

# ISOLATION OF TWO cDNAs ENCODING A TROPOMYOSIN AND AN INTERMEDIATE FILAMENT PROTEIN FROM THE SOYBEAN CYST NEMATODE *HETERODERA GLYCINES*

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## ABSTRACT

Guimarães, P. M., S. C. M. Leal-Bertioli, R. H. Curtis, E. L. Davis and D. J. Bertioli. 2003. Isolation of two cDNAs encoding a tropomyosin and an intermediate filament protein from the soybean cyst nematode *Heterodera glycines*. *Nematropica* 33:87-95.

A cDNA expression library of the soybean cyst nematode *Heterodera glycines* was screened with polyclonal antibodies raised against complete homogenate of *Globodera rostochiensis* second-stage juveniles (J2). Two cDNAs with complete Open Reading Frames were isolated and sequenced. These cDNAs showed high similarity to genes encoding intermediate filament proteins and tropomyosins of other nematodes, including *Caenorhabditis elegans*. The sequences were named *HG-if1* (filament protein) and *HG-trp1* (tropomyosin). Considering that IF proteins and tropomyosins are essential for nematode development and show high levels of allergenicity, facilitating the production of antibodies, these molecules have potential as target molecules for the production of plantibodies to control soybean cyst nematode.

**Key words:** *Heterodera glycines*, antibodies, cDNA library, tropomyosin, intermediate filament protein, soybean cyst nematode.

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## RESUMO

Guimarães, P. M., S. C. M. Leal-Bertioli, R. H. Curtis, E. L. Davis and D. J. Bertioli. 2003. Isolamento de cDNAs codificadores para tropomiosina e proteína intermediária de filamento do nematóide do cisto da soja, *Heterodera glycines*. *Nematropica* 33:87-95.

Uma biblioteca de expressão de cDNA do nematóide do cisto da soja, *Heterodera glycines* foi avaliada utilizando-se anticorpos policlonais produzidos contra o homogenado total de juvenis em segundo estágio de *Globodera rostochiensis*. Dois cDNAs completos foram isolados e seqüenciados. Ambos apresentaram altos níveis de similaridade com genes que codificam para proteínas do filamento intermediário e tropomiosinas de outros nematóides, inclusive *Caenorhabditis elegans*. As seqüências foram chamadas *HG-if1* (proteína de filamento) e *HG-trp1* (tropomiosina). Considerando que as proteínas IF são essenciais para o desenvolvimento de nematóides, e que as tropomiosinas apresentam altos níveis de alergenicidade, facilitando a produção de anticorpos, estas proteínas podem, potencialmente ser utilizadas como moléculas-alvo para produção de anticorpos para o controle do nematóide do cisto da soja.

**Palavras chaves:** *Heterodera glycines*, anticorpos, biblioteca de cDNA, tropomiosina, proteína intermediária de filamento, nematóide do cisto da soja.

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## INTRODUCTION

The soybean cyst nematode, *Heterodera glycines* Ichinohe (SCN), is one of the main

limiting factors for production of soybean in the world (Wrather, 1997). Since the first recorded occurrence in Brazil (Mendes and Machado, 1992), SCN has

become one of the country's greatest phytosanitary problems. In Brazil, soybean is cultivated mainly in the Cerrado region, where it is the main crop. SCN has now infested more than 2 million hectares in Brazil (Silva, 1999). The use of nematicides, resistant cultivars and crop rotation are currently the main control measures used (Ferraz *et al.*, 1999).

While various studies have been carried out to identify factors involved with parasitism of this nematode in host plants, and some interesting putative parasitism genes have been isolated (Smant *et al.*, 1998; Yan *et al.*, 1998; Wang *et al.*, 1999; Mahalingam *et al.*, 1999; Gao *et al.*, 2001; Gao *et al.*, 2002), little attention has been paid to structural proteins of the nematode, which are major constituents of the cytoskeleton and muscles. Intermediate filament (IF) proteins and tropomyosins are structural proteins that play an important role in the cell structure and mechanical integrity in nematodes and other animals (Goldman, 2001). In the free-living nematode *Caenorhabditis elegans*, the hypodermis contains bundles of different filaments as part of the cytoskeletal scaffold of the cell (Francis and Waterson, 1985).

Proteins of IF superfamily are part of this scaffold and are involved in transmission of tension from the muscle cell to the cuticle (Francis and Waterson, 1991; Fuchs and Weber, 1994). All IF proteins are based on a common structural principle and have a highly conserved predicted structure, consisting of a central  $\alpha$ -helical domain with coiled coil forming ability, the rod, and hypervariable head and tail domains (Erber *et al.*, 1998).

Tropomyosins are proteins found in the actin filaments in muscles. They are involved in muscle contraction, together with actin and myosin. Tropomyosin consists of two elongated strands that run along the length of the muscle filaments

containing actin and myosin. Tropomyosins belong to a family of highly conserved proteins with multiple isoforms found in both muscle and non-muscle cells of all species of vertebrates and invertebrates. In most cases, the gene of tropomyosin is alternatively spliced into tissue-specific isoforms (Smith *et al.*, 1989). The protein native structure consists of two parallel alpha-helical tropomyosin molecules that are wound around each other as a coiled-coil dimer (Reese *et al.*, 1999). These proteins have also been identified as major human allergens in invertebrates (Ishikawa & Washamin, 1989; Reese *et al.*, 1999).

The present work describes the isolation and sequencing of cDNAs from *H. glycines* that encode a tropomyosin (*HG-trp1*) and an intermediate filament protein (*HG-if1*).

## MATERIALS AND METHODS

### *Screening of the cDNA library:*

Polyclonal antibodies against *Globodera rostochiensis* were produced with rabbit immunized three times intraperitoneally with whole macerated second-stage juvenil (J2) individuals and a further booster injection three days before fusion (Robinson *et al.*, 1993). Monoclonal antibodies were produced by immunizing a Balb/c mouse three times intraperitoneally with whole J2 and excreted secreted products obtained by incubating approximately 10000 live *Heterodera avenae* J2 in 200 $\mu$ l of water or 0.2 mg/ml of 5-methoxy dimethyltryptamine oxalate (Research Biochemical Incorporation, La Jolla, CA) for 16 hours at room temperature. A further intraperitoneal booster injection was given three days before fusion. Complete adjuvant was used for the first injection only and Mabs were prepared using standard protocols (Curtis, 1996).

Immunological cross reactivity against *H. glycines* was tested by cryosections of J2

as described by Curtis (1996), and by ELISA using total protein extracts from J2 (Robinson *et al.*, 1993).

A *H. glycines* cDNA expression library was made in vector  $\lambda$ -UniZAP XR (Stratagene, La Jolla, CA) with mRNAs of hatched J2, one-tenth of which were treated with the neurotransmitter DMT (5-methoxy dimethyltryptamine oxalate, Research Biochemical Incorporation, La Jolla, CA), in order to increase salivation. The titer of the library was  $7.8 \times 10^9$  pfu/ml. The average insert size was 1.4 kb. Transformed clones were identified by blue/white selection using the *Escherichia coli* strain XL1-Blue MRF<sup>+</sup>, according to the manufacturer's instructions (Stratagene, La Jolla, CA). The excision of the pBLUESCRIPT plasmid was performed as suggested by the manufacturer using the SOLR strain and the presence of cloned fragments confirmed using the restriction enzymes *EcoRI* and *XhoI*.

For immunological screening of the expression library, polyclonal antibodies were diluted 1,000 times, followed by incubation with polyclonal anti-rabbit peroxidase-conjugated antibody at 1:500 dilution (Sigma, La Jolla, CA). Positive clones were visualized by chemoluminescence with ECL Western blotting detection reagents (Amersham Pharmacia Biotech, La Jolla, CA).

#### *Sequencing and data analysis:*

Five clones were sequenced in the forward and reverse orientations using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer, Foster City, CA) and ABI 377 automatic sequencer (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Sequence analysis was completed using the software STADEN (<http://www.mrc-lmb.cam.ac.uk/pubseq/>). Putative protein sequences were compared with those available at the Genbank (<http://www.ncbi.nlm.nih.gov/>) using the

BLAST program (Altschul *et al.*, 1990) and with the protein domains defined in the Pfam Protein Families Database (Pfam) (<http://pfam.wustl.edu/>). Alignments were made with the sequences that showed the highest BLAST scores, using ClustalX (Thompson *et al.*, 1997).

## RESULTS

#### *Selection of antibodies:*

Polyclonal antibodies produced against *G. rostochiensis* juveniles that cross-reacted with *H. glycines* antigens in ELISA assays were used for immunolocalization experiments in cryosectioned nematodes (Curtis, data not published). The polyclonal antibody PC266 reacted to the cuticle and muscle. Six monoclonal antibodies gave distinct reaction patterns: antibodies IACR-CCNj.412D to the lining of the gut and oesophagus, IACR-CCNj.610G to the end of the tail, IACR-CCNj.84E to the nervous system, IACR-CCNj.96E to the subventral glands and 94C to the cuticle.

#### *Screening of the expression library:*

The screening of the *H. glycines* cDNA expression library with PC266 identified five distinct cDNA clones, of which two were full length cDNAs. BLAST analysis with the derived putative peptide sequence of these two cDNAs against the Pfam database showed 98.7% identity to the consensus IF cDNA (gnllCDDI7419) for clone 22 and 97.8% identity to the consensus Tropomyosin cDNA (gnllCDDI826) for clone 23. These clones were named *HG-ij1* and *HG-trp1*, respectively.

#### *Sequence analysis of Intermediate Filament Protein:*

The *H. glycines* cDNA encoding *HG-ij1* (Genbank accession number AY154743)

was 2,081 bp with a 1,743 bp ORF between nucleotides 277 and 2019, and generated a putative amino acid sequence of 580 residues with a calculated molecular weight of 63.8 kDa (Fig. 1). The first three potential initiation codons for the putative protein all conform poorly with the Kozak consensus (Kozak, 1992). However, the high amino acid similarity of the peptide sequence between the first and second start codons with the closest nematode homologues of *HG-IF1* suggests that the first possible initiation codon is used. The BLAST analysis (Altschul *et al.*, 1990) of the putative amino acid sequence of *HG-IF1* matched to filament proteins of the following nematodes (accession numbers and percentage identities in brackets): *Onchocerca volvulus* (gi940941, 76%), *Dirofilaria immitis* (gi7159290, 76%), *Brugia malayi* (gi392788, 75%), *Ascaris lumbricoides* (gi784940, 73%), *A. lumbricoides* (gi1078825, 67%), and *O. volvulus* (gi556343, 65%). A schematic representation of the putative amino acid sequence of *HG-IF1* is given to indicate that it has two Pfam defined regions (Fig. 3a).

*Sequence analysis of Tropomyosin:*

The *H. glycines* cDNA *HG-trp1* (Genbank accession number AF546757) was 1,126 bp long, with an Open Reading Frame (ORF) of 855 bp between nucleotides 58 and 912 (Fig. 2). The putative protein consists of 284 amino acid residues with a molecular weight of 31.24 kDa. The first possible initiation codon conformed well to the Kozak consensus (Kozak, 1992), and there is a very high amino acid similarity with homologues at N-terminal. Therefore, there seems little doubt that the first possible initiation codon is used. The BLAST analysis (Altschul *et al.*, 1990) of the putative amino acid sequence yielded the highest scoring matches to tropomyosins from (accession numbers and percentage identities in brackets): *Trichos-*

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1 agatcaactttgtctccc
17 tacttctgatcaaatcccggttacccctctcccaattttgtctgcaaa
63 atgtcggagcactaccgcaattcgattgcccacggcctgcgctc
M S D D Y R S S I G F R F A F
108 aaccgcaactcaacagcctattcaagtacggcgccgacctgtctcg
N R N S T A Y S S T G G P V S
153 ggcgcaaacccgctgctgaaattgtcaccggagcaaatgtcatcg
G G N R V L K I V T E T M S S
198 agcaccagcggcggcgccgtggcatgtcaccggtttggcccaac
S T S G G G G G M S P F F G A N
243 gcggcacaaatccgtgactcccgatgcccggagagaagagctg
A A Q I R D S R D R E K K E L
288 agcgtattgaaagatgattagccaattacattgaaaagttccgc
S D L N D R L A N Y I E K V R
333 ttctcggaggcccaagaccgcaattgaaaggcggacctgacatg
F L E A Q N R K L K A D L D M
378 ctgcaaggacgcccggcgaaggacaccatccaccatcaagacgatg
L Q G R R G K D T I T I K T M
423 tacgagagcgagatcaagcggcckaaaaagctgctcgaaagacg
Y E S E I Q T A K K L I E D T
468 ggcgatcaaaagcagattggagcagggcgtggccaactgcga
G R S K H E L E Q D V A K L Q
513 cgtgacttggcagcagcggcccaaatcagaggaagctgtctgc
R D L D E Q R R K Y E E A V R
558 tcacggcccggtgacccgtgaaagattggacatttgcctgtccaa
S R A G D R E K L D D L L V Q
603 ttgagcaatttggagggcgaatcagcctgttgaagcgtcgcatt
L S N L E A E I S L L K R R I
648 gccctggtgagggagctgagcaaatcggcgaaggaggaactca
A L L E E E L S K L R K E N S
693 cgactcagggcagcctgcagcgcctgcctgcagagttggatcag
R L Q A D R V R A F L D Q
738 gagcgtgaaacccgcttgcatacacaagaacccagtcgagcagctg
E T L N R I D Y Q N Q V Q T L
783 ctcgaggagatcgacttcaatccgagctcgatgaccagagatc
L E E I D F I R R S H D Q E I
828 aaggacttcagcgcgatggcggccgacacgcccaagcgaanaac
K D L Q A M A A R D T T S E N
873 cgcgagttcttcgcaacgaattggcatcggccatcccgacatt
R E F F R N E L A S A I R D I
918 cgcaacagatcagcagctgttgaacaacaagaaccgtaacgacatt
R N E Y D V L N N K D R N D I
963 gactcgtgttacaagctgaagtgcaagagatgagacgcaaaat
E S W Y K L K V Q E I E T Q S
1008 gcacagacagaacatggagcaggctaccgagagatgagctgaa
A R Q N M E Q G Y Q K D E L K
1053 cggctgcgcttccagctgacgatttgcggcgaatttgcggat
R L R V Q L T D L R G K L S D
1098 ttgaaagcgggaactcgtgttgaagaagcagatccaagagcgtg
L E A R N S L L E K Q I Q E L
1143 aactaccagcttgaggatgaccagcggcttatgaggacgctgtg
N Y Q L E D D Q R A Y E D A L
1188 aatcgaaagatgcccaaatccgaagatgcggcagcagtgccaa
N A K D A Q I R K M R D E C Q
1233 gcgctgattgctgagcgtgcaaatgctgttgcacacaacaacag
A L M V E L Q M L L D T K Q T
1278 ttgtagccgaattgccatttaccgcaaaatgctggaggggcga
L D A E I A I Y R K M L E G E
1323 ggggacggcccgccctgaacagttggtggagcagtggtgcgc
G D G F G L F Q L V E Q V V R
1368 accacggcgtcaacgaaggggcgaacaacaagaagcagctgctg
T T G I N E W A D T E M R V
1413 gtcgaaggcggagcgtctctcccgcaatcgtaccaacgctggcc
V K G E T S S R Q S Y Q R S A
1458 aaggcgaatgtgccattttggagacgtcggcggagggcaaatc
K G N V S I L E T S P E G K Y
1503 gtggttggagaacacgcaccgatacaaggaggagaccattgga
V V L E N T H R S K E E T I G
1548 gaagtgaagctgaagcgtcgcattgatggcaagcggcaattgtg
E W K L K R R I D G K R E I V
1593 tacacattgcccgaactttgtgctccgcccagtgggccgctgtg
Y T L F A N F V L R P H A R L
1638 aaggtgtggccgctaacccagggcggcctgcaagcccccggatg
K V W A R N Q G G L H A P F D
1683 cagctgattctgatggcgaagaatgctcggctcggctcctcaac
Q L I F D G E E S F G V G S N
1728 gtgcaaaccttcttcaaatcggcaaggagagaacgctgcaacg
V Q T I L Y N A Q G E E R A T
1773 cacatccaagctcaagccagacagctgctgaaaggagagagag
H I Q R S S Q T A A *
1818 agataatgctgataagagtgatcaactgggggacgggaatttaaat
1863 ttttttaattttttcgaaaatcgaaaatgtccccccccccctc
1909 ctaagaattgatttttcttttaattttcaaaaaatccccattat
1953 ataaaaattgatttttcaatgtcccaaccgacatttaaccattaa
1998 ttttaccaaattggggcaaaagtcccaacaataatcgtataaattct
2043 gttgataaatttttaatttctcaaaaaaaaaaaaaaaaaaa

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Fig. 1. Complete nucleotide and deduced amino acid sequence of the *Heterodera glycines* *HG-if1* cDNA. The ATG start codon is in bold and the TGA stop codon is marked with an asterisk.



1 ccaagctccact

13 aaatttgtttttttgtgataaacagtcagcaaaaaaagcaaaa

58 atggagcccatcaagaagaatgcaggcgatgaagatcgagaag  
M D A I K K K M Q A M K I E K

103 gacaatgccatcgaccggcgagccggagggaaaagtgccgt  
D N A I D R A D A A E E K C R

148 caaacgcaggaaacggttgagcgtgtgaggaggaattgcgggac  
Q T Q E R L E R V E E L R D

193 acgcagaagaaatgatggcagcgagaaacgaattggacaaggcg  
T Q K K M M A T E N E L D K A

238 caggaggacgtgacggtgccaacaatttggaggaggaaggag  
Q E D L T A A N N L L E E K E

283 aagaaagtgcaggaggtgaagccgaagtggcgtcactgaacgt  
K K V Q E A E A E V A S L N R

328 cgcatggttctgttggagaggttgacaaagcttgaggagcgc  
R M V L L E E E L D K L E E R

373 cttaaacctgcccaccgaaaaatggaagcggacggccaattgc  
L K L A T E K M E E A T A N C

418 gacgaatcggaacggtgctgctaaagtgatgaaaaaccgttctctg  
D E S E R V R K V M E N R S L

463 caagacgaggagcgtgccaatgccatcgaagaacagctgaaggag  
Q D E E R A N A I E E Q L K E

508 gcccaatcgctggcggaagaagcggacccgaaatcgcagcaggtc  
A Q S L A E E A D R K Y D E V

553 gctcgtaaattggcaatggtgaagccgacttggaaactgcccag  
A R K L A M V E A D L E R A E

598 gagcgcgagagccggggagaacaaaattgtggagttggaggag  
E R A E A G E N K I V E L E E

643 gaattgcccgttggggcaacaacttgaagcgttggagctcagc  
E L R V V G N N L K S L E L S

688 gaagaaaaggcgttgcaccgcgaggaacgctcgaagagcaagtg  
E E K A L Q R E E A Y E E Q V

733 cgtcagatggaagtgcgctcgaaggagctgagactcgcgggaa  
R Q M E V R L K E A E T R A E

778 ttcgctgaacgttcggtacaaaagctgcagaaggaagtggacaga  
F A E R S V Q K L Q K E V D R

823 ctggaggacgaattggtccacgagaaggagcgtacaaaagcaatt  
L E D E L V H E K E R Y K A I

868 tcggaggagttggaactcgaatttccaggactttccggctactga  
S E E L D S T F Q E L S G Y \*

913 tctgttcggtcaacggatgcgagcggaaagtgtgctgccgacgga

958 cttggaattatttttattttctctgacaaaaacgaaaaaacaaca

1003 tcattctcgttaatgataccgaagagccaattgcttctctagtaaa

1048 tctgctcaccggttcttttttatttctggtgggaaattacggcaaa

1983 taataataataatgttcaaaaaaaaaaaaaaaaaa 1127

Fig. 2. Complete nucleotide and deduced amino acid sequence of the *Heterodera glycines* *HG-trp1* cDNA. The ATG start codon is in bold and the TGA stop codon is marked with an asterisk. The putative polyadenylation signal sequence (aataaa) is underlined.

*trongylus colubriformis* (gi136098 and gi1321012, 65%), *C. elegans* (gi17510803, 64%), *C. elegans* (gi1208409, 63%), *Anisakis simplex* (gi14423976, 63%), *O. volvulus* (gi773389, 62%). A schematic representation of the putative amino acid sequence of *HG-TRP1* is given to indicate that it has one Pfam defined region (Fig. 3b).

## DISCUSSION

In the current work, we present the first complete cDNA sequences of an IF protein and a tropomyosin of a plant-parasitic nematode: *Heterodera glycines*. These sequences were respectively called *HG-ift1* and *HG-trp1*. These clones were isolated from a *H. glycines* cDNA library by using heterologous antibodies. The high level of cross reactivity found between *G. rostochiensis* antibodies and *H. glycines* antigens demonstrates the feasibility of the use of heterologous antibodies for the screening of cDNA libraries with the aim of isolating genes of interest. In the present work, the polyclonal antibody PC266 from *G. rostochiensis* identified several cDNA clones from the *H. glycines* library. This was expected due to the high amounts and conservation of IF proteins and tropomyosins present in eukaryotic cells (Goldman, 2001). Cross-reaction among antibodies produced against antigens found on the surface of nematodes of distantly related taxa has been reported (Lopez de Mendoza *et al.*, 1999).

Intermediate filaments are a major component of the cytoskeleton in various vertebrate cell types and tissues. They are abundant in the epidermis as keratin filaments and fill many axons as neurofilaments. In addition, these proteins are present in several parts of the body musculature, pharynx, intestine, ovary, uterus, and testis of nematodes (Bartnik *et al.*, 1986). One function that has been assigned to IF proteins relates to cellular resistance against mechanical stress (Erber *et al.*, 1998). In the genome of *C. elegans*, there are only 11 genes encoding IF proteins (Goldman, 2001), and four out of the five IF genes analyzed were found to be essential for the normal development of these individuals (Karabinos *et al.*, 2001). All IF proteins are based on a common structure: two homologous alpha-helical domains, separated and

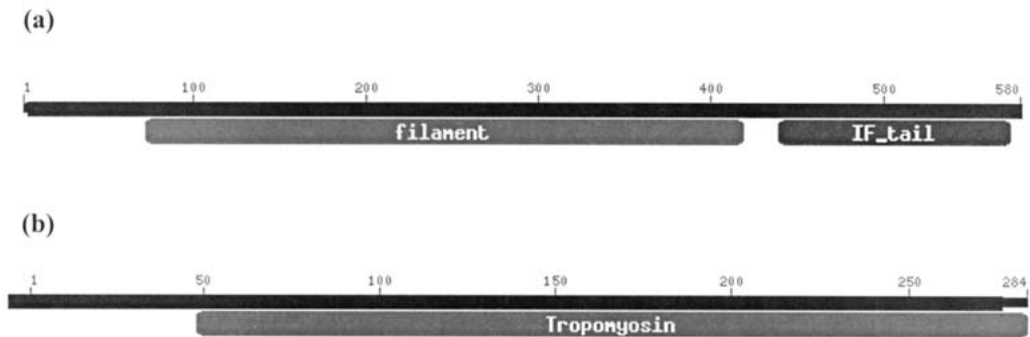


Fig. 3. Schematic representation of the conserved domains of the derived proteins of *HG-IF1* (a) and *HG-TRP1* (b) as detected by the "conserved domains from CDD" option in BLAST-P. This figure shows that the *HG-TRP1* putative protein has only one domain, whereas the *HG-IF1* putative protein has two domains: the filament and the IF tail domain.

extended by variable domains. The presence of the variable domains accounts for the high immunological diversity of IF proteins (Steinert *et al.*, 1980; Pruss *et al.*, 1981). As expected, the primary structure of the putative protein *HG-IF1* was consistent with the one defined in the Pfam database for IF proteins (Fig. 3a).

Tropomyosins belong to a family of proteins associated with thin filament in muscle, and microfilaments in many non-muscle cells. Together with actin and myosin, tropomyosin plays a functional role in the contraction of these cells. The function of tropomyosin in non-muscle cells is not well understood, but it is generally believed to participate in the regulation of cell morphology and motility (Reese *et al.*, 1999). Although the tropomyosins from different tissues are highly homologous, structural differences do exist among these isoforms. In fact, structurally and functionally different isoforms of tropomyosin are required for the regulation and contraction in the different cell types (Less-Miller and Helfman, 1991). Tropomyosins isolated from *C. elegans* have several isoforms, which are differentially expressed in various tissues (Kagawa *et al.*, 1995). According to BLAST results *HG-trp1* has the highest similarity to

the isoform CeTMI, which is expressed in body wall muscles (63% similarity). If similarity levels tended to follow patterns of expression, this could infer that *HG-trp1* is probably expressed in body cell muscles. Structurally, *HG-TRP1* follows the basic pattern defined by Pfam database (Fig. 3b).

Tropomyosins have also been identified as major allergens found in invertebrates (Ishikawa *et al.*, 1989; Reese *et al.*, 1999). There are numerous examples of antibodies in animals against major structural proteins, including tropomyosins, providing protection against various parasite infections, such as those by *Acanthocheilonema viteae*, *Schistosoma japonicum*, *Oncomelania hupensis*, *Taenia solium* and *O. volvulus* (Hartmann *et al.*, 1997; Cao and Liu, 1998; Rosalind *et al.*, 1998; Harrison and Bianco, 2000).

Several proteins from plant-parasitic nematodes have been studied with the aim of developing plantibodies to control nematode infection (Curtis, 1996). In plants, the production of antibodies as a strategy for pest control has been tried for some years with variable degrees of success for the control of some viruses (Franconi *et al.*, 1999; Kawchuk and Pruffer, 1999) and plant-parasitic nematodes (Baum *et al.*,

1996; Stiekema *et al.*, 1997; Jaeger *et al.*, 2000; Gommers *et al.*, 2000). The fact that IF proteins and tropomyosins have high abundance in the cell and show high allergenicity, in addition to their great importance for nematode development, support their potential as target molecules for the production of plantibodies. For such a strategy to be feasible the nematode target antigen must be exposed to the plantibody. In nematodes, such as the filarial parasite *B. malayi*, IF proteins are released to the environment either by being actively secreted or being passively exposed during moulting (Bisoffi and Betschart, 1996) and, in common with other nematode surface or excreted-secreted proteins, are likely to play an important role in the host-parasite interaction (Spiegel and McClure, 1995). The expression of antibodies against these proteins could prevent the successful completion of infection or of the parasite life cycle and reproduction, contributing to the control of the pest. It has been recently observed that continuous binding of monoclonal antibodies to *Meloidogyne javanica* coat proteins has affected the behaviour of pre-parasitic J2, inhibiting the infection of its host, *Arabidopsis thaliana in vitro* (Sharon *et al.*, 2002). Similar results were obtained using monoclonal antibodies reactive to the cuticle and amphids of *G. pallida* (Fioretti *et al.*, 2002). As far as tropomyosins are concerned, some are found to confer stability to actin filaments in muscle and non-muscle cells, such as the intestinal epithelium (Broschat *et al.*, 1989). In such cases, plantibodies could prevent the proper functioning of the digestive system, possibly having similar effects to lectins (Gatehouse *et al.*, 1999). Similarly, the use of ds RNAi targeting *HG-1f1* and *HG-trp1* delivered in planta to the nematode, as accomplished by Urwin and colleagues (2002), could prevent the normal nematode development

and consequently interfere in the interaction of cyst nematodes with their host.

Beyond these opportunities for novel control strategies, the isolation of genes encoding nematode structural proteins should provide a better basis for the understanding of the parasite and the whole process of the plant-parasite interaction.

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