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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, neuronal terminal differentiation, 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.

Two winged-helix transcription factors, *FoxG1* (previously called *chick brain factor1*, *CBF1*) and *FoxD1* (*chick brain factor2*, *CBF2*), are expressed specifically in the nasal and temporal regions of the developing chick retina, respectively. We previously demonstrated that *FoxG1* controls the expression of topographic molecules including *FoxD1*, and determines the regional specificity of the nasal retina. *FoxD1* is known to prescribe temporal specificity, however, molecular mechanisms and downstream targets have not been elucidated. We addressed the genetic mechanisms for establishing temporal specificity in the developing retina using an *in ovo* electroporation technique (Figure 1A). Fibroblast growth factor (Fgf) and Wnt first play pivotal roles in inducing the region-specific expression of *FoxG1* and *FoxD1* in the optic vesicle. Misexpression of *FoxD1* represses the expression of *FoxG1*, *GH6*, *SOHo1*, and *ephrin-A5*, and induces that of *EphA3* in the retina. *GH6* and *SOHo1* repress the expression of *FoxD1*. In contrast to the inhibitory effect of FoxG1 on bone morphogenic protein (BMP) signaling, *FoxD1* does not alter the expression of *BMP4* or *BMP2*. Studies with chimeric mutants of FoxD1 showed that FoxD1 acts as a transcription repressor in controlling its downstream targets in the retina. Taken together with previous findings, our data suggest that *FoxG1* and *FoxD1* are located at the top of the gene cascade for regional specification along the nasotemporal (anteroposterior) axis in the retina, and *FoxD1* determines temporal specificity (Figure 1B).

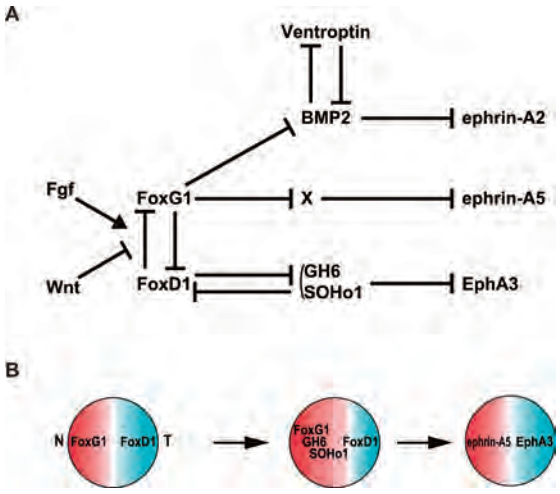


Figure 1. Expressional regulation of asymmetrically distributed molecules along the A–P axis by *FoxG1* and *FoxD1*. (A) The molecular mechanisms by which *FoxG1* and *FoxD1* control the expression of topographic molecules. Fgf8 derived from the anterior neural ridge is required for both *FoxG1* and *FoxD1* expression in the optic vesicle, but an excess of Fgf enhances *FoxG1* expression and represses *FoxD1* expression. Wnt signaling from the mesencephalon inhibits the expression of *FoxG1* and *FoxD1*. *FoxG1* and *FoxD1* counteract each other and *FoxG1* represses the transcription of negative regulators, X. When FoxG1 is absent and FoxD1 is present, X downregulates *ephrin-A5* expression and *FoxD1* represses the expression of *GH6* and *SOHo1*. When FoxG1 is present and FoxD1 is absent, X is downregulated and the expression of *ephrin-A5*, *GH6*, and *SOHo1* is induced. *GH6* and *SOHo1* inhibit the expression of *EphA3*. The inhibitory action of FoxG1 on BMP2 signaling is attributable to the change in the expression of *Ventroptin* and *BMP2* to the oblique-gradient pattern. The counteraction between *Ventroptin* and *BMP2* determines the expression of *ephrin-A2* in an oblique-gradient fashion. Arrows and T-bars indicate positive and negative effects, respectively. These effects are not necessarily induced by direct action. (B) Developmental expression of topographic molecules along the A–P axis. *FoxG1* and *FoxD1* are expressed in a counter-gradient manner in the optic vesicle. After the optic cup forms, the expression domains of *FoxG1* and *FoxD1* are separated by reducing their positive regions to a narrow region of the nasal or temporal retina, respectively. Subsequently, *GH6* and *SOHo1* are expressed specifically in the nasal retina from HH stages 12–14. The expression domains of *GH6* and *SOHo1* fill the gap between the expression areas of *FoxG1* and *FoxD1*. *EphA3* and *ephrin-A5* are homogeneously expressed in the retina

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*).

at early developmental stages and become restricted to the temporal and nasal retinae, respectively, under the control of these transcription factors at later stages. Development proceeds from left to right. Nasal (N) is left, and temporal (T) is right in the retina.

II. Mechanisms for retinotectal projection

Special attention is now devoted to the molecular mechanisms for 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*.

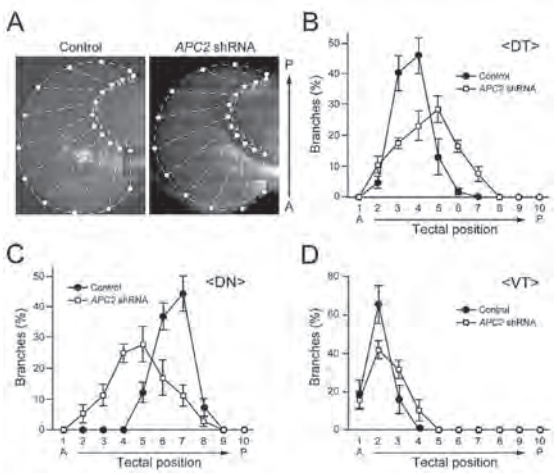


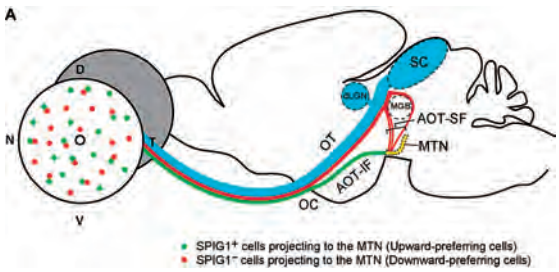
Figure 2. Distribution of interstitial branches of retinal axons on the tectum at E12.5. (A) The tectum was subdivided into 10 areas along the anteroposterior axis, and numbers of branches in each area were counted. (B–D) Quantification of branch distributions of dorsotemporal (DT), dorsonasal (DN), and ventrotemporal (VT) retinal axons, respectively. The values are shown as the mean ±SEM. In APC2 knockdown embryos, interstitial branches from the axon shaft were more diffusely distributed along the anteroposterior axis. A, Anterior; P, posterior.

Growth cones at the tip of growing axons are key cellular structures that detect guidance cues and mediate axonal growth. An increasing number of studies have suggested that the dynamic regulation of microtubules in the growth cone plays an essential role in growth cone steering. The dynamic properties of microtubules are considered to be regulated by variegated cellular factors but, in particular, through microtubule-interacting proteins. We examined the functional role of adenomatous polyposis coli-like molecule 2 (APC2) in the development of axonal projections by using the chick retinotectal topographic projection system. APC2 is preferentially expressed in the nervous system from early developmental stages through to adulthood. Immunohistochemical analysis revealed that APC2 is distributed along microtubules in growth cones as well as axon shafts of retinal axons. Overexpression of APC2 in cultured cells induced the stabilization of microtubules, whereas the knockdown of *APC2* in chick retinas with specific short hairpin RNA reduced the stability of microtubules in retinal axons. *APC2* knockdown retinal axons showed abnormal growth attributable to a reduced response to ephrin-A2 *in vitro*. Furthermore, they showed drastic alterations in retinotectal projections without making clear target zones in the tectum *in vivo* (Figure 2). These results suggest that APC2 plays a critical role in the development of the nervous system through the regulation of microtubule stability.

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 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.

The direction of image motion is coded by direction-selective (DS) ganglion cells in the retina. Particularly, the ON DS ganglion cells project their axons specifically to terminal nuclei of the accessory optic system (AOS) responsible for optokinetic reflex (OKR). We recently generated a knock-in mouse in which SPIG1 (SPARC-related protein containing immunoglobulin domains 1)-expressing cells are visualized with GFP, and found that retinal ganglion cells projecting to the medial terminal nucleus (MTN), the principal nucleus of the AOS, are comprised of SPIG1$^+$ and SPIG1$^-$ ganglion cells distributed in distinct mosaic patterns in the retina (Figure 3A, left). We examined light responses of these two subtypes of MTN-projecting cells by targeted electrophysiological recordings. SPIG1$^+$ and SPIG1$^-$ ganglion cells respond preferentially to upward motion and downward motion, respectively, in the visual field (Figure 3B). The direction selectivity of SPIG1$^+$ ganglion cells develops normally in dark-reared mice. The MTN neurons are activated by optokinetic stimuli of only vertical motion as shown by Fos expression analysis. Combination of genetic labeling and conventional retrograde labeling revealed that axons of SPIG1$^+$ and SPIG1$^-$ ganglion cells project to the MTN via different pathways (Figure 3A). The axon terminals of the two subtypes are organized into discrete clusters in the MTN. These results suggest that information about upward and downward image motion transmitted by distinct ON DS cells is separately processed in the MTN, if not independently. Our findings provide insights into the neural mechanisms of OKR; how information about the direction of image motion is deciphered by the AOS.



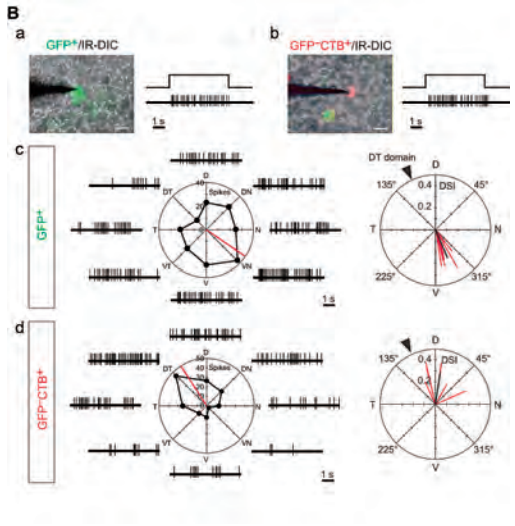


Figure 3. (A) Schematic representation of axonal connectivity between the retina and the contralateral MTN of the AOS. Information of upward and downward visual motion is conveyed to the MTN by distinct neuronal pathways. This represents our findings together with those in the previous studies on the retinal projection to the MTN in mice and rats. Upward-preferring subtype of ON DS cells (SPIG1$^+$; green) predominantly projects to the MTNd via AOT-IF, whereas downward-preferring subtype of ON DS cells (SPIG1$^-$; red) projects to the MTNv via AOT-SF. The fibers of the AOT-SF split from the OT and the brachium of the SC, then they course ventrally over the surface of the cerebral peduncle (CP) and finally terminate in the MTNv. On the other hand, the fibers of the AOT-IF leave the OT just after passing through the optic chiasm (OC), then course caudally, and terminate in the MTNd. MGB: medial geniculate body (B) SPIG1 marks the upward-preferring subtype of ON DS cells. (a, b) *Left*, the tip of a tungsten electrode was attached onto a GFP$^+$ MTN-projecting (MTN-P) cell (a) or GFP$^-$ MTN-P cell (b) to record spike discharges from the cell. Cells were identified by using IR-DIC optics. *Right*, responses of a GFP$^+$ MTN-P cell (a) and GFP$^-$ MTN-P cell (b) to a light stimulus for 5 s. Both showed sustained responses during the light stimulation. (c, d) *Left panels*, representative spike trains and their polar plots in response to eight different directions of the drifting square-wave gratings for 6 s. Spikes were recorded from a GFP$^+$ MTN-P cell (c), GFP$^-$ MTN-P cell (d), respectively. The red line indicates the preferred direction of the cells. *Right panels*, preferred direction and direction selectivity index (DSI) of individual cells are represented by the angle and length of the red line, respectively. The black line indicates the average of the preferred direction and DSI. The DSI indicates the degree of asymmetry in the polar plot of responses to the drifting gratings. The arrowhead indicates the direction of the dorsotemporal domain. Scale bars: a, b, 20 μm.

IV. Physiological roles of protein tyrosine phosphatase receptor type Z

Protein tyrosine phosphatase receptor type Z (Ptprz, also known as PTPζ/RPTPβ) is preferentially expressed in the CNS as a major chondroitin sulfate proteoglycan. Three splicing variants, two receptor isoforms (Ptprz-A/B) and one secretory isoform (Ptprz-S) are known. Receptor-type Ptprz interacts with the PSD95 family through its intracellular carboxyl-terminal PDZ-binding motif in the postsynaptic density. *Ptprz*-deficient adult mice display impairments in spatial and contextual learning. We are conducting experiments to know molecular and cellular mechanisms for the altered phenotypes in *Ptprz*-deficient mice.

V. Mechanisms of Na-level sensing in the brain for 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 *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. Very recently, we found that *Na_x*-deficient mice show normal vasopressin response to dehydration.

Publication List

〔Original papers〕

- Shintani, T., Ihara, M., Tani, S., Sakuraba, J., Sakuta, H., and Noda, M. (2009). APC2 plays an essential role in axonal projections through the regulation of microtubule stability. J. Neurosci. *29*, 11628-11640.
- Takahashi, H., Sakuta, H., Shintani, T., and Noda, M. (2009). Functional mode of FoxD1/CBF2 for the establishment of temporal retinal specificity in the developing chick retina. Dev. Biol. *331*, 300-310.
- Toychiev, A.H., Sabirov, R.Z., Takahashi, N., Ando-Akatsuka, Y., Liu, H., Shintani, T., Noda, M., and Okada, Y. (2009). Activation of maxi-anion channel by protein tyrosine dephosphorylation. Am. J. Physiol. - Cell Physiol. *297*, C990-C1000.
- Yonehara, K., Ishikane, H., Sakuta, H., Shintani, T., Nakamura-Yonehara, K., Kamiji, N.L., Usui, S., and Noda, M. (2009). Identification of retinal ganglion cells and their projections involved in central transmission of information about upward and downward image motion. PLoS ONE *4*, e4320.

〔Review articles〕

- Noda, M., Takahashi, H., and Sakuta, H. (2009). Neural patterning: Eye fields. *In* Encyclopedia of Neuroscience, Squire, L.R., ed. (Elsevier), pp. 199-204.
- Sakuta, H., Suzuki, R., and Noda, M. (2009). Retrovirus vector-mediated gene transfer into the chick optic vesicle by *in ovo* electroporation. *In* Electroporation and Sonoporation in Developmental Biology, Nakamura, H., ed. (Springer), pp. 105-116.