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Identification of a key gene *StAR-like-3* responsible for carotenoids accumulation in the noble scallop *Chlamys nobilis*

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ABSTRACT

Carotenoids play important roles in living organisms. However, animals cannot synthesize carotenoids by themselves, and they must absorb and accumulate carotenoids from their diets in which some key genes are involved. In present study, a gene named *StAR-like-3* was characterized in the noble scallop *Chlamys nobilis*, and its function was identified using golden scallops with higher carotenoids content and brown scallops with less carotenoids content by immunohistochemistry, carotenoid binding assay and RNAi. Results showed that the *StAR-like-3* encodes a 54.7 kDa transmembrane protein (named as StAR3) of 481 amino acids containing a MENTAL domain and a START (Steroidogenic acute regulatory protein-related lipid transfer) domain, and its expression level in hemocytes and intestine of golden scallops were significantly higher than those of brown ones. Subsequently, the StAR3 protein was detected in the intestinal epithelial cells of golden scallops, and recombinant StAR3 could bind lutein conjugated to protein G and antibody to form a yellow complex, suggesting it is a carotenoid binding protein involving in carotenoids accumulation in golden scallops. Furthermore, total carotenoids content of hemolymph in golden scallops was significantly decreased when the expression of *StAR-like-3* suppressed, suggesting this gene plays an important role in transport of carotenoids. Conclusion, the present results indicated that the *StAR-like-3* is a key gene responsible for the carotenoids accumulation in the scallop.

1. Introduction

Carotenoids are yellow to red isoprenoid polyene pigments widely distributed in nature synthesized by plants, algae, some bacteria and fungi (Toews et al., 2017). Carotenoids as antioxidants and colorants play important roles in living organisms. In terms of the antioxidative properties, beneficial effects of carotenoids have been reported in prevention of coronary heart diseases, certain kinds of cancers, and age-related macular degeneration in humans (Miyashita et al., 2011; Akuffo et al., 2015; Rowles and Erdman, 2020). While for its role in colorants, brightly colored traits in vertebrates and invertebrates have been shown to be pigmented by carotenoids, for example the diverse carotenoids occurring in birds with yellow, orange and red, accumulation of carotenoids in birds commonly occurs in their feather (Thomas et al., 2014); moreover, carotenoids are also the major compound in the aquatic animals' gonads and integuments which imparts their coloration to the aquatic animals (Das and Biswas, 2016; de Carvalho and Caramujo, 2017), for example, both xanthophylls and astaxanthin are good carotenoid sources for skin color improvement of large yellow croaker (Yi et al., 2014; Toews et al., 2017).

Although some arthropods, such as aphid (Moran and Jarvik, 2010), spider mite (Grbić et al., 2011), and gall midge (Cobbs et al., 2013), have been reported to be able to make their own carotenoids, these genes encoding multiple enzymes for carotenoid biosynthesis are derived from fungi. Most animals do not synthesize carotenoids by themselves and must obtain them from dietary sources (Toews et al., 2017). The carotenoids accumulation in animals is a complex biochemical process involved in absorption, transport and deposition to target tissues, however, carotenoids, as hydrophobic substances, are difficult to move into the aqueous biological environment, special systems must support their movement in the body. Previous studies have shown that lipophorins containing diacylglycerol as the predominant lipid with lesser

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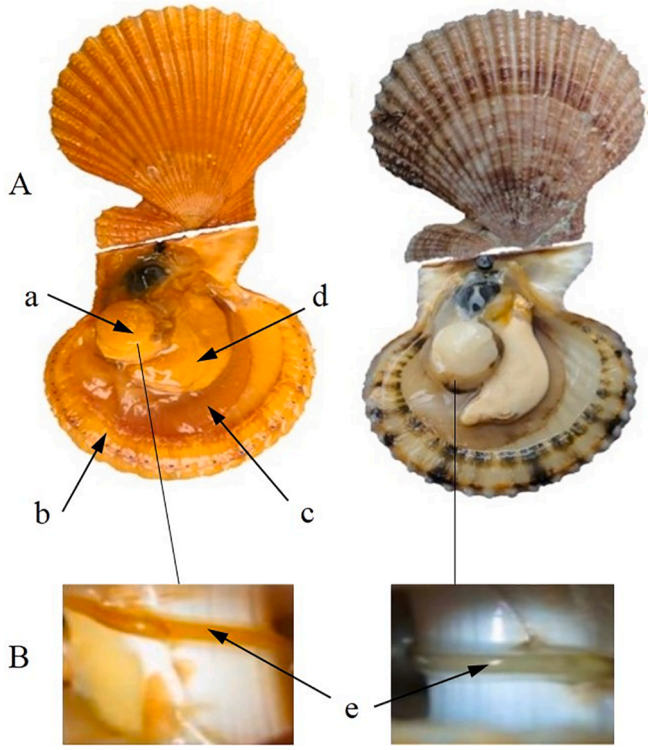

Fig. 1. The golden and brown scallop of noble scallop *Chlamys nobilis*. (A) Four types of tissues: (a) adductor, (b) mantle, (c) gill and (d) gonad. (B) The side-view of adductor shows location of intestine (e).

amounts of cholesterol, hydrocarbons, and carotenoids in the core play crucial roles in carotenoid absorption/transport in animal, and some key genes have been identified in vertebrates such as human (Li et al., 2011; Horvath et al., 2016), fish (Sundvold et al., 2011) and birds (Lopes et al., 2016; Mundy et al., 2016; Toomey et al., 2017) and invertebrates such as silkworm (Tabunoki et al., 2002; Sakudoh et al., 2007; Wang et al., 2014), fruit fly (Kiefer et al., 2002), scallops (Liu et al., 2015; Li et al., 2019) and oyster (Lei et al., 2017).

Mollusk is the second largest phylum in the animal kingdom, and many species of this phylum are important fishery and aquaculture species providing significant economic benefits to humans (Wang et al., 2017). Carotenoids are abundant in many mollusks, such as species from Polyplacophora, Gastropoda, Bivalvia, and Cephalopoda, with contents ranging from 10 μg/100 g to 140 μg/100 g (Maoka, 2011). Carotenoids in mollusks are mainly obtained from algae and then accumulated in their body, and several genes related to carotenoids accumulation have been identified in mollusk, such as *hcApo* (Li et al., 2014), *PySCD* (Li et al., 2015), *SR-BI* (Liu et al., 2015; Lei et al., 2017) and *PyBCO-like 1* (Li et al., 2019) in recent years, however, information of the *StAR* gene for carotenoids accumulation has not been found.

The *StAR* gene encodes the steroidogenic acute regulatory (StAR) protein, and the protein with a related lipid transfer domain (START) has been reported as a versatile binding interface for lipids that functions in many distinct processes (Soccio and Breslow, 2003; Swarbrick et al., 2014). In silkworm and human, the StARD3 protein, a member of the StAR protein family, has been identified as an important carotenoid binding protein responsible for absorption and transport of carotenoids (Sakudoh et al., 2005; Li et al., 2011). Therefore, it's important to explore the roles of *StAR* gene in carotenoids accumulation in mollusks.

The noble scallop *Chlamys nobilis* is an importantly economic bivalve, which has been cultured in the southern sea of China since 1980s. A new variety named as noble scallop "Nan'ao Golden Scallop" was bred by selection breeding in 2015 in China (Zheng et al., 2015; Tan et al., 2020). These golden scallops not only possess golden appearance (golden shell, golden adductor muscle, golden mantle, and golden intestine), but also enrich in carotenoids in their soft tissues compared to the common cultured scallop (brown scallops with brown shell, white mantle and adductor and poor in carotenoids) (Fig. 1). Though a key gene *SRB-like-3* responsible for carotenoid deposition in golden scallops has been identified in our previous study (Liu et al., 2015), the genetic mechanism behind the accumulation of carotenoids is still poorly understood. In our previous study, we also found the *StAR-like-3*, a member of the StAR protein family, had a significantly difference between golden scallops and brown scallops, therefore, in the present study, the role of *StAR-like-3* (member of the StAR gene family) in carotenoids accumulation was identified and characterized in noble scallop. The present results will provide insights into the molecular mechanism of carotenoids accumulation in marine mollusks.

2. Materials and methods

2.1. Experimental animals

The golden and brown scallops used in this study were originated from the same population of the noble scallop. The adult scallops were collected from Nan'ao Marine Biology Station of Shantou University, located at Nan'ao Island of Shantou, Guangdong, China. A total of 30 golden scallops and 30 brown scallops at 10-month old were randomly chosen and then taken to the laboratory with seawater. The cultured temperature and salinity of these scallops were controlled at 25℃ and 30 ppt. Tissues including adductor, mantle, gill, gonad and intestine were sampled on ice. Hemolymph was drawn from each scallop using a disposable needle with syringe (1 mL) on ice and centrifuged at 800 g for 1 min to harvest hemocytes.

Table 1
Primers used in this study.

Primer name	Sequence 5′-3′	Usage
RT-StAR3-F	GCTACGCCTTATCCGACTTGAC	qRT-PCR
RT-StAR3-R	CAAGTCTCAGCCCAGGCTAACAC	qRT-PCR
E-StAR3-F1	CCCAAGCTTGGATGTCGATAAACACGAATGAAAGAGAC	Recombinant expression
E-StAR3-R1	CCGCTCGAGAGTCTTCCTCTTTTTGTATTCCTCCAG	Recombinant expression
E-StAR3-F2	GGGGTACCCAGGATTTTGTGAGACAGGCCA	Recombinant expression
E-StAR3-R2	CCCAAGCTTGTCTTCCTCTTTTTGTATTTCCTCCAG	Recombinant expression
SiStAR3-F	GATCACTAATACGACTCACTATAGGGCCATGTCTGGTGAAGATAATT	RNAi
SiStAR3-R	CTAGTGATTATGCTGAGTGATATCCCGGTACAGACCACTTCTATTAA	RNAi
SiGFP-F	GATCACTAATACGACTCACTATAGGGGGCTACGTCCAGGAGCGCACCTT	RNAi
SiGFP-R	AAGGTGCGCTCCTGGACGTAGCCCCCTATAGTGAGTCGTATTAGTGATC	RNAi
SiRT-StAR3-F	TGGAACCCCACACTCGTAGA	qRT-PCR-RNAi
SiRT-StAR3-R	AACAAACCTCCCGCAGCCTC	qRT-PCR-RNAi
F-β-actin	5′ GGTTGCCGCCCTGGTTGTGG 3	qRT-PCR
R-β-actin	5′ GGCCGACGATGGAGGGGAAGA 3′	qRT-PCR

2.2. RNA extraction and quantification of transcripts level

Total RNA was isolated from five tissues including adductor, mantle, gill, gonad, intestine and hemocytes using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. The quality of purified RNA was determined by measuring the absorbance at 260 nm/280 nm with a Nanodrop® ND-1000 spectrophotometer (LabTech, Holliston, MA), and the RNA integrity was confirmed by electrophoresis on a 1% (w/v) agarose gel.

The sequence of *StAR-like-3* was cloned based on our transcriptome, which was not published, the accession number of *StAR-like-3* in NCBI was KP323367.1. To determine the tissue distribution of *StAR-like-3* in golden and brown scallops, qPCR was employed to analyze the expression levels in various tissues. Firstly, the first-stand cDNA was synthesized from the extracted total RNA using All-In-One RT MasterMix with AccuRT Genomic DNA Removal Kit (abm, Guangzhou, China). Quantification of transcripts was performed by RT-PCR using the first-stand cDNA as templates with Real-time PCR Super Mix (SYBRgreen, with anti-Taq) (Mei5, Guangzhou, China) and a LightCycler® 480 (Roche) under the manufacturer's protocol. The RT-PCR primer pairs for *StAR-like-3* and β-actin are listed in Table 1. Each sample was run in triplicate. Relative expression level of *StAR-like-3* was determined by the $2^{-\Delta\Delta Ct}$ algorithm with β-actin as the internal control.

2.3. Obtaining of recombinant StAR3 protein and its polyclonal antibody

The coding region of the *StAR-like-3* without transmembrane domain was amplified by PCR. The specific primers used to amplify the *StAR-like-3* region are listed in Table 1. The PCR products were cloned into the pCOLD-TF vectors cut with the same restriction enzyme, and then transformed into Transetta (DE3) chemical competent cells (TRANSGEN, Guangzhou, China). The correct clones were identified by PCR with universal primers, and further confirmed by sequencing. The bacteria cells were induced by the addition of IPTG (1 mM) and grown overnight at 18 °C at 170 rpm. Cells were harvested by centrifugation and lysed by sonication for 15 min with the program set at 3 s sonication and 4 s interval under 40 % power (BILON-250Y). The supernatant was collected by centrifuged at 12,000 g for 15 min at 4 °C and purified by Ni-NTA His Bind Resin (Transgen, Guangzhou, China). The purified protein was examined by electrophoresis on a 10 % SDS-PAGE and visualized with Coomassie brilliant blue R250. Protein concentration was determined using the BCA Protein Assay Kit (Sangon, ShangHai, China) with bovine serum albumin (BSA) as a standard.

The antibody for StAR3 immunoblotting was raised in Japanese White rabbits by subcutaneous injection of the rStAR3 protein and Freund's adjuvant. First subcutaneous injection was carried out with 0.6 mg purified protein (rStAR3) in the same volume of Freund's complete adjuvant. Twelve days later, 0.3 mg purified protein in the same volume of Freund's incomplete adjuvant was injected for a total of three times at two weeks intervals. The anti-StAR3 antiserum was confirmed by ELISA titer test. Blood was collected from the rabbit's carotid artery and purified by antigen affinity column purification. The purified antibody was mixed with phosphate-buffered saline containing 50 % glycerol and stored at −80 °C.

2.4. Tissue distribution of StAR3 protein

Hemocytes and intestine from golden and brown scallops were homogenized by tissue homogenizer and lysed using cell lysis buffer (20 mM Tris PH7.5, 150 mM NaCl, 1% TritonX-100, 2.5 mM sodium pyrophosphate, 1 mM EDTA, 1% Na_3VO_4, 0.5 μg/ml leupeptin, 1 mM PMSF). Protein (50 μg) was loaded on 10 % SDS-polyacrylamide gel for electrophoresis (SDS-PAGE) and transferred onto Millipore Immobilon^{TM}-P PVDF Transfer Membranes (Millipore, Germany). Membranes were blocked with 5 % Bovine Serum Albumin (BSA) solution at room temperature for 2 h and incubated with anti-rabbit polyclonal antibodies against StAR3 at 1: 2000 in blocking solution. After washing two times (15 min each) in TBST, membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (ABclonal, Wuhan, China) at 1: 3000 in blocking solution for 1 h at room temperature. Proteins were detected by incubating blots with ECL Reagent (Sangon, ShangHai, China). Chemiluminescence images were photographed using an Amersham Imager 600 (GE, USA).

2.5. Immunohistochemistry

The technique of immunohistochemical staining was used to determine the localization of the StAR3 protein. Intestine and adductor tissues were dissected from golden and brown scallops and washed by PBS buffer. These samples were fixed in 10 % formalin, dehydrated, cleared, embedded in paraffin and cut to sections of 3 μm thickness before staining with hematoxylin (Boster, Wuhan, China). After 10 min treatment with 3 % H_2O_2 solution, tissue sections were washed in PBS and blocked with 5 % BSA for 30 min in a humidified box at room temperature, and then incubated with anti-rStAR3 rabbit antibody (diluted 1: 250) overnight at 4 °C. Then, the sections were washed with PBS and incubated with the horseradish peroxidase-labeled anti-rabbit IgG goat antibody (diluted 1: 500) (Boster, Wuhan, China) at room temperature for 30 min. After washing three times in PBS, the sections were developed with DAB solution (CWBIO, Beijing, China) for 10 min and counterstained in hematoxylin for 3 min. Photomicrographs were taken (EVOS Cell Imaging Systems).

2.6. Carotenoid binding assay

The carotenoid binding assay was carried out following a modified method by Tabunoki et al (Tabunoki et al., 2002). Protein G-Sepharose (Beyotime, Shanghai, China) was incubated for 3 h at 4 °C with anti-His-tag mouse monoclonal antibody (Transgen, Guangzhou, China) to form Protein G-Sepharose-Antibody complexes. Next, the complexes were added into recombinant protein solution and incubated with 50 μM lutein (dissolved in ethanol) overnight at 4 °C. Sample beads were collected by centrifugation at 3000 g for 1 min. Control samples were performed using the same procedure in the absence of purified rStAR3 or in the presence of BSA. The sepharose beads were carefully washed three times with 500 mL PBS containing 1 mg/mL BSA and centrifuged gently to remove the unbound lutein. The lutein-rStAR3-Protein G-Sepharose-Antibody complexes were confirmed by the presence of yellow sediment.

2.7. RNAi of the StAR-like-3 and determination of total carotenoids content (TCC)

The siRNAs targeting *StAR-like-3* and GFP were synthesized and purified by Transcription T7 Kit (Takara, China) using synthesized primers (Table 1). Firstly, 80 golden scallops were randomly divided into two groups, siStAR-like-3 group and siGFP group, and then injected via the gonad with 50 μg of the respective siRNAs. Next, six individuals from each group were sampled at 3, 6, 12, 24 and 48 h after siRNA injection. Total RNA was extracted from hemocytes, and hemolymph was used to measure TCC according to our previous methods (Zheng et al., 2010). RT-PCR was performed as described above with 3 technical replicates for each sample.

2.8. Statistical analysis

Data are presented as the relative expression levels (mean ± S.D), and significant differences were analyzed using one-way Analysis of Variance (ANOVA). All statistical analyses were done on a SAS system for windows (SAS 8.0, SAS Institute Inc., Cary, NC, USA) and significance for all analyses was set to $P < 0.05$ unless noted otherwise.

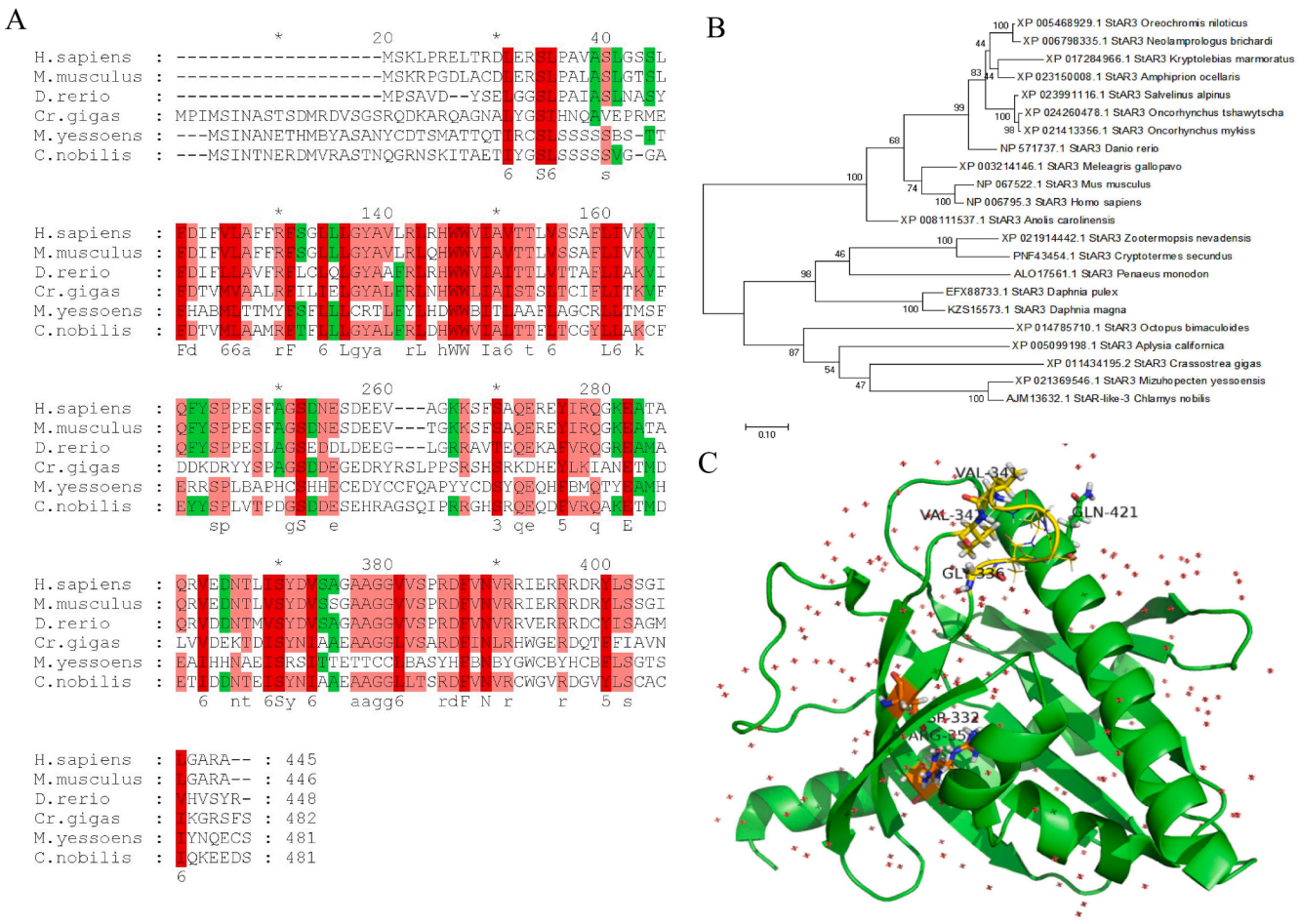

Fig. 2. Sequence analysis of *StAR-like-3*. (A) Multiple sequence alignment of StAR-3 with other species StAR. High, mediate, and low conserved amino acid residues are enclosed in black, violet and light blue shadow, respectively. Putative lutein binding sites are boxed in red. (B) Phylogenetic tree of StAR-like-3 from noble scallop and other STARD3 using MEGA 7.0 software with the ML method. 1000 bootstraps were performed to check repeatability of the results. (C) Human StARD3 protein structure. The lutein-binding function of StARD3 depends on helix-grip fold constructed around a solvent-filled cavity, which mainly bind to sites (Arg351, Asp332, Glu421, Omaga1 loop residues336-342) are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Sequence and phylogenetic tree analysis of StAR-like-3

The complete cDNA sequence of *StAR-like-3* from noble scallop is 2750 bp in length, containing a 1446 bp open reading frame encoded 481 deduced amino acids with an estimated molecular weight of 57.41 kDa and a theoretical isoelectric point of 5.39. The StAR3 protein contains a MENTAL domain (residues 65–240) and a START domain (residues 288–477), which is highly homologous to the STARD3 (steroidogenic acute regulatory protein) domain family serving as a versatile binding interface for lipids with functions in many distinct processes (Soccio and Breslow, 2003; Schrick et al., 2004; Alpy and Tomasetto, 2005). Multiple sequence alignment with other StAR proteins revealed that the StAR3 had moderate identity of 31.83%, 31.62%, 32.44% and 44.97% with *Homo sapiens* (NP_006795.3), *Mus musculus* (NP_067522.1) and *Danio rerio* (NP_571737.1) and *Crassostrea gigas* (XP_034305144.1), respectively; and a higher identity of and 87.06% with *Mizuhopecten yessoensis* (XP_021369546.1) (Fig. 2**A**), suggesting it is a novel member of STAR family.

Phylogenetic tree analysis showed StARs from vertebrates and invertebrates were clustered into three groups (Fig. 2**B**), including Mollusca, Arthropoda, and Vertebrata. The *StAR-like-3* of *C. nobilis* was clustered into the Mollusca group, and had a closer evolutional relationship with two bivalves *Mizuhopecten yessoensis* (XP_021369546.1) and *Crassostrea gigas* (XP_011434195.2), suggesting it's highly conserved in mollusks.

3.2. Protein structure prediction

The StARD3 is a versatile protein that is well known to mediate endoplasmic reticulum-to-endosome cholesterol transport (Wilhelm et al., 2017), and also functioning as carotenoid binding protein to transport carotenoids in *Bombyx mori* (Sakudoh et al., 2007) and human (Li et al., 2011). A higher-resolution structure of the lutein-binding domain of human StARD3 (Fig. 2**C**) indicates that the side chain of Arg351 and omaga1 loop (including residues 336–342) is the main contribution to lutein recognition by steric complementarity (Horvath et al., 2016). The core region of human StARD3 was defined as alternative conformation containing Asp351 to let lutein occupy the cavity, which forms a salt bridge to Asp332 and is suspected to act as an allosteric trigger point to communicate lutein binding. At the same time, the docking results indicate that Gln421 might play a crucial role in lutein binding by making a hydrogen bond with a hydroxyl group of lutein outside (Horvath et al., 2016). All these residues that are related to lutein recognition and binding are quite conserved in the StAR3, except Asp332 is replaced by another Asn, Ala336 replaced by Glu and double Vals replaced by double Leus. According to conserved sequence

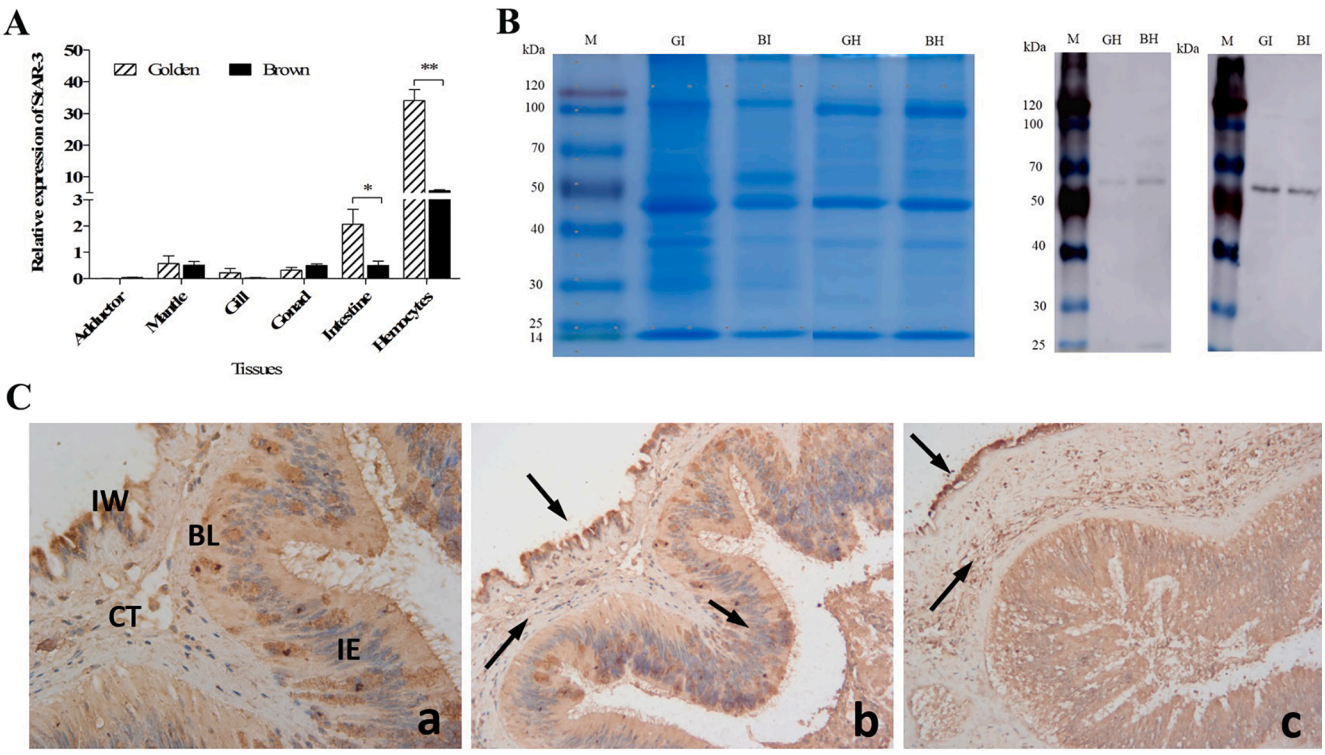

Fig. 3. (A) Tissues distribution of *StAR-like-3* transcripts in golden and brown scallops by qRT-PCR analysis. Significant differences are indicated with $P < 0.05$ or $P < 0.01$. (B) Detection of StAR3 protein in hemocytes and intestine of golden and brown scallops. Left: Gel was stained with Coomassie Brilliant Blue R-250; Right: Western blot analysis of different samples using anti-StAR3 antibody. M, molecular weight markers; GI, intestine of golden scallops; BI, intestine of brown scallops; GH, hemocytes of golden scallops; BH, hemocytes of brown scallops. (C) Immunohistochemical sections of intestine samples of golden and brown noble scallops. (a) Intestinal epithelium (IE) and intestinal wall (IW). The dark blue stains represent the nuclei. The positive stains are predominant (dark brown color) in histological sections. High magnification view (400 ×) showing details of longitudinal intestine sections. Ciliated intestinal epithelium (IE) appears as circular folds and hemocytes associated with connective tissue (CT) surrounding basal lamina (BL) and intestinal wall (IW) in intestine. (b) StAR3 distribution in the intestine of golden scallop, particularly in the intestinal epithelium (200 ×). (c) StAR3 distribution in the intestine of brown scallop (200 ×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

alignment, Asn might not affect the slat bridge formation and Glu at the beginning of the omega loop1 might not affect the loop steric complementarity. Therefore, it is predicted that StAR3 protein might be a lutein-binding protein in noble scallop.

3.3. Tissue expression of the StAR-like-3

The *StAR-like-3* was mainly expressed in hemocytes of golden and brown scallops as well as in intestines of golden scallops, but was barely detected in the other tissues (Fig. 3**A**). Furthermore, the golden scallops had significantly higher expression levels of *StAR-like-3* in hemocytes and intestine compared to brown scallops, suggesting that *StAR-like-3* may be involved in carotenoids accumulation in golden scallops. And immunoblotting analysis showed that StAR3 protein was found in intestine and hemocytes (Fig. 3**B**), which further suggest that StAR3 might be involved in carotenoids accumulation in this scallop.

Immunohistochemical sections of intestines from golden and brown scallops showed that positive stains of StAR3 protein (dark brown color) was present in connective tissue (CT) and cells of intestinal wall (IW) of both golden and brown scallops (Fig. 3**C-a**). And in the intestinal epithelium (IE), positive stains of StAR3 protein was found in intestinal epithelial cells of golden scallop but absent in brown scallop (Fig. 3**C-b and c**). From these results, it seems to suggest that StAR3 protein might be a carotenoids-binding protein related to carotenoids accumulation in golden scallops.

The above results consistently demonstrated that *StAR-like-3* might be a key gene involved in carotenoids accumulation in golden scallops. Similar results have been reported in silkworm, the carotenoid binding protein (CBP, homology to StAR) was predominant in the villi (Tabunoki et al., 2002), in which the CBP is a critical cocoon color determinant controlling the uptake of carotenoids into the intestinal mucosa and the silk glands (Sakudoh et al., 2007; Tsuchida and Sakudoh, 2015).

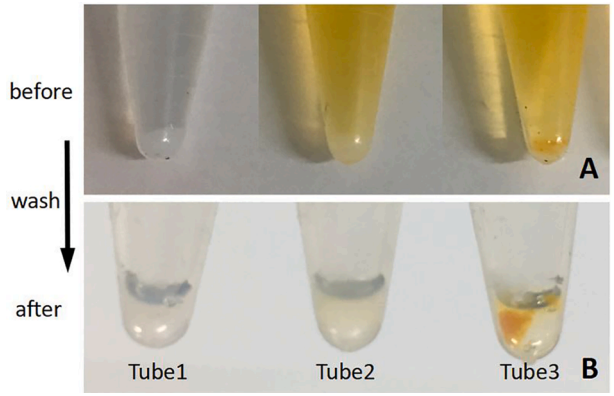

Fig. 4. Carotenoid binding assay with lutein and rStAR3. (A) Dark yellow complex at the bottom of tube 3 when samples were incubated with lutein overnight at 4 °C; (B) Samples were carefully washed three times and quickly centrifuged to show a visible yellow complex. No yellow complex was observed in tube 1 and tube 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

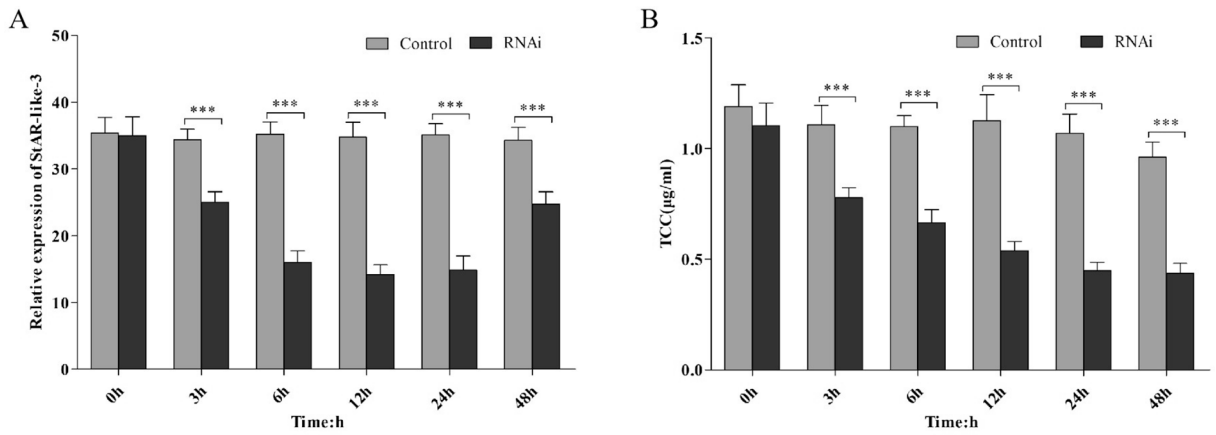

Fig. 5. RNAi experiment of StAR-like-3. (A) The mRNA expression level of StAR-like-3 after dsRNA injection. (B) Carotenoids extraction from blood after dsRNA injection. Error bars represent ± S.D. of four independent investigations. Significant differences compared with control at different time points are indicated by *** ($P < 0.001$).

3.4. Carotenoid binding assay of rStAR3

Lutein bound to the recombinant StAR3 protein by incubating with lutein conjugated to protein G and antibody to form a visible yellow complex (Fig. 4**, tube 3**). In contrast, there were no yellow complexes detected in controls (Fig. 4**, tube 1 and tube 2**). These results indicated that StAR3 protein from the noble scallop can bind to carotenoids. Although the binding carotenoids property of StAR3 protein has been documented in silkworm (Tabunoki et al., 2002; Sakudoh et al., 2005; Sakudoh et al., 2007) and human (Li et al., 2011), the present study is the first report of such binding protein in mollusks.

3.5. Expression level of StAR-like-3 and TCC in hemolymph after RNAi

qPCR analysis showed that the expression levels of *StAR-like-3* were significantly down-regulated by siStAR-like-3 (Fig. 5**A**), and the inhibition efficiency reached to 57% compared to the control group at 24 h, indicating the expression of *StAR-like-3* was significantly suppressed. Furthermore, the TCC in hemolymph of treated group was also significantly lower ($P < 0.001$) than that of the control group (Fig. 5**B**), suggesting that *StAR-like-3* may play an important role in carotenoids transportation in hemolymph.

In our previous study, *SRB-like-3*, a member of scavenger receptor class B, have been identified as a key gene that controls the carotenoid deposition in this scallop (Liu et al., 2015). And the protein function of paralog of *SRB-like-3* in other animals have been proved, which mainly responsible for selectivity uptake carotenoids, such as fly *Drosophila* (Cornelia et al., 2002) and silkworm *Bombyx mori* (Takashi et al., 2013). While, the StarD3 (also known as carotenoid-binding protein, CBP) is thought to transport carotenoids rather than cholesterol, for example, in silkworm, StarD3 is localized intracellularly in the midgut and middle silk gland cell which transport the carotenoids from midgut to middle silk gland cell (Sakudoh et al., 2005; Wang et al., 2014; Tsuchida and Sakudoh, 2015); in human, StarD3 can also transport carotenoids to the macula, playing a key role in maintaining the macular carotenoids in human ocular health (Li et al., 2011). Therefore, we are reasonable to believe that the *StAR-like-3* is an important gene responsible for carotenoids accumulation in this scallop. And the *SRB-like-3* and *StAR-like-3* may have a synergistic action in carotenoids accumulation in this scallop, which will be further studied in our future work.

4. Conclusion

In the present study, a key gene *StAR-like-3* responsible for carotenoids accumulation in noble scallop *C. nobilis* was identified. The expression levels of *StAR-like-3* in hemocytes and intestine of golden scallops were significantly higher than those of brown scallops. And immunohistochemistry analysis revealed that StAR3 protein was only present in intestinal epithelial cells of golden scallop, carotenoid binding assay indicated that StAR3 protein is a carotenoids binding protein in the scallop. Furthermore, total carotenoids content of hemolymph in golden scallops was significantly decreased when the expression of *StAR-like-3* suppressed. Collectively, these results indicate that *StAR-like-3* is a key gene responsible for the carotenoids accumulation in this scallop.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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