D114 Molecular Cloning of the Human Tyrosine Hydroxylase Gene Promoter

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Expression of tyrosine hydroxylase(TH) gene, the rate-limiting enzyme of catecholamine biosynthesis, is regulated at transcriptional level during neuronal development and in response to a variety of environmental stimuli. To investigate regulation of tissue-specific expression of TH gene, we cloned the 5'-segment of human TH gene from EMBL3T7/SP6 human genomic DNA library. A 14-kilobase (kb) genomic fragment containing part of the TH gene was isolated by screening a human genomic library with a probe derived from the most 5'-end of a human TH cDNA. A 4617-bp fragment containing sequence -3472 to +145 is subcloned to assay TH promoter activity. This 4617-bp fragment contains consensus binding sequence for basal (TATA), dopaminergic neuron-specific (NBRE A, B, C and BBE1, 2), and regulatable transcription factors (CRE). Sequence analysis showed no overall homology with the rat and mouse TH promoter region, except 2 short domains encompassing 2384 to 2323 and 123 to 65, respectively. Interestingly, upstream domain contains NGFI-B response element (NBRE) consensus sequence, but distal homology domain contain no apparent transcription factor binding consensus sequence. Nurr1, a member of the orphan nuclear receptor superfamily, is uniquely expressed in the dopaminergic neurons of the subtantia nigra pars compacta and ventral tegmental area, which together form the mesencephalic dopaminergic system. This localization and its coinciding induction of expression with the TH gene during development suggested a possible role for this transcription factor in the control of the TH gene. In 5'-flanking sequence of human TH gene, 3 consensus element for Nurr1 are located at position -2413 to -2406 and -1440 to -1433 and -833 to -824 of the human TH promoter, and designated as NBRE A, B, and C, respectively. To further localize TH promoter region responsible for activity, we are constructing and fusing a deletion series of the human TH promoter to a luciferase reporter gene, and are to assess the activity of promoter in neuronal and non-neuronal cell lines. In addition, gel mobility shift assays using nuclear extracts are to be done. (Supported by grants from KOSEF/BDRC)

D115 Differential Ligand Selectivities of Mammalian and Non-mammalian Gonadotropin-Releasing Hormone Receptors

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Mammalian gonadotropin-releasing hormone receptor (GnRHR) responds better to mammalian GnRH (mGnRH) than to chicken GnRH-II (cGnRH-II), whereas non-mammalian GnRHR is more sensitive to cGnRH-II than to mGnRH. This study attempts to identify the determinant of such differential ligand selectivities between mammalian and non-mammalian GnRHRs. To this end, the S^{331} E^{332} P^{333} motif of the rat GnRHR was mutated to the P^{331} E^{332} Y^{333} motif mimicking the bullfrog GnRHR-2 by site-directed mutagenesis. To observe the reciprocal effect, bulfrog GnRHR-2 with the S^{331} E^{332} P^{333} motif was constructed. These mutants were transfected into CV-1 cells and inositol phosphate production and CRE-Luc mediated luciferase activity in response to GnRHs were examined. Mutant rat GnRHR increased the sensitivity to cGnRH-II by 80 times. In contrast, mutant bulfrog GnRHR-2 lowered the sensitivity to

cGnRH-II by 10 times. These findings identify the S^{331} E^{332} P^{333} motif as the determinant of differential ligand selectivities between mammalian and non-mammalian GnRHRs and imply the existence of residues interacting with Arg^{8} of mGnRH and Trp^{7} and Tyr^{8} of cGnRH-II in non-mammalian GnRHRs within this motif.

D116 A Novel Protein, Src Homology Domain Binding Protein (SHOP), Suppresses a Platelet Derived Growth Factor (PDGF) BB induced Transformation in NIH3T3 cells

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The SHOP(Src Homolgy Domain Binding Protein) was initially isolated as a binding protein to the SH domain of Phospholipse Cg1 and later found to bind with adaptor proteins, Grb2 and p85a. The SHOP contains four PXXP sequence known as a SH3 domian binding motif and two YXXM sequence known as a p85a SH2 domain binding motif. In addition, SHOP contains several putative PKC phosphorylation sites. PLCg1, PI3 kinase p85a and Grb2 were all as Receptor tyrosine kinase (RTK) downstream members and have essential roles for cellular transformation upon PDGF BB stimulation. We have determined that SHOP is selectively associated with PLCg1, p85a and Grb2 upon stimulation with PDGF BB. Furthermore, constitutively overexpressed full length SHOP in NIH3T3 cells inhibits PDGF BB dependent cellular transformation (growth in soft agar). In addition, PXXP motifs of SHOP alone were able to inhibit the formation of colony in soft agar culture. Also, DT cells overexpressing SHOP underwent morphological changes resembling parental NIH3T3 cells. We also confirmed the changes in the level of PKA RIIb which induces reverse transformation in DT cells. We suggest that the inhibition of cellular transformation may be induced by reduction of JNK activity and increased p27Kip expression level. These findings show that the SHOP may have a role as a tumor suppressor protein.

D117 Ligand-Dependent Signal Transduction of Rat and Frog Gonadotropin-Releasing Hormone Receptors

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Recently, we have cloned three distinct types of GnRH receptors in the bullfrog (designated bfGnRHR-1, bfGnRHR-2, and bfGnRHR-3). In the present study, we elucidated the involvement of different signal transduction pathways mediated through these receptors in response to different ligands. With stimulation of variety GnRHs, all receptors could increase the inocitol phosphate production. Interestingly, bfGnRHR-2 induced cAMP production in response to cGnRH-II but not mGnRH while bfGnRHR-1 could induce cAMP production by mGnRH but not cGnRH-II. In consistence, either cGnRH-II/bfGnRHR-2 or mGnRH/bfGnRHR-1 mediated CRE-luc activities were inhibited by cotransfection of PKI, a PKA inhibitor, while PKI inhibited neither cGnRH-II/bfGnRHR-1 nor mGnRH/bfGnRHR-2 mediated CRE-luc activities. Taken together, these results indicate that different ligands may regulate the coupling of different G proteins with GnRHRs, which may in turn contribute to the activation of distinct second messenger systems.