Human Genetics (2019) 138:957–971
https://doi.org/10.1007/s00439-018-1931-y

REVIEW

Non-coding RNAs in retinal development and function

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Received: 6 April 2018 / Accepted: 24 August 2018 / Published online: 5 September 2018

Abstract
Accumulating evidence on the role of non-protein-coding RNA sequences in the regulation of gene expression is greatly expanding our understanding of the flow of genetic information within biological systems. The interplay between protein-coding and non-coding RNAs (ncRNAs) is essential for tissue development, homeostasis, and function. NcRNAs can be divided in short ncRNAs, whose main subtype is represented by microRNAs, and long ncRNAs, which constitute a more heterogeneous class. The retina is a light-sensitive tissue consisting of highly interconnected cell types and is the primary target of many genetic diseases. Among these, the genetically heterogeneous group of inherited retinal diseases (IRDs) represents the most frequent monogenic cause of visual impairment that can ultimately lead to blindness. Here, we provide an overview on the role of ncRNAs in retinal development and function with an emphasis on microRNAs and on different types of long ncRNAs. We also review how sequence variations in ncRNAs can play a pathogenic role in IRDs as well as in multifactorial ocular disorders. These data indicate that a comprehensive study of the contribution of ncRNAs to the mutation repertoire associated with retinal disease can shed light on previously unknown pathophysiological mechanisms and open new therapeutic avenues. We conclude that a more comprehensive dissection of the pathogenic role of non-coding RNAs in retinal function and disease will not only improve our diagnostic ability, but will allow the development of novel targeted therapies for ocular disease.

Introduction

The retina is a highly specialized ocular tissue of upmost importance for vision. All that we see depend on the way that the patterns of light are translated by the retina into neural activity. Among the highly interconnected cell types of the retina, photoreceptors are sensitive to light (Fig. 1). The photopigments in the outer segments of photoreceptors capture light and induce a cascade of molecular changes ultimately leading to the release of neurotransmitters to interneurons and retinal ganglion cells, which eventually convey visual information via the optic nerve to the brain.

Because of its complex morphogenesis, structure, and function, the retina is the target of a number of genetically diverse inherited disorders, which cause impairment and/or irreversible loss of vision (Rivolta et al. 2002; Wright et al. 2010). These disorders include diseases that affect exclusively the retina as well as conditions with extraocular phenotypes (Rivolta et al. 2002; Wright et al. 2010). Inherited retinal disorders (IRDs), such as Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP), are arguably the leading cause of inherited visual impairment in the Western population, with a cumulative prevalence of 1:3500 (Rivolta et al. 2002). Currently, no treatment is available for the vast majority of IRD patients. Great hopes reside in gene therapy. In late 2017, the first FDA-approved gene therapy for a genetic disease, LUXTURNA (Spark Therapeutics), was released. It can be prescribed to patients with mutations in RPE65, a protein expressed specifically by the retinal pigment epithelium (RPE), and represents a landmark in gene therapy for IRDs (Apte 2018; Ledford 2017).

According to the recent estimates, more than 200 genes have been implicated in IRDs (RETnet database, http://www.sph.uth.tmc.edu/Retnet/home.htm). Photoreceptor cell death represents the most common outcome of IRDs despite their remarkable genetic heterogeneity (Marigo 2007; Sancho-Pelluz et al. 2008). The majority of these disorders initiate with the degeneration of rod photoreceptors, while cones

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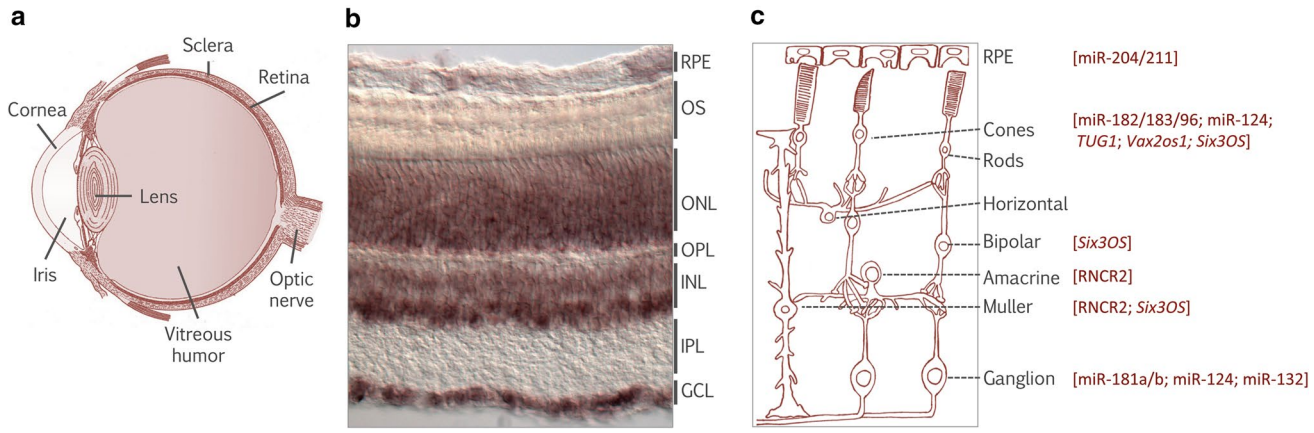

Fig. 1 Retina and examples of functionally relevant ncRNAs. **a** Drawing illustrating the main structures of the human eye. **b** Cross section of the mature mouse retina showing the laminar organization of the nuclear (ONL, INL, and GCL) and synaptic (OPL and IPL) layers. The RPE monolayer extends between the neural retina and the choroid in proximity to the photoreceptor outer segment (OS). **c** Schematic representation of the retinal cell types depicting the interconnections among the neuronal (cone and rod photoreceptors, horizontal, bipolar, amacrine, and ganglion cells) and supporting (RPE, Muller) cells. Examples of non-coding RNAs that have been suggested to regulate the specification and development of each cell type are shown. *GCL* ganglion cell layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *ONL* outer nuclear layer, *OPL* outer plexiform layer, *OS* outer segments, *RPE* retinal pigment epithelium

are affected only secondarily. The precise molecular mechanisms that lead to the death of retinal cells remain poorly defined. A large portion of IRD patients lack a genetic diagnosis (Neveling et al. 2012). This implies that, despite the substantial number of known retinal disease genes, the full catalog of causative sequence variations is far from being fully defined.

Sequencing of the human genome established that only a small part (~2%) codes for protein (Lander et al. 2001; Venter et al. 2001). With the advent of next-generation sequencing (NGS), transcriptomic analyses of different tissues, developmental stages, and conditions revealed that the vast majority of the human genome (~80%) is actively transcribed (Consortium 2012; Xie et al. 2014). The initial hypothesis that the non-protein-coding part of the transcriptome corresponded to merely spurious transcription, resulting from "leaky" RNA polymerase activity, has been revised to account for the exciting findings on the diverse functions of non-coding RNAs (ncRNAs) (Briggs et al. 2015; Kopp and Mendell 2018). Even though ncRNAs are devoid of a protein-coding capacity, it emerged that many of these transcripts are functional and represent key effectors in biological processes. In light of this, it is not surprising that the non-coding RNA component is commensurate with organism complexity more than the protein-coding content of the genome (Mattick 2011).

NcRNAs are broadly subdivided in "housekeeping" (e.g., tRNA, rRNA, snRNA, snoRNA, etc.) and "regulatory" according to their role in the flow of genetic information (Table 1). Regulatory ncRNAs, together with epigenetic marks, transcription factors and *cis*-acting elements (e.g., promoters and enhancers), are functional components of gene expression and constitute a previously undetected layer in eukaryotic gene regulation. Regulatory non-coding RNAs are a heterogeneous group of molecules, classified into two distinct categories based on their length (Table 1). The size of 200 bases is arbitrarily used to discriminate between long (e.g., lincRNAs, circRNAs, and ceRNAs) and small ncRNAs [e.g., microRNAs (miRNAs) and piRNAs]. Besides their length, long and small ncRNAs differ profoundly in aspects such as evolutionary conservation and modes of action (Table 1). LncRNAs have been shown to have very diverse functions in a range of cell types (Briggs et al. 2015; Kopp and Mendell 2018; Quek et al. 2015), e.g., by acting as decoys of transcription factors and miRNAs (Hansen et al. 2013), as regulators of epigenetic states through their interaction with chromatin modifiers (Rinn and Chang 2012), or as elements participating to nuclear topological organization (Ip and Nakagawa 2012) and to protein-complex scaffolding (Ribeiro et al. 2018). MiRNAs are the ncRNAs that have been more extensively studied, also in the retina, especially with regards to their regulatory functions. MiRNA identification and characterization has been facilitated by their extensive sequence conservation, which was soon interpreted as a strong indication of functional significance across evolution. On the other hand, the function of retina-expressed lncRNAs remains largely elusive. Nevertheless, the few examples that have been so far defined support a diversity of functions, including the establishment of large regulatory gene networks. The complexity of non-coding RNA networks is further amplified by the functional

Table 1 Main categories of non-coding RNAs

ncRNA class	Description	Molecular function
Housekeeping ncRNAs		
tRNA	Transfer RNA	Protein synthesis
rRNA	Ribosomal RNA	Component of ribosomal subunits
snoRNA	Small nucleolar RNAs	Pre-rRNA processing
snRNA	Small nuclear RNA	Splicing
Regulatory ncRNAs		
Long non-coding RNAs (> 200 nt)		
ceRNA	Competing endogenous RNA	Transcript regulation by competing for miRNA binding
circRNA	Circular RNA	miRNA decoys, transcription regulators, interference with splicing
lincRNA	Long intergenic non-coding RNAs	DNA–chromatin complex scaffolds
NATs/OS	Natural antisense transcripts/opposite strand	Transcriptional regulation in *cis* or *trans*
Small non-coding RNAs (< 200nt)		
miRNA	microRNA	Post-transcriptional silencing, translational repression
piRNA	PIWI-interacting RNA	Transposon silencing

interactions between miRNAs and lncRNAs (e.g., circRNAs) that impact on miRNA abundance and availability.

Here, we provide an overview of the current knowledge on the function of non-coding RNAs in the retina, discussing the recent findings in both model organisms and humans. We also present examples of the pathogenic role of sequence variations in ncRNAs in IRDs, highlighting how a comprehensive dissection of their role is expected to have wide implications for the understanding of retinal pathophysiology. Finally, we underline that non-coding RNAs, and miRNAs in particular, are potential therapeutic targets in IRDs because of their ability to subtly regulate the expression of multiple genes and pathways at once.

To ease the discussion, we describe in two separate sections what is presently known on lncRNAs and on the most relevant family of short ncRNAs, i.e., miRNAs.

lncRNAs in the retina: identified categories and function

Defining the lncRNA complement of the retina

The lncRNA content of the retina started to be explored before the development of high-throughput sequencing methods. The first evidence for the presence of long non-protein-coding RNAs transcripts in the retina dates back to 2004 when Cepko's group identified a number of putative lncRNAs using serial analysis of gene expression (SAGE) as part of a comprehensive study of gene expression in the developing mouse retina (Blackshaw et al. 2004). They also demonstrated, by RNA in situ hybridization (ISH), that some of these transcripts (namely, *RNCR1-3, MEG3, Xist*, and *Tsix*) have a dynamic expression across development (Blackshaw et al. 2004). Subsequent studies indicated that lncRNAs are implicated in regulatory pathways that underlie critical aspects of retinal cell-type specification (Meola et al. 2012; Rapicavoli et al. 2010, 2011; Young et al. 2005; Zhu et al. 2018) (see also section below).

The availability of high-throughput sequencing approaches has greatly facilitated the comprehensive and unbiased identification of additional lncRNAs in the retina. Palczewski and colleagues performed a transcriptomic analysis of retinas from mouse models with different photoreceptor (cone–rod) contents (Mustafi et al. 2013). They identified 18 long intergenic non-coding RNAs (lincRNAs) that were conserved from mouse to humans. Insights into their putative function came from the analysis of their genomic context and of their predicted promoters (Mustafi et al. 2013). A later study analyzed the long non-coding RNA content of the retina and of other ocular tissues in newborn and 2-month-old mice using a microarray-based transcriptome assay (Chen et al. 2017b).

CircRNAs are circular ncRNAs formed by back-splicing events that covalently join the splice donor of a downstream exon with the splice acceptor of an upstream exon. CircRNAs can act as miRNA sponges by sequestering mature miRNAs and partake in RNA–protein complexes that regulate gene transcription (Hansen et al. 2013; Memczak et al. 2013), even though it is believed that only part of the whole circRNA complement is endowed with regulatory functions. Interestingly, two studies (Legnini et al. 2017; Pamudurti et al. 2017) indicated that at least some circRNAs are translated into proteins. However, the frequency and functional role of circRNA translation remains unclear. Recently, the repertoire of circRNAs in the rat retina was investigated

by Han et al. using RNA-Seq (Han et al. 2017). A total of approximately 2500–7000 circRNAs were identified and a significant portion showed differential expression across the three post-natal stages analyzed (Han et al. 2017). On the basis of the function of their host genes, 15 differentially expressed circRNAs were postulated to be associated with physiological apoptosis taking place during retinal maturation (Han et al. 2017).

The pool of retina-expressed lncRNAs is expected to expand, as high-throughput profiling of retinal tissues from a variety of model organisms is progressively being performed. However, the availability of human retinal samples for similar analyses is more limited. To date, there are a few published reports on the transcriptome of the human retina describing novel transcripts with variable degrees of protein-coding potential (Farkas et al. 2013; Kim et al. 2018; Li et al. 2014; Pinelli et al. 2016). For example, the majority of the 116 putative novel genes identified by Farkas et al. showed limited protein-coding capacity, suggesting that they may represent novel non-coding RNAs (Farkas et al. 2013). In a study aimed at reconstructing gene networks in the human retina, Pinelli et al. identified around 2400 novel single-exon transcripts by compiling RNA-seq data from 50 human retina samples (Pinelli et al. 2016). Based on open-reading frame (ORF) analysis, the majority of these newly identified transcripts are likely to represent non-coding RNAs, consistent with the evidence that 13% of them overlap with at least one already known lncRNA (Pinelli et al. 2016).

Functional role of specific lncRNAs in the retina

The functional characterization of lncRNAs remains a key goal, yet this area of research is lagging behind their identification. Unfortunately, studies on the mechanism of action of lncRNAs are hampered by the limited conservation of their nucleotide sequence. The function of only a handful of lncRNAs has been established in the context of the retina. Table 2 reports representative examples.

Taurine upregulated gene 1 (TUG1)

The first lncRNA to be endowed with a functional role in the retina is the Taurine Upregulated Gene 1 (*TUG1*) (Young et al. 2005) (Table 2). As its name suggests, *TUG1* was identified in a screen for genes upregulated in response to taurine (Young et al. 2005). Among other functions, this amino acid stimulates rod development in vitro (Altshuler et al. 1993). *TUG1* is a spliced, poly-adenylated transcript with small ORFs that do not exceed 82 amino acids in length. *TUG1* is expressed across retinal development, reaching the highest levels in the interval between late embryonic stages and the first post-natal week. In newborns and at the preceding embryonic stages, *TUG1* expression is detected in retinal progenitor and precursor cells (Young et al. 2005). As the retina matures, *TUG1* is expressed predominantly in the ganglion cell layer (GCL) and in the inner nuclear layer (INL) where interneurons reside (Fig. 1) (Young et al. 2005). *TUG1* is implicated in normal photoreceptor development and positively regulates rod specification and survival (Young et al. 2005). *TUG1* loss-of-function in the newborn retina led to structural defects in the photoreceptor outer segment accompanied by increased apoptosis and changes in the expression of key transcription factors (e.g., *Crx* and *Otx2*) as well as of marker genes of differentiated photoreceptors (Young et al. 2005). Further evidence that lncRNAs are likely to contribute to rod differentiation was provided in a recent study that described significant changes both in lncRNA and antisense RNA profiles during rod photoreceptor development (Zelinger et al. 2017). The reported dynamic expression of the non-coding transcriptome during rod maturation is consistent with a functional role of lncRNAs in this process.

RNCR2

The retinal non-coding RNA2 [*RNCR2*; also known as myocardial infarction associated transcript (MIAT) or Gomafu] is another abundant lncRNA in the retina. *RNCR2* is a nuclear-retained lncRNA with a punctuate distribution throughout the nucleoplasm, hence, the name Gomafu (speckled, punctuated) (Sone et al. 2007). Sone and co-authors proposed that Gomafu may constitute a component of the nuclear matrix in specific cell types (Sone et al. 2007). In the neural retina, *RNCR2* is expressed in differentiating progenitor cells starting from mid-embryonic stages (Sone et al. 2007). At the early post-natal stages, *RNCR2* expression is confined in a distinct sub-set of post-mitotic cells in the INL and GCL (Rapicavoli et al. 2010; Sone et al. 2007) (Fig. 1), while no expression was detected by RNA ISH in the adult retina (Blackshaw et al. 2004). *RNCR2* knock-down was associated with an increase in the number of amacrine interneurons and Muller glial cells, implicating this lncRNA in the specification of retinal cell types (Rapicavoli et al. 2010) (Table 2).

MALAT1 and MEG3

The highly conserved, nuclear-retained Metastasis Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) is commonly dysregulated in cancer and has a key role in tumor progression and metastasis (Gutschner et al. 2013). *MALAT1* depletion in vivo impaired neonatal retina vascularization, consistent with its role in the regulation of microvascular patterning (Michalik et al. 2014). In the context of the retina, *MALAT1* is expressed in all retinal layers and is upregulated under stress conditions in Muller and retinal ganglion cells

Table 2 Representative long non-coding RNAs with a role in retinal function

LncRNA	Alias	Ensembl ID	Retinal expression domains	Function	References
MALAT1	NEAT2, PRO1073, NCRNA00047, HCN, LINC00047, mascRNA	ENSG00000251562	RPE, ONL, INL, GCL (adult)	Regulates reactive gliosis and RCG survival in neurodegeneration; regulates endothelial cell proliferation and microvascularization in DR	Yao et al. (2016)
RNCR2	*Gomafu, MIAT, LINC00066, C22orf35, FLJ25967, NCRNA00066*	ENSG00000225783	INL, GCL (P7, P8)	Specification of retinal cell types; KD associated with increase of amacrine interneurons and Muller glial cells	Blackshaw et al. (2004), Rapicavoli et al. (2010), Sone et al. (2007)
RNCR4	Retina-expressed non-coding RNA 4, Gm37046, BB283400, BF465573	ENSMUSG00000103108	PR (P5, P10, P16, P28)	Stimulates processing of pri-miR-183/96/182; proper thickness of photoreceptor and inner nuclear layers	Krol et al. (2015)
Six3OS	*RNCR1*	ENSG00000236502	INL (P8); INL, GCL (adult)	Proper specification of bipolar, photoreceptor and Muller glia cells	Alfano et al. (2005), Blackshaw et al. (2004), Rapicavoli et al. (2011)
TUG1	*FLJ20618, LINC00080, NCRNA00080*	ENSG00000253352	INL, GCL (P10)	Implicated in normal photoreceptor development; positively regulates rod specification and survival; KD led to defects in photoreceptor (PR) differentiation with structural defects in the PR OS, increased apoptosis and changes in PR gene expression	Young et al. (2005)
Vax2os1	–	ENSG00000277764	Ventral iNBL, oNBL (E12.5–E16.5); ventral ONL (P60)	Controls cell cycle progression of PR progenitors; OE leads to delays in PR differentiation	Alfano et al. (2005), Meola et al. (2012)

DR diabetic retinopathy, *E* embryonic day, *GCL* ganglion cell layer, *iNBL* inner neuroblastic layer, *INL* inner nuclear layer, *KD* knock-down, *n.d.* not described, *OE* overexpression, *oNBL* outer neuroblastic layer, *ONL* outer nuclear layer, *OS* outer segments, *P* post-natal day, *PR* photoreceptors, *RGC* retinal ganglion cell, *RPE* retinal pigment epithelium

(RGC) (Table 2) (Yao et al. 2016). *MALAT1* knock-down in retinas of optic nerve transection (ONT) animal models dampened the reactive gliosis associated with retinal neurodegeneration and improved RGC survival (Yao et al. 2016). The pro-survival effect of *MALAT1* downregulation was mediated through a CREB signaling mechanism (Yao et al. 2016). *MALAT1* was significantly upregulated also in animal models of diabetic retinopathy (Liu et al. 2014). Downregulation of *MALAT1* in these models ameliorated retinal function by attenuating the microvascular impairment and inflammation associated with diabetic retinopathy (Liu et al. 2014). Taken together, these observations suggest that *MALAT1*, under specific stress conditions, may regulate diverse expression networks in the retina in a context-dependent manner.

More recently, another lncRNA, which was previously implicated in various human cancers, the Maternally Expressed 3 (*MEG3*), was linked to the retina. *MEG3* was found to be upregulated in the mouse retina following light exposure, while the short hairpin RNA (shRNA)-mediated silencing of *MEG3* counteracted the effects of light-induced retinal damage both in vivo and in vitro models (Zhu et al. 2018).

Natural antisense transcripts: identification and functional studies

A category of retinal lncRNAs that received the early attention is represented by *cis*-natural antisense transcripts (*cis*-NATs). Eight *cis*-NATs were identified in the mouse as putative regulators of eye development because of their overlap with the mRNAs of transcription factors (*Pax6, Pax2, Six3, Six6, Otx2, Crx, Rax*, and *Vax2*) known to play a key role in vertebrate eye development (Alfano et al. 2005). These NATs were termed *Pax6OS, Six3OS, Six6OS, Vax2OS, CrxOS, Otx2OS, Pax2OS*, and *RaxOS*, where OS stands for "Opposite strand", and were devoid of protein-coding capacity (Alfano et al. 2005). This study also indicated that, even if the genomic distribution of these NATs is relatively conserved between mouse and humans, there was a little similarity in their primary sequence, with the exception of *Six6OS* (Alfano et al. 2005). These NATs were expressed in different regions of the developing and adult retina and showed either similar (e.g., for the *Six3*/*Six3OS, Six6*/*Six6OS*, and *RaxOS*/*Rax* pairs) or complementary (e.g. *CrxOS*/*Crx, Otx2OS*/*Otx2*, and *Vax2Os*/*Vax2* pairs) patterns of expression with respect to their neighboring protein-coding transcript (Alfano et al. 2005; Corbo et al. 2007; Meola et al. 2012). Besides their distinct spatial distribution, different interrelationships exist also in terms of the reciprocal modulation of expression levels (Alfano et al. 2005). Overall, it is still unclear how NATs interfere with the expression of their sense protein-coding transcript and it is envisaged that diverse mechanisms of action may be involved [reviewed in Wahlestedt (2013)].

The retina-specific function of two *cis*-NATs, namely *Vax2os1* and *Six3OS* (Table 2), has been further investigated (Alfano et al. 2005; Meola et al. 2012; Rapicavoli et al. 2011). *Vax2os1* shows a dorsal–ventral gradient of expression similar to that of its associated protein-coding transcript (Meola et al. 2012). In the developing and adult retina, *Vax2os1* expression is confined to the ventral portion and is predominantly detected in the layers where photoreceptor progenitors and differentiated cells reside (Meola et al. 2012). Consistent with this expression pattern, the AAV-mediated overexpression of *Vax2os1* in the post-natal murine retina interfered with the proper differentiation of photoreceptor progenitors. In particular, it induced proliferation of retinal progenitor cells in the outer nuclear layer (ONL), an increase of apoptosis in the photoreceptor layer, and a delay in photoreceptor differentiation (Meola et al. 2012). Combined with findings from in vitro experiments using the 661W photoreceptor cell line, the authors suggested that *Vax2os1* may directly impact cell cycle progression (Meola et al. 2012).

Six3OS is expressed in the retina from prenatal stages to adulthood (Alfano et al. 2005; Rapicavoli et al. 2011). Gain- and loss-of-function experiments demonstrated that *Six3OS* is important for the proper specification of bipolar, photoreceptor, and Muller glia cells in the developing retina, processes regulated also by Six3, the corresponding sense transcription factor (Rapicavoli et al. 2011). The *Six3OS* transcript is proposed to act by regulating Six3 function through a *trans*-mechanism that is yet to be fully defined (Rapicavoli et al. 2011).

NATs were shown to impact gene expression by acting primarily as chromatin regulators and their deregulation was described in several conditions, including cancer (Wahlestedt 2013). Very recently, Kim and co-authors compared the transcriptomic profiles of retinas from normal and age-related macular degeneration (AMD) donors, and reported that the differential antisense transcription correlated better with the disease state than the sense transcription (Kim et al. 2018).

miRNAs in the retina

The miRNA complement (miRNome) of the retina

MicroRNAs (miRNAs) represent the most abundant and best characterized class of small non-coding RNAs with a regulatory function (Table 1). miRNAs are processed from longer hairpin transcripts through two consequent cleavage events, mediated, respectively, by the ribonucleases Drosha and Dicer. Drosha processes the primary miRNA transcript

(pri-miRNA) in the nucleus to generate a precursor hairpin (pre-miRNA). The pre-miRNA is exported to the cytoplasm, where it is cleaved by Dicer to release the ~ 22 nt-long double strand RNA molecule, from which the mature miRNA is produced. The mature miRNAs, loaded onto the RNA-induced silencing complex (RISC) complex, bind to complementary sites mainly at the 3′ UTR of target genes causing translational repression or transcript degradation. As disruption of Dicer function interferes with the global miRNA processing, its conditional ablation has been extensively used as a tool to dissect miRNA function.

The early reports described the expression of a small number of miRNAs in the mammalian eye and retina (Deo et al. 2006; Karali et al. 2007; Ryan et al. 2006). Despite being limited in number, this initial sub-set of miRNAs included some of the most prominent miRNAs, in terms of abundance and significance for retinal function (e.g., miR-124, miR-182, miR-183, miR-96, miR-204, miR29c, miR-9, and miR-181). Subsequently, more comprehensive analyses using both microarray (Xu et al. 2007) and RNA ISH methods revealed miRNAs with differential expression in ocular tissues (Karali et al. 2010). A high-resolution expression atlas characterized the spatiotemporal distribution of 221 miRNAs in the developing and adult wild-type mouse eye (Karali et al. 2010). This study demonstrated that each ocular tissue (e.g., lens, cornea, retina, and RPE) exhibited notably distinct miRNA enrichment patterns with more than 100 miRNAs displaying restricted expression domains at different developmental stages (Karali et al. 2010).

The application of unbiased, high-throughput sequencing approaches unveiled the extent of complexity of the retinal miRNome in terms of sequence variation and expression (Karali et al. 2016; Soundara Pandi et al. 2013). Karali et al. analyzed the human retina miRNome from 16 individuals without any reported retinal disease (Karali et al. 2016). Although a total of 480 miRNAs were detectable, a remarkably small number of miRNAs (20 miRNAs), comprising those described in early studies (Deo et al. 2006; Karali et al. 2007; Ryan et al. 2006), accounted for almost 90% of the retina miRNome (Karali et al. 2016). In addition, the authors discovered more than 3000 miRNA variants (isomiRs) of the retinal-expressed miRNAs. A portion of these isomiRs is expected to impact miRNA functionality, because they are probably endowed with different targeting properties compared to the corresponding canonical form (Karali et al. 2016). This study also predicted a number of novel putative miRNAs in the human retina (Karali et al. 2016), supporting the notion that comprehensive tissue- and/or species-specific analyses are necessary to fully define the miRNA complement.

The identification of the spatial and temporal distribution of the miRNAs in different retinal cell types may provide further information on their biological role. To this aim, different studies reported the miRNA content of the following retinal cell types; namely, murine cone (Busskamp et al. 2014) and rod photoreceptors (Sundermeier et al. 2014), murine Muller glial cells (Wohl and Reh 2016), human RPE (Karali et al. 2016), and axons of developing RGCs in *Xenopus* (Bellon et al. 2017).

Role of microRNAs in the retina

The impact of miRNAs on gene regulation is comparable to that of transcription factors (Bartel 2018). It has been proposed that each miRNA could fine-tune the expression of an average of 200 transcripts (Bartel 2018). MiRNAs are, therefore, considered as molecular dimmers that can modulate in parallel functionally correlated genes and pathways, conferring robustness to biological processes (Ebert and Sharp 2012). The importance of miRNAs in retinal physiology became soon evident both from studies that highlighted their aberrant expression profiles in pathological conditions, such as retinal degeneration (Genini et al. 2014; Loscher et al. 2007, 2008), as well as from the impact of the global impairment of miRNA biogenesis in specific retinal cell types and developmental stages.

Insight from the global inactivation of miRNA biogenesis in the retina

The biogenesis of mature miRNAs depends on the Dicer enzyme. Several reverse genetic approaches exploited Dicer ablation to uncover the phenotype associated with global loss of miRNAs in the retina. In the early studies, global perturbation of miRNA function in the eye and retina in vertebrates was achieved using different conditional *Dicer* mutants. For instance, it was demonstrated that the global perturbation of miRNA biogenesis impairs the normal development of the retina, lens, cornea, and optic chiasm (Damiani et al. 2008; Georgi and Reh 2010; Li and Piatigorsky 2009; Pinter and Hindges 2010). Specifically, conditional *Dicer* deletion impaired the developmental transition of retinal progenitors (Georgi and Reh 2010). This phenotype was probably regulated by the miRNAs let-7, miR-125, and miR-9, since their overexpression in knockout retinas was able to rescue the developmental delay (La Torre et al. 2013). The *Rhodopsin* promoter-guided ablation of *Dicer1* in mature post-mitotic rods led to disorganization of the photoreceptor outer segments, abnormal retinal morphology, and progressive retinal degeneration, underscoring that miRNA function is indispensable for photoreceptor survival (Sundermeier et al. 2014). Similarly, the genetic ablation of *Dicer1* in retinal Muller glia interfered with the normal migration of Muller cells, causing the formation of cell aggregations. The displacement of Muller glia to other retinal layers disrupted normal retinal architecture and significantly reduced visual

acuity (Wohl et al. 2017). Along the same line, the conditional depletion of *Dicer1* in the developing RPE in mice demonstrated that the regulatory activity of miRNAs is also required for proper RPE differentiation and function, with direct repercussions for the development and survival of the adjacent photoreceptors (Ohana et al. 2015). Finally, morpholino-mediated inactivation of *Dicer* in *Xenopus* retinal progenitor cells led to defects in lamination, delays in cell cycle exit, and increased cell death in the retina (Decembrini et al. 2008; Gessert et al. 2010).

Insight into the role of individual miRNAs in retinal pathophysiology

Knowledge of the molecular mechanisms of miRNA action has allowed an easier dissection of their biological function in retina compared to lncRNAs. Currently, a number of miRNAs contribute to the specification of the different retinal cell types (Sundermeier and Palczewski 2016). We describe below examples of well-characterized miRNAs that control retinal development and function (Table 3).

The miR-204/miR-211 family

The miR-204/miR-211 is an example of how specific miRNAs can regulate multiple events in eye formation in vertebrates. MiR-204 is one of the most abundant in the retina and is expressed strongly in the RPE, the INL, and the GCL (Karali et al. 2007, 2016). It was also detected in the ONL where photoreceptors reside (Conte et al. 2015) and its expression increases upon light exposure (Krol et al. 2010). MiR-204 loss-of-function in medaka fish led to microphthalmia, abnormal lens formation, and coloboma partly due to perturbations of the Meis2/Pax6 pathway (Conte et al. 2010). Interestingly, Pax6 was shown to control miR-204 levels by directly regulating the expression of its host gene *Trpm3* (Shaham et al. 2013). In addition, disruption of miR-204 activity caused significant defects in dorso-ventral retinal patterning (Conte et al. 2010), induced apoptosis, and altered the expression of rod and cone photoreceptor markers (Conte et al. 2015). The importance of the miR-204 subfamily of miRNAs for photoreceptor cell specification, differentiation, and survival was further corroborated by the characterization of miR-211 knockout mice (Barbato et al. 2017). MiR-211, the other member of the miR-204/miR-211 subfamily, is highly similar to miR-204 in terms of expression, mature sequence, and targeting capability. In mouse, these two miRNAs have only one nucleotide difference in their mature sequence and share an identical seed sequence, the primary module in target recognition (Barbato et al. 2017). Loss of miR-211 activity in mice elicited a progressive cone dystrophy accompanied by cone loss and significant alterations in visual function (Barbato et al. 2017), suggesting that miR-204/miR-211 are indispensable for proper retinal function. Moreover, miR-204 was shown to contribute to axon guidance (Conte et al. 2014).

The photoreceptor-enriched cluster miR-183/96/182

A set of well-studied miRNAs for photoreceptor function is represented by the retina-enriched cluster miR-183/96/182. These miRNAs are very abundant in photoreceptors (Karali et al. 2007; Xu et al. 2007) and are strongly light-regulated (Krol et al. 2010). Due to their enrichment in photoreceptors, efforts from several groups focused in generating in vivo genetic tools to decipher their function (Busskamp et al. 2014; Krol et al. 2010; Lumayag et al. 2013; Xiang et al. 2017; Zhu et al. 2011). Busskamp and co-authors showed that the re-expression of miR-182 and miR-183 preserved cone outer segments and restored light responses in vitro in a mouse model in which miRNAs were specifically depleted in adult cones (Busskamp et al. 2014). Specific inactivation of the *miR-183/96/182* cluster in mouse by gene-trap approaches resulted in photoreceptor dysfunction and defects in photoreceptor synaptic transmissions, ultimately converging on progressive retinal degeneration (Lumayag et al. 2013). More recently, Fan et al. described that the deletion of the entire *miR-183/96/182* cluster in mice led to severe delays in the maturation of early post-natal ciliated sensory neurons not only in retina but also in other sensory organs (Fan et al. 2017). *miR-183/96* double knockout mice exhibited severe impairment of cone photoreceptor maturation and maintenance as well as progressive photoreceptor degeneration that severely compromised visual function (Xiang et al. 2017). This effect is mediated by the action of the two miRNAs on the taurine transporter Slc6a6, a common target of both miR-183 and miR-96 (Xiang et al. 2017). An interesting example of cross-talk between miRNAs and lncRNAs in the retina was shown for the *miR-183/96/182* cluster (Krol et al. 2015). Krol *et al*. demonstrated that the lncRNA *Rncr4* stimulates the processing of the *miR-183/96/182* cluster by antagonizing the repressive effect mediated by the RNA helicase Ddx3x at earlier developmental stages (Krol et al. 2015). Finally, miR-182 was found to be the most abundant miRNA also in the axons of developing retinal ganglion cells in *Xenopus* where it regulates axon guidance by targeting cofilin-1 in response to Slit2 (Bellon et al. 2017).

Other miRNAs

A number of additional miRNAs were shown to influence the specification of retinal cell types. For example, miR-124 is an abundant neuronal-specific miRNA and it is also important for retinal development. Analysis of an $Rncr3^{-/-}$ mouse, in which the main primary transcript of miR-124 is disrupted, revealed that miR-124 is required for the maturation and

Table 3 Representative examples of miRNAs with a well-characterized role in retinal function

miRNA	Expression ranking in the human retina[a]	Retinal expression domains	Function	References
miR-182, miR-183, miR-96	1, 2 and 20	ONL, OS (adult)	Light-regulated expression; OE preserves cone OS upon PR-specific *Dicer1* depletion; inactivation causes PR dysfunction, defects in PR synaptic transmissions, impairment of cone photoreceptor maturation and maintenance, and progressive photoreceptor degeneration; regulate RGC axon guidance	Bellon et al. (2017), Busskamp et al. (2014), Fan et al. (2017), Karali et al. (2007), Krol et al. (2010, 2015), Lumayag et al. (2013), Xiang et al. (2017), Xu et al. (2007)
miR-124	8	ONL, INL except Muller, GCL (adult)	Promotes maturation and survival of cone photoreceptors; regulates the timing of RGC growth cone sensitivity to repellents (e.g., Sema3A); proper axon guidance and pathfinding	Baudet et al. (2011), Karali et al. (2007), Sanuki et al. (2011)
miR-204, miR-211	5 and 35	RPE, ONL, INL, GCL (adult)	Medaka MO exhibited microphthalmia, abnormal lens formation, and coloboma; cell differentiation defects at the ventral retina; increased apoptosis; altered expression of PR markers; KO mice showed progressive cone dystrophy, cone loss, and alterations in visual function; Point mutation in seed associated with retinal dystrophy and coloboma in humans; Control axon guidance	Barbato et al. (2017), Conte et al. (2010, 2014, 2015), Karali et al. (2016)
miR-181a, miR-181b	3 and 23	INL and GCL	Regulate proper neuritogenesis in amacrine cells and RGCs	Carrella et al. (2015a, b)
miR-28	176	n.d	Inhibits differentiation of Muller glia-derived progenitors towards a photoreceptor lineage fate	(Ji et al. 2017)

E embryonic day, *GCL* ganglion cell layer, *iNBL* inner neuroblastic layer, *INL* inner nuclear layer, *KD* knock-down, *MO* morphants, *n.d*. not described, *OE* overexpression, *oNBL* outer neuroblastic layer, *ONL* outer nuclear layer, *OS* outer segments, *P* post-natal day, *PR* photoreceptors, *RPE* retinal pigment epithelium

[a]According to Karali et al. (2016)

survival of cone photoreceptors via *Lhx2* targeting (Sanuki et al. 2011). MiR-124 was also shown to regulate the timing of RGC growth cone sensitivity to repellents, such as Sema3A, through its action on CoREST (Baudet et al. 2011). Ablation of miR-124 in *Xenopus* interfered with axon guidance and led to pathfinding errors in vivo (Baudet et al. 2011).

Downregulation of miR-28 in cultured Muller glia-derived progenitors from mouse retinas is thought to promote their differentiation towards a photoreceptor lineage fate, probably by impacting the expression levels of *Crx*, a key transcription factor for the initial specification of the photoreceptor lineage (Ji et al. 2017). Moreover, Decembrini et al. reported that inactivation of miR-129, miR-155, miR-214, and miR-222 increases the proportion of retinal bipolar cells (Decembrini et al. 2009). Finally, miR-24a was shown to control programmed cell death during retina development in *Xenopus* by targeting proapoptotic factors such as *caspase9* and the a*poptosis protease-activating factor 1* (*apaf1*) (Walker and Harland 2009).

MiR-132 is considered to be important for visual cortex plasticity (Mellios et al. 2011). In the retina, this miRNA promotes axon formation of RGC (Marler et al. 2014). MiR-132 is expressed in the GCL and INL, and is upregulated in response to the brain-derived neurotrophic factor (BDNF) (Marler et al. 2014). MiR-132 loss-of-function in murine retinas affected the maturation of RGC termination zones, indicating that it positively controls RCG axon branching by targeting the Rho family GTPase-activating protein p250GAP, a suppressor of this process (Marler et al. 2014). Some members of the miR-181 family (i.e., miR-181a and miR-181b) are highly enriched in the retina. They regulate neuritogenesis in both amacrine cells and RGCs (Carrella et al. 2015b) through a pathway that involves TGF-β signaling (Carrella et al. 2015a). Finally, miR-7a was shown to negatively regulate the differentiation of Muller glia in the vertebrate retina by suppressing Notch3 expression (Baba et al. 2015).

A number of studies explored the role of miRNAs in the RPE, because the integrity of this epithelial monolayer is crucial for photoreceptor homeostasis and vision. For example, miR-204/miR-211 (Adijanto et al. 2012; Ohana et al. 2015; Wang et al. 2010), miR-184 (Murad et al. 2014), miR-23 (Lin et al. 2011), miR-9 (Kutty et al. 2010b), miR-34a (Hou et al. 2013), miR-155 (Kutty et al. 2010a), miR-146a, and miR-146b-5p (Kutty et al. 2013) were shown to influence diverse aspects of RPE physiology and function.

Non-coding RNAs and retinal diseases

In spite of their recent functional characterization, ncRNAs have been associated with a significant number of human diseases (Esteller 2011). However, only a limited number of inherited ocular diseases have been reported thus far to be caused by dysregulation or sequence mutations associated with ncRNAs. A mutation altering the seed region of miR-184 was linked to severe keratoconus combined with the early onset cataract (Hughes et al. 2011). Later on, another mutation in the seed region of miR-184 was reported to cause EDICT syndrome, characterized by endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning (Iliff et al. 2012). Interestingly, point mutations in miR-96, which belongs to the photoreceptor-abundant cluster *miR-183/96/182*, cause hearing loss (Mencia et al. 2009) but not retinal disorders.

The only example of the causative role of an ncRNA in a Mendelian retinal disease in humans refers to the highly expressed miR-204 (Conte et al. 2015). A point mutation in the seed region of miR-204 is responsible for an autosomal dominant form of retinal dystrophy accompanied by eye coloboma, probably through a gain-of-function mechanism (Conte et al. 2015). More recent studies (Huang et al. 2018) highlighted the presence of rare sequence variants in retina-expressed miRNAs in IRD patients whose pathogenic role, however, is still to be assessed.

ncRNAs may have a pathogenic impact also on complex, multifactorial disorders. A sequence variant in miR-182 was associated with susceptibility to a complex, multifactorial eye disease, namely primary open-angle glaucoma (Liu et al. 2016), as well as variants in the lncRNA CDKN2B-AS1 (Burdon et al. 2012; Pasquale et al. 2013). The upregulation of miR-21 has been implicated in diabetic retinopathy (DR) and its downregulation by inhibitor delivery suppressed retinal inflammation in mouse models of DR (Chen et al. 2017a). Finally, another hint to the possible involvement of ncRNA alterations in the pathogenesis of complex retinal disease derives from the observation that reduced function of Dicer1 may be linked to the geographic atrophy form of AMD, through a mechanism that does not seem to implicate impairment of miRNA processing but rather *Alu* RNA accumulation (Kaneko et al. 2011).

Assessment of the pathogenic impact of sequence variations in ncRNAs

With the increased awareness of the significance of ncRNA-associated variants in the etiology of genetic diseases, ncRNAs are starting to be probed as part of routine diagnostic protocols for IRD patients. Customized panels for targeted sequencing [e.g., RETPLEX (Di Iorio et al. 2017)] and platforms for microarray-based chromosome number variation (CNV) analysis [e.g., arrEYE (Van Cauwenbergh et al. 2017)] now enable to scrutinize retinal-expressed miRNAs with a putative role in retinal function as well as evolutionarily conserved lncRNAs expressed in retinal tissues. The current challenge is the interpretation of the biological impact

and clinical significance of the identified variants. In that respect, considerations on the potential pathogenicity of SNVs identified within non-protein-coding genes are precluded by the lack of comprehensive information on ncRNA function in the retina and/or in the specific cell types that are primarily implicated in IRDs. Insights into the role of retinal ncRNAs are, therefore, expected to empower the interpretation of ncRNA-associated variants in IRDs. Towards this end, ad hoc databases and tools have been developed to annotate disease-associated SNPs within human lncRNAs [e.g., Chen et al. (2013), Gong et al. (2015), and Ning et al. (2017)]. Similar resources are also available for miRNAs providing a useful starting point to uncover the possible functional consequences of a given sequence variation (Cammaerts et al. 2016; Gong et al. 2012).

Non-coding RNAs as therapeutic targets

MiRNAs are new therapeutic targets for human diseases (Rupaimoole and Slack 2017). Eye diseases, and in particular IRDs, are conditions that can greatly benefit from the use of miRNA-based therapies, because the eye is an ideal target for gene therapy. Being a small, compartmentalized organ, it requires the administration of low doses of gene therapy vectors. Moreover, the presence of the blood–retinal barrier prevents unintentional spreading of the vector to the general circulation. Therefore, it is feasible to restrict the desired changes in expression of a given miRNA or lncRNA, exclusively to the eye or to the retina, even with cell-type selectivity, without interfering with its function in other districts. In addition, the high genetic heterogeneity of IRDs calls for the development of gene-independent therapeutic approaches that are directed to tackle the common cellular hallmarks of retinal degeneration, such as photoreceptor cell death (Wright et al. 2010).

The accumulating evidence of the role of miRNAs in the retina, both in physiological and pathological conditions, is the basis for the selection of interesting therapeutic targets for IRDs. Insights into the contributions of specific miRNAs to retinal function and photoreceptor degeneration processes from in vitro functional analysis and in vivo studies will help to identify promising candidates and design miRNA-based therapeutic approaches aimed at delaying or, ideally, preventing retinal degeneration processes. For example, miR-125b-5p was shown to regulate the ectopic neuritogenesis of rod bipolar cells in a rat model of retinal degeneration (Royal College of Surgeon rat model) (Fu et al. 2017). Based on that evidence, the authors proposed the modulation of miR-125b-5p levels as a means of stimulating functional dendritic growth in IRDs (Fu et al. 2017). Moreover, in the light of findings implicating changes in the extent of antisense transcription with the disease state in AMD, new therapeutic considerations targeting antisense transcription may be contemplated (Kim et al. 2018). The development of new ncRNA-based approaches [reviewed in Adams et al. (2017) and Wahlestedt (2013)] may represent effective and innovative gene-independent therapeutic tools for IRDs ultimately satisfying a currently unmet medical need.

Conclusions and perspectives

Regulatory ncRNAs are potent effectors in the epigenetic, transcriptional, and post-transcriptional regulation of gene activity, and important factors for nuclear sub-domain function, translational control, genomic integrity, and plasticity. Therefore, their potential significance in the regulation of pathophysiological processes should not be overlooked. Empirical evidence on the biogenesis, maturation, function, and regulatory mechanisms of ncRNAs in the retina is still fragmented, particularly with regards to the heterogeneous class of lncRNAs. Nevertheless, the emerging picture is that ncRNAs are new pieces to the puzzle of IRDs and crucial to better understand the underlying molecular pathways and assess causative sequence variants. Another area that is currently unexplored in the retina is the possible presence of functional micropeptides that can be produced by ncRNAs (Makarewich and Olson 2017).

Because of the complexity of their molecular impact, defining the physiological role of ncRNAs that are relevant for retinal function will require comprehensive investigative approaches. These range from ncRNA annotation (which includes computational, molecular, and cellular studies) to the experimental validation in model systems in both physiological and pathological conditions.

The majority of ncRNAs studies in the retina have focused on their expression analysis and the cellular effects of altered transcript abundance. Structural approaches to evaluate functions in relation to sequence variations are limited to miRNAs (Conte et al. 2015; Hughes et al. 2011; Iliff et al. 2012; Liu et al. 2016; Mencia et al. 2009). The emerging role of lncRNAs in different human diseases suggests that also their sequence variation, and not only their deregulation (Kim et al. 2018), may contribute to some pathological retinal phenotypes. Application of high-throughput sequencing is revolutionizing our understanding of the pathogenic contribution of sequence variations in ncRNAs to inherited diseases and it will greatly support their structural analyses. This emerging area is expected to be relevant for lncRNAs, because their regulatory mechanisms may provide exciting research avenues to develop novel therapeutic approaches (Wahlestedt 2013).

For their high sequence conservation, miRNAs are promising biomarkers of diagnostic significance in various diseases, yet larger hopes come from their therapeutic

potential [reviewed in Rupaimoole and Slack (2017)]. Studies in model organisms are still critical to demonstrate the usefulness of miRNAs, whose therapeutic benefits have been observed in preclinical studies of IRDs (Karali et al., unpublished data). The vast genetic heterogeneity of IRDs has slowed down the development of therapeutic approaches that can be applied to a large number of patients. MiRNAs, as well as other types of ncRNAs, that impact retinal function could represent relevant targets for gene-independent therapeutic intervention. The recent commercialization of a gene therapy-based approach for IRDs indicates that novel molecular treatments can be translated to clinical products in the near future.

Acknowledgements We apologize to colleagues whose work could not be cited due to limitations in the number of citations. We would like to thank Enrico Maria Surace for critical reading of the manuscript. Our research is supported by the Foundation Fighting Blindness (Grant TA-NMT-0616-0701-TIGM) and Fondazione Telethon (Grant TGM16YGM02).

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ (2017) Targeting noncoding RNAs in disease. J Clin Invest 127:761–771. https://doi.org/10.1172/JCI84424

Adijanto J, Castorino JJ, Wang ZX, Maminishkis A, Grunwald GB, Philp NJ (2012) Microphthalmia-associated transcription factor (MITF) promotes differentiation of human retinal pigment epithelium (RPE) by regulating microRNAs-204/211 expression. J Biol Chem 287:20491–20503. https://doi.org/10.1074/jbc.M112.354761

Alfano G et al (2005) Natural antisense transcripts associated with genes involved in eye development. Hum Mol Genet 14:913–923. https://doi.org/10.1093/hmg/ddi084

Altshuler D, Lo Turco JJ, Rush J, Cepko C (1993) Taurine promotes the differentiation of a vertebrate retinal cell type in vitro. Development 119:1317–1328

Apte RS (2018) Gene therapy for retinal degeneration. Cell 173:5. https://doi.org/10.1016/j.cell.2018.03.021

Baba Y, Aihara Y, Watanabe S (2015) MicroRNA-7a regulates Muller glia differentiation by attenuating Notch3 expression. Exp Eye Res 138:59–65. https://doi.org/10.1016/j.exer.2015.06.022

Barbato S et al (2017) MiR-211 is essential for adult cone photoreceptor maintenance and visual function. Sci Rep 7:17004. https://doi.org/10.1038/s41598-017-17331-z

Bartel DP (2018) Metazoan MicroRNAs. Cell 173:20–51. https://doi.org/10.1016/j.cell.2018.03.006

Baudet ML et al (2011) miR-124 acts through CoREST to control onset of Sema3A sensitivity in navigating retinal growth cones. Nat Neurosci 15:29–38. https://doi.org/10.1038/nn.2979

Bellon A et al (2017) miR-182 regulates Slit2-mediated axon guidance by modulating the local translation of a specific mRNA. Cell Rep 18:1171–1186. https://doi.org/10.1016/j.celrep.2016.12.093

Blackshaw S et al (2004) Genomic analysis of mouse retinal development. PLoS Biol 2:E247. https://doi.org/10.1371/journal.pbio.0020247

Briggs JA, Wolvetang EJ, Mattick JS, Rinn JL, Barry G (2015) Mechanisms of long non-coding RNAs in mammalian nervous system development, plasticity, disease, and evolution. Neuron 88:861–877. https://doi.org/10.1016/j.neuron.2015.09.045

Burdon KP et al (2012) Glaucoma risk alleles at CDKN2B-AS1 are associated with lower intraocular pressure, normal-tension glaucoma, and advanced glaucoma. Ophthalmology 119:1539–1545. https://doi.org/10.1016/j.ophtha.2012.02.004

Busskamp V et al (2014) miRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function. Neuron 83:586–600. https://doi.org/10.1016/j.neuron.2014.06.020

Cammaerts S, Strazisar M, Dierckx J, Del Favero J, De Rijk P (2016) miRVaS: a tool to predict the impact of genetic variants on miRNAs. Nucleic Acids Res 44:e23. https://doi.org/10.1093/nar/gkv921

Carrella S, Barbato S, D'Agostino Y, Salierno FG, Manfredi A, Banfi S, Conte I (2015a) TGF-beta controls miR-181/ERK regulatory network during retinal axon specification and growth. PLoS One 10:e0144129. https://doi.org/10.1371/journal.pone.0144129

Carrella S et al (2015b) miR-181a/b control the assembly of visual circuitry by regulating retinal axon specification and growth. Dev Neurobiol 75:1252–1267. https://doi.org/10.1002/dneu.22282

Chen G et al (2013) LncRNADisease: a database for long-non-coding RNA-associated diseases. Nucleic Acids Res 41:D983–D986. https://doi.org/10.1093/nar/gks1099

Chen Q et al (2017a) Pathogenic role of microRNA-21 in diabetic retinopathy through downregulation of PPARalpha. Diabetes 66:1671–1682. https://doi.org/10.2337/db16-1246

Chen W, Yang S, Zhou Z, Zhao X, Zhong J, Reinach PS, Yan D (2017b) The long noncoding RNA landscape of the mouse eye. Invest Ophthalmol Vis Sci 58:6308–6317. https://doi.org/10.1167/iovs.17-22178

Consortium EP (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489:57–74. https://doi.org/10.1038/nature11247

Conte I, Carrella S, Avellino R, Karali M, Marco-Ferreres R, Bovolenta P, Banfi S (2010) miR-204 is required for lens and retinal development via Meis2 targeting. Proc Natl Acad Sci USA 107:15491–15496. https://doi.org/10.1073/pnas.0914785107

Conte I et al (2014) The combination of transcriptomics and informatics identifies pathways targeted by miR-204 during neurogenesis and axon guidance. Nucleic Acids Res 42:7793–7806. https://doi.org/10.1093/nar/gku498

Conte I et al (2015) MiR-204 is responsible for inherited retinal dystrophy associated with ocular coloboma. Proc Natl Acad Sci USA 112:E3236–E3245. https://doi.org/10.1073/pnas.1401464112

Corbo JC, Myers CA, Lawrence KA, Jadhav AP, Cepko CL (2007) A typology of photoreceptor gene expression patterns in the mouse. Proc Natl Acad Sci USA 104:12069–12074. https://doi.org/10.1073/pnas.0705465104

Damiani D et al (2008) Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. J Neurosci 28:4878–4887. https://doi.org/10.1523/JNEUROSCI.0828-08.2008

Decembrini S, Andreazzoli M, Barsacchi G, Cremisi F (2008) Dicer inactivation causes heterochronic retinogenesis in *Xenopus laevis*. Int J Dev Biol 52:1099–1103. https://doi.org/10.1387/ijdb.082646sd

Decembrini S et al (2009) MicroRNAs couple cell fate and developmental timing in retina. Proc Natl Acad Sci USA 106:21179–21184. https://doi.org/10.1073/pnas.0909167106

Deo M, Yu JY, Chung KH, Tippens M, Turner DL (2006) Detection of mammalian microRNA expression by in situ hybridization with RNA oligonucleotides. Dev Dyn 235:2538–2548

Di Iorio V et al (2017) Clinical and genetic evaluation of a cohort of pediatric patients with severe inherited retinal dystrophies. Genes (Basel). https://doi.org/10.3390/genes8100280

Ebert MS, Sharp PA (2012) Roles for microRNAs in conferring robustness to biological processes. Cell 149:515–524. https://doi.org/10.1016/j.cell.2012.04.005

Esteller M (2011) Non-coding RNAs in human disease. Nat Rev Genet 12:861–874. https://doi.org/10.1038/nrg3074

Fan J et al (2017) Maturation arrest in early postnatal sensory receptors by deletion of the miR-183/96/182 cluster in mouse. Proc Natl Acad Sci USA 114:E4271–E4280. https://doi.org/10.1073/pnas.1619442114

Farkas MH, Grant GR, White JA, Sousa ME, Consugar MB, Pierce EA (2013) Transcriptome analyses of the human retina identify unprecedented transcript diversity and 3.5 Mb of novel transcribed sequence via significant alternative splicing and novel genes. BMC Genom 14:486. https://doi.org/10.1186/1471-2164-14-486

Fu Y, Hou B, Weng C, Liu W, Dai J, Zhao C, Yin ZQ (2017) Functional ectopic neuritogenesis by retinal rod bipolar cells is regulated by miR-125b-5p during retinal remodeling in RCS rats. Sci Rep 7:1011. https://doi.org/10.1038/s41598-017-01261-x

Genini S, Guziewicz KE, Beltran WA, Aguirre GD (2014) Altered miRNA expression in canine retinas during normal development and in models of retinal degeneration. BMC Genom 15:172. https://doi.org/10.1186/1471-2164-15-172

Georgi SA, Reh TA (2010) Dicer is required for the transition from early to late progenitor state in the developing mouse retina. J Neurosci 30:4048–4061. https://doi.org/10.1523/JNEUROSCI.4982-09.2010

Gessert S, Bugner V, Tecza A, Pinker M, Kuhl M (2010) FMR1/FXR1 and the miRNA pathway are required for eye and neural crest development. Dev Biol 341:222–235. https://doi.org/10.1016/j.ydbio.2010.02.031

Gong J et al (2012) Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. Hum Mutat 33:254–263. https://doi.org/10.1002/humu.21641

Gong J, Liu W, Zhang J, Miao X, Guo AY (2015) lncRNASNP: a database of SNPs in lncRNAs and their potential functions in human and mouse. Nucleic Acids Res 43:D181–D186. https://doi.org/10.1093/nar/gku1000

Gutschner T, Hammerle M, Diederichs S (2013) MALAT1—a paradigm for long noncoding RNA function in cancer. J Mol Med (Berl) 91:791–801. https://doi.org/10.1007/s00109-013-1028-y

Han J, Gao L, Dong J, Bai J, Zhang M, Zheng J (2017) The expression profile of developmental stage-dependent circular RNA in the immature rat retina. Mol Vis 23:457–469

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495:384–388. https://doi.org/10.1038/nature11993

Hou Q et al (2013) Inhibitory effect of microRNA-34a on retinal pigment epithelial cell proliferation and migration. Invest Ophthalmol Vis Sci 54:6481–6488. https://doi.org/10.1167/iovs.13-11873

Huang XF, Huang ZQ, Fang XL, Chen ZJ, Cheng W, Jin ZB (2018) Retinal miRNAs variations in a large cohort of inherited retinal disease. Ophthalmic Genet 39:175–179. https://doi.org/10.1080/13816810.2017.1329448

Hughes AE, Bradley DT, Campbell M, Lechner J, Dash DP, Simpson DA, Willoughby CE (2011) Mutation altering the miR-184 seed region causes familial keratoconus with cataract. Am J Hum Genet 89:628–633. https://doi.org/10.1016/j.ajhg.2011.09.014

Iliff BW, Riazuddin SA, Gottsch JD (2012) A single-base substitution in the seed region of miR-184 causes EDICT syndrome. Invest Ophthalmol Vis Sci 53:348–353. https://doi.org/10.1167/iovs.11-8783

Ip JY, Nakagawa S (2012) Long non-coding RNAs in nuclear bodies. Dev Growth Differ 54:44–54. https://doi.org/10.1111/j.1440-169X.2011.01303.x

Ji HP et al (2017) MicroRNA-28 potentially regulates the photoreceptor lineage commitment of Muller glia-derived progenitors. Sci Rep 7:11374. https://doi.org/10.1038/s41598-017-11112-4

Kaneko H et al (2011) DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. Nature 471:325–330. https://doi.org/10.1038/nature09830

Karali M, Peluso I, Marigo V, Banfi S (2007) Identification and characterization of microRNAs expressed in the mouse eye. Invest Ophthalmol Vis Sci 48:509–515. https://doi.org/10.1167/iovs.06-0866

Karali M et al (2010) miRNeye: a microRNA expression atlas of the mouse eye. BMC Genom 11:715. https://doi.org/10.1186/1471-2164-11-715

Karali M et al (2016) High-resolution analysis of the human retina miRNome reveals isomiR variations and novel microRNAs. Nucleic Acids Res 44:1525–1540. https://doi.org/10.1093/nar/gkw039

Kim EJ, Grant GR, Bowman AS, Haider N, Gudiseva HV, Chavali VRM (2018) Complete transcriptome profiling of normal and age-related macular degeneration eye tissues reveals dysregulation of anti-sense transcription. Sci Rep 8:3040. https://doi.org/10.1038/s41598-018-21104-7

Kopp F, Mendell JT (2018) Functional classification and experimental dissection of long noncoding RNAs. Cell 172:393–407. https://doi.org/10.1016/j.cell.2018.01.011

Krol J et al (2010) Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. Cell 141:618–631. https://doi.org/10.1016/j.cell.2010.03.039

Krol J, Krol I, Alvarez CP, Fiscella M, Hierlemann A, Roska B, Filipowicz W (2015) A network comprising short and long noncoding RNAs and RNA helicase controls mouse retina architecture. Nat Commun 6:7305. https://doi.org/10.1038/ncomms8305

Kutty RK, Nagineni CN, Samuel W, Vijayasarathy C, Hooks JJ, Redmond TM (2010a) Inflammatory cytokines regulate microRNA-155 expression in human retinal pigment epithelial cells by activating JAK/STAT pathway. Biochem Biophys Res Commun 402:390–395. https://doi.org/10.1016/j.bbrc.2010.10.042

Kutty RK et al (2010b) MicroRNA expression in human retinal pigment epithelial (ARPE-19) cells: increased expression of microRNA-9 by *N*-(4-hydroxyphenyl)retinamide. Mol Vis 16:1475–1486

Kutty RK et al (2013) Differential regulation of microRNA-146a and microRNA-146b-5p in human retinal pigment epithelial cells by interleukin-1beta, tumor necrosis factor-alpha, and interferon-gamma. Mol Vis 19:737–750

La Torre A, Georgi S, Reh TA (2013) Conserved microRNA pathway regulates developmental timing of retinal neurogenesis. Proc Natl Acad Sci USA 110:E2362–E2370. https://doi.org/10.1073/pnas.1301837110

Lander ES et al (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921. https://doi.org/10.1038/35057062

Ledford H (2017) FDA advisers back gene therapy for rare form of blindness. Nature 550:314. https://doi.org/10.1038/nature.2017.22819

Legnini I et al (2017) Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. Mol Cell 66:22–37 e29. https://doi.org/10.1016/j.molcel.2017.02.017

Li Y, Piatigorsky J (2009) Targeted deletion of Dicer disrupts lens morphogenesis, corneal epithelium stratification, and whole eye development. Dev Dyn 238:2388–2400. https://doi.org/10.1002/dvdy.22056

Li M et al (2014) Comprehensive analysis of gene expression in human retina and supporting tissues. Hum Mol Genet 23:4001–4014. https://doi.org/10.1093/hmg/ddu114

Lin H, Qian J, Castillo AC, Long B, Keyes KT, Chen G, Ye Y (2011) Effect of miR-23 on oxidant-induced injury in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 52:6308–6314. https://doi.org/10.1167/iovs.10-6632

Liu JY et al (2014) Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus. Cell Death Dis 5:e1506. https://doi.org/10.1038/cddis.2014.466

Liu Y et al (2016) A common variant in MIR182 is associated with primary open-angle glaucoma in the NEIGHBORHOOD Consortium. Invest Ophthalmol Vis Sci 57:3974–3981. https://doi.org/10.1167/iovs.16-19688

Loscher CJ, Hokamp K, Kenna PF, Ivens AC, Humphries P, Palfi A, Farrar GJ (2007) Altered retinal microRNA expression profile in a mouse model of retinitis pigmentosa. Genome Biol 8:R248

Loscher CJ, Hokamp K, Wilson JH, Li T, Humphries P, Farrar GJ, Palfi A (2008) A common microRNA signature in mouse models of retinal degeneration. Exp Eye Res 87:529–534. https://doi.org/10.1016/j.exer.2008.08.016

Lumayag S et al (2013) Inactivation of the microRNA-183/96/182 cluster results in syndromic retinal degeneration. Proc Natl Acad Sci USA 110:E507–E516. https://doi.org/10.1073/pnas.1212655110

Makarewich CA, Olson EN (2017) Mining for micropeptides. Trends Cell Biol 27:685–696. https://doi.org/10.1016/j.tcb.2017.04.006

Marigo V (2007) Programmed cell death in retinal degeneration: targeting apoptosis in photoreceptors as potential therapy for retinal degeneration. Cell Cycle 6:652–655. https://doi.org/10.4161/cc.6.6.4029

Marler KJ et al (2014) BDNF promotes axon branching of retinal ganglion cells via miRNA-132 and p250GAP. J Neurosci 34:969–979. https://doi.org/10.1523/JNEUROSCI.1910-13.2014

Mattick JS (2011) The central role of RNA in human development and cognition. FEBS Lett 585:1600–1616. https://doi.org/10.1016/j.febslet.2011.05.001

Mellios N et al (2011) miR-132, an experience-dependent microRNA, is essential for visual cortex plasticity. Nat Neurosci 14:1240–1242. https://doi.org/10.1038/nn.2909

Memczak S et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333–338. https://doi.org/10.1038/nature11928

Mencia A et al (2009) Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. Nat Genet 41:609–613. https://doi.org/10.1038/ng.355

Meola N, Pizzo M, Alfano G, Surace EM, Banfi S (2012) The long noncoding RNA Vax2os1 controls the cell cycle progression of photoreceptor progenitors in the mouse retina. RNA 18:111–123. https://doi.org/10.1261/rna.029454.111

Michalik KM et al (2014) Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. Circ Res 114:1389–1397. https://doi.org/10.1161/CIRCRESAHA.114.303265

Murad N et al (2014) miR-184 regulates ezrin, LAMP-1 expression, affects phagocytosis in human retinal pigment epithelium and is downregulated in age-related macular degeneration. FEBS J 281:5251–5264. https://doi.org/10.1111/febs.13066

Mustafi D, Kevany BM, Bai X, Maeda T, Sears JE, Khalil AM, Palczewski K (2013) Evolutionarily conserved long intergenic noncoding RNAs in the eye. Hum Mol Genet 22:2992–3002. https://doi.org/10.1093/hmg/ddt156

Neveling K et al (2012) Next-generation genetic testing for retinitis pigmentosa. Hum Mutat 33:963–972. https://doi.org/10.1002/humu.22045

Ning S et al (2017) LincSNP 2.0: an updated database for linking disease-associated SNPs to human long non-coding RNAs and their TFBSs. Nucleic Acids Res 45:D74–D78. https://doi.org/10.1093/nar/gkw945

Ohana R et al (2015) MicroRNAs are essential for differentiation of the retinal pigmented epithelium and maturation of adjacent photoreceptors. Development 142:2487–2498. https://doi.org/10.1242/dev.121533

Pamudurti NR et al (2017) Translation of CircRNAs. Mol Cell 66:9–21 e27. https://doi.org/10.1016/j.molcel.2017.02.021

Pasquale LR et al (2013) CDKN2B-AS1 genotype–glaucoma feature correlations in primary open-angle glaucoma patients from the United States. Am J Ophthalmol 155:342–353 e345. https://doi.org/10.1016/j.ajo.2012.07.023

Pinelli M et al (2016) An atlas of gene expression and gene co-regulation in the human retina. Nucleic Acids Res. https://doi.org/10.1093/nar/gkw486

Pinter R, Hindges R (2010) Perturbations of microRNA function in mouse dicer mutants produce retinal defects and lead to aberrant axon pathfinding at the optic chiasm. PloS One 5:e10021. https://doi.org/10.1371/journal.pone.0010021

Quek XC et al (2015) lncRNAdb v2.0: expanding the reference database for functional long noncoding RNAs. Nucleic Acids Res 43:D168–D173. https://doi.org/10.1093/nar/gku988

Rapicavoli NA, Poth EM, Blackshaw S (2010) The long noncoding RNA RNCR2 directs mouse retinal cell specification. BMC Dev Biol 10:49. https://doi.org/10.1186/1471-213X-10-49

Rapicavoli NA, Poth EM, Zhu H, Blackshaw S (2011) The long noncoding RNA Six3OS acts in trans to regulate retinal development by modulating Six3 activity. Neural Dev 6:32. https://doi.org/10.1186/1749-8104-6-32

Ribeiro DM et al (2018) Protein complex scaffolding predicted as a prevalent function of long non-coding RNAs. Nucleic Acids Res 46:917–928. https://doi.org/10.1093/nar/gkx1169

Rinn JL, Chang HY (2012) Genome regulation by long noncoding RNAs. Annu Rev Biochem 81:145–166. https://doi.org/10.1146/annurev-biochem-051410-092902

Rivolta C, Sharon D, DeAngelis MM, Dryja TP (2002) Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. Hum Mol Genet 11:1219–1227

Rupaimoole R, Slack FJ (2017) MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 16:203–222. https://doi.org/10.1038/nrd.2016.246

Ryan DG, Oliveira-Fernandes M, Lavker RM (2006) MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. Mol Vis 12:1175–1184

Sancho-Pelluz J et al (2008) Photoreceptor cell death mechanisms in inherited retinal degeneration. Mol Neurobiol 38:253–269. https://doi.org/10.1007/s12035-008-8045-9

Sanuki R et al (2011) miR-124a is required for hippocampal axogenesis and retinal cone survival through Lhx2 suppression. Nat Neurosci 14:1125–1134. https://doi.org/10.1038/nn.2897

Shaham O et al (2013) Pax6 regulates gene expression in the vertebrate lens through miR-204. PLoS Genet 9:e1003357. https://doi.org/10.1371/journal.pgen.1003357

Sone M, Hayashi T, Tarui H, Agata K, Takeichi M, Nakagawa S (2007) The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. J Cell Sci 120:2498–2506. https://doi.org/10.1242/jcs.009357

Soundara Pandi SP, Chen M, Guduric-Fuchs J, Xu H, Simpson DA (2013) Extremely complex populations of small RNAs in the mouse retina and RPE/choroid. Invest Ophthalmol Vis Sci 54:8140–8151. https://doi.org/10.1167/iovs.13-12631

Sundermeier TR, Palczewski K (2016) The impact of microRNA gene regulation on the survival and function of mature cell types in the eye. FASEB J 30:23–33. https://doi.org/10.1096/fj.15-279745

Sundermeier TR et al (2014) DICER1 is essential for survival of postmitotic rod photoreceptor cells in mice. FASEB J 28:3780–3791. https://doi.org/10.1096/fj.14-254292

Van Cauwenbergh C et al (2017) arrEYE: a customized platform for high-resolution copy number analysis of coding and noncoding regions of known and candidate retinal dystrophy genes and retinal noncoding RNAs. Genet Med 19:457–466. https://doi.org/10.1038/gim.2016.119

Venter JC et al (2001) The sequence of the human genome. Science (New York) 291:1304–1351. https://doi.org/10.1126/science.1058040

Wahlestedt C (2013) Targeting long non-coding RNA to therapeutically upregulate gene expression. Nat Rev Drug Discov 12:433–446. https://doi.org/10.1038/nrd4018

Walker JC, Harland RM (2009) microRNA-24a is required to repress apoptosis in the developing neural retina. Genes Dev 23:1046–1051. https://doi.org/10.1101/gad.1777709

Wang FE et al (2010) MicroRNA-204/211 alters epithelial physiology. FASEB J 24:1552–1571. https://doi.org/10.1096/fj.08-125856

Wohl SG, Reh TA (2016) The microRNA expression profile of mouse Muller glia in vivo and in vitro. Sci Rep 6:35423. https://doi.org/10.1038/srep35423

Wohl SG, Jorstad NL, Levine EM, Reh TA (2017) Muller glial microRNAs are required for the maintenance of glial homeostasis and retinal architecture. Nat Commun 8:1603. https://doi.org/10.1038/s41467-017-01624-y

Wright AF, Chakarova CF, Abd El-Aziz MM, Bhattacharya SS (2010) Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. Nat Rev Genet 11:273–284. https://doi.org/10.1038/nrg2717

Xiang L et al (2017) miR-183/96 plays a pivotal regulatory role in mouse photoreceptor maturation and maintenance. Proc Natl Acad Sci USA 114:6376–6381. https://doi.org/10.1073/pnas.1618757114

Xie C et al (2014) NONCODEv4: exploring the world of long noncoding RNA genes. Nucleic Acids Res 42:D98–D103. https://doi.org/10.1093/nar/gkt1222

Xu S, Witmer PD, Lumayag S, Kovacs B, Valle D (2007) MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. J Biol Chem 282:25053–25066

Yao J et al (2016) Long non-coding RNA MALAT1 regulates retinal neurodegeneration through CREB signaling. EMBO Mol Med 8:346–362. https://doi.org/10.15252/emmm.201505725

Young TL, Matsuda T, Cepko CL (2005) The noncoding RNA taurine upregulated gene 1 is required for differentiation of the murine retina. Curr Biol 15:501–512. https://doi.org/10.1016/j.cub.2005.02.027

Zelinger L, Karakulah G, Chaitankar V, Kim JW, Yang HJ, Brooks MJ, Swaroop A (2017) Regulation of noncoding transcriptome in developing photoreceptors by rod differentiation factor NRL. Invest Ophthalmol Vis Sci 58:4422–4435. https://doi.org/10.1167/iovs.17-21805

Zhu Q, Sun W, Okano K, Chen Y, Zhang N, Maeda T, Palczewski K (2011) Sponge transgenic mouse model reveals important roles for the microRNA-183 (miR-183)/96/182 cluster in postmitotic photoreceptors of the retina. J Biol Chem 286:31749–31760. https://doi.org/10.1074/jbc.M111.259028

Zhu YX et al (2018) Long non-coding RNA MEG3 silencing protects against light-induced retinal degeneration. Biochem Biophys Res Commun 496:1236–1242. https://doi.org/10.1016/j.bbrc.2018.01.177