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# Human Estrogen Receptor β-Gene Structure, Chromosomal Localization, and Expression Pattern*

EVA ENMARK, MARKKU PELTO-HUIKKO, KAJ GRANDIEN, SVETLANA LAGERCRANTZ, JACOB LAGERCRANTZ, GABRIEL FRIED, MAGNUS NORDENSKJÖLD, AND JAN-ÅKE GUSTAFSSON

*Center for Biotechnology (E.E.) and Department of Medical Nutrition (M.P-H., K.G., J-Å.G.), Novum, S-141 57 Huddinge, Sweden; Department of Molecular Medicine (S.L., J.L., M.N.), Karolinska Hospital, CMM, L8:01 and L8:02, S-171 76 Stockholm, Sweden; Department of Anatomy (M.P-H.), Medical School, University of Tampere and Department of Pathology (M.P-H.), Tampere University Hospital, Tampere, Finland; Department of Physiology and Pharmacology (G.F.), Karolinska Institute and Department of Woman and Child Health (G.F.), Karolinska Hospital, S-171 77 Stockholm, Sweden*

**ABSTRACT**

The estrogen receptor (ER) is a ligand-activated transcription factor that mediates the effects of the steroid hormone 17β-estradiol, in both males and females. Since the isolation and cloning of ER, the consensus has been that only one such receptor exists.

The finding of a second subtype of ER (ERβ) has caused considerable excitement amongst endocrinologists. In this article, we present data regarding the genomic structure and chromosomal localization of the human ERβ gene, demonstrating that two independent ER genes do exist in the human. Furthermore, we present data regarding the tissue distribution of human ERβ, showing that this receptor is expressed in multiple tissues. For instance, ERβ is found in developing spermatids of the testis, a finding of potential relevance for the ongoing debate on the effects of environmental estrogens on sperm counts. In addition, we find ERβ in ovarian granulosa cells, indicating that estrogens also participate in the regulation of follicular growth in the human. (*J Clin Endocrinol Metab* **82:** 4258–4265, 1997)

ESTROGENS are steroid hormones that have profound effects on both the female and the male reproductive systems. They also have important roles in the cardiovascular system and in maintenance of bone tissue. These effects are all mediated by a ligand-activated transcription factor, the estrogen receptor (ER).

Our unexpected discovery of a second subtype of the estrogen receptor, ERβ, approximately 10 yr after the cloning of ERα (1) has raised a number of questions regarding the respective physiological roles of these two receptors (2). Some of the most interesting aspects of the new estrogen receptor refer to clinically important issues such as fertility, bone stability, and cardiovascular health.

It has previously been assumed that ERα, the first estrogen receptor to be cloned, was indispensable for maintenance of these functions. However, studies of ERα knock-out (ERKO) mice show that the gene deletion has little or no effect on bone stability or on the cardiovascular system (3). One might therefore speculate that ERβ has an important role in these tissues.

In this study we report on the tissue distribution of human ERβ and present several examples of tissues where ERβ might be of importance.

Characterization of the organization of a gene can give important clues to the evolutionary relationships within a gene family. Knowledge of the structure of the human ERβ gene is also important for the characterization of human ERβ in hereditary disorders, *e.g.* the hereditary forms of prostate cancer and Alzheimer's disease. We have therefore begun to characterize the genomic organization of the ERβ gene in mouse and human. Interestingly, the ERβ gene appears to be considerably shorter than the ERα gene. The possible functional implication of this difference between the ERα and ERβ genes is discussed.

## Materials and Methods

### *Cloning of human ERβ complimentary DNA (cDNA)*

Fragments from the N-terminal and hinge domain of rat ERβ were used to screen human cDNA libraries from ovary and testis (Clontech, Palo Alto, CA). Several partial cDNA clones were obtained, which were then joined by PCR and restriction enzyme digestion. The first 45 and the last 59 amino acids were obtained by PCR on human ovary cDNA, using primers derived from the rat ERβ cDNA sequence. The obtained sequence was essentially identical to a partial human ERβ sequence published recently (9).

### *Exon/intron structure*

Pairs of PCR primers corresponding to fragments in the N-terminal and ligand-binding domains were designed and used to identify a mouse P1 clone containing the translated exons of ERβ (Genome Systems Inc., St. Louis, MO). The parts containing exons were subcloned,

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Address correspondence and requests for reprints to: Jan-Åke Gustafsson, Department of Medical Nutrition, Huddinge University Hospital, NOVUM, Huddinge, Sweden S-141 86.

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and the distance between the exons was determined by subcloning of the respective intron or by PCR.

The human exon/intron structure was determined by PCR on total human genomic DNA, using primers in the respective exons, as inferred by the mouse genomic structure. The respective PCR products were subcloned, and the sequence of the respective ends determined by cycle sequencing.

### *PCR mapping*

The cell lines used to determine the chromosomal localization of the ERβ gene by PCR were human-rodent somatic hybrids (NIGMS Coriell Cell Repositories, Camden, NJ). Each cell line retains one of the human chromosomes in addition to the rodent genome. PCR screening was performed with oligonucleotides designed from the ligand-binding domain of human ERβ cDNA.

### *Fluorescence in situ hybridization*

Chromosome slides were prepared from lymphocyte cultures as previously described (4). A centromere-specific hybridization was included using a probe specific for the chromosome 14 and 22 centromeres. A human ERβ P1 clone was obtained from a reference library database, library no. 700 (P1 human), Max-Planck Institut für Molekular Genetik, Berlin, Germany. This clone was labeled with biotin-12-dUTP (Gibco BRL, Gaithersburg, MD), and the centromere-specific probe was labeled with fluoro-red-dUTP (Amersham International, Amersham, Buckingshamshire, UK) by nick translation.

The P1-DNA probe was preannealed with 3 μg human Cot-1 DNA (Gibco BRL) for 60 min at 37 C and hybridized together with the centromere 14/22 specific probe to human metaphase chromosomes. The slides were pretreated and hybridized as previously described (4). In total, 30 metaphases were analyzed, and the hybridization signals were seen in the metaphase chromosomes as two symmetrical dots on 14q22–24.

### *Digital image microscopy*

The signals were visualized using a Zeiss Axiophot fluorescence microscope equipped with a cooled CCD-camera (Photometrics Nu 200/CH 250, Tuscon, AZ) for image capturing. The results were analyzed on a Macintosh Quadra 950 computer (Macintosh, Cuppertino, CA) using the SmartCapture software (Digital Scientific, Cambridge).

### *Northern blot analysis*

The Multiple Tissue Northern blots are products of Clontech. The Northern blot contains messenger RNA (mRNA) from spleen, thymus, prostate, ovary, testis, small intestine, colon, and peripheral blood leukocytes (PBL). The filters were hybridized as recommended by the supplier, using either a probe corresponding to 300 bp in the N-terminal domain or a probe corresponding to 200 bp in the hinge domain of the human ERβ cDNA.

### *Preparation of isolated granulosa cells*

Luteinized granulosa cells were obtained (with informed consent from the patients) from follicular fluid obtained at ovum-pick-up for *in vitro* fertilization. Follicular fluid was layered on a gradient of Ficoll-Paque (Pharmacia, Uppsala, Sweden) and centrifuged at 800 *g* for 30 mins. Granulosa cells were removed from the gradient interface and resuspended in culture medium [serum-free hybridoma medium (Sigma, Stockholm, Sweden) + 4% fetal calf serum (Gibco, Stockholm, Sweden)] and plated in 8 cm dishes. Cells were cultured for 3–5 days, after which cells were lysed and isolated for RNA.

### *RT-PCR analysis*

The primer pair used for ERα was AATTCAGATAATCGACGCCAG and GTGTTTCAACATTCTCCCTCCTC, corresponding to nucleotides 457–477 and 801–779 of the human ERα open reading frame. The primer pair used for ERβ was TAGTGGTCCATCGCCAGTTAT and GGGAGCCACACTTCACCAT, corresponding to nucleotides 125–145 and 517–499 of the human ERβ ORF. The RT-PCR was performed essentially as described previously (5). cDNA-synthesis was done with 1 μg of total RNA, using Superscript RT (Life Technologies, Stockholm, Sweden). One twelth of the cCNA synthesis was used in PCR, using *Taq*-polymerase (Pharmacia). The PCR was carried out in a 9600 Thermocycler (Perkin Elmer, Foster City, CA) using the following program: 95 C 30 sec, 33 × (95 C 30 sec, 56 C 15 sec, 72 C 60 sec), 72 C 3 min. The PCR products were separated on a 2% Nusieve agarose gel (FMC, Rockland, ME) and blotted onto a HybondN + membrane (Amersham), according to the manufacturer's recommendations. The filter was then probed with internal oligonucleotide probes specific for ERα (CCAATGACAAGGGAAGTATGG), ERβ (GTTCCCACTAACCTTCCTTTTCA), or actin (GATGACCCAGATCATGTTTGA).

### *RNAse protection assay (RPA)*

The vectors used for generation of RPA probes were: pBS-hERα(1016–1268), the insert corresponding to nucleotides 1016–1268 of the human hERα ORF; pBS-hERβ P/E-T7, the insert corresponding to nucleotides 792–978 of the human ERβ ORF; and TKS-ActP/Act3-T7, the insert corresponding to nucleotides 374–492 of the human β-actin cDNA ORF.

The RNAse protection assay was performed essentially as described previously (6). The gels were exposed on film as well as analyzed using a Fujix bioimager (Fuji, Tokyo, Japan). Calculation of mRNA levels was based on the parallell quantification of known amounts of *in vitro* transcribed ERα and ERβ mRNA, respectively.

Total RNA was prepared as described previously (7). After the RNA had been dissolved, the concentration was determined spectrophotometrically, and the intregrity of the RNA was verified by agarose gel electophoresis.

### *In situ hybridization*

Four oligonucleotides derived from human ERβ cDNA (nucleotides 542–589, 1089–1136, 1326–1373, and 1384–1431) were labeled to a specific activity of $1 \times 10^9$ cpm/mg at the 3′-end with $^{33}$P-dATP (NEN, Boston, MA), using terminal deoxynucleotidyltransferase (Amersham). All probes produced similar results. Several control probes of the same length, with similar GC-content and specific activity, were used to ascertain the specificity of the hybridizations. Addition of 100 mol/L excess of the unlabeled probe abolished all hybridization signals.

Human tissues for *in situ* hybridization were obtained after surgery performed for different reasons. Unless mentioned, normal tissues were used.

The tissues were frozen and sectioned in Microm HM 500 cryostat at 14 μm and thawed onto Probe-On glass slides (Fisher Scientific, Philadelphia, PA). The sections were stored at 20 C until used. *In situ* hybridization was carried out as previously described (8). The slides were incubated in humidified boxes at 42 C for 18 h with 5 ng/mL of the labeled probe in the hybridization mixture, washed, dried, and covered with Amersham β-max autoradiography film (Amersham) for 30–60 days. Alternatively, the sections were dipped in Kodak NTB2 nuclear track emulsion (Rochester, NY) and exposed for 90 days at 4 C. The sections were examined in a Nikon Microphot-FX microscope (Alexandria, VA) equipped for dark-field and epipolarization microscopy. T-Max 100 black-and-white film (Kodak) was used for photography. Finally, the sections were stained with cresyl violet and analyzed under brightfield conditions.

## Results

A partial cDNA sequence for human ERβ was published recently (9). We have now cloned a full-length human ERβ cDNA. Human ERβ shows approximately 89% identity to rat ERβ, 88% to mouse ERβ, and 47% to human ERα, in its translated portion (Table 1).

We have cloned the translated exons from both the mouse and human ERβ gene (see Fig. 6A), thus determining the exon/intron organization.

Using PCR and genomic cloning, we have determined the

**TABLE 1.** Percent identity to human ERβ

| Receptor | Domain | | | | |
|---|---|---|---|---|---|
| | NHD | DBD | Hinge | LBD | F |
| rERβ | 79.6 | 98.5 | 85.6 | 93.4 | 78.6 |
| mERβ | 80.6 | 98.5 | 84.4 | 91.9 | 78.6 |
| hERα | 17.5 | 97.0 | 30.0 | 59.1 | 17.9 |
| rtER | 15.5 | 93.9 | 25.6 | 60.6 | 21.4 |

approximate size of the ERβ gene. The translated exons of the mouse ERβ gene, which have been characterized most carefully, span approximately 40 kb. The human gene, which has been less well characterized, seems to be of similar size.

We have mapped the chromosomal localization of human ERβ. Using PCR technique, we show that the human ERβ gene is localized on chromosome 14 (Fig. 1, A), and using the FISH technique we have mapped ERβ to 14q22–24 (Fig. 1, B). To broadly characterize the tissue distribution of human ERβ, we have employed a "spot blot" technique to detect the presence of ERβ RNA in several human tissues. Using this technique, the highest ERβ expression was found in kidney, thymus, and small intestine. High expression was also seen in lung, spleen, pituitary gland, blood leukocytes, bone marrow, colon, and uterus (not shown).

Using RT-PCR technique, we further detected human ERβ in uterus and mammary gland tissue, as well as in several breast tumors and breast tumor cell lines (Fig. 2).

Using Northern blotting, we found strong expression of human ERβ in testis and in ovary (Fig. 3). The major transcript is approximately 7 kb, but at least one weak band of higher molecular weight, around 9.5 kb, can also be seen, suggesting multiple transcriptional start sites and/or polyadenylation sites.

To be able to study the expression of ERβ at the cellular level in some of these tissues, we used *in situ* hybridization. These analyses show that the ERβ mRNA is highly expressed in the mucosa of the stomach, duodenum, colon and rectum (Fig. 4, a and b), whereas the muscle layer is devoid of labeling. In kidney, the expression seems to be strongest in the cortex (Fig. 4c), and in dipped sections a strong signal is evident in convoluted tubules in cortex (Fig. 4d). Also the transitional epithelium in renal pelvis expresses ERβ (Fig. 4c). In lung a signal is detected both in the lung parenchyma and in the blood vessels (Fig. 4f). In breast, the epithelium of the tubules expresses detectable levels of ERβ, and in some breast cancers we have detected ERβ (Fig. 4g). In the ovary, the signal is localized to the stroma of the cortex and in blood vessels of the medulla (Fig. 4h) as well as to the granulosa cells (not shown). In lymph nodes a large portion of the lymphocytes express ERβ (Fig. 4i). We can also detect ERβ signals with *in situ* hybridization in the uterus and the adrenal cortex (not shown), thus largely confirming the spot blot data. In prostate low signal is seen in the epithelium of secretory alveoli while the stroma is nonlabeled (Fig. 4j). In testis, ERβ is localized in the seminiferous epithelium, whereas the interstitial Leydig cells are nonlabelled (Fig. 4k). In dipped sections, grains demonstrating ERβ mRNA can be seen over developing spermatids, particularly primary spermatocytes and early round spermatids, whereas Sertoli cells and germ cells at earlier stages of differentiation are negative (Fig. 4l).

To quantify the ratio between ERα and ERβ in a few of the cell types mentioned above, we performed RNAse protection assays. These assays show the highest expression of ERβ mRNA in ovary and isolated granulosa cells, mammary gland and lung, whereas the ERα signal was strongest in endometrium, ovary, and in one of the breast tumor samples (Fig. 5, a and b). Calculation of the ratio between the ERα and ERβ mRNA levels shows that, in isolated granulosa cells and in cells from human umbilical vein endothelium (HUVEC), only ERβ is expressed. The analyzed prostate tumor sample clearly demonstrated higher levels of ERβ mRNA than ERα mRNA, although the total levels of both transcripts were low. In mammary gland, the two breast tumor samples analyzed, the endometrium and the endometrial carcinoma cell line Ishikawa, the amount of ERα mRNA was higher than ERβ mRNA. Finally, in lung and in ovary, the amounts of ERα and ERβ mRNA were approximately equal (Fig. 5 c). It should be noted, however, that in HUVEC cells and in lung, the levels of both receptor messages are low.

## Discussion

The analysis of the human ERα gene has shown that it is a very large gene, with the translated exons spanning more than 140 kb (10). The ER genes from fish, however, are considerably smaller spanning approximately 30–40 kb (11, 12). Our data show that the size of the ERβ gene is similar to those of the fish ERs (Fig 6, C). It has been speculated that the size of the introns might influence the transcriptional efficiency of a gene, particularly in situations of rapid cell division (13). The relevance of this phenomenon for mammalian genes has never been verified. For other paralogous genes in the nuclear receptor superfamily, the gene size has been shown to vary at least to the same extent as for the ER subtypes. The PPAR genes, for example, differ in size from 30–105 kb in the mouse (14).

All exon/intron boundaries are well conserved in the ERβ gene as compared with the human ERα gene. Notably, the only difference observed in the genomic organization of the ER genes is the intron present in the middle of the D domain of the ER isolated from rainbow trout (11) and *O. aureus*, which is absent from both ERα and ERβ (Fig. 7b). Interestingly, sequence comparison of all known estrogen receptors shows that these receptors seem to form three groups, where the receptors cloned from fish constitute a separate subgroup. The exception in the fish subgroup is the ER cloned from Japanese eel (15), which actually represents an ERβ homologue. The question whether this third subgroup represents an "ERγ" is obviously interesting. However, extensive PCR studies employing primers designed on the basis of the "fish ER" have hitherto failed to show the existence of a mammalian ERγ (E. Enmark, unpublished observations).

Using the FISH technique, we have mapped ERβ to 14q22–24. Since the human ERα gene has been mapped to the long arm of chromosome 6, this definitely excludes the possibility of differential splicing to explain the formation of the ERβ isoform. 14q22–24 represents a region homologous to mouse chromosome 12, to which the mouse ERβ has recently been

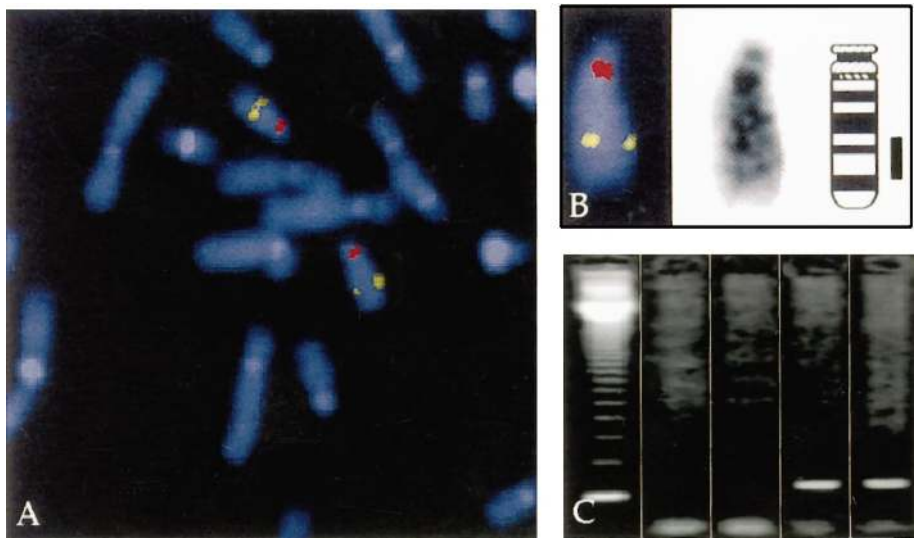


FIG. 1. Chromosomal localization of human ERβ. Part A shows a typical metaphase cell hybridized with a centromere 14/22 specific probe to human metaphase chromosomes and with a probe for human ERβ (see text). Part B shows an alignment of chromosome 14 hybridized to ERβ probe and a banded chromosome, where 14q22–24 is marked. Part C shows PCR screening of human-rodent somatic hybrids with oligonucleotides designed from the ligand-binding domain of the human ERβ gene. Lane 1, total human genomic DNA; lane 2, chromosome 14 DNA; lane 3, total hamster DNA.

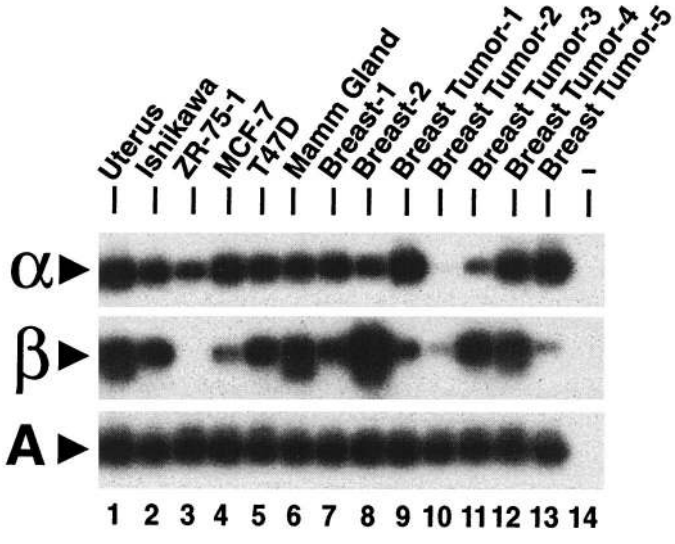


FIG. 2. Detection of ERα and ERβ expression by RT-PCR. Southern blot analysis of the RT-PCR products was carried out, as detailed in *Materials and Methods*. Ishikawa is a human endometrial carcinoma cell line, MCF7, ZR-75–1, and T47D are human breast cancer cell lines.

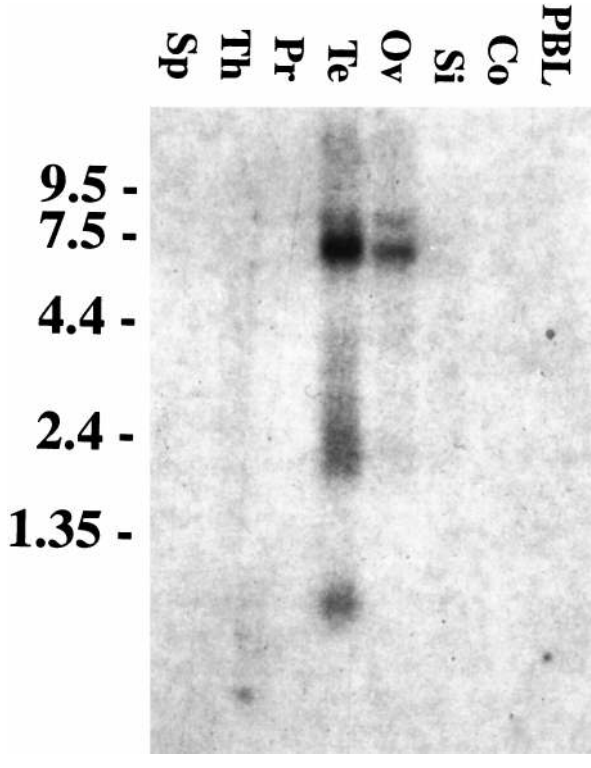


FIG. 3. Northern blot analysis of human ERβ. The RNA lanes contain from left to right: spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocytes.

mapped using interspecific backcross analysis (16). Furthermore, 14q22–24 is close to a recently identified gene associated with early onset of Alzheimer's disease (17). It has been claimed that estrogen replacement therapy reduces the risk of Alzheimer's disease in women, or improves this condition in some patients (18). Furthermore, this region of chromosome 14 is frequently involved in rearrangements in human uterine leiomyoma (19) and neoplasms of the kidney (20). A more detailed mapping of this chromosomal region, as well as studies on patient material, will in time tell whether this chromosomal localization of ERβ has any relevance to the diseases mentioned.

The human ERα has been shown to have at least three separate promoters with different but overlapping tissue distribution (5, 21). The mouse ERβ was recently shown to give at least four bands on Northern blots (16), possibly indicating that the ERβ gene is also characterized by multiple promoters. Interestingly, we and others (9) have observed multiple transcript sizes for human ERβ.

Furthermore, in the *O.aureus* ER, two alternative polyadenylation sites located approximately 300 bp apart have been found, in addition to two different transcription start sites (12).

Estrogens have important functions both in the reproductive system and in other tissues such as bone and the cardiovascular system. We have recently reported that the expression of ERβ in the rat is highest in ovary and prostate,

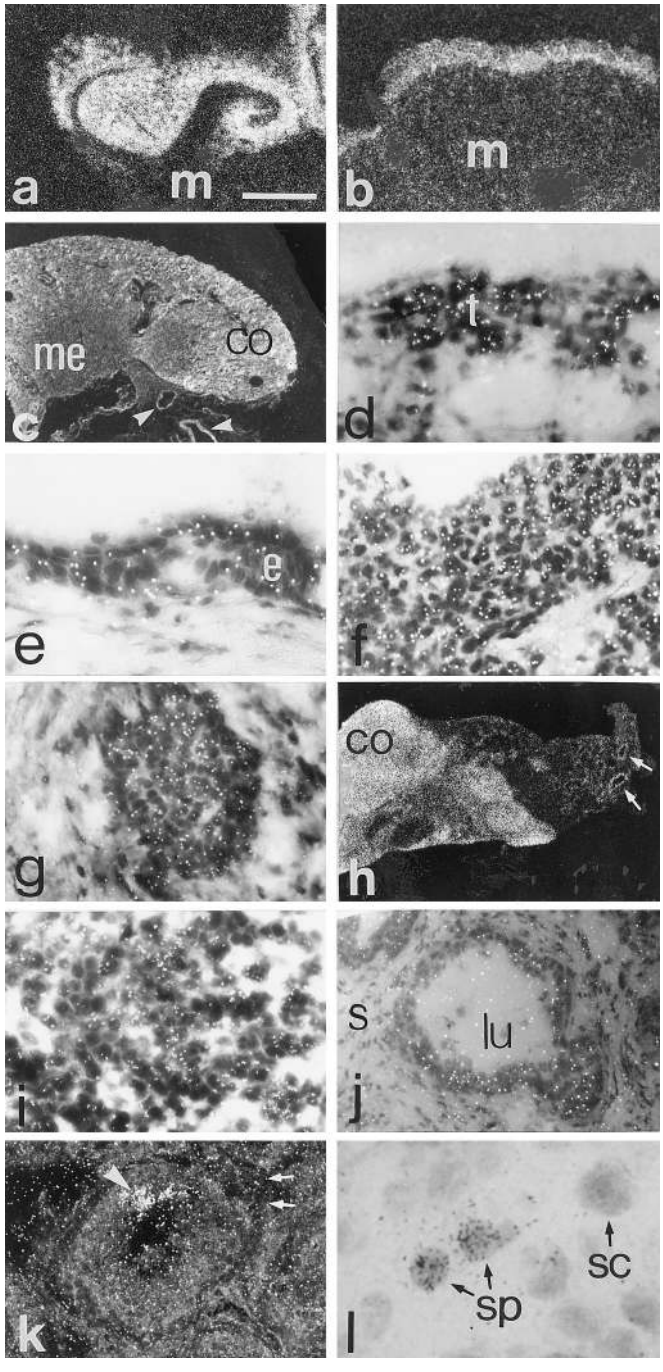


FIG. 4. Demonstration of ERβ mRNA in human tissues by *in situ* hybridization. Signal for ERβ can be seen in the mucosa of duodenum (**a**) and rectum (**b**) but the muscle layer (m) is devoid of labeling. In the kidney strongest signal can be seen in the cortex (co) and the medulla (me) shows lower levels. Blood vessels in renal pelvis are also labeled (*arrowheads*) (**c**). In dipped sections strongest signal is present in convoluted tubules (marked t) in kidney cortex (**d**) and a signal can be seen also in the transitional epithelium (e) in renal pelvis (**e**). Most of the cells in fetal lung express ERβ mRNA (**f**). Breast cancer cells show clear signal for ERβ (**g**). In the ovary a signal is present in the cortex (co) and in the blood vessels (*arrows*) of the medulla (**h**). A large portion of the lymphocytes in lymph node express ERβ (**i**). In prostata low signal is present in the epithelium of secretory alveoli (lu) while surrounding stroma (s) does not have detectable levels of ERβ (**j**). In testis, ERβ is localized in the seminiferous epithelium (*arrowhead*) but the interstitial Leydig cells (*arrows*) are nonlabeled (**k**). In dipped section, grains demonstrating ERβ mRNA can be seen over developing spermatids (sp) whereas Sertoli cells (sc) and germ cells at earlier stages of differentation are negative (l). Bar in panel a represents 0.15 mm (a, b), 0.5 mm (c), 50 μm (d), 30 μm (e, i), 160 μm (f), 100 μm (g, j), 0.2 mm (h), 150 μm (k), and 20 μm (**l**).

with lower but significant expression also in other tissues (1). The most striking difference between human and rodent is seen in the prostate, where the expression of ERβ is very high in the rat, but is relatively low in the human, as judged from Northern blot and *in situ* hybridization. In ovaries, the stroma of the cortex expresses ERβ in the human but not in the rat. Finally, the high levels of ERβ seen in the gastrointestinal tract of the human contrast to much lower levels in the rat.

Using cultured human granulosa cells we show that, just as reported in the rat (1), the granulosa cells in humans contain only ERβ mRNA. Thus, it can be concluded that ERβ is likely to play an important role in the regulation of follicular growth and oocyte development.

In the testis, ERα has previously been reported to be expressed in the Leydig cells of the testis, where no ERβ signal was detected (22). In contrast, we find ERβ expressed in the developing spermatids, where ERα is absent.

During recent years, there has been an intensive debate concerning alleged effects of different xenobiotics on the reproductive ability of animals, particularly in fish and man (reviewed in 23). A class of compounds called "environmental estrogens", including polychlorinate biphenals, has been the particular focus. We have shown that both ERα and ERβ may bind at least some of these compounds (24). Although the affinity is relatively low, ERβ binds two xenoestrogens, methoxychlor and bisphenol A, with considerably higher affinity than ERα. In this paper we show that human ERβ is expressed in the developing spermatocytes of the testis. It is tempting to speculate that some of the claimed effects of environmental estrogens on fertility might be mediated via ERβ.

We show here that both ERα and ERβ are expressed in human breast. In breast tumors the expression of the two receptors seems to vary. In future characterization of tumors from breast it might thus be relevant to determine the expression of both estrogen receptors.

It has long been known that estrogens have important effects on the immune system. One of many examples refers to pregnancy, where the immune system is significantly downregulated, leading to decreased size of both spleen and thymus. Most autoimmune diseases are also more common in women than in men (25). Many of the tissues in which we find high expression of human ERβ are related to the immune system. An exciting possibility is that some of the immunomodulatory effects of estrogen might be mediated via ERβ. Interestingly, the ERβ-containing pituitary is a common modulator of both the immune system and the endocrine system (26).

Recently, it was shown that in ERKO mice, where the ERα gene had been disrupted, the atheroprotective effect of estrogen was unchanged, using a carotid arterial injury model. The authors concluded that the protective effect was independent of ERα (27). In this study, we report that in humans,

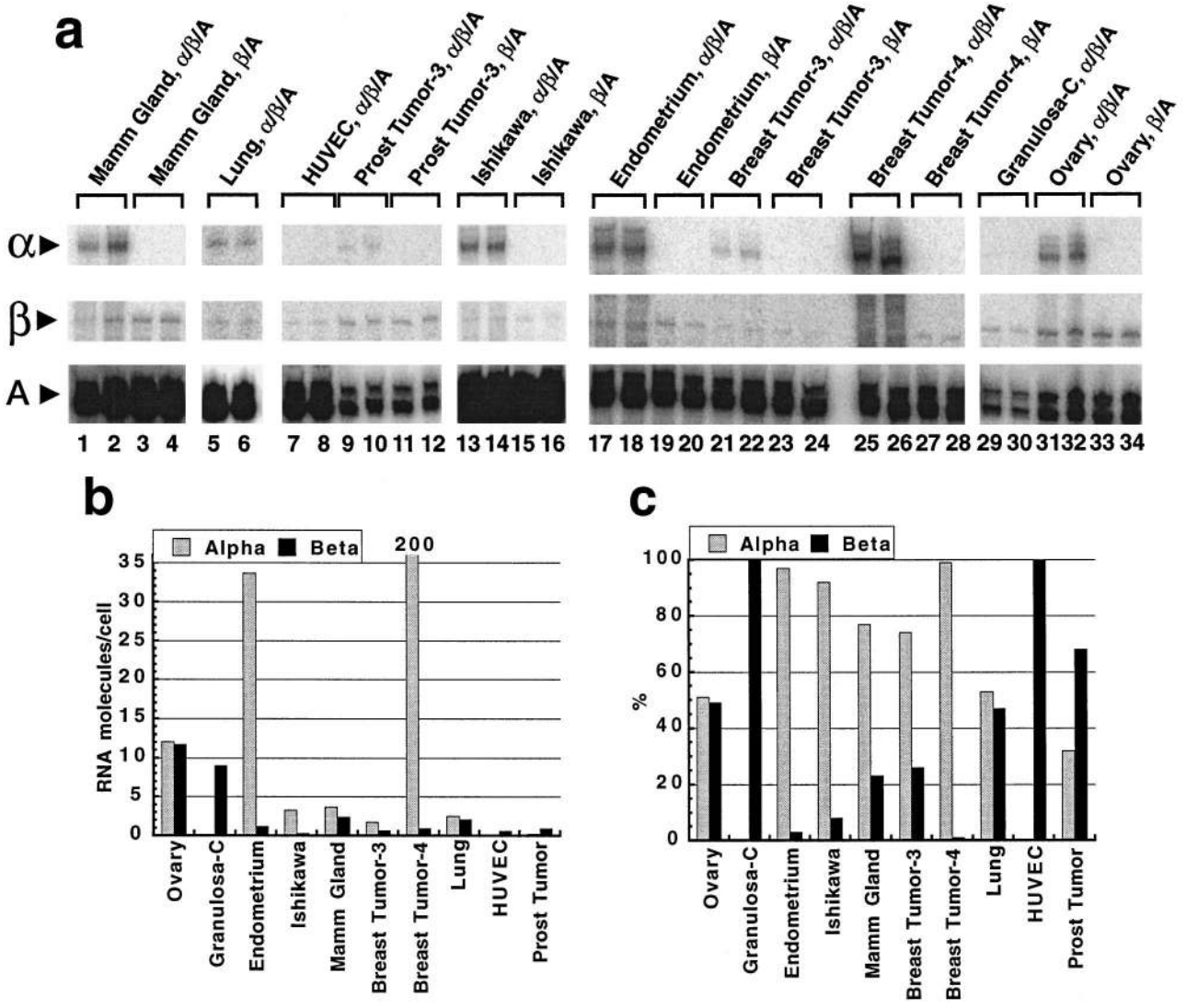


FIG. 5. A, RPA assay on tissue samples and cell lines for ERα and ERβ expression. Amount of RNA loaded in each lane: 1–4, 20 μg; 5–6, 10 μg, 7–8, 20 μg; 9–12, 10 μg; 13–20, 20 μg; 21–28, 15 μg; 29–30, 2.5 μg; 31–34, 15 μg. B, Absolute ERα and ERβ mRNA levels calculated as number of RNA molecules per cell. The amount of RNA was calculated using an ERα or ERβ *in vitro* transcribed standard mRNA with known concentration. Pure, cultured granulosa cells are denoted Granulosa-C. It should be noted that the levels for some tissues are extremely low (*e.g.* HUVEC and prostate tumor). C, Relative levels of ERα and ERβ mRNA calculated from RPA.

ERβ but not ERα is expressed in the umbilical vein endothelial cells, a finding well in line with these observations in mice. This finding may be of potential relevance for understanding the atheroprotective effects of estrogen.

We have previously shown that ERβ has a relatively high affinity for several plant-derived substances with estrogenic activity, considerably higher than that exhibited by ERα (24). It is possible that the human ERβ expressed in the gastrointestinal tract is exposed to these compounds via the diet. For several years it has been claimed that estrogens may protect against colon cancer (28). Similar claims have also been made for diets containing soy protein, a product rich in phytoestrogens (29). Estrogens have furthermore been shown to affect calcium uptake in the intestine through a poorly understood mechanism (30). Perhaps ERβ may mediate some of these effects.

In conclusion, we show in this report that human ERβ is highly expressed in many human organs, including some traditionally and probably erroneously considered "nontarget tissues" for estrogen.

The findings of high expression of ERβ in ovary, granulosa cells, and endometrium clearly indicate that many of the effects of estrogen on human female reproductive function may be mediated by this receptor. This is critically important, as the reports of absence of ERα from primate (monkey, human) granulosa cells (31, 32) have lead to an alternative model for the regulation of the human menstrual cycle. This model postulates that in primates, growth factors (activin, inhibin, and IGFs) take the role of estrogen in the rodent ovary (33). Our results indicate that estrogen may be as important for human ovarian function and reproduction as in the rodent.

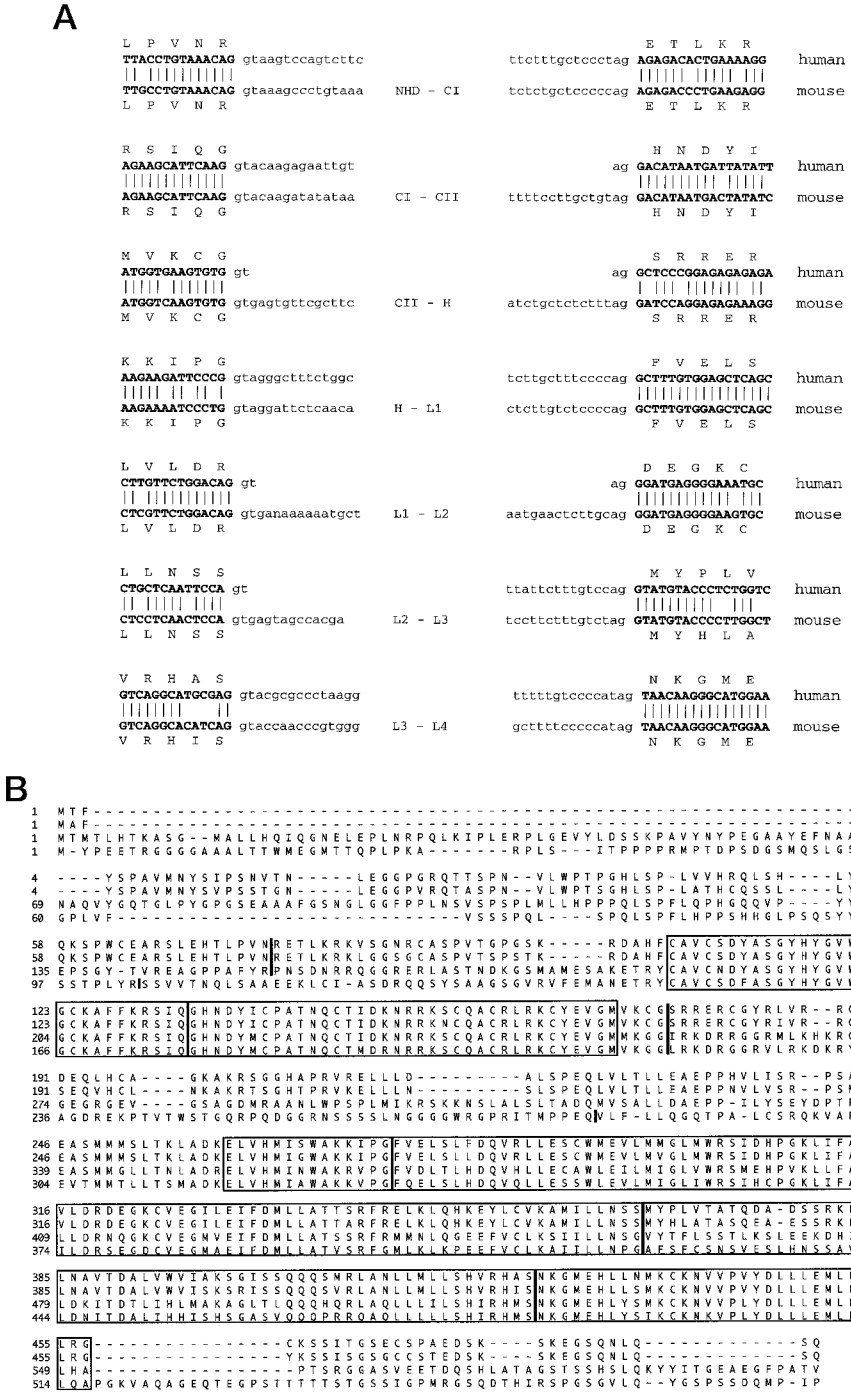


FIG. 6. A, Exon/intron borders in the human and mouse ERβ gene. In the human gene, the borders were determined by PCR. For introns bigger than approximately 8 kb, the PCR products were heterogeneous, and thus only exon sequences are shown. B, Comparison of intron positions in the human and mouse ERβ, human ERα, and rainbow trout ER. Intron borders are shown as vertical bars. For orientation, the DNA- and ligand-binding domains are boxed. The alignment was created using the Clustal alignment tool and the MegAlign program of Lasergene. C, Comparison of intron sizes (in kilobases) of ERβ, ERα, and rainbow trout ER.

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