DIVISION OF MORPHOGENESIS

Professor:	*Naoto Ueno*
Associate Professor:	*Hiroshi Shibuya*
Research Associates:	*Makoto Nakamura*
	Makoto Mochii
Technical Stuff:	*Chiyo Takagi*
	Takamasa Yamamoto
	Akiko Hyodo
JSPS Postdoctoral Fellows:	*Miho Shimizu**
	Shun-ichiro Iemura
	Kiyokazu Morita
Graduate Students:	*Michiru Nishita*[1)]
	Katsura Sugawara [2)]
	Satoru Yoshida[2)]
	Yasunori Tomoyasu[2)]
	Tomoko Kurata[2)]
	Bisei Nakano[2)]
	Masaki Takeuchi[2)]
	Jyunko Hyodo[3)]
	Shin-ichi Nagai[3)]
	Yayoi Nishida[3)]

** until August, 1999*
[1)] *Graduate School of Hokkaido University*
[2)] *Graduate University for Advanced Studies*
[3)] *Graduate School of Nagoya University*

The complex morphogenesis of organisms is achieved by consecutive cell-to-cell interactions of germ layers as well as tissues during development. Recent studies suggest that several groups of molecules called polypeptide growth factors (PGFs) are essential component controlling such intercellular communications in a variety of organisms. These cell communications via PGFs are regulated by a number of processes including secretion, activation, diffusion, reception by specific receptors and intracellualr signaling of PGFs. Our main interest is to know how pattern formation in morphogenesis is regulated by PGFs during development. We address this problem using several model animals, including frog, fly and worm with the view of extracellular and intracllular signaling of PGFs. We employ embryology, genetics, molecular and cellular biology and biochemistry. In addition, we have recently introduced a systematic and comprehensive way to screen target genes of PGFs using arrayed cDNAs.

I. Hierarchy of homeobox genes in pattening embryo

Embryos are patterned along dorso-ventral (DV) axis by the action of PGFs. Signaling triggered by PGFs leads to the activation of their target genes. Several homeobox genes are known to be induced in response to PGFs in early *Xenopus* development. In particular, Xmsx-1, an amphibian homologue of vertebrate Msx-1, is well characterized as a target gene of bone morphogenetic protein (BMP). In order to clarify molecular basis for ventalization by BMP and in vivo siginificance of Msx-1, we examined whether Xmsx-1 activity is required in the endogenous ventralizing pathway, using a dominant-negative form of Xmsx-1 (VP-Xmsx-1), which is a fusion protein with the virus-derived VP16 activation domain. Interestingly, VP-Xmsx-1 induced a secondary body axis, complete with muscle and neural tissues, when the fusion protein was overexpressed in ventral blastomeres. The result suggests that Xmsx-1 activity is necessary for both mesoderm and ectoderm to be ventralized. We also examined the epistatic relationship between Xmsx-1 and another ventralizing homeobox protein Xvent-1 and showed that Xmsx-1 is likely to be acting upstream of Xvent-1. We propose that Xmsx-1 is required in the BMP-stimulated ventralization pathway that involves the downstream activation of Xvent-1.

II. Cross-talk of growth factor signaling in early development

Signals generated by the members of the Wnt and TGF-β superfamilies mediate a diverse array of biological responses. These secreted factors, via activation of cell surface receptors, participate in the regulation of cell fate specification and control cell proliferation during development and tissue maintenance. In the early amphibian embryo (*Xenopus laevis*), both Wnt and TGF-β superfamily signaling cascades are required for establishment of a dorsal signaling center, Spemann's organizer. In *Xenopus*, as in other systems, both signaling pathways utilize extranuclear proteins which, upon activation, translocate to the nucleus to participate in transcriptional complexes. Wnt signaling is mediated by translocation of β-catenin into the nucleus together with members of the Lef1/Tcf class of HMG box transcription factors to activate gene expression. Likewise, TGF-β superfamily signaling is mediated by "pathway-specific" Smads which translocate into the nucleus together with the "universal Smad" Smad4 to activate gene expression. Recently, we have demonstrated that Lef1/Tcf, a downstream component of the Wnt signaling cascade can physically interact with Smad4. In *Xenopus*, this interaction directly affects transcriptional activity of the twin (Xtwn) gene during Spemann's organizer formation. This is the first demonstration of a physical interaction between TGF-β and Wnt signaling components, and may represent a direct connection between the TGF-β and Wnt signaling pathways.

III. Genetic dissection of TGF-β signaling in *Drosophila* model system

We have reported that the morphogen gradients of Dpp and Wg provide positional information to cells in the notum region of the wing imaginal disc. dpp is induced at A/P boundary by a Hh dependent mechanism in wing and leg discs, however, wg is induced in various ways. We revealed that initial notal wg induction is regulated by the function of two transcription factors, Pannier and U-shaped. We also found that expression of both *pnr* and *ush* is regulated by Dpp signaling. We propose that the Dpp gradient is utilized not only for anterior/posterior patterning but also contributes for dorsal/ventral patterning through the induction of wg.

We also analyzed *in vivo* function of TAK1 (dTAK1) in *Drosopihla*. We generated transgenic flies which express vertebrate or *Drosophila* homolog of TAK1. Genetic and biochemical analyses indicated that the c-Jun amino-terminal kinase (JNK) signaling pathway is specifically activated by TAK1 signaling. Expression of a dominant negative form of dTAK1 during embryonic development resulted invarious embryonic cuticle defects including dorsal open phenotypes. Our results suggested that TAK1 functions as a MAPKKK in the JNK signaling pathway and participates in such diverse roles as control of cell shape and regulation of apoptosis in *Drosophila*.

IV. TGF-β family in nematode

Nematode *C. elegans* provides powerful genetic approaches to understand the role and mechanism of TGF-β family signaling. We have identified a new member of the TGF-β superfamily, CET-1, from *C. elegans*. *cet-1* null mutants have shortened bodies and male tail abnormal phenotype resembling *sma* mutants. Genetic analysis suggested that CET-1 regulated body length and male ray pattern functioning as ligand in *sma* pathway. *cet-1* is mainly expressed in the ventral nerve cord and other neurons which are thought to secrete CET-1 ligand and induce specific gene expression in target cells. To elucidate molecular mechanism regulating *C. elegans* body length, we employed a differential hybridization of a cDNA array for identification of target genes of the CET-1 signal.

C. elegans cDNAs representing 7,584 independent genes were arrayed on a nylon membrane at high density, and hybridized with ^{33}P-labeled DNA probes synthesized from the mRNAs of wild-type, *cet-1*, *sma-2,* and *lon-2* worms (Fig. 1, top). Signals for all the spots representing hybridized DNA were quantified and compared between strains (Fig. 1, bottom). The screening identified 22 and 2 clones, which were positively and negatively regulated, respectively, by the CET-1 signal. Northern hybridization confirmed the expression profiles of most of the clones indicating good reliability of the differential hybridization using arrayed cDNAs. In situ hybridization analysis revealed the spatial and temporal expression patterns of each clone and showed that at least 4 genes, including the gene for the type I receptor for CET-1, *sma-6*, were transcriptionally regulated by the CET-1 signal.

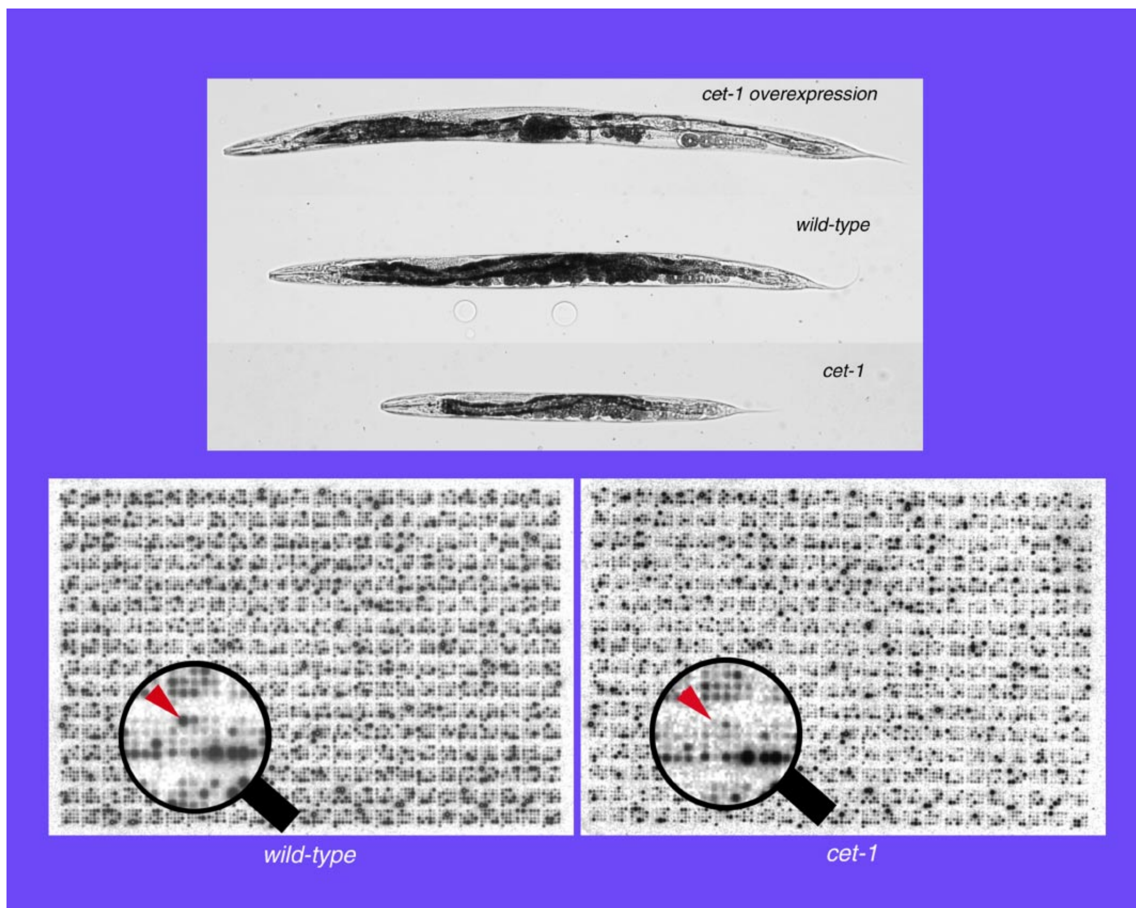

Fig. 1. Screening for TGF-β-regulated genes in *C. elegans*. (Upper panel) Change of body length depending on *cet-1* gene doses. Cet-1 overexpressing, wild-type, and *cet-1* null mutant worms from top. (Bottom panel) Macroarray of *C. elegans* cDNAs. Hybridization signals were compared between wild-type and *cet-1* null mutant worms. The arrowhead points a DNA spot down-regulated in the mutant.

Publication List:

Adachi-Yamada, T., Nakamura, M., Irie, K., Tomoyasu, Y., Sano, Y., Mori, E., Goto, S., Ueno, N., Nishiada, Y., Matsumoto, K. (1999) p38 MAP kinase participates in TGF-β superfamily signal transduction in Drosophila wing morphogenesis. *Mol. Cell. Biol.***19**, 2322-2329.

Hamada, F., Tomoyasu, Y., Takatsu, Y., Nakamura, M., Nagai, S., Fujita, F., Shibuya, H., Ueno, N. and Akiyama, T. (1999) Negative regulation of the Wingless signaling by a Drosophila homologue of Axin. *Science* **283**, 1739-1742.

Hamada, F., Murata, Y., Nishida, A., Fujita, F., Tomoyasu, Y., Nakamura, M., Toyoshima, K., Tabata, T., Ueno, N., Akiyama, T. (1999) Identification and characterization of E-APC, a novel Drosophila homologue of the tumour suppressor APC. *Genes Cells* **4**, 465-474.

Hanafusa, H., Ninomiya-Tsuji, J., Masuyama, N., Nishita, M., Fujisawa, J., Shibuya, H., Matsumoto, K. and Nishida, E. (1999) Involvement of the p38 MAP kinase pathway in TGF-β-induced gene expression. *J. Biol. Chem.* **274**, 27161-27167.

Hirota, Y., Okabe, M., Imai, T., Kurusu, M. Yamamoto, A., Miyao, S., Nakamura, M., Sawamoto, K., Okano, H. (1999) Musashi and Seven in absentia downregulate Tramtrack through distinct mechanisms in Drosophila eye development. *Mech. Dev.* **87**(1-2) 93-101.

Iemura, S., Yamamoto, T. S., Chiyo, T. Kobayashi, H. and Ueno, N. (1999) Isolation and characterization of bone morphogenetic proteins from the early Xenopus embryo. *J. Biol. Chem.* **274**, 26843-26849.

Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Baker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H. and Matsumoto, K. (1999) The TAK1-NLK MAP kinase-related pathway antagonizes β-catenin-TCF signalling. *Nature* **399**, 798-802.

Masuyama, N., Hanafusa, H., Kusakabe, M., Shibuya, H. and Nishida, E. (1999) Identification of two Smad4s in Xenopus: Their common and distinct properties. *J. Biol. Chem.* **274**, 12163-12170.

Miyama, K., Yamada, G., Yamamoto, T., Takagi, C., Miyado, K., Sakai, M., Ueno, N. and Shibuya, H. (1999) A BMP-inducible gene, Dlx-5 regulates osteoblast differentiation and mesoderm induction. *Dev. Biol.* **208**,123-133.

Mochii, M., Yoshida, S., Morita, K., Kohara, Y. and Ueno, N. (1999) Identification of transforming growth factor-β-regulated genes in *Caenohabtidis elegans* by differential hybridization of arrayed cDNAs. *Proc. Natl. Acad. Sci. USA* **96**, 15020 –15025.

Morita, K., Chow, K. and Ueno, N. (1999) Body length and male tail ray pattern formation of *Caenohabditis elegans* are regulated by a mamber of TGF-β family. Development **126**, 1337-1347.

Nagaso, H. Tada, M., Nikaido, M. and Ueno, N. (1999) Dual specificity of activin type II receptor ActRIIb in dorso-ventral patterning during zebrafish embryogeneisis. *Dev. Growth Diff.* **41**, 119-133.

Nikaido, M., Masazumi, T. and Ueno, N. (1999) Restricted expression of the receptor serine/threonine kinase BMPR-IB in zebrafish. *Mech. Dev.* **82**, 219-222.

Nikaido, M., Tada, M., Takeda, H., Kuroiwa, A. and Ueno, N. (1999) In vivo analyisis using variants of zebrafish BMPR-IA: range of action and involvement of BMP in ectoderm patterning. *Development* **126**, 181-190.

Nishita, M., Ueno, N. and Shibuya, H. (1999) Smad 8B splice variant lacking the SSXS site that inhibits Smad8 mediated signalling. *Genes Cells* **4**, 583-591.

Nishita, M., Hashimoto, M. K., Ogata, S., Laurent, M. N., Ueno, N. Shibuya, H. and Cho, K.W.Y. Interaction between Wnt and TGF-β signalling pathways during formation of Spemann's organizer. *Nature* (in press)

Shimasaki, S., Zachow, R. J., Li, D., Iemura, S., Ueno, N., Chang, R. J., Sampath, K., and Erickson, G. F. (1999) Expression of a functional bone morphogenetic protein (BMP) system in the ovary. *Proc. Natl. Acad. Sci. USA* **96**, 7282-7287.

Suzawa , M., Takeuchi, Y., Fukumoto, S., Kato, S., Ueno, N., Miyazono, K., Matsumoto, T., Fujita, T. (1999) Extracellular matrix-associated bone morphogenetic proteins are essential for differentiation of murine osteoblastic cells in vitro. *Endocrinology* **140**, 2125-2133.

Takatsu, Y., Nakamura, M., Stapleton, M. Danos, M. C., Matsumoto, K. O'Connor, M. B., Shibuya, H. and Ueno, N. TAK1 participates in JNK signaling during Drosophila development. *Mol. Cell. Biol.* (in press)

Yamaguchi, K., Nagai, S-I., Ninomiya-Tsuji, J. Noshita, M., Tamai, K., Irie, K., Ueno, N., Nishida, E., Shibuya, H. and Matsumoto, K. (1999) XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. *EMBO J.* **18**, 179-187.

Yamamoto, S.T., Takagi, C. and Ueno, N. Requirement of Xmsx-1 in the BMP-triggered ventralization of Xenopus embryos. *Mech. Dev.* (in press)