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Review

# Neuroendocrinology of protochordates: Insights from *Ciona* genomics☆

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## Abstract

The genome for two species of *Ciona* is available making these tunicates excellent models for studies on the evolution of the chordates. In this review most of the data is from *Ciona intestinalis*, as the annotation of the *C. savignyi* genome is not yet available. The phylogenetic position of tunicates at the origin of the chordates and the nature of the genome before expansion in vertebrates allows tunicates to be used as a touchstone for understanding genes that either preceded or arose in vertebrates. A comparison of *Ciona*, a sea squirt, to other model organisms such as a nematode, fruit fly, zebrafish, frog, chicken and mouse shows that *Ciona* has many useful traits including accessibility for embryological, lineage tracing, forward genetics, and loss- or gain-of-function experiments. For neuroendocrine studies, these traits are important for determining gene function, whereas the availability of the genome is critical for identification of ligands, receptors, transcription factors and signaling pathways. Four major neurohormones and their receptors have been identified by cloning and to some extent by function in *Ciona*: gonadotropin-releasing hormone, insulin, insulin-like growth factor, and cionin, a member of the CCK/gastrin family. The simplicity of tunicates should be an advantage in searching for novel functions for these hormones. Other neuroendocrine components that have been annotated in the genome are a multitude of receptors, which are available for cloning, expression and functional studies.

*Keywords: Ciona intestinalis*; Cionin; Gonadotropin-releasing hormone (GnRH); Insulin; Insulin-like growth factor (IGF); Neuroendocrinology; Hormone receptors; Tunicates

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## 1. Introduction

The genomes have been sequenced for two protochordate species, *Ciona intestinalis* (Fig. 1) and *Ciona savignyi*. The first draft of the genome for *C. intestinalis* has been published and annotated (Dehal et al., 2002) (http://www.jgi.doe.gov/ciona). The sequence of the genome for the closely related species, *C. savignyi*, has been released although not published (http://www.broad.mit.edu/annotation/ciona). This review is almost exclusively on *C. intestinalis* rather than *C. savignyi* because the genome of the latter is not yet annotated. The importance of this achievement is related in part to understanding the origin of the vertebrates from an ancestral chordate. Several hypotheses involving ancestral tunicates have been suggested for the evolutionary events leading to early vertebrates (see Gee, 1996; Lacalli, 2005). The suggestions are based in part on the resemblance of the tunicate larva to the vertebrate tadpole and on the similar development and body plan of the tunicate larva and chordate embryos. Despite such observations, the identity of the ancestor and its swimming ability remain unknown. The complexity of the vertebrates compared to the tunicates or cephalochordates (amphioxus) appears to have developed as a result of expansion of the genome through duplications in early vertebrates (Holland et al., 1994; Furlong and Holland, 2002). The mechanism of expansion is controversial as to whether whole-genome duplication or segmental duplications occurred. However, the observation that invertebrates have one *Hox* cluster, whereas mouse and human have four clusters gave rise to the inference that there were “two rounds of extensive gene duplication (perhaps by complete or partial tetraploidization)” (Holland et al., 1994). In further support for this theory, many regions of the vertebrate genome, such as the major histocompatibility complex (MHC), have expanded compared to invertebrates. However, other evidence suggests that smaller duplications may have been the driving factor in explaining the origin of vertebrates (Spring, 2002).

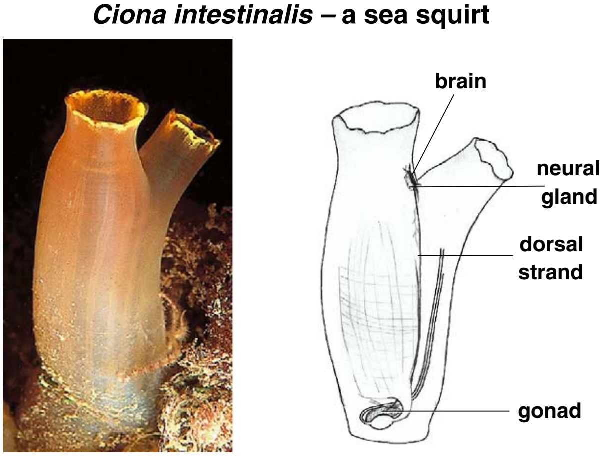

Fig. 1. *Ciona intestinalis* is shown as a photo (by Lukk Vindu) and as a drawing.

Another issue in considering tunicates as a model in evolution is that modern tunicates, both larva and adult, appear to be highly derived (Lacalli, 2005). The modern sessile asymmetrical adult may be derived from a motile, bilaterally symmetrical ancestor. Research on the organization of *Hox* genes has been very helpful in deducing evolutionary changes that have occurred in the tunicate lineage. All bilateral invertebrates have one cluster of *Hox* genes, whereas mammals have four clusters, which are likely to be duplications of the single cluster at a time near the origin of vertebrates (Holland et al., 1994). The order of the *Hox* genes (13 are identified) on the chromosome has been shown to influence the anterior–posterior axis of the body. The putative chordate ancestor is thought to have had one *Hox* cluster with 13 genes and indeed, the sea urchin (Echinodermata) and amphioxus (Cephalochordata) have relatively intact *Hox* gene clusters. However, early in the tunicate lineage there was probably a breakage of the *Hox* gene cluster and extensive shuffling of the genome (Ikuta and Saiga, 2005). As a result, the single *Hox* cluster of genes in *Ciona* (both *C. intestinalis* and *C. savignyi*) is split between two chromosomes and four genes (*Ci-Hox7*, *8*, *9*, *11*) are lost. Also, the remaining *Ciona Hox* genes have long distances between each other and the genes are expressed in an unusual spatial pattern. These changes in the tunicates after separation from the lineage leading to amphioxus and vertebrates may be responsible for the simplification of the larva and unusual body plan and sessile nature of adults. The disruption of the *Hox* cluster and genome in tunicates suggests their ancestor had a different body plan.

As a model, *Ciona* still appears to be very useful, provided one understands the changes that have occurred in modern *Ciona*. One reason for selecting the tunicates for genome sequencing was their phylogenetic position as both an