Biochemical and Biophysical Research Communications 473 (2016) 1211–1217

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Knockdown of *unc119c* results in visual impairment and early-onset retinal dystrophy in zebrafish

Nir Rainy [a], Talya Etzion [a], Shahar Alon [a, b], Adi Pomeranz [a], Yael Nisgav [c], Tami Livnat [c], Michael Bach [d], Cecilia D. Gerstner [e], Wolfgang Baehr [e, f], Yoav Gothilf [a, b], Hadas Stiebel-Kalish [g, *]

[a] Department of Neurobiology, George S. Wise Faculty of Life Sciences, Israel
[b] Sagol School of Neuroscience Tel Aviv University, Israel
[c] Laboratory of Eye Research, Felsenstein Medical Research Center Israel, Petah Tikva, Israel
[d] Eye Center, Medical Center, University of Freiburg, Killianstraße 5, 79106, Freiburg, Germany
[e] Department of Ophthalmology and Department of Neurobiology and Anatomy, University of Utah Health Science Center, Salt Lake City, UT 84132, USA
[f] Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
[g] Department of Ophthalmology at Rabin Medical Center, Petah Tikva & Sackler Faculty of Medicine, Tel Aviv University, Israel

ARTICLE INFO

Article history:
Received 6 April 2016
Accepted 9 April 2016
Available online 11 April 2016

Keywords:
Uncoordinated (UNC) 119
Morpholino knockdown
Zebrafish retina
Retina degeneration

ABSTRACT

Purpose: UNC119 proteins are involved in G protein trafficking in mouse retinal photoreceptors and *Caenorhabditis elegans* olfactory neurons. An *Unc119* null allele is associated with cone-rod dystrophy in mouse, but the mechanism leading to disease is not understood. We studied the role of Unc119 paralogs and Arl3l2 in zebrafish vision and retinal organization resulting from *unc119c* and *arl3l2* knockdown.
Methods: Zebrafish *unc119c* was amplified by PCR from retina and pineal gland cDNA. Its expression pattern in the eye and pineal gland was determined by whole-mount in-situ hybridization. *unc119c* and *arl3l2* were knocked down using morpholino-modified oligonucleotides (MO). Their visual function was assessed with a quantitative optomotor assay on 6 days post-fertilization larvae. Retinal morphology was analyzed using immunohistochemistry with anti-cone arrestin (zpr-1) and anti-cone transducin-α (GNAT2) antibodies.
Results: The zebrafish genome contains four genes encoding *unc119* paralogs located on different chromosomes. The exon/intron arrangements of these genes are identical. Three Unc119 paralogs are expressed in the zebrafish retina, termed Unc119a-c. Based on sequence similarity, Unc119a and Unc119b are orthologs of mammalian UNC119a and UNC119b, respectively. A third, Unc119c, is unique and not present in mammals. Whole mount *in-situ* hybridization revealed that *unc119a* and *unc119b* RNA are ubiquitously expressed in the CNS, and *unc119c* is specifically expressed in photoreceptive tissues (pineal gland and retina). A Unc119 interactant, Arl3l2 also localizes to the pineal gland and the retina. As measured by the optomotor response, *unc119c* and *arl3l2* knockdown resulted in significantly lower vision compared to wild-type zebrafish larvae and control morpholino (MO). Immunohistological analysis with anti-cone transducin and anti-cone arrestin (zpr-1) indicates that knockdown of *unc119c* leads to photoreceptor degeneration mostly affecting cones.
Conclusions: Our results suggest that Unc119c is the only Unc119 paralog that is highly specific to the retina in zebrafish. Unc119c and Arl3l2 proteins are important for the function of cones.

1. Introduction

Caenorhabditis elegans unc-119 was discovered based on a spontaneous mutation resulting in a nervous system defect [13]. An unc-119 ortholog was identified independently in human and rat retina (HRG4) [6], now officially termed “unc-119 lipid binding chaperone”, gene symbol *UNC119*. In mammalian retina, UNC119

* Corresponding author. Neuro-Ophthalmology Unit, Department of Ophthalmology, Rabin Medical Center, Petah Tikva, 49100, Israel.
E-mail address: kalishhadas@gmail.com (H. Stiebel-Kalish).

http://dx.doi.org/10.1016/j.bbrc.2016.04.041
0006-291X/

occurs as two paralogs, UNC119a and UNC119b [12]. UNC119a forms a stable complex with $T\alpha^{GTP}$ that is capable of diffusing from the inner segment back to the outer segment following light-induced translocation [24]. UNC119 forms an immunoglobulin-like β-sandwich fold which can accommodate the N-terminal acyl-chains of Tα and *C. elegans* olfactory G-proteins [24]. The physiological role of UNC119a is to extract Tα-GDP from inner segment membranes in the presence of GTP, forming a soluble complex. The complex was shown to play a key role in transducin's return to the outer segment following massive translocation from the outer to the inner segment in constant light [4,24].

UNC119 polypeptides are multifunctional proteins and interact with a large number of unrelated proteins – acylated Gα-subunits [24], receptor-associated src-type tyrosine kinases [3], non-receptor protein kinases and small Arf-like GTPases (ARL proteins) [9,10]. The UNC119 acyl-binding proteins derive from a supergene family whose function has been maintained through metazoan evolution. The β-sandwich structure of UNC119a is very similar to those seen in PrBP/δ [23] and RhoGDI [7]. UNC119a and UNC119b both physically interact with transducin-α as shown by pull down assays [24], but knockdown of UNC119b, but not UNC119a, prevented proper ciliary targeting of nephrocystin-3 (NPHP3) [21].

In zebrafish, three *unc119* paralogs, *unc119a-c*, were identified [1,14,19]. Knockdown of *Unc119a* resulted in Kupffer's vesicle (KV) defects and reduced startle responses to bright light stimuli, while knockdown *Unc119b* yielded only a mild KV defect with no obvious cilia-related phenotypes function [21]. Subsequently, zebrafish pineal gland transcriptome analysis revealed a previously unknown zebrafish paralog, *unc119c* [1,19]. This paralog was identified as a pineal gland-enhanced transcript, initially identified as an Expressed Sequence Tag (EST, BG3055792), with the highest fold difference as compared to its expression in other tissues [1]. Also reported in these studies is the co-expression of an Arf-like (ARL) protein, Arl3l2, in the pineal gland [1], and its interaction with Unc119c by co-immunoprecipitation [19]. Moreover, it was shown that their co-expression in the pineal gland is important for proper development of the adjacent habenular commissure [20]. These previous studies focused on the role of Unc119c in the pineal gland. In this communication, we examined the role of Unc119c and Arl3l2 in the development and organization of the zebrafish retina and in its effect on vision.

2. Materials and methods

2.1. Fish and embryos

Adult zebrafish (*Danio rerio*) were raised in a recirculation water system under 12:12 h of light–dark cycles at 28.5°C, adhering to the ARVO statement for the use of animals in Ophthalmic and Vision Research. The study was approved by the IACUC of Tel Aviv University, Israel. To produce embryos, male and female zebrafish were paired in the evening. Spawning occurred the next morning within 1 h of light. Embryos were placed in 10-cm Petri dishes with egg water (60 p.p.m. sea salts) containing methylene blue (0.3 p.p.m.) and raised in a light-controlled incubator at 28.5°C (lighting 12 W/m^2). In preparation for whole-mount *in situ* hybridization, 1-phenyl-2-thiourea (0.2 mM) was added to the water to prevent pigmentation.

2.2. Cloning of zebrafish unc119 isoforms

unc119 and *arl3l2* isoforms were PCR amplified from pineal and retinal cDNA with specific sets of primers (Table 1) and subcloned in pGEM T-easy plasmid vector (Promega, Madison, WI, USA). The identity of the resulting clones was verified by sequencing and by comparison with the zebrafish genome. These clones were used as templates to synthesize DIG-labeled antisense riboprobe for whole mount *in-situ* hybridization.

2.3. Whole mount in-situ hybridization (ISH)

Transcripts of *unc119c, unc119a, unc119b and of arl3l2* were detected by whole mount ISH using DIG-labelled antisense riboprobe RNA (DIG RNA labeling kit, Roche Diagnostics, Basel, Switzerland). Whole mount ISH analysis was carried out according to established protocol [15,18,26].

2.4. unc119c knockdown procedure

Knockdown of *unc119c* and of *arl3l2* was performed by microinjection of morpholino modified antisense oligonucleotides (MO) (Genetools, LLC, Philomath, OR, USA) as described. *unc119c* MO (5′-CAAGCCTGTTCAGAAGTTCAGTGTT-3′) was designed against the intron3-exon 4 boundary, according to the manufacturer's recommendations, to interfere with splicing. arl3l2 (5′ AAGCCCTG-GAAACAACAACACACAC-3′) was directed against the intron2-exon3 boundary to inhibit splicing. BLAST (Basic local alignment search tool) analysis was performed using UCSC genome browser (http://genome.ucsc.edu/) to verify that this MO does not bind gene sequences other than its target. Standard control MO, 5′-CCTCTTACCTCAGTTACAATTTATA-3′, which has not been reported to have other targets or generate any phenotypes in any known test, was injected as a negative control. Morpholinos were injected at a working concentration of 1–1.25 mM (approximately 8–10 mg/ml) in 0.1M KCL/0.05% phenol red. Approximately 2 nl were injected into the cytoplasm of one or two-cell-stage wild type zebrafish zygotes using micromanipulator and PV830 Pneumatic Pico Pump (World Precision Instruments, Sarasota, FL, USA). 100 to 400 embryos were injected using three to four different needles in each experiment. In rescue experiment, *unc119c* MO was co-injected with 100 ng/ml *unc119c* mature mRNA. Injected embryos kept at standard conditions as described above, until analyses.

The effect of the *unc119c* MO on splicing was validated by reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted from pools of 60 unc119c MO and control MO embryos at 2, 3 and 6 dpf, an age at which MO is still active [2], cDNA was generated and subjected to PCR using Unc119c-specific primers directed towards exons 3 and 5 [forward 5′-TGATA-GAGCTGGATGCTGGC-3'; reverse 5′-TGTCTGAGCGCGTCTCAA-3']. The PCR products were fractionated over 1.5 agarose gel, subcloned into pGEM-Teasy plasmid and sequenced. This analysis demonstrated that in addition to the normal mRNA there is a product with an additional 79 bp of intron 3 to the *unc119c* mRNA in *unc119c* MO-injected embryos, but not the control MO-injected embryos. This insertion introduced a number of premature stop codons which would lead to the production of a truncated and nonfunctional protein.

2.5. Visual testing procedure

A quantitative optomotor assay was used to measure zebrafish larvae vision [18]. The optomotor response is an innate behavior of fish (and other animals) to follow visual motion of their surround in order to stabilize location or course. Here, the larvae follow the moving stripes, if they can see them, in an attempt to stabilize their location. Repeated stimulation by moving grating stimuli (256 pixels per cycle, corresponding to 0.16 cycles/degree at a distance of 8 cm, speed 144 pixels/s corresponding to 0.56°/s) was generated using a computer-generated stimulus program which is freely