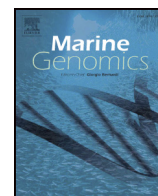




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Marine Genomics



Q1 Discovery of germline-related genes in Cephalochordate amphioxus: 2 A genome wide survey using genome annotation and transcriptome data

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ABSTRACT

The generation of germline cells is a critical process in the reproduction of multicellular organisms. Studies in animal models have identified a common repertoire of genes that play essential roles in primordial germ cell (PGC) formation. However, comparative studies also indicate that the timing and regulation of this core genetic program vary considerably in different animals, raising the intriguing questions regarding the evolution of developmental mechanisms in metazoans. Cephalochordates (commonly called amphioxus or lancelets) represent one of the invertebrate chordate groups and can provide important information about the evolution of developmental mechanisms in the chordate lineage. In this study, we used genome and transcriptome data to identify germline-related genes in two distantly related Cephalochordate species, *Branchiostoma floridae* and *Asymmetron lucayanum*. *Branchiostoma* and *Asymmetron* diverged more than 120 MYA, and the most conspicuous difference between them is their gonadal morphology. We used important germline developmental genes in several model animals to search the amphioxus genome and transcriptome dataset for conserved homologs. We also annotated the assembled transcriptome data using Gene Ontology (GO) terms to facilitate the discovery of putative genes associated with germ cell development and reproductive functions in amphioxus. We further confirmed the expression of 14 genes in developing oocytes or mature eggs using whole mount *in situ* hybridization, suggesting their potential functions in amphioxus germ cell development. The results of this global survey provide a useful resource for testing potential functions of candidate germline-related genes in cephalochordates and for investigating differences in gonad developmental mechanisms between *Branchiostoma* and *Asymmetron* species.

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1. Introduction

The germline cells of metazoan animals are segregated from somatic cell lineages during development either by the inheritance of specific maternal gene products during early cleavages (preformation) or by induction mechanisms that occur during embryogenesis (epigenesis) (Extavour and Akam, 2003; Johnson et al., 2011). Despite the great contrast between these two types of mechanism, once primordial germ cells (PGCs) are specified they usually show distinct morphological features and express a suite of conserved genes, most commonly including *Vasa*, *Nanos*, *Piwi/Ago*, *Tudor*, *Boule/DAZ*, and *bruno* (Juliano and Wessel, 2010; Voronina et al., 2011). Interestingly, many of these genes, including *Vasa*, *Nanos*, and *Piwi* are also expressed in multipotent somatic stem cells, especially in those cells that have great ability to differentiate, proliferate, self-renew and regenerate. These observations have led to the hypothesis that PGCs and somatic stem cells may have evolved from a common pluripotent progenitor cell population that

employs a conserved gene regulatory network for its development and maintenance (Juliano et al., 2010; Solana, 2013). Indeed, certain cell types that can produce both gametes and somatic cells, such as the I-cells in *Hydra* and the archeocytes in sponges (David, 2012; Funayama, 2010), have been identified in early branching metazoan animals, and these conserved genes are expressed in these pluripotent cells. In planarians, neoblasts represent totipotent stem cells that can lead to the regeneration of the entire body (including the germline); these cells also require the expression of a set of similar genes for their formation and normal function (Rink, 2013). Thus, the evolution of diverse germline developmental mechanisms in metazoans may reflect modifications of gene repertoire that interact with this core genetic network as well as changes in the timing or regulatory control to operate this core genetic network in different animals.

The phylogenetic position of Cephalochordata (commonly called amphioxus or lancelets) makes this animal group a useful reference point for studying the evolution of the genome and developmental mechanisms. Currently there is broad consensus that cephalochordates represent the earliest branching group within chordates, and tunicates are the sister group to vertebrates (Bourlat et al., 2006; Delsuc et al., 2006, 2008). Compared to tunicates, however cephalochordates retained

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more ancestral chordate characteristics and experienced less genome reorganization or gene loss after they diverged from other chordate lineages (Holland et al., 2004, 2008; Louis et al., 2012; Paps et al., 2012). Moreover, the cephalochordate genome did not undergo the two-round (2R) whole genome duplication events that occurred during early vertebrate evolution (Putnam et al., 2008). Therefore, cephalochordates provide a suitable model for comparing the evolution of genome and of gene function between invertebrate chordates and vertebrates and even between chordates and other metazoan animals (Bertrand and Escriva, 2011).

To study the evolution of germline developmental mechanisms in chordates, our group previously searched the genome of the model amphioxus *Branchiostoma floridae* (Holland et al., 2008; Putnam et al., 2008) and identified several conserved germline markers including *vasa*, *nanos*, *piwi*, and *tudor-domain-containing* genes (Wu et al., 2011; Zhang et al., 2013). We showed that transcripts of these genes are maternally deposited in the matured oocytes; after fertilization, these transcripts are asymmetrically localized in the early developing embryos and are subsequently inherited by the specific blastomere that is destined to differentiate into the germline cells in amphioxus (Wu et al., 2011; Zhang et al., 2013). Our results from studies of various *Branchiostoma* species further suggested that *Branchiostoma* amphioxus use maternal gene products to specify their germline cells during early embryogenesis (i.e. a preformation mechanism), in contrast to the previous idea that cephalochordates use an inductive mechanism (epigenesis) to form their germ cells (Extavour and Akam, 2003; Extavour, 2007; Frick and Ruppert, 1997). These new findings raised a question regarding the generality of using maternal gene products to specify germline cells in cephalochordates. Expanding our knowledge of the gene complements and mechanisms that control germline specification to cover more distantly related cephalochordate species would facilitate our understanding of how mechanisms for germline specification have evolved in chordates.

In addition to *Branchiostoma* species, which have paired gonads, there are two other groups of cephalochordates: *Epigonichthys* and *Asymmetron*, which have gonads only on the right side (Holland, 2011; Nishikawa, 2004; Ruppert, 1997). Little is known about the biology of *Asymmetron* and *Epigonichthys* amphioxus; only recently, researchers successfully obtained laboratory spawning from an *Asymmetron* amphioxus (*Asymmetron lucayanum*) and described its early development (Holland and Holland, 2010). On the basis of mitochondrial DNA, *Epigonichthys* was placed as the sister group of *Branchiostoma*, and *Asymmetron* was branched outside the *Branchiostoma* + *Epigonichthys*

clade (Kon et al., 2007; Nohara et al., 2005). According to the molecular clock based on mitochondrial DNA and on nuclear gene sequence data, divergence between *Asymmetron* and *Branchiostoma* is estimated to have occurred 120–160 million years ago (MYA), suggesting wide divergence between these two groups (Nohara et al., 2005; Yue et al., 2014).

In this study, we conducted a comprehensive survey of conserved germline-related genes in two amphioxus species, *B. floridae* and *A. lucayanum*, that represent the two diverged cephalochordate groups. We also searched for potential germline cell developmental genes within the available genome or transcriptome resources in these two species using annotated GO terms. Finally, we tested the validity of our search approach by performing *in situ* hybridization on *B. floridae* ovarian tissue and mature eggs to examine the expression patterns of a partial list of these identified genes. The identification of these germline-related genes in cephalochordates will contribute to the understanding of the evolution of mechanisms underlying germline specification in chordates and could provide a basis for investigating differences in gonad developmental processes in *Branchiostoma* and *Asymmetron* amphioxus.

2. Materials and methods

2.1. Sequence sources

B. floridae proteome sequences were downloaded from <http://genome.jgi-psf.org/Brafl1/Brafl1.home.html> following procedures described in Yue et al. (2014). The two sets of *A. lucayanum*'s likely protein-coding transcripts (asymAD and asym20h) were predicted by TransDecoder (<http://transdecoder.sourceforge.net>) based on the transcriptome assemblies of two pooled *A. lucayanum* libraries (asymAD for adults and asym20h for larvae) (NCBI BioProject accession SRP035506). The corresponding protein sequences were used as two sets of *A. lucayanum* proteomes. Details of these two transcriptome assemblies were described in Yue et al. (2014). The proteomes of four well-studied model organisms (*Drosophila melanogaster*, *Caenorhabditis elegans*, *Danio rerio* and *Mus musculus*), as well as those of six other species (*Ciona intestinalis*, *Petromyzon marinus*, *Latimeria chalumnae*, *Xenopus tropicalis*, *Gallus gallus* and *Homo sapiens*) were downloaded from Ensembl (release 78); the proteomes of *Trichoplax adhaerens*, *Amphimedon queenslandica*, *Nematostella vectensis*, *Lottia gigantea*, *Tribolium castaneum* and *Strongylocentrotus purpuratus* were downloaded

Table 1
Amphioxus homologs of conserved genes involved in germline cell development.

Gene name	Major domain encoded	<i>B. f.</i> gene models	<i>A. l.</i> transcripts (non-redundant)
<i>nanos</i>	zf-nanos	85485*	asymAD_comp37714_c0_seq1_m.14292
<i>vasa/ghl</i>	DEAD, Helicase_C	214468*	asymAD_comp42457_c0_seq2_m.23103
<i>arrest/bruno/celf2</i>	RRM_1	114402	asymAD_comp46076_c0_seq8_m.32483
<i>bgn/ythdc2</i>	HA2, OB_NTP_bind	123593	asym20h_comp78808_c0_seq2_m.38179
<i>boule/dazl</i>	RRM_1	89711	asym20h_comp73083_c0_seq1_m.25638
		89712	asym20h_comp77866_c0_seq2_m.35437
<i>csul/pmr5</i>	PRMT5	114443	asymAD_comp46212_c0_seq1_m.32865
<i>gcl</i>	BTB	209210	asymAD_comp45519_c0_seq3_m.30820
<i>gld/qki</i>	KH_1	236368	asym20h_comp78683_c0_seq2_m.37777
<i>mael</i>	Maelstrom	121310	asymAD_comp43714_c0_seq1_m.26082
<i>mago nashi</i>	Mago_nashi	114879	asymAD_comp46168_c2_seq1_m.32739
<i>mex3</i>	KH_1	126022	asymAD_comp15843_c0_seq1_m.716
<i>orb/cpeb</i>	RRM_1	85610	asymAD_comp38638_c0_seq6_m.15671
<i>ovo</i>	zf-C2H2	226363	asymAD_comp42374_c0_seq1_m.22917
<i>prdm1/blimp1</i>	zf-C2H2	120365	asym20h_comp78929_c0_seq2_m.38578
<i>prdm14</i>	zf-C2H2	240852	asym20h_comp79040_c0_seq3_m.38916
<i>pumilio/pufj/bf</i>	PUF	286861	asym20h_comp79141_c0_seq2_m.39316
<i>smB</i>	LSM	94837	asymAD_comp42845_c0_seq2_m.23966
<i>smD3</i>	LSM	114009	asymAD_comp38720_c0_seq3_m.15856
<i>smE/snr6</i>	LSM	268096	asym20h_comp72130_c1_seq26_m.23962
<i>tsunagi/rmb8a</i>	RRM_1	251578	asym20h_comp66831_c0_seq3_m.15573

Notes:

1. Gene models labeled with asterisk (*) were also identified by Wu et al. (2011).

2. We did not find orthologous genes in cephalochordates for *bam*, *buc*, *cxcl12a/sdf1a*, *cxcr4b*, *dnd1*, *dppa3/stella*, *meg*, *mex*, *nanog*, *oma*, *oskar*, *pgc*, *pie*, *pos1*, *pou5f1/oct4*, *smG/snr7*.

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