

Octopamine receptor OAMB is required for ovulation in *Drosophila melanogaster*

Hyun-Gwan Lee,^{a,1} Chang-Soo Seong,^{a,1} Young-Cho Kim,^a
Ronald L. Davis,^b and Kyung-An Han^{a,*}

^a Department of Biobehavioral Health and The Huck Institute of Life Sciences, 315 Health and Human Development East,
Pennsylvania State University, University Park, PA 16802, USA

^b Departments of Molecular and Cell Biology, and Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, TX 77030, USA

Received for publication 16 April 2003, revised 8 July 2003, accepted 28 July 2003

Abstract

Octopamine is a major monoamine in invertebrates and affects many physiological processes ranging from energy metabolism to complex behaviors. Octopamine binds to receptors located on various cell types and activates distinct signal transduction pathways to produce these diverse effects. We previously identified one of the *Drosophila* octopamine receptors named OAMB that produces increases in cAMP and intracellular Ca^{2+} upon ligand binding. It is expressed at high levels in the brain. To explore OAMB's physiological roles, we generated deletions in the OAMB locus. The resultant *oamb* mutants were viable without gross anatomical defects. The *oamb* females displayed normal courtship and copulation; however, they were impaired in ovulation with many mature eggs retained in their ovaries. RT-PCR, in situ hybridization, and expression of a reporter gene revealed that OAMB was also expressed in the thoracoabdominal ganglion, the female reproductive system, and mature eggs in the ovary. Moreover, analysis of various alleles pinpointed the requirement for OAMB in the body, but not in the brain, for female fecundity. The novel expression pattern of OAMB and its genetic resource described in this study will help advance our understanding on how the neuromodulatory or endocrine system controls reproductive physiology and behavior.

© 2003 Elsevier Inc. All rights reserved.

Keywords: Octopamine; Octopamine receptor; OAMB; cAMP; Ca^{2+} ; Ovulation; Oviposition; Egg-laying; Female reproduction; *Drosophila*

Introduction

Octopamine is a major biogenic amine in invertebrates and plays diverse roles in various physiological processes (Roeder, 1999). As a neurohormone, octopamine stimulates energy metabolism during stress responses (David and Coulon, 1985). In the central nervous system (CNS), octopamine's modulatory role has been implicated in sensory information processing, motor function, and higher order neural function. In *Drosophila*, octopamine application to decapitated flies stimulates locomotion and grooming (Yellman et al., 1997) and *inactive* mutants, which contain only 15% of

the wild-type level of octopamine, are hypoactive (O'Dell, 1993). In addition, octopamine plays a crucial role in associative learning in *Drosophila* and honeybees (Dudai et al., 1987; Hammer and Menzel, 1998; Menzel et al., 1996).

Tyramine β -hydroxylase (*t β h*) is the enzyme that converts tyramine to octopamine. Genetic studies of *Drosophila* revealed that the mutations in the *t β h* gene produce female sterility due to defective egg-laying (Monastirioti et al., 1996). This is consistent with the potential role of octopamine revealed by studies of the locust oviduct. When applied to the isolated oviduct muscle, octopamine inhibits both spontaneous and evoked muscle contractions (Cook and Wagner, 1992; Orchard and Lange, 1986), and this response is dependent on the increase in cAMP levels through octopamine receptors of unknown identity (Lange and Orchard, 1986). Thus, impaired egg laying of *t β h* fe-

* Corresponding author. Fax: +1-814-863-7525.

E-mail address: Kxh29@psu.edu (K.-A. Han).

¹ These authors contributed equally to this work.

males could be due to the absence of modulatory octopamine input on the oviduct muscle activity. However, a role of octopamine and its receptor in the *Drosophila* oviduct has not been documented. An additional phenotype associated with the *tβh* mutant is their failure to develop functional tolerance upon repetitive exposure to alcohol (Scholz et al., 2000). The *tβh* flies contain an increased level of the synthetic precursor tyramine (Monastirioti et al., 1996), which may fulfill some of octopamine's functions or produce side effects in some cells. Therefore, it may not be feasible to address certain physiological processes that involve octopamine by investigating the *tβh* mutant.

Four distinct receptor types for octopamine have been pharmacologically characterized in the locust: three receptors in the leg muscle and one found predominantly in CNS (Roeder et al., 1995). Molecular cloning identified two neuronal receptors sensitive to octopamine in *Drosophila*. One receptor is designated as a tyramine/octopamine receptor since tyramine is two orders of magnitude more potent than octopamine in inhibiting adenylyl cyclase activity (Robb et al., 1994; Saudou et al., 1990). However, both octopamine and tyramine have similar potencies for increasing intracellular Ca^{2+} (Robb et al., 1994). Another receptor named OAMB (octopamine receptor in mushroom bodies) has two orders of magnitude higher efficacy with octopamine than tyramine in activating cAMP accumulation (Han et al., 1998). Octopamine is a major stimulator of adenylyl cyclases in fly head homogenates (Dudai and Zvi, 1984; Uzzan and Dudai, 1982), suggesting that OAMB may represent a prevalent receptor type for octopamine in the *Drosophila* brain. Indeed, OAMB transcripts are present in many neurons in the brain, while OAMB proteins are highly enriched in the mushroom bodies and the ellipsoid body, neural structures crucial for learning and memory, motor control, and functional tolerance to alcohol (Davis, 1996, 2001; Han et al., 1998; Martin et al., 1999; Scholz et al., 2000). In addition to cAMP, OAMB also stimulates an increase in intracellular Ca^{2+} , implying that OAMB may utilize distinct signal transduction pathways to execute its diverse physiological functions.

To understand the physiological processes mediated by OAMB, we utilized P-element-mediated mutagenesis and obtained several excision lines containing deletions in the OAMB locus. In this report, we describe the female sterility associated with deletions in the *oamb* locus. This phenotype was attributable to defective ovulation.

Materials and methods

Drosophila strains, genetics, and fecundity, hatching, and ovulation tests

All fly stocks were reared on standard cornmeal medium at 25°C with relative humidity between 50 and 70%. The transgenic line carrying *cdc2c* cDNA was kindly provided

by Dr. Lehner (Universitat Bayreuth, Germany). The line P551 and the transposase line B1808 were obtained from the Bloomington Stock Center. The local hop and imprecise excision experiments were carried out as described (Dalby et al., 1995; Tower et al., 1993).

For fecundity tests, 2- to 3-day-old individual females were placed with three Canton-S (CS) males on grape juice medium, and eggs and larvae were counted 8 days after pairing. The same experiment was performed on cornmeal medium to count pupae and adult progeny 14 days after pairing. To measure courtship and copulation activities, 3- to 4-day-old females were individually paired with CS males in a courtship chamber (Tompkins et al., 1983) and videotaped. The percentage of time that males spent courting in the first 20 min within the chamber was measured. Once they engaged in copulation, duration of their copulation was recorded. For the hatching assay, a group of females (5) was placed with 15 CS males on a regular cornmeal medium for 1 day, and transferred to a new food vial for egg collection for 24 h. The hatched eggs from each vial were counted 48 h later (Lannutti and Schneider, 2001). To measure ovulation rates, 3- to 4-day-old virgin females were individually paired with CS males in a courtship chamber for 1 h and then placed on standard media. At various time intervals after mating, the mated females were tested on ice to determine the presence of an egg in their uteri by gently pushing the tip of their abdomens (Aigaki et al., 1991).

PCR screening and RT-PCR

Molecular techniques were performed by using standard procedures. DNA from a single fly was prepared as described (Sullivan et al., 2000), and PCRs were performed with 1/10th of a single fly crude DNA with primers representing various genomic regions as follows. Primers for initial screening for imprecise excision events: P primer: 5'-CGACGGGACCACCTTATGTTATTTTCATCATG-3'; 291U: 5'-TGGCTAACATCCTTTTGCTTCG-3'; 245C: 5'-AGAAAGCGTCGCAAATCAAAGGG-3'. Primers for mapping breakpoints in *oamb* mutants: NextCG31205U: 5'-ATTGCGGTTGCTGGATTCTTC-3'; NextCG31205D: 5'-AGCGAGGACTACCCACTGTGT-3'; 14KUU: 5'-ACTACGGACATCAACGGACTA-3'; 14KUD: 5'-TTGCCCCGTCATCATCTTACTA-3'; 5KUU: 5'-GCCCTAACATTGCTGCCTCC-3'; 5KUD: 5'-CCTCATCCGCTCCGTCCTTG-3'; 1KUU: 5'-GTTGCGGTCATTATTCACTT-3'; 1KUD: 5'-GTCAGCCCCATTCCTTTGTTC-3'; E2U: 5'-CCACCAAGCACTGAAAATA-3'; E2D: 5'-GAGATTAGCGAGAGCCCGTC-3'; I2-1U: 5'-CCAGGCGTGAA-GATTTCCATAG-3'; I2-1D: 5'-GCCCAATAACACGGACAGACTG-3'; I2-2U: 5'-TCAGAGTCGTGGAGGTGTGC-3'; I2-2D: 5'-GCTTAGGTGGGTAGCAATAAAGAT-3'; I2-3U: 5'-GCGACAAGGCGAAATCACTC-3'; I2-3D: 5'-TGGATGAGGATTGGGTCTGC-3'; I2-4U: 5'-GCGGCAACTGTGAAAAGATG-3'; I2-4D: 5'-AAACGACGAATGCTGAATGT-3'; E3U: 5'-TCAGTGTATGTG-

CCCCTAAT-3'; E3D: 5'-CCTGCCAGCCAATCAGT-AAA-3'; I3-1U: 5'-ATTAGCAGAGCCCCCACTTT-3'; I3-1D: 5'-TTGCCAGCGACTCTATTTACC-3'; I3-2U: 5'-GCAGCACTTCTCTCTACGGATT-3'; I3-2D: 5'-GTGCTGTGATTGCCCTGTGA-3'; I3-3U: 5'-GTGGAGGA-ACTGAATGAAGAGGA-3'; I3-3D: 5'-TCCCAAGTT-TCTGCCCAATG-3'; CG4000U: 5'-AAGGCAAATGGC-ACAAAG-3'; CG4000D: 5'-CGGGAGGAGCAGGTG-GAG-3'; CG5474U: 5'-CACGCACGGCTCCTACTC-3'; CG5474D: 5'-TCAACGGGCACACCTCCA-3'.

Total RNAs were prepared from heads, bodies, dissected oviducts, and the female reproductive system by using High Pure RNA Tissue Kit (Boehringer M, Indianapolis, IN). RT-PCRs were performed on total RNA with Titan One Tube RT-PCR System (Boehringer M) using primer sets representing *cdc2c* (5'-GCACACCTGATGAAACAAAT-3' and 5'-CTGGGGCTATTGGACACGA-3'), OAMB E4-E5 (5'-CGTTCTGGTCATCGGTGG-3' and 5'-CGTGGACAT-TATGCTTGG-3'), E6-E7 (5'-CACCGCCGCAGAACAG-AC-3' and 5'-CGCTACTGATTACCGCAT-3'), and E6-E8 (5'-CACCGCCGCAGAACAGAC-3' and 5'-CGCTCGTGA-CCCACATCC-3') according to the manufacturer's instruction.

DAPI and Phalloidin stain

Females were kept with CS males for 3 days in food vial containing yeast paste. The ovaries were dissected in phosphate-buffered saline (PBS) and fixed in 2% paraformaldehyde (PFA) in PBS for 20 min. After washing in PBS twice, they were incubated with 1 μ g/ml of DAPI (Molecular Probes, Eugene, OR) alone or with DAPI and FITC-Phalloidin (1:100; Molecular Probes) in PBS containing 0.1% of Triton X-100 for 10 min, and washed in PBS twice before mounting.

X-Gal stain, in situ hybridization, and immunohistochemistry

The female reproductive system was dissected in PBS, fixed in 2% PFA for 20 min, stained with X-Gal as described (Sullivan et al., 2000), and counterstained with 0.2% Nuclear Fast Red (Sigma, St. Louis, MI) in 10% of Aluminum Sulfate for 10 min. Ten-micrometer cryosections of *oamb*^{P5391} females were fixed in 2% PFA for 10 min and processed as described for the dissected tissues.

A clone containing the 5' half of the OAMB cDNA (Han et al., 1998) was used to make RNA probes with digoxigenin-UTP (Boehringer M). Ten-micrometer cryosections of CS and *oamb*²⁸⁶ flies fixed in 4% PFA were hybridized with RNA probes and processed as previously described (Han et al., 1996).

For immunostaining, flies were first fixed in 4% PFA for 3 h at 4°C. Ten-micrometer cryosections of fly heads were subjected to affinity-purified mouse anti OAMB-K3 antibody (1:50) and biotinylated horse anti-mouse IgG (1:1000;

Vector, Burlingame, CA) followed by chromatic detection for horseradish peroxidase (Han et al., 1996).

Results

Generation of *oamb* mutants

The *oamb* gene is on chromosome three at cytological position 92F (Han et al., 1998). After surveying multiple candidate lines, we identified the enhancer trap line P551 carrying a P[1ArB] element insertion approximately 50 kb upstream of the *oamb* gene (Bellen et al., 1989; Cooley et al., 1988). We subsequently mobilized the P[1ArB] in P551 by transposase-mediated dysgenesis and obtained three new insertions in the *oamb* locus (Dalby et al., 1995; Tower et al., 1993). One line, *Df(3R) 6679*, contained a P-insertion 80 bp upstream of the putative first exon (E) and deletion of genomic region between this new insertion site and the original P-insertion site in P551 (Fig. 1). *Df(3R) 6679* homozygous animals died during the second instar larval stage. This lethality was rescued by transgenic expression of *cdc2c* cDNA (data not shown), which is also deleted in *Df(3R) 6679* (Stern et al., 1993). The rescued *Df(3R) 6679* homozygous adults, nevertheless, contained OAMB immunoreactivity in the brain although the level was reduced (data not shown). Two additional lines, *oamb*^{P213} and *oamb*^{P5391}, had P-element insertions in the first intron (I) separated by 18 bp. These lines were homozygous viable with normal OAMB immunoreactivity (data not shown).

In order to generate deletions in the *oamb* coding sequence, *oamb*^{P5391} was subjected to the dysgenesis (Voelker et al., 1984). F₁ progeny with *ry* eye color that represent excision events were screened by PCR using multiple primer sets (Materials and methods). Out of 1337 chromosomes screened, 16 excision lines representing various extents of deletion in the *oamb* locus were selected for further analysis (Fig. 1). Except for *oamb*¹⁷⁴, *oamb*³¹¹, and *oamb*³⁶⁸ that removed whole P-element, the other lines had one break point in the P-element and the other either in the downstream (toward the *oamb* coding sequence) or upstream genomic region. The breakpoints of downstream excision lines, with the exception of *oamb*²⁸⁶, were confined to the *oamb* locus, clustering at I2 and I3 (Fig. 1). *oamb*²⁸⁶ had deletion of all but E1 of the *oamb* coding region and the neighboring gene CG4000 whose function is unknown (The Flybase Consortium, 2003). Consequently, *oamb*²⁸⁶ represents a null mutant for *oamb* and CG4000. The upstream lines *oamb*²³⁶ and *oamb*³⁴⁵ had breakpoints within 5 kb and between 5 and 10 kb, respectively, upstream of the *oamb* E1. Thus, these two lines may lack all or a portion of upstream regulatory sequence required for OAMB expression. The lines *oamb*¹⁷⁴, *oamb*³¹¹, and *oamb*³⁶⁸ had their downstream breakpoints in I2 and upstream breakpoints approximately 20 kb from E1. The deleted upstream genomic region contains the gene CG31205 with unknown

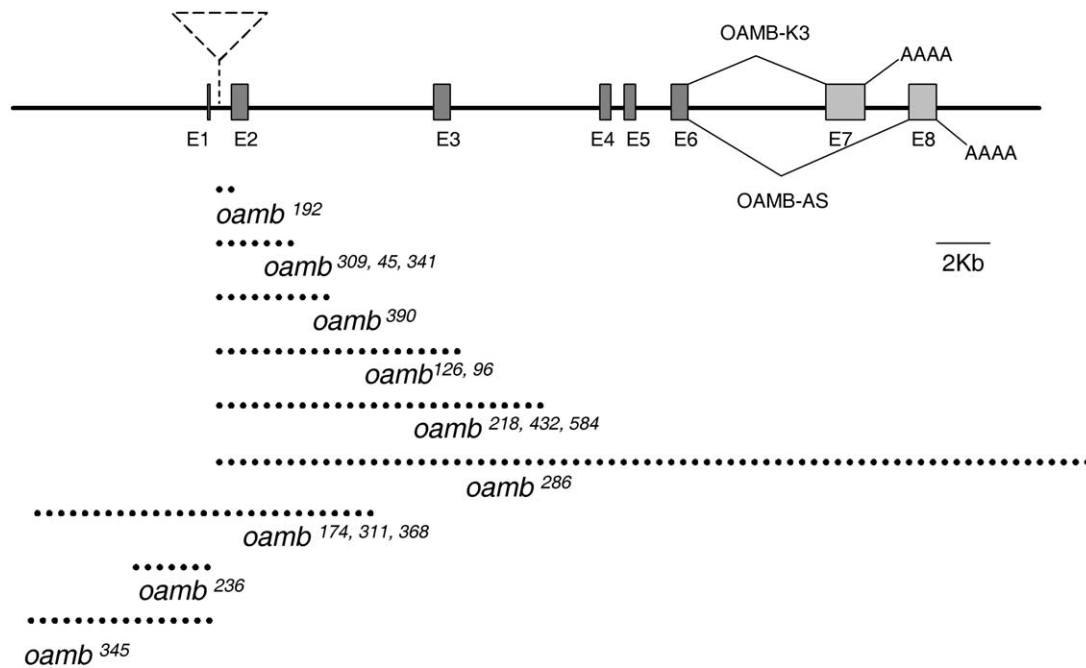


Fig. 1. A schematic map of the *oamb* locus. Boxes represent the OAMB exons and are marked with individual exon numbers, E1 to E8; the boxes filled with lines indicate the alternatively spliced exons for OAMB-K3 (E7) and OAMB-AS (E8). P-elements in *oamb*^{P5391} and *oamb*^{P213} are drawn together as a triangle in intron 1 (I1). The arrow represents the direction of *lacZ* transcription. Dotted lines delineate deleted genomic regions for *oamb* alleles.

function (The Flybase Consortium, 2003). In summary, while deletions of 12 excision lines were limited to the *oamb* locus, four lines, *oamb*²⁸⁶, *oamb*¹⁷⁴, *oamb*³¹¹, and *oamb*³⁶⁸, had additional deletions of the adjacent gene.

Female sterility of *oamb* mutants

Homozygous adults for all 16 lines were viable with normal gross external morphology; however, we were unable to establish homozygous stocks with some excision lines. When males and females from those lines were tested separately with heterozygous counterparts, sterility was associated only with homozygous females. To quantify this phenotype, individual homozygous females from each excision line were placed with three wild-type CS males on either grape juice food for counting eggs and larvae or regular cornmeal food for adults. The *ry* females, which have the same genetic background as the excision lines, were used as a control along with CS females. Overall, females of *ry*, CS, and three excision lines produced many progeny, whereas females of the remaining 13 lines produced a few or no eggs and adults (Table 1). For every genotype, the number of progeny was highly variable from vial to vial. To highlight the differences among various genotypes, we divided tested females of each genotype into two groups, one that produced more than 10 progeny ("High") and the other that produced less than 10 ("Low"). When a subset of *oamb* alleles and *ry* were examined, the percentage of females of each genotype belonging to the "High" or "Low" group was similar whether they were

grouped based on either egg/larvae or pupae/adult numbers (Fig. 2A). Moreover, when the "Low" females were divided into 2 subgroups, one that produced more than 4 progeny and the other that produced equal or more than 4, similar percentages of "Low" females of each genotype belong to the subgroups separated by eggs/larvae and pupae/adults numbers (Fig. 2A). This suggests that eggs laid by *oamb* females develop to an adult stage and the maternal OAMB is not required for this process. Therefore, the combined data of egg/larvae and pupae/adult numbers are presented in Table 1 for all the tested lines.

As shown in Table 1, all of CS females belong to the "High" group. Two upstream excision lines, *oamb*²³⁶ and *oamb*³⁴⁵, had a significant number of females belonging to the "High." While 90.1% of *oamb*³⁴⁵ females represented the "High" group, which is comparable with that of *ry* females (85.5%), the percentage of "High" *oamb*²³⁶ females was somewhat lower (67.7%). However, the difference disappeared when females of *ry* and *oamb*²³⁶ reared only on regular cornmeal food were compared (86.4 vs. 83.3%). Thus, the excision lines with deletion of only upstream genomic region exhibited fecundity comparable with that of the genetic control *ry*.

All of *oamb*¹⁹² females also belong to the "High" group. This line contained a relatively small deletion in I1. On the contrary, all females of other lines with breakpoints downstream of E2 belong to the "Low" group. The numbers of progeny produced by these "Low" females ranged from zero to nine, with the majority producing no progeny; thus,

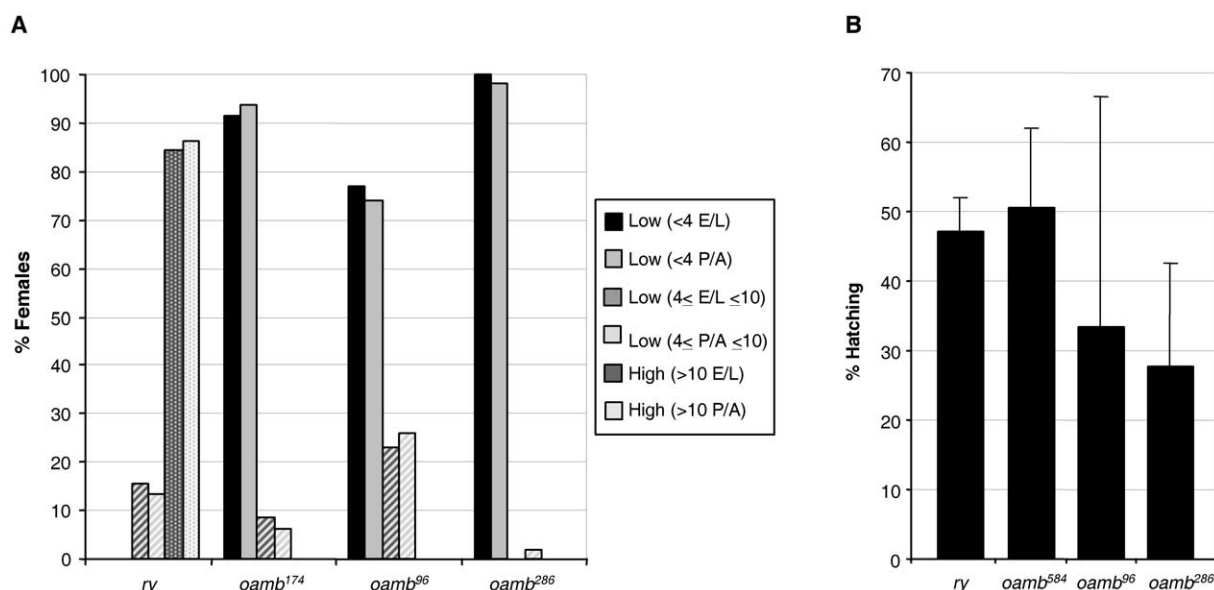


Fig. 2. (A) Female fecundity of a subset of *oamb* alleles and the control *ry*. The percentages of “Low” females that produced less than 4 eggs/larvae (E/L) and pupae/adults (P/A) are shown by solid bars; those that produced equal or more than 4 by bars filled with hatched lines; and “High” females that produced more than 10 E/L or P/A by bars filled with dots. (B) Egg hatching. Most of sterile *oamb* females did not lay eggs. When they did, they laid a few. The vials that contained eggs were only used for calculating the percentage of hatched eggs (mean \pm SEM). There was no significant difference among different genotypes ($P > 0.05$, one-way ANOVA, SPSS program). $N = 12$ (*ry*), 8 (*oamb*⁵⁸⁴), 3 (*oamb*⁹⁶), and 3 (*oamb*²⁸⁶).

the average yield was fewer than two progeny per each female (Table 1). Interestingly, sporadic egg laying observed in *oamb* females was comparable with that of virgin *ry* females, which occasionally lay unfertilized eggs (average 1.76 eggs, $N = 25$). Females transheterozygous for *oamb*⁹⁶ or *oamb*²⁸⁶ with *Df(3R) 6679* were fully fertile because all of the tested females belong to the “High” group (Table 1). Thus, the sterility of *oamb*⁹⁶ and *oamb*²⁸⁶ females was complemented by *Df(3R) 6679* containing deletion of upstream regulatory sequence. Taken together, these results suggest that an enhancer(s) for egg laying is likely located in I2 between the downstream breakpoints of *oamb*¹⁹² and *oamb*³⁰⁹. Full fecundity of the excision lines whose deletions are limited to I1 or upstream genomic region indicates that regulatory sequences residing in these areas are not essential for egg laying. For the subsequent analyses, we focused on a subset of sterile lines, including *oamb*⁹⁶ and *oamb*²⁸⁶, a fecund line *oamb*¹⁹², and the *ry* control.

To explore whether eggs laid by *oamb* females develop normally, females of *oamb*⁵⁸⁴, *oamb*⁹⁶, *oamb*²⁸⁶, or *ry* were mated with CS males en masse, and their eggs were examined for hatching. The percentages of eggs laid by *oamb*⁹⁶, and *oamb*²⁸⁶ females that hatched were highly variable due to the small number of eggs (Fig. 2B, Table 1). Nonetheless, the overall percentages of hatched eggs laid from the sterile *oamb* alleles *oamb*⁵⁸⁴, *oamb*⁹⁶, and *oamb*²⁸⁶, were comparable with that from the control *ry*, suggesting that the maternal OAMB is not required for normal embryonic development.

Abnormal egg retention in *oamb* females

Females with the sterility phenotype kept in vials were easily identifiable because their abdomens were noticeably enlarged. To investigate this abnormality further, the ovaries of *oamb*⁹⁶, *oamb*²⁸⁶, *oamb*¹⁹², or *ry* females mated with CS males were examined after DAPI staining. As shown in Fig. 3, there was a striking difference in the composition of their ovaries. While *ry* (Fig. 3A) and *oamb*¹⁹² (Fig. 3C) ovaries contained continuously developing oocytes with approximately one mature egg per ovariole as previously described in wild-type ovaries (Drummond-Barbosa and Spradling, 2001), the ovaries of *oamb*⁹⁶ (Fig. 3D) and *oamb*²⁸⁶ (Fig. 3E) had two or three mature eggs per ovariole. When the ovaries of 1-day-old *oamb*⁹⁶ and *oamb*²⁸⁶ females were examined after staining with DAPI and Phalloidin, no gross abnormalities were detectable in nurse cells, egg chambers, ring canals, and overall morphology (data not shown).

When wild-type females are not mated for several days, they often contain a higher number of mature eggs in their ovaries. Indeed, 10-day-old virgin CS (data not shown) or *ry* females (Fig. 3B) contained two or three mature eggs per ovariole, as observed in *oamb*⁹⁶ and *oamb*²⁸⁶ ovaries. To test whether abnormally retained mature eggs of *oamb* females resulted from the failure to mate, individual *oamb*⁹⁶ and *oamb*²⁸⁶ females were paired with a CS male, and the courtship index, a percentage of time that the male spends on courting, was measured for 20 min. Males paired with *oamb*⁹⁶ and *oamb*²⁸⁶ females displayed courtship indices higher than those of males paired with *ry* females [*oamb*⁹⁶,

Table 1
Fecundity of *oamb* excision lines

Genotype	Percentage of “High” females	Average number of progeny from “Low” females
CS	100 (11)	N/A
ry	85.4 (41)	7.5 ± 0.6 (6)
236	67.7 (31)	4.9 ± 1 (10)
345	90.9 (33)	2.7 ± 2.7 (3)
192	100 (18)	N/A
309	0 (13)	0.2 ± 0.1
45	0 (11)	0.3 ± 0.2
341	0 (12)	0 ± 0
390	0 (16)	0.4 ± 0.2
126	0 (26)	1.1 ± 0.3
96	0 (67)	1.6 ± 0.3
218	0 (28)	0.4 ± 0.2
432	0 (27)	0.9 ± 0.3
584	0 (27)	2.7 ± 0.5
286	0 (63)	0.2 ± 0.1
174	0 (39)	1.5 ± 0.4
311	0 (24)	0.3 ± 0.2
368	0 (22)	1 ± 0.3
96/6679	100 (15)	N/A
286/6679	100 (14)	N/A

Note. *Oamb* excision lines are represented by allelic numbers in the left column; CS and *ry* served as controls. In the middle column, “High” represents females that produced at least 10 progeny; numbers in parentheses represent the total number of females tested. The right column shows the average progeny number from “Low” females ± standard error of means. Females with less than 10 progeny for each genotype belong to the “Low” group. Total numbers of “Low” females are in parentheses for *ry*, *oamb*²³⁶, and *oamb*³⁴⁵. None of CS, *oamb*¹⁹², *oamb*⁹⁶/Df(3R) 6679, and *oamb*²⁸⁶/Df(3R) 6679 females belong to the “Low” group (N/A). For the remainder, all of the tested females belong to the “Low” group, and thus were used for calculating the average progeny number. Both developing and adult progeny were counted for this analysis.

70.6 ± 5.3% (*N* = 15); *oamb*²⁸⁶, 65.8 ± 9.3% (*N* = 8); *ry*, 42.4 ± 5% (*N* = 22)]. Moreover, the majority of females from all genotypes eventually engaged in copulation and the duration of their copulation was comparable (*oamb*⁹⁶, 18.3 ± 0.6 min; *oamb*²⁸⁶, 18.6 ± 0.6 min; *ry*, 16.6 ± 0.7 min, *N* = 15). Therefore, the sterility of *oamb* females was not due to defective courting or mating, but rather due to defective egg-laying, resulting in a higher number of mature eggs retained in their ovaries.

Egg laying is typically a two-step process: egg release by the ovary to the uterus (ovulation) and egg deposition to appropriate external environment. We have noticed from numerous dissected and sectioned reproductive systems that *oamb* females rarely contained eggs in their uteri, suggesting their defective ovulation. To quantify the ovulation rate of *oamb* females, individual females of *oamb*⁵⁸⁴, *oamb*⁹⁶, *oamb*²⁸⁶, or *ry* were mated with CS males, and the presence of an egg in their uteri was examined 4, 6, 9, 12, and 24 h after mating (Fig. 4). For all genotypes, the ovulation rate increased up to 12 h after mating and then declined at 24 h. However, the percentages of ovulating *oamb* females were dramatically lower than that of *ry* females at all time points

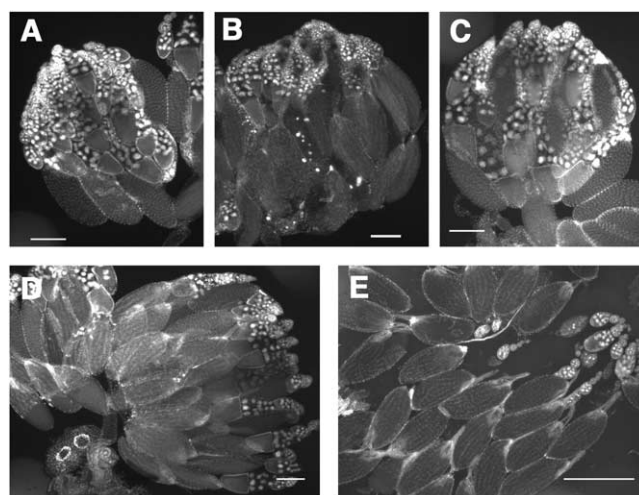


Fig. 3. Dissected ovaries stained with DAPI. (A) and (B) *ry*, (C) *oamb*¹⁹², (D) *oamb*⁹⁶, and (E) *oamb*²⁸⁶. (A) and (C–E) are from 3- to 4-day-old mated females, and (B) is from a 10-day-old virgin *ry* female. Mature eggs do not have nurse cells with large nuclei, and have dorsal appendages; many of them are visible in the ovaries of *oamb*⁹⁶ and *oamb*²⁸⁶ as well as aged virgin *ry* females.

tested. Moreover, at no time points did the tested *oamb*⁹⁶ females have eggs in their uteri (Fig. 4). These data indicate that *oamb* females were impaired in releasing mature eggs to the uterus, and this ovulation deficit may be largely responsible for their infecundity.

OAMB expression

To gain insights into how OAMB regulates ovulation in females, we took advantage of the line *oamb*^{P5391}, which carries an enhancer trap P[1ArB] element containing the reporter gene *lacZ* in II (Fig. 1). The expression pattern of *lacZ* is likely to reflect endogenous enhancer activities for *oamb* expression (O’Kane and Gehring, 1987). The *lacZ* used in P[1ArB] contains nuclear localization sequence, thus β-Gal expressed in *oamb*^{P5391} is confined in nuclei (Bellen et al., 1989). X-Gal staining of sagittal sections of

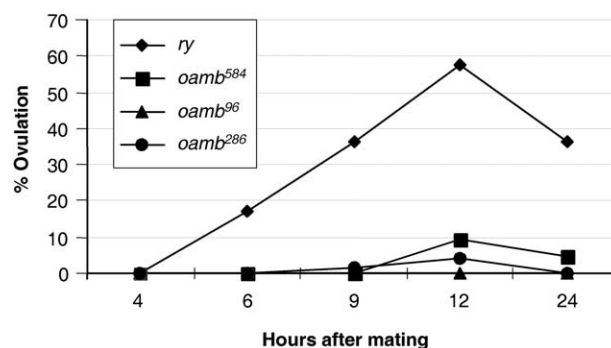


Fig. 4. Ovulation rate. Females were mated individually with CS males and were monitored for the presence of an egg in the uterus at the time intervals indicated. *N* = 33 (*ry*), 22 (*oamb*⁵⁸⁴), 35 (*oamb*⁹⁶), and 77 (*oamb*²⁸⁶).

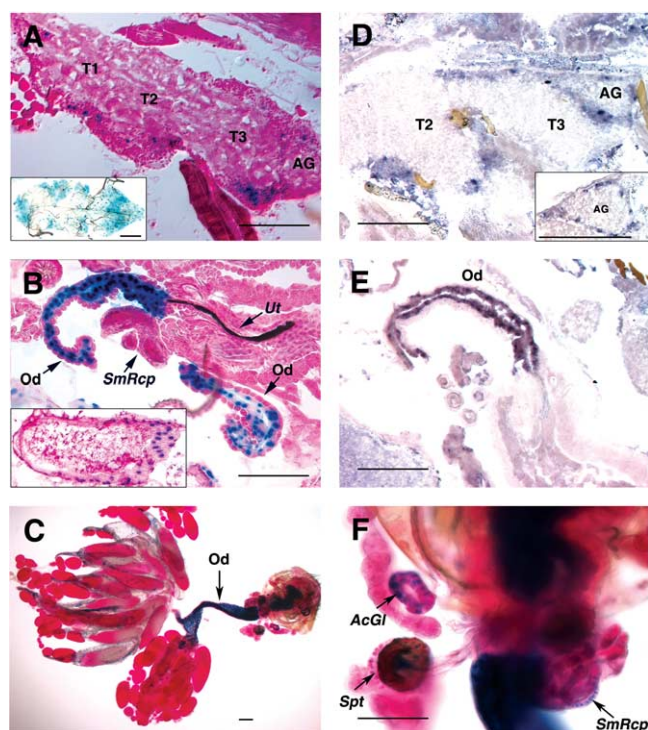


Fig. 5. OAMB expression in the female body. (A) X-Gal (blue) stain of the TAG in a sagittal section. Each thoracic neuromere (T1, T2, and T3) and the abdominal ganglion (AG) are labeled. Inset shows X-Gal stain of the whole-mount TAG. (B) X-Gal stain in epithelial cells of the oviduct (Od) in the abdomen: SmRcp, seminal receptacle; Ut, uterus. Inset shows a section of the mature egg displaying X-Gal stain in follicle cells. (C) X-Gal staining of the whole-mount female reproductive system. The lateral and common oviducts (od), and mature eggs are labeled with X-gal. (D) RNA in situ hybridization of the TAG in a sagittal section. Several cell bodies in the cellular cortex are labeled with OAMB riboprobes (purple). Inset shows the posterior ganglion (T3 and AG) from a different section. (E) RNA in situ hybridization of a common oviduct attached to the uterus. Stained cells are in the epithelial layer inside of the visceral muscle. (F) Higher magnification of the whole-mount reproductive system with X-gal stain in a subset of cells in the seminal receptacle (SmRcp), spermathecae (Spt), and accessory glands (AcGI). The *oamb*^{P5391} females were used for X-gal staining and CS females for in situ hybridization. Anterior is to left and dorsal is down. Scale bars, 100 μ m.

adult females revealed β -gal activity in the brain, the thoracic and abdominal ganglia (TAG), mature eggs, and the reproductive system (Fig. 5). In the brain, strong β -gal activity was found in two clusters of cells, corresponding to the mushroom body and ellipsoid body neurons, along with additional cells in the central brain and medulla (data not shown). This pattern was very similar to that revealed by in situ hybridization detecting OAMB transcripts in the brain (Han et al., 1998). X-Gal stain was also visible in a subset of cells in the TAG (Fig. 5A). They include both large and small cells scattered in the cellular cortex of each neuromere (Fig. 5A, inset). In the abdomen, epithelial cells in the oviduct were strongly labeled with X-Gal (Fig. 5B). Staining the dissected female reproductive system clearly revealed β -gal activity in both lateral and common oviducts, and follicle cells of the mature egg from each ovariole (Fig.

5C). In addition, subsets of cells in the seminal receptacle, spermathecae, and accessory glands were weakly stained with X-gal (Fig. 5F). Other areas in the body did not display any discernable β -gal activity.

To ascertain whether β -gal activity in the body reflected authentic OAMB expression, in situ hybridization was performed on sagittal sections of CS female bodies using riboprobes representing the 5' half of *oamb* cDNA. As shown in Fig. 5D and E, OAMB transcripts were detectable in cells scattered in the TAG cellular cortex and the oviduct in the similar pattern with X-gal stain. These signals were not found in the sections of *oamb*²⁸⁶ females (data not shown). This result suggests that β -gal expression indeed reflected endogenous enhancer activities for OAMB expression in the CNS and the oviduct. On the other hand, in situ staining of other abdominal structures was inconclusive because of nonspecific binding of the riboprobes even in *oamb*²⁸⁶ sections (data not shown). Interestingly, the upstream deletion line *oamb*³⁴⁵ contained the intact LacZ gene in the remaining P-element after imprecise excision and displayed the X-Gal stain pattern very similar to that of *oamb*^{P5391} (data not shown). This implies that the regulatory elements for OAMB expression are located downstream of E2. This is consistent with normal fecundity of the upstream excision lines, and sterility of downstream lines with deletion beyond E2. In summary, OAMB was expressed not only in the brain but also in subsets of cells in the TAG, epithelial cells in oviducts, and possibly mature eggs and other reproductive tissues.

To investigate whether the *oamb* mutants with the sterility phenotype had compromised OAMB expression in all or some of OAMB-expressing structures, we carried out RT-PCR analysis on heads, dissected oviducts, the female reproductive system (ovaries, oviducts, seminal receptacles, spermathecae, and accessory glands), and bodies that include the TAG (Fig. 6). The *oamb* locus encodes two isoforms, OAMB-K3 and OAMB-AS. They are produced by alternative splicing of the last exon (Fig. 1) to yield divergent sequences from the putative third cytoplasmic loop. RT-PCRs were performed for the common region for both isoforms, the unique sequence in OAMB-K3 or OAMB-AS, and *cdc2c* as a RNA control. As shown in Fig. 6, the control *ry* contained both OAMB isoform transcripts in every tissue sample. As expected, no OAMB transcripts were detectable in *oamb*²⁸⁶ containing a deletion of the OAMB coding sequence. Similarly, both OAMB transcripts were absent in the oviduct, the reproductive system, and the body of *oamb*⁹⁶ with a breakpoint in I3 (Fig. 6). Interestingly, the head of *oamb*⁹⁶ contained OAMB-K3, but not OAMB-AS, transcripts. The same result was obtained with *oamb*²¹⁸ with a breakpoint further downstream in I3 (data not shown). Similarly, the bodies of *oamb*³⁰⁹ females with a breakpoint in I2 had no OAMB transcripts, while their heads expressed both transcripts. In contrast, the fertile lines *oamb*¹⁹² and *oamb*²³⁶ contained both transcripts in all tissue types examined (Fig. 6). Taken together, the RT-PCR anal-

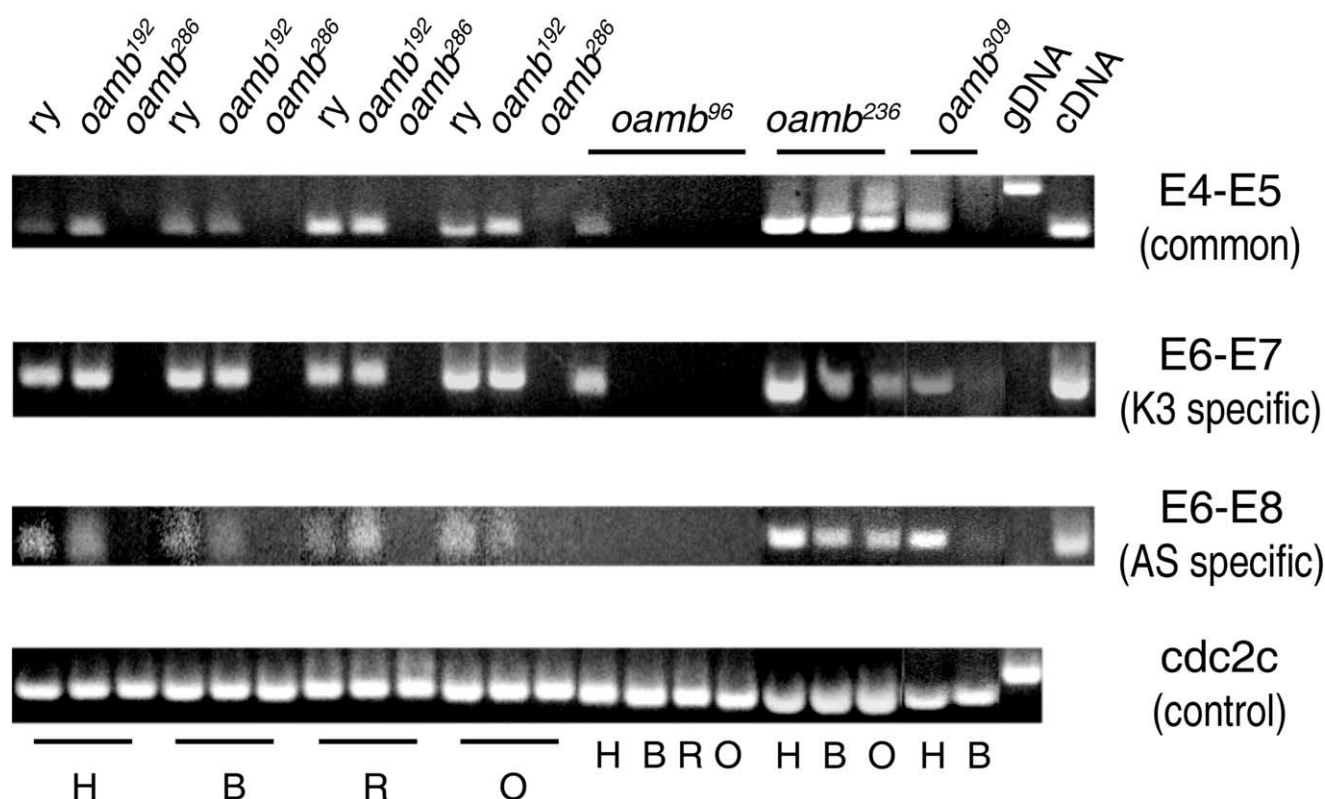


Fig. 6. RT-PCR analysis. The genotypes assayed are shown in the top panel. The areas amplified in OAMB transcripts are indicated in the right panel. OAMB cDNA clones of both isoforms (cDNA) and *ry* genomic DNA (gDNA) were used as controls for PCR, and *cdc2c* a control for RNA presence; H, male and female heads; B, female bodies; R, female reproductive systems; O, oviducts.

ysis revealed that both OAMB isoforms were normally expressed in all tissues examined. However, their expression in the brain was differently affected in *oamb* excision lines. Moreover, the absence of both OAMB isoforms in the body and the reproductive system was correlated well with the infecundity phenotype of *oamb* females.

We employed an immunohistochemical analysis to explore OAMB-K3 expression in the brain of *oamb⁹⁶* and *oamb³⁰⁹* females further. Serial frontal sections of *oamb⁹⁶* heads were compared with those of *ry* and *oamb²⁸⁶* after staining with the anti-OAMB-K3 antibody. As previously described (Han et al., 1998), OAMB-K3 was highly enriched in the mushroom bodies and the ellipsoid body in *ry* control animals (Fig. 7). The staining was totally absent not only in *oamb²⁸⁶* but also in *oamb⁹⁶* as well (Fig. 7). This suggests that *oamb⁹⁶* likely represents a protein null for OAMB-K3. On the other hand, OAMB-K3 immunoreactivity was detectable in the brain of *oamb³⁰⁹* (Fig. 7), indicating that OAMB-K3 expression in these structures was not required for ovulation. Likewise, the ovulation process may not depend on the brain expression of OAMB-AS, whose transcripts were detectable in the head of the sterile allele *oamb³⁰⁹* by RT-PCR. Therefore, OAMB-K3 and/or -AS in the body, but not in the brain, are required for normal female ovulation.

Discussion

In this report, we describe the isolation of *Drosophila oamb* mutants containing various deletions in the *oamb* locus and their impairment of female reproduction. Octopamine, an endogenous ligand of OAMB, is a major insect neuromodulator comparable to mammalian epinephrine/norepinephrine. OAMB is the only receptor identified to date that has a high specificity to octopamine for increasing cAMP, and its orthologs have been also cloned in the honeybee and other insects (Blenau and Baumann, 2001). However, mechanistic studies of its physiological functions have been hampered largely due to the lack of adequate pharmacological and genetic resources. The *oamb* mutants characterized in this study represent the first genetic variants in octopamine receptors, thus providing a unique resource to investigate specific roles of octopamine mediated by OAMB and the underlying mechanisms. The previous study (Han et al., 1998) demonstrates that OAMB transcripts are highly enriched in the head and undetectable in the body by northern analysis. More sensitive assays utilized in this study revealed additional OAMB expression in the TAG and the female reproductive system, implying wide-ranging functions for OAMB.

Deletion of the *oamb* gene beyond E2 caused female

infecundity. During copulation, males transfer not only sperm but also several seminal fluid proteins, which affect female reproductive physiology and behavior (Bloch Qazi et al., 2003; Chapman, 2001). For example, the sex peptides, Acp70A and DUP99B, are known to be crucial for decreasing receptivity of mated females and stimulating oviposition (Aigaki et al., 1991). Sex peptides have binding sites in many locations, including the brain, TAG, and reproductive system (Ottiger et al., 2000), but their action on the brain has the greatest influence on post-mating behavior (Nakayama et al., 1997). Moreover, another seminal protein ovulin (Acp26Aa) stimulates egg-laying for 1 day after mating (Herndon and Wolfner, 1995). During and right after copulation, ovulin is present in the base of the ovary where it likely enhances ovulation (Heifetz et al., 2000). Similar to sex peptides, ovulin and Acp62F also enter the female's circulatory system (Lung and Wolfner, 1999; Monsma et al., 1990). These observations suggest that various seminal proteins regulate post-mating behavior in multiple target tissues of mated females, including the brain, TAG, and reproductive system.

Studies using gynandromorphs and decapitated females demonstrate that foci controlling oviposition are located in the body (Szabad and Fajsz, 1982). This is consistent with the present study demonstrating that the absence of OAMB in the body was associated with the egg-laying defect. Oviposition is composed of two distinct steps: egg transfer from the ovary to the uterus and egg deposition. OAMB appeared to be required for the first step, egg transfer to the uterus, because the uteri of sterile *oamb* females rarely contained an egg. The focus controlling the egg transfer maps to the thorax that houses the TAG and endocrine glands. Interestingly, both male and female corpus allatum and corpus cardiacum are found in nonovipositing gynandromorphs, yet injection of glands donated from wild-type mated females induces egg laying in these animals (Szabad and Fajsz, 1982). This strongly implicates the TAG as a key focus for egg laying. Its function may be to activate endocrine glands, which, in turn, trigger oviposition. The gynandromorph study by Szabad (Szabad and Fajsz, 1982) also demonstrates that injection of the juvenile hormone analog XR-515 has the same effect as implanted glands. On the other hand, the study by Soller et al. (1997) reveals no effect of methoprene (another juvenile hormone analog) injection on oviposition of virgin females. The discrepancy between these studies could be that additional factor(s) present only in mated females is also required for oviposition. For example, as noted above, ovulin (Acp26Aa) stimulates the release of mature eggs from the ovary right after mating (Heifetz et al., 2000). Taken together, these studies suggest that seminal proteins, such as sex peptides and ovulin transferred from a male to a female during copulation, may travel to the brain to register that mating has occurred. The mating signals received in the brain may be delivered to the TAG to execute active rejection behavior (running away, kicking, and ovipositor extrusion) and to

stimulate ovulation. It is tempting to postulate that octopamine may play a role in communication between the brain and the TAG. OAMB in the ganglion, upon binding to octopamine, may be involved in executing the ovulation signal to the endocrine system, which, in turn, releases juvenile hormone and/or other factors to the reproductive system for egg laying.

OAMB expressed in the reproductive system may also play a role in oviposition. A role of octopamine in the oviduct activity has been intensely studied in locusts. While the pentapeptide proctolin stimulates contraction of the dissected oviduct, octopamine inhibits myogenic contraction as well as the basal tonus and neurally evoked contraction of oviducts in a dose response manner (Nykamp and Lange, 2000; Orchard and Lange, 1986). Octopamine and proctolin exert antagonistic effects to each other; octopamine inhibits proctolin-induced contraction by stimulating a cAMP increase and proctolin inhibits octopamine-induced relaxation by inhibiting the cAMP increase (Nykamp and Lange, 2000). Although α -adrenergic agonists can mimic the effects of octopamine (Lange and Tsang, 1993), the nature of octopamine receptor(s) mediating these effects is unknown. In vivo, octopamine may be secreted from the abdominal neurons innervating the oviduct in locusts (Lange and Orchard, 1986; Orchard and Lange, 1987). In *Drosophila*, a role of octopamine in the oviduct has not been documented; however, it is conceivable that OAMB may be involved in relaxation of the oviduct and other reproductive system for the transport of mature eggs and sperm. OAMB expressed in the TAG may directly and indirectly modulate abdominal neurons innervating the oviduct muscle. On the other hand, OAMB expressed in the oviduct epithelial cells may be involved in the secretion of proctolin or other peptides such as CCAP and allatostatin (Donini and Lange, 2002; Garside et al., 2002) to modulate the oviduct muscle activity. In the absence of OAMB, the oviduct muscle could be too constricted for an egg to pass to the uterus, causing abnormal retention of mature eggs in the oviduct.

Alternatively, OAMB in the reproductive system may be crucial for establishing proper fluid environment in the oviduct lumen for egg or sperm transport. Although biochemistry and physiology of the oviduct are documented in mammals (Menezo and Guerin, 1997), there is little information available in insects. In the rabbit oviduct, adrenaline and noradrenaline stimulate fluid formation, Cl^- transport, and electric potential difference of oviductal epithelial cells (Leese et al., 2001). These effects appear to be mediated by β -adrenergic receptors possibly by activating the intracellular Ca^{2+} rather than cAMP pathway. Indeed, β -adrenergic receptors are found in bovine oviductal epithelial cells and their expression is influenced by endocrine system (Einspanier et al., 1999). Damage to oviductal epithelial cells in human, which is often associated with pelvic inflammatory disorder, results in abnormal fluid formation and reduced fertility or infertility (Leese et al., 2001). In *Drosophila*, octopamine may play a similar role as adrenaline or nor-

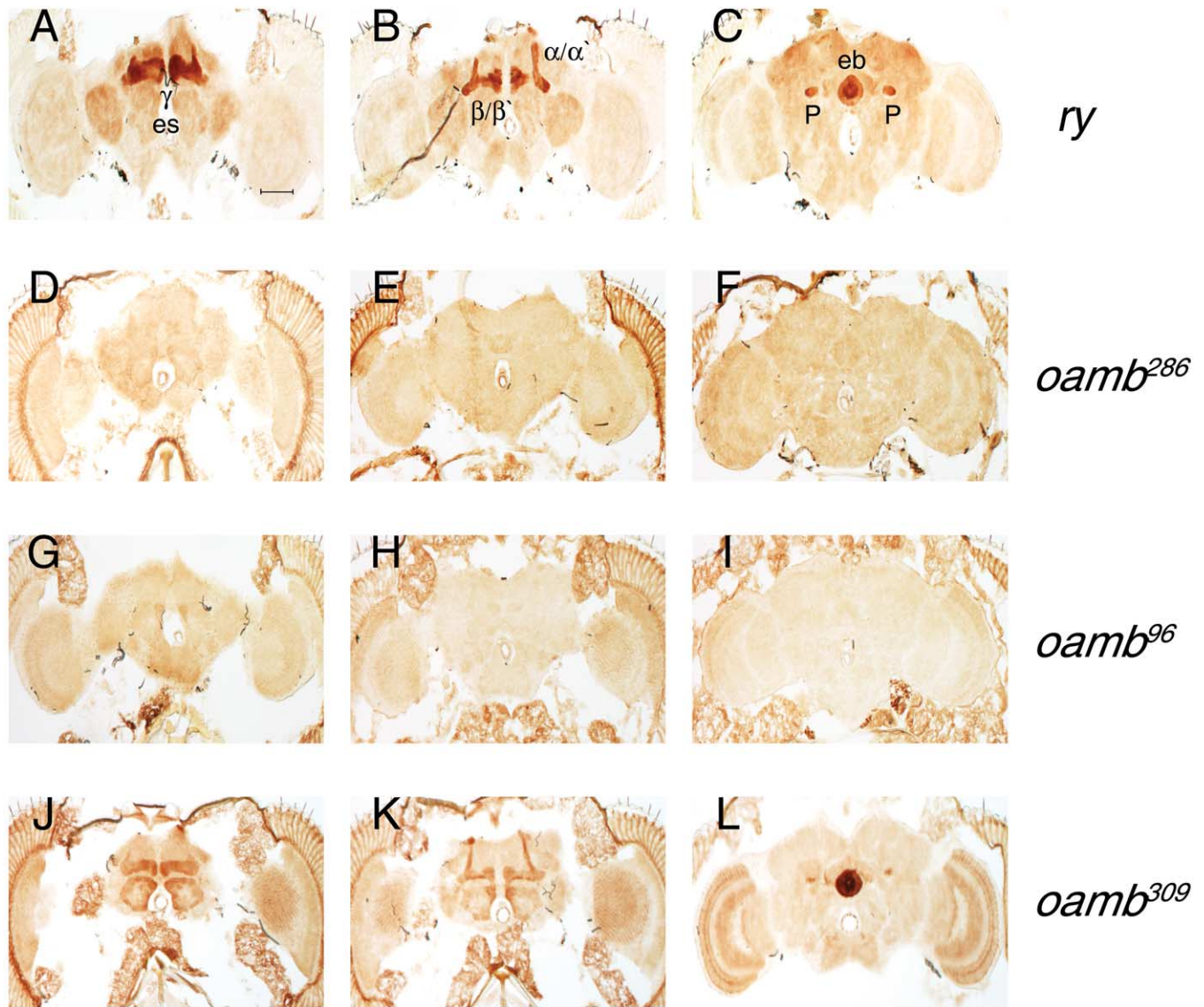


Fig. 7. OAMB-K3 immunoreactivity of *ry* (A–C), *oamb*²⁸⁶ (D–F), *oamb*⁹⁶ (G–I), and *oamb*³⁰⁹ (J–L). Frontal head sections at the levels of the mushroom body γ -lobes (γ ; A, D, G, and J), α/β lobes (α/α' , β/β' ; B, E, H, and K), pedunculi (p), and the ellipsoid body (eb; C, F, I, and L) are shown. es, esophagus. Scale bar, 100 μ m.

adrenaline through the activation of OAMB in oviductal epithelial cells. The absence of OAMB may result in abnormal fluid formation and secretion, leading to unsuccessful egg transport.

The sterility phenotype of *oamb* females appears to be relatively specific to egg laying with normal oogenesis. This is in contrast to the sterility phenotypes associated with mutations in *Gprk2* (G-protein-coupled receptor kinase) and *Dnc* (cAMP-specific phosphodiesterase), potential downstream effectors for OAMB. They display defective oogenesis with impaired cytoplasmic dumping and dying nurse cells (Lannutti and Schneider, 2001; Schneider and Spradling, 1997). Moreover, the sterility of *gprk2* females is largely rescued by the transgenic *Gprk2* expression in germline cells of the ovary (Fan and Schneider, 2003), in which OAMB expression was not detectable. These studies indi-

cate that *Gprk2* and *Dnc* may not be involved in the OAMB signaling for female fecundity. *Drosophila* has another kinase *Gprk1* for G-protein-coupled receptors (Cassill et al., 1991) and several additional cyclic nucleotide phosphodiesterases whose expression patterns and functions are yet to be determined. It is conceivable that one or several of these molecules could serve as downstream signals for OAMB. In addition, the ovulation process regulated by OAMB could utilize the Ca^{2+} -mediated signaling pathway (Han et al., 1998).

Two intriguing aspects of OAMB expression in the female reproduction system are notable. First, its expression in the oviduct epithelial cells is somewhat unexpected because octopamine's actions have been largely established on its modulatory activities on muscles. These activities are supposedly mediated by putative octopamine receptors on

the muscle to a certain extent. Such receptors have not been cloned, nor has the receptor labeling of the intact muscle been described. Second, OAMB expression in the ovariole was detectable only in the follicle cells of the fully mature egg. These cells undergo apoptosis when the egg exits the ovary (Nezis et al., 2002). No other molecular markers characterized to date share this highly restricted pattern of OAMB expression. The presence of OAMB in these cells was dispensable for the viability and development of a few eggs that were occasionally laid from *oamb* females, and could be involved in apoptosis or ovulation. Although a large body of studies has been conducted on oocyte and embryo development, there is very limited information on molecular and cellular components and mechanism involved in the egg transfer and deposit. The unique expression pattern of OAMB and its genetic resource described in this paper would help advance our understanding on these processes and other physiological functions mediated by OAMB.

Acknowledgments

We thank Lori Mancino for expert technical assistance, Dr. C. Lehner for *cdc2c* mutants and transgenic lines, Drs. E. Siegfried, B. Jones, D. Cavener, and A. Lange for their critical reading of the manuscript, and the Bloomington Stock Center for providing the fly lines. This work was supported by Grants NS17904 (to R.L.D) and NS038346 (to K.-A.H.) from the National Institute of Neurological Disorders and Stroke.

References

- Aigaki, T., Fleischmann, I., Chen, P.S., Kubli, E., 1991. Ectopic expression of sex peptide alters reproductive behavior of female *D. melanogaster*. *Neuron* 7, 557–563.
- Bellen, H.J., O’Kane, C.J., Wilson, C., Grossniklaus, U., Pearson, R.K., Gehring, W.J., 1989. P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev.* 3, 1288–1300.
- Blenau, W., Baumann, A., 2001. Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arch. Insect Biochem. Physiol.* 48, 13–38.
- Bloch Qazi, M.C., Heifetz, Y., Wolfner, M.F., 2003. The developments between gametogenesis and fertilization: ovulation and female sperm storage in *Drosophila melanogaster*. *Dev. Biol.* 256, 195–211.
- Cassill, J.A., Whitney, M., Joazeiro, C.A., Becker, A., Zuker, C.S., 1991. Isolation of *Drosophila* genes encoding G protein-coupled receptor kinases. *Proc. Natl. Acad. Sci. USA* 88, 11067–11070.
- Chapman, T., 2001. Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity* 87, 511–521.
- Cook, B.J., Wagner, R.M., 1992. Some pharmacological properties of the oviduct muscularis of the stable fly *Stomoxys calcitrans*. *Comp. Biochem. Physiol. C* 102, 273–280.
- Cooley, L., Kelley, R., Spradling, A., 1988. Insertional mutagenesis of the *Drosophila* genome with single P elements. *Science* 239, 1121–1128.
- Dalby, B., Pereira, A.J., Goldstein, L.S., 1995. An inverse PCR screen for the detection of P element insertions in cloned genomic intervals in *Drosophila melanogaster*. *Genetics* 139, 757–766.
- David, J.C., Coulon, J.F., 1985. Octopamine in invertebrates and vertebrates. A review. *Prog. Neurobiol.* 24, 141–185.
- Davis, R.L., 1996. Physiology and biochemistry of *Drosophila* learning mutants. *Physiol. Rev.* 76, 299–317.
- Davis, R.L., 2001. Mushroom bodies, Ca(2+) oscillations, and the memory gene *amnesiac*. *Neuron* 30, 653–656.
- Donini, A., Lange, A.B., 2002. The effects of crustacean cardioactive peptide on locust oviducts are calcium-dependent. *Peptides* 23, 683–691.
- Drummond-Barbosa, D., Spradling, A.C., 2001. Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* 231, 265–278.
- Dudai, Y., Buxbaum, J., Corfas, G., Ofarim, M., 1987. Formamides interact with *Drosophila* octopamine receptors alter the flies’ behavior and reduce their learning ability. *J. Comp. Physiol. [A]* 161, 739–746.
- Dudai, Y., Zvi, S., 1984. High-affinity [3H]octopamine-binding sites in *Drosophila melanogaster*: interaction with ligands and relationship to octopamine receptors. *Comp. Biochem. Physiol. C* 77, 145–151.
- Einspanier, R., Gabler, C., Kettler, A., Kloas, W., 1999. Characterization and localization of beta2-adrenergic receptors in the bovine oviduct: indication for progesterone-mediated expression. *Endocrinology* 140, 2679–2684.
- Fan, S., Schneider, L.E., 2003. The role of maternal and zygotic Gprk2 expression in *Drosophila* development. *Biochem. Biophys. Res. Commun.* 301, 127–135.
- Garside, C.S., Koladich, P.M., Bendena, W.G., Tobe, S.S., 2002. Expression of allatostatin in the oviducts of the cockroach *Diploptera punctata*. *Insect. Biochem. Mol. Biol.* 32, 1089–1099.
- Hammer, M., Menzel, R., 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learn. Mem.* 5, 146–156.
- Han, K.A., Millar, N.S., Davis, R.L., 1998. A novel octopamine receptor with preferential expression in *Drosophila* mushroom bodies. *J. Neurosci.* 18, 3650–3658.
- Han, K.A., Millar, N.S., Grotewiel, M.S., Davis, R.L., 1996. DAMB, a novel dopamine receptor expressed specifically in *Drosophila* mushroom bodies. *Neuron* 16, 1127–1135.
- Heifetz, Y., Lung, O., Frongillo Jr., E.A., Wolfner, M.F., 2000. The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. *Curr. Biol.* 10, 99–102.
- Herndon, L.A., Wolfner, M.F., 1995. A *Drosophila* seminal fluid protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. *Proc. Natl. Acad. Sci. USA* 92, 10114–10118.
- Lange, A.B., Orchard, I., 1986. Identified octopaminergic neurons modulate contractions of locust visceral muscle via adenosine 3’,5’-monophosphate (cyclic AMP). *Brain Res.* 363, 340–349.
- Lange, A.B., Tsang, P.K.C., 1993. Biochemical and physiological effects of octopamine and selected octopamine agonists on the oviducts of *Locusta migratoria*. *J. Insect Physiol.* 39, 393–400.
- Lannutti, B.J., Schneider, L.E., 2001. Gprk2 controls cAMP levels in *Drosophila* development. *Dev. Biol.* 233, 174–185.
- Leese, H.J., Tay, J.I., Reischl, J., Downing, S.J., 2001. Formation of Fallopian tubal fluid: role of a neglected epithelium. *Reproduction* 121, 339–346.
- Lung, O., Wolfner, M.F., 1999. *Drosophila* seminal fluid proteins enter the circulatory system of the mated female fly by crossing the posterior vaginal wall. *Insect Biochem. Mol. Biol.* 29, 1043–1052.
- Martin, J.R., Raabe, T., Heisenberg, M., 1999. Central complex substructures are required for the maintenance of locomotor activity in *Drosophila melanogaster*. *J. Comp. Physiol. [A]* 185, 277–288.
- Menezo, Y., Guerin, P., 1997. The mammalian oviduct: biochemistry and physiology. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 73, 99–104.
- Menzel, R., Hammer, M., Muller, U., Rosenboom, H., 1996. Behavioral, neural and cellular components underlying olfactory learning in the honeybee. *J. Physiol. Paris* 90, 395–398.

- Monastirioti, M., Linn Jr., C.E., White, K., 1996. Characterization of *Drosophila* tyramine beta-hydroxylase gene and isolation of mutant flies lacking octopamine. *J. Neurosci.* 16, 3900–3911.
- Monsma, S.A., Harada, H.A., Wolfner, M.F., 1990. Synthesis of two *Drosophila* male accessory gland proteins and their fate after transfer to the female during mating. *Dev. Biol.* 142, 465–475.
- Nakayama, S., Kaiser, K., Aigaki, T., 1997. Ectopic expression of sex-peptide in a variety of tissues in *Drosophila* females using the P[GAL4] enhancer-trap system. *Mol. Gen. Genet.* 254, 449–455.
- Nezis, I.P., Stravopodis, D.J., Papassideri, I., Robert-Nicoud, M., Margaritis, L.H., 2002. Dynamics of apoptosis in the ovarian follicle cells during the late stages of *Drosophila* oogenesis. *Cell Tissue Res.* 307, 401–409.
- Nykamp, D.A., Lange, A.B., 2000. Interaction between octopamine and proctolin on the oviducts of *Locusta migratoria*. *J. Insect Physiol.* 46, 809–816.
- O'Dell, K.M., 1993. The effect of the inactive mutation on longevity, sex, rhythm and resistance to p-cresol in *Drosophila melanogaster*. *Heredity* 70, 393–399.
- O'Kane, C.J., Gehring, W.J., 1987. Detection in situ of genomic regulatory elements in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 84, 9123–9127.
- Orchard, I., Lange, A.B., 1986. Neuromuscular transmission in an insect visceral muscle. *J. Neurobiol.* 17, 359–372.
- Orchard, I., Lange, A.B., 1987. The release of octopamine and proctolin from an insect visceral muscle: effects of high-potassium saline and neural stimulation. *Brain Res.* 413, 251–258.
- Ottiger, M., Soller, M., Stocker, R.F., Kubli, E., 2000. Binding sites of *Drosophila melanogaster* sex peptide pheromones. *J. Neurobiol.* 44, 57–71.
- Robb, S., Cheek, T.R., Hannan, F.L., Hall, L.M., Midgley, J.M., Evans, P.D., 1994. Agonist-specific coupling of a cloned *Drosophila* octopamine/tyramine receptor to multiple second messenger systems. *EMBO J.* 13, 1325–1330.
- Roeder, T., 1999. Octopamine in invertebrates. *Prog. Neurobiol.* 59, 533–561.
- Roeder, T., Degen, J., Dyczkowski, C., Gewecke, M., 1995. Pharmacology and molecular biology of octopamine receptors from different insect species. *Prog. Brain Res.* 106, 249–258.
- Saudou, F., Amlaiky, N., Plassat, J.L., Borrelli, E., Hen, R., 1990. Cloning and characterization of a *Drosophila* tyramine receptor. *EMBO J.* 9, 3611–3617.
- Schneider, L.E., Spradling, A.C., 1997. The *Drosophila* G-protein-coupled receptor kinase homologue Gprk2 is required for egg morphogenesis. *Development* 124, 2591–2602.
- Scholz, H., Ramond, J., Singh, C.M., Heberlein, U., 2000. Functional ethanol tolerance in *Drosophila*. *Neuron* 28, 261–271.
- Soller, M., Bownes, M., Kubli, E., 1997. Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*. *Eur. J. Biochem.* 243, 732–738.
- Stern, B., Ried, G., Clegg, N.J., Grigliatti, T.A., Lehner, C.F., 1993. Genetic analysis of the *Drosophila* cdc2 homolog. *Development* 117, 219–232.
- Sullivan, W., Ashburner, M., Hawley, R.S., 2000. *Drosophila* Protocols. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Szabad, J., Fajsz, C., 1982. Control of female reproduction in *Drosophila*: genetic dissection using gynandromorphs. *Genetics* 100, 61–78.
- The Flybase Consortium, 2003. The FlyBase database of the *Drosophila* genome projects and community literature. *Nucleic Acids Res.* 31, 172–175.
- Tompkins, L., Siegel, R.W., Gailey, D.A., Hall, J.C., 1983. Conditioned courtship in *Drosophila* and its mediation by association of chemical cues. *Behav. Genet.* 13, 565–578.
- Tower, J., Karpen, G.H., Craig, N., Spradling, A.C., 1993. Preferential transposition of *Drosophila* P elements to nearby chromosomal sites. *Genetics* 133, 347–359.
- Uzzan, A., Dudai, Y., 1982. Aminergic receptors in *Drosophila melanogaster*: responsiveness of adenylate cyclase to putative neurotransmitters. *J. Neurochem.* 38, 1542–1550.
- Voelker, R.A., Greenleaf, A.L., Gyurkovics, H., Wisely, G.B., Huang, S.M., Searles, L.L., 1984. Frequent imprecise excision among reversions of a P element-caused lethal mutation in *Drosophila*. *Genetics* 107, 279–294.
- Yellman, C., Tao, H., He, B., Hirsh, J., 1997. Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated *Drosophila*. *Proc. Natl. Acad. Sci. USA* 94, 4131–4136.