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# Developmental Biology

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## Tunicates: From humble sea squirt to proud model organism

Classified as mollusks by Linnaeus, it wasn't until 1866 that Kowalevsky recognized the chordate characteristics of larval tunicates. Since then, some of the most seminal ideas of developmental biology have been derived from research in this little known group. For example, in 1905 Conklin showed that a particular region of cytoplasm in the embryo of the ascidian *Styela* was associated with muscle cell specification, a key finding supporting the idea that cytoplasmic factors are involved in cell type specification (Conklin, 1905). 70 years later Whittaker and co-workers followed up with definitive experiments manipulating embryos to confirm the participation of cytoplasmic factors in the establishment of cell type (Gilbert, 2006). Meanwhile, workers in Europe and Japan were carrying out crucial research aimed at elucidating the roles of cell-cell signalling and differential gene expression in embryogenesis (Sato, 1994). However, tunicates never achieved the prominence as a developmental “model” organism of *Xenopus*, chick, or the more genetically tractable species; *Drosophila*, mouse, *C. elegans*, and zebrafish.

More recently, though, the advent of tools in molecular biology and functional genomics, along with a renewed interest in “non-model” organisms, has greatly increased the interest in tunicate developmental biology. In 2002 the draft genome of *Ciona intestinalis* (recently renamed *C. robusta*) was published, followed by the genome of its congener *C. savignyi*, and extensive in-depth genomic resources for several tunicates (Dehal et al., 2002; Hill et al., 2008; Tassy et al., 2010; Brozovic et al., 2016). No longer an “emerging model”, in the 21st century tunicates, most notably the solitary ascidians *Ciona* and *Halocynthia*, the colonial *Botryllus*, and more recently the appendicularian *Oikopleura*, have joined the company of the full-fledged model organisms. The associated advances are too numerous to list here.

This special issue of *Developmental Biology* grew out of the 9th International Tunicate Meeting at New York University in July 2017. Organized by Lionel Christiaen and one of us (Anna Di Gregorio) at NYU, the meeting attracted 135 participants from 16 countries. The work presented spanned from single-cell gene expression to marine ecology, and some of these topics are included in this special issue.

One group of papers deals with morphogenesis and pattern formation. The notochord is a chordate-specific axial structure that has become an important model for various morphogenetic processes. A review by Lu, Bhattachan and Dong, summarizes current knowledge about the assembly of the notochord, focusing on the cellular mechanisms that allow its elongation. A paper from the Veeman lab provides insights on the regulation of gene expression in secondary notochord, while the article from the Di Gregorio lab sheds light on an uncharacterized branch of the Brachyury-downstream notochord gene regulatory network. In addition to the notochord, the ascidian heart is a model for the morphogenesis of a simple chordate organ. In this vein, Kaplan, Wang, and Christiaen

describe investigations into the Wnt pathway in cardiopharyngeal development.

Recently the larvacean *Oikopleura* has attracted interest - it retains its larval chordate characteristics throughout its life and has the smallest known genome of any chordate (Nishino and Sato, 2001). Almazan et al., investigate actins in *Oikopleura* and based on their findings of two notochord-specific actins in this animal, which parallels related findings in amphioxus, suggest that the ancestral notochord expressed cytoplasmic actins, and that the expression of these genes was selectively lost from the notochord in other chordates. *Oikopleura* has another remarkable characteristic - it secretes a complex feeding structure around itself, called the “house”. The patterning of the epithelium that secretes this structure is explored in a paper from the Chourrout lab.

Regarding early development, the role that epidermal monocilia play in generating the driving force for rotation has been described in the Nishida lab paper, and the contribution of a maternal protein CiYB1 to the control of the localized mRNA in eggs and embryos has been shown in the Nishikata lab papers. A detailed investigation of the development of the sensory papillae of the *Ciona* larva, by Zeng et al. illustrates the power of cutting-edge imaging and reporter gene techniques in understanding ascidian organogenesis.

Ascidian tunicate larvae have an extremely simple nervous system, with only 177 neurons in the brain of *C. intestinalis* (Ryan et al., 2016). Despite this simplicity, the larva is able to sense light and gravity, and coordinate locomotion to find a location for settlement. Four papers in this issue deal directly with neural development in tunicates. Sharma, Wang, and Stolfi have used new single-cell RNAseq techniques to profile the transcriptomes of single cells isolated from the whole brain of *Ciona robusta* larvae, starting to reveal the compartment- and cell-specific gene expression patterns that define the organization of the *Ciona* larval brain.

Spagnuolo et al. show that a Gsx minimal CRE responds to manipulation of FGF- and Notch and Nodal signalling pathways during nervous system development, and the transcription factor Six 3/6, which is required to demarcate the anterior boundary of Gsx expression. Racioppi et al. identify Mitf as a key player in the melanogenesis of the two sensory organs found in the *Ciona* CNS, and show how this evolutionarily conserved transcription factor directly controls the expression of the single Rab 32/38 melanogenic transporter. Colonial ascidians, such as *Botryllus*, generate a nervous system in their asexual buds, without going through embryogenesis. Prünster et al. follow neurogenesis in a bud and reveal that some of the same molecular genetic mechanisms operate as in the development of a larval brain.

Solitary ascidians undergo a radical metamorphosis in which the tadpole larva settles on a substrate, resorbs its tail and most of its nervous system, and reorganizes the larval tissues to construct the adult sea

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squirt. A paper from the Chambon group reports on the involvement of Ci-hox12 in the generation of an apoptotic wave that propagates along the tail epidermis and triggers tail regression, a key early step of metamorphosis.

Not all development is embryonic, and several papers in this issue deal with non-embryonic modes: asexual budding, regeneration, and gametogenesis. The fascinating case of the asexual reproductive cycle of botryllid tunicates is introduced by Baruch Rinkevich in his commentary on the “underwater phoenix”. Manni, Anselmi, et al. fill in the details in their review of the history of the study of the cyclical budding process, termed blastogenesis. Apart from reproducing by budding, botryllids can regenerate their whole body from a fragment of the colony's shared vasculature. The review by Rodriguez et al. explores the properties of that extracorporeal vasculature. In related original research, Rosner et al. delve into the role of IAP genes in the weekly renewal of the colony as well as in whole body regeneration. Kassmer et al. review regeneration in both colonial and solitary ascidians, and original research in *Ciona* by Jeffery reveals how stem cells are mobilized to regenerate the siphons and neural complex. The larvacean *Oikopleura* appears again in a study of spermatogenesis and how it can be used to stage gonad development.

Evolutionary ideas underlie all these papers, but three articles are particularly concerned with evolution. Meedel et al. used transgenic assays to investigate the function of the unusual ascidian-specific N-terminus of *Ciona* Myogenic Regulatory Factor (MRF), and found that this puzzling region of the protein, which is not a generic transactivation domain, is required for MRF to perform its myogenic function. Two papers take a comparative evolutionary approach to cis-regulation. Ambrosino et al. used reciprocal cross-species transgenic reporter assays between zebrafish and ascidian to test for functional conservation of highly conserved non-coding elements between vertebrates and tunicates. Madgwick et al. studied the evolution of cis-regulation at a finer scale between two ascidian species, examining chromatin landscapes along with expression patterns to explore how divergent genomes can code for very similar developmental patterns.

Taken together this collection of articles span the breadth of developmental biology, demonstrating the unique insights to be gained from tunicates. These articles give a snapshot of the current state of research in tunicates, and point to new directions for the future. Many thanks to the Society for Developmental Biology and this journal for sponsoring this special issue.

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