

REVIEW

Molecular Characterization of the Inhibitory Myotropic Peptide Leucomyosuppressin¹

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BENDENA, W. G., B. C. DONLY, M. FUSE, E. LEE, A. B. LANGE, I. ORCHARD AND S. S. TOBE. *Molecular characterization of the inhibitory myotropic peptide leucomyosuppressin*. PEPTIDES 18(1) 157–163, 1997.—The myoinhibitory peptide leucomyosuppressin (LMS) (pQDVDHVFLRFamide) has been identified and characterized at the molecular level in the cockroach *Diploptera punctata* through analysis of the organization of both brain cDNA and genomic DNA. Processing of the precursor predicted from DNA sequence would release a single LMS peptide. The organization of the precursor appears to be conserved in other insects and may reflect a functional organization for this subfamily of extended FLRFamides. The expression of the LMS gene appears in numerous cells of the pars-intercerebralis of the cockroach protocerebrum as well as in numerous endocrine cells of the midgut. © 1997 Elsevier Science Inc.

Diploptera punctata FLRFamide Leucomyosuppressin Neuropeptide Myotropin Cockroach

MYOTROPINS are a diverse group of neuropeptides that either stimulate or inhibit the contractile activity of visceral muscle. The vast majority of insect myotropic peptides isolated to date stimulate muscle contraction. Several reviews have discussed the isolation, structure–activity, and function of several of these stimulatory peptides, which include FMRFamide-related peptides (FaRPs), kinins, pyrokinins, tachykinins, sulfakinins, and proctolin (4,18,24,25,42,43). The FMRFamide and sulfakinin genes have been cloned from dipterans (10,11,27,28,39) and the sequences have revealed that each precursor contains a family of related peptides that may be processed and simultaneously released. This review will examine the inhibitory myotropic peptides with emphasis on leucomyosuppressin (LMS). LMS is one of a relatively small number of characterized myotropic peptides that inhibit muscle contraction and is part of a subfamily of FMRFamide-related peptides called the myosuppressins. LMS has been characterized at the molecular level in the cockroach *Diploptera punctata* and found to be among a growing number of brain–gut expressed peptides identified in insects.

INHIBITORY MYOTROPIC PEPTIDES

The first inhibitory myosuppressin was isolated from the cockroach *Leucophaea maderae* as an inhibitor of spontaneous

hindgut contraction (17). This peptide (pQDVDHVFLRFamide), termed LMS because of its activity, belongs to a subclass of the FaRPs, a wide-ranging family of structures found throughout the Metazoa (34). Structurally and functionally equivalent peptides have now been identified in other insects including the locusts *Schistocerca gregaria* (35) and *Locusta migratoria* (32,41) (SchistoFLRFamide; PDVDHVFLRFamide), the locust *L. migratoria* (ADVGHVFLRFamide) (32), the tobacco hornworm *Manduca sexta* (ManducaFLRFamide; pQDVVHSFLRFamide) (19), and the flies *Drosophila melanogaster* (29) and *Neobellieria bullata* (14) (TDVDHVFLRFamide). A second type of myoinhibitory peptide that is structurally unique from these myosuppressins has also been isolated from *L. migratoria*. This peptide, termed locustamyoinhibiting peptide or Lom-MIP (AWQDLNAGWamide), suppresses spontaneous contractions in both hindgut and oviducts of *L. migratoria* (40). Recently, two structurally related peptides (AWQDLNSAWamide and GWQDLNSAWamide) were identified from the ventral nerve cord of adult *M. sexta* that significantly reduce or abolish peristalsis in an isolated ilea (anterior hindgut) assay (2). Other peptides, termed allatostatins, that were originally isolated for their ability to inhibit the bio-

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synthesis of juvenile hormone by corpora allata (CA) of cockroaches [reviewed in (6,44)] have also been found to be potent inhibitors of both spontaneous and proctolin-induced contractions of the hindgut of the cockroach *D. punctata* (20). The allatostatins are a family of peptides that are characterized by a common carboxy-terminus -Y/FXFLG/I-NH₂ (where X S, G, D, A, N), which also appears to be the core region required for both activities (6,44). In contrast to Lom-MIP, *D. punctata* allatostatins do not inhibit contraction of oviduct muscle from *D. punctata* or *L. migratoria* (20). *Calliphora vomitoria* also has a structurally related family of peptides known as Leu- or Met-callatostatins dependent on the terminal amino acid (8). Callatostatins do not inhibit juvenile hormone biosynthesis in *C. vomitoria* CA but rather are inhibitors of spontaneous hindgut contractions in both *C. vomitoria* (9) and *D. punctata* (20). Post-translational prolylhydroxylation of either proline in Met-callatostatin (GPPYDFGM-NH₂) increases the potency of this peptide as an inhibitor of spontaneous hindgut contraction (12).

A fourth type of inhibitory myotropin has been isolated and partially sequenced from *L. migratoria* (42,43). The sequence pE?Y?KQSAFNAV-S-NH₂ is structurally unique and exhibits activity as an inhibitor of contraction in *L. maderae* hindgut and *L. migratoria* oviduct.

MOLECULAR CHARACTERIZATION OF LEUCOMYOSUPPRESSIN IN DIPLOPTERA PUNCTATA

A peptide with the character and activity of LMS was identified in *D. punctata* brain extracts by high pressure liquid chromatography (HPLC) using three systems. After initial fractionation on a C-18 reverse-phase column, fractions that were immunoreactive with anti-FMRFamide antisera in a radioimmunoassay (31) and had elution times that corresponded with synthetic LMS were pooled. The pooled fractions were then passed through a Phenyl column run with a 18–60% acetonitrile gradient. The major FMRFamide-immunoreactive peaks coeluting with LMS from this column were pooled and then loaded onto a second Phenyl column run with a 20–29% acetonitrile gradient. The final immunoreactive peak showed the same elution profile as synthetic LMS and was found to inhibit spontaneous contractions of isolated locust oviduct with effects similar to that produced by 10⁻⁶ M LMS (7).

Having detected the presence of a peptide in the brain of this cockroach with the characteristics of LMS, two overlapping degenerate oligonucleotides were designed by reverse translation of the LMS amino acid sequence to amplify the corresponding gene using the polymerase chain reaction (PCR). PCR amplification resulted in a 400-bp DNA fragment representing the 3' portion of the LMS gene, which was then used to screen aliquots of unamplified brain cDNA libraries for homologous clones. Many of the isolated clones were found to contain an insert of approximately 3500 bp, which correlates well with the major band of about 3800 bp observed when Northern filter blots of *D. punctata* brain mRNA were hybridized with the same fragment of the LMS gene (7).

The structure of the LMS transcription unit was investigated by comparing the sizes of PCR amplification products obtained from genomic DNA to those obtained from cDNA. The locations of two introns in the LMS transcript were deduced by this method and the sequences of the resulting intron/exon boundaries determined (Fig. 1). Both introns were found to conform fully with the GT-AG rule defining splice sites of eukaryotic nuclear genes. Inverse PCR (30) was used to determine the boundaries of the first intron (greater than

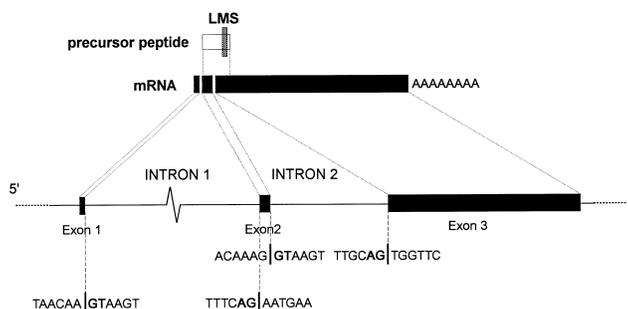


FIG. 1. Organization of the *D. punctata* LMS gene. A schematic representation of the genomic organization of the three exons of the LMS gene is shown in the lower portion of the diagram. The sequence of the splice junctions is shown for each exon/intron boundary. The location of the LMS precursor relative to the mRNA, which is derived from the genomic exon sequences, is shown above. The position of LMS within the precursor is indicated by a stippled box.

9000 bp), as a PCR product could not be generated that spanned this intron. Two overlapping genomic clones have subsequently confirmed the PCR-deduced genomic organization illustrated in Fig. 1 (Lee, unpublished). Sequencing of the LMS cDNA revealed that the 288-bp open reading frame specifying the preproLMS was localized to the 5' end of the 3800-bp transcript. In the genome, the preproLMS is interrupted between the second and third exon by a second intron of approximately 2000 bp. The coding sequence for the mature portion of the peptide is found exclusively on the third exon (Fig. 1). As the amino-terminal region of the precursor is found on a separate exon the possibility that alternative splicing might occur cannot be ruled out. However, no brain cDNAs examined thus far appear to use alternative exons to those shown (Fig. 1). Further investigation of transcripts in other tissues is currently being pursued.

MYOSUPPRESSIN PRECURSORS

The sequence derived from *D. punctata* brain cDNA was found to contain a single open reading frame encoding a preproLMS of 96 amino acids. The amino-terminus of this 10.8-kDa precursor contains either a 17- or 25-amino acid hydrophobic signal peptide (50) found in most eukaryotic secretory proteins. This signal peptide serves to target secretory protein translocation into the lumen of the endoplasmic reticulum after which time the sequence is removed by endoproteolytic cleavage (51). If the signal endoproteolytic cleavage signal sequence after residue 25 is used, further endoproteolytic cleavage at dibasic endoproteolytic cleavage sites would result in three peptides of 18, 35, and 11 amino acids, respectively, with the last peptide being LMS. In searches of Genbank and Swissprot databanks the first 18-amino acid peptide shares weak homology with the FMRFamide gene from the snail *Cepia nemoralis* but does not have the necessary sequence to be carboxy-terminally amidated, as are other FaRPs. The functional significance of the 18- and 35-amino acid peptides is unclear as their sequences have not been conserved in a similar precursor containing a *Manduca*FLRFamide from the moth *Pseudaletia unipuncta* (Fig. 2; Lee, unpublished). In contrast, the carboxy-terminal localization of the 11-amino acid LMS and *Manduca*FLRFamide is conserved between the cockroach and moth precursors (Fig. 2). Potential dibasic (KR) and tribasic (RRR) endoproteolytic cleavage sites surrounding the extended FLRFamide sequences have also been

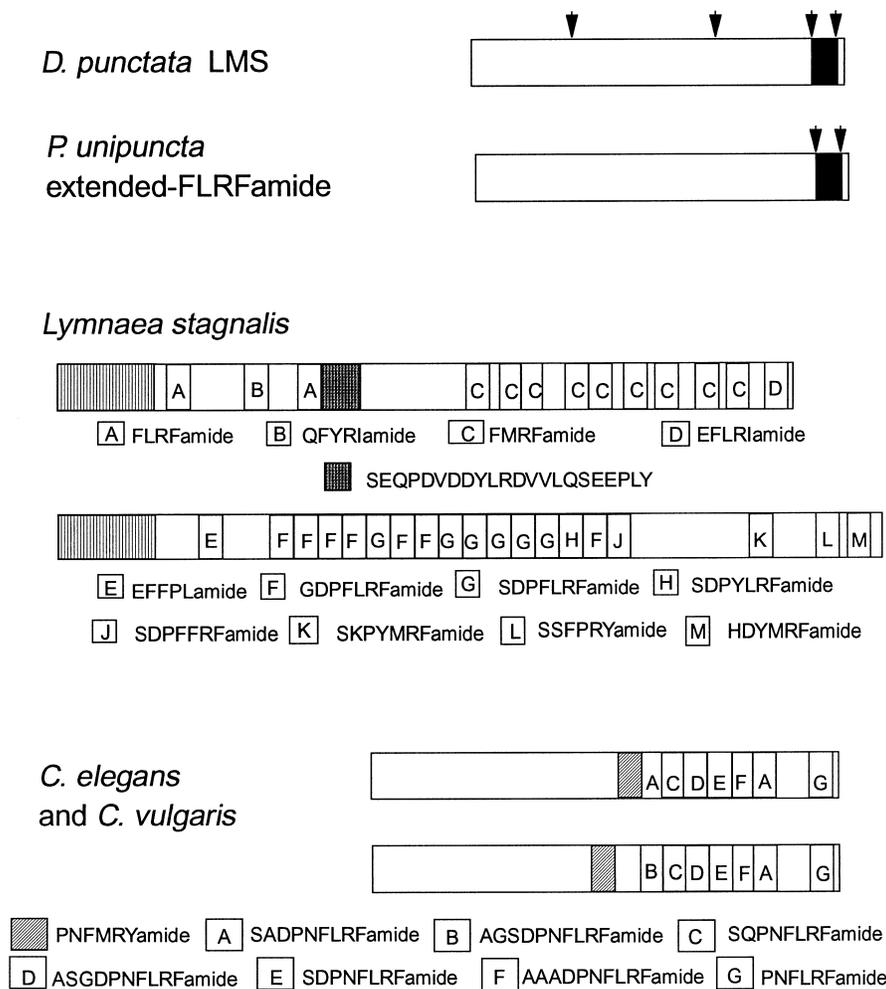


FIG. 2. Schematic representation of various precursor proteins for extended FLRFamides. The organization of the extended FLRFamide specifying precursor in the cockroach *D. punctata* and the moth *P. unipuncta* are similar. Each contains a single peptide, either unmodified LMS or *Manduca*FLRFamide, both of which are found at the C-terminus. The arrows indicate the positions of potential di- or tribasic endoproteolytic cleavage sites. The two precursors of *L. stagnalis* are derived from alternate mRNA splicing. The peptides derived from the precursor after endoproteolytic cleavage and C-terminal amidation are shown below each precursor. Alternative mRNA splicing also results in two extended FLRFamide specifying precursors in *Caenorhabditis*.

conserved. Most prohormones are cleaved at paired dibasic residue sites, primarily RR or KR, within the secretory pathway. Cleavage of the tribasic site would precede carboxyl-terminal amidation that is required for bioactivity of all FaRPs.

COMPARISON WITH OTHER FARP PRECURSORS

The presence of a single peptide, LMS or *Manduca*-FLRFamide, in both the cockroach and moth precursors is unique to this family of peptides. Other known FaRP precursors contain multiple peptides. In *Drosophila*, the FMRFamide precursor contains 10 or 13 FaRPs dependent on the species (26,38,46), and in *D. melanogaster* the drosulfakinin precursor encodes two cholecystokinin-related peptides that terminate in the sequence MRFamide (28). Similarly, the blowflies *C. vomitoria* and *Lucilia cuprina* express precursors that specify 18 FaRPs, 16 of which are potential FMRFamides

and 2 potential FIRFamides (10). Blowflies also express a sulfakinin precursor that contains two sulfakinins and resembles that expressed in *Drosophila* in both organization and peptide sequence (11). The dipteran myosuppressin, TDVDHVFLRFamide, appears to be expressed from a separate gene as this sequence is not found within the FMRFamide or sulfakinin precursors. Possibly the dipteran myosuppressin precursor will resemble that found in the cockroach and moth. Multipetide FMRFamide-containing precursors have also been characterized in the mollusks (1,3,37). Mollusks such as *Lymnaea stagnalis* produce two FaRP-containing precursors by alternate splicing of the gene transcripts (1,3). One precursor contains two FLRFamides and nine FMRFamides and the alternate precursor contains 13 extended-FLRFamides of two types, as well as other extended FaRPs (Fig. 2). *Aplysia californica*, on the other hand, produces only one precursor sharing sequence identity with the *Lymnaea* transcript that en-

codes the tetrapeptides (37). Alternate splicing also generates two transcripts in *Caenorhabditis* species that translate into precursors containing seven extended FLRFamides (36,38) (Fig 2). Genes encoding FaRPs in *Drosophila* and *Caenorhabditis* differ from that of mollusks in that both encode multiple copies of amino-terminally extended versions of the peptides but no copies of the tetrapeptides. As noted above, there is as yet no evidence to suggest that alternative splicing occurs during the expression of the cockroach LMS gene. The organizational variation found in the extended FaRP-containing precursors may reflect a physiological need for greater quantities of some peptides relative to others. Furthermore, myosuppressins appear to represent a functionally different class of extended FLRFamides compared to those identified in the *Lymnaea* and *Caenorhabditis* precursors.

STRUCTURE–ACTIVITY STUDIES

Structure–activity studies on FaRPs have shown that C-terminal amidation is generally required for biological activity (5,33). The terminal RFamide may also be an evolutionarily conserved feature of receptor activation as analogue substitution of these amino acids results in complete loss of biological activity in SchistoFLRFamide (52) and FMRFamide (16). However, exceptions do exist. The *Lymnaea* peptide EFLRFamide has a direct transmitter effect that inhibits the frequency and amplitude of the heart beat, which is directly opposite to the effect of FMRFamide (1). SchistoFLRFamide and the amidated LMS both decrease the amplitude and frequency of myogenic contraction of locust oviducts. This activity is retained by the N-terminally truncated peptide HVFLRFamide but abolished in VFLRFamide (32). Activity reversal or stimulatory activity is seen with this latter peptide. Activity reversal has also been demonstrated using a non-amidated SchistoFLRF. A similar effect results from shortening the N-terminal extension to produce either FLRFamide or FMRFamide or altering the N-terminal extension as in YGGFMRFamide or TNRNFLRFamide (31). Other studies have demonstrated that VFLRFamide is the minimum sequence capable of receptor binding. However, inhibitory biological activity appears to be dependent on the presence of the His residue in HVFLRFamide (52). Similar studies have also emphasized the importance of the His residue for inhibitory activity of LMS in the cockroach hindgut assay (25). In *Lymnaea*, the heptapeptides GDPFLRFamide and SDPFLRFamide, although lacking His, act as interneuronal inhibitory transmitters, hyperpolarizing the postsynaptic follower cell (1). Functional specialization may have selected for different critical residues, because the stimulatory peptide counterparts in this system may be the sequences HDYMRFamide and SKPYMRFamide (1). It may be that the structure–function relationships of residues in LMS and similar molecules may differ for alternate, potentially tissue-specific, functions. For example, *Manduca*FLRFamide increases the force of contraction of a skeletal muscle, the dorsal longitudinal flight muscle, and is likely to promote or sustain flight behavior (19) yet also inhibits contractions of midgut of the sphingid moth, *Agrius convolvuli* (15). Functional differences may also exist in different insect orders. In contrast to the effects on moth flight muscle, LMS was found to inhibit glutamate-mediated neuromuscular transmission in ventral longitudinal skeletal muscle fibers in the mealworm, *Tenebrio molitor* (53).

DISTRIBUTION OF LEUCOMYOSUPPRESSIN AND RELATED MOLECULES

Immunohistochemistry

The similarity of the C-terminal moiety in the FaRPs has made generating specific antisera difficult. As a result, puzzling

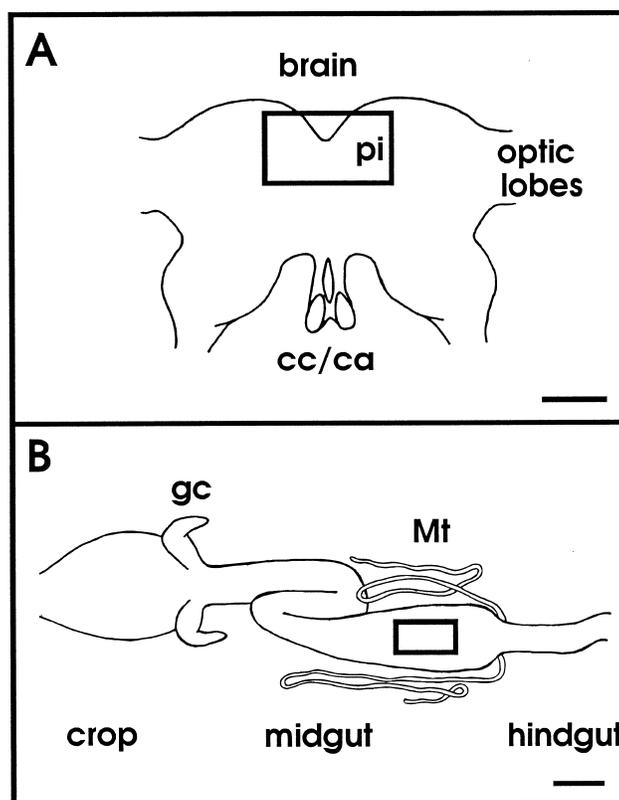


FIG. 3. Schematic representation of the cockroach brain (A) illustrating the position of the pars intercerebralis of the protocerebrum (pi) and the corpora cardiaca/corpora allata complex (cc/ca). Scale bar = 150 μ m. (B) Illustrates the three regions of the cockroach digestive tract with gastric caecae (gc) and Malpighian tubules (Mt) as indicated. Scale bar = 1 mm. The boxes on the tissues in (A) and (B) depict areas shown as photomicrographs in Fig. 4(A) and (B), respectively.

differences in distribution based on the differences in the nature of the antisera used have appeared in the literature. Specific antiserum against the amino-terminus of SchistoFLRFamide (45) produces different cell staining patterns in *S. gregaria* from that produced by an anti-FLRF antiserum (13). Similarly, an anti-SchistoFLRFamide antiserum used in studies against the CNS of *L. migratoria* appear to identify cells in various ganglia (41) that do not appear immunoreactive in *Schistocerca* (45). This may again reflect a difference in antiserum specificity. Anti-LMS antiserum stains eight pairs of medial and five pairs of lateral neurosecretory cells of the stable fly *Stomoxys calcitrans* brain but the majority of immunoreactive neurons are in the thoraco-abdominal ganglia (22). This contrasts with the pattern of expression detected by a specific antiserum raised against the amino-terminus of dromyosuppressin. In *Drosophila*, cells of the medial protocerebrum are detected by anti-TDVDHV as early as the late embryo stage. During development the number of cell types expressing dromyosuppressin increases as cells of the superior protocerebrum and ventral ganglia also participate in expression (21). Similarly, an anti-LMS antiserum detects eight pairs of LMS-immunoreactive cells in the pars intercerebralis of *L. maderae* brain and several cells in the ventro-lateral region of each protocerebral lobe (23). The ventro-lateral immunoreactivity can be competed with anti-FMRFamide and thus in this study it was unclear as to whether these cells actually express LMS.

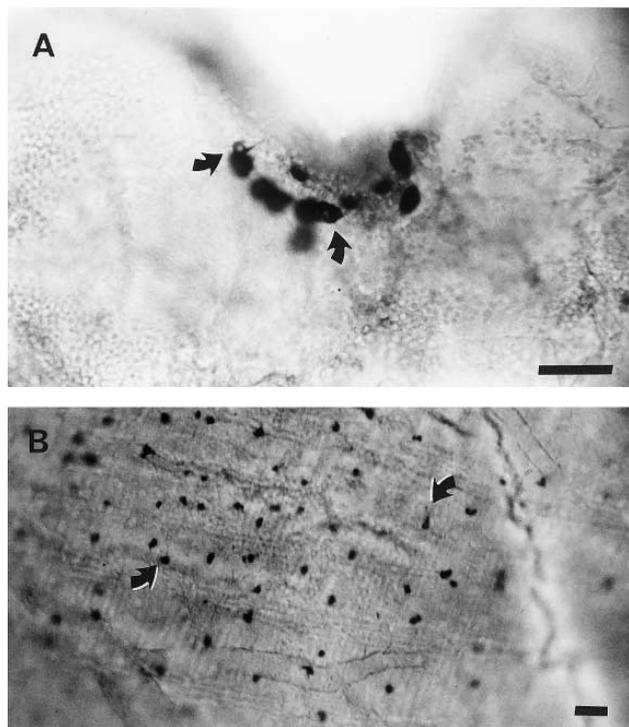


FIG. 4. In situ hybridization of whole-mount preparations of tissues from *D. punctata*. (A) Positive cell bodies (arrows) in the pars intercerebralis of the protocerebrum after hybridization with DIG-labeled *Diploptera* LMS cDNA (7). (B) Positive cell bodies (arrows) in the posterior midgut after hybridization. Scale bar is 50 μ M.

Using an anti-FMRFamide antiserum, which cross-reacts with LMS, 30 to 35 medial cells of the *D. punctata* protocerebrum, two lateral cells and three cells near the optic lobes were found to be immunoreactive (7).

In Situ Hybridization

The availability of LMS gene sequences allows detection of cells that are expressing LMS-specific mRNA transcripts through in situ hybridization (47). An LMS gene probe detects only a single gene and one major gene transcript in Southern and Northern filter-blot hybridizations, respectively (7). In situ hybridization (47) using the *D. punctata* LMS gene to detect specific mRNA expression demonstrated that approximately 15–20 cells of the pars intercerebralis express the LMS gene (7) [Figs.

3(A), 4(A)]. As transcript detection is specific to LMS sequences, the additional cells in the protocerebral region detected by anti-FMRFamide antiserum can be attributed to the expression of other FaRPs. This is further emphasized by the appearance of multiple FMRFamide-like immunoreactive peaks after HPLC separation of brain extracts. Two lateral cells and one cell near the optic lobe also express LMS mRNA. Some cells in the deutocerebra and tritocerebra appear to contain LMS mRNA but are not immunoreactive to anti-FMRFamide antiserum. This may suggest that, in these cells, the LMS precursor is not processed immediately, is processed and the products rapidly transported to other areas, or is processed in limited amounts. The presence of numerous immunoreactive axonal processes in the region of these nonimmunoreactive cell bodies may suggest that rapid processing and transport is taking place. In situ hybridization with the LMS gene also detects LMS-specific mRNA transcripts in numerous endocrine cells of the *D. punctata* posterior midgut [Figs. 3(B), 4(B)]. The LMS mRNA expression only occurred in a subfraction of endocrine cells that display FMRFamide-like immunoreactivity, indicating that there are other FaRPs likely to be present. This has recently been corroborated by the sequencing of a novel RFamide peptide from the midgut of the cockroach, *Periplaneta americana* (49). Although there is little understanding of the physiological role of the LMS-expressing endocrine cells in midgut of *Diploptera*, it is worth noting that in the weevil, *Rynchophorus ferrugineus*, LMS stimulates the release of the digestive enzyme alpha-amylase into the lumen of ligated midgut (26). A similar pattern of *D. punctata* midgut endocrine cell expression was also detected by in situ hybridization with the allatostatin gene (54). As LMS and allatostatin both have myoinhibitory activity their expression and actions may be coordinated. Interestingly, a colocalization of FaRPs and allatostatin-like immunoreactivity has been demonstrated in the lateral heart nerve of *Periplaneta* (48). Anti-FMRFamide antisera also detects the presence of FaRPs in numerous endocrine cells in *D. punctata* (not shown) and resembles the pattern shown when using a similar antisera with midguts of *Blaberus* (55) and *Manduca* (56). Surprisingly, anti-dromyosuppressin antibodies do not stain cells of the proventriculus, midgut, or hindgut in *Drosophila* but rather stain only two cells of the rectum (21). This again suggests that peptides with very similar structures may have adapted unique functions through evolution. A clearer picture of species-specific expression pattern variation will emerge as FaRP family genes become available to complement antibody studies.

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REFERENCES

1. Benjamin, P. R.; Burke, J. F. Alternative mRNA splicing of the FMRFamide gene and its role in neuropeptidergic signalling in a defined neural network. *Bioessays* 16:335–342; 1994.
2. Blackburn, M. B.; Wagner, R. M.; Kochansky, J. P.; Harrison, D. J.; Thomas-Laemont, P.; Raina, A. K. The identification of two myoinhibitory peptides, with sequence similarities to the galanins, isolated from the ventral nerve cord of *Manduca sexta*. *Regul. Pept.* 57:213–220; 1995.
3. Bright, K.; Kellett, E.; Saunders, S. E.; Brierley, M.; Burke, J. F.; Benjamin, P. R. Mutually exclusive expression of alternatively spliced FMRFamide transcripts in identified neuronal systems of the snail *Lymnaea*. *J. Neurosci.* 13:2719–2729; 1993.
4. Cook, B. J.; Wagner, R. M. Myotropic neuropeptides: Physiological and pharmacological actions. In: Menn, J. J., Ed. *Insect neuropeptides: Chemistry, biology, and action*. Washington, DC: American Chemical Society; 1991:51–64.
5. Cuthbert, B. A.; Evans, P. D. A comparison of the effects of FMRFamide-like peptides on locust heart and skeletal muscle. *J. Exp. Biol.* 144:395–415; 1989.
6. Donly, B. C.; Ding, Q.; Tobe, S. S.; Bendena, W. G. Molecular cloning of the gene for the allatostatin family of neuropeptides from the cockroach *Diploptera punctata*. *Proc. Natl. Acad. Sci. USA* 90:8807–8811; 1993.
7. Donly, B. C.; Fuse, M.; Orchard, I.; Tobe, S. S.; Bendena, W. G. Characterization of the gene for leucomyosuppressin and its expres-

- sion in the brain of the cockroach *Diploptera punctata*. Insect Biochem. Mol. Biol. (in press).
8. Duve, H.; Johnsen, A. H.; Scott, A. G.; Yu, C. G.; Yagi, K. J.; Tobe, S. S.; Thorpe, A. Callatostatin: Neuropeptides from the blowfly *Calliphora vomitoria* with sequence homology to cockroach allatostatins. Proc. Natl. Acad. Sci. USA 90:2456–2460; 1993.
 9. Duve, H.; Thorpe, A. Distribution and functional significance of Leu-callatostatins in the blowfly *Calliphora vomitoria*. Cell Tissue Res. 276:367–379; 1994.
 10. Duve, H.; Johnsen, A. H.; East, P.; Thorpe, A. Comparative aspects of the FMRFamides of blowflies: Isolation of the peptides, genes, and functions. In: Davey, K. G.; Peter, R. E.; Tobe, S. S., Eds. Perspectives in comparative endocrinology. Ottawa: National Research Council of Canada; 1994:91–96.
 11. Duve, H.; Thorpe, A.; Scott, A. G.; Johnsen, A. H.; Rehfeld, J. F.; Hines, E.; East, P. D. The sulfakinins of the blowfly *Calliphora vomitoria*. Peptide isolation, gene cloning and expression studies. Eur. J. Biochem. 232:633–640; 1995.
 12. Duve, H.; Johnsen, A. H.; Scott, A. G.; Thorpe, A. Isolation, identification and functional significance of [Hyp²]Met-callatostatin and des Gly-Pro Met-callatostatin, two further posttranslational modifications of the blowfly neuropeptide Met-callatostatin. Regul. Pept. 57:237–245; 1995.
 13. Evans, P. D.; Cournil, I. Co-localization of FLRF- and vasopressin-like immunoreactivity in a single pair of sexually dimorphic neurons in the nervous system of the locust. J. Comp. Neurol. 292:331–448; 1990.
 14. Fonagy, A.; Schoofs, L.; Proost, P.; Van Damme, J.; Bueds, H.; De Loof, A. Isolation, primary structure and synthesis of neomyosuppressin, a myoinhibiting neuropeptide from the grey fleshfly, *Neobellieria bullata*. Comp. Biochem. Physiol. [C] 102:239–245; 1992.
 15. Fujisawa, Y.; Shimoda, M.; Kiguchi, K.; Ichikawa, T.; Fujita, N. The inhibitory effect of a neuropeptide, *Manduca*FLRFamide, on the midgut activity of the sphingid moth, *Agrius convolvuli*. Zool. Sci. 10:773–777; 1993.
 16. Gerachty, R. F.; Irvine, G. B.; Williams, C. H.; Cottrell, G. A. Biological activity and receptor binding properties of some C-terminally modified analogues of FMRFamide. Peptides 15:73–81; 1991.
 17. Holman, G. M.; Cook, B. J.; Nachman, R. J. Isolation, primary structure and synthesis of leucomyosuppressin, an insect neuropeptide that inhibits spontaneous contractions of the cockroach hindgut. Comp. Biochem. Physiol. [C] 85:329–333; 1986.
 18. Holman, G. M.; Nachman, R. J.; Wright, M. S.; Schoofs, L.; Hayes, T. K.; DeLoof, A. Insect myotropic peptides. In: Menn, J. J., Ed. Insect neuropeptides: Chemistry, biology, and action. Washington, DC: American Chemical Society; 1991:40–50.
 19. Kingan, T. G.; Teplow, D. B.; Phillips, J. M.; Riehm, J. P.; Rao, K. R.; Hildebrand, J. G.; Homberg, U.; Kammer, A. E.; Jardine, I.; Griffin, P. R.; Hunt, D. F. A new peptide in the FMRFamide family isolated from the CNS of the hawkmoth, *Manduca sexta*. Peptides 11:849–856; 1990.
 20. Lange, A. B.; Bendena, W. G.; Tobe, S. S. The effect of thirteen dip-allatostatins on myogenic and induced contractions of the cockroach (*Diploptera punctata*) hindgut. J. Insect Physiol. 41:581–588; 1995.
 21. McCormick, J.; Nichols, R. Spatial and temporal expression identify dromyosuppressin as a brain–gut peptide in *Drosophila melanogaster*. J. Comp. Neurol. 338:279–288; 1993.
 22. Meola, S. M.; Wright, M. S.; Holman, G. M.; Thompson, J. M. Localization of leucomyosuppressin-like peptides in the central nervous system of the stable fly with immunocytochemistry. J. Med. Entomol. 28:712–718; 1991.
 23. Meola, S. M.; Wright, M. S.; Holman, G. M.; Thompson, J. M. Immunocytochemical localization of leucomyosuppressin-like peptides in the CNS of the cockroach, *Leucophaea maderae*. Neurochem. Res. 16:543–549; 1991.
 24. Nachman, R. J.; Holman, G. M. Myotropic insect neuropeptide families from the cockroach *Leucophaea maderae*. Structure–activity relationships. In: Menn, J. J., Ed. Insect neuropeptides: Chemistry, biology and action. Washington, DC: American Chemical Society; 1991:194–214.
 25. Nachman, R. J.; Holman, G. M.; Hayes, T. K.; Beier, R. C. Structure–activity relationships for inhibitory insect myosuppressins: Contrast with the stimulatory sulfakinins. Peptides 14:665–670; 1993.
 26. Nachman, R. J.; Favrel, P.; Sreekumar, S.; Holman, G. M. Insect myosuppressins and sulfakinins stimulate release of the digestive enzyme alpha-amylase in two invertebrates: The scallop *Pectin maximus* and insect *Rynchophorus ferrugineus*. In: Strand, F.; Beckwith, W.; Sandman, C., Eds. New York: New York Academy of Science (in press).
 27. Nambu, J. R.; Murphy–Erdosh, C.; Andrews, P. C.; Feistner, G. J.; Scheller, R. H. Isolation and characterization of a *Drosophila* neuropeptide gene. Neuron 1:55–61; 1988.
 28. Nichols, R.; Schneuwly, S. A.; Dixon, J. E. Identification and characterization of a *Drosophila* homologue to the vertebrate neuropeptide cholecystokinin. J. Biol. Chem. 263:12167–12170; 1988.
 29. Nichols, R. Isolation and structural characterization of *Drosophila* TDVDHVFLRFamide and FMRFamide-containing neural peptides. J. Mol. Neurosci. 3:213–218; 1992.
 30. Ochman, H.; Medhora, M. M.; Garza, D.; Hartl, D. L. Amplification of flanking sequences by inverse PCR. In: Gelfand, M. A.; Sninsky, D. H.; White, T. J., Eds. San Diego: Academic Press; 1990:219–227.
 31. Peeff, N. M.; Orchard, I.; Lange, A. B. The effects of FMRFamide-related peptides on an insect (*Locusta migratoria*) visceral muscle. J. Insect Physiol. 39:207–215; 1993.
 32. Peeff, N. M.; Orchard, I.; Lange, A. B. Isolation, sequence, and bioactivity of PDVDHVFLRFamide and ADVGHVFLRFamide peptides from the locust central nervous system. Peptides 15:387–392; 1994.
 33. Price, D. A.; Greenberg, M. J. The pharmacology of the molluscan cardioexcitatory neuropeptide FMRFamide. Gen. Pharmacol. 11:237–241; 1980.
 34. Price, D. A.; Greenberg, M. J. The hunting of FaRPs: The distribution of FMRFamide-related peptides. Biol. Bull. 177:198–205; 1989.
 35. Robb, S.; Packman, L. C.; Evans, P. D. Isolation, primary structure and bioactivity of SchistoFLRF-amide, a FMRF-amide-like neuropeptide from the locust, *Schistocerca gregaria*. Biochem. Biophys. Res. Commun. 160:850–856; 1989.
 36. Rosoff, M. L.; Burglin, T. R.; Li, C. Alternatively spliced transcripts of the *ftp-1* gene encode distinct FMRFamide-like peptides in *Caenorhabditis elegans*. J. Neurosci. 12:2356–2361; 1992.
 37. Schaefer, M.; Picciotto, M. R.; Kreiner, T.; Kaldany, R. R.; Taussig, R.; Scheller, R. H. *Aplysia* neurons express a gene encoding multiple FMRFamide neuropeptides. Cell 41:457–467; 1985.
 38. Schinkmann, K.; Li, C. Comparison of two *Caenorhabditis* genes encoding FMRFamide (Phe-Met-Arg-Phe-NH₂)-like peptides. Mol. Brain Res. 24:238–246; 1994.
 39. Schneider, L. E.; Taghert, P. H. Isolation and characterization of a *Drosophila* gene that encodes multiple neuropeptides related to Phe-Met-Arg-Phe-NH₂ (FMRFamide). Proc. Natl. Acad. Sci. USA 85:1993–1997; 1988.
 40. Schoofs, L.; Holman, G. M.; Hayes, T. K.; Nachman, R. J.; De Loof, A. Isolation, identification and synthesis of locustamyoinhibiting peptide (Lom-MIP), a novel biologically active neuropeptide from *Locusta migratoria*. Regul. Pept. 36:111–119; 1991.
 41. Schoofs, L.; Holman, G. M.; Paemen, L.; Velaert, D.; Amelincx, M.; De Loof, A. Isolation, identification, and synthesis of PDVDHVFLRFamide (SchistoFLRFamide) in *Locusta migratoria* and its association with the male accessory glands, the salivary glands, the heart, and the oviduct. Peptides 14:409–421; 1993.
 42. Schoofs, L.; Vanden Broek, J.; De Loof, A. The myotropic peptides of *Locusta migratoria*: structures, distribution, functions and receptors. Insect Biochem. Mol. Biol. 23:859–881; 1993.
 43. Schoofs, L.; Holman, G. M.; Nachman, R. J.; Hayes, T. K.; De Loof, A. Structure, function, and distribution of insect myotropic peptides. In: Davey, K. G.; Peter, R. E.; Tobe, S. S., Eds. Perspectives in comparative endocrinology. Ottawa: National Research Council of Canada; 1994:155–165; 1994.
 44. Stay, B.; Tobe, S. S.; Bendena, W. G. Allatostatins: Recent progress in structure, function and distribution. Adv. Insect Physiol. 25:267–338; 1994.

45. Swales, L. S.; Evans, P. D. Distribution of SchistoFLRFamide-like immunoreactivity in the adult ventral nervous system of the locust, *Schistocerca gregaria*. *Cell Tissue Res.* 281:339–348; 1995.
46. Taghert, P. H.; Schneider, L. E. Interspecific comparison of a *Drosophila* gene encoding FMRFamide-related neuropeptides. *J. Neurosci.* 10:1929–1942; 1990.
47. Tautz, D.; Pfeifle, C. A nonradioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* 98:81–85; 1989.
48. Ude, J.; Agricola, H. FMRFamide-like and allatostatin-like immunoreactivity in the lateral heart nerve of *Periplaneta americana*: Colocalization at the electron-microscope level. *Cell Tissue Res.* 282:69–80; 1995.
49. Veenstra, J. A.; Lambrou, G. Isolation of a novel RFamide peptide from the midgut of the american cockroach, *Periplaneta americana*. *Biochem. Biophys. Res. Commun.* 213:519–524; 1995.
50. von Heijne, G. A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* 14:4683–4690; 1986.
51. Walter, P.; Blobel, G. Translocation of proteins across the endoplasmic reticulum. II. Signal recognition protein (SRP) mediates the selective binding to microsomal membranes of in vitro-assembled polysomes synthesizing secretory proteins. *J. Cell Biol.* 91:551–556; 1981.
52. Wang, Z.; Orchard, I.; Lange, A. B. Binding affinity and physiological activity of some HVFLRFamide analogues on the oviducts of the locust, *Locusta migratoria*. *Regul. Pept.* 57:339–346; 1995.
53. Yamamoto, D.; Ishikawa, S.; Holman, G. M.; Nachman, R. J. Leucomyosuppressin, a novel insect neuropeptide, inhibits evoked transmitter release at the mealworm neuromuscular junction. *Neurosci. Lett.* 95:137–142; 1988.
54. Yu, C. G.; Stay, B.; Ding, Q.; Bendena, W. G.; Tobe, S. S. Immunocytochemical localization and expression of allatostatins in the midgut of *Diptera punctata*. *J. Insect Physiol.* 41:1035–1043; 1995.
55. Zitnan, D.; Sauman, I.; Sehnal, F. Peptidergic innervation and endocrine cells of insect midgut. *Arch. Insect Biochem. Physiol.* 22:113–132; 1993.
56. Zitnan, D.; Kingan, T. G.; Beckage, N. E. Parasitism-induced accumulation of FMRFamide-like peptides in the gut innervation and endocrine cells of *Manduca sexta*. *Insect Biochem. Mol. Biol.* 25:669–678; 1995.