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Ligand-induced large-scale chromatin dynamics as a biosensor for the detection of estrogen receptor subtype selective ligands

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ABSTRACT

Estrogen receptors (ER), members of the nuclear steroid receptor superfamily, act to activate transcription through ligand-dependent recruitment of coregulators and chromatin modifications. A series of synthetic A-ring reduced 19-nortestosterone-derived progestins has the capacity to selectively bind ERα for activated transcription, and to recruit coregulatory factors. In this study, we have analyzed the ability of synthetic 19-nortestosterone derivatives to visibly alter the configuration of ER-target gene chromatin using a novel mammalian promoter transcriptional biosensor (PRL-array) stably transfected into the genome of HeLa cells (PRL-HeLa cells). Results from synthetic steroid-treated cells expressing functional GFP-ERα or YFP-ERβ chimeras were compared to those obtained with estradiol (E_2) and the antiestrogen tamoxifen. In the presence of synthetic ligands or E_2 a concentration-dependent increase in area of the biosensor array was observed in GFP-ERα-expressing PRL-HeLa cells. No significant differences were found between the effects obtained with natural and synthetic steroids. Similarly, E_2 or synthetic steroids-treated PRL-HeLa cells also resulted in similar colocalization of SRC-1- and RNAPII-immunofluorescence at the array. YFP-ERβ-expressing PRL-HeLa cells treated with E_2 showed increases in array area that were similar to ERα; however, treatment of YFP-ERβ-expressing cells with synthetic ligands was indistinguishable from vehicle controls. These data indicate that A-ring reduced 19-nortestosterone derivatives have an estrogen-like effect on chromatin, including recruitment of transcription factors through selective interactions with ERα.

1. Introduction

Estrogen actions on growth, development, behavior and regulation of a number of reproductive functions are mediated by a ligand-regulated transcription factor that belongs to the nuclear receptor superfamily (Carson-Jurica et al., 1990; Beato et al., 1996). As with other nuclear receptors, estrogen receptors (ER) have three main distinct domains: an N-terminal transactivation domain, a central DNA binding domain, and a C-terminal ligand binding domain. Once bound to its ligand, the ER undergoes conformational changes (Fritsch et al., 1992; Beekman et al., 1993; Brzozowski et al., 1997) followed by specific interaction with DNA regulatory sequences allowing the receptor to regulate gene transcription by the recruitment of chromatin modifying complexes, including the basal transcriptional machinery (McKenna et al., 1999). The ER exists as two subtypes, ERα and ERβ, which are products from distinct genes that differ in their tissue distribution and ligand selectivity (Kuiper et al., 1996, 1997; Mosselman et al., 1996; Couse et al., 1997).

Results from several studies suggest that a number of compounds may exhibit differential binding and relative binding affinities for both ER subtypes (Kuiper et al., 1997; Sun et al., 1999; Stauffer et al., 2000; Harris et al., 2002). Regarding this, we have systematically characterized the estrogenic activity of a number of synthetic 19-nortestosterone derivatives that function as agonists with high potency and efficacy preferentially for ERα (Chavez et al., 1985; Larrea et al., 1987, 2001; Lemus et al., 1992, 2000; Garcia-Becerra et al., 2002, 2006). Among these compounds, the A-ring 3β,5α-reduced derivatives of norethisterone (NET), levonorgestrel (LNG) and gestodene (GSD)

Abbreviations: 3β,5α-GSD, 3β,5α-gestodene; 3β,5α-LNG, 3β,5α-levonorgestrel; 3β,5α-NET, 3β,5α-norethisterone; C-, carbon; C-terminal, carboxyl-terminal; DAPI, 4,6-diamidino- 2-phenylindole; DMEM, Dulbecco's modified Eagle's Medium; DNA, deoxyribonucleic acid; E_2, estradiol; ER, estrogen receptor; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; FBS, fetal bovine serum; GFP, green fluorescent protein; GSD, gestodene; h, hour; HTM, high throughput microscopy; LNG, levonorgestrel; M, molar; mRNA, messenger ribonucleic acid; N-terminal, amino-terminal; NET, norethisterone; Opti-MEM, growth-factor-containing minimal essential medium; p, plasmid; PBS, phosphate-buffered saline; Pit-1, a POU-class transcription factor; PRL, prolactin; RNAPII, RNA polymerase II; SD, standard deviation; SRC-1, steroid receptor coactivator -1; TAM, tamoxifen; V, vehicle; YFP, yellow fluorescent protein.

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behave as complete ERα agonists but are inactive on ERβ in terms of receptor binding, recruitment of coactivators and transcriptional activation (Larrea et al., 2001; Garcia-Becerra et al., 2006). Although, the precise molecular basis for their ERα selectivity remains unclear, these compounds may be of interest in identifying or developing new ligands for ER subtypes.

In this study, our goal was to further expand our comparisons on the intrinsically selective interactions of 19-nortestosterone derivatives with ERα or ERβ. For this purpose we used a recently developed transcriptional biosensor constructed from mammalian promoter/enhancer components to study ligand-dependent large-scale chromatin modifications at the single cell level, including recruitment of endogenous chromatin-remodeling complexes at the promoter level. In this assay, ER-mediated chromosomal dynamics can be assayed using an integrated responsive array of promoter/enhancer elements based on the mammalian prolactin (PRL) promoter/enhancer (Sharp et al., 2006; Berno et al., 2008). The PRL promoter contains both Pit-1 (a POU-class transcription factor) (Andersen and Rosenfeld, 2001) and ER binding sites (Day and Maurer, 1989; Day et al., 1990). The PRL array is visible in cells expressing fluorescently tagged Pit-1 and /or ER (Sharp et al., 2006).

2. Materials and methods

2.1. Reagents

Non-radioactive estradiol (E_2) was purchased from Sigma Chemical Co. (St. Louis, MO) and cell culture medium from Life Technologies (Gaithersburg, MD). Fetal bovine serum (FBS) was from Hyclone Laboratories, Inc. (Logan, UT). The antiestrogen tamoxifen was purchased from Zeneca Pharmaceuticals (Wilmington, DE). Authentic NET (17α-ethynyl-17β-hydroxy-4-estren-3-one) and LNG (13β-ethyl-17α-ethynyl-17β-hydroxy-4-gonen-3-one) were kindly provided by Schering Mexicana, S.A. (Mexico D.F., Mexico) and GSD (13β-ethyl-17α-ethynyl-17β-hydroxy-4,15-gonadien-3-one) from Schering AG (Berlin, Germany). Synthesis of the corresponding 3β,5α- (3β,5α-NET, 3β,5α-LNG and 3β,5α-GSD) tetrahydro derivatives, including the description of their corresponding physical and spectroscopic constants have been previously described (Vilchis et al., 1986; Lemus et al., 2000). All other solvents and reagents used were of analytical grade.

2.2. Plasmids

The expression vectors for GFP-hERα and YFP-hERβ, used for transient transfections, were generated as previously described (Stenoien et al., 2000; Muddana and Peterson, 2003). The carrier DNA pBluescript vector was purchased from Stratagene (La Jolla CA). The correct sequences of all final constructs were confirmed by sequencing.

2.3. Cell culture and hormonal treatments

The PRL-HeLa line used in this and other studies (Sharp et al., 2006; Amazit et al., 2007; Berno et al., 2008) was designed for single-cell studies of transcription factor function.

The generation and detailed description of the PRL-HeLa cell line bearing integrated rat PRL promoter-enhancer have been published in detail elsewhere (Sharp et al., 2006). The cells were maintained in Opti-MEM (growth-factor-containing minimal essential medium) (Life Technologies, Inc.) containing 5% charcoal-stripped FBS. Twenty-four hours before transfection, cells were seed onto poly-D-lysine-coated cover slips in 35 mm wells at a concentration of 1×10^5 cell/cm^2 in phenol red-free DMEM (Dulbecco's modified Eagle's medium) containing 5% charcoal-stripped and dialyzed FBS. Cells were transiently transfected with pEGFP-ERα or pEYFP-ERβ and carrier DNA vectors at 1:1 ratio using TransFectin (Bio-Rad Laboratories, Inc. Hercules, CA, USA) according to protocols provided by the manufacturer. Twenty-four hours later, the medium was replaced with fresh DMEM containing natural or synthetic hormones at different concentrations in the presence or the absence of 10 nM 4-hydroxytamoxifen or ethanol as the vehicle alone. As in a previously discussed (Sharp et al., 2006), only

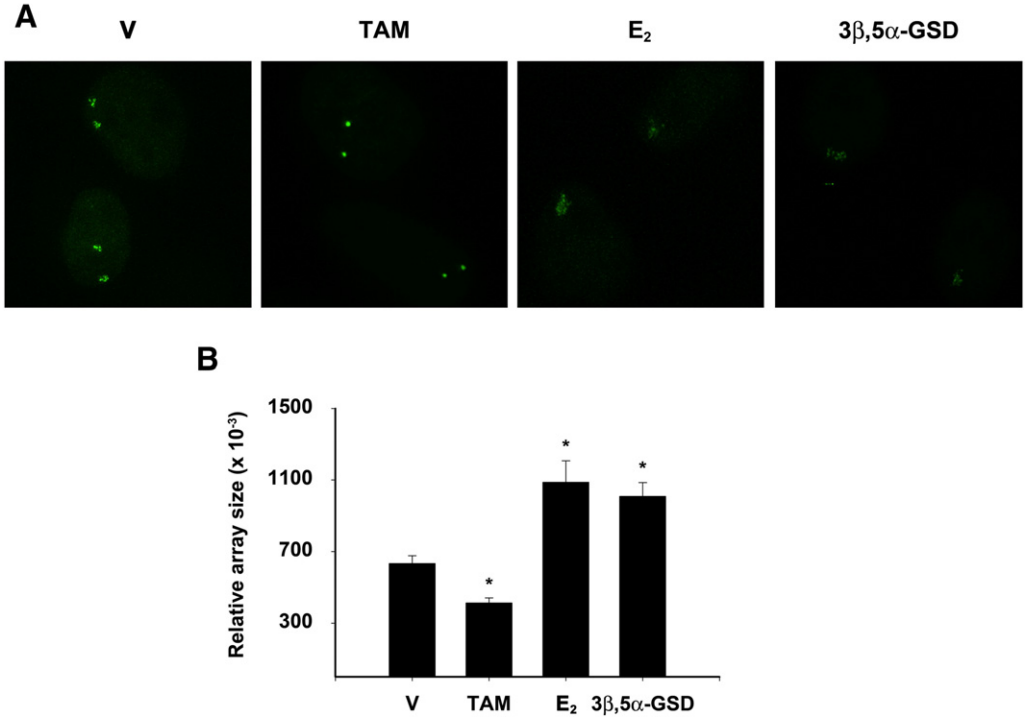

Fig. 1. (A) Large-scale chromatin changes induced by tamoxifen, estradiol (E_2) and the 3β-5α-GSD synthetic derivative. PRL-HeLa cells were transiently transfected with GFP-ERα and 24 h later cells were cultured in the presence or absence (V) of 1×10^{-8} M ligands during 2 h prior to fixation. **(B)** Automated quantification of GFP-ERα at the promoter array using high-resolution HTM. Each bar represents the mean ± SD of three experiments. $P<0.05$ vs. V.

GFP/YFP-ER-transfected PRL-HeLa cells with levels of expression similar to those found in non-transfected MCF-7 cells were used in this study in order to avoid over expression artifacts. Transcriptional responses of our chromosomally integrated PRL-based array have previously been documented by *in situ* hybridization of accumulated reporter mRNA (Sharp et al., 2006), which confirmed the biological functionality and physiological relevance of the mammalian promoter array to study transcription and chromatin regulation.

2.4. Colocalization of endogenous coactivators and RNAPII

Cells were grown on acid-etched and poly-D-lysine-coated glass cover slips. After treatment, cells were immediately rinsed in ice-cold phosphate-buffered saline (PBS) fixed in 4% formaldehyde, permeabilized in 0.5% Triton X-100, and then washed and quenched in sodium borohydride as previously described (Sharp et al., 2006; Amazit et al., 2007). After incubation for 1 h at room temperature in 5% nonfat dry milk, cells were incubated for 2 h at room temperature with primary antibodies and then with fluorophores conjugated secondary antibodies (Alexa Fluor-555 and Fluor-647; Molecular Probes, for SRC-1 and RNAPII, respectively). Affinity-purified rabbit antibodies against SRC-1 and mouse monoclonal anti-RNAPII were obtained from Abcam Inc. (Cambridge, MA). After primary and secondary antibody labeling, cells were post fixed and quenched as described above, and counterstained with DAPI (4,6-diamidino- 2-phenylindole) prior to mounting onto slides (ProLong Gold; Molecular Probes).

2.5. Imaging

Imaging of PRL-HeLa transiently expressing pEGFP-ERα/pEYFP — ERβ and immunofluorescently labeled cells were performed using a DeltaVision restoration microscopy system (Applied Precision, Inc., Issaqua, WA) as previously described (Stenoien et al., 2000). A *z* series of focal planes was digitally imaged and deconvoluted with the Delta Vision constrained iterative algorithm to generate high- resolution images. Whole nuclear volumes were collected at 0.2 μm Z-steps, and images from select focal planes or 3D projections were imported into Adobe PhotoShop. Histogram adjustments were made relevant to negative controls, which routinely included non-transfected cells and/or omission of primary antibodies. Live imaging was performed by collecting short Z-stacks (~5–10 focal planes at 300-nm increments); neutral density for the green channel was set at 50% and the images were binned 2×2. Typical exposures were for <1 s, and time points from 3–10 min per stack. Quantitative image analyses by high throughput microscopy (HTM) using the Cell Lab IC 100 Image Cytometer (Beckman Coulter, Inc) were performed as previously described (Berno et al., 2006; Sharp et al., 2006).

2.6. Statistical analysis

All results were expressed as means value ± SD. Statistical significance of differences between groups was calculated by

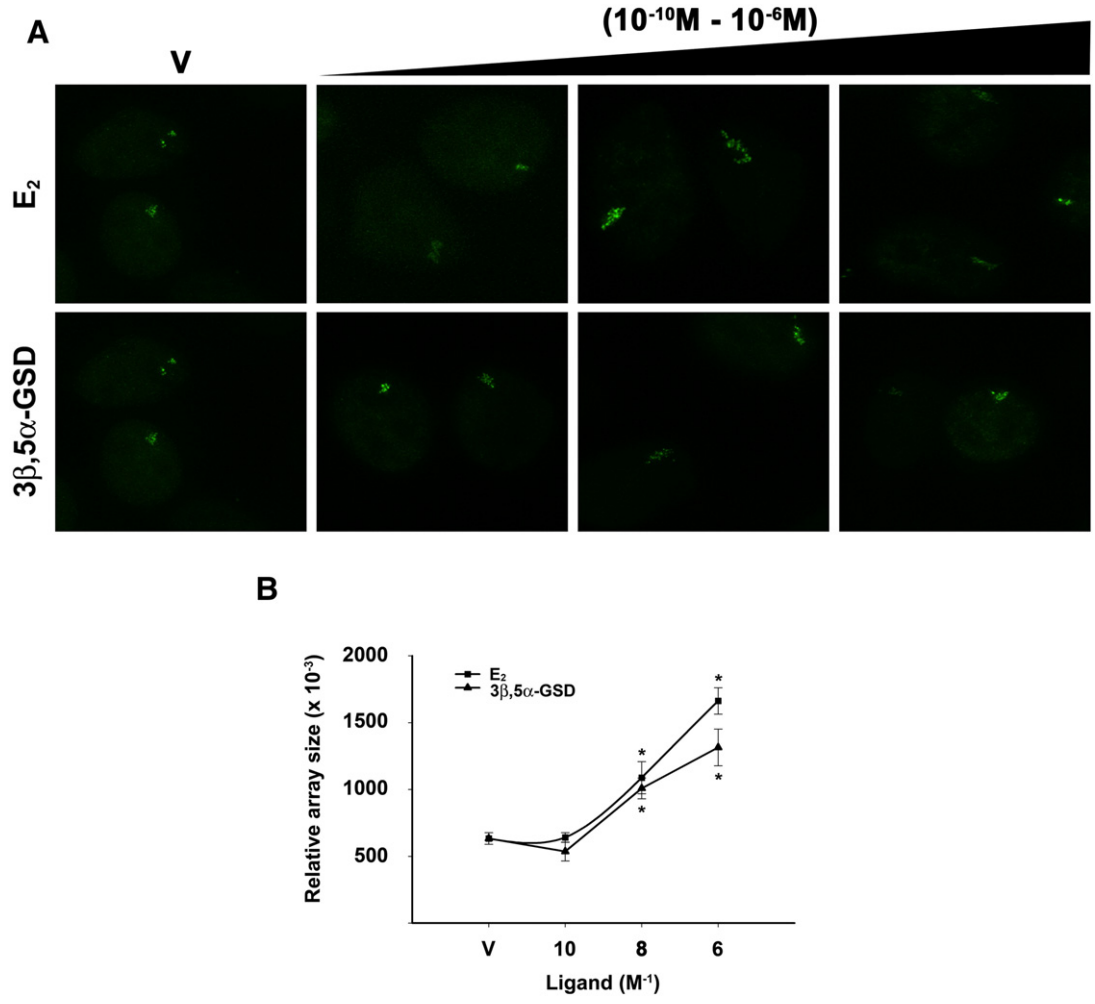

Fig. 2. (A) Changes in chromatin structure of the GFP-ERα-targeted PRL array in response to several doses (1×10^{-10} M to 1×10^{-6} M) of estradiol (E_2) or 3β-5α-GSD. Experimental conditions were as described in Fig. 1. (**B**) After fixation and staining with DAPI, cells were imaged and array size was quantified using HTM. Each point represents the mean ± SD of three experiments. $P<0.05$ vs. V.

Student's *t*-test. A *P* value equal or lower than 0.05 was considered as significant.

3. Results

3.1. ERα-dependent changes in chromatin structure in response to ligands

To address the question as to whether synthetic ER agonists elicit *in vivo* large-scale chromatin responses comparable to those previously observed for E_2, we treated GFP-ERα-expressing PRL-HeLa cells with either E_2, tamoxifen, the A-ring reduced 19-nortestosterone derivative, 3β,5α-GSD or vehicle. As previously observed (Sharp et al., 2006), treatment with tamoxifen resulted in significant (compared to vehicle) condensation of the area of fluorescence at the array demarcated by GFP-ERα (Fig. 1). E_2 treatment resulted in a significant increase of the array area, and sub-array texture (Fig. 1). Incubation of GFP-ERα-expressing PRL-HeLa cells with 3β,5α-GSD, resulted in an array area similar to that obtained with E_2.

GFP-ERα-targeted PRL array responses to variable concentrations of natural and synthetic ligands are shown in Fig. 2. As depicted, both E_2 and 3β,5α-GSD induced a significant concentration-dependent increase in array size with similar dose-response curves. GFP-ERα-targeted PRL arrays in the presence of the lowest concentration (10^{-10} M) of ligands exhibited array sizes that were no different than those obtained with vehicle alone. Similar responses were obtained with other synthetic A-ring reduced 19-nortestosterone derivatives (data not shown).

3.2. ERα- and ligand-dependent colocalization of endogenous SRC-1 and RNAPII

For this purpose, we used PRL-HeLa cells transiently expressing GFP-ERα or non-transfected controls, which were immunolabeled using antibodies specific for SRC-1 or the active form of RNAPII. In the absence of GFP-ERα neither SRC-1 nor RNAPII immunofluorescence signals showed focal concentrations consistent with array morphology (e.g., no marked increase in signal over the GFP-ERα-marked array versus the rest of the nucleoplasm). Conversely, in vehicle-treated, GFP-ERα-expressing cells, both SRC-1 and RNAPII immunofluoresecence exhibited marked colocalization at the GFP-ERα-targeted PRL array, but with an array size significantly lower to that obtained in the presence of ligands (Figs. 3 and 4, respectively). Treatment with tamoxifen resulted in condensation of the GFP-ERα-labeled array, but with minimal diminishment of SRC-1 immunofluorescence signal at the array (Fig. 3). RNAPII immunofluorescence, however, was displaced from the PRL array by tamoxifen treatment (Fig. 4). As shown also in Fig 3, incubation of PRL-HeLa cells expressing GFP-ERα with E_2 or 3β,5α-GSD resulted in SRC-1 and RNAPII recruitment, with an array area significantly greater compared to vehicle.

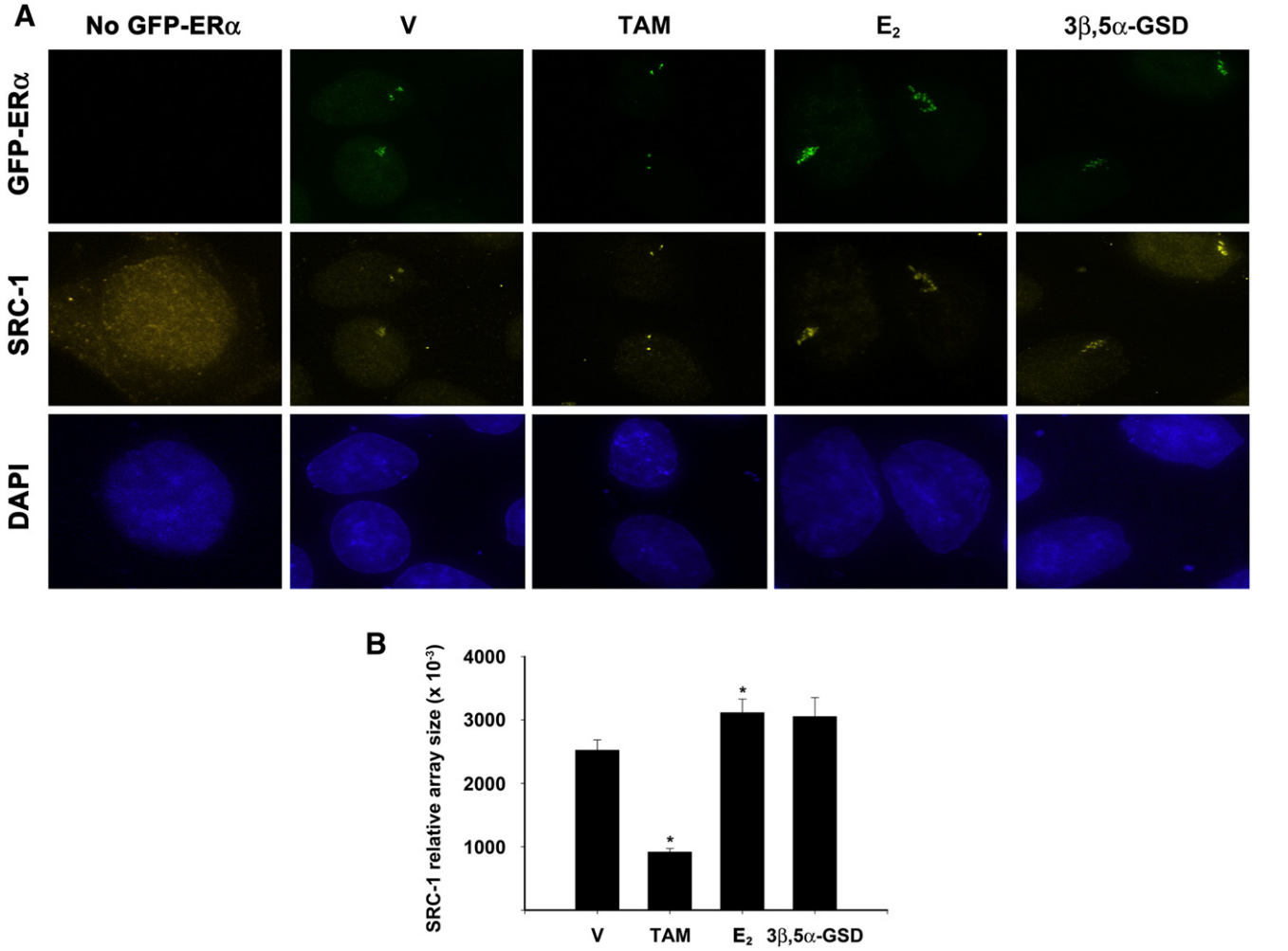

Fig. 3. (A) Colocalization of GFP-ERα and endogenous SRC-1 at the PRL-array. PRL-HeLa cells transiently transfected with or without GFP-ERα were incubated in the presence of 1×10^{-8} M of tamoxifen, estradiol (E_2), 3β,5α-GSD or the vehicle alone (V) for 2 h and immunolabeled for SRC-1 as described in Materials and methods. **(B)** Automated quantification of GFP-ERα and SRC-1 colocalized at the promoter array using high-resolution HTM. Cells were stained with DAPI. Each bar represents the mean ± SD of three experiments. $P<0.05$ vs. V.

Similar patterns of nuclear and array-associated fluorescence and SRC-1 and RNAPII immunofluorescence at the array were obtained with other synthetic A-ring 19-nortestosterone derivatives (3β,5α-NET and 3β,5α-LNG, data not shown).

3.3. ERβ-dependent chromatin effects and recruitment of endogenous transcription factors to the PRL array

We have previously reported that 19-nortestosterone A-ring reduced derivatives stimulated ERα- but not ERβ-driven reporter activity, including recruitment of coactivators (Larrea et al., 2001; Garcia-Becerra et al., 2006). We hypothesized that this receptor-specific transcription response would correlate with differential chromatin modification at the PRL-array, i.e., treatment of GFP-ERβ-expressing cells with synthetic ligands would not result in decondensed arrays.

As shown in Fig. 5 and similar to cells expressing GFP-ERα, YFP-ERβ fluorescence and SRC-1 and RNAPII immunofluorescence accumulated over the PRL promoter/enhancer array in PRL-HeLa cells incubated in the presence or absence of E_2. Also in concert with GFP-ERα-expressing cells, YFP-ERβ-positive cells incubated in the presence of E_2 demonstrated significantly increased areas of array-associated fluorescence; as with GFP-ERα, SRC-1 and RNAPII immunofluorescence was recruited to the YFP-ERβ-targeted arrays. In contrast, unlike GFP-ERα-expressing cells, when YFP-ERβ-positive cells were treated with 3β,5α-GSD or 3β,5α-LNG, both the size of the PRL array, and recruitment of SRC-1 or RNAPII were similar to vehicle control (Fig. 5).

4. Discussion

There is a critical need to identify novel ER selective ligands that provide health benefits while eliminating side effects. Potential selective ligands have been identified using steroid-receptor competition or transcription-based assays but most of them are time consuming and include significant cost and safety issues. Herein, we have taken advantage of a recently developed mammalian promoter/enhancer array system to test *in vivo* ligand-dependent nuclear receptor-chromatin interactions at the single cell level.

In this report, we extend previous observations of ERα selective interactions and transactivation of transcription by synthetic A-ring reduced 19-nortestosterone derivatives (Larrea et al., 2001; Garcia-Becerra et al., 2002, 2006). Our results are consistent with the ability of these ligands to induce ERα-dependent decondensation of chromatin encompassing the PRL-array. Our results also highlight the close correlation between selective ERα-based large-scale chromatin changes and colocalization of SRC-1 and RNAPII that are induced by synthetic ligands, suggesting a permissive state exists at the chromatin level for *trans*-acting factors to influence the responsiveness of the multicopy promoter/enhancer's response. In contrast to E_2, which induced large-scale chromatin decondensation in both ERα- and ERβ-expressing cells, 3β,5α-derivatives induced changes in chromatin structure in ERα-expressing cells, but not in those expressing ERβ. In this study, significant changes in array size are interpreted as an alteration in the chromatin and transcriptional state of the

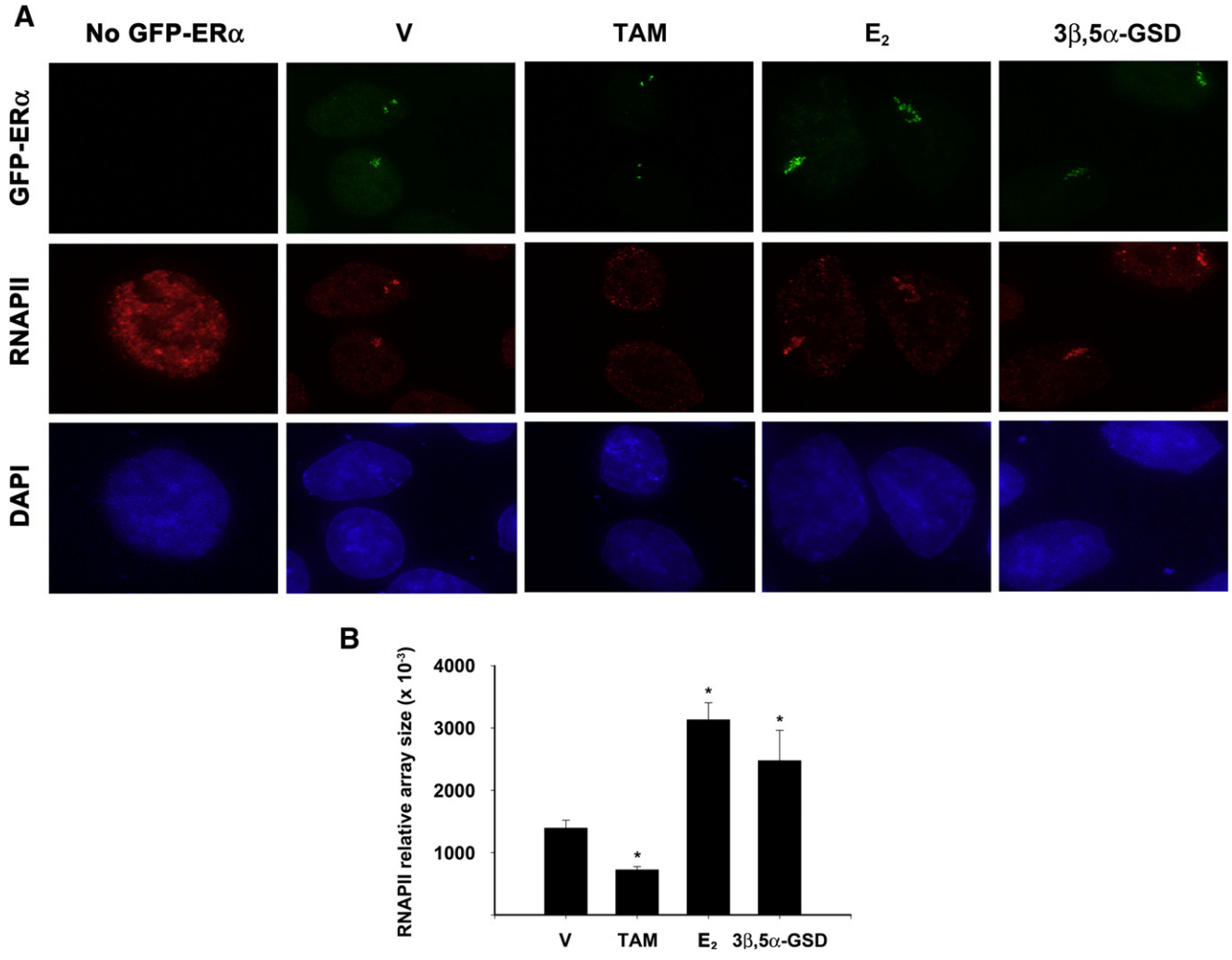

Fig. 4. Colocalization of GFP-ERα and RNAPII at the PRL array. Experimental conditions were the same as those described in Fig. 3.

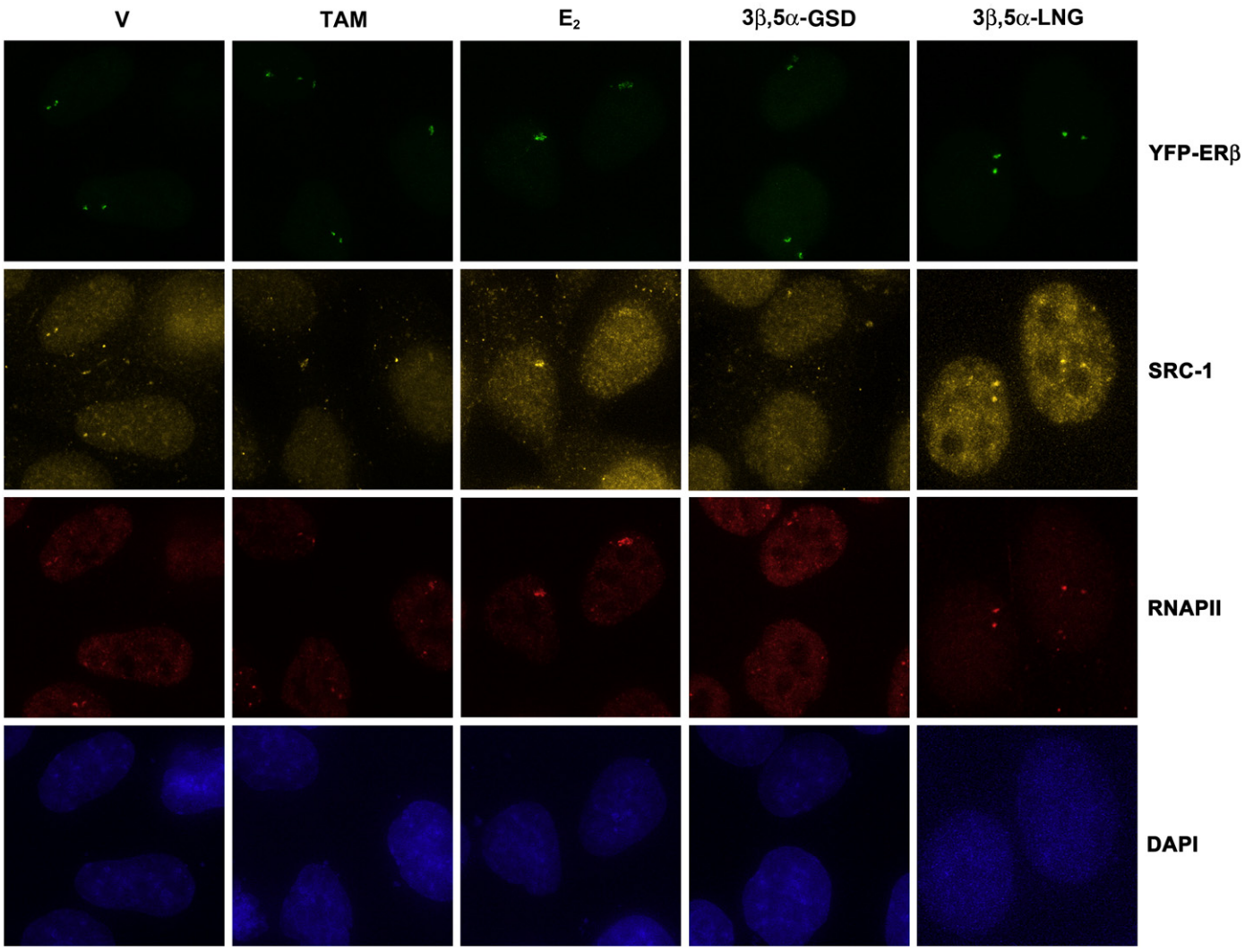

Fig. 5. Colocalization of YFP-ERβ and endogenous SRC-1 at the PRL-array. PRL-HeLa cells transiently expressing YFP-ERβ were incubated in the presence of 1×10^{-8} M of tamoxifen, estradiol (E_2), 3β,5α-GSD, 3β,5α-LNG or the vehicle alone (V) for 2 h and immunolabeled for SRC-1 and RNAPII as described in the Materials and methods.

reporter, based on ER-mediated colocalization of SRC-1 and RNAPII (Figs. 4 and 5), and colocalized reporter mRNA accumulation (Sharp et al., 2006; Berno et al., 2008). Addition of an ER agonist (E_2) to PRL-HeLa cells is followed by a visible decondensation of the PRL-promoter array and colocalization of endogenous transcription factors. In contrast, the presence of an ER antagonist results in a marked condensation pattern of the array relative to controls in the absence of ligand (Sharp et al., 2006). These changes in array size are consistent with large-scale chromatin relaxation leading to or concomitant with an increased activity of the reporter transcription units. These chromatin decondensation/condensation events likely result from ER-dependent coregulator recruitment and histone modification (Sharp et al., 2006; Amazit et al., 2007). This leads to progression or inhibition of a transcription cycle, which requires a series of integrative events comprising *cis*- and *trans*-acting factors and the basal transcription machinery, including RNAPII. Our results obtained with the synthetic ligands demonstrate an agonist pattern of chromatin dynamics and recruitment of endogenous SRC-1 and RNAPII, similar to that found with E_2. Collectively, these findings supports and extend our previous observations of the full agonist, ERα selective activity of A-ring reduced 19-nortestosterone derivatives (Lemus et al., 2000; Larrea et al., 2001; Garcia-Becerra et al., 2002).

Although the mechanistic difference by which ligands selectivity discriminates between the two ER subtypes is not well understood, the dynamic and plastic nature of the ER ligand-binding domain is presumed to play an important role. The ability of the ER to bind structurally diverse compounds might be partially attributed to the size and composition of the ligand-binding pocket (Brzozowski et al., 1997; Zhu et al., 2006). Although there are sequence differences in the ERα and ERβ ligand binding domains, similar amino-acids participate in E_2 binding. This suggests that other amino-acids, in addition to the size and flexibility of the respective binding pockets, also play a role in determining ligand binding preference (Ekena et al., 1997; Pike et al., 1999; Matthews et al., 2000). Given the interest in searching for specific ERα or ERβ ligands for therapeutic uses, a number of potential compounds with greater or lesser affinity for either ER subtype have been identified. Among the best characterized agonists for ERα are the trialkyl-substituted pyrazoles and furans (Stauffer et al., 2000, 2001). Phytoestrogens such as genistein and coumesterol have a higher affinity for ERβ (Kuiper and Gustafsson, 1997; Bowers et al., 2000; Norman et al., 2006; Ullrich et al., 2007). However, the molecular basis for ER-ligand subtype selectivity still remains to be determined.

Given the structural similarity between our selective ERα compounds with androstanediol (5α-androstan, 3β,17β-diol), an A-ring reduced derivative of testosterone, which activates transcription through both ER subtypes (Garcia-Becerra et al., 2002), raises the possibility that A-ring reduction at the C-3 and C-4 induces affinity for ER. On the other hand, the presence of steric bulk at C-17 and the

absence of methyl group at C-19, as in the case of 19-nortestosterone derived progestins, are associated with ERα ligand selectivity (Kubli-Garfias et al., 2002). However, this awaits X-ray crystallography analyses.

We have previously shown that binding affinities of 19-nortestosterone derived progestins for ERα are significantly lower than those of natural E_2 (Chavez et al., 1985; Lemus et al., 2000; Larrea et al., 2001; Garcia-Becerra et al., 2002). However, their transcriptional potencies, recruitment of coactivators or alterations in chromatin structure as shown in this study are very much similar to those obtained with equimolar doses of E_2. In fact, all responses occur at physiological concentrations of ligands. These differences in ligand-binding affinity compared to ligand potency or efficacy could have several explanations, but most likely are due to intrinsic preferential interactions of the ER subtype-ligand complex for coregulatory proteins that mediate its effects on transcription beyond the receptor-ligand affinity constants (Katzenellenbogen and Katzenellenbogen, 1996). In this regard, our laboratory is currently analyzing, by fluorescence resonance energy transfer-based biosensors, the ability of a number of potential selective ligands to induce conformational changes in the ER ligand-binding domain as part of the mechanisms involved in recruitment of coregulatory proteins and transcriptional activation (Zwart et al., 2007). We believe that these 19-nortestosterone compounds are useful tools to understand the biological roles of both ER subtypes, particularly in conjunction with both wild-type and ER-knock-out animals.

5. Conclusions

In summary, a single-cell approach allowing the direct visualization of ERα and ERβ at a physiologically regulated transcription locus, including ligand-dependent cofactor recruitment and large-scale chromatin re-modeling was used to better quantify the unique selectivity of synthetic 19-nortestosterone derived ligands for ERα. In addition, our results, which are increasingly amendable to high throughput imaging-based acquisition and analyses (Berno et al., 2006; Sharp et al., 2006; Berno et al., 2008) show the benefits of this mammalian promoter array for the evaluation of novel ligands with receptor subtype selective interactions.

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References

Amazit, L., et al., 2007. Regulation of SRC-3 intercompartmental dynamics by estrogen receptor and phosphorylation. Mol. Cell. Biol. 27, 6913–6932.
Andersen, B., Rosenfeld, M.G., 2001. POU domain factors in the neuroendocrine system: lessons from developmental biology provide insights into human disease. Endocr. Rev. 22, 2–35.
Beato, M., Chavez, S., Truss, M., 1996. Transcriptional regulation by steroid hormones. Steroids 61, 240–251.
Beekman, J.M., Allan, G.F., Tsai, S.Y., Tsai, M.J., O'Malley, B.W., 1993. Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. Mol. Endocrinol. 7, 1266–1274.
Berno, V., Hinojos, C.A., Amazit, L., Szafran, A.T., Mancini, M.A., 2006. High-resolution, high-throughput microscopy analyses of nuclear receptor and coregulator function. Methods Enzymol. 414, 188–210.
Berno, V., et al., 2008. Activation of estrogen receptor-alpha by E2 or EGF induces temporally distinct patterns of large-scale chromatin modification and mRNA transcription. PLoS ONE 3, e2286.
Bowers, J.L., Tyulmenkov, V.V., Jernigan, S.C., Klinge, C.M., 2000. Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. Endocrinology 141, 3657–3667.
Brzozowski, A.M., et al., 1997. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389, 753–758.
Carson-Jurica, M.A., Schrader, W.T., O'Malley, B.W., 1990. Steroid receptor family: structure and functions. Endocr. Rev. 11, 201–220.
Chavez, B.A., Vilchis, F., Perez, A.E., Garcia, G.A., Grillasca, I., Perez-Palacios, G., 1985. Stereospecificity of the intracellular binding of norethisterone and its A-ring reduced metabolites. J. Steroid Biochem. 22, 121–126.
Couse, J.F., Lindzey, J., Grandien, K., Gustafsson, J.A., Korach, K.S., 1997. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138, 4613–4621.
Day, R.N., Maurer, R.A., 1989. The distal enhancer region of the rat prolactin gene contains elements conferring response to multiple hormones. Mol. Endocrinol. 3, 3–9.
Day, R.N., Koike, S., Sakai, M., Muramatsu, M., Maurer, R.A., 1990. Both Pit-1 and the estrogen receptor are required for estrogen responsiveness of the rat prolactin gene. Mol. Endocrinol. 4, 1964–1971.
Ekena, K., Weis, K.E., Katzenellenbogen, J.A., Katzenellenbogen, B.S., 1997. Different residues of the human estrogen receptor are involved in the recognition of structurally diverse estrogens and antiestrogens. J. Biol. Chem. 272, 5069–5075.
Fritsch, M., et al., 1992. A ligand-induced conformational change in the estrogen receptor is localized in the steroid binding domain. Biochemistry 31, 5303–5311.
Garcia-Becerra, R., et al., 2002. The intrinsic transcriptional estrogenic activity of a non-phenolic derivative of levonorgestrel is mediated via the estrogen receptor-alpha. J. Steroid Biochem. Mol. Biol. 82, 333–341.
Garcia-Becerra, R., et al., 2006. Synthetic 19-nortestosterone derivatives as estrogen receptor alpha subtype-selective ligands induce similar receptor conformational changes and steroid receptor coactivator recruitment than natural estrogens. J. Steroid Biochem. Mol. Biol. 99, 108–114.
Harris, H.A., Katzenellenbogen, J.A., Katzenellenbogen, B.S., 2002. Characterization of the biological roles of the estrogen receptors, ERalpha and ERbeta, in estrogen target tissues in vivo through the use of an ERalpha-selective ligand. Endocrinology 143, 4172–4177.
Katzenellenbogen, J.A., Katzenellenbogen, B.S., 1996. Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses. Chem. Biol. 3, 529–536.
Kubli-Garfias, C., Vazquez, R., Cooney, A.J., Larrea, F., 2002. Ab initio electronic structure of the progestogen norethisterone and its 5 alpha-derivatives. J. Steroid Biochem. Mol. Biol. 82, 385–391.
Kuiper, G.G., Gustafsson, J.A., 1997. The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. FEBS Lett. 410, 87–90.
Kuiper, G.G., Enmark, E., Pelto-Huikko, M., Nilsson, S., Gustafsson, J.A., 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. U. S. A. 93, 5925–5930.
Kuiper, G.G., et al., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870.
Larrea, F., Vilchis, F., Chavez, B., Perez, A.E., Garza-Flores, J., Perez-Palacios, G., 1987. The metabolism of 19-nor contraceptive progestins modulates their biological activity at the neuroendocrine level. J. Steroid Biochem. 27, 657–663.
Larrea, F., et al., 2001. A-ring reduced metabolites of 19-nor synthetic progestins as subtype selective agonists for ER alpha. Endocrinology 142, 3791–3799.
Lemus, A.E., et al., 1992. Mechanism of action of levonorgestrel: in vitro metabolism and specific interactions with steroid receptors in target organs. J. Steroid Biochem. Mol. Biol. 41, 881–890.
Lemus, A.E., et al., 2000. The oestrogenic effects of gestodene, a potent contraceptive progestin, are mediated by its A-ring reduced metabolites. J. Endocrinol. 165, 693–702.
Matthews, J., Celius, T., Halgren, R., Zacharewski, T., 2000. Differential estrogen receptor binding of estrogenic substances: a species comparison. J. Steroid Biochem. Mol. Biol. 74, 223–234.
McKenna, N.J., Lanz, R.B., O'Malley, B.W., 1999. Nuclear receptor coregulators: cellular and molecular biology. Endocr. Rev. 20, 321–344.
Mosselman, S., Polman, J., Dijkema, R., 1996. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett. 392, 49–53.
Muddana, S.S., Peterson, B.R., 2003. Fluorescent cellular sensors of steroid receptor ligands. Chembiochem 4, 848–855.
Norman, B.H., et al., 2006. Benzopyrans are selective estrogen receptor beta agonists with novel activity in models of benign prostatic hyperplasia. J. Med. Chem. 49, 6155–6157.
Pike, A.C., et al., 1999. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. EMBO J. 18, 4608–4618.
Sharp, Z.D., et al., 2006. Estrogen-receptor-alpha exchange and chromatin dynamics are ligand- and domain-dependent. J. Cell Sci. 119, 4101–4116.
Stauffer, S.R., et al., 2000. Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. J. Med. Chem. 43, 4934–4947.
Stauffer, S.R., Huang, Y., Coletta, C.J., Tedesco, R., Katzenellenbogen, J.A., 2001. Estrogen pyrazoles: defining the pyrazole core structure and the orientation of substituents in the ligand binding pocket of the estrogen receptor. Bioorg. Med. Chem. 9, 141–150.

Stenoien, D.L., Mancini, M.G., Patel, K., Allegretto, E.A., Smith, C.L., Mancini, M.A., 2000. Subnuclear trafficking of estrogen receptor-alpha and steroid receptor coactivator-1. Mol. Endocrinol. 14, 518–534.

Sun, J., Meyers, M.J., Fink, B.E., Rajendran, R., Katzenellenbogen, J.A., Katzenellenbogen, B.S., 1999. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-alpha or estrogen receptor-beta. Endocrinology 140, 800–804.

Ullrich, J.W., Unwalla, R.J., Singhaus Jr., R.R., Harris, H.A., Mewshaw, R.E., 2007. Estrogen receptor beta ligands: design and synthesis of new 2-phenyl-isoindole-1, 3-diones. Bioorg. Med. Chem. Lett. 17, 118–122.

Vilchis, F., Chavez, B., Perez, A.E., Garcia, G.A., Angeles, A., Perez-Palacios, G., 1986. Evidence that a non-aromatizable metabolite of norethisterone induces estrogen-dependent pituitary progestin receptors. J. Steroid Biochem. 24, 525–531.

Zhu, B.T., Han, G.Z., Shim, J.Y., Wen, Y., Jiang, X.R., 2006. Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: Insights into the structural determinants favoring a differential subtype binding. Endocrinology 147, 4132–4150.

Zwart, W., et al., 2007. PKA-induced resistance to tamoxifen is associated with an altered orientation of ERalpha towards co-activator SRC-1. EMBO J. 26, 3534–3544.