ISJ 12: 38-45, 2015 ISSN 1824-307X

RESEARCH REPORT

Identification and characterization of the *Bombyx mori* myosin II essential light chain and its effect in BmNPV infection

LJ Hao, P Lü, L Gao, Y Zhou, Q Yao, YH Yang, KP Chen

Institute of Life Science, Jiangsu University, Zhenjiang, China

Accepted February 2, 2015

Abstract

Myosin, as a type of molecular motor, is mainly involved in muscle contraction. Recently, myosin research has made considerable progress. However, the function of *Bombyx mori* myosin remains unclear. In this study, we cloned the BmMyosin II essential light chain (BmMyosin II ELC) gene from a cDNA library of silkworm, which had an open reading frame (ORF) of 444 bp encoding 147 amino acids (about 16 kDa). After analyzing their sequences, BmMyosin II ELC was similar to the ELCs of 27 other Myosin II types, which contained EFh domain that bound Ca^{2+}. In addition, 28 sequences had five motifs, motifs 1 and 3 were relatively conserved. We constructed two vectors with BmMyosin to transfect MGC803 or BmN, monolayer wound healing of cells indicated they can promote cell migration successfully. For three fifth instar silkworms, Bm306, BmNB, $BmBC_8$, we mainly analyzed the change of BmMyosin II ELC from transcription and translation after infecting with nucleopolyhedrovirus (BmNPV). We found that gene expression of resistant strains were higher than susceptible strains at 12 h, while the result of the translation level was opposite that of the transcription level. Through *in vitro* protein interactions, we found BmMyosin II ELC can interact with BmNPV ubiquitin.

Key Words: *Bombyx mori;* cell migration; myosin; ubiquitin

Introduction

Khne (1859) proposed that myosin could be extracted from frog tissue. Myosin, as the unit of myofibril, was composed of many heavy chains and light chains and divided into three areas, spherical head, neck and tail (Kwon *et al*., 2014), (Sweeney *et al*., 2010) regarded myosin as a kind of molecular motors , which played an important role in muscle contraction, chemotaxis cytoplasmic division, cell migration and vesicular transport signal transduction and so on (Andruchov *et al*., 2006). Myosin is actually a superfamily of chemicals. To date, we have found 24 kinds of myosin, and have divided them into traditional and unconventional myosin based on the myosin source. Specifically, myosin II is considered to be a traditional form of myosin. Unconventional myosin refers to myosin not found in muscle myosin tissue, such as myosin I, III, IV, V, exists only in the muscle cells, while myosin VIII, XI and XII only exist in plants. In recent years, myosin II has been a frequent topic of research, the protein coiled into the shape much like the letter Y. Myosin II has six protein polymers, including two heavy chains of about 220 kDa, two essential light chain of about 17 kDa and two regulatory light chain of about 20 kDa (Santos *et al*., 2007). According to the study, most myosin light chains were EF-hand superfamily members (Kolodney *et al*., 1999).

Recently, some studies found that myosin plays a crucial role in cell growth and motion (Holmes *et al*., 2000), and light chain of myosin II has specific functions. Therefore, the light chain of myosin has become a hot research topic. In higher mammal cells, phosphorylation of the myosin light chain can promote cell migration (Straight *et al*., 2003).

The function of myosin in silkworm (*Bombyx mori*), a representative lepidopteran insect, remains unclear (Wang *et al*., 2007). Our previous unpublished research found that essential light chain of myosin II was related with nucleopolyhedrovirus (BmNPV). Therefore, we chose the mysin II essential light chain of the *B. mori* (BmMyosin II ELC) infected with BmNPV (T3 strain) to study change of protein expression. Then through protein interactions *in vitro*, we predicted the protein of BmNPV which interacted with BmMyosin II ELC. Simultaneously, we attempted to determine whether BmMyosin II ELC promotes migration in insect and mammalian cells.

Corresponding author:
Keping Chen
Insitute of Life Science
Jiangsu University
301 Xuefu Road, Zhenjiang 212013, China
E-mail: kpchen@ujs.edu.cn

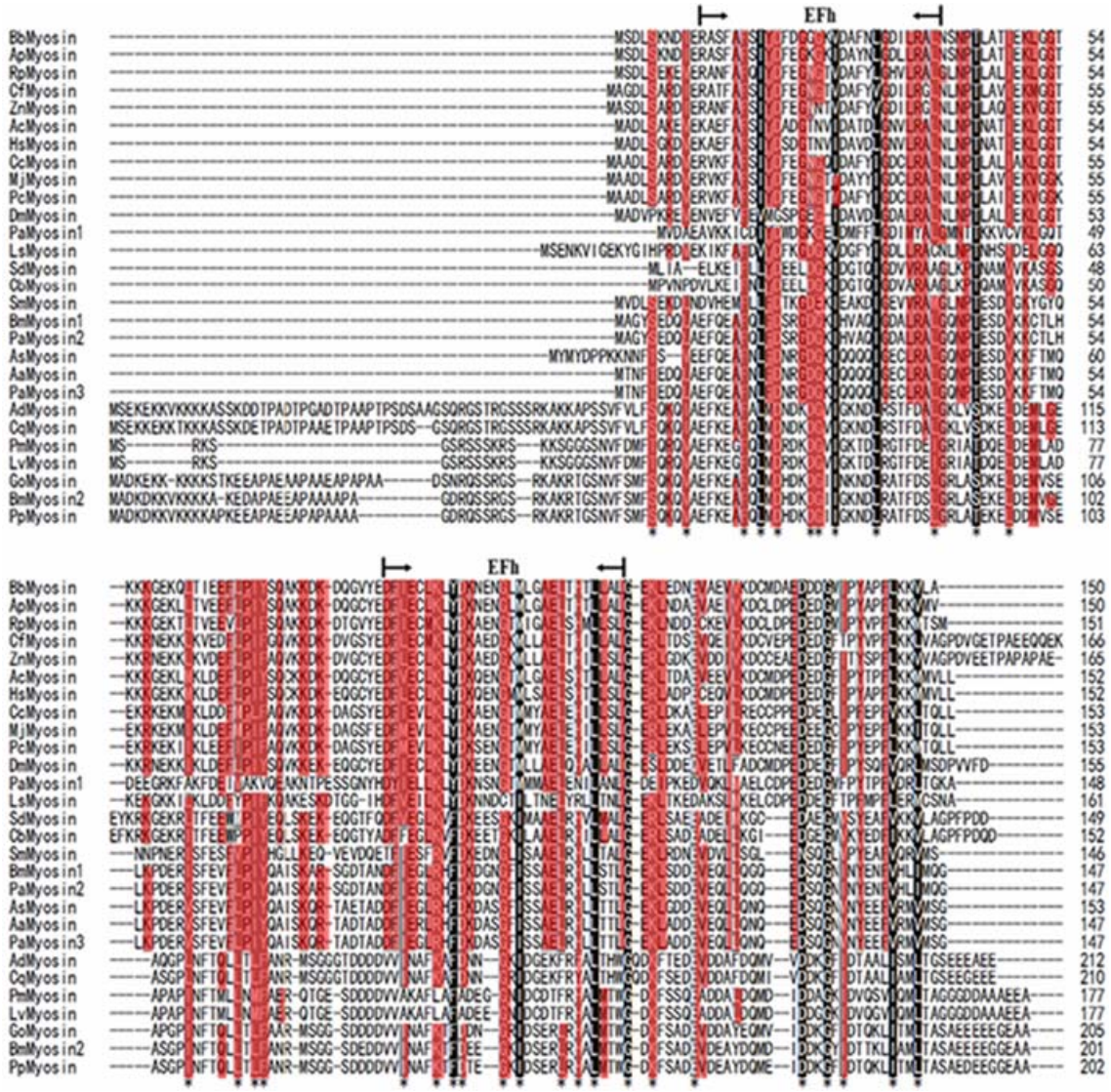

Fig. 1 Myosin II essential light chain amino acid sequence homology analysis and the related genbank for 28 species analyzed here. *Biston betularia* (AEP43792); *Antheraea pernyi* (AGL33708); *Riptortus pedestris* (BAN21409); *Riptortus pedestris* (AGM32403); *Zootermopsis nevadensis* (KDR17497); *Apis cerana* (AEY59302); *Harpegnathos saltator* (EFN85742); *Crangon spp* (ACR43477); *Marsupenaeus japonicus* (ADD70028); *Procambarus clarkii* (AFP95338); *Drosophila melanogaster* (AAA28710); *Pseudodiaptomus annandalei* (AGT28473); *Lepeophtheirus salmonis* (ACO13186); *Setaria digitata* (ACT15365); *Caenorhabditis brenneri* (ACD86909); *Schistosoma mansoni* (AAR99584); *Bombyx mori* (NP_001040547.1); *Pararge aegeria* (JAA88715); *Anopheles sinensis* (KFB36116); *Aedes aegypti* (ABF18115); *Psorophora albipes* (JAA94216); *Anopheles darlingi* (ETN67861); *Culex quinquefasciatus* (EDS45595); *Penaeus monodon* (AET87131); *Litopenaeus vannamei* (ACC76803); *Gryllotalpa orientalis* (AAW22542); *Bombyx mandarina* (ACI05250); *Papilio polytes* (BAM18881). Identical residues are shaded in black, while similar residues are shaded in red, each species contains two EFh domains,* indicates conservative sites.

Materials and Methods

Analysis of bioinformatics on the essential light chain of BmMyosin II

The essential light chain of BmMyosin II was used to find 28 similar protein sequences related to other invertebrates using the NCBI BLAST Network Service (http://au.expasy.org/tools/BLAST/). A neighbor-joining (NJ) phylogenetic tree (Dias *et al*., 2003) and the schematic distribution of conserved motifs among the defined gene clusters were found using online versions of MEGA5.0 and MEME software.

Cell culture and monolayer wound healing assay

In preparation for TransIT-2020 mediated transfections, MGC803 (human stomach cancer cells) and BmN (insect ovary cells) were plated in a

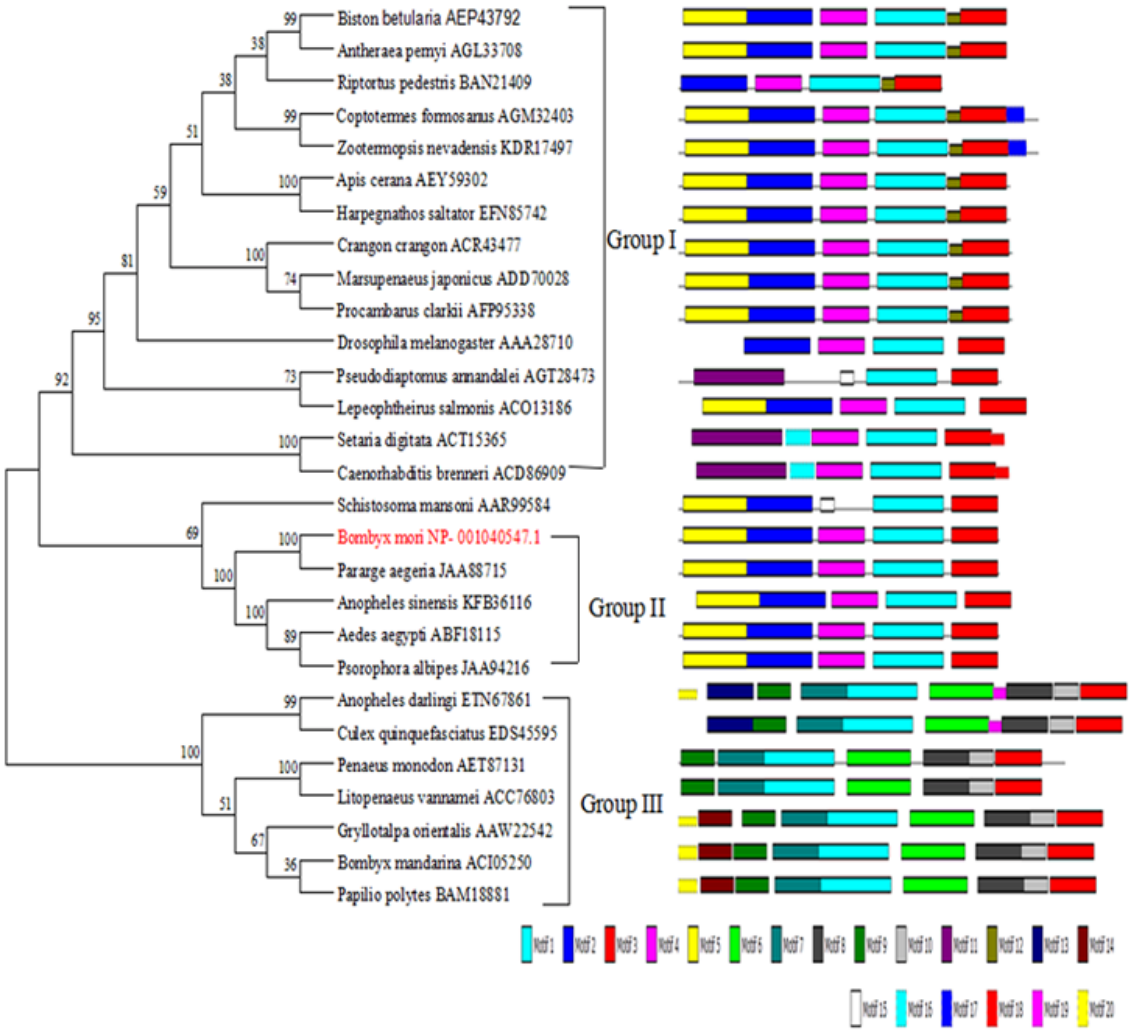

Fig. 2 Neighbor-joining (NJ) phylogenetic tree and schematic distribution of conserved motifs among the defined gene clusters. The unrooted phylogenetic tree (left side of the figure) from the complete protein sequence was depicted by the MEGA 5.0 program using the NJ method. The tree shows the 28 phylogenetic subgroups (Group I-Group III) with high bootstrap values. Motifs were identified by means of MEME software using the complete amino acid sequence of the 28 invertebrates (right side of the figure).

24-well plate at a confluency of 50 - 70 % (3 - 4×10^4 cells per well) in 500μl plating medium (DMEM or TC-100 medium supplemented with 10 % FBS). Before plating onto this plate, one black line was drawn on the underside of the well with a Sharpie marker. The line would serve as fiducial marks for the wound areas to be analyzed. In preparation for making the wound, the free serum medium was used to prevent cell growth. Two parallel scratch wounds of approximately 300μm width were made perpendicular to the marker line with a yellow P200 pipet tip (Valster *et al*., 2005). Then vectors were constructed with PCDNA3.0-C-FLAG-Myosin II ELC and PIZ-C-FLAG-Myosin II ELC to transfect corresponding the cells of MGC803 and BmN. The wounds were observed using a microscope. Images were taken at regular intervals over a 0 - 48 h period of both areas flanking the intersections of the wound and the marker lines. Images were analyzed by digitally drawing lines (using Adobe Photoshop) averaging the position of the migrating cells at the wound edges. The cell migration distance was determined by measuring the width of the wound divided by two and by subtracting this value from the initial half-width of the wound (Eccles *et al*., 2005).

The preparation of BmMyosin II essential light chain polyclonal antibody

The recombinant plasmid PGEX-5X-3-Myosin II ELC was transformed into E. coli BL21, which were incubated at 37 °C in liquid LB culture media containing 50 mg/mL ampicillin. Expression of the GSTtag fusion protein was induced by adding IPTG to a final concentration of 1 mM before another 5-h incubation. Purification of the fusion protein was accomplished using Glutathione-Sepharose beads. Polyclonal antibody of BmMyosin II ELC was prepared by immunizing a laboratory rat (*Rattus rattus*) using purified Myosin II ELC as antigen (Zhang *et al*., 2012).

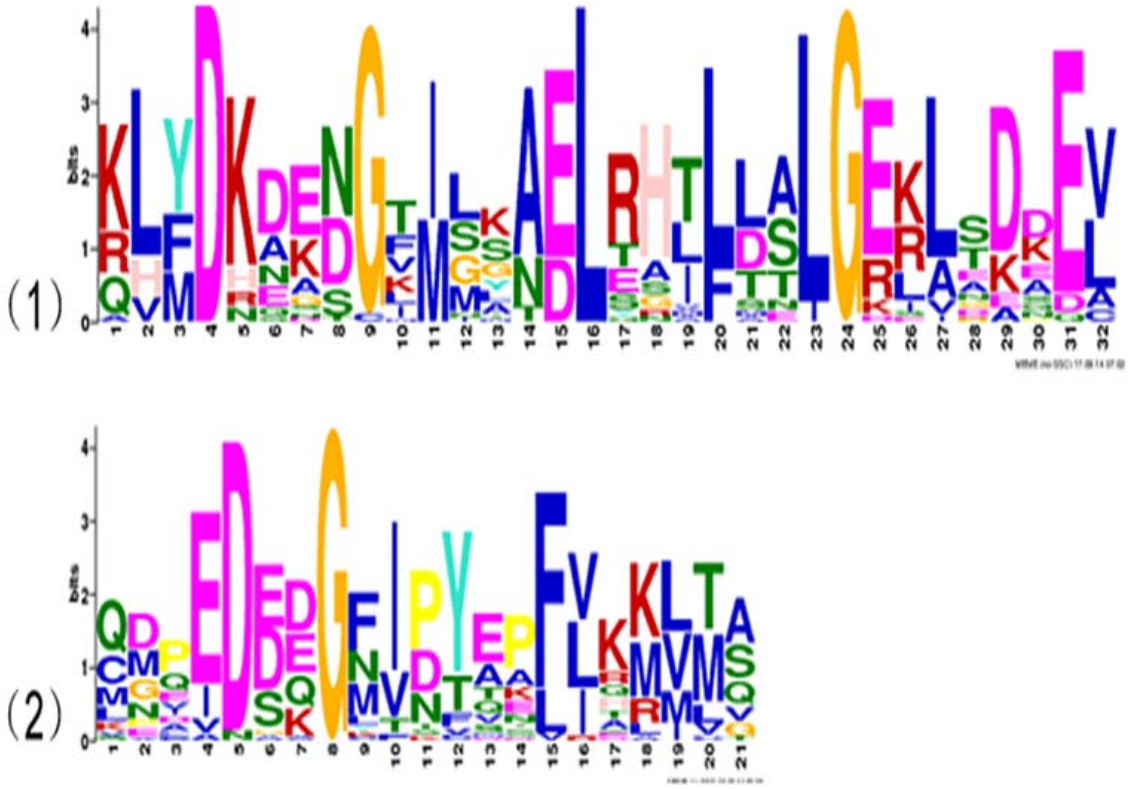

Fig. 3 The motif 1 and motif 3 are highly conserved among all 28 Myosin II ELC proteins. The overall height of each stack indicates the conservation of the sequence at that position, whereas the height of letters within each stack represents the relative frequency of the corresponding amino acid. (1) and (2) show motif 1 and 3 amino acid conservative analysis, respectively.

RNA isolation and cDNA synthesis of midguts strains of $BmBC_8$, Bm306 and BmNB infected BmNPV

Midguts of the 5th instar larvae of strains BC_8, 306 and NB infected BmNPV (1×10^9 pfu/ml) at 0 h, 6 h, 12 h, 24 h, 48 h, 72 h were collected and washed with cold PBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 and 1.4 mM KH_2PO_4). Then all samples were frozen immediately in liquid nitrogen and stored at −80 °C until extraction of RNA. Total RNA was extracted from each frozen sample with triol (GENERAY BIOTECH RnaEx). DNA was removed through digestion with RNase-free DNase I at 37 °C for 20 min. The RNA was further purified with phenol-chloroform and precipitated with ethanol. The RNAs dissolved in DEPC-treated ddH_2O were used to synthesize cDNAs with a Thermo Scientific RevertAid First strand cDNA synthesis Kit (Thermo-Fisher Scientific, New York, USA) by following the manufacturer's instructions (Zhang *et al*., 2012).

qPCR analysis

qPCR primers were designed using the Primer Select program of the PrimerSelect software. The primer pairs were as follows: (Myosin II essential light chain) Forward primer, 5'-GGACAAAATCCTACAGAG-3', Reverse primer, 5'-CCCTGAGAGTCTTCTTGTC-3'. A SYBR Premix Ex Taq II Kit (Takara Biotechnology Co., Ltd. Dalian, China) was used. qPCR was performed using an ABI 7300 Sequence Detection System (Applied Bio systems, Darmstadt, Germany) under the following conditions: an initial cycle at 95 °C for 30 sec., followed by 40 cycles of 95 °C for 5 sec., 60 °C for 31 sec., one cycle of 95 °C for 15 sec., 60 °C for 1 min., 95 °C for 15 sec., and 60 °C for 15 sec.. Each reaction was performed in triplicate in 96-well plates. The relative expression level was calculated using $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT = \Delta CT$ (target gene)- ΔCT (actin gene), $\Delta\Delta CT = \Delta CT$(target gene)- ΔCT (maximum) (Gareus *et al*., 2006).

Western blot analysis

The protein of midguts were collected and ground to powder in liquid nitrogen followed by RIPA Lysis Buffer (50 mM Tris-HCl pH7.4, 150 mM NaCl, 1 mM EDTA, 1 % NP-40, 0.5 % sodium deoxycholate, 0.1 % SDS, 1 mM PMSF). The protein concentrations were quantitated by the method of BCA, in which BSA was used as the protein standard. Protein samples were equalized and electrophoresed by 12 % SDS-PAGE and electrotransfered to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5 % milk in TBS. Following incubation with purified anti-BmMyosin IgG, the membranes were washed and incubated with HRP-labeled anti-mouse IgG.

The membranes were washed three times with TBST, and scanned using a TMB membrane peroxidase substrate system (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD, USA) or was detected by Electro-Chemi-Luminescence (ECL) and exposed to film.

GST pull-down

To directly obtain a function protein, the expression vector PGEX-5X-3 was constructed and used to express the recombinant protein GST-BmMyosin II ELC. The protein was suspended on Glutathione-Sepharose beads in wash buffer (25 mM Tirs-Hcl, 150 mM NaCl, 1 mM EDTA), BmN and BmN infected with recombinant BmNPV (His-Ubiquitin) after two days were collected. Then RIPA Lysis Buffer (50 mM Tris-HCl pH7.4, 150 mM NaCl, 1 mM EDTA, 1 % NP-40, 0.5 % sodium deoxycholate, 0.1 % SDS, 1 mM PMSF) was used and the recombinant protein was combined. They were palced in 4°C refrigerator overnight. After washing three times by washing buffer, the beads were loaded on the 12 % SDS polyacrylamide gels. Western blotting was used to analyze the expression of ubiquitin using His tag McAb.

Results

BmMyosin II essential light chain cloning and sequence analysis

Based on the BmMyosin II ELC gene sequence (GenBank: DQ534197) from NCBI, the full length cDNA was cloned. Sequencing showed that its length was 1086bp, ORF was 444bp, encoding protein was 16kDa and PI was 4.49. The sequence

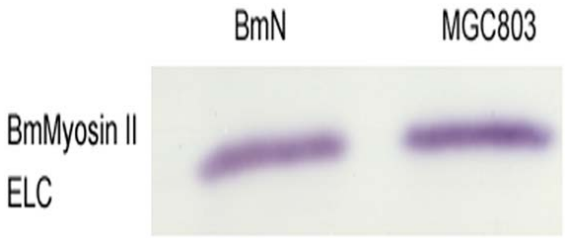

Fig. 4 The vectors of PIZ/V5-C-Flag-Myosin II ELC and PCDNA3.0-C-Flag-Myosin II ELC transfected BmN and MGC803. Analysis of Myosin II ELC by western blotting using Flag monoclonal antibodies.

found here was the same as the database sequence.

On the base of BmMyosin II ELC, we found 28 similar protein sequences of other invertebrates on the NCBI BLAST Network Service website (http://au.expasy.org/tools/BLAST/) that were used to analyze homology. According to the predicted Myosin II ELC structure domain we found that each species contained two identical EFh domains, which were Ca^{2+} binding sites (Fig. 1).

An un-rooted neighbor-joining (NJ) phylogenetic tree was generated using MEGA5 software based on the alignment of the corresponding Myosin II ELC complete protein sequences. The results showed that it was similar to the sequences of *Pararg aegeria*. For statistical reliability, we conducted bootstrap analysis with 1,000 replicates. The 28 members of the Myosin II ELC were subdivided into

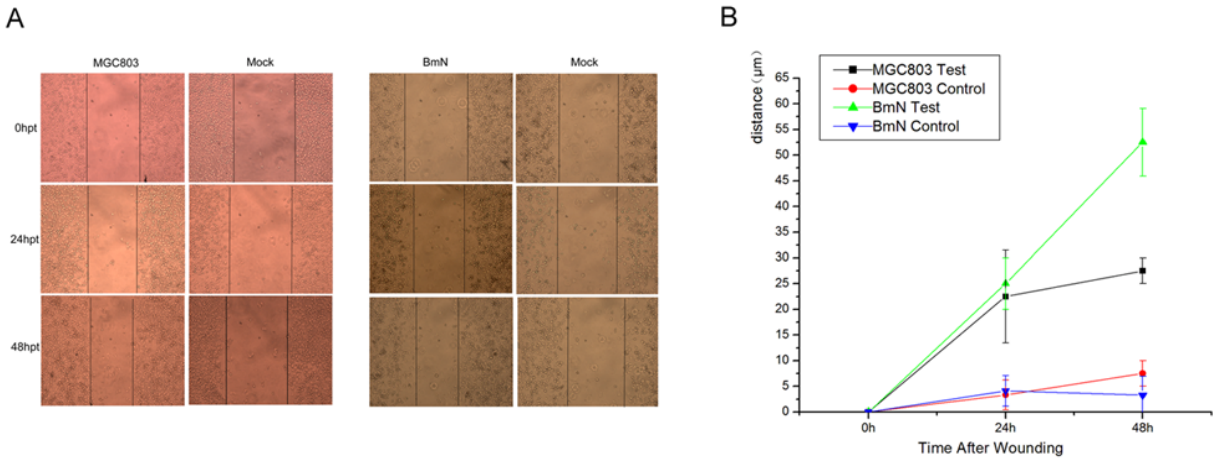

Fig. 5 (A) Monolayer wound healing of cells. Phase micrographs of MGC803 human stomach cancer cells at various times after monolayer wounding (left side of the figure). MGC803 showed that the migration of PCDNA3.0-C-Flag-Myosin II ELC transfected MGC803 at 0h, 24 h, and 48 h. Mock showed that the migration of PCDNA3.0 transfected BmN at 0 h, 24 h, and 48 h, there was no obvious migration at any time. Phase micrographs of BmN insect ovary cells at various times after monolayer wounding (right side of the figure). BmN showed that the migration of PIZ/V5-C-Flag-Myosin II ELC transfected BmN at 0 h, 24 h, and 48 h. Mock showed that the migration of PIZ/V5 transfected BmN at 0 h, 24 h, and 48 h. (B) Quantification of cell migration using the monolayer wound healing assay. The means values of three measurements are shown for each point in time and condition.

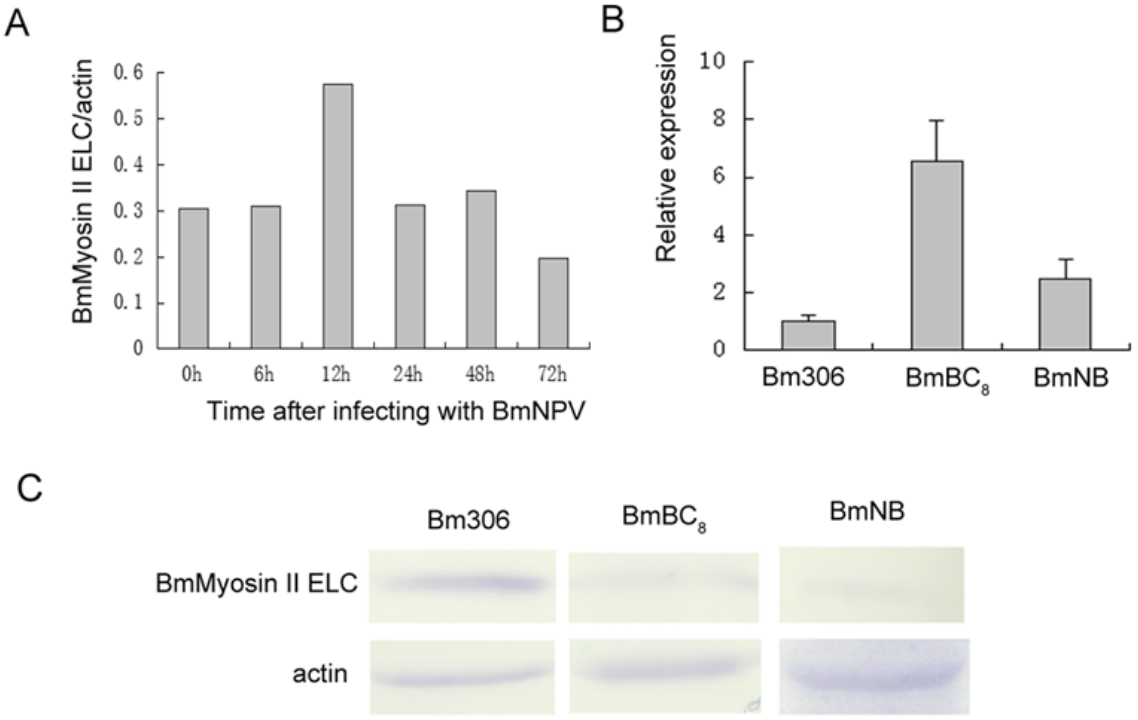

Fig. 6 (A) Analysis of gene of Myosin II ELC expression was performed by RT-PCR , optical density between myosin and actin on Bm306 at 0 h - 72 h after infecting BmNPV, indicating expression was higher at 12 h than other time. (B) Analysis of gene of Myosin II ELC expression was performed by qPCR at 12 h for three strains of *Bombyx mori* after infecting with BmNPV. Graphs depict the mean relative fold changes and are representative of three replicate plates. (C) Western blotting analysis of the expression levels of of Myosin II ELC in three strains of *Bombyx mori* after infecting with BmNPV at 12 h using its polyclonal antibody.

three subgroups, designated Groups I, II and III. BmMyosin II ELC was contained in Group II based on the clade with at least 50 % bootstrap support. According to the MEME software (Zhao *et al*., 2014 http://meme.nbcr.net/meme/cgi-bin/meme.cgi), *Bombyx mori* had five motifs, followed by motif 5 (yellow) corresponding to 3 - 31 amino acids, motif 2 (blue) corresponding to 32 - 61 amino acids, motif 4 (pink) corresponding to 65 - 85 amino acids, motif 1 (light green) corresponding to 90 - 121 amino acids, and motif 3 (red) corresponding to 126 - 146 amino acids (Fig. 2). All 28 sequences had motifs 1 and 3, and MEME prediction showed that the amino acid sequences of motif 1 (light green) and motif 3 (red) were relatively conserved (Fig. 3).

BmMyosin II essential light chain promoted BmN and MGC803 migration after the protein expressing successfully

We selected two different cell lines, MGC803 and BmN, and constructed two vectors (PCDNA3.0 and PIZ/V5) to encode BmMyosin II ELC protein fused to the Flag epitope tag at the COOH terminus (C-Flag-BmMyosin II ELC) (Clarke *et al*., 2007). Two days later after transfection, western blotting and Flag specific antibodies can be to detect whether the target protein transfection succeeded (Fig. 4). Monolayer wound healing of cells showed that the target protein can promote cell migration in two cell lines (Shimura *et al*., 2012 Fig. 5A). Through quantitative calculation, we drew the curve of cell migration and the effect of BmMyosin promotion of BmN became more apparent (Fig. 5B).

Transcription and expression Levels of the essential light chain of BmMyosin II where Bm306, BmBC8, and BmNB infected BmNPV

We selected three different silkworm strains, strain Bm306 which is susceptible to NPV and a near isogenic line $BmBC_8$ and BmNB is resistant to NPV (Kang *et al*., 2011). The 5^{th} instar larvae of $BmBC_8$, Bm306 and BmNB were infected with BmNPV virus and midguts were collected at 0 h, 6 h, 12 h, 24 h, 48 h and 72 h. The RT-PCR results showed that there exits a higher level about BmMyosin II ELC about Bm306 at 12 h (Fig. 6A). To further analyze the BmMyosin II ELC expression after Bm306, $BmBC_8$, BmNB infecting BmNPV at 12h (Zhang *et al*., 2012), we found the expressed levels in resistant strains BC_8 and NB treated with BmNPV were also significantly higher than the susceptible strain 306 by qPCR (Fig. 6B). To further confirm the result of qPCR, antibody against the BmMyosin II ELC protein was used to perform Western blot analysis of BmNPV-infected midgut of *B. mori*. The results showed that a specific band was observed clearly in controls and samples of strains Bm306, while the samples of strain BmNB and

$BmBC_8$ were relatively weak (Fig. 6C), which is opposite of the results from qPCR analysis.

BmMyosin II essential light chain interacted with ubiquitin of BmNPV

To further analyze the relationship between BmNPV and BmMyosin II ELC, we expressed fusion protein with GST-BmMyosin II ELC, interacting with BmN and BmN infected recombinant BmNPV (His-Ubiqutin) *in vitro* (Elena *et al*., 2005), we found BmMyosin II ELC was relevant with the ubiquitin of BmNPV (Fig. 7).

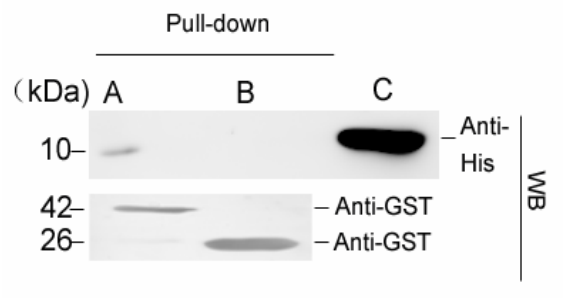

Fig. 7 Analysis of the interaction between BmMyosin II ELC and BmN infected with recombinant BmNPV (His-Ubiqutin) by pull down *in vitro*. Western blot analysis was used to check the expression of ubiquitin using His tag McAb. (A) BmN infected recombinant BmNPV (His-Ubiqutin) interacted with GST-BmMyosin II ELC, there existed ubiquitin band (~10 kDa), GST-BmMyosin II ELC was detected using GST tag McAb (~42 kDa). (B) BmN infected recombinant BmNPV (His-Ubiqutin) interacted with GST, no detectable ubiquitin band was observed. GST was detected using GSTtag McAb (~26 kDa). (C) BmN was infected with recombinant BmNPV (His-Ubiqutin) in two days, verifying the expression of BmNPV ubiquitin (~10 kDa).

Discussion

The study of myosin, as a kind of superfamily of proteins, has progressed in recent years. The myosin family contained a conserved structure domain of EFh, and a Ca^{2+} binding site was involved the domain. Experimental studies have found that when Ca^{2+} combined with Calmodulin complexes, the serine on the 19^{th} (Ser19) (Komatsu *et al*., 2007) of the Myosin Light Chain 20 will be phosphorylated, leading to activation of the Myosin head Mg^{2+}-ATPase, which hydrolysised ATP to produce energy to make the conformation change. The conformation will cause myosin to bind to actin (Spudich *et al*., 2001), which plays an important function in motion. BmMyosin II ELC had the same EFh domain based on bioinformatics prediction. Recently, the research has shown that cell migration is a process that is critical at many stages of embryonic development, and is essential for tissue repair and immune function. In this article, we proved that BmMyosin II ELC promoted cell migration in BmN and MGC803. In addition, we speculated the EFh domain may be involved in the important functions.

The experiment showed that the heavy chain of myosin was a substrate for MuRF 1 ubiquitin ligase activity, and that the protein of myosin heavy chain was ubiquitinated by MuRF 1 *in vitro* (Clarke *et al*., 2007). Previous research in our lab found BmNPV ubiquitin was relevant to BmMyosin II ELC by Co-IP (unpublished data), in this article we chose BmMyosin II ELC as bait to prove an interaction between the target protein and BmNPV ubiquitin via pull down technique.

Of the three different silkworm strains tested here, showed the expression of BmMyosin II ELC had the opposite result in transcription and translation level at 12 h. The expression of BmMyosin II ELC in resistant strain was higher than it was in the susceptible strain at the transcription level, while the result was opposite at the translation level. We speculated that silkworm infected BmNPV at 12 h belonged to late stage, in which DNA replicated and expressed viral structural protein (Faulkner *et al*., 1997). Insect cells have different abilities to detect the presence of a virus infection and initiate an apoptotic program (LaCount *et al*., 1997). In addition, baculoviruses are able to interfere with apoptosis by the expression of apoptotic inhibitors (Prikhod'ko *et al*., 1996), which induce encoding BmMyosin II ELC mRNA of silkworm which was resistant to NPV increase. Research indicated that after AcMNPV infects TN-368 cells, actin moves into nuclei and subsequently is polymerized from G-actin to F-actin, leading to reorganization of the cytoskeleton. When F-actin polymerized, some activators are called Wiskott-Aldrich syndrome proteins (WASPs) and a homolog of WASP (PP78/83) (AcMNPV orf9) is encoded by all lepidopteran NPV genomes combined with Arp2/3 complex (Goley *et al* 2006) to be involved in nucleating the formation of F-actin filaments. Studies of AcMNPV with mutations of PP78/83 suggested nuclear actin polymerization was required for the coordination of nucleocapsid development. In addition, since PP78/83 is a virion structural protein that is localized to the basal region of the nucleocapsids, it has the ability to cause actin nucleation and subsequent polymerization to release ATP to facilitate the movement of the nucleocapsids through the cytoplasm (O'Reilly *et al*., 1998). As BmNPV and AcMNPV are closely related to homologous orfs showing ~90 % nt and ~93 % aa sequence identity (Gomi *et al*.,1999), this shows that the mechanism was approximately the same. There were actin and ATP binding sites in myosin head. When the virus needs a large amount of G-actin, the host was likely to initiate prevention and control mechanisms to reduce the expression of BmMyosin II protein which was resistant to NPV, and uncombined with actin to defend itself against viral infection.

Acknowledgements

Grants from the National Basic Research Program of China “973” (2012CB114604), the National Natural Science Foundation of China (No. 31301919), and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions supported this work.

References

Andruchov O, Andruchova O, Wang Y, Galler S. Dependence of cross-bridge kinetics on myosin light chain isoforms in rabbit and rat skeletal muscle fibres. J. Physiol. 571: 231-242, 2006.

Clarke BA, Drujan D, Willis MS, Murphy LO, Corpina RA, Burova E*, et al.* The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. Cell Metab. 6: 376-385, 2007.

Dias AP, Braun EL, McMullen MD, Grotewold E. Recently duplicated maize R2R3 Myb genes provide evidence for distinct mechanisms of evolutionary divergence after duplication. Plant Physiol. 131: 610-620, 2003.

Eccles SA, Box C, Court W. Cell migration/invasion assays and their application in cancer drug discovery. Biotechnology Annu. Rev. 11: 391-421, 2005.

Elena K, Dmitri K, Irina K, Spencer MJ. Trim32 is a Ubiquitin Ligase Mutated in Limb Girdle Muscular Dystrophy Type 2H that Binds to Skeletal Muscle Myosin and Ubiquitinates Actin. J. Microbiol. Biotechnol. 354: 413-424, 2005.

Faulkner P, Kuzio J, Williams GV, Wilson JA. Analysis of p74, a PDV envelope protein of *Autographa californica* nucleopolyhedrovirus required for occlusion body infectivity in vivo. J. Gen. Virol. 78(Pt 12): 3091-3100, 1997.

Gareus R, Di Nardo A, Rybin V, Witke W. Mouse profilin 2 regulates endocytosis and competes with SH3 ligand binding to dynamin 1. J. Biol. Chem. 281: 2803-2811, 2006.

Goley ED, Ohkawa T, Mancuso J, Woodruff JB, D'Alessio JA, Cande WZ, *et al.* Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. Science 314: 464-467, 2006.

Gomi S, Majima K, Maeda S. Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. J. Gen. Virol. 80 (Pt 5): 1323-1337, 1999.

Holmes KC, Geeves MA. The structural basis of muscle contraction. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355: 419-431, 2000.

Kang LQ, Shi HF, Liu XY, Zhang CY, Yao Q, Wang Y, *et al*. Arginine kinase is highly expressed in a resistant strain of silkworm (*Bombyx mori, Lepidoptera*). Implication of its role in resistance to *Bombyx mori* nucleopolyhedrovirus. Comp. Biochem. Physiol. 158B: 230-234, 2011.

Kolodney MS, Thimgan MS, Honda HM, Tsai G, Yee HF. Ca^{2+}-independent Myosin II phosphorylation and contraction in chicken embryo fibroblasts. J. Physiol. 515: 87-92, 1999.

Komatsu S, Ikebe M. The phosphorylation of Myosin II at the Ser1 and Ser2 is critical for normal platelet-derived growth factor-induced reorganization of myosin filaments. Mol. Biol. Cell 18: 5081-5090, 2007.

Kwon TJ, Oh SK, Park HJ, Sato O, Venselaar H, Choi SY, *et al*. The effect of novel mutations on the structure and enzymatic activity of unconventional myosins associated with autosomal dominant non-syndromic hearing loss. Open Biol. 4: 140107, 2014.

LaCount DJ, Friesen PD. Role of early and late replication events in induction of apoptosis by baculoviruses. J. Virol. 71: 1530-1537, 1997.

O'Reilly DR, Hails RS, Kelly TJ. The impact of host developmental status on baculovirus replication. J. Invertebr. Pathol. 72: 269-275, 1998.

Prikhod'ko EA, Miller LK. Induction of apoptosis by baculovirus transactivator IE1. J. Virol. 70: 7116-7124, 1996.

Santos M, Moura RS, Gonzaga S, Nogueira-Silva C, Ohlmeier S, Correia-Pinto J. Embryonic essential myosin light chain regulates fetal lung development in rats. Am. J. Respir. Cell Mol. Biol. 37: 330-338, 2007.

Shimura T, Yoshida M, Fukuda S, Ebi M, Hirata Y, Mizoshita T, *et al.* Nuclear translocation of the cytoplasmic domain of HB-EGF induces gastric cancer invasion. BMC Cancer 12: 205, 2012.

Spudich JA. The myosin swinging cross-bridge model. Nature reviews Mol. Cell Biol. 2: 387-392, 2001.

Straight AF, Cheung A, Limouze J, Chen I, Westwood NJ, Sellers JR, *et al.* Dissecting temporal and spatial control of cytokinesis with a Myosin II Inhibitor. Science 299: 1743-1747, 2003.

Sweeney HL, Houdusse A. Structural and functional insights into the myosin motor mechanism. Annu. Rev. Biophys. 39: 539-557, 2010.

Valster A, Tran NL, Nakada M, Berens ME, Chan AY, Symons M. Cell migration and invasion assays. Methods 37: 208-215, 2005.

Wang YJ, Yao Q, Chen KP, Wang Y, Lu J, Han X. Characterization of the genome structure of *Bombyx mori* densovirus (China isolate). Virus Genes 35: 103-108, 2007.

Zhang W, Zhang C, Lv Z, Fang D, Wang D, Nie Z, *et al.* Molecular Characterization, Tissue Distribution, Subcellular Localization and Actin-Sequestering Function of a Thymosin Protein from Silkworm. PloS One e31040, 2012.

Zhao PP, Li Q, Li J, Wang L, Ren ZH. Genome-wide identification and characterization of R2R3MYB family in *Solanum lycopersicum*. Mol. Genet. Genomics 289: 1183-1207, 2014.