Space-time Expression Characteristics analysis of 4 ABC genes in the embryonic development of *Neocaridina denticulata sinensis*

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Abstract

ATP-binding cassette (ABC) transporter is a membrane protein superfamily. ABC transporters involve in cytology processes, such as substance transmembrane transport and cellular detoxification. The numbers of ABCG and ABCG subfamily play a role in carotenoid transmembrane transport, but there are few reports in pigmentation of crustacean. The all four types color cells of *Neocaridina denticulate sinensis* have finished differentiation and proliferation. There are huge differenece in types and quantities of color cell of varied color strains. In order to study the function of ABCG and ABCB in pigmentation of *N. denticulate sinensis* embryo, the expression of *ABCG2*、*ABCG5*、*ABCB6*、*ABCB8* in red, yellow, bule strains and wild type were analyzed by qPCR and whole mounting *in situ* hybridization. qPCR results showed that the expression levels of the four genes were relatively stable before the gastrula stage, decreased in the nauplius stage Ⅰ, and increased from nauplius stage II to the highest expression levels in the nauplius stage IV. The results of whole mounting *in situ* embryo hybridization showed that the patterns of the four ABC genes were similar. There were positive signals from cleavage stage to nauplius stage Ⅳ, whose intensity was consistent with the qPCR. The expression patterns from cleavage stage to nauplius stage III were similar, positive signals were distributed in the cells around the yolk. The positive signal was strongest in the stage without nauplius stage Ⅳ, in which the patterns were different in four color strains. The positive signals of red strain were concentrated in ommateum and cephalothorax, and the positive signal was the most obvious in ommateum. The positive signals of yellow strain were distributed in ommateum and gill. The positive signals of blue strain and wild strain were distributed in ommateum, gill and somite junctions. However, no hybridization signal appeared in all strains and periods with positive probes as negative control. The expression levels of the four genes were correlative with the pigment cells quantities of nauplius. Moreover, there were different space expression patterns in four strains. These results suggested that the four genes might play a role in pigmentation.

Introduction

Neocaridina denticulata sinensis has the characteristics of gorgeous color, easy feeding, weak fighting and short breeding cycle. Since 2003, it has been popular in the market as an ornamental shrimp.Color formation involves many cytological processes, such as pigment synthesis, transport and pigment body formation. Most of the known body color variations are caused by pigment transporter or transcription mutation. One of the most striking is the ATP binding cassette transporter family.ABC is a kind of transmembrane transport carrier, which is distributed on all membrane structures except nuclear membrane and endoplasmic reticulum. It has a wide variety of substrates, including almost all metabolites and foreign substances. Members of the G subfamily of ABC family were first found to be involved in compound eye pigment deposition in Drosophila compound eye mutants, which were named white, Scarlett and brown.In the early stage of this experiment, qPCR was used to quantitatively analyze ABC gene in embryos, so as to further understand the function and expression localization of ABC gene, track the expression localization of ABC gene during embryonic development through hybridization signals, understand the gene function and expression pattern of ABC gene during embryogenesis, and lay a foundation for further understanding the body color formation mechanism of *N. denticulate sinensis*.

Methods

1 Cultivation of red, yellow, blue and wild body colors of *N. denticulate sinensis*.
2 RNA extraction of *N. denticulate sinensis*.
3 Preparation of cDNA template.
4 Observation and collection of embryos at various developmental stage.s
5 4% PFA fixed embryo
6 Preparation of whole mounting *in situ* embryo hybridization probes for ABCG2, ABCG5, ABCB6 and ABCB8 genes.
7 whole mounting *in situ* embryo hybridization of four strains at different stages.

Resultas

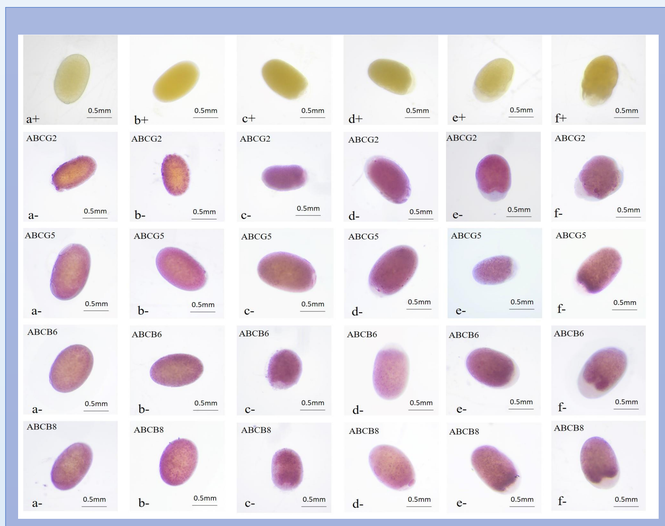

Figure. 1 Results of whole mounting *in situ* embryo hybridization of four ABC genes in red strains of *N. denticulate sinensis.*
a-—f- .antisense probe, a+—f+. sense probe; *a.* cleavage stage, b. blastula stage, c. gastrula stage, d. nauplius stage I, e. nauplius stage II, f. nauplius stage III, et sequential.

Figure. 2 Results of whole mounting *in situ* embryo hybridization of four ABC genes in yellow strains of *N. denticulate sinensis.*

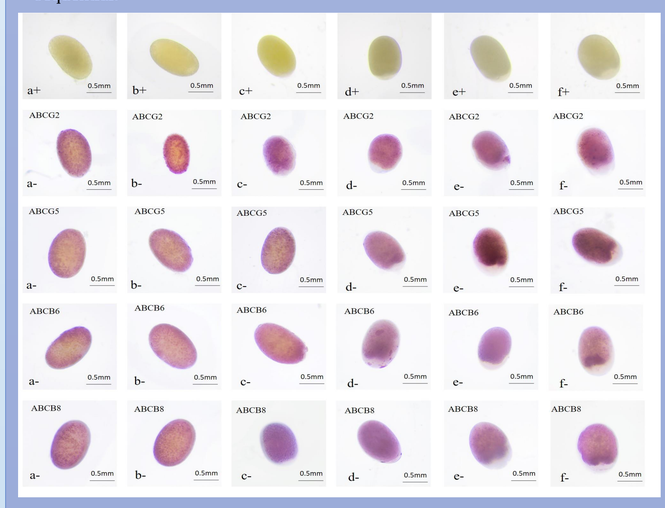

Figure. 3 Results of whole mounting *in situ* embryo hybridization of four ABC genes in blue strains of *N. denticulate sinensis.*

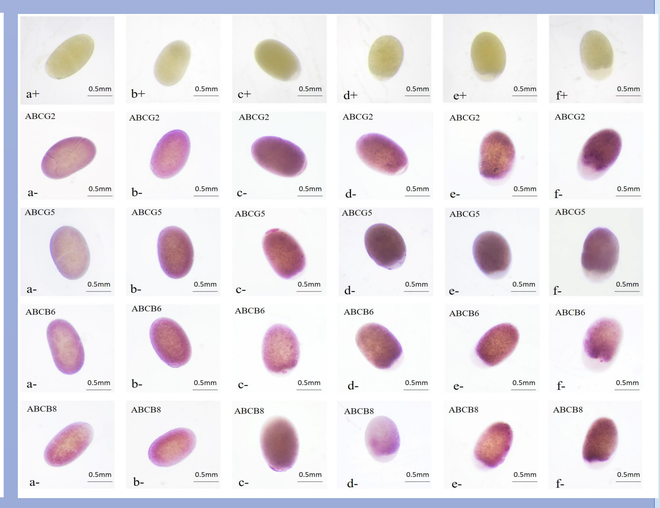

Figure. 4 Results of whole mounting *in situ* embryo hybridization of four ABC genes in wide type of *N. denticulate sinensis.*

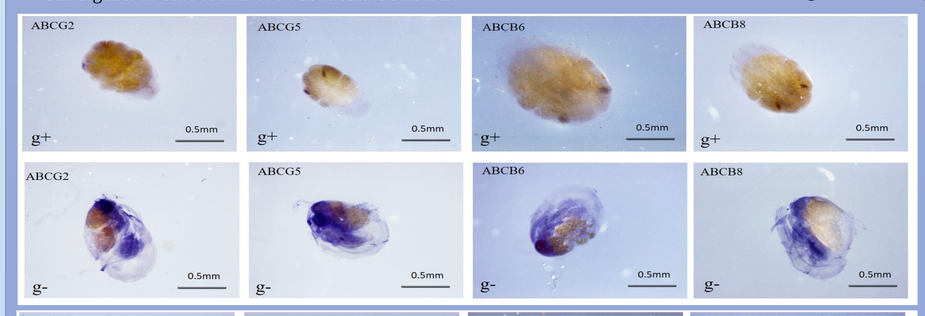

Figure. 5 Results of whole mounting *in situ* embryo hybridization of four ABC genes in nauplius stage IV of red strain of *N. denticulate sinensis.*
g-.antisense probe, g+. sense probe; g. nauplius stage IV, et sequential.

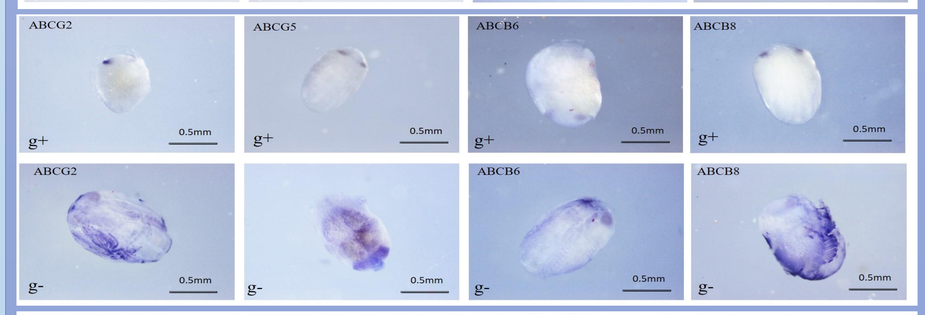

Figure. 6 Results of whole mounting *in situ* embryo hybridization of four ABC genes in nauplius stage IV of yellow strain of *N. denticulate sinensis.*

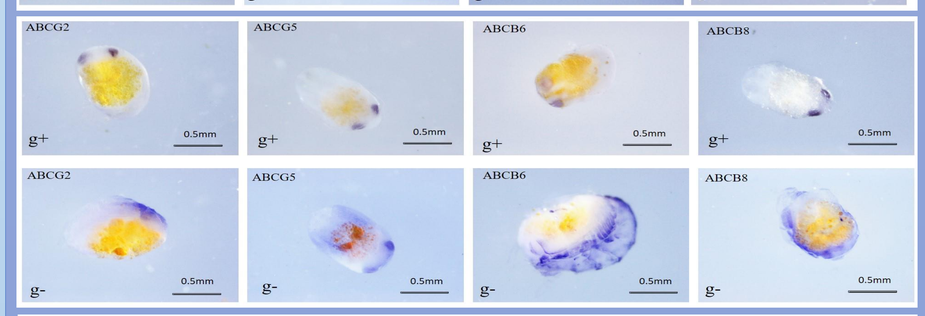

Figure. 7 Results of whole mounting *in situ* embryo hybridization of four ABC genes in nauplius stage IV of blue strain of *N. denticulate sinensis.*

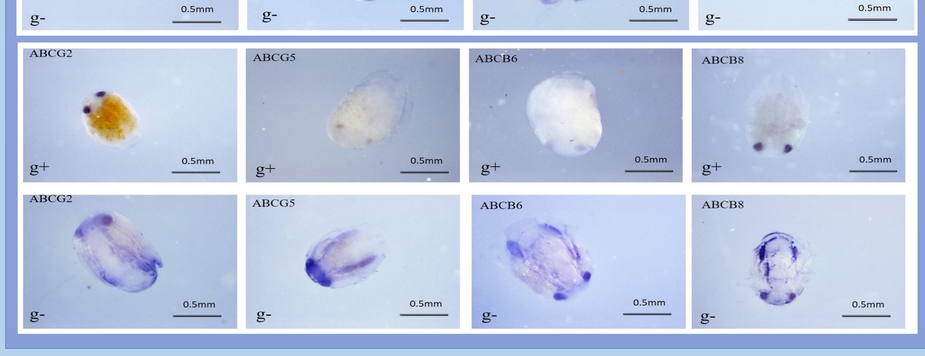

Figure. 8 Results of whole mounting *in situ* embryo hybridization of four ABC genes in nauplius stage IV of wide type of *N. denticulate sinensis.*

Conclusion

1 The results of whole mounting *in situ* embryo hybridization showed that the expression patterns of the four genes were similar; The hybridization signal increased gradually with the embryonic development of *N. denticulate sinensis*.
2 There were positive signals from cleavage stage to nauplius stage IV, whose intensity was consistent with the qPCR. The expression patterns from cleavage stage to nauplius stage III were similar, positive signals were distributed in the cells around the yolk.
3 The positive signal was strongest in the stage without nauplius stage IV, in which the patterns were different in four color strains. The positive signals of red strain were concentrated in ommateum and cephalothorax, and the positive signal was the most obvious in ommateum.
4 The positive signals of yellow strain were distributed in ommateum and gill.
5 The positive signals of blue strain and wild strain were distributed in ommateum, gill and somite junctions.
6 The expression levels of the four genes were correlative with the pigment cells quantities of nauplius. Moreover, there were different space expression patterns in four strains. These results suggested that the four genes might play a role in pigmentation.

Funding

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