## DIVISION OF MOLECULAR AND DEVELOPMENTAL BIOLOGY

*Professor*
TAKADA, Shinji

| | |
|---|---|
| *Assistant Professor:* | *YABE, Taijiro* |
| | *MII, Yusuke* |
| *Technical Staff:* | *UTSUMI, Hideko* |
| *NIBB Research Fellow* | *SHINOZUKA, Takuma* |
| *Postdoctoral Fellow:* | *TAKADA, Ritsuko* |
| *SOKENDAI Graduate Student:* | *HATAKEYMA, Yudai* |
| | *TRAN, Thi Hong Nguyen* |
| | *SUZUKI, Minako* |
| *Technical Assistant:* | *TAKASHIRO, Kayoko* |
| | *ITO, Yukiko* |
| *Secretary:* | *NOBATA, Ryuko* |

The morphology of the body and tissues is established in a spatio-temporarily regulated manner. A number of genes involved in morphogenesis have been identified, but it is still uncertain how either spatial and temporal information are established and converted to morphology during embryogenesis. Therefore, our aim is to understand the mechanism by which this spatial information is established and how temporal, or periodical, information is converted into morphology through the application of several different approaches.

Secreted signal molecules are important in forming spatial information during the development of many tissues. These molecules are secreted from the cells that produce them and transported to surrounding cells, thus resulting in the formation of concentration gradients. Given that their concentration decreases in accordance with their distance from the source, their specific signal gradient defines the relative positions of receiving cells in developing tissues. Many genetic studies have revealed that a limited number of secreted signal proteins, including Wnt, BMP, and Hedgehog, function during tissue and embryo morphogenesis. However, in spite of the accumulation of genetic evidence, the molecular mechanism that regulates their distribution in certain developing tissues remains to be elucidated. To this end, we have visualized signal proteins and monitored their movement in tissues. In addition to this, we are currently examining the biochemical characteristics and functions of these molecules, which appear to affect how they are spread.

In contrast to secreted signal proteins, the segmental subregions of several specific tissues, like somites, appear not to be simply directed by the gradient of the signal molecules, but by a unique mechanism that functions periodically. Somites are sequentially generated in an anterior-to-posterior order via the conversion of temporal periodicity, created by a molecular clock, into periodical structures. However, the molecular mechanism underlying this conversion and morphological segmentation is not yet fully understood. Therefore, another goal of our current studies is to reveal the molecular mechanism of this differing and unique mode of patterning that underlies the periodical and sequential subdivision in the development of somites.

## I. Effect of morphologic change in signal-producing cells during signal transmission in tissues

During morphogenesis, cells dynamically change their shape. Marked changes in the morphology of signal producing cells during tissue morphogenesis presumably result in the activation of signaling in neighboring cells. In early development of the vertebrate spinal cord, the most dorsal region, known as the roof plate, functions as an organizing center by secreting BMP and Wnt. These signaling proteins' concentration gradients generate interneuron subtype patterns in the dorsal spinal cord. Interestingly, as this patterning event nears completion, the spinal cord's morphology changes dynamically. Essentially, the neural tube lumen gradually shrinks, and a median septum forms along the dorsoventral axis. A recent study in zebrafish embryos revealed that the roof plate stretches during this morphologic change. This finding suggests that the processes of these stretched roof-plate cells are involved in intercellular communication and that the stretching of roof-plate cells induces changes in cells that respond to signals from the roof plate.

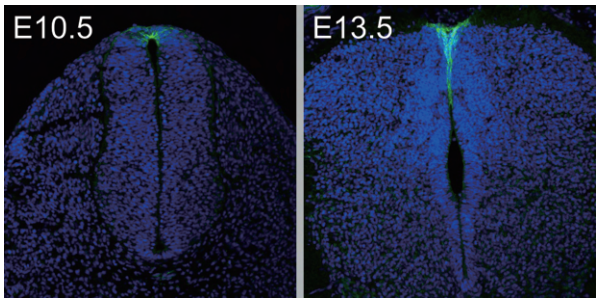


Figure 1. Stretching of Wnt expressing cells during spinal cord development in the mouse. Roof plate cells act as an organizing center in early development of spinal cord (embryonic day 10.5; E10.5). Then, these cells are stretched along the dorso-ventral axis (E13.5) and provide Wnt ligands to neural progenitor cells close to the tip of extended roof plate cells.

In adult mice, neural progenitor/stem cells localize around the shrunken lumen of the spinal cord, and this area is referred to as the central canal. The central canal is connected to the ventricle, where neural progenitor/stem cells localize in the forebrain. As the proliferation of neural progenitor/stem cells in the brain are regulated by secreted signaling molecules, including Wnt, it is plausible that spinal cord progenitor/stem cells are regulated in a similar manner. In the developing spinal cord, *Wnt1* and *Wnt3a* are specifically expressed in the roof plate and perform numerous roles, including regulating the proliferation and specification of dorsal interneurons prior to the mid-gestation stage. However, genetic studies of *Wnt1*- and *Wnt3a*-deficient mutant mouse embryos yielded no information regarding the roles of the respective proteins in later stages, as neither *Wnt1*- nor *Wnt3a*-deficient single mutants exhibit a roof plate–related phenotype, and most of the double mutants die before embryonic day 12.5 (E12.5).

Recently, we found that the expression of Wnt1 and Wnt3a is maintained in stretched roof-plate cells (Figure 1). Roof plate-specific cKO for Wls, which is essential for Wnt secretion, revealed that Wnt secretion from roof-plate cells was required for their coordinated elongation. Furthermore,

proliferation was reduced in ependymal cells in Wls cKO embryos during the development and regeneration of the spinal cord (Figure 2). Thus, dynamic change in cell extension can create novel roles for signal-producing cells.

## II. Regulation of spatial distribution of Wnt proteins in vertebrate embryos

To examine the mobility of Wnt3a in an extracellular situation, we performed FCS analysis of GFP-Wnt3a in Xenopus embryos. This examination revealed that the dynamic behavior of Wnt3a could be divided into two distinct states. The fast population appears to reflect quickly and freely diffusing molecules, and the slow population reflects slowly moving molecules because of its likely interaction with extracellular matrices. Further precise analyses revealed that HMW complexes were less mobile than relatively smaller ones.

We showed that the trimer and larger HMW complexes of Wnt3a proteins could be dissociated via interaction with their receptor Frizzled8 and with secreted Wnt binding protein, sFRP2, in vitro by utilizing AUC-FDS. Similarly, this dissociation was detected in vivo by FCS. Importantly, the results of FCS suggested that dissociation of the large assembly of Wnt3a proteins by interaction with sFRP can make Wnt more mobile, probably resulting in longer diffusion distance in the embryo. These ideas are supported by a finding that states that the distribution range of Wnt3a was expanded by the co-expression of sFRP2 in Xenopus embryos. These results showed that large assemblies of Wnt3a are less mobile, and sFRP2 can expand the diffusion range of Wnt proteins in Xenopus embryos. Based on these results, we propose a model that contends that the assembly and dissociation of dissociable oligomers modulate Wnt signaling range (Figure 3).

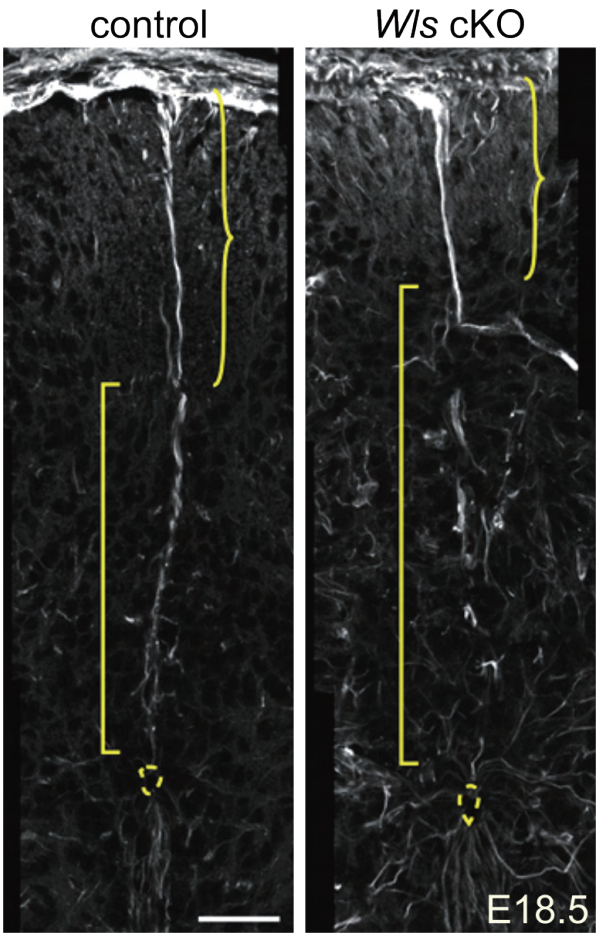


Figure 2. Impaired morphology of stretched roof plate cells in Wnt-defective embryos (Wls cKO). Extensions are indicated by brackets.

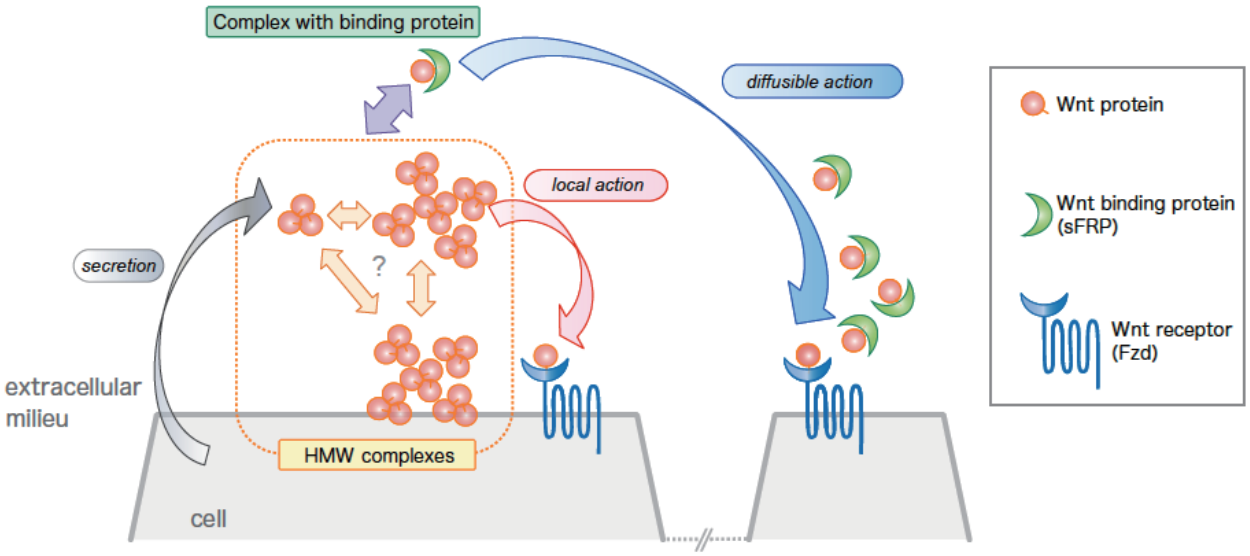


Figure 3. Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu although it is uncertain when the assembly to the HMW complex occurs during the process of Wnt secretion. The HMW complex is probably less mobile when interacting with the plasma membrane, resulting in the restriction of Wnt diffusion range. Some Wnt molecules can be dissociated by local interaction with Frizzled receptor (Fzd), resulting in a short-range signal (local action). In contrast, the HMW complex, probably as well as the trimer itself, can also be dissociated by interaction with soluble Wnt binding protein (partner protein), including sFRP. Due to this dissociation, Wnt turns to be more mobile and its diffusion range is expanded (diffusible action).

## III. Molecular mechanism of somite development

Somites are segmental epithelial blocks located symmetrically on either side of the neural tube. Somitogenesis periodically proceeds in an anterior to posterior manner from their precursor, the presomitic mesoderm (PSM), which is located at the posterior of newly formed somites. This periodic generation is achieved by a complex and dynamic mechanism within the PSM. First, the molecular clock, the so-called segmentation clock, creates oscillatory expression of particular genes, *hairy* and some *notch*-related genes, in the posterior PSM. Because the phase of oscillation is gradually shifted along the posterior-to-anterior axis, a wave of oscillation appears to move in a posterior-to-anterior fashion. This oscillatory gene expression subsequently results in periodical generation of morphologically segmented somites.

The spatial pattern of somites is defined by positioning of intersomitic boundaries and rostro-caudal patterning within them. This spatial pattern is established in the anterior PSM by converting the oscillatory gene expression into spatially restricted patterns of gene expression. Several intra-cellular molecules, as well as FGF and Wnt signaling, are involved in this conversion. It has been generally considered that Mesp of the bHLH transcriptional regulator plays a critical role in this conversion. In mice, the prospective segmentation boundary is periodically established in the anterior PSM at the rostral border of the *Mesp2* expression domain. Conversely, recent studies of this and other groups strongly suggest that Mesp2 does not directly define the position of the segmentation boundary, rather that other genes called *Ripply1* and *Ripply2* play more essential roles in this conversion in mice and zebrafish. *Ripply* genes encode ~100 amino acid proteins, that degrades Tbx6 proteins, which is involved in the positioning of the segmentation boundary. We have examined the mechanism of this conversion by focusing on the interaction between Ripply and other molecules involved in this conversion, including Hairy and FGF pathway molecules. Currently, we are visualizing the spatio-temporal shifts of these molecules in the PSM in wild-type and segmentation-defective zebrafish embryos.

### Publication List:

〔**Original papers**〕


- Ban, H., Yokota, D., Otosaka, S., Kikuchi, M., Kinoshita, H., Fujino, Y., Yabe, T., Ovara, H., Izuka, A., Akama, K., Yamasu, K., Takada, S., and Kawamura, A. (2019). Transcriptional autoregulation of zebrafish *tbx6* is required for somite segmentation. Development *146*, dev177063. doi: 10.1242/dev.177063
- Kikuchi, M., Nishimura, T., Saito, D., Shigenobu, S., Takada, R., Gutierrez-Triana, J.A., Cerdán, J.L.M., Takada, S., Wittbrodt, J., Suyama, M., and Tanaka, M. (2019). Novel components of germline sex determination acting downstream of foxl3 in medaka. Dev. Biol. *445*, 80-89. doi: 10.1016/j.ydbio.2018.10.019
- Shinozuka, T., Takada, R., Yoshida, S., Yonemura, S., and Takada, S. (2019). Wnt produced by stretched roof-plate cells is required for the promotion of cell proliferation around the central canal of the spinal cord. Development *146*, dev159343. doi: 10.1242/dev.159343