Exploring the role of two interacting phosphoinositide 3-kinases of *Haemonchus contortus*

Fa-Cai Li¹, Robin B Gasser², James B Lok³, Pasi K Korhonen², Yi-Fan Wang¹, Fang-Yuan Yin¹, Li He¹, Rui Zhou¹, Jun-Long Zhao¹ and Min Hu*¹

**Abstract**

**Background:** Phosphoinositide 3-kinases (PI3Ks) are relatively conserved and important intracellular lipid kinases involved in signalling and other biological pathways. In the free-living nematode *Caenorhabditis elegans*, the heterodimeric form of PI3K consists of catalytic (AGE-1) and regulatory (AAP-1) subunits. These subunits are key components of the insulin-like signalling pathway and play roles in the regulation of the entry into and exit from dauer. Although, in parasitic nematodes, similar components are proposed to regulate the transition from free-living or arrested stages to parasitic larvae, nothing is known about PI3Ks in relation to the transition of third-stage larvae (L3s) to parasitism in *Haemonchus contortus*.

**Methods:** An integrated molecular approach was used to investigate *age-1* and *aap-1* of *H. contortus* (*Hc-age-1* and *Hc-aap-1*) in *C. elegans*.

**Results:** The two genes *Hc-age-1* and *Hc-aap-1* were transcribed in all life stages, with the highest levels in the egg, infective L3 and adult female of *H. contortus*. The expression of these genes was localized to the intestine, contrasting the pattern of their orthologues in *C. elegans* (where they are expressed in both head neurons and the intestine). The yeast two-hybrid analysis demonstrated that the adaptor-binding domain of *Hc-AGE-1* interacted strongly with the *Hc-AAP-1*; however, this complex did not rescue the function of its orthologue in *age-1-deficient C. elegans*.

**Conclusions:** This is the first time that the PI3K-encoding genes have been characterized from a strongylid parasitic nematode. The findings provide insights into the role of the PI3K heterodimer represented by *Hc-age-1* and *Hc-aap-1* in the developmental biology of *H. contortus*.

**Keywords:** Parasitic nematode, *Haemonchus contortus*, *age-1*, *aap-1*, Development, Transgenesis

---

**Background**

Phosphoinositide 3-kinases (PI3Ks) are relatively conserved and important intracellular lipid kinases that synthesize lipid second messengers by phosphorylating the 3-hydroxyl group of the inositol head-group in the phosphatidylinositol and phosphoinositides [1,2]. This phosphorylation process gives rise to the activation of a series of downstream effector proteins, such as protein kinase, regulators of small GTPases and/or scaffolding proteins [3,4], which play key roles in lipid and cell signalling as well as membrane trafficking. In multicellular organisms, including the *Caenorhabditis elegans* (free-living nematode), *Drosophila melanogaster* (vinegar fly) and *Mus musculus* (mouse), PI3Ks play key roles in the regulation of cellular metabolism and/or growth [5-9]. Additionally, PI3Ks are involved in the regulation of human maladies, such as heart disease and cancer, and are thus considered possible therapeutic targets [10].

Based on sequence homology and lipid substrate preference, PI3Ks are classified into classes I to III [11]. In *C. elegans*, PI3K (class I) is a heterodimer consisting of two subunits encoded by *age-1* (orthologue of the
p110 catalytic subunit) and aap-1 (orthologue of the p50/p55-like regulatory subunit) [12,13]. This AGE-1/AAP-1 heterodimer is a key component of the canonical insulin-Pi3K signalling pathway, which plays an important role in dauer formation in C. elegans [14]; genetic screens have shown that Pi3K is downstream of the insulin-like receptor DAF-2 [15-17] and upstream of PDK-1 [18,19], AKT-1/AKT-2 kinases [20] and the forkhead transcriptional factor DAF-16 [21-23]. In C. elegans, loss-of-function mutations in the gene age-1 lead to dauer constitutive phenotypes [6]. Similarly, C. elegans lacking AAP-1 have phenotypes that are similar to age-1 mutants [13]. Upstream signalling via DAF-2 activates Pi3K by phosphorylation, which, in turn, negatively regulates DAF-16, a crucial regulator of dauer formation, with its characteristics of developmental arrest, longevity and stress-resistance [24,25].

Although advances have been made in understanding the mechanisms involved in the entry into and exit from dauer in C. elegans, very little is known about such mechanisms in parasitic nematodes. Some workers [26-29] have proposed that infective third-stage larvae (L3s) of parasitic nematodes represent the same or a similar developmental state to dauer in C. elegans, with comparable features (including an inability to feed, resistance to stress/environment and extended lifespan). In addition, there are indications that mechanisms controlling the transition of free-living to invasive L3s in parasitic nematodes are similar to those that regulate the recovery from dauer in C. elegans. For instance, recently, two orthologues of Ss-age-1 and Ss-aap-1 were identified in the parasitic nematode Strongyloides stercoralis [30]. The proteins and anatomical expression patterns of AGE-1s from C. elegans and S. stercoralis were shown to be similar. Additionally, LY294002 (a specific inhibitor of PI3-kinase) was shown to block L3 activation in vitro, with a cessation of feeding in treated L3s [30,31]. These findings suggested that insulin-like signalling is conserved and plays a critical role in activating L3s of parasitic nematodes during their invasion of the animal host.

The nuclear genomes and transcriptomes of H. contortus [32,33] provide a foundation to begin to explore mechanisms of key molecules involved in developmental processes. However, a lack of effective in vitro-culture and genetic methods for parasitic nematodes has hampered the study of the developmental biology of these nematodes [28,34-36]. In contrast, C. elegans provides a powerful surrogate system to shed light on gene function in parasitic nematodes, such as Ancylostoma caninum [37,38], H. contortus [39,40] and S. stercoralis [41,42].

In the present study, we isolated and characterized the C. elegans age-1 and aap-1 orthologues of H. contortus (Hc-age-1 and Hc-aap-1). Using in vivo and in vitro techniques, we characterized and compared the transcriptional profiles and expression patterns of Hc-age-1 and Hc-aap-1 in different developmental stages of the parasite. To investigate the proposed orthology of Hc-AGE-1 and Ce-AGE-1, we tested whether the adaptor-binding domains of Hc-AGE-1 and Hc-AAP-1 interacted with each other as a heterodimer in a yeast two-hybrid assay, and then assessed function using constructs containing the Ce-age-1 and Hc-age-1 regulatory and coding regions by attempting heterologous rescue of an age-1 mutant strain of C. elegans.

**Methods**

**Ethics statement**

All the experimental animals used were treated strictly in accordance with the recommendations in the Guide for the Regulation for the Administration of Affairs Concerning Experimental Animals of P. R. China. The protocol employed was approved by the Animal Ethics Committee of Hubei Province (permit no. SYXK-0029). The care and maintenance of animals were in accordance with government guidelines.

**Worm strains and their maintenance**

The N2 strain of C. elegans was obtained from the Caenorhabditis Genetics Center (CGC, University of Minnesota, USA) and maintained using standard methods [43]. C. elegans mutant strain CY246 [age-1 (mg44); sqt-1 (sc13)/mntC1 dpy-10 (e128); unc-52 (e444) II], was maintained by picking wild-type phenotypes. The age-1+/F1 progeny of this strain, which are marked in cis with the sqt-1 roller phenotype and exhibit maternal rescue, produce F2 progeny that constitutively form roller daueras [30]. The Hacon-5 strain of H. contortus was maintained in goats (raised helminth-free), which were infected intra-ruminally with 8,000 L3. Eggs, L1s, L2s and L3s were harvested from the faeces from infected goats, as described previously [40]. For the collection of L4s and adults, infected goats were euthanized with an overdose of pentobarbitone sodium (Lethobarb, Virbac) at 8 and 30 days of infection, respectively. These two developmental stages were collected from abomasum at necropsy, washed extensively in physiological saline to remove debris, and male and female worms separated prior to snap freezing in liquid nitrogen and subsequent storage at -80°C.

**DNA and RNA preparation**

Genomic DNA was extracted from mixed-stage C. elegans (N2 strain) using the EasyPure Genomic DNA Kit (TransGen Biotech, China); total RNA was isolated using the TRIZol Plus Purification kit (Life Technologies, USA). Genomic DNA of H. contortus was extracted from cultured L3s, and total RNA was isolated from various stages, including eggs, first-stage larvae (L1), second-stage larvae (L2), infective L3s (iL3s), females and males.
of fourth-stage (L4) and adults using the same kits. RNA integrity and yields were verified by electrophoresis and spectrophotometric analysis (NanoDrop Technologies), respectively. RNA was treated with RNase-Free DNase (Promega, USA). Nucleic acids were frozen and stored at -80°C.

**Isolation of genes and cDNAs**

Guided by transcriptomic and genomic datasets for *H. contortus* [32,33], we isolated full-length *Hc-age-1* and *Hc-aap-1* cDNAs and genes. Coding regions were obtained by 5′- and 3′-rapid amplification of cDNA ends (RACE) (SMARTer RACE cDNA Amplification Kit, Clontech, USA) and cloned separately into the plasmid vector pMD-19 T (Takara, Japan) and sequenced using the primer pairs *Hc-age-1* F/*Hc-age-1*R or *Hc-aap-1* F/*Hc-aap-1*R (Additional file 1). Gene sequences were available from contigs [33], and exon-intron junctions were identified using the program Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Genomic sequences upstream of the genes *Hc-age-1* and *Hc-aap-1* were sequenced as described previously [40].

**Bioinformatic analyses**

Nucleotide sequences were assembled using the program CAP3 (http://seq.cs.iastate.edu/cap3.html) and compared with those in non-redundant databases using the BLAST services/SignalP). Amino acid sequence alignments were carried out using the program Clustal W [45] and adjusted manually. Promoter elements in the 5′-untranslated region (UTR) were predicted using the transcription element search system available at http://www.dna.affrc.go.jp/PLACE/signalscan.html.

Amino acid sequences of *Hc-AGE-1* and *Hc-AAP-1* (determined in this study) and homologous sequences from other invertebrates (nematodes, *Drosophila melanogaster* and *Saccharomyces cerevisiae*) and vertebrates (human and mouse) were used for phylogenetic analyses. Sequences were aligned (as described above), and analyses conducted using the neighbor joining (NI), maximum parsimony (MP) and maximum likelihood (ML) methods, based on the Jones-Taylor-Thornton (JTT) model [46]. Confidence limits were assessed using 1000 pseudo-replicates for NJ, MP and ML trees, and other settings were obtained using the default values using the program MEGA v.5.0 [46]. A 50% cut-off value was implemented for the consensus tree.

**Transcript abundance based on RNA-seq analysis**

Transcriptomic data for different developmental stages of *H. contortus* (Haecon 5 strain, Australia), including eggs, L1s, L2s, L3s, L4 males, L4 females, adult males and adult females, were generated previously by RNA-seq [33] and made publicly available. The abundances of relevant transcripts were established as described previously [33].

**Construction of plasmids**

Two constructs *Ce-age-1p::Ce-age-1* (102 bp)::*gfp::Ce-age-1* t and *Hc-age-1p::Hc-age-1* (45 bp)::*gfp::Ce-age-1* t (designated pl-Cagep and pl-Hagep; Additional file 2) were made. Briefly, the 1,480 bp region 5′ to *Ce-age-1* and 1,900 bp region 5′ to *Hc-age-1* were each PCR-amplified from *C. elegans* and *H. contortus* genomic DNAs, respectively. The *gfp*-coding region, with introns, was amplified from the pPD95.75 vector, and the *Ce-age-1* t sequence was amplified from *C. elegans* genomic DNA. Then, the different elements were fused by overlap-extension PCR [30,47] employing primer pairs *Ce-age-gfp-F/Ce-age-gfp-R* or *Hc-age-gfp-F/Hc-age-gfp-R* (Additional file 1). The PCR products were cloned into the pGEM-T-Easy vector (Promega) and sequenced.

Using a similar strategy, two plasmid constructs *Ce-aap-1p 1515 bp::Ce-aap-1* (24 bp)::*gfp::Ce-unc-54 t* and *Hc-aap-1p 2524 bp::Hc-aap-1* (36 bp)::*gfp::Ce-unc-54 t* (designated pl-Caapp and pl-Haapp) containing the promoters, the *gfp* reporter and *Ce-unc-54 t* (terminator) were made. In brief, the 1,515 bp region 5′ to *Ce-aap-1* and 2,524 bp region 5′ to *Hc-aap-1* were PCR-amplified from *C. elegans* and *H. contortus* genomic DNAs, respectively. The *gfp* and *Ce-unc-54 t* fragments were amplified from the pPD95.75 vector. Then, the promoter, *gfp* and terminator were fused by overlap-extension PCR using primer pair *Ce-aap-gfp-F/Ce-aap-gfp-R* or *Hc-aap-gfp-F/Hc-aap-gfp-R* (Additional file 1). Finally, the amplicons were cloned into pGEM-T-Easy and sequenced.

For the gene rescue assay, the constructs *Ce-age-1p::Ce-age-1* (3,549 bp)::*Ce-unc-54 t* (designated pl-Cage1), *Ce-age-1p::Hc-age-1* (3,471 bp)::*Ce-unc-54 t* (designated pl-Hage1) and *Ce-aap-1p::Hc-aap-1* (1,284 bp)::*Ce-unc-54 t* (designated pl-Haap1) were made (Additional file 2). The inferred promoters of *Ce-age-1* (1,275 bp) and *Ce-aap-1* (1,515 bp) were amplified from genomic DNA of *C. elegans* and sequenced; *Ce-unc-54 t* was from vector.
pPV238; the coding sequences of Ce-age-1, Hc-age-1 & Hc-aap-1 were amplified from C. elegans and H. contortus cDNAs, respectively. Then, three fragments, including the Ce-age-1 promoter, Ce-age-1 coding sequence and Ce-unc-54 t were fused to create pL-Cage1 by overlap-extension PCR. The pL-Hage1 plasmid was made from the C. elegans vector pPV238. Briefly, pPV238 was cut at the PstI and BstZ171 restriction sites, and the 5’ region of Ce-age-1 added, with the MluI restriction site upstream of BstZ171 being inserted. Then, the Hc-age-1 coding region with MluI and BstZ171 was inserted to create pL-Hage1 using the primer pairs Ce-age-pstF/Ce-age-mlbstR and Hc-age-mluF/Hc-age-bstzR (Additional file 1). Similarly, vector pPV199 was cut at the endonuclease sites BamHI, AgeI or MluI, and the Ce-aap-1 promoter and Hc-aap-1 coding regions were inserted, in turn, to create the pL-Haap1 using the PCR primer pairs Ce-aap-phamF/Ce-aap-pageR and Hc-aap-ageF/Hc-aap-mluR, respectively (Additional file 1).

**Yeast two-hybrid assay**

A yeast two-hybrid system (Gal4-based Matchmaker Gold, Clontech) was used to test interactions between the adaptor-binding domain of Hc-AGE-1 and Hc-AAP-1 [48,49]. Translational fusion to the Gal4-activation domain (AD) was conducted as follows: a full-length Hc-aap-1 cDNA was PCR-amplified from H. contortus using the primer pair Hc-aap-NdeF/Hc-aap-BamR (Additional file 1) and cloned into the vector pGADT7 via the Ndel and BamHI restriction sites. To construct a translational fusion to the Gal4-binding domain (BD), a cDNA fragment encoding the adaptor-binding domain of Hc-Age-1 PCR-amplified from H. contortus using primer pair Hc-age-NdeF/Hc-age-BamR was cloned into the vector pGBK7 via Ndel and BamHI sites.

The constructs fused to Hc-aap-1 or Hc-age-1 were sequenced to verify their reading frames and then transformed into yeast strains Y2HGold and Y187. Interaction trap analysis of diploids, resulting from the mating of Y2HGold and Y187 strains, was carried out using the manufacturer’s protocol (Clontech) employing minimal medium agar plates lacking leucine, tryptophan (plasmid selection marker) and either histidine (low stringency for protein-protein interaction), histidine (in the presence of 1.5 μM 3-amino-triazole; medium stringency) or histidine plus adenine (high stringency). Colonies were assessed after 5 days of incubation at 30°C. The yeast-two hybrid assay was repeated twice.

**Transformation of C. elegans**

The N2 or CY246 (wild-type) strain of adult hermaphrodites of C. elegans were transformed using a gonad microinjection method [50]. In brief, to study the expression patterns of Ce-age-1, Hc-age-1, Hc-aap-1 and Ce-aap-1 promoters, each of the constructs was microinjected with 20 ng/μl for pL-Cage1, pL-Hage1, pL-Caapp or pL-Haapp, together with 80 ng/μl PRF4 containing the rol-6 marker gene. Injected worms were transferred to nematode growth medium (NGM) plates with Escherichia coli (OP50) lawns and incubated at 20°C. Transformed worms were identified among F1 progeny based on a “right-roller” and the green fluorescence protein (GFP) phenotype. Then, transformants were anaesthetized using 10 mM levamisole and immobilized on a 2% agar pad, and examined using a stereoscopic and compound fluorescence microscope, equipped with differential interference contrast optics (DIC) and a camera (Olympus BX51, Japan).

**Gene rescue assay in the mutant strain CY246 of C. elegans**

To assess the ability of Hc-age-1 to rescue age-1 in C. elegans (mutant strain CY246), three plasmids (pL-Cage1, pL-Hage1 and pL-Haap1; Additional file 2) were constructed. Each of the plasmids was injected with 20 ng/μl of test plasmid and co-injected with 2 ng/μl of the marker Ce-myo-2 p::mCherry:C.e-unc-54 t (pCF90) and 78 ng/μl of pUC19. Additionally, worms were injected with the same amount of the marker pCF90 and 98 ng/μl of pUC19 alone, as controls. Injected worms were transferred to NGM + OP50 plates, and transformants identified by fluorescence microscopy. Lines were established from single F2 transformants. The pL-Cage1 and pL-Hage1 lines were assessed for gene rescue based on the presence of progeny from F1 rollers and the ability of F2 rollers to passage for ≥10 generations.

**Results**

Characterization of cDNAs and phylogenetic analysis of amino acid sequence data

The Hc-age-1 cDNA was 3,471 bp in length and encoded a protein (Hc-AGE-1) of 1,156 amino acids. Hc-Age-1 had 31-44% amino acid sequence similarity to homologues from Ascaris suum, C. elegans, Loa loa, Parastrongyloides trichosuri and S. stercoralis as well as Homo sapiens and Mus musculus. Hc-Age-1 consists of five conserved domains, including an adaptor-binding domain proposed to interact with a regulatory subunit (a Ras-binding domain that mediates activation by the small GTPase Ras) and the C2-like, phosphatidylinositol kinase and catalytic domains (Figure 1a). By contrast, the Hc-aap-1 cDNA was 1,284 bp in length and encoded a protein (Hc-AAP-1) of 427 amino acids. Hc-AAP-1 had 29-40% similarity to homologues from Brugia malayi, C. breneri, C. elegans and L. loa. The Hc-AAP-1 sequence included N-terminal SH2 (Src-homolog 2), C-terminal SH2 domains and an inter-SH2 domain required for binding to the catalytic subunit [51] (Figure 1b).
The *Hc-AGE-1* sequence was aligned with 14 AGE-1 homologues (from six nematodes and three eukaryotes) and subjected to phylogenetic analyses (Figure 2a). There was concordance in topology among the MP, ML and NJ trees, which all displayed three strongly supported clusters representing classes I, II and III, respectively. *Hc-AGE-1* had the closest relationship to class I homologs from *Caenorhabditis elegans* and *C. briggsae*. The predicted amino acid sequence of *Hc-AAP-1* was aligned with ten AAP-1 homologues (from eight nematodes and two other metazoans) and subjected to phylogenetic analyses (Figure 2b). Again, there was concordance in topology among the NJ, MP and ML trees which showed that *Hc-AAP-1* has its closest relationship with *Caenorhabditis elegans*. The *AAP-1* s from the latter three species grouping together with absolute nodal support (100%). Interestingly, AAP-1 homologues (from eight nematodes and two other metazoans) and subjected to phylogenetic analyses (Figure 2b). Again, there was concordance in topology among the NJ, MP and ML trees which showed that *AAP-1* has its closest relationship with *Caenorhabditis elegans*. The *AAP-1* s from the latter three species grouping together with absolute nodal support (100%). Interestingly, AAP-1 homologues (from eight nematodes and two other metazoans) and subjected to phylogenetic analyses (Figure 2b).

**Genomic organization and putative promoter elements**

We located the full-length genes of *Hc-age-1* and *Hc-aap-1* in the *H. contortus* genome [33]. The *Hc-age-1* gene was 12,896 bp in length and had 29 exons (75-198 bp); the *Hc-aap-1* gene was 6,172 bp in length and contained 11 exons (69-179 bp) (Figure 3). All exon/intron boundaries abided by the GT-AG rule in *Hc-aap-1* (Additional file 3), with the exception of the first and fifth exons in *Hc-age-1* (Additional file 4). *Hc-age-1* and *Hc-aap-1* had more exons and introns compared with orthologous genes in *C. elegans* [12,13] and *S. stercoralis* [30]. The promoter regions predicted for *Hc-age-1* and *Hc-aap-1* were 1,900 bp and 2,524 bp in length, respectively. In the promoter regions of both genes, E- (CANNTG), inverse GATA (TTATC), inverse CAAT (ATTTG), CAAT (CCAAT) and TATA boxes were identified.

**Transcriptional profiles in *H. contortus*, and localization of expression in transgenic *C. elegans**

*Hc-age-1* and *Hc-aap-1* were transcribed in all developmental stages of *H. contortus*. Highest transcription was measured in eggs, L3s and female adults (*Hc-age-1*), or eggs, L3s, female L4s and female adults (*Hc-aap-1*) (Figure 4). Expression was localized in *C. elegans* (N2) transformed with plasmid pL-Hagep containing the 1,900 bp-predicted promoter region of *Hc-age-1* or plasmid pL-Haap containing the 2,524 bp-predicted promoter region of *Hc-aap-1*, using pRF4 as a marker. In addition, *C. elegans* (N2) transformed with *gfp* fused to each *Ce-age-1* and *Ce-aap-1* constructs were used as controls. The transgenic lines were screened for the roller phenotype and *gfp* expression. *GFP* expression driven by the *Ce-age-1* promoter was detected in amphidial neurons and the intestine, consistent with previous reports for *S. stercoralis* [30]. GFP expression driven by the *Hc-age-1* promoter was detected in the entire intestine at L1 and L2 stages, but concentrated in the anterior intestine of the L3 stage (Figure 5c-f). Similarly, GFP expression driven by the *Ce-aap-1* promoter was observed in intestine and amphidial/phasmidial neurons (Figure 5g-h) in accordance with previous report [13]. The expression of *Hc-aap-1* was also predominantly present in anterior intestine, which is highly analogous to that of *Hc-age-1* (Figure 5i-j). Despite some variation in expression among individual larvae, *C. elegans* containing *Ce-age-1 p:gfp* and *Ce-aap-1 p:gfp* showed a similar expression profile (amphidial/head neurons and intestine), as did *C. elegans* containing *Hc-age-1 p:gfp* and *Hc-aap-1 p:gfp* (anterior intestine). Based on their apparent co-location, *Hc-AGE-1* and *Hc-AAP-1* might interact as a heterodimer [52].

**Assessing the interaction of AAP-1 with AGE-1**

In multicellular organisms, binding by the adaptor subunit (*Ce-AAP-1*) can stabilize and activate the PI3K catalytic
subunit (Ce-AGE-1) [11,13,51]. The interaction of these two subunits is governed by the NH2 terminus of the catalytic subunit and the inter-SH2 domain of the adaptor subunit [51]. To assess whether there was an interaction between Hc-aap-1 and the adaptor-binding domain of Hc-AGE-1, several fusion constructs in the vectors pGADT7 and pGBKT7, respectively, were prepared for analysis in the yeast two-hybrid system. Co-expression of the full-length gene Hc-aap-1 fused to pGADT7 (GAL4 AD-Hc-AAP-1) with the adaptor-binding domain of Hc-age-1 fused to pGBK7 (GAL4 BD-Hc-AGE-1) resulted in high β-galactosidase expression activity (Additional file 5). The co-expression of pGADT7 or pGBK7 fused to the large T-antigen or murine p53 (pGADT7-T and pGBK7-T53, respectively; positive controls) displayed β-galactosidase expression (Additional file 5). In contrast, the co-expression of pGADT7-Hc-aap-1 with pGBK7-T53 or pGBK7-Hc-age-1 with pGADT7-T

Figure 2 Neighbor joining trees showing the relationship of Haemonchus contortus phosphoinositide 3-kinase (PI3K) catalytic subunit Hc-AGE-1 and regulatory subunit Hc-AAP-1. The trees were calculated using the Jones-Taylor-Thornton (JTT) model in the MEGA program version 5.0. Bootstrap values above or below the branches (1,000 iterations) are shown for robust clades (>50 %). (a) The species used in the analysis include six nematodes (Strongyloides stercoralis, Ss-AGE-1; Parascaris unicaudata, Pu-AGE-1; Brugia malayi, Bm-AGE-1; Loa loa, Li-AGE-1; Caenorhabditis elegans, Ce-AGE-1; Caenorhabditis briggsae, Cb-AGE-1; and three non-nematodes (Drosophila melanogaster, Dm-PI3K92E and Dm-PI3K59F; Homo sapiens, Hs-PI3KCA, Hs-PI3KC2 and Hs-PI3KC3; Saccharomyces cerevisiae, Sc-PI3K34)). Mus musculus EGFR AAA17899 was used as an outgroup. (b) AAP-1 used in the analysis included Caenorhabditis elegans, Ce-AAP-1; Caenorhabditis briggsae, Cb-AAP-1; Caenorhabditis remani, Cr-AAP-1; Strongyloides stercoralis, Ss-AAP-1; Ascaris suum, As-AAP-1; Brugia malayi, Bm-AAP-1; Loa loa, Li-AAP-1; Trichinella spiralis, Ts-AAP-1; Drosophila melanogaster, Dm-P60; Homo sapiens, Hs-PI3K85). C. elegans Ce-LET-23 CAA93882 was used as an outgroup. GenBank accession numbers are listed on the right of each species.
did not show such expression (Additional file 5). These findings indicated that the adaptor-binding domain of Hc-AGE-1 was capable of interacting with Hc-AAP-1 in the yeast two-hybrid system.

Attempts of cross-species complementation in C. elegans CY246 with Hc-age-1 and Hc-aap-1

C. elegans mutant strain CY246 [age-1 (mg44) mutant] could be fully rescued by the homologous gene in pL-Cage1 (Additional file 2). Rescue was evidenced based on the presence of F1 roller progeny, and F2 rollers were passaged for ≥10 generations. This result suggests that the main regulatory and coding regions are functional in age-1 (mg44) mutants, thus providing a reliable basis for comparative studies of Hc-AGE-1.

To attempt to establish the functional characteristics of the Hc-age-1 gene, the construct comprising the Ce-age-1 promoter and the Hc-age-1 coding region (pL-Hage1, Additional file 2) was transformed into the CY246 mutant strain. Hc-age-1 mRNA was detected in all lines of the CY246 strain transformed with the rescuing construct. Three lines were screened, and gene rescue was attempted. However, no individuals from the progeny of age-1−/− C. elegans transformed with pL-Hage1 were rescued.

Although the Ce-age-1 promoter was functional in the positive control, Hc-age-1 did not rescue the mutant strain. A possible explanation is that the heterodimer was processed incorrectly, either by the thermally labile AGE-1 subunit or by the inhibition of catalytic activity by a conformational disorder. To address this proposal, the plasmid (pL-Haap1) containing the Hc-aap-1 coding sequence, under the control of the Ce-aap-1 promoter, was constructed (Additional file 2). Although Hc-AAP-1 conferred a strong interaction with the Hc-AGE-1 catalytic subunit in a yeast-two hybrid assay, the transgenic
lines transformed with both the pL-Haap1 and pL-Hage1 constructs did not rescue *C. elegans* age-1 (mg44) mutants.

**Discussion**

In *C. elegans*, signalling through the phosphoinositide 3-kinases heterodimer is a key regulator of the switch from dauer to continuous development [5,13]. PI3Ks, which are relatively conserved enzymes from unicellular eukaryotes to multicellular organisms, play important roles in intracellular trafficking, cell metabolism, differentiation and/or survival [53-56]. Recently, components of insulin-like signalling have been identified in parasitic nematodes, including *A. caninum* [37,38], *H. contortus* [39,40] and *S. stercoralis* [30,41,42,57], suggesting that similar signalling is utilized by both free-living and parasitic nematodes. In the present study, two PI3K genes were identified in *H. contortus* using available data [33], including a *Hc-age-1* encoding a p110 catalytic subunit and a *Hc-aap-1* encoding a p50/p55-like regulatory subunit, consistent with those in *C. elegans* and *S. stercoralis* [12,13,30].

Sequence and structural analyses revealed that *Hc*-AGE-1 and *Hc*-AAP-1 are relatively conserved among *H. contortus, C. elegans* and other nematodes. *Hc*-AGE-1 has representative structural domains, including an N-terminal adaptor-binding domain, inferred to interact with the adaptor regulatory subunit, a Ras-binding domain predicted to regulate activation by the small GTPase Ras, a C2 domain, a phosphatidylinositol kinase homology (PIK) domain and a C-terminal catalytic domain (cf. Figure 1). *Hc*-AAP-1 has a common structural domain possessing an inter-SH2 domain, flanked by both Src-homology 2 domains (SH2) and necessary for binding to the catalytic subunit (cf. Figure 1). In bovine PI3K, although the inter-SH2 of the regulatory subunit confers a strong interaction with the catalytic subunit, more than one SH2 domain, in combination with inter-SH2 domain, are required for the conformational changes required to activate the p110 subunit through phosphotyrosine peptide binding [51]. Hence, it was reasonable to hypothesize that the *Hc*-AAP-1 regulatory subunit can bind the *Hc*-AGE-1 catalytic subunit to assemble the PI3K heterodimer. This hypothesis was tested using the yeast two-hybrid assay, revealing that the adaptor-binding domain of *Hc*-AGE-1 readily and strongly interacts with the *Hc*-AAP-1 regulatory subunit, analogous to orthologous molecules in *C. elegans* and *D. melanogaster* [13,58]. Phylogenetic analysis of inferred amino acid sequences showed that *Hc*-AGE-1 and *Hc*-AAP-1 grouped with the orthologues *Ce*-AGE-1 and *Ch*-AGE-1 and *Cr*-AAP-1 (free-living nematodes), respectively. In addition, AGE-1 and AAP-1 orthologues are present in various parasitic...
nematodes, including *A. suum*, *B. malayi* and *S. stercoralis*. Therefore, these findings suggest that the Hc-AGE-1/Hc-AAP-1 PI3 heteromer kinase is generally conserved and might, thus, have similar functional characteristics in various nematodes.

Some genes are transcriptionally regulated during the key transition associated with the substantial growth and development in nematodes, such as the switch from the free-living to parasitic stages for parasitic nematodes and the transition from the arrested to the developmentally active state in *C. elegans* [30,33]. Overall, an assessment of transcription showed that Hc-age-1 and Hc-aap-1 were transcribed at a low level throughout the life cycle of *H. contortus*, like Ce-age-1 and Ss-age-1, which is in accordance with the hypothesis that the insulin-like signalling is regulated post translationally by AGE-1, rather than at the transcriptional level. Furthermore, the Hc-age-1 and Hc-aap-1 were transcriptionally up-regulated in eggs, infective L3s and female adults of *H. contortus*, which suggests the PI3K might be involved in nematode development and/or reproduction. Significantly, up-regulation in iL3, which is akin to a developmentally arrested stage, and has a significantly reduced metabolic rate and transcription [33], hints to PI3K having a functional role in recovery from developmental arrest.

The expression of Hc-age-1 was mainly localised to the intestine in the larval stages of *C. elegans*, which is consistent with the expression pattern of the Ce-age-1, gleaned from high throughput analyses in *C. elegans* (see WormAtlas: http://gfpweb.aecom.yu.edu/strain?name=BC10837), where the Ce-age-1 promoter:gfp-fusion construct is expressed in the intestine and amphidial/head neurons. Similarly, the Hc-aap-1 promoter:gfp-fusion construct was concentrated in the anterior intestine, which

![Figure 5 Expression patterns of Hc-age-1 and Hc-aap-1.](image-url)
is in accord with the Ce-aap-1 promoter [13], where the expression of Ce-aap-1 promoter: GFP-fusion plasmid was in intestine and head/tail neurons. The expression of Hc-age-1 and Hc-aap-1 showed a strong spatio-temporal relationship with a promoter-promoter interaction [52], which may indicate a functional correlation. Indeed, Hc-AGE-1 and Hc-AAP-1 can bind tightly to form a PI3K heterodimer, as confirmed by yeast two-hybrid analysis and as predicted also for a multicellular organism, including bovine, C. elegans and mouse [13,51,59]. To some extent, the heterologous expression pattern might enable us to mine for protein-protein interaction information by determining in which tissues or cells these proteins are co-expressed. In comparison with C. elegans, the expression of Hc-age-1 and Hc-aap-1 was mainly concentrated in the intestine rather than the nervous systems, which might suggest that PI3K plays a crucial role in feeding in parasitic nematodes. Thus, like in A. ceylanicum, A. caninum and S. stercoralis, the PI3K specific inhibitor LY294002 inhibits the resumption of feeding [30,31].

Based on our findings, we suggest that Hc-age-1 is a functional orthologue of Ce-age-1, and could be activated by upstream growth factor receptor tyrosine kinases (RTKs), and negatively regulates the Foxo-class transcription factor DAF-16. To test this proposal, heterologous genetic complementation was applied in C. elegans. The C. elegans mutant strain CY246 was fully rescued by the Ce-age-1 construct, indicating that the Ce-age-1 promoter can direct suitable expression and appropriate heterodimer binding to enable signalling. However, rescue was not achieved when the Hc-age-1 construct was introduced into the CY246 strain. A possible explanation is that the exogenous Hc-AGE-1 subunit cannot bind the endogenous Ce-AAP-1 subunit in vivo to constitute the functional PI3K. It is proposed that Hc-age-1 and Hc-aap-1 bind to each other to constitute a functional PI3K in transgenic C. elegans, rather than a single thermally labile AGE-1 subunit [59]. However, we were unable to detect any transgenic lines (transformed with two plasmids containing Hc-age-1 and Hc-aap-1 together) that could be passaged.

In strongyloid nematodes, the resumption of feeding has been used as a phenotypic marker in in vitro-activation assays [30,31,60,61], and the PI3 kinase-specific inhibitor LY294002 effectively blocks the resumption of feeding of exsheathed L3s [30,31], which infers that PI3K plays a key role in the activation of L3s of parasitic nematodes, as it does the exit from dauer in C. elegans. Regarding the failure of Hc-age-1 to complement the C. elegans age-1 (mg44) mutant strain, a possible explanation is that the proposed orthologues of Hc-age-1 and Hc-aap-1 are unable to assemble the functional heterodimer and/or attain their native conformation, because of a lack of molecular chaperones, such as calnexin and calreticulin [62,63], even though they bind tightly to each other in the yeast two-hybrid system. Additionally, the evolution of gene regulation might relate to variation in the temporal and spatial control of gene expression pattern in tissues [64]. We observed that the expression patterns of Hc-age-1 and Hc-aap-1 tend to be in the intestine of C. elegans compared with those of Ce-age-1 and Ce-aap-1, with expression mainly in head neurons. This difference might suggest some functional variation of PI3K between free-living and parasitic nematodes.

The “dauer hypothesis” proposes that similar molecular signalling pathways control the entry into and exit from arrested development in C. elegans and in parasitic nematodes [65,66]. Heterologous complementation showed that the main components of insulin-like signalling, includingdaf-16, daf-2, age-1 and daf-12 in parasitic nematodes [37,39-41,67], have similar functions to C. elegans orthologues. However, it is, nonetheless, interesting to note that the roles of PI3K from H. contortus appear to be divergent. Determining the lipid and protein kinase activities of PI3K in H. contortus might help establish whether PI3Ks or PI3K signalling is conserved or not. Some new heritable, transgenic elements or tools applied in the “model nematodes” S. stercoralis and P. trichosuri, including piggyback [68], might help address this and other questions relating to the insulin-like signalling pathway.

Conclusions

In conclusion, we investigated the PI3K-encoding genes Hc-age-1 and Hc-aap-1 in the parasitic nematode H. contortus. We characterized their cDNAs, genomic DNAs and promoter sequences, and determined their transcription profiles in various developmental stages. Then, we confirmed the interaction of Hc-age-1 and Hc-aap-1 by yeast two-hybrid analysis. Finally, we predicted function by gene expression location and complementation in heterologous C. elegans. Taken together, the present results provide important insights into the characteristics of PI3K in a strongyloid nematode, particularly in relation to developmental processes.

Additional files

Additional file 1: Primers used to isolate Hc-age-1 and Hc-aap-1 genes of Haemonchus contortus and to make constructs for green fluorescent protein (GFP) localization in Caenorhabditis elegans and the heterologous genetic complementation assay.

Additional file 2: Cloning strategy for reporter and rescuing constructs. The constructs containing the Caenorhabditis elegans Ce-age-1 and Ce-aap-1 promoters (pl-Cagep and pl-Caapp) and the Haemonchus contortus Hc-age-1 and Hc-aap-1 promoters (pl-Hagep and pl-Haapp) were made based on pPD95.75 by the overlapping extension PCR. The rescuing constructs (pl-Cagep, pl-Hagep and pl-Haapp) containing the Ce-age-1, Hc-age-1 and Hc-aap-1 coding regions, respectively, were made by removing the gfp coding sequence from pPD238 or pPV199 (cf. S7) and linking the appropriate cDNA, A, B, M and P represent the restriction sites for AgeI, BstZ17I, MluI and PstI, respectively.
Additional file 3: The length of exon and intron and splice donor sequences for each exon and intron of Hc-aap-1 gene. The dinucleotide consensus sequence at the splice site is italicized and underlined. All nucleotide sequences are 5’ to 3’.

Additional file 4: The length of exon and intron and splice donor sequences for each exon and intron of Hc-age-1 gene. The dinucleotide consensus sequence at the splice site is italicized and underlined. All nucleotide sequences are 5’ to 3’.

Additional file 5: The adaptor-binding domain of Hc-AGE-1 interacted with Hc-AAP-1 in a yeast two-hybrid assay. (A) The positive control showed that pGBK7-T3 can interact with pGADT7-T; (B) The negative control showed that pGBK7-T7-Lam and pGADT7-T7 did not interact; (C) The test group pGBK7-Hc-AGE-1 and pGADT7-Hc-aap-1 did interact with QDO/X/A; (D) pGBK7-T7-Lam and pGADT7-Hc-aap-1 did not interact with QDO/X/A; (E) pGBK7-Hc-age-1 and pGADT7-T7 did not interact with QDO/X/A.

Abbreviations
DIC: Differential interference contrast optics; GFP: Green fluorescence protein; JTT: Jones-Taylor-Thornton model; L3s: The third-stage larvae; ML: Maximum likelihood; MP: Maximum parsimony; NGS: Nematode growth medium; NJ: Neighbour-joining; PCR: Polymerase chain reaction; RACE: Rapid amplification of cDNA ends; UTR: Untranslated region.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MH, JBL and FCL conceived the project. FCL and YFW carried out laboratory materials/analysis tools. FCL, RBG, JBL and MH wrote the manuscript, and all authors read and approved the final manuscript.

Acknowledgements
This study was supported by ‘National Natural Science Foundation of China (NSFC)’ (Grant no. 31173210), the National Key Basic Research Program (973 program) of China (Grant No. 2015CB151030) and ‘Special Fund for Agro-scientific Research in the Public Interest, China’ (Grant no. 201303037) to MH, by a grant from ‘The National Institutes of Health (NIH), USA (AI-50191) to JBL and by funds from the National Health and Medical Research Council (NHMRC) and the Australian Research Council (ARC) of Australia to RBG. This study was also supported by a Victorian Life Sciences Computation Initiative (VLSCI), Australia, grant number VR00077 on its Peak Computing Facility at the University of Melbourne, an initiative of the Victorian Government, Australia (RBG).

Author details
1 State Key Laboratory of Agricultural Microbiology, Key Laboratory of Development of Veterinary Diagnostic Products, Ministry of Agriculture, College of Veterinary Medicine, Huazhong Agricultural University, 1 Shizishan Street, Wuhan 430070, Hubei, China. 2 Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Corner of Flemington Road and Park Drive, Parkville, VIC 3010, Australia. 3 Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, 3800 Spruce Street, Philadelphia PA 19104, USA.

Received: 5 September 2014 Accepted: 21 October 2014
Published online: 12 November 2014

References
1. Whitman M, Downes CP, Keeler M, Keller T, Cantley L: Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. Nature 1988, 338:454–466.
2. Downes CP, Carter AN: Phosphoinoside 3-kinase: a new effector in signal transduction? Cell Signal 1991, 3:501–513.
3. Vanhaesebroeck B, Waterfield MD: Signalling by distinct classes of phosphoinoside 3-kinases. Exp Cell Res 1999, 253:239–254.
4. Hawkins PT, Anderson KE, Davidson K, Stephens LR: Signalling through class I PI3Ks in mammalian cells. Biochem Soc Trans 2006, 34:647–662.
5. Friedman DB, Johnson TE: A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics 1988, 118:75–86.
6. Kenyon C, Chang J, Gems D, Rudner A, Tabtiang R: A C. elegans mutant that lives twice as long as wild type. Nature 1993, 366:461–464.
7. Oldham S, Hafen E: Insulin/GF and target of rapamycin signaling: a TOR de force in growth control. Trends Cell Biol 2003, 13:79–85.
8. Brachmann SM, Ueki K, Engelman JA, Kahn RC, Cantley LC: Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. Mol Cell Biol 2005, 25:1596–1607.
9. Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B: Critical role for the p110 alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. Nature 2006, 441:366–370.
10. Stein RC, Waterfield MD: PI3-kinase inhibition: a target for drug development? Mol Med Today 2000, 6:347–357.
11. Engelman JA, Luo J, Cantley LC: The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006, 7:606–619.
12. Morris JZ, Tissenbaum HA, Ruvkun G: A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. Nature 1996, 382:536–539.
13. Wolkow CA, Munoz MJ, Riddle DL, Ruvkun G: Insulin receptor substrate and p55 orthologous adaptor proteins function in the Caenorhabditis elegansdaf-2/Insulin-like signaling pathway. J Biol Chem 2002, 277:49591–49597.
14. Vanhaesebroeck B, Leefers SJ, Paraytou G, Waterfield MD: Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem Sci 1997, 22:667–272.
15. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bal-3. Curr Biol 1997, 7:261–269.
16. Afoldi J, Kenyon C: Cell nonautonomy of C. elegansdaf-2function in the regulation of diapause and life span. Cell 1998, 95:199–210.
17. GEMS D, Sutton AJ, Sundermeyer ML, Albert PS, King KV, Edgley ML, Larsen PL, Riddle DL: Two pleiotropic classes ofdaf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. Genetics 1997, 147:928–942.
18. Aleksi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bal-3. Curr Biol 1997, 7:261–269.
19. Paradis S, Allon M, Toker A, Thomas JH, Ruvkun G: A PKD1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. Science 1997, 277:942–946.
20. Paradis S, Ruvkun G: Caenorhabditis elegansAkt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev 1998, 12:2488–2498.
21. Lin K, Dorman JB, Rodan A, Kenyon C:daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. Science 1997, 278:1319–1322.
22. Ogg S, Paradis S, Gottlieb S, Patterson G, Lee J, Tissenbaum HA, Ruvkun G: The forkhead transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature 1997, 389:994–999.
23. Ogg S, Ruvkun G: The C. elegansPTEnormal morpholino, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol Cell 1998, 2:887–893.
24. Yan K, Narasimhan SD, Tissenbaum HA: DAF-16/Forkhead box D transcription factor: many paths to a single fork (head) in the road. Antioxid Redox Signal 2011, 14:523–634.
25. Mukhopadhyay A, Oh SW, Tissenbaum HA: Worming pathways to and from DAF-16/FOXO. Exp Gerontol 2006, 41:928–934.
26. Blaxter ML: Caenorhabditis elegans is a nematode. Science 1998, 282:2041–2046.
27. Hashmi S, Tawe W, Lustgarten S: Caenorhabditis elegans and the study of gene function in parasites. Trends Parasitol 2001, 17:387–393.
28. Gillette JS: The use of Caenorhabditis elegans in parasitic nematode research. Parasitology 2004, 128:549–570.
29. Britton C, Murray L: Using Caenorhabditis elegans for functional analysis of genes of parasitic nematodes. Int J Parasitol 2006, 36:565–569.
30. Stoltzfus JD, Massey HC Jr, Nolan TJ, Griffith SD, Lok JB: Strongyloides stercoralis age-1: a potential regulator of infective larval development in a parasitic nematode. PLoS One 2012, 7:e38587.

31. Brand A, Hawdon JM. Phosphoinositide-3-OH kinase inhibitor LY294002 prevents activation of Anclyostoma caninum and Anclyostoma ceylonicum third-stage infective larvae. Int J Parasitol 2004, 34:909–914.

32. Laing R, Kiku-T, Martinek A, Tsa T, Beech RN, Redman E, Holroyd N, Bartley DJ, Beasley H, Britton C, Currant D, Devaney E, Gilabert A, Hunt M, Jackson F, Johnston SI, Kryouk L, Li K, Morrison AA, Reid Al, Sargisson N, Saunders G, Wasmuth JD, Wolfstanholme A, Beniman M, Gilelra J, Cotton JA: The genome and transcriptome of Haemonchus contortus, a key model parasite for drug and vaccine discovery. Genome Biol 2013, 14:888.

33. Schwartz EM, Korhonen PK, Campbell BE, Young ND, Jex AR, Jarabo A, Hall RS, Mondal A, Howe AC, Piel J, Hofmann A, Boag PR, Zhu XQ, Gregory T, Loukas A, Williams BA, Antoshechkin I, Brown C, Stemberg PW, Gasser RB: The genome and developmental transcriptome of the strongyloid nematode Haemonchus contortus. Genome Biol 2013, 14:869.

34. Kalinna BH, Brindley PJ: Phosphoinositide-3-OH-kinase inhibitor LY294002 on RNAi in nematode parasites. Trends Parasitol 2011, 27:505–513.

35. Li F, Lok JB, Gasser RB, Korhonen PK, Sandeman MR, Shi D, Zhou R, Li X, Massey HC Jr, Ranjit N, Stoltzfus JD, Lok JB: Strongyloides stercoralis daf-2 encodes a divergent ortholog of Caenorhabditis elegans daf-2 and restores partial dauer regulation. Int J Parasitol 2013, 43:515–520.

36. Weinkove D, Leevens SJ, MacDougall UK, Waterfield MD: p50 is an adaptor for the Drosophila phosphoinositide-3-kinase, Dplp. J Biol Chem 1997, 272:14606–14610.

37. Geering B, Cutillas PR, Nock G, Gharbi S, Vanhoosebroek B: Class I phosphoinositide-3-kinases are obligate p85-p110 heterodimers. Proc Natl Acad Sci U S A 2007, 104:7809–7814.

38. Hawdon JM, Volk SW, Rose R, Pritchard DJ, Beech RN, Redman E, Holroyd N, Timms J, Waterfield MD: Cellular function of phosphoinositide-3-kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 2001, 17:651–675.

39. Massey HC Jr, Ranjit N, Stoltzfus JD, Lok JB: Strongyloides stercoralis daf-2 encodes a divergent ortholog of Caenorhabditis elegans daf-2. Int J Parasitol 2013, 43:515–520.

40. Rameh LE, Cantley LC: The role of phosphoinositide-3-kinase lipid products in cell function. J Biol Chem 1999, 274:8347–8350.

41. Konko O, Okkenhaug K, Ahmadi K, White S, Timms J, Timms J, Waterfield MD: Phosphoinositide-3-kinase signalling in breast cancer: how big a role might it play? Breast Cancer Res 2003, 5:304–312.

42. Keating ME, Young ND, Jex AR, Jarabo A, Hall RS, Mondal A, Howe AC, Piel J, Hofmann A, Boag PR, Zhu XQ, Gregory T, Loukas A, Williams BA, Antoshechkin I, Brown C, Stemberg PW, Gasser RB: The genome and developmental transcriptome of the strongyloid nematode Haemonchus contortus. Genome Biol 2013, 14:869.

43. Brehm K: Identification and molecular characterization of a gene encoding a member of the insulin receptor family in <i>Haemonchus contortus</i>. Int J Parasitol 2003, 33:131–144.

44. Khayath N, Vicogne J, Ahier A, BenYounes A, Konrad C, Trolet J, Viscogliosi E, Brehm K, Diissous C: Diversification of the insulin receptor family in the helminth parasite <i>Schistosoma mansoni</i>. FEBS J 2007, 274:659–676.
Author/s:
Li, F-C; Gasser, RB; Lok, JB; Korhonen, PK; Wang, Y-F; Yin, F-Y; He, L; Zhou, R; Zhao, J-L; Hu, M

Title:
Exploring the role of two interacting phosphoinositide 3-kinases of Haemonchus contortus

Date:
2014-11-12

Citation:
Li, F.-C., Gasser, R. B., Lok, J. B., Korhonen, P. K., Wang, Y.-F., Yin, F.-Y., He, L., Zhou, R., Zhao, J.-L. & Hu, M. (2014). Exploring the role of two interacting phosphoinositide 3-kinases of Haemonchus contortus. PARASITES & VECTORS, 7 (1), https://doi.org/10.1186/s13071-014-0498-2.

Persistent Link:
http://hdl.handle.net/11343/262572

File Description:
Published version

License:
CC BY