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Bioluminescence in lanternsharks: Insight from hormone receptor localization

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ABSTRACT

As part of the study of their bioluminescence, the deep-sea lanternshark *Etmopterus spinax* and *Etmopterus molleri* (Chondrichthyes, Etmopteridae) received growing interest over the past ten years. These mesopelagic sharks produce light thanks to a finely tuned hormonal control involving melatonin, adrenocorticotropic hormone and α-melanocyte-stimulating hormone. Receptors of these hormones, respectively the melatonin receptors and the melanocortin receptors, are all members of the G-protein coupled receptor family i.e. coupled with specific G proteins involved in the preliminary steps of their transduction pathways. The present study highlights the specific localization of the hormonal receptors, as well as of their associated G-proteins within the light organs, the so-called photophores, in *E. spinax* and *E. molleri* through immunohistofluorescence technic. Our results allow gaining insight into the molecular actors and mechanisms involved in the control of the light emission in Etmopterid sharks.

1. Introduction

At present, Etmopteridae and Dalatiidae are the only two families of sharks that unambiguously contain luminous species (Claes and Mallefet, 2009a; Straube et al., 2015). Among bioluminescent Etmopteridae, *Etmopterus spinax* and *Etmopterus molleri* have been studied over the past ten years to better understand their luminescence and the associated biological functions (Claes and Mallefet for review). These deep-sea lanternsharks display intrinsic light organs, called photophores, spread over the ventral epidermis (Claes and Mallefet, 2009a, 2009b; Duchatelet et al., 2019b; Fig. 1a, b). Etmopterid photophores, ultrastructurally depicted by Renwart et al. (2014, 2015), consist of emitting cells, the photocytes, enclosed in a multicellular cup-shaped pigmented sheath and surmounted by one or several lens cells. A multilayer cell zone, the so-called iris-like structure (ILS), is composed of a complex pigmented melanophore-like cell network present between the lens cells and the photocytes and is used as a shutter of the light organ (Claes and Mallefet, 2009a; Renwart et al., 2014; Fig. 1c, d). Counterillumination appears to be the main ecological function of bioluminescence for lanternsharks but aposematism and intraspecific communication have also been suggested (Claes and Mallefet, 2008, 2009a; Claes et al., 2010a, 2013; Duchatelet et al., 2019d). Conversely to the majority of bioluminescent bony fishes presenting a nervous light emission control mainly through adrenaline, noradrenaline or nitric oxide (NO) (Anctil, 1972; Krönström and Mallefet, 2009; Zaccone et al., 2011; Mallefet et al., 2019), luminescent lanternsharks are characterized by a hormonal control of the light output: melatonin (MT) and prolactin (PRL) triggering the light emission while α-melanocyte-stimulating (α-MSH) or adrenocorticotropic (ACTH) hormones inhibit luminescence (Claes et al., 2011b; Claes and Mallefet, 2009; Duchatelet et al., 2020). Typical neurotransmitters, NO and γ-aminobutyric acid (GABA) also modulate the hormonally-triggered *E. spinax* light emission (Claes et al., 2010b, 2011a). Although Claes et al. (2010b) showed that acetylated tubulin positive nerve processes innervated the surrounding of the photophores, there is no pharmacological evidence of a direct nervous control of the light emission in lanternsharks (Claes et al., 2010b, 2011a). The ultrastructural characterization of the *E. spinax* photophore did not highlight any nervous structures within the photophore itself (Renwart et al., 2014, 2015). The role of the terminal nerves, reaching the surrounding of photophores, then remain to be investigated.

Interestingly, some hormones involved in the light emission control in Etmopteridae (i.e. PRL, α-MSH) are also involved in pigment motion regulation in shallow water shark species (Visconti et al., 1999). Despite the increasing collection of pharmacological data on the luminous control, data is lacking concerning the expression and localization of the hormone receptors involved in the light emission control (i.e. stimulation or inhibition). According to the recently available skin

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transcriptome of *E. spinax* (Delroisse et al., 2018), the presence of predicted mRNA coding for such hormonal receptors (i.e. MT and α-MSH/ACTH receptors) was pinpointed.

To date, in vertebrates: (i) four different melatonin receptors (Mel1a/MTNR1A, Mel1b/MTNR1B, Mel1c/MTNR1C, and Mel1D/MTNR1D) are known to be expressed (Shiu et al., 1996; Witt-Enderby et al., 2003; Falcon et al., 2010; Li et al., 2013); (ii) melanocortin system comprises five G protein-coupled receptors (MC1-5R) that could link melanotropic peptides (α-/ß-/γ-MSH and ACTH) (Chhajlani and Wikberg, 1992; Chakrabothy et al., 1995); and (iii) PRL receptors (PRLRa and PRLRb), closely related to growth hormone receptors (GHRa and b), appear to be expressed from gnathostomes (Ellens et al., 2013). Recently, Daza and Larhammar (2018) demonstrated a secondary loss of PRLR in cartilaginous fishes during the GHR/PRLR family evolution.

To gain insights on the molecular pathways involved in the light emission control in Etmopteridae, immunolocalizations of the hormone G-protein coupled receptors, MTNR and MCR as well as of their associated G proteins, were performed within the Etmopterid photogenic organs of the two investigated species. Based on transcriptome data, phylogenetic analyses were performed in parallel to assign new predicted receptors to known vertebrate receptors subclasses.

2. Materials and methods

2.1. Experimental specimens

Velvet belly lanternsharks (n = 12), *E. spinax,* were caught by longlines lowered at 250 m depth during field sessions in January 2017 in the Raunefjord, Norway [60°15′54″ N; 05°07′46″ E]. Specimens were maintained in a tank with running fresh seawater (6 °C) in a dark cold room at Bergen University Marine Station (Espegrend, Norway). Slendertail lanternsharks (n = 10), *E. molleri,* were captured during a field session in November 2016 by fishing rod equipped with electric reel at 500 m depth near the coast of Sesoko Island, Okinawa, Japan [26°28′94″ N; 127°41′20″ E]. Slendertail lanternsharks were brought to the Okinawa Churaumi Aquarium where they were kept alive in dark tank filled with running seawater (13 °C). Both shark species specimens were treated according to the European regulation for animal research handling and euthanized following the local rules for experimental vertebrate care. Sharks were measured, weighed and sexed before dissection occurs. Skin patches of around 3 cm^2 were dissected from the ventral luminous zone, fixed in phosphate buffer saline (PBS) containing 4% paraformaldehyde for 12 h at 4 °C, and, then, bathed and stocked in PBS at 4 °C until use.

2.2. Sequence searches

Using recently available transcriptome data of *E. spinax* (Delroisse et al., 2018) homology-based searches, i.e. local tBLASTn (Altschul et al., 1990), for MT, PRL and α-MSH receptor sequences and G-protein alpha subunits i, s, o and t sequences were performed using vertebrate reference sequences as queries. Vertebrate reference sequences are listed in Supplementary data S1 and S2. Top hit candidates were used as requests in reciprocal BLASTx searches against online NCBI databases to emphasize sequences with the highest homology to MTNR, MCR, and G-protein subunits, respectively. *In silico* translations were performed for the MTNR and MCR-like sequences retrieved from the shark transcriptome using the ExPASy online tool (http://web.expasy.org/translate; Gasteiger et al., 2005). Sequence alignment and secondary structure prediction were performed using the MEMSAT online tool (Jones et al., 1992, 1994) and allowed us to confirm their receptor status.

2.3. Receptor phylogenetic inference

Reference metazoan MTNR and MCR sequences were collected as in NCBI public databases (https://www.ncbi.nlm.nih.gov/) and are listed in Supplementary data S3a, b. For both receptors, a multiple alignment was performed with the MAFFT algorithm using the consistency-based iterative refinement method E-INS-i implemented in Geneious 2 (v11.1.2). A strict trimming was then performed using TrimAL algorithm (automated heuristic) (Capella-Gutiérrez et al., 2009) implemented in Metapiga 3.1. (Helaers and Milinkovitch, 2010). Maximum likelihood phylogenetic analyses were performed using PhyML 3.0 with SPR tree searching (Guindon et al., 2005). Before the ML analysis, automatic model selection was performed using the Smart Model Selection implemented in the PhyML environment and based on the Akaike Information Criterion (Lefort et al., 2017). The JTT + G + F model of amino acid substitution was selected for MTNR and MCR phylogeny. Bootstrap analysis (1000) was performed. A vertebrate opioid receptor was selected as an outgroup for the MTNR phylogeny based on Park et al. (2007). Based on Klovins et al. (2004), the neuropeptide Y receptor (NPYR) from *Homo sapiens* was selected to root the MCR phylogeny.

2.4. Section preparation

Photogenic shark skin patches previously preserved in PBS were bathed in the same buffer with an increasing sucrose concentration (10% for 1 h, 20% for 1 h and 30% overnight). Tissues were then embedded in Optimal Cutting Temperature O.C.T. compound (Tissue-Tek, The Netherlands) and rapidly frozen at −80 °C. Cryostat microtome (CM3050 S, Leica, Solms, Germany) was used to perform 10 µm sections that were laid on coated Superfrost slides (Thermo Scientific) and left overnight to dry.

2.5. Receptor and G-protein immunolocalizations

Commercial antibodies directed against vertebrate MCR, MTNR, Gαs, Gαi, Gαo, and Gαt subunit were selected based on their high sequence similarity with *E. spinax* predicted protein sequences. The anti-MCR antibody (sc-6881, lot number F060, Santa Cruz Biotechnology, Inc.) recognizes a sequence between amino acids 270–320 of MC4R (S-18) in *Homo sapiens* (P32245.2) but was shown to also recognize MC1,3-5R (Gantz et al., 1993; Labbe et al., 1994; Mountjoy et al., 1994). The anti-MTNR antibody (sc-30017, lot number K2906, Santa Cruz Biotechnology, Inc.) recognizes the amino acids 161-280 mapping within an internal region of MEL-1A/B-R of *H. sapiens* (P48039.1). The anti-Gαs antibody (XLalphas antibody, (PA5-22261, lot number QL2131541, Thermo Fisher) is directed against amino acids 807–1037 of the Gαs *H. sapiens* (Q5JWF2.2). G protein alpha inhibitor 1 (GTX105292, lot number 39939, GeneTex) antibody is raised against amino acids 23–354 within the centre region of human guanine nucleotide-binding protein G(i) subunit alpha (NP_002060.4). Guanine nucleotide-binding protein G(o) subunit alpha (GNAO1, (PA5-30044, lot number QL2131541E, Thermo Fisher) antibody recognizes a sequence corresponding to a region between amino acids 104 and 338 of human GNAO1 (P09471.4). Guanine nucleotide-binding protein G(t) subunit alpha-2 (GNAT2; PA5-22340, lot number QL2131541G, Thermo Fisher) antibody recognizes a sequence corresponding to a region between amino acids 154 and 354 of the human GNAT2 (P19087.4). The list of antibodies is presented in Supplementary data S4.

E. spinax and *E. molleri* 10 µm skin section slides were blocked with TTBS [Trizma base 20 mM (Sigma), NaCl 150 mM, pH 7.5 + 1% Tween 20 (Sigma)] containing 10% powder milk (Gloria, Nestlé). Sections were incubated overnight with primary antibody (anti-MCR, anti-MTNR, anti-Gαs, anti-Gαi, anti-Gαo or anti-Gαt) at 4 °C used at a dilution of 1/300 in TTBS 5% milk (Gloria, Nestlé). Revelation of the

protein immunoreactivity was done after 1 h incubation at RT of fluorescent dye labelled secondary antibody (Donkey Anti-Goat IgG H& L Alexa Fluor® 555, Abcam for the MCR and Gαs labelling and Goat Anti-Rabbit Alexa Fluor® 594, Life Technologies Limited for the MTNR, the Gαi, Gαo and Gαt protein) with a dilution of 1/200 in TTBS 5% milk. Sections have also been subjected to a DAPI (Dapi nucleic acid stain, Invitrogen) staining for 15 min before being mounted (Mowiol® 4–88, Sigma). Slides were examined using a confocal microscope (Zeiss confocal microscope LSM) equipped with the Zen software (Zeiss, Germany). Control sections were incubated in TTBS 5% milk devoid of primary antibody.

3. Results

3.1. MTNR and MCR receptors in Etmopterus spinax

Orthologous sequences corresponding to MTNR and MCR receptor mRNA were found in the ventral skin transcriptome of *E. spinax* using a tBLASTx/reciprocal BLASTx approach (Es-MTNR-like, NCBI accession number: MK923747; Es-MC3-R, MK923745; Es-MCR-like (partial), MK923746). Reciprocal BLASTx top hits highlight a clear homology between the predicted receptors found in *E. spinax* and vertebrate MTNR and MCR receptors. Considering the elasmobranch only, the *E. spinax* MTNR (Es-MTNR-like) and MCR sequences (Es-MC3R, Es-MCR-like) are similar to the melatonin-related receptor from *Callorhinchus milii* (MTNR-like), the melanocortin 3 receptor from *Squalus acanthias* (MC3R) and the melanocortin receptor-like from *Rhincodon typus* (MCR-like), respectively (Supplementary data S1). Predicted protein sequences from *E. spinax* present a high amino acid similarity with human MTNR and MCR (Supplementary data S4). Sequences were translated into amino acid sequences and predicted molecular weights and isoelectric points of these receptors were calculated (Supplementary data S5). A typical GPCR structure, with seven transmembrane helix domains, was highlighted in all three predicted receptors (Es-MTNR-like, Es-MC3R, Es-MCR-like). While predicted Es-MTNR-like and Es-MC3R are complete sequences, Es-MCR-like is partial and only contain three transmembrane helix domains. Sequences similarities of 83.43%, 91.69% and 88.66% were observed for the MTNR and both MCR, respectively.

The same *in silico* approach was applied for the search of PRLR, GHR and different G proteins. While PRLR mRNA has not been detected in the ventral skin transcriptome of *E. spinax*, the presence of a partial GHR mRNA (MK923748) was highlighted (Supplementary data S1, S6). Sequences corresponding to Gαs (MK923749), Gαi (MK923750), Gαo (MK923751) and Gαt (MK923752) were found in the *E. spinax* transcriptome (Supplementary data S2). Reciprocal BLASTx top hits matched with orthologous sequences from phylogenetically close Chondrichthyes, i.e. *Chiloscyllium punctatum*, *C. milii*, *Aptychotrema vincentiana* (Supplementary data S2).

3.2. Phylogenetic analyses of Es-MCR and Es-MTNR

Phylogeny reconstructions validated the inclusion of Es-MTNR-like, Es-MC3R and Es-MCR-like to MTNR and MCR types. Predicted chondrichthyan MTNRs are clustered between the MTNR1A-MTNR1B group and the MTNR1C group (Fig. 2a). Predicted MCRs retrieved from the ventral skin transcriptome of *E. spinax* appear to be an MC3R-like (Fig. 2b) and an MC1R-like (above mentioned as Es-MCR-like; data not shown). For both receptors, clustering is relevant according to the sequence identity with other reference sequences from other elasmobranch species (*C. milii*, *R. typus* and *S. acanthias*).

3.3. Location of MTNR, MCR and associated G proteins within the photogenic tegument of E. spinax and E. molleri

Cryosections through photophores allowed to visualize the general morphology of photophores (Fig. 1c). As depicted by Claes and Mallefet (2008) and Duchatelet et al. (2019a), autofluorescence of photocytes is visible under UV excitation (Fig. 1d). IHF experiments allowed to localize the two main GPCR involved in the light emission regulation and the two associated G protein within the tissue. For both lanternshark species, MTNRs are mainly labelled in the cell membrane of the ILS cells and around the lens cells (Figs. 3a; 4a), while MCRs are precisely immunodetected in the cell membrane of the photocytes and the lower part of the ILS cells (Figs. 3c; 4c). Co-localization of MTNR and Gαi protein (Figs. 3a, b; 4a, b), as well as of MCR and Gαs protein (Figs. 3c, d; 4c, d), is strongly supported. All these proteins are expressed within the photogenic organs, the photophores (Figs. 3; 4). Gαo protein immunoreactivity was observed in and around the lens of the light organ for all the tested sections (Figs. 3e; 4e). Finally, immunodetection using the Gαt antibody showed no labelling at the photophores (Figs. 3f; 4f). Gαt, also called transducin, a protein typically found associated with visual opsins, is mainly expressed in the shark retina (Supplementary data S7). Controls with the omission of the primary antibody do not show any staining (Supplementary data S7). Interestingly, the IHF gave the same results for both *E. spinax* and *E. molleri* lanternshark light organs (Figs. 3 and 4).

4. Discussion

Although several studies focused on the lanternshark bioluminescence hormonal control (Claes and Mallefet, 2009a, c; Claes et al., 2010b, 2011a; Duchatelet et al., 2019c, 2020), the presence of associated hormonal receptors (i.e. MTNR, MCR, PRLR) within the photophore cells has not yet been investigated. Since these hormone receptors (except PRLR) are members of GPCRs, G proteins were also investigated in parallel.

Our analyses reveal the absence of predicted PRLR mRNA sequence from the *E. spinax* transcriptome which is consistent with previous studies demonstrating the secondary loss of PRLR from cartilaginous fishes during GHR cluster evolution (Daza and Larhammar, 2018). Although physiological studies using heterologous PRL administration described an effect on the pigment motion in the smooth backed river stingray, *Pomatotrygon reticulatus* (Visconti et al., 1999), and on the light emission of various luminous sharks such as *E. spinax, E. molleri, Squaliolus aliae* (Claes and Mallefet, 2009a, c, 2015; Claes et al., 2012; Duchatelet et al., 2019c), there is no report of the identification of PRL or PRLR in Chondrichthyes (Anderson, 2016). Besides, affinity studies demonstrate that the human PRL does not bind to the human GHR, but GH binds to both the human GHR and PRLR (Cunningham et al., 1990; Sommers et al., 1994). Therefore, the absence of classical PRLR in the lanternshark skin raise questions on how PRL could cellularly be perceived by photophore to trigger light emission as demonstrated by Claes and Mallefet (2009a, c).

Transcriptomic and phylogenetic analyses highlighted the evolutionary conservation of the MTNR and MCR within the elasmobranch lineage. Our *in silico* analyses highlighted (*i*) the presence of a unique Es-MTNR-like (MK923747) and (*ii*) the presence of Es-MC1R-like and Es-MC3R (MK923746, MK923745) in the velvet belly lanternshark transcriptome. Previous studies on *Xenopus* melanophores highlighted a specific high-affinity of MT for the receptor MTNR1C (Ebisawa et al., 1994), which activates Gi protein, leading to a down-regulation of adenylate cyclase and cAMP-dependent protein kinase (White et al., 1987). According to Duchatelet et al. (2020), α-MSH as well as ACTH, two members of the melanocortin protein family, act to decrease the amount of light emitted. In vertebrates, each melanocortin protein displays a preferential affinity for an MCR (1–5); i.e. α-MSH preferentially targets MC1R, MC3R, MC4R, and MC5R, while ACTH has a higher affinity for MC2R, MC3R and MC4R (Olney et al., 2014; Butler et al., 2017). The presence of at least two predicted MCR in *E. spinax*, Es-MC1R and Es-MC3R, is consistent with our previous study on the inhibitory effects of both α-MSH and ACTH (Claes and Mallefet, 2009a,

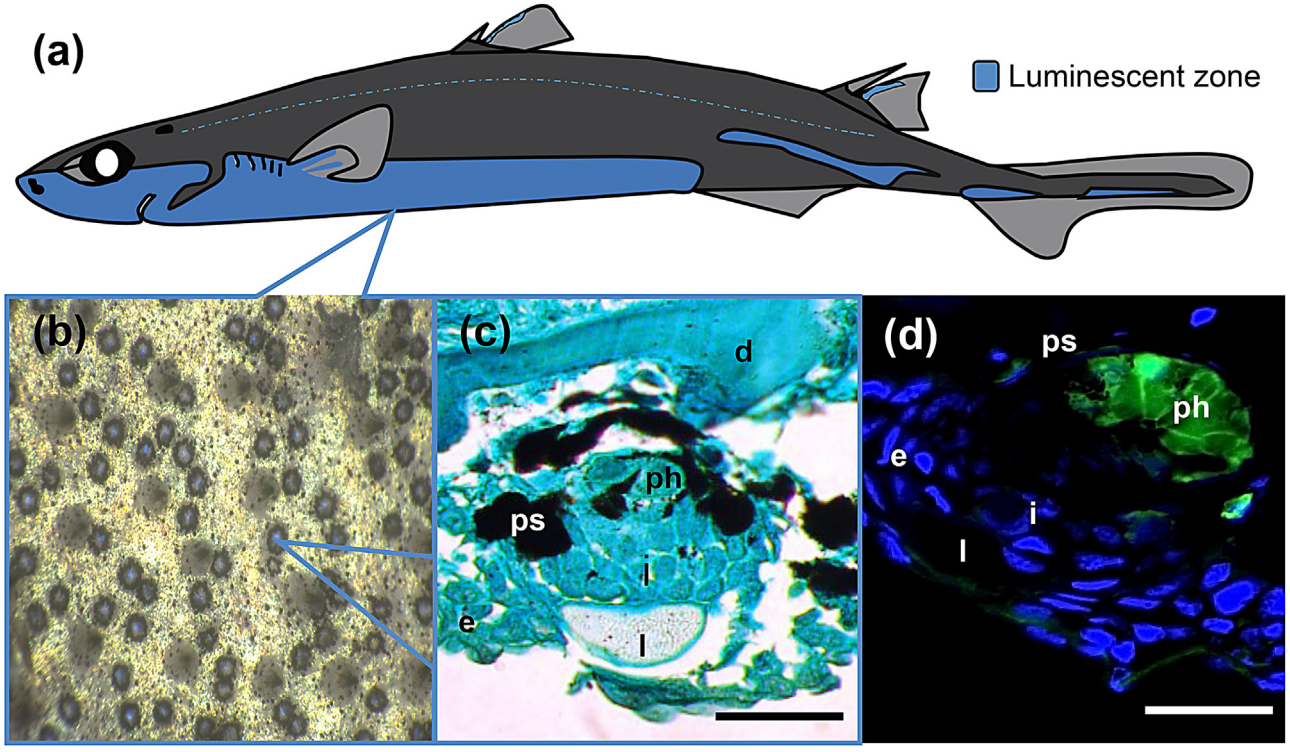

Fig. 1. The light organ of *Etmopterus spinax*. (a). Schematic view of the luminescent zones. (b). Ventral luminous area showing the photophores. (c, d). Histological section across a photophore. (d). Green autofluorescence of photocytes with nuclear DAPI blue staining on control cryosection without primary antibody. d, denticle in formation; e, epidermis; i, iris-like structure cells; l, lens cell; ph, photocytes; ps, pigmented sheath. Scale bar: 50 μm.

c; Duchatelet et al., 2020). Also, the presence and the respective melanocortin affinity of those receptors could explain the variation in term of pigment motion between skin treated with either α-MSH or ACTH [i.e. α-MSH darkens both skin melanophores and ILS-melanophores, while ACTH acts only on ILS-melanophore pigment motion (Duchatelet et al., 2020)].

The present study shows that both MTNR and MCR receptors are expressed and co-localized within the light organs of the two bioluminescent sharks, *E. spinax* and *E. molleri.* More precisely, co-expression occurs in the ILS cell membranes. Confidence on the IHF labelling is obtained via similarities of protein expression between the two studied lanternshark (Figs. 3 and 4) as well as sequence similarities between the target protein and epitope-recognized antibodies (Supplementary data S4) and the negative control with primary antibody omission (Supplementary data S7). Moreover, IHF labelling results are consistent with previous studies highlighting narrow link between MTNR and Gi protein as well as MCR and Gs protein (Potenza and Lerner, 1992; Visconti et al., 1999; Aspengren et al., 2003; van der Salm et al., 2005;

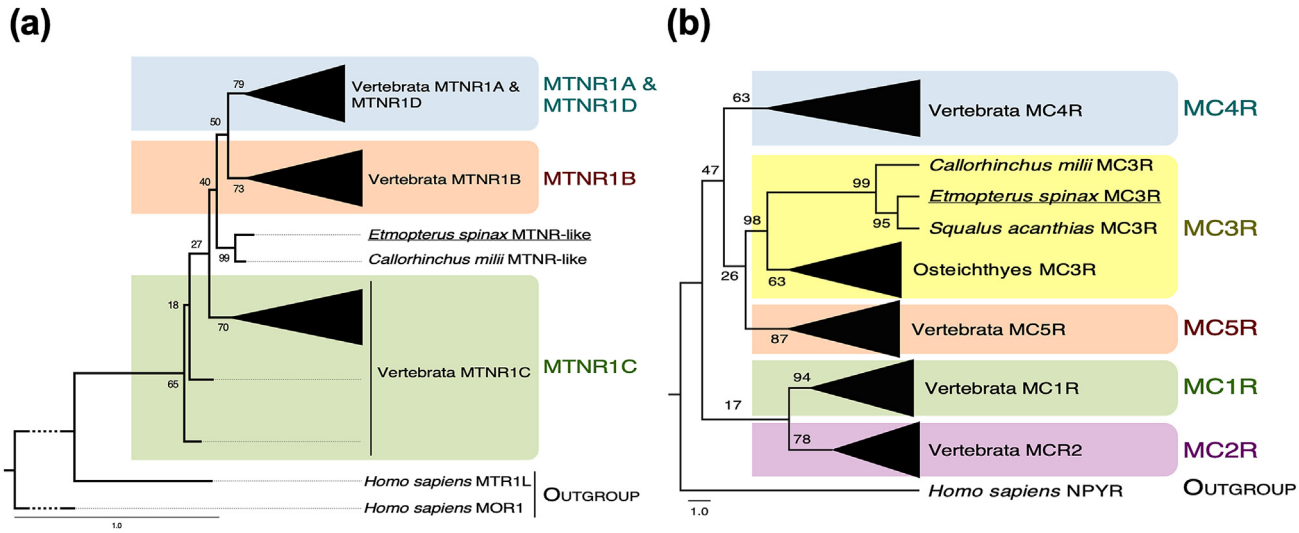

Fig. 2. Metazoan melatonin and melanocortin receptors phylogenetic trees including the *Etmopterus spinax* amino acid sequences. Maximum likelihood tree based on an amino acid sequence alignment of (a) melatonin receptors (MTNR) and (b) melanocortin receptor (MCR). Tree is calculated by PhyML using the JTT + G + F model of evolution. SPR setting was used for tree optimization. Numbers at the nodes indicate bootstrap percentages based on 1000 replicates. The scale bar represents the percentage of amino acid substitutions per site. The μ-type opioid receptor (MOR1) and the melatonin-related receptor (MTR1L) sequences from *Homo sapiens* and the neuropeptide Y receptor (NPYR) sequence from *Homo sapiens* were used to root the tree of MTNR and MCR, respectively.

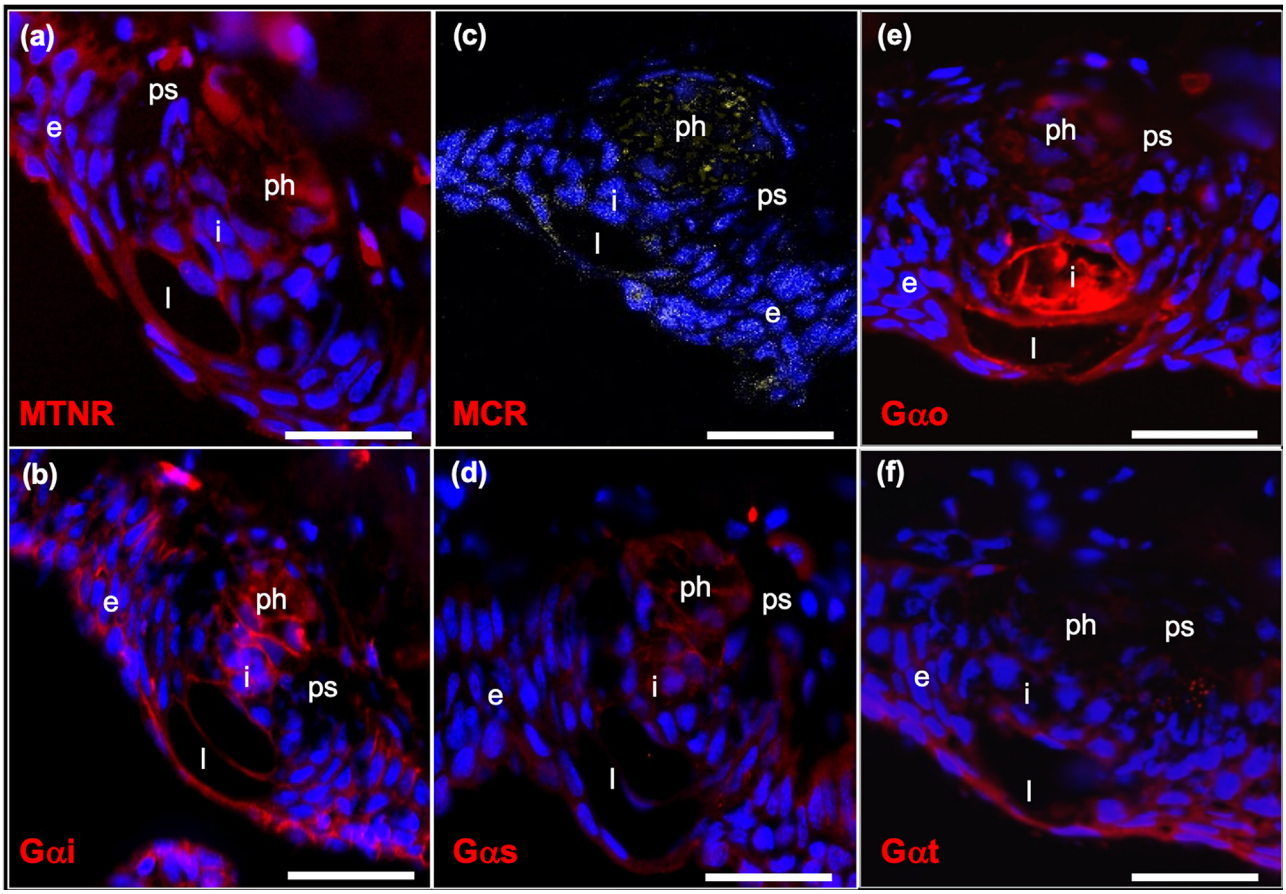

Fig. 3. Immunodetections of melatonin receptor (MTNR), melanocortin receptor (MCR), G protein αi, s, o and t subunit within the photophores of *Etmopterus spinax*. (a) Immunohistofluorescence (IHF) staining of the photophore obtained with the anti-MTNR antibody; (b) with the anti-Gαi subunit antibody. A similar staining is observed in the cell membrane of ILS cells, lens cell and photocytes. (c) IHF staining of the photophore obtained with the anti-MCR antibody; (d) with the anti-Gαs subunit antibody. A clear co-localization of MCR and Gαs subunit staining is observed within the cell membrane of the photocytes and the ILS cells. (e) IHF staining obtained with anti-Gαo subunit antibody. A strong lens immunoreactivity is observed; (f) IHF staining obtained with anti-Gαt subunit antibody showing a weak immunoreactivity of the first layer of epidermis. Nuclear blue DAPI staining was applied for all sections; secondary antibody coupled with red fluorochrome was used for all sections except for MCR labelling where a yellow fluorochrome coupled secondary antibody was used. e, epidermis; i, iris-like structure cells; l, lens cell; ph, photocytes; ps, pigmented sheath. Scale bar: 50 μm.

Tuma and Gelfand, 1999; Sköld et al., 2013).

Immunohistofluorescence results support Duchatelet et al. (2020), on a dual role of MT and α-MSH/ACTH on the light emission and ILS shutter organ control since both receptors are present at photocytes and ILS cell level. Studies about MTNR implication in physiological colour change show that this receptor is mainly coupled with a Gi protein that inhibits the cyclic adenosine monophosphate (cAMP) formation and triggers a skin lightening (Aspengren et al., 2003; Tuma and Gelfand, 1999; Sköld et al., 2013). In other studies (Potenza and Lerner, 1992; Visconti et al., 1999; van der Salm et al., 2005), MCR receptor was shown to be involved in the skin pigment movement. Indeed, according to these studies, this GPCR is mainly coupled with a Gs protein that stimulated the adenylate cyclase activity and refines trigger an increase of cAMP. Claes and Mallefet (2009c) already assumed the role of cAMP on the light emission. More recently, Duchatelet et al. (2020), demonstrated the modulation of cAMP intra-photophore concentration according to the variation of the light emission in *E. spinax* and *E. molleri* (i.e. MT triggering light emission and decreasing cAMP concentration, while α-MSH/ACTH inhibit luminescence and seems to increase cAMP concentration). All our results coincide with these previous studies since MTNR-Gi and MCR-Gs are co-localized within the light organ, respectively. Delroisse et al. (2018) and Duchatelet et al. (2019a) also demonstrated the mRNA sequence presence, ontogenic apparition and co-localization of an extraocular opsin, encephalopsin (Es-Opn3), with the light organ (i.e. at the ILS cell level). Encephalopsins (Opn3) are described as GPCRs able to detect blue light wavelength in *Danio rerio* (Sugihara et al., 2016), *H. sapiens* (Regazzetti et al., 2018), and other organisms (Koyanagi et al., 2013). The lanternshark opsin is therefore assumed to be able to perceive shark luminescence and potentially serves as a feedback regulator of the light emission process. The GABA neuromodulator, an inhibitor of lanternshark hormonally-triggered light emission, was also immunodetected at the level of the ILS cells (Claes et al., 2011a). Locating all these proteins in a single organ highlights a potential functional link between all these actors. Links can be found in the literature passing through photoreception and pigmentation regulation. Altogether, these proteins (MT, α-MSH, ACTH, GABA, Es-Opn3) are assumed to take part in both pathways involved in light emission control and ILS pigment motion regulation (Fig. 5). Work is in progress to reveal the presence of these receptors and actors at the cellular level (e.g. through immunocytology using electron microscope immunogold) as well as histochemical detection using Western Blot technics. Although not studied in the present work, insights in (i) the local action of the neuromodulators (NO and GABA) at the level of the photophore and/or surrounding cells; (ii) their potential release in the bloodstream surrounding the photophore could improve the understanding of the puzzled equation of light emission control in lanternsharks. Similarly, metabolic studies concerning the secretion and the signaling type (e.g. autocrine, paracrine or endocrine signaling, across gap junction or bloodstream) of the hormones and neuromodulators involved in the lanternshark luminescence are under consideration.

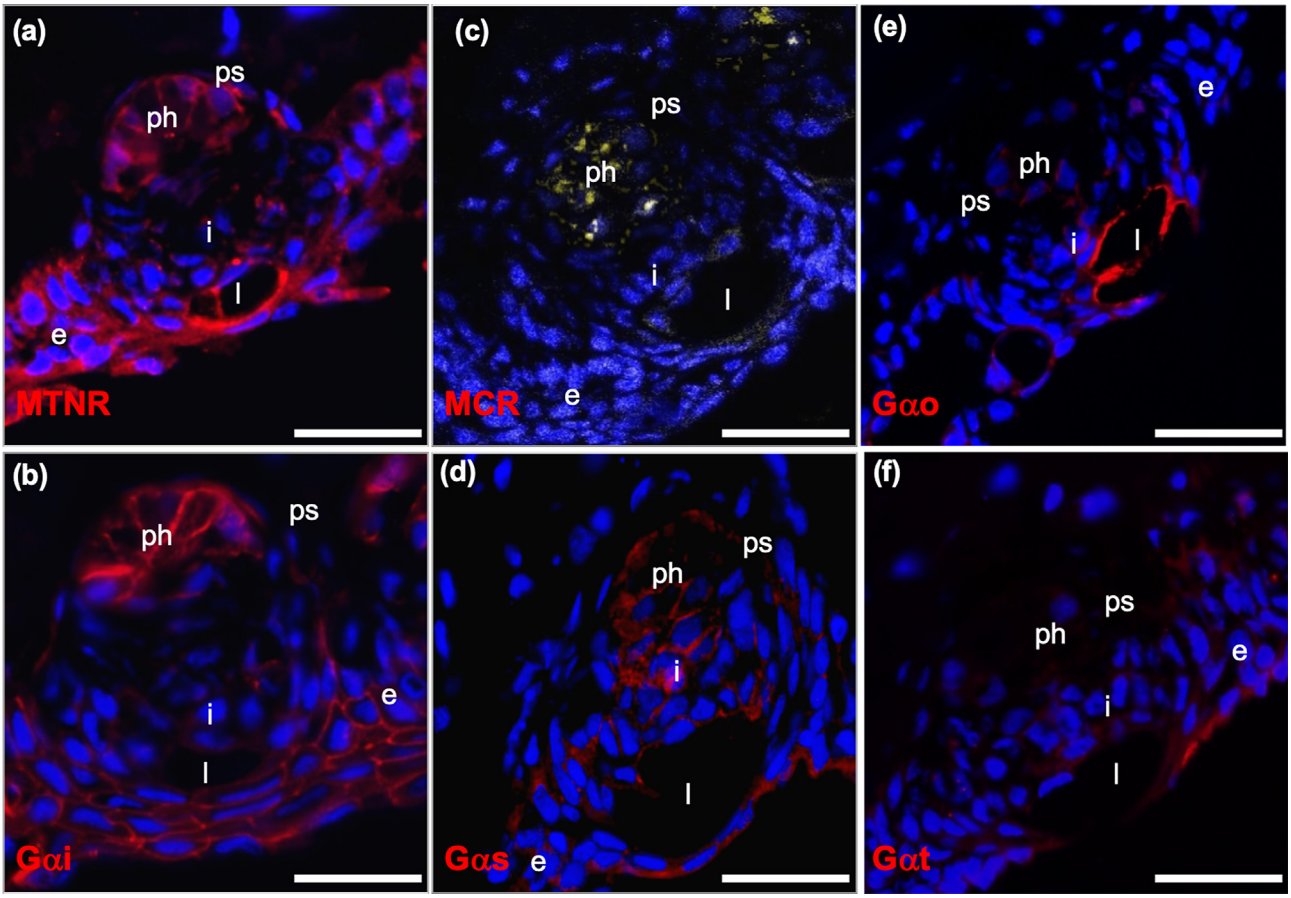

Fig. 4. Immunodetections of melatonin receptor (MTNR), melanocortin receptor (MCR), G protein αi, s, o and t subunit within the photophores of *Etmopterus molleri*. (a) Immunohistofluorescence (IHF) staining of the photophore obtained with the anti-MTNR antibody; (b) with the anti-Gαi subunit antibody. A similar signal is observed in the cell membrane of ILS cells, lens cell and photocytes. (c) IHF staining of the photophore obtained with the anti-MCR antibody; (d) with the anti-Gαs subunit antibody. A clear co-localization of MCR and Gαs subunit staining is observed within the cell membrane of the photocytes and the ILS cells. (e) IHF staining obtained with anti-Gαo subunit antibody. A strong lens immunoreactivity is observed; (f) IHF staining obtained with anti-Gαt subunit antibody showing a weak immunoreactivity of the first layer of epidermis. Nuclear blue DAPI staining was applied for all sections; secondary antibody coupled with red fluorochrome was used for all sections except for MCR labelling where a yellow fluorochrome coupled secondary antibody was used. e, epidermis; i, iris-like structure cells; l, lens cell; ph, photocytes; ps, pigmented sheath. Scale bar: 50 μm.

As developed by Duchatelet et al. (2020), regulation of skin pigment motion could have been co-opted from skin melanophore pigment motion involved in the countershading camouflage to precisely regulate light output through ILS cell shutter-like action and refine counter-illumination function for lanternsharks during evolution.

CRediT authorship contribution statement

Laurent Duchatelet: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing - original draft. **Jérôme Delroisse:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - review & editing. **Jérôme Mallefet:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - review & editing.

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Declaration of interest

None.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures

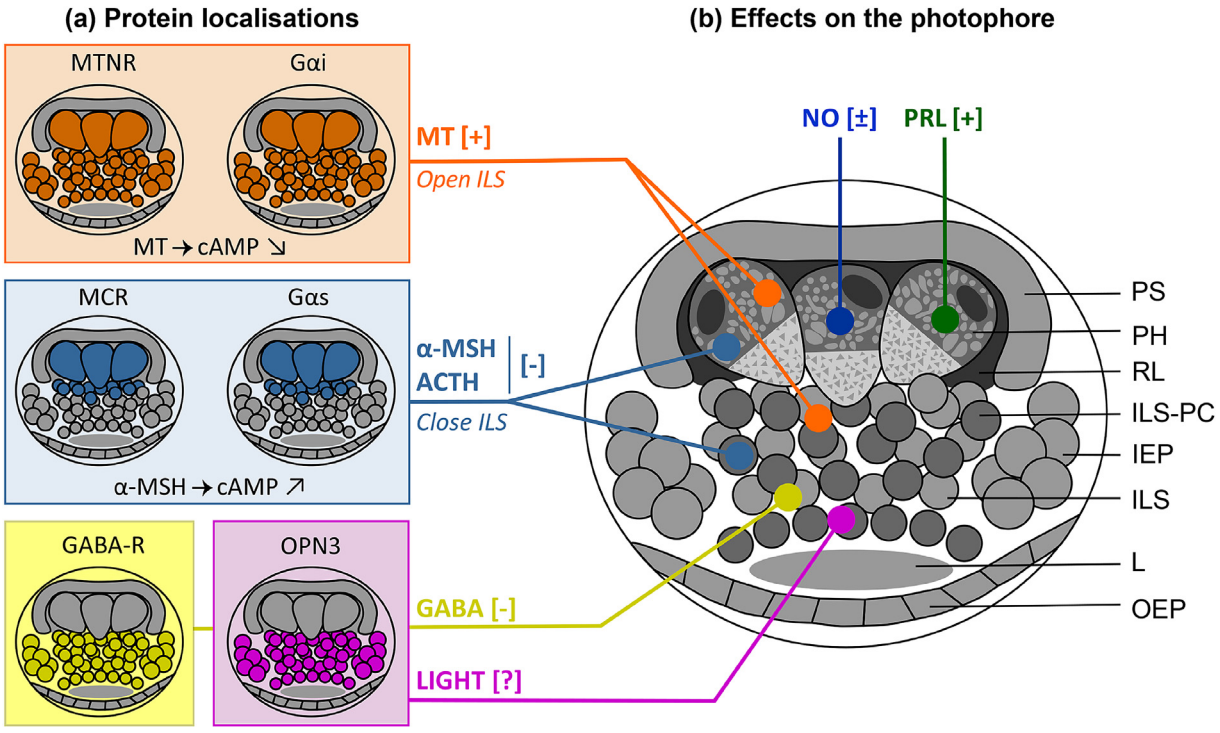

Fig. 5. Schematic view of localization and control of known actors involved in bioluminescence in lanternsharks. (a) Localizations of the different GPCR involved in the light emission control and known implication on the intracellular cAMP concentration level. (b) Effects of the known GPCR ligands on the light emission control at the photophore level. [+] activator, [±] modulator, [-] inhibitor and [?] unknown effects on the light emission. IEP, inner epidermis layer; ILS, iris-like structure cells; ILS-PC, iris-like structure pigmented cells; L, lens cell; OEP, outer epidermis layer; PH, photocytes; PS, pigmented sheath; RL, reflective layer.

performed in studies involving animals were per under the ethical standards of the institution or practice at which the studies were conducted. Animal procedures were conducted in compliance with the Belgian national guidelines and in agreement with the European directive 2010/63/UE, under the approval of the Animal Ethics Committee of the Université catholique de Louvain in Louvain-la-Neuve. This article does not contain any studies with human participants performed by any of the authors.

Data accessibility

Data not available/Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2020.113488.

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