Estrogen (E2) plays significant roles in ligand target organs such as the reproductive organs, bone and the brain. This female steroid hormone is well known also to be deeply involved in cancer development in the reproductive organs. The majority of E2 actions are mediated through its nuclear estrogen receptor (ER). ERα and ERβ are members of the steroid/thyroid hormone nuclear receptor superfamily, and they act as ligand-inducible transcription factors to control expression of a particular set of target genes for E2. In cancer development, growth factors are shown to act synergistically with E2, possibly modulating the function of ER. In this review, the molecular mechanism of this cross-talk is discussed.

**ERα AND ERβ ARE E2-INDUCIBLE TRANSCRIPTIONAL FACTORS**

ERs are members of the steroid/thyroid hormone, vitamin D, retinoic acid nuclear receptor superfamily (1-2). In addition to classical ER, a second ER (ERβ) has been recently identified from one of the orphan receptors (3). The members of this family share a common domain structure with distinct functional domains referred as A to E/F domains. The DNA binding domain (C domain) is located in the middle of the receptor molecule, and the ligand binding domain (E domain) is mapped to the C-terminal end. These two domains are highly conserved among the members of this family. As the nuclear receptors act as ligand-inducible transcription factors the signal of the ligands is transmitted into genetic information by transcriptionally activating the target genes, thereby exerting the actions of the ligands through the biological functions of the gene products (proteins). During the ligand-induced transactivations, two domains at the N-terminal A/B domains (AF-1) and the C-terminal E domain (AF-2) are prerequisite (4,5). The functions of both AF-1 and AF-2 are tissue- and species-specific, while their properties are distinct. The activity of AF-1 is ligand-independent, and constitutive, in contrast the function of AF-2 is induced by ligand binding. In the absence of ligand binding, where ER is transcriptionally inactive, the AF-1 function is supposed to be suppressed by AF-2. In other words, the ligand binding to the nuclear receptor induces the functions of both AF-1 and AF-2 in a different fashion.

**GROWTH FACTORS POTENTIATE THE HUMAN ERα THROUGH PHOSPHORYLATION OF Ser118 RESIDUE OF HUMAN ERα**

In 1993, three independent groups showed that E2 binding induces phosphorylation of the Ser118 residue of human ERα, and furthermore one of them demonstrated that this phosphorylation plays a significant role in the AF-1 function by replacing this serine residue into alanine not to be phosphorylated (6). Since the AF-1 activity was reported to be potentiated by growth factors which activate MAP kinase (MAPK) in their signaling pathways, we searched the amino acid sequences, and found a round sequence of Ser118 is well conserved among human, mouse, rat, chicken and trout ERαs, and a target phosphorylation site (PXXSP) recognized MAP kinase (MAPKK) (7).

MAPKK is activated by distinct signaling pathways, and one of these is mediated through tyrosine kinase-type membrane receptors activated by growth factors such as insulin, EGF-I, FGF and TNF-α (8). As such growth factors are shown to synergize the E2 actions in cancer development, we speculated a possibility that MAPK-phosphorylation of the Ser118 of human ERα potentiates the AF-1 of human ERα. Activated MAPK purified from the frog egg extracts phosphorylated in vitro the Ser118 of hERα. Phosphorylation of the serine residue of hERα expressed in mammalian cells was seen when the MAPK pathway was activated by either treatment of the cells with insulin and EGF or co-expression of Ras, one component constituting the MAPK signaling pathway. Under this condition, the ligand-induced transactivation function of hERα was potentiated. Thus, taken all together, we speculate that the cross-talk between growth factors and E2 is mediated at least in part, through MAPK-mediated phosphorylation of the Ser118 of hERα (9). We further found that phosphorylation by MAPK takes place in the AF-1 of hERβ, and the AF-1 function is potentiated by the activated MAPK signaling pathways, suggesting that the cross-talk occurs not only through ERα, but also through ERβ (Y. Kobayashi et al., submitted).

Furthermore, several lines of experiment suggested that known nuclear receptor AF-2 co-activators like CBP/p300 general integrators and/or the SRC-1/TIF2 family proteins potentiate also the AF-1 of ERs.
THE CBP/p300 PROTEINS FUNCTION AS CO-ACTIVATOR FOR BOTH AF-1 AND AF-2, BUT THEY ARE NOT INVOLVED IN THE ENHANCEMENT OF ERα AF-1 FUNCTION BY MAPK-MEDIATED PHOSPHORYLATION

To test the possible functions of the CBP/p300 proteins and the SRC-1/TIF2 proteins (10), we chose p300 and SRC-1. First we examined whether p300 and SRC-1 enhance the function of the ER (AF-1), since if one of these proteins mediates the interaction between AF-1 and AF-2 of ERα, it should act as a co-activator for AF-1 by direct binding. Consequently, we found that p300, but not SRC-1, potentiates the AF-1 function of ERα, though both factors enhanced the ligand-induced transactivation function of AF-2 as expected from previous reports. In agreement with the transactivation property of p300 to the ER AF-1, bacterially expressed p300 directly bound to the ER AF-1. Taken together, we concluded that p300 (and possibly CBP) is a co-activator for ER (AF-1). As p300/CBP are documented to bind the ER E domain in a ligand-binding way, it is most likely that p300/CBP mediates the ligand-dependent interaction between AF-1 and AF-2 of ERα. We tested if p300 is responsible for the enhanced transactivation function of ERα AF-1 by the MAPK-mediated phosphorylation. For this, we used a series of ER mutants to destroy Ser residues one by one in the A/B regions. p300 was effective on all of the ER mutants including ER15/457, which has no phosphorylation site for MAPK by substituting Serine into Alanine. These results indicate that the enhanced transactivation function of ERα AF-1 by MAPK-mediated phosphorylation is not mediated through p300/CBP. p300/CBP appears to stabilize the ligand-interaction of AF-1 and AF-2 acting as common co-activators for both (Y. Kobayashi et al., submitted).

PURIFICATION AND IDENTIFICATION OF p68 RNA HELICASE ACTING AS A TRANSCRIPTIONAL COACTIVATOR SPECIFIC FOR THE ACTIVATION FUNCTION 1 (AF-1) OF HUMAN ESTROGEN RECEPTOR

Thus, we found that the MAP kinase activated by growth factors phosphorylates the Ser118 residue of human ERα, resulting in potentiation of the N-terminal transactivation function (AF-1) of human ERα (10). However, unknown coactivators seemed to be involved in this regulation. Therefore, using the phosphorylated human ERα recombinant protein by MAP kinase, we purified and identified an interacting protein, p68 RNA helicase (p68), from the nuclear extracts of MCF7 cells (11). Overexpression of p68 enhanced the activity of AF-1, but not AF-2, of hERα, and it was not potent to any other nuclear receptors including hERβ, suggesting that p68 acts as the hERα AF-1 specific co-activator to constitute cross-talk with growth factor signaling.

PERSPECTIVES

For the E2-induced function of ER in gene regulation, known and unknown coactivators are essential, so that research to understand molecular mechanisms of E2 actions is moving towards identifying such ER coactivators. Most notably, future studies into ER coactivators will definitely clarify, at least in part, the mechanism of cancer development in female reproductive organs.

References