DIVISION OF MOLECULAR NEUROBIOLOGY

Professor:	*NODA, Masaharu*
Research Associates:	*SHINTANI, Takafumi*
	SAKUTA, Hiraki
	HIYAMA, Takeshi
Technical Staff:	*TAKEUCHI, Yasushi*
NIBB Research Fellow:	*TAKAHASHI, Hiroo*
Postdoctoral Fellows:	*FUJIKAWA, Akihiro*
	SUZUKI, Ryoko
	FUKADA, Masahide
	NAKAMURA, Takahiro
	YAMAMOTO, Yasunori
Graduate Students:	*IHARA, Masaru*
	ETANI, Kazuma
	SHIMIZU, Hidetada
	YONEHARA, Keisuke
	NAKAMURA, Kayo
	CHOW, Pak Hong Jeremy
	NAGAKURA, Ayano
	ZHANG, Lanfan
	AOSHIMA, Akihiro
Visiting Scientist:	*TAMURA, Hiroshi*
Technical Assistants:	*MIZOGUCHI, Masae*
	GOTOH, Megumi
	AYABE, Yuko
	KATAYAMA, Tomomi
	HATTORI, Nobuko
Secretary:	*KODAMA, Akiko*

We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system, 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 mechanisms for various functions of the mature brain, including sensation, emotion, behavior, learning and memory.

I. Mechanisms for regional specification in the developing retina

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 retina as the basis of the topographic retinotectal projection.

We can now present gene cascades for retinal patterning and region-specific expression of topographic molecules for retinotectal projection as in Figure 1. *FoxG1* and *FoxD1* are expressed in the nasal and temporal regions of the developing chick retina at an early stage (peaking at E3), respectively, and determine the regional specificity in the retina by their counteraction. Consequently, two homeobox transcription factors, *SOHo1* and *GH6*, are expressed specifically in the nasal region: it is known that the two control the retinotectal projection along the A-P axis by the repression of *EphA3* expression. Afterwards, *ephrin-A5* and *ephrin-A2* begin to show nasal-high expression and *EphA3* temporal-high expression in the retina. In this process, *FoxG1* controls *ephrin-A5* via a DNA binding-dependent mechanism, *ephrin-A2* via a DNA binding-independent mechanism (see below), and *FoxD1*, *SOHo1*, *GH6* and *EphA3* via dual mechanisms.

Along the D-V axis, counteraction between BMP4 and Ventroptin governs the regional specification in the retina. At the early stages of development from HH stage 11 to E5, dorsally-expressed *BMP4* determines the regional specificity of the dorsal retina and ventrally-expressed *Ventroptin* counteracts the activity of BMP4. Transcription factors *Tbx2/3/5* in the dorsal retina and *cVax* in the ventral retina begin to be expressed under the control of the BMP4 signal. At approximately E5, *BMP4* expression in the dorsal retina rapidly disappears. Concomitantly, *Ventroptin* turns to be expressed in an oblique-gradient fashion (V/N-high pattern from E6 onward). Then, instead of *BMP4*, *BMP2* begins to be expressed in an oblique-gradient fashion (D/T-high pattern), complementary to that of *Ventroptin,* to counteract it. The inhibitory effect of FoxG1 on the BMP signaling is thought to be responsible for turning the expression patterns of *Ventroptin* and *BMP2* about 30 degrees to the posterior side from the first D-V axis. Switching from *BMP4* to *BMP2* should occur owing to the difference in their genetic regulatory mechanisms, and this would be the basis of the tilting of the D-V axis in the developing retina. Expression of *EphBs*, *ephrin-Bs* and *ephrin-A2* are under the control of BMP2 signal. BMP2 thus appears to play an important role in the topographic projection along both axes.

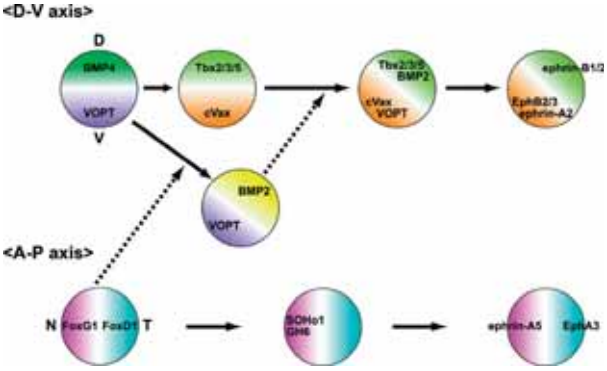

Figure 1. Developmental expression of topographic molecules in the chick retina. Regional specification along the nasotemporal (A-P) and dorsoventral (D-V) axes in the developing retina precedes the topographic retinotectal projection. The developing stage proceeds from left to right.

II. Mechanisms for the topographic retinotectal projection

Eph receptors are implicated in topographic projections in many regions of the developing nervous system, including the retinotectal (or retinocollicular) projection, where gradients of expression of Eph receptors and ephrins in the retina and tectum play essential roles. Eph receptors are activated by autophosphorylation of tyrosine residues upon the binding of their ligands, ephrins. The

protein tyrosine phosphatases (PTPs) responsible for the negative regulation of Eph receptors, however, have not been elucidated.

We identified protein tyrosine phosphatase receptor type O (Ptpro) as a specific PTP that efficiently dephosphorylates both EphA and EphB receptors as substrates. Biochemical analyses revealed that Ptpro dephosphorylates a phosphotyrosine residue conserved in the juxtamembrane region, which is required for the activation and signal transmission of Eph receptors. Ptpro thus appears to moderate the amount of maximal activation of Eph receptors. Using the retinotectal projection system, we showed that Ptpro controls the sensitivity of retinal axons to ephrins, and thereby plays a crucial role in the establishment of topographic projections. Our findings explain the molecular mechanism to determine the threshold of the response of Eph receptors to ephrins *in vivo*.

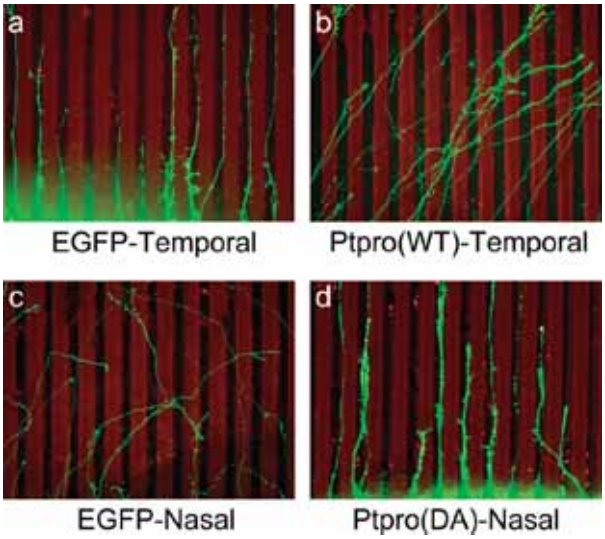

Figure 2. Regulation of the sensitivity of chick retinal axons to ephrin-A2-Fc by Ptpro. To visualize retinal axons, *egfp* was coelectroporated into the retina, and the retinal strips were subjected to the ephrin-A2-Fc stripe assay. (a) Control temporal axons preferred to grow on control Fc-containing lanes (dark) but not on ephrin-A2-Fc lanes (red). (b) Ptpro (WT)-overexpressing temporal axons randomly grew on both control Fc and ephrin-A2-Fc lanes. (c) Control nasal axons randomly grew on both control Fc and ephrin-A2-Fc lanes. (d) When the DA mutant of Ptpro, Ptpro (DA), was overexpressed, nasal axons showed preferential growth on control Fc lanes like the normal temporal axons.

III. Physiological roles of protein tyrosine phosphatase receptor type Z (Ptprz)

Ptprz (also called PTPζ/RPTPβ) is a receptor-type protein tyrosine phosphatase (RPTP) predominantly expressed in the brain as a chondroitin sulfate proteoglycan. *Ptprz*-deficient mice exhibit an age (maturation)-dependent impairment of spatial learning in the Morris water maze test and enhancement of long-term potentiation (LTP) in the CA1 region in hippocampal slices. The enhanced LTP is canceled out by pharmacological inhibition of Rho-associated kinase (ROCK), suggesting that the lack of *Ptprz* causes learning impairment due to aberrant activation of ROCK.

We found that *Ptprz*-deficient mice exhibit impairments in hippocampus-dependent contextual fear memory (Figure 3) because of abnormal tyrosine phosphorylation of p190 RhoGAP, a GTPase-activating protein (GAP) for Rho GTPase. Phosphorylation at Y1105, a major tyrosine phosphorylation site on p190 RhoGAP, was decreased 1 h after the conditioning in the hippocampus of wild-type mice, but not of *Ptprz*-deficient mice. Pleiotrophin (PTN), a natural ligand for Ptprz, increased tyrosine phosphorylation of p190 RhoGAP in B103 neuroblastoma cells. Furthermore, Ptprz selectively dephosphorylated pY1105 of p190 RhoGAP *in vitro*, and the tyrosine phosphorylation at Y1105 controled p190 RhoGAP activity *in vivo*. These results suggest that Ptprz plays a critical role in memory formation by modulating Rho GTPase activity through dephosphorylation at Y1105 on p190 RhoGAP.

RPTPs are considered to transduce extracellular signals across the membrane through changes in their PTP activity, however, our understanding of the regulatory mechanism is still limited. We revealed that PTN inactivates Ptprz through oligomerization and increases the tyrosine phosphorylation of substrates for Ptprz, G protein-coupled receptor kinase-interactor 1 (Git1) and membrane associated guanylate kinase, WW and PDZ domain containing 1 (Magi1). Oligomerization of Ptprz by an artificial dimerizer or polyclonal antibodies against its extracellular region also leads to inactivation, indicating that Ptprz is active in the monomeric form and inactivated by ligand-induced oligomerization.

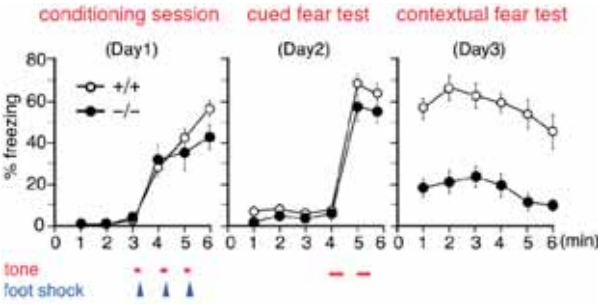

Figure 3. Impaired contextual fear memory in *Ptprz*-deficient mice. During the conditioning session, no differences were observed between the wild-type and *Ptprz*-deficient mice in freezing response on Day 1. *Ptprz*-deficient mice showed normal responses in the cued fear test on Day 2, but impairments in contextual fear conditioning on Day 3.

IV. Mechanisms for Na-level sensing in the brain and body fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluid. For Na homeostasis of the body, controls of Na and water intake and excretion are of prime importance. Although the circumventricular organs (CVOs) are thought to be involved in body-fluid homeostasis, the system for

sensing the Na level within the brain that is responsible for the control of Na- and water-intake behavior has long been an enigma.

We found that the Na_x channel is preferentially expressed in the CVOs in the brain and that *Na*$_x$-deficient mice ingest saline in excess under dehydrated conditions. Subsequently, we demonstrated that Na_x is a Na-level-sensitive Na channel. When *Na*$_x$ cDNA was introduced into the brain of the knock-out mice with an adenoviral expression vector, only animals which received a transduction of the *Na*$_x$ gene into the subfornical organ (SFO) among the CVOs recovered salt-avoiding behavior under dehydrated conditions. Based on these findings, we advocate that the SFO is the center for the control of salt-intake behavior in the brain, where the Na-level-sensitive Na_x channel is involved in sensing the physiological increase in the level of Na in body fluids.

We recently found that Na_x channels are specifically expressed in the perineuronal processes of astrocytes and ependymal cells enveloping particular neural populations in the sensory CVOs (Figure 4). These Na_x-positive glial cells were sensitive to an increase in the extracellular sodium level, indicating that glial cells – not neurons – are the primary site of sodium-level sensing. The mechanism by which the sodium signal sensed by ìinexcitableî glial cells is transferred to neurons remains to be elucidated.

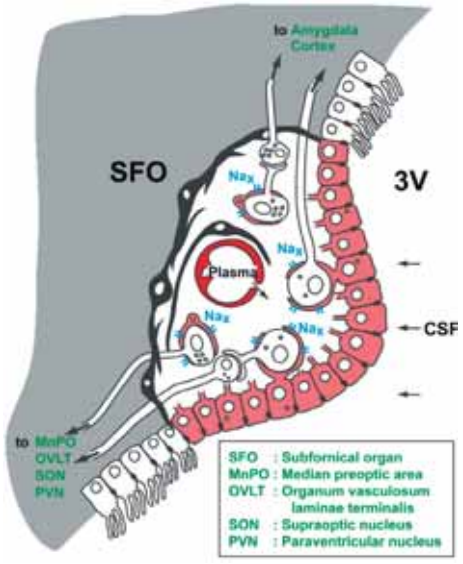

Figure 4. Schematic drawing of the SFO. Na_x is exclusively localized to perineuronal lamellate processes extended from ependymal cells and astrocytes. This suggests that glial cells bearing Na_x are the first to sense a physiological increase in the level of sodium in the body fluid, and they regulate the neural activity by enveloping neurons. 3V, 3rd ventricle.

Publication List:

Original papers

Fukada, M., Fujikawa, A., Chow, J.P.H., Ikematsu, S., Sakuma, S., and Noda, M. (2006). Protein tyrosine phosphatase receptor type Z is inactivated by ligand-induced oligomerization. FEBS Lett. *580*, 4051-4056.

Sakuta, H., Takahashi, H., Shintani, T., Etani, K., Aoshima, A., and Noda, M. (2006). Role of bone morphogenic protein 2 in retinal patterning and retinotectal projection. J. Neurosci. *26*, 10868-10878.

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Watanabe, E., Hiyama, T.Y., Shimizu, H., Kodama, R., Hayashi, N., Miyata, S., Yanagawa, Y., Obata, K., and Noda, M. (2006). Sodium-level-sensitive sodium channel Na_x is expressed in glial laminate processes in the sensory circumventricular organs. Am. J. Physiol. ñ Regul. Integr. Comp. Physiol. *290*, 568-576.

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Fukada, M., and Noda, M. (2006). Yeast substrate-trapping system for isolating substrates of protein tyrosine phosphatases. In Protein Phosphatase Protocols, Methods in Molecular Biology, vol. *365*, G. Moorhead, ed. (Totowa, NJ: Humana Press Inc.), pp. 371-381.