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Short Communication

RNA interference of PBAN receptor suppresses expression of two fatty acid desaturases in female *Plutella xylostella*

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ABSTRACT

Pheromone biosynthesis-activating neuropeptide (PBAN) stimulates sex pheromone biosynthesis by activating PBAN receptor (PBANr), which triggers a specific signal transduction in the pheromone gland cells. We have shown that RNA interference (RNAi) of PBANr of *Plutella xylostella* significantly suppressed pheromone biosynthesis and subsequent mating behavior. In order to assess molecular events occurring downstream of PBAN signaling, we cloned partial sequences of Δ9 and Δ11 fatty acid desaturases of *P. xylostella*. Phylogenetic analysis indicated that these two desaturase genes were highly clustered with other desaturases associated with sex pheromone biosynthesis in other insects. RT-PCR analysis showed that Δ9 desaturase was dominantly expressed in adult females, whereas Δ11 desaturase was expressed in all *P. xylostella* developmental stages. When PBANr expression was suppressed by PBANr-RNAi, the treated females also showed significant suppression of expression of both desaturases. These results suggest that expressions of the two desaturases are controlled by PBAN and that the two desaturases may be involved as downstream components in sex pheromone biosynthesis of *P. xylostella*.

Introduction

Sex pheromones in moths are synthesized in pheromone glands and released at specific times to attract reproductive partners (Roelofs and Rooney, 2003). Most sex pheromones in moths are linear and unsaturated fatty acids, range from 12 to 18 carbons, and have alcohol, carboxylic, aldehyde or acetate functional groups (Baker and Heath, 2005). Lepidopteran sex pheromones contain derivatives of unsaturated fatty acid precursors, which are typically produced by acyl-CoA desaturases expressed in pheromone glands (Roelofs, 1995; Tillman et al., 1999). Specific enzymes, including fatty acid synthase, desaturases, limited chain-shortening enzymes, and other modification enzymes, are involved in several crucial steps of pheromone biosynthesis.

Production of sex pheromone is hormonally regulated by pheromone biosynthesis-activating neuropeptide (PBAN) (Jurenka et al., 1994). PBAN produced in the subesophageal ganglion is released into hemolymph via the corpora cadiaca (Raina and Klun, 1984). When PBAN binds to the PBAN receptor (PBANr), the subsequent conformational change of the receptor triggers species-specific PBAN signal downstream of the pheromone biosynthesis pathway in the pheromone gland (Rafaeli, 2009). Two secondary messengers, Ca^{2+} and cAMP, are involved in the species-dependent signal pathway. Ca^{2+} influx increases cAMP level, which subsequently activates kinases or phosphatases prior to fatty acid synthesis in *Helicoverpa zea* (Jurenka et al., 1991, 1994). Ca^{2+} influx directly activates pheromone biosynthesis in *Bombyx mori* (Matsumoto et al., 1995).

The diamondback moth, *Plutella xylostella* (L.), is a very destructive pest of cruciferous crops throughout the world (Taleka and Shelton, 1993). *P. xylostella* sex pheromone is a mixture of major components ((Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate) and minor components ((Z)-11-hexadecen-1-ol and (Z)-9-tetradecenyl acetate) (Tamaki et al., 1977; Koshihara and Yamada, 1980; Chrisholm et al., 1983; Lee et al., 2005). A *P. xylostella* sex pheromone receptor gene has been identified in male antennae, which respond to a major component (Mitsuno et al., 2008). *P. xylostella* PBAN was identified along with four other putative neuropeptides. A synthetic PBAN induced biosynthesis of a major sex pheromone component (Lee and Boo, 2005). *P. xylostella* PBANr was cloned and proved to be involved in sex pheromone biosynthesis in response to PBAN (Lee et al., 2011). RNA interference (RNAi) of PBANr significantly suppressed pheromone biosynthesis and calling behavior of the treated females (Lee et al., 2011). However, it remains unknown how the PBAN signal after binding to PBANr affects downstream activation of sex pheromone biosynthesis in *P. xylostella*. In order to unravel molecular events occurring downstream of PBAN signaling, we cloned partial sequences of Δ9 and Δ11 fatty acid desaturases and examined their expressions

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Table 1
Primers used in this study and their sequences.

Primer	Sequence	Experiment
Oligo dT-	5′-TTTTTTTTTTTTTTTTTTT	cDNA synthesis
d9-1F	5′- TGGGCCYCACAAGTCHTACAAAGC	Δ9 desaturase
d9-1R	5′- TGRGCGGCRGAGTTVACVAGCCA	cloning/RT-PCR
d11-1F	5′- GATGCTGATCCCCACAATGC	Δ11 desaturase
d11-1R	5′-AAGGGTAYKGCGTAYTTTTTCTGAAA	cloning/RT-PCR
RT-PR-F	5′-GGTTTAGCATCAGAGACTTC′	dsRNA synthesis
RT-PR-R	5′-AAAGGTGTCGCTAGGATGG′	for PBANr RNAi
Tub-F	5′-AACCGCGTATCCACTTCCC′	Internal control
Tub-R	5-AACTCGCCCTCCTCCATACC′	

in females. To analyze any association of the desaturases with PBANr, their expressions were assessed in females treated with PBANr-RNAi.

Materials and methods

Insect rearing

P. xylostella larvae were reared on Chinese cabbage leaves at 25 ± 1°C with a 16:8 h light:dark cycle. Adults were fed 10% sucrose.

Total RNA extraction and cDNA synthesis

Total RNA extraction was performed following the method of a previous report (Lee et al., 2011). Briefly, total RNA from various developmental stages of *P. xylostella* was extracted with Trizol reagent (Invitrogen, USA) following the manufacturer's instructions. The total RNAs were treated with DNase I (Takara Biomedical Inc., Korea) for 1 h prior to cDNA synthesis to remove contaminating genomic DNA. No DNA contamination was confirmed by PCR using the RNA templates. First-strand cDNA was synthesized with 1 μL of the DNase-treated RNA using Oligo dT-adaptor primer (Table 1) by reverse transcription using RT-premix (Intron Biotechnology, Korea).

Cloning of desaturase genes from P. xylostella

Abdomens containing pheromone glands were isolated from one-day-old female adults and total RNA extraction and cDNA synthesis were performed as described above. To amplify partial fragments of desaturase genes, degenerate primers were used (Table 1). All PCR reactions were performed with *Pfx Taq* polymerase (Invitrogen). The following parameters were used to amplify desaturase genes: 95°C for 1 min followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, and 68°C for 1 min, plus one additional cycle at 68°C for 2 min. Five microliters of each PCR reaction was electrophoresed on 1% agarose gel containing GelStar® nucleic acid stain (BioWhittaker Molecular Applications, USA). Resulting PCR products were cloned into pGEM T-Easy vector (Promega, USA) and bidirectionally sequenced.

RNA interference of Plx-PBANr expression and semi-quantitative RT-PCR of desaturase genes

For the suppression of PBANr expression, PCR primers were designed via siRNA Template Design Tool for the Silencer™ siRNA (Invitrogen) and double-stranded RNA (dsRNA) was synthesized with Ribomax™ Large Scale RNA Production System-T7 (Promega). Nucleotide sequences of PCR primer (RT-PR-F and RT-PR-R) for dsRNA synthesis are in Table 1. The dsRNA was synthesized by annealing sense and anti-

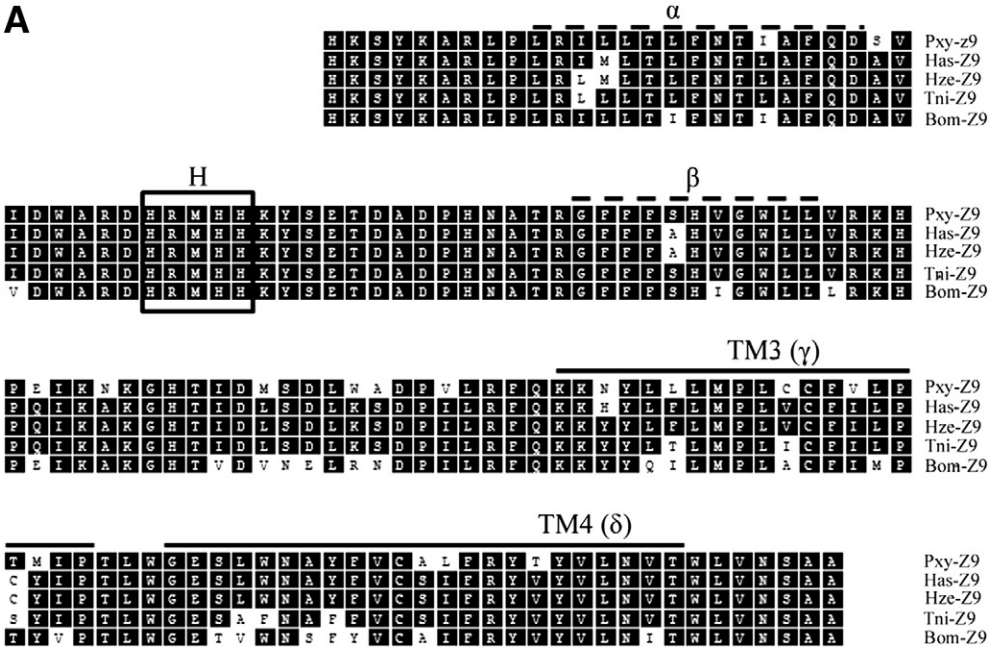

Fig. 1. Multiple alignment of lepidopteran Δ9 desaturases and phylogenetic analysis of Δ9 desaturase of *Plutella xylostella* with other desaturase genes. (A) The partial amino acid sequence of Pxy-Z9 desaturases was aligned with previously reported lepidopteran Z9 desaturases: Hze-Z9 from *Helicoverpa zea* (Rosenfield et al., 2001), Has-Z9 from *H. assulta* (Jeong et al., 2003), Bom-Z9-from *Bombyx mori* (Yoshiga et al., 2000), and Tni-Z9 from *Trichoplusia ni* (Liu et al., 1999). Identical amino acids are shaded. Amino acids of predicted transmembrane domains (TM3 and TM4) and of hydrophobic domains (α, β, γ and δ) are underlined and labeled. The Histidine box (H) is boxed. The partial nucleotide sequences of Δ9 and Δ11 desaturase genes of *P. xylostella* have been deposited in the GenBank under accession numbers JF421683 and JF421684, respectively. (B) Δ9 and Δ11 desaturase formed independent phylogenies. Asterisks indicate desaturases identified from the pheromone gland in *P. xylostella* in this study. The tree was constructed with the neighbor-joining method using multiple alignment of amino acid sequences. The numbers above branches are bootstrap values (1000 repetitions). The bar indicates the number of estimated amino acid changes per 100 amino acids. NF refers to a non-functional transcript. The accession numbers are indicated in parentheses. The abbreviated species names correspond to: Aae, *Aedes aegypti*; Ave, *Argyrotaenia velutinana*; Bmo, *Bombyx mori*; Cpa, *Choristoneura parallela*; Cpi, *Culex pipiens*; Cro, *Choristoneura rosaceana*; Dme, *Drosophila melanogaster*; Dpl, *Danaus plexippus*; Dpu, *Dendrolimus punctatus*; Dsi, *Drosophila simulans*; Epo, *Epiphyas postvittana*; Has, *Helicoverpa assulta*; Her, *Heliconius erato*; Hze, *Helicoverpa zea*; Lca, *Lampronia capitella*; Mbr, *Mamestra brassicae*; Mdo, *Musca domestica*; Mse, *Manduca sexta*; Ofu, *Ostrinia furnacalis*; Onu, *Ostrinia nubilalis*; Poc, *Planotortrix octo*; Sli, *Spodoptera littoralis*; Tni, *Trichoplusia ni;* and Tpi, *Thaumetopoea pityocampa*.