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PTL-1, a microtubule-associated protein with tau-like repeats from the nematode *Caenorhabditis elegans*

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SUMMARY

Tau, MAP2 and MAP4 are structural microtubule-associated proteins (MAPs) that promote the assembly and stability of microtubules. They share three or four imperfect tandem repeats of an amino acid motif, which is involved in the binding to microtubules. All sequences to date containing this motif are of mammalian origin. We report here the cloning and functional characterisation of a new member of this family of proteins from the nematode *Caenorhabditis elegans*. This protein exists as two isoforms of 413 and 453 amino acids with four or five tandem repeats that are 50% identical to the tau/MAP2/MAP4 repeats. Both isoforms bind to microtubules and promote microtubule assembly, with the five-repeat isoform being more effective at promoting assembly than the four-repeat isoform. When expressed in COS cells, the five-repeat isoform co-localises with microtubules and induces the formation of microtubule bundles, whereas its expression in Sf9 cells leads to the extension of long unipolar processes. In view of its length, amino acid sequence and functional characteristics, we have named this invertebrate structural MAP 'Protein with Tau-Like Repeats' (PTL-1). In *C. elegans* PTL-1 is expressed in two places known to require microtubule function. It is first seen in the embryonic epidermis, when circumferentially oriented microtubules help to distribute forces generated during elongation. Later, it is found in mechanosensory neurons which contain unusual 15 protofilament microtubules required for the response to touch. These findings indicate that MAPs of the tau/MAP2/MAP4 family are found throughout much of the animal kingdom, where they may play a role in specialised processes requiring microtubules.

Key words: Protein with tau-like repeats, Microtubule-associated protein, Tau, MAP2, MAP4

INTRODUCTION

Microtubules are required for a variety of cellular processes, such as mitosis, organelle transport and the establishment of cell shape. These diverse functions require modulation of the intrinsic lability of microtubules that derives from their dynamic instability (Mitchison and Kirschner, 1984). This is especially important in the nervous system, where stable microtubules in nerve cell processes are essential for rapid axonal transport. Structural microtubule-associated proteins (MAPs) are believed to play an important role in the stabilisation of microtubules. They are particularly abundant in the nervous system and are identified by their co-purification with tubulin and their association with microtubules both in vitro and in vivo. Functionally, they promote microtubule assembly and stability.

Structural MAPs comprise high-molecular weight proteins, such as MAP1a, MAP1b, MAP2a, MAP2b, MAP4 and big tau, as well as low-molecular weight proteins, such as MAP2c and tau (for reviews, see Müller et al., 1994; Matus, 1994; Bulinski, 1994; Goedert et al., 1994). Additional isoforms of these proteins are generated by alternative mRNA splicing. Based on shared sequence similarities, two groups of structural MAPs can be distinguished. MAP1a and MAP1b share partial sequence similarity and are synthesised as polyproteins coupled to a light chain (Noble et al., 1989; Langkopf et al., 1992). By contrast, the thermostable proteins tau, MAP2 and MAP4 are characterised by the presence of imperfect tandem repeats located in the carboxy-terminal half and a proline-rich region that precedes the repeats (Lee et al., 1988; Goedert et al., 1988, 1989a,b, 1992; Lewis et al., 1988; Aizawa et al., 1990; Kosik et al., 1989; Himmler et al., 1989; Chapin and Bulinski, 1991; West et al., 1991; Kindler et al., 1990; Couchie et al., 1992; Doll et al., 1993; Chapin et al., 1995). The homologous regions are required for microtubule binding, with the other regions of tau, MAP2 and MAP4 probably serving functions unique to each protein (Lewis et al., 1989; Kanai et al., 1989, 1992; Butner and Kirschner, 1991; Lee and Rook, 1992; Gustke et al., 1994; Olson et al., 1995). Various lines of evidence have indicated that these structural MAPs are made of a carboxy-terminal microtubule-binding domain and an amino-terminal projection domain (Hirokawa et al., 1988a,b), with the length of the projection domain determining the spacing between adjacent microtubules (Chen et al., 1992). Tau and MAP2 are expressed at high levels in nerve cells, where they have a characteristic distribution. Thus, tau is

localised predominantly in axons, whereas MAP2 is expressed specifically in dendrites (Matus et al., 1981; Binder et al., 1985). MAP4 is widely distributed and is a major MAP in dividing cells (Bulinski and Borisy, 1980). Transfection of tau, MAP2 or MAP4 cDNAs into non-neuronal cells changes the organisation of microtubules and induces the formation of microtubule bundles (Kanai et al., 1989, 1992; Lewis et al., 1989; Takemura et al., 1992; Weisshaar et al., 1992; Olson et al., 1995). Moreover, neurite-like processes emerge from Sf9 cells expressing tau or MAP2, suggesting that these proteins may play a role in neuronal morphogenesis (Knops et al., 1991; Chen et al., 1992; LeClerc et al., 1993; Frappier et al., 1994).

The sequencing of tau, MAP2 and MAP4 from a number of species has shown that they are highly conserved, especially in their microtubule-binding regions. However, to date, only sequences from mammals are known. Immunohistochemical studies have shown the presence of tau- and MAP2-like immunoreactivities in a number of non-mammalian species (Viereck et al., 1988). No information is available on invertebrates. In the present study we report the sequencing and functional characterisation of a MAP from the nematode *C. elegans*. This protein exists as two isoforms with four or five tandem repeats that are similar to the three or four repeats found in tau, MAP2 and MAP4. Both isoforms bind to microtubules and promote microtubule assembly, with the five-repeat isoform being more effective at promoting assembly than the four-repeat isoform. Upon expression in COS cells, the five-repeat isoform co-localises with fibrous microtubules and induces the formation of microtubule bundles; upon expression in Sf9 cells it induces the extension of neurite-like processes. In *C. elegans*, the protein is expressed in the embryonic epidermis and in mechanosensory neurons; in both places microtubules are known to play an important functional role. In view of its length, amino acid sequence and functional characteristics we have named this invertebrate MAP 'Protein with Tau-like Repeats' (PTL-1).

MATERIALS AND METHODS

Cloning of PTL-1A and PTL-1B

The N2 Bristol strain of *C. elegans* was grown in liquid culture and harvested as described (Sulston and Hodgkin, 1988). Total RNA and poly(A)$^+$ RNA were isolated as described (Goedert et al., 1988) and cDNA synthesised using random primers and murine reverse transcriptase. Oligonucleotides based on predicted exon sequences of PTL-1 were used to amplify a 387 bp piece of PTL-1 by PCR. The oligonucleotides were: 5′-CGAGCTCAGTGTCACCAATCATAAG-GCGGGCGGCGGA-3′ (sense) and 5′-CGAGCTCTCCACCAGGC-CGATGATTCATATTGTC-3′ (anti-sense). The PCR fragment was digested with *Sac*I, cloned into M13mp18 and sequenced using the dideoxy chain termination method. The PCR product was radiolabelled by random priming and used as the probe to screen a mixed-stage *C. elegans* cDNA library in lambdaZAP (Barstead and Waterston, 1989), as described (Goedert et al., 1988). Positive plaques were purified and Bluescript SK(–) plasmids carrying the cDNAs excised in vivo. The inserts were subcloned into M13 and sequenced on both strands using synthetic primers.

Bacterial expression and purification of PTL-1A and PTL-1B

For the expression of PTL-1A the internal *Sac*I/*Eco*RI fragment of cDNA clone PTL-116 was ligated to a PCR fragment obtained using PTL-116 DNA as the template. The sense oligonucleotide contained a *Nde*I site in the context of the initiator codon, whereas the anti-sense oligonucleotide contained the internal *Sac*I site. The resulting *Nde*I/*Eco*RI fragment was subcloned into *Nde*I/*Eco*RI digested pRK172 (McLeod et al., 1987). For the expression of PTL-1B the *Sac*I/*Eco*RI fragment of cDNA clone PTL-129 was used. The plasmids pRK172-PTL-1A and pRK172-PTL-1B were transformed into BL21(DE3) and the bacteria grown and induced as described (Goedert and Jakes, 1990).

For the purification of PTL-1A and PTL-1B, pellets from a 1 L bacterial culture were resuspended in 40 ml of 50 mM sodium phosphate buffer, pH 6.8, 1 mM EDTA, 0.1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulphonyl fluoride (PMSF) and sonicated twice for 2 minutes using a Micro Ultrasonic Cell Disrupter at maximum settings. Following a 20 minute centrifugation at 10,000 rpm the supernatant was applied to a DE52 column (3.5 cm × 6 cm), which was then washed with 50 ml starting buffer and PTL-1 eluted with 0.3 M NaCl in starting buffer. PTL-1 peak fractions were precipitated with 30% ammonium sulphate and centrifuged. The pellet was suspended in 0.5 M ammonium sulphate in 50 mM sodium phosphate buffer, pH 6.8, and re-centrifuged. The supernatant was applied to a phenylSepharose 6 column (1.5 cm × 4 cm) and eluted with a gradient of 30 ml 0.5-0 M ammonium sulphate in starting buffer. Pooled fractions were dialysed against 100 mM Tris-HCl, pH 8.0, 0.1 mM DTT, 0.1 mM PMSF and applied to a Q sepharose column (1.5 cm × 4 cm). PTL-1 was eluted using a gradient of 0-0.4 M NaCl in 50 mM Tris-HCl, pH 8.0. Peak fractions were dialysed against water and lyophilised. Purification of PTL-1A and PTL-1B was monitored by 10% SDS-PAGE. Amino acid sequencing of purified recombinant PTL-1A and PTL-1B that had been electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane was carried out using an ABI gas phase sequencer.

Antibody production and purification

Polyclonal antibodies (antisera 13 and 14) were raised against synthetic peptides KVGSMDNAAHKPAGG (residues 381-395, antiserum 13) and KSDVKIVSEKLTWQA (residues 364-378, antiserum 14) from PTL-1A. Each peptide (500 µg), coupled to keyhole limpet hemocyanin using glutaraldehyde, was mixed 1:1 with Freund's complete adjuvant and used to immunise white Dutch rabbits. Booster injections were given 2 weeks after the primary immunisation using 250 µg conjugate peptide mixed 1:1 with Freund's incomplete adjuvant. Another booster injection was given after an additional 2 weeks, and the animals were bled 10 days later. For affinity purification of PTL-1 antibodies, recombinant PTL-1A was resolved by 10% SDS-PAGE (50 µg protein across a 9 cm gel) and electroblotted onto Immobilon P. The PTL-1A strip was excised, washed in PBS and incubated with 5 ml antiserum overnight at 4°C. The Immobilon strip was then washed in 3× 10 ml PBS, followed by 1× 10 ml water. The antibodies were eluted with 2× 100 µl 0.2 M glycine, 0.1 mM sodium azide, 0.01% phenol red, pH 2.8. The eluates were aspirated and quickly neutralised with 1 M Tris, using phenol red as the indicator.

Extraction of PTL-1 from *C. elegans*

One gram of frozen *C. elegans* mixed-stage worms was thawed, mixed with 3 ml of 6 M guanidine-HCl, 0.1 mM EDTA, 1 mM DTT, and homogenised twice in a French press at 12,000 psi. The homogenate was centrifuged at 80,000 ***g*** for 30 minutes at 4°C and the supernatant dialysed against 50 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF. The dialysate was centrifuged at 40,000 ***g*** for 30 minutes at 4°C and the supernatant brought to 30% ammonium sulphate. The subsequent precipitate was dissolved in 1 ml of 6 M guanidine-HCl and applied to an Aquapore 300 reverse-phase column equilibrated in 0.1% trifluoroacetic acid. The column was run at 0.5 ml/minute with a 0-60% acetonitrile gradient over 40 minutes. PTL-1 eluted at approximately 45% acetonitrile; PTL-1-containing fractions were concentrated and subjected to SDS-PAGE and

western blotting. Bound antibody was detected by the biotin/peroxidase system (Vectastain, Vector).

Immunohistochemical distribution of PTL-1 in *C. elegans*

Affinity-purified anti-PTL-1 serum 14 was diluted 1:50 in phosphate-buffered saline + 0.2% Tween-20 (PBST) and used to stain *C. elegans* embryos and larvae. Specificity of staining was tested by incubating diluted purified serum 14 with 20 μM recombinant PTL-1A overnight at 4°C before use. In all experiments a mouse monoclonal antibody (directed either against P-granules or against pharyngeal myosin) was included as a control to ensure that animals had been permeabilised and that any competition was specific for PTL-1. Embryos and larvae were prepared for antibody staining by placing on a poly-L-lysine coated slide in water, followed by slight squashing with a coverslip by wicking liquid away. The slides were frozen on dry ice for 10 minutes, the coverslip flicked off and the slides immersed in −20°C methanol for 10 minutes, followed by −20°C acetone for 10 minutes. Tissues were quickly re-hydrated through an acetone series, immersed in PBST for 10 minutes, blocked with PBST + 1% non-fat milk for 30 minutes and washed in PBST for 5 minutes. The primary antibody was then applied overnight at 4°C, the slides washed for 2× 15 minutes in PBST, followed by Texas red-conjugated donkey anti-rabbit IgG (1:50) plus 1 μg/ml DAPI. Following a 2 hour incubation in the dark at room temperature, the slides were washed in PBST for 2× 15 minutes and then mounted using Mowiol.

Microtubule binding and assembly

Bovine brain tubulin (Cytoskeleton, Inc.) was diluted to 20 μM in assembly buffer (80 mM Pipes, 0.2 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 1 mM GTP, pH 6.8) and assembled into microtubules at 37°C in the presence of 20 μM taxol (Calbiochem). After 15 minutes different amounts of recombinant PTL-1A and PTL-1B were added and the incubations continued for a further 15 minutes. The mixtures were then loaded onto a buffered cushion (assembly buffer containing 25% glycerol and 20 μM taxol) and centrifuged for 20 minutes at 340,000 ***g***. The resulting pellets were resuspended in the same volume of assembly buffer minus GTP as that of the supernatant. Aliquots of supernatants and pellets were then subjected to SDS-PAGE. Polymerisation of tubulin was carried out in 500 μl assembly buffer containing 20 μM tubulin and 4 μM recombinant PTL-1A or PTL-1B. Tubulin polymerisation was followed over 20 minutes at 37°C by the change in turbidity at 350 nm.

COS cell transfection and indirect immunofluorescence

The insert from pRK172-PTL-1A was blunt-end ligated into the eukaryotic expression vector pSG5 (Green et al., 1988). Exponentially growing COS cells were transiently transfected with 10 μg/ml plasmid DNA using DEAE-dextran chloroquine. The binding of PTL-1A to microtubules was assessed by the co-localisation of PTL-1A staining and tubulin staining in Triton-extracted cells. Double-labelling indirect immunofluorescence was carried out 48 hours following transfection. Cells were fixed in 5 mM ethylene glycol *bis*(succinic acid *N*-hydroxysuccinimide ester) (EGS, Sigma) in PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 2 mM $MgCl_2$, pH 6.95) for 20 minutes at 37°C, as described (Takemura et al., 1992). For the first 2 minutes of fixation 0.1% Triton X-100 and 10 μM taxol (Calbiochem) were added to the fixative. Cells were then permeabilised with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 20 minutes and treated with 0.1 M glycine, pH 7.0, for 10 minutes. After blocking they were incubated with anti-PTL-1 serum 13 (1:100) for 1 hour at 37°C and washed. The cells were then incubated for 1 hour at 37°C with rat monoclonal anti-alpha tubulin antibody YL1/2 (1:300) (Kilmartin et al., 1982). Following washing they were incubated sequentially with FITC-conjugated goat anti-rabbit IgG (1:100), biotin-conjugated goat anti-rat IgG (1:100) and avidin Texas red (1:100). Following further washing, the cells were mounted for immunofluorescence.

Expression of PTL-1A and tau in Sf9 cells

PCR was used to introduce a *Not*I site upstream of the start codon and an *Eco*RI site downstream of the stop codon of PTL-1A cDNA using pRK172-PTL-1A as the template and appropriate oligonucleotides containing these restriction sites at their 5′ ends. The PCR product was cut with *Not*I/*Eco*RI and subcloned into *Not*I/*Eco*RI cut pVL1392 (Pharmingen). PTL-1A(1-286) was produced using an anti-sense oligonucleotide that introduced a stop codon at codon 287 of PTL-1A; PTL-1A(259-453) was produced by using a sense oligonucleotide that introduced an ATG start upstream of codon 259 of PTL-1A. Constructs encoding PTL-1A(1-286) and PTL-1A(259-453) were cut with *Not*I/*Eco*RI and subcloned into *Not*I/*Eco*RI cut pVL1392. PCR was used to introduce a *Bam*HI site upstream of the start codon and an *Eco*RI site downstream of the stop codon of hTau40 cDNA using pRK172-hTau40 DNA (Goedert and Jakes, 1990) as the template and appropriate oligonucleotides containing these restriction sites at their 5′ ends. The PCR product was cut with *Bam*HI/*Eco*RI and subcloned into *Bam*HI/*Eco*RI cut pVL1393. DNA from the resulting transfer vectors and linear baculovirus DNA (BaculoGold, Pharmingen) were co-transfected into Sf9 cells to obtain recombinant baculoviruses. Primary virus stocks were amplified in Sf9 cells and used in all experiments. For process formation, western blotting and indirect immunofluorescence Sf9 cells were analysed 48 hours following infection with recombinant baculovirus.

For immunoblotting cells were lysed in SDS-PAGE sample buffer and centrifuged. Equal amounts of supernatant protein were run on a 10% Tris-Tricine gel and the separated proteins transferred to Immobilon-P. PTL-1A was visualised with anti-PTL-1 serum 14 (1:100). For immunofluorescence Sf9 cells were grown and infected on poly-L-lysine-coated multispot microscope slides. The cells were fixed in 2% paraformaldehyde in PBS and permeabilised in methanol. PTL-1A was visualised using anti-PTL-1 serum 14 (1:100) and FITC-conjugated goat anti-rabbit IgG (1:100).

RESULTS

Sequencing of cDNA clones encoding PTL-1A and PTL-1B

The sequencing of cosmid F42G9 from chromosome III of *C. elegans* by the *C. elegans* Genome Consortium (Wilson et al., 1994) identified predicted exons that are homologous to the tandem repeat region of tau. We used synthetic oligonucleotides from this region to amplify a 387 bp fragment from *C. elegans* cDNA by the polymerase chain reaction (PCR). Following sequencing the PCR fragment was used as a probe to isolate 38 clones from a mixed stage *C. elegans* cDNA library (Barstead and Waterston, 1989). Two types of cDNA clones were isolated and full-length clones sequenced. They contain 9 bp of the 22 bp spliced leader SL1 at their 5′ ends. This sequence is *trans*-spliced from an unlinked exon and is found at the 5′ ends of many *C. elegans* mRNAs (Krause and Hirsh, 1987); its presence signifies that the cDNA is full-length. After the SL1 sequence, 5 bp precede the initiator methionine codon. The nucleotide sequences contain two types of open reading frames that encode predicted proteins of 453 amino acids (PTL-1A) or 413 amino acids (PTL-1B), starting at the first methionine codon of the open reading frame (Figs 1 and 2). The AUG start codon is preceded by an in-frame termination codon within the SL1 sequence. A poly(A) tail is present at the end of 3′ untranslated regions of 107 bp (PTL-1A) and 339 bp (PTL-1B) and a canonical polyadenylation sequence (AATAAA) is located 12 bp or 9 bp upstream of the poly(A) tail. Comparison of the cDNA sequences with the genomic sequence shows that the *ptl-1* gene comprises nine

exons that range from 71 to 391 bp, with intron sizes ranging from 47 bp to 3308 bp (Fig. 3). PTL-1A and PTL-1B are produced by alternative mRNA splicing; PTL-1A comprises exons I-VIII, whereas PTL-1B consists of exons I-VI and exon IX (Fig. 3). Low-stringency Southern blotting using the 387 bp PCR product from the tandem repeat region as a probe only provided evidence for a single *ptl-1* gene (data not shown).

The open reading frame of PTL-1A encodes a protein of 453 amino acids, with a predicted molecular mass of 49,386; its most striking feature is a stretch of 29 or 30 amino acids that is tandemly repeated five times (residues 287-315, 316-345, 346-374, 375-404 and 405-434) (Fig. 4A). Repeats 1, 2, 4 and 5 of PTL-1A can be aligned with repeats 1-4 of tau, MAP2 and MAP4, whereas repeat 3 is unique to PTL-1 (Fig. 4B). Repeats 1, 2, 4 and 5 of PTL-1A are 49-51% identical to repeats 1-4 of tau, MAP2 and MAP4. The sequence identities extend throughout all four repeats and include a lysine (residue 314) at the equivalent position of the lysine residue in tau, MAP2 and MAP4 that is known to make an important contribution towards the higher microtubule-binding affinity of tau isoforms with 4 repeats when compared to isoforms with 3 repeats (Goode and Feinstein, 1994). One notable difference between the PTL-1 repeats and the repeats in tau, MAP2 and MAP4 is the replacement of the cysteine residues in repeats 2 and 3 of the mammalian MAPs by valines in PTL-1.

Like the repeats in mammalian MAPs, the PTL-1A repeats have a basic character (24 positively charged residues and 9 negatively charged amino acids). Similarly, the region preceding the repeats has a basic character. This contrasts sharply with the acidic nature of the amino-terminal 188 amino acids of PTL-1A (57 negatively charged amino acids, with only 8 positively charged residues). The amino-terminal half of PTL-1A is also proline-rich. This is especially true of the 100 amino acid long exon II which comprises 35 proline and 37 glutamic acid residues, with striking 29 glutamic acid-proline dipeptide repeats.

PTL-1A contains 12 serine/threonine-proline motifs which are potential phosphorylation sites for proline-directed protein kinases. With the exception of one serine-proline sequence in the third repeat they are all located upstream of the repeats.

The open reading frame of PTL-1B encodes a protein of 413 amino acids, with a predicted molecular mass of 44,846; it is identical to PTL-1A up to residue 398 (exons I-VI). It lacks exon VII which encodes part of repeat 4 and most of repeat 5, as well as exon VIII which encodes the remainder of repeat 5 and the carboxy-terminal 19 amino acids of PTL-1A. PTL-1B contains instead 15 carboxy-terminal amino acids encoded by exon IX.

The imperfect tandem repeats constitute the only region of similarity between the two PTL-1 isoforms and the mammalian MAPs tau, MAP4 and MAP2 (Fig. 4C).

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      *       M  S  T  P  Q  S  E  P  G  S  E  P  G  S  P     15
 aagtttgaggaaaaatgtcaacccctcaatcagagcctggatccgagccaggctcccct    59

 G  E  N  E  N  S  M  E  T  Q  Y  S  Q  G  S  F  P  E  V  A     35
ggcgaaaacgaaaattcgatggaaactcagtatagtcagggctcttttccggaggttgca   119
                  ><
 V  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E     55
gtagaaccggagcccgaacctgaaccggaaccagaacctgaaccggagcctgaaccggag   179

 P  E  P  E  P  E  P  K  P  K  P  E  V  E  P  V  P  Q  P  E     75
cctgaaccggagcctgaaccgaagcctaaaccagaagttgaaccggtgccacaaccagag   239

 P  E  P  E  Y  Q  P  E  P  E  P  E  P  E  V  E  P  E  P  E     95
cctgagccagagtatcaacctgaaccggagcctgaaccagaggttgaaccggagccagaa   299

 P  E  P  V  P  E  P  E  P  E  P  E  P  E  P  E  P  V  V  E    115
cctgaacctgtgccggagccagagcctgaaccggagccagagcccgaaccagtagtcgag   359

 K  E  E  E  V  V  V  E  S  P  P  R  E  Q  E  P  E  K  S  G    135
aaggaagaagaagttgtagtcgaatctcctcctcgagaacaagaacctgaaaaatctgga   419
                  ><
 K  S  K  P  S  S  P  I  P  D  A  P  T  M  E  D  I  A  P  R    155
aaatccaagccttcgagtccgatccccgacgccccaacaatggaagatatcgctccgaga   479

 E  L  E  S  L  N  F  S  E  T  S  G  T  S  D  Q  Q  A  D  R    175
gagctcgagtctctaaacttttccgaaacgtctggtacctccgatcaacaagccgacaga   539

 I  M  Q  N  N  E  N  E  R  V  E  E  K  K  Q  M  S  P  T  P    195
attatgcagaacaatgaaaacgagagagtcgaggagaagaaacaaatgagcccaactcca   599

 S  Q  P  Q  H  K  T  P  Q  R  S  G  I  R  P  P  T  A  I  L    215
tcacaaccccaacataagaccccacaaagatctgggattcggccaccaacggcgattctg   659

 R  Q  P  K  P  I  P  A  S  L  P  R  P  A  T  A  T  P  S  S    235
cgacagccaaaaccgataccagcatctttgccaaggccagcaactgctactccctcgtct   719
                              ><
 Q  R  A  I  S  T  P  R  Q  T  A  S  T  A  P  S  P  R  P  I    255
caacgcgctattagtacaccaagacaaactgcttcaacagcgccatctccgagacccata   779

 S  K  M  S  R  E  R  S  D  V  Q  K  S  T  S  T  R  S  I  D    275
tcaaaaatgtctcgagaacggtcggatgttcaaaaatccacgtctacgcgaagcatcgat   839
 ><
 N  V  G  R  M  T  P  K  V  N  A  K  F  V  N  V  K  S  K  V    295
aatgttggaagaatgacgccaaaggttaacgccaaatttgtaaatgtgaagagcaaagtg   899

 G  S  V  T  N  H  K  A  G  G  G  N  V  E  I  F  S  E  K  R    315
ggaagtgtcaccaatcataaggcgggcggcggaaatgtggaaattttctcggagaaaaga   959
                                                          >
 L  Y  N  A  Q  S  K  V  G  S  L  K  N  A  T  H  V  A  G  G    335
ctctacaacgcccaatccaaagttggctccttgaaaaacgcaacacacgttgccggaggc  1019
<
 G  N  V  Q  I  E  N  R  K  L  D  F  S  A  A  S  P  K  V  G    355
ggaaacgttcaaatcgaaaacaggaagctagatttttcggcagcttcaccaaaggttggc  1079

 S  K  T  N  Y  Q  P  A  K  S  D  V  K  I  V  S  E  K  L  T    375
tcgaaaaccaattatcagccagcaaagtctgacgtgaagatagtttcggagaagttgact  1139

 W  Q  A  K  S  K  V  G  S  M  D  N  A  A  H  K  P  A  G  G    395
tggcaagctaaatcaaaagttgggtcaatggacaacgcagcgcataagcctgctggtgga  1199

 N  V  Q  I  L  S  Q  K  L  N  W  K  A  E  S  K  V  G  S  K    415
aatgttcagatattaagtcaaaaattgaactggaaagccgagagtaaggttgggtcaaag  1259
      ><
 D  N  M  N  H  R  P  G  G  G  N  V  Q  I  F  D  E  K  I  R    435
gacaatatgaatcatcggcctggtggtggaaatgttcaaattttcgacgaaaaaatccgc  1319
                                    ><
 Y  V  S  T  D  S  S  R  N  H  S  T  L  D  I  S  S  L  *       453
tacgtgtcgacggactcgagccggaatcattctacgctggacatcagctcgttatgatcg  1379

gaccacgctttcacttgaattttaccacacctcactgatcttcagaggttttgtatttttt  1439
tcagcaaactgatgacctctttttgtaataaatttgattatttgaaaaaaaaaaaaaaaa  1499
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Fig. 1. Nucleotide sequence of PTL-1A and deduced amino acid sequence (GenBank accession number U67966). The predicted amino acid sequence (in single-letter code) is shown above the nucleotide sequence. Numbers on the right indicate the positions of amino acids or nucleotides. The positions of introns as determined from the genomic sequence are indicated (><). The partial splice-leader sequence SL1 at the 5′ end and the putative polyadenylation sequence (AATAAA), 12 nucleotides upstream of the poly(A) stretch, are underlined. Asterisks denote in-frame termination codons.

Expression of PTL-1A and PTL-1B in *E. coli* and comparison with PTL-1 from *C. elegans*

To express PTL-1A and PTL-1B, the coding regions were cloned downstream of the T7 RNA polymerase promoter in the expression vector pRK172 (McLeod et al., 1987). Expression was then induced with isopropyl-β-D-thiogalactoside (IPTG) and PTL-1 detected by western blotting using polyclonal antibodies raised against synthetic peptides derived from the PTL-1 sequence; these antibodies recognise both PTL-1A and PTL-1B. PTL-1 isoforms were extracted and fractionated to over 95% purity using column chromatography (Fig. 5A). The amino-terminal sequence of PTL-1A and PTL-1B was determined and read as STPQSEPGSEP, indicating that the initiating methionine residue had been cleaved away. On SDS-PAGE the PTL-1 isoforms ran with apparent molecular masses of 79 kDa (PTL-1A) and 76 kDa (PTL-1B). The predicted molecular masses are 49 kDa (PTL-1A) and 45 kDa (PTL-1B), indicating that both isoforms run abnormally on SDS-PAGE. Purified recombinant PTL-1 isoforms were found to be heat-stable, but insoluble in 2.5% perchloric acid.

The pattern of recombinant PTL-1 bands was compared with that of native PTL-1 extracted from *C. elegans* extracts. PTL-1 ran as two bands with apparent molecular masses of 79 kDa and 75 kDa, respectively. The 79 kDa band was stronger in intensity than the 75 kDa band (Fig. 5B). The 79 kDa band aligned with purified recombinant PTL-1A, whereas the 75 kDa band aligned with purified recombinant PTL-1B (Fig. 5B).

Expression pattern of PTL-1 in *C. elegans*

Affinity-purified anti-PTL-1 serum 14 was used to examine the distribution of both isoforms of PTL-1 during *C. elegans* development. Staining was observed in the epidermis of embryos undergoing elongation, but not after hatching (Fig. 6A). In addition, weak staining was observed in a number of neurons within the head region (Fig. 6A). During larval and adult stages, mechanosensory neurons and their processes express PTL-1 (Fig. 6C). The number and position of immunoreactive neurons were examined in six late first or second stage larvae, when six mechanosensory cells are present. In six out of six animals, five of these neurons stained for PTL-1 (neurons ALML, ALMR, AVM, PLML and PLMR). Thus, with the exception of PVM, all mechanosensory neurons express PTL-1. Mechanosensory neurons stained for the remainder of development, but their number was not counted after the L2 stage. PTL-1 staining was specific, as it was completely abolished following pre-adsorption of the diluted antibody with recombinant PTL-1 (Fig. 6B).

Binding of PTL-1A and PTL-1B to microtubules and effects on microtubule assembly

Recombinant PTL-1A or PTL-1B were mixed with taxol microtubules and incubated for 15 minutes at 37°C, followed by sedimentation through a glycerol

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      *       M  S  T  P  Q  S  E  P  G  S  E  P  G  S  P     15
 aagtttgaggaaaaatgtcaacccctcaatcagagcctggatccgagccaggctcccct    59

 G  E  N  E  N  S  M  E  T  Q  Y  S  Q  G  S  F  P  E  V  A     35
ggcgaaaacgaaaattcgatggaaactcagtatagtcagggctctttccggaggttgca   119
                  ><
 V  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E     55
gtagaaccggagcccgaacctgaaccggaaccagaacctgaaccggagcctgaaccggag   179

 P  E  P  E  P  E  P  K  P  K  P  E  V  E  P  V  P  Q  P  E     75
cctgaaccggagcctgaaccgaagcctaaaccagaagttgaaccggtgccacaaccagag   239

 P  E  P  E  Y  Q  P  E  P  E  P  E  P  E  V  E  P  E  P  E     95
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 P  E  P  V  P  E  P  E  P  E  P  E  P  E  P  E  P  V  V  E    115
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 K  E  E  E  V  V  V  E  S  P  P  R  E  Q  E  P  E  K  S  G    135
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 I  M  Q  N  N  E  N  E  R  V  E  E  K  K  Q  M  S  P  T  P    195
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                              ><
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 W  Q  A  K  S  K  V  G  S  M  D  N  A  A  H  K  P  A  G  G    395
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 N  V  Q  R  S  G  S  K  S  T  K  N  D  D  G  H  N  N  *  *    413
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Fig. 2. Nucleotide sequence of PTL-1B and deduced amino acid sequence (GenBank accession number U67967). The predicted amino acid sequence (in single-letter code) is shown above the nucleotide sequence. Numbers on the right indicate the positions of amino acids or nucleotides. The positions of introns as determined from the genomic sequence are indicated (><). The partial splice-leader sequence SL1 at the 5′ end and the putative polyadenylation sequence (AATAAA), 9 nucleotides upstream of the poly(A) stretch, are underlined. Asterisks denote in-frame termination codons.

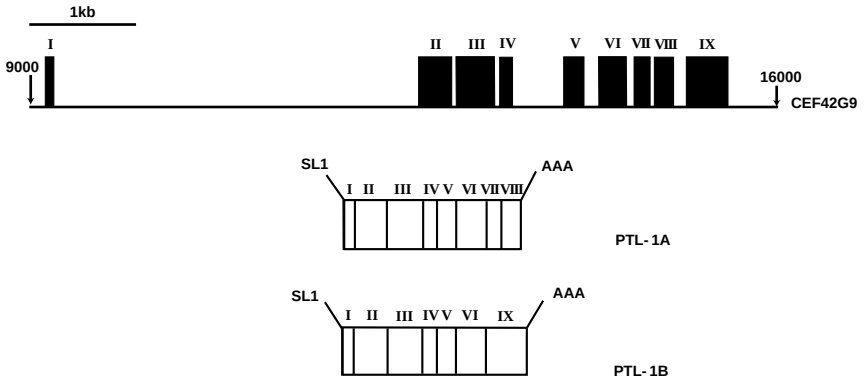

Fig. 3. *ptl-1* gene structure and exon organisation of PTL-1A and PTL-1B. Upper part: schematic representation of the sequence comprising nucleotides 9,000-16,000 of the *C. elegans* cosmid CEF42G9. This sequence was determined by the *C. elegans* Genome Consortium (Genbank Accession Number U00051). Exons in the *ptl-1* gene are represented by numbered black boxes and introns by a solid black line. The lengths of exons and introns are drawn to scale. Lower part: Exon organisation of PTL-1A and PTL-1B. PTL-1A consists of exons I-VIII, whereas PTL-1B is composed of exons I-VI and exon IX. The splice leader sequence at the 5′ end (SL1) and the poly(A) tail at the 3′ end (AAA) are indicated.

cushion. As shown in Fig. 7A, both PTL-1A and PTL-1B pelleted with microtubules at all concentrations studied, indicating that they were bound to microtubules. Polymerisation of tubulin was induced by the addition of 4 μM PTL-1A or PTL-1B to bovine brain tubulin and monitored by turbidimetry. As shown in Fig. 7B, both isoforms promoted microtubule assembly. However, PTL-1A promoted microtubule polymerisation at an approximately five times faster rate than PTL-1B.

Transfection of PTL-1A into COS cells

The full-length cDNA of PTL-1A was subcloned into the eukaryotic expression vector pSG5 (Green et al., 1988) and COS cells transiently transfected with pSG5-PTL-1A DNA. To examine the localisation of PTL-1A in relation to microtubules in vivo, we examined the transfected cells by immunofluorescence. After 48 hours the cells were fixed with EGS in the presence of detergent and taxol, permeabilised and stained with anti-PTL-1 serum 13. An antibody against alpha-tubulin was used in double immunofluorescence to identify microtubule networks. PTL-1A was expressed in some of the transfected COS cells, with a transfection efficiency of approximately 10%. PTL-1A was localised to microtubules, as indicated by the co-localisation of PTL-1 staining (Fig. 8A,C) and tubulin staining (Fig. 8B,D). In addition, some transfected cells contained PTL-1A- and tubulin-immunoreactive microtubule bundles that were never observed in non-transfected cells (Fig. 8A-D).

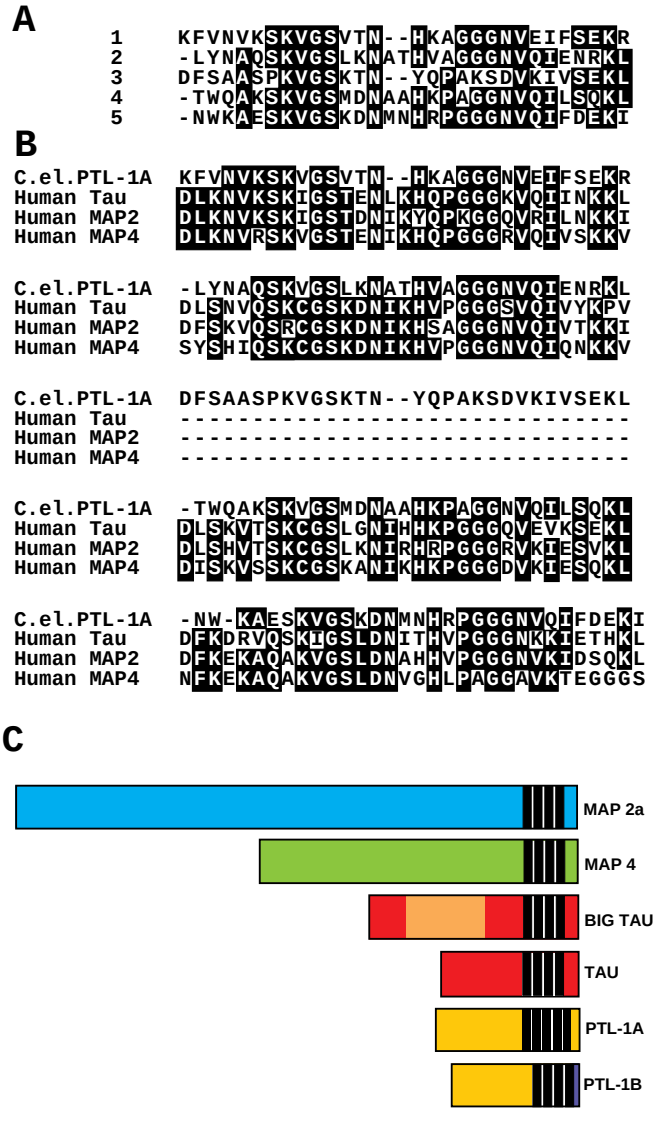

Fig. 4. Alignment of the five repeats from PTL-1A (A), comparison with the four repeats from human tau, MAP2 and MAP4 (B) and schematic representation of the tau/MAP2/MAP4/PTL-1 family of microtubule-associated proteins (C). (A) Residues 287-434 of PTL-1A, with residues that are identical between three of the five repeats indicated by black bars. (B) The sequence comparison of the five repeats from PTL-1A with the four similar repeats from human tau (Goedert et al., 1989a), human MAP2 (Kindler and Garner, 1994) and human MAP4 (Chapin et al., 1995). Residues that are identical between three of the four aligned sequences are indicated by black bars. Dashes denote gaps introduced into the sequences to maximise the alignment. (C) A schematic representation of MAP2, MAP4, big tau, tau and the PTL-1 isoforms PTL-1A and PTL-1B. All these proteins bind to microtubules via their carboxy-terminal portion that includes imperfect tandem repeats (indicated by black boxes). The rest of each protein serves as a projection domain of variable length which extends from the microtubule surface and which differs in sequence between the different proteins. MAP2, MAP4, big tau and tau exist as different isoforms that are produced from a single gene by alternative mRNA splicing (only one isoform for each protein is shown). PTL-1 exists as two isoforms with five (PTL-1A) or four (PTL-1B) tandem repeats. The lengths of the proteins are drawn to scale and identical sequences outside of the repeats are indicated by identical colours.

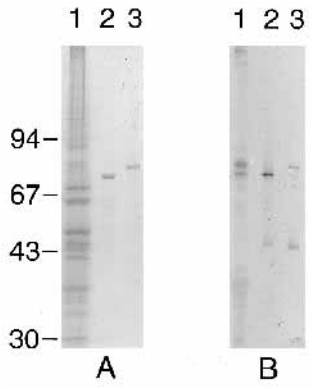

Fig. 5. Immunoblot analysis of native and recombinant PTL-1 proteins. (A) Coomassie blue-stained gel of *C. elegans* extract (lane 1), recombinant PTL-1B (lane 2) and recombinant PTL-1A (lane 3). (B) Immunoblot of *C. elegans* extract (lane 1), recombinant PTL-1B (lane 2) and recombinant PTL-1A (lane 3). Anti-PTL-1 serum 14 was used at 1:500. Size standards are shown at the left (in kDa).

Expression of PTL-1A and tau in Sf9 cells

Infection of Sf9 cells with the recombinant baculovirus containing PTL-1A resulted in the expression of large amounts of PTL-1A. On SDS-PAGE it ran at the same position as PTL-1A expressed in *E. coli* (Fig. 9). Wild-type Sf9 cells did not express any detectable PTL-1. Sf9 cells expressing PTL-1A underwent a marked change in shape: they extended long unipolar and very occasionally bipolar processes (Fig. 10). Both cell bodies and cell processes of infected cells were reactive with anti-PTL-1 serum 14 (Fig. 11). The processes were unbranched and variable in length, sometimes extending 200-300 μm, with a diameter of 1-2 μm. They were uniform in calibre and showed occasional flattening at the tips of the processes. By 48 hours, nearly 60% of the cells had processes and up to 80% were stained with the anti-PTL-1 serum. Only antibody-positive cells had processes. Infection of Sf9 cells with the recombinant baculovirus containing the longest human brain tau isoform (htau40) also resulted in the extension of neurite-like processes, in confirmation of previous reports (Fig. 10) (Knops et al., 1991; Chen et al., 1992). The number and appearance of processes found following the expression of tau were indistinguishable from those observed following the expression of PTL-1A. To determine which PTL-1A sequences mediate process extension, we expressed PTL-1A(1-286) and PTL-1A(259-453) in Sf9 cells. Expression of PTL-1A(1-286) failed to result in process formation, with the Sf9 cells being indistinguishable from wild-type cells (Fig. 10). By contrast, expression of PTL-1A(259-453) resulted in the extension of processes very similar to those formed following the expression of full-length PTL-1A (Fig. 10). SDS-PAGE and western blotting showed that PTL-1A(1-286) and PTL-1A(259-453) were expressed at levels similar to those of PTL-1A(1-453) (data not shown).

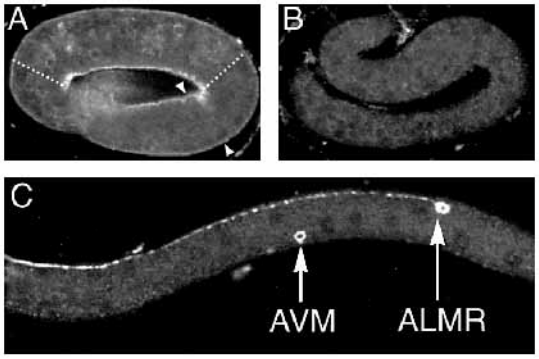

Fig. 6. Expression pattern of PTL-1 in *C. elegans*. (A,B) Threefold elongating embryos stained with (A) affinity-purified anti-PTL-1 serum 14 or (B) the same serum preincubated with recombinant PTL-1. In A, note strong staining of the epidermis (arrowheads) and weak staining of neurons in the head region (between stippled lines). In B, staining is competed away. (C) Second stage larva stained with affinity-purified serum 14 showing strong staining of mechanosensory neurons AVM and ALMR in this focal plane. Neurons ALML, PLML and PLMR also stain. Anterior, left.

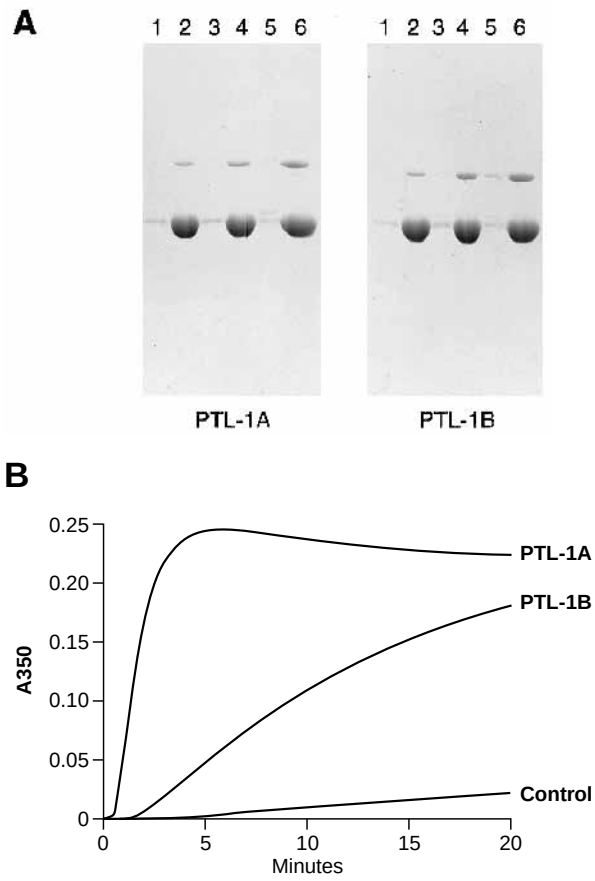

Fig. 7. Binding of PTL-1A and PTL-1B to microtubules and effects on microtubule assembly. (A) Coomassie blue-stained gel of samples from microtubule binding assay. Lanes 1, 3, 5, supernatants; lanes 2, 4, 6, pellets. PTL-1A and PTL-1B were used at 1 μM (lanes 1 and 2), 2 μM (lanes 3 and 4) and 4 μM (lanes 5 and 6). The concentration of taxol-stabilised microtubules was 10 μM. A typical experiment is shown; similar results were obtained in four separate experiments. (B) Polymerisation of tubulin was induced by addition of 4 μM PTL-1A or PTL-1B and monitored by turbidimetry. The control consisted of 20 μM tubulin without any added PTL-1. A typical experiment is shown; similar results were obtained in three separate experiments.

DISCUSSION

In this study we report the cloning and functional characterisation of PTL-1, a structural MAP from *C. elegans*. The PTL-1 isoforms PTL-1A and PTL-1B are produced from a single gene by alternative mRNA splicing. Both proteins contain repeats that are 50% identical to those found in the mammalian

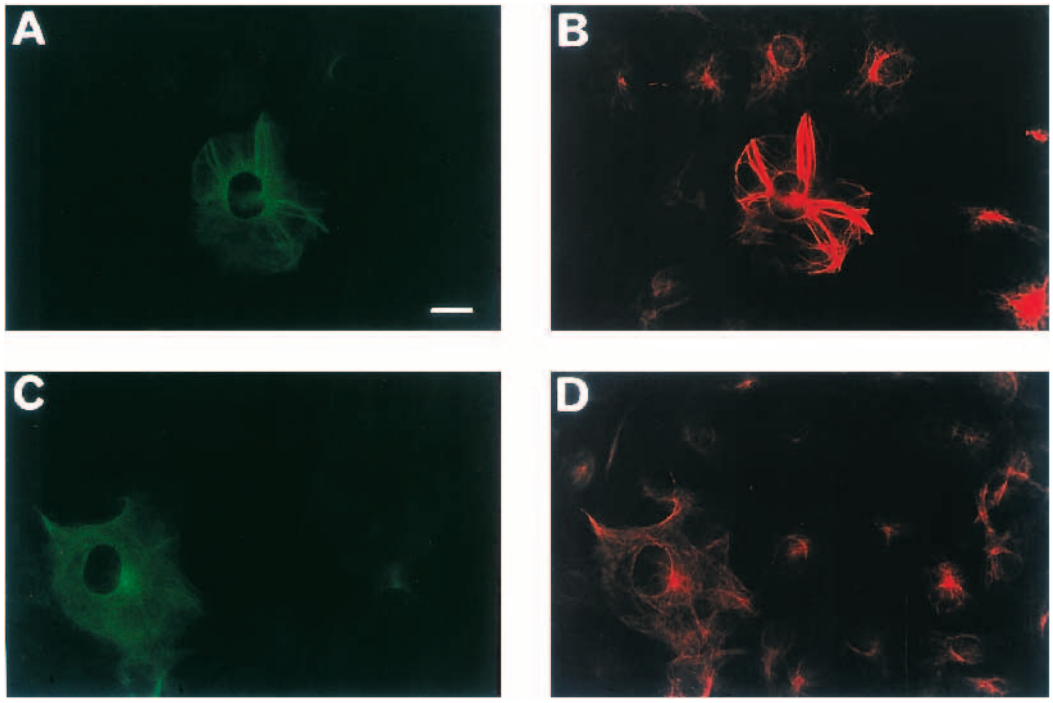

Fig. 8. Immunofluorescence following transient transfection of the PTL-1A cDNA into COS cells. After 48 hours the cells were fixed with EGS in the presence of detergent and analysed by indirect double-labelling immunofluorescence using antiserum 13 directed against PTL-1 (diluted 1:100) (A,C) and an anti-alpha tubulin monoclonal antibody (diluted 1:300) (B,D). Note the co-localisation of PTL-1A and fibrous microtubules (A-D) and the presence of microtubule bundles (A,B). Bar, 12 μm.

MAPs tau, MAP2 and MAP4; the PTL-1 proteins are otherwise unrelated in sequence to the mammalian MAPs. PTL-1A has 5 tandem repeats of 29 or 30 amino acids each, whereas PTL-1B has 4 such repeats. Tau and MAP2 exist as isoforms with 3 or 4 tandem repeats of 31 or 32 amino acids each (Lee et al., 1988; Goedert et al., 1988, 1989a,b, 1992; Lewis et al., 1988; Kosik et al., 1989; Himmler et al., 1989; Kindler et al., 1990; Couchie et al., 1992; Doll et al., 1993) that align with repeats 1, 2, 4 and 5 of PTL-1A, with repeat 3 being unique to PTL-1.

PTL-1A and PTL-1B differ by the presence or absence of the last of the 5 repeats, whereas tau and MAP2 isoforms differ by the presence or absence of the second of the 4 repeats. MAP4 also exists as isoforms with three or four tandem repeats of 31 or 32 amino acids each. However, unlike tau and MAP2, some MAP4 isoforms contain an additional poorly conserved 'pseudo-repeat' that is intercalated between repeats 1 and 2 (Aizawa et al., 1990; West et al., 1991; Chapin et al., 1995).

As in its mammalian counterparts, the repeats in PTL-1 are located close to the carboxy terminus. PTL-1A and PTL-1B have different carboxy-termini that extend 19 or 15 amino acids beyond the repeat region. It follows that PTL-1 consists of a microtubule-binding domain and a projection domain. Like the mammalian MAPs, the projection domain of PTL-1 has a bipartite charge distribution. The region preceding the repeats is positively charged, as are the repeats themselves. This contrasts with the amino-terminal region of PTL-1 which carries a strong negative charge and is proline-rich. Exon II contains a striking sequence of glutamic acid-proline dipeptide repeats, similar to the repeats found in procyclin, a protein expressed on the surface of the procyclic form of trypanosomes (Roditi et al., 1987; Mowatt and Clayton, 1987). PTL-1 may bind to other cytoplasmic proteins through these repeats which probably have an extended structure (Roditi et al., 1989). The repeats in the carboxy-terminal half are the only region of similarity between PTL-1, tau, MAP2 and MAP4, suggesting that the projection domains and the carboxy-termini of these proteins may serve functions unique to each protein. The length of the projection domain of PTL-1 is most similar to that of tau. It has been demonstrated that the length of the projection domain of tau and MAP2 determines the spacing between adjacent microtubules (Chen et al., 1992; Frappier et al., 1994).

A previous study on *C. elegans* has shown cross-bridges between adjacent microtubules, suggestive of the presence of

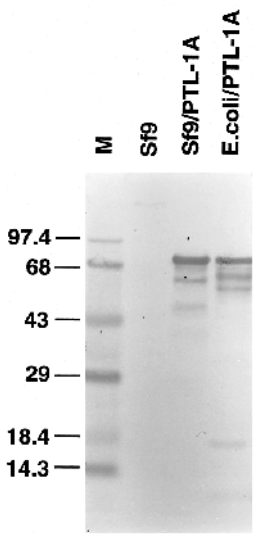

Fig. 9. Immunoblot analysis of PTL-1A expressed in Sf9 cells. Non-infected Sf9 cells (labelled Sf9), Sf9 cells infected with PTL-1A for 48 hours (labelled Sf9/PTL-1A) and PTL-1A from *E. coli* (labelled *E. coli*/PTL-1A) are shown. Anti-PTL-1 serum 14 was used at 1:100. M, size standards (kDa).

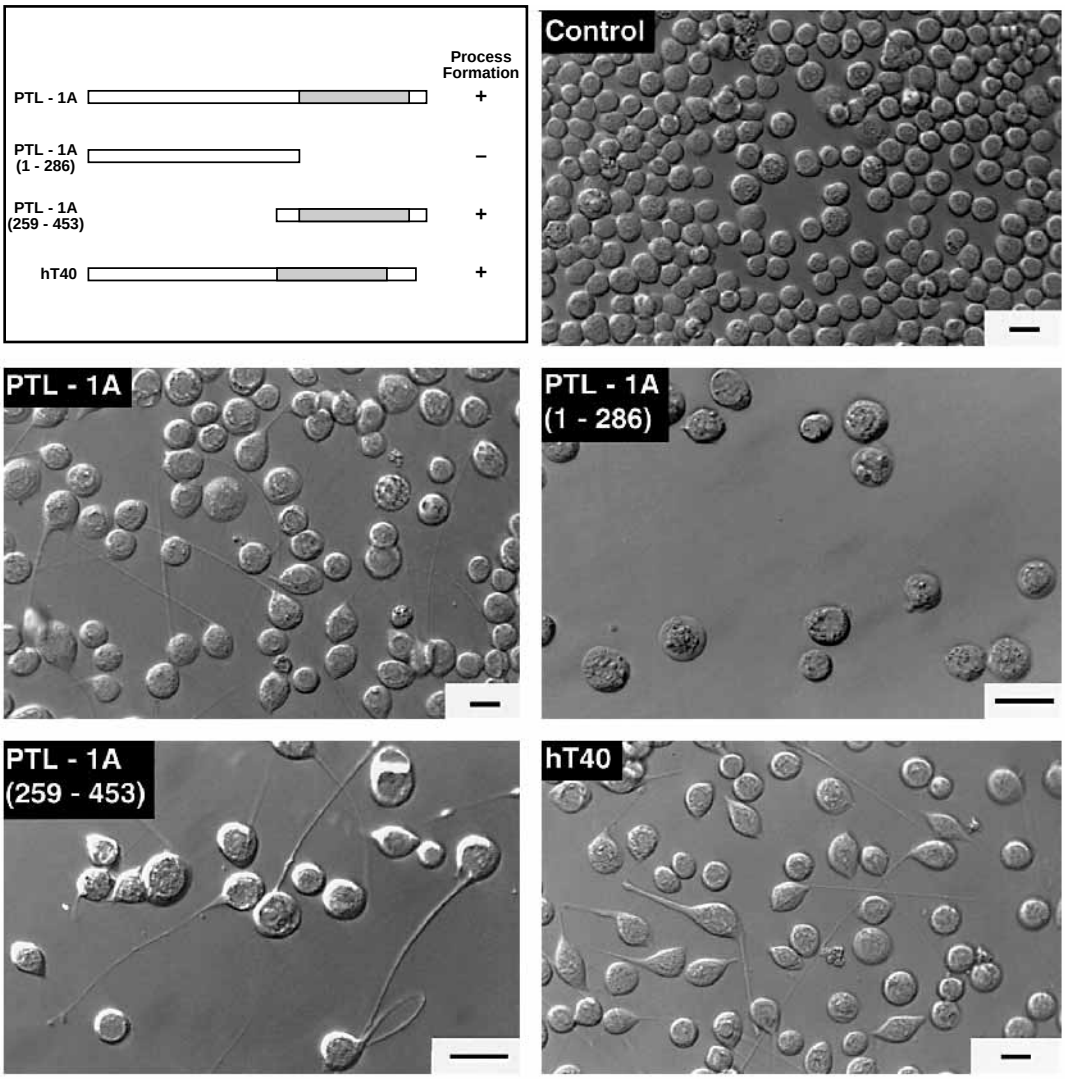

Fig. 10. Light microscopy of Sf9 cells after infection with recombinant baculovirus expressing PTL-1A(1-453), PTL-1A(1-286), PTL-1A(259-453) or the longest human brain tau isoform (hT40). See diagram for constructs and summary of results. Control Sf9 cells are devoid of processes, as are cells expressing PTL-1A(1-286). This contrasts with Sf9 cells expressing PTL-1A(1-453), PTL-1A(259-453) and hTau40 which exhibit long unipolar processes. The cells were photographed 48 hours after infection using Nomarski optics. Bars: 16 μm (for control), 20 μm (for PTL-1A and hT40), 25 μm (for PTL-1A(1-286) and PTL-1A(259-453)).

structural MAPs (Aamodt and Culotti, 1986). However, none has been characterised at the molecular level. It is well established that *C. elegans* possesses kinesin and dynein motor MAPs (Otsuka et al., 1991; Hall and Hedgecock, 1991; Patel et al., 1993; Lye et al., 1995). The present study demonstrates that this is also true of at least one structural MAP of the tau/MAP2/MAP4 family and indicates that proteins with tau-like repeats are conserved throughout much of the animal kingdom. It is unknown whether PTL-1 is an ancestral MAP or whether PTL-1 homologues exist in vertebrates. It is also not known whether additional proteins with tau-like repeats exist in *C. elegans*. All the cDNA clones we have isolated using a probe from the PTL-1 repeats corresponded to PTL-1. Moreover, low-stringency Southern blotting failed to provide any evidence for the existence of additional genes. When the *C. elegans* genome sequencing project is completed, it will become clear how many MAP genes are present.

PTL-1A and PTL-1B contain 453 and 413 amino acids, respectively, with predicted molecular masses of 49 kDa and 45 kDa. Both proteins were expressed in *E. coli* from full-length cDNA clones and purified. PTL-1A and PTL-1B ran with apparent molecular masses of 79 kDa and 76 kDa, demonstrating that they are anomalously retarded on SDS-PAGE, probably indicating an extended structure. This is also reflected in the marked heat-stability of PTL-1, a characteristic shared with the mammalian MAPs. Unlike tau, but like MAP2 and MAP4, PTL-1 is insoluble in perchloric acid. To identify the PTL-1 proteins from *C. elegans* we generated polyclonal PTL-1 antisera. By immunoblot analysis of *C. elegans* extracts these antisera recognised two bands of 79 kDa and 76 kDa apparent molecular mass which co-migrated with purified recombinant PTL-1A and PTL-1B. This demonstrates that PTL-1A and PTL-1B represent the full complement of PTL-1 isoforms.

PTL-1 is expressed in two places where microtubules have a known function. First, it is found in the epidermis of embryos that are undergoing elongation. This process transforms a spherical embryo into a long thin worm. After most cell divisions are complete, the epidermal cells migrate around the embryo to enclose it and then squeeze it circumferentially (Priess and Hirsh, 1986). Circumferentially oriented microtubules in the epidermis are thought to distribute a squeezing force that requires actin microfilaments. PTL-1 is no longer found in the epidermis after elongation is complete, suggesting that it may be involved in the force distribution mediated

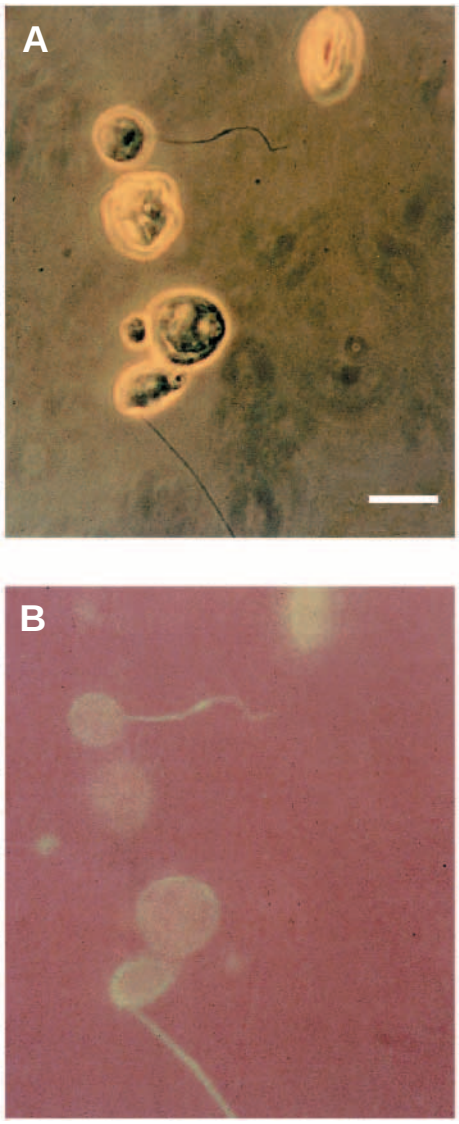

Fig. 11. Immunofluorescence of Sf9 cells infected with a recombinant baculovirus expressing PTL-1A. The cells (shown in phase-contrast in A) were stained 48 hours after infection with anti-PTL-1 serum 14, at a dilution of 1:100 (shown in B). Bar, 15 μm.

by microtubules. In embryos, weak staining was also observed in a number of nerve cells within the head region.

During the first and second larval stages, as well as in adult animals, five of the six mechanosensory neurons and their processes express PTL-1. These neurons mediate a response to touch. In addition to the 11 protofilament microtubules found in all nerve cells, the mechanosensory neurons also contain unusual 15 protofilament microtubules that are required for the touch response (Chalfie and Thomson, 1979; Chalfie and Sulston, 1981; Chalfie and Au, 1988; Savage et al., 1989, 1994). This suggests that PTL-1 may play a role in the structure and function of 15 protofilament microtubules. It is interesting to note that PVM, which fails to express PTL-1, is the only neuron with 15 protofilament microtubules that is not required for mechanosensation (Chalfie and Sulston, 1981) and that does not have gap junctions to ventral cord interneurons (Chalfie et al., 1985). These findings indicate that PTL-1 is expressed in a restricted and developmentally regulated manner in *C. elegans*, where its localisation is both non-neuronal and neuronal. The serum used for immunostaining recognises both PTL isoforms. Future studies will have to investigate whether PTL-1A and PTL-1B are differentially expressed. Within neurons of *C. elegans* PTL-1 is found in cell bodies and their processes. This contrasts with tau and MAP2 which are widely expressed in nerve cells, with an axonal localisation for tau and a dendritic localisation for MAP2 (Matus et al., 1981; Binder et al., 1985).

The functional characteristics of PTL-1A and PTL-1B were investigated using in vitro microtubule binding and assembly assays, as well as following transient transfection of the PTL-1A cDNA into COS cells and by expression of PTL-1A in Sf9 cells. Recombinant PTL-1A and PTL-1B both bound to taxol-stabilised microtubules, demonstrating that they are microtubule-binding proteins. Both proteins also promoted microtubule assembly, with the five repeat-containing PTL-1A promoting assembly at an approximately 5-fold faster rate than the four repeat-containing PTL-1B. This difference is similar to that observed previously with tau protein, where four repeat-containing isoforms promoted microtubule assembly at a 2.5- to 3.0-fold faster rate than the three repeat-containing isoforms (Goedert and Jakes, 1990). Unlike the tau isoforms, PTL-1A and PTL-1B differ not only by an extra repeat, but also have different carboxy-termini. It remains to be determined whether the carboxy-termini of PTL-1A and PTL-1B contribute to their different potencies in promoting microtubule assembly.

Following transfection, PTL-1A co-localised with fibrous microtubules in fixed and extracted COS cells. Moreover, in some transfected cells, PTL-1-immunoreactive microtubule bundles were observed. These results, which are very similar to those obtained previously with tau, MAP2 and MAP4 (Kanai et al., 1989, 1992; Lewis et al., 1989; Takemura et al., 1992; Lee and Rook, 1992; Weisshaar et al., 1992; Olson et al., 1995), establish PTL-1 as a bona fide MAP.

Infection of Sf9 cells with a baculovirus expressing PTL-1A markedly changed the morphology of the host cells. PTL-1A-expressing cells developed long processes not seen in non-infected cells, with the vast majority of cells growing a single process. The infected cells and their processes were immunoreactive with PTL-1 antibodies. By western blotting infection of Sf9 cells with the PTL-1A recombinant baculovirus resulted in the expression of a protein band with an apparent molecular mass of 79 kDa which co-migrated with purified PTL-1A expressed in *E. coli*. There were no obvious differences in the numbers or characteristics of the processes when cells expressing PTL-1A were compared with cells expressing human tau. Both tau and MAP2 have previously been shown to induce processes upon expression in Sf9 cells (Knops et al., 1991; Chen et al., 1992; LeClerc et al., 1993; Frappier et al., 1994), with MAP2-producing cells growing 4-5 processes in 10-15% of cells (Chen et al., 1992; LeClerc et al., 1993). To understand the roles of the amino-terminal and carboxy-terminal halves of PTL-1A in process formation, we expressed PTL-1A(1-286) and PTL-1A(259-453) in Sf9 cells. Cells expressing PTL-1A(1-286) were indistinguishable from control cells, whereas expression of PTL-1A(259-453) led to the formation of processes, similar to those obtained with full-length PTL-1A. This indicates that the tandem repeats in the carboxy-terminal half of PTL-1A, together with the carboxy terminus and 28

amino acids preceding the repeats, are sufficient for process formation.

Process formation in Sf9 cells is thought to be the direct consequence of microtubule stabilisation and bundling effected by the binding of tau and MAP2 to microtubules. Microtubule binding studies and transfection experiments with tau and MAP4 have indicated that the carboxy-terminal repeats and the region preceding the repeats are required for microtubule binding and stabilisation (Kanai et al., 1992; Lee and Rook, 1992; Gustke et al., 1994; Olson et al., 1995). The present results on PTL-1A are similar to these findings and indicate that the functional characteristics of PTL-1 are comparable to those of tau, MAP2 and MAP4. PTL-1 is thus a new member of this family of structural MAPs.

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