Developmental and Comparative Immunology 87 (2018) 36–50

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/devcompimm

Comparative analysis of long non-coding RNAs in Atlantic and Coho salmon reveals divergent transcriptome responses associated with immunity and tissue repair during sea lice infestation

Valentina Valenzuela-Muñoz, Diego Valenzuela-Miranda, Cristian Gallardo-Escárate*

Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research, University of Concepcion, Barrio Universitario s/n, Concepción, Chile

ARTICLE INFO

Keyswords:
LncRNA
Atlantic salmon
Coho salmon
C. rogercresseyi

ABSTRACT

The increasing capacity of transcriptomic analysis by high throughput sequencing has highlighted the presence of a large proportion of transcripts that do not encode proteins. In particular, long non-coding RNAs (lncRNAs) are sequences with low coding potential and conservation among species. Moreover, cumulative evidence has revealed important roles in post-transcriptional gene modulation in several taxa. In fish, the role of lncRNAs has been scarcely studied and even less so during the immune response against sea lice. In the present study we mined for lncRNAs in Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynkus kisutch*), which are affected by the sea louse *Caligus rogercresseyi*, evaluating the degree of sequence conservation between these two fish species and their putative roles during the infection process. Herein, Atlantic and Coho salmon were infected with 35 lice/fish and evaluated after 7 and 14 days post-infestation (dpi). For RNA sequencing, samples from skin and head kidney were collected. A total of 5658/4140 and 3678/2123 lncRNAs were identified in uninfected/infected Atlantic and Coho salmon transcriptomes, respectively. Species-specific transcription patterns were observed in exclusive lncRNAs according to the tissue analyzed. Furthermore, neighbor gene GO enrichment analysis of the top 100 highly regulated lncRNAs in Atlantic salmon showed that lncRNAs were localized near genes related to the immune response. On the other hand, in Coho salmon the highly regulated lncRNAs were localized near genes involved in tissue repair processes. This study revealed high regulation of lncRNAs closely localized to immune and tissue repair-related genes in Atlantic and Coho salmon, respectively, suggesting putative roles for lncRNAs in salmon against sea lice infestation.

1. Introduction

The increasing capacity of next generation sequencing has highlighted the presence of transcripts that do not encode proteins, denominated noncoding RNA (ncRNA). Among the ncRNA transcribed in eukaryotes, two types have been identified; housekeeping ncRNAs (ribosomal, small nuclear, small nucleolar and transfer) and regulatory ncRNAs. Among them, microRNA, piwi-interacting RNA, small interfering RNA, and long non-coding RNA (lncRNA) represent the most abundant ncRNAs (Johnsson et al., 2014; Tang et al., 2017). In particular, lncRNAs are defined as transcripts with a length higher than 200 base pairs and low coding potential. LncRNA can be located in nuclear or cytosolic fractions, can be polyadenylated or not and are transcribed in a similar way to mRNA (Cech and Steitz, 2014; Ponting et al., 2009). It is possible to observe two different classes according to their genomic position, lncRNA transcribed from intron regions in sense or antisense orientations, and lncRNA transcribed from intergenic regions located between known proteins (Mallory and Shkumatava, 2015).

LncRNAs, in contrast to coding sequences, present low degree of sequence conservation among species. In fact, from a study performed in 17 different species it was observed that more than 70% of lncRNAs have no corresponding orthologs, and less than 100 lncRNA sequences have a common ancestor between teleost fish and tetrapod (Hezroni et al., 2015). Where, the authors report only 29 lncRNA conserved between fish and mammals (Hezroni et al., 2015). In addition, lncRNA conservation between species does not only depend on sequence conservation, such as orthologous sequences of coding genes, lncRNA conservation can be given by its sequence, structure, function and syntenic loci (Diederichs, 2014; Johnsson et al., 2014).

Each cell type as a specific LncRNAs are expressed in a cell-specific way, suggesting that each cell type has a specific lncRNA repertoire involved in cell identity and function. In the case of immune cell

* Corresponding author. Universidad de Concepción, P.O. Box 160-C, Concepción, 4030000, Chile.
E-mail address: crisgallardo@udec.cl (C. Gallardo-Escárate).

https://doi.org/10.1016/j.dci.2018.05.016
Received 22 January 2018; Received in revised form 19 May 2018; Accepted 20 May 2018
Available online 24 May 2018
0145-305X/

Table 1
RNA sequencing statistics for Atlantic and coho salmon transcriptomes.

	Atlantic salmon			Coho salmon		
	skin	head kidney	*de novo* assembly	skin	head kidney	*de novo* assembly
Reads (Mb)	37.6	41.2	78.7	37	40.7	77.7
Average length (bp)	199.52	194.39	196.84	203.86	193.91	198.65
Matched (Mb)	23.88 (63.5%)	29.56 (71.7%)	65.9 (83.8%)	23.4 (63.2%)	27.8 (68.3%)	64.1 (82.5%)
Nucleotide number (Gb)	7.5	7.9	15.4	7.5	7.8	15.4
Contigs	252,614	165,171	303,898	259,389	165,879	308,992
Average length (bp)	546	596	554	542	585	547
Singletons (Mb)	13.6	11.5	12.7	13.5	12.8	13.6
Average length (bp)	203.01	198.24	196.85	207.17	191.93	199.35

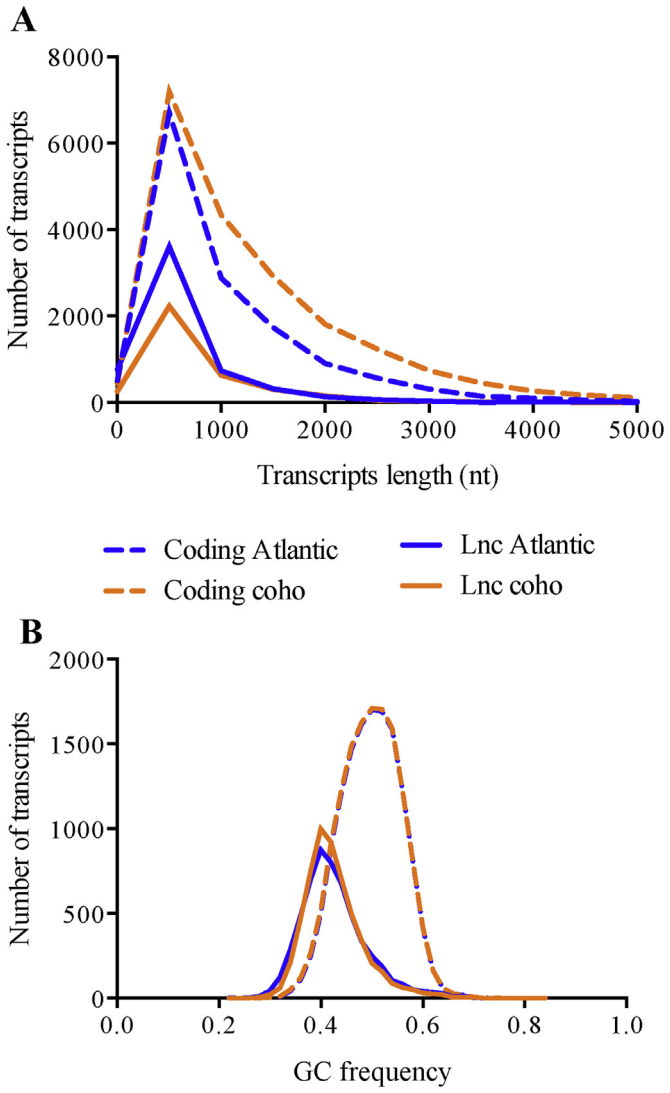

Fig. 1. Features of predicted lncRNAs from Atlantic and coho salmon transcriptomes. A) Length distribution for predicted lncRNAs and coding transcripts. B) Comparison of GC content between lncRNAs and coding transcripts.

development and differentiation, several studies reported a unique lncRNA expression profile, indicating a role of lncRNA in the immune system (Aune and Spurlock, 2016). Indeed, lncRNAs are specifically expressed in T cell linages of Th1, Th2 and Th17 responses in humans (Spurlock et al., 2015). In mammal lncRNA are responsible of myeloid and dendritic cell development as lncRNA denominated Morribid, HOTAIRM1 or lnc-DCs which participated in DC cell differentiation and regulation of STAT3 in presence of antigen (Atianand et al., 2017). Moreover, lncRNAs play important roles during pathogens infestation, for instance, a down regulation of lncRNA NRAV reduce the capacity of virus replication, working as immune component (Atianand et al., 2017). Furthermore, lncRNAs have been demonstrated to possess a regulatory role in important innate immune pathways, such as toll-like receptors (TLR) (Carpenter et al., 2013) and NF-κB (Zhou et al., 2016). The role of lncRNAs in the fish immune response has been poorly studied. However, a transcriptomic study in yellow croaker showed a high number of lncRNAs specifically expressed in fish exposed to polyinosinic-polycytidylic acid (poly I:C). (Jiang et al., 2016). Furthermore, lncRNAs annotated with immune functions were highly expressed in spleen and the upregulated lncRNAs evidenced to be activators of Toll-like receptors signals pathway. Additionally, through RNA-Seq analysis of Atlantic salmon infected with the ISA virus, 4967 putative lncRNAs were differently regulated during viral infection with a positive correlation between the lncRNA abundance and fish ISAv load, showing a putative immune role for lncRNA in Atlantic salmon (Boltaña et al., 2016). Differences in lncRNAs abundance were observed between ISAv target genes, showing a tissue-specific distribution of lncRNA during the infestation (Boltaña et al., 2016). With respect to fish bacterial infection, a study performing in Atlantic salmon infected with *Piscirickettsia salmonis* evidenced a similar expression profiles between coding RNA and lncRNA, suggesting a putative role of lncRNA in fish immune response (Valenzuela-Miranda and Gallardo-Escárate, 2016). This study revealed a positive regulation between lncRNA and neighbor genes associated to *P. salmonis* infection such as *hepcidin, clathrin and haptoglobin* (Valenzuela-Miranda and Gallardo-Escárate, 2016). Furthermore, a recent comparative study among Atlantic salmon infected with virus, bacterial and ectoparasite pathogens demonstrated high modulation of lncRNAs with immune-related genes (Tarifeño-Saldivia et al., 2017). The authors reported high number of lncRNA differently expressed in skin of Atlantic salmon infected with sea lice compared with fish infected with ISAv and *P. salmonis* (Tarifeño-Saldivia et al., 2017). However, comparative studies with respect to the conservation of lncRNAs among salmon species and their expression patterns have not yet been conducted. Herein, we characterized lncRNAs from Atlantic and coho salmon transcriptomes stimulated by the sea louse *C. rogercresseyi,* the most detrimental ectoparasite that affects the Chilean salmon industry. This ectoparasite species can infect both Atlantic and coho salmon, however, different degrees of susceptibility and immune response mechanisms have been reported. In our group, previous transcriptome studies have suggested differences between Atlantic and Coho salmon, for instance, infected Atlantic salmon increases the transcription levels of the TLR22 gene and deplete the cellular iron availability, while coho salmon displays pro-inflammatory mechanisms in response to the infestation (Valenzuela-Muñoz et al., 2017). Furthermore, differences in iron regulation has been observed between both salmon species infected with *C. rogercresseyi*, suggesting high regulation of heme degradation and iron transport genes in Atlantic salmon comparing with Coho salmon (Valenzuela-Muñoz et al., 2017). Due the differences in the responses mechanisms of Atlantic and Coho salmon to *C. rogercresseyi* infestation, we aimed to explore the