I-125: Binding Assay.

At the University of Pittsburgh: P-32: Electrophoretic mobility shift assay (EMSA).

Knowledge, Skills and Abilities (KSA's): (LY1—5)

1. Knowledge of Molecular Genetics and Molecular/Cellular Neurobiology

I started the training in molecular genetics and molecular biology in the Medical School, Graduate School for Masters Degree of Medical Science, and with the Molecular Biology/Genetics as my major for PhD in Medical Science, study of gene regulation in the biosynthesis of steroid hormones and their hereditary diseases. Under the supervision of Prof. Yutaka Shizuta who had worked with the Nobel Prize laureate, Dr. Edwin Krebs I studied the gene regulation in the biosynthesis of steroid hormone as well as molecular genetic analysis of steroid 11 β/c18-hydroxylase deficiencies in humans. I successfully analyzed the gene expressions of ***CYP11B1*** and ***CYP11B2*** in human fetal organs including the brain and examined the transcriptional regulation of *CYP11B1* and *CYP11B2* in NCI H-295, the human adrenocortical carcinoma cells. I demonstrated that the classic 11β-hydroxylase deficiency is caused by a point mutation in exon 7 of the gene for P450-11 β *(CYP11B1)* and that the mutant protein of P450-11 β lacks the hydroxylase activity in vitro, by transfecting the full-length cDNA corresponded to the *CYP11B1* of the patient into COS-7 cells and measuring steroid 11 β -hydroxylase activity in the solubilized mitochondria. My paper "Classic Steroid 11 β -hydroxylase Deficiency Caused by a C→G Transversion In Exon 7 of *CYP11B1*" has been published in the international journal. I also located an enhancer element in the region between -242 and -166 relative to the major cap site of the gene for human aromatase cytochrome P450 (***CYP 19***) and identified a nuclear factor specifically binding to the cis-acting element between -2141 and -2115 of *CYP 19* as NF-IL6 that participate in TPA-mediated transcriptional enhancement of *CYP 19* gene expression. The paper "Transcriptional Regulation of the Human Aromatase Cytochrome P-450 Gene Expression in Human Placental Cells" was published in the international Journal, J. Steroid. Biochem. Molec. Biol. I worked on the molecular genetic analysis of corticosterone methyloxidase deficiency (CMO) on an aldosterone synthase-deficient patient. Corticosterone methyloxidase I (CMO I) deficiency is an autosomal recessive disorder of aldosterone biosynthesis, resulting from mutations in ***CYP11B2***, the gene for a single multifunctional enzyme P450c18. Point mutation in *CYP11B2* completely abolishes all activities of P450c18 catalyzing the three reactions required for conversion of DOC to aldosterone, thereby causing CMO deficiency. The work was published on the 10th International Congress of Endocrinology in San Francisco in 1996. I also participated in research on "Cloning and Structural Characterization of the Human Endothelial Nitric-Oxide-Synthase Gene", "Genetic Variation in the Triploids of Japanese Fasciola Species and Relationships with Other Species in the Genus", and "Mitochondrial DNA Differentiation of Japanese Diploid and Triploid Paragoniums Westermani".

I further carried out postdoctoral research work in molecular genetics/neurobiology. In my first Postdoc work I studied the neurotrophic factor GDNF/receptor families, structure and characterization of the genes for GDNF receptor alpha 1 (GFRα1) and its homologues. I cloned the cDNA for GFRα1 from the mouse midbrain, screened out several genomic clones of the GFRα1 gene from a Lambda FIX II mouse genomic library, and devised a PCR approach and genomic walking for locating all the exon-intron boundaries and the promoter region. By performing 5'- and 3'-RACE,

studies of the genomic structure and Southern blot analysis I clarified that the 446 bp sequence of the 5'-end cDNA designated as exon 1A is located downstream of TATA box in the promoter region, the heterogeneity in 5'-UTR is generated by alternative RNA splicing of the exon 1B locating downstream of exon 1A, and the initiation codon ATG is located in exon 2. I obtained the cDNA clones showing that 7 kinds of transcripts exist in mouse brain encoding five isoforms of GFRα1 and resulted from alternative splicing in exon 1, 4, 5 and 9 of the gene that comprises 14 exons. I have cloned out the full-length cDNAs encoding three novel isoreceptors of GFRα1 named GFRα1c, GFRα1d and GFRα1e. They are splice variants from the single gene, ***GFRα1***. The cDNA encoding GFRα1c contains a 30 bp insertion in coding region, resulting in a single long open reading frame encoding a protein of 478 amino acids. The insertion of 30 bp in cDNA derives from a novel exon named exon 4A according to its upstream location of exon 4B in genomic DNA. The novel soluble isoreceptors GFRα1d and GFRα1e consist of 425 and 420 amino acids, respectively. They have different C-terminals from the former isoforms and their stop codons are located in the novel spliced exon 9B that I newly found. I have analyzed their mRNA expression patterns in developing mouse brains, various embryo and adult mouse tissues. I have also obtained the full-length cDNAs encoding human GFRα1d and GFRα1e consisted of 416 and 411 amino acids, respectively. I have constructed the full-length cDNA clones of the 5 isoreceptors for functional assay. By genomic walking I have cloned out the 2.4 kb promoter region of this gene showing two typical TATA boxes and many other cis-elements (including the binding site for p53). I have deposited 14 items (Accession Numbers), 17,000 bp novel cDNA and genomic DNA sequences in the GenBank. The discoveries of the soluble isoreceptors of GFRα1 (for the first time in the world) provide in vivo evidence that GFRα1 can mediate Ret activation by GDNF not only *in cis* but also ***in trans*** to regulate the neuronal survival and neuron-target interactions in the nervous system. My discoveries are unprecedented, which attracted the attentions of the scientists from Washington University, California Institute of Technology and from Belgium on the 28th and 29th Annual Meetings of The Society for Neuroscience. I also worked on "Molecular Cloning and Expression Analysis of ***GFRα-3***, a Novel cDNA Related to *GDNFRα* and *NTNRα*" and "Distribution of **RGS4** mRNA in mouse brain shown by in situ hybridization", etc. I have much experience in molecular cloning, analysis and characterization of new genes, gene structure and regulation of gene expression.

I started my second Postdoc work from Oct. 1999 in the Lab of Developmental Neurobiology, NICHD, NIH to study the Hebb activity-dependent synapse elimination, synaptogenesis, synaptic transmission and plasticity, and the molecular mechanisms of Autism. With an in vitro model of neuromuscular junction and the methods of eletrophysiological (twitch assay), morphological (immunocytochemistry) and molecular biology/biochemistry I studied the role and molecular mechanisms of PKC theta in synaptogenesis and plasticity in neuromuscular junction using PKC-θ knockout mice, acetylcholine receptor (AchR) phosphorylations by protein kinases, pre-and postsynaptic regulations by neurotrophic factors and the effects of **GDNF** on neuromuscular synapses. Our paper "Pre- and Post Synaptic Mechanisms in Hebbian Activity-Dependent Synapse Modification" has been published in 2002. Using a computerized image analysis system I have developed a new fluorescence double-labeling technique and simultaneously quantitated both the insertion of clustered AchRs into muscle surface membrane and the degradation of pre-existing AchR aggregates. Treatment of the primary nerve-muscle co-cultures with GDNF results in a prompt increase of the insertion rate at muscle surface AchR aggregates. Using biochemical methods of cold blocking the pre-existed AchRs with unlabeled bungarotoxin and biotinylation/precipitation of the newly inserted membrane AchRs, I further show that GDNF increases the insertion of AChRs into the

surface membrane of muscle cells. Imaging and Immunocytochemical analysis reveals that GDNF promotes the neurites outgrowth and increases the number of synaptic buttons. My findings demonstrate that in addition to its classic functions GDNF regulates not only the presynaptic differentiation in motor axon terminals but also the postsynaptic AchR insertion in muscles. I have also studied the molecular and cellular mechanisms of the effects of GDNF as a synaptotrophic modulator. With Western blotting, Northern blotting, RT-PCR and nucleotide sequence analysis I found that all GDNF receptor subunits, Ret, NCAM-140 and GFRα1 are expressed in skeletal muscles and motoneurons. By immunoprecipitation I show that Src protein-tyrosine kinase binds to the AchR of skeletal muscle. GDNF treatment of the primary cultures induces Src kinase phosphorylations differentially in primary myotubes and nerve-muscle co-cultures with a time course dependence that is slower than CREB phosphorylation. I have completed the training program (20 hours of lecture and laboratory training) on Two Hybrid Selection: Identification and Characterization of Protein-Protein Interactions and the Short Course for DNA Microarrays: The New Frontier in Gene Discovery and Gene Expression Analysis, SFN 2000. I have the practical experience in Molecular Biology/Genetics: Extraction and purification of DNA, RNA and protein; genomic restriction mapping, long PCR, PCR-SSCP, Real-time RT-PCR, Northern blot, Western and Southern blotting including semi-dry blotting, RFLP analysis, genomic Southern blot, DNA sequencing (ABI, A.L.F. sequencing and ^{32}P-sequencing) and Bioinformatics. Construction of cDNA library; Molecular cloning, 3'-and 5'-RACE, primer extension, obtaining the promoter region quickly through genomic walking and finding all the exon/intron organization and boundaries in a gene, mouse and human genomics. Site-directed mutagenesis, construction of mutant cDNA expression vector and recombinant viral vector, transfection of the gene into mammalian cells by electroporation or Lipofectamine methods and gene expression, CAT assay, luciferase assay, β-galactosidase assay, ribonuclease protection assay, transcrpition factor assay, Laser capture microdissection, functional genomics, proteomics, RNA differential display, DNA Microarrays, foot-printing analysis, electrophoretic mobility shift assay, Chromatin Immunoprecipitation, antibody supershift assay, in vitro trancription and translation, in situ hybridization; Two Hybrid Selection, Protein arrays, ELISA, radioimmunoassay, Imaging Analyzer Bas-2000, Molecular Imager System GS-525, STORM-860 and TaqMan 7900.

2. Knowledge of and Experience in Systems Neuroscience and Hearing Research

I obtained the knowledge of systems neuroscience including the vestibular system in the medical school from 1978. I started researching on developmental retardation of brain and inner ear back in the mid-1980s. I created the animal model of endemic hypothyrioidism and cretinism (fed in an area of severe iodine deficiency and 11.04% incidence of endemic cretinism) and examined the influence of iodine deficiency and hypothyroidism on the morphogenesis of brain such as auditory cortex and cerebellum, and inner ear synaptogenesis in rats. Using both physiological (BSRA: Brain Stem Response Audiometry and DANAC-7E computerized ERA system for objective hearing) and morphological (transmission and scanning electron microscopy) techniques and the animal model of iodine-deficient rats, I studied the mechanisms of deafness in cretinism. I found that iodine deficiency led to sluggish development of the cochlear structures in the developing rats with delayed formation of the inner spiral sulcus and abnormal persistence of synaptic bodies, kinocilia and the junctional complexes as well. In the 60-day-old iodine-deficient rats the organ of Corti and spiral ganglion neurons were underdeveloped and accompanied by degenerative changes. The major lesion sites of cretinous deafness are found to be the peripheral sensory apparatus and the spiral ganglia neurons (the

first neurons in the auditory pathways) leading to irreversible hearing dysfunction with dramatically elevated hearing threshold, increased wave I latency and slightly delayed I-IV interpeak interval, which provide both theoretical and experimental evidence for the etiopathogenesis of the deafness in endemic cretinism. These work were published in the paper "Influence of Iodine Deficiency on Audition of Rats" in the prestigious Chinese Journal of Endemiology in 1990, and further in the papers of "Effects of Iodine Deficiency on the Structural Development of the Organ of Corti in Rat", "Immunological localization of glial fibrillary acidic protein and synaptophysin in the cerebellum of experimental cretinism rat", "Effect of ethylnitrosourea on the rat cerebellum", "Alteration of GABA receptors in mouse cerebellum after transplacental administration of methylnitrosourea", "Ultrastructural Changes of nerve fibers of the Organ of Corti in the Developing Rat on Iodine Deficient Diet", and "Number of Synapses in the Cerebellar Glomeruli of Experimental Cretinism Rat--An Electron Microscopic Analysis with Phosphotungstic Acid Stain" in the Journal of Medicine and Biology in Japan in 1992. I also successfully created the animal models of hydrocephalus and microcephaly in rats. I have much knowledge and experience in physiology, anatomy as well as pathology of the auditory system and inner ear development/synaptogenesis, and study in the influence of iodine deficiency on the hearing ability of rats and humans. Combining my major of the scientific state-of-the-art in molecular biology/genetics with my many years of experience in hearing research, I am good at research on molecular genetics and molecular biology of hearing and balance disorders. I have worked three years in the Lab for Genes of Motor Systems in RIKEN in Japan; I have the knowledge of balance control and the dopaminergic motor systems. I am good at preparing cochlear samples of rodents for scanning and transmission electronmicroscopy and locating the hair cells in inner ear, obtaining the heads and whole brains from fetus of E10.5, E13 and E15 mice, making fine dissections of different brain regions of rodents including striatum and hippocampus and isolating synaptosomal fractions. I studied the mechanisms and influence of estrogen on brain.

3. Skill in oral and written communication

I was born in a teacher's family and my father is a biologist (Prof.). I was a teacher in the medical university in China from 1982 to 1996. I have given lectures of pathology for more than 300 hours to the medical students (200 students/year) who appreciated my teaching. I was an amateur interpreter of English-Chinese for my university from 1984, and a Bilingual-English interpreter in Kochi Medical School in Japan (June 1993-March. 1996). I functioned as an interpreter and coordinator using the three languages at once: English, Japanese as well as Chinese for the international collaborations in medical research projects between the two medical schools. I obtained systematic training and practicing in the skill of oral and written communications as a teacher and principle researcher. Because I am a person of sincerity and modesty I can communicate very well with my colleagues and work as a member of a team. In early 1992 I passed the written and oral entrance examinations in English for the Graduate School of Medicine for PhD in Japan. In 1996 the Profs Committee held my PhD open evaluation meeting in English and I presented my dissertation and the thesis debate to the public of Kochi Medical School in Japan successfully. I was once appointed to give a speech in English on the Steroid Hormone Symposium in Tokyo instead of my professor in 1995. I have made many oral presentations in English, Japanese as well as Chinese in national and international conferences including the Third National Conference of Endemic Goiter and Endemic Cretinism in 1987, the Fourth National Conference on Iodine-Deficient Disease in 1991, The 81st Annual Meeting of the Japanese Pathology Society in 1992, The 68th Annual Meeting of the Japanese Biochemical Society in1995, The 20th Annual Meeting of The Molecular Biology Society

of Japan in 1997, The 28th Annual Meeting of The Society for Neuroscience in 1998 in Los Angeles, USA, the 29th Annual Meeting of the Society for Neuroscience in Miami Beach in1999, the 31st Annual Meeting of the Society for Neuroscience in San Diego in 2001, and the 32nd Annual Meeting of the Society for Neuroscience in Orlando in 2002. I cited 95 English literatures in my thesis on hearing research for Masters Degree of Medical Science in 1987. I published a number of full translations of advanced English literatures in Pathology in China in 1991. I wrote an English article "In My Second Hometown, Kochi" in April 1992 and published in the Communications and News of Kochi Medical School in Japan. In the past two-decades long teaching/researching careers in three countries, I wrote all my first author papers, abstracts, presentations, some parts of coauthor papers, and my grant applications. I also wrote some chapters of the textbooks of Pathology for China.

4. Ability to plan and organize

During graduate study (1984—1987) I had demonstrated skills and perspectives in initiating the project, designing experimental protocols and creatively conducting independent research work. I can analyze problems well and organize my research activities to solve them with the technical competencies and flexibility. After graduate study I became a principle investigator. I wrote the applications for research grants and obtained my own grants from Chinese government. I was responsible for research on the developmental retardation of brain and inner ear and their synaptogenesis using the rat animal model of endemic cretinism (internationally confirmed). My findings in endemic cretinism "Influence of Iodine Deficiency on Audition of Rats" were unprecedented in the world at that time. I participated in and well organized the collaborative research with Kochi Medical School (a top national university in Japan) on clinical study of endemic cretinism (our patients). The work was published: "Thyroid Stimulating Hormone in The Endemic Cretinism". I also supervised the assistant professors in my department for my researches projects. I became Associate Professor of Medicine in 1994. I won "The First Prize for Research Article of Excellence" in 1991 and the award of "Achievements in Scientific Research" on the Pathogenesis of Developmental Retardation of Brain in Endemic Cretinism, in 1995. I was recognized by national and international conferences: The First National Conference of Mid-aged and Young Scientists in Pathology in 1991, the 80th Annual Meeting of the Japanese Pathology Society, the 32nd Annual Meeting of the Japanese Teratology Society in 1992, the China-Japan Medical Conference in Beijing in 1992. During the Ph.D. and postdoctoral studies I further mastered multi-disciplinary techniques, especially in molecular biology/genetics and made a number of collaborate studies in those fields. The paper "Functional analyses of mammalian protein kinase C isozymes in budding yeast and mammalian fibroblasts" was published in 1997. I am energetic and good at making several experiments at the same time by organizing them overall. I can make many experiments very proficiently. I am said having good sense for experiments. The sense consists of the nice intuition and much experience of research and it can guide me avoiding some mistakes and save much time. I can well apply any new technique to my research according to the necessity and I also like to make some modifications for it. I usually have advanced and comprehensive imagination or anticipation for my research and experiments. I have a strong background in molecular biology/genetics, cell biology, biochemistry, endocrinology, neurobiology, immunology as well as developmental biology. In addition I am a pathologist originally, I have a familiarity with many aspects of medical science. I have experience in developing rodent models of CNS or inner ear diseases. Recently I won the awards "For Recognition of Outstanding Neuropeptide Research", on the 22nd Annual Winter Neuropeptide Conference, Breckenridge, USA in 2001, and the " NIH Fellows Award for Research Excellence" In

Recognition of Excellence in Biomedical Research, 2002, USA. I was the Authorized User of Radioactive Materials at NIH and responsible for the monthly report of my lab to the Radiation Safety Branch of NIH. I have much knowledge and experience in Natural Dialectics, Philosophy, medical oversight and management of clinical researches. I have published a new first author and corresponding author paper on GDNF effect on neuromuscular synapse. Our new paper "The role of the theta isoform of protein kinase C in activity-dependent synapse elimination: evidence from the PKC theta knockout mouse in vivo and in vitro" has been published in the Journal of Neuroscience. All my research projects, for masters' degree, PhD and postdoctoral researches were initiated and written by myself. I have acquired the full abilities in initiating research projects, developing protocols, conducting studies, documenting findings, interpreting results in a published report, supervising staff and PhD students, and managing the budgets. I have the capability of inter-disciplinary research and evaluating the availability and level of resources that can be applied to program area needs, which is very important to the success of a lab. I have worked as Scientific Judge in Neuroscience--Cellular and Molecular Study Section (20 reviewing/ranking and judgments) for the NIH Fellows Award for Research Excellence 2003 Competition. I have been a reviewer for the journal of NEUROSCIENCE periodically contributing to the scientific peer review process.

5. Knowledge of Federal Grants/Contracts

As a principle investigator I wrote the applications for research grants and obtained my own research grants from the Chinese government. I was a major "Cooperative Researcher" on the Pathological Study in Developmental Retardation of Brain supported by Grants-in-Aid for International Scientific Research Program from the Ministry of Education, Science and Culture (Monbusho) of Japan for two years and finished that project or contract successfully. Therefore I could get further financial support from the Japanese government (Monbusho Scholarship in Medical Science) for four and a half years. I worked as a postdoctoral research fellow at the Laboratory for Genes of Motor System from 1996 to 1999 in the Institute of Physical and Chemical Research (RIKEN, the largest research institute under the Japanese Ministry of Science and Technology). I also worked as a Postdoc and then an intramural research fellow at NIH supported by the US government. I have learned how to apply for the federal grant from NIH. I am familiar with the intramural research program and have the knowledge of extramural research of federal grants/contracts issues. I set up all the equipments and apparatus for molecular biology and biochemistry experiments with the budgets of my lab. Through the two decades of comprehensive trainings, government-sponsored medical researches and international collaborations I acquired a good understanding of the history, interests, internal dynamics and relationships of organizations in which medical research is conducted, and the government-sponsored research projects involving complex administrative and management issues at NIH and university.

In September 1999 I came to NIH from Japan with B-1 visa (I also had other 4 postdoctoral job offers outside NIH at that time) and NIH changed my B-1 to J-1 visa. In August 2003 I obtained the O-1 visa (alien of extraordinary ability in science) authorized to work in the US for another tree years approved by the USCIS, Department of Justice and Department of State. Now my waiver of the "two-year foreign country residency" has been approved by the USCIS and Department of State. My Green Card is pending with approved Employment Authorization (EAD) Card to work in the US. So I can be employed without any additional immigration paper work.

Li-Xia Yang, MD/PhD

Presentation on the 29th Annual Meeting of Society for Neuroscience by Li-Xia Yang:

NOVEL cDNAs ENCODING THE SOLUBLE GFRα-1 ISORECEPTORS, GFRα-1d AND GFRα-1e IN MOUSE AND HUMAN 608.12 (Abstract)

L.-X. Yang* and K. Kiuchi. Lab. for Genes of Motor Systems, Bio-Mimetic Control Research Program, RIKEN; Nagoya, Aichi 4630003, Japan.

Many *in vitro* binding experiments have shown that both the membrane-bound and the exogenous soluble GFRα-1 can mediate the binding and activation of Ret tyrosine kinase by GDNF. To date, however, there is no information on the presence of an endogenous soluble form of GFRα-1. Here we report the existence of two soluble isoreceptors of GFRα-1 *in vivo*. From the cDNA library of P21 mouse midbrain we cloned out two full-length cDNAs encoding the novel proteins of 425 and 420 amino acids, termed as GFRα-1d and GFRα-1e, respectively, following the GFRα-1c reported previously (L-X. Yang and K. Kiuchi, Soc. Neurosci. Abstr. 1998), Both GFRα-1d and GFRα-1e are novel splice variants of GFRα-1. They have the same hydrophilic C-terminals lacking the hydrophobic domain for glycosyl-phosphatidylinositol (GPI) attachment, which are quite different from the former isoreceptors. With omitting exon 10 and exon 11, their stop codons locate in exon 9B, the novel spliced exon in the genomic DNA. *GFRα-1d* contains the 15 bp of exon 5 and exon 1A in the 5'-UTR, while *GFRα-1e* has the deletion of exon 5. Northern blot analysis with the specific probes of the novel cDNAs displayed the expression of 2-kb and 5-kb transcripts in fetal heart, lung, liver, skeletal muscle, kidney and in the developing brains of E10.5, E13.5, E16, E18 and P14. RT-PCR data indicated that the soluble isoreceptors also extensively express in various adult tissues: brain, heart, lung, liver, skeletal muscle, kidney, thymus and testis. From the human fetal brain cDNA library we obtained the full-length cDNAs encoding the human soluble isoreceptors GFRα-1d and GFRα-1e consisted of 416 and 411 amino acids, respectively. The discoveries of the soluble isoreceptors of GFRα-1 provide *in vivo* evidence that GFRα-1 can mediate Ret activation by GDNF not only in cis but also in trans to regulate the neuronal survival and neuron-target interactions in the nervous system. (Supported by the Frontier Research Program of RIKEN)

Key Words: Splice variants, Neurotrophic, Gene, Neuron-target
29th Annual Meeting of Society for Neuroscience, Miami Beach, Oct. 23-28, 1999

Presentation on the 28th Annual Meeting of Society for Neuroscience by Li-Xia Yang:

MOLECULAR ANALYSIS OF THE cDNA AND GENOMIC DNA ENCODING MOUSE GFRα-1 AND IDENTIFICATION

OF A NOVEL ISOFORM OF GFRα-1 611.3 **(Abstract)**

L.-X. Yang* and K. Kiuchi. Lab. for Genes of Motor Systems, Bio-Mimetic Control Res. Centre, Inst. of Phys. and Chemical Res. (RIKEN); Nagoya, Aichi 4630003, Japan.

Members of the glial cell line-derived neurotrophic factor (GDNF) new family, including GDNF and neurturin (NRTN), are important in the control of vertebrate neuron survival and differentiation. They signal via a receptor complex of a glycosyl-phosphatidylinositol (GPI)-linked ligand binding subunit (the α component) and the receptor tyrosine kinase Ret as a signaling subunit. GDNF receptor α (GFRα-1), the first member of the receptor family, was recently identified and found to be able to function as a coreceptor for both GDNF and NRTN in vitro. GFRα-1 mRNA is widely distributed in the peripheral and central nervous systems. In order to investigate the mechanisms underlying the regulation of GFRα-1 gene expression and its biological functions in vivo, we constructed the cDNA library of mouse brain and performed 5′- and 3′-RACE and RT-PCR. We also screened out several genomic clones of mouse GFRα-1 gene from a Lambda FIX II mouse genomic library using the cDNA fragments of coding region as probes and devised a PCR based approach and genomic walking for locating all the exon-intron boundaries and the promoter region. We obtained the full-length cDNA for the mouse GFRα-1 gene with two kinds of nucleotide sequences in 5′-untranslated region and a 1.9 kb 3′-untranslated region to which a poly (A) tract is attached. We identified three isoforms of GFRα-1 existing in mouse brain. The major isoform, termed as GFRα-1a shows identical sequence of coding region with that in Genebank encoding a protein of 468 amino acids with the 5′-untranslated exon 1A, connecting with or lacking the non-coding exon 1B. The second isoform, named GFRα-1b, has a deletion of 5 amino acids. The third one, designated as GFRα-1c, a novel isoform of GFRα-1, contains a 30 bp nucleotides insertion in coding region resulting in a single long open reading frame encoding a protein of 478 amino acids. Both GFRα-1b and GFRα-1c are splice variants from the single gene, mouse *GFRα-1* that comprises 13 exons. (Supported by the Frontier Research Program of RIKEN)

The 28th Annual Meeting of the Society for Neuroscience, Los Angeles, Nov. 7-12, 1998