



Molecular cloning and characterization of a novel Y-box gene from *Sepiella maindroni*

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ABSTRACT. Y-box proteins are a family of highly conserved nucleic acid binding proteins that interact with genome and transcription product to modulate the transcriptional and translational processes. In the present study, a complete mRNA of Y-box binding protein (designated SmYB) was obtained from *Sepiella maindroni* by amplification of flanking sequences. The full size of SmYB cDNA was 1502 bp, including 99 bp at the 5' untranslated region (UTR), a 3' UTR of 821 bp with a poly (A) tail, and an open reading frame of 582 bp, encoding a polypeptide of 193 amino acids with the predicted molecular weight of 16.48 kDa. The conserved cold-shock domain and two known RNA binding motifs identified in SmYB strongly suggested that SmYB was a new member of Y-box proteins. Quantitative real-time PCR was performed to examine the expression of SmYB mRNA in various tissues, embryos, and its temporal expression in liver after cold shock. The mRNA transcript of SmYB was detected in all examined tissues, with the highest expression level in testis and ovary. SmYB was abundant in early developmental stages of *S. maindroni* embryos but diminished in the late post-embryonic development. In addition, cold-shock treatment upregulated the transcription of SmYB mRNA in liver. These results

demonstrated that SmYB is involved in embryonic development of *S. maindroni* and its tolerance to acute low temperatures.

Key words: *Sepiella maindroni* (SmYB); Y-box; Gene cloning; mRNA expression; Embryonic development; Cold shock

INTRODUCTION

The Y-box proteins are a family of regulatory proteins characterized by the presence of a highly conserved nucleic acid-binding domain, which shows substantial similarity to bacterial cold shock proteins (Makino et al., 1996; Kohno et al., 2003). Y-box proteins were reported to perform a wide variety of cellular functions, including transcriptional activation, chromatin modification, translational repression, DNA repair, and RNA Packaging (Faustino and Cooper, 2003). Y-box proteins can interact with both DNA and RNA to modulate the transcriptional and translational processes (Wolffe, 1994; Lodomery and Sommerville, 1995; Matsumoto and Wolffe, 1998). In addition, the member of this family is also an oncogenic transcription/translation factor that is overexpressed in a number of cancer types including prostate (Giménez-Bonafé et al., 2004), bone (Oda et al., 1998), colon (Shibao et al., 1999), muscle (Oda et al., 2003), and most recently breast cancer (Stratford et al., 2007). Shiota et al. (2014) reported that Y-box binding protein-1 signaling improves cellular sensitivity to taxane in prostate cancer.

In cytoplasm, Y-box proteins are essential for messenger ribonucleoprotein (mRNP) particles in which mRNA is stored and may act as a mRNA chaperone or a translation repressor (Evdokimova et al., 2001; Bader and Vogt, 2005; Lu et al., 2006). Y-box proteins have also been reported to be transported into the nucleus from the cytoplasm to modulate gene expression in response to physiological and environmental stress factors (Koike et al., 1997).

Members of the Y-box protein family have been characterized in numerous phylogenetically diverse organisms (Graumann and Marahiel, 1998). Prokaryotic cold-shock proteins, consisting entirely of a cold shock domain (CSD), bind selectively to single- or double-stranded DNA (Graumann and Marahiel, 1996; Yamanaka et al., 1998). Eukaryotic Y-box proteins have an additional carboxyl-terminal tail, which facilitates nucleic acids binding and is involved in protein-protein interaction (Wolffe et al., 1992; Bouvet et al., 1995; Nambiar et al., 1998). The CSD of Eukaryota, containing two RNA binding motifs, RNP-1 and RNP-2 (Burd and Dreyfuss, 1994), shares substantial similarity (55%) to bacterial CSD proteins (Landsman, 1992). This domain in bacteria is involved in single-stranded nucleic acids binding (Schindelin et al., 1994). The C-terminal following CSD shows less homology among these proteins. In fact, there are distinctly different types of nucleic acids binding modes in the C-terminal domain. Tail domains of invertebrates have in common an enrichment in arginine and glycine, while the vertebrate Y-box proteins contain multiple independent nucleic acid-binding domains (Matsumoto and Wolffe, 1998).

Based on the broad nucleic acid-binding properties of Y-box proteins, some patterns regarding their expression and function are beginning to emerge. For example, the Y-box protein in oocytes of *Xenopus laevis* prevents translation of maternal mRNA until development is initiated (Sommerville and Lodomery, 1996; Thieringer et al., 1997). The Y-box protein in mouse is highly expressed in spermatocytes (Didier et al., 1988) and developing embryos (Lu et al., 2005). In addition, there is a subset of Y-box proteins, which are highly expressed in

actively proliferating adult tissues such as the colorectal epithelial glands and regenerating liver tissue after hepatectomy (Grant and Deeley, 1993; Ito et al., 1994; Shibao et al., 1999). Many of these proteins are reported to be in connection with growth related processes (Ladomery and Sommerville, 1995). Several Y-box proteins in bacteria are induced upon temperature downshift, and they maintain mRNA molecules in a single stranded conformation to facilitate translation at low temperature (Jiang et al., 1997). Although many eukaryotic Y-box proteins have been cloned, the question of cold inducibility of these proteins has not been addressed yet.

Sepiella maindroni is an important and valuable fishery species in China. Their life and activity are influenced by low temperatures because of their poor ability to withstand freezing (Li et al., 2010), and massive deaths by frostbite had frequently occurred after a cold snap in cultured *S. maindroni* populations. Since Y-box proteins carry out multiple functions, such as transcriptional activation, DNA repair or RNA Packaging (Faustino and Cooper, 2003; Kohno et al., 2003), studying the Y-box protein gene from *S. maindroni* will help elucidate the molecular mechanisms of embryonic development and tolerance to acute low temperature, which in turn will contribute to the cultivation of fine strains in farming. Dias et al. (2014) found that SmYB1 functions in the turnover, transport, and/or stabilization of RNA molecules during post-transcriptional gene regulation.

The main objectives of the present study were: 1) to clone the full-length cDNA of Y-box protein gene from *S. maindroni*; 2) to investigate tissue distribution of the SmYB transcript; 3) to describe the expression pattern of the *SmYB* gene during embryonic development; 4) to examine the temporal response of SmYB mRNA to acute low temperature at the mRNA level.

MATERIAL AND METHODS

Animals, embryos, acute low temperature challenge, and liver collection

S. maindroni larvae, averaging 4 cm in length, were collected in Ningbo, China, and acclimated at $22^{\circ} \pm 2^{\circ}\text{C}$ for one week before processing. One hundred and eighty cuttlefish were employed for the cold treatment experiment. The cuttlefish were randomly divided into nine groups and each group contained 20 individuals. Six groups were exposed to 14° or 18°C , and three control groups were maintained at 22°C , which was normal temperature of the aquaculture water. After the treatment, three individuals were randomly sampled at 0, 0.5, 2, 6, 12, 24, 48, and 72 h. The liver was collected and preserved in RNAlater (TaKaRa, Tokyo, Japan) for RNA extraction. Muscle, ink sac, liver, pancreas, ovary, testis, stomach, and gill from *S. maindroni* adults were collected to determine tissue distribution of SmYB transcript. Total RNA was extracted using RNAiso Plus (TaKaRa) according to the manufacturer protocol.

Fertilized eggs were collected and acclimated at $20^{\circ} \pm 1^{\circ}\text{C}$. The embryonic development of *S. maindroni* was divided into 12 stages (unpublished data), and to facilitate the research, they were combined into 5 major developmental stages: I) the zygote stage; II) the pre-embryonic stage, which occurs over a very short period of time, including cleavage stage, blastula, and gastrula; III) the organogenesis stage, when the main parts of a bodily structure or organs, like wrist, eye, heart, pigment, etc., are formed; IV) the morphological integrity stage, which corresponds to the ongoing further improvement of bodily structure, with a yolk sac still present; V) the pre-hatching stage when the yolk sac disappears, while the larvae are preparing for hatching. Embryos were collected at these five stages and used for RNA extraction.

cDNA library construction

Total RNA from the embryos of *S. maindroni* was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) at the stage V. First- and double-stranded cDNAs were synthesized according to the protocol for the SMART cDNA Library Construction Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). Subsequently, approximately 2 μ L of first strand cDNA sample was amplified using UPM primer. The first four peak fractions containing cDNA (>500 bp) were pooled together using column chromatography with a CHROMA SPIN-400 medium (Clontech). The cDNA was ligated to λ TriplEx2 vector (1:1.5), and the ligation was packaged with a Gigapack III Gold Packaging extract (Stratagene, La Jolla, CA, USA). Recombination efficiency was identified by blue/white screening in *E. coli* XL1-Blue. Colony PCR was used to confirm the size of inserted fragments in the library.

Sequencing and analysis

cDNA clones were selected randomly from the cDNA library and single-pass sequenced using random T3 primer on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). A large-scale EST sequencing project for *S. maindroni* was initiated to identify and functionally annotate as many unique transcripts as possible. BLAST search was used to compare processed cDNA sequences against ESTs and genes available in the GenBank database (<http://www.ncbi.nlm.nih.gov/blast>). A total of 860 unigenes were assembled from 1018 ESTs, which were obtained in the present study. Interestingly, one EST (GenBank No. GT618028; Contig356; length: 538 bp) was the mirror of a Y-box factor in *Aplysia californica* (P41824) in BLASTx analysis.

Cloning the full-length cDNA of SmYB

Amplification of flanking sequences was carried out by a 5'- and 3'-Full RACE kit (TaKaRa) to obtain the complete sequence of EST (GT618028). Operating steps were referenced following the manuals of these kits. Purified sequences were cloned into pMD-18T vector (TaKaRa) and transformed into *Escherichia coli* Top10F competent cells. Finally, M13-47 and RV-M universal primers (Table 1) were used to screen for three positive recombinants to be used for sequencing.

3'-RACE

The 3'-RACE was performed with a 3'-Full RACE CoreSet Ver. 2.0 (TaKaRa) following manufacturer instructions. The first-round PCR consisted of initial denaturation at 94°C for 4 min, followed by 32 cycles at 94°C for 45 s, 52°C for 45 s, and 72°C for 90 s, and a final extension for 7 min at 72°C. The second PCR with nested primers consisted of 4 min at 94°C, followed by 32 cycles at 94°C for 45 s, 51°C for 45 s, and 72°C for 90 s, and a final extension for another 7 min at 72°C.

5'-RACE

The 5'-RACE was carried out with a 5'full RACE kit (TaKaRa Code: D315) following manufacturer instructions. Total RNA was extracted from the second flower. mRNA was

dephosphorylated and the mRNA cap structure was removed. A 5'-RACE adaptor was ligated to the decapped mRNA with a 5'-full RACE kit (TaKaRa) and the first-stranded cDNA was synthesized by reverse transcription. The first-round PCR protocol consisted of initial denaturation at 94°C for 4 min, followed by 32 cycles at 94°C for 45 s, 58.7°C for 45 s, and 72°C for 60 s, and a final extension of 7 min at 72°C. The second-round PCR with nested primers consisted of 4 min at 94°C, followed by 32 cycles at 94°C for 45 s, 59°C for 45 s, and 72°C for 60 s, and a final extension of 7 min at 72°C.

Table 1. Oligonucleotide primers used in the experiment.

Primer	Sequence (5'-3')	Sequence information
P1 (reverse)	TC ACCATCTCCA ACACTCCT	SmYB specific primer
P2 (forward)	GCTCAAGATGAGGGTTCAGAAT	SmYB specific primer
P3 (forward)	CATGGCTACATGCTGACAGCCTA	Universal primer
P4 (reverse)	TACCGTCGTTCCACTAGTGATTT	Universal primer
P5 (forward)	GAGGGTGAAGTAGAGACTAC	Real-time SmYB primer
P6 (reverse)	ACTCCACCTTCTCACCATCT	Real-time SmYB primer
P7 (forward)	GACTCCTACGTAGGAGACGA	Real-time actin primer
P8 (reverse)	CGTTGAAGGTCTCGAACATGA	Real-time actin primer
M13-47	CGCCAGGGTTTTCCAGTCACGAC	Vector primer
RV-M	GAGCGGATAACAATTTACACAGG	Vector primer

Sequence analysis, multiple sequences alignment, and phylogenetic analysis

Sequence similarity of nucleotide and protein sequences was examined with BLAST (<http://www.ncbi.nlm.nih.gov/Blast.cgi>). Amino acid sequences were deduced by Expert Protein Analysis System (Artimo et al., 2012; <http://www.expasy.org>). The binding motives within proteins were identified by simple Modular Architecture Research Tool (SMART) (Letunic et al., 2014; <http://smart.embl-heidelberg.de/>). Signal peptides of amino sequences were predicted by SignalP 3.0 (Bendtsen et al., 2004; <http://www.cbs.dtu.dk/services/SignalP/>). Neighbor-joining method used to reconstruct phylogenetic network and multiple sequence alignment between amino acid sequences of SmYb and its homologs in other species were carried out with MEGA 5.0 (Tamura et al., 2011; <http://www.megasoftware.net/>).

Quantitative analysis of SmYB mRNA expression

Real-time PCRs were performed using an ABI 7300 (Applied Biosystems). The PCR was carried out in a final volume of 20 µL with 12.5 µL SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan), 4.5 µL water, 0.5 µL of each primer (P5 and P6 for SmYB and P7 and P8 for β-actin, see Table 1), and 2 µL cDNA. The reactions were performed at 95°C for 3 s, followed by 40 cycles at 95°C for 5 s, 56°C for 5 s, and 72°C for 20 s, and a final extension at 72°C for 1 min. Each reaction was repeated three times, and the same reaction mixtures without cDNA were used as negative controls. The relative amount of target mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method. Significant differences were analyzed using the SPSS software (IBM Corp., Armonk, NY, USA).

RESULTS

Identification and analysis of SmYB cDNA

The complete cDNA sequence of SmYB was obtained by overlapping the sequence of EST (GenBank No. GT618028) with the amplified fragments. The complete mRNA of this gene was deposited in the GenBank (No. HQ179101). The full-length mRNA sequence of SmYB is 1502 bp, including 682-bp code region (open reading frame, ORF), which starts at the position 100 and extends to the position 681, indicating that the 193-amino acid protein is 16.48 kDa with theoretical isoelectric point predicted at 10.06. The polyadenylation signal, AATAAA, was found at position 1289, followed by a poly-A tail in the 3'-terminal region. The deduced amino acid sequence of SmYB is shown in Figure 1. The analysis with SMART revealed that this protein sequence carried a cold-shock domain (from Ser31 to Pro99) and two putative RNA binding motifs (i.e., RNP-1 and RNP-2) presented at the N-terminus of SmYB. However, no signal peptide region was found at the N-terminus. The C-terminal tail of the protein is extremely basic with high arginine (26%) and glycine (16%) content.

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1 GGGGACAGAGGTGAACAAAGAAGAGAGGGGAGAGAAGTTAGTGAAGTCCAGTGACCGTAG
1 M S D S E K Q
61 GGTCCGGATATTAACATCTAAATTGTAACATCAACAGCCATGTCGACTCGAGAAACAA
8 P E G E V E T T E R P A T S G K K V I A
121 CCAGAGGGTGAAGTAGAGACTACTGAGAGGCCGTGCAACCAGTGGAAAGAAAGTAATAGCC
28 S K V S E T V K W F N V K S G Y G F I N
181 TCCAAAGTATCGGGGACAGTAAAATGGTTCAATGTA AAAAGCGGATATGGCTCATTAAAC
48 R E D T K E D V F V H Q T A I I K N N P
241 CGGGAAGACACAAAAGAGATGCTTTGTACATCAGACAGCTATAATTA AAAACAATCCT
68 R K Y L R S V G D G E K V E F D V V E G
301 CGTAAATATCTGAGGAGTGTGGAGATGGTGAGAAGTGGAGTTGATGTCGCGAAGGA
88 E K G N E A A N V T G P G G N H V Q G S
361 GAGAAGGTAATGAAGCTGCCAATGCTCACTGGACCTGGTGAATCATGTGCAAGGTAGC
108 K Y A A D R R R Y R R R W W N R R R R G
421 AAATATGCTGCAGACCCAGCAGATATCGACGGCATGGTGAATAGGAGGCCCTCGT
128 A A R E E G E E A K G D Q D D G E S G E
481 GCTCCAGGAGGAGGTGAAGAGCAAAGGTGACCAAGACGATGGGAAAGTGGCGAA
148 G A G S G E D R S R P P M R R R P Y W R
541 GGAGCTGGTAGTGAGAAGACCGTAGCCACCCCAATGAGGCGCAGGCTTACTGGAGA
168 R R R F F R G P R R G M P S R S I P Q D
601 CGGAGACGTTTTTCCGTGGCCCAAGACGTGGCATGCCATCTCGTAGCATCCCTCAGGAT
188 E H G G N R *
661 GAGCATGGTGGAAACCGTGTGCGGATCTCGGATGCATGGCCACCCCATTCGCGCG
721 ACGACGCCCTTCTACCGTAGTATTATCGCCACCCATGCACCGTTACTCCCAAGGTGG
781 CCCACCTCGAGATGGTGTGCTCAAGATGAGGGTTTCAAGATGGACCTCCTCCTCCCG
841 GCGACGACCCAGCGTTCTAGAGGTGGTGGAGCAGCTCAGGCCAGGCAGATGGGGATC
901 ACGAGGGGTCTGACAGGCTCTGGCCAAAGGGAGCAATCTGAAGGGGAAACGGAACGAG
961 TGACAGTGGCCATAAAGCAGAGGATTCTGTCCAGCAAATCAGAAGACAGCAGTTGAGC
1021 TCCTCCTCCTGCTCAGTCATCGACAGAAGTACTCCTGCTGCTGCTCAGGTGGCTGCTTC
1081 GACTTAATGTCCTTTTAGCACAGCTTACGTAGCAAGAAATGAAATGGGAAAGACAAATT
1141 CCACTCTCTAGGAAGATCTACTAATTTAGTAAATATGCTAAATTTCCCCCTGTAAG
1201 TTGAATGAAGCCATTTCAAGCAGCTTTTCTTTTATGGAAGAATCCAAAAATA
1261 CTCACCTCCACTTACCAGCAACAAGTTGATGGGGTGGCGATCTTCTGATTGAATGCA
1321 GTTTGTGGGGTGTAGGCGCTTTTCTTTCTTCTTCTTCTGAAAGGAGAAAGAAAAA
1381 CATGATCCAATCCATCATTATAGCCCATCTCAACATCTTCAACTTTCTATCAGG
1441 GCAGTGAAGGAAAGTAGTTTTTTTGAATAATTTCCATCTTATGCAAAAAAAA
1501 AA
    
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Figure 1. Nucleotide sequence of the *SmYB* gene with its deduced amino acid sequence. Asterisk indicates the stop codon. Polyadenylation signal is in bold and italicized. The cold-shock domain is boxed. RNP-1 (GYGFINR) and RNP-2 (EDVVFVHQT) RNA binding motifs presented in shadow bold letters.

Multiple sequences alignment and phylogenetic analysis

BLASTp and phylogenetic analysis showed that the SmYB sequence was highly homologous with other Y-box proteins: 73% homology with *Lethenteron japonicum* (ACF33226), 68% homology with *A. californica* (P41824), and 58% homology with *Saccoglossus kowalevskii* (XP_002732435). In addition, multiple alignments revealed that the CSD as well as the two RNA-binding motifs (RNP-1 and RNP-2) were highly conserved within all Y-box proteins analyzed (Figure 2). Phylogenetic tree of the Y-box proteins, constructed using MEGA 4.0 software, resolved three groups including vertebrates, invertebrates, and prokaryotes, and the sequence of SmYB was resolved within the invertebrate branch (Figure 3).

Distribution of SmYB mRNA

The qPCR was conducted to analyze expression of SmYB in various tissues, such as muscle, ink sac, liver, pancreas, ovary, testis, stomach, and gill. The results showed that SmYB expression was significantly greater in testis and ovary. Its expression in pancreas, liver, and gill was comparatively at medium levels, whereas weak signal of the SmYB transcript was detected in stomach, muscle, and ink sac. The relative expression levels in tissues of testis, ovary, liver, and pancreas were 582.43-, 102.31-, 47.57-, and 49.90-fold, respectively, of that in the ink sac with a significant difference ($P < 0.05$). The expression levels of SmYB in gill, stomach, and muscle were slightly higher than in ink sac, but the difference was not significant ($P > 0.05$) (Figure 4).

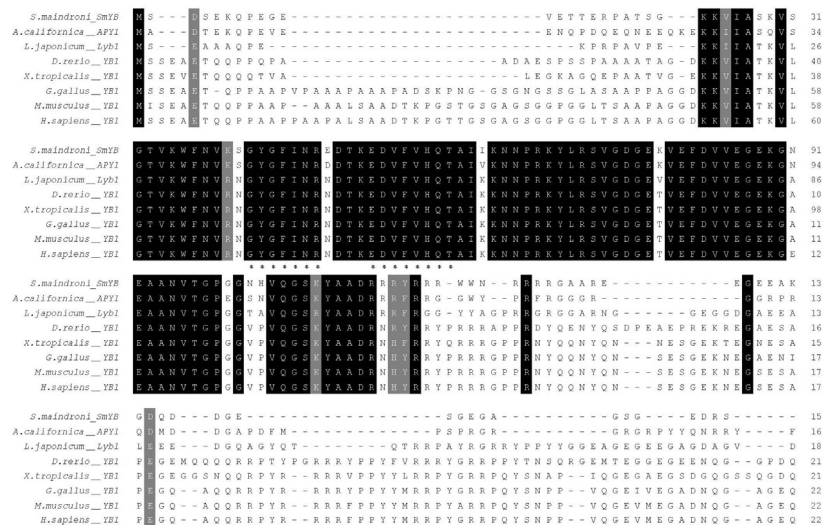


Figure 2. Multiple sequence alignment of the deduced SmYB amino acid sequence and other members of Y-box binding protein family deposited in the GenBank. The black shadow region indicates positions with the same amino acid residue. Gaps are indicated by dashes. RNP-1 (GYGFINR) and RNP-2 (EDVFNHQT) RNA binding motifs are marked by asterisks below the sequence. Abbreviations are as follows: P41824 (*Aplysia californica*_APY1), ACF33226 (*Lethenteron japonicum*_Lyb1), AAH50156 (*Danio rerio*_YB1), NP_001016677 (*Xenopus tropicalis*_YB1), NP_989745 (*Gallus gallus*_YB1), AAH49977 (*Mus musculus*_YB1), and AAA61308 (*Homo sapiens*_YB1).

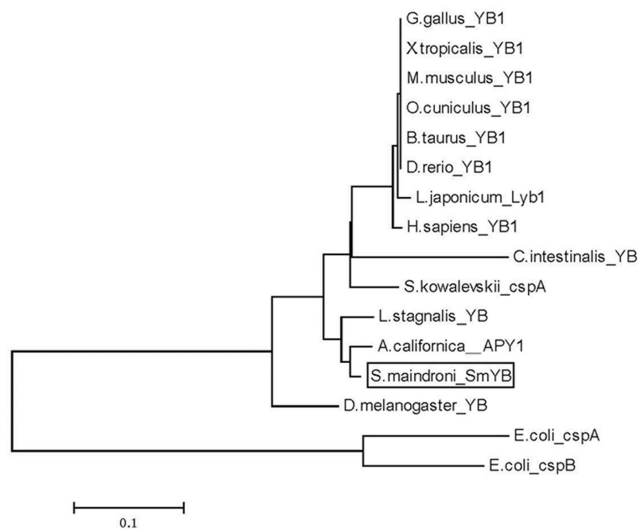


Figure 3. Consensus neighbor-joining tree based on sequences of Y-box proteins from different animals. Protein sequences used for phylogenetic analysis are as follows: P41824 (*Aplysia californica*_APY1), NP_001016677 (*Xenopus tropicalis*_YB1), ACF33226 (*Lethenteron japonicum*_Lyb1), AAT97092 (*Lymnaea stagnalis*_YB), NP_777240 (*Bos taurus*_YB1), NP_001076254 (*Oryctolagus cuniculus*_YB1), AAH49977 (*Mus musculus*_YB1), AAA61308 (*Homo sapiens*_YB1), AAB94634 (*Drosophila melanogaster*_YB), NP_989745 (*Gallus gallus*_YB1), AAH50156 (*Danio rerio*_YB1), NP_001072039 (*Ciona intestinalis*_YB), XP_002732435 (*Saccoglossus kowalevskii_cspA*), AAA23617 (*Escherichia coli_cspA*), and YP_003502592 (*E. coli_cspB*).

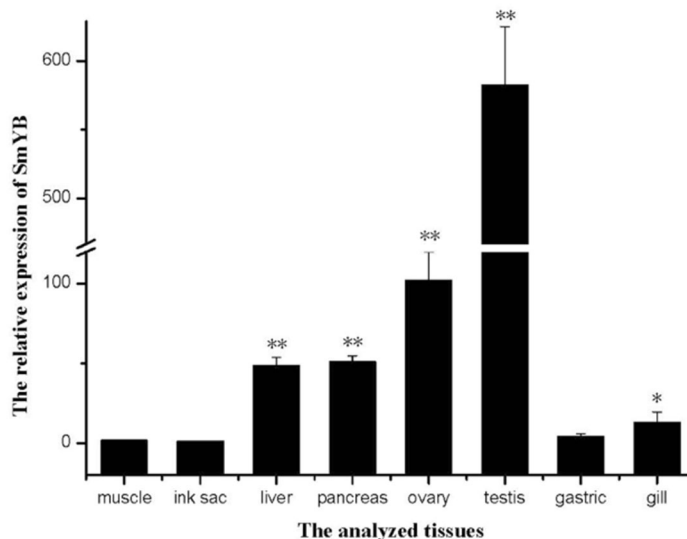


Figure 4. Tissue distribution of the SmYB transcript measured by SYBR Green Real time-PCR. The tissues, including muscle, ink sac, liver, pancreas, ovary, testis, stomach, and gill were collected from three individual cuttlefish. Vertical bars represent the mean \pm SD (N = 3). * and ** indicate significant differences across calibrator (ink sac) at $P < 0.05$ and $P < 0.05$, respectively.

Expression pattern of the *SmYB* gene during embryonic development

The mRNA expression level of *SmYB* gene in the embryos was detected by qPCR. The *SmYB* gene transcript was detected in all stages of development that were tested. The expression level decreased steadily during development. *SmYB* mRNA was detectable until the pre-hatching stage. The highest expression was found in the zygote stage, at about 13.26-fold of that in the pre-hatching stage ($P < 0.05$) (Figure 5).

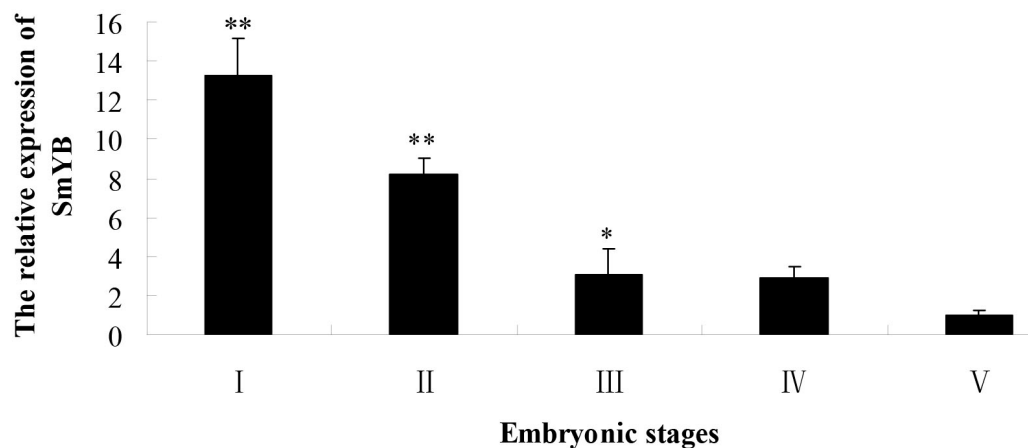


Figure 5. Expression of the *SmYB* mRNA at different embryonic stages measured by SYBR Green Real time-PCR. β -actin gene was used as an internal control to calibrate the cDNA template for all samples. * and ** indicate significant differences at $P < 0.05$ and $P < 0.05$, respectively.

Expression of *SmYB* mRNA in liver after cold shock

To characterize the response of the *SmYB* gene to external temperature stimulus, *S. maindroni* larvae were exposed to 14° or 18°C for 0.5, 2, 6, 12, 24, 48, and 72 h, while the control groups were maintained at 22°C. The expression of *SmYB* was analyzed by qPCR with β -actin as internal control. As shown in Figure 6, *SmYB* expression in the two experimental groups showed the same trends. In the first few hours after the cold shock, the *SmYB* mRNA expression was slightly downregulated, but it was not significantly different from that in the control group ($P > 0.05$). As time progressed, the expression of *SmYB* mRNA increased significantly; the expression peak at 14°C and 48 h was 3.82-fold higher than that observed in the control group at the same time period ($P < 0.05$), while the peak at 18°C and 12 h was 3.33-fold higher than that in the control group ($P < 0.05$). The *SmYB* mRNA expression of the two groups both reverted to the original levels in the last few hours of the experiment (Figure 6).

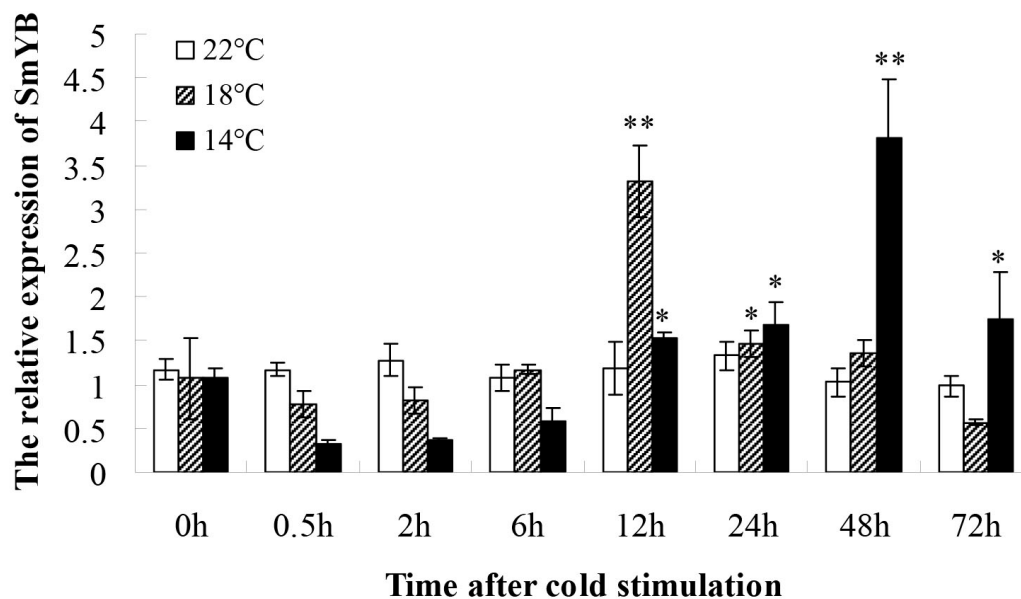


Figure 6. Temporal expression of the Y-box gene transcript in liver during the cold shock. *Sepiella maindroni* larvae were incubated at 18° or 14 °C for 0, 0.5, 2, 6, 12, 24, 48, and 72 h. Control groups were maintained at 22 °C. Each bar represents the mean value from three determinations with standard error.

DISCUSSION

Y-box proteins are widely expressed in organisms from bacteria to humans and play vital roles in multiple biological processes (Evdokimova et al., 2001; Lu et al., 2006). In this study, a full-length cDNA encoding a member of the Y-box protein family (SmYB) was identified from *S. maindroni*, 1502 bp in length. BLASTx analysis revealed that SmYB is homologous to Y-box proteins from other animals (73% to *L. japonicum*, 68% to *A. californica*, and 58% to *S. kowalevskii*). The conserved CSD and two known RNA binding motifs identified in SmYB classified it as a member of eukaryotic Y-box proteins (Graumann and Marahiel, 1998). However, the C-terminal tail of SmYB has a high frequency of arginine and glycine, which is characteristic of invertebrate Y-box proteins (Matsumoto and Wolffe, 1998), and phylogenetic analysis clustered SmYB together with invertebrates and distant to other organisms. Therefore, we conclude that SmYB belongs to the invertebrate subgroup of the Y-box protein family.

Y-box proteins regulate the utilization of maternal and paternal stores of mRNA by translational machinery in non-dividing germ cells (Matsumoto and Wolffe, 1998). For example, the mouse homolog MSY1 has been demonstrated to be implicated in masking of mRNA transcripts in spermatocytes (Tafari et al., 1993). In *Xenopus* oocytes, FRGY2 binds to maternal mRNAs and masks them from translation (Yu et al., 2001, 2004). In the present study, SmYB was constitutively expressed in all examined tissues, with the highest expression level in testis and ovary. The results indicated that SmYB is involved in the development of *S. maindroni* germ cells.

Y-box protein expression is closely associated with cell proliferation (Wolffe et al.,

1992). For instance, Y-box protein is abundantly expressed in actively proliferating adult tissues such as colorectal epithelial glands, regenerating liver tissue that develops after hepatectomy (Grant and Deeley, 1993; Ito et al., 1994; Shibao et al., 1999), and YB-1 from mouse protects cells from senescence during periods of proliferative stress (Lu et al., 2006). The mRNA for SmYB is expressed throughout the embryogenesis, and its amount declines gradually from stage I to stage V. The highest expression of SmYB was found in the zygote stage, which was 13.26-fold of that in the pre-hatching stage ($P < 0.05$). Early embryonic period can be considered to be the time of substantial proliferative stress because the early-stage embryogenesis involves massive cellular proliferation that occurs over a very short period of time (Lu et al., 2006). High expression of SmYB in early-stage embryos showed that SmYB is important for cellular stress responses and plays an important role in embryonic development.

Cold shock is the most primitive form of stress that organisms can be exposed to, from bacteria to plants and animals (Kohno et al., 2003). Three of the bacterial Y-box protein homologs were induced upon temperature downshift, and they maintained mRNA molecules in a single stranded conformation at low temperature to facilitate translation (Lee et al., 1994; Jiang et al., 1997). Whether eukaryotic Y-box binding proteins are involved in the cold-shock response or not is still uncertain. In the present study, we tested this by using liver from *S. maindroni* larvae, because liver is the major metabolizing organ (Rigato et al., 2007) and has an endogenous SmYB expression. During the first few hours after the cold shock treatment, the SmYB mRNA expression was slightly down-regulated, but no significant difference between the experimental and control groups was observed, indicating that the cold shock response leads to temporary cessation of the liver growth and reduction in SmYB expression in protein synthesis in *S. maindroni* larvae. The expression of SmYB mRNA increased significantly with the time, which is explained by the immune rejection of body or the basic defense system. The result was similar to the expression pattern of cold-shock proteins in *E. coli* treated with cold shock (Jiang et al., 1997). By contrast, the expression level increased earlier in the samples exposed to 18°C than in the samples exposed to 14°C. This indicates that the damage to the cells was milder due to slightly lower temperature, and the organisms could facilitate quicker their recovery and growth. We can conclude that SmYB is a cold-shock inducible gene, and it displays a protective role against cold shock.

In conclusion, a novel Y-box protein gene (*SmYB*) was cloned from *S. maindroni*. SmYB was constitutively expressed in muscle tissue and tissues of the ink sac, liver, pancreas, ovary, testis, stomach, and gill, and it is highly expressed in testis and ovary. The SmYB mRNA is abundant in the early stages of the development of *S. maindroni* embryos but diminishes in the late post-embryonic development. Cold-shock treatment up-regulates the transcription of SmYB mRNA in liver. The results of this study indicate that *SmYB* is a specific gene involved in cellular growth and protection by providing resistance to cold stimulus.

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