Development of mRNA knock-down system via dsRNA-mediated RNA interference in oocytes and early embryos of Pacific abalone Haliotis discus hannai

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Development of mRNA knock-down system via dsRNA-mediated RNA interference in oocytes and early embryos of Pacific abalone *Haliotis discus hannai* （エゾアワビ卵母細胞および初期胚における母性ｍＲＮＡノックダウン法の開発）		

Abalone are the top priced seafood species in the market due to their delicate flavor and rich nutritional value. During the last decade, the species has been intensively captured and natural populations have become depleted. Production of abalone mainly relied on aquaculture because of the market demand. The annual global production of farmed abalone is currently on the rise, and most of the increases are in production of Black abalone *Haliotis discus discus* and a subspecies of Pacific abalone, Ezo abalone *Haliotis discus hannai*, which are cultured in China and Korea. Globally, the highest annual production of farmed abalone species is for Pacific abalone species. However, despite these significant increases in production, several critical issues, such as slow growth rates and diseases, affect the abalone farming industry. The sterilization of artificial seeds of abalone is an ideal strategy to promote faster growth and to avoid sexual precocity. Recently, RNA interference (RNAi) is the popular method for the production of sterile animals in vertebrates and invertebrates, and identification of target gene for this technology is a crucial step to the production of sterilization.

In the present study, to obtain candidate genes involved in germ cell development as targets of RNAi experiments, we conducted transcriptome analysis of Black abalone for gene discovery in multiple tissues: brain, ovary, testis and unfertilized eggs. A total of 211,678,906 high quality reads were obtained and assembled into 234,353 unigenes, of which 24.9% were functionally annotated. Based on differences in the reads per kilobase of transcripts per million (RPKM) values of annotated unigenes, 48.6% of annotated unigenes were identified by Venn diagram analysis as having universal or tissue-specific expression. Three search strategies were carried out to identify candidate genes characterized by the Venn diagram. Twenty-three genes with gonad-biased gene ontology (GO) terms were first obtained by querying the GO database. Secondly, 36 genes were found by screening known gene names related to germ cell development, such as germ cell and meiotic markers, in the annotated unigene database. Finally, 17 genes were obtained by querying the annotated unigene database for zygotically expressed gonadal genes (ovary and testis) and maternally expressed gonadal genes (ovary, testis and unfertilized eggs) using keywords related to reproduction. To further verify expression pattern of these genes, RT-PCR of 15 candidate genes and a unigene encoding a germ cell marker, *vasa*, were performed with cDNA prepared from mature and immature gonads, unfertilized eggs and different somatic tissues. Of these genes, 5 candidate genes showing gonad-specific expression patterns and *vas*a were further selected to detect sub-cellular localization in the gonads by in situ hybridization. The results showed that *vasa* was expressed mainly in the early developmental stages of germ cells in both sexes. One of the candidate genes, *vitelline envelope zona pellucida domain protein* 12 (*ZP12*), was expressed in the primordial germ cells of immature gonad and early developmental stages of germ cells of the adult female, whereas no signals were detected at any developmental stage of germ cells from the other 4 genes. The results obtained from the present study suggest that *vasa* and *ZP12* are involved in germ cell development of Pacific abalone and that *ZP12* is an especially useful germ cell-specific marker in immature adults.

The *vasa* gene, a member of the DEAD-box gene family, has been reported to be restricted mainly to the germ cell lineage. The gene plays a crucial role in the development of germ cell lineage and is the most reliable molecular marker for studies on germ cell development in vertebrates and invertebrates. However, little is

known about the expression pattern and function of *vasa* gene in Pacific abalone. Thus, We cloned the full-length cDNA of *vasa* homologue gene of Ezo abalone and analyzed its tissue distribution and expression pattern in different developmental stages of pacific abalone. In this study, the full-length cDNA of *vasa* gene was isolated and characterized in Pacific abalone, an economically important bivalve. The cDNA sequence of *vasa* was 3866 base pairs (bp) in length and encode a peptide of 801 amino acid residues including highly conserved domains of DEAD-box protein family. It was mainly expressed in both gonads, unfertilized eggs and somatic tissues. and its mRNA was predominantly localized in oogonia and stage 1-3 oocytes of ovary, in spermatogonia and primary spermatocytes of testis. In unfertilized egg, *vasa* was detected in animal hemisphere. During embryogenesis, it was strongly expressed in the irregular patches of animal portion of 32-cell stage. Later on, it was restricted to cells of ventral paraxial bands in trochophore and the fields of visceral mass in veliger. To evaluate effects of *vasa* knockdown, the dispersion and inhibitor effect of *vasa* dsRNA in ovary were examined. After 12 hours post-injection, dsRNA with *vasa* DIG-labelled delivered to full-grown oocytes of female. Expression levels of *vasa* in RNAi group using 3 different dose of dsRNA were significantly lower than control group at 7 days post-injection (dpi) and 14 dpi. Similar inhibitor effects were observed in unfertilized eggs and 8-cell stage of embryos obtained from treated females using 100 μg *vasa* dsRNA. The results suggest that *vasa* is essential genes for germ cell development and could serve as a germ cell marker. Further, RNAi of *vasa* gene can be effectively utilized in oocytes and early embryos of pacific abalone to reduce amount of mRNA level.