Review

# Estrogen receptor transcription and transactivation
# Basic aspects of estrogen action

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## Abstract

Estrogen signaling has turned out to be much more complex and exciting than previously thought; the paradigm shift in our understanding of estrogen action came in 1996, when the presence of a new estrogen receptor (ER), ERβ, was reported. An intricate interplay between the classical ERα and the novel ERβ is of paramount importance for the final biological effect of estrogen in different target cells.

**Keywords:** breast, central nervous system, estrogen receptor β, estrogen receptor knockout mice, heterodimerization, prostate, uterus

## Introduction

Jensen and Jacobsen were the first to describe that the biological effects of estrogen are mediated by a receptor protein [1]. The cloning of the ER, today renamed ERα, was reported in 1986 [2,3]. For a long time, it was believed that only one ER existed; however, in 1995 a second ER, ERβ, was cloned from a rat prostate cDNA library by Gustafsson and colleagues [4••]. This finding has lead to a paradigm shift in our understanding of estrogen action, as will be evident from the different reviews in this issue of *Breast Cancer Research*.

## ERβ and ERα isoforms

Since the discovery of ERβ in rat prostate, several groups have reported the cloning of ERβ from other species [5–7] or different sized ERβ isoforms, some with extended N-termini and others with truncations and/or insertions in the C-terminal ligand binding domain (LBD). The original ERβ clone encodes a protein of 485 amino acids, designated ERβ-485. ERβ-503 has an 18 amino acid residue in frame insertion into the LBD, and has a considerably lower affinity for E2 than ERβ-485. Both ERβ-503 and ERβ-485 bind to a consensus estrogen response element (ERE) and heterodimerize with each other and with ERβ [8,9]. The coactivator SRC-1 interacts with both ERα and ERβ-485 in an estrogen-dependent manner but not with ERβ-503 [9]. An additional ERβ isoform, ERβcx [10], is identical to ERβ-530 except that the last 61 C-terminal amino acids (exon 8) are replaced by 26 unique amino acid residues. The ERβcx isoform shows no ligand binding activity and has no capacity to activate transcription of an estrogen-sensitive reporter gene [10]. Furthermore, ERβcx shows preferential heterodimerization with ERα rather than with ERβ, inhibiting ERα DNA binding and having a dominant negative effect on ligand-dependent ERβ reporter gene transactivation [10].

AF1 = activation function 1; AF2 = activation function 2; ER = estrogen receptor; ERE = estrogen response element; LBD = ligand binding domain.

Various alternatively spliced forms of ERα have also been reported [11–16]. Whether all isoforms or differentially spliced versions of ERα and ERβ, respectively, are expressed as proteins or have any major biological role warrants further investigation.

ERα and ERβ are similar in their architecture to the other members of the steroid/thyroid hormone nuclear receptor superfamily [17–22] in that they are composed of independent but interacting functional domains. Ligand-induced gene modulation by hormone receptors is due to ligand-induced conformational changes in the receptor. These conformational changes lead to receptor dimerization, receptor–DNA interaction, recruitment of and interaction with co-activators and other transcription factors, and the formation of a preinitiation complex [23–26].

In ERα, the N-terminal A/B domain encodes activation function 1 (AF1) [27–30]. Synthetic antiestrogens such as tamoxifen, raloxifene and ICI 164,384 induce a partial agonism on an ERE-based reporter gene in the presence of ERα but pure estrogen antagonism with ERβ [7,31•,32]. In ERα, different parts of AF1 are required to mediate the agonism of E2 and the partial agonism of tamoxifen [30], a particular function of ERα AF1 that is missing in ERβ [32]. Differences in the amino-terminal regions of ERα and ERβ thus constitute a possible explanation for the difference between ERα and ERβ in their response to various estrogens including antagonists such as tamoxifen and raloxifene.

The C or DNA binding domains of ERα and ERβ are highly homologous [6] with identical P-box sequences and, therefore, ERα and ERβ are likely to bind to different EREs with similar specificity and affinity.

Activation function 2 (AF2) in the LBD constitutes the ligand-dependent transcription activation function of nuclear receptors [26,33–37]. In the crystal structure of ERα LBD, complexed with E2 [38••], the agonist-induced positioning of H12 over the ligand-binding pocket has been shown to form the basis for the AF2 coactivator recruitment and interaction surface, together with amino acid residues in H3, H4, and H5. In contrast, in the ERα and ERβ LBD–raloxifene complexes, respectively [38••,39], H12 was displaced from its agonist position over the ligand-binding cavity and instead occupied the hydrophobic groove formed by H3, H4, and H5, foiling the coactivator interaction surface. Although E2 and raloxifene bind to the same cavity in the receptor, these ligands induce a different conformation of H12 in the LBD, discriminating an agonistic effect by E2 from estrogen antagonism by raloxifene. Surprisingly, H12 in the ERβ genistein structure did not adopt an agonist conformation [39] but a position more similar to an antagonist conformation, a finding in agreement with the partial (60–70% of E2) agonism of genistein acting via ERβ on an ERE-driven reporter gene in cells [31•]. It is evident that

different ligands induce different receptor conformations [24,40], and that different conformations of the receptor affect the agonist efficacy and potency of ligands.

An interesting difference between ERα and ERβ is also seen on an AP1 site. In the presence of ERα, typical agonists such as E2 and diethylstilbestrol as well as the antiestrogen tamoxifen function as equally efficacious agonists in the AP1 pathway, raloxifene being only a partial activator. In contrast, in the presence of ERβ, the antiestrogens tamoxifen and raloxifene behave as fully competent agonists in the AP1 pathway, while estradiol acts as an antagonist inhibiting the activity of both tamoxifen and raloxifene [41••].

## Tissue distribution of ERβ and ERα

ERβ is widely distributed in the organism. ERβ was originally cloned from rat prostate, which is one of the most ERβ dense in the body. The ovaries in the female rodent show a corresponding abundance of ERβ, mainly in the granulosa cells. The tissues that appear to be richest in ERβ are the central nervous system, the cardiovascular system, the lung, the kidney, the urogenital tract, the mammary gland, the colon, the immune system and the reproductive organs. The significance of ERβ and ERα in some tissues will now be discussed.

### Breast tissue

The importance of estrogens in the development of female breast tissue is well documented. Female aromatase deficient patients, unable to convert $C_{19}$ steroids (eg testosterone) to estrogens, showed no sign of breast development at the onset of puberty [42–44]. Administration of estrogen to the two described female patients, however, led to normal prepubertal and postpubertal breast development. ERα knockout female mice have lost their capacity to develop mammary gland tissue beyond the embryonic and fetal stages despite elevated levels of circulating estrogens (17β-estradiol).

More than 70% of primary breast cancers in women are 'ER' (actually ERα) positive and show estrogen-dependent growth [45], and undergo regression when deprived of supporting hormones. Patients whose breast tumors lack significant amounts of ER rarely respond to endocrine ablation or treatment with antiestrogens, whereas most patients with ER-containing cancers benefit from such treatment [46,47]. Immunochemical determination of ER in tumor biopsies has become a routine clinical procedure on which the choice of therapy is based. However, the currently available immunochemical procedures for ER measurements are based on ERα-specific antibodies that do not detect ERβ protein (unpublished observations).

ERβ mRNA and protein have been detected in human breast cancer biopsies and in human breast cancer cell

lines [6,48–50]. With the use of receptor specific anti-bodies, both ERα and ERβ were expressed in the normal rat mammary gland, but the presence and cellular distribution of the two receptors was distinct [51•]. Further-more, while the level and number of cells expressing ERβ were more or less constant during prepubertal and pubertal stages, and throughout pregnancy, lactation and postlactation, the level and percentage of ERα-containing cells varied dramatically. The possible role of ERβ in normal breast tissue development and physiology or in breast cancer development and/or therapy is, however, as yet unknown [52,53•].

### Urogenital tract
Estrogens are claimed to be effective in the treatment of urge incontinence in postmenopausal women (see [54,55] and references cited therein). It has recently been shown that ERβ is highly expressed in the inner epithelial cell layer of the rat bladder and urethra [56,57], which may explain the beneficial effect of estrogens in urinary incontinence and suggest that patients with urinary incontinence might benefit from ERβ-selective agonist therapy.

Estrogens have been linked with prostate pathologies. It has been shown in different species that estrogens synergize with androgens in inducing glandular hyperplasia and dysplasia, and adenocarcinoma in the prostate [58•]. Immunohistochemical studies have revealed that ERβ is the predominant ER in the prostate, located in the epithelial cells along the ductal network of the prostate. ERα has been detected only in the stromal compartment of the prostate [57,58•] (Weihua *et al*, manuscript submitted). ERβ–/– mice display signs of prostatic hyperplasia with aging [59]. This suggests that ERβ may protect against abnormal prostate growth and that ERβ-selective ligands would be of clinical relevance in the prevention and treatment of neoplasia of the prostate.

### Bone: development and homeostasis
There is compelling evidence that estrogens protect post-menopausal women from bone loss and the development of osteoporosis, maintaining a balance between bone resorption and bone formation [54,55,60–63]. As in other tissues, estrogens probably have both direct and indirect effects in maintaining a balanced bone metabolism. The likelihood of important direct effects of estrogens on bone is based on the presence of ERα in the bone-forming osteoblasts [64–66] and in the bone-resorbing osteoclasts [67]. ERβ mRNA has been found in primary rat osteoblasts and in rat osteosarcoma cells [68]. It has been described in immortalized human fetal osteoblasts that ERα and ERβ are differentially expressed during osteoblast differentiation *in vitro* [69].

### The cardiovascular system
The risk of women developing cardiovascular disease increases dramatically after the menopause, suspected to be a consequence of the cessation of estrogen production by the ovaries. Estrogen replacement therapy has a cardiovascular protective effect in postmenopausal women, significantly decreasing the risk of developing atherosclerosis and cardiovascular disease [54,55,70–74].

The estrogen receptors ERα and ERβ are expressed in vascular endothelial cells [74–76], smooth muscle cells [77–79], and in myocardial cells [56,80]. Various direct effects of estrogen on vascular tissue have been reported [73,74,80–82]: nongenomic vasodilatation as an effect of estrogen on ion channel function [83] and nitric oxide synthesis [84–87]; long-term effects by modulation of, for example, prostaglandin synthase, nitric oxide synthase and endothelin gene expression [88–93]; regulation of AT1 receptor density on vascular smooth muscle cells [94]; and inhibition of injury-induced vascular intimal thickening [95–97]. Furthermore, reduced heart contractility in ovariectomized female rats was normalized following estrogen replacement [98], an effect explained in part by estrogen mediated changes in expression of contractile proteins [80,99]. The precise functions of ERα and ERβ in protection of the vessel wall from injury-induced hyperproliferation are still under active investigation. Estrogen can inhibit hyperproliferation of the vascular smooth muscle cells after injury in both ERα knockout and BERKO (ERβ–/–) mice [100•–102•], possibly indicating that the effects of estrogen on the smooth muscle cells are not receptor mediated, but possibly also indicating that the vesssel wall is one location where ERα and ERβ have overlapping functions. The answer to the question will be found when ERα/ERβ double knockout mice are examined.

### Central nervous system and the hypothalamus–pituitary axis
Estrogens are reported to influence a variety of functions in the central nervous system such as learning, memory, awareness, fine motor skills, temperature regulation, mood, and reproductive functions [103]. Estrogens are also linked to symptoms of depression and treatment of depressive illness.

The expression patterns of ERα and ERβ, respectively, based on mRNA, autoradiographic or immunohistochemical studies of rat and mouse brain, indicate that there is selective expression of one of the two ER subtypes in certain areas of the brain, but that there are also areas where they seem to be colocalized. ERα is more abundant in the hypothalamus (preoptic, arcuate, periventricular, and ventromedial nucleus) and in selected nuclei in the amygdala (hippocampal area, medial and cortical nucleus) [104–107]. A high level of ERβ mRNA has been found in the medial preoptic, paraventricular and supraoptic nucleus of the rat hypothalamus and in the medial amygdala nucleus. Moderate to high ERβ mRNA is expressed in olfactory bulbs, the bed nucleus of the stria terminalis, the hippocampus, the cerebral cortex, the cerebellum, the midbrain raphe and the basal forebrain [103,105–111].

The hypothalamus–pituitary axis regulates overall endocrine homeostasis in the body. Estrogen, through effects on the hypothalamus–pituitary axis, modulates the expression and secretion of hormones such as luteinizing hormone, follicle stimulating hormone, growth hormone, and prolactin, from the anterior pituitary gland [112]. Both ERα and ERβ are expressed in the pituitary gland but ERα predominates [112,113], particularly in the gonadotrophs and lactotrophs. Both ER subtypes are also expressed in the preoptic area of the hypothalamus, which is involved in regulating the expression of pituitary hormones, but ERβ is predominant [105].

## Concluding remarks

Our understanding of estrogen action has undergone a radical change following the discovery of ERβ. Although not addressed in this particular review, evidence is accumulating that ERα and ERβ may indeed regulate, at least partially, separate and distinct gene networks. We are thus now beginning to have tools to grasp many of the seemingly confusing and contradictory aspects of estrogen action, particularly regarding tissue specific and cell specific effects of estrogen. Varying ratios between ERα and ERβ in different contexts seem to quite probably be of paramount importance for the finally obtained hormonal effects. This paradigm shift in our concepts of estrogen action, needless to say, will lead to many exciting new opportunities for pharmaceutical development in the field of women's health.

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