E113

Cloning and Characterization of Gene Encoding Neural Cell-specific EGF-like Repeat Domain-containing Protein NELL2

Eun Jung Choi[P], Byung Ju Lee[C]

Department of Biological Sciences, University of Ulsan, Ulsan 680-749

Our previous reports showed that NELL2 is a novel neural tissue-specific epidermal growth factor (EGF)-like repeat domain-containing protein and is involved in the development and differentiation of neural tissues, and that NELL2 mRNA level in the hypothalamuswas increased by estrogen. To determine binding sits for transcription factors including estrogen receptor (ER) and their function on NELL2 expression, we cloned NELL2 genomic DNA. NELL2 gene is composed of 20 exons sized form 71 to 223 base pairs with a total 307 kilobases. Nine transcription initiation sites were identified by primer extension and RNase protection assays. Two major transcription initiation sites were located 32 and 26 base pairs upstream from the translation start site. Its gene regulatory region contains several conserved binding domains for transcription regulators such as Sp1, ER, activator protein (AP1), Olf-1, Krox-20, and TATA box binding protein (TBP). In the C6 glioma cells, NELL2 promoter showed a estrogen-dependent expression. The present result showed that 5'-flanking region of NELL2 gene contains estrogen-response element that endorse the regulatory function of estrogen.

E114

Characterization of a Gain-of-function Mutant of Calcineurin A (*cna-1*)

Jungsoo Lee[P], Changhoon Jee[1], Joohong Ahnn[C]

Department of Life Science, K-JIST, Gwangju 500-712

Calcineurin is a calcium-calmodulin dependent protein phosphatase that is a key component of Ca2+ -related signaling pathways. Calcineurin functions as a heterodimer, consisting of a catalytic subunit (CnA) and a regulatory subunit (CnB). This two-subunit structure is well conserved from yeast to human.Previously, we have isolated and characterized a null mutant of calcineurin B [*cnb-1 (jh103)*] which showed the severe defects in locomotion, egg laying and displayed serotonin-mediated egg-laying resistance. Interestingly, very similar phenotypes were also observed in gain-of-function mutant of *unc-43*, which encodes the calcium-calmodulin dependent protein kinase CaMKII. Taken together, it has been suggested that *cnb-1* and *unc-43* are involved in the G-protein signaling pathway which regulates locomotion and egg laying.Recently, we have isolated again-of-function mutant of catalytic subunit, calcineurin A [*cna-1 (jh107)*] which is deleted in the calmodulin binding site and the autoinhibitory region of this phosphatase. *cna-1 (jh107)* shows hyperactive serotonin-mediated egg laying which is opposite phenotypes of *cnb-1 (jh103)*. Genetic interaction between *cna-1* other mutants supports the fact that calcineurin is indeed a component of G-protein signaling pathway and is involved in locomotion and egg laying in *C. elegans*.

E115

Identification and Characterization of Calcineurin Binding Protein in *C. elegans*

Changhoon Jee[P], Joohong Ahnn[C]

Department of Life Science, K-JIST, Gwangju 500-712

Calcineurin is a Ca2+/calmodulin dependent Ser/Thr protein phosphatase. To identify the molecular targets of calcineurin action in *C. elegans*, yeast two-hybrid screening was performed. One of the candidates from the screening, *cnp-1* (T12D8.4), shows 25% identity over 344 residues with human TBP-2/VDUP1, which has been recently reported as a negative regulator of Thioredoxin(TRX) function. Human TRX-binding protein 2(TBP-2), also known as Vitamin D3 up-regulated protein 1(VDUP1), was originally reported as an up-regulated gene in HL-60 cells treated with 1a, 25-dihydroxyvitamin D3, and significantly downregulated in chemically induced rat mammary tumors. We have cloned and sequenced the full cDNA from cDNA library and found that the gene structure predicted by database was incorrect. Complete DNA sequencing data showed that cnp-1 gene consists of 9 exons encoding a protein of 426 amino acid residues. Our sequencing results added one more exon, exactly 96 base pairs (32 amino acids) to the previously predicted one. To confirm in vitro interaction between calcineurin A and entire coding region of *cnp-1*, GST pull-down assay was conducted. GST fused full-length CNP-1 pulled down calcineurin A from the worm lysate. GFP expression of promoter region of *cnp-1* was seen mainly in nerve cells from embryo to adult stage, which is similar to that of *cna-1*. We have isolated a deletion mutant of *cnp-1 (jh105)* by UV-TMP mutagenesis methods in which this gene was deleted from the beginning of exon2 to the end. Based on this molecular evidence, we suggest that this mutant is a functionally null mutant.

E116

Brain-specific Homeodomain-containing Transcription Factor, TTF-1, Regulates Growth Hormone and Prolactin Gene Expression in the Rat Anterior Pituitary

Nam Oak Lee[P], Min Kyu Hur[1], Young June Son[2], Byung Ju Lee[C]

Department of Biological Sciences, University of Ulsan, Ulsan 680-749

Pit-1/GHF-1 is a pituitary-specific, POU homeodomain transcription factor specifically expressed in the anterior pituitary and regulates transcription of growth hormone (GH) and prolactin (PRL). We found that TTF-1 is also expressed in the pituitary tissue. In the present study, we examined if TTF-1 regulates GH and PRL gene expression in the rat anterior pituitary. Using transient transfection, we identified TTF-1 as a factor functionally regulating the GH and PRL promoters. In the pituitary adenoma GH3 cells, the PRL promoter was highly activated by TTF-1, whereas GH promoter was inhibited by TTF-1. GH and PRL promoters contain several binding sites for TTF-1. Electrophoretic mobility shift assays showed that TTF-1 binds to a specific subset of its consensus DNA binding sites in the GH and PRL promoters. Moreover, we found that TTF-1 and Pit-1 cooperatively regulate the GH and PRL promoters in the GH3 cells. Our results suggest that GH and PRL gene expression in the pituitary is regulated by TTF-1 in combination with Pit-1.