DIVISION OF SPECIATION MECHANISMS I

Professor:	*Tetsuo Yamamori*
Research Associates:	*Yuriko Komine*
	Akiya Watakabe
	Takashi Kitsukawa
Technical Staffs:	*Hideko Utsumi*
	Kaoru Sawada
NIBB Postdoctoral Fellow:	*Rejan Vigot*
Visiting scientist:	*Yoshinori Shirai*
Graduate student:	*Shiro Tochitani*
	Katsusuke Hata
	Yusuke Komatsu
	Shuzo Sakata
	(kyoto University)
	Ryohei Tomioka
	(Kyoto University)

Our research goal is to understand mechanisms underlying evolution of the nervous system. In order to approach this question, we are studying in two major subjects. 1) One approach is to understand informational processing in the brain underlying learning behaviors. 2) The second approach is to study the genes that are expressed in the specific areas of the monkey neocortex. Here, we report our findings of this year (1999).

I. Gene expression and cerebellar long-term plasticity

In order to know roles of the genes involved in long-term memory, we choose the cerebellum as a model system. In the cerebellum the conjunctive stimuli of parallel fibers and a climbing fiber to a Purkinje cell induce prolonged reduction of a synaptic efficacy between the paralleled fiber to the Purkinje cell (LTD; long-term depression, Ito et al., 1982). Previously, we examined the expression of 10 immediate early genes (IEGs) including all the known Fos and Jun family in cerebellar slices under the pharmacological condition that cause long-term desensitization of the Purkinje cell to AMPA (a glutamate analogue). Among the IEGs examined, Fos and Jun-B were predominantly induced under the conjunctive condition (Nakazawa et al., 1993).

We further examined Jun-B expression in vivo under a conjunctive protocol of AMPA, a pharmacological substitute for parallel fiber stimulation, and climbing fiber stimulation via electric Inferior Olive stimulation. June-B are predominantly induced around the local area where the AMPA and climbing fiber stimulation were conjunct. These results suggest that the coincidence mechanism may exist at gene expression level and lead to a cerebellar long-term plasticity. However, it has not been still clear how these newly synthesized gene products are involved in cerebellar LTD. We are currently investigating the mechanisms.

II. Gene expression under audio-visual discrimination task

We are studying the gene expression of c-Fos under audio-visual discrimination tasks in collaboration with Dr. Yoshio Sakurai (Kyoto University). We found that the visual and audio tasks enhanced the specific expression of c-Fos in the visual and audio cortex, respectively. Among the early visual and auditory pathways examined, c-Fos was specifically induced in the cortexes but not in the earlier pathways, suggesting the neural modulation of the neocortex depending on the types of the tasks (manuscript in preparation).

III. Color-defective (dichromat) monkeys

Several percent of humans, most of whom are X-chromosome linked, have been reported to be color deficient. However, despite the color pigment genes of macaque monkeys are very similar to human in the nucleotide sequence, no color-deficient monkeys have been found. We have examined 3153 macaque monkeys using an assay to identify a deletion of exon 5 of the long wave (red) pigment and middle wave (green) pigment genes by PCR amplification with a common primers in both genes and discriminating them by the following *Mbo* I restriction enzyme digestion, and found three monkeys that lacked the exon five of the long wave pigment genes. Further genetic analysis revealed that the three monkeys possessed a hybrid of long (exon 1 to 4) and middle wave (exon 5 and 6) pigment genes (Fig. 1). Analysis of photobleaching difference absorption spectra showed that the absorbency maxima (λmax) of reconstituted L, M and hybrid pigments are 564, 532 and 538 nm, respectively. The λmax for the hybrid was red-shifted by 6 nm from that of the M pigment, indicating that these monkeys are almost protanopic (insensitive to red). The ratio of dichromats in macaque monkeys (approximately 0.1%=3/3153) is significantly lower than that of 1% in humans. These results suggest that there may be some mechanisms which do not exist in humans to reduce the occurrence of color-deficiency in macaque monkeys.

IV. Brain Specific Repetitive (Bsr) RNA

During our attempt to isolate LTD-related genes in cultured cerebellar Purkinje cells, we accidentally found repetitive genes which were specifically expressed in the rat brain (Bsr: brain specific repetitive gene). Among the cells of the rat brain Bsr RNA tends to express in the relatively large cells and in phylogenetically old structures, such as the pareo- and archicortex, amygdala, thalamus and hypothalamus (see Fig. 2., for example). To our surprise, the genes are only found in the rattus but no other species so far examined including murine species. Although we do not know the function of this new type of gene at the moment, it may play some important role in the rat brain and we hope further characterization of the gene reveals it.

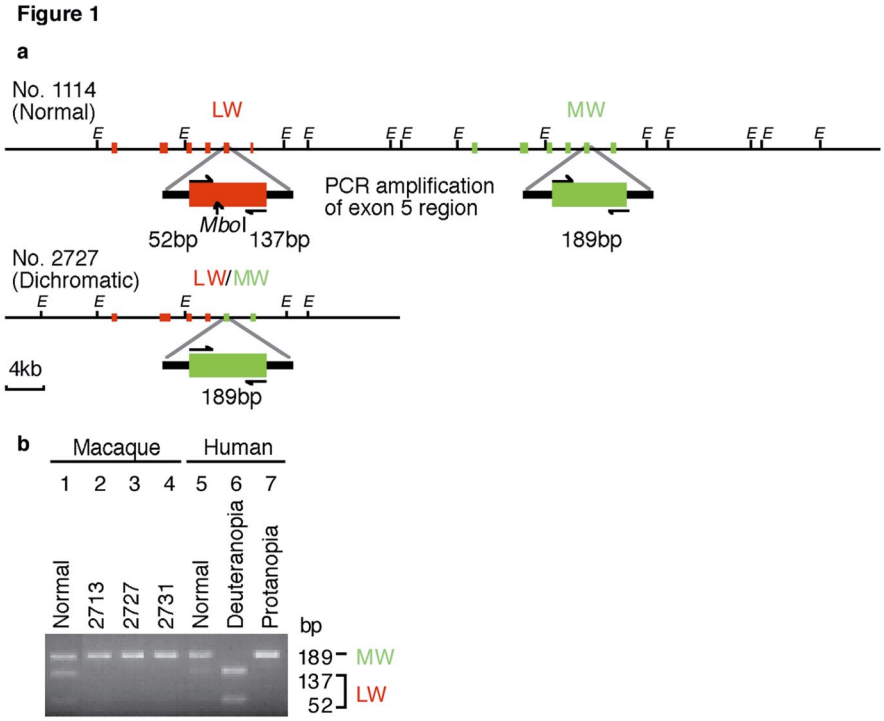

Fig. 1 Physical maps of the L and the M genes. a, Top, the 77-kilobase (kb) region of a wild-type macaque, showing exons of the L (red) and M (green) genes. Enlarged exon-5 regions are shown underneath. The PCR primers and products for exon 5 of the L and M genes are shown in red (137 and 52 base pairs (bp), cleaved by MboI digestion) and green boxes (189 bp, uncleaved), respectively. An Mbo I site specific for exon 5 of the L gene is conserved among Old World monkeys. Bottom, the 36-kb region of a dhichromatic candidate. The enlarged PCR-amplified fragment is shown underneath. B, Gel elctrophoresis analysis. The bands of 189, 137 and 52 bp shown in a are indicated as M (green) or L (red). Lane 1, macaque wild type; lanes 2-4, dichromats, showing only the exon 5 fragment of the M gene; lane 5, human wild type; lanes 6 and 7, deuteranopic (M cone absent) and protanopic (L cone absent) humans, respectively. [Published in Onishi et al., *Nature* 402, 139-140, 1999]

V. Genes expressed in specific areas of the neocortex

The neocortex is most evolved in mammals, particularly in primates, and thought to play the major role in higher functions of the brain. The neocortex in the mammal can be divided into distinct functional and anatomical areas. It has been a matter of debate what extent the areas of the neocortex are genetically and environmentally determined. To access this question, we started to isolate the genes that are expressed in specific areas of the brain. Using differential display methods, we found at least two genes that indicated the area specific expression. One, designated OCC-1 is expressed in the occipital cortex in the brain and the other gene is expressed in the motor area. We are currently examining the detailed expression pattern of the genes. We are also further isolating area specific genes with RLCS (Restriction Landmark cDNA Scanning).

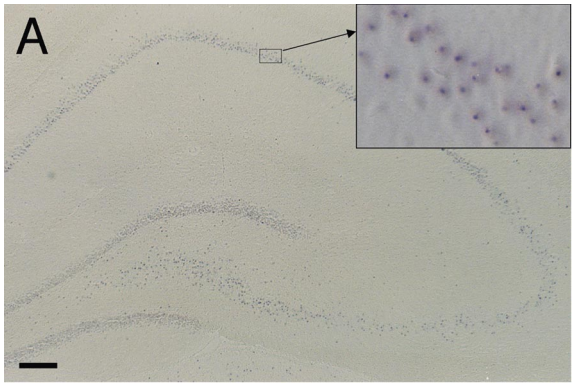

Fig. 2 Expression of Bsr in the rat hippocampus. In situ hybridization with an antisense probe of Bsr. Inset: higher magnification. [published in Komine et al., *Mol. Brain Res.*, 66, 1-13, 1999]

Publication List:

Liang, F., Hatanaka, Y., Saito, H., Yamamori, T. and Hashikawa, T. Differential expression of gamma-aminobutyric acid type B receptor-1a and 1b mRNA variants in GABA and non-GABAergic neurons of the rat brain. (2000) *J. Comp. Neurol*. 416, 475-495

Onishi, A., Koike, S., Ida, M., Imai, H., Shichida, Y., Tatenaka, O., Hanazawa, A., Komatau, H., Mikami, A., Goto, S., Suryobroto, B., Kitahara, K. and Yamamori, T. (1999) Dichromatism in macaque monkeys. *Nature* 422, 140-141.

Tochitani, S., Watakabe, A., Liang, F. and Yamamori, T. (1999) A molecular marker for a neuronal population dominantly distributed in the macaque occipital neocortex. *29th Society for Neuroscience (North America) Abstract* 2190.

Komine, Y., Tanaka, N., Yano, R., Takai, R., Yuasa, S., Shiroishi, T., Tsuchiya, K. and Yamamori, T. (1999) A novel type of non-coding RNA expressed in the rat brain. *Mol. Brain Res.*, 66, 1-13.

Matsuzawa, M., Muramatsu, T., Yamamori, T., Knoll, W. and Yano, R. (1999) Novel neuronal activities of midkine were examined on embryonic cerebellar neurons using a defined culture system. *Cell. Mol. Neurobiol.*, 19, 209-220.

Kawasaki, T., Kitsukawa, T., Bekku, Y., Matsuda, Y., Sanbo, M., Yagi, T., Fujisawa, H. (1999) A requirement for neuropilin-1 in embryonic vessel formation. *Development* 126, 4895-902