



Comprehensive non-coding RNA analysis unveils lncRNA- and circRNA-mediated ceRNA networks regulating ER stress response in *Patinopecten yessoensis* under thermal stress

HANG Yunna, WANG Yuhua, PANG Yuming, TANG Anqi, WANG Xubo, TIAN Ying, HAO Zhenlin, DING Jun, MAO Junxia*, CHANG Yaqing
Key Laboratory of Mariculture & Stock Enhancement in North China's Sea, Ministry of Agriculture and Rural Affairs
College of Fisheries and Life Sciences, Dalian Ocean University, Dalian 116023, China.)

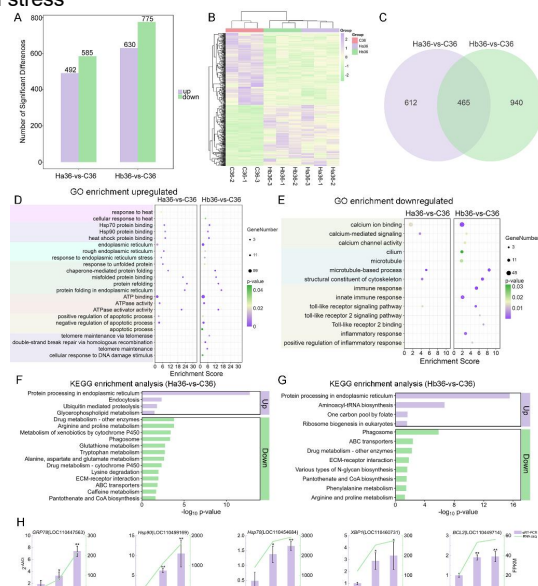
Introduction

Patinopecten yessoensis, a vital aquaculture species in North China, has recently suffered massive deaths in summer. In this study, whole transcriptome sequencing was used to analyse expression changes of non-coding RNAs in gills of *P. yessoensis* under different heat stress. There were 2017, 120, 1477 and 1722 mRNAs, miRNAs, lncRNAs and circRNAs differentially expressed after heat stress. The lncRNA/circRNA-miRNA-mRNA ceRNA networks were constructed. Functional analysis revealed processes of heat stress, Hsp binding, endoplasmic reticulum (ER) stress, protein folding, energy metabolism, apoptosis, chromosomal and DNA stability maintenance, calcium regulation, microtubule activity and immune response related functions significantly enriched, indicating *P. yessoensis* probably activated ER stress and apoptosis, and suppressed ciliary activity to adapt to high temperature, and the above processes were regulated by non-coding RNA. Further, key genes of ER stress, e.g., *GRP78*, *HSP90*, *Hsp70*, were competitively regulated by lncRNA/circRNA with miRNA. The study explored the regulatory mechanism of non-coding RNA in response to heat stress in *P. yessoensis*.

Results

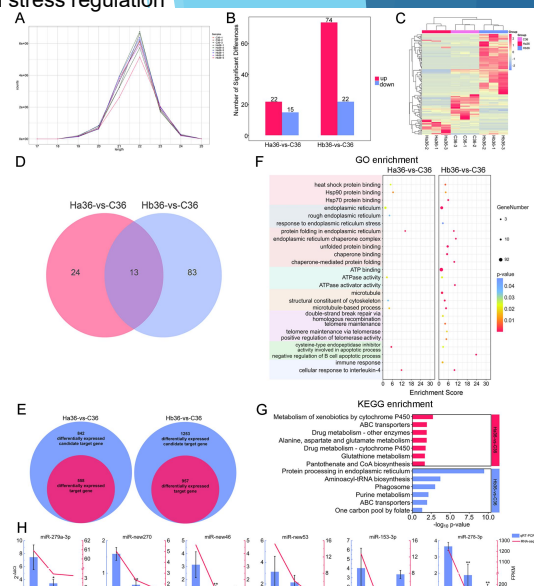
1. Expression patterns of mRNA under thermal stress

GO results demonstrated significant enrichment in the following biological processes: heat stress, Hsp binding, endoplasmic reticulum (ER) stress, protein folding, energy metabolism, apoptosis, chromosomal and DNA stability maintenance, calcium regulation, microtubule activity and immune response related functions. KEGG pathway of protein processing in endoplasmic reticulum (myi04141) was significantly upregulated.



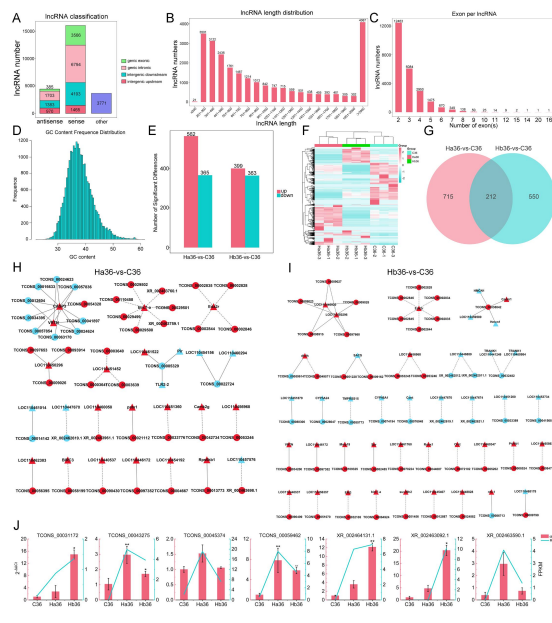
2. Identification of miRNAs involved in thermal stress regulation

Through small RNA-seq, a total of 2361 miRNAs identified, with 1564 known and 797 novel miRNAs. The identified miRNAs were mainly concentrated in the length of 22 nt. The target relationships between DemiRs and DEGs were predicted through co-expression and sequence interaction analysis. In the Ha36 group, 20 DemiRs were identified targeting 558 DEGs (DEMTs), while in the Hb36 group, 74 DemiRs were found to target 957 DEGs.



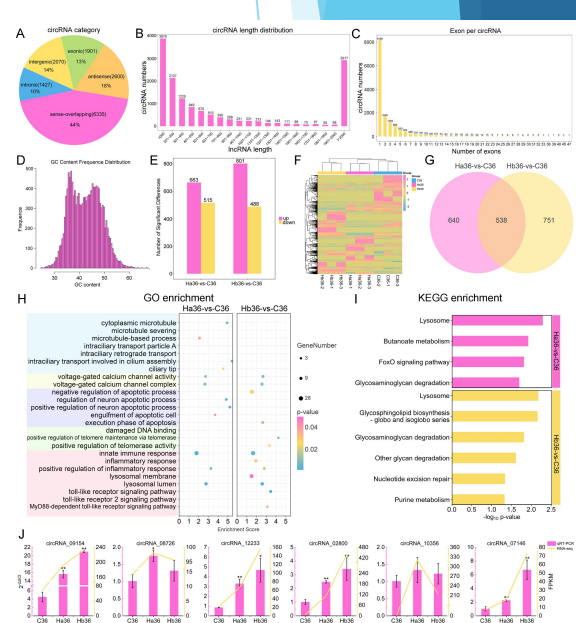
3. Identification of lncRNAs involved in thermal stress regulation

Gene co-expression analysis revealed that 912 and 741 DElncs exhibited significant correlations with 1,077 and 1,405 DEGs in the Ha36 and Hb36 groups, respectively. In the Ha36 group, 19 DElncs were located near (within 100 kb) 13 DEGs, which were considered cis-regulatory targets. Additionally, 25 DElncs demonstrated direct sequence interactions (with at least 10 interacting bases and free binding energy not exceeding -100) with 14 DEGs, classified as trans-regulatory targets. In the Hb36 group, 34 DElncs exerted cis-regulation on 27 DEGs, and 18 DElncs performed trans-regulation on 17 DEGs.



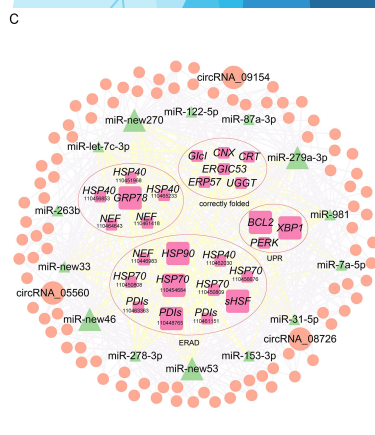
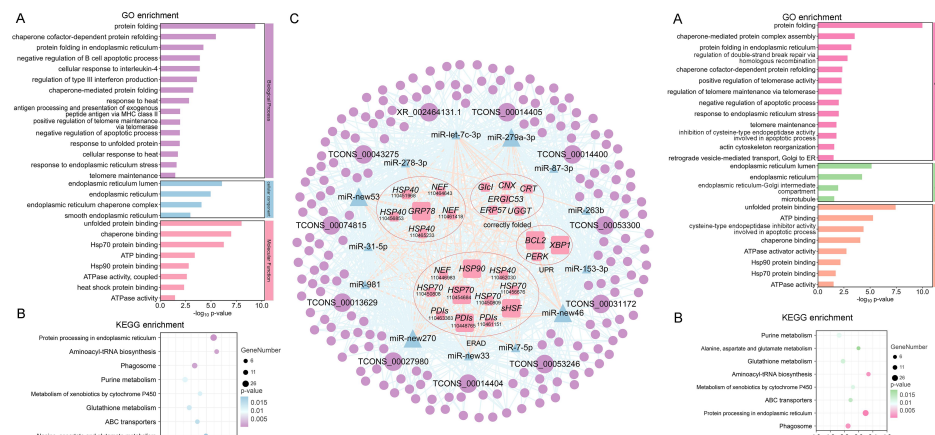
4. circRNAs identification involved in thermal stress regulation

These circRNAs were classified into five types, including sense-overlapping, antisense, intergenic, exonic, and intronic circRNAs. The histogram of length and exon number distribution showed that most circRNAs had a length less of less than 300 bp with one exon. The GC content was mainly concentrated between 35%-50%. GO and KEGG enrichment analysis with DEcirc host genes indicated these DEcircs were mainly from cilia activity, endocytosis, immune, apoptosis, lysosome, and glycan metabolism related genes.



5. lncRNA and circRNA-mediated ceRNA regulatory networks involved in thermal stress

There were 421 DElncs, 67 DemiRs, and 1281 DEGs performing lncRNA-mediated ceRNA interactive relationships in the thermal-stressed groups, and there were 198 DElncs, 13 DemiRs, and 26 DEGs exhibiting interactive relationships in the protein processing in endoplasmic reticulum (myi04141) pathway. To elucidate the regulatory function of circRNAs in Yesso scallops' response to thermal stress, interactive networks including 207 DEcircs, 80 DemiRs, and 1268 DEGs were constructed, and there were 95 DEcircs, 14 DemiRs, and 26 DEGs exhibiting complex interactive relationships to response to thermal stress.



Conclusion

- In the present study, to explore the molecular regulatory mechanisms underlying thermal stress response in the Yesso scallop, integrated analysis of coding and non-coding RNAs was performed in gill tissues of the species under different high temperatures by whole-transcriptome RNA sequencing.
- Expression profile changes of mRNA, miRNA, lncRNA, and circRNA were systematically analysed. lncRNA- and circRNA-mediated ceRNA networks were separately constructed, and ERS response related ceRNA networks were identified and validated.
- Significantly, crosstalk between lncRNA-mediated and circRNA-mediated ceRNA networks were detected. Some lncRNAs and circRNAs regulated the same gene expression by binding to the same miRNAs.
- Core genes and ncRNAs and their interactive mechanisms involved in thermal stress in the Yesso scallop were elucidated, which provided new insights into regulatory mechanisms of marine mollusks in response to thermal stress.