Binding of estrogen receptor α/β heterodimers to chromatin in MCF-7 cells

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Abstract

Estrogen receptors (ERs), ERα and ERβ, belong to a group of transcription factors that, upon ligand binding, regulate gene expression by binding to specific DNA regions in chromatin as dimers. In this article, we applied the sequential chromatin immunoprecipitation assay (Re-ChIP) to study the simultaneous presence of ERα and ERβ on various DNA-binding regions in intact chromatin. ERα/β heterodimers were isolated by precipitation with anti-ERβ antibody followed by anti-ERα antibody from a stable MCF-7-derived cell line that expresses endogenous ERα and an inducible version of ERβ. The Re-ChIP method was first validated based on the detection of ERα/β heterodimers bound to a promoter region of the pS2 gene known to bind both ERα and ERβ. We next examined 12 ER-binding sites using Re-ChIP assays for ERα/β heterodimer recruitment. Our results confirmed the recruitment of ERα/β heterodimers to all these regions. This study represents the first demonstration of binding of ERα/β heterodimers to various DNA-binding regions in intact chromatin.

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Introduction

Estrogen receptors (ERs), ERα and ERβ, mediators of the biological actions of estrogens, belong to the nuclear receptor protein family. In the classical model of estrogen action, upon ligand binding, the receptor dimerizes, and binds to DNA, followed by recruitment of co-regulatory proteins and transcriptional regulation of target genes. ERα and ERβ are highly homologous, displaying 96 and 58% homology in their DNA- and ligand-binding domains (LBD) respectively. The region that is responsible for receptor dimerization lies within the LBD (Dahlman-Wright et al. 2006). Although ERα and ERβ are widely expressed throughout the body, they exhibit distinct expression patterns in a variety of tissues. ERα is expressed primarily in the uterus, kidney, and liver, whereas ERβ is highly expressed in the prostate, lung, bladder, and central nervous system (Matthews et al. 2006).

The fact that there are two ERs suggests that heterodimerization (α/β) could play a role in estrogen signaling. The formation of ERα/β heterodimers has been demonstrated using techniques such as glutathione-S-transferase (GST) pull-down assays (Pace et al. 1997, Pettersson et al. 1997, Ogawa et al. 1998) and gel shift assays (Cowley et al. 1997, Tremblay et al. 1999). The chromatin immunoprecipitation (ChIP) assay is a powerful tool to study protein:DNA interactions in their native chromatin context (Collas & Dahl 2008). Several recent studies using ChIP assays have explored the binding of ERα and ERβ to DNA in intact chromatin in MCF-7 cells (Carroll et al. 2006, Liu et al. 2008). However, the association of ERα/β heterodimers to DNA was not investigated in these studies. Using sequential Chip with two antibodies (Re-ChIP), the simultaneous binding of two proteins to the same DNA-binding region can be demonstrated in the context of intact chromatin (Kouskouti & Talianidis 2005).

In this article, we describe our studies of the binding of ERα/β heterodimers to various DNA-binding regions in intact chromatin, applying the Re-ChIP method to a stable MCF7-derived cell line that expresses endogenous ERα and an inducible version of ERβ.

Materials and methods

Generation of tetracycline-inducible stable cell lines

MCF-7 Tet-Off cells were purchased from Clontech and maintained in DMEM/F-12 supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 μg/ml G418 (Invitrogen). The cells were transfected with either the parental plasmid (pBI-EGFP) to generate a mock cell line or a plasmid (pBI-EGFP-ERβ) expressing a flag-tagged human ERβ using the Lipofectamine 2000 reagent (Invitrogen). Cells were selected in the presence of 5 μg/ml blasticidin for 10 days. ERβ protein levels were determined by western blot analysis. In order to confirm the functionality of the ERβ protein, we examined its effect on cell proliferation.
We evaluated the cell cycle distribution by fluorescence activated cell sorter analysis (FACS) in both MCF-7 mock and MCF-7 ERβ expressing cells after 72-h stimulation with E2. The FACS analysis revealed a decrease in the percentage of cells in the S phase in MCF-7 ERβ cells, 12% compared with 18% in MCF-7 mock cells (data not shown). This finding is in line with previously published data, where overexpression of ERβ inhibited the proliferation of breast cancer cells (Chang et al. 2006, Williams et al. 2008).

**RNA purification and quantification of mRNA levels**

To assay ERα and ERβ mRNA levels, MCF-7 ERβ and MCF-7 mock cells were seeded in six-well plates and grown in the absence of tetracycline (−tetr, +ERβ) for 4 days. To assay mRNA levels of ER target genes, MCF-7 ERβ and MCF-7 mock cells were seeded in six-well plates and grown in 5% FBS dextran-coated charcoal-treated DMEM phenol red free media in the absence of tetracycline (Chang et al. 2006, Williams et al. 2008). 200 µg total RNA from each well was reverse transcribed into cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems) with random hexamer primers. Real-time (RT) PCR assays were conducted using the Applied Biosystems 7500 fast RT PCR system with SYBR green master mix RT-PCR reagent (Applied Biosystems, Stockholm, Sweden). All RT-PCRs were performed in duplicate. Acidic ribosomal phosphoprotein PO (36B4) was used as internal control gene (Akamine et al. 2007). The sequences of the primers are listed in Table 1.

**Western blot analysis of ERα and ERβ protein levels**

MCF-7 ERβ and MCF-7 mock cells were seeded in 150 mm dishes and grown in the absence of tetracycline (−tetr, +ERβ) for 4 days. Total cell extracts were prepared as previously described (Zhao et al. 2007). Aliquots corresponding to 40 µg cell extract and 1–10 ng human recombinant ERα and ERβ (Invitrogen) were separated by SDS/PAGE. For quantification of ERβ and ERα protein levels, we used the anti-ERβ rabbit polyclonal antibody LBD, developed in our laboratory (Omoto et al. 2001) and the anti-ERα rabbit polyclonal antibody HC-20 (Santa Cruz, Stockholm, Sweden). Protein bands were quantified with densitometry using Scion Image Software, Maryland, USA.

**Chromatin immunoprecipitation/re-immunoprecipitation**

MCF-7 ERβ and MCF-7 mock cells were seeded in 150 mm dishes and grown in the absence of tetracycline (−tetr, +ERβ) for 4 days. Cells were treated with 10 nM E2 for 45 min and ChIP was performed as previously described (Matthews et al. 2006, Liu et al. 2008). The anti-ERβ rabbit polyclonal antibody LBD (Omoto et al. 2001) was used to perform ChIP for ERβ and the rabbit polyclonal anti-ERα antibody HC-20 (Santa Cruz) was used for ERα ChIP. The rabbit polyclonal anti-RNA polymerase II antibody H-224 (Santa Cruz) was used for the determination of non-specific binding. For Re-ChIP, ChIP was performed with the anti-ERβ LBD antibody or the anti-ERα antibody HC-20. Beads from the first cycle of ChIP were incubated with an equal volume of 10 nM dithiothreitol at 37 °C for 30 min, centrifuged at 16 000 g for 1 min to elute DNA-bound proteins. The elution was repeated twice. The final elute was diluted 1:15 in lysis buffer containing a protease inhibitor cocktail (Roche) and re-immunoprecipitated with the anti-ERα HC-20 antibody, the anti-Pol II H-224 antibody, or normal rabbit IgG. The final pellet was processed for western blotting (ChIP protein) or, after reverse cross-linking, for PCR or RT PCR (ChIP DNA).

**Table 1** Primer pairs employed to quantify mRNA levels of estrogen receptors (ERα, ERβ), the internal control gene, acidic ribosomal phosphoprotein PO (36B4), as well as of some selected genes

<table>
<thead>
<tr>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>ERα</td>
<td>GGA TCT GCC AAG GAG ACT CGC</td>
</tr>
<tr>
<td>ERβ</td>
<td>ACT GGT TGG TGG CTG GAC AC</td>
</tr>
<tr>
<td>36B4</td>
<td>GGG CACAGCAAGACATCGAAAGAG</td>
</tr>
<tr>
<td>µS2</td>
<td>GGG CGGT TTT ATC GAT T</td>
</tr>
<tr>
<td>NBPF1</td>
<td>TGT CGT CAG CGA TTA CGG A</td>
</tr>
<tr>
<td>NBPF4</td>
<td>GAC ACC CTC CAG GAA GGC A</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>CATCGACGTCCCTCCAGAAGAG</td>
</tr>
<tr>
<td>BCL9</td>
<td>CTCTGGAGACTATACCCAGTTGCG</td>
</tr>
<tr>
<td>PRUNE</td>
<td>TGGTACACAGTGTTGTTGTTGAA</td>
</tr>
<tr>
<td>ADORA1</td>
<td>TGGTGGTGCTGGGAATCC</td>
</tr>
<tr>
<td>ADORA1</td>
<td>CAGAGATCTTGAGCAGGCAAAC</td>
</tr>
<tr>
<td></td>
<td>TGGTGACACGTCAGGATAGT</td>
</tr>
<tr>
<td></td>
<td>GGGGTCACACGCAATTGC</td>
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</tbody>
</table>

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Re-ChiP followed by western blotting, PCR or RT PCR

Total ChIP protein was eluted from beads and separated by SDS/PAGE followed by detection with the anti-flag M5 monoclonal antibody (Sigma). Equal amounts of PCR product after amplification with pS2 primers (Table 2) were analyzed by gel electrophoresis and visualized by ethidium bromide staining. Immuno-precipitated DNA was amplified by RT PCR using Platinum SYBR green quantitative PCR supermix uracil DNA glycosylase (Invitrogen). The primer sequences for the 14 genes that were investigated are given in Table 2.

Results

The MCF-7 ERβ cell line expresses similar levels of ERα and ERβ

A stable cell line, MCF-7 ERβ, that expresses an inducible version of ERβ fused to a flag tag, was used in all experiments. The MCF-7 mock cell line, a clone of MCF-7 stably transfected with the empty vector, was used as a negative control for ERβ expression. The cell lines express endogenous ERα. Initially, we measured the mRNA levels of both ERα and ERβ at conditions similar to those of the ChIP and Re-ChIP experiments. As shown in Fig. 1A, the MCF-7 mock cell line and the MCF-7 ERβ cell line express equal amounts of ERα mRNA levels. In the MCF-7 ERβ cell line, ERβ mRNA levels were similar to those of ERα, while the ERβ mRNA levels were undetectable in the MCF-7 mock cell line. Protein levels of ERα and ERβ were quantified, under the same conditions, by western blot analysis using purified ERα and ERβ proteins as standards. As shown in Fig. 1B, high levels of ERβ protein were observed in the MCF-7 ERβ cell line, whereas no expression of ERβ was detected in the MCF-7 mock cell line. In Fig. 1C, the amounts of expressed ERα and ERβ proteins are shown as derived from comparison with those of the corresponding purified proteins of known concentration. This analysis revealed that the protein ratio of ERα/ERβ is 0.7:1 in the MCF-7 ERβ cell line under the conditions used in our ChiP and Re-ChiP experiments.

Binding of ERα/β heterodimers to DNA in intact chromatin

To demonstrate binding of ERα/β heterodimers to intact chromatin, we performed Re-ChiP experiments. ERα/β heterodimers were isolated by precipitation with the anti-ERβ antibody followed by the anti-ERα antibody. DNA-bound ERα/β was confirmed by western blot analysis showing that ERβ can be detected in protein–DNA complexes after sequential precipitation with anti-ERβ and anti-ERα antibodies. The interaction of ERα/β heterodimers with specific DNA-binding regions was further assessed by conventional PCR targeting a region of the pS2 promoter known to bind ERα and ERβ (Fig. 2A). Additionally, binding to the pS2 promoter region, after sequential immunoprecipitation with ERβ and ERα antibodies, was confirmed using RT PCR. As shown in Fig. 2B, a significant recruitment of ERα to the pS2 promoter was observed in the MCF-7 mock cell line, while ERα-, and ERβ, as well as ERα/β heterodimers, were recruited to the pS2 promoter in the MCF-7 ERβ cell line.

Our group has recently identified genome-wide ERα- and ERβ-binding regions in the MCF-7 ERβ cell line by the ChIP-on-chip assay (unpublished data). Based on this information, 12 regions (each labeled by the closest gene in Fig. 3) that were identified as binding regions for both ERα and ERβ with similar signal intensity in the ChIP-on-chip analysis (ratio of ChIP sample to input sample) were selected for further studies of the

Table 2 Primer pairs for amplification of chromatin immunoprecipitation assay (ChiP) and Re-ChiP enriched regions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>18S</td>
<td>GCTTAATTTGACTCAACACGGGA</td>
<td>AGCTATCAATGTCATCTCTGTC</td>
</tr>
<tr>
<td>pS2</td>
<td>CCT CCC GGC AGG GTA AAT AC</td>
<td>CCG GCC ATC TCT CAT TAT GAA</td>
</tr>
<tr>
<td>NBPF1</td>
<td>AGC GGC ATC CCC AGT GT</td>
<td>AGG GTG CCT GGA GCT TAA GC</td>
</tr>
<tr>
<td>NBPF4</td>
<td>GGC TGC AGA GGT GGG TAT GA</td>
<td>GCC CCA TGG GCA GAT CAC T</td>
</tr>
<tr>
<td>VANGL1</td>
<td>GGT TGC GTG TGT GCG A</td>
<td>AGT TAG GTG GGC AGC AAC CTT</td>
</tr>
<tr>
<td>NOTCH2</td>
<td>CCA TTC TTG CTC TTG CCT TAG AG</td>
<td>GCT TGT GGC CAT GTC TTT C</td>
</tr>
<tr>
<td>NOTCH2NL</td>
<td>CAG CAG GTG AGA TTC CAT CGA</td>
<td>TGA GAG CCT GTT TTC TTG AGC AT</td>
</tr>
<tr>
<td>BCL9</td>
<td>TGG ACA GAA GCC CTG GAG ATG</td>
<td>GCC TGC CAG GTT TCA GGA A</td>
</tr>
<tr>
<td>NBPF15</td>
<td>CTT GTT TTA GCT CAT CTG TCG ATC A</td>
<td>GGG TGA AAA GTG AGC CTT TTA T</td>
</tr>
<tr>
<td>PRUNE</td>
<td>GAG AGG AAA AAG CCA AGG TTA CA</td>
<td>ACA GCC TCG GCA AAT TCG TTC T</td>
</tr>
<tr>
<td>PBX1</td>
<td>TGT CTC CGC GCT TTG TGG</td>
<td>GCC TGG CTC TCC TTC TA</td>
</tr>
<tr>
<td>NRS82</td>
<td>AAG CAA AGA GAT GAT GGA TGT AAC TC</td>
<td>CTT TTT TCC TCC CCA GGT GTG</td>
</tr>
<tr>
<td>ADORA1</td>
<td>GAT GGA TGG GAA CAC ATT GGT</td>
<td>TGG TGG CGG AGC ACA AA</td>
</tr>
<tr>
<td>CAPN2</td>
<td>CTG GCA TCT GCC TAC AGC AA</td>
<td>GTG TCA TTG GCG CCC AAC AG</td>
</tr>
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binding of ERα/β heterodimers to DNA. Regions from the pS2 promoter and the 18S gene, which serve as ER-bound positive control and ER-bound negative control respectively were also included in the experiments.

The binding of ERα and ERβ to these DNA-binding regions was confirmed using ChIP followed by RT PCR (Fig. 3). All the binding sites exhibited similar fold enrichment for both ERs. There was no recruitment of any ER to a region of the 18S gene in the MCF-7 ERβ cell line. In the MCF-7 mock cell line, with no expression of ERβ, there was no detectable recruitment of ERβ to any of the binding sites tested, while the recruitment of ERα was similar to that in the MCF-7 ERβ cell line (data not shown).

To study the binding of ERα/β heterodimers to these regions in the context of intact chromatin, we performed Re-ChIP experiments. Re-ChIP was performed with the anti-ERβ antibody followed by the anti-ERα antibody and precipitated DNA was analyzed using RT PCR. In the MCF-7 ERβ cell line, all analyzed binding regions from the pS2 (trefoil factor 1), neuroblasta breakdownt point family, member 4 (NBPF4), NOTCH Drosophila homolog 2 (NOTCH2), NOTCH Drosophila homolog 2 N-terminal like (NOTCH2NL), NBPF15, PRUNE (Drosophila h-prune homolog), B-cell chronic lymphoblastic leukemia/lymphoma 9 (BCL9), pre-B-cell leukemia homeobox 1 (PBX1), nuclear receptor subfamily 5, group A, member 2 (NR5A2), adenosine A1 receptor (ADORA1), calpain 2 (CAPN2), NBPF1, and Van Gogh-like 1 (VANGL1) genes exhibited significant fold enrichment of ERα/ERβ heterodimers, whereas there was no enrichment to the analyzed region of 18S (Fig. 4).

Figure 1 (A) ERα and ERβ mRNA levels in MCF-7 mock and MCF-7 ERβ cell lines. Cells were grown in the absence of tetracycline for 4 days. Data are presented as relative mRNA levels compared with acidic ribosomal phosphoprotein PO (36B4). Results represent the mean ± s.d. values of two independent experiments conducted in six replicates. (B) ERα and ERβ protein levels in total cell extracts from MCF-7 ERβ cell lines determined by western blot analysis. Cell extracts were separated by SDS/PAGE. Anti-ERβ and anti-ERα antibodies were used for detection as indicated. (C) Quantification of ERα and ERβ protein levels in MCF-7 cells were based on standard curves (1–10 ng protein) made with pure recombinant ERα and ERβ proteins. Purified and expressed protein bands were quantified by densitometry using Scion Image Software.

Figure 2 (A) Experimental design for the Re-ChIP assay. Confirmation of ERα/β heterodimers was based on the flag tag detection of the ERβ protein by western blotting and on the amplification of a fragment from the pS2 promoter by conventional PCR. (B) ERα, ERβ, and ERα/ERβ recruitment to the pS2 promoter. MCF-7 mock and MCF-7 ERβ cells were treated and processed for ChIP and Re-ChIP assays as described in Materials and methods. Binding of ERα, ERβ, and ERα/ERβ in immunoprecipitated fractions to a fragment from the pS2 promoter was confirmed by real-time PCR. Data are presented as fold enrichment of ERα, ERβ, and ERα/β binding compared with IgG (means ± s.d., n = 2).
Recruitment of RNA polymerase II to ER\(\alpha/\beta\)-bound DNA regions

To investigate the effect of ER\(\beta\) on transcriptional regulation and in particular on the recruitment of RNA polymerase II, we performed ChIP experiments using an anti-PolII antibody. As shown in Fig. 5A, PolII is recruited to the pS2 promoter in both the MCF-7 mock and the MCF-7 ER\(\beta\) cell lines. However, the PolII recruitment is significantly decreased by ER\(\beta\) expression in the MCF-7 ER\(\beta\) cell line (Fig. 5A). Re-ChIP assays (ER\(\beta\)/PolII, ER\(\alpha\)/PolII and ER\(\beta\)/ER\(\alpha\)) were used to assay co-recruitment of ERs and RNA polymerase II to the pS2 promoter. As shown in Fig. 5B, a significant co-recruitment of ER\(\alpha\) and PolII to the pS2 promoter was observed in the MCF-7 mock cell line, while, as expected, there was no co-recruitment of ER\(\beta\) and PolII or ER\(\alpha\) and ER\(\beta\) in this cell line. In the MCF-7 ER\(\beta\) cell line, significant recruitment to the pS2 promoter was observed for all three Re-ChIP assays, suggesting that PolII co-exists with ER\(\alpha\) and ER\(\beta\) on this binding site.

Estrogen treatment affects expression of genes close to ER\(\alpha/\beta\)-bound DNA regions

To assay whether the presence of ER\(\beta\) affects the expression of ER\(\alpha\)-mediated transcription, we compared the mRNA levels of seven selected genes in the MCF-7 ER\(\beta\) cell line versus the MCF-7 mock cell line in response to E\(2\) treatment and increased expression of all tested genes, following E\(2\) treatment, was observed in the MCF-7 mock cell line (Fig. 6). Interestingly, in the presence of ER\(\beta\), the expression of pS2, ADORA1, NBPF1, and NBPF4 was decreased, the expression of PRUNE and NOTCH2 not changed while the expression of BCL9 was increased.
we used a mock MCF-7 cell line. Although theous ER that express an inducible version of ER was confirmed by real-time PCR. Data are presented as fold enrichment of PolII alone or ER compared with IgG (means ± S.D., n = 2).

Discussion

In this study, we report the identification of ERα/β heterodimer-binding DNA regions in MCF-7 ERβ cells that express an inducible version of ERβ and endogenous ERα, using a Re-ChIP assay. As a negative control, we used a mock MCF-7 cell line. Although the formation of ERα/β heterodimers has been previously demonstrated using GST-pull down and gel-shift assays (Pace et al. 1997, Pettersson et al. 1997), the exact role of ERα/β heterodimers in estrogen signaling remains largely unknown. However, it is assumed that in cell types, where the two receptor subtypes are co-expressed, the formation of ERα/β heterodimers plays an important role in E2 signaling (Matthews et al. 2006).

Recent studies have explored the ERα- and ERβ-binding sites for whole genomes or for specific chromosomes (Carroll et al. 2006, Liu et al. 2008); following up on these studies, we studied ERα/β heterodimers in a cell system where both ERα and ERβ are expressed. It has been observed previously that the extent of formation of ERα/β heterodimers in a system, with both ERs present, depends upon the relative amount of contributing receptors (Li et al. 2004). Under conditions of co-expression of ERs at similar levels, we applied a Re-ChIP assay, followed by RT PCR, to characterize in a quantitative manner the recruitment of ERα/β heterodimers to various DNA-binding regions in intact chromatin. We detected, by western blotting the ERβ protein only in the ERβ–ERα and not in the ERβ–IgG immunoprecipitated samples after Re-ChIP assay (Fig. 2A). We confirmed the presence of ERα/β heterodimers on the pS2 promoter, a well-characterized promoter to which both ERα and ERβ bind in the MCF-7 ERβ cell line (Zhao et al. 2007). As shown in Fig. 2, amplification of the pS2 promoter was only detected in ERβ–ERα immunoprecipitated fractions from the MCF-7 ERβ cell line. Previously, Monroe et al. (2005) showed binding of ERα/β heterodimers to chromatin in a modified osteoblast cell line by amplifying ER-binding region of two genes, RBBP1 and DHCR7.

In order to study the simultaneous presence of ERα/β heterodimers on various binding sites in intact chromatin, we queried ER-binding sites recently mapped by the ChIP-on-chip assay (data not shown) and randomly selected 12 DNA regions, which showed similar signal intensity (ratio of ChIP sample to input sample) in the ChIP-on-chip assay for ERα and ERβ. Notably, eight binding sites are located within genes (NBPF1, NBPF4, VANGL1, NOTCH2, NOTCH2NL, BCL9, NBPF15, and PBX1), and the residual four are located in the promoter regions (NR5A2, ADORA1, CAPN2, and PRUNE).

To confirm the recruitment of both ERα and ERβ to the regions identified by the ChIP on chip experiment, we performed ChIP assays using ERα or ERβ antibodies. All the binding sites tested exhibited significant recruitment of ERα and ERβ and the level of recruitment was similar for both receptors. Next, we examined the binding of ERα/β heterodimers to the selected DNA regions using a Re-ChIP assay. In the MCF-7 ERβ cell line, all the investigated DNA-binding regions
showed significant recruitment of heterodimers. However, it is notable that different binding sites exhibit different fold enrichment of ERα/β heterodimers. This could be attributed to variations in affinity of heterodimers to various ER-binding sites depending upon the sequence of the binding regions. In a preliminary analysis, using the CONSITE program (Sandelin et al. 2004) and setting TF-score cut-off at 85%, we found that all of the selected binding sites contained half-ERE motif, 70% contained AP1-motif (pS2, NOTCH2, NOTCH2NL, PRUNE, BCL9, NR5A2, ADORA1, NBPF1, and VANGL1), but only 23% contained full-ERE (pS2, NR5A2, and ADORA1). However, the detailed dissection of the mechanism of heterodimers’ recruitment needs a larger number of such identified regions and a systematic binding motifs enrichment analysis with strict background control. Furthermore, our data showed that the ratio of enrichment in the ERα/β Re-ChIP assay versus the enrichment in the ERα and ERβ ChIP assays varied. For example, for genes such as NBPF4, NOTCH2, NBPF15, and PRUNE, this ratio is high, suggesting that heterodimer recruitment may play a more significant role in the transcriptional regulation of these genes. Our results also showed that overall there is less recruitment of heterodimers compared with ERα or ERβ alone. This may be due to the recovery being less after two rounds of ChIP (Re-Chip assay) compared with the single ChIP assay. Additionally, previous kinetic studies have revealed that heterodimers exhibit low binding affinity (Jisa & Jungbauer 2003).

Previous work has shown that the presence of ERβ can affect ERα’s transcriptional activity (Chang et al. 2006). To verify this in our cell system, we evaluated the mRNA levels of some selected genes after E2 treatment. Our data showed that mRNA levels of genes that are implicated in cell proliferation, like pS2, and Adora1 are down-regulated, when ERβ is expressed (Ribieras et al. 1998, Mirza et al. 2005). The decrease of pS2 mRNA levels in the MCF-7 ERβ cells is in accordance with the decrease of PolII recruitment to the pS2 promoter in the same cell line as compared with MCF-7 mock cells. The mRNA levels of NBPF1 and NBPF4, whose function is not fully explored, are also decreased in the presence of ERβ (Vandepoele et al. 2005). mRNA levels of BCL9, which is participating in the wnt signaling of cell proliferation and development, were up-regulated in the MCF-7 ERβ cell line compared with the MCF-7 mock cell line (Willert & Jones 2006). Finally, the mRNA levels of NOTCH2 and PRUNE were unaffected by the presence of ERβ. These genes are both implicated in the aggressiveness of breast neoplasm (Marino & Zollo 2007, Wang et al. 2008). Considering that all selected genes exhibit significant ERα/β heterodimer recruitment, our data suggest that ERβ may have an activating or inhibitory influence on ERα through heterodimerization. Furthermore, using a Re-ChIP assay and various combinations of ERα, ERβ, and PolII antibodies, we showed that PolII co-occupied the same DNA-binding regions as ERα and ERβ, consistent with a role of ERα/β heterodimers in transcriptional regulation.

In this study, we report the recruitment of ERα/β heterodimers to various ER-binding DNA regions in an intact cell system, following E2 treatment. Further experimental data are needed to elucidate the detailed mechanism of formation of ERα/β heterodimers, their function, and the influence of ligands.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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