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Invited Review

Parasite neuropeptide biology: Seeding rational drug target selection?

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ABSTRACT

The rationale for identifying drug targets within helminth neuromuscular signalling systems is based on the premise that adequate nerve and muscle function is essential for many of the key behavioural determinants of helminth parasitism, including sensory perception/host location, invasion, locomotion/orientation, attachment, feeding and reproduction. This premise is validated by the tendency of current anthelmintics to act on classical neurotransmitter-gated ion channels present on helminth nerve and/or muscle, yielding therapeutic endpoints associated with paralysis and/or death. Supplementary to classical neurotransmitters, helminth nervous systems are peptide-rich and encompass associated biosynthetic and signal transduction components – putative drug targets that remain to be exploited by anthelmintic chemotherapy. At this time, no neuropeptide system-targeting lead compounds have been reported, and given that our basic knowledge of neuropeptide biology in parasitic helminths remains inadequate, the short-term prospects for such drugs remain poor. Here, we review current knowledge of neuropeptide signalling in Nematoda and Platyhelminthes, and highlight a suite of 19 protein families that yield deleterious phenotypes in helminth reverse genetics screens. We suggest that orthologues of some of these peptidergic signalling components represent appealing therapeutic targets in parasitic helminths.

Contents

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1. Introduction

Parasitic helminths (worms classified in phyla Nematoda and Platyhelminthes) remain an insidious threat to human health, social development and economic progress worldwide. Infections by parasitic nematodes and flatworms have direct effects on human health and well being throughout the developing world and are responsible for a large proportion of the so-called 'Neglected Tropical Diseases' (Hotez et al., 2008). Additionally, helminths maintain an economic grip on the worldwide agricultural economy, a situation compounded by the spread of anthelmintic-resistance. For example, nematodes displaying multidrug-resistance to all of the well-established therapeutic compounds threaten the sustainability of livestock farming in some areas of South America, South Africa, Malaysia, New Zealand and the USA (Kaplan, 2004; Sutherland and Leathwick, 2011). Ruminant farming worldwide is threatened further by *Fasciola* spp. liver flukes, which have displayed incidences of resistance to triclabendazole, the single most effective fasciolicide on the market (Keiser and Utzinger, 2007). The latter issue illustrates a salient point in anthelmintic therapy – we have only a limited arsenal of drugs with which to combat pathogenic helminths and, particularly in human infections, many of the drugs in use are sub-optimal and suffer from our poor understanding of their pharmacology (Geary et al., 2010). Clearly, new anthelmintic treatment regimens will be essential in the short to medium term.

The development of resistance to chemotherapeutics is inevitable. However, while novel non-chemical control alternatives have been suggested (Hoste and Torres-Acosta, 2011), and given the limited availability of helminth vaccines (Hotez et al., 2010), the intelligent and sustainable use of available anthelmintics remains our best short term option for helminth control (Kaplan, 2004). While a few new drug classes active against nematodes (cyclooctadepsipeptide/emodepside, Harder et al. (2003); paraherquamide, Lee et al. (2002); aminoacetonitrile derivatives [AADs], Ducray et al. (2008)) are showing promise and progress has been reported for some new trematocidal drugs (Sayed et al., 2008; Keiser et al., 2010), to rely on these novel compounds/leads to the detriment of continued anthelmintic discovery would be to ignore the lessons of the past.

Modern drug discovery paradigms rely largely on mechanism-based screening of compounds against heterologously-expressed targets, which have been selected on the basis of biology, distinct host/pathogen pharmacology and 'druggability' (i.e. that the protein family in question is already targeted by existing drugs) (Geary et al., 1999). The quality of evidence required to support this target selection has increased as a factor of the economic and socio-political pressures experienced by the pharmaceutical industry (Knowles and Gromo, 2002; Geary et al., 2004), which attempts to discover new drugs for which profitability is a primary consideration. As such, the rationale for selecting a target must be strongly supported by evidence from basic research.

Our own interests in parasite neuropeptides evolved through the pioneering work of David W. Halton and Chris Shaw in the mid 1980s (see Halton et al., 1992, 1994) and progressed through neuropeptide localisation (Halton, 2004), characterisation (Maule et al., 1991, 1993; Marks et al., 1999) and physiology (Maule et al., 1995, 1996a,b, 2002) phases, prior to a slowing of impetus as classic approaches went out of vogue and genomic/post-genomic technologies captured attention. Recently, parasite neuropeptide research has been reinvigorated by two developments: the accumulation of transcriptomic/genomic resources for parasites (McVeigh et al., 2005a, 2008, 2009; see reviews by McVeigh et al., 2006; Marks and Maule, 2010; Mousley et al., 2010); and, the potential of RNA interference (RNAi) to facilitate gene function studies in parasites (Hussein et al., 2002; Urwin et al., 2002; Boyle et al., 2003; Skelly et al., 2003; Kimber et al., 2007; McGonigle et al., 2008; Pierson et al., 2010; Dalzell et al., 2011).

Whilst neuropeptide research continues to seed our understanding of parasite neurobiology, it also facilitates the identification and validation of candidate anthelmintic targets (e.g. Mair et al., 2004; Atkinson et al., 2010; Dalzell et al, 2010; Novozhilova et al., 2010; McVeigh et al., 2011). Targets originating from helminth neuropeptidergic signalling systems have generated intermittent interest from some of the major players in animal health drug discovery since the early 1990s. The target potential of helminth neuropeptidergic systems was recognised in particular by the successive Upjohn/Pharmacia and Pharmacia/Pfizer companies, where the animal health drug discovery research teams, initially led by Timothy G. Geary and David P. Thompson, generated significant amounts of valuable data on the basic structure, function and pharmacology of helminth neuropeptides and receptors (for reviews see Geary et al., 1992, 1995; Thompson et al., 1996; Greenwood et al., 2005; Geary and Kubiak, 2005). Since then, industry efforts in these areas appear to have waned. Here, we review current knowledge of helminth neuropeptide signalling mechanisms, and examine the evidence for drug target candidates within these pathways, including the receptors responsible for neuropeptide biological activity, and the enzymes involved in peptide synthesis, signal transduction and signal termination. We consider evidence from reverse genetics and RNAi experiments in parasites and model organisms, and whether existing inhibitors or drugs against each protein family are already in use. Also, we have attempted to illustrate those areas where further study is warranted.

2. Criteria for drug target selection and validation

It is well known that a large proportion of the anthelmintics used to control parasitic worms act on targets associated with the modulation of nerve and/or muscle systems and that these systems are pivotal to motor and sensory capabilities (Martin and Robertson, 2010). This is most evident amongst the compounds used for nematode parasite control and encompasses both long established drugs including dichlorovos, levamisole, morantel, piperazine, pyrantel and the macrocyclic lactones, and more recent additions to the anthelmintic arsenal such as the cyclooctadepsipeptides, paraherquamides and AADs. The empirical nature of anthelmintic discovery, at least to this point, largely dispels the potential of experimental prejudice having selected for neuro/myo-modulatory drugs, leaving us to ponder the preferential selection of parasite nerve/muscle targets by empirical drug screening processes. Although these anti-parasite drug screens were of empirical design, we cannot discount the possibility of bias inherent to the chemical libraries screened, and the obvious appeal of paralysis (flaccid or spastic) as a phenotypic endpoint, combining to skew the selection towards nerve/muscle targets. Nevertheless, it seems very unlikely that these alone could explain the propensity of frontline anthelmintics to act on targets associated with parasite nerve/muscle – especially as this marked bias is not as apparent amongst the platyhelminth-targeting anthelmintics. Therefore, a clear take home message from these observations is that parasite neuromuscular systems are lucrative for drug target discovery with much evidence that multiple targets from this resource are druggable. With front-line anthelmintics, much lauded for their efficacy and spectrum, facing the constant threat of resistance, we must consider sources of new targets that could facilitate the continued discovery of new drugs and so safeguard the future of helminth parasite chemotherapy.

The evolving strategies and changing pressures/expectations within the pharmaceutical sector demands mechanism-based approaches to drug discovery and strong, current market drivers,

i.e. there needs to be resistance (or strong evidence of imposing threat) in key host species and geographical regions to justify investment being channelled to anthelmintic discovery/development programmes. The mechanism-based approaches to drug discovery dictate that we mine for new targets and initiate their validation prior to screen implementation. Although helminth parasites have been late entrants to the genomics/transcriptomics era, they have well and truly arrived such that we have the luxury of rapidly expanding resources from which to select targets for validation. This late deluge of resources has exposed the absence of functional genomics tools for many/most parasites. Although RNA interference (RNAi) has facilitated reverse genetic studies in distinct parasite species (Hussein et al., 2002; Urwin et al., 2002; Boyle et al., 2003; Skelly et al., 2003; McGonigle et al., 2008; Pierson et al., 2010), it is still a developing platform and not yet robust enough to drive post-genomic screens that would validate targets in the absence of volume filters that focus efforts on small sub-sets of parasite genes. Therefore, before the initiation of validation processes we are thrust back to the position of having to make selections based on such criteria as target function/importance and 'druggability' in other (model) species, criteria that can be provided to support a huge array of parasite gene sets by scientists' enthusiastic about their individual merits.

Whilst pre-experimental validation processes claim rigour, they often rely on the assumption of functional conservation in target parasite and model species. Further, by their design, they unavoidably bias selections to those targets present in free-living, model species, leading to the relative neglect of molecules involved in host-parasite interplay. Weaknesses aside, initial target selections are founded on both biological and theoretical justifications prior to functional validation. At this time, the latter is constrained by the lack of available and rigorous functional validation tools.

There are a number of broad criteria that are commonly used to drive target selection processes, although the relative importance of each can vary with need and is open to debate. Below is a list of the most common criteria used to provide preliminary and experimental validations for parasite drug targets.

2.1. Criteria for anthelmintic target candidature

(i) *Druggability of drug target candidate*
- (a) Existing drugs act on homologous targets in other phyla, i.e. target family is already known to be 'druggable' in other systems. This may facilitate pre-selection of drug libraries/chemistries to be screened, diminishing size of initial screening efforts.
- (b) Drug target candidate is known to be amenable to heterologous expression; an obvious route to medium/high throughput screening is desirable.

(ii) *Expression/occurrence of drug target candidate*
- (c) Drug target candidate is expressed in key parasite species for which control issues are a current or potential concern.
- (d) Drug target candidate is expressed in key life stage(s) of parasite that are amenable to chemotherapy.

(iii) *Resistance, spectrum and toxicity of drug target candidate*
- (e) Drug target candidate is new, i.e. it is not known to interact with available anthelmintics.
- (f) Drug target candidate is unique to the parasite and does not occur in the host; in the case of orthologues occurring in both parasite and host, the candidate drug target must display structural differences from host protein(s).
- (g) Evidence that drug target candidate is well conserved across multiple parasite species, potentially facilitating broad spectrum action of drug leads. A non-conserved drug target candidate may warrant selection where target species merit investment in selective drugs.

(iv) *Function of drug target candidate*
- (h) Experimental evidence of functional importance to parasite, i.e. deleterious effects of either over- or under-expression of target protein; in the absence of functional data from parasite, evidence of functional importance in other (preferably related/model) species. It may be acceptable to move forward with strong theoretical support for functional importance. Obviously, evidence of critical function in parasite is needed at some stage.

With these criteria in mind, we now consider the selection of neuropeptide signalling processes as a key resource for parasite drug targets.

3. Do helminth neuropeptidergic signalling proteins represent good drug targets?

Firstly, and with respect to the first broad selection criterion of druggability, we know that nerve/muscle function has provided many of the most useful targets for broad spectrum anti-parasite chemotherapeutics. This observation extends beyond anthelmintics to encompass some of the leading nematicides, such as the carbamate aldicarb (Baron, 1994), used to control plant parasitic nematodes through the inhibition of acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of the classical neurotransmitter acetylcholine (ACh). Although most evidence supporting nerve/muscle function as a target for parasite control derives from nematodes, praziquantel, used to control schistosomes and many tapeworms, provides evidence that this target system has validity in flatworm parasites (Pax et al., 1978). Therefore, taken *in toto*, the parasite neuromuscular system is eminently 'druggable'. However, this does not mean that all proteins associated with signalling mechanisms within parasite neuromusculature have equal merit and/or are equally druggable – so whilst this can be used to support the selection of these tissue types as sources of potential drug targets, it cannot be used to vindicate the selection of individual proteins in the absence of additional justification.

Accepting the broad notion that parasite neuromuscular systems could continue to provide opportunities for novel drug target discovery, what are the targets we know of that have additional attributes which promote their selection? Since most of the current anthelmintic targets drawn from this system are ion channels that are gated by classical neurotransmitters such as ACh, γ-aminobutyric acid (GABA) and glutamate, then other ion channels associated with classical transmitter signalling would seem obvious as putative drug targets that await individual validation. Whilst past success would place ligand-gated ion channels firmly at the head of the queue of putative targets for progression to validatory screens, there are some impediments to their selection as drug target candidates. For example, performing biological studies on these channels is a major challenge and often requires highly skilled physiologists to work through single cell or cell patch-based electrophysiology experiments to derive data on protein functionality (Robertson et al., 2008; Williamson et al., 2009). A key issue is that these channels are generated through the accumulation of multiple, and often inter-changeable, proteins (channel subunits), providing for channel complexity and heterogeneity (Martin et al., 1998). This channel heterogeneity makes it difficult to evaluate which conformations of channel are functional (and important) within parasites. Even if this is determined, reconfiguring the relevant stoichiometries in heterologous expression systems suitable for high throughout screens remains a challenge, as described in the case of nicotinic acetylcholine receptors (nAChRs) (Boulin et al., 2008; Boulin et al., 2011). Further, such heterogeneity in the protein complements of ion channels seeds concern that

conformations with variable drug sensitivities will readily facilitate Darwinian-based progression towards resistance. Finally, for those making decisions on funding new drug discovery programmes, the selection of ion channels as targets raises the concern that resistance against drugs acting at similar targets is already undermining control such that the search for drugs guaranteed to have a novel mode-of-action would seem preferable. The latter approach should diminish the risk that parasites resistant to established anthelmintics would be resistant to new drug leads. This thinking does assume that the established drug resistance is due to mutation in the target protein rather than a more generic mechanism, for example, enhanced detoxification processes. Regardless, all lead compounds are tested against known anthelmintic resistant isolates at an early stage in development.

Whilst the targets of current anthelmintics centre upon classical neurotransmitter signalling, neuropeptide signalling is a similarly major, but neglected component of all helminth nervous systems. Numerous features of neuropeptide signalling can be used to provide justification for its selection as a drug target resource. Obviously, peptides themselves are not useful as chemotherapeutic targets, although their transcripts could have merit as targets for transgenic plant-based RNAi-approaches to nematode control (Huang et al., 2006; Yadav et al., 2006; Kimber et al., 2007). Narrowing focus to subsets of putative chemotherapeutic targets within neuromuscular signalling, the G-protein coupled receptors (GPCRs) step forward as proteins that are highly 'druggable' with estimates that up to 50% of human medicines are directed at these proteins (Flower, 1999; Wise et al., 2002; Jacoby et al., 2006; Overington et al., 2006; Lagerström and Schiöth, 2008). GPCRs have well established utility as drug targets, being suitable for heterologous expression and high throughput screening. Further, helminth GPCRs are documented as being amenable to heterologous expression (Bouchard et al., 2003; Kubiak et al., 2003, 2008; Rogers et al., 2003; Geary and Kubiak, 2005; Greenwood et al., 2005; Mertens et al., 2004, 2005, 2006; Carre-Pierrat et al., 2006; Omar et al., 2007; Kimber et al., 2009; Taman and Ribeiro, 2009, 2011), meaning that parasite GPCRs would meet the first two 'druggability' criteria. The 'druggability' of other candidate proteins would have to be considered on a case-by-case basis.

With a provisional selection of GPCRs as candidate drug targets, and following our scheme for selection, we would next consider their expression/occurrence within key parasite species and life stages. Unfortunately, detailed information on neuropeptide GPCR expression in helminth parasites is lacking, thus the selection of individual receptors would have to be justified based on initial evidence derived for homologues from model species such as *Caenorhabditis elegans*. In the absence of appropriate expression data for parasites, data on the occurrence and activity of neuropeptides (receptor ligands) does provide some evidence of receptor expression. An appealing feature of this widespread expression is target accessibility to drug. Whilst widespread/high expression might require higher effective concentrations in order to affect a sufficient portion of the receptor population to have a deleterious effect, requirements for drug penetrance (and potentially effective concentration) could be lower for targets that are expressed adjacent to the surface of the parasite. Further, there is ample evidence from physiology studies and some RNAi-based studies that neuropeptides modulate parasite motor function and that these receptors are expressed on nerve and muscle tissues across key parasite life stages (see Day et al., 1994; Maule et al., 1995, 2002; Moffett et al., 2003; Kimber et al., 2007; Marks and Maule, 2010; Mousley et al., 2010). Determining expression in the target life stage(s) of parasites is a pre-requisite for target selection and progression such that it needs to be established at an early stage.

With respect to resistance, spectrum and toxicity, targets associated with parasite neuropeptide signalling appear to fulfill the selection criteria. Firstly, no available anthelmintics are known to target neuropeptide signalling such that drugs acting on targets drawn from this protein subset have an *a priori* claim to a novel mode-of-action. Secondly, several BLAST surveys on neuropeptide complements have revealed marked conservation of peptide ligands across nematode parasite species and clades (see McVeigh et al., 2005a, 2008). The ability of individual peptide ligands to act across species and clade boundaries gives hope for the identification of broad spectrum drugs that act on the same receptor targets in multiple parasite species. Feeding confidence further is the fact that some helminth neuropeptide GPCRs are notably promiscuous with respect to activating ligand structure (see Greenwood et al., 2005; McVeigh et al., 2006). Indeed, some neuropeptides have been shown to display inter-phyla activities in arthropods, nematodes and platyhelminths (Marks et al., 1997; Mousley et al., 2004, 2005a,b), highlighting the possibility that drugs acting at associated receptors could have endectocide potential. Thirdly, there is much evidence that the neuropeptide complements of both nematode and platyhelminth parasites are unique and quite distinct to those seen in vertebrate/host species (see McVeigh et al., 2005a,b, 2008, 2009). Again, such differences serve to reduce concerns that toxicity would be an issue for target selective drugs.

Finally, data on functional importance are needed to underpin the initial selection of targets for progression to focussed, target validation experiments. With respect to our example selection of neuropeptide GPCRs, there are much data showing the impact of neuropeptides (in particular those from the FMRFamide-like peptide [FLP] family) on parasitic helminth tissues with evidence of physiological effects through peptide application to muscle strips/muscle fibres/nerves (Day et al., 1994; Cowden and Stretton, 1995; Maule et al., 1995; Pang et al., 1995; Marks et al., 1996; Fellowes et al., 1998; Davis and Stretton, 2001), and behavioural modulation through peptide injection (Reinitz et al., 2000) and RNAi (Kimber et al., 2007; Dalzell et al., 2010). The marked potency and longevity of neuropeptide actions described in these studies adds to the appeal of their receptors as targets in that less drug may be needed to trigger activity and impact worm behaviour. These data broadly support the potential of neuropeptide GPCRs as drug targets.

So within the context of our criteria for drug target candidate selection, targets from within neuropeptide signalling present a *prima facie* case for selection. This case appears to be particularly strong for neuropeptide-activated GPCRs. However, we still await direct validation that individual neuropeptide GPCRs represent good anthelmintic targets, especially important in light of potential concerns about their utility for this purpose. Amongst concerns raised about their validity as targets is the fact that in human medicine, most drugs acting at GPCRs serve to alter the physiological state of cells or tissues, resulting in modulatory impacts rather than dramatic alterations that could equate to spastic or flaccid paralysis/death, desirable endpoints for anthelmintics (one obvious exception is atropine, a muscarinic AChR antagonist, which can be lethal to mammals). Further, many of the neuropeptide families are diverse with numerous structurally similar peptides (this is especially evident in nematodes), leading to the charge of inherent functional redundancy. Although this is supported by evidence that some neuropeptide mutant *C. elegans* present with normal or wild-type phenotypes (Li et al., 1999), increasing the diversity of phenotype scoring methods and monitoring the consequences of both knockout and overexpression, are exposing evermore functionalities and reducing this concern (Li and Kim, 2008).

Knowing that over-expression (rather than knock-out or knock-down) is required to reveal phenotypic aberration for a particular GPCR would demand that screens were tailored to identify agonists rather than antagonists (see Geary, 2010). Conveniently, in such circumstances a primary weakness of RNAi-based screens can

become its strength – RNAi lowers the level of target protein, equating to an antagonistic impact on phenotype. Therefore, in a target validation paradigm, RNAi will select for candidate drug targets which disrupt worm biology upon knockdown, making them suitable for antagonistic drug screens.

Whilst there are data supporting the selection of candidate anthelmintic targets from neuropeptide signalling systems, these are relatively fragmented and need more data from basic research if we are to better resolve target selection issues. In an effort to facilitate progress in this area, the remainder of this review discusses the merits for what we consider the most obvious anthelmintic target candidates associated with neuropeptide signalling.

4. Putative anthelmintic targets within the helminth neuropeptidergic system

Neuropeptidergic signalling pathways can be considered in terms of three primary divisions, which are discussed separately below: (1) neuropeptide biosynthesis and synaptic release, (2) receptors and signal transduction, (3) signal termination.

4.1. Neuropeptide biosynthesis and synaptic release

Both flatworms and nematodes display an ever-growing diversity of peptidergic messengers, encoded by several distinct gene families, with different naming systems employed between phyla. Nematodes are currently understood to possess the larger complement of peptides (the free-living model nematode *C. elegans* possesses $\geqslant$250 neuropeptides, with parasitic nematodes likely displaying similar diversity; McVeigh et al., 2005a, 2006, 2008, 2009; Abad et al., 2008; Kikuchi et al., 2011). Nematode neuropeptide genes span three gene families: *flp* (FMRF-amide-like peptides), *ins* (insulin peptide-like), and *nlp* (neuropeptide-like protein) (see Marks and Maule, 2010; Li and Kim, 2010, for recent reviews). The diversity of flatworm neuropeptides has only recently been fully appreciated, with bioinformatic and proteomic descriptions of more than 100 individual peptides from 10 species (McVeigh et al., 2009; Collins et al., 2010). While increasing knowledge of these amidated bioactive peptides has illustrated the widespread nature and potential functional diversity of helminth neuropeptide signalling, the peptides are not viable drugs because of their poor in vivo instability and poor penetrance of lipid bilayers (Ho et al., 1990; Sheehy et al., 2000). Attention has instead been directed towards screening for non-peptide ligands for helminth neuropeptide receptors, although no such lead compounds have yet been described. Fortunately, neuropeptide-encoding genes are translated and processed by a conserved biosynthetic pathway with homologous proteins recognisable in both helminth phyla. If structurally or pharmacologically distinct from host homologues, these proteins associated with neuropeptide maturation and signal termination may represent valuable anthelmintic targets. However, our ability to target these components is only as good as our knowledge of basic parasite biology, and a recurring theme illustrated below is that while *C. elegans* and some other models have provided much useful knowledge, our current understanding of the specific effectors of neuropeptide signalling in parasitic helminths is extremely limited. Graphical depictions of neuropeptide processing and signalling pathways have been presented elsewhere (see McVeigh et al., 2005b, 2006; Husson and Schoofs, 2007a,b; Li and Kim, 2008; Geary, 2010), and are not reproduced here.

4.1.1. Transcription and translation of helminth neuropeptide genes

Neuropeptide genes are initially transcribed under the control of cell-specific promoters within neuronal nuclei, yielding complex gene-specific expression patterns which presumably indicate differential functionality. For example, localisation studies in both nematodes and flatworms reveal complex, distinct and sometimes overlapping neuropeptide expression patterns (Yew et al., 2005; Li and Kim, 2008, 2010; Collins et al., 2010; Jarecki et al., 2010; Nanda and Stretton, 2010). Unsurprisingly, neuropeptide genes are additionally subject to developmentally- or environmentally-regulated expression by micro-RNA (miRNA)-based post-transcriptional regulation (several *flp*-miRNAs have been described in the plant parasitic nematode *Bursaphelenchus xylophilus* (Huang et al., 2010)). Such mechanisms raise the (currently) remote possibility of anthelmintic therapy based on inhibition of miRNAs – an approach proposed as a possible future anti-cancer therapy (reviewed by Nana-Sinkam and Croce (2010, 2011)), although the applicability of these approaches to helminths remains untested.

Following nuclear export and splicing, neuropeptide mRNAs are translated directly into a precursor protein (also referred to as a prepropeptide), which may incorporate single or multiple copies of mature neuropeptides. The precursor is, during translation, translocated into the endoplasmic reticulum (ER) lumen and thus into the classical regulated secretory pathway, which involves transit through the trans-Golgi network, packaging and concentration into large dense-cored secretory vesicles (LDCVs), axonal transport, and eventual release at the synapse (Emanuelsson et al., 2007; Zhang et al., 2010). Recognition of the prepropeptide's nascent N-terminal secretory signal peptide is thought to occur upon its translational emergence from the ribosome, by a signal recognition particle (SRP), which binds the signal peptide and transports the entire ribonucleoprotein complex to an ER channel known as a translocon, through which the growing prepropeptide is inserted into the ER lumen. Reverse genetics experiments on a *C. elegans* SRP homologue (Table 1) reveal a range of aberrant motility and developmental phenotypes consistent with SRP's role at the apex of the classical secretory pathway. Although this protein represents an appealing target, it is difficult to envisage a mechanism which could therapeutically and specifically inhibit this molecule's function, short of targeting the protein–protein interaction (a wider strategy proposed by Taylor et al. (2011)) between SRP and prepropeptide. This process of classical secretion/exocytosis is thought to exist in all of the eukarya (Blobel and Dobberstein, 1975; Koch et al., 2003). Within the ER lumen, the signal peptide is first cleaved from the precursor by a membrane-bound signal peptidase complex. Molecular disruption of a *C. elegans* signal peptidase gene (*phi-20*; Table 1) demonstrates a range of aberrant phenotypes including paralysis. Similarly, when a subunit of the plant parasitic nematode *Meloidogyne incognita* signal peptidase (MiSPC3) was targeted by RNAi, a ~50% reduction in the number of *M. incognita* recovered from plant roots was reported (Charlton et al., 2010).

4.1.2. Prepropeptide processing

Once bereft of signal sequence, the peptide precursor is next cleaved by prohormone/proprotein convertase (PC) enzymes (also known as neuroendocrine convertases) situated within LDCVs. These enzymes belong to the subtilisin/kexin-like superfamily of serine proteases which are responsible for post-translational processing of a range of secretory proteins in both prokarya and eukarya. Prohormone convertase PC2 and PC1/3 families are involved in processing within the regulated secretory pathway of invertebrate neuronal and endocrine tissues (Seidah, 2011), and indeed available evidence suggests that PC2 orthologues play a conserved role in helminth neuropeptide processing (Reddien et al., 2005; Husson et al., 2006). Functionally, PC2 excises peptides from their prepropeptide by C-terminal cleavage of basic amino acid motifs which flank mature peptides in prepropeptides. Basic motifs most commonly consist of pairs of the basic amino acids lysine and/or arginine (although some variation on this pattern has been noted

Table 1
Neuropeptide pathway genes that following knockout or RNA interference induce deleterious phenotype in *Caenorhabditis elegans*. Note that some of these genes do not function exclusively within neuropeptide signalling pathways, and are involved more generally in cellular secretory processes.

Protein	*C. elegans*	*D. melanogaster*	Helminths
Neuropeptide biosynthesis			
Signal recognition particle	F08D12.1, larval arrest/late larval arrest, locomotion variant, embryonic lethal. larval lethal[1,2,3,4,5,6]	Srp72, no phenotype available	*Schmidtea mediterranea*: blastema abnormal; body lesions; lysis; photoreceptors abnormal[7]
Signal peptidase complex	*phi-20*, Paralysed, larval lethal, embryonic lethal, maternal sterile[2,4,5,8,9,10,11,12]	Spase22-23, no phenotype data available	*Meloidogyne incognita*: reduced infectivity of plant roots[13]
Prohormone convertase PC2	*egl-3*, Egg retention, egg-laying defective, coiler[14]	Amon, developmental defects, larval arrest, defective larval motility[15]	*S. mediterranea*: aberrant motility (paralysis, abnormal flipping, abnormal responses to touch, vibration and light)[7]
PC2 chaparone, 7B2	Sbt-1; no deleterious phenotypes reported	7B2; no phenotype data available.	*S. mediterranea*: blastema abnormal; behaviour abnormal (sluggish, abnormal flipping, abnormal vibration response, light response abnormal); flattened posture[7]
Carboxypeptidase E	*egl-21*, Egg retention, egg-laying defective, coiler[14]	No direct orthologue[16]	
Peptidylglycine-α-amidating monooxygenase	T19B4.1, sterile, lethal [WB]		
Large dense core vesicle kinesin,	*unc-104/klp-1*, Sterile, locomotion variant, lethal[5] [WB]	Unc-104, aberrant larval motility[17]	
Calcium-activated protein for secretion (CAPS)	*unc-31/egl-22*, Egg-laying defective, paralysed, flaccid, locomotion variant, egg-retention, sluggish[14,18]	CAPS; embryonic/larval lethal[19]	
Syntaxin	*syn-1/syx-3*, Sterile, lethal [WB]; *Syn-4/syx-4*, embryonic lethal, maternal sterile, reduced brood size[9,20]; *unc-64*, locomotion variant[21]	Syx1A, temperature-dependent paralysis, multiple developmental defects, larval paralysis and lethality[22]	
Tomosyn	*tom-1*, Locomotion variant, larval lethal, embryonic lethal[2,10,23]	Tomosyn, no deleterious phenotypes reported	
Neuropeptide signal transduction			
Insulin receptors	*daf-2*, Sterile, locomotion variant, larval lethal, reduced brood size, lethal[24]		
G-protein α subunits	*gsa*-1, *egl-30*, *goa*-1, Egg laying defective, locomotion variant, sterile progeny, maternal sterile, larval lethal, reduced life-span, sluggish[2,6,20,23]; *gpa-12*, egg laying variant, embryonic lethal, locomotion variant[25]	G-salpha60A, larval lethal[26]	
G-protein β and γ subunits	*gpb-1*, Locomotion variant, larval lethal, egg laying variant, embryonic lethal, maternal sterile[2,5]; *gpb-2*, coiler[27]; *gpc-2*, sterile, embryonic lethal, sick, lethal[28]	Gbeta13F, developmental defects[29]	
Regulators of G-protein signalling (RGS) proteins	*eat-16*, Hyperactive egg laying, sterile, body bend variant, hyperactive, early eggs laid, lethal[30]; *rgs-9*, embryonic lethal[31]; *rgs-10*, embryonic lethal, maternal sterile[32]	CG42450, no phenotype available	*S. mediterranea*: blastema abnormal; photoreceptors abnormal; light response abnormal; variably abnormal [7]
Adenylate cyclase	*acy-2*, locomotion variant[2]; *acy-4*, sterile, male mating defective, lethal [WB]	AC13E, AC76E, AC3, CG43373, Ac78C no phenotypic data available; Rut, no deleterious phenotypes reported	
Protein kinase A	*kin-1*, egg-laying variant, larval lethal[9]	Pka-C1, embryonic lethality, slow development[33]; CG32944, no phenotype data available	
Phospholipase C-β	*egl-8*, Sterile, male sterile, egg-laying defective, egg-laying variant, posterior body wall contraction defective[28,34]	norpA, no deleterious phenotypes reported	
IP_3 channel	*itr*-1, Sterile, maternal sterile, lethal[2,5] [WB]	Itp-r83A, no deleterious phenotypes reported	
Synaptic peptidases			
Neprilysin	*nep-1*, Maternal sterile[31]; C49D10.10, embryonic lethal[2]	Nep2, no phenotype data available. CG15485, no phenotype data available	

Deleterious refers to lethality, reduced lifespan, or aberrant motility or reproduction. Phenotype data from WormBase (http://www.wormbase.org; WS225 16/06/2011), FlyBase (http://www.flybase.org; FB2011_07, 21/07/2011) or as referenced: [1]Gaiser et al. (2009), [2]Simmer et al. (2003), [3]Hamamichi et al. (2008), [4]Sonnichsen et al. (2005), [5]Kamath et al. (2003), [6]Sieburth et al. (2005), [7]Reddien et al. (2005), [8]Gonczy et al. (2000), [9]Rual et al. (2004), [10]Balklava et al. (2007), [11]Nollen et al. (2004), [12]Frand et al. (2005), [13]Charlton et al. (2010), [14]Trent et al. (1983), [15]Rayburn et al. (2003), [16]Settle et al. (1995), [17]Barkus et al. (2008), [18]Speese et al. (2007), [19]Renden et al. (2001), [20]Ceron et al. (2007), [21]Brenner, 1974, [22]Littleton et al. (1998), [23]Fraser et al. (2000), [24]Malone and Thomas, 2004, [25]Yau et al. (2003), [26]Wolfgang et al. (2001), [27]Avery, 1993, [28]Lehner et al. (2006), [29]Schaefer et al. (2001), [30]Porter and Koelle, 2010, [31]Maeda et al. (2001), [32]Fernandez et al. (2005), [33]Perrimon et al. (1996), [34]Espelt et al. (2005).

(Veenstra, 2000; Duckert et al., 2004; Fricker, 2005; McVeigh et al., 2005a; Rehfeld et al., 2008). PC2 orthologues have been characterised in *C. elegans* (*egl-3*, named for its identification from a screen for egg-laying mutants), and *Schmidtea mediterranea*. In both of these species, reverse genetics analyses (Table 1) have validated disruption of PC2 as having deleterious effects on helminth biology, including effects on motility, behaviour and reproduction (Trent et al., 1983; Kass et al., 2001; Reddien et al., 2005). While such effects are not lethal in these free-living species, therapeutic effects of a similar magnitude in a parasite, resident in a more

challenging environment, may result in reduced viability or lethality for the parasite. In *C. elegans* this disruption almost completely ablates the normal profile of FLP neuropeptides seen in N2 (wild-type) worms (Husson et al., 2006), perhaps explaining the global effects described above. Disruption of PC2's chaperone protein '7B2' (a protein required for the normal transport, activation and regulation of PC2 (Van Horssen et al., 1995; Muller et al., 1997; Lee and Lindberg, 2008)) has similarly severe effects on the *C. elegans* FLP profile (Husson et al., 2007). Specific small-molecule inhibitors have been reported as active against murine PC2, demonstrating >90% inhibition with K_i values in the sub-μM range (Kowalska et al., 2009), indicating the possibility of developing similar lead compounds with therapeutic activity against helminth PC2. Although no pharmacological or structural studies have been performed on helminth PC2 enzymes, it is perhaps worth noting that nematode PC2/EGL-3 orthologues exhibit a unique sequence insert within the catalytic domain, which may indicate the possibility of nematode-specific pharmacology (Kovaleva et al., 2002), potentially permissive of pharmacological differentiation between host and helminth enzymes. A *Schistosoma mansoni* PC2 orthologue was suggested as a drug target candidate in a chemogenomics screen based on proteins displaying deleterious mutant phenotypes in both *C. elegans* and *Drosophila melanogaster* (Caffrey et al., 2009), although this was not supported by RNAi studies from the same laboratory (Stefanić et al., 2010), which described the absence of aberrant phenotypes associated with RNAi of 11 schistosome targets.

Since PC2 cleaves C-terminally to dibasic markers, its cleavage results in excision of a glycine-extended peptide intermediate which retains a C-terminal dibasic motif. Before further processing can occur, this extension must be removed by carboxypeptidase E (CPE) activity, as demonstrated by proteomics analysis of *C. elegans* mutants deficient in the CPE orthologue, *egl-21*, which displayed a peptide profile consisting almost exclusively of unprocessed, C-terminally extended peptides (Husson et al., 2007), allied to a range of aberrant phenotypes (Table 1). Although small molecule inhibitors have been described for other metallocarboxypeptidases (Fernández et al., 2010), none have yet been discovered with specific activity against CPE.

4.1.3. Neuropeptide amidation

Most helminth neuropeptides are C-terminally amidated, where the sequential actions of PC2 and CPE liberate a C-terminal glycine-extended prepeptide which is then subject to amidation (enzymatic production of a C-terminal NH_2 moiety), a process that represents the final step in neuropeptide maturation. Amidation is a two-step process in which two enzymes act sequentially: (1) peptidyl α-hydroxylating monooxygenase (PHM), a copper-, oxygen- and ascorbate-dependent enzyme, first catalyses hydroxylation of the pre-peptide C-terminal glycine, to yield an alpha-hydroxyglycine-extended peptide (Eipper et al., 1992; Kulathila et al., 1999), (2) peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL), a zinc dependant enzyme, catalyses the secondary dealkylation of the alcohol amide intermediate to form the alpha-amidated neuropeptide and a glyoxylate by-product (Eipper et al., 1992; Kulathila et al., 1999).

In vertebrates, PHM and PAL exist as a bifunctional protein designated peptidylglycine alpha-amidating monooxygenase (PAM; for review see Prigge et al., 2000). Several invertebrate species, including the flatworms *Dugesia japonica* (Asada et al., 2005), and *S. mansoni* (Mair et al., 2004; Atkinson et al., 2010), encode monofunctional PHM and PAL enzymes on distinct genes, while nematodes including *C. elegans*, encode multiple copies of bi- and monofunctional enzymes. The biological significance of such genetic complexity remains unclear. Amidating enzymes similarly have widespread expression within helminth nervous systems (Mair et al., 2004; Atkinson et al., 2010). Using an anti-PHM antiserum we have been able to visualise the entire amidated neuropeptide system of adult schistosomes (Fig., 1), revealing previously unrecognised levels of neuronal complexity and supporting hypotheses on the importance of amidated neuropeptides to schistosome biology. This importance is directly illustrated by the reduced activity of glycine-extended free-acids (<10%) in comparison to their alpha-amidated counterparts (Eipper et al., 1992; Bowman et al., 1996; Bolkenius and Ganzhorn, 1998; Kulathila et al., 1999; Prigge et al., 2000; Bowman et al., 2002; McVeigh et al., 2011). Further, C-terminal amidation was shown to be critical to the activity of a myoexcitatory peptide on dispersed schistosome muscle fibres (Day et al., 1997), supporting the importance of the C-terminal amide signature for myoactivity in schistosomes. Importantly, no alternative mechanism for the in vivo amidation of neuropeptides has been documented.

Commercially available inhibitors of PHM/PAL/PAM act by inhibition of conserved active site mechanisms, and would therefore likely be toxic to both parasite and host enzymes. Thus, the identification of differences between host and helminth enzymes is key to unearthing their potential as novel parasite drug targets. Functional data from characterised helminth enzymes such as schistosome PHM and PAL (Mair et al., 2004; Atkinson et al., 2010) indicate that while structural similarities remain evident within conserved active site motifs, marked catalytic and in some cases subtle structural differences between vertebrate and parasite enzymes may provide for successful chemotherapeutic discrimination.

On the face of it, peptide amidation, a process mandatory for the biological activity of most neuropeptides, is an appealing component of the neuropeptide signalling system for anti-parasitic intervention. As such, much of the appeal of targeting either enzyme is the likelihood that their dysfunction would compromise all forms of amidated neuropeptide signalling and, thereby, disrupt all the associated parasite processes and behaviours. One would hypothesise that this would be catastrophic to worm biology and survival. Clearly, using reverse genetic tools to probe this hypothesis is an important step in the drug target validation process. Whilst this has been attempted in *S. mansoni*, knockdown for *Sm-phm-1* and *Sm-pal-1* was inconsistent preventing valid phenotypic assessments (Atkinson et al., 2010). To date most of the phenotypic evidence from RNAi based attempts to disrupt the helminth neuropeptide amidation process comes from large scale RNAi experiments in *C. elegans*, which have reported sterile, lethal and aldicarb resistant phenotypes (see Table 1); *Drosophila phm* loss-of-function mutants display lethal phenotypes (Jiang et al., 2000). Clearly, the validation of amidating enzymes as targets would be strongly supported by RNAi-based evidence of profound phenotypic aberration in parasites. In the absence of these data the potential of PHM and PAL as anthelmintic targets remains to be established.

4.1.4. Docking and release of synaptic large dense-cored vesicles

Upon arrival in the synaptic terminal, neuropeptide-containing LDCVs are ready for release into the synapse. Available evidence from *C. elegans* suggests that in nematodes, these processes employ similar molecular mechanisms to those of higher organisms (indeed, all of the proteins mentioned below have clear orthologues in all of the major model organisms, including humans) although there is currently insufficient evidence to enable rational identification of prospects for chemical intercession within this system. Current understanding of the *C. elegans* LDCV release mechanism is limited, but implicates an UNC-104 kinesin in LDCV antero-grade axonal transport (Zahn et al., 2004) (UNC-116 may also be involved; Li and Kim, 2008). Only three proteins have been identified as specifically required for aspects of LDCV docking, priming and

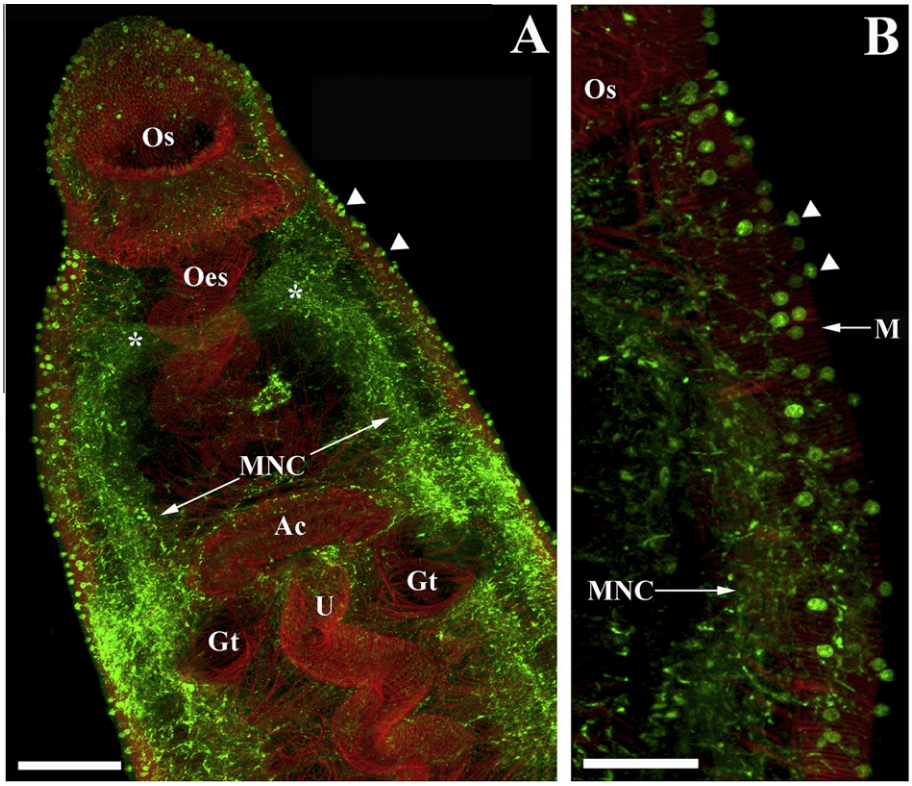

Fig. 1. The amidated neuropeptide system of an adult female *Schistosoma mansoni*. Peptidyl α-hydroxylating monooxygenase (PHM)-immunoreactivity (IR) appears green (fluorescein isothiocyanate [FITC]-labelled secondary antibody) whilst filamentous actin in muscle fibres appears red (tetramethyl rhodamine isothiocyanate [TRITC]-labelled phalloidin). (A) Extensive PHM-IR in the paired cerebral ganglia (*), main nerve cords (MNC) and nerve processes innervating the oral sucker (Os), oesophagus (Oes), acetabulum (Ac), uterus (U) and gut (Gt) in the anterior region of a female schistosome. The outer surface of the fore body region is covered in immuno positive sensory endings (arrow heads). (B) PHM-IR just posterior to the oral sucker (Os) in one of the main nerve cords (MNC) and associated nerve net that provides branches to the numerous sensory endings (arrow heads) of the anterior-lateral forebody. Note that the tegument is not visible; body wall muscle (M). Scale bars: *A* = 30 μm; *B* = 12 μm.

exocytosis: UNC-31/CAPS, a Ca^{2+}-sensing LDCV-membrane docking protein (Speese et al., 2007; Hammarlund et al., 2008; Lin et al., 2010); syntaxin, a component of the SNARE (soluble N-ethyl-maleimide-sensitive fusion protein attachment receptor) complex involved in LDCV-membrane fusion (Hammarlund et al., 2008); and tomosyn, an inhibitor of LDCV exocytosis (Gracheva et al., 2007a,b). Other membrane proteins implicated in exocytosis of synaptic vesicles (carrying classical neurotransmitters; Richmond, 2007) may also have roles in LDCV exocytosis.

4.2. Neuropeptide receptors and signal transduction mechanisms

4.2.1. Insulin-like receptor tyrosine kinases

While the vast majority of neuropeptides in helminths, and throughout nature, are thought to exert their effects via GPCRs, insulin-like peptides signal through conserved receptor tyrosine kinase (RTK)-based mechanisms, which represent proven drug targets for an existing arsenal of anti-phosphorylation drugs used in cancer chemotherapy (Levitzki and Gazit, 1995). Although only a single homologue of mammalian insulin receptors has been described in *C. elegans* (Kimura et al., 1997), this species appears to possess an additional suite of structurally-divergent insulin-like receptors (Dlakic, 2002). Currently, no insulin receptors have been characterised from parasitic nematodes, although seven insulin-like peptide-encoding gene orthologues were recently identified in the genome of the plant parasitic nematode, *B. xylophilus* (Kikuchi et al., 2011). In contrast, insulin receptors have been identified from parasitic platyhelminths with some success – an insulin-like RTK (EmIR) has been identified in the cestode *Echinococcus multilocularis* (Konrad et al., 2003). Unfortunately, similar insulin-binding kinetics of the cestode and human insulin receptors could raise concerns about the potential toxicity of therapeutic agents active at the cestode receptor. Two insulin receptors have been described in *S. mansoni* (SmIR-1, -2) and *Schistosoma japonicum* (SjIR-1, -2) (Vicogne and Dissous, 2003; Khayath et al., 2007; Ahier et al., 2008; You et al., 2010, 2011), both of which possess sequence inserts within tyrosine kinase domains that are absent from the human insulin receptor. You et al. (2010) demonstrated that these inserts do not affect the functionality of the TK fold, but are predicted to be highly antigenic and are proposed as potential structural targets for the design of anti-SmIR vaccines or inhibitors.

4.2.2. Neuropeptide-like G protein-coupled receptors

The vast majority of neuropeptide effects are mediated by GPCRs, which, upon ligand binding, recruit a heterotrimeric GTP-binding protein (aka G-protein) through which intracellular signal transduction is mediated. Neuropeptide-activated GPCRs have drawn much attention from industrial and academic drug discovery research groups thanks largely to the proven druggability of this receptor family, and perhaps by the novel anthelmintic emodepside, one target of which appears to be nematode latrophillin-like GPCRs (Saeger et al., 2001; Welz et al., 2005; Krüger et al., 2009; Mühlfeld et al., 2009). From an industrial point of view the appeal of GPCR targets is further enhanced by an array of existing heterologous expression and assay technologies (Greenwood et al., 2005; Minic et al., 2005; Woods et al., 2010). GPCRs are implicated as mediators of neuropeptide signalling events in both helminth phyla, and escape major concerns associated with anthelmintic field-resistance. The value of neuropeptide GPCR drug targets has been recognised by the Bill and Melinda Gates Foundation

which is currently funding a project led by Prof Timothy G. Geary (McGill University) in collaboration with two African universities, aiming to identify FLP GPCR ligands from natural product extracts (http://www.gatesfoundation.org/Grants-2008/Pages/McGill-University-OPP52088.aspx).

Despite the undeniable potential of helminth GPCRs as targets for novel anthelmintics, our understanding of their basic biology is limited and fragmentary, such that we can point to specific functions of only a handful of helminth peptide GPCRs, all from *C. elegans* – at the time of writing, not one peptide GPCR has been functionally characterised from a parasitic worm. Functional data from model helminths have centred on receptor deorphanisation (ligand-receptor matching) and/or reverse genetics analyses, as described below.

4.2.2.1. Peptide GPCRs in Nematoda. Estimates of the extent of the *C. elegans* peptide-GPCR complement vary widely between published studies (Bargmann, 1998; Keating et al., 2003; Fredriksson and Schiöth, 2005), with the most recent analysis suggesting that *C. elegans* possesses as many as 91 peptide receptor genes encoding 125 distinct GPCRs (Janssen et al., 2010); similar figures are not yet available for any parasitic nematode. *C. elegans* therefore represents the source of much of our current understanding of helminth GPCR function, having supported studies into both receptor deorphanisation and functional characterisation. Functional genomics studies in *C. elegans* have exposed some peptide GPCRs that associate with deleterious phenotypes such as lethality, reduced lifespan, compromised motility or aberrant reproductive function; a subset of these have been deorphanised (Table 2). Assuming functional conservation, parasite orthologues of these receptors would have obvious appeal as candidate chemotherapeutic targets. The *C. elegans* database (WormBase) identifies 21 putative peptide GPCRs that yield deleterious phenotypes following knockout or RNAi analysis (Table 2), effectively simulating antagonistic pharmacology at these receptors. Perhaps most promising, are five GPCRs for which reduced function leads to lethality and/or sterility, including one of the FLP-18 receptors (Y58G8A.4/FLP18R2). Six GPCRs yield phenotypes associated primarily with altered motility, including the FLP-15 receptor NPR-3 (C10C6.2), which yields reduced egg output, alongside aberrant locomotion and paralysis in some animals (see Keating et al., 2003). Ablation of nine more receptors triggers reduced/compromised reproductive capacity. These lethal, motility and reproductive phenotypes are consistent with deleterious anthelmintic treatment outcomes, and go some way towards validating the utility of antagonistic chemotherapy at neuropeptide GPCRs. The potential for agonist activity at nematode neuropeptide receptors has been demonstrated by a large body of literature describing the effects of FLPs on *Ascaris suum* neuromuscular bioassays, in which FLPs have been shown to have potent effects on somatic, pharyngeal and ovijector muscle preparations (Maule et al., 1995; Holden-Dye et al., 1997; Fellowes et al., 1998; Marks et al., 1999; Reinitz et al., 2000; Davis and Stretton, 2001; Purcell et al., 2002a,b; Moffett et al., 2003; Mousley et al., 2004; Papaioannou et al., 2005). Promising as these potential targets are, their further validation is hindered by our ignorance of parasite orthologues. Identification and characterisation of such orthologues should be a priority for GPCR-directed anthelmintic discovery programmes. Although there are many other *C. elegans* peptide GPCRs that correlate with more subtle functions and so lack obvious appeal as anthelmintic targets, their homologues may prove valuable as targets in parasites. More general discussions of the biology of *C. elegans* peptide GPCRs are available elsewhere (Li and Kim, 2008, 2010; Mousley et al., 2010).

4.2.2.2. Peptide GPCRs in Platyhelminthes. In flatworms, knowledge of peptide GPCRs has recently been accelerated by analysis of the trematode *S. mansoni* (Berriman et al., 2009) and planarian *S. mediterranea* (Robb et al., 2008) genomes, from which respectively at least 35 and 81 putative peptide receptors (Rhodopsin-like family GPCRs) have been described (Fitzpatrick et al., 2009; Zamanian et al., in press), as well as a divergent, flatworm-specific clade of 19 *S. mansoni* and 40 *S. mediterranea* GPCRs, known as PROF1 (*Platyhelminth*-specific, Rhodopsin-like, Orphan Family (1) receptors (Zamanian et al., in press). Although these display no homology to any other GPCRs that could inform ligand class/structure, some of them could conceivably be neuropeptide-activated. Given

Table 2
Neuropeptide-like G protein-coupled receptors (GPCRs) that following knockout or RNA interference induce deleterious phenotype in *Caenorhabditis elegans*.

GPCR	Most potent ligand	Aberrant phenotypes reported
Y58G8A.4 (FLP18R2)	FLP-18[1]	Sterile, lethal
C54A12.2		Embryonic lethal[2]
AC7.1/TAG-49		Embryonic lethal, sick, maternal sterile[3,4]
T23B3.4 (CKR-1)		Embryonic lethal, reduced life span, reduced brood size[5,6]
C30F12.6 (NMUR-4)		Larval arrest, sterile, embryonic lethal[7]
C10C6.2 (NPR-3/FLP-15R)	FLP-15[8]	Some paralysis, locomotion variant, reduced brood size, sluggish[9]
F59D12.1		Paralysed, locomotion variant, sluggish, flat locomotion path, frequency body bend reduced[9]
Y54E2A.1		Locomotion variant, maternal sterile[5,6]
T05A1.1 (NPR-2)		Locomotion variant, locomotion reduced, kinker[9]
T02E9.1		Locomotion variant, sluggish, body bend reduced[9]
C04E6.9 (SRD-16)		Locomotion variant[10]
C16D6.2 (FLP18R1)		Reduced fertility, fewer egg-laying events during active[9]
Y59H11AL.1 (VRFaR2)	FLP-7[11]	Reduced brood size[7]
T22D1.12 (NPR-12)		Reduced brood size[5]
F41E7.3		Fewer egg-laying events during active[9]
C25G6.5 (NPR-11/FLP-21R)	FLP-21[12]	Fewer egg laying events during active
F21C10.12		Egg laying defective[13]
F54D7.3 (GnRHR)	NLP-47[14]	Delayed egg laying[14]
Y39A3B.5 (CKR-2)	NLP-12[15]	Reduced life span[6]
T27D1.3 (NPR-15)		Organism development variant[16]
C26F1.6 (VRFaR1)	FLP-7[17]	Hyperactive egg laying[9]
F35G8.1		Hyperactive egg laying[9]

Deleterious refers to lethality, reduced lifespan, or aberrant motility or reproduction. Where known, most potent ligand is indicated. Note that most of these GPCRs have orthologues in *D. melanogaster*, but (with the exception of F54D7.3) all are listed on FlyBase as 'no phenotypic data available' (http://www.flybase.org; FB2011_07, 21/07/2011). Phenotype data from WormBase (http://www.wormbase.org; WS225 16/06/2011), or as referenced: [1]Kubiak et al. (2008), [2]Sonnichsen et al. (2005), [3]Kamath et al. (2003), [4]Simmer et al. (2003), [5]Rual et al. (2004), [6]Park et al. (2010), [7]Ceron et al. (2007), [8]Kubiak et al. (2003), [9]Keating et al. (2003), [10]Zugasti et al. (2005), [11]Mertens et al. (2006), [12]Lowery et al. (2003), [13]Gort et al. (2008), [14]Lindemans et al. (2009), [15]Janssen et al. (2008), [16]Byrne et al. (2007), [17]Mertens et al. (2004).

their unique existence in phylum Platyhelminthes, PROF1 GPCRs represent candidate drug targets with potentially unique pharmacology, which could promote exquisite drug selectivity between host and parasite GPCRs. An additional FLP-activated GPCR (Gt-NPR-1) has been described in the planarian flatworm, *Girardia tigrina* (Omar et al., 2007). Gt-NPR-1, heterologously expressed in Chinese hamster ovary cells, was activated by FLPs emanating from both flatworms and nematodes and as such, currently represents the sole deorphanised neuropeptide receptor from any flatworm. Notably, the existence of clear Gt-NPR-1 orthologues in both *S. mansoni* and *S. mediterranea* (Zamanian et al., in press), provides tacit support for employing planarian model systems for the study of parasitic flatworm receptors. This is an important observation, given that presently no flatworm peptide GPCR has been subject to direct functional characterisation, making it impossible to directly validate any individual flatworm receptor as an anthelmintic target – planarians represent useful tools in the push towards functional characterisation of conserved GPCRs in phylum Platyhelminthes.

4.2.3. Peptide-gated ion channels

GPCRs represent the prevalent mechanism of neuropeptide signal reception and transduction, eliciting the long-term, metabotropic, modulatory effects that are characteristic of neuropeptides. However, neuropeptide-gated ion channels have also been reported as mediators of fast synaptic transmission in molluscan (Cottrell et al., 1990; Lingueglia et al., 1995; Jeziorsky et al., 2000; Furukawa et al., 2006) and cnidarian (Golubovic et al., 2007) nervous systems. Intriguingly, similar channels may also exist in parasitic nematodes. One nematode FLP (PF4 - *Panagrellus redivivus* FLP #4; KPNFIRFamide; Maule et al., 1995), displays a unique activity profile, having been shown in patch-clamp experiments to directly gate a population of low-conductance Cl^- channels on *A. suum* somatic muscle, leading to a fast hyperpolarisation of *Ascaris* body wall muscle preparations in vitro, with consequent muscular flaccid paralysis (Maule et al., 1995; Holden-Dye et al., 1997; Purcell et al., 2002a,b). This implies the existence in parasitic nematodes of a muscle-expressed peptide-gated Cl^- channel (here referred to as a 'PF4 receptor'). Such receptor channels are not known to exist in mammalian hosts, and therefore represent a target of potentially unique, and consequently selective, pharmacology, against which agonists or antagonists could elicit deleterious effects on parasite neuromuscular function. Other helminth ligand-gated ion channels are of proven druggability since they already represent targets for the majority of current front-line anthelmintics – levamisole, morantel, pyrantel, paraherquamides and the AADs act by modulating the function of nAChRs (Atchison et al., 1992; Zinser et al., 2002; Kaminsky et al., 2008) while the macrocyclic lactones act through GABA – and glutamate-gated Cl^- channels (Arena et al., 1992; Wolstenholme and Rogers, 2005). It is clear that helminth ligand-gated ion channels have demonstrable drug-discovery potential and that the PF4 receptor, as a unique example of that group, warrants further research directed towards its identification and characterisation.

4.2.4. GPCR signal transduction mechanisms

GPCRs signal through a conserved mechanism involving recruitment of a heterotrimeric G-protein complex, an event that is triggered by ligand binding to the GPCR, through which signals are transduced in order to initiate an intracellular response. Current understanding of the intimate details of this signalling process have been reviewed elsewhere (McCudden et al., 2005; Bastiani and Mendel, 2006), and are considered only briefly here. G-protein heterotrimers, consisting of α, β and γ subunits exist at rest on the cytosolic face of the membrane, with the alpha subunit bound to GDP. Interaction of a congruent ligand with a GPCR triggers recruitment of this complex, in concert with activation of the G-protein as indicated by the Gα's exchange of GDP for GTP. The conventional view is that upon activation, the Gα subunit dissociates from the G$\beta\gamma$ complex, allowing Gα and G$\beta\gamma$ to exert separate and distinct downstream effects. Gα's intrinsic GTPase activity tends to catabolise GTP into GDP (a process that is accelerated both by interaction with effectors and specific regulatory proteins), returning Gα to its resting state following effector interaction, at which time it tends to re-associate with G$\beta\gamma$. Gα-GTP and G$\beta\gamma$ can both interact with several effector proteins, although diversity within Gα subtype (classified according to the effector enzymes with which each class interacts) is the main determining factor in G-protein functional diversity.

The *C. elegans* complement of heterotrimeric G-proteins is well characterised, encompassing 21 Gα, 2 Gβ and 2 Gγ subunits. The Gα family includes single orthologues of each of the recognised mammalian Gα subunits: G_s (*gsa-1*), $G_{i/o}$ (*goa-1*), G_q (*egl-30*) and G_{12} (*gpa-12*). *C. elegans* additionally possesses a divergent clade of 17 genes lacking clear homology to any other Gα class, but most similar to $G_{i/o}$-like genes (Bastiani and Mendel, 2006). Reverse genetics of the core *C. elegans* Gα orthologues expose a variety of aberrant phenotypes befitting of their core role in cell signalling (Table 1), while the divergent Gα genes described above do not yet appear to have been subject to detailed functional interrogation. Both of the *C. elegans* Gβ and one of the two Gγ subunit genes display aberrant mutant phenotypes. This suggests that functional redundancy is largely absent within Gβ and Gγ genes, and that these proteins may then represent an appealing metabolic 'choke-point' against which therapeutics may be targeted (Smrcka et al., 2008; Dessal et al., 2008, 2011). At present there are no descriptions of G-protein sequences from parasitic nematodes. Knowledge of flatworm G-proteins is similarly sparse, with cloned sequences available only for *S. mansoni* $G\alpha_s$, $G\alpha_i$ and $G\alpha_o$ subunits (Mansour and Mansour, 1989; Iltzsch et al., 1992). Parasite G-proteins can be targeted therapeutically by suramin, an inhibitor of Gα-GPCR coupling used in both trypanosomiasis and onchocerciasis chemotherapy, and also in human cancer therapy (although suramin is a notoriously promiscuous drug) (Voogd et al., 1993; Höller et al., 1999). Further drug targeting of Gα function seems possible given descriptions of Gα modulators, which have been proposed as drugs for use in inherited human disorders related to Gα malfunction (Chahdi et al., 1998; Ja et al., 2006). Aside from the divergent clade of *C. elegans* Gα genes described above, no evidence is currently available on the existence of pharmacological or structural differences between mammalian and helminth G-protein function, consistent with parasite-specific drug function. Indeed, cursory BLAST searches indicate high levels of amino-acid sequence identity and similarity in comparisons between human and schistosome Gα subclasses (unpublished data), such that achieving selective interference with parasitic helminth G-protein function might be a challenge. There is growing interest in a family of Gα-regulatory proteins (regulators of G-protein signalling; RGS proteins) as drug targets in human medicine (McCudden et al., 2005; Kimple et al., 2007; Sjögren et al., 2010). RGS proteins are GTPase-accelerating factors that act as desensitisers of G-protein signalling, and as such represent an attractive additional target set within GPCR-related signalling pathways. Data on RGS protein structure and function is completely lacking in parasitic helminths, although *C. elegans* RGS homologues have been well characterised (Table 1) (Chase and Koelle, 2004; Hess et al., 2004; Hiley et al., 2006; Ferkey et al., 2007; Porter et al., 2010; Esposito et al., 2010).

Physiological investigation of the signal transduction mechanisms employed by helminth neuropeptides have illuminated several potential drug targets functioning downstream of peptide GPCRs. The key postsynaptic intracellular mechanisms implicated in helminth neuropeptide actions are the adenylate cyclase and phosphatidylinositol pathways. In the adenylate cyclase pathway,

$G\alpha_s$ initially stimulates adenylate cyclase to catalyse production of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). cAMP activates protein kinase A (PKA), which can elicit cellular effects by phosphorylation of enzymes, contractile proteins or ion channels. The first step in the phosphatidylinositol pathway is activation of β-class phospholipase C (PLC) by $G\alpha_{q}$, processing phosphatidylinositol (4,5) bisphosphate (PIP_2) into the second messengers inositol (1,4,5) triphosphate (IP_3) and diacylglycerol (DAG). IP_3 liberates intracellularly sequestered Ca^{2+} from the sarcoplasmic reticulum (SR) by opening of IP_3-gated Ca^{2+} channels, while DAG simulates protein kinase C (PKC) to phosphorylate cellular effectors. It is these second messengers that trigger the ultimate cellular effects reported of helminth neuropeptides. Studies in flatworms illustrate a dichotomy between signalling pathways employed by two of the primary classes of flatworm neuropeptides: flatworm neuropeptide Fs (NPFs) share a conserved neuropeptide Y (NPY)-like mechanism, signalling through depression of cellular cAMP levels (presumably by inhibition of adenylate cyclase activity) (Humphries et al., 2004), while flatworm FLPs have been shown to signal mostly through phosphatidylinositol-based pathways involving PLC and PKC (Graham et al., 2000; Novozhilova et al., 2010). More specifically, a recent study showed that the flatworm FLP YIRFamide elicits its contractile effect on *S. mansoni* dispersed muscle fibres by indirect gating of sarcolemmal voltage-operated Ca^{2+} channels, via a PKC-dependent pathway, which is likely GPCR-mediated (Novozhilova et al., 2010). Similar peptidergic signalling mechanisms in schistosomes may also involve cytosolic liberation of sequestered intracellular Ca^{2+} from the SR (Day et al., 2000). Conversely, nematode FLPs appear largely to employ adenylate cyclase-mediated signalling pathways, where the diverse FLP effects seen in *A. suum* or *Ascaridia galli* bioassays are mediated by either upregulation or downregulation of cellular cAMP levels (Trim et al., 1998; Reinitz et al., 2000; Thompson et al., 2003). However, PKC-1 has also been implicated in the regulation of *C. elegans* peptidergic secretion (Sieburth et al., 2007). An alternative, nitric oxide (NO)-based signalling mechanism is employed by PF1 (SDPNFLRFamide), which relaxed *A. suum* somatic muscle via nitric oxide synthase (NOS) activity within the hypodermis, a Ca^{2+}-dependent enzyme that catalyses production of the inhibitory messenger, NO (Bowman et al., 1995; Bascal et al., 2001).

Several of the genes encoding components of these second messenger pathways display mutant phenotypes related to viability or reproductive function in both *C. elegans* and *D. melanogaster* (Table 1). Current evidence suggests that adenylate cyclases may be druggable, following description of an isoform-selective lead candidate inhibitor of mammalian adenylate cyclase AC1, which is bioavailable in animal models following both oral and intraperitoneal application (Wang et al., 2011). The isoform selectivity demonstrated by this compound suggests that selective targeting of parasite adenylate cyclase isoforms may be possible. Although components of helminth second-messenger signal transduction pathways appear to represent putative drug targets, knowledge of the parasite homologues of these effectors is conspicuously lacking.

4.3. Neuropeptide signal termination

Following activation of its cognate receptor(s), a neuropeptide must be removed from the receptor's binding site and cleared from the synapse if further neurotransmission is to occur, in a process designated signal termination. Neuropeptide signal termination is performed by a battery of peptidases that may destroy peptides either within the synaptic cleft, or following peptide/receptor internalisation. The importance of the general process of signal termination is well illustrated by the effects of carbamate-based pesticides on AChEs, the signal terminating enzymes that destroy synaptic ACh and lead to paralysis and death in some pest species (Baron, 1994).

Much neuropeptide breakdown in nematodes is seemingly performed by neprilysin-like zinc metalloendopeptidases (Isaac et al., 2000). *C. elegans* neprilysin knockouts display deleterious phenotypes (Table 1; Spanier et al., 2005), although the specific synaptic peptidolytic function of these mutants has not yet been examined. Endopeptidase, aminopeptidase and deamidase activities have been reported in *A. suum* muscle homogenates, where enzymes capable of metabolising FLPs have been detected (Sajid and Isaac, 1994; Sajid et al., 1996, 1997), and similar aminopeptidase activity capable of digesting vertebrate peptide hormone substrates has been reported in *C. elegans* and *P. redivivus* extracts (Masler, 2002). Comparative studies are limited to the report of differential peptidolytic potencies between extracts of *P. redivivus* and *M. incognita* such that implications for selective chemotherapy or broad-spectrum utility of novel drugs are unknown (Masler, 2010). The specific enzymes responsible for these activities have not yet been purified or cloned, although evidence supports the involvement of a deamidase resembling mammalian serine peptidase (Sajid and Isaac, 1994), a neprilysin-like endopeptidase which does not appear to be membrane-bound (Isaac et al., 2000), and an aminopeptidase with similar pharmacology to mammalian aminopeptidase *N* (Sajid et al., 1997). Dipeptidyl carboxypeptidase activity, common in other peptidergic signalling systems, has not been described in nematodes (Isaac et al., 2000). The employment of standard inhibitor profiling suggests that nematode peptidases show both similarities and differences in their pharmacology to that of orthologous vertebrate enzymes (Sajid and Isaac, 1995; Sajid et al., 1996, 1997). Further studies will be required to identify helminth-specific inhibitors that might represent novel drug lead candidates.

5. Conclusions

Functional analyses of various components of helminth neuropeptidergic signalling systems indicate their potential utility as targets for anthelmintic drugs. Despite this potential, the overwhelming theme throughout this article is how little we still know about the specific molecular players within parasitic helminths, and how this currently inhibits our ability to test hypotheses relating to drug target validation. While *C. elegans* is a useful model for many aspects of nematode biology, and has illuminated some structural and functional features of nematode neuropeptide pathways, the species' utility for elucidating neuropeptide function is limited by poor knowledge of its relationship to parasitic forms, and by clear differences in the complements of some proteins between *C. elegans* and parasites. Nevertheless, nematode biology has had a head start on flatworm studies, where genome and transcriptome projects have only recently appeared, and where we have yet to reap the understanding of basic organismal and molecular biology enjoyed by the *C. elegans* and *D. melanogaster* research communities. The potential for exploitation of drug targets within helminth neuropeptide signalling has yet to be realised. With continued funding for research into basic helminth neuropeptide biology, increased knowledge can perhaps be translated into novel, broad spectrum anthelmintics.

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References

Abad, P., Gouzy, J., Aury, J.M., Castagnone-Sereno, P., Danchin, E.G., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., Blok, V.C., Caillaud, M.C., Coutinho, P.M., Dasilva, C., De Luca, F., Deau, F., Esquibet, M., Flutre, T., Goldstone, J.V., Hamamouch, N., Hewezi, T., Jaillon, O., Jubin, C., Leonetti, P., Magliano, M., Maier, T.R., Markov, G.V., McVeigh, P., Pesole, G., Poulain, J., Robinson-Rechavi, M., Sallet, E., Ségurens, B., Steinbach, D., Tytgat, T., Ugarte, E., van Ghelder, C., Veronico, P., Baum, T.J., Blaxter, M., Bleve-Zacheo, T., Davis, E.L., Ewbank, J.J., Favery, B., Grenier, E., Henrissat, B., Jones, J.T., Laudet, V., Maule, A.G., Quesneville, H., Rosso, M.N., Schiex, T., Smant, G., Weissenbach, J., Wincker, P., 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nat. Biotechnol. 26, 909–915.

Ahier, A., Khayath, N., Vicogne, J., Dissous, C., 2008. Insulin receptors and glucose uptake in the human parasite *Schistosoma mansoni*. Parasite 15, 573–579.

Arena, J.P., Liu, K.K., Paress, P.S., Schaeffer, J.M., Cully, D.F., 1992. Expression of a glutamate-activated chloride current in *Xenopus* oocytes injected with *Caenorhabditis elegans* RNA: evidence for modulation by avermectin. Brain Res. Mol. Brain Res. 15, 339–348.

Asada, A., Orii, H., Watanabe, K., Tsubaki, M., 2005. Planarian peptidylglycine-hydroxylating monooxygenase, a neuropeptide processing enzyme, colocalizes with cytochrome b561 along the central nervous system. FEBS J. 272, 942–955.

Atchison, W.D., Geary, T.G., Manning, B., VandeWaa, E.A., Thompson, D.P., 1992. Comparative neuromuscular blocking actions of levamisole and pyrantel-type anthelmintics on rat and gastrointestinal nematode somatic muscle. Toxicol. Appl. Pharmacol. 112, 133–143.

Atkinson, L., McVeigh, P., Kimber, M.J., Marks, N.J., Eipper, B.A., Mains, R.E., Day, T.A., Maule, A.G., 2010. A PAL for *Schistosoma mansoni* PHM. Mol. Biochem. Parasitol. 173, 97–106.

Avery, L., 1993. The genetics of feeding in *Caenorhabditis elegans*. Genetics 133, 897–917.

Balklava, Z., Pant, S., Fares, H., Grant, B.D., 2007. Genome-wide analysis identifies a general requirement for polarity proteins in endocytic traffic. Nat. Cell Biol. 9, 1066–1073.

Bargmann, C.I., 1998. Neurobiology of the *Caenorhabditis elegans* genome. Science 282, 2028–2033.

Barkus, R.V., Klyachko, O., Horiuchi, D., Dickson, B.J., Saxton, W.M., 2008. Identification of an axonal kinesin-3 motor for fast anterograde vesicle transport that facilitates retrograde transport of neuropeptides. Mol. Biol. Cell 19, 274–283.

Baron, R.L., 1994. A carbamate insecticide: a case study of aldicarb. Environ. Health Perspect. 102, 23–27.

Bascal, Z.A., Cunningham, J.M., Holden-Dye, L., O'Shea, M., Walker, R.J., 2001. Characterisation of a putative nitric oxide synthase in the neuromuscular system of the parasitic nematode *Ascaris suum*. Parasitology 122, 219–231.

Bastiani, C., Mendel, J., 2006. Heterotrimeric G proteins in *C. elegans* (October 13, 2006), WormBook, (Ed.), The *C. elegans* Research Community, WormBook. Doi: 10.1895/wormbook.1.75.1, <http://www.wormbook.org>.

Berriman, M., Haas, B.J., LoVerde, P.T., Wilson, R.A., Dillon, G.P., Cerqueira, G.C., Mashiyama, S.T., Al-Lazikani, B., Andrade, L.F., Ashton, P.D., Aslett, M.A., Bartholomeu, D.C., Blandin, G., Caffrey, C.R., Coghlan, A., Coulson, R., Day, T.A., Delcher, A., DeMarco, R., Djikeng, A., Eyre, T., Gamble, J.A., Ghedin, E., Gu, Y., Hertz-Fowler, C., Hirai, H., Hirai, Y., Houston, R., Ivens, A., Johnston, D.A., Lacerda, D., Macedo, C.D., McVeigh, P., Ning, Z., Oliveira, G., Overington, J.P., Parkhill, J., Pertea, M., Pierce, R.J., Protasio, A.V., Quail, M.A., Rajandream, M.A., Rogers, J., Sajid, M., Salzberg, S.L., Stanke, M., Tivey, A.R., White, O., Williams, D.L., Wortman, J., Wu, W., Zamanian, M., Zerlotini, A., Fraser-Liggett, C.M., Barrell, B.G., El-Sayed, N.M., 2009. The genome of the blood fluke *Schistosoma mansoni*. Nature 460, 352–360.

Blobel, G., Dobberstein, B., 1975. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J. Cell Biol. 67, 835–851.

Bolkenius, F.N., Ganzhorn, A.J., 1998. Peptidylglycine alpha-amidating mono-oxygenase: neuropeptide amidation as a target for drug design. Gen. Pharmacol. 31, 655–659.

Bouchard, C., Ribeiro, P., Dubé, F., Anctil, M., 2003. A new G protein-coupled receptor from a primitive metazoan shows homology with vertebrate aminergic receptors and displays constitutive activity in mammalian cells. J. Neurochem. 86 (5), 1149–1161.

Boulin, T., Gielen, M., Richmond, J.E., Williams, D.C., Paoletti, P., Bessereau, J.L., 2008. Eight genes are required for functional reconstitution of the *Caenorhabditis elegans* levamisole-sensitive acetylcholine receptor. Proc. Natl. Acad. Sci. USA 105, 18590–18595.

Boulin, T., Fauvin, A., Charvet, C., Cortet, J., Cabaret, J., Bessereau, J.L., Neveau, C., 2011. Functional reconstitution of *Haemonchus contortus* acetylcholine receptors in *Xenopus* oocytes provides mechanistic insights into levamisole resistance. Br. J. Pharmacol. 164, 1421–1432.

Bowman, J.W., Winterrowd, C.A., Friedman, A.R., Thompson, D.P., Klein, R.D., Davis, J.P., Maule, A.G., Blair, K.L., Geary, T.G., 1995. Nitric oxide mediates the inhibitory effects of SDPNFLRFamide, a nematode FMRFamide-related neuropeptide, in *Ascaris suum*. J. Neurophysiol. 74, 1880–1888.

Bowman, J.W., Friedman, A.R., Thompson, D.P., Ichhpurani, A.K., Kellman, M.F., Marks, N., Maule, A.G., Geary, T.G., 1996. Structure-activity relationships of KNEFIRFamide (AF1), a nematode FMRFamide-related peptide, on *Ascaris suum* muscle. Peptides 17, 381–387.

Bowman, J.W., Friedman, A.R., Thompson, D.P., Maule, A.G., Alexander-Bowman, S.J., Geary, T.G., 2002. Structure-activity relationships of an inhibitory nematode FMRFamide-related peptide, SDPNFLRFamide (PF1), on *Ascaris suum* muscle. Int. J. Parasitol. 32, 1765–1771.

Boyle, J.P., Wu, X.J., Shoemaker, C.B., Yoshino, T.P., 2003. Using RNA interference to manipulate endogenous gene expression in *Schistosoma mansoni* sporocysts. Mol. Biochem. Parasitol. 128 (2), 205–215.

Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.

Byrne, A.B., Weirauch, M.T., Wong, V., Koeva, M., Dixon, S.J., Stuart, J.M., Roy, P.J., 2007. A global analysis of genetic interactions in *Caenorhabditis elegans*. J. Biol. 6, 8.

Caffrey, C.R., Rohwer, A., Oellien, F., Marhöfer, R.J., Braschi, S., Oliveira, G., McKerrow, J.H., Selzer, P.M., 2009. A comparative chemogenomics strategy to predict essential drug targets in the metazoan pathogen, *Schistosoma mansoni*. PLoS One 4, e4413.

Carre-Pierrat, M., Baillie, D., Johnsen, R., Hyde, R., Hart, A., Granger, L., Ségalat, L., 2006. Characterization of the *Caenorhabditis elegans* G protein-coupled serotonin receptors. Invertebr. Neurosci. 6 (4), 189–205.

Ceron, J., Rual, J.F., Chandra, A., Dupuy, D., Vidal, M., van den Heuvel, S., 2007. Large-scale RNAi screens identify novel genes that interact with the *C. elegans* retinoblastoma pathway as well as splicing-related components with synMuv B activity. BMC Dev. Biol. 7, 30.

Chahdi, A., Daeffler, L., Gies, J.P., Landry, Y., 1998. Drugs interacting with G protein alpha subunits: selectivity and perspectives. Fundam. Clin. Pharmacol. 12, 121–132.

Charlton, W.L., Harel, H.Y., Bakhetia, M., Hibbard, J.K., Atkinson, H.J., McPherson, M.J., 2010. Additive effects of plant expressed double-stranded RNAs on root-knot nematode development. Int. J. Parasitol. 40 (7), 855–864.

Chase, D.L., Koelle, M.R., 2004. Genetic analysis of RGS protein function in *Caenorhabditis elegans*. Methods Enzymol. 389, 305–320.

Collins 3rd., J.J., Hou, X., Romanova, E.V., Lambrus, B.G., Miller, C.M., Saberi, A., Sweedler, J.V., Newmark, P.A., 2010. Genome-wide analyses reveal a role for peptide hormones in planarian germline development. PLoS Biol. 8, e1000509.

Cottrell, G.A., Green, K.A., Davies, N.W., 1990. The neuropeptide Phe–Met–Arg–Phe–NH2 (FMRFamide) can activate a ligand-gated ion channel in *Helix* neurones. Pflugers Arch. 416, 612–614.

Cowden, C., Stretton, A.O., 1995. Eight novel FMRFamide-like neuropeptides isolated from the nematode *Ascaris suum*. Peptides 16 (3), 491–500.

Dalzell, J.J., McMaster, S., Fleming, C.C., Maule, A.G., 2010. Short interfering RNA-mediated gene silencing in *Globodera pallida* and *Meloidogyne incognita* infective stage juveniles. Int. J. Parasitol. 40, 91–100.

Dalzell, J.J., McVeigh, P., Warnock, N.D., Mitreva, M., Bird, D.McK., Abad, P., Fleming, C.C., Day, T.A., Mousley, A., Marks, N.J., Maule, A.G., 2011. RNAi effector diversity in nematodes. PLoS Negl. Trop. Dis. 5 (6), e1176.

Davis, R.E., Stretton, A.O., 2001. Structure-activity relationships of 18 endogenous neuropeptides on the motor nervous system of the nematode *Ascaris suum*. Peptides 22 (1), 7–23.

Day, T.A., Maule, A.G., Shaw, C., Halton, D.W., Moore, S., Bennett, J.L., Pax, R.A., 1994. Platyhelminth FMRFamide-related peptides (FaRPs) contract *Schistosoma mansoni* (Trematoda: Digenea) muscle fibres *in vitro*. Parasitology 109, 455–459.

Day, T.A., Maule, A.G., Shaw, C., Pax, R.A., 1997. Structure-activity relationships of FMRFamide-related peptides contracting *Schistosoma mansoni* muscle. Peptides 18 (7), 917–921.

Day, T.A., Haithcock, J., Kimber, M., Maule, A.G., 2000. Functional ryanodine receptor channels in flatworm muscle fibres. Parasitology 120 (4), 417–422.

Dessal, A.L., Prades, R., Giralt, E., Smrcka, A.V., 2008. Rational design of a selective covalent modifier of G protein $\beta\gamma$ subunits. Mol. Pharmacol. 79, 24–33.

Dessal, A.L., Prades, R., Giralt, E., Smrcka, A.V., 2011. Rational design of a selective covalent modifier of G protein $\beta\gamma$ subunits. Mol. Pharmacol. 79 (1), 24–33.

Dlakic, M., 2002. A new family of putative insulin receptor-like proteins in *C. elegans*. Curr. Biol. 12, R155–R157.

Duckert, P., Brunak, S., Blom, N., 2004. Prediction of proprotein convertase cleavage sites. Protein Eng. Des. Sel. 17 (1), 107–112.

Ducray, P., Gauvry, N., Pautrat, F., Goebel, T., Fruechtel, J., Desaules, Y., Weber, S.S., Bouvier, J., Wagner, T., Froelich, O., Kaminsky, R., 2008. Discovery of amino-acetonitrile derivatives, a new class of synthetic anthelmintic compounds. Bioorg. Med. Chem. Lett. 18, 2935–2938.

Eipper, B.A., Stoffers, D.A., Mains, R.E., 1992. The biosynthesis of neuropeptides: peptide alpha-amidation. Annu. Rev. Neurosci. 15, 57–85.

Emanuelsson, O., Brunak, S., von Heijne, G., Nielsen, H., 2007. Locating proteins in the cell using Target P, Signal P and related tools. Nat. Protoc. 2, 953–971.

Espelt, M.V., Estevez, A.Y., Yin, X., Strange, K., 2005. Oscillatory Ca^{2+} signaling in the isolated *Caenorhabditis elegans* intestine: role of the inositol-1,4,5-trisphosphate receptor and phospholipases C beta and gamma. J. Gen. Physiol. 126, 379–392.

Esposito, G., Amoroso, M.R., Bergamasco, C., Di Schiavi, E., Bazzicalupo, P., 2010. The G protein regulators EGL-10 and EAT-16, the Giα GOA-1 and the G(q)α EGL-30 modulate the response of the *C. elegans* ASH polymodal nociceptive sensory neurons to repellents. BMC Biol. 8, 138–151.

Fellowes, R., Maule, A.G., Marks, N.J., Geary, T.G., Thompson, D.P., Shaw, C., Halton, D.W., 1998. Modulation of the motility of the vagina vera of *Ascaras suumin vitro* by FMRFamide-related peptides. Parasitology 116, 277–287.

Ferkey, D.M., Hyde, R., Haspel, G., Dionne, H.M., Hess, H.A., Suzuki, H., Schafer, W.R., Koelle, M.R., Hart, A.C., 2007. *C. elegans* G protein regulatorRGS-3 controls sensitivity to sensory stimuli. Neuron 53, 39–52.

Fernandez, A.G., Gunsalus, K.C., Huang, J., Chuang, L.S., Ying, N., Liang, H.L., Tang, C., Schetter, A.J., Zegar, C., Rual, J.F., Hill, D.E., Reinke, V., Vidal, M., Piano, F., 2005. New genes with roles in the *C. elegans* embryo revealed using RNAi of ovary-enriched ORFeome clones. Genome Res. 15, 250–259.

Fernández, D., Pallarès, I., Vendell, J., Avilés, F.X., 2010. Progress in metallocarboxypeptidases and their small molecular weight inhibitors. Biochimie 92, 1484–1500.

Fitzpatrick, J.M., Peak, E., Perally, S., Chalmers, I.W., Barrett, J., Yoshino, T.P., Ivens, A.C., Hoffmann, K.F., 2009. Anti-schistosomal intervention targets identified by lifecycle transcriptomic analyses. PLoS Negl. Trop. Dis. 3 (11), e543.

Flower, D.R., 1999. Modelling G protein-coupled receptors for drug design. Biochim. Biophys. Acta 1422, 207–234.

Frand, A.R., Russel, S., Ruvkun, G., 2005. Functional genomic analysis of *C. elegans* molting. PLoS Biol. 3, e312.

Fraser, A.G., Kamath, R.S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., Ahringer, J.A., 2000. Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. Nature 408, 325–330.

Fredriksson, R., Schiöth, H.B., 2005. The repertoire of G-protein-coupled receptors in fully sequenced genomes. Mol. Pharmacol. 67, 1414–1425.

Fricker, L.D., 2005. Neuropeptide-processing enzymes: applications for drug discovery. AAPS J. 7, E449–E455.

Furukawa, Y., Miyawaki, Y., Abe, G., 2006. Molecular cloning and functional characterization of the *Aplysia* FMRFamide-gated Na^+ channel. Pflugers Arch. 451, 646–656.

Gaiser, A.M., Brandt, F., Richter, K., 2009. The non-canonical Hop protein from *Caenorhabditis elegans* exerts essential functions and forms binary complexes with either Hsc70 or Hsp90. J. Mol. Biol. 391, 621–634.

Geary, T.G., Kubiak, T.M., 2005. Neuropeptide G-protein-coupled receptors, their cognate ligands and behavior in *Caenorhabditis elegans*. Trends Pharmacol. Sci. 26 (2), 56–58.

Geary, T.G., Klein, R.D., Vanover, L., Bowman, J.W., Thompson, D.P., 1992. The nervous systems of helminths as targets for drugs. J. Parasitol. 78, 215–230.

Geary, T.G., Bowman, J.W., Friedman, A.R., Maule, A.G., Davis, J.P., Winterrowd, C.A., Klein, R.D., Thompson, D.P., 1995. The pharmacology of FMRFamide-related neuropeptides in nematodes: new opportunities for rational anthelmintic discovery? Int. J. Parasitol. 25, 1273–1280.

Geary, T.G., Thompson, D.P., Klein, R.D., 1999. Mechanism-based screening: discovery of the next generation of anthelmintic depends upon more basic research. Int. J. Parasitol. 29, 105–112.

Geary, T.G., Conder, G.A., Bishop, B., 2004. The changing landscape of antiparasitic drug discovery for veterinary medicine. Trends Parasitol. 20, 449–455.

Geary, T.G., Woo, K., McCarthy, J.S., MacKenzie, C.D., Horton, J., Prichard, R.K., de Silva, N.R., Olliaro, P.L., Lazdins-Helds, J.K., Engels, D.A., Bundy, D.A., 2010. Unresolved issues in anthelmintic pharmacology for helminthiases of humans. Int. J. Parasitol. 40, 1–13.

Geary, T.G., 2010. Nonpeptide ligands for peptidergic G protein-coupled receptors. Adv. Exp. Med. Biol. 692, 10–26.

Golubovic, A., Kuhn, A., Williamson, M., Kalbacher, H., Holstein, T.W., Grimmelikhuizen, C.J., Gründer, S., 2007. A peptide-gated ion channel from the freshwater polyp *Hydra*. J. Biol. Chem. 282, 35098–35103.

Gonczy, P., Echeverri, C., Oegema, K., Coulson, A.R., Jones, S.J.M., Copley, R.R., Duperon, J., Oegema, J., Brehm, M., Cassin, E., Hannak, E., Kirkham, M., Pichler, S.C., Flohrs, K., Goessen, A., Leidel, S., Alleaume, A.M., Martin, C., Ozlu, N., Bork, P., Hymann, A.A., 2000. Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. Nature 408, 331–336.

Gort, E.H., Van Haaften, G., Verlaan, I., Groot, A.J., Plasterk, R.H., Shvarts, A., Suijkerbuijk, K.P., van Laar, T., van der Wall, E., Raman, V., van Diest, P.J., Tijsterman, M., Vooijs, M., 2008. The TWIST1 oncogene is a direct target of hypoxia-inducible factor-2alpha. Oncogene 27, 1501–1510.

Gracheva, E.O., Burdina, A.O., Touroutine, D., Berthelot-Grosjean, M., Parekh, H., Richmond, J.E., 2007a. Tomosyn negatively regulates CAPS-dependent peptide release at *Caenorhabditis elegans* synapses. J. Neurosci. 27, 10176–10184.

Gracheva, E.O., Burdina, A.O., Touroutine, D., Berthelot-Grosjean, M., Parekh, H., Richmond, J.E., 2007b. Tomosynnegatively regulates both synaptic transmitter and neuropeptide release at the *C. elegans* neuromuscular junction. J. Physiol. 585, 705–709.

Graham, M.K., Fairweather, I., McGeown, J.G., 2000. Second messengers mediating mechanical responses to the FaRP GYIRFamide in the fluke *Fasciola hepatica*. Am. J. Physiol. Regul. Comp. Physiol. 279, R2089–R2094.

Greenwood, K., Williams, T., Geary, T., 2005. Nematode neuropeptide receptors and their development as anthelmintic screens. Parasitology 131, S169–S177.

Halton, D.W., Shaw, C., Maule, A.G., Johnston, C.F., Fairweather, I., 1992. Peptidergic messengers: a new perspective of the nervous system of parasitic platyhelminths. J. Parasitol. 78 (2), 179–193.

Halton, D.W., Shaw, C., Maule, A.G., Smart, D., 1994. Regulatory peptides in helminth parasites. Adv. Parasitol. 34, 163–227.

Halton, D.W., 2004. Microscopy and the helminth parasite. Micron 35 (5), 361–390.

Hamamichi, S., Rivas, R.N., Knight, A.L., Cao, S., Caldwell, K.A., Caldwell, G.A., 2008. Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. Proc. Natl. Acad. Sci. USA 105, 728–733.

Hammarlund, M., Watanabe, S., Schuske, K., Jorgensen, E.M., 2008. CAPS and syntaxin dock dense core vesicles to the plasma membrane in neurons. J. Cell. Biol. 180, 483–491.

Harder, A., Schmitt-Wrede, H.P., Krücken, J., Marinovski, P., Wunderlich, F., Willson, J., Amliwala, K., Holden-Dye, L., Walker, R., 2003. Cyclooctadepsipeptides – an anthelmintically active class of compounds exhibiting a novel mode of action. Int. J. Antimicrob. Agents 22, 318–331.

Hess, H.A., Röper, J.C., Grill, S.W., Koelle, M.R., 2004. RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in *C. elegans*. Cell 119, 209–218.

Hiley, E., McMullan, R., Nurrish, S.J., 2006. The Galpha12-RGSRhoGEF-RhoA signalling pathway regulates neurotransmitter release in *C. elegans*. EMBO J. 25, 5884–5895.

Ho, N.F., Geary, T.G., Raub, T.J., Barsuhn, C.L., Thompson, D.P., 1990. Biophysical transport properties of the cuticle of *Ascaris suum*. Mol. Biochem. Parasitol. 41 (2), 153–165.

Holden-Dye, L., Brownlee, D.J., Walker, R.J., 1997. The effects of the peptide KPNFIRFamide (PF4) on the somatic muscle cells of the parasitic nematode *Ascaris suum*. Br. J. Pharmacol. 120, 379–386.

Höller, C., Freissmuth, M., Nanoff, C., 1999. G proteins as drug targets. Cell. Mol. Life Sci. 55, 257–270.

Hoste, H., Torres-Acosta, J.F., 2011. Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world. Vet. Parasitol. 180, 144–154.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., Jacobsen, J., 2008. Helminth infections: the great neglected tropical diseases. J. Clin. Invest. 118, 1311–1321.

Hotez, P.J., Bethony, J.M., Diemert, D.J., Pearson, M., Loukas, A., 2010. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. Nat. Rev. Microbiol. 8, 814–826.

Huang, G., Allen, R., Davis, E.L., Baum, T.J., Hussey, R.S., 2006. Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc. Natl. Acad. Sci. USA 103, 14301–14306.

Huang, Q.X., Cheng, X.Y., Mao, Z.C., Wang, Y.S., Zhao, L.L., Yan, X., Ferris, V.R., Xu, R.M., Xie, B.Y., 2010. MicroRNA discovery and analysis of pinewood nematode *Bursaphelenchus xylophilus* by deep sequencing. PLoS One 5, e13271.

Humphries, J.E., Kimber, M.J., Barton, Y.W., Hsu, W., Marks, N.J., Greer, B., Harriott, P., Maule, A.G., Day, T.A., 2004. Structure and bioactivity of neuropeptide F from the human parasites *Schistosoma mansoni* and *Schistosoma japonicum*. J. Biol. Chem. 279, 39880–39885.

Hussein, A.S., Kichenin, K., Selkirk, M.E., 2002. Suppression of secreted acetylcholinesterase expression in *Nippostrongylus brasiliensis* by RNA interference. Mol. Biochem. Parasitol. 122 (1), 91–94.

Husson, S.J., Schoofs, L., 2007a. Altered neuropeptide profile of *Caenorhabditis elegans* lacking the chaperone protein 7B2 as analysed by mass spectrometry. FEBS Lett. 581, 4288–4292.

Husson, S.J., Schoofs, L., 2007b. Processing of neuropeptide precursors in *Caenorhabditis elegans*. Commun. Agric. Appl. Biol. Sci. 72, 199–203.

Husson, S.J., Clynen, E., Baggerman, G., Janssen, T., Schoofs, L., 2006. Defective processing of neuropeptide precursors in *Caenorhabditis elegans* lacking proprotein convertase 2 (KPC-2/EGL-3): mutant analysis by mass spectrometry. J. Neurochem. 98, 1999–2012.

Husson, S.J., Janssen, T., Baggerman, G., Bogert, B., Kahn-Kirby, A.H., Ashrafi, K., Schoofs, L., 2007. Impaired processing of FLP and NLP peptides in carboxypeptidase E (EGL-21)-deficient *Caenorhabditis elegans* as analysed by mass spectrometry. J. Neurochem. 102, 246–260.

Iltzsch, M.H., Bieber, D., Vijayasarathy, S., Webster, P., Zurita, M., Ding, J., Mansour, T.E., 1992. Cloning and characterization of a cDNA coding for the alpha-subunit of a stimulatory G protein from *Schistosoma mansoni*. J. Biol. Chem. 267, 14504–14508.

Isaac, R.E., Siviter, R.J., Stancombe, P., Coates, D., Shirras, A.D., 2000. Conserved roles for peptidases in the processing of invertebrate neuropeptides. Biochem. Soc. Trans. 28, 460–464.

Ja, W.W., Wiser, O., Austin, R.J., Jan, L.Y., Roberts, R.W., 2006. Turning G proteins on and off using peptide ligands. ACS Chem. Biol. 1, 570–574.

Jacoby, E., Bouhelal, R., Gerspacher, M., Seuwen, K., 2006. The 7 TM G-protein coupled receptor target family. ChemMedChem 1, 761–782.

Janssen, T., Meelkop, E., Lindemans, M., Verstraelen, K., Husson, S.J., Temmerman, L., Nachman, R.J., Schoofs, L., 2008. Discovery of a cholecystokinin-gastrin-like signaling system in nematodes. Endocrinology 149 (6), 2826–2839.

Janssen, T., Lindemans, M., Meelkop, E., Temmerman, L., Schoofs, L., 2010. Coevolution of neuropeptidergic signaling systems: from worm to man. Ann. NY Acad. Sci. 1200, 1–14.

Jarecki, J.L., Andersen, K., Konop, C.J., Knickelbine, J.J., Vestling, M.M., Stretton, A.O., 2010. Mapping neuropeptide expression by mass spectrometry in single dissected identified neurons from the dorsal ganglion of the nematode *Ascaris suum*. ACS Chem. Neurosci. 1, 505–519.

Jeziorsky, M.C., Green, K.A., Sommerville, J., Cottrell, G.A., 2000. Cloning and expression of a FMRFamide-gated Na(+) channel from *Helisoma trivolvis* and comparison with the native neuronal channel. J. Physiol. 526, 13–25.

Jiang, N., Kolhekar, A.S., Jacobs, P.S., Mains, R.E., Eipper, B.A., Taghert, P.H., 2000. PHM is required for normal developmental transitions and for biosynthesis of secretory peptides in Drosophila. Dev. Biol. 226, 118–136.

Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., Welchman, D.P., Ziperlen, P., Ahringer, J., 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421, 231–237.

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Weber, S.S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Mäser, P., 2008. A new

class of anthelmintics effective against drug-resistant nematodes. Nature 452, 176–180.
Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481.
Kass, J., Jacob, T.C., Kim, P., Kaplan, J.M., 2001. The EGL-3 proprotein convertase regulates mechanosensory responses of *Caenorhabditis elegans*. J. Neurosci. 21, 9265–9272.
Keating, C.D., Kriek, N., Daniels, M., Ashcroft, N.R., Hopper, N.A., Siney, E.J., Holden-Dye, L., Burke, J.F., 2003. Whole-genome analysis of 60 G protein-coupled receptors in *Caenorhabditis elegans* by gene knockout with RNAi. Curr. Biol. 13, 1715–1720.
Keiser, J., Utzinger, J., 2007. Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. Trends Parasitol. 23 (11), 555–562.
Keiser, J., Duthaler, U., Utzinger, J., 2010. Update on the diagnosis and treatment of food-borne trematode infections. Curr. Opin. Infect. Dis. 23 (5), 513–520.
Khayath, N., Vicogne, J., Ahier, A., BenYounes, A., Konrad, C., Trolet, J., Viscogliosi, E., Brehm, K., Dissous, C., 2007. Diversification of the insulin receptor family in the helminth parasite *Schistosoma mansoni*. FEBS. J. 274, 659–676.
Kikuchi, T., Cotton, J.A., Dalzell, J.J., Hasegawa, K., Kanzaki, N., McVeigh, P., Takanashi, T., Tsai, I.J., Assefa, S.A., Cock, P.J.A., Otto, T.D., Hunt, M., Reid, A.J., Sanchez-Flores, A., Tsuchihara, K., Yokoi, T., Larsson, M.C., Miwa, J., Maule, A.G., Sahashi, N., Jones, J.T., Berriman, M., 2011. Genomic insights into the origin of parasitism in the emerging plant pathogen *Bursaphelenchus xylophilus*. PLoS Pathog. 7 (9), e1002219.
Kimber, M.J., McKinney, S., McMaster, S.M., Day, T.A., Fleming, C.C., Maule, A.G., 2007. *flp* Gene disruption in a parasitic nematode reveals motor dysfunction and neuronal sensitivity to RNA interference. FASEB J. 21 (4), 1233–1243.
Kimber, M.J., Sayegh, L., El-Shehabi, F., Song, C., Zamanian, M., Woods, D.J., Day, T.A., Ribeiro, P., 2009. Identification of an Ascaris G protein-coupled acetylcholine receptor with atypical muscarinic pharmacology. Int. J. Parasitol. 39 (11), 1215–1222.
Kimple, A.J., Willard, F.S., Giguère, P.M., Johnston, C.A., Mocanu, V., Siderovski, D.P., 2007. The RGS protein inhibitor CCG-4986 is a covalent modifier of the RGS4 Galpha-interaction face. Biochim. Biophys. Acta 1774 (9), 1213–1220.
Kimura, K.D., Tissenbaum, H.A., Liu, Y., Ruvkun, G., 1997. Daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277, 942–946.
Knowles, J., Gromo, G., 2002. Target selection in drug discovery. Nat. Rev. Drug Discov. 2, 63–69.
Koch, H.G., Moser, M., Müller, M., 2003. Signal recognition particle-dependent protein targeting, universal to all kingdoms of life. Rev. Physiol. Biochem. Pharmacol. 146, 55–94.
Konrad, C., Kroner, A., Spiliotis, M., Zavala-Góngora, R., Brehm, K., 2003. Identification and molecular characterisation of a gene encoding a member of the insulin receptor family in *Echinococcus multilocularis*. Int. J. Parasitol. 33 (3), 301–312.
Kovaleva, E.S., Yakovlev, A.G., Masler, E.P., Chitwood, D.J., 2002. Human proprotein convertase 2 homologue from a plant nematode: cloning, characterization, and comparison with other species. FASEB J. 16, 1099–1101.
Kowalska, D., Liu, J., Appel, J.R., Ozawa, A., Nefzi, A., Mackin, R.B., Houghten, R.A., Lindberg, I., 2009. Synthetic small molecule prohormone convertase 2 inhibitors. Mol. Pharmacol. 75, 617–625.
Krüger, N., Harder, A., von Samson-Himmelstjerna, G., 2009. The putative cyclooctadepsipeptide receptor depsiphilin of the canine hookworm *Ancylostoma caninum*. Parasitol. Res. 105 (S1), S91–S100.
Kubiak, T.M., Larsen, M.J., Zantello, M.R., Bowman, J.W., Nulf, S.C., Lowery, D.E., 2003. Functional annotation of the putative orphan *Caenorhabditis elegans* G-protein-coupled receptor C10C6.2 as a FLP15 peptide receptor. J. Biol. Chem. 278, 42115–42120.
Kubiak, T.M., Larsen, M.J., Bowman, J.W., Geary, T.G., Lowery, D.E., 2008. FMRFamide-like peptides encoded on the *flp-18* precursor gene activate two isoforms of the orphan *Caenorhabditis elegans* G-protein-coupled receptor Y58G8A.4 heterologously expressed in mammalian cells. Biopolymers 90, 339–348.
Kulathila, R., Merkler, K.A., Merkler, D.J., 1999. Enzymatic formation of C-terminal amides. Nat. Prod. Rep. 16, 145–154.
Lagerström, M.C., Schiöth, H.B., 2008. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nat. Rev. Drug Discov. 7, 339–357.
Lee, S.N., Lindberg, I., 2008. 7B2 prevents unfolding and aggregation of prohormone convertase 2. Endocrinology 149, 4116–4127.
Lee, B.H., Clothier, M.F., Dutton, F.E., Nelson, S.J., Johnson, S.S., Thompson, D.P., Geary, T.G., Whaley, H.D., Haber, C.L., Marshall, V.P., Kornis, G.I., McNally, P.L., Ciadella, J.I., Martin, D.G., Bowman, J.W., Baker, C.A., Coscarelli, E.M., Alexander-Bowman, S.J., Davis, J.P., Zinser, E.W., Wiley, V., Lipton, M.F., Mauragis, M.A., 2002. Marcfortine andparaherquamideclass of anthelmintics: discovery of PNU-141962. Curr. Top. Med. Chem. 2, 779–793.
Lehner, B., Crombie, C., Tischler, J., Fortunato, A., Fraser, A.G., 2006. Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. Nat. Genet. 38, 896–903.
Levitzki, A., Gazit, A., 1995. Tyrosine kinase inhibition: an approach to drug development. Science 267, 1782–1788.
Li, C., Kim, K., 2008. Neuropeptides, September 25, 2008. WormBook (Ed.), The *C. elegans Research Community*, WormBook. Doi: 10.1895/wormbook.1.142.1, <http://www.wormbook.org>.
Li, C., Kim, K., 2010. Neuropeptide gene families in *Caenorhabditis elegans*. Adv. Exp. Med. Biol. 692, 98–137.
Li, C., Kim, K., Nelson, L.S., 1999. FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. Brain Res. 848 (1–2), 26–34.
Lin, X.G., Ming, M., Chen, M.R., Niu, W.P., Zhang, Y.D., Liu, B., Jiu, Y.M., Yu, J.W., Xu, T., Wu, Z.X., 2010. UNC-31/CAPS docks and primes dense core vesicles in *C. elegans* neurons. Biochem. Biophys. Res. Commun. 397 (3), 526–531.
Lindemans, M., Liu, F., Janssen, T., Husson, S.J., Mertens, I., Gäde, G., Schoofs, L., 2009. Adipokinetic hormone signaling through the gonadotropin-releasing hormone receptor modulates egg-laying in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 106 (5), 1642–1647.
Lingueglia, E., Champigny, G., Lazdunsky, M., Barbry, P., 1995. Cloning of the amiloride-sensitive FMRFamide peptide-gated sodium channel. Nature 378, 730–733.
Littleton, J.T., Chapman, E.R., Kreber, R., Garment, M.B., Carlson, S.D., 1998. Temperature-sensitive paralytic mutations demonstrate that synaptic exocytosis requires SNARE complex assembly and disassembly. Neuron 21, 401–413.
Lowery, D.E., Geary, T.G., Kubiak, T.M., Larsen, M.J., 2003. G protein-coupled receptor-like receptors and modulators thereof. Pharmacia and Upjohn Company, United States. Patent US 6632621 B1.
Maeda, I., Kohara, Y., Yamamoto, M., Sugimoto, A., 2001. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. Curr. Biol. 11, 171–176.
Mair, G.R., Niciu, M.J., Stewart, M.T., Brennan, G., Omar, H., Halton, D.W., Mains, R., Eipper, B.A., Maule, A.G., Day, T.A., 2004. A functionally atypical amidating enzyme from the human parasite *Schistosoma mansoni*. FASEB J. 18, 114–121.
Malone, E.A., Thomas, J.H., 2004. A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. Genetics 136, 879–886.
Mansour, J.M., Mansour, T.E., 1989. Identification of GTP-binding proteins in *Fasciola hepatica* and *Schistosoma mansoni* by immunoblotting. Mol. Biochem. Parasitol. 36, 11–18.
Marks, N.J., Maule, A.G., 2010. Neuropeptides in helminths: occurrence and distribution. Adv. Exp. Med. Biol. 692, 49–77.
Marks, N.J., Johnson, S., Maule, A.G., Halton, D.W., Shaw, C., Geary, T.G., Moore, S., Thompson, D.P., 1996. Physiological effects of platyhelminth RFamide peptides on muscle-strip preparations of *Fasciola hepatica* (Trematoda: Digenea). Parasitology 113, 393–401.
Marks, N.J., Maule, A.G., Halton, D.W., Geary, T.G., Shaw, C., Thompson, D.P., 1997. Pharmacological effects of nematode FMRFamide-related peptides (FaRPs) on muscle contractility of the trematode, *Fasciola hepatica*. Parasitology 114, 531–539.
Marks, N.J., Sangster, N.C., Maule, A.G., Halton, D.W., Thompson, D.P., Geary, T.G., Shaw, C., 1999. Structural characterisation and pharmacology of KHEYLRFamide (AF2) and KSAYMRFamide (PF3/AF8) from *Haemonchus contortus*. Mol. Biochem. Parasitol. 100, 185–194.
Martin, R.J., Robertson, A.P., 2010. Control of nematode parasites with agents acting on neuro-musculature systems: lessons for neuropeptide ligand discovery. Adv. Exp. Med. Biol. 692, 138–154.
Martin, R.J., Murray, I., Robertson, A.P., Bjorn, H., Sangster, N., 1998. Anthelmintics and ion-channels: after a puncture, use a patch.Int. J. Parasitol. 28 (6), 849–862.
Masler, E.P., 2002. Aminopeptidases in *Caenorhabditis elegans* and *Panagrellus redivivus*: detection using peptide and non-peptide substrates. J. Helminthol. 76, 45–52.
Masler, E.P., 2010. In vitro comparison of protease activities in preparations from free-living (*Panagrellus redivivus*) and plant-parasitic (*Meloidogyne incognita*) nematodes using FMRFa and FMRFa-like peptides as substrates. J. Helminthol. 84, 425–433.
Maule, A.G., Shaw, C., Halton, D.W., Thim, L., Johnston, C.F., Fairweather, I., Buchanan, K.D., 1991. Neuropeptide F: a novel parasitic flatworm regulatory peptide from *Moniezia expansa* (Cestoda: Cyclophyllidea). Parasitology 102, 309–316.
Maule, A.G., Shaw, C., Halton, D.W., Thim, L., 1993. GNFFRFamide: A novel FMRFamide-immunoreactive peptide isolated from the sheep tapeworm, *Moniezia expansa*. Biochem. Biophys. Res. Commun. 193, 1054–1060.
Maule, A.G., Geary, T.G., Bowman, J.W., Marks, N.J., Blair, K.L., Halton, D.W., Shaw, C., Thompson, D.P., 1995. Inhibitory effects of nematode FMRFamide-related peptides (FaRPs) on muscle strips from *Ascaris suum*. Invertebr. Neurosci. 1, 255–265.
Maule, A.G., Bowman, J.W., Thompson, D.P., Marks, N.J., Friedman, A.R., Geary, T.G., 1996a. FMRFamide-related peptides (FaRPs) in nematodes: occurrence and neuromuscular physiology. Parasitology 113, S119–S135.
Maule, A.G., Geary, T.G., Bowman, J.W., Shaw, C., Halton, D.W., Thompson, D.P., 1996b. The pharmacology of nematode FMRFamide-related peptides. Parasitol. Today 12 (9), 351–357.
Maule, A.G., Mousley, A., Marks, N.J., Day, T.A., Thompson, D.P., Geary, T.G., Halton, D.W., 2002. Neuropeptide signaling systems – potential drug targets for parasite and pest control. Curr. Top. Med. Chem. 2 (7), 733–758.
McCudden, C.R., Hains, M.D., Kimple, R.J., Siderovski, D.P., Willard, F.S., 2005. G-protein signaling: back to the future. Cell Mol. Life Sci. 62 (5), 551–577.
McGonigle, L., Mousley, A., Marks, N.J., Brennan, G.P., Dalton, J.P., Spithill, T.W., Day, T.A., Maule, A.G., 2008. The silencing of cysteine proteases in *Fasciola hepatica* newly excysted juveniles using RNA interference. Int. J. Parasitol. 38 (2), 149–155.
McVeigh, P., Kimber, M.J., Novozhilova, E., Day, T.A., 2005a. Neuropeptide signalling systems in flatworms. Parasitology 131, S41–S55.

McVeigh, P., Leech, S., Mair, G.R., Marks, N.J., Geary, T.G., Maule, A.G., 2005b. Analysis of FMRFamide-like peptide (FLP) diversity in phylum Nematoda. Int. J. Parasitol. 35, 1043–1060.
McVeigh, P., Geary, T.G., Marks, N.J., Maule, A.G., 2006. The FLP-side of nematodes. Trends Parasitol. 22, 385–396.
McVeigh, P., Alexander-Bowman, S., Veal, E., Mousley, A., Marks, N.J., Maule, A.G., 2008. Neuropeptide-like protein diversity in phylum Nematoda. Int. J. Parasitol. 38, 1493–1503.
McVeigh, P., Mair, G.R., Atkinson, L., Zamanian, M., Ladurner, P., Novozhilova, E., Marks, N.J., Day, T.A., Maule, A.G., 2009. Discovery of multiple neuropeptide families in phylum Platyhelminthes. Int. J. Parasitol. 39, 1243–1252.
McVeigh, P., Mair, G.R., Novozhilova, E., Day, A., Zamanian, M., Marks, N.J., Kimber, M.J., Day, T.A., Maule, A.G., 2011. Schistosome l/Lamides – a new family of bioactive helminth neuropeptides. Int. J. Parasitol. 41, 905–913.
Mertens, I., Vandingenen, A., Meeusen, T., Janssen, T., Luyten, W., Nachman, R.J., De Loof, A., Schoofs, L., 2004. Functional characterisation of the putative orphan neuropeptide G-protein coupled receptor C26F1.6 in *Caenorhabditis elegans*. FEBS. Lett. 573, 55–60.
Mertens, I., Meeusen, T., Janssen, T., Nachman, R., Schoofs, L., 2005. Molecular characterization of two G protein-coupled receptor splice variants as FLP2 receptors in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. 330 (3), 967–974.
Mertens, I., Clinckspoor, I., Janssen, T., Nachman, R., Schoofs, L., 2006. FMRFamide related peptide ligands activate the *Caenorhabditis elegans* orphan GPCR Y59H11AL 1. Peptides 27 (6), 1291–1296.
Minic, J., Sautel, M., Salesse, R., Pajot-Augy, E., 2005. Yeast system as a screening tool for pharmacological assessment of G protein coupled receptors. Curr. Med. Chem. 12 (8), 961–969.
Moffett, C.L., Beckett, A.M., Mousley, A., Marks, N.J., Thompson, D.P., Geary, T.G., Halton, D.W., Maule, A.G., 2003. The ovijector of *Ascaris suum*: multiple response types revealed by *C. elegans* FMRFamide-related peptides. Int. J. Parasitol. 33, 859–876.
Mousley, A., Marks, N.J., Halton, D.W., Geary, T.G., Thompson, D.P., Maule, A.G., 2004. Arthropod FMRFamide-related peptide modulate muscle activity in helminths. Int. J. Parasitol. 34, 755–768.
Mousley, A., Maule, A.G., Halton, D.W., Marks, N.J., 2005a. Inter-phyla studies on neuropeptides: the potential for broad spectrum anthelmintic and/or endectocide discovery. Parasitology 131, S143–S167.
Mousley, A., Moffett, C.L., Duve, H., Thorpe, A., Halton, D.W., Geary, T.G., Thompson, D.P., Maule, A.G., Marks, N.J., 2005b. Expression and bioactivity of allatostatin-like neuropeptides in helminths. Int. J. Parasitol. 35, 1557–1567.
Mousley, A., Novozhilova, E., Kimber, M.J., Day, T.A., 2010. Neuropeptide physiology in helminths. Adv. Exp. Med. Biol. 692, 78–97.
Mühlfeld, S., Schmitt-Wrede, H.B., Harder, A., Wunderlich, F., 2009. FMRFamide-like neuropeptides as putative ligands of latrophilin-like HC110-R from *Haemonchus contortus*. Mol. Biochem. Parasitol. 164, 162–164.
Muller, L., Zhu, X., Lindberg, I., 1997. Mechanism of the facilitation of PC2 maturation by 7B2: involvement in ProPC2 transport and activation but not folding. J. Cell Biol. 139, 625–638.
Nana-Sinkam, S.P., Croce, C.M., 2010. MicroRNA dysregulation in cancer: opportunities for the development of microRNA-based drugs. IDrugs 13 (12), 843–846.
Nana-Sinkam, S.P., Croce, C.M., 2011. MicroRNAs as therapeutic targets in cancer. Transl. Res. 157, 216–225.
Nanda, J.C., Stretton, A.O., 2010. *In situ* hybridization of neuropeptide-encoding transcripts afp-1, afp-3, and afp-4 in neurons of the nematode *Ascaris suum*. J. Comp. Neurol. 518, 896–910.
Nollen, E.A.A., Garcia, S.M., Van Haaften, G., Kim, S., Chavez, A., Morimoto, R.I., Plasterk, R.H.A., 2004. Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. Proc. Natl Acad. Sci. USA 101, 6403–6408.
Novozhilova, E., Kimber, M.J., Qian, H., McVeigh, P., Robertson, A.P., Zamanian, M., Maule, A.G., Day, T.A., 2010. FMRFamide-like peptides enhance voltage-gated calcium currents to elicit muscle contraction in the human parasite *Schistosoma mansoni*. PLoS Neg. Trop. Dis. 4, e790.
Omar, H.H., Humphries, J.E., Larsen, M.J., Kubiak, T.M., Geary, T.G., Maule, A.G., Kimber, M.J., Day, T.A., 2007. Identification of a platyhelminth neuropeptide receptor. Int. J. Parasitol. 37, 725–733.
Overington, J.P., Al-Lazikani, B., Hopkins, A.L., 2006. How many drug targets are there? Nat. Rev. Drug Discov. 5, 993–996.
Pang, F.Y., Mason, J., Holden-Dye, L., Franks, C.J., Williams, R.G., Walker, R.J., 1995. The effects of the nematode peptide, KHEYLRFamide (AF2), on the somatic musculature of the parasitic nematode *Ascaris suum*. Parasitology 110, 353–362.
Papaioannou, S., Marsden, D., Franks, C.J., Walker, R.J., Holden-Dye, L., 2005. Role of a FMRFamide-like family of neuropeptides in the pharyngeal nervous system of *Caenorhabditis elegans*. J. Neurobiol. 65, 304–319.
Park, S.K., Link, C.D., Johnson, T.E., 2010. Life-span extension by dietary restriction is mediated by NLP-7 signaling and coelomocyte endocytosis in *C. elegans*. FASEB J. 24, 383–392.
Pax, R., Bennett, J.L., Fetterer, R., 1978. A benzodiazepine derivative and praziquantel: effects on musculature of *Schistosoma mansoni* and *Schistosoma japonicum*. Naunyn Schmiedebergs Arch. Pharmacol. 304 (3), 309–315.
Perrimon, N., Lanjuin, A., Arnold, C., Noll, E., 1996. Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster*. II. Loci on the second and third chromosomes identified by P-element-induced mutations. Genetics 144, 1681–1692.
Pierson, L., Mousley, A., Divine, L., Day, T.A., Marks, N.J., Maule, A.G., 2010. RNA interference in a cestode reveals specific silencing of selected highly expressed gene transcripts. Int. J. Parasitol. 40, 605–615.
Porter, M.Y., Koelle, M.R., 2010. RSBP-1 is a membrane-targeting subunit required by the Galpha(q)-specific but not the Galpha(o)-specific R7 regulator of G protein signaling in *Caenorhabditis elegans*. Mol. Biol. Cell 21, 232–243.
Porter, M.Y., Xie, K., Pozharski, E., Koelle, M.R., Martemyanov, K.A., 2010. A conserved protein interaction interface on the type 5 G protein beta subunit controls proteolytic stability and activity of R7 family regulator of G protein signaling proteins. J. Biol. Chem. 285, 41100–41112.
Prigge, S.T., Mains, R.E., Eipper, B.A., Amzel, L.M., 2000. New insights into copper monooxygenases and peptide amidation: structure, mechanism and function. Cell Mol. Life Sci. 57, 1236–1259.
Purcell, J., Robertson, A.P., Thompson, D.P., Martin, R.J., 2002a. The time-course of the response to the FMRFamide-related peptide PF4 in *Ascaris suum* muscle cells indicates direct gating of a chloride ion-channel. Parasitology 124, 649–656.
Purcell, J., Robertson, A.P., Thompson, D.P., Martin, R.J., 2002b. PF4, a FMRFamide-related peptide, gates low-conductance Cl(−) channels in *Ascaris suum*. Eur. J. Pharmacol. 456, 11–17.
Rayburn, L.Y.M., Gooding, H.C., Choksi, S.P., Maloney, D., Kidd, A.R., Siekhaus, D.E., Bender, M., Bender, M., 2003. Amontillado, the *Drosophila* homolog of the prohormone processing protease PC2, is required during embryogenesis and early larval development. Genetics 163, 227–237.
Reddien, P.W., Bermange, A.L., Murfitt, K.J., Jennings, J.R., Sánchez Alvarado, A., 2005. Identification of genes needed for regeneration, stem cell function, and tissue homeostasis by systematic gene perturbation in planaria. Dev. Cell 8, 635–649.
Rehfeld, J.F., Zhu, X., Norrbom, C., Bundgaard, J.R., Johnsen, A.H., Nielsen, J.E., Vikesaa, J., Stein, J., Dey, A., Steiner, D.F., Friis-Hansen, L., 2008. Prohormone convertases 1/3 and 2 together orchestrate the site-specific cleavages of progastrin to release gastrin-34 and gastrin-17. Biochem. J. 415 (1), 35–43.
Reinitz, C.A., Herfel, H.G., Messinger, L.A., Stretton, A.O., 2000. Changes in locomotory behaviour and cAMP produced in *Ascaris suum* by neuropeptides from *Ascarissuum* or *Caenorhabditis elegans*. Mol. Biochem. Parasitol. 111, 185–197.
Renden, R., Berwin, B., Davis, W., Ann, K., Chin, C.T., Kreber, R., Ganetzky, B., Martin, T.F., Broadie, K., 2001. *Drosophila* CAPS is an essential gene that regulates dense-core vesicle release and synaptic vesicle fusion. Neuron 31, 421–437.
Richmond, J., 2007. Synaptic function (December 7, 2007). In: WormBook (Ed.), The *C. elegans* Research Community, Wormbook, doi: 10.1895/wormbook.1.69.1, <http://www.wormbook.org>.
Robb, S.M., Ross, E., Sánchez-Alvarado, A., 2008. SmedGD: the *Schmidtea mediterranea* genome database. Nucleic Acids Res. 36, D599–D606.
Robertson, A.P., Puttachary, S., Buxton, S.K., Martin, R.J., 2008. Electrophysiological recording from parasitic nematode muscle. Invertebr. Neurosci. 8 (4), 167–175.
Rogers, C., Reale, V., Kim, K., Chatwin, H., Li, C., Evans, P., de Bono, M., 2003. Inhibition of *Caenorhabditis elegans* social feeding by FMRFamide-related peptide activation of NPR-1. Nat. Neurosci. 6 (11), 1178–1185.
Rual, J.F., Ceron, J., Koreth, J., Hao, T., Nicot, A.S., Hirozane-Kishikawa, T., Vandenhaute, J., Orkin, S.H., Hill, D.E., van den Heuvel, S., Vidal, M., 2004. Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. Genome Res. 14, 2162–2168.
Saeger, B., Schmitt-Wrede, H.P., Dehnhardt, M., Benten, W.P., Krücken, J., Harder, A., Von Samson-Himmelstjerna, G., Wiegand, H., Wunderlich, F., 2001. Latrophilin-like receptor from the parasitic nematode *Haemonchus contortus* as target for the anthelmintic depsipeptide PF1022A. FASEB J. 15, 1332–1334.
Sajid, M., Isaac, R.E., 1994. Metabolism of AF1 (HNEFIRF–NH_2) in the nematode *Ascaris suum*. Biochem. Soc. Trans. 22, 293S.
Sajid, M., Isaac, R.E., 1995. Identification and properties of a neuropeptide-degrading endopeptidase (neprilysin) of *Ascaris suum* muscle. Parasitology 111, 599–608.
Sajid, M., Keating, C., Holden-Dye, L., Harrow, I.D., Isaac, R.E., 1996. Metabolism of AF1 (KNEFIRF–NH_2) in the nematode, *Ascaris suum*, by aminopeptidase, endopeptidase and deamidase enzymes. Mol. Biochem. Parasitol. 75, 159–168.
Sajid, M., Isaac, R.E., Harrow, I.D., 1997. Purification and properties of a membrane aminopeptidase from *Ascaris suum* muscle that degrades neuropeptides AF1 and AF2. Mol. Biochem. Parasitol. 89, 225–234.
Sayed, A.A., Simeonov, A., Thomas, C.J., Inglese, J., Austin, C.P., Williams, D.L., 2008. Identification of oxadiazoles as new drug leads for the control of schistosomiasis. Nat. Med. 14 (4), 407–412.
Schaefer, M., Petronczki, M., Dorner, D., Forte, M., Knoblich, J.A., 2001. Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system. Cell 107, 183–194.
Seidah, N.G., 2011. What lies ahead for the proprotein convertases? Ann. NY Acad. Sci. 1220, 149–161.
Settle Jr., S.H., Green, M.M., Burtis, K.C., 1995. The silver gene of *Drosophilamelanogaster* encodes multiple carboxypeptidases similar to mammalian prohormone-processing enzymes. Proc. Natl. Acad. Sci. USA 92, 9470–9474.
Sheehy, B.A., Ho, N.F., Burton, P.S., Day, J.S., Geary, T.G., Thompson, D.P., 2000. Transport of model peptides across *Ascaris suum* cuticle. Mol. Biochem. Parasitol. 105 (1), 39–49.
Sieburth, D., Ch'ng, Q., Dybbs, M., Tavazoie, M., Kennedy, S., Wang, D., Dupuy, D., Rual, J.F., Hill, D.E., Vidal, M., Ruvkun, G., Kaplan, J.M., 2005. Systematic analysis of genes required for synapse structure and function. Nature 436, 510–517.
Sieburth, D., Madison, J.M., Kaplan, J.M., 2007. PKC-1 regulates secretion of neuropeptides. Nat. Neurosci. 10, 49–57.

Simmer, F., Moorman, C., van der Linden, A.M., Kuijk, E., van den Berghe, P.V.E., Kamath, F.S., Fraser, A.G., Ahringer, J., Plasterk, R.H.A., 2003. Genome-wide RNAi of *C. elegans* using the hypersensitive *rrf-3* strain reveals novel gene functions. PLoS Biol. 1, e12.

Sjögren, B., Blazer, L.L., Neubig, R.R., 2010. Regulators of G protein signaling proteins as targets for drug discovery. Prog. Mol. Biol. Transl. Sci. 91, 81–119.

Skelly, P.J., Da'dara, A., Harn, D.A., 2003. Suppression of cathepsin B expression in *Schistosoma mansoni* by RNA interference. Int. J. Parasitol. 33 (4), 363–369.

Smrcka, A.V., Lehmann, D.M., Dessal, A.L., 2008. G protein betagamma subunits as targets for small molecule therapeutic development. Comb. Chem. High Throughput Screen. 11, 382–395.

Sonnichsen, B., Koski, L.B., Walsh, A., Marschall, P., Neumann, B., Brehm, M., Alleaume, A.M., Artelt, J., Bettencourt, P., Cassin, E., Hewitson, M., Holz, C., Khan, M., Lazik, S., Martin, C., Nitzsche, B., Ruer, M., Stamford, J., Winzi, M., Heinkel, R., Roder, M., Finell, J., Hantsch, H., Jones, S.J., Jones, M., Piano, F., Gunsalus, K.C., Oegema, K., Gonczy, P., Coulson, A., Hyman, A.A., Echeverri, C.J., 2005. Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. Nature 434, 462–469.

Spanier, B., Stürzenbaum, S.R., Holden-Dye, L.M., Baumeister, R., 2005. *Caenorhabditis elegans* neprilysin NEP-1: an effector of locomotion and pharyngeal pumping. Mol. Biol. 352 (2), 429–437.

Speese, S., Petrie, M., Schuske, K., Ailion, M., Ann, K., Iwasaki, K., Jorgensen, E.M., Martin, T.F., 2007. UNC-31 (CAPS) is required fordense-core vesiclebut not synaptic vesicle exocytosis in *Caenorhabditis elegans*. J. Neurosci. 27, 6150–6162.

Stefanić, S.S., Dvořák, J., Horn, M., Braschi, S., Sojka, D., Ruelas, D.S., Suzuki, B., Lim, K.C., Hopkins, S.D., McKerrow, J.H., Caffrey, C.R., 2010. RNA interference in *Schistosoma mansoni* schistosomula: selectivity, sensitivity and operation for larger-scale screening. PLoS Negl. Trop. Dis. 4, e850.

Sutherland, I.A., Leathwick, D.M., 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends Parasitol. 37, 176–181.

Taman, A., Ribeiro, P., 2009. Investigation of a dopamine receptor in *Schistosoma mansoni*: functional studies and immunolocalization. Mol. Biochem. Parasitol. 168 (1), 24–33.

Taman, A., Ribeiro, P., 2011. Glutamate-mediated signaling in *Schistosoma mansoni*: a novel glutamate receptor is expressed in neurons and the female reproductive tract. Mol. Biochem. Parasitol. 176 (1), 42–50.

Taylor, C.M., Fischer, K., Abubucker, A., Wang, Z., Martin, J., Jiang, D., Magliano, M., Rosso, M.N., Li, B.W., Fischer, P.U., Mitreva, M., 2011. Targeting protein–protein interactions for parasite control. PLoS One 6, e18381.

Thompson, D.P., Klein, R.D., Geary, T.G., 1996. Prospects for rational approaches to anthelmintic discovery. Parasitology 113, S217–S238.

Thompson, D.P., Davis, J.P., Larsen, M.J., Coscarelli, E.M., Zinser, E.W., Bowman, J.W., Alexander-Bowman, S.J., Marks, N.J., Geary, T.G., 2003. Effects of KHEYLRFamide and KNEFIRFamide on cyclic adenosine monophosphate levels in *Ascaris suum* somatic muscle. Int. J. Parasitol. 33, 199–208.

Trent, C., Tsuing, N., Horvitz, H.R., 1983. Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics 104, 619–647.

Trim, N., Brooman, J.E., Holden-Dye, L., Walker, R.J., 1998. The role of cAMP in the actions of the peptide AF3 in the parasitic nematodes *Ascaris suum* and *Ascaridia galli*. Mol. Biochem. Parasitol. 93, 263–271.

Urwin, P.E., Lilley, C.J., Atkinson, H.J., 2002. Ingestion of double-stranded RNA by pre-parasitic juvenile cyst nematodes leads to RNA interference. Mol. Plant Microbe Interact. 15, 747–752.

Van Horssen, A.M., van den Hurk, W.H., Bailyes, E.M., Hutton, J.C., Martens, G.J., Lindberg, I., 1995. Identification of the region within the neuroendocrine polypeptide 7B2 responsible for the inhibition of prohormone convertase PC2. J. Biol. Chem. 270, 14292–14296.

Veenstra, J.L., 2000. Mono- and dibasic proteolytic cleavage sites in insect neuroendocrine precursors. Arch. Insect Biochem. Physiol. 43, 49–63.

Vicogne, J., Dissous, C., 2003. *Schistosoma mansoni* receptor tyrosine kinases: towards new therapeutic targets. J. Soc. Biol. 197 (4), 367–373.

Voogd, T.E., Vansterkenburg, E.L.M., Wilting, J., Janssen, L.H.M., 1993. Recent research on the biological activity of suramin. Pharmacol. Rev. 45, 177–203.

Wang, H., Xu, H., Wu, L.J., Kim, S.S., Chen, T., Koga, K., Descalzi, G., Gong, B., Vadakkan, K.I., Zhang, X., Kaang, B.K., Zhuo, M., 2011. Identification of an adenylyl cyclaseinhibitor for treating neuropathic and inflammatory pain. Sci. Transl. Med. 3, 65ra3.

Welz, C., Harder, A., Schnieder, T., Hoglund, J., von Samson-Himmelstjerna, G., 2005. Putative G protein-coupled receptors in parasitic nematodes–potential targets for the new anthelmintic class cyclooctadepsipeptides? Parasitol. Res. 97, S22–S32.

Williamson, S.M., Robertson, A.P., Brown, L., Williams, T., Woods, D.J., Martin, R.J., Sattelle, D.B., Wolstenholme, A.J., 2009. The nicotinic acetylcholine receptors of the parasitic nematode *Ascaris suum*: formation of two distinct drug targets by varying the relative expression levels of two subunits. PLoS Pathog. 5 (7), e1000517.

Wise, A., Gearing, K., Rees, S., 2002. Target validation of G protein-coupled receptors. Drug Discov. Today 7, 235–246.

Wolfgang, W.J., Hoskote, A., Roberts, I.J.H., Jackson, S., Forte, M., 2001. Genetic analysis of the *Drosophila* Gs gene. Genetics 158, 1189–1201.

Wolstenholme, A.J., Rogers, A.T., 2005. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. Parasitology 131, S85–S95.

Woods, D., Butler, C., Williams, T., Greenwood, K., 2010. Receptor-based discovery strategies for insecticides and parasiticides: a review. Adv. Exp. Med. Biol. 692, 1–9.

Yadav, B.C., Veluthambi, K., Subramaniam, K., 2006. Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol. Biochem. Parasitol. 148, 219–222.

Yau, D.M., Yokoyama, N., Goshima, Y., Siddiqui, Z.K., Siddiqui, S.S., Kozasa, T., 2003. Identification and molecular characterization of the G alpha12-Rho guanine nucleotide exchange factor pathway in *Caenorhabditis elegans*. Proc. Natl Acad. Sci. USA 100, 14748–14753.

Yew, J.Y., Kutz, K.K., Dikler, S., Messinger, L., Li, L., Stretton, A.O., 2005. Mass spectrometric map of neuropeptide expression in *Ascaris suum*. J. Comp. Neurol. 488 (4), 396–413.

You, H., Zhang, W., Jones, M.K., Gobert, G.N., Mulvenna, J., Rees, G., Spanevello, M., Blair, D., Duke, M., Brehm, K., McManus, D.P., 2010. Cloning and characterisation of *Schistosoma japonicum* insulin receptors. PLoS One 5, e9868.

You, H., Gobert, G.N., Jones, M.K., Zhang, W., McManus, D.P., 2011. Signalling pathways and the host-parasite relationship: putative targets for control interventions against schistosomiasis: signalling pathways and future anti-schistosome therapies. Bioessays 33, 203–214.

Zahn, T.R., Angleson, J.K., MacMorris, M.A., Domke, E., Hutton, J.F., Scwartz, C., Hutton, J.C., 2004. Dense core vesicle dynamics in *Caenorhabditis elegans* neurons and the role of kinesin UNC-104. Traffic 5, 544–559.

Zamanian, M., Kimber, M.J., McVeigh, P., Carlson, A., Maule, A.G., Day, T.A., in press. The repertoire of G protein-coupled receptors in the human parasite *Schistosoma mansoni* and the model organism *Schmidtea mediterranea*. BMC Genomics.

Zhang, X., Bao, L., Ma, G.Q., 2010. Sorting of neuropeptides and neuropeptide receptors into secretory pathways. Prog. Neurobiol. 90, 276–283.

Zinser, E.W., Wolf, M.L., Alexander-Bowman, S.J., Thomas, E.M., Davis, J.P., Groppi, V.E., Lee, B.H., Thompson, D.P., Geary, T.G., 2002. Anthelmintic paraherquamides are cholinergic antagonists in gastrointestinal nematodes and mammals. J. Vet. Pharmacol. Ther. 25, 241–250.

Zugasti, O., Rajan, J., Kuwabara, P.E., 2005. The function and expansion of the Patched- and Hedgehog-related homologs in *C. elegans*. Genome Res. 15, 1402–1410.