LABORATORY OF NEUROCHEMISTRY

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Our major research interest is to understand the physiological role of the dopaminergic system in animal behavior, particularly locomotion and eating behaviors, using genetically altered mice, both transgenic and gene knockout mice. In addition, we have developed a novel method of conditional mutagenesis in mice in order to substitute the amino acid sequence of the target gene in particular cells. We analyze the physiological roles of the components of the dystrophin complex on the skeletal muscle membrane using genetically modified mice.

Ⅰ. Role of dopaminergic transmission in locomotion and eating behavior

The dopaminergic system is implicated in the modulation of locomotor activity, the regulation of several peptide hormones in the pituitary, the modulation of synaptic plasticity and the development of neurons. The dopaminergic system is also implicated in the control of emotion, motivation and cognition. Dysfunction of the dopaminergic system can result in several neurological and psychiatric disorders, such as Parkinson's disease and schizophrenia.

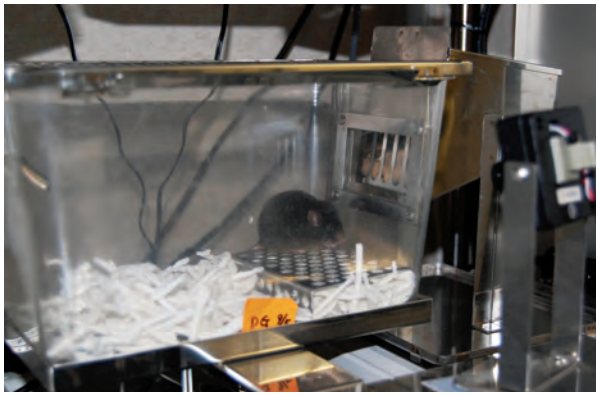

Figure 1. Experimental equipment for measurement of locomotor activity and food/water intake

In mammals, five subtypes of dopamine receptor (D1R, D2R, D3R, D4R and D5R) have been identified and divided into two subgroups, referred to as D1-like receptors (D1R, D5R) and D2-like receptors (D2R, D3R and D4R) on the basis of their gene structure and their pharmacological and transductional properties. D1R and D2R are the most abundantly and widely expressed in the brain and often play a synergistic role. D1R has an opposite property to D2R with respect to intracellular signal transduction.

In collaboration with the Laboratory of Dr. Motoya Katsuki, the former Director General, we have been investigating the involvement of dopaminergic transmission via D1R and D2R in the regulation of locomotion and eating behavior. We generated *D1R/D2R* double knockout (DKO) mice by crossing *D1R* knockout (KO) with *D2R* KO mice, and observed that *D1R/D2R* DKO mice exhibited severe impairment in locomotion, no initiation of eating, and died by 4 weeks of age. To investigate the molecular mechanism of motor control and eating behavior, we generated transgenic mice harboring tetracycline-regulated expression of the *D1R* gene on the *D1R/D2R* DKO background. Several transgenic mouse lines successfully rescued lethal phenotype of the *D1R/D2R* DKO mice and showed doxycycline (Dox) controllable expression of transgenic *D1R* gene (named as *D1R/D2R* DKO-*D1R* rescued mice). The *D1R/D2R* DKO-*D1R* rescued mice exhibited decreases in locomotion and food/water intake as well as a decrease in the amount of transgene expression after Dox administration. After the withdrawal of Dox administration, the *D1R/D2R* DKO-*D1R* rescued mice exhibited transient hyperactivity and recovered locomotor activity and food/water intake. We are analyzing these results to identify the mechanism of the relationship between the *D1R* expression and altered behavior. In addition, we are also investigating whether or not there is a critical period in development for the regulation of locomotion and eating behavior by dopaminergic transmission.

Ⅱ. Developing a novel conditional mutagenesis method in mice

In collaboration with Prof. Yo-ichi Nabeshima of Kyoto University, we developed a novel mouse developmental biotechnology of introducing an amino acid substitution into a target gene in a spatially and temporally restricted manner. The goal of the study was to overcome the limitations of the conventional mouse molecular genetic approach in the functional analysis of target genes. We substituted one critical amino acid residue (the 595th asparagines, Asp595) of N-methyl-D-aspartate receptor (NMDAR), leading to an aberrant activation of NMDAR. The NMDARs are widely expressed in the nervous system, fundamental to excitatory neurotransmission, and play a number of important roles at different brain loci and time points. The NMDARs act as a coincidence detector and are not only important for neuronal differentiation, migration, and survival, but are also critical for activity dependent synapse formation. It is suggested that the aberrant activation of NMDAR causes excitotoxicity, leading to neuronal death in various neurological diseases.

Our method is vastly applicable to the functional analysis of any desired gene and should contribute to studies on the structural and functional relationships of relevant genes.

Ⅲ. Analysis of roles of the sarcoglycan complex, dystroglycan complex and caveolin-3

Sarcoglycans (SGs) are trans-sarcolemmal glycoproteins that associate together to form sarcoglycan complex (SGC) and are present in the sarcolemma. SGC, together with dystrophin and the dystroglycan complex, comprises the dystrophin complex, which is considered to be the mechanical link between the basement membrane and the

intracellular cytoskeleton for protecting the sarcolemma from mechanical stress during muscle contraction. Each of four SG subunits (α-, β-, γ- and δ-SG) is responsible for four respective forms of SG-deficient muscular dystrophy, sarcoglycanopathy (SGP). We previously generated the β-SG KO and γ-SG KO mice and found that the dystrophin complex isolated from the SG-deficient skeletal muscles was biochemically unstable. This indicates that SGC and sarcospan play an important role in stabilizing the dystrophin complex.

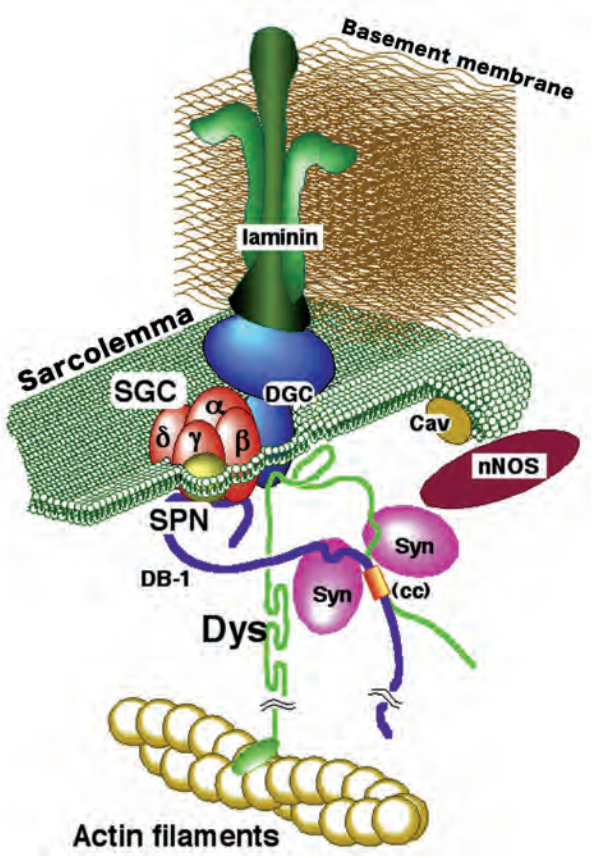

Figure 2. A schematic drawing of the dystrophin complex

Dystroglycan (DG) is a widely expressed transmembrane glycoprotein complex which plays important roles by connecting the intracellular cytoskeleton and the extracelluler matrix. DG is expressed as an 895 amino acid precursor and cleaved between amino acid residues 653 and 654 of the precursor to generate α- and β-DG subunits. In collaboration with Dr. Torahiko Tanaka of Nihon University School of Medicine, Tokyo, we performed a series of mutation analyses to determine which amino acid residues and which regions of DG are critical for cleavage in order to clarify the mechanisms involved in DG cleavage and subunit association. We transfected cultured cells with wild-type and various mutant DGs, and confirmed the DG cleavage. We found the following: (i) Disruption of the intramolecular disulfide bridge between Cys669 and Cys713 in β-DG completely abolishes cleavage; (ii) deletions in the loop region (669-713) and in the C-terminal region of α-DG (550-645) abolish the cleavage; (iii) disruption of the disulfide bridge and deletions in the loop region deteriorate the α- and β-DG association; (iv) positions P1' (Ser654) and P6' (Trp659) are critical, particularly at the cleavage site. Thus, the critical role of the Cys669-Cys713 disulfide bridge formation is, most likely, to form a specific tertiary structure, in which the α- and β-DG domains interact and the cleavage site becomes susceptible to proteolytic reactions. The Cys669 and Cys713 pair is broadly conserved in vertebrates and in some invertebrates, suggesting that the disulfide bridge formation was established early in the evolution of DG.

Caveolin-3 is a muscle-specific membrane protein, a component of the dystrophin complex, and serves as a scaffold of various molecules. Its gene mutations cause limb-girdle muscular dystrophy (LGMD1C or caveolinopathy) with mild clinical symptoms. In collaboration with Dr. Yasuko Hagiwara of Musashino University, Tokyo, we previously reported that caveolin-3 deficiency causes muscle degeneration and a decrease in sarcolemmal caveolae in *caveolin-3* gene-knockout (*Cav3-/-*) mice. To examine the pathogenic pathways and identify new or modifying factors involved in caveolinopathy, we examined the gene expression profiles of approximately 8,000 genes in the skeletal muscle of Cav3-/- mice using DNA microarray technique. We found that the gene of *osteoponin* (*OPN*), a versatile regulator of inflammation and tissue repair, was significantly down-regulated. This is in contrast to *mdx* mice showing a markedly up-regulated *OPN* gene in their skeletal muscles. Recently, *OPN* has been reported to be important in the pathogenesis of muscular dystrophy. We examined whether up-regulated *OPN* gene expression in *mdx* muscles is altered by the deficiency of caveolin-3. To this end, we developed caveolin-3 and dystrophin double-deficient mice. The levels of *OPN* mRNA and osteopontin in the double-deficient mice clearly decreased compared with those in *mdx* mice. We showed that although the level of *OPN* mRNA expressed in the double-deficient skeletal muscles was lower than that in *mdx* skeletal muscles, macrophage infiltration and muscle regeneration occurred similarly in the double-deficient and *mdx* skeletal muscles. There may still be other factors that are involved in macrophage infiltration and muscle regeneration.

Publication List

〔Original papers〕

- Ohi, Y., Ishii, Y., Haji, A., Noguchi, S., Sasaoka, T., Fujimori, T., Nabeshima, Y., Sasahara, M., and Hattori, Y. (2007). Platelet-derived growth factor (PDGF)-BB inhibits AMPA receptor-mediated synaptic transmission via PDGF receptor-beta in murine nucleus tractus solitarius. Brain Research *1159*, 77-85.
- Watanabe, N., Sasaoka, T., Noguchi, S., Nishino, I., and Tanaka, T. (2007). Cys669-Cys713 disulfide bridge formation is a key to dystroglycan cleavage and subunit association. Genes to Cells *12*, 75-88.