A CELL CYCLE REGULATOR, XBtg2 IS REQUIRED FOR *XENOPUS* NEURAL DEVELOPMENT

○Kaoru Sugimoto[1], Makoto Asashima[1,2]
[1]Department of Life Science, Graduate School of Arts and Science, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902, Japan, [2]Japan Science and Technology Corporation(JST)

The B-cell translocation gene 2 (Btg2/PC3/TIS21) is known as an anti-proliferative gene that functions in regulating both cell proliferation and differentiation. Here we report the identification and characterization of *Xenopus* homologue of Btg2 (XBtg2) in early *Xenopus* neural development. Translational inhibition of XBtg2 resulted in the impaired eye formation. At the miduneurula stage, the XBtg2-inhibited embryos showed an increase in both proliferation and apoptosis; moreover the expression patterns of early neural markers, zic3 and soxD, were found to be altered in the region that arises to the mesencephalon and diencephalon, both essential for inducing eye formation. The altered expression patterns were not reversed by co-injection of an mRNA encoding anti-apoptotic gene, human bcl2, or by treatment with a cell division inhibitor, hydroxyurea and aphidicolin (HUA). Based on these data, we proposed that XBtg2 controls cell proliferation, differentiation and survival independently, and plays an essential role in the early neural development leading to the eye formation.

ANALYSIS OF THE TRANSCRIPTIONAL MECHANISM OF THE p450 AROMATASE GENE IN THE GONAD OF *XENOPUS*

○Yuta Ohira[1], Junya Nagaki[2], Etsuko Komatsuzaki[2], Shohei Miyata[1,2]
[1]Correlative Study of Physics and Chemistry, Graduate School of Intergrated Basic Sciences, Nihon University, 3-25-40 Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan, [2]Biochemistry, Department of Chemistry, College of Humanities and Science, Nihon University, 3-25-40 Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan

We reported previously that expressional regulation of p450 aromatase gene is important for sex determination of *Xenopus*. So we isolated the promoter sequence in p450 aromatase gene, and identified CRE-like sequence and SF-1 binding sequence in the promoter. In this study, we constructed the luciferase reporter plasmid containing p450 aromatase promoter sequence, introduced the reporter gene into the gonad of *Xenopus* by electroporation and measured the activity of the reporter plasmid. We confirmed whether the reporter plasmid was electroporated into the gonad by detection of the green fluorescent protein transcripted and translated from simultaneously electroporated GFP plasmid. We could also detect the activity of promoter containing these cis element. We will report the activity of each cis element containing CREB/ATF-4 binding sequence and SF-1 binding sequence.

THE ABNORMALITIES IN *XENOPUS LAEVIS* EYES AND VARIATION OF Pax6 GENE

○Yuka Ushiromura, Takayuki Fumita, Hiroko Takasaki
Department of Biology, Graduate School of Education, Osaka-Kyoiku University, Kashiwarashi, Osaka 582-8582, Japan

We often came to obtain the embryos similar to their parent's abnormalities with lens lack or cornea nebula in *Xenopus laevis* eyes at our laboratory since 1999. Because existence of the genes which caused the abnormalities of eyes have been assumed, we started to research for specifying the genes. We performed in situ hybridization using as probes, which were several genes, that is, Pax6, Six3, Rx and so on, participating in development of eyes. Consequently, it was suggested that the abnormalities were caused by the variation of Pax6 gene as well as other animals. Therefore, by the analysis of Pax6 cDNA, we identified the known and the strange sequences of Pax6. The variety of strange sequences of Pax6 included substitution, deletion and insertion into the known sequence. Especially, it had various insertions at the Paired Domain beginning site. From the findings of other animal kinds, since it was considered that this site was the boundary of exon and intron, it was suggested that the insertions were the residual of the intron. Therefore, we performed the genome analysis. We are considering whether the insertion sequences are concerned in the abnormalities of *X. laevis* eyes.

IDENTIFICATION OF *CIS*-REGULATORY SEQUENCES ESSENTIAL FOR CNS-SPECIFIC GENE EXPRESSION IN THE ASCIDIAN LARVA (II)

○Reiko Yoshida, Motoyuki Tsuda, Takehiro Kusakabe
Department of Life Science, Graduate School of Life Science, University of Hyogo, Kamigori, Hyogo 678-1297, Japan

We analyzed the structure and function of *cis*-regulatory DNA sequences of central nervous system (CNS)-specific genes, *Ci-Gα il* and *Ci-CRALBP*, in larvae of the ascidian *Ciona intestinalis*. *Ci-Gα il* is expressed in various types of neurons, while *Ci-CRALBP* is expressed in photoreceptor cells and glial cells in the brain vesicle and visceral ganglion. The 5' flanking regions of these genes were connected to *EGFP*, and then a part of these sequences was deleted or mutated. We introduced these constructs into *Ciona* embryos, and determined genomic DNA sequences sufficient to reproduce the endogenous gene expression patterns. The 87-bp sequence between -3176 and -3090 of *Ci-Gα il* and a 40-bp sequence in the first intron and the 105-bp sequence between -161 and -57 of *Ci-CRALBP* were shown to contain *cis*-regulatory sequences necessary for the CNS-specific expression of each gene. We compared these sequences with the corresponding regions of orthologs in a closely-related species *Ciona savignyi*, and found well-conserved sequences for each gene. Functional importance of the conserved sequences in CNS-specific gene regulation was further confirmed by *in vivo* experiments.

DEVELOPMENT OF NERVOUS SYSTEMS AND SENSORY ORGANS IN *Otx*, *Pax6*, OR *Six3/6* KNOCKDOWN LARVAE OF THE ASCIDIAN *CIONA INTESTINALIS*

○Miyuki Shindo, Hiroko Kuki, Yuki Miyamoto, Takeo Horie, Motoyuki Tsuda, Takehiro Kusakabe
Department of Life Science, Graduate School of Life Science, University of Hyogo, 3-2-1 Koto, Kamigori, Akoh-Gun, Hyogo 678-1297, Japan

Transcription factors Otx, Pax6, and Six3 play pivotal roles in development of sensory organs and nervous systems of vertebrates. The ascidian larva has a simple central nervous system with two sensory organs, an ocellus and an otolith. A number of genes related to eye function and development are expressed in part of the primordial pharynx (stomodeum), suggesting that photoreceptor cells are present in this region. In the present study, we have investigated roles of Otx, Pax6, and Six3 homologues in development of nervous systems and sensory organs in the ascidian *Ciona intestinalis* by gene silencing based on antisense morpholino oligonucleotides (MO). Larvae developed from eggs injected with an Otx antisense MO lacked adhesive organ, ocellus, and otolith, but they had differentiated neurons in the brain vesicle with a distribution pattern similar to that in wild type larvae. Six3 knockdown larvae also showed deficiency in development of anterior nervous systems, but the phenotype is distinct from that of Otx knockdown larvae. Our results suggest that development of the novel stomodeum photoreceptor is controlled by a mechanism similar to that of vertebrate eyes.

DEVELOPMENTAL CHANGES OF PHOTORECEPTOR ORGANS DURING POSTEMBRYONIC DEVELOPMENT OF THE ASCIDIAN *CIONA INTESTINALIS*

○Satoshi Matsushima, Chie Hiratani, Takeo Horie, Noriko Takimoto, Takehiro Kusakabe, Motoyuki Tsuda
Department of Life Science, University of Hyogo, Graduate School of Life Science, University of Hyogo, 3-2-1 Koto, Kamigori, Akoh-Gun, Hyogo 678-1297, Japan

The ocellus (eye-spot) of the ascidian larva is responsible for the photic swimming behavior. Ascidian adults also exhibit several types of light-responsive behaviors. We have shown that photoreceptive cells are present at several different sites in adults of the ascidian. We have investigated localization of photoreceptor-specific proteins during postembryonic development of the ascidian *Ciona intestinalis*. In addition to the ocellus, the larva has at least two groups of photoreceptor cells: one in the oral siphon rudiment (stomodeum) and the other in a left-dorsal part of the brain vesicle. As the onset of metamorphosis approaches, staining of these extraocular photoreceptor cells with anti-opsin antibodies becomes more intense, while that of the ocellus becomes weaker and disorganized. The opsin staining in the stomodeum immediately disappears when the metamorphosis commences. Photoreceptor organs of the adult seem to newly develop during or after metamorphosis. These results suggest that extraocular photoreceptor cells play a role in the regulation of late larval and/or early metamorphic events, such as settlement or the onset of metamorphosis.

CELL FATE DECISION MECHANISMS IN DEVELOPMENT OF MOUSE PERIPHERAL NERVOUS SYSTEM

○Mitsunori Ota, Kazuo Ito
Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

We have examined the roles of signaling molecules and Notch signaling in development of mouse peripheral nervous system. We analyzed the regulatory mechanisms for neurogenin (ngn) expression. Ngn is a bHLH transcription factor, which is essential for choice of cell fate between sensory neurons and glia. Our data show that BMP-4 and Shh promote ngn expression and that FGF-2 activates Notch signaling and then the activation prevents the differentiation of ngn-expressing sensory neurons. Whereas BMP-4 and Shh inhibited glial differentiation by the induction of ngn, FGF-2 promoted gliogenesis through Notch activation. These results suggest that BMP-4 and Shh act as positive regulators and FGF-2 as a negative regulator of ngn expression. Thus, these signaling molecules may regulate cell fate decisions of sensory neurons and glia. Furthermore, we focused on the roles of these factors in the differentiation of autonomic neurons. The expression of Mash1 and Phox2b was monitored to analyze the differentiation of autonomic neurons. The present data indicate that the signaling molecules and Notch signaling play important roles in development of mouse peripheral nervous system.

ROLES OF NOTCH SIGNALING IN CHONDROGENESIS OF MOUSE MESENCEPHALIC NEURAL CREST CELLS

○Kouichi Nakanishi, Kazuo Ito
Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Neural crest cells respond to various signaling molecules and differentiate into a wide variety of cell types. It has been shown that FGF2 promotes chondrogenesis of cranial neural crest cells. Furthermore, this signaling molecule activates Notch signaling in development of the nervous system. In the present study, therefore, we have examined the roles of Notch signaling in chondrogenesis of mouse mesencephalic neural crest cells. The activation of Notch signaling by the addition of a Notch ligand, Delta, promoted chondrogenesis in the neural crest cell cultures. Notch activation or FGF2 exposure during the first 24 hours in culture was indispensable for the induction of chondrogenesis. The expression of SOX9, a transcription activator of type 2 collagen which is a specific marker of chondrocytes, was also upregulated by FGF2 treatment, while BMP4 had no effects