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ScienceDirect
Genomics 90 (2007) 110–120
GENOMICS
www.elsevier.com/locate/ygeno

Gene identification and evidence for expression of G protein α subunits, phospholipase C, and an inositol 1,4,5-trisphosphate receptor in *Aplysia californica* rhinophore☆

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Received 25 February 2007; accepted 28 March 2007
Available online 11 May 2007

Abstract

In the marine mollusk *Aplysia californica*, waterborne protein pheromones that are released during egg laying act in concert to stimulate mate attraction. However, molecular information concerning the cellular receptors and signaling mechanisms that may be involved in waterborne peptide and protein pheromonal communication is lacking. As a first step toward examining whether members of the G protein family and phosphoinositide signaling pathway are present in the primary peripheral chemosensory organs (i.e., rhinophores), we isolated five full-length cDNA clones from an *A. californica* central nervous system cDNA library. These clones encoded (1) the G protein α subunits of the G_q, G_i, and G_o families, (2) a protein with homology to phospholipase C (PLC) isoforms, and (3) an inositol 1,4,5-trisphosphate receptor (IP_3R). The expression of these genes was examined using laser capture microdissection/reverse transcription-polymerase chain reaction and in situ hybridization. All of them are expressed in the rhinophore sensory epithelium, suggesting that $G\alpha_q$, $G\alpha_i$, $G\alpha_o$, PLC-like protein, and IP_3R may be involved in waterborne protein pheromone detection in *Aplysia*—possibly via a phosphoinositide signaling mechanism.
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Keywords: *Aplysia*; Pheromonal communication; Signal transduction

Chemical signaling is the most ancient form of communication and is used by mollusks and most, if not all, other organisms [1–8]. For most marine organisms, pheromonal communication is essential for attracting potential mates since vision and auditory signaling are often restricted. To date, few waterborne peptide or protein pheromones have been characterized, although evidence has been accumulating for their role in *Aplysia* mate attraction. Field studies have shown that this habitually solitary animal moves into breeding aggregations, and laboratory studies have found that this is likely caused by stimulation with binary pairs of the protein pheromones attractin, enticin, temptin, or seductin [1,4,6]. The first of these, attractin, was isolated from seawater eluates of *Aplysia* egg cordons and is a 58-residue protein [1,9].

In *Aplysia* and other opisthobranchs, pheromone detection is achieved by olfactory sensory neurons (OSNs) located in specialized anterior sensory organs known as rhinophores. The rhinophores include two tentacle-like organs on the dorsal surface of the head. Rhinophores sense pheromones released during egg laying [10], and, when the rhinophores are removed, *Aplysia* spend significantly less time mating when exposed to pheromones from egg cordons [10]. The neuroanatomical organization of rhinophores has been described [11,12] and includes a rhinophore groove within which most of the sensory cells are concentrated (Fig. 1). For attraction to be initiated, released pheromones must first bind to specific receptor proteins located on the dendrites of rhinophore OSNs in *Aplysia* conspecifics and elicit pheromone signal processing. The three-dimensional nuclear magnetic resonance solution structure of attractin [3] and comparative sequence alignments of six aplysiid attractins indicate that a conserved heptapeptide region (IEECKTS) is critical for cellular receptor binding [2].

The molecular and cellular bases of pheromone detection have been well studied in a variety of vertebrates and inver-

☆ Sequence data from this article have been deposited in the GenBank/EBI Data Libraries under Accession Nos. DQ397515 ($G\alpha_q$), DQ656111 ($G\alpha_i$), DQ656112 ($G\alpha_o$), DQ397516 (phospholipase C protein), and DQ397517 (inositol triphosphate receptor).

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0888-7543/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.ygeno.2007.03.020

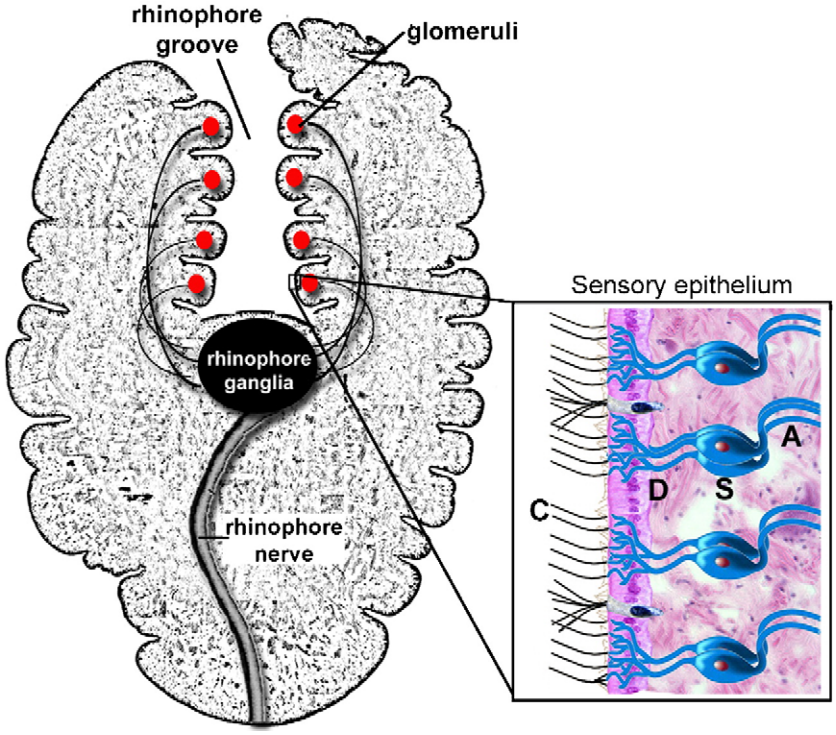

Fig. 1. Schematic diagram of the rhinophore neuroanatomy showing the location of the rhinophore groove, glomeruli underlying the sensory epithelium, rhinophore ganglia, and rhinophore nerve in *Aplysia* (modified from [12]). Enlarged box shows schematic diagram of rhinophore olfactory receptor afferent neurons located beneath the olfactory epithelium. The location of neuron soma (S), dendrites (D), axons (A), and cilia (C) are labeled. Gastropod olfactory neurons send processes to glomeruli, rhinophore ganglia, or directly to the cerebral ganglia.

tebrates [13–18]. However, unlike vertebrates, the molecular mechanisms mediating pheromone signaling in invertebrates has been poorly elucidated. In either case, G protein signaling pathways appear to be primarily responsible for transducing pheromone responses. The evolutionary importance of this system in chemosensory signal transduction is revealed in yeast, which responds to mating pheromones by activating a G protein signaling cascade [19].

Most information concerning pheromonal communication in vertebrates has been derived from rodent research. In rodents, volatile molecules and nonvolatile pheromones are primarily detected by putative pheromone receptors belonging to the heptahelical G-protein-coupled receptor (GPCR) superfamily, present on vomeronasal sensory neurons (VSNs) [20,21]. Signal transduction is driven by activation of trimeric guanine-nucleotide-binding (GTP-binding) proteins (G proteins–$G_{\alpha\beta\gamma}$), specifically $G\alpha_q$, followed by phospholipase-C (PLC)-induced production of inositol 1,4,5-trisphosphate (IP_3) and the subsequent increase in intracellular calcium (Ca^{2+}) concentration. IP_3 plays a key role in membrane depolarization by binding to the IP_3 receptor (IP_3R) located in the endoplasmic reticulum membrane, mobilizing sequestered Ca^{2+} and increasing cytosolic Ca^{2+}.

Several studies have reported that the molecular components and mechanisms of invertebrate pheromone detection are strikingly similar. For example, in insects, pheromones are detected by extremely sensitive sensory neurons localized in specialized sensory organs, the sensilla. Pheromones bind to seven-transmembrane receptors and the message is possibly relayed intracellularly via $G\alpha_q$, which in turn activates a specific PLC, resulting in a rapid and transient increase in IP_3 and intracellular Ca^{2+}. Although the precise mechanism of OSN pheromone signaling in lobsters is unknown, studies have shown that $G\alpha_q$ is expressed in the dendrites [22], and odorant exposure increases IP_3 levels leading to cellular depolarization [23,24]. A PLC and an IP_3R have also been localized to the outer dendrites of lobster OSNs [25–27]. Emerging evidence by Ache and colleagues [28] demonstrates that a calcium-sensitive transient receptor potential (TRP) channel is a downstream target of phosphoinositide signaling in lobster sensory neurons.

In contrast, prior to this study, little was known about olfactory sensory signal transduction in molluscan OSNs, and no IP_3R had been isolated. However, G proteins had been identified in the mantle and gill of the pearl oyster *Pinctada* [29], the cilia of abalone larvae [30], and the central nervous system (CNS) of *Lymnaea* [31]. Furthermore, a single retina-specific PLC isoform had been characterized in squid (*Loligo*) as being similar in structure and organization to vertebrate PLC-β members [32]. Finally, biochemical studies have implicated G-protein-induced IP_3 signaling in oocyte activation [33], and IP_3 is important for *Aplysia* neuroendocrine bag cell depolarization [34]. Taken together, the data suggest that G-protein-mediated IP_3 signaling is also important in molluscan chemosensory and perhaps pheromonal signaling. The identification by Moroz [35] of nitric-oxide-synthase-containing cells