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Olfactory regulation of mosquito–host interactions

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Abstract

Mosquitoes that act as disease vectors rely upon olfactory cues to direct several important behaviors that are fundamentally involved in establishing their overall vectorial capacity. Of these, the propensity to select humans for blood feeding is arguably the most important of these olfactory driven behaviors in so far as it significantly contributes to the ability of these mosquitoes to transmit pathogens that cause diseases such as dengue, yellow fever and most significantly human malaria. Here, we review significant advances in behavioral, physiological and molecular investigations into mosquito host preference, with a particular emphasis on studies that have emerged in the post-genomic era that seek to combine these approaches.

Keywords

Mosquito; Olfaction; Behavior; Host preference; Vector

1. Introduction

The success of mating, blood feeding and oviposition determine life history strategies of mosquitoes. Each of these behaviors is mediated by both internal and external factors. The principle external cues affecting mosquito behavior are temperature, humidity, visual objects, and most importantly, odor. Ambient temperature affects general mosquito activity and survival, whereas host temperature may affect host seeking, landing and probing (Bowen, 1991). Likewise, humidity influences general activity, survival, host seeking at close range and possibly oviposition. It is not clear to what extent mosquitoes respond to colors, but they certainly respond to shapes and they require adequate contrast for flight orientation (Bidlingmeyer, 1994). Males generally use visual markers for swarm formation (Yuval and Bouskila, 1993). Many behavioral expressions of mosquitoes are mediated by olfaction. Indeed, the chemical ecology of mosquitoes is now widely recognized as one area of investigation on which future control strategies may depend. Host seeking and feeding behaviors are much affected by host odors, and many mosquito species use olfaction for oviposition (Takken and Knols, 1999). It is not known, though, whether mating is also mediated by smell, although there is indirect evidence that for at least a few species an olfactory component is involved in mating. (Gomulski, personal communication).

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Most of the available literature on mosquito olfaction relates to host seeking and feeding behavior. Major reviews by Bowen (1991), Takken (1991), Foster (1995), Gibson and Torr (1999) and Takken and Knols (1999) cover the subject in detail, including electrophysiological work, volatiles affecting males and female host seeking. Some of this work includes studies on important vectors such as the yellow fever mosquito *Aedes aegypti* (L.), the African malaria vector *Anopheles gambiae* Giles sensu stricto and the filariasis vector *Culex quinquefasciatus* (Say). Fundamental aspects of olfactory signal transduction at the peripheral level have revealed the involvement of olfactory receptors on maxillary palpi and antennae. The detection of carbon dioxide (CO_2) and L-lactic acid by *Ae. aegypti* in particular has been well established. Studies of the responses of these species to other host odors are in progress and have not yet been reviewed. Behavioral studies in wind tunnels and olfactometers have shown that human volatiles have a role as kairomones for the three mosquito species mentioned. Lactic acid and carboxylic acids have been singled out as likely candidates involved with the attraction of these insects to humans These studies have been corroborated by field studies, mostly with *An. gambiae* and *Cx. quinquefasciatus,* which have shown that these insects are attracted to human volatiles from a distance (Costantini et al., 1996; Mboera et al., 1997).

Considerable progress has been made in the field of olfaction with respect to mosquito–host interactions. The recent publication of the *An. gambiae* genome (Holt et al., 2002) as well as ongoing sequencing projects of other important mosquito vectors offers new opportunities to advance our knowledge on mosquito olfaction by the identification of genes that regulate olfactory behavior. This may ultimately lead to a better understanding of the genetic basis of important processes such as host selection, repellent behaviors, landing and probing. The significance of this knowledge with relevance for new strategies of vector-borne disease control will be discussed.

2. Olfactory mechanisms

Vertebrates and invertebrates are remarkably similar with regard to the molecular mechanisms through which they sense chemicals and other environmental cues (Hildebrand and Shepherd, 1997). Information carried by odor molecules is encoded in electrical signals that are active in sensory and other neurons. The activation of different subsets of sensory neurons to different degrees is the basis for neural encoding and further processing of the odor information by higher centers in the olfactory pathway. Recent evidence has converged on a set of transduction mechanisms, involving G-protein-coupled second-messenger systems, and neural processing mechanisms, involving modules called glomeruli, that appear to be adapted for the requirements of different species (Hildebrand and Shepherd, 1997). These findings support the hypothesis that olfactory transduction and neural processing in the peripheral olfactory pathway involve basic mechanisms that are universal across most species in most phyla.

In all systems studied thus far, olfactory signal transduction occurs in olfactory receptor neurons (ORNs), where it is mediated by a distinctive family of seven transmembrane-containing odorant receptor proteins (ORs) that are coupled to hetero-trimeric GTP binding proteins (G-proteins) lying on the cytoplasmic face of the plasma membrane (Fig. 1). In the case of mosquito and other insect olfactory systems, odorant receptors are located on the dendrites of ORNs that extend into the hollow space formed by distinctive sensory hairs (sensilla) that populate the antennae and maxillary palps (Stocker, 1994). The accepted paradigm is that once a receptor has bound an odorant target it undergoes a conformational change that facilitates the binding and eventual activation of its cognate G-protein. The activated G-protein complex then releases one of its subunits (G_α-GTP) that in turn rapidly induces one or more downstream effector enzymes to synthesize one or more second messengers that indirectly effect the ionic balance and ultimately neuronal membrane potential. In this manner, the second messengers are responsible for relaying the signals that arise from extracellular chemicals to the neuronal components, ultimately giving rise to the electrical action potentials that are the language of nervous systems.

While the precise olfactory second messengers have yet to be conclusively established in insects, there is considerable data to suggest that molecules such as cyclic AMP (cAMP) and most importantly, inositol 1,4,5-triphosphate (IP_3) play essential roles in transducing chemical signals from the environment into neuronal activity (Boekhoff et al., 1994; Reed, 1992). Insofar as insects are concerned, mutations in several IP_3 linked components including phospholipase C (*norpA*) show impaired olfactory function in *D. melanogaster* (Riesgo-Escovar et al., 1995) and odor induced IP3 signaling has been directly measured in the cockroach (Breer et al., 1990). Regardless of the second messenger, ORN activation is largely carried out through the action of cAMP or IP_3-gated-cation channels that lie within the plasma membrane and specifically open in response to the increase in local cytoplasmic concentration of second messengers. In this manner, the rapid influx of Ca^{2+} or Na^{2+} ions into the dendrite cytoplasm of ORN results in sufficient depolarization of the membrane potential to induce an action potential that generates the electrical message that will be sent along the axons of the central nervous system (CNS).

In addition to odorant-based activation, an important component of olfactory detection leading to chemotaxis (e.g., toward a potential blood source) is the reduction of signaling that is directly related to adaptation, whereby a progressively weaker response is generated to repeated or persistent stimuli. At the molecular level, this is characterized by a process known as desensitization, which has been observed in all chemosensory systems, ranging from bacterial chemotaxis to neural transmission in humans, and can vary from complete termination of signaling, as seen in visual and olfactory systems, to graded attenuation of agonist potency in other systems (Dohlman et al., 1991). Desensitization of GPCR-mediated signal transduction is mediated through an impairment of the receptor’s ability to activate its corresponding G-protein and is carried out principally through the combined activity of two classes of proteins; G-protein-coupled serine/threonine receptor kinases (GRKs) and arrestins (reviewed in Freedman and Lefkowitz, 1996). While the role of GRKs and arrestins in desensitization pathways has been well established, it is likely that they are also intimately involved in GPCR internalization (sequestration), which is an integral component of GPCR resensitization (Ferguson et al., 1996). In *D. melanogaster*, mutations in two visual system arrestin genes (*arr1* and *arr2*) have been shown to inactivate rhodopsin in vivo (Dolph et al., 1993).

In insects, antennal ORN axons project either ipsilaterally or bilaterally to neuropilar structures of the CNS known as glomeruli that are located in antennal lobes (AL) (Stocker, 1994). The number and characterization of olfactory glomeruli in mosquitoes remains largely unknown except for *Ae. aegypti* where there are between 20 and 25 (Anton, 1996). While many insect species exhibit significant sexual dimorphism throughout their entire olfactory apparatus, this remains an unexplored aspect of mosquitoes, in spite of the high degree of female specific olfactory responses. Recent advances in the ability to stain mosquito ORN projections to the CNS (Anton et al., 2003) coupled with a heightened awareness of the need to gain a better appreciation of central processing centers in these systems will fuel important studies in the coming years.

3. Behavior and sensory physiology

Recent behavioral studies on mosquitoes have focused mostly on *Ae. aegypti* and *An. gambiae*. Both species are highly anthropophilic. In *An. gambiae* s.s. the strong dominance of anthropophily was demonstrated in choice studies in an olfactometer, with both *An. gambiae* s.s. and the more zoophilic sibling *An. quadriannulatus* (Theobald) (Pates et al., 2001a). The former expressed a strong aversion to cow odor and was significantly more attracted to human odor, while the latter species had no preference for either odor source. Human odor was offered on a worn stocking or as an ethanol extract of skin washings (Pates et al., 2001b). On the stocking, the volatiles remained active for several weeks after collection. In a related field experiment, where *An. gambiae* s.s. and *An. quadriannulatus* were given a free choice to feed on a human or a calf, >90% of *An. gambiae* s.s. fed on the human, while the choice of *An. quadriannulatus* was 50/50 for both host species. In choice studies in South Africa, *An. quadriannulatus* proved to be strongly zoophilic, selecting preferentially calf odors or CO_2 above human odors (Dekker and Takken, 1998).

In other studies, *An. gambiae* s.s. was attracted to human hosts from a distance (Costantini et al., 1996, 1998; Mboera et al., 1997) and the sibling *An. arabiensis* (Patton) expressed a lower degree of anthropophily than *An. gambiae* s.s. Unlike *An. gambiae* s.s., which is found only in association with human settlements, *An. arabiensis* can depend exclusively on wildlife and has a more opportunistic, if not zoophilic, host preference (Braack et al., 1994; Dekker and Takken, 1998; Duchemin et al., 2001). These field studies prove that host-seeking behavior of members of the *An. gambiae* complex is mainly guided by olfactory cues, and corroborate earlier reports that *An. gambiae* s.s. is anthropophilic throughout the African continent. However, it was also reported that on occasion *An. gambiae* s.s. feeds predominantly on livestock, presumably driven to this by environmental conditions (Diatta et al., 1998; Duchemin et al., 2001).

When the role of CO_2 was investigated, it was found that the turbulence of the odor plume in the laboratory greatly affected the responsiveness of *Ae. aegypti* and *An. gambiae* s.s. (Dekker et al., 2001). Plumes with high turbulence caused accurate orientation of the mosquitoes to the plume, while homogeneous or low turbulent plumes either did not affect the insects, or lead to deterrence. By contrast, responses to skin emanations released in a homogeneous fashion were greater than to an intermittent plume of skin emanations. These studies underline the different roles of both kairomone sources. CO_2 from expired air is detected from a distance whence it would be present as an intermittent plume. Skin volatiles may be detected at close range, where they will still be relatively homogeneous. In the field, CO_2 was responsible for 20% of the attraction of *An. gambiae* s.l. to human volatiles. When CO_2 was removed from the plume, catches were reduced by 20% but it was not known whether this was due to a reduction in *An. arabiensis* only (Costantini et al., 1998). Another study (Brady et al., 1997) attributed the differences in human attractiveness to less CO_2 in the expired air of the less attractive individuals. More recently, Mukabana et al. (2004) reported allomonal activity of human breath, which would explain why differences in the quantity of expired CO_2 (Brady et al., 1997) would result in variation in human attractiveness. Other factors considered to cause variation in attractiveness between individuals are differences in skin composition and microbial flora on the human skin (Knols, 1996; Takken and Knols, 1999).

3.1. Behavioral responses to single compounds and odor blends

Human skin is associated with approximately 350 different chemical compounds (Bernier et al., 2000). Although the odors affecting anthropophilic species such as *Ae. aegypti* and *An. gambiae* s.s. are likely to consist of a blend of compounds, it seems unlikely that all 350+ compounds affect host seeking, landing and probing. Lactic acid, unique for humans, is attractive for *Ae. aegypti*. Its role in *An. gambiae* has not been clarified. *Ae. aegypti* does not respond much to other chemical stimuli offered (Kline, personal communication) although it is strongly attracted to ethanol extracts of skin residues (Geier et al., 1996). Studies with *An. gambiae* s.s. have been more successful; this species is attracted to carboxylic acids (Knols et al., 1997) NH_3, (Braks et al., 1999, 2001), and to chemical compounds present in incubated human sweat but which are absent from freshly collected sweat (Braks et al., 2000). No studies on the effects of odor blends with these mosquito species have as yet been published although one study (Mboera et al., 2000) reported the attraction of *Cx. quinquefasciatus* to a blend of CO_2, acetone, 1-octen-3-ol and butyric acid the field. There was no response of *An. gambiae* to this mixture.

3.2. Human variation in attractiveness

The often-cited differences in human attractiveness for mosquitoes (Curtis, 1986) is olfactory based (Schreck et al., 1990). This behavior is most particularly expressed in anthropophilic culicids such as *Ae. aegypti* and *An. gambiae* s.s. (Schreck et al., 1990; Lindsay et al., 1993; Knols et al., 1995). Age but not gender may affect these inter-individual differences (Carnevale et al., 1978) while race also does not appear to affect these differences (Schreck et al., 1990). Young children were less attractive to anophelines than adults (Muirhead-Thomson, 1951; Thomas, 1951). Studies on the chemical composition of human volatiles (Krotoszynski et al., 1977) revealed the existence of a large number (>350) of chemicals and work is in progress to study the most important components of these volatiles regulating mosquito behavior (Van Beek, personal communication). It is already clear that quantitative differences in carbon dioxide output affects inter-individual differences in attractiveness (Brady et al., 1997). Further work should reveal the importance of selected chemicals in blends of odors as these affect mosquito behavior, similar to pheromone blends in moths and bark beetles (El-Sayed et al., 1999).

3.3. Physiology of olfactory behavior

Neurological responses of mosquitoes to host volatiles have been studied from peripheral receptors using sensory physiological techniques. Single cell responses of *Ae. aegypti* revealed a L-lactic acid sensitive receptor located in the grooved peg sensillum (Davis, 1989). Receptor cells sensitive to CO_2 are located on the maxillary palpi, and were shown to be sensitive to increasing concentrations of CO_2 in *Ae. aegypti* (Grant and O’Connell, 1996). The CO_2 responsiveness appears to be mediated from the maxillary palpi in most Culicidae (Grant and O’Connell, 1996). The olfactory receptor cells of other host volatiles are predominantly found on the antennae (McIver, 1982; Meijerink and van Loon, 1999) although several receptor cells on the maxillary palpi also encode for such volatiles (Grant and O’Connell, 1996).

In *An. gambiae* s.s., receptor cells in antennal grooved peg sensilla responded to ammonia (Meijerink et al., 2000) and sensilla trichodea contained receptor cells that were sensitive for a range of carboxylic acids, geranyl acetone, 3-methyl-1-butanol, 6-methyl-5-heptene-2-one and indole (Meijerink et al., 2001). A common dose-response activity was observed for 12 aliphatic carboxylic acids using electro-antennogram techniques (Meijerink and van Loon, 1999). Similar responses, using single cell techniques, were also observed in *An. arabiensis* Patton, *An. quadriannulatus* and *An. atroparvus* (Van Thiel) (Broek and Otter, 1999; Broek and Otter, 2000). At the peripheral level, no major differences in responses to several host volatiles were observed between these mosquito species with known differences in zoophilic and anthropophilic feeding preferences. In addition to physiological responses to host volatiles, mosquitoes also actively respond to exposure to plant and water-borne stimuli (Bowen, 1992; Blackwell and Johnson, 2000). These substances affect sugar feeding (Foster, 1995) and oviposition behavior (Allan and Kline, 1995).

4. Molecular genetics

In recent years using both molecular and genomic approaches, several proteins that are involved in fundamental aspects of olfactory signal transduction have been molecularly cloned and characterized in *An. gambiae* and other vector mosquitoes. With the recent publication of the complete genomic sequence of *An. gambiae* (Holt et al., 2002) as well as rapid advances in completing similar projects for other mosquito vectors, there can be little doubt that the coming years will see a dramatic increase in our understanding of the molecular genetics of mosquito olfaction.

One of the first classes of olfactory proteins molecularly identified in mosquitoes and other insects encompasses a broad class of water-soluble proteins that are highly expressed in the sensillar lymph. These secreted proteins, containing multiple conserved cysteine residues, are known as odorant-binding proteins (OBPs). They are hypothesized to act either as odorant carriers to facilitate solubilization, thereby raising the effective concentration of hydrophobic odorants, as a prelude to receptor binding or, alternatively, to be integral in the catalytic removal of odorants from the lymph. OBPs were first defined as having the ability to directly bind known odorants in both vertebrate (Pevsner et al., 1985) and insect (Vogt and Riddiford, 1981) systems. Indeed, in the latter case, a subset of OBPs are expressed in male-specific, pheromone-sensitive hairs and have been shown to bind pheromones in vitro. Thus, they were classified as pheromone-binding proteins (PBPs) (Vogt and Riddiford, 1981). In addition, moths have been shown to express a second group of PBP-like proteins associated with general odorant-sensitive neurons, found in both male and female antennal structures, and are known as general odorant-binding proteins (GOBPs) (Vogt et al., 1991). Despite the fact that their exact role remains elusive there is general agreement, based in part on the observation that insect OBPs constitute the most abundantly expressed proteins in olfactory tissues, that OBPs play an important function in chemosensory pathways. Compelling evidence in support of this hypothesis comes from studies that report the selective odorant-binding properties of moth PBPs (Plettner et al., 2000), as well as impaired olfactory responses to alcohols in *D. melanogaster* strains carrying a defective OBP protein encoded by the *lush* gene (Kim et al., 1998). Significantly, a member of the OBP family of proteins has recently been linked to the regulation of complex social behaviors in the fire ant, *Solenopsis invicta* (Krieger and Ross, 2002).

Several members of the OBP/PBP family of olfactory proteins have been cloned and characterized from several insects including *D. melanogaster* (Pikielny et al., 1994), *Apis mellifera* (Briand et al., 2001) and true bugs (Dickens et al., 1998). More recently several subfamilies of putative OBPs have been identified in *An. gambiae* (Biessmann et al., 2002; Vogt, 2002; Xu et al., 2003). Overall, there are a total of 57 candidate OBPs in the *An. gambiae* genome that have been broadly divided into 29 classical AgOBPs based upon amino acid sequence homology to the large number of genes encoding 14 kDa proteins with six conserved cysteines. In addition, there are 16 atypical AgOBP genes encoding considerably larger proteins of 35 kDa, which have an extended C-terminal region with several other conserved cysteine residues. Lastly, 12 distinct AgOBP genes corresponding to the Plus-C subfamily (Hekmat-Scafe et al., 2002) have been cataloged from the *An. gambiae* genome (Xu et al., 2003).

AgArr1 was the first arrestin family member molecularly cloned and characterized that displays expression in the olfactory apparatus in *An. gambiae* (Merrill et al., 2002). It is reasonable to assume that AgArr1 is involved in olfaction in *An. gambiae* in as much as its Drosophila homolog *arr1* along with a related arrestin gene (*arr2*) have been shown to be functional in olfactory as well as visual signal transduction in *D. melanogaster* (Merrill et al., 2002). More recently, using a genomics based approach, the AgArr2 gene, together with two additional non-sensory arrestins, have been identified and characterized in *An. gambiae* (Merrill et al., 2003). Because these arrestin proteins function to desensitize a broad spectrum of olfactory responses there is every possibility that they may offer attractive targets for directly impacting olfaction in vector insects.

In the first report of insect ORs outside of the model insect system, *D. melanogaster*, four candidate ORs genes (AgORs) were identified that are selectively expressed in olfactory tissues in *An. gambiae* (Fox et al., 2001). Importantly, and especially relevant for pathogen transmission, at least one these displays female-specific expression and is dramatically down-regulated following blood feeding in adult females (Fox et al., 2001). While there is a precedent in *C. elegans* for the activity-dependent regulation of chemosensory receptor ORs (Peckol et al., 2001), these observations are especially compelling in light of behavioral and electrophysiological studies in *An. gambiae* showing an inhibition of host-seeking response and olfactory sensitivity to human odorants following blood feeding (Takken et al., 2001). These latter experiments parallel similar data in another mosquito vector, *Ae. aegypti*, in which host seeking is inhibited following a blood meal and not fully restored until after the completion of the gonotrophic cycle, as indicated by the depositing of eggs (Klowden and Blackmer, 1987). In this system, the inhibition of host seeking is likely to result from two distinct mechanisms: the first caused by distension of the midgut following blood ingestion (Klowden and Lea, 1979) while the second occurs later in the reproductive cycle where a humoral factor is synthesized by the ovaries reduces the sensitivity of a subset of lactic acid receptor cells (Davis, 1984).

The recently completed genomic sequence of *An. gambiae* (Holt et al., 2002) has facilitated the use of bioinformatics and molecular approaches to describe the entire GPCR gene family in this vector insect (AgGPCRs) (Hill et al., 2002). Within the over 275 putative AgGPCRs cataloged, an additional 74 candidate AgORs have been described resulting in a total of 79 AgOR family members. Interestingly, by comparing the complete set of ORs from both *An. gambiae* and the *D. melanogaster* genomes, there is compelling evidence to support the occurrence of a dramatic species-specific gene expansion within the AgOR gene family that is consistent with the rapid evolution of a group of novel Anopheline ORs that may be associated with mosquito-specific chemosensory processes that would include, among others, host preference behaviors. From this expanded AgOR family of genes an additional set of AgORs that display female specific olfactory expression has been identified (LJZ, unpublished observation). Furthermore, an exciting set of experiments has recently utilized transgenic expression of two candidate AgORs into targeted ORNs of *D. melanogaster* to directly confirm their role in olfactory signaling in *An. gambiae* (Hallem, et al. 2004). Indeed, in one case, the female specific receptor protein AgOR1 was shown to be highly responsive to direct stimulation by physiological concentrations of 4-methyl-phenol. This is especially relevant in that previous electrophysiological studies had identified this odorant to be a stimulatory component of human sweat to female *An. gambiae* (Cork and Park, 1996). These data reinforce the hypothesis that a set of female specific AgORs are present in the olfactory apparatus of *An. gambiae* and may likely contribute to the sensory inputs that are especially relevant to host preference behaviors in these vector mosquitoes. These studies have set the stage for further investigations of additional components of this complex pathway.

5. Concluding remarks

Rapid advances along several research fronts have laid the foundation for a novel approach toward the design and development of a new generation of vector-borne disease control strategies. By combining the knowledge that is generated from the behavioral, molecular, neurological and physiological studies outlined in this review, as well as additional efforts currently underway, the fundamental elements that underlie critically important vector behaviors such as host selection may be further elucidated. In particular, by focusing on inherited differences in host preference within closely related species and within-host behavioral variations, the mechanism of strategic olfactory processes are expected to be resolved. Such an approach may also reveal the presence of olfactory control of mating and/or oviposition behavior (Takken and Knols, 1999: p. 1418). With that and other types of information (e.g. structural studies of key olfactory proteins) in hand, it should be possible to foster the implementation of high throughput approaches to identify and formulate chemicals that will act as attractants, repellents and other agents targeted against the olfactory basis of mosquito behaviors. We believe that given an increased awareness of the importance of these approaches in the fight against insect-borne diseases as well as the critical support of agencies such as the NIH, WHO and the Bill and Melinda Gates Foundation, it is reasonable to expect that these ambitious goals may be undertaken and indeed achieved within the foreseeable future.

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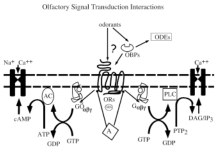

Fig. 1.

Schematic representation of the intracellular components of olfactory signal transduction pathways. Here, a 7 transmembrane odorant receptor protein (OR) lying within the ORN dendrite interacts directly with odorants or alternatively (?) in the context of odorant-binding protein (OBP) complexes. In both cases, subsequent interactions with heterotrimeric G-protein complexes (G/G′) activate downstream effector enzymes adenyl cyclase (AC) and phospholipase C (PLC). This leads to the synthesis of the second messengers cyclic AMP (cAMP), diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP_3) that regulate several cation (Na^+, Ca^{++}) channels that carry the transduction current. In addition, OBPS are hypothesized (?) to interact with odorant degrading enzymes (ODEs) to clear unbound ligand from the extracellular space. Signaling is terminated (bulbed lines) by decoupling of OR/G-protein complexes by arrestins (A) and G-protein coupled receptor kinases (GRKs).