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Characterization of vitellogenin receptor (VgR) from the Chinese oak silkworm, *Antheraea pernyi*

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Abstract

Vitellogenin receptor (VgR) mediates the uptaking of vitellogenin by oocytes and plays a critical role in egg development. Here, we first report the VgR gene in Lepidoptera insect, the Chinese oak silkworm *Antheraea pernyi* (Guerin-Meneville) (Lepidoptera Saturniidae), this gene consists of 5847 bp with a putative ORF of 5439 bp which encodes a 202.9 kD protein. Sequence analysis revealed that *A. pernyi* VgR (Ap-VgR) was highly homologous to those VgRs from other insects and contained some conservative signatures such as ligand-binding domains, epidermal growth factor (EGF)-precursor domains and *O*-linked sugar domain. The result of semi-quantitative PCR showed that the expression of Ap-VgR was found in ovary and fat body during the pupae while only in ovary at adult stage. In addition, prokaryotic expression of partial function domain from *A. pernyi* VgR was also performed, SDS-PAGE and western blot analysis demonstrated that a 31.5 KD recombinant protein was successfully expressed in *Escherichia coli* cells.

Key words: *Antheraea pernyi*; vitellogenin receptor; expression.

Introduction

Insects oocytes need accumulate plentiful yolk proteins to ensure enough supply of nutrients for the egg development (Harnish *et al*., 1982; Wyatt *et al*., 1984; Tufail *et al*., 2005). As the major yolk protein, vitellogenin (Vg) is synthesized in the fat body and taken up by vitellogenin receptors (VgRs) located on the external surfaces of the developing oocytes (Sappington and Raikhel, 1998). Vitellogenin receptors (VgRs) belong to low-density lipoprotein receptor (LDLR) superfamily and have common structural features including low-density lipoprotein receptor domain class A (LDLa), epidermal growth factor (EGF), low-density lipoprotein receptor domain class B (LDLb), O-linked sugar domain, transmembrane region and cytoplasmic domain (Tufail and Takeda, 2005).

The VgRs have been studied extensively in various animals from vertebrates to invertebrates (Bujo *et al*., 1994; Okabayashi *et al*., 1996; Li *et al*., 2003; Tiu *et al*., 2008; Tufail and Takeda, 2009). So far, the cDNA sequences of VgRs have been identified from a few insect species: *Drosophila melanogaster* Meigen (Schonbaum *et al*., 1995), *Aedes aegypti* (L.) (Sappington *et al*., 1995), *Solenopsis invicta* Buren (Chen *et al*., 2004), *Bombyx mori* L. (Lin *et al*., 2005), *Periplaneta americana* (L.) (Tufail and Takeda, 2005), *Blattella germanica* (L.) (Ciudad *et al*., 2006), *Leucophaea maderae* (F.) (Tufail and Takeda, 2007), *Spodoptera litura* (F.) (Krishnan *et al*., 2008). In addition, some other insect VgR sequences such as *Nilaparvata lugens* (Stal) (GU723297), *Anopheles gambiae* Giles (EAA06264), *Nasonia vitripennis* (Walker) (XM_001602904), *Apis mellifera* L. (XM_001121707), *Tribolium castaneum* (Herbst) (XM_963810) and *Acyrthosiphon pisum* (Harris) (XM_001944117) were also found in GenBank database. However, few VgRs were reported in Lepidopteran insects (Lin *et al*., 2005; Krishnan *et al*., 2008) as well as their biological functions.

Chinese oak silkmoth *A. pernyi* is a kind of silk-producing insect and has excellent economical values (Huang *et al*., 2002; Zhou and Han, 2006). In our previous studies, we have identified the vitellogenin gene and its function from *A. pernyi* (Liu *et al*., 2000; 2001; 2002; Zhu *et al*., 2010). To figure out the role of VgR in egg development of *A. pernyi*, the characterization and its expression were performed in this experiment and we hope these results will provide some information for the study of interaction between Ap-Vg and Ap-VgR.

Materials and methods

Experimental insects

A. pernyi was introduced from the Sericultural Research Institute of Shandong and reared on the leaves of oak.

RNA extraction and cDNA synthesis

Total RNA was extracted from 100 mg of fat body with TRIzol™ Reagent (Transgene) according to the instructions and the RevertAid™ H Minus First Strand cDNA Synthesis Kit was used to synthesize single-stranded cDNAs for RT-PCR. For RACE-PCR, single-stranded cDNAs were synthesized using the SMART™ RACE cDNA Amplification kit (Clontech).

Cloning and sequencing of Ap-VgR

Oligonucleotide primers (shown in table 1) were designed based on *B. mori* sequence with Primer premier 5.0 software to amplify the cDNA sequence of Ap-VgR gene. RT-PCR were performed using primers F_1R_1 to F_4R_4 as follows: 5 min at 94 °C; followed by 35 cycles of 94 °C for 30 s, 55 °C for 40 s, 72 °C for 1 min and a final step of 72 °C for 10 min. The primers RC3 and RC5 were used for RACE-PCR with the program consisted of 5 min at 94 °C followed by 5 cycles of 94 °C for 1 min, 60 °C for 2 min, and then 30 cycles of 94 °C for 1 min, 60 °C for 45 s, 72 °C for 1min 35 s. The PCR products were analyzed on 1% agarose gels, then subcloned into the pMD19-T simple cloning vector (Takara) and sequenced at Invitrogen, Shanghai.

Table 1. The primers used for PCR.

Primer No	Primer sequences
F1(797-816)	5'-TGGCCCGCCCTCAGTGCTCA -3'
R1(2133-2152)	5'-GTAGCGTGCGCAAGGACAAC -3'
F2(2110-2132)	5'-TCTGGCAGCGGCTACATTGAGG -3'
R2(3281-3302)	5'-TACCCGGGCTTGCATGATACG -3'
F3(2955-2976)	5'- CGGGCTCTGCGTGGCTAAGGAT-3'
R3(4379-4400)	5'-CTGGCGCATCTCCTCCTGGTGA -3'
F4(4223-4246)	5'-CGGAGTCGGGGAAGCTGATAGAAT -3'
R4(5309-5332)	5'-ACAGGCCAGCGGTACAAACAGGAC -3'
RC5(848-868)	5'- AGCCGTCGGCGCATTCACAAG -3'
RC3(5140-5161)	5'- ACGGCTTATACAGAGGTGAGGT-3'

Construction of recombinant plasmids and protein expression

To investigate the function of VgR in *A. pernyi*, the forward primer: 5'- CAGAAGCTTCTAGGAGGGAG GCGCCA-3' and reverse primer: 5'- CGCCTCGAGGA GCTCGACCCGTCCATC -3' (restriction enzyme sites *Hind* III and *Xho*I were underlined) were designed to amplify the partial function domain (residues 175-456) of VgR by PCR. The PCR product and Pet-28a vector were ligated after they were both digested with restriction enzymes *Hind* III and *Xho* I. The recombinant plasmids (Pet-VgR) were identified by sequencing and then transformed into *Escherichia coli* BL21 (DE3) cells (TransGen) for protein expression.

Western blotting

The recombinant protein was analyzed by SDS-PAGE, then transferred onto a polyvinylidene difluoride (PVDF) membrane by an electrophoretic transfer system. Membranes were blocked with phosphate-buffered saline containing 0.1% Tween-20 and subsequently incubated with anti-His tag antibodies for 2 h at room temperature, then washed by PBST and incubated with horseradish peroxidase (HRP)-conjugated sheep anti-rabbit IgG antibody (Sigma) for 1 h at room temperature (Zhu and Wu, 2008), the final detection was performed with a HRP-DAB Detection Kit (Tiangen).

Detection of Ap-VgR expression by semi-quantitative PCR

The examined tissues mid-intestine, silk gland, hemocytes, fat body, testis, integument, ovary, malpighian, antennae, wings, thorax and head were sampled from ten fifth instar larvae, pupae or adults, respectively. Semi-quantitative PCR was carried out with specific primers F_4 R_4 to determine the expression level of Ap-VgR and the actin gene (GenBank no. GU073316) was used as an internal reference (with primers F: 5′-TCTGGCACCCCACCTTCTAC-3′ and R: 5′-CCGATTGTGATGACTTGAC-3′). The amplification program for PCR was used as 94 °C for 3 min and 27 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 15 s.

Results

Sequence analysis of Ap-VgR

A full-length Ap-VgR cDNA fragment of 5847 bp (GenBank no. JN003583) was obtained by RT-PCR and RACE–PCR. Nucleotide sequence analysis revealed that Ap-VgR contains a 138 bp 5′-untranslated sequence, a putative ORF of 5439 bp, a 270 bp 3′-untranslated region and a putative polyadenylation signal upstream of the poly (A) (figure 1). Based on the entire amino acid sequence, the signal peptide, low-density lipoprotein receptor domain class A (LDLa), epidermal growth factor (EGF), low-density lipoprotein receptor domain class b (LDLb), O-linked sugar domain, transmembrane, and cytoplasmic domain were found using the ExPASy Proteomics tools. The percentage of similarity of the deduced amino acid sequence of Ap-VgR toward vitellogenin receptor sequences from *D. melanogaster*, *A. aegypti*, *S. invicta*, *B. mori*, *P. americana*, *B. germanica*, *L. maderae*, *S. litura*, *N. lugens*, *A. gambiae*, *N. vitripennis*, *A. mellifera*, *T. castaneum* and *A. pisum* was 28.3%, 29.1%, 28.1%, 98.9%, 30.1%, 29%, 28.7%, 56%, 28.2%, 30%, 28.7%, 29%, 29.6% and 28.6%, respectively. Phylogenetic analysis indicated that Ap-VgR was highly homologous to *B. mori* VgR (figure 2).

Protein expression and Western blotting

A recombinant protein with a molecular weight of about 31.5 kDa was detected by SDS-PAGE and its expression was not influenced by different IPTG concentrations (figure 3). The result of Western blotting analysis of recombinant protein showed that a 31.5 kDa consensus protein band was found in recombinant plasmids Pet-VgR while none in control group (figure 4). All this indicate the successful expression of the recombinant protein in *Escherichia coli* cells.

The expression of Ap-VgR in various tissues at different developmental stages

As the results showed (figure 5), Ap-VgR gene was differentially expressed in tissues and developmental stages. No expression was detected in various tissues at the larvae stages. The expression was found in ovary and fat body at the pupae stage while only in ovary at the adult stage.

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-138                                CGCCACTTTTTCCAAAAG
 GGGTAATAATTTAAATTTTTAAATTTAAACCCTTTTTTTTTTGTAATACATCAATCATTT
 TAATTCATATTTACCTTCACAATCGTCGATGAAGAATCGCCCTCAACACAATAGATCAGA
1 ATGAAGGTAGTTTTGTTAGCAATAGTTCTATGTACAACCTCGTGCGCGGGGCAGTTCGTT
1  M  K  V  V  L  L  A  I  V  L  C  T  T  S  C  A  G   Q  F  V
                    signal peptide (residues 1–17)
61 GACGAAATGCAAGTCTACGAGAAGGAATGCCTGGGCGAGGATGTGTTTCCGTGCATGTCC
21  D  E  M  Q  V  Y  E  K  E  C  L  G  E  D  V  F  P  C  M  S
121 GGGGGATGCATACAGCAGTCCCAGTACTGCGACGGGAAGGTGGACTGCGACGATGGAACC
41  G  G  C  I  Q  Q  S  Q  Y  C  D  G  K  V  D  C  D  D  G  T
          Low-density lipoprotein receptor domain class A   LDLa  (residues 29–67)
181 GACGAGAACTATTGTCTTGATCACAAGCCAGACGCTCAGTTCTGTAACGAGACCCACCAG
61  D  E  N  Y  C  L  D  H  K  P  D  A  Q  F  C  N  E  T  H  Q
241 TTCATGTGTCGGGATAGCAAGAAGTGCATCCCGAACCATTGGATCTGTAATAACGACATC
81  F  M  C  R  D  S  K  K  C  I  P  N  H  W  I  C  N  N  D  I
                    LDLa  (residues 74–113)
301 GATTGCGACGACGGAAGTGATGAGCTAAATTGCACTTTGGTTCCTGTGGCTACTGGTAAA
101 D  C  D  D  G  S  D  E  L  N  C  T  L  V  P  V  A  T  G  K
361 TGCAAAGGTTTTCTGTGCGGCGATGGAAAATGTATCTCCAGTCTTTGGTTATGTGATGGA
121 C  K  G  F  L  C  G  D  G  K  C  I  S  S  L  W  L  C  D  G
421 AGCTACGACTGCAAGGATAAGAGCGATGAGAATTCACCGGAAAACTGCCGTCACAGCCTC
141 S  Y  D  C  K  D  K  S  D  E  N  S  P  E  N  C  R  H  S  L
                    LDLa  (residues 120–158)
481 CTGTCCCACTCGATGCTAAGCGGATCGGATTGCCAGGATTGGCTAGGAGGGAGGCGCCAA
161 L  S  H  S  M  L  S  G  S  D  C  Q  D  W  L  G  G  R  R  Q
 541 TACAAATGCACGGACTCCTCGTTTTGCCTCCCGAGTGAAATGATGTGTGATGGCATGCAG
181 Y  K  C  T  D  S  S  F  C  L  P  S  E  M  M  C  D  G  M  Q
                    LDLa  (residues 175–214)
601 GACTGCAAGGACGGCAGTGACGAGAGATCCTTCTGTGCCAACTGGCACACGATGTGCGCG
201 D  C  K  D  G  S  D  E  R  S  F  C  A  N  W  H  T  M  C  A
661 AACCACACGTGCCTCGGTGACAAGGCCTCGTGTGTGCCGGACCGCGCCGGGCCCACGTGC
221 N  H  T  C  L  G  D  K  A  S  C  V  P  D  R  A  G  P  T  C
                epidermal growth factor (EGF)  (residues 211–259)
721 GAGTGTCTCAACCACCTCAACCTGCGTCGGTACAATACCTCGACCGGGGCCTGCGACGAC
241 E  C  L  N  H  L  N  L  R  R  Y  N  T  S  T  G  A  C  D   D
781 ATCGACGAGTGCGCGCTGGCCCGCCCTCAGTGCTCCCACTACTGCGTCAACGCGGACGGC
261 I  D  E  C  A  L  A  R  P  Q  C  S  H  Y  C  V  N  A  D  G
                    EGF-CA  (residues 260–300)
841 CATTTCACTTGTGAATGCGCCGACGGCTACTTCAAGGACGAACTTAAGTACTTGTGCTAC
281 H  F  T  C  E  C  A  D  G  Y  F  K  D  E  L  K  Y  L  C  Y
901 GCTACCGGTCCCGAACCCCTGTTGTTCTACAGTACACGAAACGAAATTAAATATCTGAAA
301 A  T  G  P  E  P  L  L  F  Y  S  T  R  N  E  I  K  Y  L  K
961 GTGAAGTCGAAGGAAGTGGTCACACTGGCGACTGGAATAAAAAAGGCTCACGGGGTCACA
321 V  K  S  K  E  V  V  T  L  A  T  G  I  K  K  A  H  G  V  T
1021 TCGAACGGAATATACGTTTACTGGGTGGAAACAGCTGAAGGTCATCAAGCCATCGTCAAA
341 S  N  G  I  Y  V  Y  W  V  E  T  A  E  G  H  Q  A  I  V  K
1081 GCTCACATAGACGACGTAGAAAACACTCGACAGGTAATAGTCGGTCTAGGTCTAGAGGAT
361 A  H  I  D  D  V  E  N  T  R  Q  V  I  V  G  L  G  L  E  D
1141 CCAGGCGATATAGCCATTGATTTCATGGCCCGCCACATTTACTTCGGCGATGCTGAAAGG
381 P  G  D  I  A  I  D  F  M  A  R  H  I  Y  F  G  D  A  E  R
          Low-density lipoprotein receptor domain class B   LDLb  (residues 371–413)
1201 GGCCTGATCTTCGTATGCTACGATAGCGGCTTCAAATGTTTTACTTTGAAAGCTGACACC
401 G  L  I  F  V  C  Y  D  S  G  F  K  C  F  T  L  K  A  D  T
1261 AAACATCCCAAGTTCATCACTCTGGACCCGGTGCACGGGAAGATGTACTGGGCCGATTGG
421 K  H  P  K  F  I  T  L  D  P  V  H  G  K  M  Y  W  A  D  W
                    LDLb  (residues 414–456)
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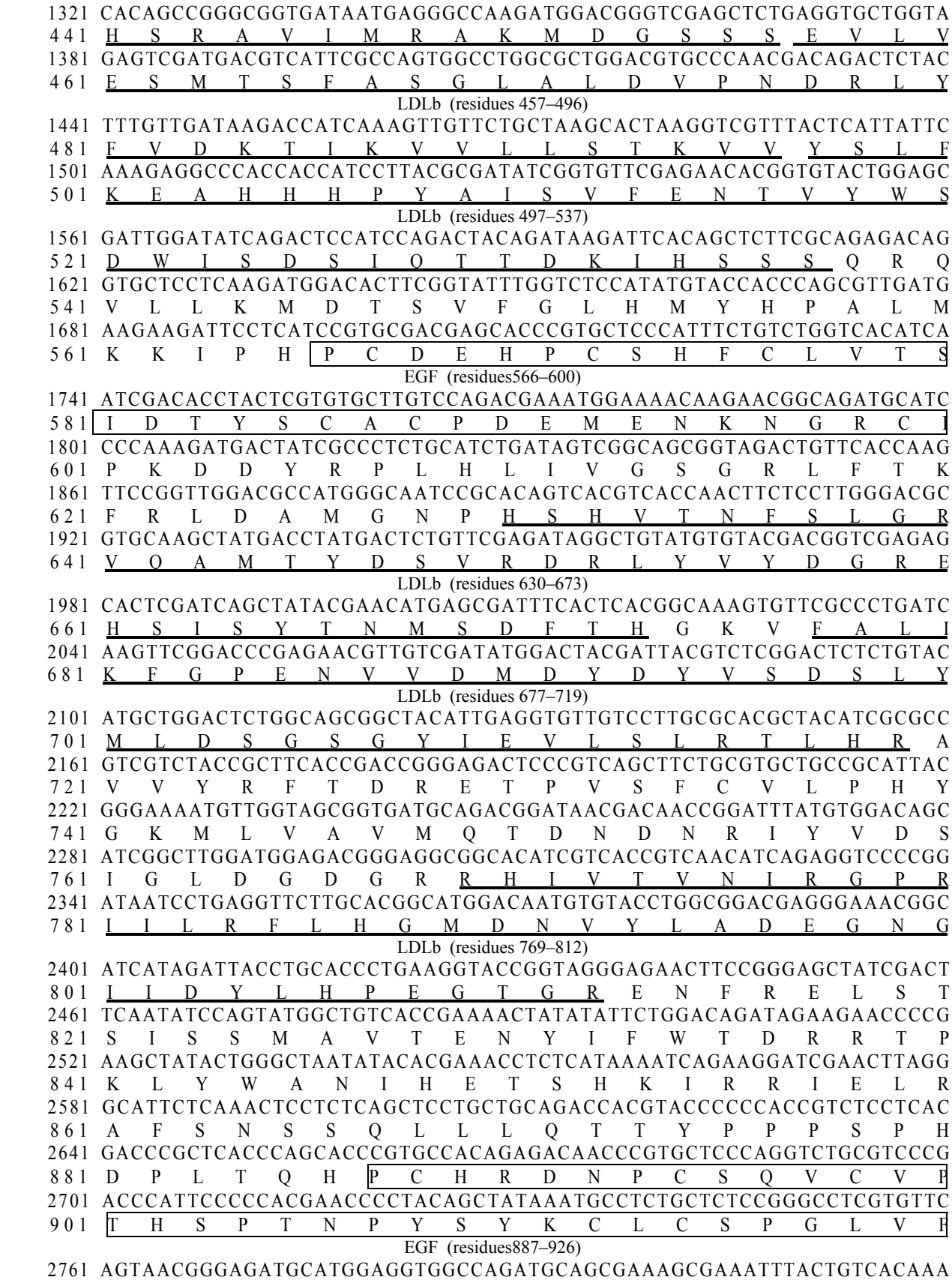
(continued)

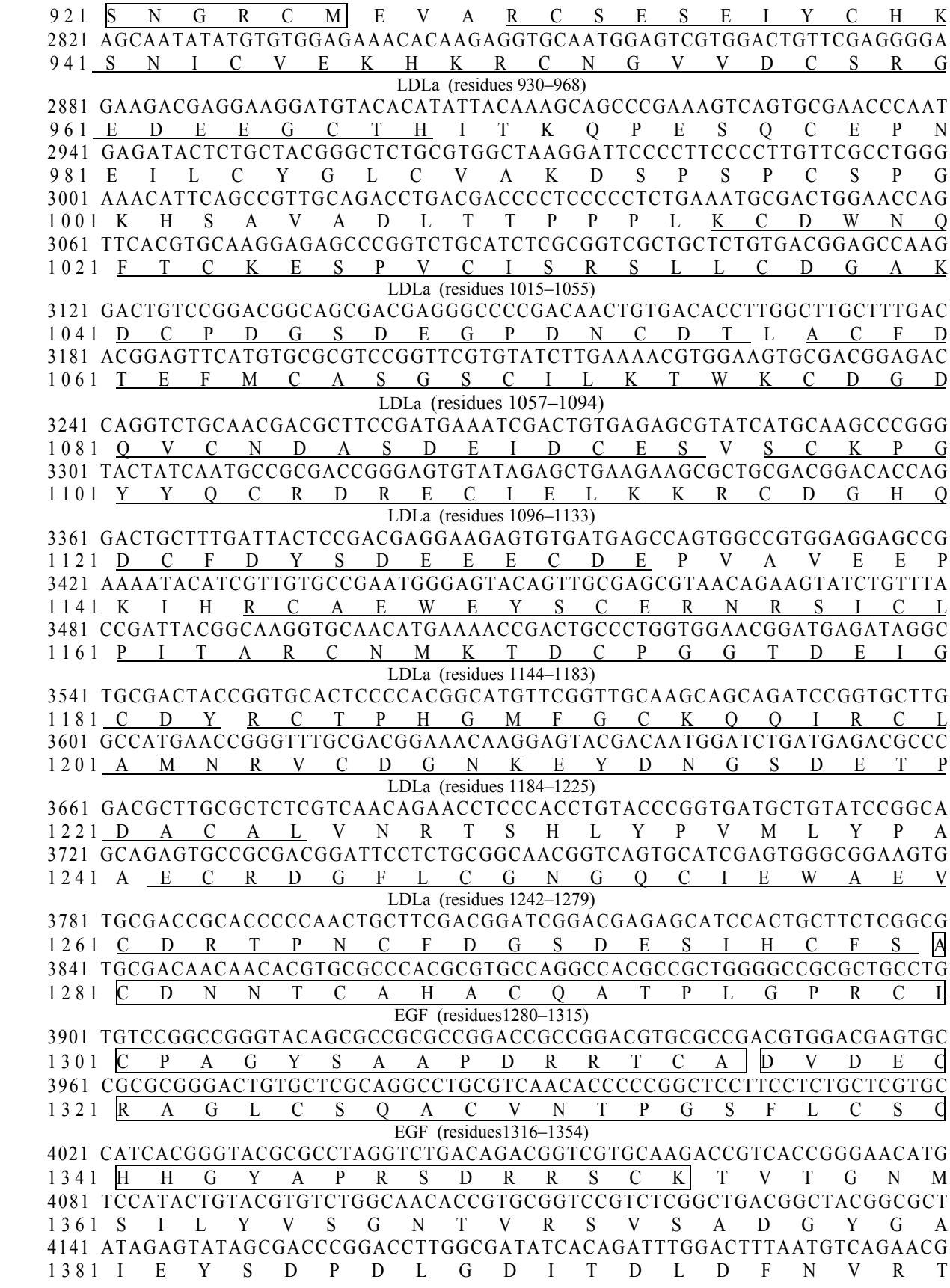
Figure 1. Nucleotide sequence and deduced amino acid sequence of vitellogenin receptor of *A. pernyi* (Ap-VgR). Termination codon (TAA) is indicated by asterisk, the polyadenylation signals AATAAA are double-underlined.

(Figure 1 continued)



(continued)

(Figure 1 continued)



(continued)

(Figure 1 continued)

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4201 AAGCGTTTGTATGTGACGTCTACGGAGTCGGGGAAGCTGATAGAATTGAACGTGACGCAT
1401 K  R  L  Y  V  T  S  T  E  S  G  K  L  I  E  L  N  V  T  H
4261 GACGTGGTCGCCGTGACGAACGTGGGACGGCCGACCAGGGTGGCAGTGGACTGGGTGACG
1421 D  V  V  A  V  T  N  V  G  R  P  T  R  V  A  V  D  W  V  T
                    LDLb (residues 1421–1465)
4321 GGCAACGTGTACTTCGCGGACAGCACGCCGGGTGCTAGCTGCGTGAGGGTCTGTGACGTC
1441 G  N  V  Y  F  A  D  S  T  P  G  A  S  C  V  R  V  C  D  V

4381 ACCAGGAGGAGATGCGCCAGGCTGCAGAAGATACCCTCTGACGCAACGGTCAAGGCATTG
1461 T  R  R  R  C  A  R  L  Q  K  I  P  S  D  A  T  V  K  A  L
4441 ATAGTGGAGCCGGCGTCACGGCGCATGTTCTACTGCGTTCAGCGCGGCCACGAGTCCGTG
1481 I  V  E  P  A  S  R  R  M  F  Y  C  V  Q  R  G  H  E  S  V
4501 GTCTGGTCCGCCTCGCTCTCCGGCCGGAGCGCCCTGGACCTCCTCCACGTGACCCAGTGC
1501 V  W  S  A  S  L  S  G  R  S  A  L  D  L  L  H  V  T  Q  C
4561 TCGGGATTAGCTGCCGATTCGTTCACGAGGAGGCTGTATGTGGCAGAGACTGCGCCCCCC
1521 S  G  L  A  A  D  S  F  T  R  R  L  Y  V  A  E  T  A  P  P
                    LDLb (residues 1510–1552)
4621 CACATCATGGTCGTCGACTTCGATGGCAAGAATCCCAAGAAGATCCTGACGGAACGTCCA
1541 H  I  M  V  V  D  F  D  G  K  N  P  K  K  I  L  T  E  R  P
4681 CAGCTGCAAGCGCCCCACGCCTTGGCGCTCTTCGAAGACCACATATACTATTTGGTGGGC
1561 Q  L  Q  A  P  H  A  L  A  L  F  E  D  H  I  Y  Y  L  V  G
                    LDLb (residues 1553–1595)
4741 GACTCGTACCGCCTCGGGCGCTGCCTGCTCCACGGCCCGAAGAACTGCGAGACCTACATC
1581 D  S  Y  R  L  G  R  C  L  L  H  G  P  K  N  C  E  T  Y  I
4801 TACAGGGTGTTCGACGCGAACACCTTCGTCATCAGACACGAGAGCATCCAGCGCGACGAC
1601 Y  R  V  F  D  A  N  T  F  V  I  R  H  E  S  I  Q  R  D  D
4861 CTGGTCAACGAGTGCGCCGCCCACGACTGCTCCAATGTGTGCGTGCTCGAGAAGGCTCCG
1621 L  V  N  E  C  A  A  H  D  C  S  N  V  C  V  L  E  K  A  P
                    EGF (residues1624–1656)
4921 GTGTGTGTCTGCGACGACGGGCACGTCCGTGACGACGGGAACTGTGACCCCAGCAGCAAA
1641 V  C  V  C  D  D  G  H  V  R  D  D  G  N  C  D  P  S  S  K
4981 AACGAGCTCCCCCTGTTCAACGGCTGGACGTACCAGGACTATCAGCGCGGTCACCGCGCC
1661 N  E  L  P  L  F  N  G  W  T  Y  Q  D  Y  Q  R  G  H  R  A
                    O-linked sugar domain (residues 1657–1683)
5041 AGCATCACCGTCGTCATCGCGGTCCTCGTGCTGTTCCTCGTGTACATAGCACTGTTTGTA
1681 S  I  T  V  V  I  A  V  L  V  L  F  L  V  Y  I  A  L  F  V
                    Transmembrane region (residues1684–1706)
5101 TATTATCACTTCGTCTATAAACCAAAGAGGAAGAGGTCCACGGCTTATACAGAGGTGAGG
1701 Y  Y  H  F  V  Y  K  P  K  R  K  R  S  T  A  Y  T  E  V  R
5161 TTCCAGAACAGCTCCGACGAAGCAGCGCAGTTGTCTTGCAGCCCGGCAGTCCAAATGAAT
1721 F  Q  N  S  S  D  E  A  A  Q  L  S  C  S  P  A  V  Q  M  N
5221 GGAAATCAACTTATCAATGGTAACGAATTCGTGAACCCGCTCCAGTACGTGCGCAACGTG
1741 G  N  Q  L  I  N  G  N  E  F  V  N  P  L  Q  Y  V  R  N  V
                    Cytoplasmic domain (residues1752–1755)
5281 TGGCAACAATCTATCAGAAGGAAGCCACGTCCTGTTTGTACAGCTGGCCTGTCAATAGCA
1761 W  Q  Q  S  I  R  R  K  P  R  P  V  C  T  A  G  L  S  I  A
5341 GTGCCTAACTCTCCACAGCAAGACTTCTCCGATACAGAGTCAGATCTAGACGATCGAGAA
1781 V  P  N  S  P  Q  Q  D  F  S  D  T  E  S  D  L  D  D  R  E
5401 ACAAAGAGGTTTATCCTCAAAAATAAATTTCTCAATTAACTTAAGTTACAGGAAATGTCG
1801 T  K  R  F  I  L  K  N  K  F  L  N  *
TTAAATCTTTTTGCTGAGCAAAAAATGGATGGCATATTCCGAATTTTATATTTTAATCCT
ACTATTTAAGATTTAGATTAGGTTATGATATAGCATAACTAACTTCTAGCTTGTTAAATT
ATTTTATTGTTTGAATGTTATCAAAATAGTTTTTATTTGCTAAATTTTATACATAAAATG
TATCGATATTGTATTGTTGAATTTTGAAAATAAAACATTGATTTATATGAAAAAAAAAAA
AAAAAAAA
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Figure 1. Nucleotide sequence and deduced amino acid sequence of vitellogenin receptor of *A. pernyi* (Ap-VgR). Termination codon (TAA) is indicated by asterisk, the polyadenylation signals AATAAA are double-underlined.

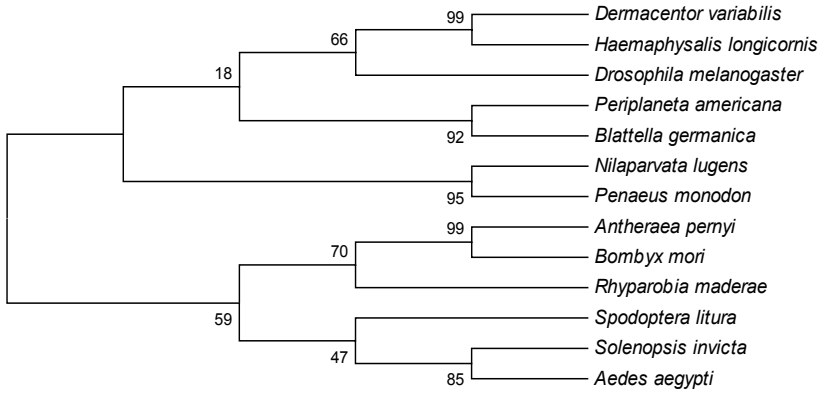

Figure 2. Phylogenetic analysis was performed by MEGA (version 4.0) program based on the VgR amino acid sequences from various species. The phylogenetic tree was constructed using the neighbor-joining algorithm method and bootstrap values (1000 repetitions) of the branches are indicated.

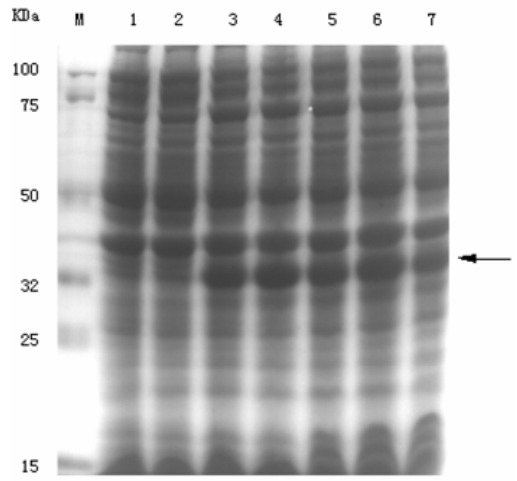

Figure 3. Analysis of recombinant Ap-VgR protein on 12% SDS-PAGE gels. The gels were revealed by Coomassie blue R-250 staining. Bacterial proteins were collected after 4 h induction with different IPTG concentration. Lane 1, *E. coli* BL21(DE3); Lane 2, before induction; Lanes 3-7, after induction with 0.2, 0.4, 0.6, 0.8, and 1.0 mM IPTG, respectively; M, molecular weight marker.

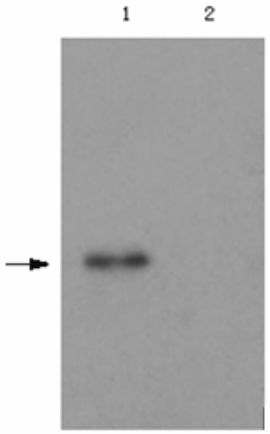

Figure 4. Western blot analysis of recombinant proteins with anti His-tag antibody. A protein band with a molecular mass of about 31.5 kDa was detected by western blotting using anti His-tag antibody. No immunoreactive band was found in the control group. Lanes 1, After IPTG induction, Lane 2, No IPTG induction.

Discussion and conclusion

In this study, a full-length cDNA encoding Ap-VgR gene has been identified from *A. pernyi*. The cDNA is 5847 bp long and encodes a 202.9 kDa protein with isoelectric point of 5.7. The size of Ap-VgR molecules was similar to those of other insect Vgs (180-214 KDa) (Sappington *et al*., 1995). Analysis of deduced amino acid sequence shows that Ap-VgR is a member of low-density lipoprotein receptor (LDLR) subfamily and contains the conservative domains as those found in other animal VgRs (Yamamoto *et al.*, 1986; Davis *et al.*, 1987; Willnow *et al.*, 1995). Different from most insects and vertebrate VgRs (Tufail and Takeda, 2009), there are eleven cysteine-rich LDLa repeats in Ap-VgR with four LDLa repeats in its first binding site and seven in its second binding site. So whether there are some rela-

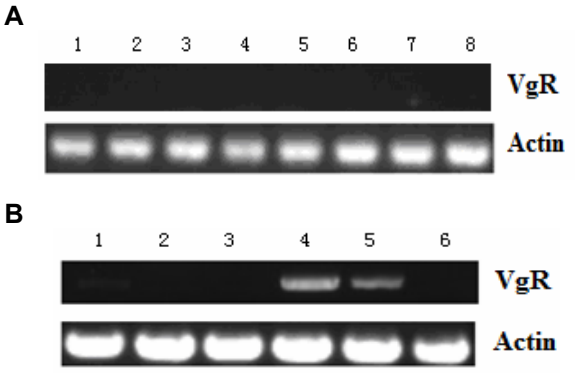

Figure 5. Expression analysis of Ap-VgR by semi-quantitative PCR. (A) Lanes 1-8, Expression of Ap-VgR in mid-intestine, silk gland, hemocytes, fat body, testis, integument, ovary and malpighian at the fifth larve stage, respectively. (B) Lanes 1-6, Expression of Ap-VgR in mid-intestine, malpighian, hemocytes, ovary, fat body and head at the pupae stage respectively. The expression of actin gene was used as a control.

tionship between the differences of the LDLa repeats and the functions of VgR remains unclear. Whatsoever, the expression of Ap-VgR was detected in ovary and fat body by RT-PCR, this result is not agree with some previous reports for it is considered that VgR was exclusively expressed in ovary tissues (Tufail and Takeda, 2005; 2007; Ciudad *et al*., 2006). However, VgR mRNA was also found in non-ovary tissues of *A. mellifera*, this maybe is relevant to the multiple functions of Vgs in biological processes (Amdam *et al*., 2003; Guidugli *et al*., 2008). Further study of the interaction between Ap-Vg and Ap-VgR will be necessary for the understanding of egg development in *A. pernyi*.

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