Insight into cephalochordate evolution from a genomic study of the Bahama amphioxus, *Asymmetron lucayanum*

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Abstract

Cephalochordates (commonly known as amphioxus or lancelets) refers to a group of marine animals that are the modern survivors of one ancestral chordate lineage whose phylogenetic position falls at the boundary between invertebrates and vertebrates. Previous study showed that they possess many primitive features in morphology, development and also genomic architecture and thus are considered as the best living proxy for ancestral chordates before the rise of vertebrates. In this study, we performed both RNA-Seq and whole genome shotgun sequencing for the Bahama amphioxus, *Asymmetron lucayanum*. The comparison between *Asymmetron lucayanum* with another distantly related cephalochordate species, *Branchiostoma floriade*, together with several representative vertebrate species was conducted for both coding and noncoding regions. For the coding regions, we found cephalochordates evolve more slowly than vertebrates, which is consistent with their apparent morphological evolutionary stasis. However, genes involved in innate immunity are notably evolving faster. For the noncoding regions, we identified 167,636 cephalochordate conserved noncoding elements (cCNEs) shared between *Asymmetron* and *Branchiostoma* using a reads-mapping based approach. We also examined the genomic distribution, functional association and evolutionary trajectories of these cephalochordate CNEs in the context of chordate evolution.

Introduction

1. *Asymmetron lucayanum*, the Bahama amphioxus, represent one of three genera of amphioxus (or lancelets). The other two genera are *Epinotichthys* and *Branchiostoma*. These three genera make up the cephalochordate sub-phyllum.

2. Both morphological and molecular data from *Branchiostoma* suggest that cephalochordates are the best living proxy for ancestral chordates.

Materials & Methods

1. Sequencing materials and methods: one adult for whole genome shotgun (WGS) sequencing; pooled adults and larvae (~20h) for RNA-Seq sequencing

2. Bioinformatics tools: de novo assembling RNA-Seq reads; Trinity; aligning WGS reads to the B. floriade ref genome: stampy; orthology detection: proteinortho; phylogenomic analysis: PhyML, MrBayes; molecular dating: MCMCTree, r8s; querying cCNE in vertebrates: Lastz

Results

1. Slow evolution of cephalochordates at the molecular level

2. Genes involved in innate immunity are fast-evolving in cephalochordates

Given considerable saturation at the synonymous sites, nonsynonymous substitution rate (Ka) was employed as the proxy to measure molecular evolutionary rates for *Asymmetron-Branchiostoma* orthologous gene pairs. Those with top 5% Ka were defined as fast-evolving genes and their associated functional categories were examined by Gene Ontology (GO) term enrichment analysis; alternative cutoffs (top 10% and top 15%) were also examined to ensure the robustness of this analysis. 61 GO terms that are overrepresented in our fast-evolving gene set regardless which RNA-Seq library or Ka cutoff was used, while >77% of them are neural/immune-related GO terms. Many genes involved in innate immunity (e.g. genes regulating neutrophil migration, Nod-like receptor genes, scavenger receptor genes, complement system’s C1q-like genes) show up on the fast-evolving gene list.

Conclusion

Comparative genomics study on different cephalochordate species can be of great value in understanding the biology and evolution of this important group of animals.