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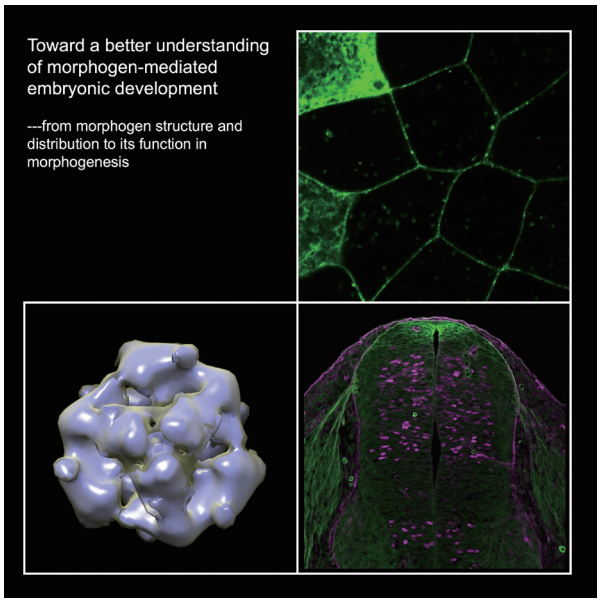
The morphology of the body and tissues is established in a spatio-temporarily regulated manner. A number of genes involved in the process of morphogenesis have been identified, but it is still uncertain how either spatial and temporal information are established and converted to morphology during embryogenesis.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



Visual overview of this lab's work.

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 is yet to be elucidated. To this end, we have visualized signal proteins and monitored their movement in tissues. Furthermore, we are also 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 research 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.

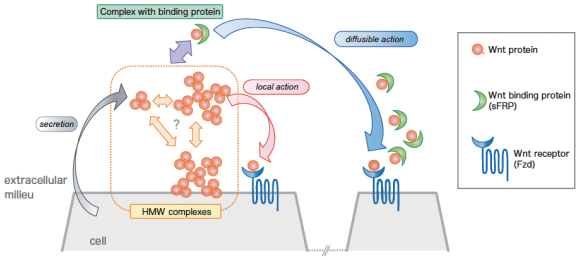


Figure 1. Model of Wnt protein diffusion: Wnt trimers are the smallest unit of the HMW complex. Both the trimer and the HMW complex appear to exist in the extracellular milieu. The HMW complex is probably less mobile when interacting with the plasma membrane, resulting in the restriction of Wnt diffusion 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 proteins (partner proteins), including sFRP. Due to this dissociation, Wnt turns out to be more mobile and its diffusion range is expanded (diffusible action).

I. Regulation of spatial distribution and function of Wnt proteins in vertebrates

By combining biochemical and structural analyses, we have already shown that Wnt3a proteins are not secreted in a monomeric form, rather in homo-trimer and larger HMW complexes. Secreted Wnt3a proteins were able to be dissociated via interaction with their receptor Frizzled8 and with a secreted Wnt binding protein, sFRP2, *in vitro*. Similarly, this dissociation was detected *in vivo* by Fluorescence

Correlation Spectroscopy (FCS). Several lines of evidence show that large assemblies of Wnt3a are less mobile, and Wnt/sFRP2 heterodimer, which is generated through the binding of dissociated Wnt with sFRP2, diffuse more freely. Based on these results, we have proposed a model which contends that the assembly and dissociation of dissociable oligomers modulate Wnt signaling range (Figure 1).

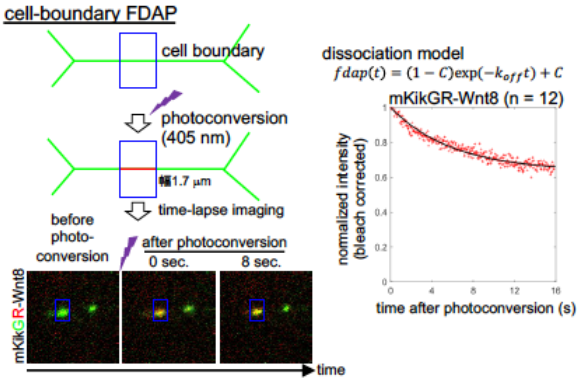


Figure 2. Fluorescence decay after photoconversion (FDAP) at cell coundaries. A Photoconvertable fluorescent protein, mKikGR can be switch its fluorescence from green to red. Decay of red (photoconverted) fluorescence can be fitted with a model of dissociation from cell surface scaffolds.

To increase our insight into the intercellular transmission of Wnt proteins in embryonic tissue, we precisely examined the extracellular dynamics of Wnt, comparing with sFRP in Xenopus embryos. Here, we focused on Wnt8 and a member of sFRPs, Frzb, both of which are involved in the anteroposterior patterning of the vertebrate embryo. While Venus-tagged Wnt8 was found on the surfaces of cells close to Wnt-producing cells, we also detected its dispersal over a long range from the source cells. We further examined their dynamics by FCS and fluorescence decay after photoconversion (FDAP)-based measurements in the embryonic tissue. In particular , we refined FDAP-based analysis by focusing on a limited area across the cell surface, which enabled us to obtain dynamics comparable to those measured by FCS (Figure 2). Combination of fluorescence correlation spectroscopy and quantitative imaging revealed that only a small proportion of Wnt8 proteins diffuse freely, whereas most of them are bound to the cell surface. FDAP analysis, that we refined by focusing on a limited area across the cell surface, showed that Wnt8 proteins that were bound to the cell surface were rapidly and exponentially decreasing, suggesting a dynamic exchange of a bound form of Wnt proteins. Based on these results and our previous findings, we have proposed a basic mathematical model to explain distribution and dispersion of secreted proteins (Figure 3). This model, which is based on the dynamic exchange of the bound form of Wnt proteins, can recapitulate a graded distribution of the bound, not free, state of Wnt proteins.

In an attempt to investigate the physiological role of Wnt signaling, we collaborated with Dr. Fujimori and Dr. Takahama at the University of Tokushima and NIH, respectively, to examine gain-of-function (GOF) and loss-of-function (LOF) of β-catenin highly specific in mouse thymic epithelial cells (TECs). GOF of β-catenin in TECs results in severe thymic dysplasia and T-cell deficiency beginning from the embryonic period. By contrast, loss-of-function of β-catenin in TECs reduces the number of cortical TECs and thymocytes modestly and only postnatally. These results indicate that fine-tuning of β-catenin expression within a permissive range is required for TECs to generate an optimal microenvironment to support postnatal T-cell development.

II. The molecular mechanism of metameric structures in vertebrate development

The segmental sub-regions of several specific tissues, including the paraxial mesoderm (or somites) and the pharyngeal pouches is not likely to be simply directed by the gradient of the signal molecules, but by a unique mechanism proceeding periodically. For instance, somites are sequentially generated in an anterior-to-posterior order by converting the temporal periodicity created by a molecular clock into periodical structures. The molecular mechanism underlying this conversion and morphological segmentation, however, is not yet fully understood.

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. The molecular clock, the so-called segmentation clock, essentially creates oscillatory expression of particular genes, *hairy* and some *notch*-related genes, in the posterior PSM.

As the oscillatory waves of the segmentation clock move from posterior to anterior PSM, the temporal dynamics of the segmentation clock are transformed into the spatial pattern

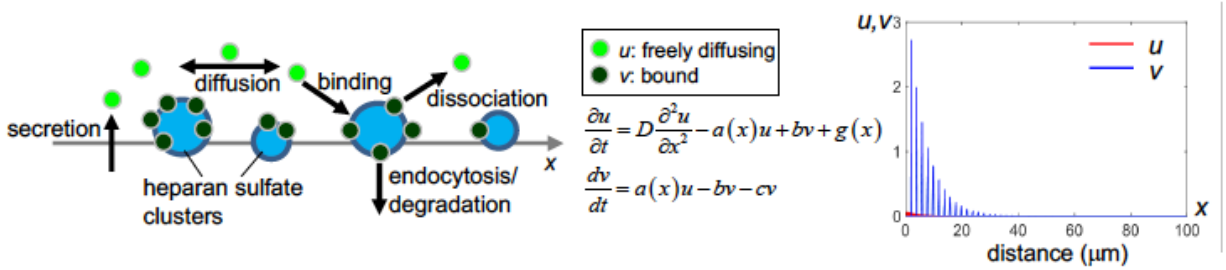


Figure 3. Mathematical model considering free diffusion and binding to heparan sulfate clusters. The free population (*u*) does not virtually contribute to spatial distribution, whereas the bound population (*v*) directly contributes to the graded distribution of a morphogen. This model suggests both populations contribute to rapid formation of a stable gradient, which gives insight into a perceived dilemma between speed and stability of gradient formation.

of somites. One of the key processes shaping the spatial pattern of somites is the generation of inter-somite boundaries. Evidences indicate that inter-somite boundary is defined by the anterior edge of the Tbx6 protein expression region. It has been reported by us and other groups that this anterior border is newly generated in each segmentation cycle, by periodic degradation of Tbx6 protein via physical interaction with *Ripply1* and *Ripply2*.

We are currently using a genetic approach in zebrafish to elucidate the mechanisms of the interaction between the segmental clock and the Tbx6/Ripply system. Based on the results obtained from the genetic approach, we provide a mathematical model showing the minimal network that forms the metameric pattern of somites and the periodic cessation of the segmentation clock.

Publication List:

〔Original papers〕

- Feng, D., Wang, J., Yang, W., Li, J., Lin, X., Zha, F., Wang, X., Ma, L., Choi, N.T., Mii, Y., Takada, S., Huen, M.S.Y., Guo, Y., Zhang, L., and Gao, B. (2021). Regulation of Wnt/PCP signaling through p97/VCP-KBTBD7-mediated Vangl ubiquitination and endoplasmic reticulum-associated degradation. Sci. Adv. *7*, eabg2099. DOI: 10.1126/sciadv.abg2099
- Fujimori, S., Ohigashi, I., Abe, H., Matsushita, Y., Katagiri, T., Taketo, M.M., Takahama, Y., and Takada, S. (2022). Fine-tuning of beta-catenin in mouse thymic epithelial cells is required for postnatal T-cell development. eLife *11*, e69088. DOI: 10.7554/eLife.69088
- Matsuda, S., Schaefer V, J., Mii, Y., Hori, Y., Bieli, D., Taira, M., Plueckthun, A., and Affolter, M. (2021). Asymmetric requirement of Dpp/BMP morphogen dispersal in the Drosophila wing disc. Nat. Commun. *12*, 6435. DOI: 10.1038/s41467-021-26726-6
- Mii, Y., Nakazato, K., Pack, C.-G., Ikeda, T., Sako, Y., Mochizuki, A., Taira, M., and Takada, S. (2021). Quantitative analyses reveal extracellular dynamics of Wnt ligands in *Xenopus* embryos. eLife *10*, e55108. DOI: 10.7554/eLife.55108
- Okubo, T., Hara, K., Azuma, S., and Takada, S. (2021). Effect of retinoic acid signaling on Ripply3 expression and pharyngeal arch morphogenesis in mouse embryos. Dev. Dyn. *250*, 1036–1050. DOI: 10.1002/dvdy.301
- Yamamoto, K., Miura, H., Ishida, M., Mii, Y., Kinoshita, N., Takada, S., Ueno, N., Sawai, S., Kondo, Y., and Aoki, K. (2021). Optogenetic relaxation of actomyosin contractility uncovers mechanistic roles of cortical tension during cytokinesis. Nat. Commun. *12*, 7145. DOI: 10.1038/s41467-021-27458-3

〔Review article〕

- Shinozuka, T., and Takada, S. (2021). Morphological and functional changes of roof plate cells in spinal cord development. J. Dev. Biol. *9*, 30. DOI: 10.3390/jdb9030030