Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome

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Abstract

Rhodopsin-type G-protein-coupled receptors (GPCRs) contribute the majority of sensory receptors in vertebrates. With 979 members, they form the largest GPCR family in the sequenced sea urchin genome, constituting more than 3% of all predicted genes. The sea urchin genome encodes at least six Opsin proteins. Of these, one rhabdomeric, one ciliary and two G_o-type Opsins can be assigned to ancient bilaterian Opsin subfamilies. Moreover, we identified four greatly expanded subfamilies of rhodopsin-type GPCRs that we call sea urchin specific rapidly expanded lineages of GPCRs (*surreal-GPCRs*). Our analysis of two of these groups revealed genomic clustering and single-exon gene structures similar to the most expanded group of vertebrate rhodopsin-type GPCRs, the olfactory receptors. We hypothesize that these genes arose by rapid duplication in the echinoid lineage and act as chemosensory receptors of the animal. In support of this, group B *surreal-GPCRs* are most prominently expressed in distinct classes of pedicellariae and tube feet of the adult sea urchin, structures that have previously been shown to react to chemical stimuli and to harbor sensory neurons in echinoderms. Notably, these structures also express different *opsins*, indicating that sea urchins possess an intricate molecular set-up to sense their environment.
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Introduction

Echinoderms are slowly moving or even sessile animals with a strong dermal skeleton armament. At first sight, elaborate sensory organs seem to be missing in many echinoderms, including the sea urchin, which has led to the common perception that these animals have only poorly developed senses. For example, referring to chemoreceptive sensation, Aristotle noted that "of the walking or creeping species the urchin appears to have the least developed sense of smell" (*Historia Animalium*, Book IV.8, quoted from the translation of D'Arcy Wentworth Thompson). In contrast to this view, many lines of evidence now indicate that echinoderms react to a wide variety of environmental stimuli, such as light, touch, as well as to chemical cues released from predators or prey (see, e.g., Goldschmid, 1996; Millott, 1975; Pisut, 2004). For example, sea urchins have been described to be able to distinguish between an active, foraging and a passive predator located upstream in the water by adapting their responses accordingly (Phillips, 1978), indicating an intricate sensory circuitry. Moreover, although echinoids are usually viewed as not possessing elaborate sensory organs, their body wall has been shown to contain a variety of sensory neurons, as it is

generally typical for echinoderms (Goldschmid, 1996). Up to 4000 sensory cells per square millimeter of skin surface have been reported in asteroids (Smith, 1937). In sea urchins, three main systems have been speculated to sense – and respond to – different cues based on behavioral and histological studies: the 'spine system', the tube feet and the pedicellariae (Campbell, 1973, 1974, 1983; Geis, 1936; Hamann, 1887; Millott, 1954; Peters and Campbell, 1987; Smith et al., 1985).

As far as photosensation is concerned, adult sea urchins exhibit a wide range of responses to light intensity, ranging from shelter seeking, covering reactions and daily migrations to light-dependent oriented movements, and spine defense reactions in response to predators shadowing an individual (reviewed in Millott, 1975; Smith, 1965, also see Barnes and Cook, 2001). In contrast to work on adult sea urchins, only scarce reports exist about the responses of sea urchin larvae to light. *Hemicentrotus* plutei prefer certain light intensities over others (Yoshida, 1966, summarized in Millott, 1975). Larvae of the echinoid *Dendraster* respond to direct sun illumination by avoiding the water surface. This descending behavior has been shown to depend on the ultraviolet light component of the sunlight and has been speculated to be the direct reason for the diel vertical migrations of these larvae (Pennington and Emlet, 1986). Whereas diffuse photoreception suffices for most of these behaviors, spatial vision, requiring more complex optical structures, has been described for the echinoid genus *Echinometra* (Blevins and Johnsen, 2004).

These reports suggest that photosensitivity is a common phenomenon in sea urchins. Some echinoderm classes, such as the Ophiuroidea, have been proposed to possess specialized photosensory organs by utilizing calcite ossicles of the dorsal arm plates as microlenses that bundle and project the beam of light to putative photoreceptors (Aizenberg et al., 2001; Döderlein, 1898; Hendler and Byrne, 1987). In principle, such photosensory structures could also exist in sea urchins but have remained unproven (Aizenberg et al., 2001). Whereas tube feet, pedicellariae, as well as spines have been shown to react in response to light, it has remained unclear if these structures are themselves photosensory or rather depend on photoreceptors located elsewhere on the animal's body (Millott, 1975).

Besides their photosensory responses, sea urchins display a rich chemosensory behavior. Chemical senses have been attributed to predator avoidance and defense, capture of prey, as well as homing (see e.g.Campbell, 1983; Phillips, 1978; Pisut, 2004). For example, the echinoid *Lytechinus variegatus* are able to detect and orient to chemicals emanating from potential food resources over a distance of 1 m, even under turbulent water flow conditions (Pisut, 2004). Similarly, *Strongylocentrotus* sp. is attracted by algae serving as its food over a distance of 1 m in a Y maze experiment (Vadas, 1977). Crushed urchins, tissue pieces of potential predators, as well as living predators located close to diverse sea urchins species trigger an activation of spines, tube feet, as well as pedicellariae (Campbell, 1973; Snyder and Snyder, 1970). Of those systems, the echinoid pedicellariae have been studied with regards to

their morphology, sensation and responses, regeneration, fossil record and development (Burke, 1980; Campbell, 1983; Dubois and Ameye, 2001; Geis, 1936; O'Connell et al., 1974; Peters and Campbell, 1987). Four major types, (1) the globiferous, (2) the tridentate, (3) the ophiocephalous and (4) the trifoliate or triphyllous pedicellariae, are commonly distinguished in the literature, which can be further subdivided into additional subclasses (Agassiz and Clark, 1907; Campbell, 1983; Geis, 1936). *Strongylocentrotus purpuratus* possesses all four major types, plus the additional claviform type, which might have arisen from the globiferous type (Burke et al., 2006; Mortensen, 1943). The responses to chemical stimuli differ between different types of pedicellariae (Campbell, 1973, 1974, 1983; Smith, 1965), suggesting that they might harbor different chemosensory receptors types. Although receptor cells have been described to be located within the jaw epithelium of all four major pedicellarian types, real chemoreceptor cells have so far only been attributed to the globiferous type (Peters and Campbell, 1987), which release a poison from their venom glands upon direct chemical contact stimulation of their sensory hillock, a small thickening of tissue rich in chemosensory neurons close to the articulation points of the valves (Campbell, 1976, 1983).

Despite the rich amount of stimuli-dependent behaviors that have been described, and some basic knowledge of putative sensory structures in sea urchins that has been gathered, neither chemo- nor photosensation of the animal are understood at the molecular level. We therefore expected that the completion of the *S. purpuratus* genome might serve as a good opportunity to investigate the molecular basis of these senses and provide hints as to their diversification.

Chemoreception and light reception are – with the main exception of the Trp channels and cryptochrome molecules – mediated by members of the G-protein-coupled receptor superfamilies in vertebrates and invertebrates (Ache and Young, 2005). Among the five main superfamilies of GPCRs distinguished by the GRAFS classification scheme (Schiöth and Fredriksson, 2005), two encode photosensory and chemosensory functions of vertebrates, namely the glutamate-receptor superfamily (pheromone and taste) and the rhodopsin-type superfamily (light, olfaction and possibly pheromones) (Bjarnadóttir et al., 2005; Liberles and Buck, 2006).

The rhodopsin-type superfamily can be further subdivided into up to more than 70 subfamilies. Among these, olfactory receptors are unique because they show the largest differences in copy number and are still rapidly evolving. Whereas orthology between other GPCRs such as the Opsins can be determined across Bilateria, olfactory receptors of invertebrates, besides belonging to the GPCRs superfamily, constitute families on their own and are not clearly related to any other GPCR subfamily (see, e.g., Fredriksson and Schiöth, 2005). Olfactory receptors of vertebrates arose by multiple gene duplication events (reviewed, e.g., in Ache and Young, 2005). Gene duplications have long been implied as a major mechanism generating evolutionary innovations (Kimura, 1983; Ohno, 1970). Moreover, increasing organismal complexity has been