Journal of Helminthology (2006) **80,** 53–58 DOI: 10.1079/JOH2005322

Changes in FaRP-like peptide levels during development of eggs from the plant-parasitic cyst nematode, *Heterodera glycines*

E.P. Masler*

Nematology Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD 20705, USA

Abstract

The plant-parasitic cyst nematode *Heterodera glycines* requires a host plant to complete its life cycle, which involves hatching of infective juveniles that parasitize through root entry. A laboratory population of *H. glycines* grown on soybean, *Glycine max*, undergoes a sharp increase in maturity between 5 and 6 weeks in culture, as measured by the proportion of eggs containing well developed pre-hatch juveniles (late development eggs) versus eggs without visible juveniles (early development eggs). The median percent of eggs classified as late development, representing all samples taken from 4 to 7 weeks in culture, was 61%. For all samples taken up to 5 weeks, 80% scored below the median. In samples taken after 5 weeks, 15% scored below the median. This shift in population maturity was accompanied by a significant increase ($P < 0.01$) in the number of hatched juveniles present in each sample. There was also a significant increase ($P < 0.02$) in amount of FaRP-like peptide detected by specific ELISA. Total FaRP levels increased from 0.18 ± 0.07 fMol FLRFamide equivalents per ng protein in early development eggs to 0.40 ± 0.17 in late development eggs. The level remained high in hatched juveniles. HPLC/ELISA detected as many as nine potential FaRPs in *H. glycines*, two of which were specifically increased ($P < 0.005$) in hatched juveniles. The association of FaRPs with maturing eggs and the possible involvement of these neuropeptides with juvenile hatching and motility are discussed.

Introduction

Communication within organisms via chemical transmission is integral to development and metabolism, and central components of this communication are neuropeptides. As a group, neuropeptides are ubiquitous, numerous, diverse, and represented by both neural and endocrine neurotransmitters (Jacob & Kaplan, 2003). Numerous families of neuropeptides have been identified in higher invertebrates, and their control of physiological processes in these organisms is well recognized (Keller, 1992; Muneoka & Kobayashi, 1992; Masler *et al.*, 1993; Nassel, 2002; Klowden, 2003). In nematodes, the FMRFamide-related peptides (FaRPs) are particularly abundant and have been widely investigated (Brownlee & Fairweather, 1999; Day & Maule, 1999). FaRPs comprise an extensive family of C-terminally amidated peptides that have profound effects upon both visceral and somatic muscular functions in invertebrates (Thompson *et al.*, 2003).

These neuromuscular effects have figured prominently in the investigation of FaRPs as leads to anthelmintic discovery (Day & Maule, 1999; Geary *et al.*, 1999; Marks *et al.*, 1999; Davis & Stretton, 2001). Eighteen FaRP sequences have been isolated and characterized from the animal parasite *Ascaris suum* (Day & Maule, 1999), and

*Fax: 301 504 5589
E-mail: maslere@ba.ars.usda.gov
Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture

electrophysiological experiments have revealed a varied and complex array of neuromuscular effects (Brownlee & Fairweather, 1999; Davis & Stretton, 2001). One gene has been isolated (*afp-1*), and codes for 6 of the 18 *A. suum* FaRPs (Edison *et al.*, 1997). Two FaRP sequences have been characterized from a second animal-parasitic nematode, *Haemonchus contortus* (Keating *et al.*, 1995; Marks *et al.*, 1999), and are identical to two *A. suum* FaRPs. In the free-living nematodes, five FaRP sequences have been isolated from *Panagrellus redivivus*. Three are unique to this species and two are identical to *A. suum* sequences (Geary *et al.*, 1992; Day & Maule, 1999). In *Caenorhabditis elegans*, 23 *flp* genes have been identified, coding for over 50 predicted FaRPs (Nelson *et al.*, 1998). Most of the *C. elegans* sequences are unique, but at least five are identical to sequences in *A. suum* and *P. redivivus*, and some may be developmentally regulated (Kim & Li, 2004). At least 11 *C. elegans* FaRPs have been isolated and characterized (Marks *et al.*, 1998, 2001).

Among plant-parasitic nematodes, *Globodera pallida* contains at least five *gpflp* genes encoding 14 different FaRP sequences, three of which are identical to sequences in both *A. suum* and *P. redivivus* (Kimber *et al.*, 2001). FaRPs were detected and quantified in all developmental stages of the soybean cyst nematode *Heterodera glycines*, and differences occurred between *H. glycines* and free-living nematodes (Masler *et al.*, 1999). Presented here is evidence that *H. glycines* contains multiple FaRPs, and that both total and specific FaRP levels change during egg and juvenile development.

Materials and methods

Heterodera glycines (population NL-RHp) were reared on soybean *Glycine max* (cv. 'Kent') at 27°C, 16 h light:8 h dark, in a constant moisture system according to Sardanelli & Kenworthy (1997). Plants were inoculated with *H. glycines* eggs at a rate of 3000 per plant, and females were harvested from 28 to 50 days after inoculation (DAI). Depending upon planting and harvest sizes, 20,000–400,000 eggs were obtained from any one collection. The total number of eggs was calculated by counting eggs in multiple representative aliquots of each collection. Juveniles (J2) in these collections were also counted. Aliquots of eggs were then examined under a light microscope (65x), and eggs were scored into one of two arbitrary categories for assignment of developmental stage. Eggs that appeared uniform, granular and opaque were scored as developmentally 'early' while those with a translucent appearance and an outline of a juvenile were scored as developmentally 'late'. For each egg collection, the proportion of eggs in the two developmental categories was determined, and was used to compare the developmental stage of all egg collections against DAI.

For the collection of freshly hatched J2 for FaRP extraction, eggs were maintained in tap water at 27°C and J2 were collected daily and frozen. Eggs used for FaRP extraction and quantification were also frozen. Eggs and J2 were extracted by disruption in a ground glass homogenizer in 50% aqueous acetonitrile (CH_3CN; EM Science, Gibbstown, New Jersey) acidified with 0.1% trifluoroacetic acid (TFA; Fluka, Sigma-Aldrich, St Louis, Missouri). Typically 20,000–25,000 eggs or 30,000–40,000 J2 were extracted in 3 ml of solvent. Extracts were agitated at room temperature for approximately 2 h and then were centrifuged (20,000 × *g*, 20 min, 20°C). The supernatants were collected and dried under vacuum.

The FaRP enzyme-linked immunosorbent assay (ELISA) was modified from Kingan *et al.* (1997). The primary antiserum prepared against FMRFamide (Marder *et al.*, 1987; gift of Eve Marder) recognizes the RFamide C-terminus. Secondary antibody (goat anti-rabbit IgG, Fc) was from American Qualex, LaMirada, California. A FLRFamide (Sigma) and horseradish peroxidase (Calbiochem, San Diego, California) conjugate was prepared using methods modified from Kingan *et al.* (1997). Peptide and enzyme were mixed in a 7.3:1 molar ratio in 0.7% glutaraldehyde (Sigma) for 1 h at room temperature. The reaction was quenched for 1 h with 180 mM glycine (Sigma), and the conjugate was purified by size exclusion chromatography (SEC; Phenomenex BioSep 3000S, 300 × 7.8 mm, 100 mM phosphate buffer, pH 7.0; Phenomenex, Torrance, California). ELISA standard curves were constructed using FLRFamide from 10 to 1000 fMol per well as the competing antigen. Data were analysed by linear regression and expressed in terms of FLRFamide equivalents.

Total protein was estimated using the microBCA protein assay (Pierce Chemical, Rockford, Illinois) using the manufacturer's protocol modified for 96-well plates.

For chromatography, dried extracts were dissolved in 50% CH_3CN/0.1% TFA and injected onto an Eclipse XDB-C8 reverse phase column (150 × 4.6 mm; Agilent Technologies, Palo Alto, California). Separations were performed at 500 μl min^{-1} using a linear gradient of 0.5% CH_3CN min^{-1} in 0.1% TFA. Fractions were dried under vacuum.

Results

During growth of the infective *H. glycines* populations on soybean plants, there was a noticeable change in the ratio of early development and late development eggs (fig. 1). Ten populations were sampled 23 times from 4 to 7 weeks after inoculation and, based upon our criteria for early and late eggs, a striking shift in development was observed between 5 and 6 weeks. The percent late eggs, as an indicator of development, increased significantly ($P < 0.01$). No significant difference was found between weeks 4 and 5 or between weeks 6 and 7. The median percent of late development eggs across all 23 observations was 61% (fig. 1, horizontal dashed line). All of the ten egg populations classified as early fell at or below the median, while 85% of the late population were above the median. The mean percent of late type eggs in early egg collections (45.6 ± 3.6) was significantly lower ($P < 0.001$) than the mean percent of late types in late egg collections (68.2 ± 2.5). Also, the number of hatched juveniles relative to the number of eggs was significantly lower ($P < 0.01$) in collections done at 5 weeks than at either 6 or 7 weeks, while no difference was detected in the juvenile/egg ratio between 6 and 7 weeks.

Direct examination of total protein per egg showed no statistical difference between the amount extracted from

https://doi.org/10.1079/JOH2005322 Published online by Cambridge University Press

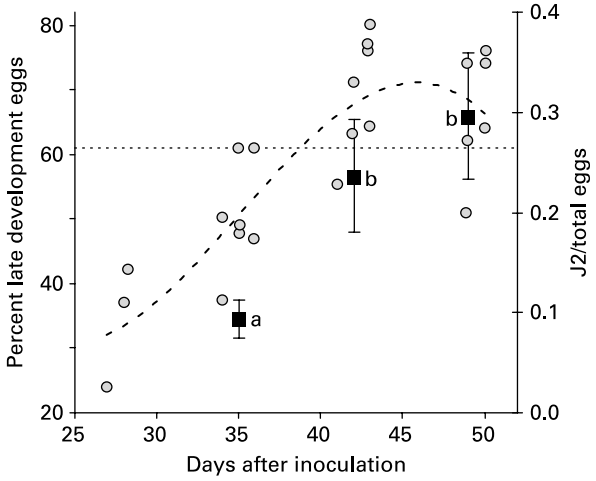

Fig. 1. Change in *Heterodera glycines* egg population maturity during culture on *Glycine max*. Eggs were harvested from females collected at various times after plant culture inoculation. Circles (○) represent the percentage of all eggs collected that were classified as late development (see text) for each of 23 individual populations. Squares (■) represent the mean ± SEM of the ratios of J2/total eggs collected at 5, 6 and 7 weeks after inoculation. Means associated with different letters are significantly different ($P < 0.01$)

the predominantly early pool (4 and 5 weeks) and the amount extracted from the predominantly late pool (6 and 7 weeks) (table 1). For comparison, J2 juveniles had significantly less protein ($P < 0.01$) than eggs. Because a main feature of cyst nematodes is muscular coordination required for hatching and infection and because FaRPs are neuromodulators, FaRP levels were compared across developmental categories in *H. glycines*. As the majority of FaRPs predicted in the isolated genes of the related cyst nematode *G. pallida* are xLRFamides, FLRFamide was chosen as the antigenic competitor in the ELISA. There was a significant ($P < 0.02$) increase in total FLRFamide-like immunoreactivity between early and late eggs (table 1). On an individual basis, FaRP levels in the J2 stage were intermediate between the levels in the two egg stages. FLRFamide specific immunoreactivity, however, was highest in both the late egg and J2 stages, 2- to 4-fold greater than in early eggs.

FLRFamide-like FaRP immunoreactivity eluted primarily between 20% and 38% CH_3CN on reverse phase HPLC (fig. 2). HPLC/ELISA profiles were similar but not identical in the two egg stages and J2. Nine distinct peaks eluted between 22 and 46 minutes in all stages (fig. 2). As a percentage of total immunoreactivity recovered from all column fractions, peak 3 was the most predominant in all three stages, representing an average of 30% of the total FLRFamide-like activity. In eggs, the second most abundant peak was peak 6, at 15% of total. Although there was an apparent decrease in this peak in J2, it was not significant. Also, no significant difference in the relative amounts of any of the nine evaluated peaks was detected between early (5 week) and late (7 week) stage eggs. In J2, seven of the nine peaks were similar in relative amounts to the corresponding peaks in the egg stages. However, two peaks (5 and 7) were significantly ($P < 0.005$) greater in juveniles than in either egg stage. Peak 5 in J2 (4.8 ± 0.7 percent of total FLRFamide-like immunoreactivity) was nearly 2-fold greater than in either early (2.5 ± 0.4) or late (2.6 ± 0.3) eggs. Peak 7 in J2 (10.6 ± 1.6 percent of total) was 1.8-fold and 2.2-fold greater, respectively, than in early (5.8 ± 1.3) or late (4.9 ± 1.5) eggs.

Discussion

Post-embryonic development in *H. glycines* includes four juvenile stages and the adult. A moult from the first to second juvenile (J2) stage occurs within the egg. The J2 that hatch are infective and invade plant roots and parasitize the plant host under suitable conditions. Mature males and females develop within the root and, after 4–5 weeks, females producing from 200–300 eggs become partially exposed on the root surface. These 200–300 eggs are distributed between an external set of eggs contained in a secreted matrix, and the majority of eggs which are retained (encysted) within the female body (Thompson & Tylka, 1997). Egg hatch occurs first in the external eggs, even if conditions for survival (e.g. presence of host plant) are not optimal. Encysted egg hatch is more restricted and occurs when environmental conditions are more favourable. This strategy protects

Table 1. Protein and FaRP-like peptide measurements for developmental stages of *Heterodera glycines* eggs and juveniles.

	Stage		
	Early[1]	Late[2]	J2
ng protein per individual	2.43 ± 1.17[a]	4.12 ± 1.17[a]	1.13 ± 0.36[b]
fMol FLRFamide eq per individual	0.22 ± 0.06[a]	1.17 ± 0.26[b]	0.61 ± 0.06[c]
fMol FLRFamide eq per ng protein	0.18 ± 0.07[a]	0.40 ± 0.17[b]	0.77 ± 0.26[b]

Eggs were obtained from *H. glycines* females harvested from *Glycine max* cultures at 4, 5, 6 and 7 weeks after inoculation. Each collection was analysed separately for total protein and FLRFamide-like immunoreactivity. Data for weeks 4 and 5 and for weeks 6 and 7 were pooled separately as early[1] and late[2] collections. Data are expressed as mean ± SEM of 5 to 6 individual collections. Means across rows followed by different letters are significantly different ($P < 0.02$).

https://doi.org/10.1079/JOH2005322 Published online by Cambridge University Press

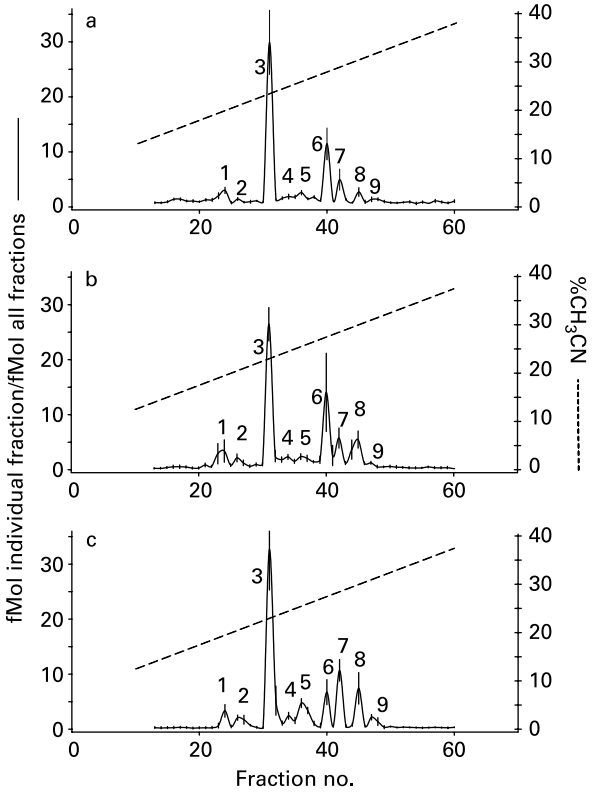

Fig. 2. FLRFamide-like HPLC/ELISA profiles from *Heterodera glycines* eggs collected at (a) 5 weeks (early) and (b) 7 weeks (late) after inoculation, and (c) from juveniles (J2). Extracts were fractionated as described in the text and individual fractions were assayed using the FLRFamide ELISA. Profiles (solid line) represent the ratio: fMol FLRFamide-like FaRP recovered in an individual fraction/total fMol FLRFamide-like FaRP recovered in all fractions. Ratios were calculated for 50 fractions, from 10–60 min, for 3–6 separate collections in each developmental category. Data are expressed as mean ± SEM for each fraction. Peaks selected for analysis are numbered (1–9). Dotted line represents CH_3CN gradient.

against environmental changes and assures successful reproduction of the species. Regardless of the conditions under which hatch might occur, however, juveniles within the eggs must all attain a required level of development to be capable of hatching. Part of this required development should be a compliment of regulatory molecules, such as FaRPs, that will coordinate hatching. Since not all eggs hatch at the same time, including those within the cyst, an accumulation of late maturity eggs should be expected. This is exactly what is observed when populations of *H. glycines*, cultured on *G. max* in a controlled environment, are sampled over time. Females available at any time following inoculation contain eggs representing a continuum of maturity. However, the distribution of eggs within this continuum clearly shifts as the culture ages. Between 35 and 42 days following inoculation, populations change significantly from containing primarily early or immature eggs to containing primarily late, mature ones. This is also expected if hatching does not occur in synchrony with egg maturation. At the same time that egg maturity of the population increases, the number of J2 relative to eggs also increases significantly, but only up to about 30 percent of total eggs. Again, this is expected given the hatch strategy of *H. glycines*.

Post-embryonic development depends on nutrition from stored egg protein, vitellin, consumed and converted by the growing juvenile (Masler, 1999). Conversion of egg protein into forms within the J2 that are less accessible to extraction methods might explain the significant drop in total protein from egg to J2. Alternatively, the J2 may not use some egg protein. Nevertheless, J2 contain 20–50% less total extractable protein than eggs. In contrast, the level of FLRFamide-like FaRPs is significantly higher in the J2 stage than it is in less mature, early eggs, and the level increases as eggs mature. It is not clear why FaRP levels in mature eggs are higher than in J2. Perhaps there is a shift in the identities of the specific FaRPs expressed. For example, pre-hatch juveniles may accumulate a FaRP pool necessary to coordinate the intense neuromuscular activity associated with hatching. Following hatch, the pool could be altered quantitatively and qualitatively (e.g. by endogenous proteases that degrade peptide messengers; increased production of different FaRPs) to provide signals more suited to the locomotion and host-seeking requirements of the hatched juvenile. Nevertheless, the level of FLRFamide-like FaRPs relative to total protein increases significantly from early to late development eggs and remains so in the J2. This supports the argument that FaRPs are necessary for the regulation of muscular coordination required for hatching and juvenile motility.

Fractionation of both egg and J2 preparations demonstrates the presence of numerous FaRP-like immunoreactive peptides in *H. glycines*. The antibody used in this study recognizes the amidated C-terminus of FaRPs, and thus the antigens detected by the ELISA should represent mature, processed neuropeptides. The possibility exists that some of the peptides detected may be degradation products, but the extraction conditions used should mitigate this. There are nine peaks of FaRP-like immunoreactivity present in each of the developmental samples tested. This is in agreement with the general observation that nematodes contain multiple FaRPs, determined by both biochemical and molecular genetic methods (Nelson *et al.*, 1998; Day & Maule, 1999). However, the actual number of FaRPs present might be greater or lesser than estimated. Different FaRPs that elute under a single peak, FaRPs not recognized by the antibody, or FaRPs present at levels below the limits of the assay, would lead to underestimates. Conversely, single peptides eluting in multiple peaks as the result of endogenous methionine oxidation would result in an overestimation. The distribution of the fractionated *H. glycines* FaRPs is retained throughout egg development. This occurs even as total amounts and specific activity increase. However, in the J2 there is an increase in the levels of two of the nine peaks. Since not all selected peaks increase, the change in FaRPs contributing to these two peaks appears to be specific to the J2 stage. It is tempting to speculate that these FaRPs are specifically involved in some component of hatching. Since hatching

https://doi.org/10.1079/JOH2005322 Published online by Cambridge University Press

involves a sustained period of locomotory movements, the increased peaks may contain peptides specific for locomotion. As noted, some of the peaks separated by HPLC may be composed of more than one peptide, since the antibody does not distinguish between sequences ending in the RFamide signature. The use of alternative competing antigens and different conjugates might be helpful in revealing different developmental changes.

Few non-FaRP neuropeptides have been defined in nematodes, in contrast to the numerous families of neuropeptides with specific physiological functions described in the higher invertebrates (Muneoka & Kabayashi, 1992; Masler *et al.*, 1993; Nassel, 2002; Klowden, 2003). In nematodes, however, the number of genes coding for FaRPs and the number of unique sequences are greater than in higher invertebrates (Taussig & Scheller, 1986; Merte & Nichols, 2002). Perhaps specific FaRPs or FaRP combinations have multiple roles in addition to muscular coordination (Jacob & Kaplan, 2003). Evidence suggests links between FaRPs and both feeding behaviour and carbohydrate metabolism in nematodes (Rogers *et al.*, 2003; Rex *et al.*, 2004).

At least three *gpflp* homologs have been reported in *H. glycines* coding for FaRP precursors (AAO92291, AAO92292, AAP02990) that contain sequences for three different FaRPs (KHEYLRFamide, KSAYMRFamide, KNKFEFIRFamide). KHEYLRFamide is also present in *A. suum* and *P. redivivus*, and KSAYMRFamide is present in *A. suum*, *H. contortus* and *P. redivivus*. KNKFEFIRFamide so far has been detected only in cyst nematodes (Kimber *et al.*, 2002), although the biochemical homolog RNKFEFIRFamide has been characterized from *A. suum* (Davis & Stretton, 1996) and has been predicted in *C. elegans* (Nelson *et al.*, 1998).

Evidence presented here suggests that additional sequences are likely to be present in *H. glycines*. Further progress in the analysis of FaRPs in *H. glycines* will be made through molecular genetics and the identification of sequences through mass spectroscopy.

References

Brownlee, D.J.A. & Fairweather, I. (1999) Exploring the neurotransmitter labyrinth in nematodes. *Trends in Neurosciences* **22**, 16–24.

Davis, E.E. & Stretton, A.O.W. (1996) The motornervous system of *Ascaris*: electrophysiology and anatomy of the neurones and their control by neuromodulators. *Parasitology* **113**, S97–S117.

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

Day, T.A. & Maule, A.G. (1999) Parasitic peptides. The structure and function of neuropeptides in parasitic worms. *Peptides* **20**, 999–1019.

Edison, A.S., Messinger, L.A. & Stretton, A.O.W. (1997) Afp-1: a gene encoding multiple transcripts of a new class of FMRFamide-like neuropeptides in the nematode *Ascaris suum*. *Peptides* **18**, 929–935.

Geary, T.G., Price, D.A., Bowman, J.W., Winterrowd, C.A., MacKenzie, C.D., Garrison, R.D., Williams, J.F. & Friedman, A.R. (1992) Two FMRFamide-like peptides from the free-living nematode *Panagrellus redivivus*. *Peptides* **13**, 209–214.

Geary, T.G., Marks, N.J., Maule, A.G., Bowman, J.W., Alexander-Bowman, S.J., Larsen, M.L., Kubiak, T.M., Davis, J.P. & Thompson, D.P. (1999) Pharmacology of FMRFamide-related peptides (FaRPs) in helminths. *Annals of the New York Academy of Sciences* **897**, 212–227.

Jacob, T.C. & Kaplan, J.M. (2003) The EGL-21 carboxypeptidase E facilitates acetylcholine release at *Caenorhabditis elegans* neuromuscular junctions. *Journal of Neuroscience* **23**, 2122–2130.

Keating, C., Thorndyke, M.C., Holden-Dye, L., Williams, R.G. & Walker, R.J. (1995) The isolation of a FMRFamide-like peptide from the nematode *Haemonchus contortus*. *Parasitology* **111**, 515–521.

Keller, R. (1992) Crustacean neuropeptides: structures, functions and comparative aspects. *Experientia* **48**, 439–448.

Kim, K. & Li, C. (2004) Expression and regulation of am FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. *Journal of Comparative Neurology* **475**, 540–550.

Kimber, M.J., Fleming, C.C., Bjourson, A.J., Halton, D.W. & Maule, A.G. (2001) FMRFamide-related peptides in potato cyst nematodes. *Molecular and Biochemical Parasitology* **116**, 199–208.

Kimber, M.J., Fleming, C.C., Prior, A., Jones, J.T., Halton, D.W. & Maule, A.G. (2002) Localisation of *Globodera pallida* FMRFamide-related peptide encoding genes using in situ hybridisation. *International Journal for Parasitology* **32**, 1095–1105.

Kingan, T.G., Zitnan, D., Jaffe, H. & Beckage, N.E. (1997) Identification of neuropeptides in the midgut of parasitized insects: FLRFamides as candidate paracrines. *Molecular and Cellular Endocrinology* **133**, 19–32.

Klowden, M.J. (2003) Contributions of insect research toward our understanding of neurosecretion. *Archives of Insect Biochemistry and Physiology* **53**, 101–114.

Marder, E.R., Calabrese, R.L., Nusbaum, M.P. & Trimmer, B. (1987) Distribution and partial characterization of FMRFamide-like peptides in the stomatogastric nervous systems of the rock crab, *Cancer borealis*, and the spiny lobster, *Palinurus interruptus*. *Journal of Comparative Neurology* **259**, 150–163.

Marks, N.J., Maule, A.G., Halton, D.W., Li, C., Geary, T.G., Thompson, D.P. & Shaw, C. (1998) KSAYFMRFamide (PF3/AF8) is present in the free-living nematode *Caenorhabditis elegans*. *Biochemical and Biophysical Research Communications* **248**, 422–424.

Marks, N.J., Sangster, N.C., Maule, A.G., Halton, D.W., Thompson, D.P., Geary, T.G. & Shaw, C. (1999) Structural characterization of KHEYLRFamide (AF2) and KSAYMRFamide (PF3/AF8) from *Haemonchus contortus*. *Molecular and Biochemical Parasitology* **100**, 185–194.

Marks, N.J., Shaw, C., Halton, D.W., Thompson, D.P., Geary, T.G., Li, C. & Maule, A.G. (2001) Isolation and preliminary biological assessment of AADGAPLIRFamide and SVPGVLRFamide from *Caenorhabditis elegans*. *Biochemical and Biophysical Research Communications* **286**, 1170–1176.

https://doi.org/10.1079/JOH2005322 Published online by Cambridge University Press

Masler, E.P. (1999) Detection and partial characterization of egg proteins from *Heterodera glycines*. *Journal of Nematology* **31**, 305–311.

Masler, E.P., Kelly, T.J. & Menn, J.J. (1993) Insect neuropeptides: discovery and application in insect management. *Archives of Insect Biochemistry and Physiology* **22**, 87–111.

Masler, E.P., Kovaleva, E.S. & Sardanelli, S.S. (1999) FMRFamide-like immunoactivity in *Heterodera glycines* (Nemata: Tylenchida). *Journal of Nematology* **31**, 224–231.

Merte, J. & Nichols, R. (2002) *Drosophila melanogaster* FMRFamide-containing peptides: redundant or diverse functions? *Peptides* **23**, 209–220.

Muneoka, Y. & Kobayashi, M. (1992) Comparative aspects of structure and action of molluscan neuropeptides. *Experientia* **48**, 448–456.

Nassel, D.R. (2002) Neuropeptides in the nervous system of *Drosophila* and other insects: multiple roles as neuromodulators and neurohormones. *Progress in Neurobiology* **68**, 1–84.

Nelson, L.S., Kim, K., Memmott, J.E. & Li, C. (1998) FMRFamide-like gene family in the nematode, *Caenorhabditis elegans*. *Molecular Brain Research* **58**, 103–111.

Rex, E., Harmych, S., Puckett, T. & Komuniecki, R. (2004) Regulation of carbohydrate metabolism in *Ascaris suum* body wall muscle: a role for the FMRFamide AF2, not serotonin. *Molecular and Biochemical Parasitology* **13**, 311–313.

Rogers, C., Reale, V., Kim, K., Chatwin, H., Li, C., Evans, P. & deBono, M. (2003) Inhibition of *Caenorhabditis elegans* social feeding by FMRFamide-related peptide activation of NPR-1. *Nature Neuroscience* **6**, 1178–1185.

Sardanelli, S. & Kenworthy, W.J. (1997) Soil moisture control and direct seeding for bioassay of *Heterodera glycines* on soybean. *Journal of Nematology (Supplement)* **29**, 625–634.

Taussig, R. & Scheller, R.H. (1986) The *Aplysia* FMRFamide gene encodes sequences related to mammalian brain peptides. *DNA* **5**, 453–461.

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 KNEFIRFamide on cyclic adenosine monophosphate levels in *Ascaris suum* somatic muscle. *International Journal for Parasitology* **33**, 199–208.

Thompson, J.M. & Tylka, G.L. (1997) Differences in hatching of *Heterodera glycines* egg-mass and encysted eggs *in vitro*. *Journal of Nematology* **29**, 315–321.

(Accepted 13 July 2005)

https://doi.org/10.1079/JOH2005322 Published online by Cambridge University Press