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Cloning and expression profile of ionotropic receptors in the parasitoid wasp *Microplitis mediator* (Hymenoptera: Braconidae)

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ABSTRACT

Ionotropic receptors (IRs) mainly detect the acids and amines having great importance in many insect species, representing an ancient olfactory receptor family in insects. In the present work, we performed RNAseq of *Microplitis mediator* antennae and identified seventeen IRs. Full-length MmedIRs were cloned and sequenced. Phylogenetic analysis of the Hymenoptera IRs revealed that ten MmedIR genes encoded "antennal IRs" and seven encoded "divergent IRs". Among the IR25a orthologous groups, two genes, MmedIR25a.1 and MmedIR25a.2, were found in *M. mediator*. Gene structure analysis of MmedIR25a revealed a tandem duplication of IR25a in *M. mediator*. The tissue distribution and development specific expression of the MmedIR genes suggested that these genes showed a broad expression profile. Quantitative gene expression analysis showed that most of the genes are highly enriched in adult antennae, indicating the candidate chemosensory function of this family in parasitic wasps. Using immunocytochemistry, we confirmed that one co-receptor, MmedIR8a, was expressed in the olfactory sensory neurons. Our data will supply fundamental information for functional analysis of the IRs in parasitoid wasp chemoreception.

1. Introduction

Chemoreception is very important for insects to locate their mates, food sources, and oviposition sites, as well as to avoid natural enemies (Bruce et al., 2005; Quicke, 1997; Vet and Dicke, 1992; Visser, 1986; Wyatt, 2003). In insects, three families of ligand-gated receptors are known to participate in various aspects of chemosensation: ionotropic receptors (IRs), odorant receptors (ORs), and gustatory receptors (GRs) (Benton et al., 2009; Dahanukar et al., 2005; Joseph and Carlson, 2015; Leal, 2013; Sato et al., 2008; Sparks et al., 2015; Su et al., 2009; Suh et al., 2014; Touhara and Vosshall, 2009; Wicher et al., 2008). Among these receptor families, IRs represent the most ancient chemosensory receptors and are evolutionarily unrelated to ORs and GRs (Benton et al., 2009; Croset et al., 2010; Groh-Lunow et al., 2014; Missbach et al., 2014; Rytz et al., 2013).

IRs are homologous to ionotropic glutamate receptors (iGluRs) and are characterized by the presence of a conserved ligand-gated ion channel domain. They share conserved transmembrane domains with iGluRs but contain large variations within the ligand-binding domains (Benton et al., 2009; Croset et al., 2010). Insect IRs are subdivided into two groups on expression and sequence based characteristics in *Drosophila melanogaster*: conserved "antennal IRs" which are involved in olfaction, and species-specific "divergent IRs" which play roles in taste (Abuin et al., 2011; Benton et al., 2009; Croset et al., 2010; Koh et al., 2014; Rytz et al., 2013). In *D. melanogaster*, antennal IRs were found to be expressed in olfactory sensory neurons (OSNs) housed in the sensilla coeloconica and specifically responded to acids or amines (Benton et al., 2009; Menuz et al., 2014; Min et al., 2013; Silbering et al., 2011; Yao et al., 2005). IRs are supposed to function as ion channels and to act as dimers or trimers of subunits that are co-expressed in the same neuron (Abuin et al., 2011; Benton et al., 2009). Two widely expressed IR genes, IR25a and IR8a, are most closely related to iGluRs, retaining their domain organization and

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acting as co-receptors (Abuin et al., 2011; Ai et al., 2013; Benton et al., 2009). They form complexes individually with other IRs, and are essential for trafficking to ciliary membranes and mediating odor responses (Abuin et al., 2011). For example, IR8a and IR64a form ligand-gated cation channels to mediate odor detection, and the loss of IR8a causes a significant reduction in IR64a protein levels (Ai et al., 2013). To date, highly conserved antennal IRs have been identified in many insect taxa through genome analyses or transcriptomic approaches (Benton et al., 2009; Croset et al., 2010; Latorre-Estivalis et al., 2015; Liu et al., 2014; Olivier et al., 2011; Poivet et al., 2013). It is likely that other insect antennal IRs are also involved in acid and amine sensing based on functional characterizations of *D. melanogaster* IRs (Abuin et al., 2011; Sparks et al., 2015; Suh et al., 2014).

Microplitis mediator (Hymenoptera: Braconidae), a generalist parasitoid of caterpillars of a wide range of lepidopteran species (Khan, 1999; Lauro et al., 2005; Li et al., 2006a,b), including *Helicoverpa armigera* (Hübner), *Mythimna separata* (Walker), *Mamestra brassicae* and several other moth species. In our previous study, six IRs highly expressed in *M. mediator* female antennae were identified. We suspected that these IRs may play important roles in chemoreception behavior of female *M. mediator*, such as host finding or host recognition (Wang et al., 2015). However, till now the repertoire of IRs expressed in the antennae has not been established.

Here, we perform further identification of IRs by re-sequencing the adult antennae transcriptome using the Illumina HiSeq 2500 platform. The gene structure of MmedIR25a was analyzed in the present study. Using RT-PCR and qRT-PCR, we explored the expression features of IRs in different tissues of adult *M. mediator*. The development-specific expression of the MmedIRs in larvae, pupae and adults were also determined by RT-PCR. In addition, we also characterized IR protein expression in adult antennae. Our work provides an extensive molecular basis for further research on the functional characterizations of IRs in parasitic wasp chemoreception.

2. Methods

2.1. Insect culture

M. mediator cocoons were reared in a growth chamber maintained at 28 ± 1 °C, 60 ± 10% R.H. and a 16L: 8D photoperiod. The emerged adults were fed on a 10% sucrose solution. Mated female parasitoids were offered 2nd instar larvae of *M. separata*. Parasitized hosts were fed on corn leaves. The wasp larvae were directly dissected from the parasitized host body.

2.2. Illumina library preparation and sequencing

Female and male antennae (400 from each sex) were dissected from 1- to 3-day-old wasps. Total RNA was extracted from the female antennae and male antennae by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. The mRNA-seq libraries were constructed by using standard Illumina protocols of Novogene (Beijing, China). The library preparations were sequenced on an Illumina Hiseq 2500 platform, and paired-end reads were generated. The data generated in this project have been in the NCBI SRA database, under the accession number of SRR2912666 (Female antennae) and SRR2912670 (Male antennae).

2.3. Data processing, assembly and annotation

The raw reads were cleaned by removing reads containing adapters, reads containing ploy-N, and low quality reads. The trimmed reads from the female and male antennae were assembled using Trinity (v2012-10-05) (Grabherr et al., 2011). All of the assembled transcripts were searched against the Nr (NCBI non-redundant protein sequences) database to identify the putative mRNA functions using the BLAST algorithm (Altschul et al., 1997) with an E-value cut-off of 10^{-5}.

2.4. Identification of the ionotropic receptor

A FASTA file of the assembled sequences from Illumina sequencing of the male and female transcriptome was formatted as a local nucleotide database file using the BioEdit Sequence Alignment Editor 7.1.3.0 (Hall, 1999). A subset of *D. melano*gaster, *Nasonia vitripennis*, *Apis mellifera*, *Harpegnathos saltator*, and *Camponotus floridanus* IR sequences (Croset et al., 2010; Zhou et al., 2012) was used for the queries in tBLASTn searches to identify sequences with homology to known insect IRs.

2.5. RACE-PCR

Non-full-length transcripts encode new putative IRs were extended by rapid amplification of cDNA ends (RACE-PCR). The 5′ and 3′ RACE-Ready cDNAs were synthesized from the female antennae RNA by using the SMARTer™ RACE cDNA amplification kit (Clontech, Mountain View, CA, USA) following the manufacturer's instructions. RACE-PCRs were performed using Advantage™ 2 polymerase mix (Clontech, Mountain View, CA, USA) combined with touchdown PCR following the manufacturer's protocol. The RACE PCR products were cloned into the pEasy-T3 vector (TransGen, Beijing, China), and both strands were sequenced. The gene-specific primers were as follows:

5′RACE primer IR316 5′-ATCAGTGCTGACCTTAACG TACGGCTCTCG-3′;
5′RACE primer IR319 5′-GATCCTGGAGATGCGTTTAAAGTGAC GAGG-3′;
3′RACE primer IR312 5′-GTCGTACTAATTGTCAGCAGTTGCAC GAGTG-3′;
3′RACE primer IR316 5′-AGAGTCATCACATGATGCTGGGTAATTC GAG-3′.

2.6. Verification of IR sequences by cloning and sequencing

Gene-specific primers were designed to clone the ORF of the new IR genes (Supporting Information S1). cDNAs were synthesized from 2 μg of female antennal RNA using the Fast Quant RT kit (TIANGEN, Beijing, China). PCRs were conducted using the KOD DNA polymerase (Taihe, Beijing, China) under the following conditions: denaturation at 95 °C for 2 min, 40 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s and extension at 68 °C for 2 min. The final extension step was at 68 °C for 10 min. The PCR products were cloned into the pEasy-T3 vector (TransGen, Beijing, China) and were sequenced.

2.7. Phylogenetic analysis

A Hymenoptera-based phylogenetic analysis was performed, including the MmedIR protein sequences and orthologues in other species (Croset et al., 2010; Zhou et al., 2012). Amino acid sequences were aligned using the program ClustalW (Thompson et al., 1994). The neighbor-joining tree was constructed using the MEGA 5.0 program (Tamura et al., 2011) with a p-distance model and pairwise deletion gaps. Bootstrapping was performed by re-sampling the amino acid positions of 1000 replicates.