The morphology of the body and tissues is established in a spatio-temporally 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.

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 sub-regions 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.

I. Regulation of spatial distribution of Wnt proteins in vertebrate embryos

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).

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.

Firstly, we visualized their localization in Xenopus embryos by fusing them with fluorescent proteins. Monomeric Venus (mV) fused with Wnt8 and Frzb were visualized on the cell surface when they were expressed in Xenopus embryos. By contrast, we found that only the secreted form of mV (sec-mV), which was expected to have no specific binding to the cell surface, was hardly visible along the cell boundary under the same conditions. This difference in protein distribution can be explained by difference of affinity to docking molecules on the cell surface because the addition of heparin binding peptides resulted in a significant increase of the localization of sec-mV on the cell surface.

We subsequently examined the dispersion of these proteins by expressing an artificial protein, called morphotrap, that can capture Venus-tagged proteins on the cell surface (Figure 2). Because mV-Wnt8 and mV-Frzb were trapped by morphotrap, a population of mV-tagged Wnt8 and Frzb deemed not likely to associate tightly with the cell surface, and were able to be dispersed over a long range from the source cells.

We then 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. 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. 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.

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 peri-
odically 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. After this, the spatial pattern of somites is defined based on the temporal periodicity created by the clock. We are currently investigating the transitory mechanism concerning the temporal periodicity in the spatial patterns of somites using zebrafish embryos.

In addition to somites, we examined the development of another metameric structure, the pharyngeal arches (PA), which give rise to skeletal elements of jaws and gills. This metamerism is brought about by the segmental development of the pharyngeal pouches (PP), which are generated by outpocketing of the pharyngeal endoderm. Interestingly, the two most anterior PAs are likely to have different characteristics from the posterior PAs. The anterior PAs express genes involved in myogenesis and chondrogenesis whereas the posterior PAs do not undergo myogenesis and chondrogenesis in chick and mouse embryos. Furthermore, several studies have suggested that distinct segmentation mechanisms for anterior and posterior PAs may cooperate in establishing the entire series of PAs. However, development of this complex PA segmentation has been poorly understood until now. It has been quite puzzling how the seamless array of pharyngeal arches is generated by a combination of anterior and posterior PAs, which are formed by distinct mechanisms.

To address this issue, we examined the development of zebrafish pharyngeal endoderm, focusing in particular on the formation of PP2, which is located at the boundary between the anterior and posterior PAs. Precise examination by live-imaging and cell-tracing experiments performed in zebrafish showed that PP2 was formed in an unexpected manner. Rostral and caudal aspects of PP2 were initially formed separately, then subsequently established contact through the dynamic remodeling of the endoderm epithelium, upon which they finally became integrated (Figure 3). These results provide an answer to the question of how a seamless array of PAs are generated.

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