

# Euro Chordates: Ascidian Community Swims Ahead. The 4th International Tunicate Meeting in Villefranche sur Mer

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A cohort of 150 biologists from 14 countries working with ascidians and appendicularians gathered on the French Riviera last June to enjoy good science and the perfect azure weather. At a time when molecular phylogeny studies tout ascidians and appendicularians as “our closest chordate ancestors,” *Ciona*, *Botryllus*, and *Oikopleura* are emerging as model systems with a growing number of research aficionados. Tunicates are particularly attractive models to study the “mosaic” type of development based on localized determinants and the differentiation of a small set of founder cells to form the seven tissues in a simple tadpole made of 2,500 cells whose lineages are well described. These models have benefited from the exploration and exploitation of their non-duplicated genomes (approximately 16,000 genes) and the increased availability of effective tools (transgenics by electroporation, knockdown by antisense oligos, microarrays, micromanipulations, and so on), and remarkable databases (see below). This

progress is due to the collaborative efforts of a dynamic worldwide scientific community numbering in the 500s, including many talented young biologists who will have a decisive effect on the future of urochordate model systems. In fact, Bob Goldstein (UNC Chapel Hill), the invited “outsider” plenary speaker, reminded the participants that experiments on cell fate specifications in the *Caenorhabditis elegans* published in the 1980s were directly modeled after classic ascidians fate-specification experiments. He also pointed out that this is a crucial time for the *Ciona* model, which is presently at a stage similar to that of *C. elegans* worm model in the early 1990s, that is just before siRNA gene interference was discovered, leading to an explosive growth in worm publications.

The large Japanese contingent who came to Villefranche sur Mer was a mixture of major contributors, including Nori Satoh who reflected on “30 years of ascidian research” and many young investigators who recently

joined a collective effort to contribute to a “Special Focus on *Ciona*” published at the time of the meeting (Satoh, 2007). Active American laboratories who have championed the ascidian model (William Jeffery from University of Maryland, Mike Levine and Bill Smith’s labs from University of California, Billie Swalla from University of Washington, and Arendt Sidow and Tony De Tomaso from Stanford University) were also well represented and led lively discussions about transgenics, ascidian culture facilities, the regulation of gene networks, the comparative use of different solitary or colonial ascidian species and the evolution of chordates. The biggest contingent came from all over Europe where in the past decade laboratories close to European coasts (Villefranche sur Mer, Marseille, Naples, Padova, Bergen, Plymouth, . . .) and more recently inland (Gif sur Yvette, Paris, Cambridge, . . .) have amplified a European tradition of studying ascidian embryos since Alexandre Kowalevsky (1866) described the chor-

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date tadpole larvae. Nearly 20 years later, Chabry (1887) performed the first embryological experiment on an ascidian embryo and in the 1940s, the Italian monk Reverberi, and his collaborators Minganti, and Ortolani pursued this line of work (Chabry, 1887; Reverberi and Minganti, 1946).

Studying these amazing embryos and larvae is still a dynamic and fruitful research endeavor. Tunicate researchers are already looking forward to the next Tunicate meetings in Japan.

### CELL BIOLOGY OF FERTILIZATION AND CORTICAL-CYTOPLASMIC REORGANIZATIONS OF EGGS

Ascidians are hermaphrodites, releasing sperms and eggs simultaneously, but many species exhibit self-sterility. This finding has been a long-standing enigma since T.H. Morgan (1923) investigated the issue using *Ciona* in Naples, Italy. The molecular basis of self-sterility is being tackled by Hitoshi Sawada (Nagoya University) who reported that self/nonself-recognition involves the polymorphic sperm receptor VC70 protein on the vitelline coat of *Halocynthia roretzi* oocytes and a binding partner for VC70, Urabin-1, a sperm membrane protein (Harada and Sawada, 2007). Yoshito Harada (Nagoya University) approached this issue in *Ciona*, taking advantage of its short life cycle (2–3 months) and sequenced genome. He presented evidence that self-recognition is genetically controlled, and the identification of the responsible genes is now anticipated.

Between fertilization and first cleavage, ascidian eggs show two main phases of “ooplasmic segregation”—cytoplasmic and cortical reorganizations—which polarize the developmental determinants (Sardet et al., 2007). Christian Sardet (CNRS / UPMC, Villefranche) detailed the cytoskeleton-driven movements that polarize the many cortically localized maternal RNAs (*postplasmic/PEM* RNAs). Many of these RNAs (including the muscle determinant *macho-1*; Nishida and Sawada, 2001) are attached to a network of cortical endoplasmic reticulum (cER) forming the

cER-mRNA domain, others, such as *Vasa*, are localized into granules. The mRNAs move with the cER to segregate to the future posterior pole (Prodon et al., 2007). Twenty-three online videos show the various phases of cytoplasmic and cortical rearrangements on the BioMarCell Web site (see the list of Web sites at end of manuscript). It is presumed that translation of these developmentally important *postplasmic/PEM* RNAs will be under strict spatial and temporal control. Alexandre Paix from the Villefranche lab provided evidence that specific phosphoproteins that are known regulators of translation initiation are localized on the cER-mRNA domain, suggesting a mechanism for translational control of *postplasmic/PEM* RNAs in the cortex.

### MATERNAL DETERMINANTS AND EARLY CLEAVAGES

A primary animal–vegetal axis preexists in ascidians eggs before fertilization. The cER-mRNA domain and the mitochondria-rich myoplasm domain form a 10-micron-thick layer in the periphery of the GV-stage oocyte. The peripheral domains polarize during oocyte maturation and establish a gradient distribution, forming a cup-shaped layer situated in the vegetal hemisphere. Using imaging of fixed and live *Halocynthia* maturing oocytes François Prodon (Osaka University) has analyzed microfilament-driven cortical and cytoplasmic flows, which polarize the cER-mRNA and myoplasm domains along the animal–vegetal axis (Prodon et al., 2008).

Additional maternal determinants are present in the periphery of the vegetal region of the eggs. During cleavage stages, in ascidians, as in sea urchins, nuclear accumulation of  $\beta$ -catenin, which is required for specification of the vegetal hemisphere, starts in the vegetal blastomeres (Imai et al., 2000). Ute Rothbächer (CNRS, University of Marseilles) demonstrated that expression of a reporter gene in the vegetal cells from the 16-cell stage was driven by binding sites of TCF, a downstream transcription factor of  $\beta$ -catenin. She also analyzed regulatory sequences of the FOG (Friend Of GATA) gene, which is

expressed in the animal hemisphere, and found that suppression of GATAa activity by  $\beta$ -catenin signaling was essential for proper specification of the animal hemisphere, although GATAa mRNA is present maternally and ubiquitously (Rothbächer et al., 2007).

Positioning of cleavage planes is crucial for stereotypic partitioning of cortical and cytoplasmic determinants localized in the egg, the adequate spatial arrangement of interacting blastomeres, as well as the size control of blastomeres. Remarkable progress has been made in France and Japan in modeling *Ciona* embryos, including surface contacts and interactions between blastomeres as they cleave and differentiate. (See ANISEED the great database assembled by the Lemaire lab [Tassy et al., 2006] and FABA, the Web-based interactive developmental table for the ascidian *Ciona intestinalis* from Kohji Hotta at Keio University [Hotta et al., 2007]).

In ascidian embryos, three rounds of cleavages result in smaller blastomeres formed at the posterior pole from the 8- to 64-cell stage (Nishida, 2005; Sardet et al., 2007). Before each unequal cleavage, a posterior centrosome in the posterior-most blastomere is attracted toward the centrosome-attracting body (CAB), a macroscopic subcellular structure in which the cER-mRNA domain and germ plasm granules are highly compacted. Takefumi Negishi (Osaka University) presented evidence that the PEM (Posterior-End Mark) protein, the product of the most abundant *postplasmic/PEM* RNA, is required for the unequal cleavages because its knockdown by means of morpholino injections results in equal cleavages and perturbation of the formation of a microtubule bundle, which connects the centrosome and the CAB (Negishi et al., 2007). Gaku Kumano (Osaka University) reported that *macho-1* and  $\beta$ -catenin work in collaboration with PEM for generation of unequal cleavages at the posterior pole.

### CELLULAR AND MOLECULAR BASIS OF EARLY CELL FATE SPECIFICATION

In addition to partitioning of maternal determinants, the stereotyped cleav-

ages of the ascidian embryos generate multiple inductive interactions between blastomeres. In ascidians, asymmetric cell division of mesenchyme/muscle and notochord/nerve cord precursor blastomeres are induced by extracellular FGF9 signal (*Fgf9/16/20* gene product) in the posterior and anterior marginal zones, respectively. Each asymmetric division separates the two kinds of cell fates and restricts the daughter blastomere fates to give rise to a single tissue. In each asymmetric division, the induced fates are mesenchyme and notochord, and the default fates are muscle and nerve cord, respectively (Nishida, 2005). Gil Jung Kim (Kangneung University) examined how mother cells are polarized by the extracellular signals. Using micromanipulation such as blastomere transplantation combined with knockdown and misexpression of FGF in identified blastomeres of *Halocynthia*, he presented evidence that the polarity of the asymmetric division of mesenchyme/muscle mother precursor is determined solely by the direction from which the FGF signal is presented (namely from vegetal endoderm blastomeres). In contrast, polarity of notochord/nerve cord precursor is determined by possible antagonistic action between the FGF signal and a signal from anterior ectoderm. The ectoderm signal suppresses MAPK activation in the nerve cord lineage (Kim et al., 2007). Vincent Picco in the Yasuo lab (CNRS/UPMC, Villefranche) independently reached the same conclusion in *Ciona* and identified the ectoderm signal as Ephrin, a membrane-anchored signal molecule (Picco et al., 2007). It is remarkable that near identical mechanisms at the level of—polarization of oocytes and embryos—patterns and role of cleavages—specification of tissue progenitors—are operating in solitary ascidian species (*Halocynthia*, *Ciona*) that diverged millions of years ago.

The specification of the cardiac mesoderm begins with the induction of *Mesp* expression in the posterior vegetal B7.5 blastomeres of 110-cell embryos. Brad Davidson (University of Arizona, Tucson) presented evidence that this induction depends on a localized FGF9 signal. Once activated, *Mesp* triggers the expression of *EtsP2*, which encodes a transcriptional effec-

tor of FGF signaling (Davidson et al., 2006). Although expressed in both the anterior and posterior descendants of B7.5 blastomere, *EtsP2* is active only in the anterior cells where it induces the expression of *FoxF* (Beh et al., 2007). The localized expression of *FoxF* is critical for the directed migration of the heart precursor cells from anterior regions of the tail into posterior–ventral regions of the trunk. Understanding the basis for the specification of distinct anterior and posterior B7.5 lineages is an interesting problem in asymmetric cell divisions. How does the *FoxF* transcription factor control cell migration? Lionel Christiaen (UC Berkeley) described the use of cell-specific gene disruptions, fluorescent-activated cell sorting (FACS), and comprehensive microarray screens for the identification of *FoxF* target genes required for migration. Christiaen presented evidence that most of the genes required for the different phases of cell migration—polarity, protrusions, chemotaxis, adhesion—are constitutively expressed in the heart cells before and after migration. Just a small set of critical effector genes are activated by the *Mesp-FoxF* heart specification network, including two genes implicated in the formation of membrane protrusions (*RhoD/F* and *Fascin*).

### NOTOCHORD MORPHOGENESIS: CONVERGENCE AND EXTENSION

The notochord is a unifying character of the phylum Chordata and plays a major role in the organization of the body plan of all the animals present in the phylum. During early vertebrate development, in addition to providing structural support to the body, the notochord patterns the neural tube by inducing its ventral-most region, the floor plate, and influences the formation of the paraxial mesoderm and its derivatives (Halpern et al., 1993), as well as the formation of heart and blood vessels (Reese et al., 2004). Also the development of organs of endodermal origin, such as liver and pancreas, are profoundly affected by the notochord in vertebrates (Cleaver and Krieg, 2001).

During the morphogenesis of the

notochord in solitary ascidian embryos, 40 notochord cells undergo mediolateral intercalation to transform the notochord plate into a single row of cells. The single *Ciona* FGFR receptor is strongly expressed in the notochord during and after the intercalary stage (Keys et al., 2002). Weiyang Shi (UC Berkeley) showed by morpholino knock down and by ectopic expression of FGF3 (*Fgf3/7/10/22* gene product) the failure of notochord intercalation and the occurrence of animals with shortened tails, demonstrating that during late neurula stage, the FGF3 ligand is emitted from the ventral nerve cord and signals to the underlying notochord plate, slightly biasing the notochord cells to intercalate along the mediolateral axis, resulting in eventual mediolateral convergence of the notochord plate. In ascidians, *brachyury* is specifically expressed in notochord lineage after the 64-cell stage. To understand the gene regulation in the differentiating notochord, Anna Di Gregorio (Cornell Medical School, NY, NY) undertook the comprehensive identification of *Ci-Bra* target genes and their associated *cis*-regulatory sequences. Several of these target enhancers were shown to contain specific arrangements of T-box (*Ci-Bra*) recognition sequences. This analysis offers the promise of elucidating a complete gene regulatory network for notochord differentiation and morphogenesis.

### PATTERNING THE NERVOUS SYSTEM

Ascidian tadpoles have a simple central nervous system (CNS), made of approximately 100 neurons of approximately 350 neuronal cells (Nicol and Meinertzhagen, 1991). Despite its simplicity, the neural tube of ascidians appears remarkably similar to its vertebrate counterparts, with broadly conserved gene expression profiles along the anterior–posterior and dorsal–ventral axis.

Clare Hudson (CNRS/UPMC, Villefranche) demonstrated the importance of extracellular signaling molecules for early neural plate patterning and, in particular, the essential and combinatorial roles of Nodal, Delta/Notch, and FGF/ERK signaling pathways. After asymmetric division of the

four notochord/nerve cord precursors, the nerve cord precursors align in a single row at the anterior edge of the vegetal hemisphere. These cells give rise to the posterior neural tube. They first divide along the medial–lateral axis to give rise to a single row of 8 cells and then along the anterior–posterior axis to give rise to 2 rows  $\times$  8 columns of cells. Each of these 16 cells expresses a unique combination of neural genes that can be used to distinguish each cell identity. The lateral cells, columns 3 and 4, acquire their identity by means of a Nodal signal coming from lateral blastomeres outside of the neural plate. Then columns 2 and 4 are specified by a Delta/Notch signal. Finally, FGF/ERK signaling distinguishes row I and row II cells. Thus, every cell in the posterior neural plate is individualized by the combination of three extracellular signaling pathways, a demonstration that, in ascidians neural patterning can be investigated at a single cell level (Hudson, et al., 2007).

The anterior part of the neural tube develops into a simplified brain (or sensory vesicle). Dr. Steven Irvine, a new lab in the ascidian field from the University of Rhode Island, showed that *Pax-6* is turned on in the fore-brain, as in other vertebrates, and that there are separate enhancer elements driving expression in the fore-brain and in the ocellus, the light sensing organ. Pierre Khoueiry (CNRS, University of Marseilles) used a bioinformatic approach to find the targets of FGF9, the brain inducer in ascidian. The *otx* gene is one of the targets whose expression is driven by GATA and ETS binding sites (Bertrand et al., 2003). Khoueiry and the Lemaire lab developed a program to search for syntactically constrained clusters of transcription factor binding sites in noncoding regions of *Ciona intestinalis* genome. They identified 13,000 clusters containing two GATA and two ETS sites. Among them, 42 clusters were also conserved in the genome of *Ciona savignyi*. An initial set of 7 clusters was tested to see whether they could drive the expression of a reporter gene in the neural precursors. The fact that two of them were positive suggests that this strategy may be also used for identification of

regulatory elements of other transcription factors.

### METAMORPHOSIS TO SESSILE FILTER FEEDING ADULTS AND COLONIES

The ascidian tadpole is a nonfeeding swimming life form, which settles on substrates and adheres with papilla at the anterior end. It metamorphoses into a sessile juvenile with chordate-type organs and secretes a tunic with siphons to filter feed. Jean Philippe Chambon (CNRS / UPMC, Villefranche) described the nervous signals and the role of ERK and JNK pathways responsible for triggering a wave of apoptosis starting at the tip of the tadpole tail. He also summarized the efforts made with the Satoh lab to understand the gene networks involved using microarrays (Chambon et al., 2007). Perhaps the most remarkable discovery reported at the meeting came from Yasunori Sasakura's laboratory (Shimoda Marine Research Center). They described extraordinary cases of metamorphosis in *Ciona* mutants called *sj* (swimming juvenile; Sasakura et al., 2005; Sasakura, 2007) and *trf* (tail regression failed). Although these mutants show normal juvenile morphology, they do not start papilla and tail retraction or sensory vesicle breakdown, yet still initiate stalk formation, body axis rotation, and adult organ formation. The mutant develops into a juvenile-like trunk structure propelled by a normal tadpole tail. "This recalls the morphology of appendicularians" exclaimed Nori Satoh (Kyoto University), and it may be that the natural occurrence of such metamorphosis mutants could be key to the evolution of new life forms.

Adults of ascidians have regenerative potential. William Jeffery (University of Maryland) building on classic studies of pigment regeneration in siphons showed the importance of nerve signals and FGF and Hedgehog signaling in pigment organ regeneration, suggesting similarities with vertebrate limb regeneration. Yuval Rinkevich (Technion, Haifa) presented an analysis of how colonial zooids of *Botryllus leachi* regenerate the whole body from circulating blood stem cells (Rinkevich et al., 2007).

The cosmopolitan colonial ascidian

*Botryllus schlosseri* is an attractive model for studying histocompatibility or allorecognition (De Tomaso et al., 2005). Because RNAi works in *Botryllus*, this colonial organism "easy to find in the field, rear in the laboratory and to reproduce both sexually and asexually" may become a remarkable model to understand development, evolution, colonial organization, and ecological adaptations. *Botryllus* was the center of attention of an international community that gathered for 2 more days after the Villefranche Meeting at the nearby Italian Riviera where active groups studying colonial ascidians are located in University of Padova.

### THE APPENDICULARIAN OIKOPLEURA DIOICA: AN ATTRACTIVE MODEL ANIMAL

Appendicularians, also called larvaceans, are planktonic tunicates/urochordates that retain a tadpole shape throughout their life and live in a floating house they secrete. *Oikopleura dioica* is characterized by a miniature genome (70 Mb) and short life cycles (5 days). It can be maintained in the laboratory over many generations (Seo et al., 2001). These advantages make this organism a suitable experimental model animal that has shown its utility in studying epidermis pattern formation that leads to secretion from patterned polyploid epidermal cells of intricate net/house. They are a favorable model to study ecological adaptations to a varying amount of resources by modulating their fecundity. Phillippe Ganot (Sars, Bergen) described a novel mode of oogenesis in this organism where the entire germline is contained in a multinucleated germ cyst, called the coenocyst. In the coenocyst, half of the nuclei are specified to give rise to nurse cells and the other half remain meiotic nuclei. Then, depending on resources, a smaller or larger number of the meiotic nuclei are eventually selected to be oocytes. The cytoplasm of the coenocyst is introduced through a ring canal into the growing oocyte, whose maturation requires the MAPK pathway (Ganot et al., 2007).

Appendicularian embryogenesis is amazingly fast and tadpoles are ready

to hatch in 3 hr. Cristian Canestro (University of Oregon) investigated the role of retinoic acid (RA) in body patterning along the anterior–posterior (A-P) axis. RA is thought to be an innovation essential for the A-P organization of the chordate body plan. However, the *Oikopleura* genome lacks genes for RA synthesis, degradation, and reception. Using pharmacological inhibitors of RA, and regional marker genes along the A-P axis, he demonstrated that RA does not cause homeotic posteriorization (Canestro and Postlethwait, 2007). A comparison of *Oikopleura* and ascidians suggests that the lack of RA response is a shared and derived feature of tunicates. Similarly, *Oikopleura* and ascidians lack several members of the *Hox* clusters. This finding suggests that at least some chordates evolved a basic chordate body plan (namely, the tadpole-shaped larvae) without RA and full complement of *Hox* cluster.

Daniel Chourrout (Sars, Bergen) provided an overview of the current state of the larvacean *Oikopleura dioica* genome project in collaboration with the Genoscope (Paris). This compact genome has now been sequenced at 14-fold coverage and 140,000 ESTs have been generated toward genome annotation ([http://www.genoscope.cns.fr/externe/Francais/Projets/Projet\\_HG/](http://www.genoscope.cns.fr/externe/Francais/Projets/Projet_HG/)). Work on embryonic fate mapping is ongoing in both the Chourrout lab and that of Nishida (Osaka University). Semiautomation of the *Oikopleura* culture system has been attained in Bergen, and the Nishida lab has developed successful culture in defined artificial seawater.

## TAXONOMY AND PHYLOGENY OF TUNICATES

Most tunicate biologists have embraced the new concatenated phylogenies published in 2006 that show tunicates as a sister group to vertebrates (Bourlat et al., 2006; Delsuc et al., 2006) with cephalochordates being the more basal chordate, but this phylogeny also suggests that the tunicate tadpole larvae is derived, not ancestral, as presented by Billie Swalla (University of Washington; Swalla, 2006). There are still several groups of tunicates that are difficult to place

phylogenetically, especially the aplousbranch ascidians, which are actively being studied as shown by several talks in the Ecology and Evolution session. The 18S rDNA data still make it difficult to place this group (Georgia Tsagkogeorga, University of Montpellier). Tunicate taxonomists are still an important and vital part of the tunicate field, as shown by the talk by Maria de las Mercedes Varela (University Al Cristo de la Paz, Spain) on the rich number of species (67 species in 10 families) discovered in the Antarctic benthic tunicates. Although the fascinating planktonic appendicularians including *Oikopleura dioica* are clearly tunicates, the rearrangements and fast evolution of their genomes also makes them difficult to place phylogenetically within the tunicates. As more information becomes available and is analyzed in different ways, we hope to be able to resolve these uncertainties in tunicate phylogenies.

## GENOMICS, PROTEOMICS, AND GENETICS: TECHNOLOGICAL INNOVATIONS

In the session “Emerging approaches: from genetics to proteomics,” speakers from Europe, United States, and Japan described how the field benefited from the draft genome sequences of *Ciona intestinalis* and *C. savignyi* (Dehal et al., 2002). With the aid of genome sequences and easy way to analyze *cis* regulatory sequences by electroporation, *Ciona* provides a fascinating experimental system for studying transcriptional regulations. The genome project has shown that the *Ciona* genome is highly polymorphic, which suggests that highly conserved sequences or “motifs” in the *cis* elements may be critical for transcriptional regulations (Brown et al., 2007), in line with Kimura’s neutral theory. Arend Sidow’s group (Stanford University) performed detailed comparisons of polymorphisms in regulatory sequences of six *C. savignyi* genes, to show the relationships between conservation and functional importance of these motifs.

Recent studies of the *Ciona* transcriptome detail the peculiar characteristics of posttranscriptional regula-

tion and the occurrence of *trans*-splicing (Vandenbergh et al., 2001). Kenneth Hastings (McGill University) has identified 7,437 *trans*-spliced genes using 454 pyrosequencing technology and classified them in several categories. *Trans*-splicing has been described in several organisms, but tunicates are, for the moment, the only deuterostomes known to perform *trans*-splicing a process whose function remains enigmatic. The availability of large amounts of synchronous embryos and the completion of the genome project of *Ciona intestinalis* has stimulated a proteomic approach of dynamic changes triggered during fertilization and early development. Kazuo Inaba’s group (University of Tsukuba) showed that most of the 500 major proteins present in unfertilized eggs, were also major proteins in cleaving stage embryos and swimming larvae based on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and MALDI-TOF-mass spectrometry (MS) (Inaba et al., 2007). Lixy Yamada (Tokushima University) used a different approach based on liquid chromatography (LC)-coupled tandem MS, a technique, which does not require to isolate single proteins using 2D electrophoresis. Approximately 1,000 proteins were identified, with some showing dramatic changes during fertilization. Yamada could also analyze various protein modifications, such as phosphorylations at fertilization and during embryogenesis. This powerful approach will soon yield essential information about protein synthesis and posttranslational regulations.

*Ciona intestinalis* is a very suitable model to conduct developmental genetics with its relatively short life cycles (2–3 months), established inland culture systems and germline transgenic technologies based on the Tc1/*mariner* transposon, *Minos* (Sasakura, 2007). Satoko Awazu (Kyoto University and now SARS Center) described efficient remobilization of *Minos* in the *Ciona* genome, achieved by microinjection of transposase mRNA into eggs of transgenic lines. Enhancer detection resulted in the production of GFP expression in the cells of larval and adult tissues, a powerful way to understand tissue organization and cell behavior and to identify tissue-specific

enhancers and transcriptional regulations in *Ciona* adults (Awazu et al., 2007).

## IMPROVED RESOURCES FOR UROCHORDATE RESEARCH

A round table on "Tunicate models, genomes and databases" discussed ascidian resources, including ANISEED, GHOST, and FABA (see below). These databases now provide an impressive integrated array of genomic, molecular, and developmental tools. ANISEED supports three *Ciona* gene model sets: JGI v1.0, KyotoGrail 2005, and ENSEMBL, and a single *Halocynthia* gene set. Genes and cDNA clones can be found through ANISEARCH, and genomes can be explored through BLAST searches or the ANISEED genome browser (*Ciona intestinalis* genome). Fate maps, anatomical ontologies, and anatomical territories are provided for a range of developmental stages, and a 3D virtual embryo can be downloaded. A database of in situ expression patterns is also provided. The Lemaire group has further developed a series of Gateway vectors that can be used for high throughput dissection of *cis*-regulatory sequences, and overexpression of wild-type or tagged proteins in a variety of chordate systems (Roure et al., 2007).

In addition to the genome exploration provided at ANISEED, the GHOST site provides chromosome mapping and the possibility to download; extensive collections of ESTs; and genome and in situ expression databases. Gene expression profiles of transcription factors and signaling molecules in *Ciona intestinalis* embryos and a clone request service are also provided. The FABA site provides a standardized developmental table for *Ciona intestinalis*. A 4D Ascidian Body Atlas includes ascidian 3D and cross-section images through 26 distinct developmental stages from fertilized egg to hatching larva.

## FUTURE PROSPECTS

Ascidian research is experiencing exponential growth, largely due to the combined application of sophisticated imaging technology, micromanipulations, and the availability of whole-

genome assemblies. Discovery of polarized cortical maternal determinants have already provided a broad understanding of the "mosaic" type of development typical of ascidians and how the polarized maternal information is read and relayed by inductive interactions among a small number of precursor blastomeres. Studies on cell lineage specification and differentiation offer the promise of elucidating complete gene regulatory networks for the morphogenesis of the *Ciona* tadpole and an approach of metamorphic transformations. It should be possible to apply such information to the neuronal and muscular circuits underlying the rudimentary swimming behavior of the tadpole and its settlement and metamorphosis. There are few systems that offer the combination of genome and cellular simplicity required for the detailed understanding of complex morphogenetic processes. The emergence of additional tunicate models, such as *Phallusia*, *Halocynthia*, *Botryllus*, and *Oikopleura*, provides a foundation for understanding the remarkable diversity of the tunicates and their adaptation to different environmental situations.

## USEFUL WEB SITES AND DATABASES

TunicatePortal: <http://www.tunicateportal.org/index.htm>

BioMarCell, Ascidians Film Archive (Sardet et al., 2007): <http://biodev.obs-vlfr.fr/recherche/biomarcell/>

ANISEED, Ascidians Network for In Situ Expression & Embryological Data (Tassy et al., 2006): <http://aniseed-ibdm.univ-mrs.fr/>

GHOST Site (Satou et al., 2005): <http://ghost.zool.kyoto-u.ac.jp/>

FABA, Four-dimensional Ascidians Body Atlas (Hotta et al., 2007): <http://chordate.bpni.bio.keio.ac.jp/faba/1.1/top.html>

DBTGR, DataBase of Tunicate Gene Regulation (Sierro et al., 2006): <http://dbtgr.hgc.jp/>

JGI *Ciona* genome browser: <http://genome.jgi-psf.org/Cioin2/Cioin2.home.html>

Genoscope *Oikopleura* genome browser: [http://www.genoscope.cns.fr/externe/Francais/Projets/Projet\\_HG/](http://www.genoscope.cns.fr/externe/Francais/Projets/Projet_HG/)

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