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The body and tissues of the developing embryo are repeatedly divided into sub-regions specified by characteristic gene expression and morphology. The process that gives rise to these sub-regions, according to a defined pattern, is called “pattern formation” or “patterning.” The most popular model to explain the patterning process is the “morphogen gradient and threshold” theory. Many genetic results indicate that secreted signal proteins such as Wnt, BMP, and Hedgehog function as morphogens in many aspects of the patterning process. In spite of the accumulation of genetic evidence, however, the biochemical characteristics of morphogens, including modification and higher order structure, remain to be elucidated. Thus, one of our major goals is to reveal *the real image* of morphogens and the molecular mechanism underlying the formation of morphogen gradients, including the secretion and extracellular transport of these morphogens.

The segmental sub-regions of the paraxial mesoderm (or somites), by contrast, appear not to be simply directed by the morphogen gradient and threshold, but by a unique mechanism proceeding periodically. Somites are sequentially generated in an anterior-to-posterior order by converting oscillatory gene expression into periodical structures. The molecular mechanism underlying this conversion and morphological segmentation, however, is not yet fully understood. Thus, another goal of our current studies is to reveal the molecular mechanism of *this other and unique mode of patterning* that underlies the periodical and sequential sub-division in the development of somites and pharyngeal arches.

I. Lipid modification and extracellular trafficking of Wnt proteins

The Wnt family of secreted signal proteins plays a key role in numerous aspects of embryogenesis. Most Wnt proteins transmit their signals locally, presumably since their secretion and transport are under tight control. One important step regulating the extracellular transport of various secreted signal proteins involves post-translational modification with lipid moieties. We found that murine Wnt-3a is modified with a mono-unsaturated fatty acid, palmitoleic acid, at a conserved Ser residue. Wnt-3a defective in this modification is not secreted from cells in culture or in Xenopus embryos, but is retained in the endoplasmic reticulum (ER). Furthermore, Porcupine (Porcn), a protein with structural similarities to membrane-bound O-acyltransferases, is required for this Ser-dependent modification, as well as for Wnt-3a transport from the ER for secretion. These results strongly suggest that Wnt protein requires a particular lipid modification for proper intracellular transport during the secretory process. We expect that the discovery of this unexpected lipid modification might provide a clue regarding the higher-order structure of secreted Wnt proteins and their gradient formation (Figure 1).

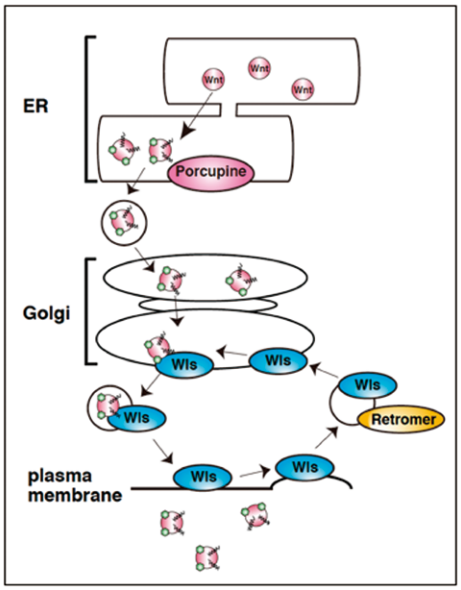

Figure 1. Wnt secretion pathway: Wnt proteins require specific machinery for their secretion. Wntless (Wls), a seven-pass membrane protein, is required for intracellular transport of Wnt proteins. After release of Wnt proteins into the extracellular space, Wls is recycled from the plasma membrane back to the Golgi apparatus by the retromer complex. Prior to the association with Wls, Wnt proteins are modified with a fatty acid, palmitoleoylate, by Porcupine in the ER.

Until now, the role of Porcn *in vivo* has been examined in only a few vertebrate studies. For instance, in humans, mutations in *PORCN* cause focal dermal hypoplasia (FDH), which is an X-linked dominant disorder, characterized by patchy hypoplastic skin and malformations of a wide variety of tissues. However, it is unclear whether the FDH phenotypes are actually caused by abnormalities in Wnt signaling. Furthermore, although some of the FDH phenotypes look similar to those caused by impaired Wnt signaling, only a few of the many defects caused by impaired Wnt-signaling components are observed in FDH patients. Thus, it is unclear whether vertebrate *Porcn* is actually

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

required for Wnt signaling *in vivo*, or whether such a requirement is equivalent for all the members of the Wnt family proteins.

To investigate these questions, we used zebrafish as a model system and examined the effects of defects in Porcn function on Wnt signals in early embryonic stages because the roles of embryonic Wnt signals have been precisely characterized. In addition, we are trying to reveal the molecular mechanism underlying the extracellular transport of Wnt proteins during embryogenesis using frog embryos.

II. Roles of Ripplys in establishment of segmental patterns of somites.

The molecular mechanism underlying the periodical formation of somites is coupled to an internal oscillator, referred to as the "segmentation clock," which has been evidenced by the cyclic expression of genes in the presomitic mesoderm (PSM). For example, *hairy/Enhancer of split* (*Espl)*-related bHLH genes, including *her1* and *her7* in zebrafish, are expressed in a dynamic pattern of stripes across the PSM in a posterior to anterior direction. The oscillating and anteriorly propagating wave of gene expression, which is maintained in the posterior PSM, becomes fixed to cause

segmentation in the anterior PSM. Prior to morphological segmentation, a segmental pre-pattern, characterized by the periodical borders between neighboring somites and by the rostro-caudal within a somite, is established in the anterior PSM. We have already shown that a gene identified by our *in situ* hybridization screening, *ripply1,* is required for the maintenance of the rostro-caudal patterning. In addition to Ripply1, another structurally related-protein, Ripply2, is required for the rostro-caudal patterning.

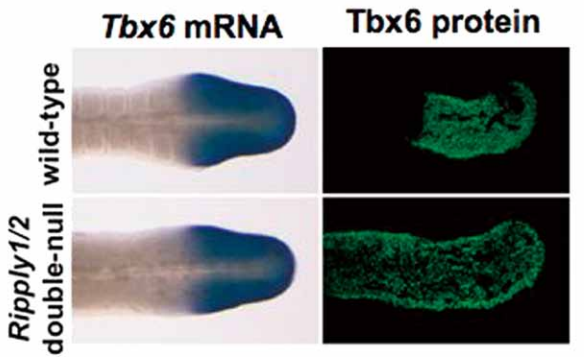

Figure 2. Ripply1 and Ripply2 determine the positions of somite boundaries through degradation of Tbx6 proteins. In Ripply1/2-deficient mouse embryos, the anterior border of the Tbx6 domain is expanded anteriorly.

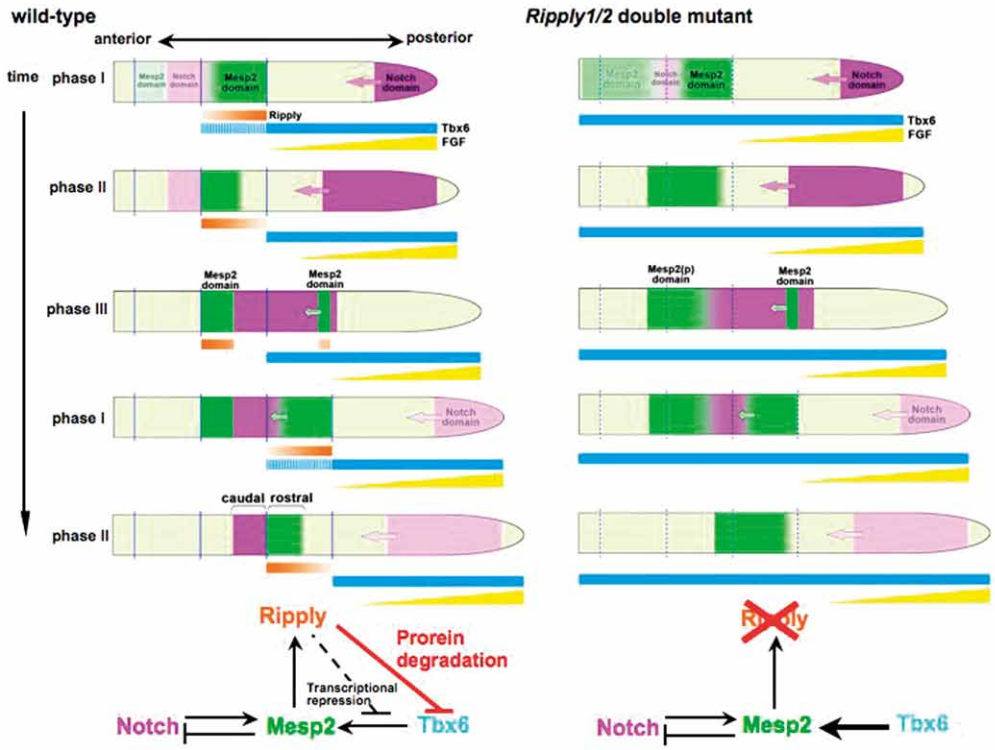

Figure 3. A model of somite segmentation. The Notch active domain travels in the posterior PSM in a posterior-to-anterior direction in phase I. Then, the anterior front of the progressing Notch domain encounters the Mesp2 expression domain, which was already generated in the previous segmentation cycle at late phase II. At this time, progression of the Notch active domain appears to be arrested at the posterior edge of the Mesp2-expressing domain, because Mesp2 inhibits Notch activity. On the other hand, a new cycle of expression of Mesp2, which is induced by traveling Notch activity, emerges in the middle-to-posterior side of the Notch active domain (phase III). In accordance with the anterior progression of the Notch domain, Mesp2 expression subsequently turns on in the Notch domain, resulting in anterior expansion of the newly formed Mesp2 domain in Tbx6 positive area (phase I in the next segmentation cycle). Ripply1/2 regulates anterior expansion of the Mesp2 domains by inhibition of Tbx6. Because of misregulation of Tbx6, boundary formation and rostro-caudal patterning were disturbed in Ripply1/2 mutant embryos.

Interestingly, mouse embryos lacking both *Ripply1* and *Ripply2* exhibit complete lack of somite boundaries, in addition to severe rostralization of somites. The positions of somite boundaries are defined by the anterior limit of the Tbx6 protein domain, which is regulated by degradation of Tbx6 proteins. A recently study showed that a transcription factor, Mesp2, is required for this degradation although it is uncertain how Mesp2 induces this degradation. Interestingly, expression of mouse *Ripply1* and *Ripply2* is dependent on Mesp2 and *Ripply1/2*-deficient embryos exhibit anterior expansion of Tbx6 protein domain (Figure 2), suggesting that *Ripply1/2* expression by *Mesp2* is important in the establishment of somite boundaries by regulating Tbx6 degradation. In addition, Ripply1/2 regulates the rostro-caudal patterning within a somite through interaction with Mesp2, Notch signaling and Tbx6. Based on these results, we proposed a model to explain the boundary formation and the rostro-caudal patterning of somites (Figure 3).

III. The role of Ripply3 in the development of pharyngeal arches.

The pharyngeal apparatus is a transient structure formed ventrolateral to the hindbrain in vertebrate embryos. This structure consists of bilaterally segmented arches, and ectodermal grooves and endodermal pouches, both of which are formed between the arches. The pharyngeal arches comprise mesodermal cells, neural crest-derived mesenchyme, an outer ectodermal cover and an inner endodermal lining. Components of the pharyngeal apparatus give rise to distinct tissues in later stages of development. For instance, the pharyngeal arteries and neural crest cells in the caudal pharyngeal arches contribute to the cardiovascular development, while the endodermal cells located in the caudal pouches give rise to several organs, including the thymus and parathyroid gland. Thus, the pharyngeal development is a key process in the generation of these organs.

We found that *Ripply3*, another member of the Ripply family, is expressed in the caudal endoderm and ectoderm of the pharyngeal apparatus in the mouse embryo. Interesitngly, in the caudal pharyngeal endoderm, *Ripply3* is co-expressed with *Tbx1, which* is essential for pharyngeal development (Figure 4). A number of mouse genetic studies and mutational analyses in human patients have indicated that *Tbx1* is the most likely gene responsible for the phenotype of Chromosome 22q11 deletion syndrome (22q11DS). 22q11DS includes the DiGeorge syndrome (DGS), conotruncal anomaly face syndrome (CAFS) and velocardiofacial syndrome (VCFS), is characterized by the abnormal development of the pharyngeal apparatus in the form of thymic hypoplasia or aplasia, hypocalcemia arising from parathyroid hypoplasia and cardiac outflow defects. We show that Ripply3 can modulate Tbx1 activity in in vitro reporter assays. Furthermore, *Ripply3*-kock out mouse, generated by ourselves, exhibit abnormal development of pharyngeal derivatives, including ectopic formation of the thymus and the parathyroid gland, as well as cardiovascular malformation. Corresponding with these defects, *Ripply3*-deficient embryos show hypotrophy of the caudal pharyngeal apparatus. Ripply3 represses Tbx1-induced expression of *Pax9* in *in vitro* luciferase assays, and *Ripply3*-deficient embryos exhibit up-regulated *Pax9* expression. Together, our results show that Ripply3 plays a role in pharyngeal development probably by regulating Tbx1 activity.

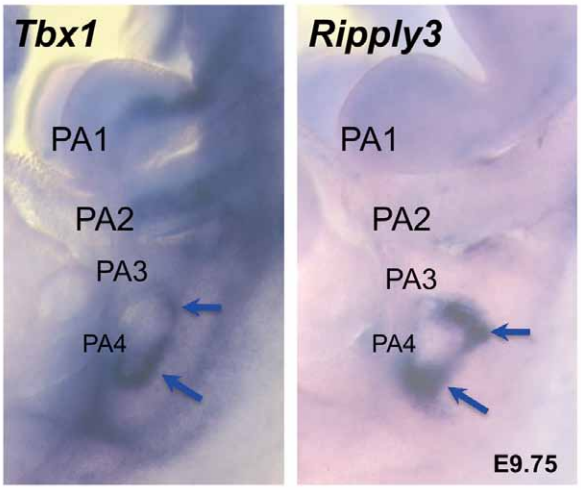

Figure 4. Expression of Ripply3 with its target, Tbx1, in the pharyngeal region of a mouse embryo.

Publication List

〔Original papers〕

- Hashimoto, H., Shinohara, K., Wang, J., Ikeuchi. S., Yoshiba, S., Meno, C., Nonaka, S., Takada, S., Hatta, K., Wynshaw-boris, A., and Hamada, H. (2010). Planar polarization of node cells determines the rotational axis of node cilia. Nat. Cell Biol. *12*, 170-176.
- Katayama, R., Ishioka, T., Takada, S., Takada, R., Fujita, N., Tsuruo, T., and Naito, M. (2010). Modulation of Wnt signaling by the nuclear localization of cellular FLIP-L. J. Cell Sci. *123*, 23-28.
- Miyaoka, Y., Tanaka, M., Imamura, T., Takada, S., and Miyajima, A. (2010). A novel regulatory mechanism for Fgf18 signaling involving cysteine-rich FGF receptor (Cfr) and delta-like protein (Dlk). Development *137*, 159-167.
- Nishita, M., Itsukushima, S., Nomachi, A., Endo, M., Wang, Z., Inaba, D., Qiao, S., Takada, S., Kikuchi, A., and Minami, Y. (2010). Ror2/Frizzled complex mediates Wnt5a-induced AP-1 activation by regulating dishevelled polymerization. Mol. Cell. Biol. *30*, 3610-3619.
- Takahashi, J., Ohbayashi, A., Oginuma, M., Saito, D., Mochizuki, A., Saga, Y., and Takada, S. (2010). Analysis of Ripply1/2-deficient mouse embryos reveals a mechanism underlying the rostro-caudal patterning within a somite. Dev. Biol. *342*, 134-145.
- Yoshinaga, Y., Kagawa, T., Shimizu, T., Inoue, T., Takada, S., Kuratsu, J., and Taga, T. (2010). Wnt3a promotes hippocampal neurogenesis by shortening cell cycle duration of neural progenitor cells. Cell. Mol. Neurobiol. *30*, 1049-1058.