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Review

# Integration of membrane and nuclear estrogen receptor signaling ☆

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**Abstract**

The classical mechanism of estradiol (E2) action is mediated by the nuclear estrogen receptors ERα and ERβ, which function as ligand-dependent transcription factors that regulate transcription of target genes containing the consensus estrogen response element (ERE) in their promoter regions. However, accumulating evidence indicates that E2 can also exert its actions through a unique membrane estrogen receptor (mER). Upon activation of the mER, various signaling pathways (i.e. $Ca^{2+}$, cAMP, protein kinase cascades) are rapidly activated and ultimately influence downstream transcription factors. Some target genes of the mER pathway may be activated independently of the nuclear estrogen receptor (nER). Additionally, it has been shown that classical nER action can be modulated by mER-initiated signaling through phosphorylation of nER and its coactivators, and by induction of third messengers (i.e. cyclin D1 and c-fos). Based on current evidence, we propose a model for E2 action integrating distinct membrane receptor and nuclear receptor signaling. This membrane receptor–nuclear receptor interaction is likely to exist for other hormones. Steroid hormones and other hormones acting through hormone receptors in the steroid receptor superfamily (i.e. thyroid hormones) also activate many of the same intracellular signaling cascades, which provides the basis for extensive crosstalk networks between hormones. The model proposed serves as a framework to investigate the diverse actions of hormones and endocrine disrupting chemicals (EDCs).


*Keywords:* Estradiol; Membrane receptor; Nuclear receptor; Signaling; Gene regulation; Hormone crosstalk

**Contents**



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## 1. Introduction

The main estrogenic hormone, 17β-estradiol (E2), is synthesized by aromatization of testosterone (Bulun et al., 2000). It plays important regulatory roles in a wide variety of biological processes including reproduction, differentiation, cell proliferation, apoptosis, inflammation, metabolism, homeostasis and brain function (Tsai and O'Malley, 1994). The primary mechanism of E2 action is mediated by transcriptional actions of the nuclear estrogen receptors, ERα and ERβ. Upon binding with a ligand, ERα and ERβ in the nucleus are released from inactive complexes containing heat-shock proteins and immunophilins (Ylikomi et al., 1998), homodimerize or heterodimerize (Cowley et al., 1997), and bind to a specific DNA estrogen response element (ERE) located in the promoters of target genes. In turn, DNA-bound ERs modulate target gene expression by interacting with a series of coactivators. In addition to direct binding to the ERE, nuclear estrogen receptor (nER) can affect gene expression through protein–protein interaction with other classes of transcriptional factors, such as AP-1 (Gaub et al., 1990) or Sp-1 (Castro-Rivera et al., 2001).

In contrast with classical nER action, the discovery of the rapid biochemical and physiological effects of E2 indicates the existence of novel mER-mediated actions. In this process, various protein kinase cascades, including mitogen activated protein kinase (MAPK), protein kinase A (PKA), protein kinase C (PKC), and phosphatidylinositol 3-OH kinase (PI3K) are activated after a short exposure time (seconds to minutes) to E2. In addition, the classical ERα is present and can also signal from the level of plasma membrane in rat pituitary tumor cell line (Pappas et al., 1995; Norfleet et al., 1999). Very recently two laboratories have shown that a G-protein-coupled receptor (GPCR30) can act as a distinct mER in human SKBR3 breast tumor cell line (Thomas et al., 2005; Revankar et al., 2005).

Since rapid signaling cascades can ultimately influence the phosphorylation state of downstream transcription factors, induction of membrane receptor-mediated action provides an alternative way for E2 to regulate gene expression. Moreover, signaling pathways activated following binding of E2 to mER can influence nER-dependent transcription. Here we will review the existing data and propose a dynamic model of E2 action that integrates both mER and nER actions. Furthermore, such a model may be extended to the action of other hormones, and the molecular mechanism of hormone crosstalk between E2 and other hormones is discussed.

## 2. Nuclear receptor mediated action of E2: the classical mechanism

Nuclear actions of E2 are mediated by two main nuclear estrogen receptor subtypes, ERα and ERβ, which are members of a superfamily of nuclear receptors that function as ligand- or hormone-dependent transcription factors (McKenna and O'Malley, 2002). Two important functional domains in ERα and ERβ, the DNA binding domain (DBD) and the ligand binding domain (LBD), are well conserved both at the amino acid sequence and the structural levels, whereas there is considerable divergence in the N-terminus between receptor subtypes. Comparative aspects of nER structure and function in various species have been discussed previously and will not be considered here (Martyniuk et al., in press; Menuet et al., 2002; Thornton et al., 2003). The two major nER subtypes have distinct expressions patterns in tissues (Couse et al., 1997; Shughrue et al., 1997, 1998) and ER knockout mice models (αERKO and βERKO) exhibit different phenotypes (Couse and Korach, 2001; Hess, 2003; Hewitt et al., 2005a) indicating distinct biological functions of the receptors. In contrast to the functional deficiencies in the reproductive system in both sexes of αERKO mice, male βERKO mice are fertile while females are subfertile.

The efficient transcriptional activity of nER requires recruitment of other transcription factors and multiple coactivators including SRC-1 (steroid receptor coactivitor-1), GRIP-1 (glucocorticoid receptor-interacting protein-1), AIB1 (amplified in breast cancer 1), CBP/p300 (cAMP response element binding protein-binding protein), PGC-1 (peroxisome proliferators-activated receptor gamma coactivator 1α) and p68 RNA helicase (McKenna and O'Malley, 2002; Metivier et al., 2003). Therefore, the availability of coactivators can greatly influence nER action. For example, in rodent brain reduction of SRC-1 and CBP protein with antisense oligonucleotides disrupted nER-mediated induction of progestin receptor gene (Molenda et al., 2002). In male Japanese quail, inhibition of SRC-1 significantly affected the estrogen-dependent male-typical sexual behavior, and is correlated with reduction in the volume of the preoptic medial nucleus and the expression of aromatase and vasotocin proteins (Charlier et al., 2005). Moreover, the transcription factor FoxA1, whose expression was induced via the nER, was found to be necessary for further nER action in human MCF-7 cancer cells (Laganiere et al., 2005; Carroll et al., 2005). The binding sites for FoxA1 are enriched in the nER binding regions of promoters of many genes. Small interfering RNA-mediated knockdown of FoxA1 blocks the transcription of other estrogen regulated genes, namely TFF-1 and vitellogenin-B1. These observations reveal an important aspect of the mode of action of E2: induction of transcription factors via the nER, then modulation of subsequent nER action.

Cellular signaling pathways can also modulate nER actions. Different kinases, such as PKA (Chen et al., 1999), mitogen activated protein kinase (MAPK) (Tang et al., 2004) and cyclin A-CDK2 (Rogatsky et al., 1999) are induced by extracellular signals (e.g. growth factors) which leads to phosphorylation of several N-terminal residues of ERα, for example, serines 104, 106, 118 and 167 (Lannigan, 2003). The phosphorylation of the serine residues of nER modulates receptor functions, including nER downregulation by the ubiquitin–proteasome pathway (Marsaud et al., 2003), nuclear localization of nER (Lee and Bai, 2002), nER dimerization (Chen et al., 1999), and transcriptional activity (Ali et al., 1993; Le Goff et al., 1994). Besides targeting nER, signaling pathways can also exert their effects on nER actions through the coregulators. For example,