Biochemical and Biophysical Research Communications **282,** 124–130 (2001)
doi:10.1006/bbrc.2001.4565, available online at http://www.idealibrary.com on IDEAL®

Molecular Cloning, Genomic Organization, and Expression of a C-Type (*Manduca sexta*-Type) Allatostatin Preprohormone from *Drosophila melanogaster*[1]

Michael Williamson,* Camilla Lenz,* Åsa M. E. Winther,†
Dick R. Nässel,† and Cornelis J. P. Grimmelikhuijzen*,[2]

**Department of Cell Biology, Zoological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark; and †Department of Zoology, Stockholm University, SE-10691, Stockholm, Sweden*

Received January 31, 2001

The insect allatostatins are a diverse group of neuropeptides that obtained their names by their inhibitory actions on the *corpora allata* (two endocrine glands near the insect brain), where they block the biosynthesis of juvenile hormone (a terpenoid important for development and reproduction). Chemically, the allatostatins can be subdivided into three different peptide groups: the large group of A-type (cockroach-type) allatostatins, which have the common C-terminal sequence Y/FXFGLamide; the B-type (cricket-type) allatostatins, which have the C-terminal sequence W(X_6)Wamide in common; and a single allatostatin that we now call C-type allatostatin that was first discovered in the moth *Manduca sexta,* and which has a nonamidated C terminus, and a structure unrelated to the A- and B-type allatostatins. We have previously cloned the preprohormones for the A- and B-type allatostatins from *Drosophila melanogaster*. Here we report on the cloning of a *Drosophila* C-type allatostatin preprohormone (DAP-C). DAP-C is 121 amino acid residues long and contains one copy of a peptide sequence that in its processed form has the sequence <EVRYRQCYFNPISCF (drostatin-C). This drostatin-C sequence is only one amino acid residue different (F → Y in position 4) from the *Manduca sexta* C-type allatostatin. The DAP-C gene has three introns and four exons and is located at position 32D2-3 on the left arm of the second chromosome. Northern blots show that the gene is strongly expressed in larvae and adult flies, but less in pupae and embryos. *In situ* hybridizations of larvae show that the gene is expressed in various neurons of the brain and abdominal ganglia and in endocrine cells of the midgut. This is the first publication on the structure of a C-type allatostatin from insects other than moths and the first report on the presence of all three types of allatostatins in a representative of the insect order Diptera (flies). © 2001 Academic Press

The allatostatins are an important group of insect neurohormones that obtained their names, because of their inhibitory actions on the *corpora allata* (two endocrine organs near the insect brain), where they block the biosynthesis of juvenile hormone (a terpenoid regulating development and reproduction) (1, 2). Structurally, the allatostatins can be subdivided into three fully different peptide groups: the A-type allatostatins, first discovered in cockroaches (3, 4) that have the C terminus Y/FXFGLamide in common; the B-type allatostatins, first discovered in crickets (5) that all have the C terminus W($X)_6$Wamide; and a single allatostatin, first discovered in the moth *Manduca sexta* (6), that we now call the C-type allatostatin. This C-type (or *Manduca sexta*-type) allatostatin has a nonamidated C terminus and a structure unrelated to the A- and B-type allatostatins. We have recently cloned the preprohormones for the A- and B-type allatostatins from *Drosophila* (7, 8) and also, together with another group, two potential receptors for the *Drosophila* A-type allatostatins (9–11). In the present report, we describe the cloning of a C-type allatostatin preprohormone from *Drosophila*.

MATERIALS AND METHODS

Drosophila (Canton S strain) poly(A)$^+$ RNA and cDNA was prepared as previously described (9, 12). The initial oligonucleotide primers used in PCR were sense 5′-TATTGTGCTACGGCCTACTCC-3′ (corresponding to positions 23 to 43 of Fig. 2) and antisense 5′-CTAACTTGACGTTTGCTCTCG-3′ (corresponding to positions 300 to 320 of Fig. 2). cDNA form 3rd instar larvae was used as a template. The PCR program was 94°C for 3 min, then touchdown PCR for 10 cycles, 95°C for 30 s, 65°C for 45 s, decreasing 1°C for each cycle and 68°C for 2 min,

[1] The nucleotide sequences reported in this paper have been submitted to the GenBank Data Bank under Accession Nos. AF312939 and AF346433.

[2] To whom correspondence should be addressed. Fax: +45-35321200. E-mail: cgrimmelikhuijzen@zi.ku.dk.

0006-291X/01 $35.00

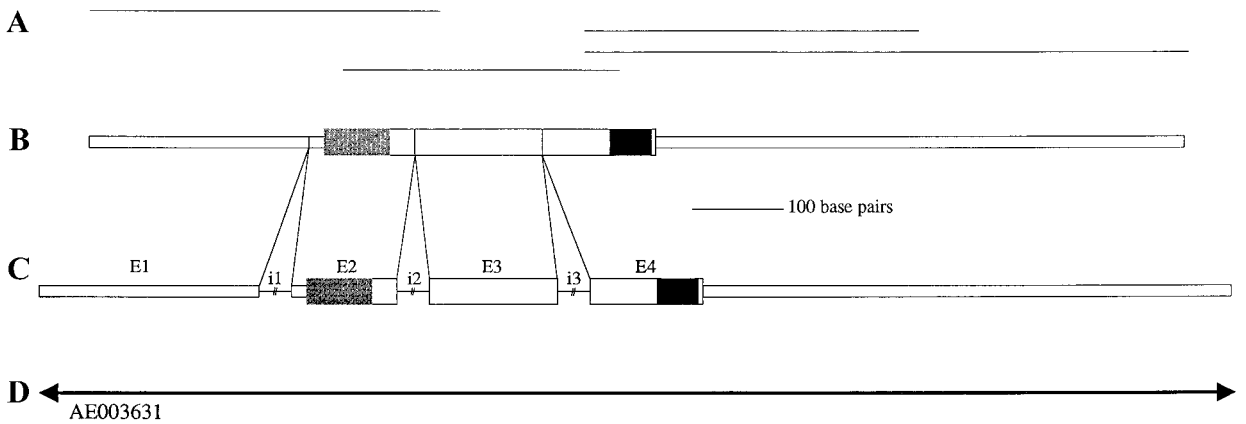

FIG. 1. Schematic representation of the *Drosophila* C-type allatostatin preprohormone cDNA, the intron/exon organization of its gene and the positions of the genomic and PCR clones. (A) The position of the PCR clones. The lowest clone is the one obtained in the original PCR, the two middle clones were obtained by 3′-RACE and the upper clone by 5′-RACE. (B) The cDNA coding for the *Drosophila* C-type allatostatin preprohormone. The drostatin-C sequence is highlighted in black and the signal peptide in grey. (C) The gene coding for the *Drosophila* C-type allatostatin preprohormone. The introns are given as lines and marked i1–i3. They are not drawn to scale. The exons are given as bars, being broad for the coding regions and small for the noncoding regions, and are marked E1–E4. (D) Position of the genomic clone from the "*Drosophila* Genome Project" with Accession No. AE003631.

followed by 35 cycles of 95°C for 30 s, 55°C for 45 s, 68°C for 2 min and a final extension of 68°C for 10 min. 3′-RACE reactions were made with the above sense primer and the nested sense primer 5′-ACAGCGCCTATTTGAGGAGTC-3′ (corresponding to positions 242 to 262 of Fig. 2). 5′-RACE reactions were made with the above antisense primer and nested antisense primer 5′-TGGCCATCAAGTCCATCGGAG-3′ (corresponding to positions 102 to 122 of Fig. 2). pCR4-TOPO (Invitrogen) was used for the cloning. Sequencing reactions were performed as described in the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing kit (Amersham). Northern blots were carried out as in (12), using a cDNA probe corresponding to nucleotide positions 19–324 of Fig. 2. cDNA probes coding for RP49 were obtained as described in (13). *In situ* hybridizations were carried out as in (14), using digoxigenin-labeled antisense ribonucleotide probes, corresponding to positions 241–654 of Fig. 2. Staining was with alkaline phosphatase-coupled antidigoxigenin Fab fragments, followed by color development with nitroblue tetrazoleum.

RESULTS

Cloning of the Drosophila C-type allatostatin preprohormone. We screened the "*Drosophila* Genome Project" database with an "electronic probe" corresponding to the unprocessed *Manduca sexta* C-type allatostatin (QVRFRQCYFNPISCF) (6). This resulted in the alignment with sequences from gene CG14919 (Accession No. AAF53062, located on a BAC clone with Accession No. AE003631), of which the function was mentioned to be unknown. We subsequently designed primers against the presumed exons of this gene and carried out PCR, using *Drosophila* cDNA as a template. This resulted in the amplification of a part of the gene, showing that the gene was indeed expressed (Fig. 1A). Finally, after 3′- and 5′-RACE PCR, we obtained the complete cDNA coding for an allatostatin preprohormone (Figs. 1A and B).

The cDNA coding for *Drosophila* C type allatostatin preprohormone (DAP-C) consists of two species, one being 912 nucleotides long (nucleotide positions −258 to 654 of Fig. 2), the other 1202 nucleotides long (nucleotide positions −258 to 944 of Fig. 2) [both numbers, excluding the poly(A^+) tail]. Both cDNA species could be amplified using 3′-RACE PCR and are identical in the coding region (which is 363 nucleotides long), and 5′-untranslated region (which is 258 nucleotides long), but differ in their 3′-(noncoding) regions by the use of two different polyadenylylation signals (Fig. 2).

The cDNA codes for a protein of 121 amino acid residues (Fig. 2). We propose that the first ATG triplet in the open reading frame is the start codon, because it is preceded by several in-frame stop codons and followed by a signal sequence for RER membrane translocation. This signal sequence is probably cleaved off at Ala-24 (15). The protein contains the unprocessed sequence of a C-type allatostatin that is located at the very C terminus (Fig. 2). After endoproteolytic cleavage at a dibasic (KR) cleavage site, removal of the two C-terminal basic residues (RK) by a carboxypeptidase specific for basic residues, and cyclization of the N-terminal Gln residue into a <Glu (<E) group (16–18), the mature structure of the C-type allatostatin would be <EVRYRQCYFNPISCF (Fig. 2). This *Drosophila* C-type allatostatin (drostatin-C) is only one amino acid residue different from the *Manduca sexta* C-type allatostatin, the Phe residue in position 4 being exchanged for another aromatic residue, Tyr (Table 1).

The DAP-C gene. Alignment of the DAP-C cDNA (Fig. 2) with the genomic BAC clone AE003631 from the "*Drosophila* Genome Project," reveals the presence of three introns and four exons in the DAP-C gene (Fig. 1C: Table 2). This alignment also reveals that the original intron/exon organization of the putative Gene

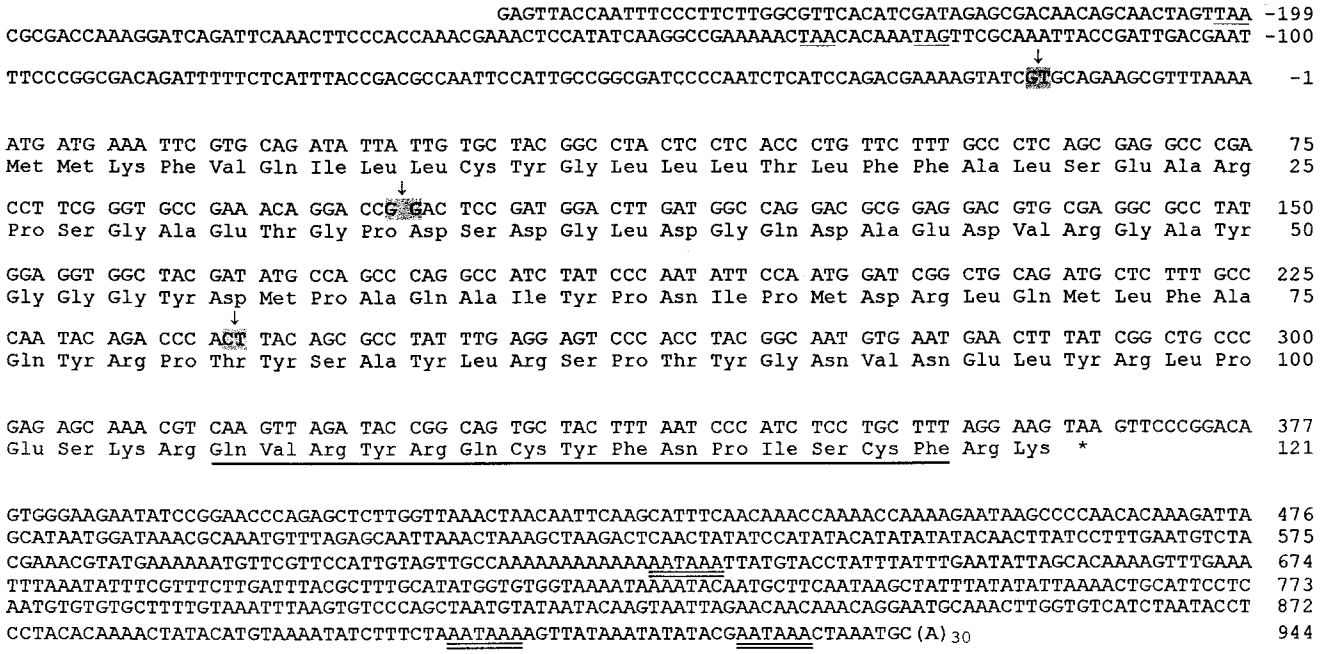

FIG. 2. cDNA and deduced amino acid sequence of the *Drosophila* C-type allatostatin preprohormone. The cDNA is composed of several clones (Fig. 1A). Nucleotides are numbered from 5′- to 3′-end and the amino acid residues are numbered starting with the first ATG codon in the open reading frame. The three introns are indicated by arrows and the exon nucleotides, bordering these introns, are highlighted in grey. The drostatin-C sequence is underlined by a bold line. The translation termination codon is indicated by an asterisk. In-frame stop codons in the 5′-noncoding region are underlined by thin lines. Three putative polyadenylylation signals in the 3′-noncoding region that are part of two cloned cDNAs [one comprising nucleotide positions −258 to 654, the other −258 to 944 of Fig. 2; both having an additional poly(A^+) tail] are underlined twice.

CG14919, annotated by the "*Drosophila* Genome Project" (Accession No. AAF53062), was not fully correct. Furthermore, this comparison reveals the existence of a small number of nucleotide differences between the cDNA and genomic DNA coding for DAP-C. These differences, however, do not lead to changes in amino acid residues in the preprohormone (Table 3).

The "*Drosophila* Genome Project" has located the genomic BAC clone AE003631, which contains the DAP-C gene, to position 32D2-3 on the left arm of the second chromosome from *Drosophila*.

Comparison of DAP-C with other proteins. The first discovered C-type allatostatin peptide (Mas-AST; see Table 1) has been isolated and sequenced from the moth *Manduca sexta* (6), but its preprohormone has never been cloned from this insect. However, from a related moth, the true armyworm *Pseudaletia unipuncta,* a C-type allatostatin preprohormone has been cloned (19). This C-type *Pseudaletia* allatostatin preprohormone (PAP-C) contains an immature C-type allatostatin sequence that, after processing, would be identical to the *Manduca sexta* C-type allatostatin (Table 1). Alignment of the DAP-C preprohormone from *Drosophila* and PAP-C from *Pseudaletia,* shows that the two C termini, containing the C-type allatostatin sequences and their processing sites, are nearly the same (Fig. 3). The two preprohormones are about equally long, but do not contain other regions with

TABLE 1

Alignment of Drostatin-C with the C-type Allatostatins from the Moths *Manduca sexta* and *Pseudaletia unipuncta*

Structure	Name
<Gln-Val-Arg-Tyr-Arg-Gln-Cys-Tyr-Phe-Asn-Pro-Ile-Ser-Cys-Phe	Drostatin-C
<Glu-Val-Arg-Phe-Arg-Gln-Cys-Tyr-Phe-Asn-Pro-Ile-Ser-Cys-Phe	*Manduca sexta* allatostatin (Mas-AST)
<Gln-Val-Arg-Phe-Arg-Gln-Cys-Tyr-Phe-Asn-Pro-Ile-Ser-Cys-Phe	*Pseudaletia unipuncta* allatostatin

Note. The common amino acid residues between mature drostatin-C (derived from DAP-C; Fig. 2), the isolated and sequenced *Manduca sexta* allatostatin (MAS-AST) (6), and the mature *Pseudaletia unipuncta* allatostatin sequence (derived from its preprohormone; Fig. 3) (19), are highlighted by boxes. From Mas-AST it is known, that the two Cys residues form a bridge (6), so it can be assumed that the other two peptides are cyclic as well.

TABLE 2

Intron/Exon Boundaries and Intron Sizes of the DAP-C Gene

Intron	5′-Donor	Intron size (bp)	3′-Acceptor	Intron phase
1	TCT gtaagta.....	3915	.....ctttcag TGC	—
2	CCG gtgagta.....	56	.....atttaag GAC	3
	Pro		Asp	
3	AC gtaaggc.....	65	.....atagcag T	2
	Thr		Thr	

Note. The nucleotide sequence of each of the intron/exon boundaries is shown, as well as the resulting amino acid residues. Uppercase and lowercase letters represent nucleotides in the exons and introns, respectively. The sequences of the introns can be retrieved from the GenBank Data Bank, Accession No. AE003631. The overall position of the introns are shown in Figs. 1C and 2.

significant sequence identities. No significant sequence identities exist between the sequences of DAP-C and that of other proteins stored in the Swissprot or GenBank databases.

Expression of the DAP-C gene. Northern blot analyses show that the DAP-C gene is expressed in all developmental stages, with highest expression in larvae and adult flies, followed by pupae and embryos (Fig. 4). The Northern blots also show that adult flies and larvae express a higher MW (1.3 kb) mRNA species slightly stronger than a lower MW species (1.0 kb), whereas in pupae and especially in embryos the lower MW species is almost absent. Finally, the blots also show that the two hybridizing mRNA species correspond very well in size to our two cloned DAP-C cDNAs (Fig. 2).

In situ hybridizations of 3rd instar larvae show about 9 pairs of symmetrically localized nerve cells in the protocerebral and 3 pairs in the tritocerebral parts of the brain, expressing the DAP-C gene (Figs. 5A and 5E). About 15 nerve cell pairs are localized in different parts of the abdominal ganglia (Figs. 5A and 5E). Strong expression of the DAP-C gene was also observed in endocrine cells of the posterior part of the midgut. These cells are located in the basal part of the epithelium and extend towards the gut lumen (Figs. 5B–5D). Controls, using sense probes, gave no staining.

DISCUSSION

The only known representative of the *Manduca sexta*-type (or C-type) allatostatins has, so far, been isolated and sequenced from the moth *Manduca sexta* (6), and its preprohormone has subsequently been cloned from a related moth, the true armyworm *Pseudaletia unipuncta* (19) (Table 1). Our present paper on the existence of a C-type allatostatin preprohormone in the fruitfly *Drosophila*, therefore, is the first report on the structure of a C-type allatostatin (drostatin-C) in an insect other than moths (Table 1). The actions of drostatin-C in *Drosophila* are unknown, but one obvious possible role of this peptide may be that it inhibits the biosynthesis of juvenile hormone in the *corpora allata*. Drostatin-C is only one amino acid residue different from the *Manduca sexta* C-type allatostatin, the difference being the exchange of the aromatic Phe for the aromatic Tyr residue (Table 1). This amazing sequence conservation between the *Manduca* and *Drosophila* neuropeptides, makes it almost certain that drostatin-C will act as an allatostatin in *Manduca*.

Whether drostatin-C acts as an allatostatin in *Drosophila* itself, has yet to be determined, but its localization in neurons that are potentially neurosecretory (Figs. 5A and 5E) could point in this direction. *In situ* hybridization, unfortunately, does only visualize cell bodies (the location of mRNA) and can, therefore, not determine whether some of the labeled neurons project to the brain neurohaemal (neurohormone releasing) organs or *corpora allata* and, thus, possibly inhibit the release of juvenile hormone. However, a previous immunocytochemical study, using an antiserum against *Manduca sexta* allatostatin, has visualized ventral neurons in the abdominal ganglia of *Drosophila* 3rd instar larvae that project to various ganglionic neurohaemal sites (20). Drostatin-C, therefore, could be a *Drosophila* neurohormone, circulating in the haemolymph. It is interesting that the immunocytochemical study stained several nerve cell groups (SLP2, PVOL, MT, VMab) that were also labeled in our *in situ* hy-

TABLE 3

Nucleotide Differences between the DAP-C cDNA (Fig. 2) and the Corresponding Genomic Sequence in BAC clone AE003631 from the "*Drosophila* Genome Project"

Position of the nucleotide in the cDNA	Type of nucleotide in the gene	Type of nucleotide in the cDNA	Change in amino acid residue
−179	C	T	—
−152	C	T	—
−124	A	G	—
−90	C	A	—
−60	T	C	—
−46	T	C	—
180	A	C	Ala → Ala
453	A	G	—
462	A	C	—
615	Absent	A	—
616	Absent	A	—
926	T	A	—

Note. The position of the nucleotide in the cDNA (Fig. 2) is given in the first column, the type of nucleotide present in the genomic DNA in the second column, and the type of nucleotide present in the cDNA in the third column. The fourth column gives the amino acid residue encoded by the genomic (first row) and cDNA (second row) sequence. None of the nucleotide differences between genomic DNA and cDNA leads to differences in amino acid residues.

```
DAP-C   M---MKFVQILLCYGLLLTLFFALSEARPSGAE-TGPDSDGLDGQDAEDVR-GAYGGGYDMPA  58
PAP-C   MKTNVCNVYLAIVAATLAMLFVTIRAA-PMEAEDEQADNTLVAHPDGDMEMTGPWDTINTAAL  62

DAP-C   QAIYPNIPMDRLQMLFAQYRPTYSAYLRSPTYGNVNELYRLPESKRQVRYRQCYFNPISCFRK 121
PAP-C   RKLLLQLDAEERMGRVSRSWPQAEPRGWGLRALDGRLARQWRADKRQVRFRQCYFNPISCFRK 125
```

FIG. 3. Alignment of the *Drosophila* C-type allatostatin preprohormone (DAP-C) with the *Pseudaletia* C-type allatostatin preprohormone (PAP-C). Amino acid residues that are identical between the two preprohormones are highlighted in grey. The position of the unprocessed drostatin-C sequence in DAP-C is marked by a bold line. The corresponding *Pseudaletia* C-type allatostatin sequence on PAP-C is nearly identical to the drostatin-C sequence. It lies at an identical position and is flanked by identical processing sites. Both preprohormones are about equally long. The PAP-C sequence is from [19].

bridizations (Fig. 5E) (20). Other cells, however, were only stained in the immunocytochemical study. It has to be stressed, however, that *in situ* hybridization is a more specific labeling technique than immunocytochemistry.

The presence of C-type allatostatins in gut endocrine cells (Figs. 5B–5D) has not been described previously for any insect. This presence suggests that drostatin-C has a regulatory (probably inhibitory) role in gut motility. Drostatin-C, therefore, is a brain-gut peptide, similar to the *Drosophila* A- and B-type allatostatins and tachykinin-related peptides (8, 14, 21).

We have cloned two DAP-C cDNAs that differ from each other by the length of their untranslated 3′-ends and, thereby, by the positions of their poly(A^+) tails (Figs. 2 and 4). Differential RNA cleavage and polyadenylylation is also known from other organisms, e.g., mammals (22). In mammals, alternative polyadenylylation contributes to differences in mRNA stability and, thus, to differential protein expression (22). One possible reason for the differential polyadenylylation that we now find in *Drosophila* might be that one group of cells (perhaps neurons?) uses a DAP-C mRNA that is different from the DAP-C mRNA used by another group of cells (perhaps endocrine cells?) (Fig. 5), and that there is a need for differential protein expression between these cell types.

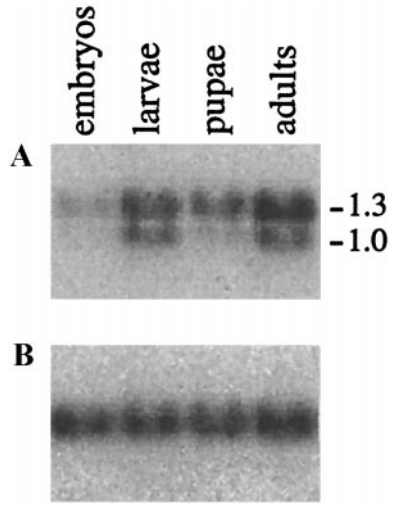

FIG. 4. Northern blots of different developmental stages from *Drosophila*. Each vertical lane contained 2.5 μg mRNA from embryos (0–24 h); 3rd instar larvae; pupae; and pooled adult male and female flies. (A) Hybridization with a cDNA probe corresponding to nucleotide positions 19–324 of Fig. 2. The number to the right gives the estimated sizes in kb. There are two hybridizing mRNA species in larvae and adult flies, each corresponding to a cloned cDNA (Fig. 2). Embryos and pupae have mainly the larger mRNA species. (B) Hybridization of the A blot with a cDNA probe corresponding to ribosomal protein 49 (RP49). This blot gives the loading efficiency.

The A-type allatostatins appear to occur ubiquitously in all insect orders (1, 2), but until recently the B-type allatostatins have only be found in a few select insect groups (5, 23–26). We have very recently cloned both the A- and B-type allatostatin preprohormones from *Drosophila* (7, 8), and in the present paper we have cloned the *Drosophila* C-type allatostatin preprohormone. These findings would suggest that all insect species may contain representatives of all three types of allatostatins.

It is unlikely that all three types of allatostatins in an insect would only be involved in the blocking of the biosynthesis of juvenile hormone in the *corpora allata*. Already some years ago, it has been found that the A-type allatostatins have pleiotropic actions, blocking the *corpora allata* in some insects (e.g., cockroaches), whereas it is inactive in these organs in other insects (e.g., flies) (1, 2). However in many (or perhaps all) insects, A-type allatostatins block muscle contraction in several regions of the gut (1, 2). Also the B-type allatostatins have multiple functions. In crickets, they inhibit juvenile hormone biosynthesis in the *corpora allata* (5), but this effect is absent in stick insects, where the hormone must have a different function (24). In locusts, the B-type allatostatins inhibit muscle contraction of the oviduct (25), whereas in the silkworm *Bombyx mori*, they inhibit the biosynthesis of ecdysone (a steroid hormone important for molting) by the prothoracic gland (26). The three types of insect allatostatins therefore, may be regarded as generally inhibitory neurohormones. Depending on the presence or absence of their specific receptors, they may or may not block specific endocrine organs or muscle tissues in an insect.

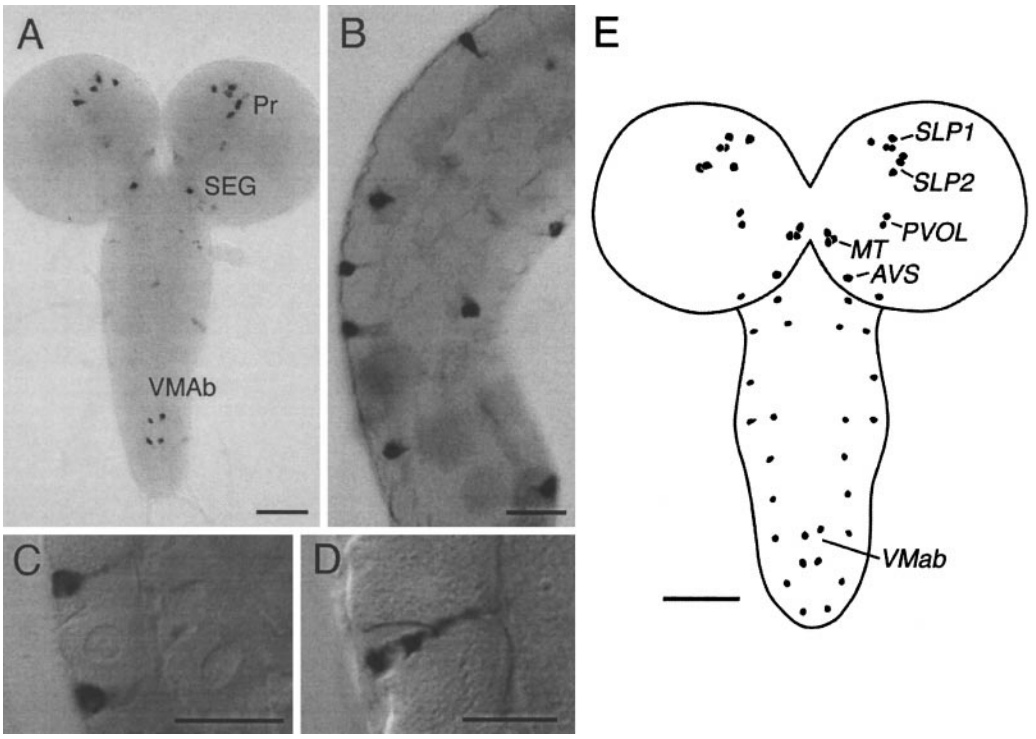

FIG. 5. Whole-mount *in situ* hybridizations of brain and gut from 3rd instar *Drosophila* larvae, using an antisense probe, corresponding to nucleotide positions 241–654 of Fig. 2. The brains are viewed dorsally. (A) Neuronal cell bodies in the brain and abdominal ganglia, expressing the DAP-C gene. Not all cells are in the focal plane and in some cells the signal is more intense than in others. Pr, a cluster of protocerebral cell bodies. SEG, a pair of cells in the subesophageal ganglion. VMAb, two pairs of ventral cells in the abdominal ganglia (scale, 100 μm). (B) Endocrine cells in the posterior midgut, strongly expressing the DAP-C gene (scale, 50 μm). (C) Two endocrine cells in the midgut at higher magnification. The gut lumen is to the right (scale, 50 μm). (D) Another endocrine cell of the midgut, expressing the DAP-C gene. The unlabeled nucleus is visible, as well as the projection of the cell to the lumen at the right (scale, 25 μm). (E) Drawing of DAP-C expressing neurons in the larval central nervous system. Only those cells are drawn that display strong hybridization signals. The same acronyms are used as in [20]: Different nerve cell clusters in the protocerebrum (SLP1, SLP2, PVOL), tritocerebrum (MT), subesophageal ganglion (AVS), ventral cells (VMab) and dorsal cells (unlabeled) from the abdominal ganglia, express DAP-C (scale, 100 μm).

ACKNOWLEDGMENTS

We thank Lotte Steffensen for typing the manuscript and Lundbeck Foundation, the Danish Biotechnological Research and Development Program of the Danish Research Agency, Novo Nordisk Foundation, Director Ib Hendriksen Foundation, and the Swedish Natural Science Research Council for financial support.

REFERENCES

1. Bendena, W. G., Garside, C. S., Yu, C. G., and Tobe, S. S. (1997) *Ann. N.Y. Acad. Sci.* **814,** 53–66.
2. Bendena, W. G., Donly, B. C., and Tobe, S. S. (1999) *Ann. N.Y. Acad. Sci.* **897,** 311–329.
3. Woodhead, A. P., Stay, B., Seidel, S. L., Khan, M. A., and Tobe, S. S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5997–6001.
4. Pratt, G. E., Farnworth, D. E., Siegel, N. R., Fok, F. K., and Feyereisen, R. (1989) *Biochem. Biophys. Res. Commun.* **163,** 1243–1247.
5. Lorentz, M. W., Kellner, R., and Hoffmann, K. H. (1995) *J. Biol. Chem.* **270,** 21103–21108.
6. Kramer, S. J., Toschi, A., Miller, C. A., Kataoka, H., Quistad, G. B., Li, J. P., Carney, R. L., and Schooley, D. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9458–9462.
7. Lenz, C., Williamson, M., and Grimmelikhuijzen, C. J. P. (2000) *Biochem. Biophys. Res. Commun.* **273,** 1126–1131.
8. Williamson, M., Lenz, C., Winther, Å. M. E., Nässel, D. R., and Grimmelikhuijzen C. J. P. (2001) *Biochem. Biophys. Res. Commun.* **281,** 544–550.
9. Lenz, C., Søndergaard, L., and Grimmelikhuijzen, C. J. P. (2000) *Biochem. Biophys. Res. Commun.* **269,** 91–96.
10. Lenz, C., Williamson, M., and Grimmelikhuijzen, C. J. P. (2000) *Biochem. Biophys. Res. Commun.* **273,** 571–577.
11. Birgül, N., Weise, C., Kreienkamp, H.-J., and Richter, D. (1999) *EMBO J.* **18,** 5892–5900.
12. Eriksen, K. K., Hauser, F., Schiøtt, M., Pedersen, K.-M., Søndergaard, L., and Grimmelikhuijzen, C. J. P. (2000) *Genome Res.* **10,** 924–938.
13. Hauser, F., Nothacker, H.-P., and Grimmelikhuijzen, C. J. P. (1997) *J. Biol. Chem.* **272,** 1002–1010.
14. Siviter, R. J., Coast, G. M., Winther, Å. M. E., Nachman, R. J., Taylor, C. A. M., Shirras, A. D., Coates, D., Isaac, J. E., and Nässel, D. R. (2000) *J. Biol. Chem.* **275,** 23273–23280.
15. Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997) *Prot. Eng.* **10,** 1–6.
16. Eipper, B. A., Stoffers, D. A., and Mains, R. E. (1992) *Ann. Rev. Neurosci.* **15,** 57–85.

17. Fischer, W. H., and Spiess, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3628–3632.
18. Pohl, T., Zimmer, M., Mugele, K., and Spiess, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10059–10063.
19. Jansons, I. S., Cusson, M., McNeil, J. N., Tobe, S. S., and Bendena, W. G. (1996) *Insect. Biochem. Mol. Biol.* **26,** 767–773.
20. Zitnan, D., Sehnal, F., and Bryant, P. J. (1993) *Dev. Biol.* **156,** 117–135.
21. Yoon, J. G., and Stay, B. (1995) *J. Comp. Neurol.* **363,** 475–488.
22. Münch, C., Schwalenstöcker, B., Hermann, C., Cirovic, S., Stamm, S., Ludolph, A., and Meyer, T. (2000) *Mol. Brain. Res.* **80,** 244–251.
23. Lorentz, M. W., Kellner, R., and Hoffmann, K. H. (1999) *Eur. J. Entomol.* **96,** 267–274.
24. Lorentz, M. W., Kellner, R., Hoffmann, K. H., and Gäde, G. (2000) *Insect Biochem. Mol. Biol.* **30,** 711–718.
25. Schoofs, L., Holman, G. M., Hayes, T. K., Nachman, R. J., and De Loof, A. (1991) *Regul. Pept.* **36,** 111–119.
26. Hua, Y.-J., Tanaka, Y., Nakamura, K., Sakakibara, M., Nagata, S., and Kataoka, H. (1999) *J. Biol. Chem.* **274,** 31169–31173.