

The tau-like protein in silkworm (*Bombyx mori*) induces microtubule bundle formation

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1. ABSTRACT

Tau proteins are major microtubule-associated proteins (MAPs), which promote polymerization of tubulin and determine spacings between microtubules in axons of both the central and peripheral nervous systems (CNS and PNS). Here, we cloned and identified a tau-like protein BmTau from silkworm, *Bombyx mori* (GenBank accession number FJ904935). The coding sequence of BmTau is 723 bases long and encodes an approximate 30kDa protein. In the C-terminus of BmTau are contained four predicted microtubule-binding domains, which share strong sequence homology to its ortholog in *Drosophila melanogaster*. Relative real-time PCR analysis showed ubiquitous expression of BmTau in both neurons and non-neural cells, with its mRNA abundantly expressing in brain but significantly less detected in trachea, fat body, and silkgland. Furthermore, immunocytochemical studies in BmN cells transfected with EGFP-BmTau indicated that BmTau functioned as microtubule bundling protein as its orthologues.

2. INTRODUCTION

Tau proteins, as neuronal heat-stable microtubule-associated proteins (MAPs), promote assembly of tubulin, bind and stabilize microtubules (MTs). Tau proteins are short rod-like molecules and are components of short cross-bridge structures between MTs (1-6). Together with higher molecular mass proteins MAP1B and MAP2, tau proteins, with lower molecular mass (55,000-62,000), are expressed in both the central and peripheral nervous systems (CNS and PNS, respectively) (7). Tau proteins localize predominantly in axons and show synergistic functions in MT organization and axonal elongation with MAP1B (8). Moreover, considerable studies have revealed that tau proteins play a crucial role in axonal morphogenesis. They are thought to determine the MT spacing in axons, whereas MAP2 in dendrites, respectively (2). Disruption of Tau gene in mice leads to the decrease of MT number in small-caliber axons, weak muscle and defect memory (3, 9).

Tau proteins were first identified in mammalian species, including mouse (10), human (11, 12), and rat (13). The tau encoding gene *MAPT* encodes six isoforms (1). According to the length of N-terminal residues after alternative splicing, which form the projection domain of tau, three main types of tau isoforms, L (long), M (medium), and S (short), are identified. Each main type contains two isoforms with one having three repeat microtubule-binding domains (MTBDs) (type 3) and the other having four (type 4) (1). Therefore, the six tau isoforms were named L4, M4, S4, L3, M3, and S3, respectively. With alternative mRNA splicing in a stage- and cell-type-specific manner, the six tau isoforms are all expressed in the adult brain, but only S3 isoform in the juvenile (1, 14-16).

The MTBDs of tau proteins are all near the C-terminus of the amino acid sequences and positively charged, allowing them to interact with the negatively-charged MTs. Moreover, tau protein of type 4 has stronger ability to polymerize MTs than that of type 3 (17, 18). Also, several phosphorylation sites in the C-terminal region are reported, being regulated by a host of kinases, such as PKN and GSK3 beta (19-22). Therefore, the differential expression pattern and specific phosphorylation of tau proteins play significant roles in the neuronal development (23, 24).

In many neurodegenerative diseases, such as Alzheimer's disease and frontotemporal dementia, tau abnormalities are present. Pathological hyperphosphorylation of tau is believed to cause detachment of tau from MTs, leading to MT breakdown and disruption of axonal transport (25-31). These tau proteins, abnormally hyperphosphorylated on more sites than the normal tau in brain, aggregate into paired-helical filaments (PHFs) (25, 26, 32). Furthermore, tau protein mutations are also confirmed as neuron killers, which are thought to disrupt normal splicing balance, promote tau aggregation, or enhance tau hyperphosphorylation (33-35). Suppressing expression of the mutant tau gene in transgenic tau mice improves memory function and protects against neuron loss (36). Also, reducing endogenous tau levels can impede the behavioral deficits and excitotoxicity caused by APP (37).

Recently, the cDNA of tau homolog in two model organisms, *Drosophila melanogaster* and *Danio rerio* have been isolated (38, 39). *Drosophila* tau (*DmTau*) is shown to colocalize with MT in larval and adult tissues (38); while in zebrafish, there are two tau paralogous genes, *mapta* and *maptb*, which can be spliced into 4R-6R and 3R isoforms, respectively (39). Although both zebrafish and *Drosophila* have been proposed as transgenic or genetic model organisms for tauopathy investigation (38, 40), little progress has been made to elucidate the molecular mechanisms underlying the tau-related neurodegenerative diseases.

Silkworm, *Bombyx mori*, an evolutionary partner of *Drosophila melanogaster*, is considered as a highly successful host system to produce silk on a large scale.

Because of its suitable large-scale culture, convenience for molecular cloning, obvious phenotypes and the abundance of mutants, it is considered one of the best-characterized models for biochemical analysis and genetic research (41). Moreover, the RNAi technology by injection of dsRNA, BmNPV baculoviruses transfection system, transgenic silkworm technique (42) and micromanipulation tool in silkworm cells (43) make silkworm an excellent model to study the physiological functions of target genes. Here, to better understand the tau protein family in the evolutionary context, we cloned silkworm tau-like protein and named *Bombyx mori* tau-like protein (*BmTau*) (GenBank accession number NM_001161718). We investigated the evolutionary status and examined its relative mRNA expression levels in different tissues and in silkglands of different stages. Besides, we overexpressed *BmTau* in silkworm BmN cells, and found that *BmTau* induced the MT bundle formation in BmN cells. These results suggest that *BmTau* shares many similar characteristics with its mammalian counterparts and possess the MT-bundling activity as its tau homologs.

3. MATERIALS AND METHODS

3.1. *Bombyx mori* strain and cell culture

The *B. mori* strain (p50) was offered by Chinese Academy of Agricultural Sciences. The embryos were placed in a humidified chamber at room temperature. After hatching, the larvae were reared on the mulberry leaf or artificial diets.

3.2. Bioinformatic analysis

We first predicted the nucleotide/amino acid sequence of tau-like protein in silkworm, comparing sequences between *D. melanogaster* and *B. mori*, or utilizing Basic Local Alignment Search Tool (BLAST) in silkworm cDNA database (<http://papilio.ab.a.u-tokyo.ac.jp/silkbase/>) (44) and genome database (<http://silkworm.genomics.org.cn/>) (45, 46). The secondary structure of *BmTau* was predicted according to previous report (18). The whole amino acid sequences of 15 tau family members, which were obtained from NCBI (<http://www.ncbi.nlm.nih.gov/>), and the amino acid sequence of the newly cloned silkworm tau-like protein were aligned using MAFFT software (<http://align.bmr.kyushu-u.ac.jp/mafft/software/>) (47). The neighboring-joining tree, arbitrarily rooted using *Caenorhabditis elegans* Tau (48), was inferred and decorated by MEGA 3.1 (49).

3.3. RNA isolation and cDNA cloning

Silkworm larvae were anesthetized and dissected under stereomicroscope (ZEISS). The dissected tissues were homogenized in TRIzol reagent (Invitrogen) and the total RNA was isolated as described on Molecular Cloning, 3rd Edition. We used 0.25 volume of isopropanol and 0.25 volume of RNA precipitation solution (0.8M disodium citrate, 1.2M NaCl) instead of 0.5 volume of isopropanol to precipitate RNA from aqueous phase (50). This modification greatly eliminated the contamination by proteoglycans and polysaccharides, promoted solubilization of precipitated RNA, and facilitated reverse transcription-PCR reaction. Subsequently, total RNA and Superscript III

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Reverse Transcriptase (Invitrogen) were used to synthesize the first-strand cDNA according to the manufacturer's instructions (Invitrogen). The prepared cDNAs of different tissues were used for cDNA cloning and real-time PCR.

We synthesized a pair of oligonucleotides (*EcoRI* - F: 5'-GGG GAA TTC ATG GCC GAA GTC AAC GAC GGA G-3', and R-*SalI* 5'-TTT GTC GAC ACT CTG CTC TTC GTT CAA GTT TTC-3') for PCR according to the predicted coding sequence of tau-like protein in silkworm. *BmTau* was amplified from the cDNA of silkworm brain or PSG (30 cycles of 94 degree C. for 30s, 60 degree C. for 30s and 72 degree C. for 1min using Ex Taq DNA polymerase (Takara). Then, the PCR products were cloned into *EcoRI/SalI* site on pEGFP-N3 vector (CLONTECH Laboratories), pEGFP-C2 vector (CLONTECH Laboratories), pGEX-6P-1 (GE Healthcare) and the pFastBac-1-based pFastBac-hr5/IE1 promoter vector. Vector pFastBac-hr5/IE1 promoter was constructed with both IE-1 promoter and hr5 enhancer amplified from the *B. mori* nucleopolyhedrovirus (BmNPV) bacmid (51, 52) and sequentially inserted into pFastBac-1 vector (Invitrogen) between *SnaBI* and *BamHI*. The rat Tau (*RnTau*) used in our experiment was amplified as previously reported (13) and inserted into the vectors above.

3.4. Real-time quantitative PCR

Reverse transcription products of eight different tissues from the fifth-instar day-3 larvae and silk glands from day-0 to day-7 of the fifth-instar larvae were amplified by ABI 7300 Detection System (Applied Biosystems) according to the manufacturer's instructions. SYBR Green PCR Master Mix (Applied Biosystems) was used. Pairs of specific primers of *BmTau* (F-RT-*BmTau* 5'-ATG GAC CGT CGC CAA ATT TA-3'; R-RT-*BmTau* 5'-TAT GTG GCG TTA TCC AGA GAT CCT A-3') and silkworm ribosomal protein L3 (*BmRpL3*) (F-RT-*BmRpL3* 5'-GAG GTC CCG TCG TCA TCGT-3'; R-RT-*BmRpL3* 5'-CAG GTT TGC TAG GGT CGT CTT T-3') were designed and selected according to the dissociation curve analysis with no primer dimer or unspecific amplification detected. Every cDNA samples were amplified in triplicate. The relative expression levels of *BmTau* were normalized to *BmRpL3*, a reference control gene. The relative expression level was calculated by $2^{-(\Delta\Delta C_T)}$ method (53).

3.5. Cell culture, transfection and immunofluorescence

The BmN cell line was cultured at 27 degree C. in TC-100 insect medium (Sigma Aldrich) which was supplemented with 10% fetal bovine serum (FBS) (GIBCO BRL). HeLa cells were maintained at 37 degree C. with 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO BRL) which was supplemented with 10% FBS (GIBCO BRL).

HeLa cells were transiently transfected by calcium phosphate precipitation as previously reported (54), while BmN cells were transfected by Cellfectin II Reagent (Invitrogen) as the manufacturer's instruction. 24-48 hours post-transfection, cells were fixed with fresh

prepared 4% paraformaldehyde in PBS for 15 minutes at room temperature and then subjected to immunofluorescence experiment. The cells were permeabilized with 0.2% Triton X-100 in PBS for 15 minutes and blocked by 3% BSA in PBS for 30 minutes. Primary antibody (anti-alpha-tubulin Mouse monoclonal antibody, DM1A, Sigma) and secondary antibody (Alexa Fluor 568- conjugated goat anti-mouse IgG, Molecular Probes) were used to incubate with the cells. The prepared samples were observed with IX71 inverted fluorescence microscope (OLYMPUS) or the TCS SP2 confocal microscope (Leica) equipped with a 100x/1.4 numerical aperture oil immersion objective lens.

4. RESULTS AND DISCUSSION

4.1. cDNA cloning of *BmTau*

Drosophila and silkworm are evolutionary partners and the nucleotide/amino acid sequences of many proteins share high sequence similarities. Therefore, to identify tau homologues in *B. mori*, we utilized Basic Local Alignment Search Tool (BLAST) and searched in the silkworm genome database (46) and EST database (44) to look for those conserved nucleotide sequences of *DmTau* protein (38). Then we located some partial conserved nucleotide sequences from silkworm and further extended these sequences forward and backward by BLAST or sequence alignment in silkworm cDNA database. Consequently, we succeeded in predicting the whole nucleotide sequence of tau-like protein in silkworm (1031 base pairs), including the coding region (723 base pairs), the 5'-flanking untranslated region (UTR, 137 base pairs) and the 3'-UTR (171 base pairs). To verify this prediction, we amplified this predicted gene by reverse transcription PCR (RT-PCR) from cDNA of both silkworm brain and posterior silk gland (PSG). A single band of the predicted coding sequence (723 base pairs) was observed by electrophoresis. Both the nucleotide sequences of PCR products from silkworm brain and PSG were identical with each other. The cloned sequence has been submitted to GenBank (GenBank accession number NM_001161718) and named *B. mori* tau-like protein (*BmTau*). So far, we identified only one mRNA transcript of *BmTau*. However, we noticed that in mammals (*Homo sapiens*, *Mus musculus* and *Rattus Norvegicus*) and nematode (*Caenorhabditis elegans*), more than one isoforms have been identified (10-13, 48).

4.2. Bioinformatic characterization of *BmTau*

To initially characterize *BmTau*, we bioinformatically analyzed its amino acid sequence. We collected the amino acid sequences of tau proteins from seven other model organisms, including six tau isoforms from *Homo sapiens* (GenBank accession number NM_001123066, NM_001123067, NM_005910, NM_016834, NM_016835, and NM_016841), the longest isoforms from the CNS of *Mus musculus* and *Rattus Norvegicus* (NM_001038609 and NM_017212), four proteins with tau-like repeats (ptl-1) from *Caenorhabditis elegans* (NM_001027405, NM_001027406, NM_001027407, and NM_001027408), and only one tau isoform so far identified in *Danio rerio* (XM_001920640),

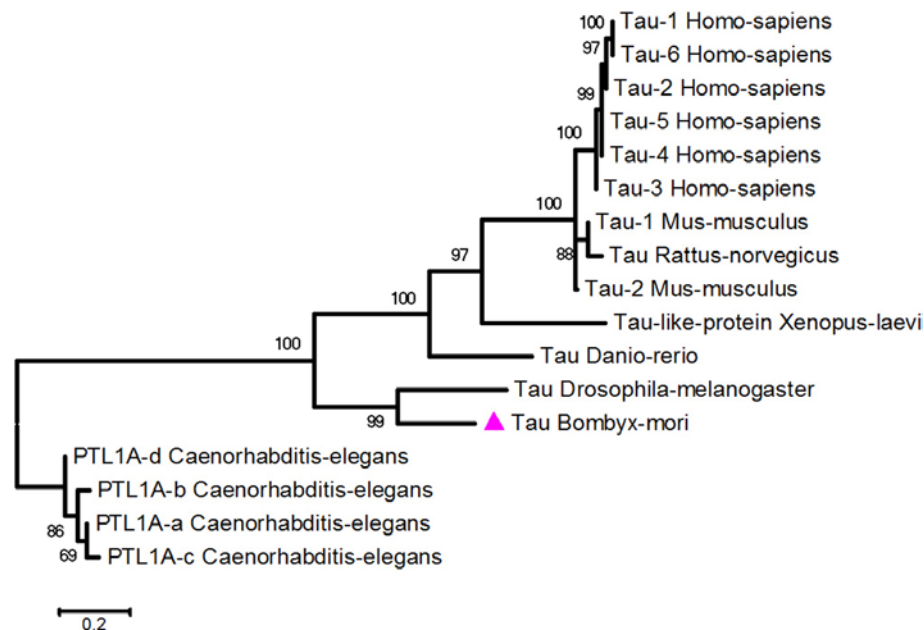


Figure 1. Molecular phylogenetic tree of tau proteins. The tree representing the evolutionary relationships among eight organisms, seventeen tau family proteins, includes six tau isoforms from human (*Homo sapiens* tau-1 NM_016835; tau-2 NM_005910; tau-3 NM_016834; tau-4 NM_016841; tau-5 NM_001123067; tau-6 NM_001123066), four tau isoforms from nematode (*Caenorhabditis elegans* PTL1A-a/b/c/d NM_001027405-NM_001027408), isoform from mouse (*Mus musculus* PNS-tau NM_001038609), one from rat (*Rattus norvegicus* tau-H NM_017212), fruit fly (*Drosophila melanogaster* NM_143318), zebrafish (*Danio rerio* XM_001920640) and African clawed frog (*Xenopus laevis* NM_001088761) respectively, and our cloned *BmTau* from silkworm (*Bombyx mori* NM_001161718), which is marked by red triangle. Close evolutionary relationship is observed between *BmTau* and *DmTau* protein. The tree is arbitrarily rooted using *Caenorhabditis elegans* Tau.

Xenopus laevis (NM_001088761), and *Drosophila melanogaster* (NM_143318).

Amino acid sequence alignment showed that *BmTau* shared more sequence similarities with *DmTau* in *Drosophila* (38), nearly 34%. Meanwhile, the percentage of protein sequence identity between *BmTau* and the six human tau isoforms (1) were much lower, only 9%, 15%, 17%, 15%, 16% and 8%, respectively. Moreover, we constructed the phylogenetic tree as previous report (55). As shown in the phylogenetic tree (Figure 1), silkworm *BmTau* and *Drosophila DmTau* had a close evolutionary relationship, which was consistent with the strong sequence similarity between the two genes.

Sequence alignment and secondary structure analysis (18) (Figure 2) showed that the *BmTau* sequence was relatively conserved in its C-terminal domain, where four MTBDs were located. These MTBDs were marked by four red rectangles with each MTBD containing eighteen amino acids. This molecular architecture of *BmTau* was similar to the secondary structure of tau proteins in other organisms. Previous studies identified that tau isoforms from different organisms possessed distinct number of MTBD repeats and promoted MT bundling[0]. In human, among six isoforms, three of them have three-repeat MTBDs, while the other half have four (1). All of them are active in promoting MT assembly, with the rates of assembly being 2.5-3.0 times faster for four-repeat tau isoforms than isoforms containing three repeats (17). In *C.*

elegans, four- or five-repeat MTBDs are consisted in tau homologs, with the five-repeat isoform promoting MT assembly more effectively than the four-repeat isoform (48). In fruit fly and African clawed frog, only isoforms containing four MTBDs have been discovered, while the tau protein isoform in zebrafish so far identified contained only three MTBDs (38, 39). Therefore, the numbers of MTBD repeats could be various in different organisms and their biological activities to promote MT assembly are dependent on the number of the MTBD repeats: the more MTBDs contained the better ability to promote MT assembly. Here, *BmTau* contains four predicted MTBDs, indicating its MT-binding ability and potential activity to promote MT bundling.

Interestingly, we noticed that several amino acids in the MTBDs of *BmTau*, the same as those in *DmTau*, were radically different from those in other organisms (Figure 2). For example, in the first MTBD, the Leucine site in *Drosophila* and silkworm (Figure 2, red arrow) was changed to Tyrosine in mammals and other organisms (SKIGSL to SKIGST). In the fourth MTBD (Figure 2, green arrow), changes were also observed (GSTAN to GSLDN).

4.3. Tissue distribution and developmental expression patterns

To determine the tissue expression pattern of *BmTau*, we performed real-time quantitative PCR analysis in eight different tissues from the fifth-instar day-3 larvae,

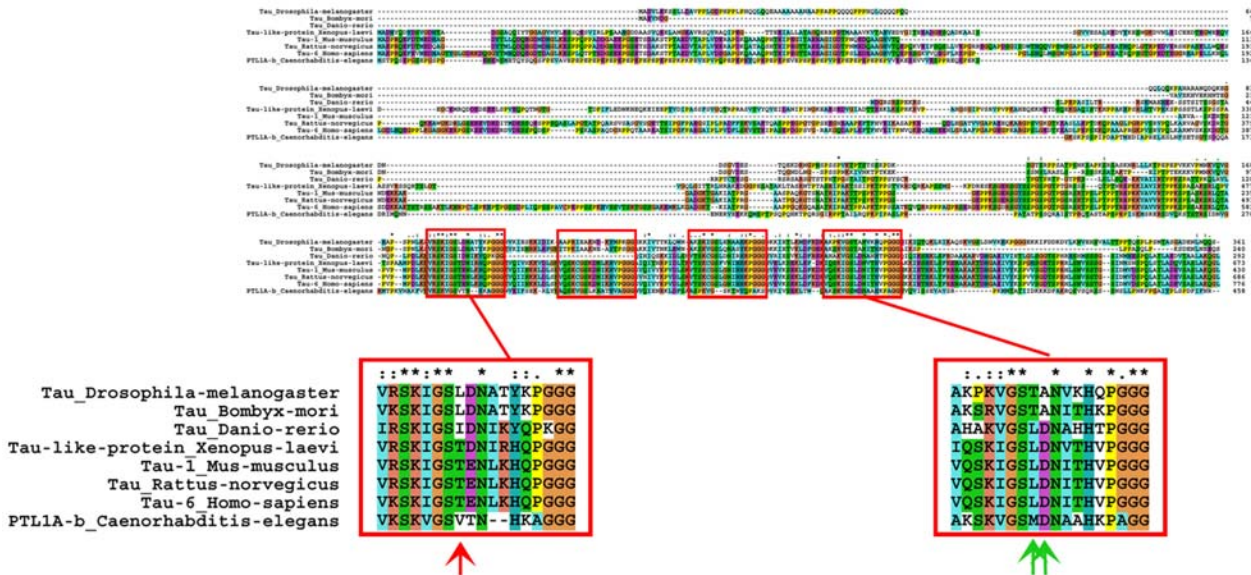


Figure 2. Sequence alignment and secondary structure analysis. Tau orthologues from eight different organisms are aligned and four conserved motifs (MTBDs) are marked by red line rectangle, consistent with previous report by Kanai, Y (18). The first and fourth MTBDs are magnified. Red and green arrows indicate the amino acids, which are different in *Drosophila* and silkworm.

including brain, Malpighian tubule, trachea, fat body, anterior silk gland (ASG), middle silk gland (MSG), posterior silk gland (PSG) and midgut (Figure 3A). The relative quantification was normalized against a reference gene called *BmRpL3*, which showed the most ubiquitous expression in silkworm (56). *BmTau* was extraordinarily abundant in brain, nearly 800-fold higher than in silk gland, which is consistent with abundance of tau proteins in CNS and PNS (2, 7, 57). Its most similar ortholog, *DmTau*, also shows obvious expression within the developing brain and ventral nerve cord in late-stage *Drosophila* embryos (38). Meanwhile, the relative mRNA expression levels of *BmTau* in Malpighian tubule and midgut were also abundant, nearly 400-fold higher compared to the amount in silk gland, which showed the lowest expression level of *BmTau* in these eight different tissues.

According to previous research, tau is primarily expressed in the CNS and PNS (2, 38, 57). During *Drosophila* embryogenesis, *DmTau* was confirmed to express in clusters of PNS cells, such as lateral and dorsal chordotonal organs. However, tau proteins have also been demonstrated to be localized in the nuclei of both neurons and some non-neuronal cells (58-62), and to bind the minor groove of the double-stranded DNA (26, 63-66). In nucleus, tau protein may participate in nucleolar organization and heterochromatinization, or prevent DNA from damage by peroxidation (64, 65). Therefore, we did not exclude the possibility that *BmTau* existed in the nuclei of silkworm non-neuronal tissues, such as silk gland.

Silk gland plays significant role in silk production and is critical for metamorphosis and development of silkworm (67). PSG cells, loaded with a complex and extensive endomembrane system, are responsible for the tremendous and indispensable intracellular transport (68).

Although the mRNA expression level of *BmTau* in MSG and PSG is low, we would like to further investigate whether the expression level changes during the maturation of MSG and PSG. In the fifth-instar MSG and PSG, the mRNA levels of *BmTau* displayed similar trend of accumulation during the eight days of the fifth instar before pupal metamorphosis (Figure 3B and 3C). As reported previously, during the larval fifth instar, a great deal of silk fibroin is synthesized and secreted into lumen of PSG, and then transported to MSG, where sericin is produced (69). Once entering the fifth-instar period, these MSG and PSG cells do not divide but instead enlarge themselves dramatically and secrete substantial protein components for silk production (67, 70). Therefore, we hypothesized that the increase of *BmTau* mRNA in the fifth-instar silk glands maybe related to cell enlargement or substantial secretion.

4.4. C-terminal MTBDs are essential for subcellular localization

We further examined the subcellular localization of *BmTau*. First, we constructed both N- and C-terminal EGFP-tagged fusion protein, EGFP-*BmTau* and *BmTau*-EGFP. To observe their subcellular localization, we overexpressed both EGFP-*BmTau* and *BmTau*-EGFP under human cytomegalovirus immediate early promoter (CMV, mammalian cells expression promoter) in HeLa cells, an immortal cell line from human cervical cancer cells. Moreover, we transfected BmN cells, a cell line established from an ovarian tissue of the silkworm, overexpressed the fusion proteins under silkworm expression promoter hr5-enhancer/ IE-1 (hr5/IE1) (51, 52). Both in HeLa and BmN cells, the CMV-driven and hr5/IE1-driven expression revealed that the C-terminal EGFP-tagged fusion protein *BmTau*-EGFP was dispersed throughout the whole cells, including the nucleus (Figure 4A and 4C), while the N-terminal EGFP-tagged fusion protein EGFP-*BmTau* was

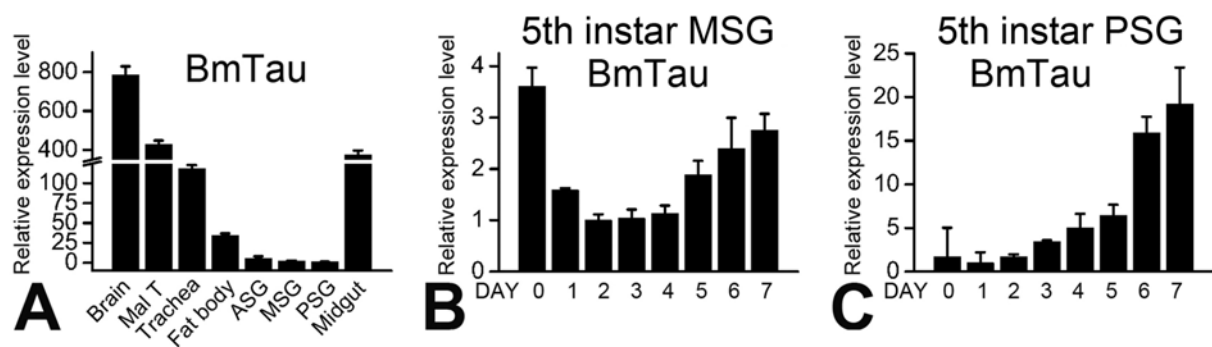


Figure 3. Relative mRNA quantification. (A) Relative expression levels of *BmTau* mRNA in eight different tissues of silkworm: brain, Malpighian tubule (Mal T), trachea, fat body, anterior silk gland (ASG), middle silk gland (MSG), posterior silk gland (PSG) and midgut. (B) Relative mRNA expression levels of *BmTau* in different developmental stages of MSG. (C) Relative mRNA expression levels of *BmTau* in PSG development. Day 0-7 indicates day 0-7 of the fifth-instar larvae.

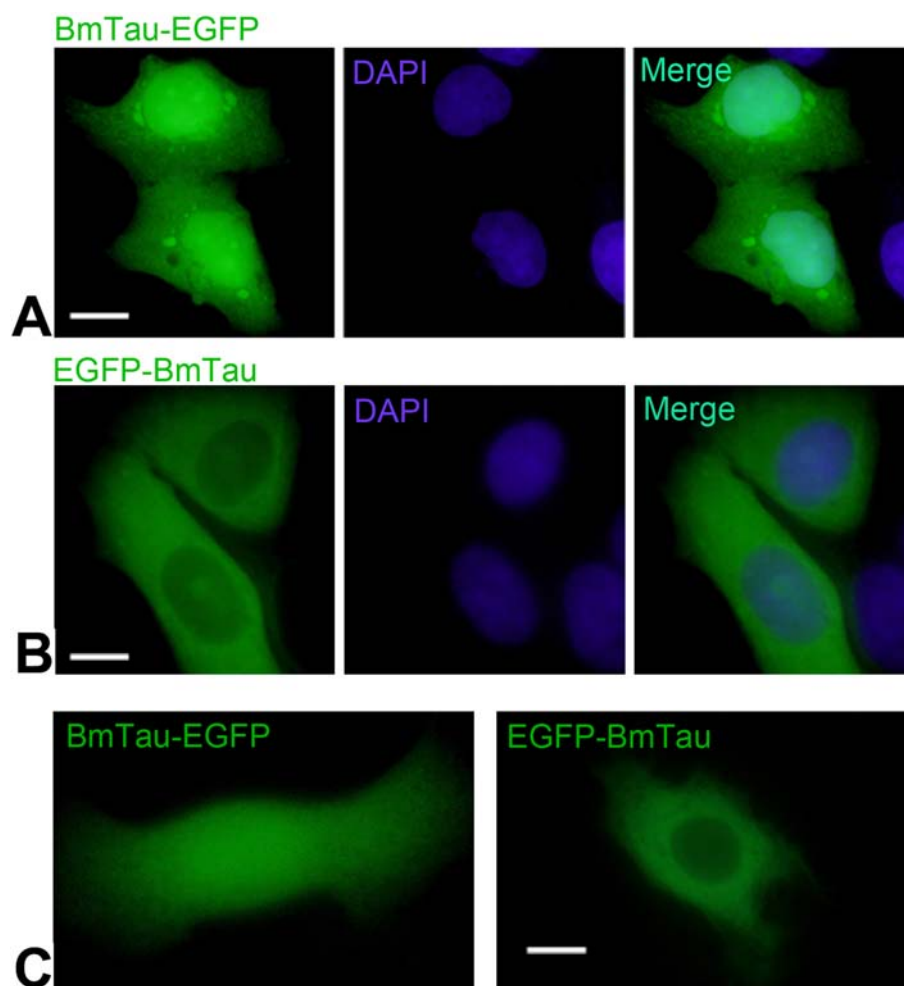


Figure 4. Overexpression of *BmTau* and EGFP fusion protein in HeLa cells and BmN cells. (A) The constructed vector pEGFP-N3-*BmTau* is transfected into HeLa cells and the C-terminal tagged *BmTau*-EGFP fusion proteins are expressed under human cytomegalovirus immediate early promoter (CMVp) and dispersed throughout the whole cytoplasm including the nucleus. (B) The constructed vector pEGFP-C2-*BmTau* is transfected into HeLa cells and the N-terminal tagged EGFP-*BmTau* fusion proteins are also expressed and distributed in cytoplasm but not in nucleus. (C) The BmN cells are transfected by constructed vector pFastBac1-hr5-IE1-*BmTau*-EGFP. Both N- and C-terminal EGFP tagged *BmTau* fusion proteins are expressed in BmN cells under hr5 enhancer and IE1 promoter (hr5-IE1). DAPI marks the nucleus. Bar presents 10 μ m.

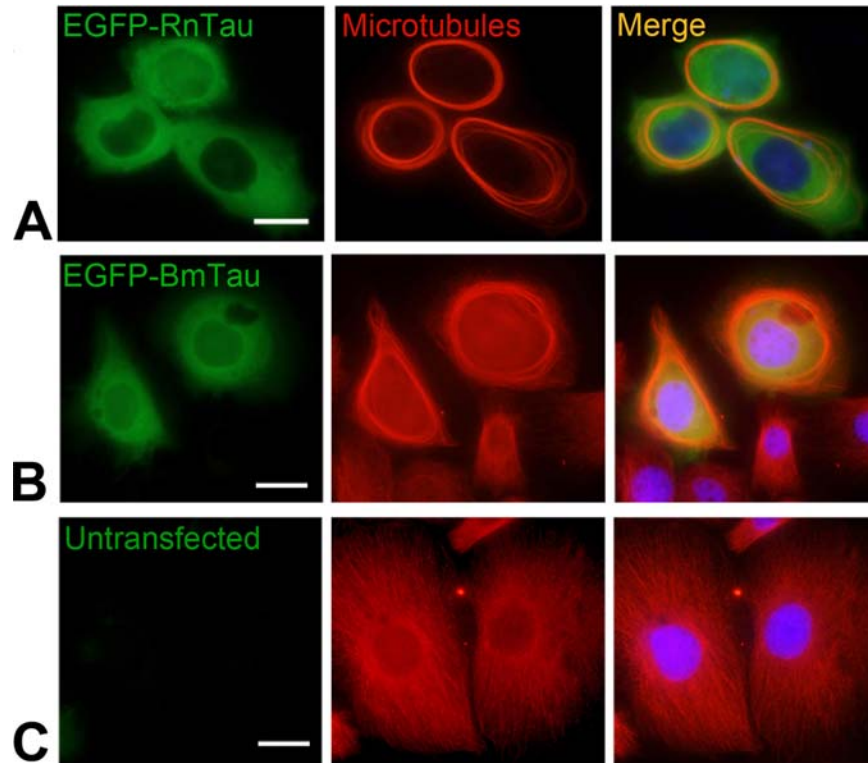


Figure 5. N-terminal EGFP-tagged *BmTau* induce MT bundle formation in BmN cells, but not in HeLa cells. (A) In HeLa cells, the overexpressed EGFP-*RnTau* (rat Tau) induces MT bundling. (B) N-terminal EGFP-tagged EGFP-*BmTau* is overexpressed under hr5/IE1 promoter in BmN cells. The MTs are bundled in transfected BmN cells overexpressing EGFP-*BmTau* compared to the untransfected cells (C). MTs are immunostained by DM1A mouse monoclonal antibody (red), and DNA by DAPI (blue). Bar presents 10 μ m.

localized only in cytoplasm but not in nucleus (Figure 4B and 4C).

According to the previous report, the tau proteins could be both nuclear and cytoplasmic (1, 2, 38, 48, 58-62). The molecular structure of exogenously expressed *BmTau* could be disrupted by the N- or C-tagged EGFP. Therefore, further experiments were needed to determine the subcellular localization of *BmTau* *in vivo*.

4.5. Microtubule-bundling ability of *BmTau*

Tau proteins promote MT assembly and further induce MT bundle formation (1, 2). The N-terminal EGFP-tagged rat tau protein EGFP-*RnTau* (*Rattus norvegicus* Tau) induced MT bundling (Figure 5A) as reported previously by immunofluorescence staining (2, 13). To test whether *BmTau* still possess the MT-bundling activity as its family members, we overexpressed the N-terminal-tagged EGFP-*BmTau* in BmN cells under the hr5/IE1 promoter, and immunostained alpha-tubulin protein with the monoclonal antibody to observe whether the MTs were bundled. Microscopy observation revealed that MT bundles formed in EGFP-*BmTau*-transfected BmN cells but not in untransfected cells, and that EGFP-*BmTau* and MTs were partially colocalized around the periphery of the nucleus (Figure 5B and 5C). From these results, we could conclude that *BmTau* is associated with MTs, and can bundle MTs.

Most members of tau protein family have been demonstrated to possess the physiological activity to promote polymerization of tubulin and MT bundling (1, 2, 13, 17, 38, 39, 48). Here, we found that *BmTau* induced MT bundle formation in BmN cells, which is consistent with the secondary structure prediction and phylogenetic tree analysis. However, much more details about *BmTau* need further characterization, such as its potential phosphorylation sites regulated by a host of kinases (19-22), the physiological function of *BmTau* during silkworm development in different tissues, and whether there are more tau isoforms in silkworm. Moreover, in mammals, several MAPs, such as tau, MAP1B, and MAP2, cooperate to regulate MT dynamics in different compartments (8, 71, 72), thus to search for other potential MAPs related to MT dynamics in silkworm seems interesting.

5. CONCLUSION

Based on the results above, we cloned the tau-like protein *BmTau* from silkworm and added a novel member into the tau protein family. We demonstrated that *BmTau* shared sequential similarity with its orthologs of other organisms and could induce MT bundling in *BmTau*-transfected BmN cells.

6. ACKNOWLEDGEMENT

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Abbreviations: ASG: anterior silk gland; BLAST: basic local alignment search tool; *BmTau*: *Bombyx mori* tau-like protein; CNS: central nervous system; EGFP: enhanced green fluorescent protein; MAP: microtubule-associated protein; MSG: middle silk gland; MT: microtubule; MTBD: microtubule-binding domain; PNS: peripheral nervous system; PSG: posterior silk gland; RT-PCR: reverse transcription PCR; UTR: untranslated region.

Key Words: Tau, Microtubule-Binding Domain, Microtubule Bundling, Silkworm, Silk gland

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