ARTICLE IN PRESS

Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Insect taste receptors relevant to host identification by recognition of secondary metabolite patterns of non-host plants

Mayu Kasubuchi [1], Fumika Shii [1], Kana Tsuneto, Takayuki Yamagishi, Satomi Adegawa, Haruka Endo, Ryoichi Sato*

*Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo, 184-8588, Japan*

ARTICLE INFO

*Article history:*
Received 2 April 2018
Accepted 3 April 2018
Available online xxx

*Keywords:*
Gustatory receptor
Bitter
Host plant selection
*Bombyx mori*
Calcium imaging

ABSTRACT

The taste sensing system is crucial for food recognition in insects and other animals. It is commonly believed that insect gustatory receptors (Grs) expressed in gustatory organs are indispensable for host plant selection. Many behavioral studies have shown that mono- or oligo-phagous lepidopteran insects use the balance between feeding attractants and feeding deterrents in host plants and that these are sensed by taste organs for host plant recognition. However, the molecular mechanism underlying taste recognition, especially of feeding deterrents, remains to be elucidated. To better understand this mechanism, we studied orphan Grs, including *Bombyx mori* Gr (BmGr) 16, BmGr18, and BmGr53, from the mono-phagous insect, *Bombyx mori*. Using Calcium imaging in mammalian cells, we first confirmed in lepidoptera insects that three of the putative bitter Grs widely responded to structurally different feeding deterrents. Although the phylogenetic distance of these Grs was considerable, they responded to partially overlapping deterrents of plant secondary metabolites. These findings suggest that not only these three Grs but also most of the Grs that have been assigned to putative bitter Grs are feeding-deterrent receptors that play a role in host plant recognition.

© 2018 Elsevier Inc. All rights reserved.

## 1. Introduction

Based on a variety of external environmental data received through their senses (e.g., olfaction, vision, and taste), animals search for food and judge whether the food is edible. Taste is important because it is involved in the final decision to feed. For example, insects have a functional gustatory sensing system, which plays a crucial role in identifying their host plants [1,2]. The taste is classified according to whether it is a feeding attractant [3] or a feeding deterrent [4], and the recognition of combinations of these two types of tastants is considered to be relevant to host identification. Furthermore, the recognition of attractants and deterrents is also involved in host plant recognition in the oviposition of swallowtail butterflies. In particular, deterrents have been reported to have a strong influence on oviposition behavior [5]. These findings suggest that understanding the mechanism that underpins deterrence is indispensable for understanding the mechanism by which insects choose host plants. Because many crop pests are lepidopteran insects, understandings of this mechanism can be directly applied to increase agricultural productivity. However, the molecular mechanisms underlying the recognition involved in host selection are not yet fully known.

In lepidopteran insect larvae, several sensory organs are located primarily on mouthparts and legs. Sensilla on the maxillary galea, maxillary palp, and epipharynx on mouthparts play a particularly important role in acceptance of taste substances [6–10]. Gustatory receptor neurons (GRNs) are located on these sensilla, and taste information is transmitted by GRNs to the subesophageal ganglion, which is the primary taste center [11,12] of the central nervous system. Several GRNs exist in each sensory nerve, and each GRN responds to sugars, deterrents, water, and salt [13–15]. In these GRNs, the gustatory receptors (Grs) that directly recognize chemical substances are expressed, and the firing of GRNs is caused by the Grs response to taste substances from host plants. Studies on insect Grs have been the most extensive in *Drosophila melanogaster*. When the phylogenetic tree regarding *D. melanogaster* Grs was drawn, they were classified into sugar [16–18], Gr43a [19], $CO_2$ [20], and bitter clades [21,22]. For insects, the word “bitter taste”

*Abbreviations:* Grs, gustatory receptors; GRNs, gustatory receptor neurons.

* Corresponding author.
*E-mail address:* ryoichi@cc.tuat.ac.jp (R. Sato).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.04.014
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Please cite this article in press as: M. Kasubuchi, et al., Insect taste receptors relevant to host identification by recognition of secondary metabolite patterns of non-host plants, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.014

has been used because most feeding deterrents evoke a bitter taste in humans. Similarly, in Lepidoptera, Grs are classified into sugar, Gr43a orthologue, $CO_2$ clades, and other receptors [23,24]. Several groups of molecules that cannot be assigned to any of the sugar, Gr43a orthologues, and $CO_2$ clades are referred to as the putative bitter taste receptor clade [23,24]. Recently, it was reported that the putative bitter taste receptors of lepidopterous insects can be classified into three types based upon the gene structures [25]. Among them, some of the Type 3 receptors of *Helicoverpa armigera* respond to the crude extracts of the host plants of *H. armigera,* and one of them responds to an amino acid, proline. However, whether these receptors are truly responding to feeding deterrents (bitter substances) has not yet been clarified. Therefore, it is necessary to carefully examine whether the receptors belonging to the putative bitter taste receptor clade of Lepidoptera are feeding-deterrent receptors.

As an important part of the promotional activities of the sericulture industry, research on the substances affecting the feeding preferences of *B. mori* have been actively investigated in Japan [15,26]. These studies reported the existence of deterrent GRNs that detect feeding deterrents in the sensilla of maxilla and epipharynx in the silkworm larvae. It was confirmed that deterrent GRNs of the silkworm larvae respond to coumarin, caffeine, pilocarpine, and nicotine [15,27]. Furthermore, the silkworms are mono-phagous insects that eat mulberry leaves, but there is a feeding mutant that eats apples and cabbage. The mutant is dominated by one gene, and the responsiveness of deterrent GRNs in maxillary sensilla is decreased to salicin [28]. No mulberry leaf specific substance that can function as a host plant marker has been reported. Thus, in the silkworm, the responsiveness of the deterrent plays a pivotal role in distinguishing non-host plants. Of the 69 kinds of Grs that have been reported in *B. mori*, 59 have been putatively assigned to bitter taste receptors.

The purpose of the present study was to clarify whether molecules assigned to the putative bitter taste receptor clade really recognize deterrents and to identify their role in host recognition. Of the receptors assigned to the putative bitter taste receptor clade, we focused on three BmGrs assigned to type 1 and 2 in the classification by Xu (2016). Calcium imaging was used as a ligand assay to investigate whether HEK293T cells transfected with three BmGrs responded to coumarin, caffeine, pilocarpine, and nicotine, which belong to plant secondary metabolites and to inositol, sucrose, and isoquercitin, which are known feeding stimulants. We further determined whether coumarin was a feeding deterrent of the silkworm larvae by using a feeding assay. Our results showed, for the first time in lepidopteran insects, that three BmGrs were deterrent receptors. Furthermore, we discussed the possible function of type 1 and type 2 BmGrs as deterrent receptors functioning in host plant recognition.

## 2. Materials and methods

### 2.1. Insect

*Bombyx mori* eggs of a hybrid race, Kinshu x Showa (Ueda Sanshu Ltd. Japan) were hatched and reared on an artificial diet, Silkmate (Nihon-Nosan Co. Ltd., Kanagawa, Japan) with long-day lighting conditions (16 h Light, 8 h Dark) at 25 °C.

### 2.2. Total RNA isolation and cDNA cloning

The mouthparts were collected from the fifth instar larvae of *B. mori* using forceps. Total RNA was isolated using ISOGEN II (NIPPON GENE, Tokyo, Japan). Using ReverTra Ace® (TOYOBO, Osaka, Japan), cDNA was synthesized from the total RNA with oligo dT-primers. BmGr16, 18, 53 and 10 cDNAs were amplified using a high-fidelity DNA polymerase, PrimeSTAR HS® (TaKaRa Bio, Shiga, Japan). mRNA sequence was obtained from NCBI database (accession number, BmGr16, BK006599.1; BmGr18, BK006601.1; BmGr53, BK006609.1; BmGr10, LC061862.1).

### 2.3. Transient expression of BmGr in HEK293T cells

Human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco's modified Eagle medium (D-MEM, Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine serum (FBS, BioWest, Nuaillé, France), 4 mM GlutaMAX™ (Thermo Fisher Scientific), 100 units/ml penicillin (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), and 100 μg/ml streptomycin (Meiji Seika Pharma Co., Ltd.) in an atmosphere of 5% $CO_2$ and 95% relative humidity at 37 °C. BmGr16, 18, 53 and 10 were directly cloned into the mammalian cell expression vector pcDNA3.1 (Thermo Fisher Scientific), respectively, and were transfected to the cells using Opti-MEM® (Thermo Fisher Scientific) including a transfection reagent, polyethylenimine (PEI Max, Polysciences, Inc., Warrington, PA). The primers were shown in Table S1. The fluorescence imaging analyses were performed at 48 h after the transfection.

### 2.4. $Ca^{2+}$ imaging

The fluorescence intensities were obtained using MetaView® imaging system under the fluorescence microscope BX53. HEK293T cells were loaded with Fluo-4 AM (Thermo Fisher Scientific) for 10 min. Cells were rinsed with a fresh Hank's buffered salt solution (HBSS; 137 mM NaCl, 5.4 mM KCl, 0.3 mM $Na_2HPO_4$, 0.4 mM $KH_2PO_4$, 4.2 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 0.4 mM $MgSO_4$, pH 7.4) including Probenecid (Thermo Fisher Scientific), and the cells were kept at 37 °C for 1 h. The time-lapsed images were obtained every 500 msec following the substances perfusion.

### 2.5. Quantitative RT-PCR

First strand cDNA was synthesized from 500 ng total RNA using the PrimeScript™ RT reagent Kit (TaKaRa Bio). Quantitative RT-PCR was performed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus, TaKaRa Bio) with a StepOnePlus™ (Thermo Fisher Scientific). Real-time PCR reactions were performed in technical triplicate. The primers were shown in Table S2.

### 2.6. Feeding suppression assay

Freshly molted fifth instar larvae were previously starved for about four or five days after moulting. The tests were carried out with the use of agar-diets (Table S3). Three larvae each were placed on one 10 cm dish and the experiments were performed at 25 °C. The amount of diet actually eaten was estimated by the difference in the weight of the larvae before and 3 h after testing.

## 3. Results

### 3.1. BmGr16, BmGr18, and BmGr53 respond to feeding deterrents but not to feeding stimulants

BmGr16, BmGr18, and BmGr53 have been reported to be putative bitter receptors based on the results of phylogenetic analyses. Therefore, we performed calcium imaging assays using HEK293T cells expressing each BmGr to indirectly measure the response of the receptors to feeding deterrents. We chose three of the putative bitter clade receptors after considering their positions on the phylogenetic tree. BmGr16 and BmGr18 are both type 1 receptors

Please cite this article in press as: M. Kasubuchi, et al., Insect taste receptors relevant to host identification by recognition of secondary metabolite patterns of non-host plants, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.014

Download English Version:

https://daneshyari.com/en/article/8292984

Download Persian Version:

https://daneshyari.com/article/8292984

Daneshyari.com