I. Mechanisms for neural circuit formation

Topographic maps are a fundamental feature of neural networks in the nervous system. We have long studied the molecular mechanisms for regional specification in the developing retina as the basis of the topographic retinotectal pathway. In the homozygous mutant mice, robust defects in neuronal lamination were observed in the cortex (Figure 1A), hippocampus, cerebellum (Figure 1B), and olfactory bulb. In vivo BrdU labeling and immunohistochemical analyses with specific markers revealed that the laminar abnormalities are a result of dysregulated neuronal migration by a cell-autonomous mechanism. Dissociated Apc2-deficient cerebellar granule cells showed no obvious alterations in migration under non-stimulated conditions, however, Brain-derived neurotrophic factor (BDNF)-stimulated directional migration was perturbed.

Recently, we generated Apc2-deficient mice by a gene-targeting technique. In the homozygous mutant mice, robust defects in neuronal lamination were observed in the cortex (Figure 1A), hippocampus, cerebellum (Figure 1B), and olfactory bulb. In vivo BrdU labeling and immunohistochemical analyses with specific markers revealed that the laminar abnormalities are a result of dysregulated neuronal migration by a cell-autonomous mechanism. Dissociated Apc2-deficient cerebellar granule cells showed no obvious alterations in migration under non-stimulated conditions, however, Brain-derived neurotrophic factor (BDNF)-stimulated directional migration was perturbed.

Total internal reflection fluorescence (TIRF) microscopy revealed that Apc2 is distributed along actin fibers as well as microtubules. BDNF-stimulated F-actin formation at the leading edge was impaired in migrating Apc2-deficient neurons, along with dysregulation of Rho GTase activity. Thus Apc2 is an essential mediator of the cytoskeletal regulation at leading edges in response to extracellular signals. The phenotypes observed in Apc2-deficient mice suggest that mutations in APC2 in humans may cause a neurodevelopmental disorder.

II. Regeneration of retinal axons

CNS neurons in fish can regrow their axons after nerve transection, while CNS neurons in mammals lose their capacity to regenerate. Thus, we have been investigating genes involved in optic nerve regeneration using fish visual systems. We found that expression of coagulation factor XIII...
A subunit (FXIII-A), a protein cross-linking enzyme, is upregulated in the goldfish optic nerve and retina during regeneration. The cells producing FXIII-A were astrocytes/microglial cells and retinal ganglion cells (RGCs) in the optic nerve and retina, respectively. Overexpression of FXIII-A in RGCs and addition of extracts of optic nerves with injury to retinal explants induced significant neurite outgrowth from the retina. From these observations, we concluded that the increase of FXIII in RGCs promotes neurite sprouting from injured RGCs, whereas the increase of FXIII in optic nerves facilitates elongation of regrowing axons.

III. Physiological roles of protein tyrosine phosphatase receptor type Z

Protein-tyrosine phosphatase receptor type Z (Ptprz, also known as PTPrζ/RPTPβ) is a member of the R5 receptor-like protein tyrosine phosphatase (RPTP) subfamily. Ptprz is predominantly expressed in glial and neuronal cells in the central nervous system (CNS) and its physiological importance has been demonstrated through studies with Ptprz-deficient mice. Ptprz modulates hippocampal synaptic plasticity: adult Ptprz-deficient mice display impairments in spatial and contextual learning. Ptprz is expressed also in the stomach, where it is used as a receptor for VacA, a cytotoxin secreted by Helicobacter pylori: Ptprz-deficient mice are resistant to gastric ulcer induction by VacA.

This year, we revealed that Ptprz plays a negative role in oligodendrocyte differentiation in early CNS development and remyelination in demyelinating CNS diseases, through the dephosphorylation of its substrates such as p190RhoGAP. We first found an early onset of the expression of myelin basic protein (MBP), a major protein of the myelin sheath, and early initiation of myelination in vivo during development of the Ptprz-deficient mouse, as compared with the wild-type (Figure 2A). In addition, oligodendrocytes appeared earlier in primary cultures from Ptprz-deficient mice than wild-type mice.

We subsequently found that adult Ptprz-deficient mice are less susceptible to experimental autoimmune encephalomyelitis (EAE) induced by active immunization with myelin/oligodendrocyte glycoprotein (MOG) peptide than wild-type mice. However, the number of T-cells and macrophages/microglia infiltrating into the spinal cord were not decreased in Ptprz-deficient mice after MOG immunization, suggesting that the reduced tissue damage is not attributable to an inhibition of infiltration by inflammatory cells.

It is known that Fyn tyrosine kinase-mediated down-regulation of Rho activity through activation of p190RhoGAP is crucial for oligodendrocyte differentiation and myelination. Here, p190RhoGAP is one of the physiological Ptprz substrates. After EAE induction, the tyrosine phosphorylation of p190RhoGAP increased significantly, and the EAE-induced loss of MBP was markedly suppressed in the white matter of the spinal cord in Ptprz-deficient mice (Figure 2B).

Thus, selective inhibition of Ptprz signaling could be an effective and plausible therapeutic strategy for treating demyelinating diseases.

IV. Brain systems for body-fluid homeostasis

Sodium (Na) is a major electrolyte of extracellular fluids and the main determinant of osmolality. Na homeostasis is essential to life and Na+ concentrations in plasma and cerebrospinal fluid (CSF) are continuously monitored to maintain a physiological level of Na+ in body fluids. We have previously shown that Na_, which structurally resembles voltage-gated sodium channels (Na,1.1–1.9), is a concentration-sensitive Na channel with a threshold of ~150 mM for extracellular Na+ concentration [Na+] in vitro.

In the brain, Na_ channels are specifically expressed in
astrocytes and ependymal cells in the sensory circumventricular organs, such as the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), where Na₉-positive glial cells are involved in sensing an increase in [Na⁺] in body fluids. Na₉-deficient mice do not stop ingesting salt even when dehydrated, while wild-type mice avoid salt. This behavioral defect of Na₉-deficient mice is recovered by a site-directed transfer of the Na₉ gene with an adenoviral vector into the SFO. Na₉ thus functions as the brain’s Na⁺-level sensor for the homeostatic control of [Na⁺] in body fluids.

Na₉ has a putative PSD-95/Disc-large/ZO-1 (PDZ)-binding motif at the carboxyl-terminus. We thus hypothesized that the Na₉ channel may be regulated by PDZ-scaffold proteins. Very recently, we found that several PDZ proteins bind to Na₉ by PDZ-array overlay assay. Among them, synapse-associated protein 97 (SAP97/DLG1) was coexpressed with Na₉ in the SFO. In C6 glioblastoma cells, destruction of the PDZ-binding motif of Na₉, Na₉(T1679A), resulted in a decrease in cell-surface Na₉, which was attenuated with inhibitors of endocytosis (Figure 3A). Depletion of SAP97 also led to the reduction in the surface expression of wild-type Na₉ (Figure 3B). Next, functional relevance of the binding of Na₉ with SAP97 was confirmed by Na⁺-imaging studies (Figure 3C). When the extracellular Na⁺ concentration, [Na⁺], was increased from 145 mM to 170 mM, both C6M16 cells expressing wild-type Na₉ and C6M(TA)8 cells expressing the Na₉(T1679A) mutant showed increases in the intracellular Na⁺ concentration, [Na⁺], and the level eventually reached the same equilibrium point between Na⁺ influx by Na₉ and Na⁺ export by Na⁺/K⁺-ATPase. However, importantly, C6M(TA)8 cells took longer to reach this equilibrium level than control C6M16 cells. This is probably because the reduction in the number of surface Na₉ channels diminished the Na⁺ influx. Thus, SAP97 appears to play an important role in sensing body-fluid [Na⁺] in the SFO through regulation of the surface expression of the sensor channel.

Figure 3. SAP97 promotes the stability of Na₉ channels at the plasma membrane. A, Decrease in cell-surface expression of the Na₉ mutant with Thr-1679 changed to Ala (mutation in the PDZ-binding motif) in C6 cells and its improvement by treatment with endocytosis inhibitors, wortmannin and dynasore. After the induction of the expression of Na₉(T1679A) channels, cells were treated with 100 nM wortmannin or 200 μM dynasore for 6 h. Then the cells were fixed, permeabilized, and stained with anti-Na₉. Scale bars, 10 μm. B, Reduction in the cell-surface expression of Na₉ with depletion of SAP97. Non-treated C6M16 cells, or C6M16 cells transfected with siRNA for SAP97 or scrambled siRNA were immunostained with anti-SAP97 and anti-Nax. The fluorescence intensity profiles along the white lines are shown in the right panel. Scale bars, 10 μm, a.u., arbitrary unit. C, Reduced sodium influx in C6 cells in the absence of any association between Na₉ and SAP97. Left: Na⁺ imaging of C6M16 (wild-type Na₉) and C6M(TA)8 (T1679A mutant Na₉) cells upon elevation of the extracellular Na⁺ concentration from 145 mM to 170 mM. The coordinate gives the fluorescence ratio (AF340/380 nm) in Na⁺ imaging with sodium-binding benzofuran isophthalate acetoxymethyl ester (SBFI/AM), representing the intracellular Na⁺ concentration. The physiological 145 mM Na⁺ solution was changed to a 170 mM solution at 0 min. Right: Summary of the time to reach 95% of the maximum fluorescence ratio. Data represent the mean ± SE (n = 40 for each); *P < 0.01, two-tailed t-test.

Publication List

Original papers


