loop could have implications for transcriptional assays as one generally expresses the receptor at much higher levels than normally found inside the cell, thus potentially disrupting a normal equilibrium. The physical interaction of Esrra and Esrrg, and the ability of the resulting heterodimer to repress is reminiscent of the Esra and Esrb story [Hall and McDonnell, 1999] and thus may make the Esrrs more related to the Esrs than previously appreciated.

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REFERENCES

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. 1987. Current protocols in molecular biology. In: Chanda VB, editor. Current protocols. New York: John Wiley and Sons, Inc.

Bain DL, Heneghan AF, Connaghan-Jones KD, Miura MT. 2007. Nuclear receptor structure: Implications for function. Annu Rev Physiol 69:201–220.

Barry JB, Laganiere J, Giguere V. 2006. A single nucleotide in an estrogen-related receptor alpha site can dictate mode of binding and peroxisome proliferator-activated receptor gamma coactivator 1alpha activation of target promoters. Mol Endocrinol 20:302–310.

Bonnelye E, Aubin JE. 2002. Differential expression of estrogen receptor-related receptor alpha and estrogen receptors alpha and beta in osteoblasts in vivo and in vitro. J Bone Miner Res 17:1392–1400.

Bonnelye E, Aubin JE. 2005. Estrogen receptor-related receptor alpha: A mediator of estrogen response in bone. J Clin Endocrinol Metab 90:3115–3121.

Bonnelye E, Aubin JE. 2013. An energetic orphan in an endocrine tissue: A revised perspective of the function of estrogen receptor-related receptor alpha in bone and cartilage. J Bone Miner Res 28:225–233.

Bonnelye E, Merdad L, Kung V, Aubin JE. 2001. The orphan nuclear estrogen receptor-related receptor alpha (ERRalpha) is expressed throughout osteoblast differentiation and regulates bone formation in vitro. J Cell Biol 153:971–984.

Bonnelye E, Kung V, Laplace C, Galson DL, Aubin JE. 2002. Estrogen receptor-related receptor alpha impinges on the estrogen axis in bone: Potential function in osteoporosis. Endocrinology 143:3658–3670.

Bonnelye E, Zirngibl RA, Jurdic P, Aubin JE. 2007. The orphan nuclear estrogen receptor-related receptor-alpha regulates cartilage formation in vitro: Implication of Sox9. Endocrinology 148:1195–1205.

Boskey AL, Maresca M, Ullrich W, Doty SB, Butler WT, Prince CW. 1993. Osteopontin-hydroxyapatite interactions in vitro: Inhibition of hydroxyapatite formation and growth in a gelatin-gel. Bone Miner 22:147–159.

Couse JF, Korach KS. 1999. Estrogen receptor null mice: What have we learned and where will they lead us? Endocr Rev 20:358–417.

Giguere V. 2002. To ERR in the estrogen pathway. Trends Endocrinol Metab 13:220–225.

Giguere V, Yang N, Segui P, Evans RM. 1988. Identification of a new class of steroid hormone receptors. Nature 331:91–94.

Greschik H, Wurtz JM, Sanglier S, Bourguet W, van Dorsselaer A, Moras D, Renaud JP. 2002. Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3. Mol Cell 9:303–313.

Hall JM, McDonnell DP. 1999. The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 140:5566–5578.

Hentschke M, Susens U, Borgmeyer U. 2009. Transcriptional ERRgamma2-mediated activation is regulated by sentrin-specific proteases. Biochem J 419:167–176.

Hong H, Yang L, Stallcup MR. 1999. Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3. J Biol Chem 274:22618–22626.

Horard B, Vanacker JM. 2003. Estrogen receptor-related receptors: Orphan receptors desperately seeking a ligand. J Mol Endocrinol 31:349–357.

Hunter GK, Kyle CL, Goldberg HA. 1994. Modulation of crystal formation by bone phosphoproteins: Structural specificity of the osteopontin-mediated inhibition of hydroxyapatite formation. Biochem J 300(Pt3):723–728.

Huppunen J, Aarnisalo P. 2004. Dimerization modulates the activity of the orphan nuclear receptor ERRgamma. Biochem Biophys Res Commun 314: 964–970.

Huppunen J, Wohlfahrt G, Aarnisalo P. 2004. Requirements for transcriptional regulation by the orphan nuclear receptor ERRgamma. Mol Cell Endocrinol 219:151–160.

Hyatt SM, Lockamy EL, Stein RA, McDonnell DP, Miller AB, Orband-Miller LA, Willson TM, Zuercher WJ. 2007. On the intractability of estrogen-related receptor alpha as a target for activation by small molecules. J Med Chem 50:6722–6724.

Jeong BC, Lee YS, Park YY, Bae IH, Kim DK, Koo SH, Choi HR, Kim SH, Franceschi RT, Koh JT, Choi. HS. 2009. The orphan nuclear receptor estrogen receptor-related receptor gamma negatively regulates BMP2-induced osteoblast differentiation and bone formation. J Biol Chem 284:14211–14218.

Kallen J, Schlaeppi JM, Bitsch F, Filipuzzi I, Schilb A, Riou V, Graham A, Strauss A, Geiser M, Fournier B. 2004. Evidence for ligand-independent transcriptional activation of the human estrogen-related receptor alpha (ERRalpha): Crystal structure of ERRalpha ligand binding domain in complex with peroxisome proliferator-activated receptor coactivator-1alpha. J Biol Chem 279:49330–49337.

Kallen J, Lattmann R, Beerli R, Blechschmidt A, Blommers MJ, Geiser M, Ottl J, Schlaeppi JM, Strauss A, Fournier B. 2007. Crystal structure of human estrogen-related receptor alpha in complex with a synthetic inverse agonist reveals its novel molecular mechanism. J Biol Chem 282:23231–23239.

Kraus RJ, Ariazi EA, Farrell ML, Mertz JE. 2002. Estrogen-related receptor alpha 1 actively antagonizes estrogen receptor-regulated transcription in MCF-7 mammary cells. J Biol Chem 277:24826–24834.

Lammi J, Huppunen J, Aarnisalo P. 2004. Regulation of the osteopontin gene by the orphan nuclear receptor NURR1 in osteoblasts. Mol Endocrinol 18:1546–1557.

Lu D, Kiriyama Y, Lee KY, Giguere V. 2001. Transcriptional regulation of the estrogen-inducible pS2 breast cancer marker gene by the ERR family of orphan nuclear receptors. Cancer Res 61:6755–6761.

Nuclear Receptors Nomenclature Committee. 1999. A unified nomenclature system for the nuclear receptor superfamily. Cell 97:161–163.

Pacifici R. 2008. Estrogen deficiency, T cells and bone loss. Cell Immunol 252:68–80.

Razzaque MA, Masuda N, Maeda Y, Endo Y, Tsukamoto T, Osumi T. 2004. Estrogen receptor-related receptor gamma has an exceptionally broad specificity of DNA sequence recognition. Gene 340:275–282.

Riggs BL, Khosla S, Melton LJ III. 2002. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302.

Sato M, Morii E, Komori T, Kawahata H, Sugimoto M, Terai K, Shimizu H, Yasui T, Ogihara H, Yasui N, Ochi T, Kitamura Y, Ito Y, Nomura S. 1998. Transcriptional regulation of osteopontin gene in vivo by PEBP2alphaA/CBFA1 and ETS1 in the skeletal tissues. Oncogene 17:1517–1525.

Shigeta H, Zuo W, Yang N, DiAugustine R, Teng. CT. 1997. The mouse estrogen receptor-related orphan receptor alpha 1: Molecular cloning and estrogen responsiveness. J Mol Endocrinol 19:299–309.

Susens U, Hermans-Borgmeyer I, Borgmeyer U. 2000. Alternative splicing and expression of the mouse estrogen receptor-related receptor gamma. Biochem Biophys Res Commun 267:532–535.

Tanabe N, Wheal BD, Kwon J, Chen HH, Shugg RP, Sims SM, Goldberg HA, Dixon SJ. 2011. Osteopontin signals through calcium and nuclear factor of activated T cells (NFAT) in osteoclasts: A novel RGD-dependent pathway promoting cell survival. J Biol Chem 286:39871–39881.

Tremblay AM, Wilson BJ, Yang XJ, Giguere V. 2008. Phosphorylation-dependent sumoylation regulates estrogen-related receptor-alpha and -gamma transcriptional activity through a synergy control motif. Mol Endocrinol 22:570–584.

Vanacker JM, Delmarre C, Guo X, Laudet V. 1998. Activation of the osteopontin promoter by the orphan nuclear receptor estrogen receptor related alpha. Cell Growth Differ 9:1007–1014.

Vanacker JM, Pettersson K, Gustafsson JA, Laudet V. 1999. Transcriptional targets shared by estrogen receptor- related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. EMBO J 18:4270–4279.

Wang L, Zuercher WJ, Consler TG, Lambert MH, Miller AB, Orband-Miller LA, McKee DD, Willson TM, Nolte RT. 2006. X-ray crystal structures of the estrogen-related receptor-gamma ligand binding domain in three functional states reveal the molecular basis of small molecule regulation. J Biol Chem 281:37773–37781.

Yang N, Shigeta H, Shi H, Teng. CT. 1996. Estrogen-related receptor, hERR1, modulates estrogen receptor-mediated response of human lactoferrin gene promoter. J Biol Chem 271:5795–5804.

Zhang Z, Teng. CT. 2007. Interplay between estrogen-related receptor alpha (ERRalpha) and gamma (ERRgamma) on the regulation of ERRalpha gene expression. Mol Cell Endocrinol 264:128–141.

Zhang Y, Ma K, Sadana P, Chowdhury F, Gaillard S, Wang F, McDonnell DP, Unterman TG, Elam MB, Park. EA. 2006. Estrogen-related receptors stimulate pyruvate dehydrogenase kinase isoform 4 gene expression. J Biol Chem 281:39897–39906.

Zirngibl RA, Chan JS, Aubin JE. 2008. Estrogen receptor-related receptor alpha (ERRalpha) regulates osteopontin expression through a non-canonical ERRalpha response element in a cell context-dependent manner. J Mol Endocrinol 40:61–73.