**Knockdown of Phosphoethanolamine Transmethylation Enzymes Decreases
Viability of *Haemonchus contortus***

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Abstract

The phosphobase methylation pathway, in which phosphoethanolamine *N*-methyltransferases (PMTs) successively catalyze the methylation of phosphoethanolamine to phosphocholine, is essential in the free-living nematode *Caenorhabditis elegans*. Two PMT-encoding genes (HcPMT1 and HcPMT2) cloned from *Haemonchus contortus* have been shown, by *in vitro* assays, to possess enzymatic characteristics similar to those of *C. elegans* PMTs, but their physiological significance in *H. contortus* is yet to be elucidated. Therefore, in this study, we endeavored to determine the importance of HcPMT1 and HcPMT2 in the survival of *H. contortus* by adapting the use of phosphorodiamidate morpholino oligomers (PPMO) antisense approach to block the translation of HcPMT1 and HcPMT2 in the worms. We found that PPMOs targeting HcPMT1 and HcPMT2 down-regulated the expression of HcPMT1 and HcPMT2 proteins in adult *H. contortus*. Analysis of the effect of HcPMT1 and HcPMT2 knockdown showed that it significantly decreased worm motility and viability, thus validating HcPMT1 and HcPMT2 as essential enzymes for survival of *H. contortus*. Studies of gene function in *H. contortus* have been constrained by limited forward and reverse genetic technologies for use in *H. contortus*. Thus, our success in adaptation of use of PPMO antisense approach in *H. contortus* provides an important reverse genetic technological advance for studying this parasitic nematode of veterinary significance.

Keywords: *Haemonchus contortus*; Gene knockdown; Phosphorodiamidate morpholino oligomers; Phosphoethanolamine *N*-methyltransferase

1. Introduction

Haemonchus contortus is a nematode of economic significance in small ruminant production. The use of anthelmintic drugs is currently the only mode of control for *H. contortus* infections in livestock, but the intensive use of and virtual total reliance on anthelmintics has led to wide-spread development of anthelmintic-resistant *H. contortus* populations (Kaplan, 2004; Gilleard, 2006). This situation requires concerted efforts to identify novel molecular targets for developing new effective anthelmintics.

Phospholipids are major integral and structural components of eukaryotic cell membranes. However, phospholipid metabolic pathways are currently poorly understood in *H. contortus*. Mammalian cells utilize the Kennedy pathway to synthesize phosphatidylcholine from choline, and an alternative Bremer-Greenberg pathway that methylates phosphatidylethanolamine to phosphatidylcholine (Kent et al., 1995; Carman and Henry, 1989). In *Plasmodium falciparum* and *Caenorhabditis elegans*, a plant-like phosphobase methylation pathway involving the three-step methylation of phosphoethanolamine for the biosynthesis of phosphatidylcholine, has been characterized and found to be essential for their survival (Witola et al., 2008; Bobenchik et al., 2013; Palavalli et al., 2006). The *P. falciparum* phosphoethanolamine methyltransferase (PMT) possesses a single methyltransferase domain that methylates all three phosphobases (Pessi et al., 2004; Pessi et al., 2005), while *C. elegans* possesses two PMTs (PMT-1 and PMT-2) that sequentially methylate phosphoethanolamine to phosphocholine (Palavalli et al., 2006; Brendza et al., 2007). Because these PMTs are not found in mammals, they are attractive potential targets for the development of a new class of antiparasitic drugs of medical and veterinary importance (Elabbadi et al., 1997).

Two PMT-encoding genes (HcPMT1 and HcPMT2) have been cloned from *H. contortus*, and by *in vitro* enzymatic assays, they have been found to possess similar enzymatic

characteristics to the PMTs in *C. elegans*, with HcPMT1 catalyzing the first methylation step of phosphoethanolamine, and HcPMT2 catalyzing the two subsequent methylation steps (Lee et al., 2011). However, the physiological roles of HcPMT1 and HcPMT2 in *H. contortus* worms are yet to be elucidated. Thus, in the present study, we endeavored to adapt the use of phosphorodiamidate morpholino oligomers antisense approach to determine the significance of HcPMT1 and HcPMT2 in *H. contortus*.

2. Materials and Methods

2.1. Experimental animals and isolation of H. contortus

The experimental animal procedures in this study were performed following the Tuskegee University Institutional Animal Care and Use Committee's approved protocol. Animals used in the experiment were one-year old Boer-kiko cross goats that were confirmed to be infected with *H. contortus* based on clinical manifestations characteristic of anemia, weight loss, submandibular edema and unthriftiness, as well as the presence of high counts (>2000 eggs per gram of fecal sample) of *H. contortus* eggs in their fecal samples. The abomasum from each goat was immediately excised after euthanasia and placed in a container in a water bath maintained at 37°C. Adult female *H. contortus* worms, identified in the abomasal contents based on their characteristic barber's pole appearance, were gently transferred using a spatula to petri dishes containing PBS at 37°C. The worms were washed repeatedly in PBS until no abomasal debris were visible. The worms were then washed three times in supplemented RPMI-1640 medium (pH 6.8, containing 2% glucose, 500 units/ml penicillin, 500 µg/ml streptomycin, 1.25 µg/ml amphotericin and 1mM chloramphenicol) (Rhoads and Fetterer, 1995). For culture assays, the parasites were resuspended in 10 ml of the supplemented RPMI medium in 65 x 15 mm petri

dishes and incubated at 37°C with 5% CO₂. For RNA extraction, the parasites were kept frozen at -80°C until use.

2.2. cDNA synthesis

Following three cycles of freeze-thaw, female *H. contortus* worms were homogenized in Trizol reagent and total RNA extracted. DNase-I (Invitrogen) treatment of 1 µg of total RNA was performed to remove residual genomic DNA after which the RNA was reverse-transcribed using the iScript Select cDNA Synthesis kit (BIO-RAD).

2.3. Cloning and sequencing of HcPMT1 and HCPMT2 open reading frames

To amplify the open reading frames of HcPMT1 and HcPMT2, the gene sequences reported by Lee et al. (2011) were used to design primers. For HcPMT1 the primer pair was 5'-CTCGAGATGACGGCTGAGGTGCGACGGGATT-3' (forward, with added *XhoI* restriction site underlined and the start codon italicized) and 5'-GGATCC*TTAAAGTGAAGCCTTGATCA*-3' (reverse, with added *BamHI* site underlined and stop codon italicized). For HcPMT2 the forward primer was 5'-CTCGAGATGCCTGCCGTTGAACGACAACTGATT-3' (*XhoI* site underlined and start codon italicized), while the reverse primer was 5'-GGATCC*TTATTGTGGCTTGACAGCAGCGAA*-3' (*BamHI* site underlined and stop codon italicized). The *XhoI* and *BamHI* restriction sites were included for directional cloning in the pET15b expression vector (Novagen). High fidelity DNA polymerase (Affymetrix) was used to generate the coding gene fragments from *H. contortus* cDNA. The fragments were first cloned in the pGEMT-Easy vector (Promega) for sequencing and then transferred into the pET15b

expression vector in-frame with the hexahistidine tag at the N-terminal. For protein expression, the vectors were transformed into *E. coli* BL21-CodonPlus-DE3-RIL (Stratagene).

2.4. Production of recombinant HcPMT1 and HcPMT2 proteins

The HcPMT1 and HcPMT2 protein expression *E. coli* (strain BL21-CodonPlus--DE3-RIL) was inoculated in Luria broth medium containing 100 µg/ml ampicillin and 34 µg/ml chloramphenicol and grown to an A_{600} of 0.6 at 37°C. Isopropyl-β-D-thiogalactopyranoside was then added to a final concentration of 1 mM and the cultures incubated for further 3 hours. The bacteria was harvested by centrifugation and proteins extracted using the BugBuster reagent (Novagen) for native protein extraction to which a protease inhibitor cocktail (Roche Diagnostics) and 10 µg/ml lysozyme were added. Nickel-affinity column chromatography (Novagen) was performed on the soluble extracts to purify His-tagged HcPMT1 and HcPMT2. After elution from the columns, the proteins were dialyzed in 5 mM Hepes–KOH (pH 7.8) buffer containing 0.5 mM DTT and concentrated using spin columns. Samples of the purified proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis to determine purity while the protein concentration was measured by the Bradford method.

2.5. Production of HcPMT1 and HcPMT2 antisera

To raise specific antibodies against HcPMT1 and 2, the purified recombinant proteins were emulsified with Freund's Complete Adjuvant (Sigma-Aldrich) and injected into rats at 40 µg of protein per rat. Two subsequent booster immunizations were administered at two weeks intervals with 20 µg of the recombinant proteins emulsified in Freund's Incomplete Adjuvant. The rats were sacrificed and blood collected by cardiac puncture and sera isolated from the blood. Pre-immune

and immune sera were used in Western blotting assays to assess the specificity of the antisera against the recombinant proteins.

2.6. Cloning and sequencing of 5'-UTR fragments

To obtain the 5'-untranslated region (5'-UTR) sequences for HcPMT1 and HcPMT2 genes, the universal splice leader sequence primer for *H. contortus*, 5'-GGTTTTAACCCAGTATCTCAAG-3' (Redmond and Knox, 2001) as forward primer was used with either HcPMT1 or HcPMT2 reverse primer. The HcPMT1 (5'-AGTTTCTGCTAGCACAGTAGTGA-3') and HcPMT2 (5'-TGAGTCAACTTCCTTGCAGAACG-3') reverse primers were designed from the reported coding sequences of HcPMT1 and 2 genes, respectively (Lee et al., 2011). The 5'-UTR fragments were amplified from *H. contortus* cDNA and cloned into pGEMT-Easy vector for sequencing.

2.7. Design of peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs)

PPMOs are synthetic chains of six-membered ring deoxyribonucleic acid bases with neutral and hydrophilic phosphorodiamidate groups (Moulton et al., 2004; Lai et al., 2012). Because of this, they are much more stable than RNA molecules. They are conjugated, at their 3'-ends, to a transductive peptide that allows PPMOs to readily penetrate cellular membrane barriers (Li and Morcos, 2008). PPMOs disrupt target mRNA translation through Watson/Crick base-pairing mechanisms. In this study, PPMOs were designed to inhibit gene products following the strategy that we have previously reported (Lai et al., 2012; Witola et al., 2014). The specific sequence for the PPMO targeting HcPMT1 mRNA was 5'-CGTCGCACTTCAGCCGTCATTGCTC -3' (with the start codon underlined), while the

HcPMT2 PPMO sequence was 5'-CGTTCAACGGCAGGCCATCTTTCTGT -3' (with the start codon underlined). An off-target standard control PPMO sequence was 5'-CCTCTTACCTCAGTTACAATTTAT-3'. The PPMOs were synthesized conjugated to a transductive peptide by Gene Tools.

2.8. PPMO toxicity assay

To determine the toxic concentrations of PPMOs in *H. contortus* worms, freshly isolated female adult *H. contortus* worms were cultured in triplicate in 10 ml of supplemented RPMI medium in 65 x 15 mm petri dishes with the off-target standard control PPMO at concentrations of 0 μ M, 20 μ M, 40 μ M, 60 μ M, 80 μ M, 90 μ M, 100 μ M, 110 μ M and 120 μ M. The motile activity of the worms was analyzed every 24 hours for a total of 72 hours by using a dissection light microscope and counting 50 worms in each culture. Of the 50 worms counted, the percentages of those that were very motile, sluggishly motile and immotile were determined.

2.9. HcPMT1 and HcPMT2 knockdown assays

Freshly isolated *H. contortus* female parasites were cultured in triplicate in 10 ml of supplemented RPMI medium in the presence of either 90 μ M of off target PPMO or a combination of 45 μ M HcPMT1 and 45 μ M HcPMT2 PPMO at 37°C with 5% CO₂ for 72 hours. The motile activity of the worms was analyzed every 24 hours for a total of 72 hours by using a dissection light microscope and randomly counting 50 worms in each culture. Of the 50 worms counted, the percentages of those that were very motile, sluggishly motile and immotile were determined.

2.10. Western blot analyses

To determine the expression levels of HcPMT1 and HcPMT2 proteins after 72 hours of culture with either 90 μ M of off-target PPMO or a combination of 45 μ M HcPMT1 and 45 μ M HcPMT2 PPMOs, equal numbers of parasites from each treatment were homogenized and boiled in SDS sample buffer. Equal protein amounts from each treatment were fractionated by SDS-PAGE and transferred onto nitrocellulose membranes followed by immunoblotting using rat anti-HcPMT1, rat anti-HcPMT2 or monoclonal antibody against mouse anti- β tubulin which recognizes *H. contortus* β -tubulin protein (Invitrogen) as primary antibodies, while conjugated goat anti-rat, or goat anti-mouse were used as the secondary antibodies. Signal generation was performed using an ECL chemiluminescence kit (PerkinElmer Life Sciences). Densitometry analysis of the intensity of bands (signals) was done using ImageJ version 1.37v software (NIH). The band intensity values of HcPMT1 and HcPMT2 were divided by the samples' respective β -tubulin band intensity values in order to derive the relative expression levels of HcPMT1 and HcPMT2.

2.11. Statistical analysis

Statistical analyses were performed using two-tailed Student's *t* test. *P* values of 0.05 or less were considered significant.

3. Results

3.1. Cloning, sequencing and expression of HcPMT1 and HcPMT2 genes

To ascertain that HcPMT1 and HcPMT2 genes were cloned from *H. contortus*, the cDNA from which the coding sequences were amplified was synthesized from RNA that was extracted

from adult female *H. contortus* worms with visible characteristic barber's pole appearance. For HcPMT1, a 1047 bp coding sequence was amplified that coded for a 349 amino acids protein (Fig. 1). When aligned with the HcPMT1 sequence reported by Lee et al., (2011), the HcPMT1 sequence in the present study lacked a 333 bp fragment positioned at 859 bp to 1192 bp of the coding sequence reported by Lee et al., (2011). Amino acid sequence alignment showed that the two genes coded for identical amino acids sequences except that the sequence of Lee et al (2011) had additional 111 amino acids coded for by the 333 bp extra gene fragment. This indicated that the 333 bp in the sequence reported by Lee et al., (2011) was an intron. The HcPMT2 coding sequence we cloned from the same cDNA was of the same size (1291 bp) as that reported by Lee et al (2011) and encoded an identical amino acids sequence.

In vitro expression of the HcPMT1 and HcPMT2 coding sequences sub-cloned in pET-15b vector and transformed into *E. coli* BL21, produced His-tagged recombinant proteins of about 41 and 52 kDa, respectively, consistent with the expected molecular masses. Polyclonal antibodies were generated by immunization of rats with the affinity-purified recombinant HcPMT1 and HcPMT2. By Western blot analysis, the anti-sera recognized their respective protein bands in *H. contortus* worm lysates consistent with the expected molecular masses of ~41 kDa and ~52 kDa for HcPMT1 and HcPMT2, respectively (Fig. 2A).

PCR amplification followed by sequencing of the HcPMT1 and HcPMT2 gene fragments using the universal splice leader sequence primer for *H. contortus* (Redmond and Knox, 2001) with a gene-specific reverse primer, yielded 5'-UTR sequences that were 27 bp and 39 bp upstream of the start codon for HcPMT1 and HcPMT2, respectively. These 5'-UTRs were used to design target PPMOs for HcPMT1 and HcPMT2.

3.2. *HcPMT1* and 2 PPMOs down-regulate *HcPMT1* and *HcPMT2* expression in *H. contortus*

Prior to testing the effect of *HcPMT1* and *HcPMT2* PPMOs, freshly isolated adult *H. contortus* worms were incubated with varying concentrations of control standard PPMO in order to determine the toxic concentration levels of PPMOs. The highest concentration of control PPMO at which 50% of the treated worms were non-motile after 72 hours of incubation was 110 μ M when compared to untreated worms. Up to a concentration of 90 μ M of control PPMO in culture medium, no difference in worm activity between the untreated and the control standard PPMO-treated worms was observed. Thus, 90 μ M was chosen as the highest final concentration of PPMOs to be used for *HcPMT1* and *HcPMT2* knockdown assays. Incubation of adult female *H. contortus* worms with a mixture of 45 μ M of *HcPMT1* PPMO and 45 μ M of *HcPMT2* PPMO for 72 hours resulted in significant reduction of *HcPMT1* (Fig. 2B) and *HPMT2* (Fig. 2C) protein levels when compared to treatment with 90 μ M of control PPMO. In all Western blotting analyses, anti- β -tubulin was used to check for protein loading (bottom panels of Fig. 2B and C). By densitometry analysis, the relative expression of *HcPMT1* in the *HcPMT1*-PPMO-treated parasites was about 15-fold lower than in the control parasites. Similarly, in *HcPMT2*-PPMO-treated parasites, the relative expression of *HcPMT2* was about 12-fold lower than in the control parasites.

3.3. *HcPMT1* and *HcPMT2* knockdown decreases the viability of *H. contortus*

To determine the effect of *HcPMT1* and *HcPMT2* knockdown, *H. contortus* adult female worms were incubated in the presence of either a combination of *HcPMT1* and *HcPMT2* PPMOs or control standard PPMO. After 72 hours of culture a total of 50 worms per well were randomly counted to establish the percentage of worms that were actively motile, sluggishly motile or non-

motile. In wells treated with control PPMO, an average of 90% worms were actively motile, 7% were sluggishly motile and about 3% were non-motile (Fig 3). In the wells treated with a combination of HcPMT1 and HcPMT2 PPMOs, about 52% worms were actively motile, 28% were sluggishly motile and 20% were non-motile (Fig. 3). Statistically, the number of actively motile worms in the wells treated with HcPMT1 and HcPMT2 PPMOs were significantly lower than for the wells treated with control PPMO. Additionally, the HcPMT1 and HcPMT2 PPMO-treated wells had significantly ($P < 0.05$) higher percentages of sluggishly motile and non-motile worms in comparison to the control wells (Fig. 3). This indicated that the knockdown of HcPMT1 and HcPMT2 significantly lowered the viability of *H. contortus* worms. The 72 hours of incubation of the worms was chosen because this was the time point at which significant reduction in the expression of HcPMT1 and HcPMT2 were notable when compared to earlier time points (data not shown). Moreover, we had earlier observed that maintaining *H. contortus* worms in culture beyond 72 hours affected the viability of the worms.

4. Discussion

While targeted gene silencing by RNA interference (RNAi) has been used successfully to study gene function in the free-living nematode, *C. elegans*, this approach has shown very limited success in most parasitic nematodes including *H. contortus* (Samarasinghe et al., 2011; Britton et al., 2012; Zawadzki et al., 2012). Thus, studying gene function in *H. contortus* using reverse genetic approaches has been seriously constrained. In the present study, we adapted the use of transductive peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) antisense knockdown approach to determine the significance of HcPMT1 and HcPMT2 in adult

H. contortus worms. This approach has successfully been used to study gene function in *Toxoplasma gondii* (Witola et al., 2014; Lai et al., 2012) and *P. falciparum* (Garg et al., 2015).

PPMOs are non-ionic DNA analogues attached at the 3'-end to a membrane-penetrating transductive peptide that allows the oligomers to readily cross multiple membrane barriers (Li and Morcos, 2008). They are designed to block translation of target mRNA through Watson/Crick base-pairing with the 5'-UTR through to 25 nucleotides downstream of the translation start codon of target mRNA, thus preventing the assembly of the ribosome at the AUG translational start site (Summerton, 1999). The phosphorodiamidate groups on PPMOs are neutral and hydrophilic, which enhances PPMO's stability and solubility, thus facilitating the use of multiple routes of administration (Moulton et al., 2004). *H. contortus* adult worms cannot be propagated in culture, but if freshly extracted from the abomasum immediately after sacrificing the animal host, they can be transiently maintained alive in culture in supplemented RPMI medium for several days, facilitating the use of PPMOs *in vitro* to study gene function in *H. contortus*.

In the present study, targeted-silencing of HcPMT1 and HcPMT2 using PPMOs led to significant reduction in the expression of HcPMT1 and HcPMT2 proteins in adult *H. contortus* parasites. This resulted in significant loss of worm motility, indicating that HcPMT1 and HcPMT2 are essential for the survival of the *H. contortus*. Likewise, silencing of *C. elegans*' phosphoethanolamine methyltransferase genes (PMT-1 and PMT-2) by RNAi has been demonstrated to lead to arrested L4 larval development and lethality in the F1 progeny at the L1/L2 stage, and in some progeny at the L3 stage (Brendza et al., 2007), suggesting that PMT enzymes in nematodes have a conserved essential role. Based on their functions, silencing of HcPMT1 and HcPMT2 genes in *H. contortus* would lead to abrogation of the methylation of

phosphoethanolamine to phosphocholine, which in turn would affect the synthesis of phosphatidylcholine. Therefore, our findings indicate that the phosphobase pathway for biosynthesis of phosphatidylcholine is essential for the survival of *H. contortus*. These results are corroborated by our previous findings in which we found that a specific chemical inhibitor for the enzymatic activity of HcPMT1 and HcPMT2 had *in vitro* anthelmintic effect against L3 larvae and adult *H. contortus* worms (Witola et al., 2016).

In conclusion, this study has successfully adapted the use of PPMO antisense approach in *H. contortus*, thus providing an important reverse genetic technological advance for studying this parasitic nematode of veterinary significance. Knockdown of HcPMT1 and HcPMT2 has demonstrated that they are essential for the viability and survival of the *H. contortus* and are, therefore, potential molecular targets for development of novel anthelmintics.

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Legends to Figures

Figure 1: Alignment of *H. contortus* HcPMT1 protein sequence translated from the HcPMT1 coding sequence cloned in this study (HcPMT1-W) with the HcPMT1 coding sequence (HcPMT1-L) reported by Lee et al. (2011). The region indicated by dashes (---) illustrates the additional 111 amino acids sequence in HcPMT1-L presumed to be encoded by an intron.

Figure 2: (A) Western blotting analysis of *H. contortus* lysate to determine the specificity of rat anti-sera generated against HcPMT1 (Anti-PMT1) and HcPMT2 (Anti-PMT2) proteins. The anti-sera against HcPMT1 and HcPMT2 recognized protein bands of ~41 kDa and 52 kDa, respectively, in the parasite lysate. (B.) Analysis of HcPMT1 (MT1) and Control (Cont) PPMOs' knockdown effect on the expression of HcPMT1 protein in *H. contortus* adult female worms after 72 hours of culture. (C.) Effect of HcPMT2 (MT2) and Control (Cont) PPMO on the expression of HcPMT2 protein after 72 hours of culture. β -Tubulin was used to check for protein loading (lower panels in B and C).

Figure 3: Effect of HcPMT1 and HcPMT2 knockdown on the motile activity of *H. contortus* adult female worms maintained in culture for 72 hours. For each assay, 50 worms were counted and the motile activity determined as *Very Motile* (if the worms were vigorously wiggling), *Sluggishly Motile* (if the worms were wiggling slowly) and *Non-motile* (if the worms were not

409 showing any sign of movement). Black columns represent control PPMO-treated worms while
410 white columns represent HcPMT1 and HcPMT2 PPMO-treated worms. The data shown
411 represent means of three independent experiments with standard error bars and levels of
412 statistical significance depicted by asterisks (*, $P < 0.05$).

FIGURES

HcPMT1-W	MTAEVRRDSFKTFWDKYSDKPDNSMMLNQTAQDLEASDRADILSSSLPHLTNKDVVDIGA	60
HcPMT1-L	MTAEVRRDSFKTFWDKYSDKPDNSMMLNQTAQDLEASDRADILSSSLPHLTNKDVVDIGA	60
HcPMT1-W	GIGRFTTVLAETARWVLSTDFIESFIEKNQERNAHMGNISYQIGDAVHLQMDEKSVDLVF	120
HcPMT1-L	GIGRFTTVLAETARWVLSTDFIESFIEKNQERNAHMGNISYQIGDAVHLQMDEKSVDLVF	120
HcPMT1-W	TNWLMMYLSDREVIEFLLNAMRWLRADGYIHLRESCSEPSTGRLKTATMHSAVDANPTHY	180
HcPMT1-L	TNWLMMYLSDREVIEFLLNAMRWLRADGYIHLRESCSEPSTGRLKTATMHSAVDANPTHY	180
HcPMT1-W	RFSSLYIKLLRAIRYGDSDGKMWKFDVQWSCSVPTYIRRCNNWRQVHWLTKKVPAVGDEE	240
HcPMT1-L	RFSSLYIKLLRAIRYGDSDGKMWKFDVQWSCSVPTYIRRCNNWRQVHWLTKKVPAVGDEE	240
HcPMT1-W	TSVDDLLNLFSQLWPAEQKTWDEKLDNEKYSWTDKIFSNAIDDEVV-----	286
HcPMT1-L	TSVDDLLNLFSQLWPAEQKTWDEKLDNEKYSWTDKIFSNAIDDEVVPKNSTAYVFTPRQR	300
HcPMT1-W	-----	286
HcPMT1-L	SPFLHVNSHLLAEKFTCNVWNVETKEYLYRTSLTKANNQKDQRVRFGWNESSLSPIDYWN	360
HcPMT1-W	-----LLEPVSGIDETSVRQRMTCGFK	309
HcPMT1-L	QRDASFDCMVATELLATCDDSVKSIASIMKPEAKVVLLEPVSGIDETSVRQRMTCGFK	420
HcPMT1-W	NITIVDVTQESLNAEVSFIDHNLDVELSGCNYLLIKASL	349
HcPMT1-L	NITIVDVTQESLNAEVSFIDHNLDVELSGCNYLLIKASL	460

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