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The genome of *Strongyloides* spp. gives insights into protein families with a putative role in nematode parasitism.

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SUMMARY

Parasitic nematodes are important and abundant parasites adapted to live a parasitic lifestyle, with these adaptations all aimed at facilitating their survival and reproduction in their hosts. The recently sequenced genomes of four *Strongyloides* species, gastrointestinal parasites of humans and other animals, alongside transcriptomic and proteomic analysis of free-living and parasitic stages of their life cycles have revealed a number of protein families with a putative role in their parasitism. Many of these protein families have also been associated with parasitism in other parasitic nematode species, suggesting that these proteins may play a fundamental role in nematode parasitism more generally. Here, we review key protein families that have a putative role in *Strongyloides*' parasitism – acetylcholinesterases, astacins, aspartic proteases, prolyl endopeptidases, proteinase inhibitors (trypsin inhibitors and cystatins), SCP/TAPS and transthyretin-like proteins – and the evidence for their key, yet diverse, roles in the parasitic lifestyle.

Key words: *Strongyloides,* helminths, acetylcholinesterases, astacins, aspartic proteases, prolyl oligopeptdases, proteinase inhibitors, SCP/TAPS, transthyretin-like proteins

INTRODUCTION

The genomes of four *Strongyloides* species (*S. ratti, S. stercoralis, S. papillosus* and *S. venezuelensis*), and two closely related species from the same evolutionary clade, the parasite *Parastrongyloides trichosuri* and the free-living species *Rhabditophanes* sp. have recently been sequenced. The genomes of each these six species have been formed into 42 – 60 Mb assemblies, with each containing 12,451 – 18,457 genes (Hunt *et al.*, 2016). The genomes of *S. ratti, S. stercoralis* and *P. trichosuri* each comprises two autosomes and an X chromosome (Bolla and Roberts, 1968; Hammond and Robinson, 1994), while in *S. papillosus* and *S. venezuelensis* chromosomes I and X have fused (Albertson *et al.*, 1979; Hino *et al.*, 2014); *Rhabditophanes* sp. has 5 chromosomes (Hunt *et al.*, 2016). All six genomes are AT rich, with GC values ranging from 21 - 25% in the four *Strongyloides* spp. to 31 and 32 % in *P. trichosuri* and *Rhabditophanes,* respectively (Hunt *et al.*, 2016). The genomes have been assembled to a high quality – the reference genome *S. ratti* into single scaffolds for each of the two autosomes, and the X chromosome assembled into 10 scaffolds. The high quality assembly of these genomes makes the *Strongyloides* species an excellent model in which to understand nematode genomics. For *Strongyloides* spp., the parasitic and free-living adult female stages of their life cycle are genetically identical because the parasitic female reproduces by a genetically mitotic parthenogenesis (Viney, 1994). The differences between the parasitic and free-living adult stages are therefore due solely to differences in expression of genes of the *Strongyloides* genome. Because the parasitic and free-living adult stages are genetically identical, the genes and proteins upregulated in the parasitic stage, compared to the free-living stage, can be directly attributed to a putative role in parasitism. Most parasitic nematode species do not have genetically identical parasitic and free-living adult stages. *Strongyloides* therefore offers a uniquely tractable system to study the genetic basis of nematode parasitism.

Specific gene and protein families are associated with different stages of the *Strongyloides* lifecycle (**Figure 1**) (Hunt *et al,* 2016). Comparisons of the transcriptome and proteome of parasitic females with the transcriptome and proteome of free-living adult female stages of the *Strongyloides* life cycle have revealed genes and proteins upregulated in the parasitic

female, with a putative role in parasitism (Hunt *et al.*, 2016). Genes encoding two protein families – astacins and SCP/TAPS – are the most commonly upregulated gene families in the transcriptome of the parasitic adult female, when compared with the genetically identical free-living female adult stage of the life cycle. Together the astacins and SCP/TAPS gene families account for 23% of all of the genes significantly upregulated in parasitic females. An expansion in the number of the astacin and SCP/TAPS genes in *Strongyloides* and *Parastrongyloides,* when compared to a range of nematode species, coincides with the evolution of parasitism in the *Strongyloides-Parastrongyloides-Rhabditophanes* clade of nematodes, further highlighting their likely role in parasitism. Several other gene and protein families including acetylcholinesterases, aspartic proteases, prolyl endopeptidases, proteinase inhibitors (trypsin-inhibitors and cystatins) and transthyretin-like proteins, are also commonly upregulated in the proteome and transcriptome of the parasitic female suggesting that these proteins also have a putative role in *Strongyloides* parasitism (**Figure 2**). Many of these protein families with a putative role in *Strongyloides* parasitism are also expressed and sometimes secreted by the parasitic stage of the life cycles of many other species of parasitic nematodes, further suggesting these families more a general role in nematode parasitism. For example, acetylcholinesterases, astacins, aspartic proteases, prolyl endopeptidases, proteinase inhibitors, SCP/TAPS and transthyretin-like proteins have all been associated with parasitism in nematodes or other parasites (Lee, 1996; Williamson *et al.*, 2002, 2006; Knox, 2007; Asojo, 2011; Fajtová *et al.*, 2015; Lin *et al.*, 2016)**.** Possible role(s) of the protein families upregulated in parasitic females, compared to the free-living stage, are summarised in Figure 1. These roles are based on evidence for putative roles of members of these protein families in other parasite species, as discussed for each protein family, below.

While identification of protein families with putatively important roles in nematode parasitism is a major advance, understanding the specific role that they each play in parasitism is still a substantial challenge. Many of these protein families also occur in a diverse range of nematode species, including free-living nematode species, as well as a diverse range of non-nematode taxa. The members of a given protein family have likely evolved diverse functions which may include conserved roles important for a range of

species and life cycle stages, as well as more specialised roles specific to nematode parasitism. Uncovering the roles of these proteins will be essential for understanding parasitism by nematodes. Understanding the role of these proteins is also important for identifying and developing drugs and other treatments to control nematode populations. Here, we consider in turn key protein families that have a putative role in parasitism in *Strongyloides* – acetylcholinesterases, astacins, aspartic proteases prolyl endopeptidases, proteinase inhibitors (trypsin inhibitors and cystatins), SCP/TAPS and transthyretin-like proteins (**Table 1**). Identification of these key protein families is based on the genomic, transcriptomic and proteomic data presented by Hunt *et al.*, (2016). We consider the possible roles of these proteins in nematode parasitism and we present new analyses of these protein families, expanding on this recently published work.

PROTEIN FAMILIES WITH A PUTATIVE ROLE IN PARASITISM

PROTEINASES

Parasites secrete proteinases and peptidases into their environment that will act on both host- and nematode-derived proteins (Tort *et al.*, 1999). Proteinases and peptidases make up a significant proportion of these upregulated in the parasitic adult female stage of the *S. ratti* and *S. stercoralis* life cycle (Hunt *et al.*, 2016). The majority of these proteinases and peptidases can be categorised into key protein families. Here we discuss the largest of these families that are associated with a putative role in *Strongyloides* parasitism: astacins, aspartic proteases and prolyl oligopeptidases.

Astacins

Astacins are zinc-metallopeptidases that require the binding of zinc in their active site for catalytic activity. They are found in diverse taxa, ranging from bacteria to mammals and have been associated with a range of functions including digestion in the crayfish (after whom they are named, *Astacus astacus*) and a bone-inducing factor (*e.g.* bone morphogenic protein) in vertebrates (Wang *et al.*, 1988; Bond and Beynon, 1995). In nematodes, astacins have been best characterised in *C. elegans* where they have roles in cuticle biosynthesis,

moulting and hatching (Hishida *et al.*, 1996; Suzuki *et al.*, 2004; Davis *et al.*, 2004; Novelli *et al.*, 2006). Nematode cuticles are predominantly made up of collagens, and astacins play a key role in cuticle biosynthesis where they are involved in processing collagen molecules for cuticle formation (Page and Winter, 2003; Stepek *et al.*, 2015). Astacins play these roles in both free-living and parasitic species and life cycle stages. However, many of the astacins coded for by the genomes of parasitic species are differentially expressed across the life cycle, suggesting that in parasitic species they have wider roles too. For example, specific sets of astacin coding genes are upregulated in infective larval stages and adult parasitic stages compared with other parasitic and free-living stages of the *Strongyloides* life cycle, suggesting astacins have also evolved roles specific to the parasitic lifestyle. The role of astacins in parasitic stages has been best characterised for infective larval stages where the gene expression and protein secretion of astacins has been reported in many parasitic nematodes including *Haemonchus contortus* (Gamble *et al.*, 1996), *Onchocerca volvulus* (Borchert *et al.*, 2007), *Ancylostoma caninum* (Williamson *et al.*, 2006), *Dictyocaulus viviparous* (Cantacessi *et al.*, 2011), *Teladorsagia circumcincta* (Menon *et al.*, 2012), *S. ratti* and *S. stercoralis* (Hunt *et al.*, 2016). Most of the evidence for the role of astacins in nematode parasitism points to them functioning in infective larvae penetrating host skin and in subsequent tissue invasion (Williamson *et al.*, 2006). For example, the astacin *Ac*-MTP-1 occurs in secretory granules of the glandular oesophagus of L3s of the dog hookworm, *A. caninum* suggesting that it plays a role in host infection. Recombinant *Ac*-MTP-1 protein (expressed in a baculovirus / insect cell system) was able to digest a range of connective tissues, including collagen, and inhibition of *Ac*-MTP-1 function with either antiserum or the metalloprotease inhibitors EDTA and 1,10-phenanthroline greatly reduced tissue penetration by infective larvae (Williamson *et al.*, 2006). Similarly, a role of the *S. stercoralis* secreted astacin, Ss40, also has tissue degradation properties (McKerrow *et al.*, 1990; Brindley *et al.*, 1995).

In the genomes of *Strongyloides* spp. and *Parastrongyloides* the astacin gene family has expanded to some 184-387 astacin coding genes per species, shown by comparison with eight other species (*Trichinella spiralis, Trichuris muris, Ascaris suum, Brugia malayi*, *Bursaphelenchus xylophilus, Meloidogyne hapla*, *Necator americanus, Caenorhabditis*

elegans – spanning four evolutionary clades). The astacin coding gene family is the single most upregulated gene family in *S. ratti* and *S. stercoralis* parasitic adult females compared with both free-living adult females and infective third stage larvae (iL3s) (Hunt *et al.*, 2016). The *Strongyloides* parasitic females reside in the mucosa of the small intestine, but rather little is known about the role of astacins at this life cycle stage. Many of the astacins expressed by parasitic females are likely to be secreted based on the prediction of a signal peptide (Hunt *et al.*, 2016), and the identification of astacins in the secretome of *Strongyloides* parasitic females (Brindley *et al.*, 1995; Soblik *et al.*, 2011; Hunt *et al.*, 2016). The secretion of these proteins into their host implies that their target substrate is external to the nematode. Because astacins have key roles in tissue degradation in larvae infecting hosts, it is probable that their role in the gut of their host is also involved in tissue degradation. One possibility is that astacins are required to break down the mucosa through which the parasitic females burrow, presumably feeding as they do so. A second, non-mutually exclusive, possibility is that astacins may have evolved a role in immunomodulation, where their proteolytic role may be directed against protein components of the host immune response. Astacins present in the secretome of the hookworm *Necator americanus* are believed to be responsible for proteolysis of eotaxin, a potent eosinophil chemo-attractant (Culley *et al.*, 2000). Further investigation is required to ascertain if astacins have a similar role in immunomodulation by *Strongyloides* and other parasitic nematode species (Maizels and Yazdanbakhsh, 2003).

Aspartic proteases

Aspartic proteases (AP), characterised by the presence of aspartic acid residues in their active site clefts, have been identified in a diverse range of organisms (Dash *et al.*, 2003). In parasitic nematodes, such as *H. contortus, A. caninum* and *N. americanus,* they are thought to play a role in the digestion of host haemoglobin (Brindley *et al.*, 2001; Williamson *et al.*, 2002; Jolodar *et al.*, 2004; Sharma *et al.*, 2005; Balasubramanian *et al.*, 2012). Genes encoding APs are upregulated in the parasitic adult female of *S. ratti* and *S. stercoralis* compared to free-living adult female stage (Hunt *et al.*, 2016). At this stage of the *Strongyloides* life cycle, parasitic adults inhabit the host's intestine, an environment that likely requires degradation of haemoglobin and other tissue. Previously, ten AP genes had

been identified in *S. ratti* (Mello *et al.*, 2009). Using the recently sequenced *S. ratti* and *S. stercoralis* genome data, we have determined that there are in fact a total of 53 genes encoding proteins containing an AP domain (InterPro domain IPR021109). Twelve and 17 of these genes are differentially expressed in the parasitic and free-living *S. ratti* adult females (6 upregulated in parasitic and 6 upregulated in free-living females) and *S. stercoralis,* respectively (15 upregulated in parasitic females and two in free-living females). This indicates that different sets of aspartic proteases genes are expressed at different stages of the life cycle in *Strongyloides*, possibly reflecting the diversification of APs in parasitic and free-living adults. Two paralogous *S. ratti* APs, ASP-2A and ASP-2B, which are highly expressed in parasitic and free-living females, respectively, have previously been described (Mello *et al.,* 2009) and molecular modelling of these suggests that they have maintained specificity for similar substrates but differ in their electrostatic charge. These differences may be an adaptation to the differences in environmental conditions encountered by parasitic and free-living stages of the life cycle (Mello *et al.*, 2009).

Many of the *S. ratti* and *S. stercoralis* genes that code for a protein with an AP domain and are not differentially expressed between parasitic and free-living adult females are associated with retrotransposons. Specifically, in *S. ratti* and *S. stercoralis* most of the genes coding for gene products with AP domains typically are associated with retroviruses and retrotransposons including ribonuclease H-like domain, peptidase A2A, zinc finger CCHC-type domain and intergrase catalystic core domains (**Table 2**). Retroviral-like and retrotransposon-associated aspartic proteases are homodimeric, and an AP residue located on each molecule come together to form the active site cleft (Koelsch *et al.*, 1994). The predicted folding structure of retroviral-like aspartic proteases of platyhelminth and of *Leishmania* retroviral-like aspartic peptidase have a quaternary structure similar to that of HIV (Santos *et al.*, 2013; Wang *et al.*, 2015). Because the aspartic protease inhibitors currently used as treatment against HIV (*e.g.* nelfinavir) have also been shown to inhibit the activity of *Leishmania* and *Trypanosoma* retroviral-like aspartic proteases (Santos *et al.*, 2013), AP inhibitors offer a potential control treatment for nematode parasites.

Prolyl oligopeptidases

Prolyl oligopeptidase (POP, also known as prolyl endopeptidase or PEP) enzymes are 70-80 kDa in size and belong to the S9 family of serine peptidases that cleave peptides at internal proline residues (Gass and Khosla, 2007). A common feature of POP enzymes is that they only cleave peptides up to 30 residues in length, such as neurotransmitters and hormones (Camargo *et al.*, 1979). In *S. ratti* and *S. stercoralis* there are 23 and 17 genes, respectively, predicted to code for a POP, based on the predicted presence of a peptidase S9 prolyl oligopeptidase domain (Pfam ID PF00326). Of these genes, 18 and 11 are upregulated in the parasitic female transcriptome, compared to the free-living transcriptome for *S. ratti* and *S. stercoralis,* respectively. Several of these upregulated POPs have the greatest differential levels of expression between parasitic and free-living stages (**Table 1**). The role of POPs in parasitism is best characterised in *Trypanosoma*, *Leishmania* and *Schistosoma* (Bastos *et al.*, 2013). For example, the *T. cruzi* POP, Tc80, is thought to have a role in facilitating entry of the trypanosomes into the mammalian host cells (Grellier *et al.*, 2001; Bastos *et al.*, 2005). In *Schistosoma* POPs are also important in parasitism, shown by *in vitro* experiments where recombinant *S. mansoni* POP*,* smPOP, cleaves small host-derived peptides such as the vascoregulatory hormones angiotensin I and bradykinin. This suggests a possible role of POPs in modulating the host vascular system (Fajtová *et al.*, 2015). Less is known about the role of POPs in nematodes, and especially in parasitic species.

We have identified genes predicted to encode a peptidase S9 prolyl oligopeptidase domain (Pfam ID PF00326) in 14 nematode species (four *Strongyloides* spp., *Parastrongyloides, Rhabditophanes*, and eight species, as above), with between 5-18 such genes in each of the eight other species. In the *C. elegans* genome we identified six genes that are predicted to encode a POP, but the function of POPs in *C. elegans* and other nematodes is not understood. In, the *Strongyloides-Parastrongyloides-Rhabditophanes* clade each species had 8-23 POP. Genes encoding POPs have previously been associated with parasitism based on genomic, transcriptomic and proteomic data of parasitic nematode species including *S. ratti* (Soblik *et al.*, 2011; Hunt *et al.*, 2016) *S. stercoralis, P. trichosuri, Dirofilaria immitis* (Mitreva *et al.*, 2004) and *Trichuris muris* (Mitreva *et al.*, 2011). The presence of a β-propeller domain (InterPro domain IPR004106) has previously been associated with genes encoding a POP-

domain in parasitic species (*S. ratti, P. trichosuri* and *D. immitis*) (Mitreva *et al.*, 2004). The β-propeller domain is associated with ligand binding, oxidoreductase and hydrolase (Chen *et al.*, 2011) and, more generally, β-propeller domains are thought to exclude larger peptides from the POP active site, thus protecting them from proteolysis in the cytosol (Fülöp *et al.*, 1998). Among the *S. ratti* and *S. stercoralis* POPs, 21 and 14, respectively, are predicted to contain a β-propeller domain, most of which (15 of 16 and all 11 in *S. ratti* and *S. stercoralis*, respectively) are upregulated in the parasitic female stage when compared to free-living adult females. A β-propeller domain has also been identified in the *Schistosoma* smPOP (Fajtová *et al.*, 2015) and Tc80 of *T. brucei.* It is not known if POPs with a β-propeller have a specific role in parasitism and, if so, what this role is.

PROTEINASE INHIBITORS

Proteinases are an important component of the host immune defence against parasites. There is strong evidence that proteinase inhibitors are secreted by parasitic nematodes to counteract attack by host proteinases, therefore providing the parasite with protection from the effects of the host digestive system and the immune response (Knox, 2007). Proteinase inhibitors are secreted by hematophagous parasites to facilitate feeding within the host environment – for example *Ancylostoma caninum* secretes the anticoagulant proteinase inhibitors anticoagulant peptide (AcAP) and hookworm platelet inhibitor (HPI) (Cappello *et al.*, 1995; Stassens *et al.*, 1996; Del Valle *et al.*, 2003). In *Strongyloides* proteinase inhibitors including trypsin inhibitors and cystatins are upregulated in the transcriptome and proteome of parasitic females, compared with free-living adult females. These proteinase inhibitors potentially have a role in parasitism in *Strongyloides*, possibly modulating the host immune response.

Proteinase inhibitors can be categorised into two classes based on their interaction with a target proteinases: (i) irreversible high-binding interactions and (ii) reversible interactions (Rawlings *et al.*, 2004; Knox, 2007). Proteinase inhibitors that irreversibly interact with their targets are endopeptidases and include the serine proteinase inhibitors (serpins) and the nematode-specific smapins family. Serpins are found in a diverse range of animals, plants and viruses (Irving, 2000) and are the largest known family of proteinase inhibitors (Law *et*

al., 2006; Rawlings *et al.*, 2010). The serpins are named after their most common function which is to inhibit serine proteases. However, some serpins also inhibit other proteases such as caspases and cysteine proteases (Ray *et al.*, 1992; Irving *et al.*, 2002), or have non-inhibitory roles such as hormone transporters in inflammation (Pemberton *et al.*, 1988). Serpins interact with their target substrate *via* conformational change which inhibits the protease (Huntington *et al.*, 2000). This involves a scissile bond on a reactive-site loop near the C-terminus that traps the protease. The protease cleaves this loop but remains attached to the serpin via an active site serine. In this process the structure of the protease is disrupted and becomes susceptible to degradation (Huntington *et al.*, 2000; Zang and Maizels, 2001). Because these inhibitors act irreversibly and can only inhibit a protease once they are often referred to as 'suicide' or 'single use' inhibitors (Law *et al.*, 2006).

Little is known about the role of serpins in nematode parasitism. The *B. malayi* serpin Bm-SPN-2 is expressed in the blood-dwelling microfilariae stage. Screening of potential target mammalian serine proteinases revealed that Bm-SPN-2 inhibits human neutrophil-derived proteinases cathepsin G and elastase (Zang *et al.*, 1999). Similarly, the serpin *Anisakis simplex* serpin (ANISERP) also inhibits cathepsin G, as well as the human blood clotting serine protease thrombin and the digestive serine protease trypsin (Valdivieso *et al.*, 2015). In *S. ratti,* and *S. stercoralis* there are 12 and 10 genes, respectively, encoding serpins belonging to the trypsin-inhibitor protein family which are significantly upregulated in the parasitic female transcriptome when compared to the free-living female transcriptome. These genes have some of the highest relative differences in expression levels suggesting they have an important role in parasitism. Further investigation is required to identify the targets of *Strongyloides* serpins and if the target is host-derived.

The second type of proteinase inhibitor are those that reversibly interact with their targets, characterised by binding to the active site of a protease with high affinity. These proteinase inhibitors are commonly found in the secretome of parasitic nematodes (Knox *et al.,* 2007). For example, cystatins are a subfamily of the cysteine proteinase inhibitors, which reversibly block the active site of a target cysteine protease. In parasitic nematodes there is evidence

that cystatins play a role in the modulation of the host anti-nematode response (Hartmann *et al.*, 2003; Maizels and Yazdanbakhsh, 2003), including antigen presentation, T cell responses and nitric oxide production (Manoury *et al.*, 2001; Dainichi *et al.*, 2001; Pfaff *et al.*, 2002; Shaw *et al.*, 2003; Knox, 2007). For example, in *Heligmosomoides polygyrus,* a recombinant cystatin protein is able to modulate differentiation and activation of bone-marrow-derived $CD11c^{+}$ dendritic cells (Sun *et al.*, 2013). In the host antigen-presenting cells there are two key pathways involving cystatins (cysteine protease-dependent degradation of proteins to antigenic peptides, and the presentation of the MHC class II complex to T cells, which is partly regulated by cysteine proteases that cleave the MHC class II associated invariant chain), and these pathways are potential targets for parasite-derived cystatins to modulate the host immune response *via* the (Hartmann and Lucius, 2003). Using the genome and transcriptome data for *S. ratti* and *S. stercoralis* (Hunt *et al,* 2016) we have identified three *S. ratti* genes and one *S. stercoralis* gene encoding cystatin proteins which are upregulated in parasitic females compared with free-living adult females suggesting they have an important role in the parasitic life style. The *Strongyloides* upregulated cystatin-encoding genes were identified by the predicted presence of a cystatin domain (InterPro domain IPR000010), but they otherwise have no significant sequence homology with known nematode cystatins. Further investigation is required into these *Strongyloides* proteins to identify if they represent a novel group of immunomodulators.

SCP/TAPS

The sperm-coating protein (SCP)-like extracellular proteins, also known as SCP/Tpx-1/Ag5/PR-1/Sc7 (SCP/TAPS) belong to the cysteine-rich secretory proteins (CRISP) superfamily of proteins. The SCP/TAPS are characterised by the presence of one or two cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins (CAP) domains (InterPro domain IPR014044, Pfam ID PF00188). These proteins are found in a diverse range of organisms, including parasitic nematodes, where they are also known by various other names including the venom allergen-like (VAL) (Hewitson *et al.*, 2011) and *Ancylostoma*-secreted proteins (ASP) (Asojo, 2011). In *S. ratti* and *S. stercoralis*, the SCP/TAPS are the second most commonly upregulated protein family in the adult female

parasitic female after the astacins (Hunt *et al.*, 2016) and are secreted by the parasitic female stage (Soblik *et al.*, 2011; Hunt *et al.*, 2016).

The first reported nematode SCP/TAPS protein was *Ac*-ASP-1 identified in the secretome of *A. caninum* infective larvae (Hawdon *et al.*, 1996). SCP/TAPS have since been identified in the secretome of a range of parasitic nematodes suggesting they have an important role in parasitism (Cantacessi and Gasser, 2012). The role of SCP/TAPS in parasitism is poorly understood. The best example for a role of SCP/TAPS in nematode parasitism is in hookworms, where there is evidence for a role in immunomodulation. Specifically, in *A. caninum*, the SCP/TAPS protein known as neutrophil inhibitory factor inhibits neutrophil adhesion to vascular endothelial cells *in vitro* by selective binding to the integrin CD11b/CD18, thus inhibiting neutrophil function and the release of hydrogen peroxide from activated neutrophils (Moyle *et al.*, 1994). Secondly, hookworm platelet inhibitor (HPI), inhibits *in vitro* platelet aggregation and adhesion by impeding the function of cell surface intergins (Del Valle *et al.*, 2003). In *N. americanus,* the SCP/TAPS protein, *Na*-ASP-2, has structural and charge similarities to CC-chemokines, which are involved in inducing the migration of immune cells such as monocytes and dendritic cells (Asojo *et al.*, 2005).

Among four *Strongyloides* spp. a total of 89-205 genes are predicted to encode SCP/TAPS coding proteins. Comparing these to eight outgroup species (as above), there is an expansion of the SCP/TAPS coding gene family in *Strongyloides* and *Parastrongyloides* but not in *Rhabditophanes* suggesting an expansion of SCP/TAPS protein coding genes coincided with the evolution of parasitism in *the Strongyloides* clade (Hunt *et al.*, 2016). Interestingly, as for the astacin gene family, the SCP/TAPS gene family is also expanded in *N. americanus* (Tang *et al.*, 2014; Schwarz *et al.*, 2015) but phylogenetic analysis suggests that the hookworm expansion of the number of SCP/TAPS coding genes is independent to that of the *Strongyloides-Parastrongyloides* clade (Hunt *et al.*, 2016).

TRANSTHYRETIN-LIKE PROTEINS

Transthyretin-like proteins (TLP) have some of the highest relative levels of gene expression in *S. ratti* and *S. stercoralis* adult parasitic females, compared to that of free-living adult females (**Table 1**) (Hunt *et al.*, 2016). This protein family, also sometimes referred to as TTL and TTR-like proteins, is only found in nematodes and is distinct from the transthyretin-related proteins (TRPs, and sometimes also referred to as TLPs) which are found across a wide range of prokaryotic and eukaryotic taxa (Sauer-Eriksson *et al.*, 2009). The TLPs are distantly related to transthyretins (TTRs), plasma proteins in vertebrates that transport thyroid hormones (Power *et al.*, 2000), though only a low level of sequence homology has been found between the TTRs, TRPs and TLPs. TRPs, along with uricase and 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU) decarboxylase, have been associated with a role in the oxidation of uric acid to allantoin (Ramazzina *et al.*, 2006), however, less is known about the role of TLPs. TLPs were originally discovered in *C. elegans* (Sonnhammer and Durbin, 1997) where the TLP, TTR-52, is involved in the recognition and subsequent engulfment of apoptopic cells. Use of TTR-52 fluorescent protein constructs and *C. elegans* mutant analysis has shown that TTR-52 aggregates around apoptopic cells and binds to surface-exposed phosphatidylserine, a known 'eat me' signal for apoptopic cells. TTR-52 also binds to the CED-1 phagocyte receptor, acting as a bridging molecule to mediate phagocytosis of apoptopic cells (Wang *et al.*, 2010). Similarly, the TTRs in vertebrates also have a binding role, together suggesting that binding to molecules could be a conserved role of TLPs, although the target molecules they have evolved to bind to has diverged among different taxa. One can envisage that in parasitic nematodes, binding of host molecules is likely to be an important function in a parasitic lifestyle.

The TLPs have been identified in the secretome, and are upregulated in the transcriptome, of parasitic stages for a number of nematodes species including *A. caninum* (Mulvenna *et al.*, 2009), *B. malayi* (Hewitson *et al.*, 2008), *A. suum* (Wang *et al.*, 2013), *H. contortus* (Schwarz *et al.*, 2013), *Radopholus similis* (Jacob *et al.*, 2007) and *Litomosoides sigmodontis* (Armstrong *et al.*, 2014). This suggests that TLPs are universally important in nematode parasitism. Little is known about the role of TLPs in nematodes other than *C. elegans*, and it is not known if the TLPs associated with nematode parasitism have a similar molecular-binding role. The plant parasite nematode *Meloidogyne javanica* secretes the transthyretin-

like protein MjTTL5. Yeast two-hybrid protein-protein interaction screens have revealed that MjTTL5 interacts with the *Arabidopsis thaliana* protein ferredoxin:thioredoxin reductase catalytic subunit (AtFTRc), the expression of which is induced by infection with *M. javanica*. The AtFTRc protein is an important component of the host antioxidant system that is deployed by plants as part of their infection defence mechanism. It is hypothesised that MjTTL5 causes a conformational change in AtFTRc that results in the reduction of reactive oxygen species in the host, enhancing nematode parasitism (Lin *et al.*, 2016). MjTTL5 is the only parasitic nematode TLP that has been characterised to date. More research is required to identify the binding targets, if any, of TLPs in parasitic nematodes that infect animals. This is particularly important because TLPs are potentially interesting as a vaccine or drug target due because they only occur in nematodes, thereby facilitating chemotherapeutic specificity.

ACETYLCHOLINESTERASES

Cholinesterases are serine esterases that preferentially hydrolyse choline esters, and are members of a large family of related esterases and lipases which share a core arrangement of eight beta sheets connected by alpha helices (the alpha/beta hydrolase fold) (Ollis *et al.*, 1992). Vertebrate cholinesterases can be distinguished by their substrate specificity; for example, acetylcholinesterase (AChE) hydrolyses acetylcholine (ACh), the major excitatory neurotransmitter which is common to most animals, including nematodes (Selkirk *et al.*, 2005). A key feature of AChEs is that the catalytic triad (S200, H440, E327, numbering based on the *Torpedo californica* enzyme) is located at the base of a 20 angstrom-deep narrow gorge which extends halfway into the enzyme (Sussman *et al.*, 1991). The gorge is lined by the rings of fourteen aromatic residues, most of which can be assigned functional roles. Of these, W84 is critical for enzymatic activity, as both it and F330 form part of the 'anionic' (choline) binding site which orients the substrate in the active site.

ACh controls motor activities such as locomotion, feeding and excretion in nematodes (Segerberg and Stretton, 1993; Rand and Nonet, 1997), and thus AChE plays a crucial role in regulation. Among nematodes, AChE coding genes have been most thoroughly

characterised in the free-living nematode *C. elegans,* which has four AChE coding genes: *ace-1-4.* Homozygous mutants of individual *ace* loci result in slight defects in locomotion, or have no phenotypic effect. However, *ace*-1 *ace*-2 double mutant worms are severely uncoordinated, and *ace-1-3* triple mutants have a lethal phenotype, suggesting that although the *ace* genes have essential functions and are required for viability it is possible that *ace-1* and *ace-2* can assume the function normally carried out by *ace-3* (Culotti *et al.*, 1981; Johnson *et al.*, 1981, 1988; Kolson and Russell, 1985). GFP localisation studies of the *ace-1-3* products shows that each gene has a distinct pattern of expression, though with some areas where expression is overlapping (Culetto *et al.*, 1999; Combes *et al.*, 2000). There is little evidence of expression for *ace-4* and it is likely to code for a redundant protein.

Neuromuscular AChEs of parasitic nematodes are much more ill-defined. In addition to expression of neuromuscular AChEs, many parasitic nematodes release variants of these enzymes from specialised secretory glands (Ogilvie et al. 1973), and this property appears to be restricted to parasitic nematodes which colonise mucosal surfaces (Selkirk *et al.*, 2005). These secretory enzymes have been best characterised in *Nippostrongylus brasiliensis* and *D. viviparus*. *N. brasiliensis* secretes three distinct AChEs encoded by separate genes (Hussein *et al.*, 1999, 2000, 2002). Two secreted AChEs from *Dictyocaulus viviparus* have been characterized (Lazari *et al.*, 2003), although this parasite secretes variants which may be encoded by additional genes (McKeand *et al.*, 1994). All of the secreted AChEs take the form of hydrophilic monomers, and analysis of substrate specificity and sensitivity to inhibitors indicates that they can all be classified as true AChEs (Selkirk *et al.*, 2005). The rationale for secretion of multiple forms is unclear, since they display similar substrate specificities and pH optima.

The exact role of AChEs in nematode parasitism is still unclear. In mammals, ACh is a key neurotransmitter involved in the regulation of gut peristalsis. It is likely that parasitic nematodes secrete AChE in order to neutralise host cholinergic signalling, with this signalling normally promoting physiological responses (smooth muscle contraction, mucus

and fluid secretion by goblet cells and enterocytes, and effects on the immune system) which contribute to parasite expulsion (Selkirk *et al.*, 2005). For example, it has been proposed that *N. brasiliensis*' release of AChE into the host gut reduces local intestinal wall spasm and reduces peristalsis, thus providing a more stable environment for *N. brasiliensis* to inhabit (Lee, 1970, 1996). Parasitic nematode-derived AChE may inhibit the release of host intestinal mucus. ACh is also involved in inflammatory immune responses (Wang *et al.*, 2003; Tracey, 2009) and has recently been shown to act as a co-stimulatory signalling molecule for CD4+ T cell activation and cytokine production in mice during *N. brasilensis* infection (Darby *et al.*, 2015). The host anti-nematode immune response is therefore also a potential target of parasitic-nematode derived AChEs.

S. ratti and *S. stercoralis* have 24 and 28 AChE coding genes, respectively, of which 17 and 24 were upregulated in the adult parasitic female stage (whereas only 1 and 0 were comparatively upregulated in the free-living female) (**Table 1**). We have resolved the phylogeny of AChE genes in 14 nematode species, including four *Strongyloides* species, *P. trichosuri* and *Rhabditophanes* sp. (from the same evolutionary clade) and 8 outgroup species (as above) encompassing four further nematode clades (**Figure 3**). AChEs coding genes were identified by the presence of a predicted cholinesterase protein domain (InterPro domain IPR000997) and the phylogeny was determined using the method described by Hunt *et al.,* 2016. The phylogeny shows an expansion of the cholinesterase gene family in *Strongyloides* and *Parastrongyloides* compared to the other nematode species (**Figure 3**). The expanded cholinesterase-like genes are all predicted to encode proteins with truncated C-termini, characteristic of secreted AChEs. The number of these genes in *Strongyloides* and *Parastrongyloides* are remarkable: 178 genes between 5 *Strongyloides* and *Parastrongyloides* species, with 80 representatives for *P. trichosuri* alone.

The expansion of the AChE family has not occurred in the closely related free-living nematode *Rhabditophanes,* which only possess two AChE coding genes. Many of the genes in the expanded AChE gene family are upregulated in the parasitic stage of *S. ratti and S. stercoralis* suggesting that, like the astacins and SCP/TAPS, the expansion of the AChE coding

gene family has coincided with the evolution of parasitism in the *Strongyloides-Parastrongyloides-Rhabditophanes* clade and has a role in parasitism. All *Strongyloides* and *Parastrongyloides* species were observed to possess one sequence encoding a protein closely related to *C. elegans* ACE-1, ACE-2 and ACE-3/4 respectively. The nematode sequences which cluster with *C. elegans* ACE-1 show features characteristic of this enzyme and tailed forms in general. The sequences which cluster with ACE-2 show similar conservation of the key amino acids required for disulphide bonds, the catalytic triad, and generally thirteen of the fourteen aromatic residues which line the active site gorge. A key feature of these predicted proteins is that many have a hydrophobic C-terminus with signals for peptide cleavage and GPI addition, analogous to *C. elegans* ACE-2. The same is true of the sequences which cluster with ACE-3/ACE-4, and key residues are particularly well conserved in this group. In summary, most parasitic nematodes examined appear to mirror *C. elegans* in possessing genes for one tailed and two hydrophobic AChEs, and in all likelihood these represent neuromuscular enzymes which perform analogous functions to their *C. elegans* counterparts. In comparison to the AChE coding genes that have expanded in *Strongyloides* and *Parastrongyloides,* the expression of the *S. ratti* and *S. stercoralis* AChE coding genes most closely related to the *C. elegans ace 1-4* genes were not upregulated in the parasitic or free-living stages of the *Strongyloides* life cycle (**Figure 3**).

We classified the members of the *Strongyloides-Parastrongyloides* expanded cholinesterase-like gene family into 8 groups, based on the phylogeny shown in **Figure 3**. The vast majority have cysteine residues in the correct position to form the 3 intramolecular disulphide bonds, and features of the active site gorge characteristic of cholinesterases. However closer examination reveals that almost all of the predicted proteins are likely to be enzymatically inactive, with the exception of those in group 1. The group 1 predicted protein sequences possess the necessary key residues for AChE activity. Groups 2, 3 and 4 lack S200 or have modifications around this residue that would render the enzymes inactive. The consensus sequence around the active site serine in cholinesterases is FGESAG. Mutation of S200 generates loss of activity (Gibney *et al.*, 1990; Shafferman *et al.*, 1992), and mutation of E199 reduces it . Most *Parastrongyloides* genes in group 2 also lack E327, approximately half of the sequences in group 3 lack H440, and mutations of these residues also render AChEs

inactive (Radić *et al.*, 1992; Shafferman *et al.*, 1992). Almost all group 6 sequences are predicted to retain F330 in the choline binding site, but show altered residues around S200: many sequences predict the consensus FGTSSG rather than FGESAG, which would likely severely reduce catalytic efficiency (Radić *et al.*, 1992). Group 5 sequences show relatively poor conservation of active site gorge aromatic residues. All group 6 predicted sequences have a W84Y substitution. W84 is considered a signature of cholinesterases, as the quaternary nitrogen groups of the substrate are stabilised by cation-aromatic interactions. Nevertheless, cholinesterases substituted with smaller aromatic residues such as tyrosine and phenylalanine at this site retain activity, albeit reduced several-fold (Ordentlich *et al.*, 1995; Loewenstein-Lichtenstein *et al.*, 1996). Proteomic analysis has identified twenty cholinesterase-like sequences in the secretome of adult female parasitic *S. ratti*, none of which were present in secreted products of free-living adult females (Hunt *et al.*, 2016). Of these, only SRAE_1000291800 from group 1 is predicted to encode an active AChE. Ten of the sequences are from group 6: of these, five have the sequence FGTSSG and five have FGTGTG around the active site serine and are thus most likely enzymatically inactive. The other secreted proteins are from groups 2, 4 and 5.

The presence of large numbers of these genes in the genomes of *Strongyloides* and *Parastrongyloides*, that these genes are transcribed and (from *S. ratti* and *S. stercoralis*, data) specifically in the parasitic female stage, and (from *S. ratti* proteomic data) that many result in proteins, sits oddly with most of these not coding for functional cholinesterases. It is possible that these proteins could have quite different, perhaps novel, roles. It is notable that cholinesterases are thought to have a wider variety of non-enzymatic roles, such as in cellular differentiation, adhesion and morphogenesis (Grisaru *et al.*, 1999; Johnson *et al.,* 2008), and such roles might be compatible with how *Strongyloides* exploits host mucosal tissue during its parasitic phase.

DISCUSSION

The continued advances in next generation sequencing has led to a great increase in the number of sequenced parasitic nematodes genomes that are available. These data, along with high quality data on the transcriptomes and proteomes of different life cycle stages of parasitic nematodes has enabled predictions of genes and proteins with possible roles in parasitism. We have discussed the protein families with a putative role in parasitism based on evidence that these proteins are upregulated in the transcriptome and proteome of the parasitic stage of the life cycle, compared to the genetically identical free-living stage. Some of these proteins are now under further investigation as potential vaccine targets against nematode infection, including members of the astacin (Nisbet *et al.*, 2013; Page *et al.*, 2014), SCP/TAPS (Goud *et al.*, 2005), aspartic protease (Loukas *et al.*, 2005) and cystatin (Arumugam *et al.*, 2014) protein families. Proteins with a role in parasitism are also key targets for the development of anthelminthic drugs (Page *et al.*, 2014), and as immunomodulatory drugs for autoimmune disease such as asthma and colitis (Wilson *et al.*, 2010; Whelan *et al.*, 2012; Maizels *et al.*, 2014).

There are still big gaps in our knowledge. Our understanding of the role played by many of these proteins in the parasitic nematode lifestyle, and their targets, if any, within the host are still largely unknown. With the exception of the transthyretin-like protein family, which is only found in nematodes, many of the protein families likely to play an important role in nematode parasitism have been identified across a diverse array of nematode and non-nematode taxa. In many cases these protein families have a diversity of roles; for example, the SCP/TAPS are not only found in nematodes, but also in plants and animals, where they have been implicated in plant pathogen defence, as components of insect and snake venom, in sperm maturation and male reproduction in insects and mammals, and the development of fibrosis (Cantacessi *et al.*, 2009). Thus identifying the family to which a protein belongs is not necessarily informative about the roles that they might have in nematode parasitism. The proteins involved in nematode parasitism may have evolved new functions which may be novel to a particular species, genus or clade of parasitic nematodes.

Many *Strongyloides* proteins, especially belonging to the protein families discussed here, have the largest relative protein or transcript expression levels in the parasitic compared with the free-living stage, and these proteins are key candidates for proteins involved in parasitism. Of these proteins, many are likely to be secreted into their host based on the prediction of a signal peptides in their protein sequence and the presence of these proteins in the secretome (Hunt *et al.*, 2016). The secretion of these proteins is important because proteins that have a role in parasitism are likely to interact directly with their host, for example interacting with components of the host's immune system or the host's gut (Hewitson *et al.*, 2009). Secreted proteins that are highly expressed in parasitic females compared with free-living females, therefore offer the best candidates for further investigation into their role in parasitism.

Empirical studies are required to investigate the role, if any, that these *Strongyloides* proteins have in parasitism. For example, measuring the effect of parasite proteins on immune responses will identify if a protein has immunomodulatory properties (McSorley *et al.*, 2013). Another avenue of research that will be important for uncovering the functions and roles of parasite proteins, is transgenic studies. Transgenic techniques have been established in few parasitic nematode species. Recent developments in transgenic techniques for *S. ratti* and *S. stercoralis* will now enable the expression patterns of proteins involved in parasitism to be investigated (Li *et al.*, 2011; Shao *et al.*, 2012). *Strongyloides* spp. therefore offer exciting opportunities to further explore the role of protein families with a putative role in parasitism. Furthermore, the potential application of knock-down and knock-in techniques for parasitic nematodes, which are especially available for *Strongyloides* (Lok, this issue; Ward, 2015), will provide important tools for exploring the function and importance of these proteins in parasitism.

Comparison of the genetically identical parasitic and free-living stages of *Strongyloides* offers a powerful tool to study nematode parasitism. However, when comparing these two life cycle stages it is also important to consider the other phenotypic differences that exists between parasitic and free-living *Strongyloides* adult nematodes, which will also be

determined by differential gene expression. In addition to their considerable differences in life style *i.e* parasitic *vs.* free-living, they also differ in their longevity, morphology and method of reproduction. Parasitic adults live for around a month in the gut of their host and this is extended up to a year in immunocompromised hosts. By comparison free-living adults are short-lived and survive for around 5-6 days as adults. Parasitic adults are approximately 2mm long, twice the size of free-living females which also differ in their oesophagus and gut morphology (Gardner *et al.*, 2004, 2006). The protein and gene families that are differentially expressed between parasitic and free-living females may therefore also represent differences in longevity, morphology and reproductive strategy.

Here, we have compared the acetyl cholinesterase protein families across fourteen nematode species including four *Strongyloides* species, and in a previous study (Hunt *et al.*, 2016) we compared the astacin and SCP/TAPS protein families cross the same 14 species. These comparisons have revealed that the *Strongyloides* and *Parastrongyloides* nematodes have undergone a massive expansion of genes encoding protein families with a putative role in parasitism, compared to most other parasitic nematode species spanning four nematode clades. The exception is *N. americanus* which also has a greater number of both astacins and SCP/TAPS genes, though phylogenetic analysis indicates that these possible gene family expansions are independent to the expansions observed in *Strongyloides* and *Parastrongyloides* (Hunt *et al.*, 2016). Future studies including greater numbers of parasitic and free-living nematode species are needed to identify if a similar phenomenon of major expansions of gene families with a putative role in parasitism has occurred in parasitic nematode species more widely. Within *Strongyloides* the high level of sequence similarity of genes within a given expanded gene family indicates that these genes have probably arisen through tandem duplication (Hunt *et al.*, 2016). Many of these genes are also physically clustered in the genome, often located in a region at the end of chromosome II suggesting this particular region has been subject to multiple tandem duplication events, where genes from different gene families with a putative role in parasitism have duplicated. The clustering of these genes could imply that they are under selective pressures to maintain close proximity. For example genes in the same region of a chromosome may share

regulatory elements or allow accessibility to certain transcription factors (Lercher *et al.*, 2003).

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Figure legends

Figure 1. The *Strongyloides* life cycle and the protein families associated with the parasitic adult female, free-living adult female and third stage infective larvae (iL3), and their putative roles in nematode parasitism. Adapted from Hunt *et al.* (2016)

Figure 2. Key gene and protein families with a putative role in parasitism. Gene and protein families that are significantly upregulated in the proteome and transcriptome of parasitic adult female compared to free-living adult females *S. ratti* nematodes.

Figure 3. The phylogeny of acetylcholinesterases from 14 nematode species including four *Strongyloides* species (red) and two species, *Parastrongyloides* (orange) and *Rhabditophanes* (pink), from the same evolutionary clade as *Strongyloides*. The four acetylcholinesterases, ACE 1-4, in the *C. elegans* genome are marked on the phylogeny. Acetylcholinesterases from *Strongyloides* and *Parastrongyloides* were categorised into eight distinct groups based on clustering in the phylogeny. The scale bar represents the number of amino acid substitutions. The phylogeny also includes genes predicted to encode acetylcholinesterases for eight further species of nematode including *Caenorhabditis elegans*(black), *Necator americanus* (brown), *Bursaphelenchus xylophilus* (light green), *Trichinella spiralis* (light blue), *Trichuris muris* (blue), *Brugia malayi* (navy blue), = *Ascaris suum* (purple), *Meloidogyne hapla* (dark green).

REFERENCES

Albertson, D. G., Nwaorgu, O. C. and Sulston, J. E. (1979). Chromatin diminution and a chromosomal mechanism of sexual differentiation in *Strongyloides papillosus*. *Chromosoma* **75**, 75–87.

Armstrong, S. D., Babayan, S. A., Lhermitte-Vallarino, N., Gray, N., Xia, D., Martin, C., Kumar, S., Taylor, D. W., Blaxter, M. L., Wastling, J. M. and Makepeace, B. L. (2014). Comparative analysis of the secretome from a model filarial nematode (*Litomosoides sigmodontis*) reveals maximal diversity in gravid female parasites. *Molecular & Cellular Proteomics* **13**, 2527–44.

Arumugam, S., Zhan, B., Abraham, D., Ward, D., Lustigman, S. and Klei, T. R. (2014). Vaccination with recombinant *Brugia malayi* cystatin proteins alters worm migration, homing and final niche selection following a subcutaneous challenge of Mongolian gerbils (*Meriones unguiculatus*) with *B. malayi* infective larvae. *Parasites & Vectors* **7**, 43.

Asojo, O. A. (2011). Structure of a two-CAP-domain protein from the human hookworm parasite *Necator americanus*. *Acta crystallographica., Biological Crystallography* **67**, 455–62.

Asojo, O. A., Goud, G., Dhar, K., Loukas, A., Zhan, B., Deumic, V., Liu, S., Borgstahl, G. E. O. and Hotez, P. J. (2005). X-ray structure of Na-ASP-2, a pathogenesis-related-1 protein from the nematode parasite, *Necator americanus*, and a vaccine antigen for human hookworm infection. *Journal of Molecular Biology* **346**, 801–14.

Balasubramanian, N., Toubarro, D., Nascimento, G., Ferreira, R. and Simões, N. (2012). Purification, molecular characterization and gene expression analysis of an aspartic protease (Sc-ASP113) from the nematode *Steinernema carpocapsae* during the parasitic stage. *Molecular and Biochemical Parasitology* **182**, 37–44.

Bastos, I. M. D., Grellier, P., Martins, N. F., Cadavid-Restrepo, G., de Souza-Ault, M. R., Augustyns, K., Teixeira, A. R. L., Schrével, J., Maigret, B., da Silveira, J. F. and Santana, J. M. (2005).

Molecular, functional and structural properties of the prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80), which is required for parasite entry into mammalian cells. *The Biochemical Journal* **388**, 29–38.

Bastos, I. M. D., Motta, F. N., Grellier, P. and Santana, J. M. (2013). Parasite prolyl oligopeptidases and the challenge of designing chemotherapeuticals for Chagas disease, leishmaniasis and African trypanosomiasis. *Current Medicinal Chemistry* **20**, 3103–15.

Bolla, R. I. and Roberts, L. S. (1968). Gametogenesis and chromosomal complement in *Strongyloides ratti* (Nematoda: Rhabdiasoidea). *The Journal of Parasitology* **54**, 849–55.

Bond, J. S. and Beynon, R. J. (1995). The astacin family of metalloendopeptidases. *Protein science* **4**, 1247–61.

Borchert, N., Becker-Pauly, C., Wagner, A., Fischer, P., Stöcker, W. and Brattig, N. W. (2007). Identification and characterization of onchoastacin, an astacin-like metalloproteinase from the filaria *Onchocerca volvulus*. *Microbes and Infection* **9**, 498–506.

Brindley, P. J., Gam, A. A., McKerrow, J. H. and Neva, F. A. (1995). Ss40: the zinc endopeptidase secreted by infective larvae of *Strongyloides stercoralis*. *Experimental Parasitology* **80**, 1–7.

Brindley, P. J., Kalinna, B. H., Wong, J. Y., Bogitsh, B. J., King, L. T., Smyth, D. J., Verity, C. K., Abbenante, G., Brinkworth, R. I., Fairlie, D. P., Smythe, M. L., Milburn, P. J., Bielefeldt-Ohmann, H., Zheng, Y. and McManus, D. P. (2001). Proteolysis of human hemoglobin by schistosome cathepsin D. *Molecular and Biochemical Parasitology* **112**, 103–12.

Camargo, A. C., Caldo, H. and Reis, M. L. (1979). Susceptibility of a peptide derived from bradykinin to hydrolysis by brain endo-oligopeptidases and pancreatic proteinases. *The Journal of Biological Chemistry* **254**, 5304–7.

Cantacessi, C. and Gasser, R. B. (2012). SCP/TAPS proteins in helminths - where to from now? *Molecular and Cellular Probes* **26**, 54–9.

Cantacessi, C., Campbell, B. E., Visser, A., Geldhof, P., Nolan, M. J., Nisbet, A. J., Matthews, J. B., Loukas, A., Hofmann, A., Otranto, D., Sternberg, P. W. and Gasser, R. B. (2009). A portrait of the "SCP/TAPS" proteins of eukaryotes-developing a framework for fundamental research and biotechnological outcomes. *Biotechnology advances* **27**, 376–88.

Cantacessi, C., Gasser, R. B., Strube, C., Schnieder, T., Jex, A. R., Hall, R. S., Campbell, B. E., Young, N. D., Ranganathan, S., Sternberg, P. W. and Mitreva, M. (2011). Deep insights into *Dictyocaulus viviparus* transcriptomes provides unique prospects for new drug targets and disease intervention. *Biotechnology Advances* **29**, 261–71.

Cappello, M., Vlasuk, G. P., Bergum, P. W., Huang, S. and Hotez, P. J. (1995). *Ancylostoma caninum* anticoagulant peptide: a hookworm-derived inhibitor of human coagulation factor Xa. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 6152–6.

Chen, C. K.-M., Chan, N.-L. and Wang, A. H.-J. (2011). The many blades of the β-propeller proteins: conserved but versatile. *Trends in Biochemical Sciences* **36**, 553–561.

Combes, D., Fedon, Y., Grauso, M., Toutant, J. P. and Arpagaus, M. (2000). Four genes encode acetylcholinesterases in the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*. cDNA sequences, genomic structures, mutations and in vivo expression. *Journal of Molecular Biology* **300**, 727–42.

Culetto, E., Combes, D., Fedon, Y., Roig, A., Toutant, J. P. and Arpagaus, M. (1999). Structure and promoter activity of the 5' flanking region of ace-1, the gene encoding acetylcholinesterase of class A in *Caenorhabditis elegans. Journal of Molecular Biology* **290**, 951–66.

Culley, F. J., Brown, A., Conroy, D. M., Sabroe, I., Pritchard, D. I. and Williams, T. J. (2000). Eotaxin is specifically cleaved by hookworm metalloproteases preventing its action *in vitro* and *in vivo*. *Journal of immunology* **165**, 6447–53.

Culotti, J. G., Von Ehrenstein, G., Culotti, M. R. and Russell, R. L. (1981). A second class of

acetylcholinesterase-deficient mutants of the nematode *Caenorhabditis elegans. Genetics* **97**, 281–305.

Dainichi, T., Maekawa, Y., Ishii, K., Zhang, T., Nashed, B. F., Sakai, T., Takashima, M. and Himeno, K. (2001). Nippocystatin, a cysteine protease inhibitor from *Nippostrongylus brasiliensis*, inhibits antigen processing and modulates antigen-specific immune response. *Infection and Immunity* **69**, 7380–6.

Darby, M., Schnoeller, C., Vira, A., Culley, F., Bobat, S., Logan, E., Kirstein, F., Wess, J., Cunningham, A. F., Brombacher, F., Selkirk, M. E. and Horsnell, W. G. C. (2015). The M3 Muscarinic Receptor Is Required for Optimal Adaptive Immunity to Helminth and Bacterial Infection. *PLoS Pathogens* **11**, 1–15.

Dash, C., Kulkarni, A., Dunn, B. and Rao, M. (2003). Aspartic peptidase inhibitors: implications in drug development. *Critical Reviews in Biochemistry and Molecular Biology* **38**, 89–119.

Davis, M. W., Birnie, A. J., Chan, A. C., Page, A. P. and Jorgensen, E. M. (2004). A conserved metalloprotease mediates ecdysis in *Caenorhabditis elegans*. *Development* **131**, 6001–8.

Del Valle, A., Jones, B. F., Harrison, L. M., Chadderdon, R. C. and Cappello, M. (2003). Isolation and molecular cloning of a secreted hookworm platelet inhibitor from adult *Ancylostoma caninum*. *Molecular and Biochemical Parasitology* **129**, 167–77.

Fajtová, P., Štefanić, S., Hradilek, M., Dvořák, J., Vondrášek, J., Jílková, A., Ulrychová, L., McKerrow, J. H., Caffrey, C. R., Mareš, M. and Horn, M. (2015). Prolyl Oligopeptidase from the Blood Fluke *Schistosoma mansoni:* From Functional Analysis to Anti-schistosomal Inhibitors. *PLOS Neglected Tropical Diseases* **9**, e0003827.

Fülöp, V., Böcskei, Z. and Polgár, L. (1998). Prolyl oligopeptidase: an unusual beta-propeller domain regulates proteolysis. *Cell* **94**, 161–70.

Gamble, H. R., Fetterer, R. H. and Mansfield, L. S. (1996). Developmentally regulated zinc

metalloproteinases from third- and fourth-stage larvae of the ovine nematode *Haemonchus contortus. The Journal of Parasitology* **82**, 197–202.

Gardner, M. P., Gems, D. and Viney, M. E. (2004). Aging in a very short-lived nematode. *Experimental Gerontology* **39**, 1267-1276.

Gardner, M. P., Gems, D. and Viney, M. E. (2006). Extraordinary plasticity in aging in *Strongyloides ratti* implies a gene-regulatory mechanism of lifespan evolution. *Aging Cell* **5**, 315–23.

Gass, J. and Khosla, C. (2007). Prolyl endopeptidases. *Cellular and Molecular Life Sciences* **64**, 345–55.

Gibney, G., Camp, S., Dionne, M., MacPhee-Quigley, K. and Taylor, P. (1990). Mutagenesis of essential functional residues in acetylcholinesterase. *Proceedings of the National Academy of Sciences* **87**, 7546–7550.

Goud, G. N., Bottazzi, M. E., Zhan, B., Mendez, S., Deumic, V., Plieskatt, J., Liu, S., Wang, Y., Bueno, L., Fujiwara, R., Samuel, A., Ahn, S. Y., Solanki, M., Asojo, O. A., Wang, J., Bethony, J. M., Loukas, A., Roy, M. and Hotez, P. J. (2005). Expression of the *Necator americanus* hookworm larval antigen Na-ASP-2 in *Pichia pastoris* and purification of the recombinant protein for use in human clinical trials. *Vaccine* **23**, 4754–64.

Grellier, P., Vendeville, S., Joyeau, R., Bastos, I. M. D., Drobecq, H., Frappier, F., Teixeira, A. R. L., Schrevel, J., Davioud-Charvet, E., Sergheraert, C. and Santana, J. M. (2001). *Trypanosoma cruzi* prolyl oligopeptidase Tc80 is involved in nonphagocytic mammalian cell invasion by trypomastigotes. *Journal of Biological Chemistry* **276**, 47078–47086.

Grisaru, D., Sternfeld, M., Eldor, A., Glick, D. and Soreq, H. (1999). Structural roles of acetylcholinesterase variants in biology and pathology. *European Journal of Biochemistry* **264**, 672–686.

Hammond, M. P. and Robinson, R. D. (1994). Chromosome complement, gametogenesis, and

development of *Strongyloides stercoralis*. *The Journal of Parasitology* **80**, 689–95.

Hartmann, S. and Lucius, R. (2003). Modulation of host immune responses by nematode cystatins. *International Journal for Parasitology* **33**, 1291–1302.

Hartmann, S., Schönemeyer, A., Sonnenburg, B., Vray, B. and Lucius, R. (2002). Cystatins of filarial nematodes up-regulate the nitric oxide production of interferon-gamma-activated murine macrophages. *Parasite Immunology* **24**, 253–62.

Hawdon, J. M., Jones, B. F., Hoffman, D. R. and Hotez, P. J. (1996). Cloning and characterization of Ancylostoma-secreted protein. A novel protein associated with the transition to parasitism by infective hookworm larvae. *The Journal of Biological Chemistry* **271**, 6672–8.

Hewitson, J. P., Harcus, Y. M., Curwen, R. S., Dowle, A. A., Atmadja, A. K., Ashton, P. D., Wilson, A. and Maizels, R. M. (2008). The secretome of the filarial parasite, *Brugia malayi:* proteomic profile of adult excretory-secretory products. *Molecular and Biochemical Parasitology* **160**, 8–21.

Hewitson, J. P., Grainger, J. R. and Maizels, R. M. (2009). Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. *Molecular and Biochemical Parasitology* **167**, 1–11.

Hewitson, J. P., Harcus, Y., Murray, J., van Agtmaal, M., Filbey, K. J., Grainger, J. R., Bridgett, S., Blaxter, M. L., Ashton, P. D., Ashford, D. A., Curwen, R. S., Wilson, R. A., Dowle, A. A. and Maizels, R. M. (2011). Proteomic analysis of secretory products from the model gastrointestinal nematode *Heligmosomoides polygyrus* reveals dominance of venom allergen-like (VAL) proteins. *Journal of Proteomics* **74**, 1573–94.

Hino, A., Tanaka, T., Takaishi, M., Fujii, Y., Palomares-Rius, J. E., Hasegawa, K., Maruyama, H. and Kikuchi, T. (2014). Karyotype and reproduction mode of the rodent parasite *Strongyloides venezuelensis*. *Parasitology* **141**, 1736–45.

Hishida, R., Ishihara, T., Kondo, K. and Katsura, I. (1996). hch-1, a gene required for normal hatching and normal migration of a neuroblast in *C. elegans*, encodes a protein related to TOLLOID and BMP-1. *EMBO journal* **15**, 4111–22.

Hunt, V. L., Tsai, I. J., Coghlan, A., Reid, A. J., Holroyd, N., Foth, B. J., Tracey, A., Cotton, J. A., Stanley, E. J., Beasley, H., Bennett, H. M., Brooks, K., Harsha, B., Kajitani, R., Kulkarni, A., Harbecke, D., Nagayasu, E., Nichol, S., Ogura, Y., Quail, M. A., Randle, N., Xia, D., Brattig, N. W., Soblik, H., Ribeiro, D. M., Sanchez-Flores, A., Hayashi, T., Itoh, T., Denver, D. R., Grant, W., Stoltzfus, J. D., Lok, J. B., Murayama, H., Wastling, J., Streit, A., Kikuchi, T., Viney, M. and Berriman, M. (2016). The genomic basis of parasitism in the *Strongyloides* clade of nematodes. *Nature Genetics* **48**, 299-307.

Huntington, J. A., Read, R. J. and Carrell, R. W. (2000). Structure of a serpin-protease complex shows inhibition by deformation. *Nature* **407**, 923–6.

Hussein, A. S., Chacón, M. R., Smith, A. M., Tosado-Acevedo, R. and Selkirk, M. E. (1999). Cloning, expression, and properties of a nonneuronal secreted acetylcholinesterase from the parasitic nematode *Nippostrongylus brasiliensis*. *The Journal of Biological Chemistry* **274**, 9312–9.

Hussein, A. S., Smith, A. M., Chacon, M. R. and Selkirk, M. E. (2000). Determinants of substrate specificity of a second non-neuronal secreted acetylcholinesterase from the parasitic nematode *Nippostrongylus brasiliensis*. *European Journal of Biochemistry* **267**, 2276–2282.

Hussein, A. S., Kichenin, K. and Selkirk, M. E. (2002). Suppression of secreted acetylcholinesterase expression in *Nippostrongylus brasiliensis* by RNA interference. *Molecular and Biochemical Parasitology* **122**, 91–94.

Irving, J. A. (2000). Phylogeny of the Serpin Superfamily: Implications of Patterns of Amino Acid Conservation for Structure and Function. *Genome Research* **10**, 1845–1864.

Irving, J. A., Pike, R. N., Dai, W., Brömme, D., Worrall, D. M., Silverman, G. A., Coetzer, T. H. T.,

Dennison, C., Bottomley, S. P. and Whisstock, J. C. (2002). Evidence that serpin architecture intrinsically supports papain-like cysteine protease inhibition: engineering alpha(1)-antitrypsin to inhibit cathepsin proteases. *Biochemistry* **41**, 4998–5004.

Jacob, J., Vanholme, B., Haegeman, A. and Gheysen, G. (2007). Four transthyretin-like genes of the migratory plant-parasitic nematode *Radopholus similis*: members of an extensive nematode-specific family. *Gene* **402**, 9–19.

Johnson, C. D., Duckett, J. G., Culotti, J. G., Herman, R. K., Meneely, P. M. and Russell, R. L. (1981). An acetylcholinesterase-deficient mutant of the nematode *Caenorhabditis elegans*. *Genetics* **97**, 261–79.

Johnson, C. D., Rand, J. B., Herman, R. K., Stern, B. D. and Russell, R. L. (1988). The acetylcholinesterase genes of *C. elegans*: identification of a third gene (ace-3) and mosaic mapping of a synthetic lethal phenotype. *Neuron* **1**, 165–73.

Jolodar, A., Fischer, P., Büttner, D. W., Miller, D. J., Schmetz, C. and Brattig, N. W. (2004). *Onchocerca volvulus*: expression and immunolocalization of a nematode cathepsin D-like lysosomal aspartic protease. *Experimental Parasitology* **107**, 145–56.

Knox, D. P. (2007). Proteinase inhibitors and helminth parasite infection. *Parasite Immunology* **29**, 57–71.

Koelsch, G., Mares, M., Metcalf, P. and Fusek, M. (1994). Multiple functions of pro-parts of aspartic proteinase zymogens. *FEBS letters* **343**, 6–10.

Kolson, D. L. and Russell, R. L. (1985). A novel class of acetylcholinesterase, revealed by mutations, in the nematode *Caenorhabditis elegans*. *Journal of Neurogenetics* **2**, 93–110.

Law, R. H. P., Zhang, Q., McGowan, S., Buckle, A. M., Silverman, G. A., Wong, W., Rosado, C. J., Langendorf, C. G., Pike, R. N., Bird, P. I. and Whisstock, J. C. (2006). An overview of the serpin superfamily. *Genome biology* **7**, 216.

Lazari, O., Hussein, A. S., Selkirk, M. E., Davidson, A. J., Thompson, F. J. and Matthews, J. B. (2003). Cloning and expression of two secretory acetylcholinesterases from the bovine lungworm, *Dictyocaulus viviparus*. *Molecular and Biochemical Parasitology* **132**, 83–92.

Lee, D. L. (1970). The fine structure of the excretory system in adult *Nippostrongylus brasiliensis* (Nematoda) and a suggested function for the "excretory glands". *Tissue & cell* **2**, 225–31.

Lee, D. L. (1996). Why do some nematode parasites of the alimentary tract secrete acetylcholinesterase? *International Journal for Parasitology* **26**, 499–508.

Lercher, M. J., Blumenthal, T. and Hurst, L. D. (2003). Coexpression of neighboring genes in *Caenorhabditis elegans* is mostly due to operons and duplicate genes. *Genome Research,* 13, 238–243.

Li, X., Shao, H., Junio, A., Nolan, T. J., Massey, H. C., Pearce, E. J., Viney, M. E. and Lok, J. B. (2011). Transgenesis in the parasitic nematode *Strongyloides ratti*. *Molecular and biochemical parasitology* **179**, 114–9.

Lin, B., Zhuo, K., Chen, S., Hu, L., Sun, L., Wang, X., Zhang, L.-H. and Liao, J. (2016). A novel nematode effector suppresses plant immunity by activating host reactive oxygen species-scavenging system. *The New Phytologist* **209**, 1159–73.

Loewenstein-Lichtenstein, Y., Glick, D., Gluzman, N., Sternfeld, M., Zakut, H. and Soreq, H. (1996). Overlapping Drug Interaction Sites of Human Butyryicholinesterase Dissected by Site-Directed Mutagenesis. *Molecular Pharmacology* **50**, 1423-31.

Loukas, A., Bethony, J. M., Mendez, S., Fujiwara, R. T., Goud, G. N., Ranjit, N., Zhan, B., Jones, K., Bottazzi, M. E. and Hotez, P. J. (2005). Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection in dogs. *PLoS medicine* **2**, e295.

Maizels, R. M. and Yazdanbakhsh, M. (2003). Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology* **3**, 733–44.

Maizels, R. M., McSorley, H. J. and Smyth, D. J. (2014). Helminths in the hygiene hypothesis: sooner or later? *Clinical and Experimental Immunology* **177**, 38–46.

Manoury, B., Gregory, W. F., Maizels, R. M. and Watts, C. (2001). Bm-CPI-2, a cystatin homolog secreted by the filarial parasite *Brugia malayi*, inhibits class II MHC-restricted antigen processing. *Current Biology* **11**, 447–451.

McKeand, J. B., Knox, D. P., Duncan, J. L. and Kennedy, M. W. (1994). The immunogenicity of the acetylcholinesterases of the cattle lungworm *Dictyocaulus viviparus*. *International Journal for Parasitology* **24**, 501–510.

McKerrow, J. H., Brindley, P., Brown, M., Gam, A. A., Staunton, C. and Neva, F. A. (1990). *Strongyloides stercoralis*: identification of a protease that facilitates penetration of skin by the infective larvae. *Experimental Parasitology* **70**, 134–43.

McSorley, H. J., Hewitson, J. P. and Maizels, R. M. (2013). Immunomodulation by helminth parasites: defining mechanisms and mediators. *International Journal for Parasitology* **43**, 301–10.

Mello, L. V, O'Meara, H., Rigden, D. J. and Paterson, S. (2009). Identification of novel aspartic proteases from *Strongyloides ratti* and characterisation of their evolutionary relationships, stage-specific expression and molecular structure. *BMC genomics* **10**, 611.

Menon, R., Gasser, R. B., Mitreva, M. and Ranganathan, S. (2012). An analysis of the transcriptome of Teladorsagia circumcincta: its biological and biotechnological implications. *BMC Genomics* **13** S10.

Mitreva, M., McCarter, J. P., Martin, J., Dante, M., Wylie, T., Chiapelli, B., Pape, D., Clifton, S. W., Nutman, T. B. and Waterston, R. H. (2004). Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans.* *Genome research* **14**, 209–20.

Mitreva, M., Jasmer, D. P., Zarlenga, D. S., Wang, Z., Abubucker, S., Martin, J., Taylor, C. M., Yin, Y., Fulton, L., Minx, P., Yang, S.-P., Warren, W. C., Fulton, R. S., Bhonagiri, V., Zhang, X., Hallsworth-Pepin, K., Clifton, S. W., McCarter, J. P., Appleton, J., Mardis, E. R. and Wilson, R. K. (2011). The draft genome of the parasitic nematode Trichinella spiralis. *Nature genetics* **43**, 228–35. doi:10.1038/ng.769.

Moyle, M., Foster, D. L., McGrath, D. E., Brown, S. M., Laroche, Y., De Meutter, J., Stanssens, P., Bogowitz, C. A., Fried, V. A. and Ely, J. A. (1994). A hookworm glycoprotein that inhibits neutrophil function is a ligand of the integrin CD11b/CD18. *The Journal of Biological Chemistry* **269**, 10008–15.

Mulvenna, J., Hamilton, B., Nagaraj, S. H., Smyth, D., Loukas, A. and Gorman, J. J. (2009). Proteomics analysis of the excretory/secretory component of the blood-feeding stage of the hookworm, Ancylostoma caninum. *Molecular & Cellular Proteomics* **8**, 109–21.

Nisbet, A. J., McNeilly, T. N., Wildblood, L. A., Morrison, A. A., Bartley, D. J., Bartley, Y., Longhi, C., McKendrick, I. J., Palarea-Albaladejo, J. and Matthews, J. B. (2013). Successful immunization against a parasitic nematode by vaccination with recombinant proteins. *Vaccine* **31**, 4017–23.

Novelli, J., Page, A. P. and Hodgkin, J. (2006). The C terminus of collagen SQT-3 has complex and essential functions in nematode collagen assembly. *Genetics* **172**, 2253–67.

Ollis, D. L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Sybille, M., Harel, M., James Remington, S., Silman, I., Schrag, J., Sussman, J. L., Koen, H. G. V. and Goldman, A. (1992). The alpha/ beta hydrolase fold. *Protein Engineering, Design and Selection* **5**, 197–211.

Ordentlich, A., Barak, D., Kronman, C., Ariel, N., Segall, Y., Velan, B. and Shafferman, A. (1995). Contribution of aromatic moieties of tyrosine 133 and of the anionic subsite tryptophan 86 to catalytic efficiency and allosteric modulation of acetylcholinesterase. *Journal of Biological Chemistry* **270**, 2082–2091.

Page, A. P. and Winter, A. D. (2003). Enzymes involved in the biogenesis of the nematode cuticle. *Advances in Parasitology* **53**, 85–148.

Page, A. P., Stepek, G., Winter, A. D. and Pertab, D. (2014). Enzymology of the nematode cuticle: A potential drug target? *International Journal for Parasitology. Drugs and drug resistance* **4**, 133–41.

Pemberton, P. A., Stein, P. E., Pepys, M. B., Potter, J. M. and Carrell, R. W. (1988). Hormone binding globulins undergo serpin conformational change in inflammation. *Nature* **336**, 257–8.

Pfaff, A. W., Schulz-Key, H., Soboslay, P. T., Taylor, D. W., MacLennan, K. and Hoffmann, W. H. (2002). *Litomosoides sigmodontis* cystatin acts as an immunomodulator during experimental filariasis. *International Journal for Parasitology* **32**, 171–8.

Power, D. M., Elias, N. P., Richardson, S. J., Mendes, J., Soares, C. M. and Santos, C. R. (2000). Evolution of the thyroid hormone-binding protein, transthyretin. *General and Comparative endocrinology* **119**, 241–55.

Radić, Z., Gibney, G., Kawamoto, S., MacPhee-Quigley, K., Bongiorno, C. and Taylor, P. (1992). Expression of recombinant acetylcholinesterase in a baculovirus system: kinetic properties of glutamate 199 mutants. *Biochemistry* **31**, 9760–9767.

Ramazzina, I., Folli, C., Secchi, A., Berni, R. and Percudani, R. (2006). Completing the uric acid degradation pathway through phylogenetic comparison of whole genomes. *Nature Chemical Biology* **2**, 144–148.

Rand, J. B. and Nonet, M. L. (1997). Neurotransmitter assignents for specific neurons In *C. elegans II.* Ed. Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess J.R. Cold Spring Harbor Laboratory Press.

Rawlings, N. D., Tolle, D. P. and Barrett, A. J. (2004). Evolutionary families of peptidase inhibitors. *The Biochemical Journal* **378**, 705–16.

Rawlings, N. D., Barrett, A. J. and Bateman, A. (2010). MEROPS: the peptidase database. *Nucleic Acids Research* **38**, D227–33.

Ray, C. A., Black, R. A., Kronheim, S. R., Greenstreet, T. A., Sleath, P. R., Salvesen, G. S. and Pickup, D. J. (1992). Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. *Cell* **69**, 597–604.

Santos, L. O., Garcia-Gomes, A. S., Catanho, M., Sodre, C. L., Santos, A. L. S., Branquinha, M. H. and d'Avila-Levy, C. M. (2013). Aspartic peptidases of human pathogenic trypanosomatids: perspectives and trends for chemotherapy. *Current medicinal chemistry* **20**, 3116–33.

Sauer-Eriksson, A. E., Linusson, A. and Lundberg, E. (2009). Transthyretin-related and transthyretin-like proteins. In *Recent Advances in Transthyretin Evolution, Structure and Biological Functions* Ed. Richardson, S. J. and Cody, V.

Schwarz, E. M., Korhonen, P. K., Campbell, B. E., Young, N. D., Jex, A. R., Jabbar, A., Hall, R. S., Mondal, A., Howe, A. C., Pell, J., Hofmann, A., Boag, P. R., Zhu, X.-Q., Gregory, T., Loukas, A., Williams, B. A., Antoshechkin, I., Brown, C., Sternberg, P. W. and Gasser, R. B. (2013). The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus*. *Genome Biology* **14**, R89.

Schwarz, E. M., Hu, Y., Antoshechkin, I., Miller, M. M., Sternberg, P. W. and Aroian, R. V (2015). The genome and transcriptome of the zoonotic hookworm *Ancylostoma ceylanicum* identify infection-specific gene families. *Nature genetics* **47**, 416–22.

Segerberg, M. A. and Stretton, A. O. W. (1993). Actions of cholinergic drugs in the nematode *Ascaris suum*. Complex pharmacology of muscle and motorneuron. *J.Gen.Physiol.* **101**, 271–286.

Selkirk, M. E., Lazari, O. and Matthews, J. B. (2005). Functional genomics of nematode acetylcholinesterases. *Parasitology* **131**, S3–18.

Shafferman, A., Velan, B., Ordentlich, A., Kronman, C., Grosfeld, H., Leitner, M., Flashner, Y.,

Cohen, S., Barak, D. and Ariel, N. (1992). Substrate inhibition of acetylcholinesterase: residues affecting signal transduction from the surface to the catalytic center. *EMBO Journal* **11**, 3561–8.

Shao, H., Li, X., Nolan, T. J., Massey, H. C., Pearce, E. J. and Lok, J. B. (2012). Transposon-mediated chromosomal integration of transgenes in the parasitic nematode Strongyloides ratti and establishment of stable transgenic lines. *PLoS Pathogens* **8**, e1002871.

Sharma, A., Eapen, A. and Subbarao, S. K. (2005). Purification and characterization of a hemoglobin degrading aspartic protease from the malarial parasite *Plasmodium vivax*. *Journal of Biochemistry* **138**, 71–8.

Shaw, R. J., McNeill, M. M., Maass, D. R., Hein, W. R., Barber, T. K., Wheeler, M., Morris, C. A. and Shoemaker, C. B. (2003). Identification and characterisation of an aspartyl protease inhibitor homologue as a major allergen of *Trichostrongylus colubriformis*. *International Journal for Parasitology* **33**, 1233–43.

Silman, I. and Sussman, J. L. (2005). Acetylcholinesterase: "Classical" and "non-classical" functions and pharmacology. *Current Opinion in Pharmacology* **5**, 293–302.

Soblik, H., Younis, A. E., Mitreva, M., Renard, B. Y., Kirchner, M., Geisinger, F., Steen, H. and Brattig, N. W. (2011). Life cycle stage-resolved proteomic analysis of the excretome/secretome from *Strongyloides ratti*-identification of stage-specific proteases. *Molecular & Cellular Proteomics* **10**, M111.010157.

Sonnhammer, E. L. and Durbin, R. (1997). Analysis of protein domain families in *Caenorhabditis elegans*. *Genomics* **46**, 200–16.

Stassens, P., Bergum, P. W., Gansemans, Y., Jespers, L., Laroche, Y., Huang, S., Maki, S., Messens, J., Lauwereys, M., Cappello, M., Hotez, P. J., Lasters, I. and Vlasuk, G. P. (1996). Anticoagulant repertoire of the hookworm *Ancylostoma caninum*. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 2149–54.

Stepek, G., McCormack, G., Winter, A. D. and Page, A. P. (2015). A highly conserved, inhibitable astacin metalloprotease from Teladorsagia circumcincta is required for cuticle formation and nematode development. *International Journal for Parasitology* **45**, 345–55.

Sun, Y., Liu, G., Li, Z., Chen, Y., Liu, Y., Liu, B. and Su, Z. (2013). Modulation of dendritic cell function and immune response by cysteine protease inhibitor from murine nematode parasite *Heligmosomoides polygyrus*. *Immunology* **138**, 370–81.

Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldmant, A., Toker, L. and Silman, I. (1991). Acetyicholinesterase from *Torpedo calfornica* : A prototypic acetyicholine-binding protein. *Science* **253**, 872–879.

Suzuki, M., Sagoh, N., Iwasaki, H., Inoue, H. and Takahashi, K. (2004). Metalloproteases with EGF, CUB, and thrombospondin-1 domains function in molting of *Caenorhabditis elegans*. *Biological chemistry* **385**, 565–8.

Tang, Y. T., Gao, X., Rosa, B. A., Abubucker, S., Hallsworth-Pepin, K., Martin, J., Tyagi, R., Heizer, E., Zhang, X., Bhonagiri-Palsikar, V., Minx, P., Warren, W. C., Wang, Q., Zhan, B., Hotez, P. J., Sternberg, P. W., Dougall, A., Gaze, S. T., Mulvenna, J., Sotillo, J., Ranganathan, S., Rabelo, E. M., Wilson, R. K., Felgner, P. L., Bethony, J., Hawdon, J. M., Gasser, R. B., Loukas, A. and Mitreva, M. (2014). Genome of the human hookworm *Necator americanus. Nature Genetics* **46**, 261–9.

Tort, J., Brindley, P. J., Knox, D., Wolfe, K. H. and Dalton, J. P. (1999). Proteinases and associated genes of parasitic helminths. *Advances in Parasitology* **43**, 161–266.

Tracey, K. J. (2009). Reflex control of immunity. *Nature Reviews Immunology* **9**, 418–428.

Valdivieso, E., Perteguer, M. J., Hurtado, C., Campioli, P., Rodríguez, E., Saborido, A., Martínez-Sernández, V., Gómez-Puertas, P., Ubeira, F. M. and Gárate, T. (2015). ANISERP: a new serpin from the parasite *Anisakis simplex*. *Parasites & Vectors* **8**, 399.

Viney, M. E. (1994). A genetic analysis of reproduction in *Strongyloides ratti*. *Parasitology* **109**, 511–5.

Wang, E. A., Rosen, V., Cordes, P., Hewick, R. M., Kriz, M. J., Luxenberg, D. P., Sibley, B. S. and Wozney, J. M. (1988). Purification and characterization of other distinct bone-inducing factors. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 9484–8.

Wang, H., Yu, M., Ochani, M., Amella, C. A., Tanovic, M., Susarla, S., Li, J. H., Wang, H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C. J. and Tracey, K. J. (2003). Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* **421**, 384–388. doi:10.1038/nature01339.

Wang, X., Li, W., Zhao, D., Liu, B., Shi, Y., Chen, B., Yang, H., Guo, P., Geng, X., Shang, Z., Peden, E., Kage-Nakadai, E., Mitani, S. and Xue, D. (2010). *Caenorhabditis elegans* transthyretin-like protein TTR-52 mediates recognition of apoptotic cells by the CED-1 phagocyte receptor. *Nature Cell Biology* **12**, 655–64.

Wang, T., Van Steendam, K., Dhaenens, M., Vlaminck, J., Deforce, D., Jex, A. R., Gasser, R. B. and Geldhof, P. (2013). Proteomic analysis of the excretory-secretory products from larval stages of *Ascaris suum* reveals high abundance of glycosyl hydrolases. *PLoS Neglected Tropical Diseases* **7**, e2467.

Wang, S., Wei, W., Luo, X., Wang, S., Hu, S. and Cai, X. (2015). Comparative genomic analysis of aspartic proteases in eight parasitic platyhelminths: insights into functions and evolution. *Gene* **559**, 52–61.

Ward, J. D. (2015). Rendering the Intractable More Tractable: Tools from *Caenorhabditis elegans* ripe for import into parasitic nematodes. *Genetics* **201**, 1279–1294.

Whelan, R. A. K., Hartmann, S. and Rausch, S. (2012). Nematode modulation of inflammatory bowel disease. *Protoplasma* **249**, 871–86.

Williamson, A. L., Brindley, P. J., Abbenante, G., Prociv, P., Berry, C., Girdwood, K., Pritchard, D. I., Fairlie, D. P., Hotez, P. J., Dalton, J. P. and Loukas, A. (2002). Cleavage of hemoglobin by hookworm cathepsin D aspartic proteases and its potential contribution to host specificity. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **16**, 1458–60.

Williamson, A. L., Lustigman, S., Oksov, Y., Deumic, V., Plieskatt, J., Mendez, S., Zhan, B., Bottazzi, M. E., Hotez, P. J. and Loukas, A. (2006). *Ancylostoma caninum* MTP-1, an astacin-like metalloprotease secreted by infective hookworm larvae, is involved in tissue migration. *Infection and Immunity* **74**, 961–7.

Wilson, M. S., Taylor, M. D., O'Gorman, M. T., Balic, A., Barr, T. A., Filbey, K., Anderton, S. M. and Maizels, R. M. (2010). Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. *European Journal of Immunology* **40**, 1682–96.

Zang, X. and Maizels, R. M. (2001). Serine proteinase inhibitors from nematodes and the arms race between host and pathogen. *Trends in biochemical sciences* **26**, 191–7.

Zang, X., Yazdanbakhsh, M., Jiang, H., Kanost, M. R. and Maizels, R. M. (1999). A novel serpin expressed by blood-borne microfilariae of the parasitic nematode *Brugia malayi* inhibits human neutrophil serine proteinases. *Blood* **94**, 1418–28.

Table 1. Summary of the key protein families with a putative role in parasitism in *Strongyloides.*

Species	Protein family	Total no. of genes in the genome	No. of genes upregulated in parasitic stage	No. of genes upregulated in free-living stage	Fold change range (log_2FC)[a]
S. ratti	Acetylcholinesterase	24	17	1	2.06 - 10.01
	Astacin	184	106	10	2.34 - 12.00
	SCP/TAPS	89	63	5	2.75 - 12.93
	Trypsin-like inhibitor	18	11	1	1.17 - 11.24
	Transthyretin-like	40	13	4	2.12 - 12.61
	Prolyl endopeptidase	23	17	1	1.02 - 10.86
	Aspartic protease	53	6	6	1.87 - 9.81
S. stercoralis	Acetylcholinesterase	28	24	0	3.80 - 12.98
	Astacin	237	146	13	2.02 - 17.32
	SCP/TAPS	113	64	8	1.85 - 14.25
	Trypsin-like inhibitor	17	10	2	3.88 - 13.60
	Transthyretin-like	52	20	4	3.28 - 17.94
	Prolyl endopeptidase	17	11	2	2.66 - 13.88
	Aspartic protease	53	15	2	3.28 - 12.71
Rhabditophanes	Acetylcholinesterase	2			
	Astacin	36			

	SCP/TAPS	12
	Trypsin-like inhibitor	31
	Transthyretin-like	34
	Prolyl endopeptidase	8
	Aspartic protease	33
C. elegans	Acetylcholinesterase	4
	Astacin	40
	SCP/TAPS	36
	Transthyretin-like	59
	Prolyl oligopeptidase	6
	Aspartic peptidase	18

[a] FC – Fold change. Values represent the range of $\log_2$ fold change values for expression in the parasitic female compared with the free-living adult female transcriptome.

Table 2. Domain combinations for the genes encoding an aspartic peptidase domain in *S. ratti* and *S. stercoralis.* Both species have a total of 53 aspartic peptidase coding genes.

Domain combinations	*S. ratti*	*S. stercoralis*
Aspartic peptidase domain	15	15
Integrase, catalytic core domain - PAN-1 domain - Apple-like domain - Ribonuclease H-like domain - Peptidase A2A, retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	0	1
Integrase, catalytic core domain - Peptidase A2A, retrovirus, catalytic domain - Ribonuclease H-like domain - Peptidase A2A, retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	0	1
Integrase, catalytic core domain - Ribonuclease H-like domain - Aspartic peptidase domain-containing protein	0	1
Peptidase A2A - retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	0	3
Retroviral aspartyl protease domain - Aspartic peptidase domain-containing protein	0	1
Reverse transcriptase domain - Integrase catalytic core domain - Ribonuclease H-like domain- Retroviral aspartyl protease domain - Aspartic peptidase domain-containing protein	1	0
Reverse transcriptase domain - Aspartic peptidase domain-containing protein	2	2
Reverse transcriptase domain - Integrase catalytic core domain - Ribonuclease H-like domain - Aspartic peptidase domain-containing protein	8	4
Reverse transcriptase domain - Integrase catalytic core domain - Ribonuclease H-like domain - Peptidase A2A , retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	1	1
Reverse transcriptase domain - Integrase catalytic core domain - Ribonuclease H-like domain - Peptidase A2A , retrovirus RVP subgroup domain - AT hook-like family - Aspartic peptidase domain-containing protein	1	0
Reverse transcriptase domain - Integrase catalytic core domain - Zinc finger, CCHC-type domain - Peptidase A2A , retrovirus, catalytic domain - Ribonuclease H-like domain - Peptidase A2A ,retrovirus RVP subgroup domain -	3	1

Aspartic peptidase domain-containing protein		
Reverse transcriptase domain - Integrase catalytic core domain - Zinc finger, CCHC-type domain - Protein of unknown function DUF1759 family - Ribonuclease H-like domain - Aspartic peptidase domain-containing protein	1	0
Reverse transcriptase domain - Integrase catalytic core domain - Zinc finger, CCHC-type domain - Ribonuclease H-like domain - Aspartic peptidase domain-containing protein	9	5
Reverse transcriptase domain - Peptidase A2A - retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	2	0
Reverse transcriptase domain - Zinc finger, CCHC-type domain - Aspartic peptidase domain-containing protein	2	1
Reverse transcriptase domain - Zinc finger, CCHC-type domain - Peptidase A2A, retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	1	0
Reverse transcriptase domain- Integrase catalytic core domain - Ribonuclease H-like domain - AT hook-like family - Aspartic peptidase domain-containing protein	3	1
Reverse transcriptase domain - Integrase, catalytic core domain - Peptidase A2A, retrovirus, catalytic domain - Ribonuclease H-like domain - Aspartic peptidase domain-containing protein	0	1
Reverse transcriptase domain - Integrase, catalytic core domain - Zinc finger, CCHC-type domain - Ribonuclease H-like domain - Peptidase A2A, retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	0	1
Reverse transcriptase domain - Peptidase A2A, retrovirus, catalytic domain - Aspartic peptidase domain-containing protein	0	2
Reverse transcriptase domain - Retroviral aspartyl protease domain - Aspartic peptidase domain-containing protein	0	1
Reverse transcriptase domain - Zinc finger, CCHC-type domain - Peptidase A2A, retrovirus, catalytic domain - Aspartic peptidase domain-containing protein	0	1

Reverse transcriptase domain - Zinc finger, CCHC-type domain - Peptidase A2A, retrovirus, catalytic domain - Peptidase A2A, retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	0	1
Reverse transcriptase domain - Zinc finger, CCHC-type domain - Ribonuclease H-like domain - Aspartic peptidase domain-containing protein	0	1
Thioredoxin-like fold domain - Aspartic peptidase domain-containing protein	0	1
Zinc finger, CCHC-type domain - Aspartic peptidase domain-containing protein	2	2
Zinc finger, CCHC-type domain - Aspartic peptidase, DDI1-type domain - Aspartic peptidase domain-containing protein	0	1
Zinc finger, CCHC-type domain - Peptidase A2A, retrovirus RVP subgroup domain - Aspartic peptidase domain-containing protein	2	1
Zinc finger, CCHC-type domain - Peptidase A2A, retrovirus, catalytic domain - Aspartic peptidase domain-containing protein	0	3