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Research article

Molecular cloning and characterisation of scavenger receptor class B in pearl oyster *Pinctada fuctada martensii*

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ABSTRACT

Background: Molluscs can accumulate carotenoids in their body tissues by predominantly feeding on aquatic plant sources. Carotenoid transport and absorption are determined by the regulation of various proteins such as Scavenger receptor class B(*SR-BI*). We report the identification and characterisation of pearl oyster *Pinctada fuctada martensii SR-BI* (*PmSR-BI*). The correlation between total carotenoid content (TCC) and gene expression was also estimated.
Results: The full-length cDNA of *PmSR-BI* was 1828 bp, including an open-reading frame encoding of 1518 bp with a *pI* value of 5.83. PmSR-BI protein contains a hydrophobic CD36 domain and four centrally clustered cysteine residues for the arrangement of disulphide bridges. The deduced amino acid sequence had an identity of 30% to 60% with the SR-B of other organisms. Reverse transcription polymerase chain reaction analysis showed that mRNA transcripts were expressed in multiple tissues of adult pearl oyster. A higher expression of *PmSR-BI* gene was observed in the hepatopancreas than in the adductor muscle, gill and mantle. The TCC and gene expression of *PmSR-BI* were significantly correlated ($P < 0.05$), with a correlation coefficient of 0.978.
Conclusions: The results suggested that *PmSR-BI* is involved in the absorption of carotenoids in the pearl oyster *P. fuctada martensii*.

1. Introduction

Carotenoids are yellow to red C40 hydrophobic isoprenoid pigments and are widely distributed in nature. More than 600 structural distinct carotenoids have been isolated [1]. Carotenoid pigments play important physiological functions in many organisms. In higher plants and photosynthetic microorganisms, they protect the tissues and cells against photosensitised oxidation, in addition to their function as accessory pigments in light harvesting [2,3]. Moreover, carotenoids are considered precursors of phytohormone and abscisic acid [3]. In animals, the pigments are the precursors (provitamins) for the formation of vitamin A [4,5]; and are active oxygen quenchers with potential anti-cancer activities [6,7]. Carotenoids are beneficial for the prevention of coronary heart diseases, certain kinds of cancer, and age-related macular degeneration in humans [8].

Plants, fungi and bacteria can synthesise carotenoids. However, carotenoids cannot be synthesised de novo by animals, except for aphids and spider mites [9]. Therefore, carotenoids in many animals are a result of carotenoid accumulation from the diet or from specific chemical modifications by metabolic reactions [10]. Marine shellfish, such as scallops, mussels and abalones, can accumulate a variety of carotenoids [9]. The principal carotenoids in marine shellfish are β-carotene, lutein A, zeaxanthin, diatoxanthin, pectenolone, pectenol and mytiloxanthin. Like other animals, marine shellfish species must obtain carotenoids from food and subsequently transport them to the cells of target tissues.

The delivery of carotenoids to cells can be divided into three categories: enzyme-mediated processes, receptor-mediated endocytosis and selective lipid transport [11]. Carotenoid transport and absorption are determined by the regulation of various proteins involved in the process [12] that are mainly involved in ATP-binding cassette A1 (ABCA1), scavenger receptor class B type I (SR-BI) and cluster-determinant 36 (CD36) [13]. SR-BI and CD36 belong to the B class scavenger receptor family (SR-B). SR-B is a type III transmembrane receptor with two transmembrane domains, an extracellular loop with multiple glycosylation sites and two short intracellular tails [14]. As a scavenger receptor, the highly glycosylated extracellular domain has numerous substrate binding sites [15] for mediating the cellular uptake of carotenoids [7], those involved in immune response defence [16], those that participate in signal transduction and apoptosis and phagocytosis of apoptotic cells [17,18].

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The pearl oyster *Pinctada fuctada martensii* is naturally distributed along the coast of southern China. The species is one of the most important commercial shellfish in the south of China and is mainly cultured for its round nucleated pearls. Moreover, the shellfish is edible and highly nutritious [19]. Basing on the transportisome dataset of the pearl oyster mantle [20], we screened and characterised lipid metabolism-related genes, such as apolipoprotein and SR-B. Herein, we describe a *P. fuctada martensii* SR-BI gene, termed *PmSR-BI*, which shares a high structural and functional homology with the SR-BI, to understand the carotenoid metabolism in pearl oyster.

2. Material and methods

2.1. Experimental animals and sample collection

Adult pearl oysters were obtained from a stock farmed in Leizhou (Zhanjiang, Guangdong Province, China) and preconditioned for 2 days at 25°C to 30°C in a 1000 L tank with circulating seawater. Various tissues, including adductor muscle, gill, hepatopancreas and mantle, were collected and frozen in liquid nitrogen for the subsequent studies.

2.2. RNA extraction and cDNA synthesis

Total RNA was isolated from the mantle tissue using the TRIzol reagent (Invitrogen). The cDNA first-strand synthesis was performed based on M-MLV RT usage information (Promega) using RQI DNase (Promega)-treated total RNA as template. cDNA mix was diluted to 1:50 and stored at -80°C for subsequent fluorescent real-time PCR.

2.3. Cloning the full-length cDNA of PmSR-BI

PmSR-BI cDNA was obtained using reverse transcription PCR (RT-PCR) and RACE technique. Degenerate primers were designed based on SR-B unigenes, which were selected from the transcriptome dataset of our library [20]. The intermediate fragment PCR reaction was implemented in a total volume of 10 μL, including 5 μL of Premix Taq, 0.4 μL of template cDNA, 0.4 μL of each primer (10 μmol L^{-1}) and 3.8 μL of double-distilled water. The PCR temperature profile was 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min and a final extension step at 72°C for 10 min. The band of the expected size (1200bp) was excised and purified by agarose gel DNA fragment recovery kit (TaKaRa), subcloned into pMD-18 T vector (TaKaRa) and transformed into competent *Escherichia coli* cells DH5α. Bacteria were grown in ampicillin-containing Luria–Bertani plates, and the recombinants were selected and sequenced using the blue–white colour selection and screened with M13 forward and reverse primers from Sangon (Shanghai, China) [21].

The 5′- and 3′-ends of the *PmSR-BI* cDNA were obtained by RACE technique. The 5′-end and PCR reaction were implemented in a total volume of 10 μL, including 5 μL of Premix Taq, 0.4 μL of template cDNA, 0.4 μL of each primer (*PmSR-BI*-5′ outer and UPM) and 3.8 μL of water. The reaction was performed at 94°C for 5 min, 35 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 2 min and 72°C for 10 min, with storage at 4°C. A nested PCR was performed using NUP and *PmSR-BI*-5′ inner. The amplification reactions of the 3′-end, *PmSR-BI*-5′ outer and *PmSR-BI*-5′ inner changed to *PmSR-BI*-3′ outer and *PmSR-BI*-3′ inner. The reaction procedure was followed. Table 1 shows the primer sequence used in the cloning and real-time PCR of *PmSR-BI* gene.

Table 1
Primer sequence used in the cloning and real-time PCR of *PmSR-BI* gene.

Primer name	Primer sequence	Action
5′-inner	GGTCTCACGATACGCAATGGTTC	5′RACE
5′-outer	GTTGCTATCACCGCCTATGTCTA	5′RACE
3′-inner	TCCATCCAGACCCTGAGCAACAT	3′RACE
3′-outer	TTTCTCAAGTAATGAAGGACCCG	3′RACE
UPM	CTAATACGACTCACTATAGGGC	RACE
NUP	AAGCAGTGGTATCAACGCAGAGT	RACE
S	TGATGTCATAAATCCAGAGGAAGTA	Middle fragment PCR
A	ATTTCACAACCTCTTCATCATCCTC	Middle fragment PCR
GAPDH-S	CACTCGCCAAGATAATCAACG	Reference genes
GAPDH-A	CCATTCCTGTCAACTTCCCAT	Reference genes
M13F(-47)	CGCCAGGGTTTTCCCAGTCACGAC	Colony PCR
M13R(-48)	AGCGGATAACAATTTCACACAGGA	Colony PCR
RT-1s	AACTGAAAAAGCAGCCAACGAT	Real-time PCR
RT-1a	ACAGATGAGAATAAAAGCACCGA	Real-time PCR

2.4. Sequence analysis of PmSR-BI

The *PmSR-BI* gene cDNA sequence was analysed by the BLAST algorithm at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast) and the deduced amino acid sequence was analysed with the Expert Protein Analysis System (http://www.expasy.org/). Characteristic domains or motifs were identified using the PROSITE profile database. The Clustal W program (http://www.ebi.ac.uk/clustalw/) was used for multiple alignments of SR-BI. An unrooted phylogenetic tree was constructed according to amino acid sequences of the selected SR-BI using the neighbour-joining algorithm embedded in the MEGA6.0 program. The bootstrap trials were replicated 1000 times to derive the confidence value for the phylogeny analysis.

2.5. Quantitative analysis of PmSR-BI mRNA expression

PmSR-BI mRNA expression was determined by quantitative real-time RT-PCR (qRT-PCR) with GAPDH as a reference gene. qRT-PCR was performed in a total volume of 10 μL, containing 5 μL of SYBR Green Master Mix (Rox), 0.4 μL pf cDNA, 0.4 μL of each primer (10 mM) and 3.8 μL of laboratory-grade water. The qRT-PCR program was 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s according to the manufacturer's instructions [22]. Dissociation analysis of amplification products was performed at the end of each PCR reaction to confirm that only one PCR product was amplified and detected. The comparative CT method ($2^{-\Delta\Delta CT}$ method) was used to analyse the expression level of the candidate genes.

2.6. PmSR-BI gene expression in yellow- and white-coloured strains

Expression levels of selected transcripts were investigated in the adductor muscle tissues of 10 yellow-coloured and 10 white-coloured pearl oysters. All oysters used in this experiment were sampled from the third-generation selected lines [23]. Total RNA was extracted and quality and quantity were determined using a Nanodrop spectrophotometer. A 1 μg of mRNA was used to synthesise cDNA by PrimeScript RT reagent kit with gDNA Eraser (TaKaRa). qRT-PCR was conducted in a LightCycler®480 System using the SYBR Premix Ex Taq II qRT-PCR Kit (TaKaRa). Each assay was performed with GAPDH mRNA as the internal control.

2.7. Total carotenoid contents of yellow- and white-coloured strains

The protocols for total carotenoid content extraction were detailed by Lei et al. [24]. Total carotenoid content (μg/g) $= D_{480} \times 10^4 \times V/(E \times m)$, where D_{480} indicates the absorbance at 480 nm. V represents extracting liquid (mL), E represents molar extinction coefficient (2500) and m represents sample quality.

2.8. Statistical analyses

All data were expressed as mean ± standard deviation. Total carotenoid content (TCC) between yellow- and white-coloured strains was compared by *T*-test. The correlation between *PmSR-BI* gene

expression levels and TCC was estimated. Statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The significant level was set at $P < 0.05$.

3. Results

3.1. cDNA and the deduced amino acid sequence of PmSR-BI gene

Using RACE, a full-length cDNA of *PmSR-BI* was obtained. The *PmSR-BI* cDNA consisted of 1828bp (Fig. 1), with an open-reading frame (ORF) of 1518bp that encoded a putative protein of 505 amino acids, a 5′-UTR of 88bp and a 3′-UTR of 222bp, with a predicted molecular mass of 56.70 kDa and a theoretical p*I* of 5.72. The aliphatic index was 96.65 and the grand average of hydropathicity was -0.040. The instability index was 34.51, which belonged to the stable protein.

3.2. Homology analyses of PmSR-BI genes

Multiple alignments of the deduced amino acid sequences of PmSR-BI showed that this sequence shared several protein features with other species. As shown in Fig. 2, PmSR-BI possessed high homology in *Mus musculus* (NP_031670.1), *Danio rerio* (NP_944603.2), *Crassostrea gigas* (XP_011432626.2) and *Mimachlamys nobilis* (AJM13628.1). Multiple sequence alignments showed that PmSR-BI possessed 60% and 52% identity with the molluscs *C. gigas* and *M. nobilis*, respectively, and relatively low identity with other invertebrates.

A phylogenetic tree of SR-B proteins was constructed using MEGA software 6 and the neighbour-joining method. This phylogenetic tree based on the deduced amino acid sequence of PmSR-BI and other species was constructed from 16 species. As shown in Fig. 3, three distinct clusters of the SR-B family could be identified. This agrees with the model that the SR-B family may be classified into three groups, namely, SR-BI, CD36 and lysosome membrane protein 2 (LIMPII, or SCARB2) [25]. We found that *C. gigas* (XP_011428974.1) belongs to SCARB2, as analysed by the BLAST algorithm at the NCBI. But on the phylogenetic tree, the *C. gigas* (XP_011432626.2) and *PmSR-BI* be classified into the SR-BI. Basing on the phylogenetic tree, we concluded that PmSR-BI belongs to SR-BI.

3.3. Expression of PmSR-BI gene in different tissues

The expression of the *PmSR-BI* mRNA in different tissues was analysed by RT-PCR analysis. As shown in Fig. 4, a strong tissue-specific *PmSR-BI* expression of pearl oyster *P. fuctada martensii* was found. *PmSR-BI* gene was highly expressed in the hepatopancreas, followed by adductor muscle, gill and mantle. Significant differences in *PmSR-BI* gene expression were found among the tissues ($P < 0.05$).

```
gacacttcctaccacttgatttgatctaaatttagatagttaggcacacgatgaagtagtacttagatctaaattttggggtgtcacg
ATGTGTTTGAAATGGATAACGAAATGCAACATCATTTTCTTGATTGTTGGCGCTGTTGTTTTGGCGGTCGGTTGTGCCTTAATAAAGGTG
 M  C  L  K  W  I  T  K  C  N  I  I  F  L  I  V  G  A  V  V  L  A  V  G  C  A  L  I  K  V
TTCCATGACATGATTATATCTAAAGTAAAATCTACCCTTCCATTAGTTCAAGGAACGTCCACGTACAATGATTGGGTTGAGCCTCCAGTG
 F  H  D  M  I  I  S  K  V  K  S  T  L  P  L  V  Q  G  T  S  T  Y  N  D  W  V  E  P  P  V
CCAATTTATTTCCAAATTTGGGTATATGATGTCATAAATCCAGAGGAAGTAGTAAACAAGGGAGAGAGACCAGCTGTCGTACAGAGAGGT
 P  I  Y  F  Q  I  W  V  Y  D  V  I  N  P  E  E  V  V  N  K  G  E  R  P  A  V  V  Q  R  G
CCATATACGTACAGGGAGAAGCGACACAAACAGAACATTACATCCTATGATAATGGAACCATTGCGTATCGTGAGACCAGAACATTCATC
 P  Y  T  Y  R  E  K  R  H  K  Q  N  I  T  S  Y  D  N  G  T  I  A  Y  R  E  T  R  T  F  I
TTTGACAGAGCAAAGTCTGTGGGACCAGAGACGGACAACTTTACGACAGCTAACATACTCATGGTGACAATAGCTGATCTCCTCGAAAGA
 F  D  R  A  K  S  V  G  P  E  T  D  N  F  T  T  A  N  I  L  M  V  T  I  A  D  L  L  E  R
GAGTATAGCTTCATCCAGGAGTTAGCAGAGATTATACTAGACATAGGCGGTGATAGCAACCTGTTTACGACTCTCTCTGTTAGGGGCATT
 E  Y  S  F  I  Q  E  L  A  E  I  I  L  D  I  G  G  D  S  N  L  F  T  T  L  S  V  R  G  I
ATGTGGGGTTACGAGGACCCATTGCTGAAAACGGTCAATGATATTTTAAAGAGATATAATCAGTCGACTATTGATGACCGTTTTGGACTT
 M  W  G  Y  E  D  P  L  L  K  T  V  N  D  I  L  K  R  Y  N  Q  S  T  I  D  D  R  F  G  L
TTCTATAAACAAAATGGTACAGATGATGGGTTGTATACAATAGACTCCGGGACAAAATCTGTCGATCATTTTGTAGAAATTCAGCGATGG
 F  Y  K  Q  N  G  T  D  D  G  L  Y  T  I  D  S  G  T  K  S  V  D  H  F  V  E  I  Q  R  W
AATGGAGAAAATAAGCTTAGTTTTTGGACCACCCCTACATGCAATATGATCAATGGAACAGATGGTACCCTATTTCCACCATTTGTAGAT
 N  G  E  N  K  L  S  F  W  T  T  P  T  C  N  M  I  N  G  T  D  G  T  L  F  P  P  F  V  D
AAAAGTCAGACATTGTATATATTTTCTTCAGATATCTGTAGATCTATTTATACTACATTCCACAAAGAGGATACGCTGAGAGGAATTGAT
 K  S  Q  T  L  Y  I  F  S  S  D  I  C  R  S  I  Y  T  T  F  H  K  E  D  T  L  R  G  I  D
CTTCTAGAGTTCCGAGTCCCGCCTAATGTTTTTTTAAATGTGACAGCAAATCCAGCGAACACAGGATTTTGTACTCCGGCTGATAACTGC
 L  L  E  F  R  V  P  P  N  V  F  L  N  V  T  A  N  P  A  N  T  G  F  C  T  P  A  D  N  C
TTTCCTTCAGGTTTATTGAACGTAGCATCGTGTAGGAGTGGTGCCCCTGTGATTATGTCTCAGCCTAATTTCTTGGCAGCAGATCCAAAA
 F  P  S  G  L  L  N  V  A  S  C  R  S  G  A  P  V  I  M  S  Q  P  N  F  L  A  A  D  P  K
GTTATAGACTCTGTCGTGGGTATCCATCCAGACCCTGAGCAACATTCCACATTAATAGCTGTGGAACCGATGACTGGTGTGGTCATGAAT
 V  I  D  S  V  V  G  I  H  P  D  P  E  Q  H  S  T  L  I  A  V  E  P  M  T  G  V  V  M  N
GCACAGAAGAAATTACAAGTCGACATATTGATAAAGAAAATCAATCACATACGTGATACAAGAAATCTGAAGTTGATGTTTTTTCCAATA
 A  Q  K  K  L  Q  V  D  I  L  I  K  K  I  N  H  I  R  D  T  R  N  L  K  L  M  F  F  P  I
CTTTGGCTGAATGAGAGTGCTGTTATAACTGAAAAAGCAGCCAACGATTTCAAAAGTGCAGTACAGACCCCATTAAAGATTACTCAGACA
 L  W  L  N  E  S  A  V  I  T  E  K  A  A  N  D  F  K  S  A  V  Q  T  P  L  K  I  T  Q  T
GTGCAGTATGGTCTGATCATTATCGGTGCTTTTATTCTCATCTGTACTCTAGCCCTCATTATAAAGAATAAGTTTTCGTACAATGATGAG
 V  Q  Y  G  L  I  I  I  G  A  F  I  L  I  C  T  L  A  L  I  I  K  N  K  F  S  Y  N  D  E
GATGATGAAGAGGTTGTGAAATGTACAAAGAAACCAGCGAGTGTAAATCCCTCGGAATCAACCAAGCTGCTATCCTAG
 D  D  E  E  V  V  K  C  T  K  K  P  A  S  V  N  P  S  E  S  T  K  L  L  S  *
tgcttatgtactgaaacttgtcaactgatttatcccttagtgtaagggagtgtggagatgggtggttggtttgcatgagacagattcttg
acatgaatcaatacataatggatacctatttatttttgtatactaaagcagttttgcaaatttggtgcctttgcctattattatcataat
ctttcaattttcaaagaaaaaaaaaaaaaaaaaaaaaaaaa
```

Fig. 1. The full-length cDNA and amino acid sequence of *PmSR-BI*. Open-reading fragment and the deduced amino acid sequences are indicated with capital letters; 5′ and 3′ UTR are indicated with small letters; nucleotides with bold letters represent the initiation codon (ATG) and stop codon (TGA). The sequences with grey backgrounds represent the typical CD36 domain; the underlined sequences are the transmembrane regions.

```
D. rerio              MWKDIPVPFFMSVYFFHIVNPDEILK-GERPMVIQRGPYVYRENRWKDNITFHENHT-VS
M. musculus           SWEKPPLPVYIQFYFFNVTNPEEILQ-GEIPLLEEVGPYTYRELRNKANIQFGENGTTIS
P. fucata martensii   DWVEPPVPIYFQIWVYDVINPEEVVNKGERPAVVQKGPYTHREKRHKQNITSYDNGT-IA
C. gigas              TWRDLPIPIYMQFYMFDCLNPEEVLN-GAKPYVVEKGPYTYIEKRIKYDIIHNSNGT-VT
M. nobilis            IWKDTPIPIYMQFYMFDVRNPEEVLQ-GKKPFVVQRGPYTYREVRTKFNITHNDNGT-IS
                       * .  *:*.::.:.:.  **:*::: *  * : : ***.: * * * :*   .* * ::
D. rerio              YKEYRQYFFEESMSVGDESDV-VTIPNMLVLGASVMMENMPFPVRLLLSTTFKTF-NEGP
M. musculus           AVTNKAYVFERNQSVGDPNVDLIRTINIPLLTVVDLAQ--LTLLRELIEAMLKAY-QQKL
P. fucata martensii   YRETRTFIFDRAKSVGPETDN-FTTANILMVTIADLLEREYSFIQELAEIILDIGGDSNL
C. gigas              YKQNRTFHFVEKLSVGPESDR-FTTPNPVYWSLVSALRYENKDINWLINLLTTFK-QEYV
M. nobilis            YRQNRTFYFVPSMSVGNENDT-FTIPNPVYWSLISSLKWMYPEIRKIIRLVTSFE-KESV
                          : : *    *** .   .   *          .     :. :         ..
D. rerio              FLTKPVGELMWGYDSKLVDFLNKYLPGMLP——SSGKFGLFADFNNSNTGLFTIFTGRDDI
M. musculus           FVIHTVHELLWGYKDEILSLVHIFKPDV-----SPNFGLFYERNGTNDGEYVFLTGEDNY
P. fucata martensii   FTTLSVRGIMWGYEDPLLKTVNDILKRYNQSTIDDRFGLFYKQNGTDDGLYTIDSGTKSV
C. gigas              FMNRTVKEILWGYQDPALHFAKAYDPEW---FYTDIAGYFINKNATNDGVYTIFTGETDI
M. nobilis            FMTRSMYDIIWGYEDKMLTAAQVLDNEW---FYTNTIGYFMNKNATNDGVYTIYTGETNI
                      *    :  ::***..  :   :              * * . * :: * :.: :*  .
D. rerio              RKVHMVDSWNGLKNVDYWRSDQCNMINGTAGQMWPPFMTKETTMPFYSPDACRSMELVYQ
M. musculus           LNFSKIVEWNGKTSLDWWTTDTCNMINGTDGDSFHPLISKDEVLYLFPSDLCRSVHITFS
P. fucata martensii   DHFVEIQRWNGENKLSFWTTPTCNMINGTDGTLFPPFVDKSQTLYIFSSDICRSIYTTFH
C. gigas              TKLGHIDQYNGSRYLNFWTTKWANMINGTDATVNPPFAKKSRVSYAFASDVCRSIKGVFA
M. nobilis            QFLEGIDRYNGSSHLDFWSTPCANMINGTDGTVGPPYRGKNQPSPVFSSDACRSAAGLYH
                        .  :  :**   :.:* :  .****** .    *   *.     :  * ***    :
D. rerio              RPLV-SSGIPVYRYVSPKTLFANGADFPPNEGFC-----PCRQSGLLNVSSCRH----GSP
M. musculus           SFE-NVEGLPAFRYKVPAEILANTS---ENAGFCIPEGNCMDSGVLNISICKN----GAP
P. fucata martensii   KEDT-LRGIDLLEFRVPPNVFLNVTANPANTGFCTPADNCFPSGLLNVASCRS----GAP
C. gigas              EEVTDSHGFTLWRYTSPDSYVANATVNPDNIGFCTP—NCLDKGLFNMSTCQIIDFFHIP
M. nobilis            GDVTSPQGIKLWRYIAPPSYLANATENPDNIGFCTPESKCLDTGMLNISTCQQVDFFHIP
                            *:   .:  *   . * :    * ***     *  .*:*:*:: *:       *
D. rerio              TFISQPHFYNADPQLLDTVSGLSPSEDEHGLFIDIHPETGVPVNVSVRLQLNLFLKKVSG
M. musculus           IIMSFPHFYQADEKFVSAIKGMHPNKEEHESFVDINPLTGIILRGAKRFQINTYVRKLDD
P. fucata martensii   VIMSQPNFLAADPKVIDSVVGIHPDPEQHSTLIAVEPMTGVVMNAQKKLQVDILIKKINH
C. gigas              AAISLPHFYLGAERYQKAVIGMRPNKEEHQTIIDAEPTIGWVLRAAKRLQINLYIQPIKG
M. nobilis            VALSFPHFNLAAKEYQDSVEGLDPVVEEHQTTVDKEPYTGLVLQAAKKLQLNMYIQPIGE
                        :* *:*  .  .  .:: *: *  ::*   :  .*  *  :.   ::*::  :: :
D. rerio              ISETGNIAEVVMPMLWFEESGYIDGPVLHTFRTNLVVLPKVMEYMQYIFIALGLAAILTA
M. musculus           FVETGDIRTMVFPVMYLNESVLIDKETANQLKSVINTTLVVTNI-PYIIMALGVFFGLVF
P. fucata martensii   IRDTRNLKLMFFPILWLNESAVITEKAANDFKSAVQTPLKITQTVQYGLIIIGAFILICT
C. gigas              FELTTKITPVFLPVLWLNESAVIDEKNAQMLQNMLFTPLKVVHVVEIVLIAAGSVLLVAT
M. nobilis            FEATKDITPVFVPLFWVNESAVVDDENADLLKSMLFTPLLVVHVIEIALISLGCLVLVGT
                      :  * .:  :..*:::.:**  :    . ::. : .   : .     ::  *    :
```

Fig. 2. Multi-alignment of SR-BI. "*" indicates the conserved amino acid; ":" indicates amino acids with strong similarities; "." indicates amino acids with weak similarities.

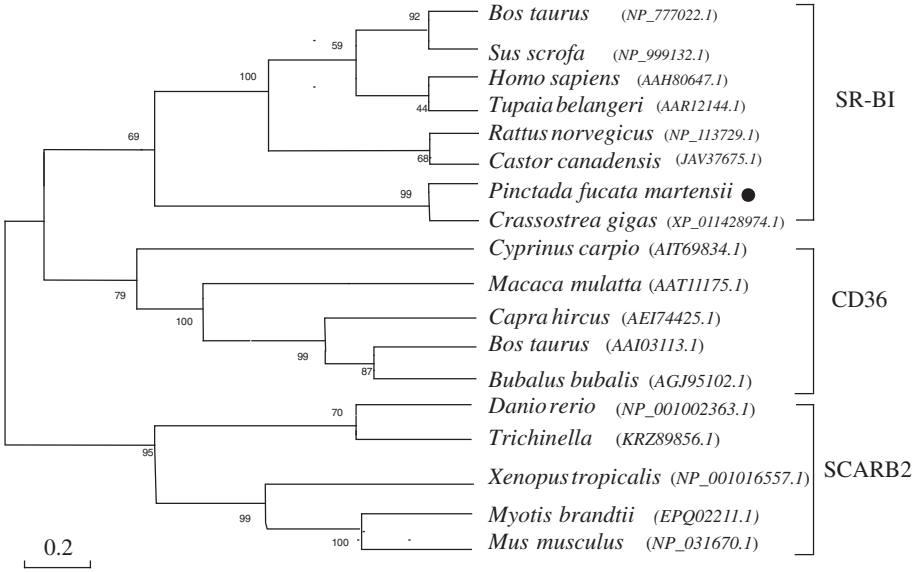

Fig. 3. Cluster analysis of SR-BI protein.

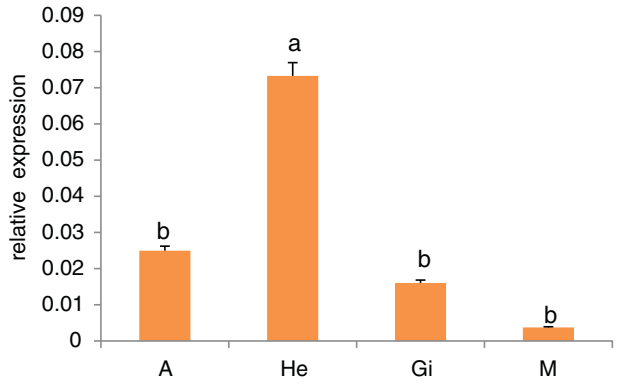

Fig. 4. Relative expression of *PmSR-BI* mRNA in the different tissues of *P. fuctada martensii*. A, Adductor muscle; Gi, Gill; He, Hepatopancreas; M, Mantle; Mean values with different letters are significantly different ($P < 0.05$).

3.4. Correlations between PmSR-BI gene expression and TCC in adductor muscle

The differences in TCC and gene expressions of yellow- and white-coloured strains are separately shown in Fig. 5. Significant differences were found in TCC and gene expressions between yellow- and white-coloured strains ($P < 0.05$). A significant correlation ($R^2 = 0.978$, $P < 0.05$) was found between the amount of *PmSR-BI* gene expression and TCC in yellow- and white-coloured strains.

4. Discussion

In this study, a novel *PmSR-BI* gene was isolated and characterised from the pearl oyster *P. fuctada martensii*. To our knowledge, this is the first *PmSR-BI* gene identified from pearl oyster species. The full-length cDNA of *PmSR-BI* gene is 1828 bp, including a perfect ORF of 1518 bp encoding a polypeptide of 505 amino acids (accession no: KY952763). PmSR-BI is predicted to possess two transmembrane domains spanning residues 9–31 and 449–471, two short cytoplasmic tails at both the N- and C-terminal ends with residues 1–8 and 472–505, respectively, and a large highly glycosylated extracellular domain comprising residues 32–448, but does not contain a signal peptide. PmSR-BI might be a transmembrane protein. This conforms to the characteristics of the B class scavenger receptor family. Basing on the sequence characteristics, the researchers found that PmSR-BI is a member of the B class scavenger receptor family.

Multiple alignments suggested that the CD36 amino acid sequence of *P. fuctada martensii* is highly identical with its homologues.

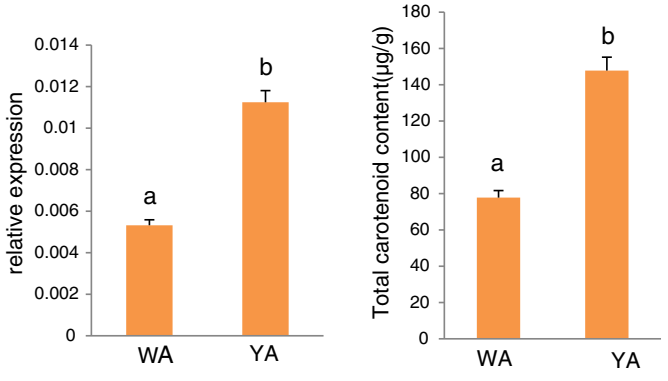

Fig. 5. Total carotenoid content and *PmSR-BI* expression of animals sampled from yellow- and white-coloured strains; Note: WA and YA are the white- and yellow-coloured strains.

Particularly, high-sequence identity was observed between two shellfish species *P. fuctada martensii* and *C. gigas*. Homology analysis revealed that the deduced amino acid sequence of PmSR-BI shares a high similarity with known SR-B. In the phylogenetic tree, *P. fuctada martensii* and *C. gigas* were located on the same branch. In the traditional taxonomy, *P. fuctada martensii* and *C. gigas* belong to the same genus and should be located on the branch. Basing on these results, the researchers found that the PmSR-BI was speculated to be the same or have similar physiological functions with SR-BI.

SR-BI is an enterocyte apical membrane transporter, which is involved in the absorption of lutein [11], β-carotene [7,12,13], b-cryptoxanthin [13] and lycopene [14]. Early studies showed that carotenoids were absorbed by passive transport. In recent years, however, several studies have found that the absorption of carotenoids is a promotional process in which SR-BI is involved [26]. For instance, SR-BI has been reported in the silk gland of *Bombyx mori* responsible for selective uptake of carotenoids [27]. Moreover, SR-BI is involved both in β-carotene uptake by brush border membrane vesicles [12]. In *Drosophila melanogaster*, carotenoids are stored in the adipose tissues, in pupal stages, and R-BI homologues (nina D) redistribute the carotenoids from the adipose tissue to the developing eyes [25]. The present results indicated that *PmSR-BI* might mediate the uptake of carotenoids in the pearl oyster *P. fuctada martensii*.

qRT-PCR analysis revealed a strong tissue-specific expression of the *PmSR-BI* gene in the present study. The *PmSR-BI* gene was ubiquitously expressed in the tissues analysed. The highest mRNA level was detected in the hepatopancreas, followed by the adductor muscle, gill and mantle. The results were consistent with other shellfish species studied. For example, high carotenoid content was found in the hepatopancreas of most freshwater species, the authors suggested that this tissue may serve as a storage site for pigments [28]. This supports the hypothesis that the hepatopancreas play an important physiological role in the storage, transport and protection of carotenoids.

Previous studies of model organisms have indicated that CD36 gene is a key regulator of carotenoid metabolism [29]. As a membrane protein receptor, mutation of SR-BI blocks the cellular uptake of carotenoids and results in blindness in *Drosophila* [8]. In the present study, differential expression of *PmSR-BI* gene in the hepatopancreas and mantle may reflect differences in the efficiency of material transportation and absorption between the two-coloured lines. Our present results indicated that *PmSR-BI* gene mRNA expression is associated with carotenoids absorption in the pearl oyster. Total carotenoid content and *PmSR-BI* gene expression levels were higher in the yellow-coloured line than in the white-coloured line.

The study found that colours of mollusc shells were determined by the organic pigments incorporated into their skeletal structures; mainly including carotenoids [30]. By predominantly feeding on aquatic plant sources, molluscs accumulate carotenoids in their body tissues and transform it themselves when needed [31]. Shell (prismatic layer) colouration of pearl oyster include brown, black, red, white and yellow, of which the brown prismatic layer is common and the yellow and white prismatic layers are rare in natural or cultured populations. During pearl oyster breeding programs, we obtained yellow and white shell colourations in the population. The muscle tissue and mantle of yellow-shelled oyster were orange, relative to the white-shelled oyster. Our previous studies found that the yellow-shelled oyster had higher carotenoid content than the white-shelled oyster [32]. Other types of bivalve shells have a similar situation. In *Hyriopsis cumingii*, TCC in gonad, gill, hepatopancreas, kidney and mantle of purple mussels was significantly higher than that of white mussels [33]. In *Patinopecten yessoensis*, carotenoids of reddish-orange shells are 40 times higher than that in the brown shells, and the results suggested that the accumulation of carotenoids contributes to the formation of reddish-orange shells [34]. In the present studies, we detected that the carotenoid content in the yellow-coloured strain was higher than in the

white-coloured strain. Furthermore, we found a significantly positive correlation between the amount of *PmSR-BI* gene expression and TCC in yellow- and white-coloured lines. These results above suggested that *PmSR-BI* is involved in the carotenoid intake and accumulation of pearl oyster.

In conclusion, we successfully cloned and sequenced the complete *PmSR-BI* gene from the pearl oyster *P. fuctada martensii*. *PmSR-BI* mRNA was highly expressed in hepatopancreas, followed by the mantle, gill and adductor muscles. A positive correlation was found between *PmSR-BI* expression levels and TCC in yellow- and white-coloured pearl oyster. The results suggested that *PmSR-BI* is involved in the absorption of carotenoids in the pearl oyster *P. fuctada martensii*.

Conflict of interest

The authors declare no conflict of interest.

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