© 2021. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2021) 224, jeb238816. doi:10.1242/jeb.238816

RESEARCH ARTICLE

Characterization of *Halyomorpha halys* TAR1 reveals its involvement in (*E*)-2-decenal pheromone perception

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ABSTRACT

In insects, tyramine receptor 1 (TAR1) has been shown to control several physiological functions, including olfaction. We investigated the molecular and functional profile of the *Halyomorpha halys* type 1 tyramine receptor gene (*HhTAR1*) and its role in olfactory functions of this pest. Molecular and pharmacological analyses confirmed that the *HhTAR1* gene codes for a true TAR1. RT-qPCR analysis revealed that *HhTAR1* is expressed mostly in adult brain and antennae as well as in early development stages (eggs, 1st and 2nd instar nymphs). In particular, among the antennomeres that compose a typical *H. halys* antenna, *HhTAR1* was more expressed in flagellomeres. Scanning electron microscopy investigation revealed the type and distribution of sensilla on adult *H. halys* antennae: both flagellomeres appear rich in trichoid and grooved sensilla, known to be associated with olfactory functions. Through an RNAi approach, topically delivered *HhTAR1* dsRNA induced a 50% downregulation in gene expression after 24 h in *H. halys* 2nd instar nymphs. An innovative behavioural assay revealed that *HhTAR1* RNAi-silenced 2nd instar nymphs were less susceptible to the alarm pheromone component (*E*)-2 decenal as compared with controls. These results provide critical information concerning the role of TAR1 in olfaction regulation, especially alarm pheromone reception, in *H. halys*. Furthermore, considering the emerging role of TAR1 as target of biopesticides, this work opens the way for further investigation on innovative methods for controlling *H. halys*.

KEY WORDS: Brown marmorated stink bug, TAR1 receptor, Antennae, Olfaction, Behaviour, RNAi

INTRODUCTION

Identifying volatile compounds through the olfactory system allows insects to find food sources, avoid predators and localize putative partners and oviposition habitats (Gadenne et al., 2016). Furthermore, olfactory modulation by volatile molecules with repellent activity could be a promising strategy for pest control (Carey and Carlson, 2011). The basic organization of the olfactory system begins with the antennae, organs possessing cuticular structures, the sensilla, innervated by olfactory sensory neurons (OSNs) (Amin and Lin, 2019). The OSNs recognize different molecules through special olfactory receptors. Each OSN expresses only one type of olfactory receptor, ensuring the specificity of signal for a single odour (Zhao and McBride, 2020). When an OSN is activated, it sends the output signal through the axon to the antennal lobe. Here, excitatory projection neurons (PNs) transport the olfactory information to brain centres such as the mushroom body and the lateral horn (Tanaka et al., 2012). The mushroom body plays an important role in olfactory learning and memory (Caron et al., 2013) whereas the lateral horn controls innate olfactory response functions (Jefferis et al., 2007). In insects, the olfactory system can be modulated by exogenous (photoperiod, temperature) and endogenous (hormones) factors.

The biogenic amines tyramine (TA) and octopamine (OA) are present in high levels in the nervous tissue of insects, suggesting a role as neurotransmitters (Roeder, 2005). Furthermore, TA and OA act also as neurohormones and neuromodulators in a wide variety of physiological processes, acting in a paracrine, endocrine and autocrine way on the cells of the organism (Pauls et al., 2018).

Initially, TA was considered only as a biosynthetic intermediate of OA (Lange, 2009), but later numerous studies showed that TA is indeed an important neurotransmitter (Blenau and Baumann, 2003; Roeder, 2005, 2020; Lange, 2009). Among invertebrates, TA is the endogenous agonist of the tyramine receptors (TARs). Structurally, TARs are part of the superfamily of G protein-coupled receptors (GPCR) sharing a typical structure with seven transmembrane domains (Ohta and Ozoe, 2014). Several studies have highlighted that TARs can by coupled with both G_q (increasing intracellular calcium levels) and G_i proteins (decreasing cAMP levels) (Saudou et al., 1990; Blenau et al., 2000; Enan, 2005; Rotte et al., 2009). Based on the rank order of potency of agonists, TARs have been classified into three different types (Wu et al., 2014): TAR1, coupled with G_q and G_i proteins, and TAR2, coupled only with G_i protein, while TAR3 has so far been described only in *Drosophila melanogaster* (Bayliss et al., 2013; Wu et al., 2014). The first TAR1 was characterized in *D. melanogaster* (Saudou et al., 1990). The receptor, called Tyr-dro, showed higher affinity (12-fold) for TA than for OA and was mainly expressed in the heads. Since then the same receptor has been characterized in several orders of insects: Hymenoptera (Blenau et al., 2000), Orthoptera (Poels et al., 2001), Lepidoptera (Ohta et al., 2003), Hemiptera (Hana and Lange, 2017a) and Diptera (Finetti et al., 2020).

Several physiological and behavioural functions are controlled by TAR1, including olfaction. Kutsukake and colleagues (2000) characterized *honoka*, a *D. melanogaster* strain that presented a TAR1 mutation and a compromised olfactory profile. These insects were not able to localize repellent stimuli, suggesting that TAR1 could be involved in this physiological response. Furthermore, RNAi-mediated modulation of TAR1 expression was shown to affect the gregarious and solitary phase change of locusts through a different olfactory sensibility to attractive and repulsive volatiles (Ma et al., 2015). In honeybee antennae, an upregulation of TAR1 was observed during the transition from nurses to pollen foragers, suggesting TAR1 regulation of their behavioural plasticity (McQuillan et al., 2012). High TAR1 levels were also found in

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Received 5 October 2020; Accepted 2 March 2021