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Research review article

Serine/threonine phosphatases in socioeconomically important parasitic nematodes – prospects as novel drug targets?

Bronwyn E. Campbell^a, Adam McCluskey^b, Andreas Hofmann^c, Robin B. Gasser^{a*}

^a*Department of Veterinary Science, The University of Melbourne, Werribee, Victoria 3030, Australia*

^b*Chemistry, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, New South Wales 2308, Australia*

^c*Structural Chemistry Program, Eskitis Institute for Cell & Molecular Therapies, Griffith University, Brisbane, Queensland 4111, Australia*

* Corresponding author. Tel.: +61 3 97312000; fax: +61 3 97312366.
E-mail address: robinbg@unimelb.edu.au (R.B. Gasser).

ABSTRACT

Little is known about the fundamental biology of parasitic nematodes (= roundworms) that cause serious diseases, affecting literally billions of animals and humans worldwide. Unlocking the biology of these neglected pathogens using modern technologies will yield crucial and profound knowledge of their molecular biology, and could lead to new treatment and control strategies. Supported by studies in the free-living nematode, *Caenorhabditis elegans*, some recent investigations have provided improved insights into selected protein phosphatases (PPs) of economically important parasitic nematodes (Strongylida). In the present article, we review this progress and assess the potential of serine/threonine phosphatase (STP) genes and/or their products as targets for new nematocidal drugs. Current information indicates that some small molecules, known to specifically inhibit PPs, might be developed as nematocides. For instance, some cantharidin analogues are known to display exquisite PP-inhibitor activity, which indicates that some of them could be designed and tailored to specifically inhibit STPs of nematodes. This information provides prospects for the discovery of an entirely novel class of nematocides, which is of paramount importance, given the serious problems linked to anthelmintic resistance in parasitic nematode populations of livestock, and has the potential to lead to significant biotechnological outcomes.

Keywords:

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1. Introduction

Nematodes are one of the most diverse groups of organisms on the planet. Some of them are free-living, but many are parasitic and cause health and economic problems worldwide. For example, nematodes of livestock cause substantial production losses to farmers due to poor productivity, failure to thrive and deaths (; ; ; Sackett and Holmes, 2006; Gasser et al., 2008). In particular, strongylid nematodes are of paramount importance as pathogens in livestock (including sheep, goats, cattle and pigs), causing gastro-enteritis and associated complications, often leading to death in severely affected animals (Gasser et al., 2008; Fig. 1). Currently, strongylid nematodes are controlled predominantly through the use of anthelmintics, but widespread resistance against a range of compounds (of three distinct classes) has compromised their efficacy (, ; ; Kaplan, 2004; ; Besier, 2007). Thus, there is an urgent need to work toward identifying new drug targets and developing new nematocides. The ability to define rational drug targets in strongylids is dependent on knowledge of the molecular biology of nematodes, which, at present, is still very limited.

The application of genomic and bioinformatic technologies provides prospects for gaining insights into the molecular biology of parasitic nematodes. Nonetheless, although there have been substantial advances in expressed sequence tag (EST) and genomic sequencing of a number of metazoan organisms, such as mammals, limited progress has been made on socio-economically important parasitic nematodes. Although there is a draft sequence for the filarial nematode, *Brugia malayi* (see Ghedin et al., 2007), there is no genome sequence for any of the socioeconomically important strongylid nematodes. By contrast, the completion of the full genome sequence (with ~20,000 genes) of the free-living nematode *C. elegans* and associated data (available from WormBase; <http://www.wormbase.org>; release WS213) has provided an extremely valuable resource to the scientific community for comparative genome analyses for various organisms, including nematodes, particularly strongylid nematodes, because they are considered to belong to the same clade as *C. elegans* (see Blaxter et al., 1998). *C. elegans* is also a powerful system for functional genomic, biochemical and many other molecular and biological investigations, as it has a rapid life-cycle and is easy to maintain *in vitro*. The karyotype is $2n = 12$ (five pairs of autosomes and one pair of sex chromosomes), which appears to be consistent with a range of strongylid nematodes, including the barber's pole worm, *Haemonchus contortus* (see Redman et al. 2008). Also based on molecular phylogenetic analysis, strongylid nematodes are considered to be relatively closely related to *C. elegans* (see Blaxter et al., 1998), supported by findings from EST projects carried out on 40 nematode species other than *Caenorhabditis*, including 24 parasitic nematodes of mammals, 14 plant parasites and two free-living bacteriovores (Mitrevu et al., 2005). Indeed, the strongylid datasets (Parkinson et al., 2004) have the highest genetic similarity to *C. elegans* compared with the other taxa examined to date, based on the cumulative number of new genes found in each species. Both the distribution of homology matches and their relative scores also support a close relationship between strongylids and *C. elegans* (see Parkinson et al., 2004). Therefore, the extrapolation from the biology of the well-studied nematode, *C. elegans*, can be of significant benefit when investigating strongylids; the chance that a cloned gene from a strongylid nematode has a homologue/orthologue in *C. elegans* is high, with the exception of genes associated with host-parasite interactions. As there are no reliable culturing systems available for the propagation and maintenance of the entire life cycle of strongylid nematodes *in vitro*, *C. elegans* also provides a useful surrogate system to test the function of orthologous/homologous genes (e.g., Britton and Murray, 2006; Massey et al., 2006; Hu et al., 2010), although some caution is required in the interpretation of experimental findings (Nikolaou and Gasser, 2006).

Over the years, we have been pursuing the characterization of a number of gender-enriched molecules in strongylid nematodes, using a comparative genomic-bioinformatic

approach and using *C. elegans* as a reference organism, with a perspective of discovering new drug targets (e.g., Cottee et al., 2006; Nisbet et al., 2004, 2008; Cantacessi et al., 2009; Ranganathan et al., 2009; Doyle et al., 2010; Hu et al., 2007a,b, 2008, 2010a,b,c; Campbell et al., 2008a,b, 2010). Through a number of investigations, we have provided insights into genes encoding protein phosphatases (PPs) for selected strongylid nematodes (Boag et al., 2003; Hu et al., 2007a; Campbell et al., 2010). In the present article, we review this progress and assess the potential of these phosphatase genes or their products as targets for new nematocidal drugs.

2. Background on protein phosphatases (PPs) – with a perspective on nematodes

The phosphorylation/dephosphorylation of proteins is a fundamental, post transcriptional process regulating their function. While protein kinases transfer a phosphate from ATP to a protein (i.e. phosphorylate), protein phosphatases (PPs) catalyse the removal of phosphate groups from specific residues of proteins (i.e. dephosphorylate) (Barford, 1996; Barford et al., 1998; Oliver and Shenolikar, 1998; Cohen, 2002; Gallego and Virshup, 2005). PPs are involved in key biological processes, such as cell division, ion channel electrophysiology, neuronal activity, apoptosis and exocytosis (Cohen, 1997; Oliver and Shenolikar, 1998; Herzig and Neumann, 2000; Klumpp and Krieglstein, 2002; Berndt, 2003; Sim et al., 2003). Based on function, PPs can be divided into tyrosine phosphatases (usually membrane-bound and often involved in receptor-mediated signal transduction) (Sarmiento et al., 1998) and serine/threonine phosphatases (STPs; usually located to the cytoplasm of the cell and often involved in signal transduction and/or transcriptional activation) (Barford et al., 1998). Based on substrate and inhibitor specificities, protein phosphatases can also be divided into four classes, PP1, PP2A, PP2B and PP2C (Barford et al., 1998). Classes PP1 and PP2A are holoenzymes, requiring the catalytic protein to be complexed with regulatory proteins linked to the targeting and regulation of their activity (Barford et al., 1998). Some of these PPs are known to be involved in reproductive processes in various animals (e.g., Kitagawa et al., 1990; Armstrong et al., 1995; Chin-Sang and Spence, 1996; Chun et al., 2000; Choi et al., 2002). Specifically, some are associated with spermatogenesis and/or the regulation of sperm motility (Smith et al., 1996; Varmuza et al., 1999). Interestingly, genome-wide profiling of transcription for *C. elegans* has shown that a large percentage ($\geq 50\%$) of protein phosphatases are enriched in the germline tissues producing spermatozoa (Reinke et al., 2000).

3. Insights into PPs from economically important strongylid nematodes

3.1. Identification and initial characterisation

In a number of previous EST and microarray studies (Boag et al., 2001; Nisbet and Gasser, 2004; Cottee et al., 2006; Campbell et al., 2008a, 2010), we identified male-enriched sequence tags encoding PPs from *Oesophagostomum dentatum*, *Trichostrongylus vitrinus* and *Haemonchus contortus* (Strongylida). Preliminary examination of these tags showed that they had (at the amino acid level) high sequence similarities/identities to yeast *glc* seven-like (*gsp*) phosphatases from *Caenorhabditis elegans*. Functional data available through WormBase (www.wormbase.org) indicated that several *C. elegans gsp* homologous/orthologous (including *gsp-1*, *gsp-2* and *gsp-3*) could be silenced by double-stranded RNA interference (RNAi), resulting in phenotypes, such as progeny with Egl (egg laying deficit), Emb (embryonic lethal), Gro (slow growth), Lvl (larval lethal) and/or Sck (sick) and/or Ste (maternal sterile). Effective silencing of such genes in *C. elegans* showed that they are central to the development, reproduction and/or survival of this free-living nematode, and stimulated our investigations of the orthologues in related strongylids.

Therefore, we isolated and characterized full-length complementary and genomic DNAs encoding STPs from selected strongylid nematodes, conducted comparative analyses at the genomic level, investigated transcription in different developmental stages, localised expression and/or predicted genetic interaction networks for these molecules (Boag et al., 2003; Hu et al., 2007a; Campbell et al., 2010).

3.2. Detailed characterisation of *stp* genes and inferred gene products

The cDNAs encoding STPs isolated from *O. dentatum*, *T. vitrinus* and *H. contortus* were ~1000 nucleotides in length (accession nos. AF496635, AM691045 and AF496634; Fig. 2). The uninterrupted ORFs of 933-951 nucleotides encoded predicted proteins of 311-316 amino acids (aa), which all contained a specific STP pattern [LIVMN]-[KR]-G-N-H-E; the Prosite motif PS00125 was consistent with the gene C47A4.3 from *C. elegans* (see Fig. 2). Comparisons of STPs with sequences in non-redundant databases by BLASTx analysis showed that inferred STPs from strongylid nematodes had significant homologies to sequences from a range of organisms, including other nematodes, protists, vertebrates and even plants (see Table 1). However, the greatest protein sequence homology (E-values: 1e-98 to 4e-104) was to sequences inferred from the *C. elegans* genes *gsp-4* and *gsp-3*, *gsp-2* and *gsp-1*, C47A4.3 and C09H5.7, all of which encode the PP1 class of PPs (Barford et al., 1998). An alignment of the predicted STP sequences from the three strongylid nematodes (*O. dentatum*, *T. vitrinus* and *H. contortus*) with selected representatives from different eukaryotic groups (Fig. 2) demonstrated 53-56% identity with plant, animal and yeast proteins. In spite of relatively high homology among the STPs inferred from the strongylids, there were significant differences in gene structure as well as size and number of exons/introns (Fig. 3), suggesting variation in gene regulation and transcription. For example, the genes *Od-mpp-1* (10271 bp) and *Tv-stp-1* (5041-5362 bp) were substantially longer than the *Hc-stp-1* gene (2854 bp). Nonetheless, the numbers of exons (10) and introns (nine) for the genes *Hc-stp-1* and *Tv-stp-1* from trichostrongylid nematodes were the same (see Fig. 3).

Phylogenetic analysis of protein sequence data showed clearly that the STPs from strongylid nematodes grouped (with strong support) with *C. elegans* GSP-3 and GSP-4 to the exclusion of related molecules from protists, vertebrates and plants, and also to the exclusion of both GSP-1 and GSP-2 of *C. elegans* (Fig. 4). Clearly, sequence homology among STPs (Fig. 2) was most pronounced in the central region of the molecules, where their catalytic activity is predicted to be modulated by eight conserved residues (Asp 61, His 63, Asp 92, Asp 95, Asn 121, His 171, His 246 and Tyr 270; Fig. 2), known to coordinate the binding of two metal ions (Mn^{2+} and Fe^{2+}) for a range of species (Barford et al., 1998). An additional residue (His 122) acts as a proton donor to catalyse the cleavage of the phosphate group from phospho-serine or -threonine (Barford et al., 1998). The alignment also revealed that the N- and C-terminal regions of the proteins are more variable than the catalytic part. These regions are proposed to interact with proteins that regulate the phosphatase activity (Egloff et al., 1997).

3.3. Three dimensional structural modelling

Comparative protein structure modelling indicate that catalytic residues and structural features around the catalytic site are conserved for nematode STPs (Campbell et al., 2010; cf. Fig. 5). Structural differences were predicted only in the ligand-binding interface, which supports the concept that a specific regulatory subunit confers the unique characteristics of an individual STP holoenzyme. For example, STPs from strongylids have 38-56% amino acid similarity/identity to *C. elegans* proteins GSP-3 and GSP-4, for which some biochemical and functional information is available (see <http://www.wormbase.org/>). These latter two

molecules belong to a group of at least eight PP1s (*gsp-1*, *gsp-2*, *gsp-3*, *gsp-4*, C06A1.3, 09H5.7, C47A4.8 and F58G13) in *C. elegans*, some of which are known to be involved in spermatogenesis in this free-living nematode (Hanazawa et al., 2001; Jiang et al., 2001).

Specific transcription in adult male strongylids (Boag et al., 2003; Hu et al., 2007a; Campbell et al., 2010) also suggests a key role for this protein in male-specific biological processes, such as spermatogenesis. Although no functional data are yet available for these molecules in strongylids, in *C. elegans*, proteins GSP-3 and GSP-4 are expressed abundantly in sperm, predominantly around meiotic and mature DNA (Chu et al., 2006). In particular, these two PP1s have been associated specifically with chromatin in the pachytene-diakinesis transition, meiosis (MI and MII), anaphase I and in mature sperm cells (Chu et al., 2006). An assessment of functionality using RNAi has indicated aberrant chromosome segregation, resulting in male sterility and in embryos surrounded by soft egg-shells (Hanazawa et al., 2001; Chu et al., 2006).

3.4. Genetic interaction networking

Probabilistic functional genetic interaction data for *C. elegans* orthologues (*gsp-3* and *gsp-4*) also supports their involvement in male-specific biological processes (summarized in Table 2). The gene *gsp-3* is predicted to interact with *gsp-4*, a serine/threonine kinase (*akt-2*) gene and also with various genes encoding major sperm and sperm-specific proteins, a glutathione S-transferase as well as a glutathione synthetase (Table 2). The *akt-2* gene and its product have been well characterized in *C. elegans* and are integral in the regulation of DNA damage-induced apoptosis in the male germline (Quevedo et al., 2007).

The serine/threonine kinases AKT-1 and AKT-2, encoded by *akt-1* and *akt-2*, respectively, are integral in the insulin-like growth factor 1 signalling pathway, which is relatively conserved among a range of organisms (Padmanabhan et al., 2009; Shaw and Dillin, 2009). This pathway regulates essential processes, such as growth, development, reproduction and longevity (Padmanabhan et al., 2009; Shaw and Dillin, 2009). Given that AKT-2 and its interactor AKT-1 are both linked to anti-apoptotic activity, GSP-3 and its homologues/orthologues in strongylid nematodes might have a role in dephosphorylating the proteins that the two kinases phosphorylate and in regulating apoptosis in the male germline. AKT-2 is known to be expressed in somatic and vulval muscles as well as the spermatheca (Padmanabhan et al., 2009). RNAi and co-immunoprecipitation studies indicate that a regulatory subunit of a PP2A holo-enzyme (designated PPTR-1) interacts directly with AKT-1 and, in turn, modulates processes, such as dauer formation and longevity in *C. elegans* (Padmanabhan et al., 2009; Shaw and Dillin, 2009). Both AKT-1 and AKT-2 are also implicated in the regulation of lifespan and dauer formation in nematodes via the repression of the forkhead transcription factor DAF-16 (Vanfleteren et al., 1999; Shaw and Dillin, 2009), inducing adult development and a short lifespan, whilst inhibiting dauer formation and a long lifespan. This information indicates that GSP-3 homologues in nematodes might also play an indirect, albeit significant role in these crucial biological processes. Similarly, the GSP-4 is predicted to interact with GSP-3, as well as major sperm protein, sperm-specific family protein (groups S, P and Q), indicating that the two STPs are essential for male germline development.

3.5. Profiling transcription, and comparisons among nematodes

Additional, indirect support for a male-specific role of some STPs of strongylid nematodes can be suggested based on findings from previous microarray analyses of *C. elegans* (Reinke et al., 2000; Jiang et al., 2001). For instance, Reinke et al. (2000) reported that ~50% of the phosphatases and ~30% of the kinases predicted to be encoded are linked to sperm production in both hermaphrodites and males of this nematode. These

studies have suggested that the abundance of these molecules could explain the observation that the terminal differentiation of spermatids into motile spermatozoa occurs without gene expression, as ribosomes are discarded during spermatid development (Nelson et al., 1982). This information would suggest that all of the proteins needed for this differentiation are produced before the ribosomes are lost. Thus, it is possible that the functions/activities of these proteins might be regulated by other means, such as through dephosphorylation and phosphorylation via the involvement of phosphatases and kinases, respectively. Another possibility is that some of these enzymes relate specifically to the fertilization of oocytes, perhaps being involved in signal transduction cascades and/or in the modification of oocyte proteins. A study (Hanazawa et al., 2001) of the transcription profiles of two mutant strains of *C. elegans* hermaphrodites, one (*fem-3*) producing sperm but not oocytes, and another strain (*fem-1*) producing oocytes but not sperm, showed that the levels of transcription for two PP1 genes (*gsp-3* and *gsp-4*) was significantly higher in sperm-producing hermaphrodites compared with those producing only oocytes. In addition, a number of investigations of various, related *stp* genes (Reinke et al., 2000; Jiang et al., 2001; Boag et al., 2003; Hu et al., 2007a; Campbell et al., 2010) suggest that they are associated with 'male' germline tissues/processes and with either sperm maturation, motility and/or the capacity of sperm to fertilize oocytes. Previous studies (Pilgrim et al., 1995; Chin-Sang and Spence, 1996; Mehra et al., 2006) have also demonstrated that the negative regulation of the genes *fem-1*, *fem-2* and *fem-3*, required for male development in *C. elegans* (via the product of the *tra-2* gene) leads to the normal development of female worms. The *fem-2* gene encodes a type 2C STP, indicating that this molecule plays a key role in sex-determination in *C. elegans*. Moreover, this phosphatase has been shown to be important in spermatogenesis and normal somatic development of the male worm (Kimble et al., 1984; Hansen and Pilgrim, 1998). The *tra-2* gene is predicted to encode a membrane protein, whereas *fem-1* codes for a novel protein containing ANK (amino acid code) repeats. The *fem-3* gene product is also a novel protein (cf. Pilgrim et al., 1995; Chin-Sang and Spence, 1996; Mehra et al., 2006) with no homology to any known protein in current databases.

The genes encoding STPs in the three strongylids studied to date are transcribed in developing and reproductively active males, which reflects the transcription profiles for homologues in *C. elegans* (which are also restricted to reproductively-active stages) (Boag et al., 2003; Hu et al., 2007a; Campbell et al., 2010). This information suggests that these genes and their *C. elegans* homologues/orthologues have similar biological functions in males and sperm-producing hermaphrodites, respectively. In addition, the apparent conservation of two closely linked GATA transcription factor-binding motifs in the promoters of selected *stp* genes in *C. elegans* and in some other male-specific genes (such as *msps* in nematodes; Klass et al., 1988; Scott et al., 1989) suggests that there is a common element regulating components of germline expression in male nematodes. Hence, detailed molecular and biochemical analyses in *C. elegans* could also have important implications for understanding developmental and reproductive processes in strongylid nematodes of economic importance in animals, although caution is required in the interpretation of findings, given the biological differences between free-living and parasitic nematodes (Nikolaou and Gasser, 2006).

3.6. Inference of gene function and expression

As there is currently no effective approach for the perturbation of gene expression *in vivo* in strongylid nematodes (reviewed by Geldhof et al., 2007; Knox et al., 2007), comparative functional genomic studies might assist in inferring function(s) for STPs from parasitic nematodes using *C. elegans* as a surrogate system. Some results for *O. dentatum* (Boag et al., 2003) using homologues/orthologues in *C. elegans* (see Reinke et al., 2000; Hanazawa et al., 2001; Jiang et al., 2001) have already indicated that STPs can be

functionally involved in reproductive processes, including spermatogenesis, and appear to reduce the capacity of the sperm to fertilize oocytes, although the precise mechanism by which this occurs remains to be elucidated. Interestingly, some *stp* genes (e.g., C47A4.3) subjected to high throughput RNAi analysis do not have 'non-wildtype' phenotypes (Maeda et al., 2001; Kamath et al., 2003; Rual et al., 2004; Sonnichsen et al., 2005). The reason for this discrepancy is not yet clear, but it is possible that there is some functional redundancy in STPs or that the RNAi phenotype was not sufficiently evident to be detected in large-scale screens, or that the *C. elegans* strains and/or different RNAi approaches used produced phenotypes of differing penetrance. In spite of distinct differences in genomic organization between *stp* genes in strongylids and orthologues in *C. elegans*, genetic complementation studies, similar to that performed recently for *daf-16* (Hu et al., 2010c), might be attempted to restore *gsp-3* and *gsp-4* functions in a *C. elegans* strain carrying a null mutation at these loci.

Linking gene function to the localisation of expression is likely to be central to understanding the biological roles of STPs. In a study of transgenic *C. elegans* (promoter::reporter gene constructs), Boag et al. (2003) showed that the expression of *C. elegans* PP1s (encoded by genes *gsp-3* and *gsp-4*), which are related to *Od-MPP1*, *Tv-STP-1* and *Hc-STP-1*, was not restricted to the germline. Indeed, expression in neuronal or neuron-associated cells and musculature was inferred. The promoters directed green fluorescent protein (GFP) expression of the translational fusion-constructs throughout the cell bodies, indicating that the proteins were not localised to any particular subcellular organelle, which suggested indirectly that they were localised in the cytoplasm. The *gsp-3* promoter directed reporter-gene expression to the tail regions of adult males *C. elegans*, inferred to be linked to either the HOA or HOB neurons. Interestingly, a large serine/threonine-rich protein, LOV-1, has been identified in *C. elegans* (see Barr and Sternberg, 1999). LOV-1 is expressed in various male neurons, including the HOA and HOB, and associated with aspects of male mating behaviour, such as locating the vulva (Barr and Sternberg, 1999). Although the function of this protein has not been unequivocally determined, it might be involved in the signal transduction of chemo-sensation and/or mechano-sensation during the mating process. If the *gsp-3* and homologous genes are expressed in these or similar neurons, it is possible that the serine/threonine-rich domain present in the LOV-1 protein represents a substrate for the phosphatase activity linked to this gene. This information suggests that, in addition to their involvement in spermatogenesis, some male-enriched PP1s could function in neural signalling linked to mating behaviour of the male nematode. Although the neural anatomy of strongylid nematodes is not yet known in detail, it is possible that there is similarity to that of *C. elegans* (see Brownlee and Fairweather, 1999; Nikolaou and Gasser, 2006). The inferred localisation to and/or function of key STPs in the germline, neuronal tissue, neuron-associated cells and musculature suggest prospects for the development of specific inhibitors of spermatogenesis and/or mating as anthelmintic drugs.

4. Conclusions and biotechnological prospects

The present review shows that some STPs are quite conserved between parasitic and free-living nematodes, whereas others are distinctly different, perhaps reflecting their roles in distinct pathways required for the growth, development, survival and/or reproduction in nematodes. Importantly, phylogenetically, some STPs are specific to nematodes and group to the exclusion of related molecules in other invertebrates and vertebrates. Future work should focus on improved functional aspects of STPs in parasitic nematodes, using, for example, gene knockout/complementation in *C. elegans* as the surrogate system. Although some progress in functional genomics has been made in parasitic nematodes (Grant et al., 2006; Geldhof et al., 2007; Lok, 2007, 2009), no reliable and practical gene knockout/complementation method is yet available for any strongylid nematode. Therefore,

developing a technique for the functional analysis of STPs and related PPs would enable fundamental insights into their biology, which could also assist in defining one or more novel anthelmintic targets amenable to the design of specific inhibitors of essential reproductive and/or developmental pathways in nematodes. Current literature does indicate some prospect for small molecules, known to specifically inhibit PPs, to be developed as nematocides. For example, inhibitors, such as cantharidin (from the blister beetle, *Mylabris*; see McCluskey et al., 2002, Hill et al., 2007, Stewart et al., 2007) and a number of analogues with the same pharmacophoric units but no adverse toxic effects on well-defined, cultured human cells (Sakoff et al., 2002; McCluskey et al., 2003; Hart et al., 2004; Hill et al., 2007), show unique potential for the development of nematocides. The latter characteristic is important, as the focus must be on identifying compounds that are lethal to the nematode or block reproduction but have no adverse effect on mammals (i.e. the host). Some cantharidin analogues are known to display exquisite PP1 and PP2A inhibitor activity, which indicates that some of them could be designed to specifically inhibit STPs of nematodes (Fig. 5). Indeed, preliminary work conducted by us has shown that some cantharidin analogues, which have no toxic effect on human cell lines, kill nematode larvae. Although the mechanisms of action are not yet known in parasitic nematodes, homology modelling and *in silico* docking studies have suggested that some prototype molecules designed bind specifically to pockets in the STPs of strongylid nematodes (A. McCluskey et al., unpublished results). Clearly, these initial findings indicate an exciting opportunity for the discovery of an entirely novel class of nematocides. This prospect is of paramount importance, given the serious problems linked to anthelmintic resistance in parasitic nematodes, and could lead to the development of a new anthelmintic and major biotechnological outcomes.

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LEGENDS

Fig. 1. Life cycle representing a number of parasitic nematodes (order Strongylida) of the alimentary tract of livestock, such as sheep, goats, cattle and pigs (adapted from Gasser et al., 2008). The adult worms exist as females and males (inset shows the typical spicules in the tail of the male). The females produce relatively large numbers of typically ovoid, strongylid eggs (70-150 μm), which are excreted in the faeces into the external environment. The first-stage larva (L1) develops inside the egg to then hatch (within 1-2 days, depending on environmental conditions) and develops through to the second-stage larva (L2). The L1s and L2s feed on bacteria and other microorganisms in the external environment (faeces). After the moults, the ensheathed third-stage larva (L3) develops (usually within 1-2 weeks, depending on species, temperature, humidity, pH and/or other factors). The cuticular sheath around the L3 prevents it from feeding but protects it from relatively harsh environmental conditions. After the L3 is ingested by the animal and passes through the stomach(s), it exsheaths (xL3) and (after a tissue phase) develops through to the fourth-stage larva (L4) and then the adult at the predilection site in the alimentary tract. The time from the xL3 to the production of eggs by the adult female is usually 3-4 weeks. Specific processes or features of the development of such strongylid nematodes include: embryogenesis (E); sexual differentiation (S), sexual reproduction (R); microbial feeding phase (F1); feeding phase inside the alimentary tract (F2); rapid growth phases (G); the potential to undergo hypobiosis (arrested development) (*). Clinical signs, such as diarrhoea and/or anaemia, can be caused by the parasitic stages of these nematodes (see images, top left).

Fig 2. Alignment of the inferred amino acid sequence for the *Haemonchus contortus* serine/threonine phosphatase (Hc-STP-1) with related molecules from *Trichostrongylus vitrinus* (Tv-STP-1, accession no. CAM84506), *Oesophagostomum dentatum* (Od-MPP-1, accession no. AF496634), *Caenorhabditis elegans* (Ce) genes C47A4.3, *gsp-4* and *gsp-3*, *gsp-2* and *gsp-1* (accession nos. CAB62794, NP_491237, NP_491429, NP_001022616 and NP_505733 respectively), and *Homo sapiens* protein phosphatase 1 (Hs-PP1; accession no. AAV38549). Amino acids predicted to be involved in the catalytic pocket (metal binding) of the enzyme are indicated by asterisks, and the histidine residue predicted to be involved in proton donation is indicated with an arrow. Bold, black and blue letters define amino acids that are conserved, conserved/semi-conserved substitutions and non-conserved residues, respectively. The Prosite motif PS00125 is indicated with a horizontal line.

Fig. 3. Organization of individual *stp* genes from *Haemonchus contortus*, *Trichostrongylus vitrinus* and *Oesophagostomum dentatum* (Strongylida) as well as related genes from *Caenorhabditis elegans*. The organization was determined by aligning the cDNA and genomic DNA sequences, with intron-exon boundaries defined using the AG-GT rule (Breathnach and Chambon, 1981). Numbers above the boxes indicate the length (nucleotides) of exons, whereas numbers below the lines indicate the intron lengths. Black boxes represent exons, whilst lines represent introns.

Fig. 4. Phylogenetic tree displaying the relationships of serine/threonine phosphatases (STPs) from *Haemonchus contortus*, *Trichostrongylus vitrinus* and *Oesophagostomum dentatum* (Strongylida; bold-type) with those of *Caenorhabditis elegans* and other organisms for which full-length protein sequences are available in current databases (<http://www.ncbi.nlm.nih.gov> and <http://www.ebi.ac.uk/parasites/parasite-genome>), using serine-threonine phosphatase sequences from *Aspergillus* (fungus) as outgroups. Nodal support of $pp \geq 0.80$ is indicated. The area shaded in grey indicates a group of STPs that is inferred to be specific to nematodes ($pp = 0.97$).

Fig. 5. Left panel: Inference of the inhibition of the active site of a serine-threonine phosphatase (STP) by cantharidin. The ligand binding mode shown was generated by a super-position of the complex of human PP5C with cantharidin (PDB accession code 3h62; Bertini et al., 2009) using the homology model of serine-threonine phosphatase-1 (called *Hc*-STP-1) from the nematode *H. contortus* (see Campbell et al., 2010). Right panel: For comparison, the same ligand-binding mode was generated for human PP1 (*Hs*-PP1; PDB accession code 3e7a; Kelker et al., 2009). The images were prepared using PyMol (DeLano, 2002). The protein surfaces are coloured according to electrostatic potentials (red: electro-negative; blue: electro-positive); the metal ions in active sites are indicated as magenta spheres. Features of the clefts (shape and electrostatics) in the active sites differ between the proteins from nematode and human, indicating that a nematode-specific inhibitory effect might be achieved using one or more cantharidin analogues.

Table 1

Serine/threonine phosphatase sequences (and relevant information from BLASTx analysis) used for phylogenetic analysis (cf. Fig. 4)

Group	Species	Protein accession no.	Gene accession no.	Gene name/locus tag	Length	Identity (%)
Amoeba	<i>Dictyostelium discoideum</i>	XP 643639	DOBO185058	PPPB	321	53
Amphibian	<i>Xenopus laevis</i>	AAH72730.1	BC072730	MGC79074	327	53
Arthropod	<i>Apis mellifera</i>	XP 393296.2	XM 393296	L0C409804	328	52
Choanoflagellate	<i>Monosiga brevicollis</i>	XP 0017485	XM_001748529.1	-	307	54
Chordate	<i>Oikopleura dioica</i>	AAS21337.1	OO2-15	<i>gsp-1</i>	322	53
Echinoderm	<i>Strongylocentrotus purpuratus</i>	XP 001175801	LOC752338	-	324	51
Fish	<i>Carassius auratus</i>	ABQ18261	ABQ18261	-	327	53
	<i>Danio rerio</i>	CAD61270.1	AL929207	-	327	52
	<i>Scophthalmus maximus</i>	ABC94584	DQ364569.2	-	327	53
Fungus	<i>Ashbya gossypii</i>	NP985713	4621968	AGOS_AFR166C	314	51
	<i>Aspergillus clavatus</i>	XP001269665	4700735	ACLA_029690	324	53
	<i>Aspergillus fumigatus</i>	XP750244	3507427	AFUA_1G04950	323	52
	<i>Aspergillus nidulans</i>	XP658014	2876189	AN0410.2	323	53
	<i>Aspergillus terreus</i>	XP001211774	4317084	ATEG_02596	324	53
	<i>Coccidioides posadasii</i>	AAW70557	AY742892.1	PP1	325	53
	<i>Coprinopsis cinerea okayama</i>	XP_001832383.1	XM_001832331.1	CC1G_07643	333	53
	<i>Cryptococcus neoformans</i>	XP569133	3255713	CNB02030	328	51
	<i>C. neoformans</i>	XP569131	3255713	CNB02030	314	52
	<i>Laccaria bicolor</i>	XP_001881229	XM_001881194.1	LACBIDRAFT_294311	331	53
	<i>Phaeosphaeria nodorum</i>	XP0017908	XM_001790816.1	-	321	51
	<i>Ustilago maydis</i>	XP759227	3631163	UM03080.1	331	52
Mammals	<i>Homo sapiens</i>	BAA82664	AB030255.2	-	294	56
	<i>Macaca mulatta</i>	XP001108364	709935	LOC709935	326	52
	<i>Mus musculus</i>	BAC40733.1	AK089067.1	-	327	53
	<i>M. musculus</i>	AAC53385.1	AAC53385	PP1cgamma	337	52
	<i>Pan troglodytes</i>	XP001144460	462795	PPP1CC	304	54
Nematode	<i>Ascaris suum</i>	CAJ98743	AM261207.1	-	308	60
	<i>Brugia malayi</i>	XP001902172	6105592	Bm1_53525	317	61
	<i>B. malayi</i>	XP001897890	6101334	Bm1_32220	304	62
	<i>B. malayi</i>	XP001894042	6097494	Bm1_12875	334	54
	<i>Caenorhabditis briggsae</i>	XP_002632333	XM002632287	CBG00341	304	55
	<i>C. briggsae</i>	CAE57617	CAE57617	-	333	52
	<i>C. briggsae</i>	XP_002631681	XM_002631635	CBG20874	305	56
	<i>C. briggsae</i>	XP_002642146	XM_002642100	CBG18099	308	55
	<i>Caenorhabditis elegans</i>	CAB62794	C47A4.3	-	333	55
	<i>C. elegans</i>	NP505733	F29F11.6	<i>gsp-1</i>	329	54
	<i>C. elegans</i>	NP001022616	F56C9.1	<i>gsp-2</i>	333	52
	<i>C. elegans</i>	NP491429	W09C3.6	<i>gsp-3</i>	305	56
	<i>C. elegans</i>	NP491237	T03F1.5	<i>gsp-4</i>	305	56
	<i>C. elegans</i>	CE35227	C09H5.7	-	333	55
	<i>C. elegans</i>	CE02116	C06A1.3	-	364	39
	<i>C. elegans</i>	CE35562	F58G1.3	-	364	38
	<i>Oesophagostomum dentatum</i>	AAO85519	AF496635.1	<i>Od-mpp1</i>	311	54

Plant	<i>Trichostrongylus vitrinus</i>	CAM84504-9	AM691044-9.1	<i>Tv-stp1</i>	316	90
	<i>Arabidopsis thaliana</i>	NP200724	836034	TOPP2	312	53
	<i>Malus pumila</i>	AAD56010	AF178530.1	PP1	316	54
	<i>Oryza sativa</i>	EAZ25104	CM000139.1	-	344	51
	<i>Vitis vinifera</i>	CAO65054	CAO65054	-	345	53
Platyhelminth Protozoa	<i>Zea mays</i>	NP001105341	542269	prh1	316	51
	<i>Schistosoma japonicum</i>	AAW24965	AY813233.1	-	314	53
	<i>Chlamydomonas reinhardtii</i>	AAD38856	AF156101.1	-	304	55
	<i>Cryptosporidium hominis</i>	XP667490	3415346	Chro.70303	320	52
	<i>Cryptosporidium parvum</i>	XP001388388	3371830	cgd7_2670	320	52
	<i>Leishmania braziliensis</i>	XP001566103	5416977	LbrM28_V2.0710	301	54
	<i>Leishmania infantum</i>	XP001470105	5070290	LinJ28.0700	301	54
	<i>Leishmania major</i>	XP001684347	5653275	LmjF28.0690	301	54
	<i>Toxoplasma gondii</i>	ABD96038	DQ437871.1	-	306	53
	<i>Trypanosoma brucei</i>	EAN79771	CH464491.1	-	303	54
Yeast	<i>Trypanosoma cruzi</i>	XP815599	3547356	Tc00.1047053508815.110	303	54
	<i>Candida albicans</i>	XP711142.1	3647257	<i>glc-7</i>	330	52
	<i>Saccharomyces cerevisiae</i>	NP0110	NC_001137.2	-	312	51
	<i>Schizosaccharomyces pombe</i>	NP596	2542632	<i>cdc25</i>	327	53

Table 2

Genetic interactions and gene ontology terms inferred for *Caenorhabditis elegans* orthologues (*gsp-3* and *gsp-4*) of STPs from strongylid nematodes studied to date (*Haemonchus contortus*, *Trichostrongylus vitrinus* and *Oesophagostomum dentatum*). Molecules include those associated with reproduction, protein phosphorylation, embryonic and larval development. Molecular functions include serine/threonine kinase, hydrolase and structural molecule activities. Cellular locations include nucleus, cytoplasm, membrane and both intracellular and extracellular spaces. No information available (-)

<i>gsp-3</i>	Gene Ontology (GO) terms		
Gene code (name)	Biological process	Cellular component	Molecular function
B0379.2	-	-	-
B0379.7	-	-	-
C04G2.8	-	-	-
C04G2.9	-	-	-
C05B5.2	-	-	-
C06A8.6	Antibacterial humoral response (sensu Protostomia)	Extracellular region	-
C10G11.8	Protein catabolic process	Nucleus, cytoplasm	Nucleotide binding, ATP binding, hydrolase activity, nucleoside-triphosphatase activity
C10G11.9	-	-	-
C14C10.1	Transport	Membrane	Binding
C14C11.1	-	-	-
C16A11.7	-	-	-
C24D10.7 (<i>nspd-3</i>)	-	-	-
C25A8.1	-	-	-
C25G4.6	Reproduction	-	Protein binding
C35D10.2	-	-	Protein binding
C45G9.9	-	-	-
C48E7.7	-	-	-
C55C2.2 (<i>ssp-19</i>)	-	-	Structural molecule activity
F17E9.5	-	-	-

F21H7.5	-	-	Structural molecule activity
F26G1.7 (<i>msp-3</i>)	-	-	Structural molecule activity
F27C1.1	-	-	-
F28H6.1 (<i>akt-2</i>)	Determination of adult life span, insulin receptor signaling pathway, dauer entry, protein amino acid phosphorylation	Intracellular	Protein serine/threonine kinase activity, ATP binding, protein tyrosine kinase activity
F32A11.3	Reproduction	-	-
F32B6.5 (<i>sss-1</i>)	-	-	-
F36D3.4	-	-	Structural molecule activity
F36H12.11 (<i>rmd-4</i>)	-	-	Binding
F36H12.9	Protein amino acid phosphorylation	-	Protein kinase activity, ATP binding
F44D12.4	-	-	Protein binding
F44D12.7	-	-	Structural molecule activity
F47B8.11 (<i>sss-2</i>)	-	-	-
F52H3.6	-	-	Hydrolase activity
F53B6.4	-	-	Structural molecule activity
K05F1.7 (<i>msp-63</i>)	-	-	Structural molecule activity
K06A5.2	-	-	-
K07F5.2 (<i>msp-10</i>)	-	-	Structural molecule activity
R09E10.6	Reproduction	-	-
R10E9.2	-	-	-
R13H9.1 (<i>rmd-6</i>)	-	-	Binding
T03F1.5 (<i>gsp-4</i>)	-	-	Hydrolase activity
T04A6.3	Metal ion transport	-	Metal ion binding
T08B2.12	-	-	-
T10E9.4	-	-	-
T13F2.9	-	-	-
T16A9.5	-	-	-
T16H12.6 (<i>kel-10</i>)	-	-	Protein binding
T21G5.4	Larval development (<i>sensu</i> Nematoda), gametogenesis, growth, hermaphrodite genitalia development	-	Protein binding
T23B3.5	-	-	-

T23F11.2	-	-	-
T27A3.3 (<i>ssp-16</i>)	-	-	Structural molecule activity
T27A3.4	-	-	-
T28H11.7	-	-	-
W03D8.10	-	-	-
W03D8.9	-	-	-
Y38H8A.3	Protein amino acid phosphorylation	-	Protein kinase activity, protein serine/threonine kinase activity, ATP binding
Y57G11A.2	-	-	-
Y69E1A.1	-	-	-
Y69E1A.2	Embryonic development (<i>sensu</i> Metazoa)	-	-
ZK1248.5	-	-	-
ZK1248.6 (<i>msp-64</i>)	-	-	Structural molecule activity
ZK265.3	-	-	-
ZK484.5	-	-	-
ZK484.8 (<i>nspd-1</i>)	-	-	-
ZK546.6 (<i>msp-152</i>)	Embryonic development (<i>sensu</i> Metazoa)	-	Structural molecule activity
ZK938.1	-	-	Hydrolase activity

<i>gsp-4</i>	Gene Ontology (GO) terms		
Gene code (name)	Biological process	Cellular component	Molecular function
C01G10.14	-	Membrane	G-protein coupled receptor activity, structural molecule activity,
C04G2.8	-	-	-
C04G2.9	-	-	-
C09B9.6 (<i>msp-55</i>)	-	-	Structural molecule activity
C10G11.8	Protein catabolic process	Nucleus, cytoplasm	Nucleotide binding, ATP binding, hydrolase activity, nucleoside-triphosphatase activity
C10G11.9	-	-	-
C14C10.1	Transport	Membrane	Binding
C25G4.6	Reproduction	-	Protein binding
C35D10.2	-	-	Protein binding
C45G9.4	-	-	-
C55C2.2 (<i>ssp-19</i>)	-	-	Structural molecule activity

F07A5.2	-	-	-
F17E9.5	-	-	-
F26G1.7 (<i>msp-3</i>)	-	-	Structural molecule activity
F32A11.3	Reproduction	-	-
F36H12.11 (<i>rmd-4</i>)	-	-	Binding
F44D12.4	-	-	Protein binding
F47B8.11 (<i>sss-2</i>)	-	-	-
F53B6.4	-	-	Structural molecule activity
F58A6.8 (<i>msp-45</i>)	-	-	Structural molecule activity
F58A6.9	-	-	Structural molecule activity
K03H1.1 (<i>gln-2</i>)	Glutamine biosynthetic process, nitrogen compound metabolic process	-	Glutamate-ammonia ligase activity
K05F1.9	-	-	Structural molecule activity,
K06A5.2	-	-	-
K07F5.2 (<i>msp-10</i>)	-	-	Structural molecule activity
K08C9.2	Reproduction	-	-
R05F9.13 (<i>msp-31</i>)	Embryonic development (sensu Metazoa)	-	Structural molecule activity
R10E9.2	-	-	-
R13H9.1 (<i>rmd-6</i>)	-	-	Binding
R13H9.4 (<i>msp-53</i>)	-	-	Structural molecule activity
T08B2.12	-	-	-
T16A9.5	-	-	-
T21G5.4	Larval development (sensu Nematoda), gametogenesis, growth, hermaphrodite genitalia development	-	Protein binding
T23F11.2	-	-	-
T27A3.3 (<i>ssp-16</i>)	-	-	Structural molecule activity
T27A3.4	-	-	-
T27A3.5	Protein amino acid dephosphorylation	-	Phosphoprotein phosphatase activity, protein tyrosine phosphatase activity
T27E7.1	-	-	-
T28H11.1 (<i>ssq-4</i>)	-	-	-
T28H11.7	-	-	-
W03D8.10	-	-	-

W09C3.6 (<i>gsp-3</i>)	Reproduction	-	Hydrolase activity,
Y57G11A.2	-	-	-
Y69E1A.1	-	-	-
Y69E1A.2	Embryonic development (sensu Metazoa)	-	-
ZK1248.17	-	-	Structural molecule activity
ZK1251.6 (<i>msp-76</i>)	-	-	Structural molecule activity
ZK484.5	-	-	-
ZK484.8 (<i>nspd-1</i>)	-	-	-
ZK546.3	-	-	Structural molecule activity
ZK546.7	-	-	-
ZK637.12	-	-	-

Fig. 1 - Campbell et al.

Fig. 2 - Campbell et al.

<i>Hc-STP-1</i>	M-----DPTQLITNLLNVGLPDKGLTKTVSENDIMEVLGKAREMFLSQPPMVELDSPVKICGDIHGQYIDLLRLFNKG	[80]
<i>Tv-STP-1</i>	M-----DTTQLITNLLSVGLPDKGLTKTVSENDIMEVLGKAREMFLSQPMVLELDSPVKICGDIHGQYIDLLRLFNKG	
<i>Od-MPP-1</i>	M----AQLDVSLSRLNVGMAGGRLTTSVSEQELQCCCFVARQVFISQSSLIICEPPLVVCGDIHGQYSDLLRIFDKN	
<i>Ce-C47A4.3</i>	M----QNNVVDSSIIDVLSASTHEKPLCKVITEERVKLKLLDALGVFKAQKPMVEVNAPIKVCGDIHGQFPDLLRLFHRG	
<i>Ce-GSP-4</i>	M---TATIDVDNLM SRLNVGMSSGRLTTSVNEQELQCCCAVAKSVFASQASLLEVEPPIIVCGDIHGQYSDLLRIFDKN	
<i>Ce-GSP-3</i>	M---TAPMDVDNLM SRLNVGMSSGRLTTSVNEQELQCCCAVAKSVFASQASLLEVEPPIIVCGDIHGQYSDLLRIFDKN	
<i>Ce-GSP-2</i>	M--DVEKLNLDNIISRLEVRGSKPGKNVQLTESEIKGLCQKSREIFLSQPIILLEAPLKIICGDIHGQYIDLLRLFYEG	
<i>Ce-GSP-1</i>	M-SNDGDLNIDNLTIRLLEVRGCRPGKPVMTSEAEIRALCHKSREIFLSQPIILLEAPLKIICGDIHGQYIDLLRLFYEG	
<i>Hs-PP1</i>	M--ADGELNVDSLITRLEVRGCRPGKIVQMTEAEVRGLCIKSREIFLSQPIIPLEAPLKIICGDIHGQYIDLLRLFYEG	
* *		
<i>Hc-STP-1</i>	GFPPLSNYLFLGDYVDRGKQNLLEVILLMIAYKLRFKPNFFLLRGNHECANVNRA YGFYECCNRRYQSQRMWQAFQDVL CV	[160]
<i>Tv-STP-1</i>	GFPPLSNYLFLGDYVDRGKQNLLEVILLVIAYKLRFKPNFFLLRGNHECANVNRA YGFYDECNRRYQSQRMWQLFQDVF CV	
<i>Od-MPP-1</i>	GFPPE TNYLFLGDYVDRGRQNIETICLMFCYRIKYPE SFFMLRGNHECPAINRIYGFYECCNRRYHSSRLWSSFDTFNW	
<i>Ce-C47A4.3</i>	GWPP TANYLFLGDYVDRGRFSIETIVLLLAYKVKFPCNF FLLRGNHECFVNKTYGFYECCKRRYQSQRMYAAFQDVFNW	
<i>Ce-GSP-4</i>	GFPDPINFLFLGDYVDRGRQNIETICLMFCFKIKYPENFFMLRGNHECPAINRVYGFYECCNRRYKSTRLWSIFQDTFNW	
<i>Ce-GSP-3</i>	GFPDPVNF LFLGDYVDRGRQNIETICLMFCFKIKYPENFFMLRGNHECPAINRVYGFYECCNRRYKSTRLWSIFQDTFNW	
<i>Ce-GSP-2</i>	GFPPE SNYLFLGDYVDRGKQSL ETICLLLAYKIKYPENFFLLRGNHECASNRIYGFYDECKRRYN- IKLWKTFTDCFCNC	
<i>Ce-GSP-1</i>	GFPPE ANYLFLGDYVDRGKQSL ETICLLLAYKIKYPENFFLLRGNHECASNRIYGFYDECKRRFS- IKLWKTFTDCFCNC	
<i>Hs-PP1</i>	GFPPE ANYLFLGDYVDRGKQSL ETICLLLAYKIKYPENFFLLRGNHECASNRIYGFYDECKRRFN- IKLWKTFTDCFCNC	
* *		
<i>Hc-STP-1</i>	MPLTALVSDKILCMHGGLSPHLQ---SLDQLRNITRPTDALGATLEM DLLWADPVIGLNGFQANIRGASYGFGPDILAKY	[240]
<i>Tv-STP-1</i>	MPLTALVGEKILCMHGGLSPHLE---SLDQLRNIPRPT EATGATLEM DLLWADPVIGLNGFQANMRGASYGFGPDILAKY	
<i>Od-MPP-1</i>	MPLCGFIAGRILCMHGGLSPQLT---SIDQLRNLRFPQDPPNPSMGI DLLWADPDQWVKGWQANTRGVSYVFGQDVVFET	
<i>Ce-C47A4.3</i>	LPLTGLIATKILCMHGGLSPMLTKFETLDTLRKIERPTEGK-EGLVADLLWADPISGLSGFMNNGRGACCGFGRDSVLNL	
<i>Ce-GSP-4</i>	MPLCGLIGSRILCMHGGLSPHLQ---TLDQLRQLFRPQDPPNPSIGI DLLWADPDQWVKGWQANTRGVSYVFGQDVVADV	
<i>Ce-GSP-3</i>	MPLCGLIGSRILCMHGGLSPHLQ---TLDQLRQLFRPQDPPNPSIGI DLLWADPDQWVKGWQANTRGVSYVFGQDVVADV	
<i>Ce-GSP-2</i>	LPVAAIID EKIFCCHGGLSPDLQ---SMEQIRIRIMRPTDVPDQGLLCDLLWSDPDKDVTDGWGENDRGVSFTFGPEVVAKF	
<i>Ce-GSP-1</i>	LPIAALID EKIFCCHGGLSPDLQ---NMEQIRRVMRPTDVPDTGLLCDLLWSDPDKDVTDGWGENDRGVSFTFGPDVVAKF	
<i>Hs-PP1</i>	LPIAAIVDEKIFCCHGGLSPDLQ---SMEQIRIRIMRPTDVPDTGLLCDLLWSDPDKDVTDGWGENDRGVSFTFGADVVS KF	
* *		
<i>Hc-STP-1</i>	CQLLNIDLVARAHQVVQDGYEFFGGRKLVITIFSAPHYCGQFDNAAMMTVDENLQCSFDFRPSCAKQP KVIATSMGSP	[320]
<i>Tv-STP-1</i>	CQALNIDLVARAHQVVQDGYEFFGGRKLVITIFSAPHYCGQFDNAAMMTVDENLQCSFEILRPSVGKQP KKIPTTIGSP	
<i>Ce-C47A4.3</i>	CSEFQLDLVCAHQQVVQDGYEFFAGRKLVITIFSAPHYCGQFDNCAAFMSCDEKLQCSFEILRPTTGRLEIREKPLLKDET	
<i>Ce-GSP-4</i>	CSRLDIDLVARAHQVVQDGYEFFASKKMVTIFSAPHYCGQFDNSAATMKVDENMVCTFVMYKPTPKSLRKG-----	
<i>Ce-GSP-3</i>	CSRLDIDLVARAHQVVQDGYEFFASKKMVTIFSAPHYCGQFDNSAATMKVDENMVCTFVMYKPTPKSMRRG-----	
<i>Ce-GSP-2</i>	LHKHDLDLICRAHQVVEDGYEFFAKRQLVITIFSAPNYCGEFDNAGGMMSVDETLMC SFQILKPADKKKYPYAGGVGSNR	
<i>Ce-GSP-1</i>	LNRHDLDLICRAHQVVEDGYEFFAKRQLVITIFSAPNYCGEFDNAGGMMSVDETLMC SFQILKPSEKKAK-YQYQGMNSGR	
<i>Od-MPP-1</i>	CQKLNIDLIAHQQVVQDGYEFFASKKMVTIFSAPHYCGQFDNFAATMKVSEDLVCNFAMYKPTAKALRMAAGVSRAS--	
<i>Hs-PP1</i>	LNRHDLDLICRAHQVVEDGYEFFAKRQLVITIFSAPNYCGEFDNAGGMMSVDETLMC SFQILKPSEKKAK-YQYGGLN SGR	
<i>Hc-STP-1</i>	GAPPCQ-----	[316]
<i>Tv-STP-1</i>	AAPPCQ-----	[316]
<i>Od-MPP-1</i>	-----	[311]
<i>Ce-C47A4.3</i>	N-----	[316]
<i>Ce-GSP-4</i>	-----	[305]
<i>Ce-GSP-3</i>	-----	[305]
<i>Ce-GSP-2</i>	PVTPPRNAPAAQP---KKGAKK	[333]
<i>Ce-GSP-1</i>	PAVGGGRPGTTAGKK-----	[329]
<i>Hs-PP1</i>	PVTPPRTANPPKKR-----	[327]

Fig. 3 - Campbell et al.

Fig. 4 - Campbell et al.