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A cathepsin L-like protease from *Strongylus vulgaris*: An orthologue of *Caenorhabditis elegans* CPL-1 ☆

Sinéad Nic An Ultaigh [a], James C. Carolan [a], Collette Britton [b], Linda Murray [b], Michael F. Ryan [a,c,*]

[a] School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland
[b] Division of Veterinary Infection and Immunity, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, Scotland, UK
[c] Department of Forestry and Natural Resources, Clemson University, SC 29634-0317, USA

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ABSTRACT

Cathespin L-like proteases (CPLs), characterized from a wide range of helminths, are significant in helminth biology. For example, in *Caenorhabditis elegans* CPL is essential for embryogenesis. Here, we report a cathepsin L-like gene from three species of strongyles that parasitize the horse, and describe the isolation of a *cpl* gene (*Sv-cpl-1*) from *Strongylus vulgaris*, the first such from equine strongyles. It encodes a protein of 354 amino acids with high similarity to other parasitic Strongylida (90–91%), and *C. elegans* CPL-1 (87%), a member of the same Clade. As *S. vulgaris cpl-1* rescued the embryonic lethal phenotype of the *C. elegans cpl-1* mutant, these genes may be orthologues, sharing the same function in each species. Targeting Sv-CPL-1 might enable novel control strategies by decreasing parasite development and transmission.

1. Introduction

Nematode parasites of the horse include more than 50 species of Strongylida from the subfamilies Strongylinae (large strongyles) and Cyathostominae (small strongyles). *Strongylus vulgaris* is the most pathogenic of the large strongyles, the migrating larval stages causing verminous arteritis and thrombosis, and adult stages anemia and poor performance. The use of modern anthelmintics has decreased the prevalence of *S. vulgaris* but has increased anthelmintic resistance and prevalence of cyathostomes (Kaplan, 2002). Indeed, multiresistant cyathostomes (to all three types of anthelmintics used to control equine nematodes) has recently been reported in Brazil (Molento et al., 2008). Clearly, alternative control strategies and/or novel candidates for vaccines against equine strongyles are required. *S. vulgaris* is the most pathogenic and most widely studied species, and novel control strategies for it might apply to other species of equine strongyles.

As parasite proteases, especially cathepsins, affect a broad range of biological processes including nutrition, digestion, blood coagulation, host immune invasion and tissue penetration (Tort et al., 1999), they are appropriate targets for chemotherapy and vaccines (McKerrow, 1999; Sajid and McKerrow, 2002). Cathepsin B-like protease genes, a large multigene family in both free-living and parasitic nematodes, are expressed in the intestine of *Haemonchus contortus* and *Caenorhabditis elegans* and may enable nutrient digestion (Ray and McKerrow, 1992; Larminie and Johnstone, 1996; Jasmer et al., 2001). Cathepsin L- and Z-like proteases are associated with larval moulting in parasitic nematodes and may have multifunctional roles in worm development (Guiliano et al., 2004; Lustigman et al., 2004). A cathepsin Z-like protease has a multifunctional role in development, and a cathepsin L-like (CPL) protease is essential for early embryogenesis, in *C. elegans* (Hashmi et al., 2002, 2004).

As recent molecular phylogenetic studies have placed *C. elegans* and the *Strongylida* in the same Clade (Blaxter et al., 1998; Holterman et al., 2006), *C. elegans* is an appropriate model organism for these parasites (Blaxter et al., 1998; Brooks and Isaac, 2002). The ability of an *H. contortus* cathepsin L-like gene to functionally rescue a *C. elegans cpl-1* mutant phenotype suggests that the parasite *cpl* may be an orthologue of *C. elegans cpl-1* (Britton and Murray, 2002).

As cathepsin L-like activity predominates in *S. vulgaris* excretory–secretory products (Caffrey and Ryan, 1994), we now report the isolation of a *cpl* gene from *S. vulgaris* and demonstrate that it rescues the *C. elegans cpl-1* genetic mutant phenotype. This is the first *S. vulgaris* gene to be characterized at genomic and functional levels and it indicates conservation of cathepsin L gene function in *Strongylida*.

Abbreviations: CPL, cathepsin L; GFP, green fluorescent protein.

☆ *Note:* Nucleotide sequence data presented here is available in the GenBank™ database under the accession numbers **EU000410, EU000411, EU884414 and EU884415.**

* Corresponding author. Address: School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland. Fax: +353 1 7161152.
E-mail address: mfryan@ucd.ie (M.F. Ryan).

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2. Materials and methods

2.1. Nematode material

Strongylus vulgaris adults removed from the caecum of freshly slaughtered horses (Caffrey and Ryan, 1994) were stored in RNA later (Invitrogen). *Strongylus edentatus* and *Cyathostominum catinatum* specimens were available from previous studies in the laboratory. *C. elegans* strains used in this study were either wild type N2 Bristol strain, or *C. elegans* genetic mutant (allele ok360) from the *C. elegans* Gene Knockout Consortium, maintained as a homozygous mutant, and rescued with *C. elegans cpl-1* (Britton and Murray, 2004). Both strains were cultured as described previously (Epstein and Shakes, 1995).

2.2. Isolation of genomic cpl gene fragments

Genomic DNA (gDNA) was extracted from single adult *S. vulgaris* using DNeasy Extraction Kit (Qiagen). Nested degenerative PCR was carried out on gDNA using the degenerate forward primers cpl-DF1, (5′-GGIATGTG[T/C]GGITCITG[T/C]TGGGGC-3′) and cpl-DF2 (5′-GA[A/G]CA[A/G]AA[T/C]CTGT IGA[T/C]TG-3′) in combination with the degenerate reverse primer cpl-DR1 (5′-AIGC[T/C] TC[T/C]TC[A/G]TCICC[T/C]TCIGG-3′). The amino acid sequence encoded by these primers is highly conserved in nematode CPL genes (Britton and Murray, 2004). PCR was performed in 25 μl volumes using 1 μl template, 0.8 μM each degenerate primers cpl-DF1 and cpl-DR1, and the following thermocycling parameters: 94 °C for 3 min, 30 cycles of (94 °C for 1 min, 45 °C for 1 min, 72 °C for 1 min), with a final extension of 72 °C for 7 min. After cleaning by a Novagen PCR clean-up kit, 1 μl of this PCR product served as a template in the 2nd round nested PCR. Conditions were as for the 1st round except for an annealing temperature of 48 °C, and use of primers cpl-DF2 and cpl-DR1, both at 0.8 μM.

Cleaned amplified fragments were sequenced (Macrogen, Korea). As the genomic segment had greatest identity with other nematode cathepsin L-like genes (BLAST; http://www.ncbi.nlm.nih.gov/blast/), internal gene specific primers SvF1 (5′-TCATG GAATTGACACTGAAG-3′) and SvR1 (5′-GTCCTCGGCACCGATATC-3′) were designed for use with cDNA.

Isolation of genomic DNA fragments from *S. edentatus* and *C. catinatum* was carried out as detailed above.

2.3. RNA isolation and cpl cDNA cloning

Total RNA was isolated from individual *S. vulgaris* adults by homogenisation in TRIzol (Invitrogen). Approximately 4 μg RNA was treated with DNAse (Invitrogen) prior to single strand cDNA synthesis using oligo d(T) primer and Superscript II reverse transcriptase (Invitrogen). One microlitre of the resulting cDNA served as template in subsequent PCRs. The 3′ end of the *Sv-cpl-1* gene was obtained using SvF1 and oligo d(T) primers (0.4 μM), at the same reagent concentrations as described above, and the following PCR cycling conditions; 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min, 30 cycles. The 5′ end of the *Sv-cpl-1* gene was determined using internal gene specific reverse primer SvR1 and the nematode splice leader primer, SL1 (5′-GGTTTAATTACCCAAGTTT-GAG-3′) under permissive conditions (annealing temperature of 50 °C). The less stringent nature of this PCR resulted in multiple fragments that resolved on a 1.8% agarose gel run for 1 hr and were visualized briefly under UV light. Sterile syringe needles were used to transfer each fragment to a prepared PCR reaction mix containing the SL1 and SvR1 primers. Products of successful PCRs, represented by fragments of the same size as the initial PCR fragment, were gel-purified using the Wizard® Sv Gel and PCR clean-up kit (Promega) and were cloned into PCR2.1 TOPO Vector (Invitrogen). One shot *E. coli* cells (Invitrogen) were transformed with product, and miniprep plasmid DNA (Spin Miniprep kit, Qiagen) from positive colonies was sequenced (Macrogen) using vector specific M13 forward and reverse primers.

Gene specific primers, designed to amplify the entire *Sv-cpl-1* cDNA gene, contained restriction sites to facilitate directional cloning into PCR2.1 TOPO expression vector and had the following sequences; SvF2 (5′-GAGTCTAG*AATG*TTCCGGCTGTTATC-3′), and SvR3 (5′-ATCAGTCGACT*TCA*AAACAAGTGGGTAAC-3′) (the underline indicates *Sal*I and *Xba*I sites, respectively, start and stop codons are in italics). PCR cycling conditions were as above except for an annealing temperature of 50 °C. The resulting PCR products were sequenced to confirm correct DNA sequence.

2.4. Isolation of the genomic Sv-cpl-1 sequence

The *Sv-cpl-1* genomic sequence was ascertained using a primer walking strategy as primers designed from cDNA sequence repeatedly gave PCR products much shorter than expected (data not shown). Accordingly, primers were designed with a 4 or 5 bp intron anchor where possible (Table 1). This strategy is illustrated in Fig. 1. Bands were gel-purified, cloned into a PCR2.1 TOPO vector and sequenced. PCR reagent concentrations and cycling parameters were as described previously.

2.5. Sequence and phylogenetic analysis

Forward and reverse sequence reads were assembled using Sequencher™ Version 3.1 (Gene Codes Corporation, 1999). Homology searches with the full *Sv-cpl-1* cDNA sequence were performed using BLAST, and CPL amino acid sequences for a number of species were aligned using CLUSTAL W (http://www.ebi.ac.uk/clustatlW/). Putative signal peptide cleavage sites were identified using Signal P (Nielsen et al., 1997) at www.expasy.org/tools/signalP and TargetP (Emanuelsson et al., 2000) at www.expasy.org/tools/targetP. Percentage similarity was determined using mean character differences calculated in PAUP (Swofford, 2002).

2.6. Generation of the S. vulgaris cpl-1 rescue clone

A construct containing the *S. vulgaris cpl-1* cDNA was generated using restriction digest and cloning steps (Britton and Murray,

Table 1
PCR primers used in primer walking strategy to isolate the genomic sequence of *Sv-cpl-1*. Nucleotides in italics indicate bases anchored in an intron. Exon positions are based on the cDNA sequence.

	Primer combination	Forward (F) primers and positions	Reverse (R) primers and positions
5′ end	SvF3 and SvR5	5′-CAATGTTCCGGCTGTTATC-3′-2 – 17 cDNA	5′-*CTTAC*AAGATCAGCAATGCTG-3′*Intron* 3/exon 3
	SvF3 and SvR7	5′-CAATGTTCCGGCTGTTATC-3′-2 – 17 cDNA	5′-*CTTAC*CAAAGGACTCCTTG-3′*Intron* 2/exon 2
	SvF4 and SvR1	5′-ACTTTTGAGATGGGATTG-3′Exon 3	5′-GTCCTCGGCACCGATATC-3′Exon 7
3′ end	SvF5 and SvR4	5′-*CAT TTATCCTTTCAG*GAGAC -3′*Intron* 6/exon 7	5′-CAAACAAGTGGGTAACTG-3′1098-1130 cDNA
	SvF5 and SvR8	5′-*CATTTATCCTTTCAG*GAGAC-3′*Intron* 6/exon 7	5′-*GACTTAC*CTGTTCTTGATTAG-3′*Intron* 9/exon 9

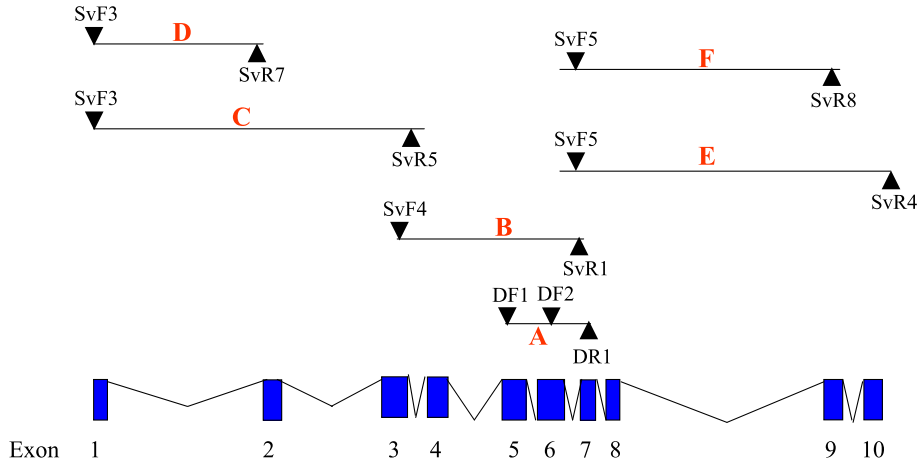

Fig. 1. The genomic organisation of *Sv-cpl-1*. The boxes represent exons and the connecting lines introns. A, B, C, D, E, and F represent the overlapping PCR products produced using the primer walking strategy. Primer details are in Table 1.

2002). The starting construct contained 1.76 Kb of *Ce-cpl-1* upstream sequence, cDNA coding sequence of the *C. elegans cpl-1* gene, and 512 bp *Ce-cpl-1* 3′ untranslated region. This construct was digested with *Sal*I and *Xba*I restriction enzymes to remove the *Ce-cpl-1* cDNA sequence. The resulting fragment containing the 1.76 kb of *Ce-cpl-1* upstream sequence and the 3′ untranslated region was gel-purified and ligated overnight with the *Sv-cpl-1* coding sequence, which had also been digested with *Sal*I and *Xba*I restriction enzymes and gel-purified. The resulting product was transformed into *E. coli* X1 cells by standard methods. Selected clones were screened by direct colony PCR using gene specific primers SvF2 and SvR3. Plasmid DNA from positive colonies was purified using the Spin Miniprep Plasmid kit (Qiagen) and an additional ethanol precipitation step. Insert DNA was sequenced to confirm replacement of the *Ce-cpl-1* cDNA with *Sv-cpl-1* cDNA (MWG, Germany).

2.7. Transformation and rescue of Ce-cpl-1 genetic mutant

The *C. elegans cpl-1* genetic mutant strain used for rescue studies was maintained as a homozygous mutant rescued with the *Ce-cpl-1* cDNA together with *dpy-7: GFP* as a transformation marker (Britton and Murray, 2004). Homozygous *Ce-cpl-1* mutant hermaphrodites produce 95-100% dead embryos and cannot be maintained. The *Ce-cpl-1* transgene was maintained as an extrachromosomal array, with around 70% of worms in each generation carrying the transgene. The *Sv-cpl-1* rescue construct (described above) was microinjected with *C. elegans* marker gene *rol-6* (at final concentrations of 25 ng/μl and 100 ng/μl, respectively) into the distal arm of *Ce-cpl-1/dpy-7: GFP* hermaphrodites using a standard procedure (Mello et al., 1991). Transformed worms, exhibiting the roller phenotype and loss of the *GFP* marker gene, were maintained as discrete lines.

To verify that transformants expressing the *rol-6* phenotype (rollers) contained the *Sv-cpl-1* construct and had lost the *Ce-cpl-1* array, single worm PCR was carried out. Briefly, single adult rollers were picked into 4 μl of lysis buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20, 0.01% gelatin, freshly added 60 μg/ml proteinase K), incubated at −70 °C for 10 min, at 60 °C for 1 h, followed by 95 °C for 15 min to inactivate proteinase K. PCR was carried out using *S. vulgaris* gene specific primers, SvF2 and SvR3, with all of the lysate as template. PCR cycling conditions were as detailed above. Control PCRs were carried out with non-roller adults from each transformed line and N2 Bristol wild type strain. *C. elegans cpl-1* specific primers (Britton and Murray, 2004) were used to confirm the presence of the mutant *Ce-cpl-1* gene within the genome and loss of the wild type *Ce-cpl-1* extrachromosomal array.

2.8. Immunoblotting

The expression of Sv-CPL-1 only in transformed lines was confirmed by immunoblotting. Approximately 75 adult rollers, transferred to eppendorf tubes and washed in PBS, were centrifuged and most of the supernatant was removed except for approximately 20 μl. SDS-loading buffer (4 × concentration; 5% SDS, 1 M Tris–HCl pH 7.5, 80% glycerol, 0.1% Bromophenol blue) was added and the worms were frozen overnight at −20 °C. Samples were incubated with 5% mercaptoethanol, boiled at 95 °C for 10 min, and centrifuged at 13,000 rpm for 5 min. Extracts were then resolved on 12.5% acrylamide gels using the Atto Corporation mini-slab system, and transferred onto a PVDF membrane that was immersed for 1 h in blocking solution (BS, Tris-buffered saline (TBS), pH 7.4, with 5% Marvel, non-fat dried milk). The membrane was incubated overnight at 4 °C in BS containing antibody against the mature region of *C. elegans* CPL-1 (diluted 1:300) (Britton and Murray, 2004). After washing in TBST (Tris-buffered saline containing 0.1% Tween 20), 6 × 5 min, membranes were incubated for 1 h with HRP-conjugated anti-rabbit IgG (Pierce), diluted 1:1500 in BS. They were then washed, incubated in SuperSignal® West Pico Chemiluminescent substrate (Pierce), and developed using Kodak® BioMax light film. Non-rollers and pre-immune serum served as controls.

3. Results and discussion

3.1. Isolation and characterisation of cathepsin L-like cDNA

An initial genomic fragment (approximately 280 bp) amplified from adult *S. vulgaris* served in the design of two internal gene specific primers. These were used in conjunction with either the nematode splice leader SL-1 or Oligo d(T) primers to amplify the complete cDNA sequence in two overlapping cDNA fragments. Gene specific primers, designed to the putative start and stop codons, amplified the entire coding sequence of the *S. vulgaris* cathepsin L cDNA (designated *Sv-cpl-1*), which was identical to the sequence assembled from the 5′ and 3′ fragments (Fig. 2).

Sv-cpl-1 encodes a protein of 354 amino acids with a predicted mass of 39,965 Da, and a calculated pI of 5.93, comprising a signal peptide, a pro-domain and a mature domain. The putative hydro-

Fig. 2. Nucleotide sequence of the cathepsin L cDNA isolated from *S. vulgaris* and the predicted amino acid sequence. The putative signal peptide as deduced by Signal P (Nielsen et al., 1997) is underlined. Putative dibasic residues and a potential *N*-glycosylation site are shown by a dotted and dashed underline, respectively. Vertical lines indicate intron positions. Potential cleavage sites between the pro-peptide and mature enzyme are indicated by arrowheads and are based on comparison with human and other nematode CPLs.

phobic signal peptide sequence has a predicted cleavage site between residues 16 and 17 (Ala-Ser).

Two putative cleavage sites between the pro-domain and the mature domain were identified as Leu^{130} and Ala^{131} and Glu^{136} and Ala^{137}, revealing pro-domains of 114 and 120 amino acid residues, respectively (Fig. 2). Dibasic residues are present approximately 14 amino acids *N*-terminal to the first proposed pro-domain cleavage site. A putative *N*-glycosylation site is present in Sv-CPL-1 at position 124 (Fig. 2 numbering) just preceding the potential pro-domain cleavage sites. The ERFNIN motif, characteristic of non-cathepsin B papain-like proteases, occurs in the pro-domain of Sv-CPL-1. The sequence motif GXNXFXD is highly conserved in Sv-CPL-1, although the Phe is replaced by Ile, as with other nematode CPLs. It occurs in proregions of cysteine proteases of the papain superfamily, and may serve intramolecular processing of papain (Vernet et al., 1995).

The predicted mature domain of 224 amino acids contains the catalytic triad cysteine, histidine and asparagine residues characteristic of cysteine proteases. The predicted amino acid sequence of the mature domain of Sv-CPL-1 had greatest homology to CPLs from *Haemonchus contortus* (91%), *Dictyocaulus viviparus* (90%), *C. briggsae* (88%) and *C. elegans* (87%) respectively of 20 nematode CPLs screened (Fig. 3A). Corresponding values for trematodes are *Fasciola hepatica* (48%), *Schistosoma japonicum* (52%), *S. mansoni* (56%). Phylogenetic analysis showed that Sv-CPL-1 clustered with CPLs from the strongylids, *H. contortus* and *D. viviparus* (Fig. 3B).

3.2. Characterisation of genomic sequence

Obtaining the genomic sequence of *Sv-cpl-1* was problematical using primers designed from the cDNA coding sequence as they consistently amplified two bands; a larger band of expected size inclusive of introns and a smaller band of expected size without introns. Sequencing established the larger band as the *Sv-cpl-1* sequence inclusive of introns, whilst the smaller band had the same sequence as the cDNA.

As the first detailed genomic information on any protease gene from *S. vulgaris*, *Sv-cpl-1* contains 9 introns, the positions of which are conserved between *H. contortus*, *D. viviparus* and *S. vulgaris*. In the relatively small genomic fragment isolated from *S. edentatus* the position of introns 5 and 6 are similarly conserved. Intron size in *S. vulgaris* ranged from 50 bp to 1083 bp, with intron 8, the largest intron, containing 1083 bp. All intron/exon boundaries conformed to the GT/AG rule (Breathnach and Chambon, 1981).

From a *cpl* fragment of approximately 280 bp amplified from *S. edentatus* gDNA, an internal gene specific reverse primer was designed and used in combination with cpl-DF1, which amplified a *cpl* fragment of approximately 400 bp containing two introns. Attempts to amplify any additional genomic sequence were unsuccessful.

A product <300 bp without introns was amplified from *C. catinatum* using *S. vulgaris*-specific reverse primer, SvR1, in combination with cpl-DF1. As with *S. edentatus*, subsequent PCRs failed to amplify additional sequence, and a primer walking strategy would be required to amplify the remaining sequence.

Although the presence of the cDNA copy in genomic DNA indicated possible RNA contamination in all of the above species, RNAse-treated DNA and the use of high quality proof-reading Taq produced similar results. This is consistent with the presence of a processed pseudogene (a possible retrotransposon) in *S. vulgaris*. Retropseudogenes occur in a range of animal and plant species (Harrison et al., 2001) and up to 20% of annotated genes in *C. elegans* may be pseudogenes (Mounsey et al., 2007).

3.3. Rescue of C. elegans genetic mutants with S. vulgaris cpl-1

In the present study *C. elegans* homozygous *cpl-1* mutant hermaphrodites were transformed with the *Sv-cpl-1* transgene, using *rol-6* as a co-transformation marker. Four discrete transgenic lines were established with a mixture of roller and non-roller worms in each line. The *C. elegans* genetic mutant phenotype is embryonically lethal and the *Sv-cpl-1* plasmid resulted in 99% rescue of

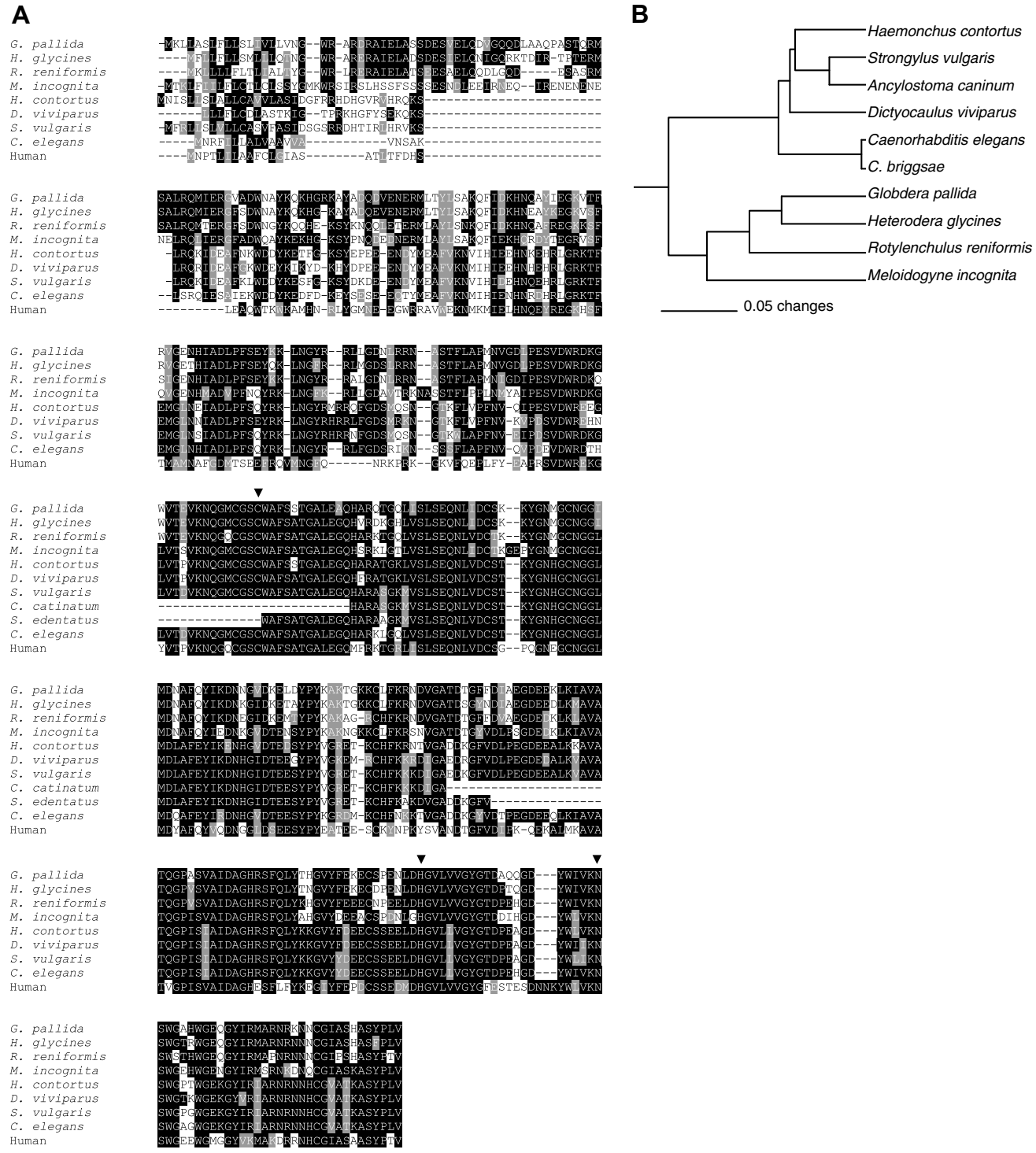

Fig. 3. (A) Amino acid sequence alignment of cathepsin L proteases from human and animal/plant parasitic nematodes. Alignment was carried out using CLUSTAL W programme, with gaps to allow maximum alignment. The cysteine, histidine and asparagine of the catalytic triad are indicated by black arrowheads. (B) Unrooted UPGMA tree based on the deduced amino acid sequences of the mature region of several nematode CPL proteases deposited in Genbank. Sequence alignment was carried out in ClustalW and analyzed in PAUP. Accession numbers for sequences shown are as follows; *Ancylostoma caninum* (AW181523), *C. briggsae* (CBG22599), *C. catinatum* (EU884415) *C. elegans* (Z92812), *D. viviparus* (AY039743), *Globodera pallida* (DQ000609), *H. contortus* (AY004155), *Heterodera glycines* (Y09498), Human, M20496, *Meloidogyne incognita* (AJ557572) *Rotylenchulus reniformis* (AY999066), *S. edentatus* (EU884414), *S. vulgaris* (EU000410).

the mutant phenotype. Non-roller worms, taken from each line, exhibited 100% embryonic lethality.

Sv-cpl-1 was confirmed in the transformed worms by single worm PCR on roller and non-roller worms using *S. vulgaris* gene specific primers SvF2 and SvR3. All rollers contained the *S. vulgaris* cDNA coding sequence whereas non-rollers and wild type N2 Bristol strain did not (Fig. 4A). Western blot analysis with anti-Ce-CPL confirmed expression of CPL-1 protein only in rescued roller worms (Fig. 4B). A band of approximately 42 kDa was evident in all four established roller lines (only lines 1 and 2 are shown in Fig 4B). No band was evident in non-roller worms from each line. Pre-immune serum or only secondary antibody controls did not re-

act with the detected band. These results are similar to that of another study where *H. contortus cpl-1* rescued the *C. elelgans cpl-1* genetic mutant phenotype (Britton and Murray, 2002).

A second band of approximately 41 kDa was also intermittently detected and is most likely due to a post-modification event (such as glycosylation), or processing of the CPL protease. As activation of cathepsin L in both human and fluke is a multi-step process (Ishidoh et al., 1998; Collins et al., 2004), this band could represent another form of the mature enzyme; intermolecular processing of human cathepsin L entails cleavage at two positions (Ménard et al., 1998).

Cathepsin L gene families identified in Trematodes such as *Schistosoma japonicum*, *S. mansoni*, *Fasciola hepatica* and *F. gigantica*, enable feeding/digestion of host tissue (Smith et al., 1993; Day et al., 1995; Dowd et al., 1997; Yamasaki et al., 2002). Trematode CPLs may also suppress or modulate the host immune response (Dalton et al., 2003). A family of CPLs isolated from the filarial nematodes *Brugia malayi* and *B. pahangi*, are associated with larval moulting and multifunctional roles during development (Guiliano et al., 2004). RNA interference (RNAi) has revealed an essential role for cathepsin L- and Z-like proteases in larval moulting in *Onchocerca volvulus* (Lustigman et al., 2004). Larvae exposed to *cpl* and *cpz* double-stranded RNA did not moult, suggesting that cathepsin L was involved in the digestion of old cuticle, degradation of cuticular anchoring proteins, or activation of other enzymes involved in moulting.

CPL proteases are associated with the digestive processes in J2 stages of the plant parasite *Meloidogyne incognita* and may influence the host-parasite relationship (Neveu et al., 2003). RNAi targeting of *M. incognita* cathepsin L (*Mi-cpl-1*), decreased cysteine protease activity in J2s, and decreased the number of egg-laying females (Shingles et al., 2007). In *C. elegans*, CPL-1 is essential for embryogenesis and is required for the correct processing of yolk proteins (Hashmi et al., 2002; Britton and Murray, 2004). A vaccine from *F. hepatica* cathepsin L proteases elicited significant protection (mean, 54%) against infection in cattle, and decreased the number of flukes developing to maturity and egg production (Dalton et al., 1996; Mulcahy et al., 1998).

However, recent work using *C. elegans*-expressed *H. contortus* CPL (Hc-CPL-1) as a vaccine in protection studies in sheep did not decrease egg development or hatching in immunised animals perhaps because the induced antibody did not adequately access the developing embryos (Murray et al., 2007). Localisation of Sv-CPL-1 would be necessary to determine if it is suitable as a vaccine target. Alternatively, this enzyme might be targeted using specific inhibitors of cathepsin L, as some cysteine protease inhibitors are viable antiparasitics (Choe et al., 2005). The cysteine protease inhibitor *N*-Methyl-Pip-F-hF-VSΦ cured infection by *Trypanosoma cruzi* in immunodeficient mice (Doyle et al., 2007).

Novel anthelmintics with different modes of action and novel drug targets are now required due to the prevalence of anthelmintic resistance, especially in small strongyles. As these data suggest a conservation of cathepsin L-like gene function in *Strongylida*, a drug specifically inhibiting strongylid CPLs could generally serve if it inhibited embryogenesis thereby decreasing the incidence of and re-infection by the larva.

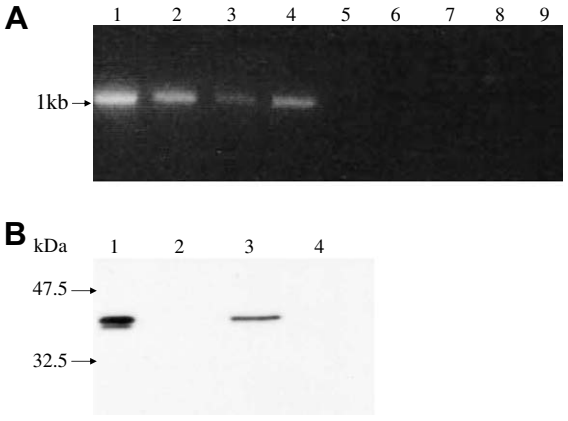

Fig. 4. (A) Single Worm PCR verified the presence of *Sv-cpl-1* in roller worms whereas non-roller and wild type (N2 strain) did not contain the *Sv-cpl-1* gene. Lanes 1–4, *Sv-cpl-1* gene amplified with *S. vulgaris* gene specific primers SvF3 and SvR3, from single roller worms of established lines 1–4, respectively. Lanes 5–8, non-roller worms of established lines 1–4, respectively, and Lane 9, wild type worm amplified with SvF3 and SvR3. (B) Western blot analysis with anti-Ce-CPL-1 antibody on roller (lanes 1 and 3) and non-roller (lanes 2 and 4) worm extracts. CPL was expressed in rescued worms and not in non-transformed worms.

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