

## DIVISION OF MOLECULAR NEUROBIOLOGY



Professor  
NODA, Masaharu

Assistant Professors	SHINTANI, Takafumi SAKUTA, Hiraki HIYAMA, Takeshi
Technical Staff	TAKEUCHI, Yasushi
NIBB Research Fellow	IHARA, Masaru
Postdoctoral Fellows	FUJIKAWA, Akihiro SUZUKI, Ryoko SHIMIZU, Hidetada NISHIHARA, Eri YOSHIDA, Masahide YONEHARA, Keisuke
Graduate Students	YONEHARA, Keisuke* CHOW, Pak Hong Jeremy NAGAKURA, Ayano ZHANG, Lanfan TANI, Sachiko SAKURABA, Jyuichi
Visiting Scientist	YONEHARA, Kayo
Technical Assistants	MIZOGUCHI, Masae KATAYAMA, Tomomi HATTORI, Nobuko TOMITA, Nao ISOSHIMA, Yoshiko MIURA, Seiko
Secretaries	KODAMA, Akiko YATAGAI, Hiroko

We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system (CNS), mainly using the visual systems of chicks and mice. This research covers many developmental events including the patterning of the retina, axonal navigation, branching and targeting, synapse formation, refinement and plasticity. The scope of our interests also encompasses the mechanisms for various functions of the mature brain, including sensation, emotion, behavior, learning, and memory.

### I. Mechanisms for retinal patterning during development

Topographic maps are a fundamental feature of neural networks in the nervous system. The retinotectal projection of lower vertebrates including birds has been used as a readily accessible model system of the topographic projection. We have been studying the mechanisms for regional specification in the developing retina as the basis of the topographic retinotectal projection.

Fifteen years ago, we performed a large-scale screening using differential hybridization and restriction landmark cDNA scanning (RLCS) on the embryonic day 8 (E8) chick retina, and successfully identified 33 asymmetric molecules along the nasotemporal (N-T) axis and 20 along the dorsoventral (D-V) axis. We have almost revealed gene cascades of topographic molecules for retinal patterning and

for the topographic retinotectal projection (see Annual Report 2006). We are now conducting experiments to elucidate the functional modes of CBF2, a winged-helix type transcription factor, which specifies the temporal character in the retina.

### II. Mechanisms for the topographic retinotectal projection

Special attention is now devoted to the molecular mechanisms for the axon branching and arborization and their selective elimination. Among the region-specific molecules in the developing retina, we have already identified several molecules which induce abnormal branching and arborization when their expression was experimentally altered *in vivo*. We have obtained promising results indicating that our research will continue to shed light on these issues.

### III. Development of retinal ganglion cell subtypes

Visual information is transmitted to the brain by roughly a dozen distinct types of retinal ganglion cells (RGCs) defined by a characteristic morphology, physiology, and central projections. However, because few molecular markers corresponding to individual RGC types are available, our understanding of how these parallel pathways develop is still in its infancy. Previously, we reported a secretory protein, *SPIG1* (clone name; D/Bsp120I #1), preferentially expressed in the dorsal region in the developing chick retina (Shintani *et al.*, 2004). Subsequently, we generated knock-in mice to visualize *SPIG1*-expressing cells with green fluorescent protein. We found that the mouse retina is subdivided into two distinct domains for *SPIG1* expression and *SPIG1* effectively marks a unique subtype of the retinal ganglion cells during the neonatal period (Figure 1).

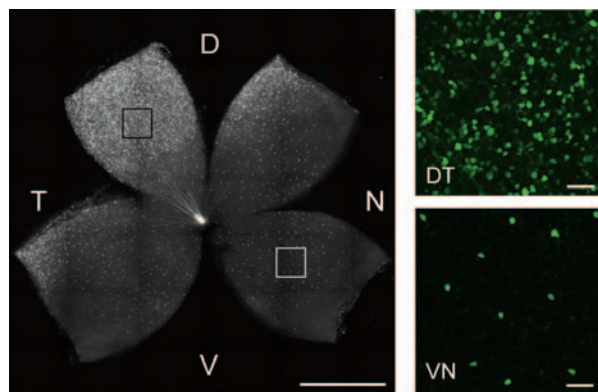


Figure 1. *SPIG1* expression in the mouse retina at P5. *SPIG1*-positive retinal ganglion cells are densely distributed in the dorsotemporal retina. In the remaining region of the retina, only a subtype of the retinal ganglion cell appears to express *SPIG1*, showing a mosaic distribution. Enlargements of the boxed regions are shown on the right. N, T, D, and V indicate nasal, temporal, dorsal, and ventral, respectively. Scale bars: 1 mm (left panel), 50 $\mu$ m (right panels).

*SPIG1*-positive RGCs in the dorsotemporal domain project to the dorsal lateral geniculate nucleus (dLGN), superior colliculus, and accessory optic system (AOS). In contrast, in the remaining region, here named the pan-ventronasal domain, *SPIG1*-positive cells form a regular mosaic and project exclusively to the medial terminal nucleus (MTN) of the AOS that mediates the optokinetic nystagmus as early as P1. Their dendrites costratify with ON cholinergic amacrine strata in the inner plexiform layer as early as P3. These findings suggest that these *SPIG1*-positive cells are the ON direction selective ganglion cells (DSGCs). Moreover, the MTN-projecting cells in the pan-ventronasal domain are apparently composed of two distinct but interdependent regular mosaics depending on the presence or absence of *SPIG1*, indicating that they comprise two functionally distinct subtypes of the ON DSGCs. The formation of the regular mosaic appears to commence at the end of the prenatal stage and be completed through the peak period of the cell death at P6. *SPIG1* will thus serve as a useful molecular marker for future studies on the development and function of ON DSGCs.

#### IV. Physiological roles of protein tyrosine phosphatase receptor type Z

Protein tyrosine phosphatase receptor type Z (Ptpz, also known as  $PTP\zeta$  /  $RPTP\beta$ ) is preferentially expressed in the CNS as a major chondroitin sulfate proteoglycan. Three splicing variants, two receptor isoforms (Ptpz-A/B) and one secretory isoform (Ptpz-S) are known. Receptor-type Ptpz interacts with the PSD95 family through its intracellular carboxyl-terminal PDZ-binding motif in the postsynaptic density. *Ptpz*-deficient adult mice display impairments in spatial and contextual learning.

##### 4-1 Plasmin mediated processing of Ptpz

We identified the proteolytic processing of Ptpz by plasmin in the mouse brain, which is markedly enhanced after kainic acid (KA)-induced seizures. We mapped plasmin cleavage sites in the extracellular region of Ptpz by cell-based assays and *in vitro* digestion experiments with recombinant proteins (Figure 2). These findings indicate that Ptpz is a physiological target for activity-dependent proteolytic processing by the tPA/plasmin system, and suggest that the proteolytic cleavage is involved in the functional processes of the synapses during learning and memory.

##### 4-2 Metalloproteinase- and $\gamma$ -secretase-mediated processing of Ptpz

We also found that the extracellular region of the receptor isoforms of Ptpz are cleaved by metalloproteinases, and that the membrane-tethered fragment is subsequently cleaved by presenilin/ $\gamma$ -secretase, releasing its intracellular region into the cytoplasm (Figure 2). Notably, the intracellular fragment of Ptpz shows nuclear localization. Administration of GM6001 (an inhibitor of metalloproteinases) to mice demonstrated the metalloproteinase-mediated cleavage of Ptpz under physiological conditions. Furthermore, we identified the

cleavage sites in the extracellular juxtamembrane region of Ptpz by tumor necrosis factor- $\alpha$  converting enzyme (TACE) and matrix metalloproteinase 9 (MMP-9). This is the first evidence of the metalloproteinase-mediated processing of a receptor-like protein-tyrosine phosphatase in the central nervous system.

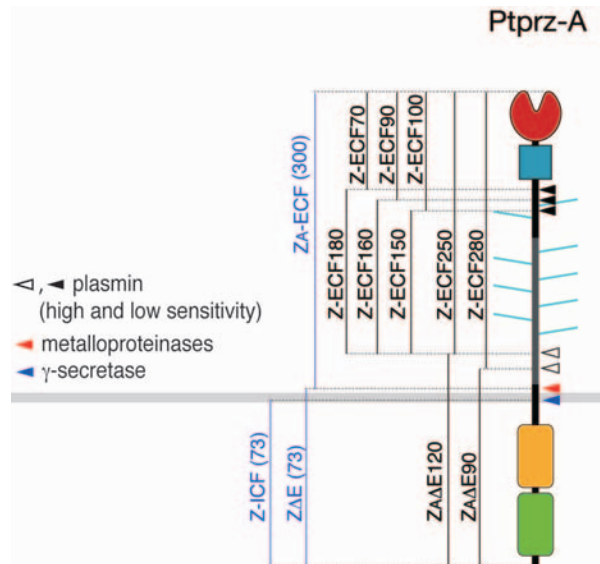


Figure 2. Proteolytic processing of Ptpz. Generated fragments are shown with their molecular size. Ptpz is a target for activity-dependent processing, which is supposedly involved in the synaptic remodeling during learning and memory.

##### 4-3 Identification of TrkA as a substrate for Ptpz

Tropomyosin-related kinases (Trks) are single-pass transmembrane molecules that are highly expressed in the developing nervous system. Upon the ligand binding of neurotrophins, Trk receptors are activated through autophosphorylation of tyrosine residues; however, the PTPs responsible for the negative regulation of Trk receptors have not been fully elucidated. We identified Ptpz as a specific PTP that efficiently dephosphorylates TrkA as a substrate (Figure 3). Co-expression of Ptpz with Trk receptors in 293T cells showed that Ptpz suppresses the ligand-independent tyrosine phosphorylation of TrkA, but not of TrkB or TrkC, and that Ptpz attenuates TrkA activation induced by nerve growth factor (NGF). Co-expression analyses with TrkA mutants revealed that Ptpz dephosphorylates phosphotyrosine residues in the activation loop of the kinase domain, which are requisite for activation of the TrkA receptor. Consistent with these findings, forced expression of Ptpz in PC12D cells markedly inhibited neurite extension induced by a low dose of NGF. In addition, an increment in the tyrosine phosphorylation of TrkA was observed in the brain of *Ptpz*-deficient mice. Ptpz thus appears to be one of the PTPs which regulate the activation and signaling of TrkA receptors.

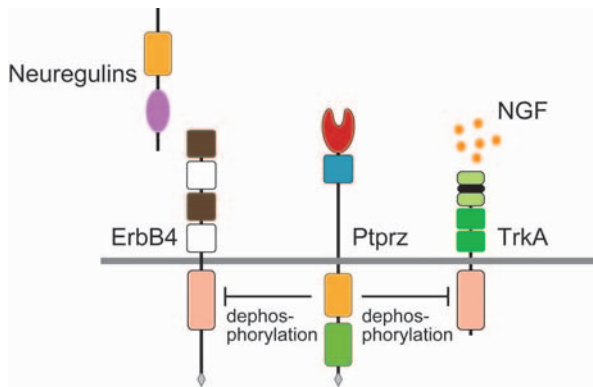


Figure 3. Schematic drawing of regulation of TrkA and ErbB4 by Ptpz. ErbB4 and TrkA are activated by their specific ligands, neuregulins and NGF, respectively. Ptpz attenuates the activation of ErbB4 (Fujikawa *et al.*, 2007) and TrkA (Shintani *et al.*, 2008) through dephosphorylation.

## V. Mechanisms of Na-level sensing in the brain for the body-fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluids. For Na homeostasis of the body, control of Na and water intake and excretion are of prime importance. Although it was suggested that the circumventricular organs (CVOs) are involved in body-fluid homeostasis, the system for sensing Na levels within the brain, which is responsible for the control of Na- and water-intake behavior, has long been an enigma.

$Na_x$  is an atypical sodium channel that is assumed to be a descendant of the voltage-gated sodium channel family. Our studies on the  $Na_x$ -knockout mice revealed that  $Na_x$  channels are localized to the CVOs and serve as a sodium-level sensor of body fluids.  $Na_x$ -knockout mice do not stop ingesting salt when dehydrated, while wild-type mice avoid salt.

$Na_x$  is exclusively localized to perineuronal lamellate processes extending from ependymal cells and astrocytes in the organs. Therefore, glial cells bearing  $Na_x$  channels are the first to sense a physiological increase in the level of sodium in body fluids. We revealed that Na-dependent activation of the metabolic state of the glial cells leads to extensive lactate production and that lactate stimulated the activity of GABAergic neurons in the subfornical organ (SFO). Thus, information of a physiological increase of the Na level in body fluids sensed by  $Na_x$  in glial cells is transmitted to neurons by lactate as a mediator to regulate the neural activity of the SFO to control salt-intake behavior.

Very recently, we found that  $Na_x$ -deficient mice show a reduced water intake compared with wild-type mice under non-feeding conditions. We suppose that animals deficient in the sodium-sensing system are apt to develop hypernatremia.

## Publication List

### [Original papers]

- Chow, J.P.H., Fujikawa, A., Shimizu, H., and Noda, M. (2008). Plasmin-mediated processing of protein tyrosine phosphatase receptor type Z in the mouse brain. *Neurosci. Lett.* *442*, 208-212.
- Chow, J.P.H., Fujikawa, A., Shimizu, H., Suzuki, R., and Noda, M. (2008). Metalloproteinase- and  $\gamma$ -secretase-mediated cleavage of protein tyrosine phosphatase receptor type Z. *J. Biol. Chem.* *283*, 30879-30889.
- Shintani, T., and Noda, M. (2008). Protein tyrosine phosphatase receptor type Z dephosphorylates TrkA receptors and attenuates NGF-dependent neurite outgrowth of PC12 cells. *J. Biochem.* *144*, 259-266.
- Tsuboi, N., Utsunomiya, T., Roberts, R.L., Ito, H., Takahashi, K., Noda, M., and Takahashi, T. (2008). The tyrosine phosphatase CD148 interacts with the p85 regulatory subunit of phosphoinositide 3-kinase. *Biochem. J.* *413*, 193-200.
- Yonehara, K., Shintani, T., Suzuki, R., Sakuta, H., Takeuchi, Y., Nakamura-Yonehara, K., and Noda, M. (2008). Expression of SPIG1 reveals development of a retinal ganglion cell subtype projecting to the medial terminal nucleus in the mouse. *PLoS ONE* *3*, e1533.

### [Review articles]

- Noda, M., Takahashi, H., and Sakuta, H. (2008). Neural patterning: Eye fields. In *Encyclopedia of Neuroscience*, Squire, L.R., ed. (Elsevier), pp. 199-204.
- Sakuta, H., Suzuki, R., and Noda, M. (2008). Retrovirus vector-mediated gene transfer into the chick optic vesicle by *in ovo* electroporation. *Dev. Growth Differ.* *50*, 453-457.