Two Subtypes of Ecdysis-triggering Hormone Receptor in Drosophila melanogaster*

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Insect ecdysis is a hormonally programmed physiological sequence that enables insects to escape their old cuticle at the end of each developmental stage. The immediate events leading to ecdysis, which are initiated upon release of ecdysis-triggering hormones (ETH) into the bloodstream, include respiratory inflation and sequential stereotypic behaviors that facilitate shedding of the cuticle. Here we report that the Drosophila gene CG5911 encodes two functionally distinct subtypes of G protein-coupled receptors through alternative splicing (CG5911a and CG5911b) that respond preferentially to ecdysis-triggering hormones of flies and moths. These subtypes show differences in ligand sensitivity and specificity, suggesting that they may play separate roles in ETH signaling. At significantly higher concentrations (>10 nM), certain insect and vertebrate peptides also activate these receptors, providing evidence that CG5911 is evolutionarily related to the thyrotropin-releasing hormone and neuromedin U receptors. The ETH signaling system in insects is a vital system that provides opportunities for the construction of models for the molecular basis of stereotypic animal behavior as well as a target for the design of more sophisticated insect-selective pest control strategies.

A defining feature of growth and metamorphosis in insects is the periodic shedding of cuticle known as ecdysis (1). This complex process, by which the insect escapes its outer shell and sheds the cuticular lining of the foregut, hindgut, and respiratory system, is mediated by a peptide hormone-signaling cascade that results in a sequence of precisely timed physiological and behavioral events. Initiation of the ecdysis sequence coincides with the appearance of ecdysis-triggering hormones (ETHs) in the bloodstream, which act directly on the central nervous system to elicit patterned motor output characteristic of pre-ecdysis and ecdysis behaviors (2, 3). In the moth Manduca sexta, two peptides called MasPETH and MasesETH activate these receptors in concert to trigger successive phases of the ecdysis behavioral sequence. Remarkably, the temporal features of these motor patterns recorded from the isolated nervous system closely reflect the behavior observed in whole animals. Related peptides in Drosophila melanogaster known as ETH1 and ETH2 trigger ecdysis, although their respective roles in the recruitment of different parts of the ecdysis sequence are less clear. As yet the molecular and cellular targets of ETHs have not been described. Additional signaling molecules operating downstream of ETH within the central nervous system include the eclosion hormone and the crustacean cardioactive peptide (2, 4–7).

Identification of ETH receptors would be of great help in defining the cellular elements involved in ecdysis behaviors. With this objective, we took advantage of the Drosophila genome project to investigate G protein-coupled receptors (GPCRs) that are likely to respond to ETH (8, 9). The search for ETH receptors was narrowed to a small number of candidates on the basis of an assumption of ligand-receptor co-evolution, in which receptors for peptides having a C-terminal amino acid motif consisting of PRX amide would have high sequence homology. An exhaustive analysis of the Drosophila genome yielded two groups of GPCRs, the neuromedin U (NMU) group and the vasopressin group (10). Functional analysis revealed that the NMU group likely arose through ligand-receptor co-evolution, because it responds to several groups of peptides with a C-terminal PRX amide motif characteristic of the ETHs. In contrast, the vasopressin group responds to unrelated peptides, and thus the PRX amide in this group appears to have arisen through convergent evolution.

The monophyletic group of NMU receptors was found to be activated by all three categories of insect PRX amide peptides (10): pyrokinins or pheromone biosynthesis-activating neuropeptide (PBAN)-like peptides ending with the amino sequence motif -FPRXa (where final “a” means amide) (11, 12), cardioactive CAP2b-like peptides with the -FPRXa motif (13, 14), and ecdysis-triggering hormones having a -PRXa motif (2, 5, 10, 15, 16). Surprisingly, some receptors exhibited sensitivity to multiple peptides. In particular, CG8795 was activated by DrmPK2, HUGγ, and ETH1, with sensitivity to DrmPK2 and HUGγ being far greater than to ETH1 (DrmPK2 > HUGγ > ETH1).

The responsiveness of CG8795 to only relatively high concentrations of ETH1 suggested that it is unlikely to be the physiological ETH receptor. We therefore expanded the search profile for ETH receptors to include CG5911, which encodes a receptor related to the NMU group. As predicted (8), CG5911 gene products occur as two alternative splice variants termed CG5911a and CG5911b. CG5911b responds to ETH1 and the

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank accession(s) AT220741 and AT220742.

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The abbreviations used are: ETH, ecdysis-triggering hormone; PETH, pre-ecdysis-triggering hormone; GPCR, G protein-coupled receptor; NMU, neuromedin U; ORF, open reading frame; RT-PCR, reverse transcriptase PCR; CHO, Chinese hamster ovary; TRH, thyrotropin-releasing hormone; PBAN, pheromone biosynthesis-activating neuropeptide.
related peptides MasETH and MasPETH from moths at sub-nanomolar concentrations while showing relative insensitivity to other peptides sharing C-terminal PRX amide motifs. A pattern of cross-reactivity in a second set of peptides provides further support for the hypothesis that CG5911 encodes the authentic ETH receptor.

EXPERIMENTAL PROCEDURES

Cloning of CG5911—cDNA was synthesized using Superscript (Invitrogen) with mRNA isolated by Dynabeads (Dynal) from whole flies (50 individuals of Canton S) in both larval and adult stages by priming at poly(A) sites. PCR reactions conducted in 20-μl volumes contained 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2 mM MgCl₂, 0.2 mM dNTP, 0.5 μM of each primer, 0.5 unit of Taq polymerase (Invitrogen), and 0.5 unit of Pfu polymerase (Stratagene). The reaction mixture was denatured initially for 5 min at 94°C and then subjected to 35 cycles of 94°C for 1 min, 53°C for 1 min and 30 s. The RT-PCR products were cloned in pGEM-T-EZ vector (Promega) and sequenced. Start and stop codons of the open reading frames (ORF) were confirmed by the primers in the RT-PCR designed on the outside of the in-frame start and stop codons in the predicted open reading frames. The 5' and 3' ends of the transcript were not determined and are depicted as slanted ends of exon 1 (5') and exon 4 (3').

Transfected cells were observed under an epifluorescence microscope 1 day later to determine transfection efficiency as measured by the number of cells expressing enhanced green fluorescent protein. Before functional assays, cell suspensions were incubated in coelanterazine h (Molecular Probes) according to previously defined protocols (20).

Functional Analysis—Luminescence assays were performed in opaque 96-well microplates (Corning) using a Beckman model LD400 LuminoStar. After the addition of cells to a well, luminescence was monitored using an 0.5-s sampling time for 20 s. Each 96-well microplate contained multiple wells for positive controls (100 and 300 μM ETH1) and negative controls (buffer only). Luminescence at each ligand concentration was integrated during the 20-s response interval and normalized to the largest positive control response in each plate after the subtraction of background values obtained from negative controls. Luminescence measured in multiple replica wells (2–4 wells) for one concentration of ligand was averaged for the analysis. Data collected from at least three replica plates were used for analysis using the Origin analysis program (OriginLab Corp., Northampton, MA). The sources of peptides were described previously (10), and additional vertebrate peptides (NMU-8, TRH, and ghrelin) were purchased from the American Peptide Company. MasETHacid was synthesized at the University of California, Riverside, using standard Fmoc (N-(9-fluorenyl)-methoxycarbonyl) chemistry and automated solid state techniques. A list of peptides used in this study is provided in Table 1.

RESULTS

Molecular Cloning of CG5911a and CG5911b—The CG5911 gene has homologous mutually alternative exons 4a and 4b encoding the protein sequence from the middle of transmembrane segment 4 to the C terminus (Figs. 1 and 2). The clones used for the expression contain the coding sequence from nucleotide position −24 upstream of the Met site to +368 nucleotides after the 3' stop codon for CG5911a and from −490 to +8 for CG5911b (see GenBank™ accession numbers AY220741, AY220742, and Celera Genomics sequence GenBank™ accession number AE003734) (Fig. 2). The ORF is supported by the in-frame stop codon in the 5'-untranslated region (Fig. 1).

Initial BLASTP searches and phylogenetic analyses identified the translation of CG5911 (GenBank™ accession number AAF55872) as a Drosophila GPCR closely related to the monophyletic group of NMU receptors responding to PRX amide peptides (10). Putative transcripts generating CG5911a and -b are grouped with thyrotropin-releasing hormone (TRH) receptor in a distance tree with a bootstrapping value of 83.8% (Fig. 3).

The prediction of gene structure revealed the presence of homologous mutually alternative exons 4a and 4b from transmembrane segment 4 to the C terminus encoding two splice variants of the receptor (Figs. 1 and 2). Stop codons occurring at C-terminal ORFs for CG5911a and CG5911b were identified by RT-PCR using primers located after the stop codon. RT-PCR with the primer covering a stop codon at the 5' end of the ORF for CG5911b revealed a transcript supporting the ORF prediction.

CG5911a and CG5911b Are Preferentially Sensitive to ETHs—Ecdysis-triggering hormones of both Drosophila and...
**FIG. 2.** Amino acid sequence alignment of G protein-coupled receptors in the clade including the neuromedin U receptor. Sequences are from the GH secretagogue receptor (GHSR, NP_114464), neurotensin receptor (NTR, JH0164), CG8784 (AF522189), CG8785 (AF522190), CG14767 (AF522191), CG14762 (AF522190), GHSR (NP_114464), and CG5911a and CG5911b. The location of mutually alternative exons 4a and 4b in CG5911a and b is indicated in TM4. Inverted and shaded letters indicate identical and similar sequences, respectively, in more than 50% of the taxa. ‘‘‘‘ and ‘‘‘‘ indicate gaps introduced for sequence alignment in the middle of the sequences and for the N-terminal end, respectively. Transmembrane domains are indicated by prediction from the NMUR1 sequence. The location of mutually alternative exons 4a and 4b in CG5911 is indicated in TM4.
Manduca elicited robust luminescent responses in the CHO-WTA11 cell line stably expressing Go16 and aequorin transfected with pXOON-CG5911a and pXOON-CG5911b (Fig. 4). The time course of cellular responses was concentration-dependent, with delayed slow responses beginning at 0.1 nM and rapid peak responses occurring at 1 μM for CG5911b (Fig. 4A). At the lowest effective concentrations (0.1 nM), the luminescence response was delayed more than 10 s. Integration of each response shown in Fig. 4A during a 20-s sampling period is shown in Fig. 4B.

Both CG5911a and CG5911b showed preferential sensitivity to ETHs, with CG5911b showing a significantly higher apparent affinity for all peptides tested (Fig. 4, C and D). Drosophila ETH1 was the most active ligand for both receptors. For CG5911b, responses were evident at 0.25 nM and reached peak values at 10 nM with an EC50 value of 0.9 nM. ETH2 and Manduca PETH were only slightly less active, with EC50 values of ~2 nM. Interestingly, Manduca ETH was approximately 5 times less active on CG5911b than PETH.

CG5911a was markedly less sensitive to all peptides tested compared with CG5911b. The most active peptide again was ETH1, with an EC50 of 414 nM, making it ~400-fold less active on this receptor than on CG5911b. ETH2, MasPETH, and MasETH had EC50 values greater than 2 μM.

Cross-reactivity of CG5911 to Other Peptide Ligands—CG5911b showed significant sensitivity to a range of ETH-related peptides having the signature PRXa C-terminal amino acid motif (Fig. 5). These peptides include SCPβ, HUGγ, CAP2b-1, CAP2b-2, PBAN, and MasETH acid. The most active of these peptides were SCPβ, MasETH acid, HUGγ, and CAP2b-1; the EC50 values of these peptides were in the range of 300 nM to 3 μM. Furthermore, the vertebrate peptides NMU and TRH caused low but significant receptor activation when applied at 10 μM as did the molluscan peptide myomodulin (p < 0.001 in Student’s t test; Fig. 5B). No activity was observed for a number of other peptide ligands, including some with the C-terminal PRXa motif. These ligands include Drm-PK-2, CAP2b-3, and Hex PMP (Fig. 5B).

**DISCUSSION**

Ecdysis-triggering hormones are vital regulatory signals that govern the stereotypic physiological sequence leading to cuticle shedding in insects. In previous work we identified ETHs in two moth species, Manduca sexta and Bombyx mori, and in the fruit fly Drosophila melanogaster (2, 15, 16). Using genetic tools in Drosophila to excise the eth gene, lethal ecdysis deficits were demonstrated at the end of the first larval instar (21). Lethality was reversed by the injection of ETH peptides (21). The ETH signaling system therefore has been demonstrated to be both necessary and sufficient for insects to survive the earliest ecdysis. To expand our understanding of the molecular basis for ETH action, we conducted a search for its likely receptor in Drosophila.

In this paper we report on the cloning and functional expression of the Drosophila gene CG5911, predicted by Hewes and Taghert (8) to encode splice variants called CG5911a and CG5911b. Our results show that both splice variants of CG5911 are highly selective for ETH1 and related peptides from both fly and moth, with CG5911b responding to subnanomolar con-
centrations of *Drosophila* ETH1. For each receptor subtype the highest sensitivity was observed to *Drosophila* ETH1 and ETH2, but comparable sensitivity also was observed for MasPETH and MasETH. Other insect and vertebrate peptides, particularly those having C-terminal PRX amide motifs, also activated CG5911b, but only at $\sim100$-fold higher concentrations. The selectivity and high sensitivity of CG5911 to ETHs alone strongly supports the hypothesis that this gene encodes the authentic ETH receptor.

The gene structure of CG5911 with the results of RT-PCR revealed two homologous exons presenting the mutually alternative splice variants CG5911a and CG5911b. Phylogenetic analysis suggests that divergent exons 4a and 4b are the product of duplication. The portion of the receptor protein predicted by exon 4 extends from the middle of transmembrane segment 4 (T4) to the C terminus and therefore includes two extracellular loops (T4-T5 and T6-T7), one intracellular loop (T5-T6), and the C-terminal intracellular tail. Clearly, alternative splicing of exons 4a and 4b to create these receptor isoforms could affect both their ligand and G protein-coupling specificities.

Functional differences in CG5911a and CG5911b are suggested by several observations. CG5911b is activated by sub-nanomolar concentrations of ETH1 (EC$_{50} = 0.9$ nM), whereas the sensitivity of CG5911a is more than 400-fold lower (EC$_{50} = 410$ nM). The lower sensitivity of CG5911a to all ligands may be a consequence of poor coupling efficiency to the $G_{11}$ used in our assay system. Indeed, the smaller size of peak luminescence responses recorded for CG5911a as compared with CG5911b (data not shown) supports this idea. Further investigation of CG5911a sensitivity using different G proteins will be necessary to resolve this question. Nevertheless, differences in G protein-coupling and downstream signal transduction steps could provide a basis for functionally distinct roles for these two receptor subtypes in ETH signaling.

In addition to these differences, the ligand sensitivity for the two receptor subtypes is significantly different. The relative potency of ligands on CG5911a (ETH1 $>$ MasETH $>$ ETH2 $>$ MasPETH; see Fig. 4) reflects the same order of biological activity observed for the induction of ecdysis behavior (16). In the case of CG5911b, we observed a somewhat different order of potency (ETH1 $>$ ETH2 $>$ MasPETH $>$ MasETH). The reversals in receptor sensitivity to MasPETH and MasETH suggest that these receptor subtypes discriminate between MasPETH and MasETH and raises the intriguing possibility that CG5911 orthologs in the *Manduca* system encode receptors that are ligand-specific. Indeed, preliminary findings involving the *Manduca* ortholog of CG5911b confirm the preference for MasPETH. The possible ligand specificity of the two receptor subtypes may account at least in part for divergent activities of these two ligands in the induction of natural pre-ecdysis behavior (2, 3). Further study of *Manduca* orthologous isoforms of CG5911 is under way to test this hypothesis.

Responses of CG5911b to a second tier of ligands that are active only at $\gg100$-fold higher concentrations reveal further insights into its properties and the likely physiological role of CG5911 as the ETH receptor. All ETHs are C-terminally amided, and this post-translational modification is essential for biological activity in the silkworm, *B. mori* (15). Although the injection of synthetic *Bombyx* ETH elicits the entire ecdysis behavioral sequence in silkworm larvae, the free acid form proved to be largely inactive (15). In the present study, the free acid form of MasETH (MasETHacid, Fig. 5) was found to be more than 100 times less active than native MasETH. CG5911b therefore discriminates between these subtly different forms of the peptide just as we observed previously in behavioral assays (2).

We tested a variety of both invertebrate and vertebrate peptides on CG5911b, and most were inactive. Nevertheless, some interesting patterns of activity are apparent among the few peptides that showed some biological activity. Of the invertebrate peptides tested, the peptide HUG$_{GH}$, encoded by the ortholog of the moth PBAN gene as well as PBAN itself, were active at several hundred-fold higher concentrations than authentic ETHs. The low affinity response of CG5911b to HUG$_{GH}$ and its high affinity response to ETH1 and ETH2 complement our previous results, which showed that the related GPCR encoded by CG8795 is highly sensitive to HUG$_{GH}$ but considerably less sensitive to ETHs (10). These results confirm our phylogenetic analyses, which indicate the close evolutionary relationship between CG8795 and CG5911. The question remains as to whether the cross-reactivities of these two ligands (ETH and HUG$_{GH}$) against CG8795 and CG5911b reflect genuine biological phenomena. Evidence in support of this comes from Roos and colleagues (22), who showed that ectopic expression of the *hugin* gene produced lethal ecdysis defects.

The mammalian peptides neuromedin and TRH, the receptors of which are related phylogenetically to CG5911, also activate CG5911b, albeit at relatively high concentrations. The significant bioactivity of these peptides, with the complete absence of responses to neurotensin and ghrelin (assayed at 10 $\mu$M), strongly support our phylogenetic analysis, which indicates that CG5911 evolved from a GPCR ancestral to the NMU/TRH receptors.

Myomodulin (PMSMRLamide), a neuropeptide first identified in molluscs, was also found to activate CG5911b. We were unable to find myomodulin-like peptides in the *Drosophila* genomic data base, but a similar sequence occurs in the *Caenorhabditis elegans* data base (Y4510A.5). Myomodulin could play an analogous role to ETH in nematodes, which with the arthropods have been newly classified in the Ecdysozoa (23). Indeed, antibodies raised against myomodulin cross-react with ETHs in endocrine Inka cells (24), indicating structural similarities between these peptides.

During preparation of this manuscript, independent evidence appeared to support the hypothesis that CG5911 functions as the ETH receptor (25). These findings confirm that two functional subtypes of CG5911 are produced by alternative splicing and that ETH1 is a more potent agonist against both receptors. These authors (25) also demonstrated the presence of an additional 5’-untranslated exon. However, our results demonstrate a higher sensitivity of CG5911 to both ligands and a considerably greater divergence in receptor sensitivity between the two subtypes. These findings could indicate that CG5911a and CG5911b interact with different G proteins and may activate different signal transduction pathways. Our pharmacological and phylogenetic analyses also indicate that ETH and the pyrokinin/CAP2b groups of insect neuropeptides constitute a set of ligands that have evolved with CG5911 and the neuromedin U group of vertebrate receptors and their respective ligands.

In summary, we have demonstrated that alternative splice variants of CG5911 encode two functional receptor subtypes of ETH receptor in *Drosophila*. Although further work is necessary to confirm their physiological roles in natural ETH signaling, the pronounced ligand sensitivity and specificity of CG5911 gene products along with the phylogenetic relationships of CG5911 to both *Drosophila* and mammalian receptors for PRX amide peptides provide strong evidence for their roles as the physiological ETH receptors.

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2 Y. J. Kim and M. E. Adams, unpublished data.

3 Y. Park and M. E. Adams, unpublished data.
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REFERENCES

1. Truman, J. W., Ewer, J., and Ball, E. E. (1996) J. Exp. Biol. 199, 749–758
2. Zitnan, D., Ross, L. S., Zitanova, I., Hermesman, J. L., Gill, S. S., and Adams, M. E. (1999) Neuron 23, 1–20
3. Zitnan, D., and Adams, M. E. (2000) J. Exp. Biol. 203, 1329–1340
4. Ewer, J., and Truman, J. W. (1996) J. Comp. Neurol. 370, 330–341
5. Zitnan, D., Kingan, T. G., Hermesman, J., and Adams, M. E. (1996) Science 271, 88–91
6. Gammie, S. C., and Truman, J. W. (1999) J. Exp. Biol. 202, 343–352
7. McNabb, S. L., Baker, J. D., Agapite, J., Steller, H., Riddiford, L. M., and Truman, J. W. (1997) Neuron 19, 813–823
8. McNabb, S. L., Baker, J. D., Agapite, J., Steller, H., Riddiford, L. M., and Truman, J. W. (1997) Neuron 19, 813–823
9. Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Sutton, G. G., Wortman, J. R., Yandell, M. D., Zhang, Q., Chen, L. X., Brandon, R. C., Rogers, Y.-C., Blazewicz, K. G., Champe, M., Pfeiffer, B. D., Wan, K. H., Doyle, C., Baxter, E. G., Helt, G., Nelson, C. R., Gabol Miklos, G. L., Abri, J. F., Abayani, A., An, H.-J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R. M., Basu, A., Baxendale, J., Beissler, L. E., Beisson, K. Y., Berman, B. P., Bhandari, D., Bobshakov, S., Borkova, D., Botchan, M. R., Bouck, J., Bruckstein, P., Brotter, P., Burtis, K. C., Busam, D. A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J. M., Cawley, S., Dahlke, C., Davenport, L. E., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A. D., Dew, I., Dietz, S. M., Doudna, K., Droop, L. E., Downs, M., Dugan-Rocha, S., Dunkov, B. C., Durbin, K., Evans, J., Evangelista, C. C., Ferraz, C., Ferrerias, S., Fleischmann, W., Foster, C., Gabrielian, A. E., Garg, N. S., Gelbart, W. M., Glasser, K., Goleck, A., Gong, F., Gorrell, J. H., Gu, Z., Guan, P., Harris, M., Harris, N. L., Harvey, D., Heiman, T. J., Hernandez, J. R., Houck, J., Hostin, D., Houston, K. A., Howland, T. J., Wei, M.-H., and Beegwari, C. (2000) Science 287, 2185–2195
10. Park, Y., Kim, Y. J., and Adams, M. E. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 11423–11428
11. Raina, A. K., Jaffe, H., Kempe, T. G., Keim, P., Blacher, R. W., Fales, H. M., Riley, C. T., Klas, J. A., Ridgway, R. L., and Hayes, D. K. (1989) Science 244, 796–798
12. Matsumoto, S., Kitamura, A., Nagasawa, H., Kataoka, H., Okakoba, C., Mitsu, T., and Suzuki, A. (1996) J. Insect Physiol. 36, 427–432
13. Huesmann, G. R., Cheung, C. C., Loi, P. K., Lee, T. D., Swiderek, K. M., and Tublinz, N. J. (1996) FEBS Lett. 371, 311–314
14. Morris, H. R., Panico, M., Karplus, A., Lloyd, P. E., and Rink, F. (1982) Nature 300, 643–645
15. Adams, M. E., and Zitnan, D. (1999) Biochem. Biophys. Res. Commun. 300, 188–191
16. Park, Y., Zitnan, D., Gill, S. S., and Adams, M. E. (1999) FEBS Lett. 36, 359–363
17. Jespersen, T., Grunnet, M., Angel, K., Klaerke, D. A., and Olesen, S. P. (2002) BioTechniques 32, 536–538
18. Rizzuto, R., Simpson, A. W., Brini, M., and Pozzan, T. (1992) Nature 358, 325–327
19. Offermann, S., and Simon, M. I. (1995) J. Biol. Chem. 270, 21575–21580
20. Le Poul, E., Hisada, S., Mizuguchi, Y., Dupriez, V. J., Burgon, E., and Deloche, M. (2002) J. Biomol. Screen. 7, 57–65
21. Park, Y., Filipov, V., Gill, S. S., and Adams, M. E. (2002) Development 129, 493–503
22. Meng, X., Wahlstrom, G., Immonen, T., Kolmer, M., Tirronen, M., Predel, R., Kalkkinen, N., Heino, T., Sarola, H., and Ross, C. (2002) Mech. Dev. 117, 5
23. Aguinaldo, A. M., Turbeville, J. M., Linford, L. S., Rivera, M. C., Greve, J. R., Raff, R. A., and Lake, J. A. (1997) Nature 387, 489–493
24. O'Brien, M. A., and Taghert, P. H. (1996) J. Exp. Biol. 201, 193–209
25. Iversen, A., Cazzamali, G., Williamson, M., Hauser, F., and Grimmelshuijzen, C. J. P. (2002) Biochem. Biophys. Res. Commun. 299, 924–931