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Review

An overview of odorant-binding protein functions in insect peripheral olfactory reception

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ABSTRACT. Insect olfactory perception involves many aspects of insect life, and can directly or indirectly evoke either individual or group behaviors. Insect olfactory receptors and odorant-binding proteins (OBPs) are considered to be crucial to insect-specific and -sensitive olfaction. Although the mechanisms of interaction between OBPs or OBP/ligand complex with olfactory receptors are still not well understood, it has been shown that many OBPs contribute to insect olfactory perception at various levels. Some of these are numerous and divergent members in OBP family; expression in the olfactory organ at high concentration; a variety of combinational patterns between different OBPs and ligands, but exclusive affinity for one OBP to specific binding ligands; complicated interactions between OBP/ligand complex and transmembrane proteins (olfactory receptors or sensory neuron membrane proteins). First, we review OBPs' ligand-binding property based on OBP structural research and ligand-binding test; then, we review current progress around the points cited above

to show the role of such proteins in insect olfactory signal transmission; finally, we discuss applications based on insect OBP research.

Key words: Insect olfaction; OBP/ligand complex; Odorant-binding proteins; Olfactory signal transmission

INTRODUCTION

Insects are different from higher animals. They depend on olfaction to estimate overall situation of habitats, which is essential for their survival and reproduction, whereas the animals use their highly developed vision to define the outer world. Insect olfactory perception involves individual aspects such as the spotting of food, host, mate or prey, and group communication aspects such as aggregation and avoidance (intraspecific or interspecific). Besides, the recognition of other signals around the habitat environment, which may evoke no behavioral responses, helps insects to distinguish, comprehend and estimate the overall situation. For example, the aphid alarm pheromone (E)-β-farnesene (EβF) is secreted by at least 16 aphid species (Francis et al., 2005a) but to which the *Brevicoryne brassicae* does not show their aposematism. Therefore, it has been suggested that EβF role is to distinguish conspecifics from host plant rather than as an alarm signal by *B. brassicae* (Francis et al., 2005b). As a huge and diverse group, insects have been estimated to have 4-5 million species (Novotny et al., 2002) and occupy numerous different habitats. Adaptive evolution has resulted not only in their morphological variation but also in their organ functional specialization. For example, insect antennas are grouped in more than 10 basic morphological types but have a common function, olfaction.

Lots of sensilla are located on the surface of the insect antennae with olfactory neurons being protected inside. There are 1-4 dendrites per olfactory sensilla and they are immersed in the hemolymph. Therefore, to trigger the olfactory signal transduction, ligands in the habitat should pass through the hemolymph to stimulate specific dendrites.

Indeed, insects have diverse olfactory organs and a different mechanism of peripheral signal inception and processing, but the resemblances in neuroanatomical logic and physiological coding properties compare with those in mammals, which have been reviewed by Silbering and Benton (2010). Also, there is a comparable function among members in their relative stages of olfactory signal transduction pathways. For example, although odorant-binding proteins (OBPs) in mammals and insects are not homologous, they are both expressed in high concentration around olfactory dendrites, and play a critical role at the first step of olfactory signal transmission. Recently, it has been demonstrated (Buck and Axel, 1991; Benton et al., 2006) that the locating patterns of olfactory receptors (ORs) and the olfactory signal transduction mechanisms between mammals and insects are alien (Neuhaus et al., 2005; Benton et al., 2006; Sato et al., 2008; Wicher et al., 2008). However, only their physiological function is the same, transforming an extracellular chemical signal into an intracellular electronic signal. Then, signals from dispersive olfactory neurons are centralized into a primary processing system, called the olfactory bulb in mice (Friedrich and Korsching, 1997; Mori et al., 1999; Uchida et al., 2000) and antennal lobe, which is analogous to the vertebrate bulb in the fruit fly (Gao et al., 2000; Vosshall et al., 2000). Numbers of glomeruli working in this primary processing system are formed by mitral cells in mice or projection neurons in the fruit fly (Hildebrand and Shepherd, 1997; Strausfeld and Hildebrand, 1999). They concentrate the same olfactory signals from receptors and send them separately to a higher central nervous system. The fruit fly has about 50 (Laissue and Vosshall, 2008)

glomeruli, which is much less than 1800-2400 glomeruli in mammals depending on the species. The simpler olfactory system of insects is still worth being considered as a model to investigate and compare the regulation of olfactory perception with other organisms.

In insects, some functional components are involved in odor recognition and transduction such as OBPs and ORs. Insect-specific and -sensitive olfactory responses are widely considered to contribute through a combinatorial action of these two protein families as two odorants filters for other flying animals. This paper focuses on the OBP of insect olfactory signal transduction system. We attempt to explain how OBPs work as the first filter for odor recognition and trigger the olfactory perception.

Although OBPs have been identified as the first protein family involved in the olfactory system since 1980s (Vogt and Riddiford, 1981; Pelosi et al., 1981, 1982), scientists could only find few ways to explain the mechanism of olfactory conception during the decade with this isolated protein. This was the situation until the adjacent component, the OR, was identified in mammals (Buck and Axel, 1991). Later, insect ORs were characterized as G protein-coupled receptor (GPCR) according to the mammal receptor (Clyne et al., 1999; Vosshall et al., 1999) in *Drosophila*, but it was finally revised by Benton et al. (2006) as a different protein family.

To date, more than 300 putative OBP genes have been registered (Zhou et al., 2010a) in GenBank® at the National Center for Biotechnology Information (NCBI) and many OBPs from at least eight orders have been identified (Pelosi et al., 2006) (Table 1).

Table 1. Number of odorant-binding proteins (OBPs) annotated from insect genomes.

Species	Common name	OBPs	References
Drosophila melanogaster	Fruit fly	51	Hekmat-Scafe et al., 2002
Anopheles gambiae	Malaria mosquito	66	Xu et al., 2003; Zhou et al., 2008
Aedes aegypti	Yellow fever mosquito	66	Zhou et al., 2008
*Bombyx mo*ri	Silkworm moth	45	Gong et al., 2009
Tribolium castaneum	Red flour beetle	46	Foret and Maleszka, 2006
Apis mellifera	Honey bee	21	Foret and Maleszka, 2006
Acyrthosiphon pisum	Pea aphid	15	Zhou et al., 2010a
Culex quinquefasciatus	Southern house mosquito	53 (classic OBP only)	Pelletier and Leal, 2009

Supplementary base on Zhou et al. (2010a).

OBPs have been divided into four groups according to their primary protein sequences (Hekmat-Scafe et al., 2002; Xu et al., 2003; Pelosi et al., 2006; Zhou et al., 2010a) (Table 2). They are classical OBPs, Plus-C OBPs, which were recently identified in *Acyrthosiphon pisum* as well as expressed in dipteran insects (Zhou et al., 2010b), Minus-C OBPs and Atypical OBPs, which are only found in blood-sucking insects.

Table 2. Classification of odorant-binding proteins (OBPs) from insects

Classification	Cysteine residue	Other description	Example
Classical OBPs	6	3 disulfide bonds formed by 6 cysteine residues ~14 kDa	BmorPBP (Vogt and Riddiford, 1981)
Plus-C OBPs	6 + 2/3	1 highly conserved proline residue at least 2 conserved cysteines 17-25 kDa	AgamOBP48 Putative Plus-C alignment GenBank® accession No., ALIGN_000581 (Zhou et al., 2004a)
Minus-C OBPs	<6	-	*Drosophila* Obp99a, Obp99b and Obp99d (Hekmat-Scafe et al., 2002)
Atypical OBPs	≥6	a long C-terminus up to 38 kDa	*Anopheles* OBP35 (Xu et al., 2003)

Adaptive evolution in diverse habitats with diverse odor molecules has resulted in a large OBP family with variant proteins (Hekmat-Scafe et al., 2002). Most of insect-OBP sequences are divergent among different orders, even less than 20% identity from different insect genera (Zhou et al., 2010a). OBP phylogenetic relationship from several model insect species shows a very restricted conservation between different orders (Zhou et al., 2010a). Therefore, it seems that homology cloning is not an effective tool to obtain OBPs among species (Jacobs et al., 2005), which is similar to insect classic olfactory receptor cloning. Using available insect genome information is still the prior option for identifying OBPs.

Diversity of OBPs indicates they distinguish between different ligands or bind to the same ligand in different ways. In contrast, very few highly conserved OBPs indicate common but crucial physiological function in species. LUSH is a pheromone-binding protein (PBP) that prevents sequence conservation from orders, and is required for 11-cis vaccenyl acetate (cVA)-induced behavior and normal cVA sensitivity of the T1 neurons in *D. melanogaster* (Kim et al., 1998; Xu et al., 2005; Ha and Smith, 2006; Kurtovic et al., 2007; Jin et al., 2008; Laughlin et al., 2008). Further investigation is of interest on this conserved protein in other species. As another example, it has been demonstrated in some OBPs in aphids that the differences of sequences were limited to no more than 2-3 amino acids among species (Qiao et al., 2009). OBP3 is one of these OBPs, which was first identified in *A. pisum* (Apis OBP3) and has an affinity to EβF. This is the first and only identified component involved in insect EβF perception to date. Either as the alarm pheromone to aphids or as the kairomone to natural enemies, EβF is one of the shared semiochemicals released by most of aphid species and plays an important role in their chemical communication. This suggests that OBP homology cloning could work on OBPs, especially the common OBPs shared by insects.

THE BINDING PROPERTIES OF OBPs

As a binding protein expressed by a multigene family, insect OBPs have an overwhelming and common feature, namely their binding activity. Proper designed recombinant insect OBPs expressed in the heterologous system, being identical to natural OBPs *in vivo*, allow ligand binding assays *in vitro*. Taking a panoramic view of the OBP-binding test history, three OBP-binding approaches have contributed to the study of OBP-binding affinity with different ligands. However, drawbacks as well as advantages in each of the three methods have been summarized in excellent reviews (Tegoni et al., 2004; Pelosi et al., 2006).

Analysis on the protein caught by tritium-labeled pheromone (E,Z)-6,11-hexadecadienyl incubated with *Antheraea polyphemus* antennal extract identified the first insect OBP (Vogt and Riddiford, 1981). This method, based on a radioactive-labeled ligand, has been popular for the binding of radioactive pheromone or radioactive photoaffinity. The labeled structural analog shows direct evidence of olfactory specificity *in vivo* and does not require purified OBPs. On the other hand, radioactivity loss through electrophoresis renders it impracticable to lead to a final conclusion.

Fluorescence-binding tests are currently being chosen for OBPs as a binding measurement, and could be indirectly evaluated by intensity of fluorescence under equilibrium, besides it is simple, safe and fast. Although it requires a fluorescent probe with a binding affinity, tests with OBP could be competed and replaced by ligand. This technique was first performed in insects to demonstrate unspecific binding capacity of PBPs with several ligands

in *A. polyphemus* and *Mamestra brassicae* through a competitive experiment of fluorescent probe, 1-amino-anthracene (AMA), and ligands (Campanacci et al., 2001). Identification of EβF-binding protein (Apis OBP3) in *A. pisum* is another remarkable competitive experiment and example of another widely used probe, N-phenyl-1-naphthylamine (1-NPN), and EβF or its structural analogs to Apis OBP3 (Qiao et al., 2009). Regarding the binding with some ligands, Trp37 has been proposed to be directly involved in change of fluorescence (Campanacci et al., 2001; Bette et al., 2002; Leal et al., 2005), also inducing significant intrinsic fluorescence change, by which one more detail, based on three ApolPBP1-binding ligands, has revealed that there are different interaction modes between ApolPBP1 and the three ligands, (E6,Z11)-hexadecadienal (6E,11Z-16:Al), (E6,Z11)-hexadecadienyl-1-acetate (6E,11Z-16:Ac) and (E4,Z9)-tetradecadienyl-1-acetate (4E,9Z-14:Ac) (Bette et al., 2002).

Another method called cold-binding assay, performed by Leal et al. (2005), shows its unique advantages, which are multiple ligand competitive experiments for best ligand identification and the availability of more accurate determination of constant dissociation based on accurate measurements of the concentrations of free or bound ligand and protein. The binding complex is separated from free ligand filtrate by rapid ultrafiltration. Then, an evaluation of the extraction of bound ligands released from the complex by gas chromatography-mass spectrometry (GC-MS) analysis is performed.

THE THREE-DIMENSIONAL (3-D) STRUCTURE OF OBPs

Binding tests show unspecific binding properties of OBPs with ligands at times, which can be better understood by further studies at a microscopic level. For example, only 4E,9Z-14:Ac of the three ApolPBP1-binding ligands induces a conformational change, which is monitored by circular dichroism measurements on ApolPBP1 secondary structure (Mohl et al., 2002). Microscopic study on OPBs, especially 3-D structure of OBP/ligand complex, reveals more information on explaining what OBPs contribute to insect-specific and -selective olfaction.

X-ray diffraction analysis and nuclear magnetic resonance (NMR) on crystal and solution of protein, respectively, are powerful tools for protein 3-D structure alone or with its ligand. To date, crystal or NMR structures of OBPs or PBPs, from more than 10 species belonging to four orders of insect, are available in Entrez's 3-D structure database at NCBI, and over 20 papers explaining their details are in Table 3.

All published structures show that both OBPs and PBPs consist of at least 6 α-helixes, which are held together by three disulfide bridges, cys1-cys3, cys2-cys5, and cys4-cys6, when only label cysteine residues form orderly disulfide bridges. In contrast, different mechanisms of ligand binding, release, and receptor recognition have been revealed by the diversity of key sites related to function, 3-D structure, and physicochemical property among OBPs.

pH-dependent conformational change associated with change of binding affinity is reported to be common to OBPs in distinct insects. Moreover, it also shows a special character to different groups. For the moth, pH dependence has been demonstrated initially in *Bombyx mori* (Sandler et al., 2000). Asp-132 and Glu-141 are two well-conserved amino acid residues in moth PBPs, which form two salt bridges with protonated His-70 (Damberger et al., 2007; Xu and Leal, 2008) in both *A. polyphemus* and *B. mori* or His-80 (Xu et al., 2010) in *Amyelois transitella* and His-95, respectively, at low pH (4.5). The formation of two salt bridges together are referred to as histidine protonation switch (Xu et al., 2010), as they are demonstrated to pro-

mote a C-terminal seventh α-helix formation, and proposed to help these α-helixes to disrupt the binding site of pheromone and replace the pheromone after a rapid ligand release to ORs. Deprotonation of the two His residues at pH 7 has been demonstrated to cause the abolishment of salt bridges, promoting the withdrawal of the helix from the binding pocket and making the hydrophobic cavity available for sex pheromone.

Table 3. List of registered odorant-binding protein (OBP) 3-D structures (published with original paper)

OBPs name	Species	Accession No. (MMDB ID)	Reference	Determined by
CquiOBP1	*Culex quinquefasciatus*	85954	Mao et al., 2010	X-ray 1.3 Å
AaegOBP1	*Aedes aegypti*	78632	Leite et al., 2009	X-ray 1.85 Å
OBP76a (LUSH)	*Drosophila melanogaster*	32732 62291-62295	Thode et al., 2008	X-ray 2 Å
OBP76a (LUSH)	*Drosophila melanogaster*	46137	Laughlin et al., 2008	X-ray 1.4 Å
OBP76a (LUSH)	*Drosophila melanogaster*	23921-23924	Kruse et al., 2003	X-ray 2.04 Å
Atrapbp1	*Amyelois transitella*	79681	Xu et al., 2010	NMR
BmorGobp2	*Bombyx mori*	75865-75871	Zhou et al., 2009	X-ray 1.5 Å
BmPBP	*Bombyx mori*	21174	Lee et al., 2002	NMR
BmPBP	*Bombyx mori*	18041	Horst et al., 2001	NMR
BmPBP	*Bombyx mori*	15475	Sandler et al., 2000	X-ray 1.8 Å
BmorPBP	*Bombyx mori*	45969 45970	Lautenschlager et al., 2007	X-ray 2.01 Å
ApolPBP	*Antheraea polyphemus*	35432	Zubkov et al., 2005	NMR
ApolPBP	*Antheraea polyphemus*	26871	Mohanty et al., 2004	NMR
ApolPBP1	*Antheraea polyphemus*	60086	Damberger et al., 2007	NMR
ASP1	*Apis mellifera*	78405-78407 64848-64850 61456 64867 64870	Pesenti et al., 2008	X-ray 1.75 Å
ASP1	*Apis mellifera*	71369-71372 72879-72884	Pesenti et al., 2009	X-ray 1.6 Å
ASP2	*Apis mellifera*	34972	Lescop et al., 2001	NMR
LmaPBP	*Rhyparobia maderae*	24088 24093 24102	Lartigue et al., 2003	X-ray 1.7 Å

NMR = nuclear magnetic resonance.

For the mosquito, AgamOBP1, AaegOBP1 and CquiOBP1 are highly conserved OBPs expressed in *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*, respectively. Their affinities for ligands are also pH-dependent (Wogulis et al., 2006; Leite et al., 2009; Mao et al., 2010), whereas a C-terminal loop lacks a C-terminus region, which is also identical to Lma PBP with a direct end after the sixth helix in *Leucophaea maderae* (Lartigue et al., 2003) comparing with moth OBPs, folding into an extra α-helix at low pH. Hydrogen bonds involving the C-terminal loop, which forms part of the binding pocket, will be broken at low pH and make the loop open, releasing further ligands. Under several conditions, dimers dynamically trap pheromone at high concentrations to mitigate the stimulus saturation (Hon-

son et al., 2003). This has been previously suggested, and almost all determined OBP crystal structures show a dimeric structure. However, the fashion of CquiOBP1/(5R,6S)-6-acetoxy-5-hexadecanolide (MOP) was found to be novel in *C. quinquefasciatus* (Mao et al., 2010). Helices 4 and 5 not only form a hydrophobic tunnel but also a dimeric interface, which is also identified in AgamOBP1 and AaegOBP1. The lactone head of the MOP binds in the central cavity of CquiOBP1 and its tail is buried in the tunnel created by the dimmer, which shows no hydrogen bonds, but exclusively hydrophobic and van der Waals interactions. In solution, the binding is retained even after the dimmer is dissociated.

For the honey bee, the affinity of another OBP, the antennal-specific protein 1 (ASP1), for 9-keto-2(E)-decenoic acid (9-ODA) is opposite to the pH-dependent affinity of the insects mentioned above (Pesenti et al., 2008, 2009). At low pH (4), ASP1 binds to 9-ODA with higher affinity than at neutral pH. This apparent paradox is explainable, considering that under physiological environment pH is not categorically acidic, basic or neutral, but it is different from local microenvironments in any organism. It seems that pH dependence of ligand release has been adapted to local pH around the membrane of the OBP's corresponding neuron cell. The Asp35 residue is demonstrated to be essential for the formation of dimmer, which has 10-fold lower affinity for ligand at neutral pH than monomeric ASP1 at pH 4 (Pesenti et al., 2009).

Ligand-dependent conformational change is another mechanism of ligand release (Mohl et al., 2002; Zhou et al., 2004b; Lautenschlager et al., 2005; Laughlin et al., 2008). It has been demonstrated that it plays a role in at least two different ways. BmorPBP keeps unavailable the conformation with an ordered C-terminal helix inside the binding pocket when the ligand is absent, even at neutral pH (high pH) (Lautenschlager et al., 2005). For LUSH, it shows a binding affinity with more than one ligand (Kim et al., 1998; Xu et al., 2005), but it mediates the activation of or67d through a conformational change only when cVA is present. The interaction between Phe121 of LUSH and cVA triggers LUSH-activated conformation transformation by disrupting a salt bridge between Asp118 and Lys87, and then the activated LUSH is able to independently stimulate T1 neuron as a ligand with or without the presence of cVA (Laughlin et al., 2008).

PHYSIOLOGICAL CHARACTERISTICS

To stimulate olfactory neuron, hydrophobic odorants such as pheromones need carriers to help them to pass through the aqueous hemolymph in which the neuron is bathed. The first OBP as a putative pheromone-binding protein, ApolPBP1, was identified in the polyphemus moth, *A. polyphemus* (Vogt and Riddiford, 1981). All the OBPs under olfactory reception investigation are specifically expressed or at least highly expressed in insect olfactory organs and more functions of OBPs have been proposed to date. They are triggers of an activated corresponding receptor with the ligand as the OBP/ligand complex; deactivation or protection from enzymatic degradation of the pheromone, and a solubilizer and transporter of specific ligands. There are some experimental or model proofs for the functions based on OBP-binding properties as well as 3-D structure studies.

SOLUBILIZER AND TRANSPORTER OF SPECIFIC LIGAND

Some OBPs are required as solubilizer and/or transporter and/or specific-ligand selector like filter in insect olfactory perception.

Although pheromone-binding capability of OBPs has been demonstrated in various species since 1981 (Vogt and Riddiford, 1981), the first indication that OBPs solubilize ligands and are required for olfactory perception *in vivo* originated from Van den Berg and Ziegelberger (1991). These authors showed that replacing the lymph in relatively empty sensilla of *A. polyphemus*, the induced-pheromone electrophysiological response was abolished.

Several investigations have indicated that the final specific perception to some ligands is contributed by recognition and selection of the OBPs located around ORs, but not ORs. In the moth, *A. polyphemus*, ApolPBP1 and ApolPBP2 bound to two sex pheromones (E,Z)-6,11-hexadecadienyl acetate (ACl) and (E,Z)-6,11-haxadecadienal (AL), respectively. However, the exchanged combination of ACl/ApolPBP2 and AL/ApolPBP1 excited neuron cells corresponded with PBP, but no longer with their specific ligands (Pophof, 2002). The same team later obtained a similar result in *B. mori* (Pophof, 2004).

Another remarkable finding is that the transgenic *Drosophila*, lacking the *lush* gene, was reported to be anosmic to the male pheromone cVA (Xu et al., 2005). Furthermore, or67d from *Drosophila*, missexpressed in other trichoid neurons, conferred cVA sensitivity in the presence of LUSH (Ha and Smith, 2006).

All the above indicate that some OBPs select ligand for downstream ORs as a ligand selector or filter, as well as, transporters in insect olfactory perception, which contributes to insect-specific olfactory perception.

However, some investigations have shown that OBPs are not necessary to mediate the interaction between ligands and ORs. Odorants could directly stimulate several receptors expressed in selected cell lines (Wetzel et al., 2001; Hallem et al., 2004). Compared with insect fast behavioral reaction (milliseconds), the systems mentioned above showed much slower response speed (several seconds), which was reviewed by Pelosi et al. (2006). These indicate that some ligands do not need OBPs as carriers but as solubilizers in lymph, leaving the obligation of olfactory specificity to the corresponding downstream ORs.

With further research, some OBPs reviewed in this section were also found to play a direct role in chemical signal transmission.

ACTIVATION OF CORRESPONDING RECEPTOR THROUGH OBP/LIGAND COMPLEX

To date, available information remains limited but it reveals an interesting diversity of probable mechanisms of how the OBP/ligand complex interacts with downstream OR.

pH-DEPENDENT OBP/LIGAND COMPLEX RELEASE TO ORs

OBPs may be involved as ligand selectors, transporters or deactivators after triggering olfactory signal transduction but they are not required for a trigger event, which means OBP/ligand complex will release its ligand after they arrive at specific ORs. This process may occur by a pH change, as released pheromone independently activates ORs. pH-dependence was investigated first to explain “extra” peaks shown by NMR from a highly pure BmorPBP expressed in *B. mori.* Its tertiary structure exhibited a conformational transition between pH 5 and 6 (Wojtasek and Leal, 1999). Then, it became clear that a single form exists at pH below 4.9, whereas it will change into another form at pH above 6 (Damberger et al., 2000).

BmorPBP binds to bombykol when it is in the lymph, whose pH is 6.5 (Kaissling and Thorson, 1980), but not when at the surface of dendrites, which has a lower pH induced by a negatively charged surface (Blomquist and Vogt, 2003). The occurrence of pH-dependent conformational change was strongly supported by structural biology (see the last Section).

ACTIVATED OBPs INDEPENDENTLY STIMULATE NEURON

As mentioned above, LUSH is required for cVA-induced behavior and normal cVA sensitivity of the T1 neurons (Xu et al., 2005; Ha and Smith, 2006). Further research has shown more details in this process. LUSH activated by cVA plays a role as ligand to activate its neuron (Laughlin et al., 2008) (see more in the last section). Sensory neuron membrane proteins (SNMPs) and or67d are required in this process. Here, PBP changes its conformation after binding to the ligand, and PBP/ligand complex mediates the related neuron activation *in vivo*.

OBP/LIGAND COMPLEX RELEASE TO SNMPs

The results of other studies have indicated another interaction pattern among LUSH/cVA complex, SNMPs and or67d (Benton et al., 2007; Jin et al., 2008). They concluded that SNMP may act downstream from OBP (LUSH) and upstream from odorant receptors (or67d) in the generation of action potentials. And another olfactory receptor, HR13, shares less than 15% amino acid identity with or67d, and could be activated by a similar pheromone of cVA, which has suggested that it is the fatty acid-derived hydrocarbon tail common to these pheromones that needs SNMP, which is a conserved protein among species. In other words, as a conserved protein, SNMP but not ORs may interact with ligand released by OBPs here.

PHEROMONE DEACTIVATION AND PROTECTION FROM ENZYMATIC DEGRADATION

The expected pheromone half-life (in the range of 1 s) is about 300-fold shorter than the half-life of the pheromone measured on the living antenna (4-5 min). Here, an unclear process (degradation or inactivation) must exist in the organism to stops pheromone from stimulating receptor neuron. It was hypothesized that fast inactivation of pheromones is achieved by pheromone-degrading enzymes (Kaissling, 1972). However, the enzymatic degradation *in vivo* has been considered too slow (on a minute timescale) (Kasang et al., 1988) to account for the fall of the receptor potential (Kaissling, 2001). It has also been suggested that the discrepancy between data from *in vivo* and *in vitro* experiments is due to the involvement of PBPs avoiding pheromone degradation (Kaissling, 2001). This result has been shown *in vitro* (Vogt and Riddiford, 1986; Kaissling, 2009). Major attention on modeling has been devoted to the unknown mechanism of odorant deactivation, and modeling also helps to understand the multiple functions of the PBP (Kaissling, 2001). A study suggested that the fall of the receptor potential after stimulus offset is not caused by pheromone degradation and that pheromone deactivation must occur and could be associated with PBP. This study is a combination of model N (Kaissling, 1998a, 2001), model R (Kaissling, 1998a,b) and it is compared with another model in which deactivation is a spontaneous, non-enzymatic process (Kaissling, 2009). More data (structural and kinetic) are available from *B. mori* and *A. polyphemus*.

Increased PBP-binding capacity at high pheromone concentrations was observed, and it has been suggested to play a role of trapping pheromone for mitigating the stimulus saturation (Honson et al., 2003). Considering a suggestion in the elephant (Lazar et al., 2002), Vogt (2005) proposed a conjecture that some odor-OBP complexes may sequester ligand as a deactivator. Together with the high concentration of OBP, which is up to 10 mM *in vivo* (Klein, 1987), the protection of ligand from enzyme degradation and the ability of transformation from different conformations, it is worth considering that OBPs can be a candidate for ligand deactivator. The process of pheromone deactivation is an essential postulate that needs to be experimentally verified.

PERSPECTIVES FOR APPLIED RESEARCH

Making the insect olfactory molecular mechanisms clearer aids in a better understanding of behaviors of various agricultural pests and pathogenic insects. Moreover, it would be useful to find novel tools for pest control strategies. OBPs are the functional component located at the start of olfactory signal transmission, and insect OBPs belong to a distinct protein family from other organisms. Target insect OBPs as a point of penetration to stop or interfere with insect olfactory conception will alter their reproduction or feeding and, therefore, it will artificially avoid risky behaviors and help natural enemies detect their preys. On the other hand, it will prevent threats for humans or animals and is also eco-friendly. Insect OBPs generally show their sensitivity and selectivity to at least a group of species-specific ligands but they also show their plasticity in some cases. For example, *B. mori* PBP1 has higher affinity for non-pheromone than for its natural pheromone (Hooper et al., 2009). A translational research combining molecular basis of olfaction and chemical ecology, using CquiOBP1 as a molecular target to identify its unnatural ligands, has contributed to the commercially available oviposition attractant for *C. quinquefasciatus* (Leal et al., 2008), which is a mixture of nonanal and trimethylamine. It can be concluded that OBP is the target of transgenic plant (Beale et al., 2006) synthesizing the aphid alarm pheromone, EβF, as EβF-binding OBP (Apis OBP3) has been identified in *A. pisum*.

WHAT MORE

Multiple OBPs encoded by overlapping genes *obp99a*, *obp99b*, and *obp99d* contribute to the same odorant reception but show different olfactory response independent of acetophenone (promotion or inhibition) in *Drosophila* (Wang et al., 2010). Here, different OBPs interact with the same ligand but induce a different response, which indicates a more complicated unclear interaction pattern among OBPs or between OBP/ligand complex and ORs.

Additionally, *obp* genes contribute to different responses to the same odorant. *Obp57e* is proposed to be responsible for interspecies differences of response to hexanoic acid contained in the ripe fruit of *M. citrifolia*, which is the toxin to *D. melanogaster* and *D. simulans*, but harmless to *D. sechelliaheld*. However, the fact that *obp57e* ORF shows no alteration in different species suggests that the interspecies difference of host plant preference is in gene expression rather than in the structure of gene product (Matsuo et al., 2007). Further, polymorphisms including single-nucleotide polymorphisms (SNPs) of *obp* genes contribute to individual variation olfactory behavior (Keller and Vosshall, 2007; Wang et al., 2007, 2010).

CONCLUSION

Following the conventional approach, homology cloning is an available strategy to identify OBP in different species that share the same semiochemical reception. It is worth paying more attention to modeling in order to enrich and develop comparisons of the very complicated physiology condition of insect olfactory system *in vivo*.

Although in the past 20 years many studies have contributed to the understanding of insect OBPs, further knowledge of insect olfactory perception is still lacking. The major puzzle is how exactly OBPs play their roles in insect olfactory perception. On the contrary, available information, such as diverging primary sequences coupled with selective expression; numbers of intraspecies OBPs; diverse but specific combinational patterns of OBP/ligand complexes; different interactions between OBP/ligands and transmembrane proteins (ORs and SNMPs), and the dimer of OBPs, indicate that insect OBPs may have adapted from different levels to contribute to insect-sensitive and -specific odorant perception.

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