THE WOOD CHIPPER PROBLEM:

By

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ABSTRACT

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Abstract goes here

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Acknowledgements go here

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Background

Chordates are a branch of deuterostome that are characterized by a dorsal nervous system, pharyngeal gill slits, and defined by the presence of a notochord. Tunicates are one of the three subphyla of chordates and are grouped because of their outer covering known as a tunic. During development tunicates form a tailed larvae that closely resembles the vertebrate body plan [12] and this tadpole larvae is typical of ˜3000 tunicates *cite*. Out of these 3000 species 16 are known to have independently lost their larval tail, with the majority of them being *Molgula* [1, 34]. During this time, known as the free-swimming stage, the elongation and mobility of the tail is depended upon the proper formation of the notochord and muscle cells [29]. As a tissue the notochord is closest related to cartilage and serves as the axial skeleton of the embryo in addition to a source patterning signaling [14]. In ascidians and in lower vertebrae the improper formation of the notochord leads to severely shortened larva that cannot swim or feed properly [6, 15, 31]. We present a comparative study of the tailed *M. oculata* and the tail-less *M. occulta* through gene expression in order to understand the underlying factors behind tail development and tail loss.

Ascidians are a simpler system to study developmental processes, their development is well studied, they have invariant early cell lineages, a small number of cells [18] and there has been no documentation of ascidians developing without an invariant cell lineage [19]. Although, this study present the first *Molgula* genomes assemblied, there the assembled and annotated genome of *Ciona intestinalis* which serves as a In *Ciona intestinalis* there are

2,600 cells, 36 of them being muscle, 40 of them being notochord and many of these cells have be traced starting at fertilization. Tunicates have a small number of cells compared to vertebrates, they also have rapid embryogenesis, compact genomes, few larval tissue types, simplified larval body plans and shallow gene networks [4, 12, 5]. For all of these reasons tunicates make great models for both tail development and loss, in addition to several Molgulids independently losing their tail and two of the Molgulids, a tailed and tailless species having the ability to hybridize [13].

Sequence technology has continued to advance and become cheaper. Technology such as Ion Torrent, Roche 454 and Illumina has made genome or transcriptome wide analysis more readily available for non-model species. These technologies have several advantages over the prior standard mircoarray; they have a wider scope, are more precise and are able for find novel genes []. With the advances in technology when can now sequence the transcriptomes of both species and their hybrid. This always use to look at pivotal time points in tail development and compare across closely related species. This type of study has yet to be done. Genes have been identified by both hybridization and subtractive screening (hotta, swalla, satoh, etc).

Tail development has been previously studied in ascidians and other chordates, with no one factor being the cause of a improperly form tail or lack of tail.

Tail development and lost has been studied on We started this project with the mRNA of the two Molgula species and their hybrid. mRNA was collected for three time points in all

genes have been idenitified in C. intestinalis, holycythia rorzi using substrative methods and microarrays. In molgula a global view has yet to be examined. However, several genes have been identified, using the tailed, tail-less and the hybrid.

We started this project with RNA-seq data which presented us with the problem of determining which assembly was the best and what metrics should be used to analysis them.

Experiemential techniques have yet to be adapted to *M. occult* and *M. oculata* because of there short gustation period, not being able to be cultured in lab conditions, although this is being currently developed amongst embryo specific difficulties. Most of the studies for tail development have been done in *C. intestinalis* and *H. roretzi*

Chapter 2

Literature Review

2.1 Ascidian tail development

Ascidians are known for the bilateral and invariant cell cleavage. Their development well described up to the gastrulation stage[26, 27, 25]. Like vertebrate chordates such as xenopus ascidians depend on maternally localized determinants to regulate cell moments and division, however the location and identity of these determinants are different although the development of the early body plans are similar [19]. In ascidians the first cell division is coordinated by β-catenin which activates the vegetal gene and restrict GATA4/5 [] and determines the axis of division []. The notochord is one of the most distinguishing characteristics of chordates. Solitary ascidians notochords typically come from two cell lineages, the primary notochord coming from the "A" blasomere and the secondary notochord comes from the "B" blastomere [26]. At this stage the blastomeres are labeled in Conklin [3] convention; "a" and "A" for the anterior animal and vegetal blastomeres, respectively and "b" and "B" for the posterior animal and vegetal blastomeres, respectively. Although the notochords cells have been traced back to this point, notochord inductions does not occur until the 32-cell stage, where the notochord/nerve chord precursors are activated by fiber growth factor (FGF) and without FGF activation the cells lose competency and the notochord can no longer from [24, 23]. By the 64-cell stage there are 10 notochord cell precursors, the 8 primary precursor notochord cells are identifiable and no longer multipotent, while the 2

secondary notochord cells are not restricted until the 110-cell stage [27, 41, 42, 17]. Two addition stages of cell division occur, one at gastrulation and one at neurulation, ending with 40 notochord cells, which is typical of most solitary ascidian tadpole larvae [3]. At the onsite of neurulation the notochord begins to form, this process includes the closing of the neural tube and posterior movement of the notochord and muscle cells, followed by the polarization and intercalate mediolaterally to the midline through a process known as convergence and extension where the cells [32]. At this point the larval tail is constructed of a notochord flanked by 3 rows of muscles on each side, and both notochord and muscle cell derive from the same blastomeres [27]. The arrangement of the notochord cells is a stochastic process, the anterior 32 cells—primary notochord cells—are always formed by the A7.3 and A7.7 blastomere and the posterior most 8—secondary—notochord cells are always formed by the B8.6 blastomere, but the ordering of the 32 most anterior is not determinate, cells from both the A7.3 and A7.7 intercalate in a random order [26, 27, 22, 32**?**]. This process, along with muscle cell are the causes the larval tail to form [22, 11, 32]. Although a tailed larvae is typical of most ascidians, several species with in the Stolidobranchia order have individually undergone tail-loss, many of which fall in the Molgulidae [1, 14, 10, 20]. The tail-less—anural—species develop in a similar manner and are indistinguishable from the tailed—urodele—counterparts up to late gastrulation [1, 34, 11]. Anural ascidians lack several urodele features including a converged and extended notochord, muscle cells and the otilith sensory organ. The absence of differentiated muscles cells and intercalated notochord are the cause for the lack of tail in these species [22, 34]. *M. tectiformis* notochord cells do not divide again after the 10 precursor cells are formed and *M. occulta* stops dividing after 20 cells [14]. The same occurs in *M. bleizi*, however after the 20 notochord cells are formed, the embryo attempts to make a tail but never does so [37]. It has also been shown that chordate

embryos without fully developed notochord and/or muscle cells do not fully elongate or fail completely to develop a tail [14, 38, 31]. Seeing that most ascidians have tailed larvae and that the tail can be restored through the use of interspecies hybrids, the lack of tail has been shown to be a loss of function. *M. oculata* (urodel) and *M. occulta* (anural) both of the Roscovita clade have been shown to produce hybrids in lab conditions. Of the known *Molgula* species *M. occulta* and *M. oculata* are the only two that can hybridize. Although *M. occulta* and *M. oculata* have been found to dwell in the same habitat, hybrids have not been found in nature and have only been produced in lab conditions, and no other crosses are known to produce hybrids. Fertilizing *M. oculata* eggs with *M. occulta* sperm in most cases produce embryos with fully formed tails. The reciprocal hybrid produces an embryo with 20 notochord cells like *M. occulta*, however the notochord cells converge and extent like *M. oculata* [34]. The ascidian tail has been shown to form in the presence of notochord and the absence of muscles cells [22] and the hybrid tail is not flaked by muscles as that of tail species [37], however in hybrids embryos that express the p58 which is associated with cytoskeleton develop urodele features. Hybrid embryos that develop urodele features are batch specific and features are only restored in embryos that express p58 [33, 11]. It was also shown that in hybrid embryos in which urodele features were restored, the number of cells that express acetylcholinesterase (AChE) in a vestigial muscle cell lineage increased [13].

2.2 Brachyury has been shown to be the

The induction of the notochord begins at the 32 cell stage by fibroblast growth factor (FGF) in the A6.2 and A6.4 notochord/nerve cord precursors[28] after the 7th cleavage. FGF transducer FGF receptor, Ras, MEK and MAPK. MAPK promotes Ets which promotes

Bra at the 64 cell stage. It was observed from isolation experiments that notochord/nerve cord precursors that loss FGF competence at the 32 cell stage assume the default nerve cord cell fate [21]If FGF is not present at the 32 cell stage competence is loss and *bra* is not induced. This is because is be MAPK which is down stream in the cascade is not activated and the induction of bra and repression of FoxB are not carried out [8]. And in the absence of bra notochord cell become nerve chord cells (Yasuo and Satoh 1998 Consevation of the developmental role of bra).As stated above the notochord is specific at the 64 cell stage. At this point *brachyury* is expressed first weakly in the at the 64-cell stage in the notochord/nerve chord precursors [41] and unlike other chordates, in ascidians *bra* is only expressed in the notochord cells [40, 4, 9, 38]. Without *bra* the ascidian tail does not form. Although *bra* is necessary, its presence does not guarantee a tail. *M. occulta* and *M. tectiformis*, two tailless *Molgula*, both express *bra*. In both cases *bra* expression stop earlier than that of *M. oculata*, but produce different results. *Bra* is expressed in the 10 precursor notochord cells in *M. occulta*, another round of cell division occurs which does not in *M. tectiformis*. In these two species of *Molgula* muscle actin became pseudo genes, however the mutation in the muscle actin genes are not the same [37, 14]. *Manx* is another gene identified to be important for tell development in *Molgula*, however, not in all ascidians. *Manx* is lowly expressed in *M. occulta*, and has been shown to restore the hybrid tail, but there is no homolog for *manx* in *C. intestinalis* [35, 36].

It was shown in *H. roretzi* that *FoxB* is represses the activation of *bra* predominately through the binding of Fox BS1 (GCACTGA*ACAAACA*TACATAG). *FoxB* is activated by *ZicN* and present in both nerve cord and notochords precursors, however is repressed by MAPK in the notochord cell lineage at the 64-cell stage [8]. MAPK is thought to be repressed by Ephin which is one of the key differences between notochord and nerve cord

determination. Ephin and FoxB have redundant roles in the repression of the notochord fate, but differ in that ephin is spatial and FoxB is mediates temporal restriction of *Bra* induction.

The Planar Cell Polarity (PCP) pathway is involved in cell movement during this process and mutations in *prickle*—a known PCP gene—have shown to cause a shortened ascidian tail affecting both the mediolateral intercalation and the elongation of the ascidan tail[15]. The *pk* mutant *aimless* produces a truncated tail, however the polarity of the nuclei are present, showing that prickle does not establish polarity with in the cell but polarity between cells, acting in a local manner and perhaps their is a global organizer [15**?**]. However, even in the absence of the PCP pathway considerable convergence and elongation of the notochord was observed in Ciona, driven by a presumed boundary effect" [39].

On larger scale subtractive screening was done to identify genes downstream of *bra*, 39 genes were initially found.

Oikopleura did not exhibit the same mechanism for tail development as Ciona, of the 50 bra target genes previously identified cite only 26 of them had orthologs in Oikopleura [16] of those genes expression ranged from notochord specific to tail including possible notochord, to tissues that were clearly not the notochord.

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there are 3 major pathways in chordates: FGF, BMP and Nodal.

2.3 Assembling and analyzing data

One of the major advances in science in the past 20 years was the implementation of sequencing technologies. These technologies allowed us to examine problems in ways not previously

possible. The first wave were Sanger sequencing in the 1986, but was not used until 10 years later. and mircoarrays which became popular starting in the mid '90s. Mircoarrays allow us to look at a wide spectrum of genes and understand relative expression within a sample. Sanger sequencing allowed us to sequence whole genomes and

Outside of this project there are four tunicata genomes assembled; *C. intestinalis*, *C. savignyi*, *Oikopleura dioica*, *Botryllus schlosseri*, *Halocynthia uranium*, *H. foretzi*, *Phallusia fumigata*, and *P. mammilata*, but no *Molgula* genomes. Of those genome *C. intestinalis* is the best assembled and most well annotated. In addition to long reads (Sanger) scaffolding was done using experimental data.

Kobayashi et al. [**?**] isolated and analyzed gene expression in notochord (A7.3+A7.7) and nerve cord (A7.4+A7.8) precursors using microarrays. This study was able to identify 106 genes expressed in the notochord precursor and 68 expressed in the nerve cord precursor at the 64-cell stage. Of these the genes, 36 notochord genes and 25 nerve chord genes were confirmed via Whole Mount In Situ Hybridization in the respective cells.

Currently both 2^{nd} and 3^{rd} generation technologies are in use, with Roche 454, Ion Torrent, Illumina and PacBio are the most wide spread. There are many trade-offs for each of the technologies, cost per MB, sequencing time, prep cost, error rate and sequencing bias; 454 and PacBio have longer reads than Illumina and Ion Torrent, 800 bp and 1+kbp, respectively. However, both Illumina and Ion Torrent's short reads are cheaper to generate, produce more reads and better for counting, in addition to PacBio having a high error rate. Illumina and Ion Torrent have the best error rates and while Ion Torrent calls more more Single nucleotide polymorphisms, it also calls more false positives. For this reason, amongst other Illumina is the most used because it is the most versatile and preforms the best in general [7**?**]. This drop in price and produced many of the assembled genomes within the

Tuncata phyla.

Chapter 3

Genome assembly and characterization

3.1 Materials and methods

3.1.1 Genomic DNA library preparation and sequencing

Genomic DNA was phenol/chloroform extracted from dissected gonads of *Molgula occulta* (Kupffer) and *Molgula oculata* (Forbes) adults from Roscoff, France, and a *Molgula occidentalis* (Traustedt) adult from Panacea, Florida, USA (Gulf Specimen Marine Lab). Genomic DNA was sheared using an M220 Focused-ultrasonicator (Covaris, Woburn, MA). Sequencing libraries were prepared using KAPA HiFi Library Preparation Kit (KAPA Biosystems, Wilmington, MA) indexed with DNA barcoded adapters (BioO, Austin, TX). Size selection was performed using Agencourt (Beckman?Coulter, Brea, CA) AMPure XP purification beads (300?400 bp fragments), or Sage Science (Beverly, MA) Pippin Prep (650?750 bp and 875?975 bp fragments). For *M. occulta* and *M. occidentalis* libraries, 6 PCR cycles were used. For *M. oculata* libraries, 8 cycles were used for the 300?400 bp library, and 10 cycles were used for the 650?750 and 875?975 bp libraries. Libraries of different species but same insert size ranges were multiplexed for sequencing in three 100 100 PE lanes on a HiSeq 2000 sequencing system (Illumina, San Diego, CA) at the Genomics Sequencing Core Fa-

cility, Center for Genomics and Systems Biology at New York University (New York, NY). Thus, each lane was dedicated to a mix of species, specifically barcoded libraries of a given insert size range. Raw sequencing reads were deposited as a BioProject at NCBI under the ID# PRJNA253689.

3.1.2 Genome sequence assembly

All genomes were assembled on Michigan State University High Performance Computing Cluster (http://contact.icer.msu.edu). Prior to assembly, read quality was examined using FastQC v0.10.1. Reads were then quality trimmed on both the 5? and 3? end using seqtk trimfq (https://github.com/lh3/seqtk) which uses Phred algorithm to determine the quality of a given base pair. Seqtk trimfq only trims bases, so no reads were discarded. Each library per species was then abundance filtered using 3-pass digital normalization to remove repetitive and erroneous reads [2, 30], Howe et al., 2014). Genome assembly was done using velvet v1.2.08 (Zerbino and Birney, 2008) with k-mer overlap length ('k') ranging from 19 to 69 and scaffolding was done by Velvet, by default. Velvet does not produce separate files for contigs and scaffolds; because Velvet scaffolded conservatively, contigs dominated the assemblies so we refer to both contigs and scaffolds as contigs. CEGMA scores were then computed to evaluate genome completeness (Parra et al., 2007). The latest versions of three species' genome assemblies have been deposited on the ANISEED (Ascidian Network for In Situ Expression and Embryological Data) database for browsing and BLAST searching at http://www.aniseed.cnrs.fr/ (Tassy et al., 2010). Scripts for genome assembly and CEGMA analysis can be found in the following github repository: https://github.com/elijahlowe/molgula_genome_assemblies.git

3.2 Results

In addition to N50 lengths, we also used CEGMA (Core Eukaryotic Genes Mapping Approach) scores, in order to evaluate the assemblies' representative completeness (Parra et al., 2007). CEMGA reports scores for complete and partial alignments to a subset of core eukaryotic genes. An alignment is considered ?complete? if at least 70% of a given protein model aligns to a contig in the assembly, while a partial alignment indicates that a statistically significant portion of the protein model aligns. The partial alignment scores are ?97% or higher for all assemblies. *M. oculata* has the best complete alignment score at ?90%. *M. occidentalis* and *M. occulta* have complete alignment scores of 81% and 77% respectively (Table 1). These scores indicate that our assemblies contain at least partial sequences for the vast majority of protein-coding genes in the genomes of these species.

3.2.1 Gene complexes

When studying development it is important to characterize the genome for particular gene cluster/families. There are 13 HOX clusters, in humans

Chapter 4

Another chapter

Chapter 5

Conclusions

APPENDIX

BIBLIOGRAPHY

[1] N. J. BERRILL. Studies in tunicate developnent. *Society*, 219:281–346, 1931.

[2] C. T. Brown, A. Howe, Q. Zhang, A. B. Pyrkosz, and T. H. Brom. A reference-free algorithm for computational normalization of shotgun sequencing data. arXiv e-print 1203.4802, Mar. 2012.

[3] E. G. Conklin. *The organization and cell-lineage of the ascidian egg.* Philadelphia : [Academy of Natural Sciences], 1905.

[4] J. C. Corbo, M. Levine, and R. W. Zeller. Characterization of a notochord-specific enhancer from the brachyury promoter region of the ascidian, ciona intestinalis. *Development (Cambridge, England)*, 124(3):589–602, Feb. 1997.

[5] P. Dehal, Y. Satou, R. K. Campbell, J. Chapman, B. Degnan, A. D. Tomaso, B. Davidson, A. D. Gregorio, M. Gelpke, D. M. Goodstein, N. Harafuji, K. E. M. Hastings, I. Ho, K. Hotta, W. Huang, T. Kawashima, P. Lemaire, D. Martinez, I. A. Meinertzhagen, S. Necula, M. Nonaka, N. Putnam, S. Rash, H. Saiga, M. Satake, A. Terry, L. Yamada, H.-G. Wang, S. Awazu, K. Azumi, J. Boore, M. Branno, S. Chin-bow, R. DeSantis, S. Doyle, P. Francino, D. N. Keys, S. Haga, H. Hayashi, K. Hino, K. S. Imai, K. Inaba, S. Kano, K. Kobayashi, M. Kobayashi, B.-I. Lee, K. W. Makabe, C. Manohar, G. Matassi, M. Medina, Y. Mochizuki, S. Mount, T. Morishita, S. Miura, A. Nakayama, S. Nishizaka, H. Nomoto, F. Ohta, K. Oishi, I. Rigoutsos, M. Sano, A. Sasaki, Y. Sasakura, E. Shoguchi, T. Shin-i, A. Spagnuolo, D. Stainier, M. M. Suzuki, O. Tassy, N. Takatori, M. Tokuoka, K. Yagi, F. Yoshizaki, S. Wada, C. Zhang, P. D. Hyatt, F. Larimer, C. Detter, N. Doggett, T. Glavina, T. Hawkins, P. Richardson, S. Lucas, Y. Kohara, M. Levine, N. Satoh, and D. S. Rokhsar. The draft genome of ciona intestinalis: Insights into chordate and vertebrate origins. *Science*, 298(5601):2157–2167, Dec. 2002.

[6] A. Di Gregorio, R. M. Harland, M. Levine, and E. S. Casey. Tail morphogenesis in the ascidian, ciona intestinalis, requires cooperation between notochord and muscle. *Developmental biology*, 244(2):385–95, Apr. 2002.

[7] T. C. Glenn. Field guide to next-generation DNA sequencers. *Molecular Ecology Resources*, 11(5):759–769, Sept. 2011.

[8] H. Hashimoto, T. Enomoto, G. Kumano, and H. Nishida. The transcription factor FoxB mediates temporal loss of cellular competence for notochord induction in ascidian embryos. *Development*, 138(14):3091–3091, June 2011.

[9] K. Hotta, H. Takahashi, a. Erives, M. Levine, and N. Satoh. Temporal expression patterns of 39 brachyury-downstream genes associated with notochord formation in the ciona intestinalis embryo. *Development, growth & differentiation*, 41(6):657–64, Dec. 1999.

[10] J. L. Huber, K. B. da Silva, W. R. Bates, and B. J. Swalla. The evolution of anural larvae in molgulid ascidians. *Seminars in cell & developmental biology*, 11(6):419–26, Dec. 2000.

[11] Jeffery, R. billie, and J. Swalla. Factors necessary for restoring an evolutionary change in an anural ascidian embryo. *Developmental biology*, 153:194–205, 1992.

[12] W. R. Jeffery. Minireview ascidian gene-expression profiles. *Genome biology*, 3(10):1–4, 2002.

[13] W. R. Jeffery and B. J. Swalla. An evolutionary change in the muscle lineage of an anural ascidian embryo is restored by interspecific hybridization with a urodele ascidian. *Developmental Biology*, 337:328–337, 1991.

[14] W. R. Jeffery, B. J. Swalla, N. Ewing, and T. Kusakabe. Evolution of the ascidian anural larva: evidence from embryos and molecules. *Molecular biology and evolution*, 16(5):646–54, May 1999.

[15] D. Jiang, E. M. Munro, W. C. Smith, S. Barbara, and F. Harbor. Ascidian prickle regulates both mediolateral and anterior-posterior cell polarity of notochord cells. *Current biology*, 15:79–85, 2005.

[16] J. E. Kugler, P. Kerner, J.-M. Bouquet, D. Jiang, and A. Di Gregorio. Evolutionary changes in the notochord genetic toolkit: a comparative analysis of notochord genes in the ascidian ciona and the larvacean oikopleura. *BMC evolutionary biology*, 11(1):21, Jan. 2011.

[17] P. Lemaire. Unfolding a chordate developmental program, one cell at a time: invariant cell lineages, short-range inductions and evolutionary plasticity in ascidians. *Developmental biology*, 332(1):48–60, Aug. 2009.

[18] P. Lemaire. Evolutionary crossroads in developmental biology: the tunicates. *Development*, 138(11):2143–2152, May 2011.

[19] P. Lemaire, W. C. Smith, and H. Nishida. Ascidians and the plasticity of the chordate developmental program. *Current biology : CB*, 18(14):R620–31, July 2008.

[20] M. E. Maliska and B. J. Swalla. Molgula pugetiensis is a pacific tailless ascidian within the roscovita clade of molgulids. *The Biological Bulletin*, 219(3):277–282, Dec. 2010.

[21] T. Minokawa, K. Yagi, K. W. Makabe, and H. Nishida. Binary specification of nerve cord and notochord cell fates in ascidian embryos. *Development*, 128(11):2007–2017, June 2001.

[22] D. Miyamoto and R. Crowther. Formation of the notochord in living ascidian embryos. *Journal of Embryology and Experimental Morphology*, VOL. 86:1–17, 1985.

[23] Y. Nakatani and H. Nishida. Duration of competence and inducing capacity of blastomeres in notochord induction during ascidian embryogenesis. *Development, Growth & Differentiation*, 41(4):449–453, Aug. 1999.

[24] Y. Nakatani, H. Yasuo, N. Satoh, and H. Nishida. Basic fibroblast growth factor induces notochord formation and the expression of as-t, a brachyury homolog, during ascidian embryogenesis. *Development (Cambridge, England)*, 122(7):2023–2031, July 1996.

[25] H. Nishida. Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme: III. up to the tissue restricted stage. *Developmental Biology*, 121(2):526–541, June 1987.

[26] H. Nishida and N. Satoh. Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme: I. up to the eight-cell stage. *Developmental Biology*, 99(2):382–394, Oct. 1983.

[27] H. Nishida and N. Satoh. Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme: II. the 16- and 32-cell stages. *Developmental Biology*, 110(2):440–454, Aug. 1985.

[28] N. Satoh. Ascidian embryos as a model system to analyze expression and function of developmental genes. *Differentiation; Research in Biological Diversity*, 68(1):1–12, Aug. 2001.

[29] N. Satoh. The ascidian tadpole larva: comparative molecular development and genomics. *Nature reviews. Genetics*, 4(4):285–95, Apr. 2003.

[30] E. M. Schwarz, P. K. Korhonen, B. E. Campbell, N. D. Young, A. R. Jex, A. Jabbar, R. S. Hall, A. Mondal, A. C. Howe, J. Pell, A. Hofmann, P. R. Boag, X.-Q. Zhu, T. R. Gregory, A. Loukas, B. A. Williams, I. Antoshechkin, C. T. Brown, P. W. Sternberg, and R. B. Gasser. The genome and developmental transcriptome of the strongylid nematode haemonchus contortus. *Genome Biology*, 14(8):R89, Aug. 2013.

[31] D. L. Stemple. Structure and function of the notochord: an essential organ for chordate development. *Development (Cambridge, England)*, 132(11):2503–12, June 2005.

[32] B. J. Swalla. Mechanisms of gastrulation and tail formation in ascidians. *Microscopy research and technique*, 26(4):274–84, 1993.

[33] B. J. Swalla, M. R. Badgett, and W. R. Jeffery. Identification of a cytoskeletal protein localized in the myoplasm of ascidian eggs: localization is modified during anural development. *Development (Cambridge, England)*, 111(2):425–436, Feb. 1991.

[34] B. J. Swalla and W. R. Jeffery. Interspecific hybridization between an anural and urodele ascidian: differential expression of urodele features suggests multiple mechanisms control anural development. *Developmental biology*, 142(2):319–34, Dec. 1990.

[35] B. J. Swalla and W. R. Jeffery. Requirement of the manx gene for expression of chordate features in a tailless ascidian larva. *Science (New York, N.Y.)*, 274(5290):1205–8, Nov. 1996.

[36] B. J. Swalla, M. a. Just, E. L. Pederson, and W. R. Jeffery. A multigene locus containing the manx and bobcat genes is required for development of chordate features in the ascidian tadpole larva. *Development (Cambridge, England)*, 126(8):1643–53, Apr. 1999.

[37] B. J. Swalla, K. W. Makabe, N. Satoh, and W. R. Jeffery. Novel genes expressed differentially in ascidians with alternate modes of development. *Development*, 318:307–318, 1993.

[38] N. Takada, J. York, J. M. Davis, B. Schumpert, H. Yasuo, N. Satoh, and B. J. Swalla. Brachyury expression in tailless molgulid ascidian embryos. *Evolution & development*, 4(3):205–11, 2002.

[39] M. T. Veeman, Y. Nakatani, C. Hendrickson, V. Ericson, C. Lin, and W. C. Smith. Chongmague reveals an essential role for laminin-mediated boundary formation in chordate convergence and extension movements. *Development (Cambridge, England)*, 135(1):33–41, Jan. 2008.

[40] H. Yasuo and N. Satoh. Function of vertebrate t gene. *Nature*, 364(6438):582–583, Aug. 1993.

[41] H. Yasuo and N. Satoh. An ascidian homolog of the mouse brachyury (t) gene is expressed exclusively in notochord cells at the fate restricted stage. *Development, Growth & Differentiation*, 36(1):9–18, Feb. 1994.

[42] H. Yasuo and N. Satoh. Conservation of the developmental role of brachyury in notochord formation in a urochordate, the ascidian halocynthia roretzi. *Developmental Biology*, 200(2):158–170, Aug. 1998.