



SYMPOSIUM

“Insights of Early Chordate Genomics: Endocrinology and Development in Amphioxus, Tunicates and Lampreys”: Introduction to the symposium

Linda Z. Holland* and Stacia A. Sower^{1,†}

*Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0202, USA; [†]Center for Molecular and Comparative Endocrinology, University of New Hampshire, Durham, NH 03824, USA

From the symposium “Insights of Early Chordate Genomics: Endocrinology and Development in Amphioxus, Tunicates and Lampreys” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2010, at Seattle, Washington.

¹E-mail: sasower@unh.edu; lzholland@usc.edu

Synopsis This symposium focused on the evolution of chordate genomes, in particular, those events that occurred before the appearance of jawed vertebrates. The aim was to highlight insights that have come from the genome sequences of jawless chordates (lampreys, tunicates, and amphioxus) not only into evolution of chordate genomes, but also into the evolution of the organism. To this end, we brought together researchers whose recent work on these organisms spans the gap from genomics to the evolution of body forms and functions as exemplified by endocrine systems and embryonic development.

The three subphyla of the phylum Chordata: Cephalochordata (amphioxus or lancelets), Tunicata (ascidians, appendicularians and thaliaceans), and Vertebrata (agnathans plus gnathostomes) radiated over 500 mya. The chordates represent contrasts in genome size, from 2.3 Gb for the sea lamprey *Petromyzon marinus* to 520 Mb for amphioxus *Branchiostoma floridae*, 168 Mb for the ascidian tunicate *Ciona intestinalis* and 60 Mb for the appendicularian tunicate *Oikopleura dioica*. Sequencing of the genome of the sea lamprey began in 2005 and that of the amphioxus genome in 2003. Both are essentially completed (<http://genome.ucsc.edu/cgi-bin/hgGateway?db=pemMar1>; <http://genome.jgi-psf.org/Brafl1/Brafl1.info.html> [Holland et al. 2008; Putnam et al. 2008]). The genome of the ascidian *C. intestinalis* was published in 2002 (Dehal et al. 2002), and an improved version 2.0 has been released (<http://genome.jgi-psf.org/Cioin2/Cioin2.home.html> [Satou et al. 2008]). Sequencing of the genome of

the appendicularian tunicate *O. dioica* is underway and initial results are available at (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Oikopleura/>). As stated by Henry Gee (2008), “The genome sequence of a species of amphioxus, an iconic organism in the history of evolutionary biology, opens up a fresh vista on the comparative investigation of invertebrate chordates and vertebrates.” In that paper, Gee nicely summarized the state of research on amphioxus, essentially stating that during most of the 20th century, the amphioxus was neglected as a subject of study, . . . now this “eldritch” organism is set to re-enter public life.

Because of these advances in genomics, our symposium brought together the latest findings from invertebrate chordate genomes and agnathan genomes in regard to the evolution of developmental mechanisms and the neuroendocrine systems that are already challenging several current hypotheses and providing directions for new comparative studies.

This was the first symposium to examine the evolution of both developmental mechanisms and of neuroendocrine systems in these jawless chordates.

“Amphioxus and tunicates” are examples of contrasts. Although they share the fundamental chordate body plan with a dorsal, hollow nerve cord, paraxial muscles and notochord, they exemplify the extremes of evolutionary rates and developmental modes. Amphioxus, which is basal in the chordates, is evolving relatively slowly and its genome has retained considerable synteny with those of vertebrates (Holland et al. 2008; Putnam et al. 2008). Thus, amphioxus is proving to be an excellent model for the chordate ancestor. On the other hand, tunicates are evolving rapidly (Holland and Gibson-Brown 2003). Their small genomes have discarded many genes (e.g. several Hox genes, *Gbx*) and duplicated others (two Pax2/5/8 genes, two for gonadotropin-releasing hormone [GnRH] encoding six peptides) (Sherwood et al. 2006). Tunicates also have reduced larval body plans (e.g. only about 330 neurons in the central nervous system of *Ciona* and even fewer in that of *Oikopleura* versus 20,000 in that of amphioxus and millions or more in vertebrates (Nicol and Meinertzhagen 1991). Tunicates are an excellent example of how many genes and how much structure can be thrown away and still have a viable organism. Tunicates show what evolution can do and raise the question of why amphioxus, in particular, has conserved so much.

The genome projects for the three invertebrate chordates are, for the first time, allowing a good look into the neuroendocrine systems. For example, amphioxus has a functional thyroid hormone receptor, and vertebrate triiodothyronine (T3, a thyroid hormone) induces metamorphosis in amphioxus as in the frog (Paris et al. 2008). However, as yet no gene homologous to the vertebrate T3 precursor, thyroglobulin has been found, suggesting that the active compound may be 3,3',5-triiodo-thyroacetic acid (TRIAC) which, in vertebrates, is a breakdown product of T3 (Paris et al. 2008). Amphioxus does have homologs of the primary vertebrate endocrine organs (pineal gland, ovary, testis, endostyle/thyroid, brain, gut, and pituitary), although they are structurally more simple than in their vertebrate counterparts. For example, although the pituitary homolog is considered a homolog of the adenohypophysis, a counterpart of the vertebrate neurohypophysis is lacking. Moreover, analyses of genomes indicate that amphioxus probably lacks homologs of several key pituitary hormones. Thus, pituitary control of reproduction is considered a vertebrate innovation (Holland et al. 2008; Sower et al. 2009).

Analyses of the *Ciona* genome have revealed four GnRH receptors, a corticotropin-releasing hormone (CRH) receptor, and genes for insulin and insulin growth factor and their receptors as well as genes for several somatostatin receptors, calcitonin receptor, and the receptors for CRH, calcitonin and somatostatin (Campbell et al. 2004; Freamat et al. 2006). The emerging picture from amphioxus and tunicates reveals a core neuroendocrine system present at the base of the chordates to which early vertebrates added additional complexity.

Lampreys: as an agnathan, the oldest extant lineage of vertebrates, the sea lamprey has become a model system for analysis of the evolution of many genes and systems, including the evolution of the neuroendocrine regulation of reproduction (Sower 2003; Freamat et al. 2006; Kawauchi and Sower 2006; Silver and Sower 2006; Sower et al. 2009) and the evolution of development (Kuratani et al. 2002, Kuraku et al. 2009). Moreover, lampreys have been key to the question of when the two rounds of whole genome duplications occurred in the vertebrate lineage. Comparisons of vertebrate genomes with those of amphioxus and tunicates were in general agreement in that one whole genome duplication occurred at the base of the vertebrates. However, these data have failed to resolve whether the second duplication occurred before, or after, the agnathan/gnathostome split (Putnam et al. 2008). Recently, Kuraku et al. (2009) concluded from an analysis including 55 gene families in the sea lamprey that both whole genome duplications occurred before the agnathan/gnathostome split. Moreover, although lampreys have not invented such gnathostome characteristics as an immunoglobulin-based adaptive immune system, limbs or jaws, they have many vertebrate features that are not present in amphioxus and tunicates including paired eyes, a complex brain with a telencephalon, and otic and lateral line placodes.

For the lamprey genome, it is estimated that the current coverage of the genome is about 5.9×, which infers that much of the genome has been sequenced. However, given a high level of polymorphism in the sea lamprey, this coverage may not suffice for complete assembly of the genome. Sequences are available for analysis using the trace archives and the partially assembled genome by Ensembl (http://pre.ensembl.org/Petromyzon_marinus/index.html or the site listed above). The phylogenetic position of lampreys as a basal vertebrate establishes them as a basis for understanding the molecular evolution of the genes encoding receptors and hormones that arose in the vertebrate lineage.

The acquisition of a hypothalamic–pituitary axis was a seminal event in vertebrate evolution leading to the neuroendocrine control of many complex functions including growth, reproduction, osmoregulation, stress and metabolism (Sower et al. 2009). To date, biochemical, molecular, immunocytochemical, and functional studies on the structure and function of the GnRHs in lampreys have established that similar to all other vertebrates, the lamprey has a hypothalamic–pituitary–gonadal axis and that there is a high degree of conservation of the mechanisms of GnRH action (Sower 2003; Kavanaugh et al. 2008). Generally, gnathostomes have one or two GnRHs that act as hypothalamic hormones, two pituitary gonadotropins (GTHs) (luteinizing hormone [LH] and follicle stimulating hormone [FSH]), and one gonadal FSH receptor and one LH receptor compared to the lamprey that has three hypothalamic GnRHs, only one pituitary GTH and one gonadal glycoprotein receptor (Sower et al. 2009). Recently, two additional type-2 GnRH receptors have been identified (Aquilina-Beck, A., C. MacDonald, and S.A. Sower, unpublished); all three GnRH receptors are expressed in the pituitary (Silver et al. 2005). The identification of a novel GnRH (Kavanaugh et al. 2008), lamprey GTH beta (Sower et al. 2006) and two glycoprotein receptors—a GTH-like receptor and a TSH-like receptor (Freamat et al. 2006; Freamat and Sower 2008), along with the GnRH receptors, provides an opportunity for comparative and evolutionary analysis of the neuroendocrine system in vertebrates in comparison to basal chordates. Sower et al. (2009) hypothesized that the glycoprotein hormone/glycoprotein hormone receptor systems emerged as a link between the neuro-hormonal and peripheral control levels during the early stages of divergence of the gnathostomes. The significance of the results obtained by analysis of the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–thyroid axes in sea lamprey may transcend the limited scope of the corresponding physiological compartments by providing important clues in respect to the interplay between genome-wide events (duplications), coding sequence (mutation), and expression–control–level evolutionary mechanisms in definition of the chemical control pathways in vertebrates.

Overview of the symposium

The constraints of a single-day symposium necessarily limited the number of papers, although the contributing oral and poster sessions nicely complemented the symposium. The first paper by Holland and Short provides a review of the evolution

and roles of alternative splicing in embryogenesis and the endocrine system. Particular emphasis was on the Pax family of transcription factors, which play key roles in development of many organs, including the endostyle, homologous to the vertebrate thyroid. The importance of alternative splicing in development and physiology/endocrinology is only just coming to light. In the next paper, Kano describes genomic and developmental approaches to identify an ascidian homolog of the vertebrate adenohypophysis. Although an extensive *in silico* survey of the genome of *C. intestinalis* (tunicate) did not have any vertebrate pituitary-hormone genes within the ascidian neural complex, the author argues that based on immunohistochemistry, the ciliated funnel, may be considered a proto-pituitary.

Kubokawa et al. provide the most updated information for amphioxus on the expression and synteny of thyrostimulin (ancestral type of vertebrate glycoprotein hormones) and sex steroidogenic enzymes. The authors discuss the evolution of the neuroendocrine control of these hormones and relate it to reproductive function. Next, Paris et al. describe the latest findings on the role of thyroid hormone metabolism in regulating metamorphosis in amphioxus. These authors provide key information that the thyroid hormone derivatives, TRIAC, are involved in upregulation or downregulation of thyroid hormone receptor and deiodinases during metamorphosis.

The paper by Zeller reports a computational analysis of the genes that comprise the *Ciona* operons. The results from his study suggest that like *Caenorhabditis elegans*, operons in *Ciona* are composed of several genes that are not necessarily related in function. The genes in only 50% of the operons appear to be co-regulated, suggesting that more complex gene regulatory mechanisms are likely operating. Roch and Sherwood's paper describes the identification and analyses of three stanniocalcins from two invertebrates, *C. intestinalis* (tunicate) and *B. floridae* (amphioxus). The genes and deduced proteins share several key structural features found in all previously described homologs. The data suggest that the amphioxus stanniocalcin is most similar with the common ancestor of vertebrate stanniocalcins.

The final papers discuss the development and neuroendocrine systems in lampreys. Retaux and Kano investigate the possibility that embryonic midline signaling systems have been a driving force for the evolution of the forebrain in vertebrates by examining the *Sonic Hedgehog/Hedgehog* (*Shh/Hh*) signaling in lampreys. Their results show that the

Shh/Hh locus is one of the best loci for studying genomic evolution with regard to developmental events. The paper by Freamat and Sower examines the evolution of the four domains of the vertebrate glycoprotein receptor using lamprey and rat chimeric receptors. Their data show that the functional roles of each of the lamprey glycoprotein-receptor 1 are conserved with its gnathostome homologs.

Karaku's paper provides an overview of the difficulties in deciphering the timing of whole genome duplications in the early-branching lineages, lampreys and hagfish, in vertebrates. He proposes that comparative studies involving molecules should be based on solid assessment of orthology/paralogy. He provides three examples that point out potential problems involved in molecular phylogeny after whole genome duplication. He concludes that it is crucial to employ sound molecular phylogenetic approaches and include as many relevant sequences as possible in examining whole genome duplications.

Finally, Smith and Amemiya provide unique evidence that lamprey and hagfish, unlike gnathostomes, undergo extensive programmed genome rearrangements during embryonic development. These authors show that the adaptive immune receptors and the mechanisms of rearrangement that are used by extant agnathans clearly evolved independently of the gnathostome system.

Acknowledgments

The organizers thank the Society for Integrative and Comparative Biology (SICB) staff and especially SICB Program Officer, Dr Eduardo Rosa-Molinar, for their excellent support of our effort.

Funding

The symposium was supported by National Science Foundation Grant IOS 0938420 and the Divisions of Comparative Endocrinology and Developmental and Evolutionary Biology, Society of Integrative and Comparative Biology.

References

- Campbell RK, Satoh N, Degnan BM. 2004. Piecing together evolution of the vertebrate endocrine system. *Trends Genet* 20:359–66.
- Dehal P, et al. 2002. The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* 298:2157–67.
- Freamat M, Kawachi H, Nozaki M, Sower SA. 2006. Identification and cloning of a glycoprotein hormone receptor from sea lamprey, *Petromyzon marinus*. *J Mol Endocrinol* 37:135–46.
- Freamat M, Sower S. 2008. A sea lamprey glycoprotein hormone receptor similar with Gnathostome thyrotropin hormone receptor. *J Mol Endocrinol* 41:219–28.
- Gee H. 2008. Evolutionary biology: the amphioxus unleashed. *Nature* 453:999–1000.
- Holland L, Gibson-Brown J. 2003. The *Ciona intestinalis* genome: when the constraints are off. *Bioessays* 25:529–32.
- Holland LZ, et al. 2008. The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Res* 18:1100–11.
- Kavanaugh SI, Nozaki M, Sower SA. 2008. Origins of gonadotropin-releasing hormone (GnRH) in vertebrates: identification of a novel GnRH in a basal vertebrate, the sea lamprey. *Endocrinology* 149:3860–69.
- Kawachi H, Sower SA. 2006. The dawn and evolution of hormones in the adenohypophysis. *Gen Comp Endocrinol* 148:3–14.
- Kuraku S, Meyer A, Kuratani S. 2009. Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before, or after? *Mol Biol Evol* 26:47–59.
- Kuratani S, Kuraku S, Murakami Y. 2002. Lamprey as an evo-devo model: lessons from comparative embryology and molecular phylogenetics. *Genesis* 34:175–83.
- Nicol D, Meinertzhagen IA. 1991. Cell counts and maps in the larval central nervous system of the ascidian *Ciona intestinalis* (L.). *J Comp Neurol* 309:415–29.
- Paris M, et al. 2008. Amphioxus postembryonic development reveals the homology of chordate metamorphosis. *Curr Biol* 18:825–30.
- Putnam NH, et al. 2008. The amphioxus genome and the evolution of the chordate karyotype. *Nature* 453:1064–71.
- Satou Y, et al. 2008. Improved genome assembly and evidence-based global gene model set for the chordate *Ciona intestinalis*: new insight into intron and operon populations. *Genome Biol* 9:R152.
- Sekiguchi T, Kawashima T, Satou Y, Satoh N. 2007. Further EST analysis of endocrine genes that are preferentially expressed in the neural complex of *Ciona intestinalis*: receptor and enzyme genes associated with endocrine system in the neural complex. *Gen Comp Endocrinol* 150:233–45.
- Sherwood NM, Tello JA, Roch GJ. 2006. Neuroendocrinology of protochordates: insights from *Ciona* genomics. *Comp Biochem Physiol A Mol Integr Physiol* 144:254–71.
- Silver MR, Nucci NV, Root AR, Reed KL, Sower SA. 2005. Cloning and characterization of a functional type II gonadotropin-releasing hormone receptor with a lengthy carboxy-terminal tail from an ancestral vertebrate, the sea lamprey. *Endocrinology* 146:3351–61.
- Silver MR, Sower SA. 2006. Functional characterization and kinetic studies of an ancestral lamprey GnRH-III selective type II GnRH receptor from the sea lamprey, *Petromyzon marinus*. *J Mol Endocrinol* 36:601–10.

- Sower SA. 2003. The endocrinology of reproduction in lampreys and applications for male lamprey sterilization. *J Great Lakes Res* 29:50–65.
- Sower SA, Freamat M, Kavanaugh SI. 2009. The origins of the vertebrate hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) endocrine systems: new insights from lampreys. *Gen Comp Endocrinol* 161:16120–29.
- Sower SA, Moriyama S, Kasahara M, Takahashi A, Nozaki M, Uchida K, Dahlstrom JM, Kawauchi H. 2006. Identification of sea lamprey GTHbeta-like cDNA and its evolutionary implications. *Gen Comp Endocrinol* 148:22–32.