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Molecular cloning of estrogen receptor alpha and its transcriptional activity on promoter regions of masu salmon gonadotropin subunit genes

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Introduction

Estrogens play a role not only in growth, differentiation, and homeostasis of male and female reproductive organs, but also in liver, and cardiovascular system. It is generally accepted that the action of estrogen is mediated by a specific estrogen receptor (ER) that is present in the nucleus of target cells. The effects of estrogens are mediated by at least two receptors, i.e., ERα and ERβ, which are members of the nuclear receptor family (Katzenellenbogen and Korach, 1997).

In order to better understand the function of estrogen receptor in the pituitary of fish species, an ERα cDNA containing the A - F domains from the pituitary gland of masu salmon (*Oncorhynchus masou*) was cloned. In addition, transcriptional activity of the msERα was examined in a mouse gonadotrope-derived cell line, LβT2.

Materials and Methods

1. cDNA amplification and cloning of masu salmon ERα (msERα)

Degenerated ERα primers were based on a highly conserved region common to salmon species ERα (GenBank Accession No. AJ242740). After reverse transcription of pituitary ERα, polymerase chain reaction (PCR) was performed in 50 ul final volume containing 5 ul of 10x reaction buffer, 2 mM MgCl2, 200 uM dNTP, 2 uM of each primer, and 2.5 U LA *Taq* DNA polymerase (Takara Biomedicals, Japan). After an initial 5 min denaturing step at 94C, 30 cycles of amplification were performed using a cycle profile of 94C for 30 sec, 55C for 30 sec, and 72C for 1.5 min.

2. Transient transfections and luciferase assay

LβT2 cells were cultured at 37C with 5% CO2 in DMEM medium (GIBCO BRL, USA) containing 10% fetal bovine serum and 1% antibiotic-antimycotics. The cells were grown in 24-well plates with medium supplemented with 10% charcoal-stripped serum. Cells were transfected 24 hr later with 100 ng of a luciferase reporter plasmid (pGL3; Promega, USA) containing various size of gonadotropin subunit gene promoters of masu salmon (GTHα, FSHβ, LHβ) or an estrogen responsive element (ERE) of vitellogenin promoter in xenopus, and 50 - 100 ng of msERα in pcDNA3 plasmid vector (Invitrogen, USA) by liposome-mediated method (Lipofectamine, Invitrogen). After 24 hr, cells were treated for 20 hr with estradiol-17β (E2) or equal volume of ethanol as a control. Cell lysis and luciferase assay was performed as previously reported (Sohn, 2004).

Results and conclusion

The msERα cDNA contains 1863 bp and an open reading frame encoding 620 amino acid residues, and the molecular weight of this protein was calculated to be about 68,200 Dalton. When the overall nucleotide sequence of the msERα was compared with those of fish species, there were sequence similarities ranging from 85 to 98%. The ERE reporter gene was significantly activated by msERα and E2. Among the promoters of masu salmon GTHα, FSHβ and LHβ, we observed a estrogenic repression region (-1422 ~ -2799) in FSHβ promoter.

References

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