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Identification and Characterization of Main Genetic Components Involved in Phototransduction and Vision of the Cubozoan Jellyfish *Tripedalia Cystophora*

Identifikace a charakterizace hlavních genetických komponent účastnících se fototransdukce a zrakové percepce u medúzy čtyřhranky *Tripedalia cystosphora*

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OBSAH/CONTENT

ABSTRAKT (ČESKY)

Mnoho živočichů vnímá světlo pomocí fotoreceptorů, obsahujících světločivný pigment, jehož základ tvoří protein opsin. Žahavci jsou bezesporu první živočišný kmen, u kterého můžeme nalézt dobře vyvinutý a komplexní zrakový systém (komplexní oči morfologicky podobné očím obratlovců). Evoluční historie fototransdukce a vzniku jednotlivých zrakových komponent (od světločivných opsinů po strukturální geny čoček) zůstává dodnes sporná. V této práci jsme se rozhodli k tématu přistoupit s využitím širokého multidisciplinárního přístupu, kombinujícího moderní metody molekulární biologie a bioinformatiky. Podrobná celogenomová analýza čtyřhranky trojité (*Tripedalia cystophora*), byla doplněna analýzami genové exprese, funkčními testy v buněčných kulturách a farmakogenetickým testováním (behaviorální testy).

Za prvé, v genomu byl odhalen překvapivě velký počet genů pro opsiny se zřetelnou tkáňově a vývojově specifickou expresí. Rozsáhlá fylogenetická analýza vedla k vymezení opsinů čtyřhranek jako sesterské větve k c-opsinům a ke zmapování expanze opsinů v této živočišné linii. Funkční testy v buněčných kulturách odhalily, že Gs-cAMP signalizace je typická pouze pro malou podskupinu opsinů a naznačily, že většina opsinů čtyřhranek signalizuje odlišnou a doposud neidentifikovanou kaskádou. Dále funkční testy odhalily jemné rozdíly mezi jednotlivými opsiny, což naznačuje možné "vylaďovaní" pro specifické úlohy daných fotoreceptorů. Analýzy exprese genů vedly k identifikaci dvou odlišných fotoreceptorů v sítnici *T. cystophora* a poukázaly tak na další úroveň komplexity jejích očí. Data, získaná z výzkumu exprese genů vedla také k odhalení zcela nových domén exprese opsinů. V neposlední řadě studium genomu vedlo k odhalení dalších fototransdukčních komponent (připomínajících fototransdukční kaskádu obratlovců) a nových strukturních proteinů čoček.

ABSTRACT (ENGLISH)

Many of the metazoan phyla sense light by an opsin-based photopigment present in a photosensitive receptor cell (photoreceptor), with Cnidaria being arguably the earliest branching phylum containing a well-developed and complex visual system (advanced eyes morphologically similar to those of vertebrate). The evolutionary history of phototransduction and visual components (ranging from light-sensing opsins to structural genes of the lenses) is a long standing question. In this work, we decided to address this issue by applying a comprehensive multidisciplinary approach combining modern molecular biology methods with bioinformatics. Comprehensive genome-wide inspection of a cubozoan jellyfish *Tripedalia cystophora*, was complemented with gene expression analyses, together with functional (cell culture based assays) and behavioural (pharmacogenetics) testing.

First, genome analysis uncovered the presence of a surprisingly large number of opsin genes with distinct tissue- and stage-specific expression. Our extensive phylogenetic analysis classified cubozoan opsins as a sister group to c-type opsins and documented a lineage-specific expansion of opsin gene repertoire. Functional tests in cell cultures provided evidence for the use of Gs-cAMP signalling pathway only in a small subset of opsins, indicating that the majority of cubozoan opsins likely signal by a distinct, yet unidentified pathway. In addition, these functional tests uncovered subtle differences among individual cubozoan opsins, suggesting a possible fine-tuning for specific photoreceptor tasks. The opsin expression data led to identification of two distinct photoreceptors in the retinas of *T. cystophora*, revealing yet another level of complexity of cubozoan advanced eyes. Furthermore, novel opsin expression domains were documented for the first time. Finally, genome analysis revealed the presence of vertebrate-like phototransduction cascade components, together with additional structural proteins of the lenses.

ENGLISH SECTION

INTRODUCTION

Many of the metazoan species sense light for vision and nonvisual photoreceptions. The importance of the ability to detect spatial differences in ambient light levels could be documented by the fact that 96% of the known species alive today possess image-forming eyes (Land and Fernald 1992). A common indispensable basis of all animal eyes are the photoreceptor cells, containing a photopigment connected to a downstream phototransduction cascade. Based on their morphology they could be classified into rhabdomeric or ciliary photoreceptors. Rhabdomeric photoreceptors bear visual pigments in the membrane protrusions as a part of the apical cell surface, while the ciliary photoreceptors fold the membrane of the cilium (Arendt 2003). On the molecular level, both types of photoreceptors use a vitamin-A-based light sensitive photopigment, comprising of a chromophore retinal and of the apoprotein opsin.

The process of phototransduction activation always requires the binding of a photoactivated opsin to the corresponding G alpha subunit of a trimeric G protein (Kuhn et al. 1981), as well as the subsequent deactivation of the involved cascade by e.g. rhodopsin kinase, which phosphorylates the photoactivated opsin, or by arrestin which competes with the G alpha subunit of the corresponding G protein for binding to opsin (Krupnick et al. 1997).

The protein moiety of the photopigments are opsins. They are members of the G protein coupled receptor (GPCR) superfamily of proteins, with seven transmembrane helices that are involved in a diverse set of signalling functions. Opsins have been discovered in a wide variety of tissues and cell types, where they also serve other functions than image formation (Terakita 2005). Consistent differences in the structure between photoreceptors, as well as in the corresponding opsins primary sequences between vertebrates and invertebrates led to the conceptual division of opsins into two distinct classes: rhabdomeric type - r-opsin and ciliary type - c- opsins (Arendt and Wittbrodt 2001). Four major monophyletic subgroups of opsins can be recognized, namely the c-type, the cnidopsins, the r-type and group 4 opsins (Terakita 2005).

Cubozoa (box jellyfish) belong to the phylum Cnidaria, probably the earliest branching phylum containing a well-developed visual system. Their phylogenetic position, simple nervous system and elaborate set of many eyes (Nilsson et al. 2005) render their visual system important for understanding the early evolution of vision as well as the basic biology of box jellyfish (Garm et al. 2011). Surprisingly, eyes of box jellyfish share many features with those of vertebrates. Morphologically, by the overall design comprising ciliary photoreceptors, retina, lens (Nilsson et al. 2005) and based on recent characterisation of some of the molecular components, it was suggested that the box jellyfish visual system could be more closely related to vertebrate than to that of invertebrates (Piatigorsky and Kozmik 2004, Kozmik et al. 2008a). The box jellyfish investigated in our study, *Tripedalia cystophora* (Conant 1897), has four equally spaced rhopalia (sensory structures), hanging from stalks and situated within open cavities surrounding the

bell. Each of the rhopalia bears six separate eyes. The lens containing eyes have sophisticated visual optics (lenses created by multiple crystallin proteins) as do molluscs and vertebrates (Nilsson et al.2005).

MOTIVATION AND AIMS OF THE STUDY

Vision is one of the most crucial senses in many animals and perhaps the most important sense for humans. Eye morphogenesis and phototransduction have been studied for a long time, however the function of involved genes began to be elucidated in the last two decades. One of the most striking findings over the past few years is the discovery of vertebrate-like components (e.g. ciliary-type opsin) in the phototransduction of the non-vertebrate cnidarian box jellyfish, *T. cystophora.*

The aim of this study is the identification and characterization of the main genetic components of *T. cystophora* phototransduction cascade and vision, with the main focus on opsins, and inspection of the putative similarities to phototransduction of other species, particularly vertebrates. Elucidation of the biological role and function of *T. cystophora* opsins and other phototransduction genes used for its "pioneer vision" will enhance our knowledge of specific aspects of eye and phototransduction evolution. Last but not least this study aims to identify and characterize novel cnidarian opsin sequences as a potential source for novel optogenetic tools development.

To solve these questions a comprehensive multidisciplinary approach combining modern molecular biology methods with bioinformatics was applied.

The aims could be summarized as follows:

1. Identification and cloning of *T. cystophora* opsin genes
2. Sequence analysis of *T. cystophora* opsin genes
3. Phylogenetic analysis of *T. cystophora* opsin genes
4. Analysis of *T. cystophora* opsin genes expression patterns and dynamics
5. Identification and functional testing of a possible *T. cystophora* opsin coupling partner
6. Behavioural testing of *T. cystophora* visual navigation ability after treatments with pharmacological inhibitors of the opsin´s coupling partner
7. Identification of other possible phototransduction cascade components by *T. cystophora* genome analysis *in silico*, complemented with immunohistochemical screening with commercially available antibodies *in situ*
8. Identification of novel crystallin genes in the sequenced *T. cystophora* genomic library

MATERIAL AND METHODS

In this work, a comprehensive multidisciplinary approach combining modern molecular biology methods with bioinformatics was applied. Comprehensive genome-wide inspection of a cubozoan jellyfish *T. cystophora*, was complemented with characterization and cloning of the identified genes, gene

expression analyses by immunohistochemistry and an extensive qRT-PCR, together with functional (cell culture based light-response assay) and behavioural (pharmacogenetics) testing.

RESULTS

Identification, cloning and sequence analysis of *T. cystophora* opsin genes

In addition to the previously annotated *T. cystophora* c-opsin (Kozmik et al. 2008a) another seventeen opsin sequences (named Tcop1-17 - JQ968416-JQ968432) were identified in the *T. cystophora* genomic library. All of the seventeen novel opsins are intronless genes, showing overall sequence homologies to other cnidarian opsins as well as to bilaterian rhodopsins. Next, we focused on the identity of the most important amino acid residues and the putative G protein binding tripeptide (Marin et al. 2000) of the cnidarian opsins. The tripeptides have shown to be conserved between closely related opsin branches within each species but are apparently not conserved between the species across cnidarian lineages. Furthermore, Tcop1 tripeptide was found to be identical with vertebrate rhodopsin NKQ motive.

Phylogenetic analysis of *T. cystophora* opsin genes

Our phylogenetic analysis of a large and diverse set of 779 opsin sequences recovered the four major clades described in earlier studies (e.g. Suga et al. 2008, Porter et al. 2012) – the c-type opsins, cnidopsins, r-type opsins and group 4 opsins. The relationship between cnidopsins and the c-type opsin subfamily had the strongest support. All *T. cystophora* opsins (Tcops) fell into the cnidopsins subfamily, clustering with the hydrozoan opsins, which was consistent with the relationship among cnidarian classes. In the phylogenetic tree Tcops clearly fell into two distinct sub-groups: Tc-group-1 and Tc-group-2.

Analysis of *T. cystophora* opsin genes expression patterns and dynamics

To inspect the expression patterns of Tcops, qRT-PCR analysis on mRNA isolated from different jellyfish life stages and various adult tissues was performed. For the majority of Tcops, rhopalium was the tissue with the highest expression detected. Other Tcops shown to be mainly expressed in male gonads, manumbrium or tentacles. Two consistent features were revealed in the results. First, Tcops with highest expression in the adult rhopalium, significantly increased their expression during the metamorphosis into medusa stage (when the rhopalia emerge and develop). Second, many group-1 Tcops were highly expressed in the larval stage, in contrast to group-2 Tcops (established as Gs-coupled receptors with a major role in adult lens-containing eyes), whose expression was absent at this stage.

To gain further insight into the possibly diverse roles of group-1 and group-2 Tcops in *T. cystophora* eyes, we analysed expression of key representatives of each of the Tcops sub-group by immunohistochemical (IHC) staining of the rhopalia cryosections *in situ*. According to analysis of the immunolabelled rhopalia sections, *T. cystophora* retinas contain at least two morphologically distinct photoreceptor cell types: ciliary photoreceptor type-1 (expressing Tcop13) and photoreceptor type-2 (expressing Tcop18). Tcop18 was found to be expressed in the minor eyes (pit and slit eye) of *T. cystophora* and is the only known opsin to be expressed in the lesser eyes so far.

Identification and functional testing of the possible *T. cystophora* opsins coupling partner

In silico search for G protein alpha subunits in the sequenced *T. cystophora* genomic library resulted in identification of four putative genes, representing three distinct G alpha subunit guanine nucleotide binding protein alpha (GNA) subfamilies. We performed cross-species IHC staining of the rhopalia sections with commercially available anti-GNA antibodies and the only detectable signal was obtained for the Gs alpha subunit in the retinas of major and minor *T. cystophora* eyes.

In order to get a deeper insight into the functional diversification of opsins identified in *T. cystophora* we used a Gs protein-coupled signalling assay (light-response assay) to investigate their biochemical properties. The activity of luciferase in GloSensor cAMP HEK293 cells, transfected with individual opsin constructs, was determined before and after repeated light stimulations. The light-response assay was performed for the entire set of Tcops. Only Tcop5 and Tcop13 (both from group-2) activated the Gs-cAMP signalling pathway.

To investigate the role of the tripeptide (putative contact between opsin and G protein) in cnidopsin signalling, we replaced this tripeptide region in Tcop13 with tripeptides of Tcop1, Tcop14, and Tcop18 (all goup-1 Tcops; none of these Tcops activated the Gs signalling cascade in the light-response assay by itself). Surprisingly, the mutation of Tcop13 tripeptide did not disrupt Gs activation (as expected), but rather modulated the response of Tcop13 to the light stimulation.

Behavioural testing of *T. cystophora* visual navigation after pharmacological inhibition

To investigate whether group-2 Tcops (signal via Gs) serve as the main visual pigments, we performed a behavioural assay focused on the positively phototactic behaviour of the medusae in the absence or presence of pharmacological inhibitors. Positive phototaxis in *T. cystophora* was significantly decreased after treatment with Gs signalling inhibitor.

Identification of other putative phototransduction cascade components

We screened the sequenced *T. cystophora* genomic library for seven other candidate phototransduction components previously identified as a part of the vertebrate phototransduction cascade (or involved in photopigment regeneration) and combined this *in silico* approach with *in situ* data by parallel IHC staining on rhopalia cryosections with some of the commercially available antibodies. We identified arrestin-like protein with high similarity to vertebrate visual arrestins and inspected the possible expression pattern by using anti-bovine visual-arrestin antibody and indeed obtained a strong signal in the retinas of *T. cystophora* major lens eyes. Arrestin is the first of the phototransduction deactivation components identified in cnidarian eyes so far.

Identification of novel crystallins in the *T. cystophora* genomic library

We screened the *T. cystophora* genomic database for the structural proteins of the lenses – crystallins. Three putative crystallin genes, namely J1D, J1E and J2B, were identified and isolated. The novel

crystallins strongly cluster with the previously reported and annotated sequences in *T. cystophora* – J1 and J2 respectively, whose expression was confirmed in the lenses (Piatigorsky et al. 1993).

DISCUSSION

Scenario for cnidarian opsins rapid expansion and retro-gene origin

Spectrally rich and diverse aquatic environment provides strong selective pressures on photoreception evolution, as shown in fish (Hofmann et al. 2012, Cortesi et al. 2015), where the opsin gene diversity in the genome is similarly high as in the genome of *T. cystophora*. The large complement of opsins found in freely swimming species seems to be a result of sensory adaptation to this spectrally diverse environment.

Based on our data, confirming once again the clustering of cnidopsins as sister clade to c-opsins, we conclude that cnidarian intronless opsin genes are derived via retrotransposition from an ancient eumetazoan ciliary opsin with introns. Once the intronless variant appeared in the genome, it was subjected to rapid duplications across cnidarian lineages, followed by subsequent species-specific duplications resulting in the present day diversity of cnidopsins, with modified sub-functions and spatio-temporal expression within the individual animal. This diversity probably provided a substrate for the evolution of cnidarian photoreception.

Rhopalia-specific opsin expression in *T. cystophora*

In addition to the photoreceptors within the retinas of all the *T. cystophora* eyes, each rhopalium accommodates over a thousand various neurons with approximately half of them being retina-associated (Parkefelt et al. 2005, Skogh 2006). Based on our mRNA and protein expression profiles of *T. cystophora* opsins, most of the Tcops identified are indeed expressed in rhopalia. Our data suggest that all group-2 Tcops and at least one Tcop from each of the group-1 sub-groups (group-1A and 1B) are rhopalium-specific. The expression of rhopalium-specific Tcops goes far beyond the expression in the retinas of the eyes, as many of retina-associated neurons already proved to be photosensitive as well (Garm and Mori 2009). We thus confirmed that the rhopalium is a complex organ, which serves for integration and processing of diverse light cues (enabled by the diverse set of opsins) and transformation of these signals into various behavioural responses.

Retina-specific opsin expression in *T. cystophora*

Our IHC analysis revealed the presence of at least two types of photoreceptors (each expressing distinct opsin) in both of the lens eyes of *T. cystophora*. According to our data Tcop13 serves as the main visual opsin in *T. cystophora* major lens eyes. Moreover, Tcop18 is the only known opsin to be expressed in the minor (pit and slit) eyes so far. Our IHC data indicate, that retinas of both eye types (major with lenses and minor) express different Tcops combinations according to their task, providing another level for visual tuning.

Larval and tissue-specific opsins

Our gene expression analyses imply that cnidarians indeed utilize opsins extensively, not only for visual but also for extraocular photosensitivity. By analysing all the Tcops expression in various body parts and live stages by qRT-PCR and with clear support from the phylogenetic data, it was revealed that all the Tcops could be classified into two subgroups. Group-2 Tcops, all of which are rhopalia specific with Tcop13 as the main visual opsin for the major lens eyes, and group-1 Tcops. The group-1 Tcops, being probably more ancient, have broad expression ranging from larvae to male gonads, however a trend for specialization and increased tissue or organ specificity could be observed in the 1A and 1B subgroups, being in agreement with the proposed scenario of subfunctionalization after subsequent duplication events. Expression data from adult tissues identified common sites of expression (likely reflecting a common gene origin), yet a clear tendency for specialization is apparent, as huge differences in expression levels and unique sites of expression were identified. For the first time, we identified opsins being expressed in the cubozoan planula larvae.

Phototransduction by cubozoan opsins

Our cell culture based light-response assay revealed that the Gs-cAMP signalling pathway is used only by a small set of Tcops and the majority of cubozoan opsins likely signal by a distinct pathway. Our behavioural assay confirmed that visually guided behaviour is impaired after Gs pharmacological inhibition, confirming the conclusion that Tcop13 is the main visual pigment.

From our data, we assume that variable sensitivity and bleaching properties of the individual Tcops depend on their primary amino acid sequence. Our data indicate that tripeptide mutation in cnidopsins might contribute to subtle tuning of the opsin response to light stimulation, rather than being determinative for the G alpha subunit coupling. These non-conventional properties and distinct characteristics of Tcops make these opsins valuable potential candidates for novel optogenetic tools development.

As aforementioned, some of the Tcops show some intriguing similarities to vertebrate visual opsins, this resemblance is intensified by some of the previously identified necessary intermediary vertebrate-like cascade components in *T. cystophora* (Kozmik et al.2008a). In agreement with this scenario is the presence of vertebrate-like visual arrestin in the eyes.

Novel lens crystallins

With addition of the three crystallins identified in this study, at least eight crystallin genes are present in *T. cystophora* genome. Since several variants of J1 and J2 crystallin genes were found in the genome, multiplication (most likely via DNA based duplications) might be perceived as an important step for the highly up-regulated lens-specific expression in the present-day lenses.

In summary, the case of *T. cystophora* provides an example where recruitment of crystallins for structural function, via gene sharing, was probably followed by gene duplication and subsequent partial separation of both functions, as clearly documented by multiplication within members of the two unrelated

groups of J1 and J2 crystallins. From this view gene sharing strategy seems to be universally applicable throughout the animal kingdom and probably represents a common evolutionary strategy, as is well documented by the example of lens history shared between Cubomedusae and other invertebrates and vertebrates (Piatigorsky et al. 1993, Kozmik et al. 2008b)

CONCLUSIONS

In this work we integrated approaches of phylogenetics, gene expression analyses, functional studies in cell cultures and behavioural pharmacogenetics to provide compelling evidence for the existence of multiple general- and visual- photosystems in cubozoan jellyfish *T. cystophora*. In addition, we identified seventeen novel non-conventional opsins with a potential to be co-opted in optogenetics.

The key conclusions of our study and my thesis, focused on identification and characterization of the genetic components necessary for cubozoan phototransduction and vision, are summarized as follows:

1. A surprisingly large number of functional opsin genes is present in the *T. cystophora* genome.

2. Some of these opsins show intriguing sequence similarities to vertebrate opsins.

3. Extensive phylogenetic analysis clearly classifies cubozoan opsins as a sister group to c-type opsins and documents lineage-specific expansion of the opsin gene repertoire in the cubozoan genome.

4. Detailed opsin expression analyses uncovered both redundancy and specialization in the use of the opsin gene repertoire. Multiple opsins with presumably similar molecular characteristics are apparently utilized in the same stage/tissue while a clear tendency to establish unique expression patterns exists both within the opsin subfamilies (group-1 and group-2) and between the two subfamilies.

5. There are at least two types of photoreceptors in the retinas of the major lens eyes of *T. cystophora*, expressing distinct opsins.

6. Gs type alpha subunit is expressed in the photoreceptors of the major and minor eyes and probably serves as a direct coupling partner at least for the main visual opsin (based on IHC data and functional tests in cell cultures), however most of the cubozoan opsins probably signal via distinct yet unidentified phototransduction cascade.

7. Pharmacological inhibition by Gs antagonist abrogates visual navigation in *T. cystophora in vivo*.

8. Vertebrate-like arrestin mediates phototransduction quenching in the eyes of *T. cystophora.*

9. The crystallin repertoire of *T. cystophora* is even larger than anticipated, as documented by identification of three novel crystalline genes.

ČESKÁ SEKCE

ÚVOD

Oko s čočkou se vyskytuje u překvapivě širokého spektra živočichů a představuje významné zdokonalení primitivního typu oka skládajícího se z receptorové buňky v těsné blízkosti buňky pigmentové. Zatímco obratlovci využívají fotoreceptorové buňky ciliárního typu, bezobratlí v naprosté většině využívají fotoreceptory rabdomerické (Arendt 2003). Rozdíl mezi těmito dvěma základními typy receptorů lze sledovat nejen v jejich morfologii, ale také na molekulární úrovni. Každý z obou typů receptorů má svou vlastní specifickou fototransdukční kaskádu. Společným znakem těchto kaskád je využití opsinu jako světločivného pigmentu. Navzdory společnému původu lze v primární struktuře opsinů nalézt specifické záměny aminokyselin, které určují příslušnost daného opisnu k dráze ciliární nebo rhabdomerické. Ostatní komponenty jednotlivých kaskád jsou obvykle také specifické pro daný typ fotoreceptorové buňky (Arendt 2003). Ciliární fototransdukční kaskáda v doposud zkoumaných případech využívá trimerní G protein s α-podjednotkou spadající do podskupiny GNAI (Gi), enzym fosfodiesterázu a cyklický GMP, zatímco rabdomerická kaskáda využívá výhradně G protein s α-podjednotkou patřící do podskupiny GNAQ (Gq) a enzym fosfolipázu C.

Velkým překvapením byl objev opsinu ciliárního typu u zástupce kmene žahavci, medúzy čtyhranky trojité *Tripedalia cystophora* (Kozmik et al. 2008a). Čtyřhranky žijí v mangrovových porostech karibských vod a lze je charakterizovat jako aktivně lovící predátory morfologicky přizpůsobené k rychlému pohybu. V každé ze čtyř stěn zvonu medúzy se nachází komplexní orgán nazývaný ropálium, který nese šest očí čtyř různých typů. Nejsložitější jsou dvě komplexní oči s čočkou a rohovkou velmi podobná komorovému oku obratlovců. Čočky těchto komplexních očí dokonce obsahují gradient refrakčního indexu, který vylepšuje optické vlastnosti tohoto oka (Nilsson et al. 2005). Ropálium dále obsahuje dva typy jednoduchých očí jamkového typu, z nichž každé je v ropáliu přítomno dvakrát. Každá jednotlivá medúza tedy nese celkem dvacet čtyři očí. Ukázalo se, že některé z genů *T. cystophora* kódujících komponenty fototransdukční kaskády (c-opsin) jsou podobné genům obratlovců (Kozmik 2008a). Je tedy možné, že kromě ciliárního opsinu by *T. cystophora* mohla při fototransdukci využívat také další z komponent typických pro fototransdukci obratlovců (Gi, fosfodiesteráza, guanylátcykláza, CNG kanál, atd.). Nedávný výzkum poukazuje na vysokou míru konzervace mnoha genů mezi žahavci a obratlovci. Vyvstává tedy otázka, zda evoluce očí probíhala u obou skupin paralelně anebo byl zakonzervován starobylý „oční program“ právě u žahavců a obratlovců. Problematika se dále komplikuje nedávným objevením odlišného typu zpracování světelné informace u blízce příbuzného druhu - čtyřhranky tichomořské (*Carybdea rastonii*) (Koyanagi 2008). U tohoto živočišného druhu byl popsán nový typ fototransdukční kaskády využívající také opsin ciliárního typu, avšak spřažený s α-podjednotkou G proteinu spadající do podskupiny GNAS (Gs) a využívající enzym adenylátcyklázu. V současné době tedy není zřejmé, zda v rámci kmene žahavci (Cnidaria) neexistují paralelně dva na sobě nezávislé fototransdukční systémy.

CÍLE PRÁCE

Cílem této vědecké práce je identifikace a charakterizace hlavních genetických komponent fototransdukční kaskády a zrakové percepce u čtyřhranky trojité (*T. cystophora*), a objasnění případné podobnosti s komponentami fototransdukční kaskády obratlovců. Objasnění biologické role a funkce opsinů a jiných fototransdukčních genů u tohoto druhu žahavce, rozšíří naše znalosti o specifických aspektech evoluce světločivných komponent a zraku. V neposlední řadě je cílem této práce identifikace a charakterizace nových opsinů žahavců jako potenciálního zdroje pro vývoj nástrojů využitelných v optogenetice.

Blíže specifikované cíle:

1. Identifikace a izolace opsinů *T. cystophora*
2. Sekvenční analýza opsinů
3. Fylogenetická analýza opsinů
4. Analýza exprese genů pro opsiny
5. Identifikace proteinu spřaženého v signalizační kaskádě s opsinem a ověření jeho funkce
6. Behavioralní testy fototaxe po inhibici fototransdukční kaskády farmakologickými sloučeninami
7. Identifikace dalších možných fototransdukčních komponent *in silico*, doplněné IHC skríninkem komerčně dostupnými protilátkami *in situ*
8. Identifikace strukturálních genů čoček (krystaliny) *in silico*

MATERIÁL A METODY

Pro splnění vytyčených cílů jsme použili multidisciplinární přístup s využitím mnoha různých experimentálních metod. Rozsáhlá analýza *in silico* osekvenovaného genomu *T. cystophora*, byla doplněna izolací příslušných genů, analýzou exprese těchto genů histochemickými metodami a rozsáhlou kvantitativní RT-PCR. Pro odhalení kooperace a návaznosti jednotlivých komponent bylo provedeno testování interakce nově identifikovaných opsinů s α-podjednotkou Gs jejich kotransfekcí v buněčné kultuře (light-response assay). Pro ověření kooperace opsinů s příslušným G proteinem na úrovni celého organismu byly využity komerčně dostupné farmakologické inhibitory, které umožňují rozlišit mezi signalizací zprostředkovanou Gs a Gi. Za tímto účelem byla vyvinuta jednoduchá aparatura pro behaviorální studie umožňující kvantifikovat změny ve vizuální odpovědi *T. cystophora* po přidání inhibitorů fototransdukční kaskády.

VÝSLEDKY

1. V genomu čtyřhranky je přítomen překvapivě vysoký počet opsinových genů (18 opsinů).
2. Některé z těchto opsinů svými sekvenčními motivy nápadně připomínají opsiny obratlovců.
3. Rozsáhlá fylogenetická analýza vedla k vymezení opsinů žahavců jako sesterské větve k c-opsinům a zdokumentovala expanzi opsinových genů v genomu *T. cystophora*. Podskupiny opsinů s podobnými

molekulárními charakteristikami se uplatňují ve stejných vývojových stádiích (případně tkáních), zároveň je zde však zjevná tendence k ustanovení unikátních expresních domén jak uvnitř obou podskupin (Tcop group-1, Tcop group-2) opsinů čtyřhranek, tak mezi nimi.

4. Sítnice velkých očí *T. cystophora* je tvořena minimálně dvěma typy fotoreceptorů, exprimujících různé opsiny.
5. α-podjednotka Gs je spřažena s hlavním zrakovým opsinem *T. cystophora* (IHC data a funkční testy v buněčné kultuře), zatímco naprostá většina opsinů pravděpodobně signalizuje doposud neidentifikovanou kaskádou.
7. Farmakologický inhibitor Gs signalizace zcela potlačuje fototaktické chování medúz.
8. Arrestinu podobný protein obratlovců zajišťuje zhášení fototrasdukční signalizace v očích *T. cystophora.*
9. Soubor krystalinů tvořících čočky velkých očí je ještě obsáhlejší než se předpokládalo, jak dokládá identifikace dalších tří krystalinových genů v genomu *T. cystophor*a.

ŽIVOTOPIS/CURRICULUM VITAE

Michaela Liegertova, M. Sc.

Date and place of birth: 23 January 1983 / Litoměřice, Czech Republic

Education:

2009 – present	Ph.D. degree at Charles University, Department of Cell and Developmental Biology
2007	Semester Study Abroad at University of Cape Town, Department of Zoology, S. Africa
2002 – 2009	M.Sc. degree in Biology and Chemistry, Faculty of Education at J. E. Purkinje University

Research and teaching experience:

2013 – to date:	Assistant lecturer at J. E. Purkinje University Courses: General Zoology, Model Animals in Molecular Biology
2009 – 2014	Laboratory of Transcriptional Regulation, Institute of Molecular Genetics of the Academy of Sciences, Czech Republic; supervisor: RNDr. Zbyněk Kozmik, CSc Thesis research topic: Identification and characterization of main genetic components involved in phototransduction and vision of the cubozoan jellyfish *Tripedalia cystophora*

Conferences:

2012 Euro Evo Devo Lisbon. Poster presentation: Liegertová M., Pergner J., Kozmiková I., Fabian P.: Multiple opsin genes in cubozoan jellyfish *T. cystophora*

2010 Euro Evo Devo Paris. Poster presentation: Liegertová, M., Kozmik, Z.: Characterization of phototransduction cascade components in cnidarian *Tripedalia cystophora*

Courses:

2016 Zebrafish genome editing workshop - 13th Transgenic Technology Meeting Prague, Czech Republic

2014 ELLS LearningLAB "Bioinformatics in the classroom – a complementary approach to teaching biology" European Learning Laboratory for the Life Sciences by EMBL – Prague, Czech Republic

2010 Handling methods - *Branchiostoma floridae* (material collection and processing) at University of South Florida - Tampa, Florida, USA

2009 Handling Multicellular Model Organism *C. elegans* (RNAi method) at University of South Bohemia in České Budějovice

Awards:

IMG Prize for the Best Paper in 2012: Molecular analysis of the amphioxus frontal eye unravels the evolutionary origin of the retina and pigment cells of the vertebrate eye. (Vopalensky P, Pergner J, Liegertova M, Benito-Gutierrez E, Arendt D, Kozmik Z.)

SEZNAM PUBLIKACÍ/LIST OF PUBLICATIONS

1) **Liegertová M**, Pergner J, Kozmiková I, Fabian P, Pombinho AR, Strnad H, Pačes J, Vlček Č, Bartůněk P, Kozmik Z. (2015). **Cubozoan genome illuminates functional diversification of opsins and photoreceptor evolution.** Sci Rep. 5:11885. (IF 5.578)

2) Vopalenský P, Pergner J, **Liegertová M**, Benito-Gutierrez E, Arendt D, Kozmik Z. (2012). **Molecular analysis of the amphioxus frontal eye unravels the evolutionary origin of the retina and pigment cells of the vertebrate eye**. Proc Natl Acad Sci U S A. 109(38):15383-8. (IF 9.674)

Kumulovaný impakt faktor autora/ Cumulative Impact Index of the Author: 2,73

3) Manuscript in preparation:
Marková K., Sekereš J., Goliáš V., **Liegertová M**., Vlček Č., Kozmik Z.
The lenses of box jellyfish in space and time: Crystallins as lens creators.

DALŠÍ VÝZKUMNÉ PROJEKTY V RÁMCI STUDIA/OTHER RESEARCH PROJECTS

Molecular analysis of the frontal eye pigmented cells in cephalochordate *Branchiostoma floridae* (results published in Vopalensky et al. 2012)
Methods: Collection of animals, laboratory spawning, larvae culturing, antibody design and IHC

Regulation of pigmentation genes in *Branchiostoma floridae*
Methods: Meganuclease-mediated transgenesis to test promoter activity of *B. floridae* pigmentation genes in a heterologous system (*Oryzias latipes*)

Regulation of opsin genes in *Tribolium castaneum*
Methods: RNAi knock-down, larval phenotype analyses, qRT-PCR

POUŽITÁ LITERATURA/REFERENCES

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