Effects of Certain cis-Regulatory Elements on Stage-Specific vitellogenin Expression in the Bombyx mori (Lepidoptera: Bombycidae)

Guanwang Shen1,2, Hongling Liu1,2, Ying Lin1,2, Dongxu Xing3, Yujing Zhang1,2 and Qingyou X1,2,4

1Biological Science Research Center of Southwest University, Chongqing 400716, China, 2Chongqing Key Laboratory of Sericulture Science, Chongqing 400716, China, 3Sericulture and Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510610, China, and 4Corresponding author, e-mail: xiaqy@swu.edu.cn

Received 7 April 2020; Editorial decision 19 May 2020

Abstract

Bombyx mori vitellogenin (BmVg) is highly upregulated during pupation, and the 20-hydroxyecdysone and amino acids may regulate stage-specific BmVg expression. However, previous studies showed that other factors may also affect stage-specific BmVg expression. Here, we characterized effective BmVg transcription factors by identifying the corresponding cis-regulatory elements (CREs). We prepared transgenic B. mori, in which DsRed was driven by various lengths of BmVg promoter. qRT-PCR analysis showed that DsRed expression driven by a 1.0-kb BmVg promoter (VgP1.0K) was consistent with endogenous BmVg. VgP1.0K specificity was closer to the endogenous BmVg promoter than that of VgP0.8K. These results suggest that CREs affecting stage-specific BmVg expression were localized to the 1.0-kb BmVg promoter. We investigated the effects of certain CREs that could influence the stage specificity of BmVg promoter on BmVg expression in transgenic B. mori. The relative DsRed expression was significantly reduced in transgenic female B. mori and the peak in DsRed expression was delayed after E-box CRE mutation. These results demonstrate that the E-box element enhanced BmVg expression and also affected stage-specific BmVg expression. Moreover, the relative DsRed expression was significantly increased in transgenic female of B. mori after 3xBD CRE mutation in BmVg promoter. However, the stage specificity of the mutated promoter was consistent with that of the endogenous BmVg promoter. The 3xBD element downregulated BmVg but had no effect on stage-specific BmVg expression. The present study promoted the process of elucidating the regulatory network for stage-specific BmVg expression and furnished a theoretical basis for the application of BmVg promoter.

Key words: vitellogenin, promoter, regulation, transgenic, B. mori

The Vg gene is unique in oviparous animals, which plays a significant role in yolk protein synthesis during the embryo formation (Valle 1993). Vg is essential for insect reproduction. When the Vg content is reduced, the embryo fails to develop normally (Lin et al. 2013, Yang et al. 2014a). Vg is synthesized mainly in the fat bodies of female insects at specific developmental stages (Valle 1993, Hirai et al. 1998, Raikhel et al. 2002). The mechanisms regulating Vg expression have been widely investigated. The stages at which Vg is expressed vary with insect species (Hirai et al. 1998, Tuflai et al. 2010, Yang et al. 2014a). Stage-specific Vg expression is relatively complex and regulated mainly by juvenile hormones, ecdysone, amino acids, and insulin (Bownes 1982, Hansen et al. 2014, Gujar and Palli 2016, Lu et al. 2016, Roy et al. 2018, Asad et al. 2020).

Bombyx mori (B. mori) is an economically important lepidopteran insect that synthesizes and secretes abundant silk proteins (Luan et al. 2018). It is completely metamorphic. Previous studies showed that B. mori Vg (BmVg) expression has definite sex, tissue, and stage specificity (Yang et al. 2014a). BmVg expression starts in the fat body on day 2 after spinning in female B. mori (Yang et al. 2014a). Stage-specific BmVg expression is regulated mainly by nutritional and 20-hydroxyecdysone (20E) signals (Yang et al. 2014b; Lin et al. 2017; Shen et al. 2018; Liu et al. 2019a,b). After spinning is complete, the increase of amino acid in the hemolymph induces BmGATA β4 to upregulate BmVg (Liu et al. 2019a,b). 20E titer is also increased in the hemolymph at this time to induce the early gene BmBrC-Z2 and the homodomain transcription factor BmPOU-M2, then BmBrC-Z2 and BmPOU-M2 to upregulate the expression of BmVg (Lin et al. 2017). During spinning, BmGATAβ4 is expressed at a certain level and 20E has a small titer peak but BmVg is not expressed. Therefore, other unidentified transcription factors may regulate stage-specific BmVg expression.

© The Author(s) 2020. Published by Oxford University Press on behalf of Entomological Society of America.
Gene expression is regulated mainly by promoters including their cis-regulatory elements and their corresponding trans-acting factors. There are many predicted cis-regulatory elements in the BmVg promoter (Yang et al. 2014b). The regulatory regions −42 to −152 bp and −752 to −952 bp in the BmVg promoter significantly influence its activity. There is a predicted binding site for the Drosophila transcriptional repressor hairy in the −39 to −54 region of the BmVg promoter. Hairy belongs to the bHLH family, which could recognize E-box or N-box sites in target gene promoters. Thus, hairy could recruit a co-suppressor to inhibit target gene expression (Davis and Turner 2001, Jindra 2016, Saha et al. 2016). Hairy could also recruit a co-suppressor to inhibit target gene expression (Sasai et al. 1992). Hairy may also affect BmVg expression. Moreover, three tandem repeat sequences in the −836 bp to −887 bp region of the BmVg promoter are the predicted binding sites for DREF (DNA replication-related element factor) or BEAF (boundary element-related factor). DREF elements promote gene expression (Hart et al. 1999, Fernández-Moreno et al. 2009). BEAF requires three tandem repeats to block the enhancer-upstream fragments of the gene promoter (Hart et al. 1999). The core motifs of DREF and BEAF are similar; therefore, DREF and BEAF may be antagonistic (Hart et al. 1999). However, there are no prior reports on DREF or BEAF in B. mori. It remains to be determined whether DREF or BEAF affects stage-specific BmVg expression.

Previous studies used −0.8-kb upstream region of the BmVg promoter to drive green fluorescent protein expression and prepare transgenic B. mori. RT-PCR assays revealed that the promoter of 0.8 kb in length had the same sex, tissue, and stage specificity as the endogenous BmVg promoter (Xu et al. 2014). This discovery may imply that the cis-regulatory elements influencing stage-specific BmVg expression mainly reside within this 0.8-kb BmVg promoter.

For this reason, here we focused on the cis-regulatory elements in the BmVg promoter to identify those regulating stage-specific BmVg expression. We used the promoter 0.8 kb in length as a control and extended it. We first prepared transgenic B. mori with BmVg promoters of various lengths to drive DsRed expression and analyzed the expression characteristics of the different BmVg promoters. And we comprehensively analyzed the regulatory regions affecting stage-specific BmVg expression. Then we applied this transgenic method to explore the effects of certain cis-regulatory elements in the BmVg promoter on stage-specific BmVg expression. This study initiated the process of elucidating the regulatory network of stage-specific BmVg expression and provided a theoretical basis for the application of the BmVg promoter.

Materials and Methods

Construction of Vectors for Cell Line Transfection

Various lengths of BmVg promoter were amplified from the B. mori genome and ligated to the pGL3-basic vector (Promega, Madison, WI) using the restriction endonucleases XhoI and HindIII. The vector constructs were named pGL3-VgP0.8K Luc, pGL3-VgP1.0K Luc, and pGL3-VgP1.7K Luc. The BmVg promoter was truncated and used to construct the pGL3-VgP34 Luc vector with the E-box element, the pGL3-VgP39 Luc vector without the E-box element, the pGL3-VgP888 Luc vector with the 3×BD element, and the pGL3-VgP34 Luc vector without the 3×BD element. The E-box element was connected to the upstream regulatory sequence from −38 to +40 of BmVg (basic promoter VgP78ML), the VgP78ML was unresponsive to the induction of ecdysone, juvenile hormone, or their transcription factors (Liu et al. 2019b) stored in the laboratory and the product was named VgP78ML+E-box Luc. The amplification primers are listed in Table 1.

Cell Transfection

The B. mori embryonic cell line BmE-SWU1 stored in the laboratory was maintained at 27°C in Grace’s insect medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone, Thermo Fisher Scientific, Waltham, MA). X-tremeGENE HP DNA transfection reagent (Roche, Basel, Switzerland) was used for the cell transfection assays. The PRL-VgP78M vector was transferred as an internal control (Liu et al. 2018). After 36 h transfection, the cells were harvested and assayed for luciferase activity. The cell transfection experiments and luciferase activity measurements were conducted as previously described (Deng et al. 2012).

Construction of Vectors for Germline Transformation

BmVgP1.0K with a mutant E-box element was named as BmVgP1.0M. The BmVgP1.0M sequence was amplified with a unique primer containing the mutated E-box sequence. The core site of the E-box element, CGCGTG, was changed to TATACA. Three repeated DREF and BEAF elements in series on the BmVg promoter were named 3×BD. BmVgP1.7K with a deletion 3×BD element was named BmVgP1.7K-3×BD. The BmVgP1.7K-3×BD sequence was amplified with a unique primer deleting the 3×BD element.

The pBac [3xp3-EGFP-SV40] stored in the laboratory served as a framework vector expressing enhanced green fluorescent protein (EGFP) in the B. mori eyes (Chen et al. 2018). It is used to screen for positive transgenic B. mori. DsRed was the target gene driven by the BmVg promoter. The BmVg promoter was ligated with BamHI and EcoRI into the plasmid pSL1180-A4-DsRed-SV40 stored in the laboratory. The vector product was pSL1180-VgP-DsRed SV40. The plasmid containing VgP-DsRed-SV40 was digested with AscI and inserted into the vector pBac [3xp3-EGFP-SV40] to generate pBac [3xp3-EGFP-SV40; VgP-DsRed-SV40]. Transgenic vectors were constructed with promoters of various length and mutated CRE and named pBac [3xp3-EGFP-SV40; VgP0.8K-DsRed-SV40], pBac [3xp3-EGFP-SV40; VgP1.0K-DsRed-SV40], pBac [3xp3-EGFP-SV40; VgP1.7K-DsRed-SV40], pBac [3xp3-EGFP-SV40; VgP1.0M-DsRed-SV40], and pBac [3xp3-EGFP-SV40; VgP1.7K-3×BD-DsRed-SV40]. The primers used are listed in Table 1.

Production of Transgenic B. mori

The nondiapause B. mori dazao strains were used to prepare transgenic B. mori and the transgenic B. mori were generated as previously described (Chen et al. 2018). The injected G0 embryos were reared to the moth stage for G1 oviposition. The G1 embryos were screened under a fluorescence microscope (Olympus, Tokyo, Japan) after growth for ~6–7 d. The G1 embryos positive were intercrossed and reared to adult stage. Individuals positive were intercrossed and reared to the moth stage for G1 oviposition. The G0 embryos were used to construct the transgenic method. The nondiapause B. mori dazao strains were used to prepare transgenic B. mori and the transgenic B. mori were generated as previously described (Chen et al. 2018). The injected G0 embryos were reared to the moth stage for G1 oviposition. The G1 embryos were screened under a fluorescence microscope (Olympus, Tokyo, Japan) after growth for ~6–7 d. The G1 individuals were selected and reared to adult stage. Individuals positive were intercrossed and reared to the moth stage for G1 oviposition. The G1 individuals were selected and reared to adult stage. Individuals positive were intercrossed and reared to the moth stage for G1 oviposition.
China). The sequencing results were blasted in the *B. mori* genome database SilkDB (http://www.silkdb.org/silkdb/) to identify the insertions in the transgenic *B. mori*. The primers are listed in Table 1.

### Collection of Fat Body Samples and RNA Isolation
Numerous factors affect exogenous *DsRed* expression in transgenic *B. mori* such as the *DsRed* copy number and the homozygous and hybrid transgenic lines. To compare *BmVg* promoter activity accurately and eliminate the influences of the aforementioned factors, the transgenic lines with single insertion site were selected. The transgenic *B. mori* lines were also backcrossed with the wild type to generate more hybrid transgenic *B. mori* for subsequent experiments.

The last-instar *B. mori* larvae began spinning silk on days 7–8. The period from spinning to pupation is the wandering stage and it lasts for 2–3 d. The fat bodies of 5–10 *B. mori* were collected for each group between day 1 of wandering to day 3 of pupation. TRIzol reagent (Invitrogen) was used to extract total RNA according to the manufacturer’s protocol.

### RT-PCR and qRT-PCR
The corresponding cDNA was synthesized with Moloney murine leukemia virus reverse transcriptase (Promega). For the RT-PCR analysis, the *BmVg* and *DsRed* fragments were amplified from the cDNA samples using gene-specific primers. *BmActin3* was the housekeeping gene. The PCR program was as follows: 95°C, 4 min; 95°C, 30 s; 55°C, 30 s; 72°C, 30 s, *n* cycles; and 72°C, 10 min. The amplified samples were maintained at 4°C.

*Here, *n* = 20 for *BmVg* in females, *n* = 25 for *DsRed* in females, *BmVg* in males, and *BmActin3*, and *n* = 30 for *DsRed* in males. The PCR products were separated by agarose gel electrophoresis.

For qPCR, SYBR Green kits (TaKaRa Biotech, Osaka, Japan) were used according to the manufacturer’s instructions. The relative mRNA levels of the target gene were normalized to the housekeeping gene translation initiation factor 4A. The expression level was determined by the 2−ΔΔCt method (Livak and Schmittgen 2001). Three independent replicates were performed for each experiment. The RT-PCR and qPCR primers are listed in Table 1.

### Statistical Analysis
The data are presented as means ± standard error. The relative *DsRed* expression values were calculated as follows: *DsRed* mRNA level/*BmVg* mRNA level. Independent-sample t-tests were applied to evaluate the promoter activity and gene expression data. Differences

---

Table 1. Primers used in the study

| Purpose/primer name | Nucleotide sequence (5′–3′) |
|---------------------|-----------------------------|
| **Cell transfection** |                             |
| VgPcell-0.8K-F (XhoI) | CCGCTCGAGCCCGATCCATTAACAGTGCT |
| VgPcell-1.0K-F (XhoI) | CCGCTCGAGAGCACAGCTTACTAACAAGA |
| VgPcell-1.7K-F (XhoI) | CGCGATATCTGAGATTCCAGGCTGTC |
| VgPcell888-F (XhoI)  | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| VgPcell834-F (XhoI)  | CATACCCGATCCCGTAGACCGA |
| VgP54-F (XhoI)       | CTGGGTCGGCTTAGACGATC |
| VgP39-F (XhoI)       | CCGCGATGACGATCCGAG |
| 78ML+E-box-F (XhoI)  | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| E-box-M1-F           | CTGGGACGCGTGTACCCTCCCTAATCCAGG |
| Transgenic vectors   |                             |
| VgP0.8K-F (EcoRI)    | CCGGAATTCCTAGAGCTTTATAGCT |
| VgP1.0K-F (EcoRI)    | CCGGAATTCCTAGAGCTTTATAGCT |
| Vg-R (BamHI)         | CCGGAATTCCTAGAGCTTTATAGCT |
| E-box-M1-F           | CCGGAATTCCTAGAGCTTTATAGCT |
| E-box-M1-R           | CCGGAATTCCTAGAGCTTTATAGCT |
| 3×BD-M1-F            | CCGGAATTCCTAGAGCTTTATAGCT |
| 3×BD-M1-R            | CCGGAATTCCTAGAGCTTTATAGCT |
| RT-PCR               |                             |
| *DsRed*-F            | TTGGTATGACGAGCTTTATAGCT |
| *DsRed*-R            | CGCGATGACGATCCGAG |
| *BmVg*-F             | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| *BmVg*-R             | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| qRT-PCR              |                             |
| Bmsu22934-F          | ATCGATGATCAGACGAGCTTTATAGCT |
| Bmsu22934-R          | CGCGATGACGATCCGAG |
| *DsRed*-F            | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| *DsRed*-R            | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| *BmVg*-F             | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| *BmVg*-R             | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| Inverse PCR           |                             |
| pBacl-F              | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| pBac1-R              | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| pBacR-F              | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| pBacR-R              | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| *BmVg*-F             | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| *BmVg*-R             | TGGGACGCGTGTACCCTCCCTAATCCAGG |
| pBac-R               | TGGGACGCGTGTACCCTCCCTAATCCAGG |

Journal of Insect Science, 2020, Vol. 20, No. 3
between group means were considered significant at \( P < 0.05; \) *\( P < 0.05; \) **\( P < 0.01. \)

**Results and Discussion**

**BmVgP1.0K Stage Specificity Was Closer to the Endogenous BmVg Promoter**

Previous studies showed that BmVgP0.8K expression is tissue-, stage-, and sex-specific (Xu et al. 2014). To verify whether all elements determining stage-specific BmVg expression are located in the 0.8-kb region of the BmVg promoter, we used BmVgP0.8K as a control to analyze promoters of different lengths. The luciferase activity assay in cells revealed that BmVgP1.0K activity was significantly higher than those of BmVgP0.8K and BmVgP1.7K (Fig. 1A). Thus, there are some elements upregulate promoter activity from −0.8 to −1.0 kb.

We then constructed the transgenic vector for BmVg promoters of different lengths (Fig. 1B). These vectors were injected into B. mori embryos to gain transgenic B. mori. The compound eyes of the positive moths presented with specific EGFP emission (Fig. 1C) and the transgenic insects with only one insertion site (Fig. 1D) were backcrossed of wild-type B. mori to collect fat bodies for detection. RT-PCR showed that exogenous DsRed was not expressed in female or male wild-type B. mori (Fig. 2A). The expression patterns of DsRed were consistent with those of BmVg in BmVgP0.8K (Fig. 2B), BmVgP1.0K transgenic B. mori (Fig. 2C), and BmVgP1.7K transgenic B. mori (Fig. 2D). DsRed was highly expressed only in the fat bodies of transgenic B. mori (Fig. 2E). This discovery was consistent with previous reports demonstrating that BmVgP0.8K had BmVg promoter specificity (Xu et al. 2014). Our results showed that BmVgP1.0K and BmVgP1.7K also had BmVg promoter specificity. These results indicated that the elements that determine the stage-specific expression are mainly in the 0.8-kb BmVg promoter.

In this region, the DSX, BrC-Z2, POUm2, ERR, and GATA elements could regulate BmVg expression (Suzuki et al. 2003, Yang et al. 2014b, Lin et al. 2017, Shen et al. 2018, Liu et al. 2019b). Moreover, ABBD, FTZ, and hairy elements have been predicted for this region (Yang et al. 2014b). However, we found that the onecut, ABBD, and FTZ elements had no apparent impact on BmVg promoter activity (data not shown). Therefore, we selected a Drosophila hairy binding site E-box element that could inhibit the BmVg promoter in theory (Davis and Turner 2001) for analyzing the effects on BmVg stage-specific expression in this study.

Then we used qPCR to further compare promoter activity among the three types of transgenic B. mori. In BmVgP0.8K transgenic B. mori, the DsRed expression peak was delayed (Fig. 3A).
However, DsRed expression was consistent with endogenous BmVg expression in BmVgP1.0K (Fig. 3B) and BmVgP1.7K (Fig. 3C) transgenic B. mori. These results suggest that the elements determining stage-specific BmVg expression resided mainly within 0.8 kb. However, there were some elements between −0.8 and −1.0 kb of the BmVg promoter that affected stage-specific BmVg expression. The typical elements in the range from −0.8 to −1.0 kb are three tandem repeats of the BEAF or DREF element (Yang et al. 2014b), so we then detected the effect of 3×BD CREs on the stage-specific expression of BmVg promoter. And we calculated relative exogenous DsRed expression value and found that it was significantly higher in BmVgP1.0K transgenic B. mori than it was in BmVgP0.8K and BmVgP1.7K transgenic B. mori on day 3 after wandering (Fig. 3D), suggesting the BmVgP1.0K activity was higher than that of BmVgP0.8K and BmVgP1.7K. Therefore, some cis-regulatory elements may enhance BmVg promoter activity in the region from −0.8 to −1.0 kb and inhibit BmVg promoter activity in the region from −1.0 to −1.7 kb.

The E-Box Element Affects the Stage-Specific Expression of BmVg Promoter

The E-box element was between −39 and −54 bp in the BmVg promoter (Fig. 4A). We transfected the vectors for BmVg promoter with and without E-box into cells and found that the E-box element could significantly enhance BmVg promoter activity (Fig. 4B). We then connected the E-box element to the basic promoter VgP78ML and also confirmed that the E-box element enhanced promoter activity (Fig. 4C). While the promoter activity was significantly downregulated after mutating the E-box element (Fig. 4D).
We then constructed a transgenic vector by VgP1.0K with the E-box mutation (Supp Fig. S1A [online only]) and screened the stable heritable transgenic individuals (Supp Fig. S1B [online only]). We selected single-insertion moths in subsequent analyses (Supp Fig. S1C [online only]). RT-PCR revealed that the expression profile of exogenous DsRed driven by VgP1.0M was consistent with that of endogenous BmVg (Supp Fig. S1D [online only]). DsRed was also highly expressed in the fat bodies (Supp Fig. S1E [online only]). Therefore, VgP1.0M had essentially the same expression characteristics as the BmVg promoter.

Similarly, we also analyzed DsRed expression by qPCR. The DsRed expression peak was delayed and occurred on day 1 after pupation in VgP1.0M transgenic B. mori (Fig. 4E), indicating the E-box element affected the stage specificity of BmVg expression. Meanwhile, we found that the relative DsRed expression value was significantly lower after mutating the E-box element (Fig. 4F). This finding was consistent with the observed results in the transfected cells. Nevertheless, this E-box element had essentially the same expression characteristics as the BmVg promoter.

3xBD Elements Have No Effects on the Stage-Specific Expression of BmVg Promoter

Previous studies showed certain elements might affect the stage specificity of the promoter in the upstream 0.8- to 1.0-kb region of the BmVg promoter. There are three tandem repeat elements in this sequence (Fig. 5A). They may be the binding elements of BEAF and DREF (Fig. 5B). DREF could significantly upregulate the promoter activity and BEAF could suppress upstream enhancer in theory (Hart et al. 1999). To confirm whether 3xBD affects BmVg promoter stage-specific expression, we firstly analyzed its effects on promoter activity in cells and found the 3xBD element could inhibit promoter activity (Fig. 5C). So, the 3xBD element may be a boundary blocking the enhancer upstream of this element. Thus, we choose the 1.7-kb BmVg promoter to examine the effects of 3xBD CRE in transgenic individuals. The transgenic vector VgP1.7K-3xBD (Supp Fig. S2A [online only]) was injected into B. mori embryos and obtained stable heritable transgenic individuals (Supp Fig. S2B [online only]). Similarly, the single-insertion transgenic individuals were selected for the subsequent analyses (Supp Fig. S2C [online only]). RT-PCR revealed that the stage and tissue expression profiles of DsRed driven by VgP1.7K-3xBD were consistent with endogenous BmVg (Supp Fig. S2D and E [online only]). Thus, it retained the BmVg promoter expression characteristics even after the 3xBD element was deleted.
We ran qPCR to analyze the VgP1.7K-3×BD expression characteristics and found that the expression of DsRed driven by it was consistent with that of endogenous BmVg (Fig. 5D). Therefore, the 3×BD element did not affect BmVg promoter stage specificity. Consistent with cell results, DsRed expression was significantly increased after 3×BD CRE deletion in transgenic B. mori (Fig. 5E). Thus, the transcription factor binding 3×BD CRE was not related to DREF. 3×BD CRE does not affect BmVg promoter stage specificity, but only inhibited BmVg expression. The stage specificity and the activity of the 1.0-kb BmVg promoter were greater than those of the 0.8-kb BmVg promoter. These results suggest that in this sequence interval, there were other elements influencing BmVg promoter activity and stage specificity. Nevertheless, these elements remain to be identified. However, physiological significance of the element inhibiting BmVg expression during vitellogenesis is obscure.
Conclusion

The present study compared the activity of BmVg promoters of different length and examined the effects of certain elements on BmVg promoter stage specificity and activity in transgenic B. mori. This study indicated that the elements that affect the stage-specific BmVg expression were basically localized to the 1.0-kb BmVg promoter and the E-box element could affect the stage-specific BmVg expression, while the 3xBD element could not. This information forms the theoretical basis for the transformation and application of the BmVg promoter.

Supplementary Data

Supplementary data are available at Journal of Insect Science online.

Acknowledgments

This work was supported by the National Natural Science Foundation of China 31530071 and 31772532.

References Cited

Asad, M., Munir, F., Xu, X., Li, M., Jiang, Y., Chu, L. and Yang, G. 2020. Functional characterization of cis-regulatory region for vitellogenin gene in Plutella xylostella. Insect Mol. Biol. 29: 137–147.

Bownes, M. 1982. Hormonal and genetic-regulation of vitellogenesis in Drosophila. Q. Rev. Biol. 57: 247–274.

Chen, W., F. Wang, C. Tian, Y. Wang, S. Xu, R. Wang, K. Hou, P. Zhao, L. Yu, Z. Lu, et al. 2018. Transgenic silkworm-based silk gland bioreactor for large scale production of bioactive human platelet-derived growth factor (PDGF-BB) in silk cocoons. Int. J. Mol. Sci. 19: 2533.

Davis, R. L., and D. L. Turner. 2001. Vertebrate hairy and enhancer of split-related proteins: transcriptional repressors regulating cellular differentiation and embryonic patterning. Oncogene. 20: 8342–8357.

Deng, H. M., J. L. Zhang, Y. Li, S. C. Zheng, L. Liu, L. H. Huang, W. H. Xu, S. R. Palli, and Q. L. Feng. 2012. Homeodomain POU and Abd-A proteins regulate the transcription of pupal genes during metamorphosis of the silkworm, Bombyx mori. Proc. Natl. Acad. Sci. USA. 109: 12598–12603.

Fernández-Moreno, M. A., F. Bruni, C. Adán, R. H. Sierra, P. L. Polosa, P. Cantatore, R. Garesse, and M. Roberiti. 2009. The Drosophila nuclear
factor DREF positively regulates the expression of the mitochondrial transcription termination factor DmTTF. Biochem. J. 418: 453–462.

Gujar, H., and S. R. Palli. 2016. Juvenile hormone regulation of female reproduction in the common bed bug, Cimex lectularius. Sci. Rep. UK. 6:35546.

Hansen, I. A., G. M. Attardo, S. D. Rodriguez, and L. L. Drake. 2014. Four-way regulation of mosquito yolk protein precursor genes by juvenile hormone-, ecdysone-, nutrient-, and insulin-like peptide signaling pathways. Front. Physiol. 5:203.

Hart, C. M., O. Cuvier, and U. K. Laemmli. 1999. Evidence for an antagonistic relationship between the boundary element-associated factor BEAF and the transcription factor DREF. Chromosoma. 108: 375–383.

Hirai, M., D. Watanabe, A. Kiyota, and Y. Chinzei. 1998. Nucleotide sequence of vitellogenin mRNA in the bean bug, Riptortus clavatus: analysis of processing in the fat body and ovary. Insect Biochem. Mol. Biol. 28: 537–547.

Jindra, M. 2016. Something “hairy” in juvenile hormone signaling for mosquito reproduction. Proc. Natl. Acad. Sci. USA. 113: 1474–1476.

Lin, Y., H. Liu, C. Yang, J. Gu, G. Shen, H. Zhang, E. Chen, C. Han, Y. Zhang, Y. Xu, et al. 2017. The POU homeodomain transcription factor POUM2 and broad complex isoform 2 transcription factor induced by 20-hydroxyecdysone collaboratively regulate vitellogenin gene expression and egg formation in the silkworm Bombyx mori. Insect Mol. Biol. 26: 496–506.

Liu, H., Y. Lin, G. Shen, J. Gu, H. Zhang, J. Wu, Y. Xu, W. Long, and Q. Xia. 2018. Establishing of a suitable control reporter plasmid of a dual luciferase reporter gene system for hormone research in silkworm cell lines. Sheng Wu Gong Cheng Xue Bao. 34: 1631–1641.

Liu, H., Y. Lin, J. Gu, Y. Ruan, G. Shen, Y. Zhang, H. Wang, Z. Meng, K. Li, and Q. Xia. 2019a. The increase of amino acids induces the expression of vitellogenin after spinning in the silkworm Bombyx mori. J. Insect Physiol. 118: 103913.

Liu, H. L., Y. Lin, G. W. Shen, J. J. Gu, Y. Ruan, J. X. Wu, Y. J. Zhang, K. R. Li, W. Long, L. B. Jia, et al. 2019b. A novel GATA transcription factor GATA beta 4 promotes vitellogenin transcription and egg formation in the silkworm Bombyx mori. Insect Biochem. Mol. Biol. 107: 10–18.

Lin, Y., Y. Meng, Y. X. Wang, J. Luo, S. Katsuma, C. W. Yang, Y. Banno, T. Kusakabe, T. Shimada, and Q. Y. Xia. 2013. Vitellogenin receptor mutation leads to the oogenesis mutant phenotype “scanty vitellin” of the silkworm, Bombyx mori. J. Biol. Chem. 288: 13345–13355.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods. 25: 402–408.

Lu, K., X. Chen, W. T. Liu, and Q. Zhou. 2016. TOR pathway-mediated juvenile hormone synthesis regulates nutrient-dependent female reproduction in Nilaparvata lugens (Stal). Int. J. Mol. Sci. 17:438.