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Gene Cloning and Tissue-Specific Expression of G Protein β Subunit in *Microplitis mediator* (Hymenoptera: Braconidae)

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Abstract

A gene encoding a novel G protein β subunit of β1 subclass, *G*β*Mmed* was isolated from *Microplitis mediator* (Hymenoptera: Braconidae). The full-length sequence of *G*β*Mmed* is 1 119 bp, the cDNA contains a 1 023 bp open reading frame that encodes a protein with 340 amino acids, and the predicted molecular weight of GβMmed is 37.23 kDa and isoelectric point is 5.86. By the quantitative real-time RT-PCR method, the tissue-specific expression and quantitative changes in the developmental expression profile of *G*β*Mmed* were detected. It was found that *G*β*Mmed* was abundantly expressed in *M*. *mediator* antennae, head (without antennae), thorax, abdomen, legs and the wings, and especially at high levels in abdomen. In antennae, expression varied through 1st day before emergence to 5-d-old adults, and had equal expression levels detected in females and males in total. In head, *G*β*Mmed* expresses while initially high in females, and have another peaked in stage 4 and 1st day, in males showed a peak of *G*β*Mmed* expression prior to emergence and relatively low levels after emergence. In female abdomen *G*β*Mmed* expression levels have two peaks in stage 1 and the 5th d, but just have one peak in male abdomen in stage 1. In all other tissues expression was low and stable.

Key words: *Microplitis mediator*, G protein β subunit, quantitative real-time RT-PCR, expression pattern

INTRODUCTION

Insects have an enormous impact on global agriculture both as pests and as beneficial organisms, and olfaction of insects plays a crucial role in modulating their behavior. Therefore, it is very important to understand the interplay of the molecular components of the olfactory system. Numerous sensory stimuli exert their biological effects through trans-membrane receptors which couple to heterotrimeric guanine nucleotide binding proteins (G proteins) (Offermanns 2003), and G proteins consist of α subunit and βγ dimers. Both α subunit and βγ dimers play key roles in various signal transduction pathways. So far, 5 isoforms of β subunit have been identified, which show 50-90% identity in primary structure (Sprang 1997). The effectors regulated by βγ dimers are adenylyl cyclases (Chakrabarti and Gintzler 2003), phospholipase C-β (Barr *et al*. 2000), ion channel proteins (Mirshahi *et al*. 2002; Zhao *et al*. 2003), mitogen-activated protein kinase (Ptashne and Gann 2003), and calmodulin (Liu *et al*. 1997; Chen *et al*. 2005).

G protein β subunits must depend upon γ subunits to fold correctly and its signals transduction *in vitro* also bound to γ subunits (Sprang 1997). The crystal structure of the βγ dimers revealed that the β subunit

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consists of an N-terminal α helix followed by a symmetrical seven-bladed propeller structure based on WD-repeat sequences, repeat motifs of about 40 amino acids (Sondek *et al*. 1996). The β1-subunit of heterotrimeric G proteins undergoes phosphorylation on His^{266} which is apparently involved in receptor-independent G protein activation (Wieland *et al*. 1992). G β subunits also have other functions, yeast G β subunits also participate in adaptive processes that promote recovery from pheromone action (Coleg and Reed 1991).

G proteins play important roles in arthropods, where they mediate responses to an array of chemical messengers, such as hormones, neurotransmitters, odorants, and tastants (Krieger and Breer 1999; Hardie and Raghu 2001; Jiang *et al*. 2005; Su *et al*. 2006). As for the G protein function in insect olfaction, Sato *et al*. (2008) ruled out any role of G proteins. Through various experiments, Wicher *et al*. (2008) implicated G-protein-mediated signalling production the cyclic nucleotide cAMP was generated in response to odours. Other data showed that though odour-evoked neuronal responses were observed in neurons lacking a chemosensory G α subunit, Gαq, they were reduced in intensity (Kain *et al*. 2008).

In the present study, *G*β*Mmed* was cloned and characterized in *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae), which is an important endoparasitoid of *Helicoverpa armigera* in North China (Liu *et al*. 2005). Quantitative real-time RT-PCR (qRT-PCR) was conducted to invesgate the expression pattern of *G*β*Mmed* mRNA in *M. mediator*, particularly in the antennae and head along the 9 different developmental stages.

MATERIALS AND METHODS

Insects

M. mediator was obtained from the Institute of Plant Protection, Hebei Academy of Agriculture and Forestry in China. The parasitoids emerge from a silk cocoon inside which they pupate. Emergent adult parasitoids were fed on a 10% honey solution and mated in culture cages (20 cm × 20 cm × 10 cm) at (26 ± 1)°C, 14 L: 10 D (Wang *et al*. 1984). Antennae, head (without antennae), thorax, abdomen, legs, and wings were dissected from 4 stages prior to emergence and from 1-5 d old adults respectively. The 4 stages during the pupal process were as follows: (1) about 24 h before emergence (black thorax and abdomen); (2) about 16 h before emergence (darker thorax with slime); (3) about 8 h before emergence (wings can be seen); and (4) about 4 h before emergence (able to move freely). About 100 antennae, legs and wings and about 10 heads (without antennae), thoraxes and abdomens were collected and used. Tissues were immediately flash frozen in liquid nitrogen. All tissues were stored at -70°C for future use.

RNA manipulation

Total RNA was extracted by homogenizing antennae or other tissues in Trizol™ reagent (Invitrogen, USA) following the manufacturer's instructions. Total RNA was transcribed into single-strand cDNA that served as templates for PCR amplification by extension of an oligo $(dT)_{18}$ primer using MMLV reverse transcriptase (Promega, USA) at 42°C for 30 min. The reaction was stopped by heating at 95°C for 5 min.

PCR and sequencing

The cDNAs encoding G protein β subunit (Gβ) of *M. mediator* were amplified from antennae cDNA of males. A pair of degenerate primers listed in Table was

Table Oligonucleotide primers used for isolation and expression analysis of the G protein β subunit (Gβ) in *M. mediator*

Purpose/primer name	Sequence (5´→3´)
cDNA isolation (RT-PCR)	
Sense	AAAATCTA(C/T)GCCATGCA(T/C)TGGGG
Anti sense	ACACGGTTGTC(G/A)TGGCCAGCGAG
5´ and 3´ cDNA end isolation (rapid-amplification of cDNA ends)	
5 *G*β S1	GTCCACGCCATACCTCTGCGA
5 *G*β S2	TGCGATCATCATGGGTAATG
5 *G*β R1	CTTGGGATGCGGACACCAGAT
5 *G*β R2	GAGTCGCTGCCCCAGTGCAT
*G*β rt	PGGCACAGGTCAT
3 *G*β S1	GGGCTCTTGCAAACAGACTT
Expression analysis (real-time PCR)	
18SFP	CGGAGAGGGAGCCTGAGAA
18SRP	CCGGGAGTGGGTAATTTGC
18S probe	FAM-TACCACATCCAAGGAAGGCAGCAGG-TAMRA
GβFP	TCCTTGCGGGTCACGAT
GβRP	GGCCATGCCGTCTTCAGTT
Gβprobe	FAM-ATCGTGTGTCCTGCCTTGGAG-TAMRA

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