*Exp. Anim.* 58(5), 459–469, 2009

**—Review—**
**Review Series: Animal Bioresource in Japan**

# *Ciona intestinalis* and *Oxycomanthus japonicus*, Representatives of Marine Invertebrates

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**Abstract:** The study of marine invertebrates is useful in various biological research fields. However, genetic analyses of these animals are limited, mainly due to difficulties in culturing them, and the genetic resources of marine invertebrates have not been organized. Recently, advances have been made in the study of two deuterostomes, an ascidian *Ciona intestinalis* and a feather star *Oxycomanthus japonicus*. The draft genome sequence of *Ciona intestinalis* has been determined, and its compact genome, which has less redundancy of genes compared with vertebrates, provides us with a useful experimental system for analyzing the functions of genes during development. The life cycle of *Ciona intestinalis* is approximately 2–3 months, and the genetic techniques including a perfect inland culture system, germline transformation with a transposon *Minos*, enhancer detection and insertional mutagenesis, have been established. The feather star *Oxycomanthus japonicus* conserves the characteristics of the basic echinoderm body plan with a segmented mesoderm, which is a fascinating characteristic for understanding the evolution of echinoderms. *Oxycomanthus japonicus* shows strong regeneration ability and is a suitable subject for analysis of the mechanisms of regeneration. In consideration of these features, the National BioResource Project (NBRP) has started to support the supply of wild-types, transgenic lines and inbred lines of *Ciona intestinalis* and *Oxycomanthus japonicus*.

**Key words:** ascidian, chordate, echinoderm, feather star

## Introduction

Marine invertebrates include most of the phyla of metazoans, and studying them is necessary to understand animal evolution and phylogeny. In addition, they include animal characteristics that are of interest in terms of physiology, development, cell biology, and reproductive biology. The study of marine invertebrates is promoting innovation and advances in various fields in biology. However, these advances have one limitation in that most of marine invertebrates are not suitable for use in genetic approaches, which are powerful ways of studying functions of genes. The culture of marine invertebrates requires specific conditions and is difficult to

*(Received 4 June 2009 / Accepted 17 August 2009)*
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carry out in inland laboratories. The life cycle of marine invertebrates is basically long, and cannot practically be subjected to genetic analyses, for which animals with shorter life cycles are favored. Because of the absence of inland sources and cultivating systems, the supply of marine invertebrates is dependent on wild populations with different genetic backgrounds. The growth of wild populations is affected by weather conditions that interrupt the constant supply of marine invertebrates, and the different genetic backgrounds of wild populations often limit the ability to perform sophisticated functional analyses of genes. The absence of an inland culture system is a critical limitation in the creation of genetically modified organisms (GMOs), because GMOs have to be isolated in laboratories; thus, a closed inland culture system is necessary for creating them. The discovery of marine invertebrates with a short life cycle and the establishment of cultivation and inland culture systems for them are necessary to solve these problems.

Recently, a cultivation system and inland culture of two marine invertebrates, a basal chordate *Ciona intestinalis* and an echinoderm *Oxycomanthus japonicus*, were reported [21, 52]. A constant supply of wild-type animals of these species has been achieved with these cultivation systems. As for *Ciona intestinalis*, methods of generating transgenic lines with transposons have been established with the aid of a perfect inland culture system [36, 37]. These technological innovations are helping the study of these organisms, and the culture, preservation and supply of these organisms are supported by the "National BioResource Project (NBRP)". In this review, we focus on the characteristics of *Ciona intestinalis* and *Oxycomanthus japonicus* as biological materials, the current status of their NBRP project, and useful studies involving them that have been carried out.

## *Ciona intestinalis* as Subjects in the Study of Genetic Functions

*Ciona intestinalis* is a kind of tunicate. Tunicates are included in the phylum of chordates with cephalochordates (amphioxus) and vertebrates. Recent genomic analyses have shown that tunicates are closer to vertebrates than amphioxus [12, 34]. Therefore, tunicates are regarded as the invertebrates that are closest to vertebrates (Fig. 1A). Tunicates consist of three groups, namely ascidians, thaliaceans, and appendicularians. *Ciona intestinalis* is a member of the ascidians group.

As the phylogenetic position suggests, ascidians share basic body plans with vertebrates [43]. The larvae of ascidians are typical tadpoles (Fig. 1B). They have a notochord in the tail and a hollowed neural tube at the dorsal side. Although ascidian larvae share a tadpole body plan with vertebrates, their bodies are much simpler than those of vertebrates. Some ascidian larvae consist of approximately 2,600 cells. The small number of cells in the body enables us to observe developmental processes at the cellular level. Ascidian larvae change their body structure drastically through metamorphosis. After metamorphosis, ascidians acquire an adult body plan and begin a sessile life style (Fig. 1C). Adult ascidians have a body structure that is superficially distinct from that of vertebrates (Fig. 1D). However, they share common characteristics with vertebrates such as gills at the pharynx and an endostyle, an organ homologous to the thyroid gland [29].

*Ciona intestinalis* (hereafter referred to as "*Ciona*") is used as a representative of ascidians in the study of genetic functions. It is supported by the availability of the draft genome sequence that was reported in 2002 [11]. The genome sequence allows us to obtain the sequence information of almost all genes including their *cis* regulatory sequences. A large amount of EST information is useful for determining the rough temporal expression pattern of the genes, and a set of cDNA is available from RIKEN BioResource Center DNA bank (http://www.brc.riken.jp/lab/dna/ja/) which eliminates the need for time-consuming cloning procedures [46, 50]. The latest version of the full ORF Gateway-compatible cDNA collection can presently be purchased as collective batch orders from the company Cogenics Europe (http://www.cogenics.com/). The *Ciona* genome size is approximately 160 Mbp per haploid, and the genome contains 15,852 protein-coding genes. The genome size and number of genes are similar to those of the protostome *Drosophila melanogaster*, and represent a much more compact genome than those found in vertebrates. In addition, the annotation of developmentally relevant genes in *Ciona* suggests that *Ciona* genes are

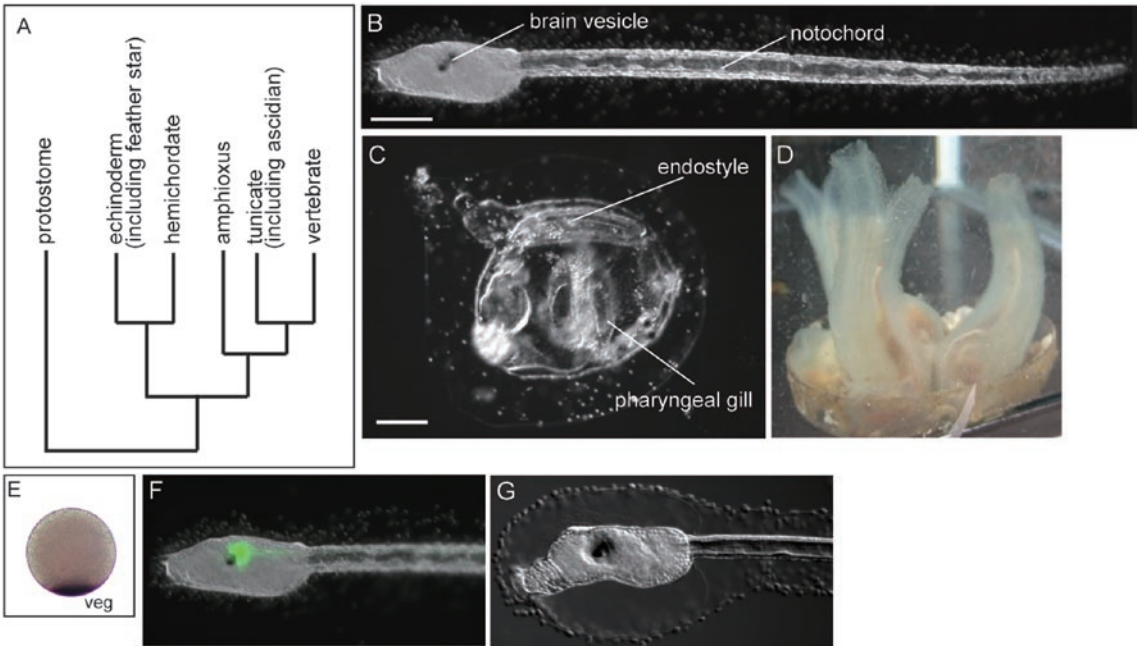


**Fig. 1.** An ascidian *Ciona intestinalis*. (A) Relationships among phyla of deuterostomes, showing phylogenetic position of feather star, ascidian and vertebrate. (B) A larva of *Ciona intestinalis*. Three photographs with different focuses were merged to construct this figure. Larvae of ascidians are typical tadpoles and have a notochord in the tail and a dorsal neural tube with a brain vesicle. Bar, 100 $\mu$m. (C) A *Ciona* after metamorphosis. Through metamorphosis, *Ciona* loses its tail and starts a sessile life style. A pharyngeal gill and endostyle are evident at this stage. Ascidians rotate their body axis during metamorphosis, and this animal is in the same orientation as in (B). Bar, 100 $\mu$m. (D) Adults of *Ciona intestinalis*. In our NBRP project, *Ciona* adults settled on Petri dishes are shipped. (E) Localization of *Ci-pem* mRNA at the vegetal pole (veg) of a fertilized egg, as revealed by whole-mount *in situ* hybridization (WISH). (F) A larva of the transgenic line (Tg[MiCiNutG]3) showing GFP expression in the central nervous system. (G) A *swimming juvenile* mutant larva, whose trunk underwent some metamorphic events, thus making it distinct from wild-type larvae.

less redundant than those of vertebrates [41, 42]. During the evolution of vertebrates from a common ancestor of chordates, the two-time occurrence of genomic duplication was estimated [31]. Therefore, the vertebrate genome contains several closely related genes that have similar functions. This is a disadvantage for analyses of genetic functions; disruption of a gene is sometimes compensated for by another related gene, and no phenotype will result. Genomes of ascidians did not experience genome-wide duplication; therefore the *Ciona* genome has a basic set of genes for the chordate body plan. The compact genome with less redundancy of genes suggests that mutations introduced into the genome are more frequently hit genes that generate mutants. This is an advantageous characteristic of *Ciona* for the purpose of genetic analyses. Techniques of molecular biology have been introduced in *Ciona*. Expression patterns of genes can be analyzed by whole-mount *in situ* hybridization (WISH) during embryogenesis and later developmental stages (Fig. 1E) [30, 49]. Knockdown and overexpression of genes of interest have been established in *Ciona* by microinjecting antisense morpholino oligonucleotides (MOs) and *in vitro*-synthesized mRNAs into eggs [17, 45]. The compact genome indicates that the *cis* regulatory element controlling gene expression is relatively small, and the short upstream sequences of genes isolated with a polymerase chain reaction (PCR) can mimic the expression pattern of endogenous genes when fused with reporter genes [6]. Such artificial DNAs can be introduced simultaneously into hundreds of embryos by electroporation. These techniques of developmental and molecular biology enhance functional analyses of genes. The genome sequence indicates the amino-acid sequences of proteins

consisting of a *Ciona* body. This information has been used to perform proteomic approaches in this organism [20]. For example, the proteins constituting sperm were analyzed using two-dimensional electrophoresis tagged with mass spectrometry, and the proteins showing changes during sperm activation were identified [16].

*Ciona* has another advantage for the use of genetic approaches in that its generation time is approximately 2–3 months. This generation time is similar to that of the zebrafish and *Arabidopsis thaliana*. This relatively short generation time enables us to culture *Ciona* in laboratories and to carry out genetic analyses. In fact, the culture of *Ciona* in a perfect inland system has been reported [21]. With the aid of this closed culture system, the generation of stable transgenic lines of *Ciona* has been established with a Tc1/*mariner* superfamily transposon *Minos* [36, 37]. The *Minos* system has been applied to sophisticated genetic techniques such as enhancer detection and insertional mutagenesis [3, 40]. These techniques are reviewed later.

## NBRP Supports the Breeding and Supply of Wild-Type and Stable Transgenic Lines of *Ciona*

*Ciona intestinalis* is a useful organism for understanding genetic functions in the basic chordate body plans with the established techniques described above. This ascidian is a cosmopolitan species and is most commonly used by researchers both in Japan and abroad. The NBRP project supports research in three ways. The first is the cultivation and supply of wild-type *Ciona*. This project is conducted mainly by Kyoto University. Wild-type *Ciona* eggs are fertilized and cultured until they reach the juvenile stage in plastic Petri dishes. Juveniles of *Ciona* are settled on the dish, and they are cultivated in the ocean at the Field Science Education and Research Center, Kyoto University, for 2–3 months. During this period they grow to the reproductive stage, and adult animals are shipped to researchers upon their request. Usually *Ciona* researchers use embryos; they collect eggs and sperm from the supplied animals, and the gametes are used for each experiment. *Ciona* adults have also been used as materials to study physiology, regeneration and biochemistry [7, 22]. In 2009, the Misaki Marine Biological Station of the University of Tokyo, began supporting part of this project. Detailed information is available at the website of NBRP (http://www.shigen.nig.ac.jp/marinebio/wild.jsp).

The second project is the collection, preservation and supply of transgenic lines and mutants of *Ciona* (Figs. 1F and 1G). This project is mainly conducted by the Shimoda Marine Research Center (SMRC), University of Tsukuba. With *Minos* transposons, many stable transgenic lines that express fluorescent proteins in specific tissues have been created [35, 40]. These lines are valuable markers for studying the development of these tissues. For example, marker transgenic lines expressing GFP in the major larval tissues, namely epidermis, notochord, muscle, and neural tissues, are useful for observing the differentiation of these tissues during early development (Fig. 1F) [21]. To share these useful lines, SMRC collects and cultures them in the inland culture system, and supplies them according to requests from researchers. Adults at the reproductive stage of these transgenic lines are usually available so that users' requests can be accepted quickly. Information on transgenic lines, including the expression pattern of fluorescent proteins, is available at the CITRES database website (http://marinebio.nbrp.jp/ciona). The transgenic line resource at NRBP is now the biggest *Ciona* resource center in the world. A method of cryopreservation of sperm has been designed for *Ciona*, and the sperm bank of the transgenic lines is under construction at Misaki Marine Biological Station in order to maintain the transgenic lines permanently.

The third project is the establishment of inbred lines. The wild population of *Ciona intestinalis* maintains many polymorphisms. The genome project showed approximately 1.2% polymorphism between haplotypes [11]. For refined genetic analyses, such a high frequency of polymorphism presents a problem, and the utilization of an inbred line is ideal to circumvent this disadvantage. Ascidians are generally hermaphrodites, and *Ciona intestinalis* is self-fertile. This is advantageous for creating inbred lines, because repeated progeny isolation by self-fertilization leads to the isolation of an inbred line much more quickly than in dioecious animals.

## *Ciona intestinalis* Is an Excellent Subject for Studying the Functions of Transcription Factors and Signaling Molecules during Development

Mechanisms of embryogenesis are central subjects of developmental biology. During embryogenesis, genes encoding transcriptional factors and signaling molecules play conductive roles in gene expression. It is necessary to understand the regulative mechanisms of gene expression conducted by such regulatory genes. For this purpose, the characterization of regulatory genes, such as their repertoire in the genome, expression patterns and functions, is required. Because *Ciona* has a compact genome with sequence information, the number of genes encoding regulatory genes is limited. Therefore, acquisition of the characteristics of all regulatory genes is possible.

The genome-wide annotations of genes encoding regulatory genes during development were reported in 2003 [15, 48], and that project constructed a catalogue of regulatory genes in the *Ciona* genome. By utilizing this catalogue, the expression patterns of these regulatory genes were described extensively with the WISH technique [18]. The results of that report are available from the Ghost database at Kyoto University (http://hoya.zool.kyoto-u.ac.jp/cgi-bin/gbrowse/ci).

Antisense techniques are useful for gene disruption. In *Ciona*, the microinjection of antisense morpholino oligonucleotide (MO) into embryos interferes with the translation of genes and results in disruption of function [45]. Knockdown of all regulatory genes showing conspicuous zygotic expression during early embryogenesis was conducted, and resulted in the construction of gene regulatory networks of analyzed regulatory genes [19]. The upstream and downstream regulation of regulatory genes can be visualized by the networks. The regulatory networks become a powerful tool for studying the functions of regulatory genes during embryogenesis together with their sequence information, cDNA/EST resources and expression pattern profiles. *Ciona intestinalis* is an excellent model organism for analyzing regulatory genes owing to the availability of information, resources, and established techniques.

## Germline Transformation, Enhancer Detection, and Insertional Mutagenesis of *Ciona intestinalis* with a Transposon

*Minos* is a kind of Tc1/*mariner* superfamily transposon [14]. This transposon has excision and transposition activity in *Ciona* [36]. Transposition of *Minos* occurs in the genome of *Ciona*, and germline transformation with this transposon has been achieved [21, 35, 37, 39]. The *Minos* transposon and transposase can be introduced by both the microinjection and electroporation methods [24]. Approximately 30–35% of *Minos*-introduced *Ciona* becomes a founder and transmits *Minos* insertions to progeny. *Minos* insertion sites can be identified by a simple PCR technique [23]. So far *Minos* is the only transposon in *Ciona* with which germline transformation has been achieved.

Enhancer detection is a useful application of transposons [32]. When a transposon vector with a minimal promoter and a reporter gene is inserted near an endogenous enhancer, the expression of the reporter gene is affected by the enhancer. This local effect is called enhancer detection or enhancer trap. In *Ciona*, this technique has been established with *Minos* and a promoter of a gene encoding thyroid peroxidase [2]. Because the *Ciona* genome is compact, enhancers are frequently present in the genome. Therefore, efficient enhancer detection can be carried out. In fact, a high-throughput method of enhancer detection has been established by utilizing transposon-donor lines and transgenic lines expressing transposase in the germ cells [38]. This method has an advantage in that new enhancer detection lines can be created through the crossing of transposon-donor and transposase-expressing lines; no time-consuming founder creation is required. With enhancer detection, various marker transgenic lines have been created, and these lines are maintained at NBRP in addition to the transposon-donor and transposase expressing lines.

Transposons cause gene disruption when they are inserted into critical regions of genes, such as exons and the regulatory elements of transcription. Insertional mutagenesis with transposons has an advantage over chemical mutagens in that the mutated sites can easily be identified by using transposon sequences as tags of

mutations. In *Ciona*, an insertional mutant has been isolated with *Minos* in which a gene encoding cellulose synthase is disrupted [39]. This mutant, *swimming juvenile* (*sj*), shows defects in cellulose synthesis as well as metamorphosis (Fig. 1G). This mutant is a useful resource for investigating the mechanisms of animal cellulose biosynthesis and the mechanisms of the metamorphosis of ascidians. The *sj* mutant has been provided to NBRP and is now available.

Wild populations of *Ciona intestinalis* frequently contain spontaneous mutants, and these are useful sources for mutant screening [53]. Recently, a mutant named *tail regression failed* (*trf*), that shows defects during metamorphosis, was isolated [28]. Analyses of *trf* and *sj* mutants revealed that ascidian metamorphic events can be classified into four groups in which initiation is conducted by different pathways [28]. The mutants that show defects during metamorphosis are valuable for analyzing the mechanisms of ascidian metamorphosis. The *trf* mutant is available from NBRP.

## The Feather Star, *Oxycomanthus japonicus*, Is a New Model for Studying Marine Invertebrates

Echinoderms are placed in the evolutionary tree as a member of the deuterostomes, the group of animals that lead to the vertebrates, as *Ciona intestinalis* does, but lack the chordate. They are exclusively marine invertebrates with a wide range of habitats and show distinctive characters in their morphology. As the name indicates, they are spiny-skinned, having mesodermally derived calcareous ossicles that make up their skeletons. They employ a unique water vascular system consisting of radial canals which form a network, used for respiration, feeding, locomotion etc. The body plan and development of echinoderms are also interesting: larvae showing bilateral symmetry transform into adult whose body is basically radial with pentamerous symmetry characterized by five or more radiating areas. Because of these characteristics, echinoderms have been an attractive group of animals for evolutionary and embryological research. They have been used for other areas of research as well, for example, cyclin was first identified using sea urchins [13] and Mechnikov first demonstrated phagocytosis in sea stars for which he was awarded the Nobel Prize in 1908. Thus, echinoderms have contributed to our understanding of many fundamental biological processes.

Sea urchins, sea stars, and sea cucumbers have been studied as representative echinoderms because of their high availability and ease of maintaining in the laboratory. Nevertheless, crinoids are potentially important model organisms for research.

Among the extant echinoderms, crinoids constitute the earliest-branching class. Crinoids show physical characteristics somewhat different from the other echinoderms. They are generally divided into two groups, the stalked and stalkless crinoids. From rich fossil records, it is known that the first crinoids were stalked, originated in the Ordovician, about 450 million years ago, and flourished during the Paleozoic era. The feather star, *Oxycomanthus japonicus*, is shown in Fig. 2. Comatulids lose the stalk during development, and stick to the substratum with a cluster of appendages called cirri that replace the stalk. The mouth is in the upward position, which differs from other echinoderms, and a difference in the nervous system is also observed. While echinoderms in general have a poorly developed nervous system and lack a central nervous system, crinoids possess a comparatively well-developed aboral nerve system and a ganglion where there are particularly numerous nerve cells. It is unknown whether the ganglion corresponds to the brain in other metazoans, but crinoids fail to swim with coordinated beating movements of their arms when this structure is disrupted.

Since most crinoids are found in the deep sea, they are the least well-known of the echinoderms. In fact, the difficulty of collection and maintenance of crinoids has been an obstacle to their use in research. For example, the sea lily *Metacrinus rotundus*, had to be collected by fishnet from a depth of 100–150 m in Suruga Bay, Japan, to elucidate its larval development [27]. In comparison, feather stars are more accessible, since there are those that live in shallower waters. The feather star *O. japonicus* inhabits shallow rocky seashores around Japan, and can be collected by scuba divers near the Misaki Marine Biological Station (MMBS), the University of Tokyo, situated at the tip of the Miura Peninsula facing Sagami Bay, at around 5 m depth [52]. Such wild specimens are maintained in cages hung at a depth of 3 m in the cove of the MMBS.

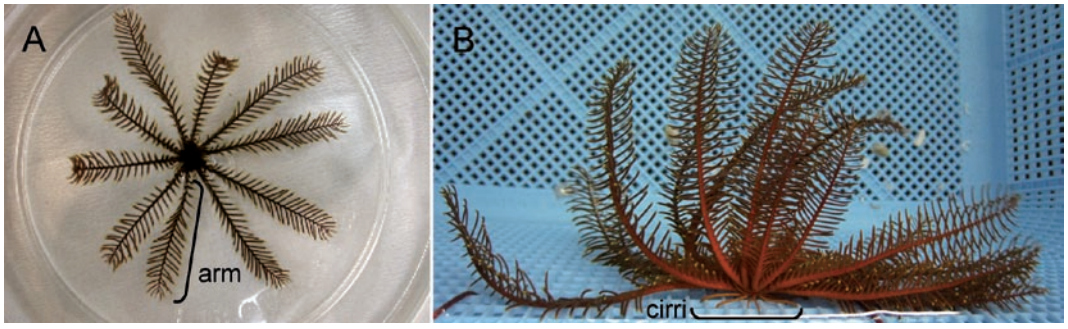


**Fig. 2.** The feather star, *Oxycomanthus japonicus*. (A) A young feather star observed from above. This individual has 10 arms, showing the pentameral symmetry of the basic body plan. The arms grow out from the central part of the body and the mouth is positioned upward at the center. The small branches on the arms are called pinnules. The arms are about 3 cm in length. (B) A young adult feather star observed from the side. The number of arms increase during growth. The cirri are seen at the base of the body. The arms are about 5 cm in length.

## *O. japonicus* as a Model Species for Developmental and Regenerative Studies

Though the spawning season of most crinoids is not well known, for *O. japonicus*, information has been accumulating since 1937 [9, 10]. Their early development was also reported [8]. These studies also used the specimens from the MMBS. Recently, Shibata *et al*. reported that sexually mature females and males captured at MMBS spawn naturally on a neap-tide day in middle to late October [52]. Eggs and sperm were collected and fertilized eggs were reared into larvae, from which juveniles were obtained. They could be further reared until sexual maturity [52]. Thus, the complete cultivation method for *O. japonicus* on a large scale, from larvae up to sexually mature adults, has been established. In addition, a large number of embryos are collected and fixed for use around the year. The availability of larvae allows *O. japonicus* to serve as a model animal for developmental studies.

Crinoids are known to possess a strong capacity for regeneration, and have been studied using a variety of crinoids, such as the sea lily *M. rotundus* [1, 26] and the comatulid *Antedon mediterranea* [5]. Comatulids regenerate most of their organs and many studies have focused on arm regeneration [5]. Regeneration is dependent on the nervous system and goes through three main phases: a repair phase, an early regenerative phase and an advanced regenerative phase [5]. The regeneration of an arm of *O. japonicus* takes a month. For this short term, maintenance is not difficult, and individuals may be kept in an aquarium in the laboratory, in seawater.

Taking advantage of the cultivation system/method of *O. japonicus* in MMBS, the hypothesis that the increase in arm number during growth of comatulids is caused by autotomy of one arm, followed by regeneration of two arms, was proven [51]. This arm autotomy and regeneration is a natural ontogenetic process.

## Ongoing Analyses of *O. japonicus* at the Molecular Level

Until now most analysis on comatulid regeneration or any other biological phenomenon has been done at the anatomical or histological level. Only a modest number of crinoid genes have been registered in the Genbank database, including *Anbmp2/4*, a member of the transforming growth factor-b of *Antedon bifida* [33].

In order to promote studies of crinoids at the molecular level, cDNA or cDNA libraries of embryos and adult tissue such as the ganglion-like structure have been constructed from *O. japonicus*. From these resources, genes involved in anterior-posterior (A-P) patterning of embryos, for example, *Otx*, *Pax6* etc. have been cloned, and their expression during development has been analyzed by *in situ* hybridization (Omori, in preparation). For expression analyses during embryogenesis, fixed and

stored embryos were used.

Since echinoderms show characteristic body plans, expression of *Hox* genes, which are considered an indicator of the body axis, is of great interest. The structure of the sea urchin *Hox* cluster has been elucidated and compared to the *Hox* gene cluster of vertebrates or *Drosophila*, missing members and translocation and/or inversion of some of the genes were found [4]. To elucidate if this unprecedented order of *Hox* genes is a common trait of echinoderms or if it underlines the individual unique body plans observed in echinoderms, and to look at evolution of the body plan through the analysis of *Hox* genes, studies are currently being conducted with *O. japonicus*. Short sequences corresponding to the homeobox domains have been published [25], and using this information the full lengths of some of the *Hox* genes have been cloned and their expression during embryogenesis is being analyzed by RT-PCR (Tsurugaya, personal communication). The results show a difference in expression of the *Hox* gene orthologs of the sea urchin and *O. japonicus* during embryogenesis. To reveal the genomic organization of *Hox* genes, genomic BAC libraries constructed from sperm DNA are being screened for clones containing *Hox* genes and the clones are being analyzed. Preliminary results show, as expected, linkage of several *Hox* genes (Tsurugaya, personal communication), and it would be interesting to know the relationship between spatial expression and gene order.

## NBRP Support for Research through Distribution of *O. japonicus* Material

Although comatulids are a very interesting group of animals to study, access to this animal has been very limited due to a lack of breeding methods. The NBRP for *O. japonicus* based in MMBS supports researchers by providing all sorts of materials. Adults are constantly kept and maintained, and thus may be provided as live specimens. Because embryos can only be obtained during a certain period of the year, embryos may be provided as live material when available, but fixed material, which we have confirmed can be used for RNA expression analyses, may be provided round the year. RNA, cDNA and genomic DNA, either unprocessed or in the form of a library are also distributed upon request. Recently, a microarray of EST from the ganglion has been generated, and analyses are awaited. We also developed a method of introducing DNA/RNA into fertilized eggs, which would be useful for analyzing cis-regulatory elements and the function of the genes (Kurokawa, personal communication). Comatulids should be able to expand our knowledge of echinoderms and deuterostomes from its position in evolution and phylogeny. The NBRP aims to shed light on *O. japonicus* and encourages researchers to use this animal. Detailed contact information is available at the website of MMBS, the University of Tokyo (http://www.mmbs.s.u-tokyo.ac.jp/index-e.html).

## Conclusions

*Ciona intestinalis* and *Oxycomanthus japonicus* provide excellent experimental systems for studying the mechanisms of development, genetic functions, regeneration, and evolution of deuterostomes. Such studies are based on established techniques of molecular biology, physiology, biochemistry, embryology, and genetics in these organisms. To support research on these two species, our NBRP project collects, preserves and provides wild-type, transgenic and inbred lines of both species. Currently, NBRP possesses the most well-developed *Ciona* and *Oxycomanthus* resources in both quality and quantity in the world. Presently, our NBRP project aims to make the resources more sophisticated by adding more useful lines and refining the information databases. To achieve this, utilization of the resources and feedback from users are necessary and are always welcomed.

## Acknowledgment(s)

The authors would like to thank Yutaka Satou, Kazuko Hirayama, Yasuyo Kasuga, Yoko Kitagawa, Yasutaka Tsuchiya, Toshihiko Sato, Hideo Shinagawa, Takeo Horie, Ryoko Shinki, Narudo Kawai, Reiko Yoshida, Kaoru Mita, Yosuke Ogura, Takako Iitsuka, Yoshiko Harada, Hiromi Takahashi, Ute Rothbacher, and Patrick Lemaire for their kind cooperation with this review. We would also like to thank Drs. Tomoko F. Shibata and

Daisuke Kurokawa, along with Akihito Omori and Toko Tsurugaya, of the Misaki Marine Biological Station, the University of Tokyo. We thank all members of the Maizuru Fishery Research Station of Kyoto University, the International Coast Research Center of the Ocean Research Institute of the University of Tokyo, and the Education and Research Center of Marine Bioresources of Tohoku University, and Dr. Shigeki Fujiwara for the collection of *Ciona* adults. This study was further supported by grants from the National BioResource Project.

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