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Odorant receptor-based discovery of natural repellents of human lice

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ABSTRACT

The body louse, *Pediculus humanus humanus*, is an obligate blood-feeding ectoparasite and an important insect vector that mediates the transmission of diseases to humans. The analysis of the body louse genome revealed a drastic reduction of the chemosensory gene repertoires when compared to other insects, suggesting specific olfactory adaptations to host specialization and permanent parasitic lifestyle. Here, we present for the first time functional evidence for the role of odorant receptors (ORs) in this insect, with the objective to gain insight into the chemical ecology of this vector. We identified seven putative full-length ORs, in addition to the odorant receptor co-receptor (Orco), and expressed four of them in the *Xenopus laevis* oocytes system. When screened with a panel of ecologically-relevant odorants, PhumOR2 responded to a narrow set of compounds. At the behavior level, both head and body lice were repelled by the physiologically-active chemicals. This study presents the first evidence of the OR pathway being functional in lice and identifies PhumOR2 as a sensitive receptor of natural repellents that could be used to develop novel efficient molecules to control these insects.

1. Introduction

The human body louse, *Pediculus humanus humanus*, is an important vector of human pathogens responsible for the transmission of epidemic typhus, trench fever and relapsing fever (Fournier et al., 2002) (Bonilla et al., 2013). The impact of these diseases has been dramatically reduced in recent times, simply because efficient control measures and better hygiene standards in developed countries allowed a near-eradication of the vector (Badiaga et al., 2008) (Brouqui, 2011). However, the reemergence of body lice has occurred in specific areas and populations, maintaining a high epidemiological risk (Raoult and Roux, 1999) (Badiaga et al., 2008) (Brouqui, 2011). Since there are currently no commercial vaccines against louse-borne diseases, control and/or elimination of lice are considered as the best methods available to combat the transmission of these diseases to humans (Bonilla et al., 2013). However, conventional insecticides present several limitations: (1) they do not prevent re-infestation (Mumcuoglu et al., 1996); (2) they promote the development of resistance mechanisms in lice (Bonilla et al., 2013); (3) they might cause health problems when applied at high doses to humans (Semmler et al., 2012). In this context, identifying molecules that efficiently repel lice from humans, which protect against re-infestation and have no negative impact on human health, is critical. Several types of repellent products have been considered for louse control, including broad-spectrum synthetics such as N,N-diethyl-3-methylbenzamide (DEET) and plant-derived compounds such as essential oils and some of their constituents (e.g. citronellal) (Peock and Maunder, 1993) (Burgess, 1993) (Mumcuoglu et al., 1996) (Mumcuoglu et al., 2004) (Toloza et al., 2006a) (Toloza et al., 2006b) (Toloza et al., 2008) (Canyon and Speare, 2007) (Semmler et al., 2010). Nevertheless, no specific anti-louse repellent has been discovered to date (Semmler et al., 2012) (Burgess et al., 2014).

A main limitation regarding the development of molecules with repellent activity lies in the very limited comprehension of their mode of action at the insect level. Such strategies would likely benefit from a better understanding of the interactions between chemicals and the sensory system of the insect. Most insects rely heavily on chemoreception as it provides a highly valuable link between volatile cues (odorants) from the environment and critical behaviors such as attraction and avoidance (Touhara and Vosshall,

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2009) (Carey and Carlson, 2011) (Leal, 2013). In insects, olfaction takes place in olfactory receptor neurons (ORNs) housed principally in the antennae, the main olfactory organ. At the molecular level, a diverse array of odorant receptors (ORs), ionotropic receptors (IRs), odorant binding proteins (OBPs) and chemosensory proteins (CSPs) have been shown to interact with specific sets of ligands and to play major roles in odorant detection, contributing to the enormous evolutionary success of insects (Benton et al., 2009) (Carey and Carlson, 2011) (Leal, 2013). The publication of the genome sequence of the human body louse showed very limited repertoires of chemosensory genes when compared to other insect species with sequenced genomes, with only ten ORs, twelve IRs, five OBPs, and seven CSPs identified (Kirkness et al., 2010) (Croset et al., 2010). This drastic reduction of the chemoreception machinery is thought to reflect the particular lifestyle of this insect as an obligate ectoparasite which lives in the cloths and feeds solely on the blood of human hosts. This extreme ecological specialization likely goes along with a reduced capacity to locate alternative food sources, prospect for oviposition substrates or detect a variety of natural enemies, but nothing is known about the olfactory adaptations underlying the ecology and behavior of this insect.

To understand the contribution of the louse olfactory system to host specificity and ecological specialization, we took advantage of the genome sequence to identify, clone and functionally characterize OR genes. Odorant receptors represent valuable targets since they ensure the direct interaction with odor ligands, eliciting signal transduction mechanisms that will ultimately lead to specific behaviors (Touhara and Vosshall, 2009) (Carey and Carlson, 2011) (Leal, 2013). In addition, insect ORs have been shown to undergo rapid evolution (Robertson et al., 2003) (Sanchez-Gracia et al., 2009), which is consistent with a role in the adaptation to different ecological environments. A search of the genome data revealed eight full-length putative OR genes, confirming the limited range of this family in this insect. Four ORs were cloned and expressed in the *Xenopus laevis* oocytes system where they were challenged with a set of ecologically-relevant odorants. Three ORs remained non-responsive but PhumOR2 responded to a narrow set of odorants. Both head and body lice were repelled by the physiologically-active molecules, with two compounds showing high biological activity. This work marks the first step towards a better understanding of chemical communication mechanisms in lice and demonstrates the potential of an odorant receptor as a biological repellent detector towards the development of novel control strategies against this insect vector.

2. Materials and methods

2.1. Identification and cloning of P. h. humanus odorant receptors

2.1.1. Genome search

Search for ORs in the *P. h. humanus* genome (Assembly PhumU2, Gene set Phum2.1) was performed by Blast homology search using known *Drosophila melanogaster* OR sequences as queries. Blast algorithm was used to identify putative ORs in the predicted peptide sequences database in VectorBase. The putative lice OR sequences were screened for the presence of typical OR domains in the NCBI Conserved Domains Database (CDD) and aligned with known insect OR sequences to assess sequence integrity. The original Vectorbase annotation names for putative *P. h. humanus* ORs were used in this study (Table 1).

2.1.2. Cloning

Total RNA was extracted from body louse females (whole-body) using Trizol (MRC, Cincinnati, OH), following the manufacturer's instructions. First-strand cDNA was synthesized from the RNA template (5 μg) using Superscript™ III reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo(dT)$_{12\text{-}18}$ primers in a 20 μl reaction. The following gene-specific primers were designed to amplify the coding sequences of seven putative full-length OR genes (Table 1): PHUM213810-F (PhumORco): 5′ ATGGGAAAG-TACAAACCTCACGGATTGG-3′; PHUM213810-R (PhumORco): TTATTTCAGTTGAACTAAAACCATGAAATA-3′; PHUM225140-F: 5′-ATGAAAAATCATATAGATCTTCACATACAT-3′; PHUM225140-R: 5′-TCAAGGAATATATTTTTTAGAATTGTTCAG-3′; PHUM430460-F: 5′-ATGGAGGAGTTTACCGGATACGAAAAAT-3′; PHUM430460-R: 5′-TTATTTTTCGTCTCTGATTTGAAGTAAAAA-3′; PHUM318760-F: 5′-ATGAGTTTTTTCAATTTGGATTATTTTAAA-3′; PHUM318760-R: 5′-TTAATGTTTTCTCGCCGTTTGATATGCA-3′; PHUM318770-F: 5′-ATGGAAAAAAATTTTGAAAATCACGTTTAT-3′; PHUM318770-R: 5′-CTAATTGTTATTTTTTTCTCTCATTATTTG-3′; PHUM080360-F: 5′-ATGAAATCAAATTTTAACGAATTTTTTTTTTC-3′; PHUM080360-R: 5′-TTACTTGATTTCAAATTGTCTTATTAACAT-3′; PHUM600410-F: 5′-ATGGAAGAAAATAATAATTTATCTAATTCT-3′; PHUM600410-R: 5′-TTATTTAGATTCCAATTGCCAAAAAAAC-3′. Full-length ORs were amplified by PCR (Pfu Ultra II polymerase, Agilent Technologies, Santa Clara, CA) in 25 μl reactions containing 1 μl of a whole-body cDNA template and 100 nM of each primer. The following cycling conditions were used: 95 °C for 1 min for the initial denaturation step, followed by 40 cycles at 95 °C for 1 min, 54 °C for 30 s, 72 °C for 1 min and a final 72 °C for 5 min elongation step. PCR products were purified from agarose gel (QIAquick Gel Extraction Kit, Qiagen, Valencia, CA) and ligated into blunt-end EcoRV-digested pBlueScript SK+ (T4 DNA ligase, Promega, Madison, WI). Ligation products were used to transform competent cells (One Shot OmniMAX, Invitrogen, Carlsbad, CA), positive clones were grown in LB medium containing ampicillin and plasmids were purified (QIAprep Spin Miniprep Kit, Qiagen, Valencia, CA) and sequenced (Davis Sequencing Inc, Davis, CA). Several independent clones were obtained for 5 putative full-length ORs. Clones for PHUM213810 (PhumOrco), PHUM225140 (PhumOR2) and PHUM600410 (PhumOR7) were identical to database sequences at the amino acid level, whereas clones for PHUM318760 (PhumOR4) and PHUM080360 (PhumOR6) differed slightly from the database versions at the amino acids level. The sequences for PhumOrco, PhumOR2, PhumOR4, PhumOR6 and PhumOR7 were deposited into GenBank under the accession numbers KT369093, KT369094, KT369095, KT369096 and KT369097, respectively.

2.1.3. Sub-cloning

Putative full-length ORs were amplified by PCR (Pfu Ultra II polymerase) from pBluescript SK+ plasmid templates using gene-specific primers containing restriction enzyme recognition sites, PCR products were digested with appropriate combinations of restriction enzymes (XmaI, BamHI and/or EcoRI, New England Biolabs, Ipswich, MA), purified from agarose gel (QIAquick Gel Extraction Kit) and ligated into pre-digested pGEMHE plasmids (T4 DNA ligase, Promega). Ligation products were used to transform competent cells (One Shot OmniMAX, Invitrogen), positive clones were grown in LB medium containing ampicillin and plasmids were purified and sequenced (Davis Sequencing Inc, Davis, CA).

2.2. Expression of odorant receptors in X. laevis oocytes

Using the pGEMHE-PhumOR plasmids as templates, capped cRNAs were synthesized with mMACHINE T7 Kit following the manufacturer's instructions (Xu et al., 2013). Purified OR cRNAs were re-suspended in nuclease-free water at 200 ng/μl and 18.4 nl of cRNAs were microinjected with the same amount of PhumOrco cRNA into stage V or VI *X. laevis* oocytes (purchased from EcoCyte Bioscience, Austin, TX). Injected oocytes were kept at 18 °C for 3–7