DOI: 10.1002/arch.21449

WILEY ARCHIVES OF INSECT BIOCHEMISTRY AND PHYSIOLOGY

ARTICLE

Effects of overexpression and inhibited expression of thymosin, an actin-interacting protein from *Bombyx mori*, on BmNPV proliferation and replication

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Grant sponsor: Public Technology Application Research Project from Science Technology Bureau of Zhejiang Province; Grant numbers: 2016C33080 and 2016C32012; Grant sponsor: National Natural Science Foundation of Zhejiang Province; Grant number: LY16C170002; Grant sponsor: Science Foundation of Zhejiang Sci-Tech University; Grant number: 16042187-Y.

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Abstract

Previous study showed that exogenously applied recombinant thymosin from *Bombyx mori* (BmTHY) reduces *B. mori* nucleopolyhedrovirus (BmNPV) proliferation in silkworm. Which stands to reason that BmTHY in *B. mori* is crucial for the defense against BmNPV. However, little is known about the effect of endogenously overexpressed or repressed BmTHY on *B. mori* resistance to virus infection. To study this issue, we constructed an overexpression and inhibited expression systems of BmTHY in BmN cells. The viral titer and the analysis from the quantitative real-time polymerase chain reaction (PCR) revealed that overexpression of BmTHY decreased the copies of BmNPV gene *gp41*, which goes over to inhibit the proliferation of BmNPV in BmN cells, while the inhibited expression of BmTHY significantly enhanced viral proliferation in infected BmN cells. These results indicated that endogenous BmTHY can inhibit BmNPV proliferation and replication in infected BmN cells. Furthermore, Co-IP showed that BmTHY could bind to actin in BmN cells. Also, the overexpression or inhibited expression of BmTHY shifted the ratio of F/G-actin in infected BmN cells. Lastly, the BmTHY, an actin-interacting protein, might be one of the key host factors against BmNPV, which inhibits viral proliferation and replication in BmN cells.

KEYWORDS

actin, *Bombyx mori*, *Bombyx mori* nucleopolyhedrovirus, thymosin

1 | INTRODUCTION

Silkworm is a model insect of Lepidoptera, and sericulture is a crucial industry of agriculture economy in some developing countries (Konala, Abburi, Bovilla, & Mamillapalli, 2013). However, the silkworm baculovirus disease caused by *Bombyx mori* nucleopolyhedrovirus (BmNPV) has done severe damage to sericulture, which is highly contagious and difficult to control. Its infection does result in about 50% annual loss of sericulture in the world (Subbaiah et al., 2013). Therefore, it is very urgent and necessary for researchers to explore some new effective antivirus agents against BmNPV.

Thymosins (THY), one member of the thymic hormone family associated with immunity, were originally isolated and purified from calf thymus by Goldstein and White in 1966 (Goldstein, Slater, & White, 1966; Hadden, 1992; Shi, Shi, Zhao, Zhao, & Wang, 2015). Thymosins are classified into three main groups (α, β, γ) based on their isoelectric points (Low & Goldstein, 1979) and are evolutionarily conserved either vertebrate or invertebrate (Zhang et al., 2012). Vertebrate thymosins have been developed into pharmacological agents to treat some diseases including HIV, viral hepatitis, herpes virus keratitis, and certain types of clinically observed cancer (Dube, Bollini, Smart, & Riley, 2012; He et al., 2012; Matteucci et al., 2017; Sarin, Sun, Thornton, Naylor, & Goldstein, 1986; Wang, Zheng, Guo, & Ding, 2010). For *B. mori* thymosin (BmTHY), we first screened and identified the *BmTHY* gene from a *cDNA* library of silkworm pupae (GenBank accession: FJ602790) (Zhang et al., 2012). The homology of BmTHY to human thymosin beats (Tβ) is 74% (Zhang et al., 2012). BmTHY is located mainly in the nucleus and its rarely found in the cytoplasm of BmN cells, and it is also distributed in most tissues of fifth instar (Zhang et al., 2012). These suggest that BmTHY play an essential role in many biological events of *B. mori*.

In a recent study, we found out that exogenously applied recombinant BmTHY does not only increase the viability of BmNPV-infected BmN cells, but also it improves the survival rate of *B. mori* infected with BmNPV. So, BmTHY exerts immunomodulatory effects on *B. mori*, rendering them resistant to viral infection (Zhang et al., 2016). However, the effect of endogenous BmTHY on *B. mori* resistance to virus infection remains unknown. In this study, we constructed the over expression and inhibited expression systems of BmTHY in BmN cells to further explore the influence of endogenous BmTHY on BmNPV proliferation and replication, also the interaction of BmTHY with actin in vivo was investigated.

2 | MATERIALS AND METHODS

2.1 | Materials

Bombyx mori larval ovarian cell line (BmN cell) was preserved in our laboratory and cultured at 27°C in Sf-900II medium (Sigma, Shanghai, China) supplemented with 10% fetal bovine serum (FBS, Gibco BRL). Wild-type BmNPV and recombinant BmNPV-RFP (maintained in our laboratory) were propagated in BmN cells. Anti-BmTHY IgG was preserved in our laboratory. Antibodies of Tubulin (AT819-1) (Beyotime, Shanghai, China), goat anti-rabbit IgG H&L (HRP) (ab97051), and goat anti-mouse IgGH&L (HRP) (ab97023) were purchased from Abcam (Shanghai, China). Antibody of actin (ET1701-80) was bought from HUABIO (Hangzhou, China). Primers used in this work (Table 1) were synthesized by Biotech Corporation (Hangzhou, China).

2.2 | BmTHY overexpression and RANi

BmTHY-coding sequence (NCBI GenBank accession: FJ602790) was cloned into pIEX-1 expression vector using primer set in Table 1. Si-BmTHY (siRNA to BmTHY) or si-nc (siRNA to negative control) was designed and synthesized in vitro by GenePharma (Shanghai, China). Plasmids or siRNA were transfected into BmN cells using X-terme GENE transfection reagent (Roche, Switzerland) following the manufacturer's instruction. The expression level of BmTHY was subjected to western blotting analysis.

TABLE 1 Sequence of primers used in this work

GENE	Sequence of Primers (5′-3′)
BmTHY	Forward: CGCGGATCCATGGCCTGCTCCGTGAGTG
	Reverse: CCGCTCGAGTTAAGCTGATTTCTCTTGCTCAATGAC
Si-BmTHY	Forward: GAAGUCUUUAUUCGACGGUdTdT
	Reverse: ACCGUCGAAUAAAGACUUCdTdT
GP41	Forward: CGTAGTGGTAGTAATCGCCGC
	Reverse: ATGCGAGTCGCGTCGCTTT
BmGAPDH	Forward: CATTCCGCGTCCCTGTTGCTAAT
	Reverse: GCTGCCTCCTTGACCTTTTGC

2.3 | MTT assay

Two hundred microliters of the BmN cells suspension (2×10^4 cells/ml) was added into flat-bottomed 96-well plates in eight wells. After 24 h, BmN cells were transfected with pIEX-1, pIEX-1-BmTHY, si-nc, and si-BmTHY for 48 and 72 h, while the controls received phosphate buffered solution (PBS). At each time point, cells were stained with 3-(4,5Dimethylthiazol-yl)-2,5Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) dye (0.5 mg/ml) for 4 h at 27°C, followed by the removal of the culture medium and addition of 150 μl dimethyl sulfoxide. The absorbance was measured at 490 nm. All experiments were performed in triplicate.

2.4 | Viral titer assay

To determine the infection of BmNPV, BmN cells were challenged with BmNPV at a multiplicity of infection (MOI) of 1 for 48 h in each group. The supernatant of BmN cells was harvested and serially diluted 10-fold from 10^{-1} to 10^{-8} and 100 μl of each dilution was inoculated in flat-bottomed 96-well plates in eight wells. Then, 100 μL of the BmN cells suspension (1×10^3 cells/ml) was added per well, while the control received PBS. The infection status of BmN cell wells were observed under microscope to record the accounts of wells with damaged or dead cells for each dilution every day for seven consecutive days to calculate the viral titer using TCID50 analysis (Martin & Croizier, 1997; Zhang et al., 2016). Each assay was replicated three times.

2.5 | Quantitative real-time PCR analysis

Total DNA was extracted from cells using FavorPrep Tissue Genemic DNA Extraction Mini Kit (Taiwan, China) according to the manufacturer's protocol at different time range post BmNPV infection. Quantitative real-time PCR (qRT-PCR) analysis was conducted with 2× SYBR Green qPCR Master Mix (Bimake, Shanghai, China) using ABI Prism 7300 Sequence Detection System (Applied Bio Systems). BmNPV *gp41* and *GAPDH* were amplified with primer sets according to Table 1, respectively. The expression level of BmNPV *gp41* genes was normalized to the control *GAPDH*. The relative gene transcription level was calculated using $2^{-\triangle\triangle C}{}_T$, where $\Delta C_T = C_{T(gp41)} - C_{T(\mathrm{GAPDH})}$, $\Delta\Delta C_T = \Delta C_{(gp41)} - \Delta C_{T(\mathrm{maximum})}$. Each assay was performed in triplicate.

2.6 | Western-blotting analysis

The total proteins were extracted from BmN cells by cell lysis buffer (50 mM tris (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate) containing protease inhibitor phenylmethanesulfonyl fluoride (PMSF) (Sigma, Temecula, USA). After centrifugation at 12,000 rpm for 10 min at 4°C, the supernatant was collected and the protein concentration in each cell lysate was determined by BCA Protein Assay Kit (Beyotime, Shanghai, China). Equal amounts of protein were loaded and separated by 10% SDS–polyacrylamide gel, and then transferred to

a poly(vinylidene fluoride) (PVDF) membrane (Millipore, Cork, Ireland). The membranes were blocked in tris-buffered saline and tween 20 (TBST) containing 5% nonfat milk for 120 min at room temperature, then incubated with the primary antibodies at 4°C overnight. The membranes were washed with TBST for three times (10 min each time) on the following day and after that incubated with the secondary antibodies (conjugated with horseradish peroxidase) for 1 h at room temperature. Then, washing with TBST for 3 × 10 min, the membrane was then detected by enhanced chemiluminescence. Western blot images were measured using ImageJ software.

2.7 | Co-immunoprecipitation

Co-immunoprecipitation (CoIP) assay was performed according to the manufacturer's instruction of immunoprecipitation Kit Dynabeads® Protein G (Thermo Fisher, Rockford, USA). The total proteins were extracted from the BmN cells and the protein lysates were centrifuged at 12,000 rpm for 10 min. Equal amounts of supernatant were incubated with Protein G agarose at 4°C overnight. Then, the indicated antibody was added, followed by incubation for 4 h at 4°C. Control immunoprecipitations were carried out using both beads with lack of antibody but with IgG. Immunoprecipitated proteins were examined with western blotting.

2.8 | Separation of G-actin and F-actin

The ratio of F-actin to G-actin was separated by triton solubility and measured by western blotting techniques as previously described (Parreno et al., 2014). After the experiment, BmN cells were rinsed with PBS and then the cells were lysed with 150 μl extraction buffers (0.1% Triton X-100 in PBS containing protease inhibitor PMSF) for 5 min under slight agitation. After centrifugation at 12,000 rpm for 5 min at 4°C, the soluble portions (supernatant; predominantly G-actin) were harvested. For insoluble portions (predominantly F-actin), the pellets were resuspended in 150 μl RIPA buffer. After centrifugation at 12,000 rpm for 10 min at 4°C, the supernatant was harvested to measure F-actin. Samples from the supernatant (G-actin) and pellet (F-actin) fractions were proportionally loaded and analyzed by western blotting.

3 | RESULTS

3.1 | Overexpression and knockdown of BmTHY in BmN cells

To investigate the physiological function of endogenous BmTHY in *B. mori*, we first upregulated the BmTHY expression in BmN cells by transfecting cells with pIEX-1-BmTHY vector (Figure 1A). The expression level of BmTHY in BmN cells transfected with pIEX-1-BmTHY was significantly increased at 48 h, which was about 130% of that of the corresponding control group (BmN cells transfected with pIEX-1). Then, we performed dsRNA-mediated RNAi to deplete the expression of BmTHY in BmN cells. As shown in Figure 1B, the expression level of BmTHY was significantly downregulated at 48 h, which declined to 59% of the corresponding control group values (BmN cells transfected with si-nc). The above data show the system of overexpression and also the successful construction of knockdown BmTHY in BmN cells.

MTT assay revealed that after transfected with pIEX-1, pIEX-1-BmTHY, si-nc, si-BmTHY for 48 h, the viability of BmN cells were 93.2, 94.7, 90.6, and 93.9%, respectively, as compared to the control (Figure 1C), which were 92, 92.7, 91.9, and 90.9% (Figure 1D) for 72 h, respectively. These results showed that overexpressed or repressed BmTHY had no significantly influence on the viability of BmN cells.

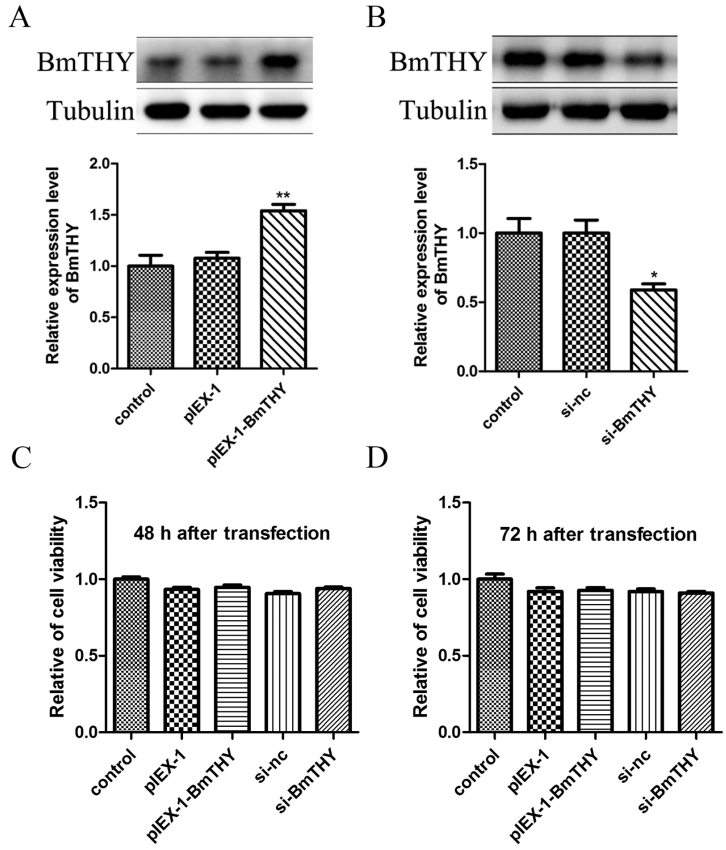

FIGURE 1 Overexpression and knockdown of BmTHY in BmN cells

Notes. (A) Overexpression efficiency of BmTHY in BmN cells treated with pIEX-1 and pIEX-1-BmTHY for 48 h were measured by western blotting. (B) Knockdown efficiency of BmTHY in BmN cells transfected with si-nc and si-BmTHY for 48 h were analyzed by western blotting, and tubulin was used as the control. Error bars represent the standard error of mean (SEM) in the three replicates. (C and D) MTT assay showed that effects of overexpression and knockdown of BmTHY on the viability of BmN cells. Asterisk indicates significant difference between the control group and experimental group (*$P < 0.05$ vs. control,**$P < 0.01$ vs. control. $n = 6$, one-way ANOVA).

3.2 | Effects of overexpression and knockdown of BmTHY on BmNPV proliferation in BmN cells

To test the effects of overexpression of BmTHY on BmNPV proliferation in BmN cells, BmN cells were transfected with pIEX-1 and pIEX-1-BmTHY expressing plasmid for 48 h, then after they were infected with wild-type BmNPV or BmNPV-RFP for 48 h, and the viral proliferation was analyzed by the method of TCID50 or fluorescence microscopy. To detect the viral titer, the mean value of TCID50 at 144 h postinfection of wild BmNPV in BmTHY overexpressing cells treated with pIEX-1-BmTHY and the control cells treated with pIEX-1 were 0.74×10^6 and 1.33×10^6, respectively. At 168 h postinfection of wild BmNPV in BmN cells, overexpression of BmTHY resulted in an over 50% decrease in the viral titer compared to the control (Figure 2A). Moreover, fluorescence was observed at 48 h postinfection of BmNPV-RFP. The red fluorescence intensity in the overexpression of BmTHY group treated with pIEX-1-BmTHY was remarkably reduced than the control group treated with pIEX-1 (Figure 2B). These results indicated that overexpression of BmTHY can inhibit BmNPV proliferation in BmN cells.

Interestingly, the knockdown of BmTHY can promote BmNPV proliferation in BmN cells. For instance, the viral titer analysis showed that the mean value of TCID50 at 144 h postinfection of wild BmNPV in the knockdown of BmTHY group (treatment with si-BmTHY) and the corresponding control group (treatment with si-nc) were 1.8×10^6 and

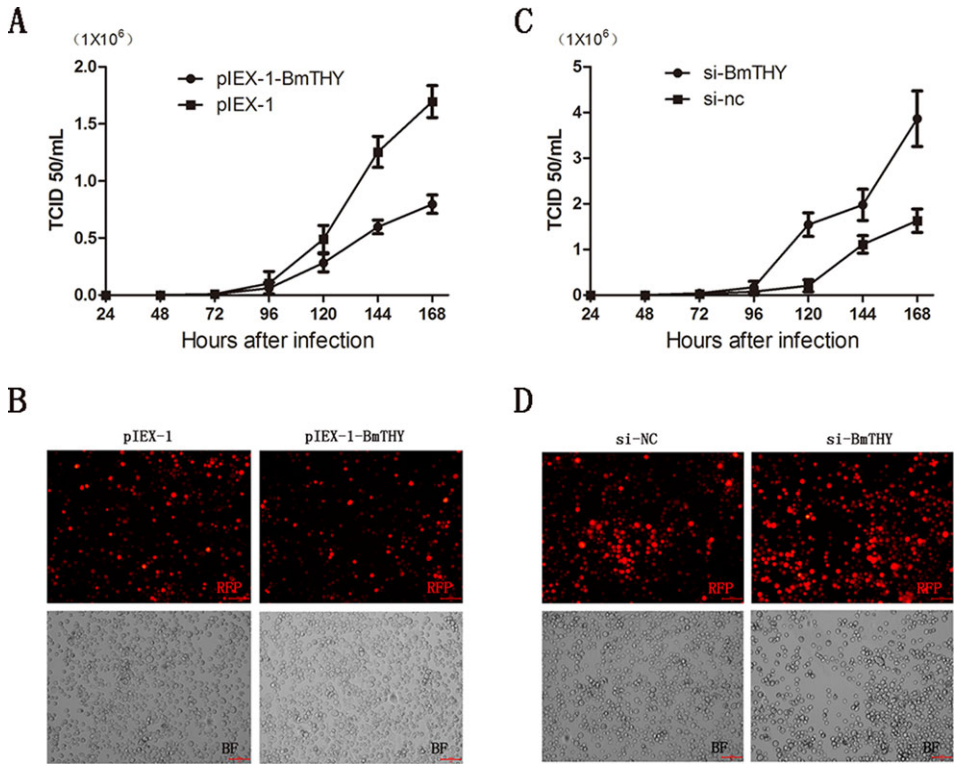

FIGURE 2 Effects of overexpression and knockdown of BmTHY on BmNPV proliferation in BmN cells

Notes. (A) BmN cells were transfected with pIEX-1 and pIEX-1-BmTHY expressing plasmids for 48 h before BmNPV infection. The media was collected at 48 h postinfection with the BmNPV for the detection of viral titer. The titer was analyzed by the method of TCID50. (B) Fluorescence (RFP) and bright field (BF) images were observed in BmN cells (treated with pIEX-1 and pIEX-1-BmTHY) at 48 h postinfection with BmNPV-RFP. (C) BmN cells were transfected with si-nc and si-BmTHY dsRNA for 48 h before BmNPV infection. The supernatant titer was measured by the method of TCID50. (D) Fluorescence (RFP) and bright field (BF) images were observed in BmN cells (treated with si-nc and si-BmTHY) at 48 h postinfection with BmNPV-RFP. Data represents mean + SEM of the three independent experiments.

1.3×10^6, respectively. At 168 h postinfection of wild-type BmNPV, TCID50 mean value in the knockdown of BmTHY group climbed up, with an increase of 2.37-fold to that of the control group (Figure 2C). In addition, fluorescence result showed that the red fluorescence intensity in the knockdown BmTHY group significantly increased as compared with the control group (Figure 2D). These results suggested that, the downregulation of BmTHY can improve BmNPV proliferation in BmN cells.

3.3 | Effects of overexpression and knockdown of BmTHY on BmNPV replication in BmN cells

The gp41 gene exists in all baculovirus and is highly conserved, which plays a very important role in baculovirus replication (Liu & Maruniak, 1999). Therefore, gp41 gene copies in BmTHY overexpressing cells or in BmTHY knockdown cells were analyzed by qRT-PCR to further investigate the role of BmTHY in BmNPV replication in BmN cells. For the overexpressing of BmTHY in BmN cells infected with BmNPV, we found out that the expression level of gp41 was significantly decreased at 48 and 72 h postinfection, which were declined to 71.3 and 75.1% of the corresponding control group, respectively (Figure 3A). However, for the knockdown BmTHY in BmN cells infected with BmNPV, as shown in Figure 3B, the expression level of gp41 was significantly increased in 48 and 72 h postinfection and was of 1.46- and

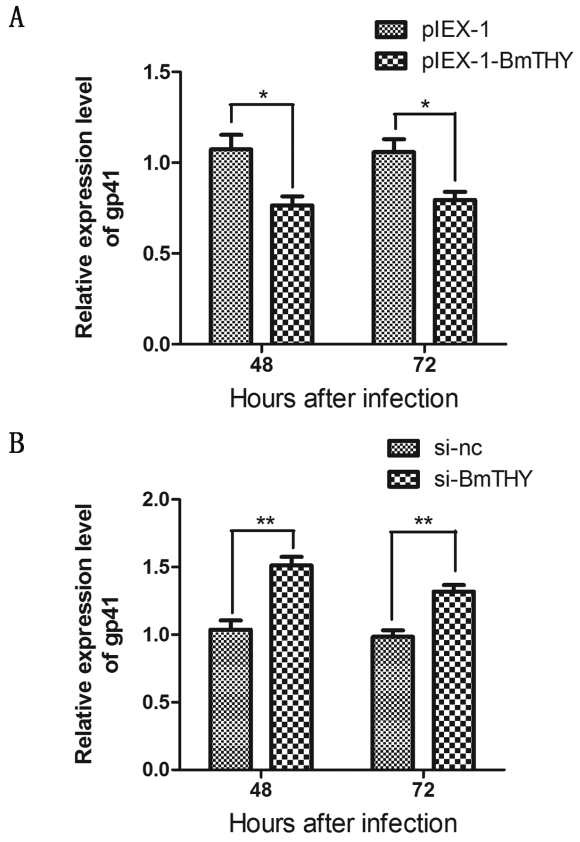

FIGURE 3 Effects of overexpression and knockdown of BmTHY on BmNPV replication in BmN cells
Notes. (A) BmN cells were transfected with pIEX-1 and pIEX-1-BmTHY expressing plasmid for 48 h. Then, the cells were infected with BmNPV for 48 and 72 h, respectively. The relative replication level of virus, determined by BmNPV gp41 gene copies were measured by qRT-PCR analysis. (B) BmN cells were transfected with si-nc and si-BmTHY dsRNA for 48 h. Then, the cells were infected with BmNPV for 48 and 72 h. Relative replication level of viruses determined by BmNPV gp41 copies were measured by qRT-PCR analysis. The mRNA level of target genes was normalized to the internal control (BmGAPDH). Data represent mean ± SEM of the three independent experiments (*t*-test,*$P < 0.05$ vs. control,**$P < 0.01$ vs. control).

1.34-fold of the corresponding control group, respectively. These results indicated that BmTHY can inhibit BmNPV replication in BmN cells.

3.4 | BmTHY binding to actin in BmN cells

In the present study, after the total protein was extracted from BmN cells, co-immunoprecipitation assay was carried out using BmTHY antibody as the bait, with rabbit IgG as a negative control. As shown in Figure 4, BmTHY and actin were coprecipitated by antibodies against BmTHY. This testified that BmTHY could bind to actin in vivo. The result of Co-IP suggested a physical interaction between BmTHY and actin in BmN cells.

3.5 | BmTHY shifted the ratio of F/G-actin (filamentous/monomeric actin) in BmN cells infected with BmNPV

To further investigate whether and how BmTHY interacts with actin in BmN cells in case of pathogens invasion, the ratio of F/G-actin was analyzed after the infection of the overexpressed and knockdown BmTHY in BmN cells for

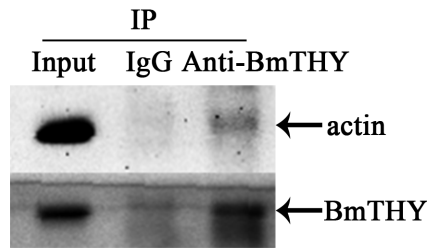

FIGURE 4 BmTHY interacted with actin in BmN cells
Notes. Interaction between BmTHY and actin was confirmed by Co-IP assay using BmTHY antibody, with rabbit IgG as a negative control.

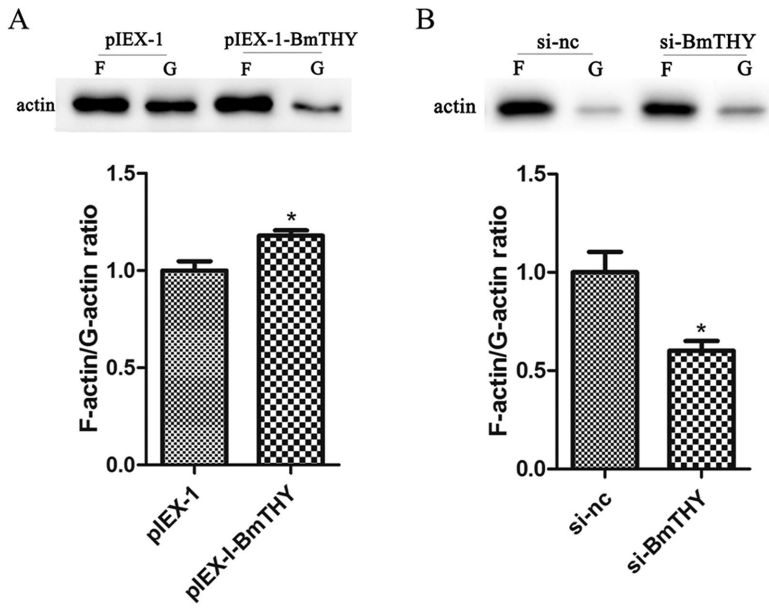

FIGURE 5 BmTHY shifted the ratio of F/G-actin in BmN cells infected with BmNPV
Notes. (A) BmN cells were transfected with pIEX-1 and pIEX-1-BmTHY expressing plasmid for 48 h, then the cells were infected with BmNPV for 48 h. (B) BmN cells were transfected with si-nc and si-BmTHY dsRNA for 48 h, then the cells were infected with BmNPV for 48 h. The protein of G-actin and F-actin were extracted from cells in each group. Western blotting of actin was performed on fractions of soluble actin (G-actin) in supernatant (G) and insoluble actin (F-actin) in the pellet (F), separated from the BmN cells by centrifugation. Supernatant and pellet fractions from each sample were proportionally loaded for all conditions and the ratio of F- to G-actin was calculated. Data represent mean ± SEM of three independent experiments (*t*-test,$^*P < 0.05$ vs. control).

48 h. For BmTHY overexpressing in BmN cells infected with BmNPV, the ratio of F/G-actin was increased to 20.4% of the corresponding control group (Figure 5A). However, knockdown BmTHY in BmN cells infected with BmNPV showed a 39.8% reduction in the ratio of F/G-actin compared to the control group (Figure 5B). These results showed that BmTHY shifted the ratio of F/G-actin in BmN cells infected with BmNPV, and further demonstrated that BmTHY interacted with actin in vivo.

4 | DISCUSSION

BmNPV, a typical group of baculovirus, has caused great economic losses in sericulture (Dong et al., 2015). However, silkworm and BmNPV have always coexisted mutually in the nature. In the process of *B. mori* evolution, some inherent

antiviral genes have been optimized and rejuvenated to maintain the survival and reproduction of silkworm. Therefore, it is possible to excavate these antiviral genes to create new silkworm strains to withstand BmNPV. In recent 20 years, scientists have found some endogenous genes, such as Bmlipase-1 (Jiang et al., 2012), BmSpry (Jin et al., 2014), BmSP-2 (Nakazawa et al., 2004), Bmtryp (Ponnuvel, Nithya, Sirigineedi, Awasthi, & Yamakawa, 2012), and V-ATPase (Lu et al., 2013), are involved in silkworm defense response against BmNPV. A transgenic silkworm was generated via embryo microinjection with a vector overexpressing the Bmlipase-1 gene (Jiang et al., 2012). The mortality of the transgenic line decreased to approximately 33% compared with the nontransgenic line challenged with BmNPV (Jiang et al., 2012). These evidences demonstrate that overexpressing endogenous antiviral genes can enhance the antiviral resistance of silkworms.

In our laboratory, we also found a new gene, *BmTHY*, involved in silkworm defense response against BmNPV. A previous study showed that exogenously applied recombinant BmTHY renders *B. mori* resistant to viral infection (Zhang et al., 2016). We hypothesize that BmTHY might be a new potential antiviral factor in silkworm, but should be supported by further experimental data on the endogenous BmTHY antiviral activity against BmNPV. Therefore, in this study, overexpression and inhibited expression systems of BmTHY were constructed in BmN cells, and cells were then infected with wild-BmNPV. Data for the viral titer revealed that an overexpression of BmTHY inhibited proliferation of BmNPV in BmN cells, while knockdown of BmTHY significantly enhanced viral proliferation. These results indicated that BmTHY is an important antivirus gene in *B. mori*.

Further investigation showed that the copies of BmNPV gp41 gene decreased when the expression level of BmTHY was upregulated, and increased with BmTHY downregulation in BmN cells infected with BmNPV. This suggests that BmTHY could resist BmNPV invasion by affecting replication of BmNPV. Because the abundance of gp41 gene is indicative of viral DNA replication, which can also be buttressed with the titers of BmNPV to show that when BmTHY is overexpressed or suppressed, one can conclude that the BmTHY is blocked during or prior to viral DNA replication.

Actin cytoskeleton is a key factor in BmNPV infectivity in that the viral manipulation of actin cytoskeleton is at the core of successful replication (Volkman, 2007). In this study, Co-IP assay demonstrated that BmTHY can bind to actin in BmN cells. Moreover, overexpression or inhibited expression of BmTHY shifted the ratio of F/G-actin in BmN cells infected with BmNPV, which suggest that the structure and function of actin cytoskeleton were affected. Converging lines of such evidence preliminarily implied that BmTHY may exert influence in viral manipulation of the actin cytoskeleton during BmNPV infection in BmN cells. Considering the effects of overexpression and the knockdown of BmTHY on BmNPV proliferation in BmN cells together, we speculate BmTHY interacting with actin in vivo might be involved in the resistance to virus infection.

Taken together, the overexpression of BmTHY, which is an actin-interacting protein from *B. mori*, can inhibit BmNPV proliferation and replication in BmN cells. The results provide a solid foundation to promote BmTHY gene for the creation of a new silkworm strain to defend against BmNPV.

ACKNOWLEDGMENTS

This work was supported by the Public Technology Application Research Project from Science Technology Bureau of Zhejiang Province (2016C33080, 2016C32012), the National Natural Science Foundation of Zhejiang Province (LY16C170002), the Science Foundation of Zhejiang Sci-Tech University (16042187-Y). And also my profound gratitude to Obeng Enoch who contributed to the editorial section.

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REFERENCES

Dong, X. L., Liu, T. H., Wang, W., Pan, C. X., Wu, Y. F., Du, G. Y., … Pan, M. H. (2015). BmREEPa is a novel gene that facilitates BmNPV entry into silkworm cells. *Plos One*, *10*(12), e0144575.

Dube, K. N., Bollini, S., Smart, N., & Riley, P. R. (2012). Thymosin beta4 protein therapy for cardiac repair. *Current Pharmaceutical Design*, *18*(6), 799–806.

Goldstein, A. L., Slater, F. D., & White, A. (1966). Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proceedings of the National Academy of Sciences of the USA*, *56*(3), 1010–1017.

Hadden, J. W. (1992). Thymic endocrinology. *International Journal of Immunopharmacology*, *14*(3), 345–352.

He, G., Lei, W., Wang, S., Xiao, R., Guo, K., Xia, Y., … Wang, Y. (2012). Overexpression of tumor suppressor TSLC1 by a survivin-regulated oncolytic adenovirus significantly inhibits hepatocellular carcinoma growth. *Journal of Cancer Research and Clinical Oncology*, *138*(4), 657–670.

Jiang, L., Wang, G., Cheng, T., Yang, Q., Jin, S., Lu, G., … Xia, Q. (2012). Resistance to *Bombyx mori* nucleopolyhedrovirus via overexpression of an endogenous antiviral gene in transgenic silkworms. *Archives of Virology*, *157*(7), 1323–1328.

Jin, S., Cheng, T., Jiang, L., Lin, P., Yang, Q., Xiao, Y., … Xia, Q. (2014). Identification of a new sprouty protein responsible for the inhibition of the *Bombyx mori* nucleopolyhedrovirus reproduction. *Plos One*, *9*(6), e99200.

Konala, N., Abburi, P., Bovilla, V. R., & Mamillapalli, A. (2013). The effect of bovine milk on the growth of *Bombyx mori*. *Journal of Insect Science*, *13*, 98. doi:10.1673/031.013.9801

Liu, J. C., & Maruniak, J. E. (1999). Molecular characterization of genes in the GP41 region of baculoviruses and phylogenetic analysis based upon GP41 and polyhedrin genes. *Virus Research*, *64*(2), 187–196.

Low, T. L., & Goldstein, A. L. (1979). The chemistry and biology of thymosin. II. Amino acid sequence analysis of thymosin alpha1 and polypeptide beta1. *Journal of Biological Chemistry*, *254*(3), 987–995.

Lu, P., Xia, H., Gao, L., Pan, Y., Wang, Y., Cheng, X., … Yao, Q. (2013). V-ATPase is involved in silkworm defense response against *Bombyx mori nucleopolyhedrovirus*. *Plos One*, *8*(6), e64962.

Martin, O., & Croizier, G. (1997). Infection of a *Spodoptera frugiperda* cell line with *Bombyx mori* nucleopolyhedrovirus. *Virus Research*, *47*(2), 179–185.

Matteucci, C., Grelli, S., Balestrieri, E., Minutolo, A., Argaw-Denboba, A., Macchi, B., … Garaci, E. (2017). Thymosin alpha 1 and HIV-1: Recent advances and future perspectives. *Future Microbiology*, *12*, 141–155.

Nakazawa, H., Tsuneishi, E., Ponnuvel, K. M., Furukawa, S., Asaoka, A., Tanaka, H., … Yamakawa, M. (2004). Antiviral activity of a serine protease from the digestive juice of *Bombyx mori* larvae against nucleopolyhedrovirus. *Virology*, *321*(1), 154–162.

Parreno, J., Raju, S., Niaki, M. N., Andrejevic, K., Jiang, A., Delve, E., & Kandel, R. (2014). Expression of type I collagen and tenascin C is regulated by actin polymerization through MRTF in dedifferentiated chondrocytes. *FEBS Letters*, *588*(20), 3677–3684.

Ponnuvel, K. M., Nithya, K., Sirigineedi, S., Awasthi, A. K., & Yamakawa, M. (2012). In vitro antiviral activity of an alkaline trypsin from the digestive juice of *Bombyx mori* larvae against nucleopolyhedrovirus. *Archives of Insect Biochemistry and Physiology*, *81*(2), 90–104.

Sarin, P. S., Sun, D. K., Thornton, A. H., Naylor, P. H., & Goldstein, A. L. (1986). Neutralization of HTLV-III/LAV replication by antiserum to thymosin alpha 1. *Science*, *232*(4754), 1135–1137.

Shi, X. Z., Shi, L. J., Zhao, Y. R., Zhao, X. F., & Wang, J. X. (2015). Beta-thymosins participate in antiviral immunity of red swamp crayfish (*Procambarus clarkii*). *Developmental and Comparative Immunology*, *51*(2), 213–225.

Subbaiah, E. V., Royer, C., Kanginakudru, S., Satyavathi, V. V., Babu, A. S., Sivaprasad, V., … Mauchamp, B. (2013). Engineering silkworms for resistance to baculovirus through multigene RNA interference. *Genetics*, *193*(1), 63–75.

Volkman, L. E. (2007). Baculovirus infectivity and the actin cytoskeleton. *Current Drug Targets*, *8*(10), 1075–1083.

Wang, F., Zheng, Z., Guo, J., & Ding, X. (2010). Correlation and quantitation of microRNA aberrant expression in tissues and sera from patients with breast tumor. *Gynecologic Oncology*, *119*(3), 586–593.

Zhang, C., Wang, Y., Fang, Q., Xu, M., Lv, M., Liao, J., … Zhang, W. (2016). Thymosin from *Bombyx mori* is down-regulated in expression by BmNPV exhibiting antiviral activity. *Journal of Insect Science (Online)*, *16*(1). doi:10.1093/jisesa/iew039

Zhang, W., Zhang, C., Lv, Z., Fang, D., Wang, D., Nie, Z., … Zhang, Y. (2012). Molecular characterization, tissue distribution, subcellular localization and actin-sequestering function of a thymosin protein from silkworm. *Plos One*, *7*(2), e31040.

How to cite this article: Liao J, Zhang C, Ru W, Wang D, Zhang W. Effects of overexpression and inhibited expression of thymosin, an actin-interacting protein from *Bombyx mori*, on BmNPV proliferation and replication. *Archives of Insect Biochemistry and Physiology*. 2018;e21449. https://doi.org/10.1002/arch.21449