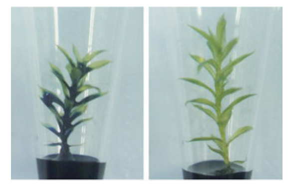
Selected Press Releases from NIBB in 2018

9 January

The origin of flower making genes

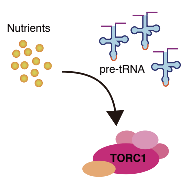


Flowers are reproductive organs, which are formed by a group of genes called MADS-box genes. Although non-flowering plants are also known to have these genes, it was previously unclear how they worked. Consequently, a research team at NIBB using the moss *Physcomitrella patens* revealed that MADS-box genes control sperm motility, cell division, and elongation of gametophore stems. This finding indicates that MADS-box genes extensively changed their functions during the evolution of land plants (see p. 49).

Koshimizu, S., Kofuji, R., Sasaki-Sekimoto, Y., Kikkawa, M., Shimojima, M., Ohta, H., Shigenobu, S., Kabeya, Y., Hiwatashi, Y., Tamada, Y., Murata, T. and Hasebe, M. (2018). Physcomitrella MADS-box genes regulate water supply and sperm movement for fertilization. Nat. Plants 4, 36-45. doi: 10.1038/s41477-017-0082-9

18 January

tRNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway

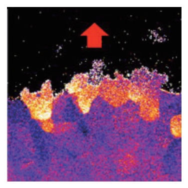


Cells control growth and differentiation in response to their changing external environments. TOR complex 1 (TORC1) is a kinase complex conserved throughout eukaryotes and plays a key role in this control system. In the fission yeast *Schizosaccharomyces pombe*, TORC1 has been known to control the switch between asexual and sexual growth, but the mechanism of how TORC1 recognizes nutrient conditions has remained mostly unknown. NIBB researchers revealed that the precursors of tRNA, which is one of the types of classical non-coding RNA that are indispensable in protein synthesis, operate in the *S. pombe* TORC1 pathway to switch a cell's growth mode from vegetative to reproductive in response to the nutrient conditions (see p. 23).

Otsubo, Y., Matsuo, T., Nishimura, A., Yamamoto, M., and Yamashita, A. (2018). tRNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway. EMBO Rep. *19*, e44867. doi: 10.15252/embr.201744867

7 February

Controlling cell movement during gastrulation: elevation of intracellular calcium at the leading edge is critical for cell movement

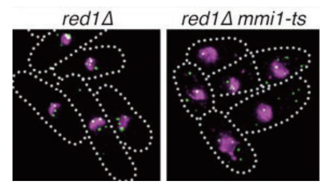


Gastrulation is a large-scale cell movement in the process of animal body formation, and is quite important in the positioning of various body structures. NIBB researchers succeeded in observing changes in intracellular calcium concentrations in moving cell groups during gastrulation in a *Xenopus* embryo. They subsequently found that calcium oscillates only at the leading edge of the cell group, and revealed that this localized elevation of intracellular calcium controls cellular movement during gastrulation (see p. 30).

Hayashi, K., Yamamoto, T.S., and Ueno, N. (2018). Intracellular calcium signal at the leading edge regulates mesodermal sheet migration during *Xenopus* gastrulation. Sci. Rep. *8*, 2433. doi: 10.1038/s41598-018-20747-w

13 February

A novel control mechanism of the expression of genes related to germ cell formation: the transcripts of genes promoting meiosis are tethered to nuclear foci and sequestered from the translation machinery during somatic mitoses

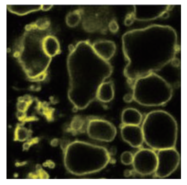


During the formation process of germ cells such as sperm and eggs, a set of genes that differ from somatic cells are expressed. The genes controlling meiosis, which is a form of cell division essential in the formation of germ cells, are known to be regulated not only at the transcriptional level but also at the stability of the transcripts. NIBB researchers revealed that the transcripts of genes promoting meiosis in fission yeast are transcribed at a low level during the mitotic cell cycle but degraded selectively. Even when selective RNA degradation is impaired, these transcripts are tethered to focal structures in the nucleus and inhibited from being translated into protein products (see p. 23).

Shichino, Y., Otsubo, Y., Kimori, Y., Yamamoto, M., and Yamashita, A. (2018). YTH-RNA-binding protein prevents deleterious expression of meiotic proteins by tethering their mRNAs to nuclear foci. eLife *7*, e32155. doi: 10.7554/eLife.32155

20 February

Discovery of the mechanism of plant-specific control of vacuole transport routes

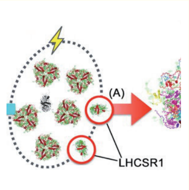


Plant vacuoles have a variety of functions. As such, they have diverse vacuolar transport pathways. An NIBB research team demonstrated that a plant-specific vacuole transport pathway is controlled by CORVET: one of the tethering factors acting in the transport pathway to the vacuole. They also showed that the combination of a factor from a common ancestor and a factor newly acquired by plants has resulted in the diversification of the process of transportation routes to vacuoles. In particular, the group initially examined the relationship between RAB GTPase, CORVET and HOPS using *Arabidopsis thaliana*. It was found that RAB 5 acts on CORVET and endosomes, and that RAB 7 works on HOPS and vacuolar membranes (see p. 14).

Takemoto, K., Ebine, K., Askani, JC., Krüger, F., Ito, E., Goh, T., Schumacher, K., Nakano, A., and Ueda, T. (2018). Distinct sets of tethering complexes, SNARE complexes, and Rab GTPases mediate membrane fusion at the vacuole in Arabidopsis. Proc. Natl. Acad. Sci. USA *115*, E2457-E2466. doi: 10.1073/pnas.1717839115

20 March

Discovery of a novel mechanism that allows plants to release excess light energy

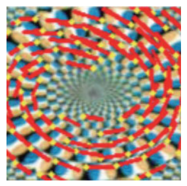


Plants utilize light energy during the process of photosynthesis to convert carbon dioxide and synthesize sugar molecules. An environment with strong light therefore may seem to be preferable for plants, but in many cases the solar light on the earth's surface exceeds the endurable limit of the photosynthetic apparatus. This means that direct exposure to sunlight may have a deleterious effect on plants. Plants have evolved to have mechanisms that intentionally release energy from excessively strong light. As such, NIBB researchers have endeavored to elucidate one of these mechanisms, qE quenching, at the molecular level. They utilized the green alga *Chlamydomonas reinhardtii* and found a novel mechanism that releases the excess energy to Photosystem I through a protein called LHCSR1 (see p. 68).

Kosuge, K., Tokutsu, R., Kim, E., Akimoto, S., Yokono, M., Ueno, Y., and Minagawa, J. (2018). LHCSR1-dependent fluorescence quenching is mediated by excitation energy transfer from LHCII to photosystem I in *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA *115*, 3722-3727, doi: 10.1073/pnas.1720574115

20 March

Illusory motion reproduced by deep neural networks trained for prediction



Deep neural networks (DNNs) have achieved notable success in a broad range of fields in which they have produced results comparable to the performance of human experts. In line with this, a research team at NIBB successfully reproduced illusory motions by DNNs trained for prediction. This research supported the idea that the mechanism assumed by the predictive coding theory is a basis of motion illusion generation. The DNNs were trained with videos of motion from the point of view of someone watching them, and the motion prediction ability of the obtained computer model was verified using a rotating propeller in unlearned videos. The computer model accurately predicted the magnitude and direction of motion of said rotating propeller in these videos (see p. 48).

Watanabe, E., Kitaoka, A., Sakamoto, K., Yasugi, M. and Tanaka, K. (2018). Illusory motion reproduced by deep neural networks trained for prediction. Front. Psychol. *9*, 345. doi: 10.3389/fpsyg.2018.00345

4 April

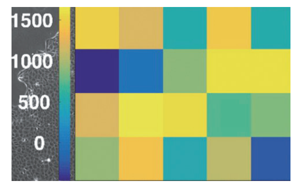
Predicting the presence of a new diffusible factor during the formation of a regular pattern of phyllotaxis: a simulation utilizing a mutual interaction model between auxin and PIN1

Phyllotaxis, the arrangement of leaves on a plant stem, is well known because of its beautiful geometric configuration, which is derived from the constant spacing between leaf primordia. This regular pattern has been reported to be formed by the mutual interaction between a plant hormone, auxin, and its membrane transporter protein PIN1, but its regulation mechanisms remain mostly unknown. NIBB researchers revealed through a computer simulation that a realistic model introducing the extracellular space between neighboring cells does not produce a regular pattern only with auxin and PIN1, but that by assuming the presence of a hypothetical diffusible factor, the regular pattern is reproduced. They also predicted that auxin does not directly regulate PIN1, but needs to indirectly regulate it through a diffusible factor (see p. 52).

Fujita, H., and Kawaguchi, M. (2018). Spatial regularity control of phyllotaxis pattern generated by the mutual interaction between auxin and PIN1. PLoS Comput. Biol. *14*, e1006065. doi: 10.1371/journal.pcbi.1006065

5 April

Non-invasive measurement of the tissue stiffness with live-cell time-lapse imaging

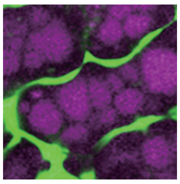


When a fertilized egg transforms into an animal's body during development, cell populations forming tissues can be easily deformed. Cells also generate mechanical forces during development, shaping the tissues and organs. In order to understand the process of tissue and organ formations, it is of particular importance to understand how the physical characteristics of tissues responding to mechanical stresses influence the process of organogenesis. To tackle this problem, a joint National Institute for Basic Biology (NIBB)/Kyoto University research team has proposed a unique non-invasive way of measuring the stiffness of tissues by combining physical modeling of the tissues with statistical estimation. The research team developed methodology for estimating tissue stiffness based on the tissue deformation and the mechanical forces applied over the tissue (see p. 17).

Kondo, Y., Aoki, K., and Ishii, S. (2018). Inverse tissue mechanics of cell monolayer expansion. PLoS Comput Biol. *14*, e1006029. doi: 10.1371/journal.pcbi.1006029

15 May

The generality and diversity of life explored through the transportation mechanisms in plant cells: a discovery of a new regulatory mechanism in plant membrane traffic

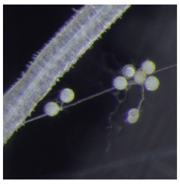


All life forms are composed of cells and their activities are mostly similar throughout different species. However, the set of component proteins are different and the mechanisms that distribute proteins within a cell also differ between species. NIBB researchers have studied the mechanism of transporting proteins to the plant-specific organelle, the vacuole, and found that plant-specific proteins called ARA6 and PUF2 suppress or activate RAB5, which is also present in animal and fungi. It then accurately controls transportation to the vacuole. The plant devised a unique controlling factor and has succeeded in providing diversity to cellular activity also present in other life forms (see p. 14).

Ito, E., Ebine, K., Choi, S.-w., Ichinose, S., Uemura, T., Nakano, A., and Ueda, T. (2018). Integration of two RAB5 groups during endosomal transport in plants. eLife 7, e34064. doi: 10.7554/eLife.34064

10 July

Greatly improved decoding of the genome of Arbuscular mycorrhizal fungus (AMF) led to the identification of nutrients that AMF receives from plants and the discovery of a unique gene structure

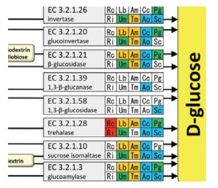


AMFs penetrate their hyphae into the soil and also into the roots of plants, which enables them to deliver nutrients deep within the soil to the plants that live in it, and receive photosynthesis products from plants. NIBB researchers decoded the genome of a representative AMF, *Rhizophagus irregularis,* at a considerably higher resolution and confirmed that AMFs lack enzyme genes necessary to synthesize nutrients such as fatty acids or vitamin B1. This information on nutrient requirements may lead to a large-scale culture of AMFs. They also found a unique gene structure in the ribosome DNA gene (see p. 52).

Maeda, T., Kobayashi, Y., Kameoka, H., Okuma, N., Takeda, N., Yamaguchi, K., Bino, T., Shigenobu, S., and Kawaguchi, M. (2018). Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*. Commun. Biol. *1,* 87. doi: 10.1038/s42003-018-0094-7

11 July

Comparative genome analysis of Arbuscular mycorrhizal fungi (AMFs) revealed a common genetic basis for auxotrophy among AMFs



AMFs are representative symbionts with land plants and require nutrients from plants to grow (auxotrophy). To analyze this requirement, NIBB researchers decoded the sequence of an AMF, *Rhizophagus clarus*, and compared it with the genome of a representative AMF, *R. irregularis*. These two AMFs lack the enzymes necessary to synthesize fatty acids or thiamine, and they also lack the enzymes required to degrade polysaccharides into glucose. This indicates that AMFs depend on the supply of fatty acids, thiamine, and glucose from host plants and also suggests the possibility of culturing AMFs, which in turn will enable detailed analyses of the mechanisms of AMF's growth and may lead to agricultural applications (see p. 52).

Kobayashi, Y., Maeda, T., Yamaguchi, K., Kameoka, H., Tanaka, S., Ezawa, T., Shigenobu, S., and Kawaguchi, M. (2018). The genome of *Rhizophagus clarus* HR1 reveals a common genetic basis for auxotrophy among arbuscular mycorrhizal fungi. BMC Genomics *19*, 465. doi: 10.1186/s12864-018-4853-0

13 July

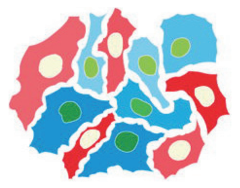
Development of a cryopreservation method of ladybird beetle ovaries

A study led by a research team at NIBB found an effective way to cryopreserve and transplant the ovaries of the multicolored Asian ladybird beetle, *Harmonia axyridis*. This study was the first to report a successful ovary cryopreservation method in a small non-model insect, or an insect that had not previously been widely studied in this manner. The team successfully preserved ladybird beetle ovaries by first slowly cooling them down to -80ºC in a freezing container at a rate of 1ºC per minute. The frozen ovaries were then placed into liquid nitrogen, where they were stored until further use. Immediate thawing at 37ºC was done at the time of transplant, and thawed donor ovaries were then placed in the recipient larva's body (see p. 55).

Kawaguchi, H., and Niimi, T. (2018). A method for cryopreservation of ovaries of the ladybird beetle, *Harmonia axyridis*. J. Insect Biotechnol. Sericol. *87*, 35-44. doi: 10.11416/jibs.87.2_035

5 September

Cell-to-cell heterogeneity in molecular activity leads to cell fate decisions regarding whether they will live or die: variation of negative regulation between cells leads to heterogeneity



When cells of multicellular organisms suffer from stress and are irretrievably damaged, apoptosis (cell death) is actively induced and cells 'commit suicide' so to speak. Once the switch of apoptosis is turned on, cell fate is irreversibly determined. The switch of apoptosis is known to include stress-activated protein kinases (SAPKs) such as p38 and JNK, but its detailed mechanism has been unknown. NIBB researchers observed the activities of p38 and JNK in individual live cells under various stresses, and found that JNK is negatively regulated by p38. Furthermore, it was discovered that the variation of this negative regulation between cells leads to the variation of JNK activity. They also confirmed that an above-threshold JNK activity in individual UV-stressed cells leads to apoptotic cell deaths (see p. 17).

Miura, H., Kondo, Y., Matsuda, M., and Aoki, K. (2018). Cell-to-cell heterogeneity in p38-mediated cross-inhibition of JNK causes stochastic cell death. Cell Reports *24*, 2658-2668. doi: 10.1016/j.celrep.2018.08.020

21 September

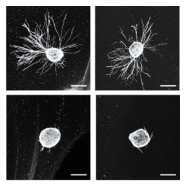
A mechanism of color pattern formation in ladybird beetles

Many ladybirds have attractive red and black color patterns. This color pattern is thought to be used a warning to predators that they are unpalatable. A research team from NIBB has identified a single gene, *pannier*, that regulates such highly diverse ladybird color patterns found in the Asian ladybird beetle *Harmonia axyridis* which lives mainly in Siberia and East Asia, and shows >200 color patterns within a species. *pannier* was expressed in the black pigmented regions where the red pigment was not deposited. Functional inhibition of the *pannier* gene during pupal development resulted in the loss of the black color patterns and ectopic red pattern formation in the forewing (see p. 55).

Ando, T., Matsuda, T., Goto, K., Hara, K., Ito, A., Hirata, J., Yatomi, J., Kajitani, R., Okuno, M., Yamaguchi, K., Kobayashi, M., Takano, T., Minakuchi, Y., Seki, M., Suzuki, Y., Yano, K., Itoh, T., Shigenobu, S., Toyoda, A., and Niimi, T. (2018). Repeated inversions within a *pannier* intron drive diversification of intraspecific colour patterns of ladybird beetles. Nat. Commun. *9*, 3843. doi: 10.1038/s41467-018-06116-1

25 September

Identification of enzymes important for the visual circuit formation in mice: the control of retinal axonal projections by PTPRJ

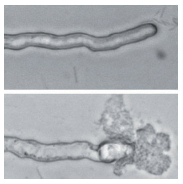


It is well known that Eph receptor-type protein tyrosine kinases play crucial roles in the formation of visual circuits. NIBB researchers previously reported that protein tyrosine phosphatase R3 subfamily members (PTPRB, PTPRH, PTPRJ, and PTPRO) dephosphorylated Eph receptors in cultured cells. In the present study, they revealed that *Ptprj*-knockout mice exhibited abnormal projections of retinal axons through the enhancement of the Eph receptor activity. They also showed that PTPRJ inhibited a non-receptor-type tyrosine kinase, c-Abl, a downstream target of Eph signaling in retinal axons. PTPRJ thus regulates retinal axonal projections by inhibiting Eph receptors and c-Abl (see p. 42).

Yang, Y., Shintani, T., Takeuchi, Y., Shirasawa, T., and Noda, M. (2018). Protein tyrosine phosphatase receptor type J (PTPRJ) regulates retinal axonal projections by inhibiting Eph and Ab1 kinases in mice. J. Neurosci. *38*, 8345-8363. doi: 10.1523/JNEUROSCI.0128-18.2018

1 October

Discovery of a mechanism of membrane transport that is necessary in elongating pollen tubes

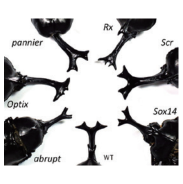


During plant reproduction, sperm cells are delivered to ovules through elongating pollen tubes. This elongation requires several receptor proteins such as ANXUR being localized at the tip of the pollen tube. However, the mechanism of localization had been unknown to date. To this end, NIBB researchers found molecules (PICALM5a and PICALM5b) required for this localization. The molecules belong to ANTH proteins related to the transport of membrane embedded proteins and play a role in loading ANXUR protein to membrane vesicles at the sub-apical region of the pollen tube. The molecules were not necessary in loading another receptor protein necessary for pollen tube guidance. The diversification of the functions of ANTH proteins was shown to be closely related to the evolution of reproduction mechanisms in plants (see p. 14).

Muro, K., Matsuura-Tokita, K., Tsukamoto, R., Kanaoka, MM., Ebine, K., Higashiyama, T., Nakano, A., and Ueda, T. (2018). ANTH domain-containing proteins are required for the pollen tube plasma membrane integrity via recycling ANXUR kinases. Commun. Biol. *1*, 152. doi: 10.1038/s42003-018-0158-8

5 October

The identification of the genes necessary in horn formation in rhinoceros beetles

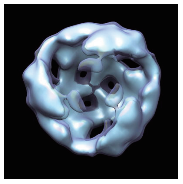


Many are fascinated by rhinoceros beetles because of their spectacular horns but the genes related to the formation of these horns have been unknown. NIBB researchers have identified eleven genes related to horn formation by extensive genome analyses using next-generation DNA sequencers. Inhibition of these genes resulted in the disappearance, shortening, or malformation of the horn or the formation of excessive horns. The comparison of these genes and already reported horn formation genes in dung beetles suggested the presence of a common mechanism between the two beetle groups. These results are important in understanding the evolutionary process of the beetle horn (see p. 55).

Ohde, T., Morita, S., Shigenobu, S., Morita, J., Mizutani, T., Gotoh, H., Zinna, R.A., Nakata, M., Ito, Y., Wada, K., Kitano, Y., Yuzaki, K., Toga, K., Mase, M., Kadota, K., Rushe, J., Lavine, L.C., Emlen, D.J., and Niimi, T. (2018). Rhinoceros beetle horn development reveals deep parallels with dung beetles. PLoS Genet. *14*, e1007651. doi: 10.1371/journal.pgen.1007651

12 October

Determining the diffusion range of information by assembly and dissociation of the Wnt protein complex: how information is distributed among the society of cells



Cell-to-cell communication is indispensable in the formation and maintenance of tissues and organs made up of many cells. Signal proteins such as Wnt are secreted by specific cells in the tissue and received by surrounding cells thereby establishing cell-to-cell communication. How the diffusion range of the Wnt is determined has been unknown. NIBB researchers found that the Wnt proteins excreted outside of the cell are assembled into high-molecular-weight complexes whose smallest unit is the trimer of the Wnt protein. They also proposed a mechanism that the balance between assembly and dissociation of the Wnt protein complex determines the range of diffusion of the Wnt signal (see p. 33).

Takada, R., Mii, Y., Krayukhina, E., Maruyama, Y., Mio, K., Sasaki, Y., Shinkawa, T., Pack, C.-G., Sako, Y., Sato, C., Uchiyama, S., and Takada, S. (2018). Assembly of protein complexes restricts diffusion of Wnt3a proteins Commun. Biol. *1*, 165. doi: 10.1038/s42003-018-0172-x

16 October

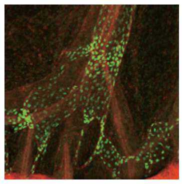
Firefly genomes disclosed the evolution of bioluminescence genes in fireflies

The genome of the Japanese firefly, *Aquatica lateralis*, was decoded at NIBB, and was compared with the genome of the North American firefly, *Photinus pyralis*, and the genome of a Caribbean click beetle, *Ignelater luminosus*, both decoded by researchers listed among those found in the reference list. The light of fireflies is emitted by the reaction of an enzyme called luciferase and the substrate called luciferin. The genome comparison showed that the luciferase gene has evolved from the acyl-CoA synthetase gene ubiquitously found in non-light emitting organisms. The luciferase gene of the click beetle was shown to be evolved from the same gene, but the evolution was independent from that found in fireflies (see p. 98 and 81).

Fallon, T.R., Lower, S.E., Chang, C.-H., Bessho-Uehara, M., Martin, G.J., Bewick, A.J., Behringer, M., Debat, H.J., Wong, I., Day, J.C., Suvorov, A., Silva, C.J., Stanger-Hall, K.F., Hall, D.W., Schmitz, R.J., Nelson, D.R., Lewis, S., Shigenobu, S., Bybee, S.M., Larracuente, A.M., Oba, Y., and Weng, J.-K. (2018). Firefly genomes illuminate parallel origins of bioluminescence in beetles. eLife *7*, e36495. doi: 10.7554/eLife.36495

27 November

Lymphatic valve formation requires mechanosensor channel PIEZO1

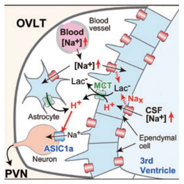


Valves are essential within the cardiac and lymphatic systems, ensuring proper blood and lymph circulation. However, little is known how they are formed within the body. Following up previous research that showed that human patients with *PIEZO1* loss-of-function mutations are associated with congenital lymphedema, a team led by biologists at The National Institute for Basic Biology (NIBB), The Scripps Research Institute and Howard Hughes Medical Institute, USA, discovered through the analysis of mouse models lacking PIEZO1 (a mechanically activated cation channel in endothelial cells) that lymphatic valve formation requires the abovementioned cation channel (see p. 36).

Nonomura, K., Lukacs, V., Sweet, D.T., Goddard, L.M., Kanie, A., Whitwam, T., Ranade, S.S., Fujimori, T., Kahn, M.L., and Patapoutian, A. (2018). Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proc. Natl. Acad. Sci. USA *115*, 12817-12822. doi: 10.1073/pnas.1817070115

30 November

Elucidation of central mechanisms of salt-induced hypertension through activation of sympathetic nerve activities

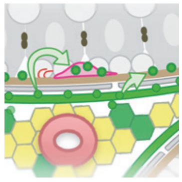


Hypertension is a major risk factor for cardiovascular diseases worldwide, and a positive correlation between salt intake and blood pressure has long been postulated. However, underlying mechanisms responsible for [Na^+] sensing and signaling pathways to induce sympathetically mediated blood pressure elevations have yet to be elucidated. An NIBB research team has previously reported that Na_x channels expressed in specific glial cells in the sensory circumventricular organs (SFO and OVLT) are the sensor for monitoring of increases in [Na^+] in body fluids to control salt/water-intake behaviors. They have now found that sympathetic activation leading to blood pressure increases was not induced in Na_x-knockout mice by mandatory high salt intakes or the intraperitoneal/intracerebroventricular infusions of hypertonic NaCl solutions. The study showed that Na_x channels in the OVLT function as the brain Na^+-level sensor for blood pressure control. The research team further demonstrated that the Na_x signal is transferred from glial cells to OVLT (→PVN) neurons harboring acid-sensitive ion channels (ASIC1a) by using H^+ as a gliotransmitter (see p. 42).

Nomura, K., Hiyama, T.Y., Sakuta, H., Matsuda, T., Lin, C.-H., Kobayashi, K., Kobayashi, K., Kuwaki, T., Takahashi, K., Matsui, S., and Noda, M. [Na^+] increases in body fluids sensed by central Na_x induce sympathetically mediated blood pressure elevations via H^+-dependent activation of ASIC1a. Neuron 2018 Nov 29. doi: 10.1016/j.neuron.2018.11.017

21 December

How sperm stem cells maintain their number



Steady sperm production relies on a constant number of sperm stem cells in the testis. Regarding this phenomenon, researchers from both NIBB and the University of Cambridge in the UK revealed a novel mechanism for stem cell number control. Gained through quantitative analysis, their results showed that constant sperm stem cell numbers are achieved in mouse testes through a self-organized process in which they actively migrate and compete for a limited supply of self-renewal-promoting fibroblast growth factors (FGFs). In this study, the researchers found that a subset of lymphatic endothelial cells produce FGFs which promote stem cell self-renewal. The research illustrated a competitive scenario where stem cells that consumed more FGFs were likely to duplicate, while those that consumed less were inclined to differentiate (see p. 39).

Kitadate, Y., Jörg, D.J., Tokue, M., Maruyama, A., Ichikawa, R., Tsuchiya, S., Segi-Nishida, E., Nakagawa T., Uchida, A., Kimura-Yoshida, C., Mizuno, S., Sugiyama, F., Azami, T., Ema, M., Noda, C., Kobayashi, S., Matsuo, I., Kanai, Y., Nagasawa, T., Sugimoto, Y., Takahashi S., *Simons, B.D., and *Yoshida, S. Competition for mitogens regulates spermatogenic stem cell homeostasis in an open niche. Cell Stem Cell. 2018 Dec 20. (*: Co-corresponding authors) doi: 10.1016/j.stem.2018.11.013