

Sea urchin tube feet are photosensory organs that express a rhabdomeric-like opsin and PAX6

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All echinoderms have unique hydraulic structures called tube feet, known for their roles in light sensitivity, respiration, chemoreception and locomotion. In the green sea urchin, the most distal portion of these tube feet contain five ossicles arranged as a light collector with its concave surface facing towards the ambient light. These ossicles are perforated and lined with pigment cells that express a PAX6 protein that is universally involved in the development of eyes and sensory organs in other bilaterians. Polymerase chain reaction (PCR)-based sequencing and real time quantitative PCR (qPCR) also demonstrate the presence and differential expression of a rhabdomeric-like opsin within these tube feet. Morphologically, nerves that could serve to transmit information to the test innervate the tube feet, and the differential expression of opsin transcripts in the tube feet is inversely, and significantly, related to the amount of light that tube feet are exposed to depending on their location on the test. The expression of these genes, the differential expression of opsin based on light exposure and the unique morphological features at the distal portion of the tube foot strongly support the hypothesis that in addition to previously identified functional roles of tube feet they are also photosensory organs that detect and respond to changes in the underwater light field.

Keywords: sea urchin; opsins; photosensory; tube feet; echinoderm

1. INTRODUCTION

Green sea urchins (*Strongylocentrotus droebachiensis*) from the Gulf of Maine, and other echinoderms, undergo an annual reproductive cycle that is regulated by changing autumn (Northern Hemisphere) photoperiod that results in a decrease in the total number of daylight hours. Studies on sea urchins suggest that these changes in photoperiod are the signal that causes the mobilization of nutrients from somatic cells and initiation of gametogenesis in the gonads of both sexes [1–3]. The photoperiod control of gametogenesis requires photosensory capabilities on the surface of the sea urchin to detect and respond to the low irradiances of light in their underwater habitat, which is dominated by the green portion of the spectrum [3]. Sea urchins are clearly capable of sensing changes in the underwater light field of their environment and using this information to modulate both their behaviour [4–6] and physiology [3]. How sea urchins detect these changes has been unclear, because they lack image-forming eyes and light-sensitive eyespots. In the echinoderms, there are several studies that describe the location, distribution and morphology of putative photosensory organs or extra-ocular photoreceptors, while the functional and molecular characteristics are largely unknown or poorly

described. Several anatomical features of the sea urchin that might serve as photoreceptors include the spines, pedicellariae and tube feet (i.e. podia), and sea urchins are known to respond to both the visible and ultraviolet components of solar radiation [1,4,6,7], suggesting that their photosensory capability is on the surface of the test because the transmission of visible and ultraviolet radiation through the sea urchin test is less than 1 per cent of the available ambient underwater irradiance [3]. Additionally, recent work on the calcitic microlenses found in light-sensitive brittlestars [8] suggests that the ossicles within their radially arranged arms and underlying tissues may be involved in photoreception, and sea urchins also have ossicles in the distal portion of their tube feet [9].

The study of photoreception in echinoderms, and echinoids in particular, has been of historical interest, and in the past was more commonly referred to as the ‘dermal light sense’ [5,10–13]. Millot & Yoshida [14] described putative photoreceptors in the epithelium of the sea urchin *Diadema antillarum* and the photosensitivity of its radial nerve. The spectral sensitivity of these photoreceptors peaks between 530 and 550 nm [11], which points towards the involvement of a specific chromophore or pigment. In this study, it was also suggested that the pigments associated with echinoderm photoreceptors might be carotenoids [11], while another study on the sea urchin *Centrostephanus longispinus* shows peak sensitivity of its isolated chromatophores between 430 and 450 nm based on an action spectrum of chromatophore contraction and expansion [15]. In

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this latter case, however, the chromatophores were not functionally linked to the nervous system and were not interpreted as being involved in photoreception [15].

Recently, putative opsin proteins have been identified in the optic cushion of asteroids and arm tissue of ophiuroids using both western blots and immunohistochemistry [10]. Opsins have been characterized for many photosensitive prokaryotic and eukaryotic organisms, including sea urchins [16,17]. In vertebrates, multiple opsins are involved both in vision and in extra-ocular photoreception, which regulate circadian clocks and gametogenesis [17,18]. Opsins involved in extra-ocular photoreception share structural features with opsins involved in image-forming eyes, including a lysine retinal attachment site and the Schiff base counterion, but also possess unique features at the gene and protein level. Light appears to be a very important stimulus for a variety of sea urchin functions that may require multiple opsins to confer photosensitivity on time scales from short-term sensitivity (which governs rapid behavioural responses) to long-term circadian rhythms (which control processes such as gametogenesis). Determining which structures in this eyeless organism are capable of photoreception and can express specific opsins should provide useful information on the photobiology of sea urchins generally, and the evolution of photoreceptors and image-forming eyes.

2. MATERIAL AND METHODS

(a) Preparation of tube feet for microscopy and immunocytochemistry

Sea urchins ($n = 3-5$) were immersed in chilled 7 per cent MgCl solution to relax and extend their tube feet. From these sea urchins, 50–75 tube feet were excised for the procedures described below. Several individual tube feet were used to isolate ossicles for scanning electron microscopy (SEM) by fixing in 70 per cent ethanol and macerating in 2.0 per cent KOH for more than 3 days. Additional samples of tube feet for microscopy were placed in primary fixative (2% glutaraldehyde in 0.2 M cacodylate buffer) for 2 h. Fixed tube feet were then dehydrated in an ethanol series, decalcified using 5 per cent ethylenediaminetetra-acetic acid (EDTA) in water and prepared for light (stained with Azure B), SEM or transmission electron microscopy (TEM) or for immunocytochemistry. For SEM and TEM, standard protocols for echinoderm tissues were used as described by Crawford & Burke [19]. For immunocytochemistry, dehydrated tube feet were embedded in Immuno-Bed (Polysciences, Inc., Warrington, PA, USA) overnight under vacuum. Sections (1 μm) were prepared on a JB-4 microtome and mounted on poly-L-lysine coated slides. Immunocytochemical detection and localization of PAX6 protein were performed using a goat PAX6 polyclonal antibody (C-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) to the C-terminus of human PAX6, and either directly conjugated to QDots (525 green fluorescence; Quantum Dot Corporation, Hayward, CA, USA) or visualized by labelled secondary antibody (Elite Goat IgG ABC kit, Vector Laboratories, Burlingame, CA, USA) and photographed on a Zeiss Axioplan II microscope equipped with epifluorescence and AXIOVISION v. 4.4 software (Carl Zeiss, Inc., Thornwood, NY, USA). This commercial antibody has been shown to be specific to PAX6 purified protein and cross-reacts with both sea urchin tube feet and sea star optic cushion using western blots and the appropriate negative controls [20].

(b) Green sea urchin opsin primer design and gene amplification

Sequences from the *Strongylocentrotus purpuratus* Genome Project were searched for matches against the term 'opsin'. The returned sequences were aligned using LASERGENE v. 6 software (DNASTAR, Inc., Madison, WI, USA) and run through BLASTx to match the sequence against the GenBank 'nr' database. Two non-degenerate primers were then designed and designated as follows: Opsin Forward (5'-ACCTGCTCG TTGCGACTACG) and Opsin Reverse (5'-GGCCTTCC TGAACCTTGGAAATT). Approximately 30–50 tube feet were extracted for total RNA using TRIzol Reagent (Sigma-Aldrich, St Louis, MO, USA), and the mRNA population reverse transcribed using a polyT primer and Superscript II (Invitrogen, Carlsbad, CA, USA). The resulting cDNA was used in a polymerase chain reaction (PCR) with the primers described above, and all PCR products were TA cloned (Promega pGEM-T kit) with individual clones grown overnight and minipreped to produce plasmid DNA for sequencing using an ABI 377 gel sequencer. Rapid amplification of cDNA ends methodology was used in order to obtain full-length amplification of the complete gene. The same cDNA sequence was obtained when the mRNA was pretreated with DNase, suggesting that the extra sequence observed in the cytoplasmic loop is actually present in the mRNA transcript and not the result of contamination from unspliced DNA.

(c) Phylogenetic analysis of green sea urchin opsin

An alignment of 45 opsin amino acid sequences was created using the MAFFT alignment program [21]. GenBank accession numbers for the alignment are as follows: *Ciona intestinalis* opsin (BAB68391); *Danio rerio* vertebrate ancient long (VAL) opsin (NP_571661) and teleost multiple tissue (TMT) opsin (NP_001112371); *Salmo salar* vertebrate ancient opsin (O13018); *Petromyzon marinus* pineal opsin (O42490); bovine rhodopsin (AH001149); *Gallus gallus* SWS1 (AAA49141), SWS2 (AAA48633), RH2 (AAA48786), LWS (AAA49137), RH1 (BAA00610) and pinopsin (P51475); *Geotria australis* SWS1 (AAR14684), SWS2 (AAR14681), RhA (AAR14682), RhB (AAR14683) and LWS (AAR14680); *Homo sapiens* opsin-3 (NP_055137), melanopsin or opsin-4 (NP_150598), opsin-5 (NP_859528) and peropsin (NP_006574); *Mus musculus* opsin-3 (NP_034228), melanopsin or opsin-4 (NP_038915), opsin-5 (NP_861418) and peropsin (NP_033128); *Platynereis dumerilii* c-opsin (AAV63834) and r-opsin (CAC86665); *Drosophila melanogaster* RH1 (NP_524407), RH2 (NP_524398), RH3 (NP_524411), RH4 (NP_476701), RH5 (NP_477096) and RH6 (NP_524368); *Limulus polyphemus* opsin (P35361); *Mizohupecten yessoensis* Gq coupled opsin (O15973); *Octopus rhodopsin* (P09241); *Gadus morhua* melanopsin (AAO20043); *Xenopus laevis* melanopsin (AAC41235) and chlamyopsin 4 (XP_001701725). In addition, we included the five opsins identified from the *Strongylocentrotus purpuratus* genome [16].

The alignment was checked by eye to ensure the characteristic functional regions of the proteins were aligned (e.g. the counterion₋₁₁₃ and lysine₋₂₉₆ residues). The alignment was converted to phylip format using CLUSTALX [22] and used in ProtTest to identify the best model of protein evolution using the Akaike information criterion [23]. A maximum-likelihood tree was then constructed in genetic algorithm for rapid-likelihood inference [24] by Grid computing [25] through the Lattice Project [26]. The consensus bootstrap tree was then calculated from 1000

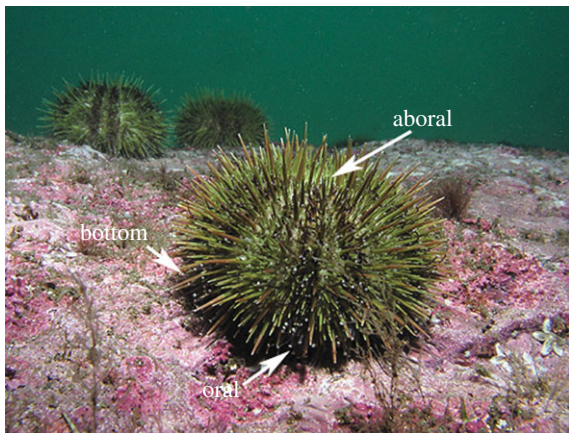


Figure 1. Underwater photograph of a green sea urchin showing the aboral, bottom (at interface of urchin test and substrate) and oral locations where tube feet were collected. See also fig. 4*a,m* in [30].

bootstrap replicates using PAUP v. 4.0b10 (Sinauer Associates, Sunderland, MA, USA [27]).

A hydrophobicity plot analysis was carried out in order to determine potential transmembrane regions of the green sea urchin opsin protein. The Kyte–Doolittle scale with a window size of 19 was used for delineating the hydrophobic character of the protein [28]. Green sea urchin opsin was analysed along with a subset of opsins including sea urchin, invertebrate and vertebrate opsins with previously characterized transmembrane domains to determine the most likely transmembrane regions of the green sea urchin opsin [29].

(d) Differential gene expression of the green sea urchin opsin gene

qPCR was used to determine the relative levels of mRNA expression of opsin in the tube feet located on different areas of the test of *S. droebachiensis* ($n = 3$ urchins). Multiple tube feet ($n = 25–50$) from the aboral, bottom and oral portions of each individual urchin test (figure 1) were collected and total RNA extracted. The mRNA population was then reverse transcribed as described above and the resulting cDNA was used in a qPCR reaction using the Taqman Fast System (Applied Biosystems, Carlsbad, CA, USA), where triplicate reactions for each set of tube feet from each urchin were run on the 7500 Fast RT-PCR System (Applied Biosystems), at 45 cycles of 95°C with an automatically set critical cycle threshold (Ct). To quantify the opsin transcripts of *S. droebachiensis*, forward and reverse primers were designed from the sequence data (see above) in combination with a Taqman probe as follows: TB opsin F 5'-AAGCTCCATA GCCCATCAA, TB opsin R 5'-CGAA-TAACCA CCGACCGTAGAA and TB opsin probe 5'-CGTTAACC TCTCCGCCAGCGACC. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene that is constitutively expressed, was used as an internal control to normalize all data. Primer efficiencies, calculated from the raw data, were used to calculate the maximum expression value so that calculations from raw qPCR data used the formula $P^{\Delta Ct}$, where P = primer efficiency and ΔCt = cycle threshold difference with GAPDH for the sample. Specifically, the ratio of the target gene and the control gene can be determined using the equation

$$\frac{G_1}{G_2} = \frac{(1 + E_2)^{Ct_2}}{(1 + E_1)^{Ct_1}}$$

and the cycle numbers at which each gene (G) becomes detectable by calculating the Ct and E that is the PCR efficiency for the target and reference genes. The variability between qPCR runs was very low and the biological variability of the relative expression of opsins between tube foot locations is presented.

3. RESULTS

In adult urchins, approximately 1500 tube feet are present and serve as a radially distributed sensory array for these shallow water marine invertebrates (figure 2*a*). At the distal tip of each tube foot is a group of five independent calcareous ossicles circularly arranged with their concave surfaces facing the ambient underwater light environment (figure 2*b,c*). When illuminated at any single point on the ossicle using a visible spectrum (400–700 nm) fiberoptic probe, the ossicles scatter light across the entire ossicle (figure 2*d*). Similarly, when illuminated at any single point on the ossicle using a red (approx. 685 nm) laser the ossicles scatter light across the ossicle (figure 2*e*). The ossicles of the tube feet are perforated (figure 2*c*) and contain cells that express PAX6 protein (figure 2*f* and inset, using PAX6 antibody conjugated to QDots; ossicles removed by the de-calcification process). Note the presence of the pigment cells, probably containing opsin, in and around the ossicles (figure 2*g*). Nerves are also present along the entire length of the tube foot under the epithelium, forming a nerve plexus (figure 2*h*) that terminates in a ganglion at the distal portion of the tube foot as previously described for sea urchins [30–32].

Using primers specific for opsins, a 1.563 Kbp cDNA was amplified from the green sea urchin tube foot. The predicted protein from the corresponding mRNA encodes an opsin protein that has a 1.116 Kbp open reading frame and 371 amino acids (figure 3, GenBank DQ285097). The identity of this protein as an opsin was confirmed by a phylogenetic analysis of the amino acid sequence data (see below). The Kyte–Doolittle hydrophobicity plot analysis of green sea urchin opsin showed seven segments greater than one in the hydrophathy score, which represent potential transmembrane domains. These transmembrane domains matched those found in other opsins, including opsins from invertebrates [17,33,34]. The green sea urchin tube foot opsin shares many additional features with known opsins, the most conserved of which is the lysine in the seventh transmembrane domain (TM; K₂₉₆/K₃₃₁), which serves as an attachment site for the chromophore retinal [35] and two cysteine residues (C₁₀₀ and C₁₇₈) that together form a disulphide bridge essential for stability of opsin tertiary structure [36]. The cytoplasmic carboxy tail of the urchin opsin is only 25 amino acids long and is similar in this respect to the shorter tails of vertebrate visual opsins [37]. Most vertebrate opsins have a negatively charged glutamic acid residue (E₁₁₃) in TM III that acts as a counterion for the protonated Schiff base [17]. The green sea urchin opsin, however, has a neutrally charged tyrosine residue (E₁₁₃/Y₁₀₃) that is common for most non-vertebrate opsins (figure 3) [33] and probably does not act as the counterion. The green sea urchin opsin does have a negatively charged aspartic acid residue (D₁₈₁) and there is strong evidence that this amino acid acts as the counterion for the protonated Schiff base in the ancestral, non-vertebrate opsins [17,33].

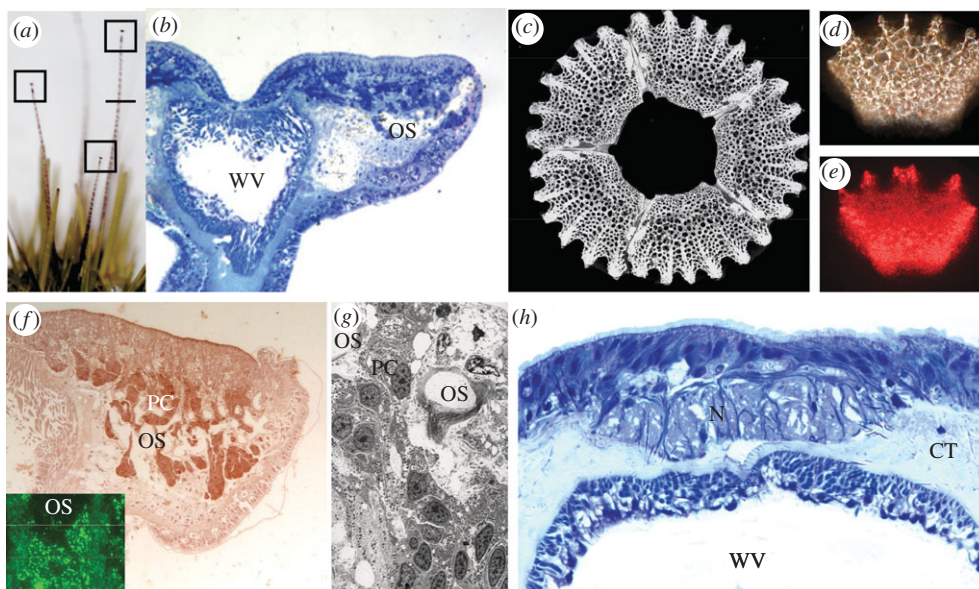


Figure 2. Green sea urchin tube feet. (a) Extended tube feet of a green sea urchin (maximum length of adult tube feet is 7–8 cm). Boxes indicate the distal tips shown in (b) and the line indicates the position of the cross-section shown in (h). (b) Structure of the right-hand side of the distal tip of a tube foot, showing position of ossicles (OS) seen in (c). (c) Array of five single crystalline ossicles removed from the tube foot. (d) Single ossicle under polarized light. (e) Single ossicle showing scattering of laser light directed at lower left corner. (f) Immunocytochemical localization of PAX6 protein in the pigment cells (PC) passing through perforations in the ossicles; inset shows the same PAX6 antibody conjugated to Qdots within pigment cell nuclei. (g) Electron micrograph of pigment cells passing through ossicle. (h) Cross-section at the level shown in figure 1a, indicating the nerve (N) running down one side of the tube foot (CT, connective tissue; WV, water vascular system of tube foot).

A phylogenetic analysis was performed on 45 opsin sequences, resulting in a bootstrap-supported, maximum-likelihood tree that provides insight into the relationship of this molecule to other opsins (figure 4). ProtTest found the Wheland and Goldman (WAG) [39] protein model to be best, with empirical amino acid frequencies (F option) and four categories of amino acid rate classes (G option). However, including invariant sites (I option) did not improve the model and so was not used. Bootstrap analyses give high support for the majority of the nodes on the tree.

This analysis included the green sea urchin sequence, as well as sequence data for five urchin genes that encode for opsins from the *S. purpuratus* purple sea urchin genome project [16]. One of the *S. purpuratus* opsins (Sp-opsin5) is similar to the green sea urchin opsin, varying only 4 per cent at the nucleotide level and 5.4 per cent in their amino acid sequences. In our analyses and the Raible *et al.* [16] study, the green sea urchin sequence and Sp-opsin5 are intermediate between the ciliary and rhabdomeric opsins. Interestingly, comparisons of the *S. droebachiensis* cDNA with the genomic *S. purpuratus* sequences revealed that the open reading frame of the *S. purpuratus* homologue occurs without introns. This suggests that this gene is an inserted transcript with the extra sequence in the third cytoplasmic loop, probably resulting from a retrotransposon event.

Primer efficiencies for the qPCR of green sea urchin opsin were 99 per cent. The differential expression of the green sea urchin rhabdomeric opsin from tube feet located on different parts of the sea urchin test, relative to GAPDH, showed a significant effect (ANOVA: $F = 217.6$, $p < 0.0001$) of tube foot location (figure 5). The tube feet on the oral side of the urchin test, which are exposed to the

lowest irradiances of visible light (approx. $5 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; M. Lesser 2006, unpublished data), expressed significantly (Tukey's HSD, $p < 0.05$) more opsin transcripts than either tube feet from the bottom (approx. $35 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; M. Lesser 2006, unpublished data) or aboral (approx. $65 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$; M. Lesser 2006, unpublished data) portions of the test that were exposed to increasing amounts of downwelling irradiance (figure 5) and were not significantly different from each other (Tukey's HSD, $p > 0.05$).

4. DISCUSSION

The tube feet of the green sea urchin include morphological and molecular features consistent with a role in photoreception. Morphologically, the arrangement of the ossicles at the distal portion of the tube feet could function as a light collector in photoreception just as the calcitic microlenses of brittlestars refract and focus light as part of their ability to detect light [8]. As shown here, the presence, structure and morphological arrangement of the ossicles facilitates the scattering of light and the potential for increasing the path length of any individual photon, with a subsequent increase in the ability for light absorption within the ossicle by pigment cell opsins within the ossicle. A nerve plexus is also present along the entire length of the tube foot and extends under the ossicles, providing ultrastructural evidence of nerve transduction to the test and radial nerve. Additionally, the tube feet are used for adhesion and locomotion for which there is evidence of cholinergic-based motor control along the nerve [32,40]. The multiple lines of evidence provided here strongly suggest that tube feet are also photosensory organs [30,41].

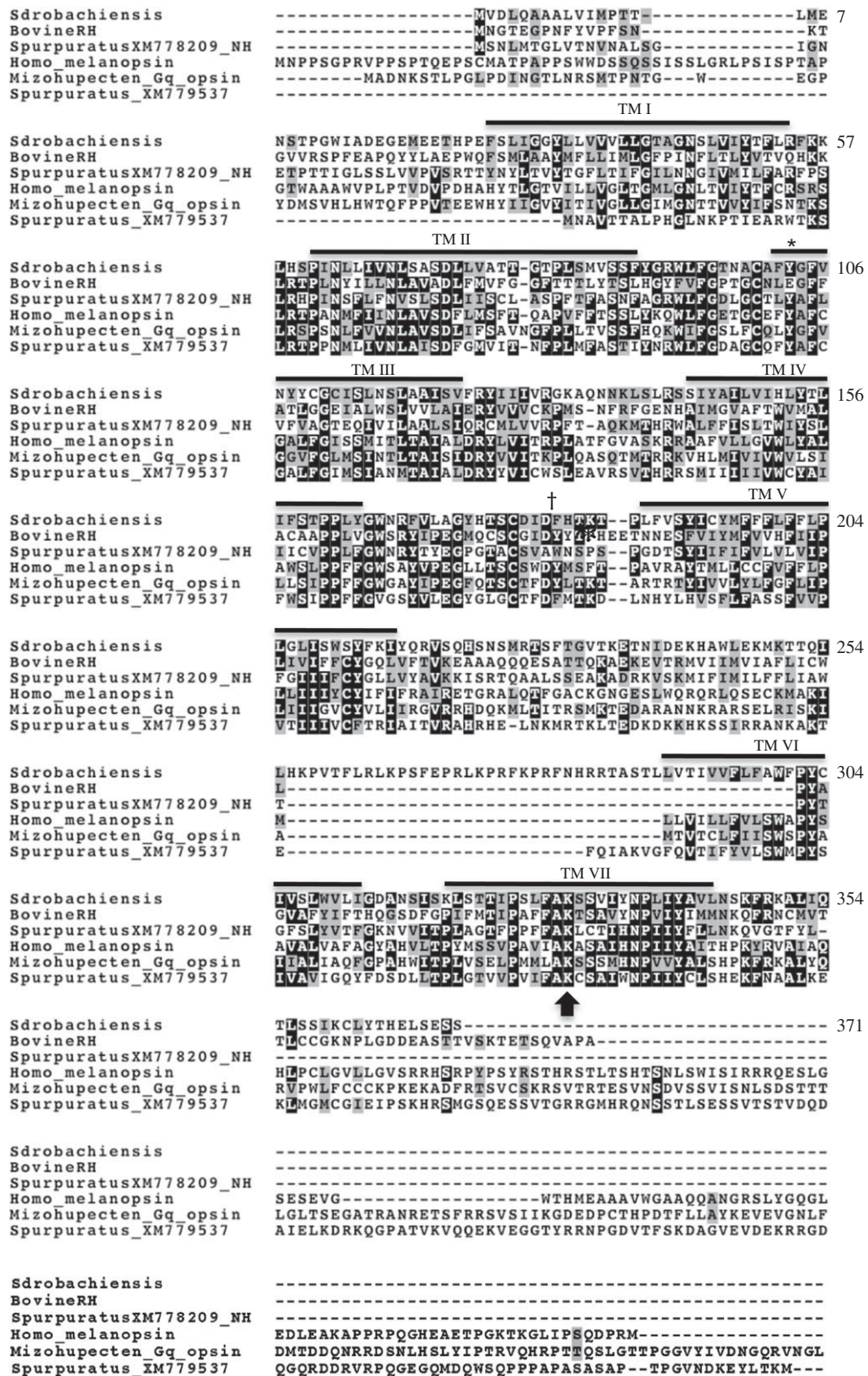


Figure 3. Amino acid alignment of green urchin opsin and several other opsins. Identical residues between species share a black background and conserved residues share a grey background. Transmembrane domains (TM) are indicated with a solid bar, lysine attachment site for retinal is marked with an arrow, the tyrosine residue (E₁₁₃/Y₁₀₃) is marked with an asterisk and the aspartic acid at the proposed counterion position (D₁₈₁) for the green sea urchin opsin is indicated with a dagger.

The cells within the ossicles also express the transcriptional activator PAX6 protein, a product of the homeotic gene known to control eye development in other organisms [42]. PAX6 is expressed during the development of eyespots in planarians [43] and of structurally advanced image-forming eyes like those in mammals and squid

[44,45]. Studies on the fruitfly *D. melanogaster* suggest that the evolutionarily ancestral role of PAX6 may have been to regulate structural genes involved in photoreception and that it later functioned to regulate the morphogenesis of divergent and complex eye structures, including image-forming eyes, and regulating the

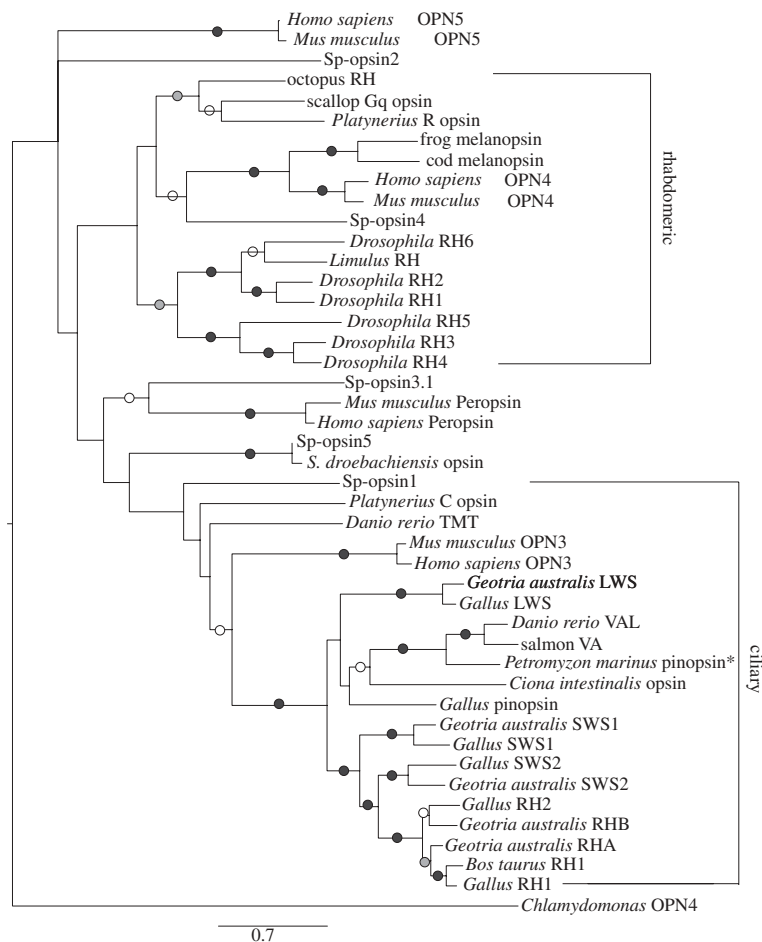


Figure 4. Phylogenetic analysis: neighbour-joining tree based on amino acid distances that are Poisson-corrected for multiple hits. Scale bar is substitutions per amino acid residue. The range of bootstrap values for significant nodes is indicated. LWS = long-wavelength sensitive, SWS = short-wavelength sensitive, VA = vertebrate ancient, VAL = vertebrate ancient long, RH = rhodopsin, OPN3 = panopsin or encephalopsin, OPN4 = melanopsin, OPN5 = neuropsin, TMT = teleost multiple tissue. An asterisk is located on the pinopsin of the lamprey, *Petromyzon marinus*, because there is a suggestion that it is actually a VA opsin [38], but the sequence is still listed as a pinopsin. Open circles, 50–75%; filled grey circles, 75–90%; filled black circles, more than 90%.

expression of rhodopsin [46]. *PAX6* has been cloned from the gastrulae of the sea urchin *Paracentrotus lividus* [47] and is highly conserved with respect to vertebrate genes. A Northern blot analysis of adult *P. lividus* tissues (including ovary, testis, tube foot, intestine and coelomocytes) demonstrated *PAX6* expression only in tube feet, including the tips of the tube feet [47] as we have reported here for the green sea urchin protein. *PAX6* sequences from *S. purpuratus* [30], as well as green sea urchin *PAX6* (GenBank DQ230536), are also highly conserved relatives of vertebrate *PAX6* genes, including human *PAX6*.

The most unique feature of green sea urchin tube foot opsin is the extended third cytoplasmic loop between TM V and TM VI, which is 28 amino acid residues longer than the corresponding cytoplasmic loop of all other opsins. Together with amino acids in the third cytoplasmic loop and others in the cytoplasmic carboxy tail, these amino acids are the ones that interact with the α subunit of heterotrimeric G-proteins to initiate the phototransduction cascade [36,48]. All G-protein coupled receptors share a five-residue motif with unknown function in TM VII consisting of the amino acids NPXXY. Green sea urchin opsin has this motif as NPLIY (residues

348–352). The phylogenetic analysis of the green sea urchin opsin suggests that a $G_q \alpha$ is the most likely G-protein involved in the tube foot phototransduction cascade. Alternatively, the unique long third cytoplasmic loop may point to a novel G-protein, as is the case in phototransduction of the scallop, a protostome bivalve mollusc [48]. It is possible that the green sea urchin opsin has no known function or is a pseudogene as we have no protein expression data, but we believe this to be unlikely given the multiple lines of evidence presented here.

The phylogenetic position of the green sea urchin opsin sequence places it between the invertebrate G_q -coupled opsins found in rhabdomic photoreceptors and the vertebrate G_t α -coupled opsins found in ciliary photoreceptors. However, many features of the green sea urchin opsin—including the presence of a tyrosine at the counterion position (E₁₁₃/Y₁₀₃), which is common to non-vertebrate opsins, and the proposed evolutionary history of photoreceptors and associated opsins [33,49]—support the classification of the green sea urchin opsin described here as a rhabdomic-like opsin similar to Sp-opsin5 from the *S. purpuratus* urchin genome project, which is homologous to the green sea

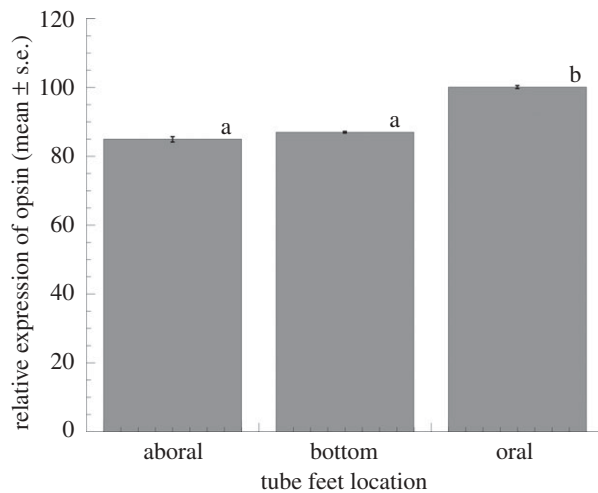


Figure 5. Maximum relative expression of the rhabdomeric-like opsin in tube feet from different areas of urchin test (figure 1) compared with the expression of GAPDH in the green sea urchin *Strongylocentrotus droebachiensis* (mean \pm s.e.). As these values are proportions they were *a priori* transformed ($\log+1$) before analysis by ANOVA and were determined to be normally distributed and homoscedastic. All values were then back-transformed for presentation. Common letters above bars indicate samples that are not significantly different ($p < 0.05$) from each other using Tukey's HSD multiple comparison testing.

urchin opsin described here (figure 4). However, in addition to this rhabdomeric-like opsin, *S. purpuratus* does express a rhabdomeric opsin (Sp-opsin4) that groups with invertebrate G_q -coupled opsins and vertebrate melanopsins, as well as a ciliary opsin (figure 4, Sp-opsin1) that groups with the vertebrate opsins [16]. This expression of rhabdomeric and ciliary opsins is similar to a study by Arendt *et al.* [33] where they found that the polychaete worm, *Platynereis dumerilii*, has both vertebrate ciliary and rhabdomeric opsins that are associated with specific photoreceptor cells. Their finding confirms that both types of photoreceptors with distinct opsins are found in the Urbilateria (the last common ancestor of the Bilateria) and that in the evolutionary line leading to vertebrates, which includes sea urchins, both photoreceptor types were selected for, and incorporated into, the evolving vertebrate retina. The rhabdomeric photoreceptor cells may have transformed into ganglion cells expressing melanopsin, acquiring a new role in light detection and signal transduction [49]. First described in *Xenopus*, melanopsin is now known to confer photosensitivity on non-photosensitive cells types [50,51]. It has been hypothesized that melanopsin sets the circadian clock through non-ocular light detection [52], and is also expressed in sea urchins [30]. Additionally, an encephalopsin homologue has been sequenced from sea urchins [30,41], which is expressed at the tips of the larval arms of plutei and tube feet of adults [16,41], and potentially aids in the detection of light by planktonic larvae vertically migrating in the water column.

The inverse relationship between tube foot position on the test, which equates to differences in light exposure, and expression of the green sea urchin rhabdomeric-like opsin, is phenomenologically similar to the well-known inverse relationship between light and photosynthetic

pigments universally expressed from microalgae to higher plants. This inverse relationship between light and opsin expression also occurs in the multi-cellular alga *Volvox carteri*, where the expression of an opsin (i.e. channelrhodopsin) homologous to animal opsins is inversely related to the amount of light the alga is exposed to, and is also used for phototaxis by the alga [53]. Similarly, the differential expression of the green sea urchin opsin in tube feet may be an important component of a sea urchin's ability to photoacclimatize to different irradiances on multiple timeframes to regulate the phototransduction of this external stimulus for important organismal functions, such as gametogenesis [3]. This may not be the case for all opsin classes in sea urchins, however, as the expression of encephalopsin in the tube feet of the sea urchin *Hemicentrotus pulcherrimus* showed a more direct relationship with light exposure, where encephalopsin expression decreased under dark conditions [41].

The green sea urchin, like many species of sea urchin, has unique hydraulic structures called tube feet. Within the tube feet there are calcareous ossicles, arranged in such a manner that they could serve as a light collector, and a unique, rhabdomeric-like opsin that is expressed along with PAX6, the master control gene for the development of eyes and photosensory organs in many organisms, including sea urchins [47]. In response to changes in the underwater light field, the light absorbed by the opsins of the pigment cells could potentially stimulate the nerves beneath them and conduct electrical information along the major nerve that passes along one side of each tube foot towards the test of the urchin. We now know that a full array of genes encoding neurotransmitters is present in sea urchins, which could facilitate the transmission of these signals [30]. For temperate invertebrates like the green sea urchin, opsins are well suited to mediate this transduction of an external environmental signal because of their absorption maxima in the blue-green portion of the spectrum that dominates in these underwater habitats [3,54]. The data presented here show that green sea urchins have a rhabdomeric-like opsin. While we have no direct evidence that this opsin is functional, it could be conferring an important capability to detect and transmit differences in light on varying time scales, from seconds and minutes used in the behavioural responses of echinoderms (e.g. [4]) to longer-term changes in irradiance occurring on the scale of months, which are important for processes such as seasonal gametogenesis [3]. Additional work is required to determine the various phototransduction pathways involving these opsins, any additional structures or tissues, where they are expressed, and the temporal scales over which they provide information to modify urchin behaviour and physiology. The simultaneous expression of an opsin gene and the unique morphological features at the distal portion of the tube foot shows that sea urchin tube feet, among their many functions, have also evolved into a unique photosensory organ to sense and respond to changes in the underwater light field, and may also provide useful information related to the origin of eyes in other organisms.

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