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Vision from next generation sequencing: Multi-dimensional genome-wide analysis for producing gene regulatory networks underlying retinal development, aging and disease

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ABSTRACT

Genomics and genetics have invaded all aspects of biology and medicine, opening uncharted territory for scientific exploration. The definition of "gene" itself has become ambiguous, and the central dogma is continuously being revised and expanded. Computational biology and computational medicine are no longer intellectual domains of the chosen few. Next generation sequencing (NGS) technology, together with novel methods of pattern recognition and network analyses, has revolutionized the way we think about fundamental biological mechanisms and cellular pathways. In this review, we discuss NGS-based genome-wide approaches that can provide deeper insights into retinal development, aging and disease pathogenesis. We first focus on gene regulatory networks (GRNs) that govern the differentiation of retinal photoreceptors and modulate adaptive response during aging. Then, we discuss NGS technology in the context of retinal disease and develop a vision for therapies based on network biology. We should emphasize that basic strategies for network construction and analyses can be transported to any tissue or cell type. We believe that specific and uniform guidelines are required for generation of genome, transcriptome and epigenome data to facilitate comparative analysis and integration of multi-dimensional data sets, and for constructing networks underlying complex biological processes. As cellular homeostasis and organismal survival are dependent on gene–gene and gene–environment interactions, we believe that network-based biology will provide the foundation for deciphering disease mechanisms and discovering novel drug targets for retinal neurodegenerative diseases.

Contents

Abbreviations: AMD, Age-related macular degeneration; ChIP, Chromatin immunoprecipitation; ChIP-seq, Chromatin immunoprecipitation-sequencing; CLIA, Clinical laboratory improvement amendments; eQTL, Expression quantitative trait locus; GA, Geographic atrophy; GRN, Gene regulatory network; GWAS, Genome-wide association study; H3K4me2(3), Histone H3 lysine 4 di(tri)methylation; H3K27me3, Histone H3 lysine 27 trimethylation; M cone, Medium wavelength sensitive cone; MIAME, Minimum information about a microarray experiment; NGS, Next generation sequencing; ONL, Outer nuclear layer; PCA, Principal component analysis; RDD, Retinal degenerative disease; RNA-seq, RNA-sequencing; RPE, Retinal pigment epithelium; S cone, Short wave length sensitive cone; SNP, Single nucleotide polymorphism; WES, Whole exome sequencing; WGS, Whole genome sequencing.

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1. Introduction

Ramon y Cajal was the first to recognize the retina as 'a true nervous center, as a peripheral extension of the central nervous system' (Ramon y Cajal, 1893 La retine des Vertebres; translation taken from Piccolino 1989 Santiago Ramon Y Cajal, the retina and the neuron theory). The retina has thus attained the distinction of being an attractive model to investigate fundamental biology and therapy of the nervous system. The vertebrate retina is composed of six major neuronal cell types that are organized in three cellular layers forming exquisite neuronal circuits for detection of visual information (Fig. 1A). Light is captured by photoreceptors; visual signals then undergo enhancement, integration and processing through bipolar, horizontal and amacrine cells and by varied usage of parallel synaptic circuits, before eventually being transmitted via ganglion cells to the brain (Lamb et al., 2007; Masland, 2001). This remarkable complexity makes the retina extremely vulnerable to degeneration caused by genetic defects that eventually lead to vision loss. In fact, a vast majority of incurable blindness is caused by dysfunction or death of retinal photoreceptors (Wright et al., 2010). Thus, knowledge-based design of therapies for blinding retinal degenerative diseases (RDDs) depends on better understanding of pathways that are associated with: (i) normal development of the retinal neurons (specifically photoreceptors) from progenitors and stem cells; (ii) assembly of synaptic circuits; (iii) cellular adaptation and homeostasis; (iv) response to aging and inherited genomic changes; and (v) disease pathogenesis.

The human genome of almost three billion nucleotides contains the complete instruction for generating over 100 billion neurons and 150 trillion synapses (Pakkenberg et al., 2003; Williams and Herrup, 1988). Despite tremendous cellular heterogeneity and functional complexity, our genome encompasses only approximately 20,000 protein-coding genes (Ezkurdia et al., 2014) (http://www.gencodegenes.org), fewer than some of the "apparently" less-evolved organisms (Carninci et al., 2005; de Laat and Duboule, 2013; Pruitt et al., 2009). However, alternative splicing and use of alternate promoters can produce unique gene expression patterns associated with fate determination and cell-type specific functions (Wang et al., 2008). Furthermore, the non-protein-coding genomic DNA [the so-called "junk DNA" (Balakirev and Ayala, 2003; Brosius and Gould, 1992; Ohno, 1972)] is increasingly being recognized as an important "regulator" of the coding information (Palazzo and Gregory, 2014). *Cis*-regulatory sequences include the genomic code for binding of transcription complexes that dictate quantitatively precise as well as cell type- and stage-specific gene expression (Levine et al., 2014; Shlyueva et al., 2014). In addition, though the coding transcripts are relatively small in number, a plethora of small and long non-coding RNAs are detected in different cell types and believed to modulate gene expression and homeostasis (Morris and Mattick, 2014; Slack, 2006). Normal gene expression patterns, established by combinatorial action of multiple regulatory modules, can be disrupted in response to changes in microenvironment and/or by inherited genomic variations/mutations, resulting in altered physiology and phenotype (including disease) (Lagha et al., 2012). Thus, comprehensive understanding of integrated gene regulatory networks (GRNs) is critical for deciphering normal development and homeostatic mechanisms as well as pathways leading to disease.

Remarkable advances have been made in elucidating molecules and pathways that control retinal cell fate specification and differentiation (Agathocleous and Harris, 2009; Bassett and Wallace,

2012). Defects in over 200 genes have been associated with inherited monogenic RDDs (RetNet; https://sph.uth.edu/retnet/). We have also begun to dissect the complexities of multifactorial retinal diseases that afflict large segments of the population (Fritsche et al., 2014; Kuo et al., 2014). Impressive gene- and stem cell-based approaches are being developed for treatment of retinal disease; nonetheless, individual research projects have generally centered on single genes or molecules or on a single functional pathway, and therefore the biomedical progress has not been able to keep pace with public expectations (Bull and Martin, 2011; Cuenca et al., 2014; Lindvall and Kokaia, 2010; Rowe-Rendleman et al., 2014). We must recognize that genes/RNAs/proteins are part of complex molecular networks and biochemical pathways and that the disruption of one creates a domino effect leading to multiple changes (including in gene expression patterns) not only within the affected cell(s) but also in neighboring cells and tissues. Therefore, holistic assessment of biological components and of interaction networks constitutes essential tasks in exploring retinal development and disease in the post-genomic era (Barabasi and Oltvai, 2004; Hwang et al., 2012; Kitano, 2002; Schadt, 2009; Vidal et al., 2011; Yu et al., 2004b; Zhou et al., 2014).

The emergence of next generation sequencing (NGS) technology has dramatically broadened the scope in which diverse cellular processes can be interrogated, setting the stage for system-level approaches to comprehend retinal GRNs. In this review, we will describe the current status of genome-wide strategies as applied to the retina and discuss in which direction the field appears to be moving. As the task ahead is daunting yet feasible, we believe that a collaborative consortium-like approach, elegantly demonstrated by genetic studies of age-related macular degeneration (AMD) (Cipriani and International AMD Genomics Consortium, 2014; Fritsche et al., 2013; Fritsche and International AMD Genomics Consortium, 2014), is required to elucidate retinal GRNs that control development, maintain homeostasis and modulate responses to aging, environment and inherited disease-causing variants or mutations. We have therefore taken the liberty to put forward a framework for integrative and comparative analysis of NGS data, with a goal to build comprehensive GRNs pertaining to the retina. Here, we have primarily focused on the photoreceptors because of their association with incurable blinding retinal diseases. Rod photoreceptors, in particular, constitute nearly 80% of retinal neurons in many mammalian species, including mouse and human (Hendrickson et al., 2008; Lamb et al., 2007; Rapaport et al., 2004); thus NGS data sets from retina are especially rich in rod photoreceptor-related information. We envision the photoreceptors as an ideal paradigm for initiating multi-dimensional, system-level studies that can be widely applicable to other cell types, especially in the retina but broadly for the central nervous system.

2. Systems biology approaches

Biomedical research is mostly driven from phenotypic observations and curiosity to understand biological phenomena. For example, forward genetic screening using animal models, such as *Drosophila melanogaster* (fruit fly), *Caenorhabditis elegans* and *Mus musculus* (mouse), have led to identification of many essential genes and their function. Similarly, human genetic studies have focused on identifying genes linked to specific phenotypes or traits, thereby providing significant insights into biological basis of the disease. In addition, biochemical, genetic and molecular biology approaches have further elucidated mechanism(s) of action of specific molecules and cellular components. Though biological pathways can be deciphered by compiling individual molecular functions and binary relationships, current approaches are not optimal in mapping complex regulatory interactions among discrete constituents. Systems biology takes a bird's eye view of cellular function with a goal of delineating molecular interactions and crosstalk among the pathways (Ideker et al., 2001; Ryan et al., 2013; Sorger, 2005), complementing the reductionist approach (Fig. 1B). Although the concept of "systems biology" is not new (Trewavas, 2006), it has had only limited success because of unavailability of extensive data sets.

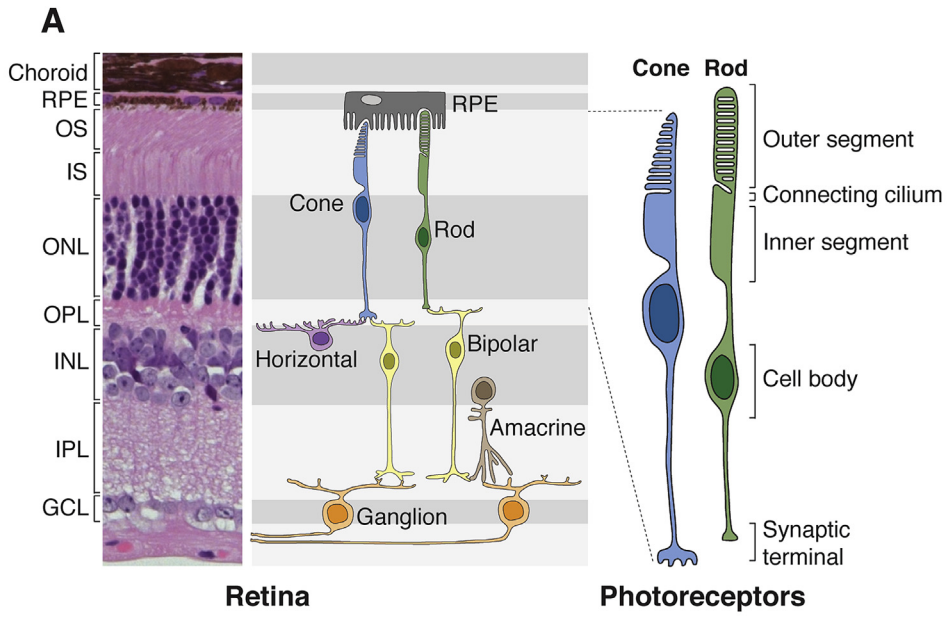
Recent advent of NGS and computational methodology has permitted biologists to generate system-wide data sets, leading to rapid advances in this field. Three major components can be assigned to systems biology, especially when we discuss GRNs (Fig. 2A):

(i) Prediction of regulatory networks via generation of various high throughput data sets and by computational analysis
(ii) Extension and/or refinement of the networks by superimposing measurements made at multiple levels of cellular constituents such as DNA, transcribed sequences, chromatin state, proteins, and metabolites.
(iii) Assessment of system's response to time, risk factors or interaction with its microenvironment (e.g., neighboring cells/tissues)

A comprehensive system-level understanding of a cell/tissue/organism requires integrated analysis of all intracellular molecular interactions and pathways, including data sets from proteomic and metabolomic studies. However, high throughput data from such investigations is not readily obtainable for most tissues including the retina (or other ocular cell types). We therefore limit our discussion to genetic and epigenetic control networks that can be measured by NGS.

2.1. High throughput data generation

NGS is a versatile technology that can be coupled with an endless list of classical assay strategies (Table 1), enabling genome-wide measurements of DNA sequence variations as well as components of GRNs at both transcriptional and epigenetic levels. High throughput genome-wide data generation requires a careful study design, which can be summarized in two complementary strategies (Fig. 2A). In the first approach, the output is a nearly complete catalog of components of a given class of biomolecules with relevant quantitative information. For example, GRNs associated with rod photoreceptors can be constructed by integrating a comprehensive and quantitative catalog of mRNA transcripts (using RNA-seq), target genes for key transcription factors (using chromatin immunoprecipitation-sequencing, termed ChIP-seq), global profiles of epigenome [histone modifications using ChIP-seq, and DNA methylation using different NGS-based methods such as reduced representation bisulfite sequencing (RRBS)], and other regulatory molecules (such as miRNA, using small RNA-seq). In this review, we will not discuss NGS-based methods (summarized in Table 1), which have been subjects of excellent reviews recently (Furey, 2012; Metzker, 2010; Park, 2009; Telese et al., 2013; Wang et al., 2009). With these data, regulatory networks can be inferred based on co-expression and prior knowledge of interactions though additional experiments are needed for validation. In the second strategy, a 'seed network' is employed for the experimental design. A known molecular interaction or functional hub is altered experimentally, and the consequences of system perturbation are interrogated genome-wide. For example, gene knock-out studies followed by mRNA or epigenomic profiles would greatly assist in deciphering the associated GRN. While the former approach charts all the possible physical or regulatory interactions, the latter refines the networks by directly examining functional relationships.



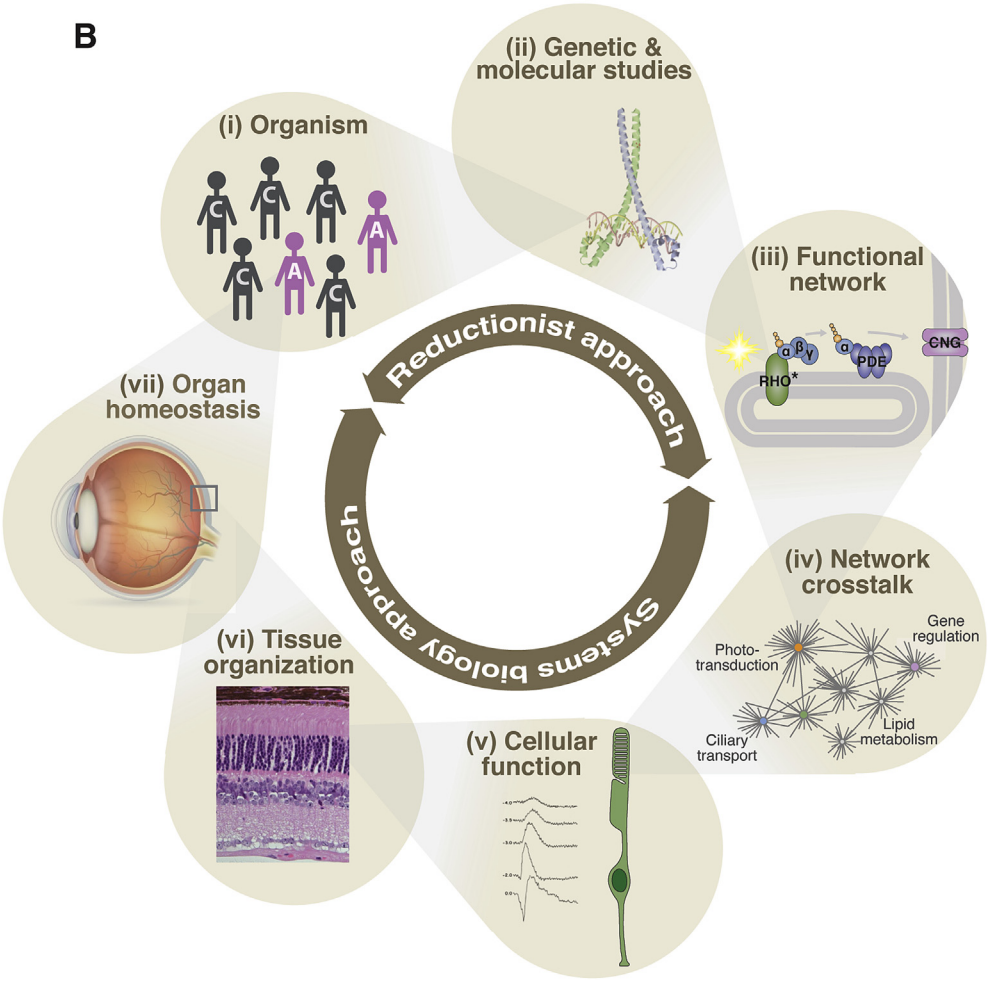


Fig. 1. The mammalian retina and systems biology approaches. **A**, A representative retinal anatomy is shown by hematoxylin and eosin stained cross section of an adult mouse retina (left) and by schematics (center and right). In the mammalian retina, six main neuronal classes are organized into three nuclear layers [outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL)] and form synaptic connections in two plexiform layers [outer plexiform layer (OPL) and inner plexiform layer (IPL)]. Cone and rod photoreceptors comprise the outer retina with their cell bodies situated in ONL and their inner and outer segments (IS and OS, respectively) located between ONL and the retinal pigment epithelium (RPE). RPE microvilli ensheath the outer segments, supporting phototransduction and photoreceptor survival. Photoreceptors transfer visual information through retinal interneurons to ganglion cells. **B**, To date, population-based genetic analyses (i) and genetic and molecular studies of known genes (ii) have been major strategies in retinal research.

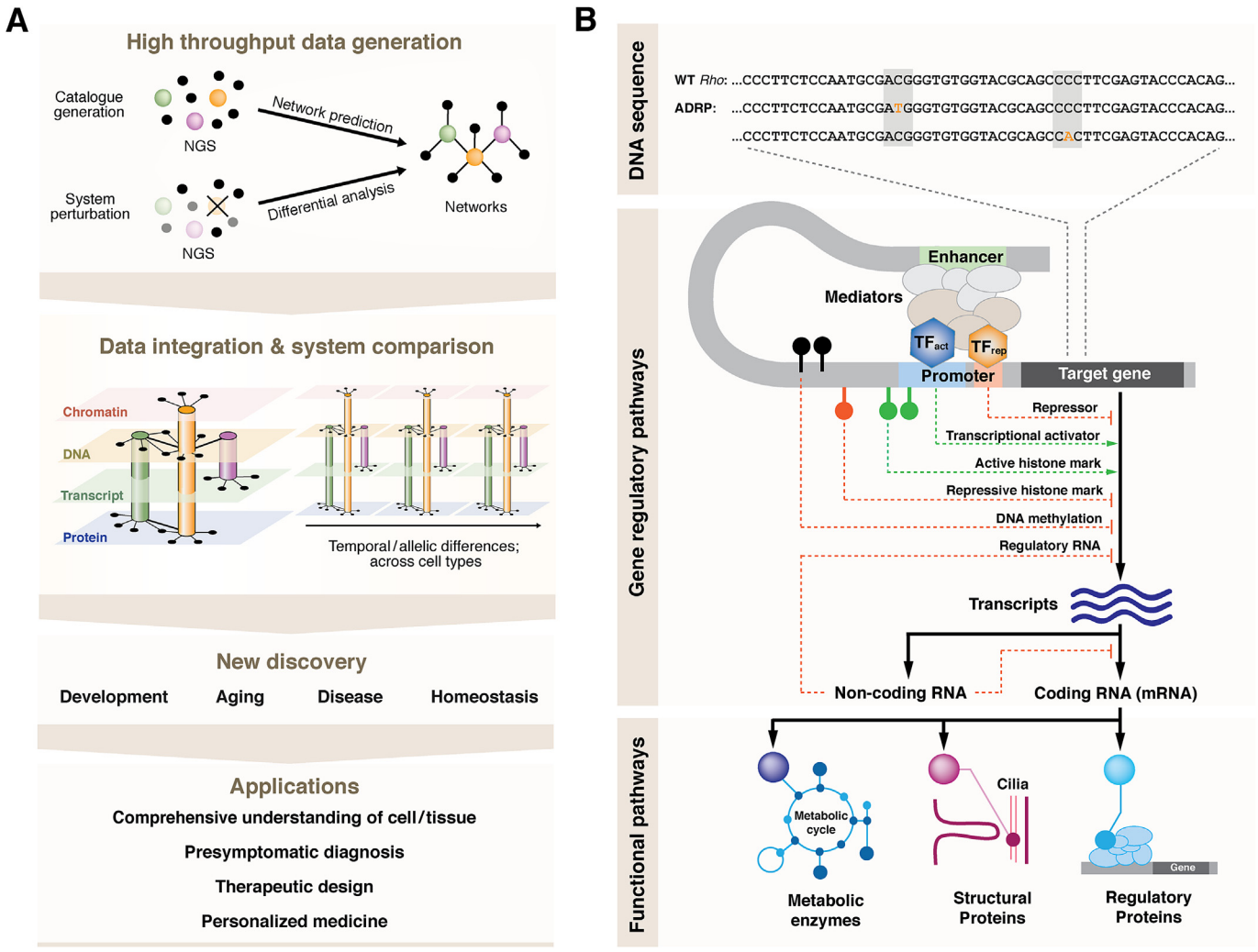


Fig. 2. Strategies and aims of system-wide, multi-dimensional data analysis. **A**, Networks of a tissue or a cell type of interest can be inferred from high throughput data analysis. Next generation sequencing (NGS) allows cataloging cellular constituents at a steady state and functional interactions when combined with system perturbation and differential analysis. Molecular interactions are not confined to only one molecular type such as DNA, transcripts, chromatin marks or proteins. Thus, multi-dimensional data integration further refines the networks. In addition, comparative analysis is critical as discrete cells are subjected to temporal changes (i.e., development and aging) as well as interactions with neighboring cells and the microenvironment, which evoke physiological modulation of the tissue and eventually of the organism. These holistic approaches will lead to new discoveries of the biological systems and offer broad application. **B**, Cellular function is regulated at multiple levels. The DNA sequence contains the instructions of protein coding and gene regulation, and diverse gene regulatory mechanisms ensure expression of a unique set of components highly specialized for each cell identity. Intrinsic and/or exogenous damage to any level can lead to deleterious effects on function and survival of the system. TF_{act}, transcription activator; TF_{rep}, transcription repressor.

2.1.1. *Gene expression profiling*

Transcriptome analysis is central to comprehensive understanding of complex biological systems such as photoreceptors (Swaroop and Zack, 2002). In RNA-seq assays, total RNA isolated from a cell type or a tissue of interest is converted to cDNA and deep-sequenced to obtain a comprehensive catalog of transcripts (Mortazavi et al., 2008). Although mRNA-seq is most commonly conducted, genome-wide profiling of regulatory RNAs is also possible and provides an additional layer of information regarding gene regulation (Morin et al., 2008). RNA-seq offers many advantages over hybridization-based expression profiling (such as microarrays), permitting greater sensitivity and dynamic range in transcript detection (Zhao et al., 2014) and more accurate identification of alternatively spliced transcripts (Shen et al., 2014). As detection and quantification of transcripts is not limited to the current annotation, RNA-seq analysis enables discovery of novel genes and of new transcripts from annotated genes (Wang et al., 2009). Finally, RNA-seq can be performed even at the level of a

Such studies have long been a major driving force in identifying retinal disease genes and in revealing the function of the disease genes and their functional or structural associates. The "bottom up" approaches have been undertaken to build functional networks (iii) that are critical for retinal function (e.g., phototransduction pathway) by compiling functional/structural relationships among individual molecules. Considering widespread crosstalk between functional and/or regulatory networks (iv), system-wide measurement of various biomolecules is critical in constructing a comprehensive map of complex intermolecular regulatory interactions. Systems biology approach thus complements the traditional reductionist methodologies. The recent advent of next generation sequencing technologies has enabled system-level assessment of various biological processes. Computational analysis of next generation sequencing and other types of high throughput data, ideally by integrating multiple data sets, allows a holistic approach to elucidate cellular function (v) as well as homeostasis of tissues (vi), organs (vii), and organisms (i). RHO, rhodopsin; PDE, phosphodiesterase; CNG, cyclic nucleotide gated channel.

Table 1
High throughput techniques used in system-level data generation at multiple levels of biological pathways.

	Method	Information	Strategy	Reference
Genome	WGS (whole genome sequencing)	Genetic variation in the entire genome	High throughput sequencing of the complete genome	
	WES (whole exome sequencing)	Genetic variations in the exome	Exome capture followed by high throughput sequencing.	(Ng et al., 2009)
	Repli-seq (replication sequencing)	Temporal mapping of DNA replication	BrdU labeling of newly replicated DNA and FACS-mediated fractionation of cell populations according to cell cycle phases followed by high throughput sequencing.	(Hansen et al., 2010)
	ChIP-seq (chromatin immunoprecipitation sequencing), ChIP-exo, or DamID	Genome-wide protein binding sites	Genome-wide profiling of occupancy by a protein of interest. A specific antibody is used to isolate protein-bound chromatin areas (ChIP-seq), and the resolution of the assay can be further enhanced by employing exonuclease digestion of protein-bound DNA ends (ChIP-exo). In cases that the specific ChIP quality antibody is not available, the protein of interest that is fused to *E. Coli* DNA adenine methyltransferase (Dam) and therefore methylates DNA adjacent to the binding sites can be used instead.	(Barski et al., 2007), (Rhee and Pugh, 2011)
Transcriptome	RNA-seq	Gene expression profile	Genome-wide profiling of transcripts. Typically, polyadenylated RNAs are enriched for subsequent cDNA synthesis and high throughput sequencing.	(Mortazavi et al., 2008)
	small RNA-seq	Regulatory RNA profile	Genome-wide profiling of small RNA species by high throughput sequencing with modified cDNA synthesis protocols.	(Morin et al., 2008)
	Deep CAGE (cap analysis of gene expression)	Transcription start sites	Capturing cDNA tags corresponding 5′ ends of transcripts by cap trapping system followed by high throughput sequencing.	(de Hoon and Hayashizaki, 2008)
	RNA-PET (RNA paired end tag) sequencing	5′ and 3′ ends of transcripts	Profiling of 5′ and 3′ end tags of transcripts. This method is especially powerful in detection of fusion transcripts.	(Ruan and Ruan, 2012)
	GRO-seq (global run-on sequencing)	Nascent transcripts	Global mapping of transcriptionally active polymerase density by genome-wide profiling of nascent RNA.	(Core et al., 2008)
	Ribo-seq (ribosome profiling)	Transcripts engaged with translation	Profiling of ribosome-protected mRNA fragments by deep sequencing.	(Ingolia et al., 2009)
	CLIP-seq (Cross-linking immunoprecipitation sequencing) or RIP-seq (RNA immunoprecipitation sequencing)	Transcripts bound by a protein of interest	High throughput sequencing of cDNA made from RNA pulled down with antibody against a protein of interest. RNA-protein complexes are cross-liked with UV in CLIP-seq. In RIP-seq, no cross-linking is necessary although formaldehyde-mediated cross-linking can be included.	(Yeo et al., 2009) (Zhao et al., 2010)
Epigenome & chromatin conformation	Histone modification ChIP-seq	Genome-wide map of histone modification	ChIP-seq can be also applied for genome-wide profiling of histone modifications. For histones, ChIP can be also performed without cross-linking of proteins to DNA (native ChIP).	(Barski et al., 2007)
	MeDIP-seq (methylated DNA immunoprecipitation sequencing), WGBS (whole genome bisulfite sequencing) or RRBS (reduced representation bisulfite sequencing)	Genome-wide map of DNA methylation	Genome-wide mapping of methylated cytosine (5 mC) of DNA. Methylated DNA can be detected by various methods such as immunoprecipitation using 5 mC-specific antibody (MeDIP-seq), MBD2b/MBD3L1 protein complexes with high affinity to 5 mC (MIRA-seq) and sodium bisulfite conversion of unmethylated C to U (WGBS or RRBS).	(Berman et al., 2012; Gu et al., 2011; Lister et al., 2009; Weber et al., 2005)
	hMeDIP-seq (hydroxymethylated DNA immunoprecipitation sequencing), TAB-seq (Tet-assisted bisulfite sequencing)	Genome-wide map of DNA hydroxy-methylation	Genome-wide mapping of hydroxymethylated cytosine (5 hmC) of DNA. 5 hmC is either isolated by immunoprecipitation (hMeDIP-seq) or by glucosylation-mediated protection of 5 hmC sites from the subsequent Tet enzyme assisted conversion of 5 hmC or MspI digestion (TAB-seq and RRHP-seq, respectively).	(Jin et al., 2011; Song et al., 2011a; Wu et al., 2011)
	MNase-seq (micrococcal nuclease sequencing)	Nucleosome positioning	MNase digestion of chromatin followed by high throughput sequencing. MNase preferentially cuts at the linker DNA between nucleosomes.	(Henikoff et al., 2011)
	4C-seq (circular chromosome conformation capture sequencing) or HiC-seq	Chromatin–chromatin interaction	Variations of chromatin conformation capture (3C) assays to detect intra- or interchromosomal interaction genome-wide. 4C-seq detects chromatin areas interacting with one genomic locus of interest in genome-wide manner, whereas HiC-seq captures all detectable chromatin-to-chromatin interactions.	(Lieberman-Aiden et al., 2009; Rao et al., 2014; Splinter et al., 2012)
	ChIA-PET (chromatin interaction analysis by paired-end tag sequencing)	Chromatin–chromatin interaction mediated by a protein of interest	Chromatin immunoprecipitation and ligation of the adjacent DNA ends followed by high throughput sequencing.	(Zhang et al., 2012)
	DNase-seq (DNase I hypersensitivity sequencing), FAIRE-seq (formaldehyde assisted isolation of regulatory sequences), or ATAC-seq (assay for transposase-accessible chromatin sequencing)	Open chromatin	Genome-wide mapping of open chromatin. Open chromatin can be selectively isolated by the following characteristics: high sensitivity to DNase I digestion (DNase-seq), segregation in aqueous phase upon phenol/chloroform extraction (FAIRE-seq), or preferential transposon integration.	(Crawford et al., 2006) (Giresi et al., 2007)

single cell (Shapiro et al., 2013). With increasing accessibility and affordability, RNA-seq analysis is fast becoming a routine procedure replacing microarrays.

2.1.2. Transcriptional and epigenetic regulation

Gene expression is controlled by combinatorial action of diverse regulatory programs (Fig. 2B). Interaction of transcription factors with specific *cis*-regulatory motifs is one of the primary mechanisms underlying temporal and cell type-specific gene regulation. ChIP, a widely used procedure to study transcription factor occupancy *in vivo* (Gilmour and Lis, 1985; Weinmann and Farnham, 2002), can now be applied in conjunction with tiling array (ChIP-on-chip) (Ren et al., 2000) or NGS (ChIP-seq) (Barski et al., 2007) to examine genome-wide binding of a specific protein. NGS-based methods ('-seq' assays) are now being used to delineate global profiles of chromatin states (such as histone modification, DNA methylation, nucleosome positioning and chromatin accessibility) and to identify novel fundamental mechanisms underlying gene transcription (see Table 1).

2.1.3. DNA sequence variation

The human genome includes millions of sequence variations, primarily in the non-coding regions; of these, tens of thousands may be unique to an individual (Schaibley et al., 2013), and many can even be linked to disease phenotype. Linkage analysis has traditionally been successful in identifying the disease variant/gene in monogenic RDDs, and genome-wide association studies (GWAS) have identified variants associated with complex traits. More recently, profiling of genetic variants using NGS methods, such as whole exome sequencing (WES) and targeted or whole genome sequencing (WGS), have transformed human genetic research. The identified variants/mutations can provide valuable insights into normal biology as well as elucidate mechanism(s) of disease pathogenesis. For instance, gene ontology analysis of 142 genes implicated in photoreceptor degeneration has identified a handful of biological pathways including those associated with cilia biogenesis, lipid metabolism, and phototransduction (Wright et al., 2010). Similarly, genetic variants associated with susceptibility to AMD have revealed the involvement of complement regulation, cholesterol transport and extracellular matrix remodeling in disease pathogenesis (Fritsche et al., 2014).

2.2. Data integration

Once high throughput data is collected, computational tools are employed to predict and construct GRNs. Although system-level measurements are made at discrete levels such as transcriptome, DNA-protein interaction, epigenome and genome, intermolecular interactions that form a network are generally not confined to a single category. Thus, the experimental data pertaining to distinct aspects of the network must be analyzed simultaneously and assimilated to identify new patterns of a biological system, which single data sets fail to detect.

Generation of networks by integrating multiple data sets necessitates that data are acquired under conditions of minimal variability; e.g., from a single cell type since cellular heterogeneity in the retina would create high transcriptional noise affecting the generation of a viable and verifiable GRN across development and disease states. Ideally, transcriptional and regulatory data for network formation should be obtained from a specific cell type at distinct stages of differentiation, aging or disease. Efforts have been made to isolate individual neuronal cells of the retina for genome-wide analysis. Such attempts, however, have been few and primarily limited to gene expression profiling (Akimoto et al., 2006; Siegert et al., 2012; Trimarchi et al., 2007). Table 2 lists public databases containing high throughput data relevant to studies of the retina.

2.3. System comparison

Biological systems are highly dynamic and undergo progressive transition over time (Hwang et al., 2012). During development, intrinsic and extrinsic factors sequentially restrict cell fates and specify morphology and function most suited for a given cellular identity. Cumulative adaptive responses to internal and/or environmental challenges over a lifespan also impact spatio-temporal architecture of a system. Therefore, comparisons along time series data sets represent valuable strategies to assess system dynamics. Additionally, continuous fluctuations imposed by microenvironment and/or intrinsic genetic variations may induce changes beyond the cell tolerance level and manifest as a disease. The use of animal models for retinal traits or diseases provides a powerful tool to evaluate direct influence of genetic mutation(s) or experimental stress on networks as relatively little variation is expected in congenic strains raised under uniform conditions. Another important factor in system dynamics is variability among cell types exhibiting functional interaction such as between photoreceptors and retinal pigment epithelium (RPE); for example, defects in RPE are associated with photoreceptor dysfunction or death (Strauss, 2005). Even cells of the same neuronal type may display high variability based on temporal context and spatial organization (Trimarchi et al., 2007).

Change in system architecture can be ascertained from human population genetics using control individuals and patients with a specific disease/trait (e.g., AMD). Such genetic association studies exploit naturally occurring differences that cannot necessarily be generated by experimental means. However, it should be noted that the strength of data sets depends on the size of the cohort and penetrance of a specific variant/mutation. Elucidation of multifactorial complex biological phenomena, such as aging and pathogenesis of complex disease, can especially benefit from comparative system-level analysis using large NGS-based genomic data.

In the following sections, we will elaborate on system-level analysis of rod photoreceptors in three disciplines: development (Section 3), aging (Section 4) and degenerative disease (Section 5).

3. System-level analysis of retinal photoreceptor development

Early attempts for system-wide assessment of photoreceptor development included gene expression profiling of the retina using expressed sequence tags (ESTs), serial analysis of gene expression (SAGE) and microarrays (Fig. 3A) (Akimoto et al., 2006; Blackshaw et al., 2001; Gieser and Swaroop, 1992; Livesey et al., 2000; Sharon et al., 2002; Yoshida et al., 2004; Yu et al., 2003b). NGS-based methods have dramatically expedited the pace of expression profiling and permitted the application of gene regulation assays to genome-wide scale (Fig. 3B). In addition to examining the whole retina, we can now develop quantitatively precise expression profiles of individual cell types and obtain global data on transcription factor binding and epigenomic marks. Multi-dimensional data integration should therefore enable us to identify novel patterns in GRNs that control functional architecture of individual retinal cells, such as photoreceptors.

In the developing vertebrate retina, rod and cone photoreceptors differentiate from common pools of retinal progenitor cells with distinct temporal profiles. While cone generation ceases prenatally in rodents, rod birth spans a long time window from embryonic day (E)12 to postnatal day (P)10 with peak at P0–P2

Table 2
Public databases containing clinical or high throughput retina data sets.

Database	URL	Description	Reference
Allen Brain Atlas	http://www.brain-map.org/	Public resources integrating comprehensive gene expression and anatomical data from developing or adult mouse and human brain	(Hawrylycz et al., 2012; Lein et al., 2007)
BrainCloud	http://braincloud.jhmi.edu/plots/	Database of temporal gene expression dynamics in the human prefrontal cortex across the lifespan	(Colantuoni et al., 2011)
EyeSAGE	http://neibank.nei.nih.gov/EyeSAGE/index.shtml	Resource for retina, RPE and trabecular meshwork transcriptomes	(Bowes Rickman et al., 2006)
Gene Expression Omnibus (GEO)	http://ncbi.nlm.nih.gov/geo	Public data repository of functional genomics data supporting MIAME-compliant data submissions	
Gene Expression Profile Database	http://www.fmi.ch/roska.data/index.php	Microarray data across diverse adult mouse retinal cell types	(Siegert et al., 2012)
High resolution fundus image database	https://www5.cs.fau.de/research/data/fundus-images/	Database of fundus images of healthy eyes and eyes with diabetic retinopathy or glaucoma	
Human Retinal Transcriptome	http://ocular genomics.meei.harvard.edu/index.php/ret-trans	The human retina transcriptome data generated from three normal human retinas using RNA-seq	(Farkas et al., 2013)
mirNEYE	http://mirneye.tigem.it	Expression atlas of 221 miRNAs in the developing and adult wild type mouse eye	
Mouse Retina SAGE library	http://cepko.med.harvard.edu	Database of gene expression profiling of developing and adult mouse retina and a few other non-ocular tissues. *In situ* hybridization data are also available for select genes.	(Blackshaw et al., 2001; Blackshaw et al., 2004)
NEIbank	http://neibank.nei.nih.gov/index.shtml	Database of assembled EST data from ocular tissues of various organisms	
Retina Central (WEBER)	http://www.retinacentral.org/	Database of genes experimentally shown to be expressed in the retina/the retinal pigment epithelium.	(Schulz et al., 2004)
Retina International	http://www.retina-international.org/sci-news/databases/mutation-database/	Database of retinal diseases, mutations, animal models and disease-associated proteins	
Retinal Express	http://odin.mdacc.tmc.edu/RetinalExpress/	E14.5 mouse retina cDNA/EST database	
RetinoBase	http://alnitak.u-strasbg.fr/RetinoBase/	Microarray database generated from all publicly available retina-related gene expression profiles	(Kalathur et al., 2008)
RetNet (Retinal information network)	https://sph.uth.edu/retnet/	Database of genes and loci causing inherited retinal diseases	
RP Gene Expression Atlas	http://rpexp.tigem.it/	Collection of *in situ* hybridization data for retinitis pigmentosa genes in mouse and human retina	
STARE (Structured analysis of retina)	http://www.ces.clemson.edu/~ahoover/stare/	Database of retinal images of various clinical manifestations	(Hoover and Goldbaum, 2003; Hoover et al., 1998)

(Carter-Dawson and LaVail, 1979; Rapaport et al., 2004). Photoreceptor identity is largely dictated by an intrinsic transcriptional program (Swaroop et al., 2010) that involves a number of transcription factors, including cone-rod homeobox protein CRX (Chen et al., 1997; Furukawa et al., 1997), neural retina leucine zipper protein NRL (Mears et al., 2001; Swaroop et al., 1992) and thyroid hormone receptor THRB (also called TRβ2) (Ng et al., 2001). CRX plays an essential role in photoreceptor development (Furukawa et al., 1999) by controlling the expression of both rod and cone genes (Corbo et al., 2010; Hennig et al., 2008). NRL and THRB, however, determine the genesis of rods (Mears et al., 2001) and medium wavelength sensitive (M) cones (Ng et al., 2011), respectively, from photoreceptor precursors that seem to be otherwise specified by "default" as short wavelength sensitive (S) cones (Swaroop et al., 2010). Other essential regulators of photoreceptor development include OTX2 (Nishida et al., 2003; Roger et al., 2014), RORB (Jia et al., 2009; Srinivas et al., 2006) and NR2E3 (Chen et al., 2005; Cheng et al., 2006, 2011; Haider et al., 2000; Oh et al., 2008; Peng et al., 2005). These key transcriptional regulators constitute central nodes ("hubs") in the GRN that controls photoreceptor development.

3.1. Cell type-specific approaches for generating GRN

Although few studies have been performed using purified photoreceptor cells, rod photoreceptor-specific information can be predicted by genome-wide data generated from the predominantly rod-containing mouse or human retina. Considering that NRL and CRX expression are largely photoreceptor-specific, ChIP-seq data for these two key transcription factors would broadly reflect photoreceptor biology. The same is not true for transcriptome and epigenome data obtained from the whole retina. Isolation of photoreceptors is necessary to acquire true photoreceptor-specific information. Cell type-specific approaches are even more critical for developmental studies since the proportion of photoreceptors in mouse retina continuously increases until P10, making it difficult to discern true cellular changes in gene expression or epigenetic marks. The first example demonstrating the importance of cell type-specific studies comes from microarray-based expression profiling of flow-sorted rod photoreceptors from *Nrl*p-GFP mice (Akimoto et al., 2006). The resulting rod-specific transcriptome data effectively detected many low level transcripts that had not been observed in the whole retina transcriptome data. Although many transgenic mouse lines expressing fluorescence reporters in specific retinal cell types have been described and are being used for transcriptome analysis (Siegert et al., 2012), identification of new cell type-specific markers (Koso et al., 2009) would greatly facilitate NGS studies of retinal neuronal subtypes.

An array of subtypes, each with unique morphology and function, can be recognized within major classes of retinal neurons (Masland, 2012). Single cell gene expression profiling has demonstrated a high degree of heterogeneity among individual retinal cells (Trimarchi et al., 2008). A better understanding of biological events thus requires delineation of GRNs specific not only at the level of individual subtypes but perhaps even at a single cell level. Due to limitations in obtaining sufficient material, conventional methods need to be

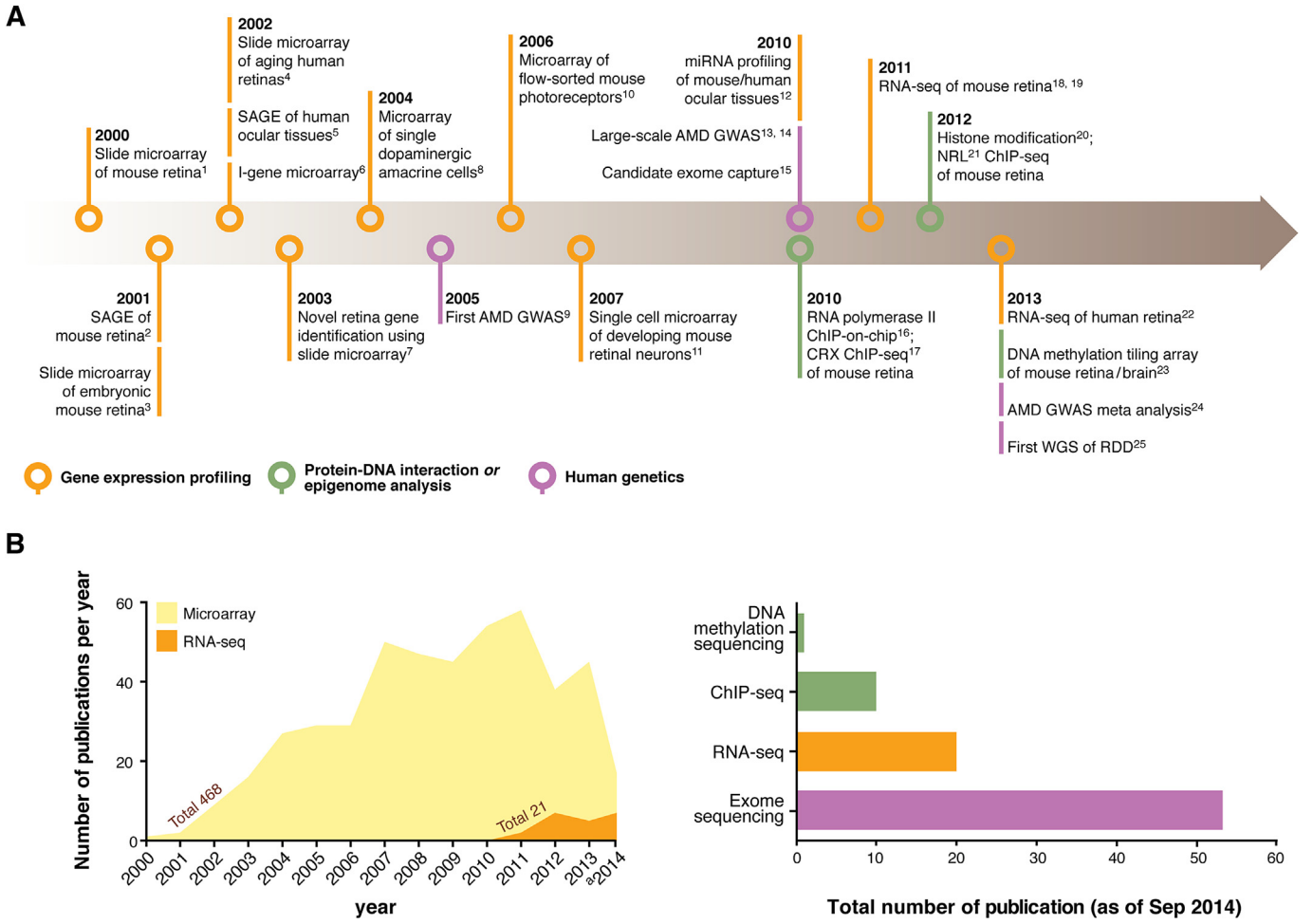


Fig. 3. Timeline of genome-wide studies of the retina biology and disease pathogenesis. The advent of genome-scale profiling technologies has been a critical step for systems biology approaches. **A**, From the pioneering high throughput transcript analysis, such as the initial application of microarray and serial analysis of gene expression (SAGE), to whole genome sequencing, an ever-growing number of genome-wide studies have advanced our knowledge about healthy retina and disease pathogenesis. Highlights of such innovative genome-scale studies were selected and presented chronologically. **B**, For more than a decade, microarray has been a widely used methodology of choice for gene expression profiling, yielding a substantial number of publications each year. RNA-seq, deep sequencing of cDNA using NGS technology, is becoming more accessible and affordable and thus expected to be applied more widely. In addition to transcriptome analysis, NGS is applicable to a variety of other conventional research techniques and has already generated numerous data sets surveying whole exomes for genetic variation (whole exome sequencing), transcription factor targetome (ChIP-seq) and epigenome (ChIP-seq for histone modifications and various DNA methylome sequencing methodologies). [1](Livesey et al., 2000), [2](Blackshaw et al., 2001), [3](Mu et al., 2001), [4](Yoshida et al., 2002), [5](Sharon et al., 2002), [6](Farjo et al., 2002), [7](Chowers et al., 2003b), [8](Gustincich et al., 2004), [9](Klein et al., 2005), [10](Akimoto et al., 2006), [11](Trimarchi et al., 2007), [12](Arora et al., 2010; Hackler et al., 2010; Karali et al., 2010; Wang et al., 2010), [13](Chen et al., 2010b), [14](Neale et al., 2010), [15](Otto et al., 2010), [16](Tummala et al., 2010), [17](Corbo et al., 2010), [18](Grant et al., 2011), [19](Brooks et al., 2011; Mustafi et al., 2011), [20](Popova et al., 2012), [21](Hao et al., 2012), [22](Farkas et al., 2013), [23](Oliver et al., 2013b), [24](Fritsche et al., 2013), [25](Nishiguchi et al., 2013), [a] number of publications until September 2014.

miniaturized to gain enough sensitivity for single cells or cell types. However, technical hurdles including sample loss, degradation and contamination can have more pronounced impact on quality and robustness of data generated from single cells or small quantities versus large pools of cells. In addition, many rounds of PCR amplification may generate increased noise and inaccurate quantification. Nevertheless, cell type-specific or even single cell level analyses are necessary to extract gene regulatory information relevant to the cell of interest. Single cell collection methods based on micro-fluidic systems (Shalek et al., 2014), high-resolution imaging (Lubeck and Cai, 2012), and better protocols for constructing NGS libraries from small amount of starting RNA (Brooks et al., 2012; Tang et al., 2009; Tariq et al., 2011) have made it possible to attempt more robust expression profiling of individual retinal neurons. However, a major challenge remains for the generation of epigenomic and other NGS data sets with small number of cells. Our laboratory and others are currently developing such protocols (Adli et al., 2010; Guo et al., 2013; Smallwood et al., 2014).

The importance of using a purified single cell type for system-level analysis cannot be over-emphasized since biological processes such as development and aging have broad and concurrent impact on multiple cell types in a tissue and on the organism as a whole. Deciphering photoreceptor GRN from the whole retina studies would be complicated by transcriptional noise and differences in epigenome and regulatory molecules among different cell types. This is especially true for dissecting changes in GRNs during the pathogenesis of photoreceptor degeneration. As photoreceptor-specific data are not available for all NGS-based assays, we also cover studies from the whole retina in the discussion below.

3.2. Construction of photoreceptor GRN

Gene expression requires context-specific interaction among *cis*-regulatory DNA elements, basal transcriptional machinery and transcription regulatory proteins. In addition, transcriptional activity is under the influence of chromatin architecture as well as

regulatory RNAs. Thus, basic elements needed to generate a rod photoreceptor GRN include:

(i) Global mRNA profiling by RNA-seq (Section 3.2.1)
(ii) Identification of target genes for cell specific transcription factors by ChIP-seq (Sections 3.2.2 and 3.2.3)
(iii) Chromatin state such as histone modifications by ChIP-seq, DNA methylation profiling, and chromatin accessibility by DNase I hypersensitivity sequencing (Section 3.2.4)
(iv) Regulatory RNA profiling (Section 3.2.5)

Transcriptional profiles are also needed at multiple stages of rod development in order to build a developmental GRN. Gene expression profiling can be used in conjunction with gain- or loss-of function mutations of relevant "hub" transcription factors to further validate GRNs. For example, known hubs in the photoreceptor network such as NRL and CRX can be perturbed (e.g., in mouse mutants) before genome-wide gene expression analysis. The genes showing altered expression in the absence of a regulatory factor would represent direct or indirect (i.e., through secondary regulatory nodes) transcriptional targets. Additionally, GRN can also be inferred from co-expression data. Groups of genes that share similar expression signatures in response to experimental manipulation and/or during development would likely represent common gene regulatory pathways. Although such information is only suggestive, co-expression data allow for the identification of novel testable regulatory patterns.

3.2.1. Expression profiling

Global profiling of gene expression in the developing and mature retina was first accomplished by Affymetrix™ microarrays (Dorrell et al., 2004; Yoshida et al., 2004). Expression profiles have also been generated from flow-sorted rod photoreceptors (Akimoto et al., 2006; Parapuram et al., 2010) and from single retinal cells (Ma et al., 2013; Roesch et al., 2008; Trimarchi et al., 2007; Xue et al., 2011). However, NGS-based RNA-seq profiling has yielded more quantitatively precise information on mRNA transcripts in the mammalian retina (Brooks et al., 2011; Farkas et al., 2013; Gamsiz et al., 2012; Kandpal et al., 2012; Kozhevnikova et al., 2013; Roger et al., 2014). More recently, we have produced extensive RNA-seq-based temporal expression profiles of purified rod photoreceptors that reveal novel insights in cellular pathways (Fig. 4A) (Kim et al., manuscript in preparation) as well as additional modes of gene regulation, such as alternative splicing (Fig. 4B).

3.2.1.1. Perturbation studies. For elucidating GRNs, it is valuable to generate gene profiles after disrupting one of the key regulatory nodes. This strategy was first applied to *Crx*$^{-/-}$ mice (Blackshaw et al., 2001; Hennig et al., 2008; Livesey et al., 2000). CRX is essential for both cone and rod development (Furukawa et al., 1999); however, its role in cell fate determination is debatable because of overlapping functions with another homeodomain protein OTX2, which regulates the expression of CRX as well as NRL (Nishida et al., 2003; Omori et al., 2011; Roger et al., 2014; Terrell et al., 2012). A majority of genes with altered expression in *Crx*$^{-/-}$ mice showed photoreceptor enriched expression, and functional categorization of differentially expressed genes has revealed an array of CRX-regulated cellular functions, including those involved in phototransduction, metabolism, signal transduction and cytoskeletal components.

Another extensively studied gene regulatory program is the NRL-centered transcriptional network. The first observation that NRL is the master switch for generating rod cell fate came from studies of *Nrl*$^{-/-}$ mice as their retina completely lacks rod photoreceptors, with concurrent enhancement of S cone function (Mears et al., 2001). In concordance, ectopic expression of NRL in photoreceptor precursors or in early-born S-cones resulted in formation of functional rods (Oh et al., 2007). Gene profiling of *Nrl*$^{-/-}$ retina revealed little or no expression of rod-specific genes, including those associated with rod phototransduction, and a concomitant increase in the expression of S cone photopigment (encoded by *Opn1sw*) and other cone genes (Brooks et al., 2011; Yoshida et al., 2004; Yu et al., 2004a). Global expression analysis of purified photoreceptors from *Nrl*$^{-/-}$ retina has validated the essential role of NRL in activating the expression of rod genes and suppressing cone genes (Akimoto et al., 2006).

NR2E3, an orphan nuclear receptor, constitutes another important hub in photoreceptor GRN and is a direct transcriptional target of NRL (Bumsted O'Brien et al., 2004; Oh et al., 2008). Mutations in *NR2E3* cause enhanced S cone syndrome in humans (Haider et al., 2000; Sharon et al., 2003; Wright et al., 2004), similar to the retinal phenotype exhibited by *Nrl*$^{-/-}$ mice (Mears et al., 2001). Retinal and rod gene expression profiling of *rd7* mutant mice, in which *Nr2e3* function is ablated, further validates NR2E3 function primarily as a suppressor of cone genes and as a co-activator (with NRL and CRX) of rod genes (Chen et al., 2005; Cheng et al., 2006, 2011, 2004; Corbo and Cepko, 2005; Haider et al., 2009; Oh et al., 2008; Peng et al., 2005).

3.2.1.2. Co-expression network analysis. Target genes of the a transcriptional regulator likely display similar expression patterns and may participate in common or related cellular functions (Yu et al., 2003a). Clusters of co-expressed genes can thus be used to model gene regulatory events. The inferred GRNs enable the detection of putative protein-DNA and protein–protein interactions without requirement of prior knowledge. Mathematical tools have been developed to predict GRNs from gene expression profile data, and putative regulatory relationships can then be validated experimentally (Hecker et al., 2009). Co-expression network inference thus represents a powerful analytical tool for expression profiling data, complementing the perturbation studies discussed above. As activity of a given transcriptional regulator is confined only to the same cell as its target, only the information obtained from a single cell or cell type provides accurate prediction without analytical noise caused by sample heterogeneity. Transcriptome analysis of flow-sorted rod photoreceptors from wild type and *Nrl*$^{-/-}$ retina has revealed multiple clusters of co-expressed genes, which likely represent diverse regulatory events (Akimoto et al., 2006). Co-expression analysis of newer and deeper RNA-seq data sets should provide valuable insights, including those relevant for identification of disease genes and pathways (see also Section 5.4).

3.2.2. Transcription targets ("targetome") of NRL and CRX

Differential gene expression analysis does not distinguish direct transcriptional regulation from secondary regulatory events. ChIP-seq analysis, on the other hand, assays direct physical interactions between a transcription factor and the cognate DNA elements. Recent studies have superimposed transcriptome and transcription factor targetome data to identify biologically relevant target genes of CRX (Corbo et al., 2010) and NRL (Hao et al., 2012). ChIP-seq analysis of mouse retina demonstrated CRX binding to 67% of mis-regulated genes in *Crx*$^{-/-}$ retina, likely representing direct CRX targets (Corbo et al., 2010). Similarly, only a subgroup (15%) of genes differentially expressed in *Nrl*$^{-/-}$ retina exhibited NRL ChIP-seq peaks and thus are direct transcriptional targets of NRL (Hao et al., 2012).

One of the challenges in deciphering targetome data is assigning transcription factor binding to relevant genes. A majority of NRL- and CRX-bound genomic DNA regions are in close proximity of or within the genes. However, binding of regulatory factors far away from genes is not uncommon and may exert transcriptional control

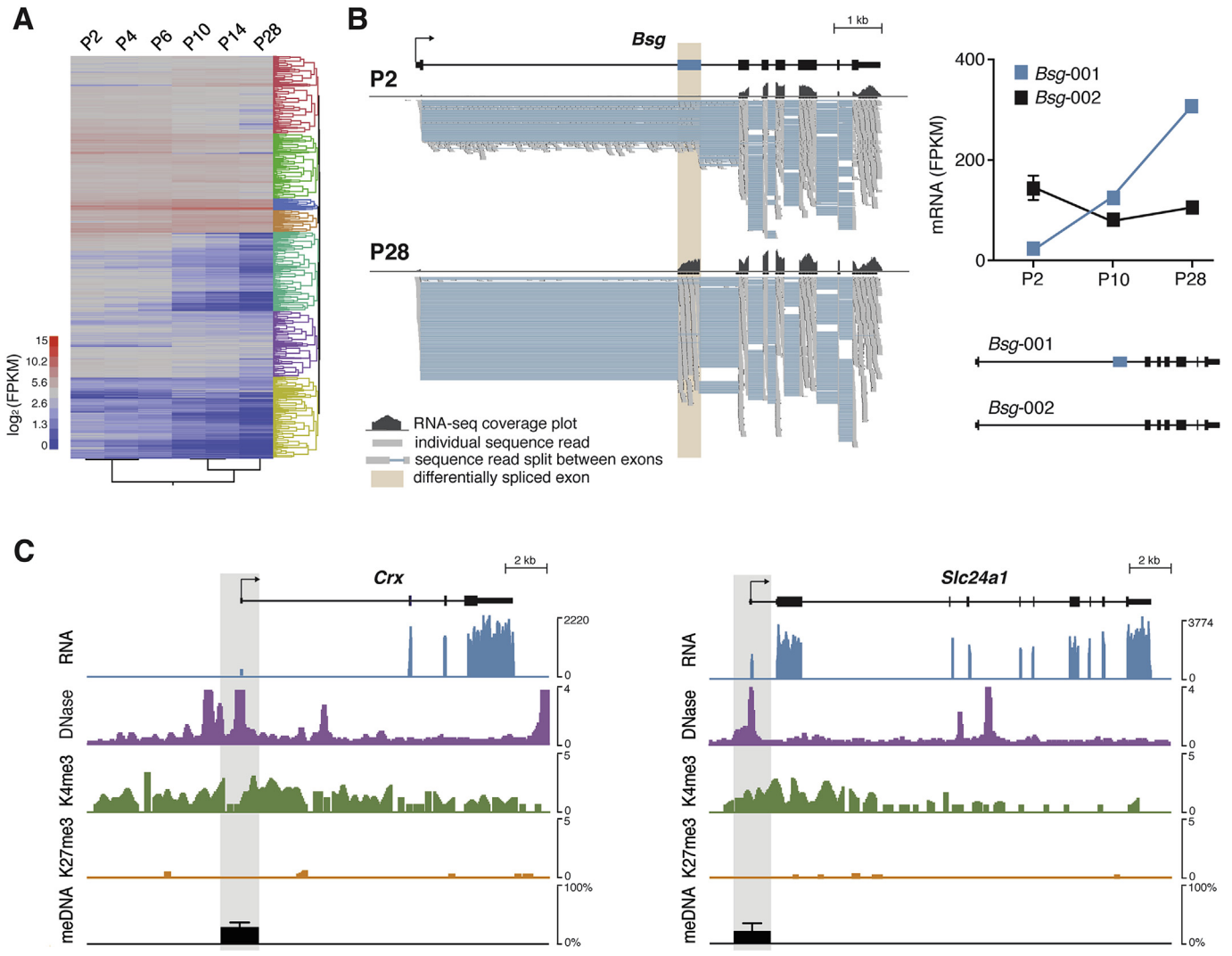


Fig. 4. Cell type-specific System-level analysis. **A**, Heat map of time course RNA-seq data generated from isolated, developing (P2 to P14) and mature (P28) mouse rod photoreceptors. Dynamic gene regulation during rod photoreceptor maturation and clusters of genes with similar temporal expression patterns are apparent. Heat map was generated based on $\log_2$FPKM, and individual co-expression clusters were highlighted with different colors in the dendogram. FPKM, fragments per kilobase of exon per million reads. **B**, Transcript-level analysis of RNA-seq data enables detection of complex gene regulatory programs such as distinct splicing events during development. Alternative splicing of *Bsg* (basigin) gene during rod photoreceptor development is shown as an example. Coverage plots (dark gray histogram) and read alignments (gray blocks indicating individual sequence reads with thin blue horizontal lines connecting portions of sequence reads that are split between exons) show differential inclusion of the second exon (brown shade) in P2 and P28 rod photoreceptors. Expression level of each splice variant during rod development is plotted and shown on the right. **C**, System-level profiling of diverse chromatin signatures, including chromatin accessibility [DNase-seq (DNase I hypersensitivity sequencing)], active and repressive promoter histone modifications (H3K4me3 and H3K27me3, respectively; mini-ChIP-seq) and DNA methylation (bisulfite sequencing). Application of genome-wide analysis of chromatin states used to be limited to *in vitro* samples and pooled tissues of heterogeneous cell types. Bisulfite sequencing and ChIP-seq have now been miniaturized for flow-sorted single type of neurons, which are available in small amounts. Shown are active gene expression and hallmark of active chromatin state of two select photoreceptor-specific genes, *Crx* and *Slc24a1*, in P28 flow-sorted rod photoreceptors. Scale on y-axis of RNA, DNase, K4me3 and K27me3 tracks indicates RPM (reads per million reads). Average percent 5-methylcytosine (meDNA) within promoter (±1 kb from the transcription start site, highlighted with a gray shade) was plotted as a bar graph.

by chromatin looping. Recently, high throughput reporter gene assays have successfully validated the enhancer activity of over 1000 CRX interacting genomic loci (White et al., 2013). Similar large-scale enhancer assays and chromatin state mapping are expected to confirm the functional significance of distant ChIP-seq peaks. Integrated analysis with gene expression data and long distance chromatin association profiling [by chromatin conformation capture (3C) assay (Peng and Chen, 2011) and its NGS applications such as 4C-seq and HiC-seq] (see Table 1) will further facilitate the identification of novel transcription targets of key photoreceptor transcription factors.

ChIP-seq analyses have identified thousands of genomic loci (sequence regions) that are occupied by NRL or CRX *in vivo*, and both transcription factors bind to common enhancer regions that specify photoreceptor genes (Hao et al., 2012). In embryonic stem cells (ESCs), master transcriptional regulators are reported to form super-enhancers, atypical long enhancer clusters that are associated with unusually high level of mediators as well as enhancer-associated epigenetic marks (Whyte et al., 2013). Subsequent studies have demonstrated the importance of super-enhancers in many differentiated cell types (Hnisz et al., 2013) and their implications in developmental and neurological disease (Lee and Young, 2013). Further investigations are necessary to evaluate whether NRL- and CRX-bound enhancer elements exhibit characteristics of super-enhancers and whether variants in these elements are associated with retinal disease.

3.2.3. Dynamicity and combinatorial action of transcription factors

Most of the transcription factor targetome analyses have so far been conducted at a single time point, revealing only a snapshot of gene regulation. The function of transcriptional regulators, however, is highly dynamic (Davidson and Levine, 2008; Hwang et al., 2012). Transcriptional complexes are under continuous modulation by availability of co-factors and by chemical modifications at the level of protein, DNA and/or chromatin (Berger, 2007; Voss and Hager, 2014). Thus, the regulatory outcome can vary depending on cellular context such as developmental stage, cell identity and fitness. Comparative transcriptome analysis of the retina and of flow-sorted rod photoreceptors across developmental stages has revealed a striking variation in expression kinetics among photoreceptor genes controlled by NRL (Akimoto et al., 2006; Yoshida et al., 2004; Yu et al., 2004a). *Nrl* expression is initiated immediately after the final mitosis of photoreceptor precursors, leading to expression of a large number of target genes including *Nr2e3*. Notably, a number of NRL target genes, including visual pigment rhodopsin (encoded by *Rho*), exhibit substantial delay in expression during rod photoreceptor development.

Although underlying factors for differential onset of expression of NRL target genes are not completely understood, analyses of multiple data sets suggest additional control mechanisms. One of the possible scenarios can be inferred by linking NRL targetome data with gene expression profiles and with previously identified retinal disease loci (Hao et al., 2012). NRL target genes with putative functional relevance can be selected based on the assumption that such genes are likely associated with rod function and cause retinal degeneration when mutated. Such analyses have identified histone demethylase KDM5B as a secondary regulatory node, and *Kdm5b* knock-down partially phenocopies the rod-to-cone transition observed in $Nrl^{-/-}$ retina (Hao et al., 2012). KDM5B is one of the histone demethylases implicated in gene repression through demethylation of H3 lysine 4 trimethylation (H3K4me3), a histone mark associated with transcriptional activation. Thus, it can be hypothesized that NRL function is mediated through multiple as yet unidentified secondary nodes (including epigenetic factors) and that other regulatory mechanisms may define precise temporal control of gene expression. Functional interaction between NRL-centered transcriptional network(s) and the epigenetic program will be further elaborated in the following section 3.2.4.

Another important feature of GRNs is combinatorial actions of transcriptional regulators (Davidson and Levine, 2008). Numerous pre-genomic approaches have reported cooperative functions among photoreceptor transcription factors. Interaction between NRL and CRX exerts synergistic effects on transcriptional activation of rod-specific genes (Mitton et al., 2000; Pittler et al., 2004; Yoshida et al., 2004). As part of the same complex, NR2E3 exhibits dual function by inducing rod and suppressing cone gene expression (Oh et al., 2008; Webber et al., 2008), thereby constituting a critical secondary transcriptional regulatory node (Chen et al., 2005; Cheng et al., 2006, 2004; Haider et al., 2000; Peng et al., 2005). Compiling system-wide surveys of putative target genes for these three regulators has revealed significant overlaps in their targets (Hao et al., 2012; Qian et al., 2005; Yu et al., 2006), suggesting that combinatorial functions among transcription factors exert tight regulation of photoreceptor development and homeostasis. Salient features of NRL transcriptional regulatory network have been assembled (Hao et al., 2012), and further computational analyses of existing and new data sets are expected to provide a comprehensive map of rod photoreceptor GRNs (Hwang et al., 2012; Wan et al., 2013).

Combinatorial action of regulatory factors is highly context-dependent and cell type-specific, and the composition of transcriptional complexes is expected to influence the selectivity of downstream target genes. For instance, although CRX expression is common to both rods and cones, transcriptional regulatory outcomes show a striking difference. CRX cooperates with RORB to induce S cone photopigment (*Opn1sw*) expression in cone precursors (Srinivas et al., 2006), whereas, in the presence of NRL and NR2E3, CRX enhances rod-specific gene expression in rod photoreceptors (Hao et al., 2012). ChIP-seq analysis has shown that OTX2-bound *cis*-regulatory elements are quite different in RPE and neural retina (Samuel et al., 2014). Only retina-specific OTX2 target sites, but not RPE-specific ones, show redundancy with CRX occupancy, further demonstrating the importance of combinatorial transcriptional programs in cell fate specification.

3.2.4. Chromatin state and gene regulation

DNA within a cell is packaged into DNA-protein complexes (termed chromatin), which constitute a dynamic structure. DNA itself and the histones, which are primary protein components in the chromatin, are subjected to chemical alterations that can influence transcriptional activity. Regulation of chromatin state is an important mechanism for modulating biological states, including lineage restriction and cell fate specification during development (Hirabayashi and Gotoh, 2010; Meshorer and Misteli, 2006; Sasaki and Matsui, 2008; Wan et al., 2013). Studies of epigenetic marks such as DNA methylation and posttranslational modifications of histones in the retina have revealed cell type- and stage-specific chromatin states, recapitulating context-dependent differential gene regulation.

3.2.4.1. DNA methylation. DNA methylation at gene promoters has been negatively associated with gene expression and is considered a key mechanism for maintenance of chromatin state (Suzuki and Bird, 2008). Integration of global DNA methylation profiles with gene expression is critical for deciphering the overall structure of a GRN. System-level analysis of DNA methylome has implicated differential 5-methylcytosine in altering gene expression during photoreceptor degeneration in *rd1* retina (Farinelli et al., 2014) as well as in tissue-specific splicing in the retina and the brain (Wan et al., 2013). More restricted approaches examining selected genes further support the association of DNA methylation with photoreceptor gene regulation. Bisulfite sequencing of targeted genomic areas from WERI and Y9 human retinoblastoma cell lines and laser-capture micro-dissected mouse photoreceptors and non-photoreceptor cell types has revealed photoreceptor-specific cytosine hypomethylation within opsin genes such as *Rho*, *Opn1sw* and *Opn1mw* (Merbs et al., 2012), demonstrating cell type-specific patterns of DNA methylation. Conditional knock-out of DNA methyltransferase 1 (*Dnmt1*) shows lack of outer segments despite nearly normal expression of phototransduction and cilia genes in photoreceptors (Nasonkin et al., 2013), suggesting a broad impact of DNA methylation on morphological specification.

Contrary to rapidly growing number of gene expression profiling studies, only few genome-wide DNA methylome profiles have been generated for the retina (Fig. 3B). New highly sensitive detection assays for 5-methylcytosine and 5-hydroxymethylcytosine (see Table 1) (Oliver et al., 2013b; Smallwood et al., 2014) and with single cell-type resolution (Powell et al., 2013) are expected to facilitate such investigations at the level of a single retinal neuron. We have recently completed the generation of DNA methylome for flow-sorted rod photoreceptors and are examining its relationship to RNA-seq and histone modification data sets (Fig. 4C) (Yang et al., manuscript in preparation). Further technical improvements should therefore unravel system-wide interactions between DNA methylation and cell type-specific dynamics of gene expression.

3.2.4.2. Histone modifications. More than 60 amino acid residues on different histones undergo diverse posttranslational modifications including acetylation and methylation (Kouzarides, 2007; Zentner and Henikoff, 2013). While histone acetylation is almost strictly associated with active gene expression, methylation represents more complex modes in gene regulation. Transcriptional activity is shown to depend on the degree of methylation – mono-, di-, and trimethylation, the specific modified amino acid residue and associated DNA elements (e.g., promoter, gene body, enhancers). Among multitudes of reported histone methylations, H3K4 and H3K27 methylation are closely correlated with transcription status and thus constitute the most commonly examined histone modifications. Promoters of active and silent genes are enriched with H3K4me2/me3 and H3K27me3, respectively, while active and repressive enhancers are demarcated with H3K4me1/H3K27ac and H3K4me1/H3K27me3, respectively. Dynamic regulation of histone methylation patterns in rod or cone photoreceptors is expected to have significant impact on photoreceptor GRNs. ChIP-seq is fast becoming a routine procedure for genome-wide profiling of histone modifications. Histone signature, however, is highly heterogeneous across distinct cell types (ENCODE Project Consortium, 2012); thus, cell type-specific histone modification profiling is necessary to obtain useful information on the chromatin state. Purified cells do not easily meet the requirement of sample size for reported epigenome profiling assays as yet.

The whole retina histone ChIP-seq performed with appropriate control tissue such as *rd1* mutant retina, a degenerative mouse model that lacks photoreceptors, has offered significant photoreceptor-specific epigenomic information as rod photoreceptors account for a vast majority of retinal neurons in mature retina (Popova et al., 2012). In this study, genome-wide signatures of H3K4me2 and H3K27me3 in the mouse retina were mapped and compared with transcriptome at various developmental stages. Distinct clusters of chromatin modification dynamics were identified among rod-specific genes (Popova et al., 2012). Surprisingly, rod genes showed varied H3K4me2 signature. While some rod genes have a detectable level of H3K4me2 as early as E17.5 and at all subsequent developmental stages, another group of rod genes including phototransduction genes shows delayed *de novo* accumulation of H3K4me2 from P7 to P15. Concordantly, phototransduction genes gain full chromatin accessibility only after P10 as revealed by DNase I hypersensitivity sequencing (DNase-seq) of the mouse retina (ENCODE, www.mouseencode.org). ChIP experiments have demonstrated a limited interaction of NRL with cognate DNA binding site in *Rho* at P2 (Hao et al., 2011), suggesting that inactive chromatin state at *Rho* and other phototransduction genes may interfere with NRL binding to its target genes in newborn rods. These results, however, should be interpreted with caution as the detected change in histone marks, chromatin accessibility and NRL recruitment may simply reflect a dramatic increase in the proportion of rod photoreceptors in the retina from P2 to P28. Assays using purified rod photoreceptors offer an ideal alternative. Our initial analysis of histone ChIP-seq data from purified rod photoreceptors has revealed promising insights into rod-specific histone signature (Fig. 4C) (Yang et al., manuscript in preparation).

3.2.5. miRNA and other transcribed sequences

Non-coding RNAs (ncRNAs) constitute as much as 80% of the human transcriptome (Rosenbloom et al., 2013) and contribute additional complexity to GRNs (reviewed in Mortimer et al., 2014). Small ncRNAs (<200 bp) include microRNAs (miRNAs) of 18–24 nucleotides. Long ncRNAs (lncRNAs) are over 200 bp in length but can be up to hundreds of kb. Each of the reported 2000–3000 miRNAs (miRBASE Release 21, June 2014) can regulate hundreds of target genes, and each mRNA can be targeted by several miRNAs, fine tuning gene expression of an estimated 30–80% of the human genome (John et al., 2004; Lim et al., 2005; Lu and Clark, 2012). Tens of thousands of lncRNAs are expressed at low levels in a tissue-restricted manner and believed to regulate the expression of related genes associated with most biological processes in a given cell (Li and Chang, 2014).

Although photoreceptor-specific profiling of ncRNA species is yet to be conducted, dynamic regulation of specific ncRNAs has been reported during retinal development and disease. *In silico*, RT-PCR and *in situ* hybridization studies have revealed spatio-temporal expression of miRNAs in the developing and mature retina (Arora et al., 2010; Deo et al., 2006; Hackler et al., 2010; Karali et al., 2010; Ryan et al., 2006; Xu, 2009). In addition, miRNAs exhibit altered expression during circadian cycle (Krol et al., 2010; Xu et al., 2007), cell survival (Damiani et al., 2008; Lumayag et al., 2013; Sundermeier et al., 2014), and in disease conditions (Loscher et al., 2008; Shen et al., 2008). The effect of miRNAs at the intersection of several GRN is exemplified by the "miR-96, miR-182 and miR-183" cluster, which contributes to circadian regulation of gene expression in the retina and has a more general role in neurosensory cell-specific GRNs (Xu et al., 2007).

Eighteen lncRNAs are highly conserved in mammalian eyes; of these, fourteen are expressed in the macular region and others in rest of the retina (Mustafi et al., 2013). Furthermore, lncRNAs are shown to play a role in retinal development (Rapicavoli and Blackshaw, 2009); e.g., in regulation of cell cycle (Vax2os1), modulation of transcription factor activity (Six3OS), and specification of cell fate (RNCR2) (Meola et al., 2012; Rapicavoli et al., 2010, 2011), highlighting their potential involvement in GRNs. As transcriptome databases expand, functional studies of existing and newly-identified small and lncRNAs will provide novel insights into their role in fine-tuning GRNs.

3.2.6. Proteome analysis

Transcriptome analysis does not accurately represent protein expression in a cell or tissue since protein content is also regulated by translational and post-translational events and reflects the equilibrium between synthesis, stability and degradation. Complex protein–protein interactions add another level of complexity to how cellular physiology and homeostasis are controlled. Protein profiles of the retina (Barnhill et al., 2010; Finnegan et al., 2008) have been generated using mass spectrometry, validating the presence of a multitude of retinal proteins as previously predicted by microarray results. Studies have also reported photoreceptor-enriched proteome acquired from planar cryosections of the photoreceptor layer (McKay et al., 2004) or rod outer segment disk purification (Elliott et al., 2008; Kwok et al., 2008; Panfoli et al., 2008). However, global proteomic methodologies are more qualitative than quantitative and not suitable for the analyses that require high detection sensitivity and comprehensiveness as in genomics approaches. Micro-scale detection techniques are being developed for protein analysis. Quantification of protein expression using nanopore technology (Wei et al., 2012b), single protein molecule counting on a two-dimensional surface (Tessler et al., 2009), and single cell western blotting (Hughes et al., 2014) are among promising new technologies, which can potentially be adapted for increased throughput.

A targeted proteomic approach has now begun to provide insights into photoreceptor gene regulation. A recent study focused on NRL-containing transcriptional complexes and identified the transcription-splicing protein, NonO, and other binding partners of NRL as important regulators of rod gene expression (Yadav et al., 2014). We should note that translation of mRNA into protein has been successfully monitored indirectly through ribosomal profiling

(Ribo-seq) (Ingolia et al., 2009). Application of evolving proteomic approaches would also be valuable to complement gene expression profiling and epigenome data. More targeted or global proteomic analysis is expected to completely define photoreceptor GRNs.

4. System-level analysis of retinal aging

Aging in higher organisms reflects the interplay of genetic factors with the environment and influence of stochastic events (Fig. 5); this requires investigation(s) of interaction networks underlying biological processes rather than focus on a single gene or protein (Kriete et al., 2006). Although aging does not cause a clinical disease *per se*, phenotypes of aging can resemble disease phenotypes. Conversely, aging-associated changes increase vulnerability and instability of the system, making it more susceptible to further damage and to the effects of genetic mutations. Thus, aging is a leading risk factor for several common late-onset diseases. In this section, we explore genome-wide studies of aging as a time-dependent event relevant for eye function and pathology, with focus on the retina.

Each component of the eye contributes differently to overall decline in visual function with aging. Progressive deterioration in retinal anatomy (Curcio, 2001; Curcio et al., 1993; Gao and Hollyfield, 1992) and psychophysical parameters (Birch and Anderson, 1992; Bonnel et al., 2003; Freund et al., 2011; Shinomori and Werner, 2012) is observed with advanced age. Structural and functional changes have also been reported in photoreceptors (Curcio, 2001; Gresh et al., 2003; Kolesnikov et al., 2010; Parapuram et al., 2010; Samuel et al., 2011).

4.1. Expression profiling of aging retina

Systems biology of the aging retina starts with gene expression profiling to detect chronological events that alter cellular homeostasis (Table 3). Early studies were performed using SAGE (Sharon et al., 2002) or microarray platforms (Cai et al., 2012; Chowers et al., 2003b; Yoshida et al., 2002) on post-mortem human retinas, which have inherent variability associated with individual genetic variations, pre- and post-mortem conditions, and quality of RNA. Little to no concordance among studies was observed at the gene level, due to differences in samples, platforms and analyses. However, when genes are considered in the context of ontologies and gene networks, characteristic patterns begin to emerge in the aging retina, suggesting a general metabolic slow down (Cai et al., 2012; Chowers et al., 2003b; Yoshida et al., 2002) and decrease in expression of phototransduction genes (Chowers et al., 2003b; Sharon et al., 2002). Genes associated with stress response and inflammation are generally up-regulated (Cai et al., 2012; Chowers et al., 2003b; Yoshida et al., 2002), thus highlighting manifestations of retinal aging that are shared with the brain (Lee et al., 2000). Notably, there appears to be a trend towards increased expression in older age of extracellular matrix genes, with relevance to the ontogenesis of AMD (Chowers et al., 2003b) and differential aging mechanisms in the macula compared to the peripheral retina (Cai et al., 2012).

Some of the variability reported by the human studies can be overcome by molecular studies of aging in mouse. Comparison of two senescence-accelerated mouse prone strains (SAMP8 and SAMP10) to the senescence-accelerated mouse resistant strain (SAMR1) and the commonly inbred C57Bl/6J strain using Affymetrix™ oligonucleotide microarrays (Table 3) has highlighted the strong genetic background effect on the transcriptional response to aging (Carter et al., 2005). Expression of genes involved in inflammation and response to injury/stress is higher in aging mouse retina (Chen et al., 2010a), in concordance with the studies on human aging and with SAMR mice.

A limitation of gene expression data in the aging retina is that it represents average changes in six neuronal and two glial cell types, which contribute differently to tissue homeostasis. While these data may provide insights into the behavior of the system at the tissue level, expression profiling of the aging mouse rod photoreceptors (Parapuram et al., 2010) and of microglia (Ma et al., 2013) has expanded our understanding at the cellular level. Notably, genes associated with inflammatory signaling and stress response pathways constitute predominant hubs in network analysis of photoreceptor aging data (Parapuram et al., 2010). Furthermore, expression of genes involved in oxidative phosphorylation is reduced in aging photoreceptors (Barb et al., manuscript in preparation), highlighting an age-related pathway that was not detected by whole retina studies but is a common feature of aging (de Magalhaes et al., 2009). Finally, angiogenesis and retinoid/lipid metabolism (regulated through signaling from the retinoic acid receptor) have emerged as unique pathways to rod photoreceptor aging (Parapuram et al., 2010). Focusing on photoreceptors as a single cell type, the question is whether gene expression changes observed in aging are intrinsic or an adaptive response to modification of retinal microenvironment, and whether these changes are protective, permissive or causative of the functional decline (Parapuram et al., 2010). Transcriptome analysis of isolated aging microglia shows changes in genes involved in cell metabolism, shape and motility, and in neurotrophic signaling, which could compromise the supporting role of microglia in the retina (Ma et al., 2013). Furthermore, expression changes in genes associated with microglia activation and inflammatory signaling suggest a contribution to the general retinal neuro-inflammatory process (von Bernhardi et al., 2010; Xie et al., 2003). As in case of rod photoreceptors, expression changes also affect genes involved with lipid metabolism and angiogenesis. The latter underscores the complexity of angiogenic events in AMD, in which signaling from multiple cell types, including RPE, photoreceptors and microglia, may converge to determine pathological changes, further demonstrating the importance of approaching retinal aging from a systems biology perspective.

From existing expression data, it is reasonable to assume that aging involves small changes in several genes that contribute to common pathways rather than major changes in individual genes. It becomes therefore critical to detect even small expression shifts with good statistical confidence. RNA-seq using NGS, with much higher reproducibility and greater dynamic range compared to microarray, is the ideal platform for more in depth molecular studies of retinal aging. RNA-seq has been employed in expression studies of human and mouse retina, however, not in relation to aging (Brooks et al., 2012; Farkas et al., 2013; Gamsiz et al., 2012; Li et al., 2014). One RNA-seq study so far published in the aging retina investigates the senescence-accelerated OXYS rats compared to Wistar controls (Kozhevnikova et al., 2013). Because of the profound pathological phenotype observed in the OXYS rats, it is difficult to distinguish aging from overt strain-dependent disease. However, differential expression of genes between 3 and 18 month old Wistar rats shows significant down-regulation of genes associated with extracellular matrix, response to nutrient levels and aminoacyl-tRNA biosynthesis, together with up-regulation of genes encoding negative regulators of transcription and components of synaptic transmission pathways. Immune response genes appear in both up-regulated and down-regulated gene ontologies, suggesting potentially impaired immune function in the aging rat retina, similar to the findings by microarrays in mouse and human.

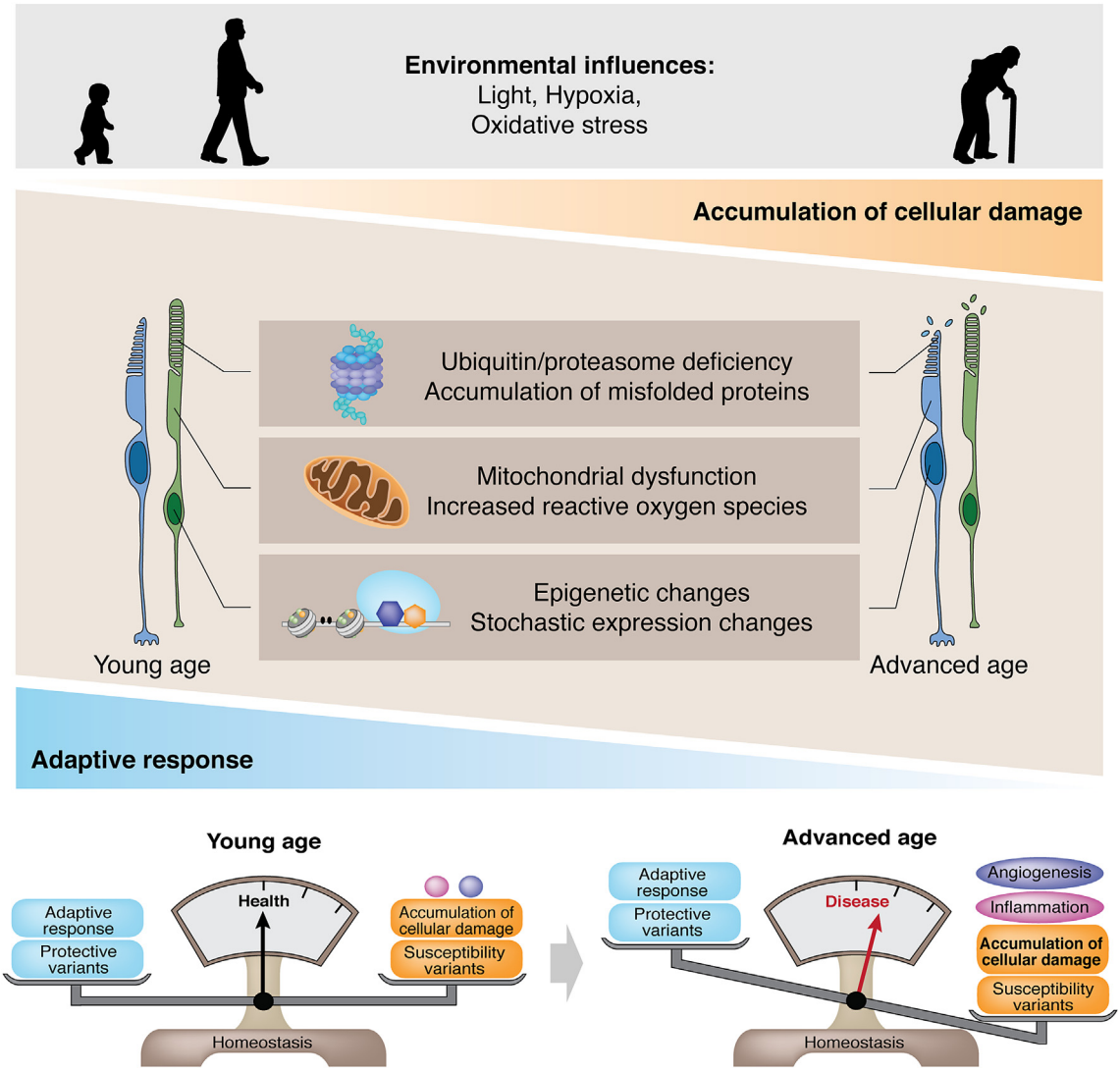


Fig. 5. Interface between aging and disease. The environment influences photoreceptor homeostasis throughout life. The cell adaptive response helps maintain a balanced homeostasis. As the adaptive response becomes insufficient to overcome "insults" to the system, damage accumulates in aging photoreceptors. Major metabolic failure is observed for the ubiquitin-proteasome system and mitochondria. Epigenetic changes and stochastic changes in gene expression combined with the presence of susceptibility variants, eventually tilt the equilibrium towards the disease state. At which point, inflammation and angiogenesis become pathologic, further aggravating disease manifestations.

4.2. Limitations and potential of aging studies in the retina

The scenario emerging from gene expression analyses of the aging retina is one of a tissue that is metabolically slowing down, activates stress and signaling pathways, and presents a complex immune and inflammatory activity. Because of the nature of aging, data collection from the same individual over time would be ideal but it is not a viable option for the retina. For human studies, a random selection of subjects from different age groups (post-mortem donors) suffers from large individual variations that can only be overcome if enough replicates can be collected and if experimental variables are minimally altered. This underscores the importance of strict collection and transportation protocols that minimize the time from death to enucleation and processing. Eliminating technical variability becomes even more critical if one wants to take an unbiased approach and use principal component analysis (PCA) to partition the samples between young and old on the first component.

RNA-seq has revealed the expression of novel transcripts and genes in the retina (Farkas et al., 2013; Gamsiz et al., 2012; Li et al., 2014) suggesting the need to extend this analysis to aging individual cell types in mouse and, where possible, in human. In depth analysis of RNA-seq data will allow the identification and study of emerging modulators of aging such as regulatory ncRNAs described in Section 3.2.5 (reviewed in Montano and Long, 2011). Reproducibility of RNA-seq will also facilitate compilation of different studies to increase sample number and statistical significance. Finally, comprehensive cross-comparisons of existing databases (Table 2) should provide deeper insight than that achieved by individual analyses.

5. Systems biology of retinal degeneration

Retinal degenerative diseases (RDDs) are genetically heterogeneous, ranging from monogenic forms such as retinitis pigmentosa to complex disorders such as AMD. Since the first linkage analysis that identified a genomic locus associated with X-linked retinitis pigmentosa (Bhattacharya et al., 1984), we have witnessed a remarkable progress in elucidating the genetic basis of retinal diseases (Swaroop and Sieving, 2013; RetNet, http://sph.uth.edu/

Table 3
Gene expression profiling studies of aging ocular tissues.

Organism	Tissue/cell type	Platform	Number of genes	Age range	Reference
Human	Neural retina/macula and periphery	SAGE	320,998 tags	44–88 yr	(Sharon et al., 2002)
Human	Neural retina	Microarray, Micromax	2400 probes	13–72 yr	(Yoshida et al., 2002)
Human	Neural retina	Microarray, Custom	10,034 genes	29–90 yr	(Chowers et al., 2003a)
Human	Neural retina/macula and periphery	Microarray, Affymetrix Human Genome U133 plus 2	54,600 gene probes	18–79 yr	(Cai et al., 2012)
Mouse	Neural retina	Microarray, Agilent Technologies, Whole Mouse Genome Oligo Microarrays 4 × 44K	43,379 gene features	3–20 mo	(Chen et al., 2010a)
Mouse -senescence	Neural retina	Microarray, Affymetrix MG_U74Av2 GeneChip	36,000 full-length genes and EST clusters	3–21 mo	(Carter et al., 2005)
Mouse	Rod photoreceptors (flow-sorted *Nrl*p-EGFP retina)	Microarray, Affymetrix Mouse Exon 1.0ST Array GeneChip	>28,000 coding, >7000 non-coding transcripts	1.5–12 mo	(Parapuram et al., 2010)
Mouse	Microglia (flow- sorted, CD11b-immunopositive cells)	Microarray, Affymetrix Mouse Exon 1.0ST Array GeneChip	>28,000 coding, >7000 non-coding transcripts	3–24 mo	(Ma et al., 2013)
Rat - senescence	Neural retina	RNA-Seq, Illumina GA IIx	15,300 identified transcripts[a]	3–18 mo	(Kozhevnikova et al., 2013)

[a] Approximate number of transcripts detected in at least three samples with FPKM > 1.

retnet/). Photoreceptor cell death remains central to many RDDs (Wright et al., 2010). Functional analyses of the identified disease genes through cell-based and animal models have enhanced our understanding of their normal function in ocular development and homeostasis. However, elucidation of underlying mechanism(s) has not kept pace with disease gene identification.

The advent of NGS is now transforming, at an unprecedented rate, the way we study genetic basis of human disease. Holistic approaches to RDDs have become possible through a combination of NGS-based strategies including disease-associated variant identification, transcriptome analysis, and high throughput search for biomarkers (Fig. 6). We can learn about regulatory networks, disease-relevant pathways and their crosstalk, which can be invaluable for translational and personalized medicine. In this section, we discuss the following areas, which will provide the necessary impetus:

(i) Whole exome and whole genome sequencing (WES and WGS) of patients with monogenic RDDs for better genotype–phenotype correlation and search for genetic modifiers
(ii) Fine mapping and functional dissection of associated loci
(iii) Genetics of RDDs beyond genetic variants

5.1. NGS applications for disease variant/mutation discovery

NGS methods have expedited the process of cataloging human genetic variation. While precise correlation of variants associated with disease phenotypes is still challenging, NGS approaches, such as WES, WGS and targeted sequencing, have become preferred methods for disease gene discovery (Fig. 7A). A number of novel genes have been identified through WES in small Mendelian RDD families and in sporadic cases that were not suitable for customary linkage studies (Ratnapriya and Swaroop, 2013).

Progress in dissecting genetic architecture of complex RDDs has been relatively slow since these multifactorial diseases are more common in the population and often do not exhibit familial segregation. Furthermore, non-genetic factors, such as environment, can contribute to disease onset and severity. We have come a long way since the initial association of *CFH* variant with AMD (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Jakobsdottir et al., 2005; Zareparsi et al., 2005a). A total of 19 AMD susceptibility loci have been detected so far, including 7 new loci identified in the meta-analysis of 17,000 cases and 60,000 controls (Fritsche et al., 2013). These discoveries have provided clues about the underlying disease processes and have implicated complement cascade, high-density lipoprotein cholesterol and extracellular matrix pathways in AMD pathogenesis (Fritsche et al., 2014; Priya et al., 2012). However, associated locus variants are usually not causal and often reside outside the gene region. Thus, more than one gene at each associated locus can be potential disease gene. The nature of causal variants/genes at these loci remains debatable and needs further explorations as the causal variants could be an associated variant, another common variant, a rare variant or even a structural variant (Fig. 7B).

Identification of rare variant(s) in a specific gene at the associated locus can provide functional clues. For AMD, a rare penetrant mutation, R1210C was first identified at the *CFH* locus (Raychaudhuri et al., 2011). Subsequently, rare variants in several complement pathway genes have been associated with AMD through targeted and whole genome sequencing (Helgason et al., 2013; Seddon et al., 2013; van de Ven et al., 2013; Yu et al., 2014; Zhan et al., 2013). However, the search for rare variants in RDDs has been limited to known loci and candidate association regions. Whole exome and whole genome sequencing studies in large patient cohorts may facilitate cataloging of rare and common causal variants, identification of novel disease genes and assist in biological investigations. However, rare variant association study design requires a large sample size since, as predicted, not many individuals will harbor a rare variant. Genetic refinement of associated signals with improved imputation methods can further help in identifying potential causal variants. Recently, a new SNP array ("exome-chip") was developed to explore the role of common as well as rare variants in complex traits (Huyghe et al., 2013). Sixteen additional AMD loci have now been identified by exome-chip analysis and imputation of variants in approximately 26,000 cases and 22,000 controls (Cipriani and International AMD Genomics Consortium, 2014; Fritsche and International AMD Genomics Consortium, 2014). Notably, only a small number of rare variants were identified in this study, and whole genome sequencing might be necessary for evaluating the role of rare variants in AMD.

5.2. Less-recognized complexities associated with genetic variations and human retinal diseases

Human genome harbors a large number of genetic variations, and NGS has made it possible to systematically catalog genome-wide sequence variants. However, methods for distinguishing a neutral variant from the causal one are not yet fully established.

The current criteria include nature of change, allele frequency and nucleotide conservation across species. However, these criteria do not seem sufficient as we are learning more about the architecture of variants in the human genome. A single human genome can harbor thousands of variants (10,000–11,000 non-synonymous and 10,000–12,000 synonymous variations) (Genomes Project Consortium et al., 2012), with as many as 300 loss-of-function alleles having little or no clinical consequence (Kukurba et al., 2014; MacArthur et al., 2012). Incorrect assignment of variants can have immediate implication for diagnosis, therapeutic advice and research direction. The recommendations for identifying causal genes/variants associated with specific phenotypes through NGS studies have been reviewed recently (Chakravarti et al., 2013; MacArthur et al., 2014; Samocha et al., 2014). Here, we revisit some of the issues that have implications for retinal genetics.

5.2.1. Are mutations rare genetic variations?

Mutations causing monogenic diseases are believed to be rare, and often a blanket minor allele frequency cut-off is applied to filter out disease variants. Such an approach can miss cases where disease variants are common and/or likely modify the disease phenotype (Ebermann et al., 2010; Khanna et al., 2009; Louie et al., 2010; Venturini et al., 2012). Notably, carrier frequency of the null mutations responsible for RDDs is unusually high in general population (Nishiguchi and Rivolta, 2012). A vast majority of known genetic variants associated with complex diseases (such as AMD) are common and yet some may have functional relevance to pathology (such as Y402H in *CFH*). As investigations of genetic variation underlying complex traits continue to mature, we are seeing examples of rare and common regulatory as well as copy number variants associated with disease. Thus, no general analysis pipeline should be applied to identify the variants of interest, and one must customize based on genetic architecture of the disease under study.

5.2.2. Do disease-causing mutations have to be conserved?

Computational prediction tools incorporate evolutionary conservation across species as a criterion when assigning causality to variants. While a majority of disease-related variants follow this pattern, less conserved residues, silent variants or changes in the non-coding region have also been implicated in disease (Cartegni et al., 2002; Sauna and Kimchi-Sarfaty, 2011). Furthermore, the effect of a variant is likely to be influenced by size, polarity, codon-usage, neighboring amino-acid residues and location with respect to the overall protein conformation. Prediction programs fail to account for many of these important parameters. Thus, filtering out any "potentially interesting" variant based on prediction criteria should not be a common practice. Development of more sophisticated analytical tools is therefore necessary for better predictive power.

5.2.3. Are monogenic diseases caused by defects in only one gene?

Until recently, a majority of disease genes for monogenic traits have been identified through traditional linkage approach, where loci that segregate with the disease phenotype are screened for mutation(s) in candidate genes. Once a segregating disease mutation is identified, the researchers usually no longer search for mutations/variations. However, extensive genetic and even phenotypic heterogeneity observed in RDDs has been an obstacle to diagnosis and counseling. Different RDDs also share overlapping clinical findings; thus, mutations in many different genes may result in similar phenotypes and/or mutations in one gene can lead to distinct phenotypes. In Mendelian diseases, inheritance of a genetic defect implies that one will get the disease. However, many individuals carrying a mutation may show no clinical phenotype (incomplete penetrance) or variable expressivity. As every individual carries thousands of unique variants, one can envisage compensatory variations in interacting genes (epistasis) that can alter the clinical phenotype(s)

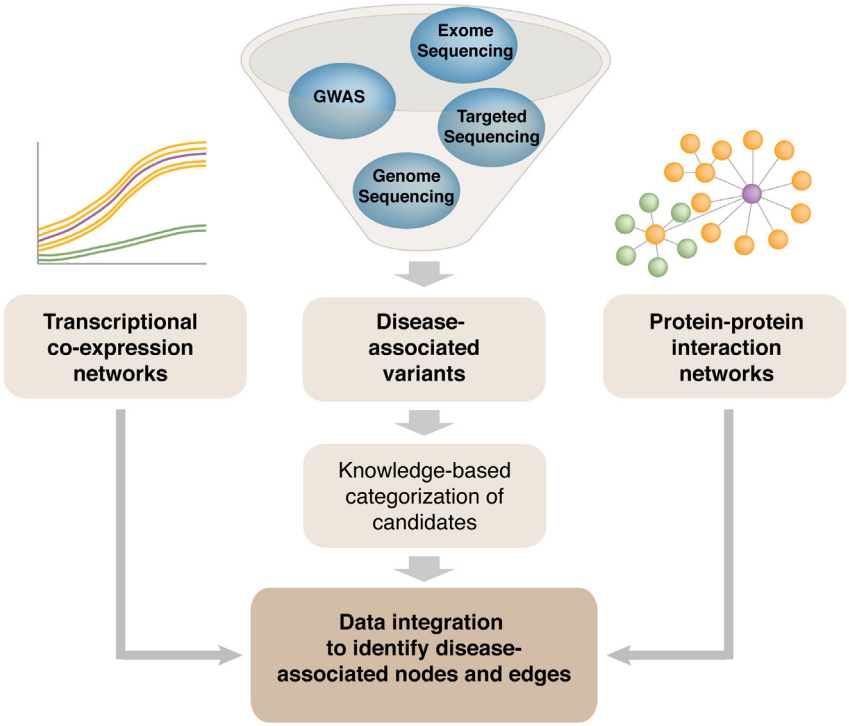


Fig. 6. Integration of multi-level data sets for system-level understanding. Simultaneous analysis of genomics, transcriptomics and proteomics data can provide a comprehensive multi-dimensional view of the system and can help identify pathways and networks that are specific to development and disease. This can lead to better understanding of the biology as well as to identification of disease associated nodes, which can be potential drug targets (e.g., hub depicted as a purple node in the example network).

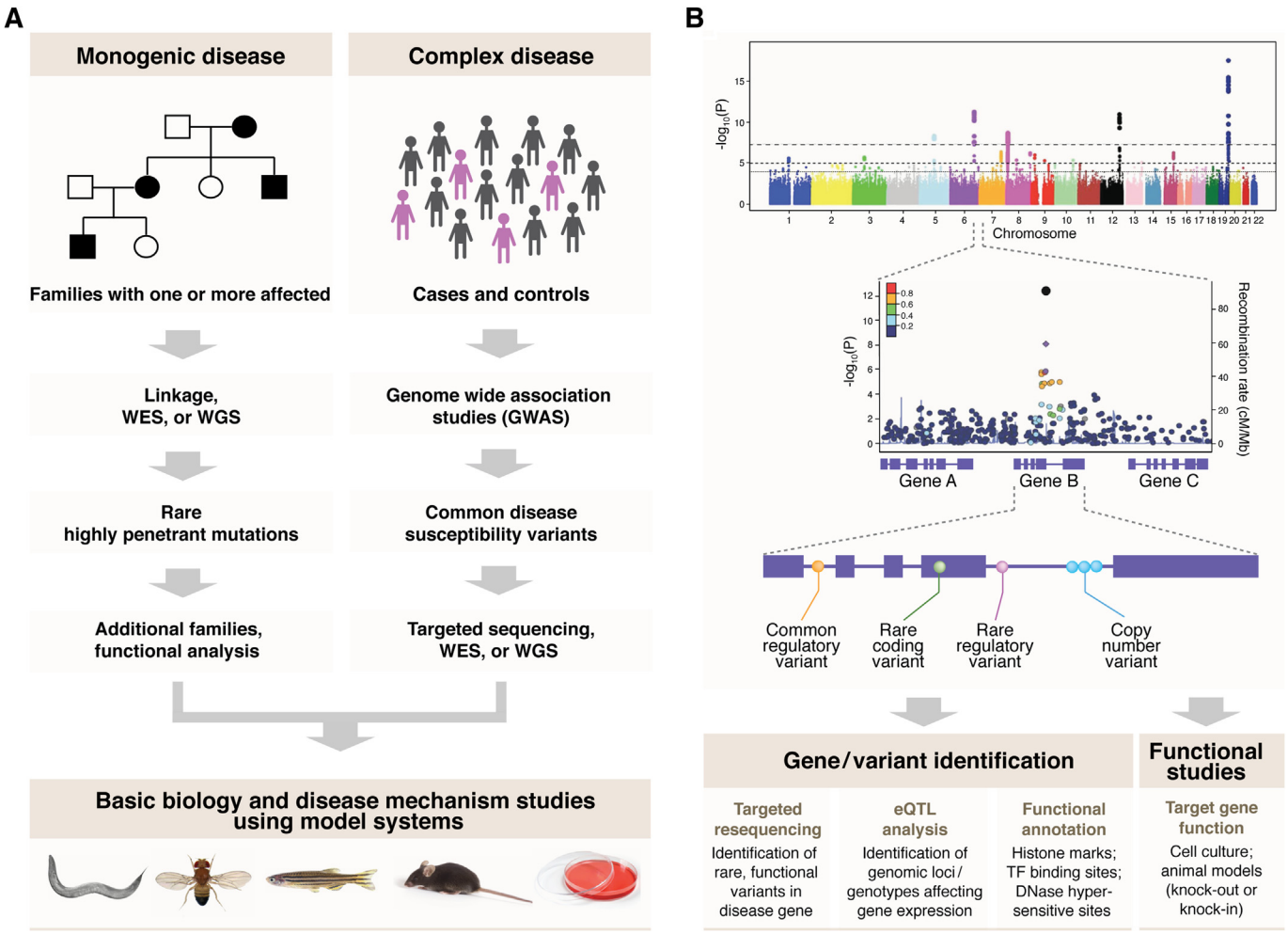


Fig. 7. General recommendations for genetic analysis of monogenic and complex diseases using next-generation approaches. **A**. Families with a monogenic (i.e., Mendelian) disease, even those with a small number of individuals, can be analyzed by whole exome or genome sequencing. In case of identification of mutation(s) in a novel gene, additional validation is required, including genetic analysis of more families/patients as well as functional analysis using animal models to understand the role in disease causation. For complex diseases, genome wide association study is performed on a large number of samples including patients and population-matched controls, to identify common susceptibility variants associated with the disease. Targeted sequencing, whole exome or genome sequencing can be applied to identify the causal variant at the susceptibility locus. Finally, multiple molecular genetic and cellular assays as well as studies in animal models are required to identify functional variants underlying trait association. WES, whole exome sequencing; WGS, whole genome sequencing, GWAS, genome-wide association study. **B**. Schematic representation of follow-up on a GWAS hit to identify the causal gene/variant. Causal variants could be common regulatory variants, rare coding variants or copy number variants. Targeted sequencing around the associated locus can help identify the causal variant. Generally, more than one candidate gene is found at an associated locus, and eQTL analysis in disease relevant tissues can help identify the specific genotypes affecting gene expression within the causal gene. Additional functional annotation, including histone modifications, transcription factor binding sites and DNase I hypersensitivity sites can also help in identifying the causal gene. Ultimately, a high throughput functional assay in animal and/or cell-based models is highly recommended to understand the role of the gene in disease pathophysiology. cM/Mb, centimorgan (cM) per megabase pair (Mb); eQTL, expression quantitative trait locus, TF, transcription factor.

(Lehner et al., 2006). Thus, in addition to the "primary" gene defect, many patients may harbor alleles that can modify the disease phenotype (modifier alleles). For example, a common allele in the *RPGRIP1L* gene, associated with Joubert and Meckel syndromes (Arts et al., 2007; Delous et al., 2007), is shown to contribute to retinal degeneration phenotype in individuals with ciliopathies caused by mutations in other genes (Khanna et al., 2009). In other instances, mutations in more than one gene might be necessary to observe a disease phenotype (e.g., "digenic" RP caused by *ROM1* and *RDS* alleles) (Kajiwara et al., 1994). NGS offers an unbiased approach for unveiling genetic modifiers of retinal degeneration across the genome and can help in refining the genetic definition of phenotypic variations in RDD patients.

The spectrum of potentially disease-causing variants makes the correlation of the variant with the phenotype challenging. Given that causative variants could be rare, common, regulatory, intronic, silent or compensatory, establishing the role of a gene or a variant in disease pathogenesis is not straightforward. Genes that are identified through genetic studies should be referred as "candidates" until their association with the disease phenotype is validated by additional evidence including functional data. Further support can emerge from enrichment of genetic variants (gene-level evidence) in patients/families compared with controls. Disruption of the gene in a model system can, in some instances, reproduce the relevant human phenotype. In addition, one should be able to rescue the phenotype with the wild type allele but not with mutant allele(s), thereby mimicking the "Koch's postulates" (Chakravarti et al., 2013). While mouse (or zebrafish) models do not always recapitulate the human phenotype, such studies could provide disease mechanisms and opportunities for evaluation of treatments (Veleri et al., 2015). Alternative models using patient-derived human induced pluripotent stem (iPS) cells also appear promising.

5.2.4. Missing heritability of complex traits

In addition to common and rare genetic variants, other components including copy number variation, epigenetic signature and gene–environment interaction contribute to complex disease phenotype. While many studies have looked at copy number variations in candidate genes with limited success and replication (Cantsilieris and White, 2013; Liu et al., 2012), genome-wide surveys have not been useful in identifying such variations associated with major risk of AMD (Fritsche et al., 2013; Meyer et al., 2011; Neale et al., 2010; Sobrin et al., 2012).

Epigenetics has gained immense interest because of its role in human diseases such as cancer (Feinberg et al., 2006). Disease-related changes include stochastic modifications in epigenetic marks as well as local gene-specific alterations (Pujadas and Feinberg, 2012). Beyond cancer, epigenetic changes have been widely associated with human diseases involving chromosomal abnormalities and intellectual disability (Brookes and Shi, 2014). Retinal diseases have also been explored for possible involvement of epigenetic alterations, and genes such as *IL17RC* (Wei et al., 2012a) and *ApoJ* (Suuronen et al., 2007) have been suggested to contribute to the pathogenesis of AMD. However, such studies require further validation (Oliver et al., 2013a). More extensive analysis of the epigenome must be performed using normal or disease retina (or target cell types).

Complex diseases, such as AMD, exhibit tremendous phenotypic heterogeneity, and multiple classification and grading systems have been put forward (Bird et al., 1995; Ferris et al., 2005, 2013). However, much of the genetic analysis of AMD has been performed on cohorts with advanced stage of disease, with phenotypes broadly classified as geographic atrophy and choroidal neovascularization. While an *ARMS2* risk variant is reportedly more strongly associated to neovascularization compared to geographic atrophy (Chen et al., 2010b; Sobrin et al., 2011), it has been difficult to identify genes and pathways associated with distinct clinical subtypes (Fritsche et al., 2014). The recently completed exome-chip analysis of the large case-control cohort may provide additional insights on subtype specific genetic variants. Whole genome sequencing of 6000 AMD cases and controls is currently in progress (Kwong et al., 2014) and should be valuable for deciphering susceptibility and protective alleles for AMD subtypes.

5.3. Integration of transcriptome with genetic data

Though heritable mutations are present in every cell of an individual, disease manifestation is often tissue or cell specific because of differences in spatio-temporal expression or function of the responsible gene(s). Thus, it would be important to correlate genetic variations to gene expression in the retina (or photoreceptors) to obtain insights into the regulatory network/pathways perturbed in RDDs. NGS methods allow high throughput analysis of genotypes as well as provide an opportunity to sequence all species of RNA, including the splice variants and relatively less studied miRNAs and lncRNAs.

A majority of variants associated with complex diseases often reside outside the coding region of a gene or even in non-transcribed regions. It is assumed that such variants would have subtle effects on gene expression or function. The expression quantitative trait locus (eQTL) analysis combines gene expression data with genotype information and can assist in identifying risk alleles (Cheung et al., 2003; Lappalainen et al., 2013). Expression profiling of disease-relevant tissue or cells in case-control samples may reveal functional variants with biological relevance to the phenotype. eQTLs are primarily *cis*-acting, suggesting a common mechanism at many associated loci (Edwards et al., 2013). Identification of target genes modulated by *cis*-regulatory risk variants will allow better understanding of disease network(s) and/or mechanism(s) through which they act. In fact, ENCODE project has demonstrated that a large proportion of associated GWAS SNPs appear to map to functional regulatory elements (Schaub et al., 2012). As noted earlier, gene expression patterns are cell type- and tissue-specific, and eQTL analyses are more useful in cells/tissue types relevant to the human disease of interest. The current databases do not include eQTL information on the retina or other ocular cell types owing to limitations in obtaining human tissues.

GWAS studies have yielded a large number of new genetic loci associated with AMD susceptibility. However, several genes that seem to have biological relevance to AMD pathology have not been validated even in large GWAS meta-analyses, in part because of false positive results or phenotypic overlap together with etiological heterogeneity; these include *ABCA4* (Allikmets, 2000), *TLR4* (Zareparsi et al., 2005b), *TLR3* (Kleinman et al., 2008; Yang et al., 2008), *CX3CR1* (Tuo et al., 2004), *DICER* (Kaneko et al., 2011), and *AHR* (Choudhary et al., 2014; Kim et al., 2014). Another explanation could be that the effect size of these variants is small, and much larger sample size is needed to achieve genome-wide significance. We should also note that the biological significance of many genes/variants identified by GWAS is not yet clear. The genetic variants identified through different approaches may have subtle yet meaningful influence on specific pathways and networks that when disrupted lead to AMD pathology. We recently suggested that cumulative impact of few strong or multiple weaker alleles must reach a threshold of disruption for producing a clinically identifiable phenotype (Fritsche et al., 2014). An integrated approach of spatial and temporal mapping of disease genes and variants onto the expression data generated from disease-relevant tissues would be necessary to generate disease-specific networks. Distinct candidates and pathways may converge in networks of co-expressed genes in disease-relevant cell/tissue at a specific time. Such disease networks can help in identifying novel regulatory nodes and therapeutic targets.

5.4. Approaches for elucidating disease networks

Identifying disease-associated genetic variants is an important piece of the puzzle in understanding pathogenic mechanism(s). Rather than examining one gene (and its function) at a given time, our goal must be to understand the “disease” itself and elucidate complex interplay among multiple factors (genes, epigenetic changes, and interacting environment). Initially, one can investigate all genes associated with a retinal disease phenotype (such as Leber Congenital Amaurosis or AMD) to deduce relevant biological pathways and then integrate genes and pathways into networks. For examples, 19 AMD loci, published so far, have implicated complement, extracellular matrix and lipid metabolism pathways (Fritsche et al., 2014; Priya et al., 2012). Other studies on GWAS loci have hinted towards localization of cis-regulatory variation at enhancer elements (Pennacchio et al., 2013). Integrating high throughput functional annotations, such as histone marks, transcription factor binding sites and DNase I hypersensitive sites (DHSs), relevant to AMD cell types can help in prioritization of non-coding variants at associated loci for subsequent functional evaluation. Combining this information with the genetic data can provide novel insights into disease mechanisms; for example, exome sequencing in small families can identify large number of rare and novel potential disease variants, and ChIP-seq data can allow filtering of genetic variants, as shown in case of *MAK* (Ozgul et al., 2011) where CRX Chip-seq data was used to rank the candidate genes. Combined systems level analyses of multiple datasets have already begun to reveal novel insights in disease mechanisms and

targets for drug discovery (Cohen et al., 2006; Dadu and Ballantyne, 2014; Sahebkar, 2014).

Rare variants or common loci with small effect often require large sample size to reach genome-wide statistical significance. However, there could be alternate ways to extract meaningful information about the disease process. For example, exome sequencing of families where the disease is clustered can identify rare segregating variants that can impact susceptibility. Enrichment analysis of candidate variants can either link to existing disease-associated pathways or reveal novel insights into the disease process (Ratnapriya et al., 2014a). Alternatively, pathogenic mechanisms associated with a specific phenotype within the complex disease (such as AMD) can also be derived from delineating the biology of Mendelian RDDs that include overlapping clinical features (such as Stargardt disease) (Ratnapriya et al., 2014b).

6. Next generation diagnosis and drug development

The promise of next generation genomics to medicine is exciting and unprecedented; yet, we have a long way before incorporating NGS into routine clinical practice. Meanwhile, NGS has created new opportunities for faster genetic diagnosis that can help in improved disease management. Given the allelic heterogeneity of RDDs, the current sequencing methods are not very efficient. Decreasing cost of sequencing and approval from U.S. Food and Drug Administration allowing NGS methods for clinical diagnosis constitute steps in the rapid realization of personalized medicine. NGS-based screening methods are now being utilized for RDDs as well (Booij et al., 2011; Bowne et al., 2011; Neveling et al., 2012; Song et al., 2011b). The National Ophthalmic Disease Genotyping and Phenotyping Network (eyeGene), a CLIA certified community resource designed to bring together patients, providers, molecular testing laboratories and researchers, is incorporating NGS tools for investigating disease causing mutations (Blain et al., 2013).

Another major application of NGS is in the area of pharmacogenomics. Patients with the same disease often respond differently to drug efficacy and safety because of unique genetic variations they inherit. Identification of large number of susceptibility alleles has propelled the field of pharmacogenomics to a potential future in personalized medicine. In AMD, the effect of GWAS susceptibility loci, mainly *CFH* and *ARMS2* variants on treatment outcome of Avastin (bevacizumab) and Lucentis (ranibizumab), has received a lot of attention, though such studies have not yielded consistent results (Hagstrom et al., 2013; Kanoff and Miller, 2013). Small sample size, incorporation of only few disease markers, and short term-follow up represent some of the challenges. More comprehensive, large-scale prospective studies are necessary to establish definitive associations between genotypes and drug response/efficacy.

Relevance of NGS datasets to development of translational strategies has been limited so far because of inadequate understanding of underlying biology of the disease. GWAS hits are rarely direct drug targets (Teslovich et al., 2010). Limited predictive value of the disease models and inability to accurately estimate the efficacy, toxicity and long-term side effects have made discovery of new drugs very expensive and time consuming (Plenge et al., 2013). NGS technology has remarkable promise for genomic medicine, impacting both predictive and preventive medicine. Few studies have already shown novel means for drug discovery using genetic data and bioinformatics tools, such as functional annotation, *cis*-acting eQTLs and pathway analyses (Okada et al., 2014). We need to better define and map interactome networks with its components (nodes) and interactions (edges) (Vidal et al., 2011). These biological networks can be integrated with known drug and disease information to build drug-target networks (Yildirim et al., 2007). As NGS technology becomes widely available, pharmacological interventions will have personalized focus on disease treatment. Identification of clinically important variants, together with transcriptome and epigenetic profiling, should permit dissection of human disease genome that is amenable to pharmacological interventions.

7. Challenges and further considerations

Here, we have examined new developments in photoreceptor biology stemming from NGS technologies. Despite much progress, challenges remain; these include development of effective bioinformatics tools for multi-layer data integration, standardization of procedures to facilitate collaborative works, data sharing within the research community and application of novel findings from system-level analysis to develop new hypothesis.

7.1. Multi-layer data integration

Most high throughput data generation and analysis have so far been conducted at one or two layers of biological information. For instance, transcriptome analysis has been the most dominant form of genome-wide information, and other types of data such as transcription factor targetome or epigenome profiling have been individually compared to transcriptome data but not to one another. With increasing amount of studies examining various layers of biological pathways, including regulatory RNAs, epigenome and proteome, the resulting higher order integrative analysis is expected to further refine the current knowledge of molecular networks. To this end, applying robust computational analysis of the data sets is critical. The large-scale ENCODE (encyclopedia of DNA elements) consortium probably provides the best model. Upon completion of the human genome project, ENCODE was launched to investigate functional elements of human and mouse genome (http://encodeproject.org). By employing more than two-dozen assays, a large number of data sets have been generated from 147 distinct cell types or tissues (ENCODE Project Consortium, 2012). Several powerful software tools were developed with a goal of effective data integration across heterogeneous data sets (http://encodeproject.org/ENCODE/encodeTools.html). Additional open source bioinformatics tools are freely available.

7.2. Standardization of procedures and quality control

Given the significant amount of expertise, workloads, and funding required for high throughput data collection, the practice of comprehensive and multi-dimensional data analysis demands collaboration among various disciplines. Independently generated data sets will often have to be compiled together for integrative analysis. The vision research community, therefore, needs standardized procedures for experimentation and data analysis. Detailed instructions for high throughput assay protocols and quality control are also provided as a part of the ENCODE project (ENCODE Project Consortium, 2011), and all the upcoming data generation should refer to those. The retina, however, consists of highly specialized sets of neurons and supporting cells; thus, additional guidelines specific to the vision field must be implemented. Directions and relevant resources for retinal sample preparation should be established. As retina is rich with tissue-specific transcript isoforms, genome annotations tailored for the retina should be established for standardized data analysis.

7.3. Data sharing

A fundamental consideration for systems approaches is to facilitate data sharing. High throughput studies are unique in that the generated data can be re-analyzed to discover new patterns and to test new hypotheses distinct from those posed in the original study. Therefore, after the initial analysis and publication, high throughput data sets should be readily accessible for any research group to view, verify and reanalyze. More than 200 retina-related data series can be retrieved from public databases, such as Gene Expression Omnibus (GEO), which archive genome-wide information generated by microarray and NGS technologies. Microarray data deposited to GEO follows, for the most part, the guidelines from Minimum Information About a Microarray Experiment (MIAME) (Brazma et al., 2001) put forward by the microarray gene expression data (MGED) society. However, standardized directions specific for NGS data have not been implemented yet, nor the strict measures for quality control are in place. We have recently undertaken the establishment of a web-based database for retinal NGS data repository with the following goals:

(i) Implementation of uniform guidelines for sample collection, assay procedures and data analysis
(ii) Ensure the quality of the deposited data and encourage data sharing in a timely manner
(iii) Consolidation of high throughput data sets related to retina (or other ocular tissues) commonly affected in retinal neurodegenerative diseases
(iv) Provide structured and comprehensive annotation of each data set

Our archive of high throughput data sets should provide a critical resource to facilitate higher-order integrated data analysis and/or novel discovery through re-analysis.

7.4. From in silico analysis to the bench

The observations made from system-wide analyses, in most instances, are descriptive and hypothesis-generating rather than hypothesis-testing. Wet bench research is needed to verify and further develop the knowledge obtained from systems level computational analyses. Networks and/or patterns implicated in retinal development, aging or disease must be tested by traditional *in vivo* and *in vitro* model systems. The three-dimensional retinal cultures derived from human or mouse stem cells (Eiraku et al., 2011; Nakano et al., 2012) provide a useful model for human retina in the context of evaluating the role of microenvironment and genetic pathways. Patient-specific induced pluripotent stem cells provide hope for the development of personalized medicine.

8. The future of systems biology approaches: biology of the organism as a whole

In this review, we have primarily focused on GRNs that maintain photoreceptor function and on RDDs; nonetheless, we do realize that a comprehensive systems biology strategy would also incorporate population-wide genetic variations, protein interaction networks and metabolomics. In general, we believe that reproducible topologies in the networks with principal regulators would constitute the hubs of sub-networks. It is not difficult to imagine that perturbation of such primary hubs (e.g., key regulators, rate-limiting substrates and enzymes) likely imposes more pronounced impact on photoreceptor health than the defect in locally confined nodes with fewer interactions (e.g., those with limited physiological reach) (Fig. 8). Targeting “key” hubs would therefore represent an attractive strategy for drug discovery.

One of the major bottlenecks in therapeutic development has been the overwhelming number of disease-associated nodes (i.e., disease genes) due to heterogeneity of RDDs. As suggested earlier

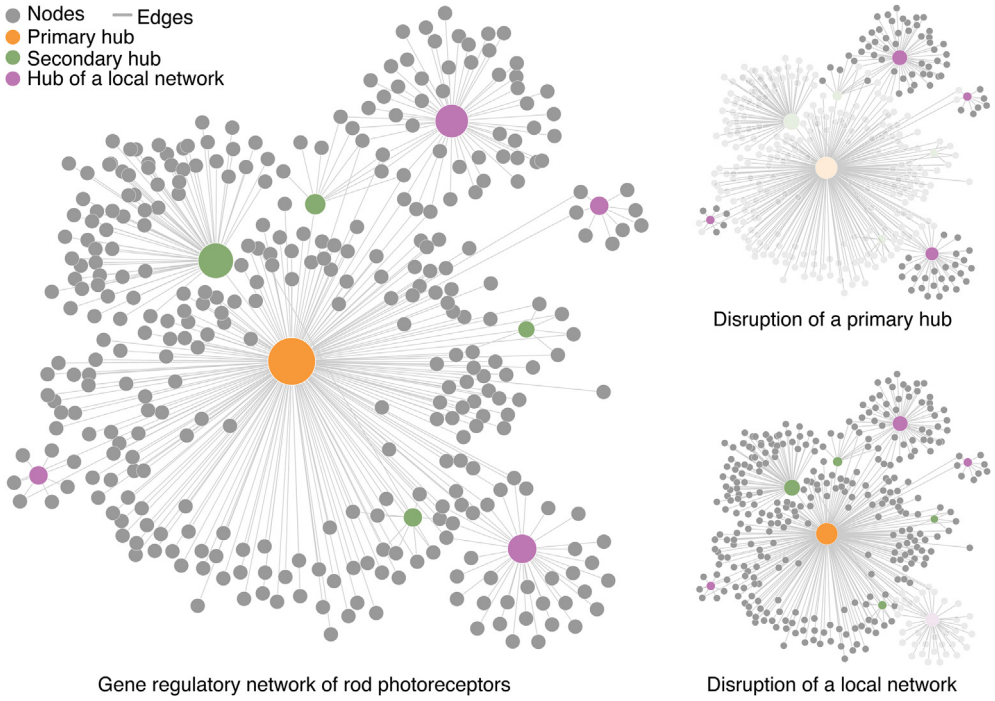


Fig. 8. Model of multi-dimensional network of photoreceptors. Networks consist of nodes and edges, representing cellular components (genes, transcripts, proteins, etc.) and their interactions, respectively. The nodes with the greatest number of interactions constitute primary hubs. Secondary hubs themselves are the interacting partners of primary hubs and simultaneously connect with many other nodes, thereby partially mediating the function of the primary hubs. Local sub-networks are also prevalent. Perturbation of primary or secondary hubs of the network likely poses detrimental impact to the system, while perturbation of a more locally confined network or a node with few interacting partners may not exert substantial effect on the system integrity. Identification of commonly affected networks in diverse retinal diseases will allow the better drug design.

(Swaroop et al., 2010; Yu et al., 2004b), it is conceivable that distinct mutations resulting in a broad spectrum of retinal diseases converge onto a manageable number of "key" hubs (with overlapping disease-associated pathways/networks) prior to photoreceptor death. Thus, one may not need to target individual disease causing genes to rescue photoreceptor integrity. Identification of common retinal disease-related networks and associated hubs may now be within reach for discovering better drug targets.

We have come a long way in defining the biology of individual genes. Nonetheless, we now must provide an appropriate cellular context to individual genes and their functional products. We have already started to examine genetic, transcriptional and epigenetic architectures and await integrated analysis to incorporate proteome and metabolome data. Networks constructed from multi-dimensional data analysis will simulate functional and physiological outcome of photoreceptors in response to alteration in the microenvironment and variations in the genetic makeup. Human genomes are highly polymorphic, and even monozygotic twins may carry genetic differences (Bruder et al., 2008). Notably, individuals are estimated to carry over 2500 functionally relevant genetic variations (Genomes Project Consortium et al., 2012). Such variability is what makes each of us unique and would be expected to influence the network structure. Genetic polymorphism, together with aging and environmental stress, may have profound impact on susceptibility to diseases and drug response. The holistic approaches thus have the key to accurate diagnosis and personalized medicine.

In this review, we have primarily discussed system-level analysis of the whole cell (rather than focusing on a gene or a pathway in isolation). We recognize that cells in higher organisms do not function independently but constitute higher order structures that are morphologically and functionally distinct (e.g., retina, lens, cerebellum), which in turn are in constant communication with structures farther away (e.g., via systemic circulation or neuronal connectivity). Thus, aberrant function of a gene in a specific cell may have wide-ranging impact on other tissues and the whole organism. A true system-level analysis will eventually incorporate both limited and long-range effects on GRNs and cell function. The retinal research has an exciting future, as we are closer to better predictive and treatment strategies for every individual.

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References

Adli, M., Zhu, J., Bernstein, B.E., 2010. Genome-wide chromatin maps derived from limited numbers of hematopoietic progenitors. Nat. Methods 7, 615–618.
Agathocleous, M., Harris, W.A., 2009. From progenitors to differentiated cells in the vertebrate retina. Annu. Rev. Cell. Dev. Biol. 25, 45–69.
Akimoto, M., Cheng, H., Zhu, D., Brzezinski, J.A., Khanna, R., Filippova, E., Oh, E.C., Jing, Y., Linares, J.L., Brooks, M., Zareparsi, S., Mears, A.J., Hero, A., Glaser, T., Swaroop, A., 2006. Targeting of GFP to newborn rods by Nrl promoter and temporal expression profiling of flow-sorted photoreceptors. Proc. Natl. Acad. Sci. U. S. A. 103, 3890–3895.
Allikmets, R., 2000. Further evidence for an association of ABCR alleles with age-related macular degeneration. The International ABCR Screening Consortium. Am. J. Hum. Genet. 67, 487–491.
Arora, A., Guduric-Fuchs, J., Harwood, L., Dellett, M., Cogliati, T., Simpson, D.A., 2010. Prediction of microRNAs affecting mRNA expression during retinal development. BMC Dev. Biol. 10, 1.
Arts, H.H., Doherty, D., van Beersum, S.E., Parisi, M.A., Letteboer, S.J., Gorden, N.T., Peters, T.A., Marker, T., Voesenek, K., Kartono, A., Ozyurek, H., Farin, F.M., Kroes, H.Y., Wolfrum, U., Brunner, H.G., Cremers, F.P., Glass, I.A., Knoers, N.V., Roepman, R., 2007. Mutations in the gene encoding the basal body protein RPGRIP1L, a nephrocystin-4 interactor, cause Joubert syndrome. Nat. Genet. 39, 882–888.
Balakirev, E.S., Ayala, F.J., 2003. Pseudogenes: are they "junk" or functional DNA? Annu Rev. Genet. 37, 123–151.
Barabasi, A.L., Oltvai, Z.N., 2004. Network biology: understanding the cell's functional organization. Nat. Rev. Genet. 5, 101–113.
Barb, J.J., Gotoh, N., Brooks, M., Kim, J.W., Gieser, L., Villasmil, R., Bhagwat, M., Kooragayala, K., Gupta, N., Ratnapriya, R., Munson, P.J., Cogliati, T., Swaroop, A., 2015. Hallmarks of Mammalian Rod Photoreceptor Aging and Risk for Neurodegeneration. Manuscript in preparation.
Barnhill, A.E., Hecker, L.A., Kohutyuk, O., Buss, J.E., Honavar, V.G., Greenlee, H.W., 2010. Characterization of the retinal proteome during rod photoreceptor genesis. BMC Res. Notes 3, 25.
Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., Zhao, K., 2007. High-resolution profiling of histone methylations in the human genome. Cell 129, 823–837.
Bassett, E.A., Wallace, V.A., 2012. Cell fate determination in the vertebrate retina. Trends Neurosci. 35, 565–573.
Berger, S.L., 2007. The complex language of chromatin regulation during transcription. Nature 447, 407–412.
Berman, B.P., Weisenberger, D.J., Aman, J.F., Hinoue, T., Ramjan, Z., Liu, Y., Noushmehr, H., Lange, C.P., van Dijk, C.M., Tollenaar, R.A., Van Den Berg, D., Laird, P.W., 2012. Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. Nat. Genet. 44, 40–46.
Bhattacharya, S.S., Wright, A.F., Clayton, J.F., Price, W.H., Phillips, C.I., McKeown, C.M., Jay, M., Bird, A.C., Pearson, P.L., Southern, E.M., et al., 1984. Close genetic linkage between X-linked retinitis pigmentosa and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28. Nature 309, 253–255.
Birch, D.G., Anderson, J.L., 1992. Standardized full-field electroretinography. Normal values and their variation with age. Arch. Ophthalmol. 110, 1571–1576.
Bird, A.C., Bressler, N.M., Bressler, S.B., Chisholm, I.H., Coscas, G., Davis, M.D., de Jong, P.T., Klaver, C.C., Klein, B.E., Klein, R., et al., 1995. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv. Ophthalmol. 39, 367–374.
Blackshaw, S., Fraioli, R.E., Furukawa, T., Cepko, C.L., 2001. Comprehensive analysis of photoreceptor gene expression and the identification of candidate retinal disease genes. Cell 107, 579–589.
Blackshaw, S., Harpavat, S., Trimarchi, J., Cai, L., Huang, H., Kuo, W.P., Weber, G., Lee, K., Fraioli, R.E., Cho, S.H., Yung, R., Asch, E., Ohno-Machado, L., Wong, W.H., Cepko, C.L., 2004. Genomic analysis of mouse retinal development. PLoS Biol. 2, E247.
Blain, D., Goetz, K.E., Ayyagari, R., Tumminia, S.J., 2013. eyeGENE(R): a vision community resource facilitating patient care and paving the path for research through molecular diagnostic testing. Clin. Genet. 84, 190–197.
Bonnel, S., Mohand-Said, S., Sahel, J.A., 2003. The aging of the retina. Exp. Gerontol. 38, 825–831.
Booij, J.C., Bakker, A., Kulumbetova, J., Moutaoukil, Y., Smeets, B., Verheij, J., Kroes, H.Y., Klaver, C.C., van Schooneveld, M., Bergen, A.A., Florijn, R.J., 2011. Simultaneous mutation detection in 90 retinal disease genes in multiple patients using a custom-designed 300-kb retinal resequencing chip. Ophthalmology 118, 160–167 e161–163.
Bowes Rickman, C., Ebright, J.N., Zavodni, Z.J., Yu, L., Wang, T., Daiger, S.P., Wistow, G., Boon, K., Hauser, M.A., 2006. Defining the human macula transcriptome and candidate retinal disease genes using EyeSAGE. Investig. Ophthalmol. Vis. Sci. 47, 2305–2316.
Bowne, S.J., Sullivan, L.S., Koboldt, D.C., Ding, L., Fulton, R., Abbott, R.M., Sodergren, E.J., Birch, D.G., Wheaton, D.H., Heckenlively, J.R., Liu, Q., Pierce, E.A., Weinstock, G.M., Daiger, S.P., 2011. Identification of disease-causing mutations in autosomal dominant retinitis pigmentosa (adRP) using next-generation DNA sequencing. Investig. Ophthalmol. Vis. Sci. 52, 494–503.
Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J., Ansorge, W., Ball, C.A., Causton, H.C., Gaasterland, T., Glenisson, P., Holstege, F.C., Kim, I.F., Markowitz, V., Matese, J.C., Parkinson, H., Robinson, A., Sarkans, U., Schulze-Kremer, S., Stewart, J., Taylor, R., Vilo, J., Vingron, M., 2001. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat. Genet. 29, 365–371.
Brookes, E., Shi, Y., 2014. Diverse epigenetic mechanisms of human disease. Annu. Rev. Genet. 48, 237–268.
Brooks, M.J., Rajasimha, H.K., Roger, J.E., Swaroop, A., 2011. Next-generation sequencing facilitates quantitative analysis of wild-type and Nrl(-/-) retinal transcriptomes. Mol. Vis. 17, 3034–3054.
Brooks, M.J., Rajasimha, H.K., Swaroop, A., 2012. Retinal transcriptome profiling by directional next-generation sequencing using 100 ng of total RNA. Methods Mol. Biol. 884, 319–334.
Brosius, J., Gould, S.J., 1992. On "genomenclature": a comprehensive (and respectful) taxonomy for pseudogenes and other "junk DNA". Proc. Natl. Acad. Sci. U. S. A. 89, 10706–10710.
Bruder, C.E., Piotrowski, A., Gijsbers, A.A., Andersson, R., Erickson, S., Diaz de Stahl, T., Menzel, U., Sandgren, J., von Tell, D., Poplawski, A., Crowley, M., Crasto, C., Partridge, E.C., Tiwari, H., Allison, D.B., Komorowski, J., van

Ommen, G.J., Boomsma, D.I., Pedersen, N.L., den Dunnen, J.T., Wirdefeldt, K., Dumanski, J.P., 2008. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. Am. J. Hum. Genet. 82, 763–771.

Bull, N.D., Martin, K.R., 2011. Concise review: toward stem cell-based therapies for retinal neurodegenerative diseases. Stem Cells 29, 1170–1175.

Bumsted O'Brien, K.M., Cheng, H., Jiang, Y., Schulte, D., Swaroop, A., Hendrickson, A.E., 2004. Expression of photoreceptor-specific nuclear receptor NR2E3 in rod photoreceptors of fetal human retina. Investig. Ophthalmol. Vis. Sci. 45, 2807–2812.

Cai, H., Fields, M.A., Hoshino, R., Priore, L.V., 2012. Effects of aging and anatomic location on gene expression in human retina. Front. Aging Neurosci. 4, 8.

Cantsilieris, S., White, S.J., 2013. Correlating multiallelic copy number polymorphisms with disease susceptibility. Hum. Mutat. 34, 1–13.

Carninci, P., Kasukawa, T., Katayama, S., Gough, J., Frith, M.C., Maeda, N., Oyama, R., Ravasi, T., Lenhard, B., Wells, C., Kodzius, R., Shimokawa, K., Bajic, V.B., Brenner, S.E., Batalov, S., Forrest, A.R., Zavolan, M., Davis, M.J., Wilming, L.G., Aidinis, V., Allen, J.E., Ambesi-Impiombato, A., Apweiler, R., Aturaliya, R.N., Bailey, T.L., Bansal, M., Baxter, L., Beisel, K.W., Bersano, T., Bono, H., Chalk, A.M., Chiu, K.P., Choudhary, V., Christoffels, A., Clutterbuck, D.R., Crowe, M.L., Dalla, E., Dalrymple, B.P., de Bono, B., Della Gatta, G., di Bernardo, D., Down, T., Engstrom, P., Fagiolini, M., Faulkner, G., Fletcher, C.F., Fukushima, T., Furuno, M., Futaki, S., Gariboldi, M., Georgii-Hemming, P., Gingeras, T.R., Gojobori, T., Green, R.E., Gustincich, S., Harbers, M., Hayashi, Y., Hensch, T.K., Hirokawa, N., Hill, D., Huminiecki, L., Iacono, M., Ikeo, K., Iwama, A., Ishikawa, T., Jakt, M., Kanapin, A., Katoh, M., Kawasawa, Y., Kelso, J., Kitamura, H., Kitano, H., Kollias, G., Krishnan, S.P., Kruger, A., Kummerfeld, S.K., Kurochkin, I.V., Lareau, L.F., Lazarevic, D., Lipovich, L., Liu, J., Liuni, S., McWilliam, S., Madan Babu, M., Madera, M., Marchionni, L., Matsuda, H., Matsuzawa, S., Miki, H., Mignone, F., Miyake, S., Morris, K., Mottagui-Tabar, S., Mulder, N., Nakano, N., Nakauchi, H., Ng, P., Nilsson, R., Nishiguchi, S., Nishikawa, S., Nori, F., Ohara, O., Okazaki, Y., Orlando, V., Pang, K.C., Pavan, W.J., Pavesi, G., Pesole, G., Petrovsky, N., Piazza, S., Reed, J., Reid, J.F., Ring, B.Z., Ringwald, M., Rost, B., Ruan, Y., Salzberg, S.L., Sandelin, A., Schneider, C., Schonbach, C., Sekiguchi, K., Semple, C.A., Seno, S., Sessa, L., Sheng, Y., Shibata, Y., Shimada, H., Shimada, K., Silva, D., Sinclair, B., Sperling, S., Stupka, E., Sugiura, K., Sultana, R., Takenaka, Y., Taki, K., Tammoja, K., Tan, S.L., Tang, S., Taylor, M.S., Tegner, J., Teichmann, S.A., Ueda, H.R., van Nimwegen, E., Verardo, R., Wei, C.L., Yagi, K., Yamanishi, H., Zabarovsky, E., Zhu, S., Zimmer, A., Hide, W., Bult, C., Grimmond, S.M., Teasdale, R.D., Liu, E.T., Brusic, V., Quackenbush, J., Wahlestedt, C., Mattick, J.S., Hume, D.A., Kai, C., Sasaki, D., Tomaru, Y., Fukuda, S., Kanamori-Katayama, M., Suzuki, M., Aoki, J., Arakawa, T., Iida, J., Imamura, K., Itoh, M., Kato, T., Kawaji, H., Kawagashira, N., Kawashima, T., Kojima, M., Kondo, S., Konno, H., Nakano, K., Ninomiya, N., Nishio, T., Okada, M., Plessy, C., Shibata, K., Shiraki, T., Suzuki, S., Tagami, M., Waki, K., Watahiki, A., Okamura-Oho, Y., Suzuki, H., Kawai, J., Hayashizaki, Y., FANTOM Consortium; RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group), 2005. The transcriptional landscape of the mammalian genome. Science 309, 1559–1563.

Cartegni, L., Chew, S.L., Krainer, A.R., 2002. Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat. Rev. Genet. 3, 285–298.

Carter, T.A., Greenhall, J.A., Yoshida, S., Fuchs, S., Helton, R., Swaroop, A., Lockhart, D.J., Barlow, C., 2005. Mechanisms of aging in senescence-accelerated mice. Genome Biol. 6, R48.

Carter-Dawson, L.D., LaVail, M.M., 1979. Rods and cones in the mouse retina. II. Autoradiographic analysis of cell generation using tritiated thymidine. J. Comp. Neurol. 188, 263–272.

Chakravarti, A., Clark, A.G., Mootha, V.K., 2013. Distilling pathophysiology from complex disease genetics. Cell 155, 21–26.

Chen, S., Wang, Q.L., Nie, Z., Sun, H., Lennon, G., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Zack, D.J., 1997. Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. Neuron 19, 1017–1030.

Chen, J., Rattner, A., Nathans, J., 2005. The rod photoreceptor-specific nuclear receptor Nr2e3 represses transcription of multiple cone-specific genes. J. Neurosci. 25, 118–129.

Chen, M., Muckersie, E., Forrester, J.V., Xu, H., 2010a. Immune activation in retinal aging: a gene expression study. Investig. Ophthalmol. Vis. Sci. 51, 5888–5896.

Chen, W., Stambolian, D., Edwards, A.O., Branham, K.E., Othman, M., Jakobsdottir, J., Tosakulwong, N., Pericak-Vance, M.A., Campochiaro, P.A., Klein, M.L., Tan, P.L., Conley, Y.P., Kanda, A., Kopplin, L., Li, Y., Augustaitis, K.J., Karoukis, A.J., Scott, W.K., Agarwal, A., Kovach, J.L., Schwartz, S.G., Postel, E.A., Brooks, M., Baratz, K.H., Brown, W.L., Brucker, A.J., Orlin, A., Brown, G., Ho, A., Regillo, C., Donoso, L., Tian, L., Kaderli, B., Hadley, D., Hagstrom, S.A., Peachey, N.S., Klein, R., Klein, B.E., Gotoh, N., Yamashiro, K., Ferris Iii, F., Fagerness, J.A., Reynolds, R., Farrer, L.A., Kim, I.K., Miller, J.W., Corton, M., Carracedo, A., Sanchez-Salorio, M., Pugh, E.W., Doheny, K.F., Brion, M., Deangelis, M.M., Weeks, D.E., Zack, D.J., Chew, E.Y., Heckenlively, J.R., Yoshimura, N., Iyengar, S.K., Francis, P.J., Katsanis, N., Seddon, J.M., Haines, J.L., Gorin, M.B., Abecasis, G.R., Swaroop, A., 2010b. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc. Natl. Acad. Sci. U. S. A. 107, 7401–7406.

Cheng, H., Khanna, H., Oh, E.C., Hicks, D., Mitton, K.P., Swaroop, A., 2004. Photoreceptor-specific nuclear receptor NR2E3 functions as a transcriptional activator in rod photoreceptors. Hum. Mol. Genet. 13, 1563–1575.

Cheng, H., Aleman, T.S., Cideciyan, A.V., Khanna, R., Jacobson, S.G., Swaroop, A., 2006. In vivo function of the orphan nuclear receptor NR2E3 in establishing photoreceptor identity during mammalian retinal development. Hum. Mol. Genet. 15, 2588–2602.

Cheng, H., Khan, N.W., Roger, J.E., Swaroop, A., 2011. Excess cones in the retinal degeneration rd7 mouse, caused by the loss of function of orphan nuclear receptor Nr2e3, originate from early-born photoreceptor precursors. Hum. Mol. Genet. 20, 4102–4115.

Cheung, V.G., Conlin, L.K., Weber, T.M., Arcaro, M., Jen, K.Y., Morley, M., Spielman, R.S., 2003. Natural variation in human gene expression assessed in lymphoblastoid cells. Nat. Genet. 33, 422–425.

Choudhary, M., Kazmin, D., Hu, P., Thomas, R.S., McDonnell, D.P., Malek, G., 2014. Aryl hydrocarbon receptor knockout exacerbates choroidal neovascularization via multiple pathogenic pathways. J. Pathol. 235, 101–112.

Chowers, I., Gunatilaka, T.L., Farkas, R.H., Qian, J., Hackam, A.S., Duh, E., Kageyama, M., Wang, C., Vora, A., Campochiaro, P.A., Zack, D.J., 2003a. Identification of novel genes preferentially expressed in the retina using a custom human retina cDNA microarray. Investig. Ophthalmol. Vis. Sci. 44, 3732–3741.

Chowers, I., Liu, D., Farkas, R.H., Gunatilaka, T.L., Hackam, A.S., Bernstein, S.L., Campochiaro, P.A., Parmigiani, G., Zack, D.J., 2003b. Gene expression variation in the adult human retina. Hum. Mol. Genet. 12, 2881–2893.

Cipriani, V., International AMD Genomics Consortium, 2014. Unravelling the Complex Genetics of Age-related Macular Degeneration, 64th Annual Meeting of the American Society of Human Genetics. Personal communication.

Cohen, J.C., Boerwinkle, E., Mosley Jr., T.H., Hobbs, H.H., 2006. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N. Engl. J. Med. 354, 1264–1272.

Colantuoni, C., Lipska, B.K., Ye, T., Hyde, T.M., Tao, R., Leek, J.T., Colantuoni, E.A., Elkahloun, A.G., Herman, M.M., Weinberger, D.R., Kleinman, J.E., 2011. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. Nature 478, 519–523.

Corbo, J.C., Cepko, C.L., 2005. A hybrid photoreceptor expressing both rod and cone genes in a mouse model of enhanced S-cone syndrome. PLoS Genet. 1, e11.

Corbo, J.C., Lawrence, K.A., Karlstetter, M., Myers, C.A., Abdelaziz, M., Dirkes, W., Weigelt, K., Seifert, M., Benes, V., Fritsche, L.G., Weber, B.H., Langmann, T., 2010. CRX ChIP-seq reveals the cis-regulatory architecture of mouse photoreceptors. Genome Res. 20, 1512–1525.

Core, L.J., Waterfall, J.J., Lis, J.T., 2008. Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science 322, 1845–1848.

Crawford, G.E., Holt, I.E., Whittle, J., Webb, B.D., Tai, D., Davis, S., Margulies, E.H., Chen, Y., Bernat, J.A., Ginsburg, D., Zhou, D., Luo, S., Vasicek, T.J., Daly, M.J., Wolfsberg, T.G., Collins, F.S., 2006. Genome-wide mapping of DNase hypersensitive sites using massively parallel signature sequencing (MPSS). Genome Res. 16, 123–131.

Cuenca, N., Fernandez-Sanchez, L., Campello, L., Maneu, V., De la Villa, P., Lax, P., Pinilla, I., 2014. Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. Prog. Retin Eye Res. 43C, 17–75.

Curcio, C.A., 2001. Photoreceptor topography in ageing and age-related maculopathy. Eye (Lond) 15, 376–383.

Curcio, C.A., Millican, C.L., Allen, K.A., Kalina, R.E., 1993. Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. Investig. Ophthalmol. Vis. Sci. 34, 3278–3296.

Dadu, R.T., Ballantyne, C.M., 2014. Lipid lowering with PCSK9 inhibitors. Nat. Rev. Cardiol. 11, 563–575.

Damiani, D., Alexander, J.J., O'Rourke, J.R., McManus, M., Jadhav, A.P., Cepko, C.L., Hauswirth, W.W., Harfe, B.D., Strettoi, E., 2008. Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. J. Neurosci. 28, 4878–4887.

Davidson, E.H., Levine, M.S., 2008. Properties of developmental gene regulatory networks. Proc. Natl. Acad. Sci. U. S. A. 105, 20063–20066.

de Hoon, M., Hayashizaki, Y., 2008. Deep cap analysis gene expression (CAGE): genome-wide identification of promoters, quantification of their expression, and network inference. Biotechniques 44, 627–628, 630, 632.

de Laat, W., Duboule, D., 2013. Topology of mammalian developmental enhancers and their regulatory landscapes. Nature 502, 499–506.

de Magalhaes, J.P., Curado, J., Church, G.M., 2009. Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 25, 875–881.

Delous, M., Baala, L., Salomon, R., Laclef, C., Vierkotten, J., Tory, K., Golzio, C., Lacoste, T., Besse, L., Ozilou, C., Moutkine, I., Hellman, N.E., Anselme, I., Silbermann, F., Vesque, C., Gerhardt, C., Rattenberry, E., Wolf, M.T., Gubler, M.C., Martinovic, J., Encha-Razavi, F., Boddaert, N., Gonzales, M., Macher, M.A., Nivet, H., Champion, G., Bertheleme, J.P., Niaudet, P., McDonald, F., Hildebrandt, F., Johnson, C.A., Vekemans, M., Antignac, C., Ruther, U., Schneider-Maunoury, S., Attie-Bitach, T., Saunier, S., 2007. The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. Nat. Genet. 39, 875–881.

Deo, M., Yu, J.Y., Chung, K.H., Tippens, M., Turner, D.L., 2006. Detection of mammalian microRNA expression by in situ hybridization with RNA oligonucleotides. Dev. Dyn. 235, 2538–2548.

Dorrell, M.I., Aguilar, E., Weber, C., Friedlander, M., 2004. Global gene expression analysis of the developing postnatal mouse retina. Investig. Ophthalmol. Vis. Sci. 45, 1009–1019.

Ebermann, I., Phillips, J.B., Liebau, M.C., Koenekoop, R.K., Schermer, B., Lopez, I., Schafer, E., Roux, A.F., Dafinger, C., Bernd, A., Zrenner, E., Claustres, M., Blanco, B.,

Nurnberg, G., Nurnberg, P., Ruland, R., Westerfield, M., Benzing, T., Bolz, H.J., 2010. PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. J. Clin. Investig. 120, 1812–1823.
Edwards, A.O., Ritter 3rd, R., Abel, K.J., Manning, A., Panhuysen, C., Farrer, L.A., 2005. Complement factor H polymorphism and age-related macular degeneration. Science 308, 421–424.
Edwards, S.L., Beesley, J., French, J.D., Dunning, A.M., 2013. Beyond GWASs: illuminating the dark road from association to function. Am. J. Hum. Genet. 93, 779–797.
Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., Sasai, Y., 2011. Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472, 51–56.
Elliott, M.H., Nash, Z.A., Takemori, N., Fliesler, S.J., McClellan, M.E., Naash, M.I., 2008. Differential distribution of proteins and lipids in detergent-resistant and detergent-soluble domains in rod outer segment plasma membranes and disks. J. Neurochem. 104, 336–352.
ENCODE Project Consortium, 2011. A user's guide to the encyclopedia of DNA elements (ENCODE). PLoS Biol. 9, e1001046.
ENCODE Project Consortium, Bernstein, B.E., Birney, E., Dunham, I., Green, E.D., Gunter, C., Snyder, M., 2012. An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57–74.
Ezkurdia, I., Juan, D., Rodriguez, J.M., Frankish, A., Diekhans, M., Harrow, J., Vazquez, J., Valencia, A., Tress, M.L., 2014. Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes. Hum. Mol. Genet. 23, 5866–5878.
Farinelli, P., Perera, A., Arango-Gonzalez, B., Trifunovic, D., Wagner, M., Carell, T., Biel, M., Zrenner, E., Michalakis, S., Paquet-Durand, F., Ekstrom, P.A., 2014. DNA methylation and differential gene regulation in photoreceptor cell death. Cell. Death Dis. 5, e1558.
Farjo, R., Yu, J., Othman, M.I., Yoshida, S., Sheth, S., Glaser, T., Baehr, W., Swaroop, A., 2002. Mouse eye gene microarrays for investigating ocular development and disease. Vis. Res. 42, 463–470.
Farkas, M.H., Grant, G.R., White, J.A., Sousa, M.E., Consugar, M.B., Pierce, E.A., 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 Genomics 14, 486.
Feinberg, A.P., Ohlsson, R., Henikoff, S., 2006. The epigenetic progenitor origin of human cancer. Nat. Rev. Genet. 7, 21–33.
Ferris, F.L., Davis, M.D., Clemons, T.E., Lee, L.Y., Chew, E.Y., Lindblad, A.S., Milton, R.C., Bressler, S.B., Klein, R., 2005. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. Arch. Ophthalmol. 123, 1570–1574.
Ferris 3rd, F.L., Wilkinson, C.P., Bird, A., Chakravarthy, U., Chew, E., Csaky, K., Sadda, S.R., 2013. Clinical classification of age-related macular degeneration. Ophthalmology 120, 844–851.
Finnegan, S., Robson, J.L., Wylie, M., Healy, A., Stitt, A.W., Curry, W.J., 2008. Protein expression profiling during chick retinal maturation: a proteomics-based approach. Proteome Sci. 6, 34.
Freund, P.R., Watson, J., Gilmour, G.S., Gaillard, F., Sauve, Y., 2011. Differential changes in retina function with normal aging in humans. Doc. Ophthalmol. 122, 177–190.
Fritsche, L.G., International AMD Genomics Consortium, 2014. The Role of Rare TIMP3 Mutations in Age-related Macular Degeneration, 64th Annual meeting of the American Society of Human Genetics. Personal communication.
Fritsche, L.G., Chen, W., Schu, M., Yaspan, B.L., Yu, Y., Thorleifsson, G., Zack, D.J., Arakawa, S., Cipriani, V., Ripke, S., Igo Jr., R.P., Buitendijk, G.H., Sim, X., Weeks, D.E., Guymer, R.H., Merriam, J.E., Francis, P.J., Hannum, G., Agarwal, A., Armbrecht, A.M., Audo, I., Aung, T., Barile, G.R., Benchaboune, M., Bird, A.C., Bishop, P.N., Branham, K.E., Brooks, M., Brucker, A.J., Cade, W.H., Cain, M.S., Campochiaro, P.A., Chan, C.C., Cheng, C.Y., Chew, E.Y., Chin, K.A., Chowers, I., Clayton, D.G., Cojocaru, R., Conley, Y.P., Cornes, B.K., Daly, M.J., Dhillon, B., Edwards, A.O., Evangelou, E., Fagerness, J., Ferreyra, H.A., Friedman, J.S., Geirsdottir, A., George, R.J., Gieger, C., Gupta, N., Hagstrom, S.A., Harding, S.P., Haritoglou, C., Heckenlively, J.R., Holz, F.G., Hughes, G., Ioannidis, J.P., Ishibashi, T., Joseph, P., Jun, G., Kamatani, Y., Katsanis, N., C, N.K., Khan, J.C., Kim, I.K., Kiyohara, Y., Klein, B.E., Klein, R., Kovach, J.L., Kozak, I., Lee, C.J., Lee, K.E., Lichtner, P., Lotery, A.J., Meitinger, T., Mitchell, P., Mohand-Said, S., Moore, A.T., Morgan, D.J., Morrison, M.A., Myers, C.E., Naj, A.C., Nakamura, Y., Okada, Y., Orlin, A., Ortube, M.C., Othman, M.I., Pappas, C., Park, K.H., Pauer, G.J., Peachey, N.S., Poch, O., Priya, R.R., Reynolds, R., Richardson, A.J., Ripp, R., Rudolph, G., Ryu, E., Sahel, J.A., Schaumberg, D.A., Scholl, H.P., Schwartz, S.G., Scott, W.K., Shahid, H., Sigurdsson, H., Silvestri, G., Sivakumaran, T.A., Smith, R.T., Sobrin, L., Souied, E.H., Stambolian, D.E., Stefansson, H., Sturgill-Short, G.M., Takahashi, A., Tosakulwong, N., Truitt, B.J., Tsironi, E.E., Uitterlinden, A.G., van Duijn, C.M., Vijaya, L., Vingerling, J.R., Vithana, E.N., Webster, A.R., Wichmann, H.E., Winkler, T.W., Wong, T.Y., Wright, A.F., Zelenika, D., Zhang, M., Zhao, L., Zhang, K., Klein, M.L., Hageman, G.S., Lathrop, G.M., Stefansson, K., Allikmets, R., Baird, P.N., Gorin, M.B., Wang, J.J., Klaver, C.C., Seddon, J.M., Pericak-Vance, M.A., Iyengar, S.K., Yates, J.R., Swaroop, A., Weber, B.H., Kubo, M., Deangelis, M.M., Leveillard, T., Thorsteinsdottir, U., Haines, J.L., Farrer, L.A., Heid, I.M., Abecasis, G.R., AMD Gene Consortium, 2013. Seven new loci associated with age-related macular degeneration. Nat. Genet. 45, 433–439, 439e431-432.
Fritsche, L.G., Fariss, R.N., Stambolian, D., Abecasis, G.R., Curcio, C.A., Swaroop, A., 2014. Age-related macular degeneration: genetics and biology coming together. Annu. Rev. Genomics Hum. Genet. 15, 151–171.
Furey, T.S., 2012. ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions. Nat. Rev. Genet. 13, 840–852.
Furukawa, T., Morrow, E.M., Cepko, C.L., 1997. Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. Cell 91, 531–541.
Furukawa, T., Morrow, E.M., Li, T., Davis, F.C., Cepko, C.L., 1999. Retinopathy and attenuated circadian entrainment in Crx-deficient mice. Nat. Genet. 23, 466–470.
Gamsiz, E.D., Ouyang, Q., Schmidt, M., Nagpal, S., Morrow, E.M., 2012. Genome-wide transcriptome analysis in murine neural retina using high-throughput RNA sequencing. Genomics 99, 44–51.
Gao, H., Hollyfield, J.G., 1992. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. Investig. Ophthalmol. Vis. Sci. 33, 1–17.
Genomes Project Consortium, Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T., McVean, G.A., 2012. An integrated map of genetic variation from 1,092 human genomes. Nature 491, 56–65.
Gieser, L., Swaroop, A., 1992. Expressed sequence tags and chromosomal localization of cDNA clones from a subtracted retinal pigment epithelium library. Genomics 13, 873–876.
Gilmour, D.S., Lis, J.T., 1985. In vivo interactions of RNA polymerase II with genes of Drosophila melanogaster. Mol. Cell. Biol. 5, 2009–2018.
Giresi, P.G., Kim, J., McDaniell, R.M., Iyer, V.R., Lieb, J.D., 2007. FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) isolates active regulatory elements from human chromatin. Genome Res. 17, 877–885.
Grant, G.R., Farkas, M.H., Pizarro, A.D., Lahens, N.F., Schug, J., Brunk, B.P., Stoeckert, C.J., Hogenesch, J.B., Pierce, E.A., 2011. Comparative analysis of RNA-Seq alignment algorithms and the RNA-Seq unified mapper (RUM). Bioinformatics 27, 2518–2528.
Gresh, J., Goletz, P.W., Crouch, R.K., Rohrer, B., 2003. Structure-function analysis of rods and cones in juvenile, adult, and aged C57bl/6 and Balb/c mice. Vis. Neurosci. 20, 211–220.
Gu, H., Smith, Z.D., Bock, C., Boyle, P., Gnirke, A., Meissner, A., 2011. Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. Nat. Protoc. 6, 468–481.
Guo, H., Zhu, P., Wu, X., Li, X., Wen, L., Tang, F., 2013. Single-cell methylome landscapes of mouse embryonic stem cells and early embryos analyzed using reduced representation bisulfite sequencing. Genome Res. 23, 2126–2135.
Gustincich, S., Contini, M., Gariboldi, M., Puopolo, M., Kadota, K., Bono, H., LeMieux, J., Walsh, P., Carninci, P., Hayashizaki, Y., Okazaki, Y., Raviola, E., 2004. Gene discovery in genetically labeled single dopaminergic neurons of the retina. Proc. Natl. Acad. Sci. U. S. A. 101, 5069–5074.
Hackler Jr., L., Wan, J., Swaroop, A., Qian, J., Zack, D.J., 2010. MicroRNA profile of the developing mouse retina. Investig. Ophthalmol. Vis. Sci. 51, 1823–1831.
Hageman, G.S., Anderson, D.H., Johnson, L.V., Hancox, L.S., Taiber, A.J., Hardisty, L.I., Hageman, J.L., Stockman, H.A., Borchardt, J.D., Gehrs, K.M., Smith, R.J., Silvestri, G., Russell, S.R., Klaver, C.C., Barbazetto, I., Chang, S., Yannuzzi, L.A., Barile, G.R., Merriam, J.C., Smith, R.T., Olsh, A.K., Bergeron, J., Zernant, J., Merriam, J.E., Gold, B., Dean, M., Allikmets, R., 2005. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc. Natl. Acad. Sci. U. S. A. 102, 7227–7232.
Hagstrom, S.A., Ying, G.S., Pauer, G.J., Sturgill-Short, G.M., Huang, J., Callanan, D.G., Kim, I.K., Klein, M.L., Maguire, M.G., Martin, D.F., Comparison of A.M.D.T.T.R.G, 2013. Pharmacogenetics for genes associated with age-related macular degeneration in the Comparison of AMD Treatments Trials (CATT). Ophthalmology 120, 593–599.
Haider, N.B., Jacobson, S.G., Cideciyan, A.V., Swiderski, R., Streb, L.M., Searby, C., Beck, G., Hockey, R., Hanna, D.B., Gorman, S., Duhl, D., Carmi, R., Bennett, J., Weleber, R.G., Fishman, G.A., Wright, A.F., Stone, E.M., Sheffield, V.C., 2000. Mutation of a nuclear receptor gene, NR2E3, causes enhanced S cone syndrome, a disorder of retinal cell fate. Nat. Genet. 24, 127–131.
Haider, N.B., Mollema, N., Gaule, M., Yuan, Y., Sachs, A.J., Nystuen, A.M., Naggert, J.K., Nishina, P.M., 2009. Nr2e3-directed transcriptional regulation of genes involved in photoreceptor development and cell-type specific phototransduction. Exp. Eye Res. 89, 365–372.
Haines, J.L., Hauser, M.A., Schmidt, S., Scott, W.K., Olson, L.M., Gallins, P., Spencer, K.L., Kwan, S.Y., Noureddine, M., Gilbert, J.R., Schnetz-Boutaud, N., Agarwal, A., Postel, E.A., Pericak-Vance, M.A., 2005. Complement factor H variant increases the risk of age-related macular degeneration. Science 308, 419–421.
Hansen, R.S., Thomas, S., Sandstrom, R., Canfield, T.K., Thurman, R.E., Weaver, M., Dorschner, M.O., Gartler, S.M., Stamatoyannopoulos, J.A., 2010. Sequencing newly replicated DNA reveals widespread plasticity in human replication timing. Proc. Natl. Acad. Sci. U. S. A. 107, 139–144.
Hao, H., Tummala, P., Guzman, E., Mali, R.S., Gregorski, J., Swaroop, A., Mitton, K.P., 2011. The transcription factor neural retina leucine zipper (NRL) controls photoreceptor-specific expression of myocyte enhancer factor Mef2c from an alternative promoter. J. Biol. Chem. 286, 34893–34902.
Hao, H., Kim, D.S., Klocke, B., Johnson, K.R., Cui, K., Gotoh, N., Zang, C., Gregorski, J., Gieser, L., Peng, W., Fann, Y., Seifert, M., Zhao, K., Swaroop, A., 2012. Transcriptional regulation of rod photoreceptor homeostasis revealed by in vivo NRL targetome analysis. PLoS Genet. 8, e1002649.
Hawrylycz, M.J., Lein, E.S., Guillozet-Bongaarts, A.L., Shen, E.H., Ng, L., Miller, J.A., van de Lagemaat, L.N., Smith, K.A., Ebbert, A., Riley, Z.L., Abajian, C., Beckmann, C.F., Bernard, A., Bertagnolli, D., Boe, A.F., Cartagena, P.M., Chakravarty, M.M., Chapin, M., Chong, J., Dalley, R.A., Daly, B.D., Dang, C., Datta, S., Dee, N.,

Dolbeare, T.A., Faber, V., Feng, D., Fowler, D.R., Goldy, J., Gregor, B.W., Haradon, Z., Haynor, D.R., Hohmann, J.G., Horvath, S., Howard, R.E., Jeromin, A., Jochim, J.M., Kinnunen, M., Lau, C., Lazarz, E.T., Lee, C., Lemon, T.A., Li, L., Li, Y., Morris, J.A., Overly, C.C., Parker, P.D., Parry, S.E., Reding, M., Royall, J.J., Schulkin, J., Sequeira, P.A., Slaughterbeck, C.R., Smith, S.C., Sodt, A.J., Sunkin, S.M., Swanson, B.E., Vawter, M.P., Williams, D., Wohnoutka, P., Zielke, H.R., Geschwind, D.H., Hof, P.R., Smith, S.M., Koch, C., Grant, S.G., Jones, A.R., 2012. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 489, 391–399.

Hecker, M., Lambeck, S., Toepfer, S., van Someren, E., Guthke, R., 2009. Gene regulatory network inference: data integration in dynamic models-a review. Biosystems 96, 86–103.

Helgason, H., Sulem, P., Duvvari, M.R., Luo, H., Thorleifsson, G., Stefansson, H., Jonsdottir, I., Masson, G., Gudbjartsson, D.F., Walters, G.B., Magnusson, O.T., Kong, A., Rafnar, T., Kiemeney, L.A., Schoenmaker-Koller, F.E., Zhao, L., Boon, C.J., Song, Y., Fauser, S., Pei, M., Ristau, T., Patel, S., Liakopoulos, S., van de Ven, J.P., Hoyng, C.B., Ferreyra, H., Duan, Y., Bernstein, P.S., Geirsdottir, A., Helgadottir, G., Stefansson, E., den Hollander, A.I., Zhang, K., Jonasson, F., Sigurdsson, H., Thorsteinsdottir, U., Stefansson, K., 2013. A rare nonsynonymous sequence variant in C3 is associated with high risk of age-related macular degeneration. Nat. Genet. 45, 1371–1374.

Hendrickson, A., Bumsted-O'Brien, K., Natoli, R., Ramamurthy, V., Possin, D., Provis, J., 2008. Rod photoreceptor differentiation in fetal and infant human retina. Exp. Eye Res. 87, 415–426.

Henikoff, J.G., Belsky, J.A., Krassovsky, K., MacAlpine, D.M., Henikoff, S., 2011. Epigenome characterization at single base-pair resolution. Proc. Natl. Acad. Sci. U. S. A. 108, 18318–18323.

Hennig, A.K., Peng, G.H., Chen, S., 2008. Regulation of photoreceptor gene expression by Crx-associated transcription factor network. Brain Res. 1192, 114–133.

Hirabayashi, Y., Gotoh, Y., 2010. Epigenetic control of neural precursor cell fate during development. Nat. Rev. Neurosci. 11, 377–388.

Hnisz, D., Abraham, B.J., Lee, T.I., Lau, A., Saint-Andre, V., Sigova, A.A., Hoke, H.A., Young, R.A., 2013. Super-enhancers in the control of cell identity and disease. Cell 155, 934–947.

Hoover, A., Goldbaum, M., 2003. Locating the optic nerve in a retinal image using the fuzzy convergence of the blood vessels. IEEE Trans. Med. Imaging 22, 951–958.

Hoover, A., Kouznetsova, V., Goldbaum, M., 1998. Locating blood vessels in retinal images by piece-wise threshold probing of a matched filter response. Proc. AMIA Symp. 931–935.

Hughes, A.J., Spelke, D.P., Xu, Z., Kang, C.C., Schaffer, D.V., Herr, A.E., 2014. Single-cell western blotting. Nat. Methods 11, 749–755.

Huyghe, J.R., Jackson, A.U., Fogarty, M.P., Buchkovich, M.L., Stancakova, A., Stringham, H.M., Sim, X., Yang, L., Fuchsberger, C., Cederberg, H., Chines, P.S., Teslovich, T.M., Romm, J.M., Ling, H., McMullen, I., Ingersoll, R., Pugh, E.W., Doheny, K.F., Neale, B.M., Daly, M.J., Kuusisto, J., Scott, L.J., Kang, H.M., Collins, F.S., Abecasis, G.R., Watanabe, R.M., Boehnke, M., Laakso, M., Mohlke, K.L., 2013. Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. Nat. Genet. 45, 197–201.

Hwang, W., Hackler Jr., L., Wu, G., Ji, H., Zack, D.J., Qian, J., 2012. Dynamics of regulatory networks in the developing mouse retina. PLoS One 7, e46521.

Ideker, T., Galitski, T., Hood, L., 2001. A new approach to decoding life: systems biology. Annu. Rev. Genomics Hum. Genet. 2, 343–372.

Ingolia, N.T., Ghaemmaghami, S., Newman, J.R., Weissman, J.S., 2009. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science 324, 218–223.

Jakobsdottir, J., Conley, Y.P., Weeks, D.E., Mah, T.S., Ferrell, R.E., Gorin, M.B., 2005. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am. J. Hum. Genet. 77, 389–407.

Jia, L., Oh, E.C., Ng, L., Srinivas, M., Brooks, M., Swaroop, A., Forrest, D., 2009. Retinoid-related orphan nuclear receptor RORbeta is an early-acting factor in rod photoreceptor development. Proc. Natl. Acad. Sci. U. S. A. 106, 17534–17539.

Jin, S.G., Wu, X., Li, A.X., Pfeifer, G.P., 2011. Genomic mapping of 5-hydroxymethylcytosine in the human brain. Nucleic Acids Res. 39, 5015–5024.

John, B., Enright, A.J., Aravin, A., Tuschl, T., Sander, C., Marks, D.S., 2004. Human MicroRNA targets. PLoS Biol. 2, e363.

Kajiwara, K., Berson, E.L., Dryja, T.P., 1994. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264, 1604–1608.

Kalathur, R.K., Gagniere, N., Berthommier, G., Poidevin, L., Raffelsberger, W., Ripp, R., Leveillard, T., Poch, O., 2008. RETINOBASE: a web database, data mining and analysis platform for gene expression data on retina. BMC Genomics 9, 208.

Kandpal, R.P., Rajasimha, H.K., Brooks, M.J., Nellissery, J., Wan, J., Qian, J., Kern, T.S., Swaroop, A., 2012. Transcriptome analysis using next generation sequencing reveals molecular signatures of diabetic retinopathy and efficacy of candidate drugs. Mol. Vis. 18, 1123–1146.

Kaneko, H., Dridi, S., Tarallo, V., Gelfand, B.D., Fowler, B.J., Cho, W.G., Kleinman, M.E., Ponicsan, S.L., Hauswirth, W.W., Chiodo, V.A., Kariko, K., Yoo, J.W., Lee, D.K., Hadziahmetovic, M., Song, Y., Misra, S., Chaudhuri, G., Buaas, F.W., Braun, R.E., Hinton, D.R., Zhang, Q., Grossniklaus, H.E., Provis, J.M., Madigan, M.C., Milam, A.H., Justice, N.L., Albuquerque, R.J., Blandford, A.D., Bogdanovich, S., Hirano, Y., Witta, J., Fuchs, E., Littman, D.R., Ambati, B.K., Rudin, C.M., Chong, M.M., Provost, P., Kugel, J.F., Goodrich, J.A., Dunaief, J.L., Baffi, J.Z., Ambati, J., 2011. DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. Nature 471, 325–330.

Kanoff, J., Miller, J., 2013. Pharmacogenetics of the treatment response of age-related macular degeneration with ranibizumab and bevacizumab. Semin. Ophthalmol. 28, 355–360.

Karali, M., Peluso, I., Gennarino, V.A., Bilio, M., Verde, R., Lago, G., Dolle, P., Banfi, S., 2010. miRNeye: a microRNA expression atlas of the mouse eye. BMC Genomics 11, 715.

Khanna, H., Davis, E.E., Murga-Zamalloa, C.A., Estrada-Cuzcano, A., Lopez, I., den Hollander, A.I., Zonneveld, M.N., Othman, M.I., Waseem, N., Chakarova, C.F., Maubaret, C., Diaz-Font, A., MacDonald, I., Muzny, D.M., Wheeler, D.A., Morgan, M., Lewis, L.R., Logan, C.V., Tan, P.L., Beer, M.A., Inglehearn, C.F., Lewis, R.A., Jacobson, S.G., Bergmann, C., Beales, P.L., Attie-Bitach, T., Johnson, C.A., Otto, E.A., Bhattacharya, S.S., Hildebrandt, F., Gibbs, R.A., Koenekoop, R.K., Swaroop, A., Katsanis, N., 2009. A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. Nat. Genet. 41, 739–745.

Kim, J.W., Yang, H.J., Barb, J.J., Brooks, M., Chaitankar, V., Cogliati, C., Gieser, L., Gotoh, N., Munson, P.J., Anand Swaroop, A., 2015. Time-varying Gene Regulatory Network Controls Stepwise Functional Maturation of Mammalian Rod Photoreceptors. Manuscript in preparation.

Kim, S.Y., Yang, H.J., Chang, Y.S., Kim, J.W., Brooks, M., Chew, E.Y., Wong, W.T., Fariss, R., Rachel, R., Cogliati, T., Qian, H., Swaroop, A., 2014. Deletion of aryl hydrocarbon receptor AHR in mice leads to subretinal accumulation of microglia and RPE atrophy. Investig. Ophthalmol. Vis. Sci. 55, 6031–6040.

Kitano, H., 2002. Systems biology: a brief overview. Science 295, 1662–1664.

Klein, R.J., Zeiss, C., Chew, E.Y., Tsai, J.Y., Sackler, R.S., Haynes, C., Henning, A.K., SanGiovanni, J.P., Mane, S.M., Mayne, S.T., Bracken, M.B., Ferris, F.L., Ott, J., Barnstable, C., Hoh, J., 2005. Complement factor H polymorphism in age-related macular degeneration. Science 308, 385–389.

Kleinman, M.E., Yamada, K., Takeda, A., Chandrasekaran, V., Nozaki, M., Baffi, J.Z., Albuquerque, R.J., Yamasaki, S., Itaya, M., Pan, Y., Appukuttan, B., Gibbs, D., Yang, Z., Kariko, K., Ambati, B.K., Wilgus, T.A., DiPietro, L.A., Sakurai, E., Zhang, K., Smith, J.R., Taylor, E.W., Ambati, J., 2008. Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. Nature 452, 591–597.

Kolesnikov, A.V., Fan, J., Crouch, R.K., Kefalov, V.J., 2010. Age-related deterioration of rod vision in mice. J. Neurosci. 30, 11222–11231.

Koso, H., Minami, C., Tabata, Y., Inoue, M., Sasaki, E., Satoh, S., Watanabe, S., 2009. CD73, a novel cell surface antigen that characterizes retinal photoreceptor precursor cells. Investig. Ophthalmol. Vis. Sci. 50, 5411–5418.

Kouzarides, T., 2007. Chromatin modifications and their function. Cell 128, 693–705.

Kozhevnikova, O.S., Korbolina, E.E., Ershov, N.I., Kolosova, N.G., 2013. Rat retinal transcriptome: effects of aging and AMD-like retinopathy. Cell. Cycle 12, 1745–1761.

Kriete, A., Sokhansanj, B.A., Coppock, D.L., West, G.B., 2006. Systems approaches to the networks of aging. Ageing Res. Rev. 5, 434–448.

Krol, J., Busskamp, V., Markiewicz, I., Stadler, M.B., Ribi, S., Richter, J., Duebel, J., Bicker, S., Fehling, H.J., Schubeler, D., Oertner, T.G., Schratt, G., Bibel, M., Roska, B., Filipowicz, W., 2010. Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. Cell 141, 618–631.

Kukurba, K.R., Zhang, R., Li, X., Smith, K.S., Knowles, D.A., How Tan, M., Piskol, R., Lek, M., Snyder, M., Macarthur, D.G., Li, J.B., Montgomery, S.B., 2014. Allelic expression of deleterious protein-coding variants across human tissues. PLoS Genet. 10, e1004304.

Kuo, J.Z., Wong, T.Y., Rotter, J.I., 2014. Challenges in elucidating the genetics of diabetic retinopathy. JAMA Ophthalmol. 132, 96–107.

Kwok, M.C., Holopainen, J.M., Molday, L.L., Foster, L.J., Molday, R.S., 2008. Proteomics of photoreceptor outer segments identifies a subset of SNARE and Rab proteins implicated in membrane vesicle trafficking and fusion. Mol. Cell. Proteomics 7, 1053–1066.

Kwong, A.Z.X., Fritsche, L.G., Bragg-Gresham, J., Branham, K.E., Othman, M., Boleda, A., Gieser, L., Ratnapriya, R., Stambolian, D., Chew, E.Y., Swaroop, A., Abecasis, G., 2014. Whole-genome Sequencing Study of ~6,000 Samples for Age-related Macular Degeneration.

Lagha, M., Bothma, J.P., Levine, M., 2012. Mechanisms of transcriptional precision in animal development. Trends Genet. 28, 409–416.

Lamb, T.D., Collin, S.P., Pugh Jr., E.N., 2007. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. Nat. Rev. Neurosci. 8, 960–976.

Lappalainen, T., Sammeth, M., Friedlander, M.R., tHoen, P.A., Monlong, J., Rivas, M.A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G., Barann, M., Wieland, T., Greger, L., van Iterson, M., Almlof, J., Ribeca, P., Pulyakhina, I., Esser, D., Giger, T., Tikhonov, A., Sultan, M., Bertier, G., MacArthur, D.G., Lek, M., Lizano, E., Buermans, H.P., Padioleau, I., Schwarzmayr, T., Karlberg, O., Ongen, H., Kilpinen, H., Beltran, S., Gut, M., Kahlem, K., Amstislavskiy, V., Stegle, O., Pirinen, M., Montgomery, S.B., Donnelly, P., McCarthy, M.I., Flicek, P., Strom, T.M., Geuvadis, C., Lehrach, H., Schreiber, S., Sudbrak, R., Carracedo, A., Antonarakis, S.E., Hasler, R., Syvanen, A.C., van Ommen, G.J., Brazma, A., Meitinger, T., Rosenstiel, P., Guigo, R., Gut, I.G., Estivill, X., Dermitzakis, E.T., 2013. Transcriptome and genome sequencing uncovers functional variation in humans. Nature 501, 506–511.

Lee, T.I., Young, R.A., 2013. Transcriptional regulation and its misregulation in disease. Cell 152, 1237–1251.

Lee, C.K., Weindruch, R., Prolla, T.A., 2000. Gene-expression profile of the ageing brain in mice. Nat. Genet. 25, 294–297.

Lehner, B., Crombie, C., Tischler, J., Fortunato, A., Fraser, A.G., 2006. Systematic mapping of genetic interactions in Caenorhabditis elegans identifies common modifiers of diverse signaling pathways. Nat. Genet. 38, 896–903.

Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., Chen, L., Chen, L., Chen, T.M., Chin, M.C., Chong, J., Crook, B.E., Czaplinska, A., Dang, C.N., Datta, S., Dee, N.R., Desaki, A.L., Desta, T., Diep, E., Dolbeare, T.A., Donelan, M.J., Dong, H.W., Dougherty, J.G., Duncan, B.J., Ebbert, A.J., Eichele, G., Estin, L.K., Faber, C., Facer, B.A., Fields, R., Fischer, S.R., Fliss, T.P., Frensley, C., Gates, S.N., Glattfelder, K.J., Halverson, K.R., Hart, M.R., Hohmann, J.G., Howell, M.P., Jeung, D.P., Johnson, R.A., Karr, P.T., Kawal, R., Kidney, J.M., Knapik, R.H., Kuan, C.L., Lake, J.H., Laramee, A.R., Larsen, K.D., Lau, C., Lemon, T.A., Liang, A.J., Liu, Y., Luong, L.T., Michaels, J., Morgan, J.J., Morgan, R.J., Mortrud, M.T., Mosqueda, N.F., Ng, L.L., Ng, R., Orta, G.J., Overly, C.C., Pak, T.H., Parry, S.E., Pathak, S.D., Pearson, O.C., Puchalski, R.B., Riley, Z.L., Rockett, H.R., Rowland, S.A., Royall, J.J., Ruiz, M.J., Sarno, N.R., Schaffnit, K., Shapovalova, N.V., Sivisay, T., Slaughterbeck, C.R., Smith, S.C., Smith, K.A., Smith, B.I., Sodt, A.J., Stewart, N.N., Stumpf, K.R., Sunkin, S.M., Sutram, M., Tam, A., Teemer, C.D., Thaller, C., Thompson, C.L., Varnam, L.R., Visel, A., Whitlock, R.M., Wohnoutka, P.E., Wolkey, C.K., Wong, V.Y., Wood, M., Yaylaoglu, M.B., Young, R.C., Youngstrom, B.L., Yuan, X.F., Zhang, B., Zwingman, T.A., Jones, A.R., 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature 445, 168–176.

Levine, M., Cattoglio, C., Tjian, R., 2014. Looping back to leap forward: transcription enters a new era. Cell 157, 13–25.

Li, L., Chang, H.Y., 2014. Physiological roles of long noncoding RNAs: insight from knockout mice. Trends Cell. Biol. 24, 594–602.

Li, M., Jia, C., Kazmierkiewicz, K.L., Bowman, A.S., Tian, L., Liu, Y., Gupta, N.A., Gudiseva, H.V., Yee, S.S., Kim, M., Dentchev, T., Kimble, J.A., Parker, J.S., Messinger, J.D., Hakonarson, H., Curcio, C.A., Stambolian, D., 2014. Comprehensive analysis of gene expression in human retina and supporting tissues. Hum. Mol. Genet. 23, 4001–4014.

Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., Sandstrom, R., Bernstein, B., Bender, M.A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, L.A., Lander, E.S., Dekker, J., 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326, 289–293.

Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S., Johnson, J.M., 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433, 769–773.

Lindvall, O., Kokaia, Z., 2010. Stem cells in human neurodegenerative disorders–time for clinical translation? J. Clin. Investig. 120, 29–40.

Lister, R., Pelizzola, M., Dowen, R.H., Hawkins, R.D., Hon, G., Tonti-Filippini, J., Nery, J.R., Lee, L., Ye, Z., Ngo, Q.M., Edsall, L., Antosiewicz-Bourget, J., Stewart, R., Ruotti, V., Millar, A.H., Thomson, J.A., Ren, B., Ecker, J.R., 2009. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462, 315–322.

Liu, M.M., Chan, C.C., Tuo, J., 2012. Genetic mechanisms and age-related macular degeneration: common variants, rare variants, copy number variations, epigenetics, and mitochondrial genetics. Hum. Genomics 6, 13.

Livesey, F.J., Furukawa, T., Steffen, M.A., Church, G.M., Cepko, C.L., 2000. Microarray analysis of the transcriptional network controlled by the photoreceptor homeobox gene Crx. Curr. Biol. 10, 301–310.

Loscher, C.J., Hokamp, K., Wilson, J.H., Li, T., Humphries, P., Farrar, G.J., Palfi, A., 2008. A common microRNA signature in mouse models of retinal degeneration. Exp. Eye Res. 87, 529–534.

Louie, C.M., Caridi, G., Lopes, V.S., Brancati, F., Kispert, A., Lancaster, M.A., Schlossman, A.M., Otto, E.A., Leitges, M., Grone, H.J., Lopez, I., Gudiseva, H.V., O'Toole, J.F., Vallespin, E., Ayyagari, R., Ayuso, C., Cremers, F.P., den Hollander, A.I., Koenekoop, R.K., Dallapiccola, B., Ghiggeri, G.M., Hildebrandt, F., Valente, E.M., Williams, D.S., Gleeson, J.G., 2010. AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. Nat. Genet. 42, 175–180.

Lu, J., Clark, A.G., 2012. Impact of microRNA regulation on variation in human gene expression. Genome Res. 22, 1243–1254.

Lubeck, E., Cai, L., 2012. Single-cell systems biology by super-resolution imaging and combinatorial labeling. Nat. Methods 9, 743–748.

Lumayag, S., Haldin, C.E., Corbett, N.J., Wahlin, K.J., Cowan, C., Turturro, S., Larsen, P.E., Kovacs, B., Witmer, P.D., Valle, D., Zack, D.J., Nicholson, D.A., Xu, S., 2013. Inactivation of the microRNA-183/96/182 cluster results in syndromic retinal degeneration. Proc. Natl. Acad. Sci. U. S. A. 110, E507–E516.

Ma, W., Cojocaru, R., Gotoh, N., Gieser, L., Villasmil, R., Cogliati, T., Swaroop, A., Wong, W.T., 2013. Gene expression changes in aging retinal microglia: relationship to microglial support functions and regulation of activation. Neurobiol. Aging 34, 2310–2321.

MacArthur, D.G., Balasubramanian, S., Frankish, A., Huang, N., Morris, J., Walter, K., Jostins, L., Habegger, L., Pickrell, J.K., Montgomery, S.B., Albers, C.A., Zhang, Z.D., Conrad, D.F., Lunter, G., Zheng, H., Ayub, Q., DePristo, M.A., Banks, E., Hu, M., Handsaker, R.E., Rosenfeld, J.A., Fromer, M., Jin, M., Mu, X.J., Khurana, E., Ye, K., Kay, M., Saunders, G.I., Suner, M.M., Hunt, T., Barnes, I.H., Amid, C., Carvalho-Silva, D.R., Bignell, A.H., Snow, C., Yngvadottir, B., Bumpstead, S., Cooper, D.N., Xue, Y., Romero, I.G., Genomes Project Consortium, Wang, J., Li, Y., Gibbs, R.A., McCarroll, S.A., Dermitzakis, E.T., Pritchard, J.K., Barrett, J.C., Harrow, J., Hurles, M.E., Gerstein, M.B., Tyler-Smith, C., 2012. A systematic survey of loss-of-function variants in human protein-coding genes. Science 335, 823–828.

MacArthur, D.G., Manolio, T.A., Dimmock, D.P., Rehm, H.L., Shendure, J., Abecasis, G.R., Adams, D.R., Altman, R.B., Antonarakis, S.E., Ashley, E.A., Barrett, J.C., Biesecker, L.G., Conrad, D.F., Cooper, G.M., Cox, N.J., Daly, M.J., Gerstein, M.B., Goldstein, D.B., Hirschhorn, J.N., Leal, S.M., Pennacchio, L.A., Stamatoyannopoulos, J.A., Sunyaev, S.R., Valle, D., Voight, B.F., Winckler, W., Gunter, C., 2014. Guidelines for investigating causality of sequence variants in human disease. Nature 508, 469–476.

Masland, R.H., 2001. The fundamental plan of the retina. Nat. Neurosci. 4, 877–886.

Masland, R.H., 2012. The neuronal organization of the retina. Neuron 76, 266–280.

McKay, G.J., Campbell, L., Oliver, M., Brockbank, S., Simpson, D.A., Curry, W.J., 2004. Preparation of planar retinal specimens: verification by histology, mRNA profiling, and proteome analysis. Mol. Vis. 10, 240–247.

Mears, A.J., Kondo, M., Swain, P.K., Takada, Y., Bush, R.A., Saunders, T.L., Sieving, P.A., Swaroop, A., 2001. Nrl is required for rod photoreceptor development. Nat. Genet. 29, 447–452.

Meola, N., Pizzo, M., Alfano, G., Surace, E.M., Banfi, S., 2012. The long noncoding RNA Vax2os1 controls the cell cycle progression of photoreceptor progenitors in the mouse retina. RNA 18, 111–123.

Merbs, S.L., Khan, M.A., Hackler Jr., L., Oliver, V.F., Wan, J., Qian, J., Zack, D.J., 2012. Cell-specific DNA methylation patterns of retina-specific genes. PLoS one 7, e32602.

Meshorer, E., Misteli, T., 2006. Chromatin in pluripotent embryonic stem cells and differentiation. Nat. Rev. Mol. Cell. Biol. 7, 540–546.

Metzker, M.L., 2010. Sequencing technologies – the next generation. Nat. Rev. Genet. 11, 31–46.

Meyer, K.J., Davis, L.K., Schindler, E.I., Beck, J.S., Rudd, D.S., Grundstad, A.J., Scheetz, T.E., Braun, T.A., Fingert, J.H., Alward, W.L., Kwon, Y.H., Folk, J.C., Russell, S.R., Wassink, T.H., Stone, E.M., Sheffield, V.C., 2011. Genome-wide analysis of copy number variants in age-related macular degeneration. Hum. Genet. 129, 91–100.

Mitton, K.P., Swain, P.K., Chen, S., Xu, S., Zack, D.J., Swaroop, A., 2000. The leucine zipper of NRL interacts with the CRX homeodomain. A possible mechanism of transcriptional synergy in rhodopsin regulation. J. Biol. Chem. 275, 29794–29799.

Montano, M., Long, K., 2011. RNA surveillance-an emerging role for RNA regulatory networks in aging. Ageing Res. Rev. 10, 216–224.

Morin, R.D., O'Connor, M.D., Griffith, M., Kuchenbauer, F., Delaney, A., Prabhu, A.L., Zhao, Y., McDonald, H., Zeng, T., Hirst, M., Eaves, C.J., Marra, M.A., 2008. Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. Genome Res. 18, 610–621.

Morris, K.V., Mattick, J.S., 2014. The rise of regulatory RNA. Nat. Rev. Genet. 15, 423–437.

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods 5, 621–628.

Mortimer, S.A., Kidwell, M.A., Doudna, J.A., 2014. Insights into RNA structure and function from genome-wide studies. Nat. Rev. Genet. 15, 469–479.

Mu, X., Zhao, S., Pershad, R., Hsieh, T.F., Scarpa, A., Wang, S.W., White, R.A., Beremand, P.D., Thomas, T.L., Gan, L., Klein, W.H., 2001. Gene expression in the developing mouse retina by EST sequencing and microarray analysis. Nucleic Acids Res. 29, 4983–4993.

Mustafi, D., Kevany, B.M., Genoud, C., Okano, K., Cideciyan, A.V., Sumaroka, A., Roman, A.J., Jacobson, S.G., Engel, A., Adams, M.D., Palczewski, K., 2011. Defective photoreceptor phagocytosis in a mouse model of enhanced S-cone syndrome causes progressive retinal degeneration. FASEB J. 25, 3157–3176.

Mustafi, D., Kevany, B.M., Bai, X., Maeda, T., Sears, J.E., Khalil, A.M., Palczewski, K., 2013. Evolutionarily conserved long intergenic non-coding RNAs in the eye. Hum. Mol. Genet. 22, 2992–3002.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., Sasai, Y., 2012. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell. Stem Cell. 10, 771–785.

Nasonkin, I.O., Merbs, S.L., Lazo, K., Oliver, V.F., Brooks, M., Patel, K., Enke, R.A., Nellissery, J., Jamrich, M., Le, Y.Z., Bharti, K., Fariss, R.N., Rachel, R.A., Zack, D.J., Rodriguez-Boulan, E.J., Swaroop, A., 2013. Conditional knockdown of DNA methyltransferase 1 reveals a key role of retinal pigment epithelium integrity in photoreceptor outer segment morphogenesis. Development 140, 1330–1341.

Neale, B.M., Fagerness, J., Reynolds, R., Sobrin, L., Parker, M., Raychaudhuri, S., Tan, P.L., Oh, E.C., Merriam, J.E., Souied, E., Bernstein, P.S., Li, B., Frederick, J.M., Zhang, K., Brantley Jr., M.A., Lee, A.Y., Zack, D.J., Campochiaro, B., Campochiaro, P., Ripke, S., Smith, R.T., Barile, G.R., Katsanis, N., Allikmets, R., Daly, M.J., Seddon, J.M., 2010. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc. Natl. Acad. Sci. U. S. A. 107, 7395–7400.

Neveling, K., Collin, R.W., Gilissen, C., van Huet, R.A., Visser, L., Kwint, M.P., Gijsen, S.J., Zonneveld, M.N., Wieskamp, N., de Ligt, J., Siemiatkowska, A.M., Hoefsloot, L.H., Buckley, M.F., Kellner, U., Branham, K.E., den Hollander, A.I., Hoischen, A., Hoyng, C., Klevering, B.J., van den Born, L.I., Veltman, J.A., Cremers, F.P., Scheffer, H., 2012. Next-generation genetic testing for retinitis pigmentosa. Hum. Mutat. 33, 963–972.

Ng, L., Hurley, J.B., Dierks, B., Srinivas, M., Salto, C., Vennstrom, B., Reh, T.A., Forrest, D., 2001. A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat. Genet. 27, 94–98.

Ng, S.B., Turner, E.H., Robertson, P.D., Flygare, S.D., Bigham, A.W., Lee, C., Shaffer, T., Wong, M., Bhattacharjee, A., Eichler, E.E., Bamshad, M., Nickerson, D.A., Shendure, J., 2009. Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461, 272–276.

Ng, L., Lu, A., Swaroop, A., Sharlin, D.S., Swaroop, A., Forrest, D., 2011. Two transcription factors can direct three photoreceptor outcomes from rod precursor cells in mouse retinal development. J. Neurosci. 31, 11118–11125.

Nishida, A., Furukawa, A., Koike, C., Tano, Y., Aizawa, S., Matsuo, I., Furukawa, T., 2003. Otx2 homeobox gene controls retinal photoreceptor cell fate and pineal gland development. Nat. Neurosci. 6, 1255–1263.
Nishiguchi, K.M., Tearle, R.G., Liu, Y.P., Oh, E.C., Miyake, N., Benaglio, P., Harper, S., Koskiniemi-Kuendig, H., Venturini, G., Sharon, D., Koenekoop, R.K., Nakamura, M., Kondo, M., Ueno, S., Yasuma, T.R., Beckmann, J.S., Ikegawa, S., Matsumoto, N., Terasaki, H., Berson, E.L., Katsanis, N., Rivolta, C., 2013. Whole genome sequencing in patients with retinitis pigmentosa reveals pathogenic DNA structural changes and NEK2 as a new disease gene. Proc. Natl. Acad. Sci. U. S. A. 110, 16139–16144.
Nishiguchi, K.M., Rivolta, C., 2012. Genes associated with retinitis pigmentosa and allied diseases are frequently mutated in the general population. PLoS One 7, e41902.
Oh, E.C., Khan, N., Novelli, E., Khanna, H., Strettoi, E., Swaroop, A., 2007. Transformation of cone precursors to functional rod photoreceptors by bZIP transcription factor NRL. Proc. Natl. Acad. Sci. U. S. A. 104, 1679–1684.
Oh, E.C., Cheng, H., Hao, H., Jia, L., Khan, N.W., Swaroop, A., 2008. Rod differentiation factor NRL activates the expression of nuclear receptor NR2E3 to suppress the development of cone photoreceptors. Brain Res. 1236, 16–29.
Ohno, S., 1972. So much "junk" DNA in our genome. Brookhaven Symp. Biol. 23, 366–370.
Okada, Y., Wu, D., Trynka, G., Raj, T., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Yoshida, S., Graham, R.R., Manoharan, A., Ortmann, W., Bhangale, T., Denny, J.C., Carroll, R.J., Eyler, A.E., Greenberg, J.D., Kremer, J.M., Pappas, D.A., Jiang, L., Yin, J., Ye, L., Su, D.F., Yang, J., Xie, G., Keystone, E., Westra, H.J., Esko, T., Metspalu, A., Zhou, X., Gupta, N., Mirel, D., Stahl, E.A., Diogo, D., Cui, J., Liao, K., Guo, M.H., Myouzen, K., Kawaguchi, T., Coenen, M.J., van Riel, P.L., van de Laar, M.A., Guchelaar, H.J., Huizinga, T.W., Dieude, P., Mariette, X., Bridges Jr., S.L., Zhernakova, A., Toes, R.E., Tak, P.P., Miceli-Richard, C., Bang, S.Y., Lee, H.S., Martin, J., Gonzalez-Gay, M.A., Rodriguez-Rodriguez, L., Rantapaa-Dahlqvist, S., Arlestig, L., Choi, H.K., Kamatani, Y., Galan, P., Lathrop, M., RACI consortium, GARNET consortium, Eyre, S., Bowes, J., Barton, A., de Vries, N., Moreland, L.W., Criswell, L.A., Karlson, E.W., Taniguchi, A., Yamada, R., Kubo, M., Liu, J.S., Bae, S.C., Worthington, J., Padyukov, L., Klareskog, L., Gregersen, P.K., Raychaudhuri, S., Stranger, B.E., De Jager, P.L., Franke, L., Visscher, P.M., Brown, M.A., Yamanaka, H., Mimori, T., Takahashi, A., Xu, H., Behrens, T.W., Siminovitch, K.A., Momohara, S., Matsuda, F., Yamamoto, K., Plenge, R.M., 2014. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506, 376–381.
Oliver, V.F., Franchina, M., Jaffe, A.E., Branham, K.E., Othman, M., Heckenlively, J.R., Swaroop, A., Campochiaro, B., Vote, B.J., Craig, J.E., Saffery, R., Mackey, D.A., Qian, J., Zack, D.J., Hewitt, A.W., Merbs, S.L., 2013a. Hypomethylation of the IL17RC promoter in peripheral blood leukocytes is not a hallmark of age-related macular degeneration. Cell. Rep. 5, 1527–1535.
Oliver, V.F., Wan, J., Agarwal, S., Zack, D.J., Qian, J., Merbs, S.L., 2013b. A novel methyl-binding domain protein enrichment method for identifying genome-wide tissue-specific DNA methylation from nanogram DNA samples. Epigenetics Chromatin 6, 17.
Omori, Y., Katoh, K., Sato, S., Muranishi, Y., Chaya, T., Onishi, A., Minami, T., Fujikado, T., Furukawa, T., 2011. Analysis of transcriptional regulatory pathways of photoreceptor genes by expression profiling of the Otx2-deficient retina. PLoS One 6, e19685.
Otto, E.A., Hurd, T.W., Airik, R., Chaki, M., Zhou, W., Stoetzel, C., Patil, S.B., Levy, S., Ghosh, A.K., Murga-Zamalloa, C.A., van Reeuwijk, J., Letteboer, S.J., Sang, L., Giles, R.H., Liu, Q., Coene, K.L., Estrada-Cuzcano, A., Collin, R.W., McLaughlin, H.M., Held, S., Kasanuki, J.M., Ramaswami, G., Conte, J., Lopez, I., Washburn, J., Macdonald, J., Hu, J., Yamashita, Y., Maher, E.R., Guay-Woodford, L.M., Neumann, H.P., Obermuller, N., Koenekoop, R.K., Bergmann, C., Bei, X., Lewis, R.A., Katsanis, N., Lopes, V., Williams, D.S., Lyons, R.H., Dang, C.V., Brito, D.A., Dias, M.B., Zhang, X., Cavalcoli, J.D., Nurnberg, G., Nurnberg, P., Pierce, E.A., Jackson, P.K., Antignac, C., Saunier, S., Roepman, R., Dollfus, H., Khanna, H., Hildebrandt, F., 2010. Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. Nat. Genet. 42, 840–850.
Ozgul, R.K., Siemiatkowska, A.M., Yucel, D., Myers, C.A., Collin, R.W., Zonneveld, M.N., Beryozkin, A., Banin, E., Hoyng, C.B., van den Born, L.I., European Retinal Disease, C., Bose, R., Shen, W., Sharon, D., Cremers, F.P., Klevering, B.J., den Hollander, A.I., Corbo, J.C., 2011. Exome sequencing and cis-regulatory mapping identify mutations in MAK, a gene encoding a regulator of ciliary length, as a cause of retinitis pigmentosa. Am. J. Hum. Genet. 89, 253–264.
Pakkenberg, B., Pelvig, D., Marner, L., Bundgaard, M.J., Gundersen, H.J., Nyengaard, J.R., Regeur, L., 2003. Aging and the human neocortex. Exp. Gerontol. 38, 95–99.
Palazzo, A.F., Gregory, T.R., 2014. The case for junk DNA. PLoS Genet. 10, e1004351.
Panfoli, I., Musante, L., Bachi, A., Ravera, S., Calzia, D., Cattaneo, A., Bruschi, M., Bianchini, P., Diaspro, A., Morelli, A., Pepe, I.M., Tacchetti, C., Candiano, G., 2008. Proteomic analysis of the retinal rod outer segment disks. J. Proteome Res. 7, 2654–2669.
Parapuram, S.K., Cojocaru, R.I., Chang, J.R., Khanna, R., Brooks, M., Othman, M., Zareparsi, S., Khan, N.W., Gotoh, N., Cogliati, T., Swaroop, A., 2010. Distinct signature of altered homeostasis in aging rod photoreceptors: implications for retinal diseases. PLoS One 5, e13885.
Park, P.J., 2009. ChIP-seq: advantages and challenges of a maturing technology. Nat. Rev. Genet. 10, 669–680.
Peng, G.H., Chen, S., 2011. Active opsin loci adopt intrachromosomal loops that depend on the photoreceptor transcription factor network. Proc. Natl. Acad. Sci. U. S. A. 108, 17821–17826.
Peng, G.H., Ahmad, O., Ahmad, F., Liu, J., Chen, S., 2005. The photoreceptor-specific nuclear receptor Nr2e3 interacts with Crx and exerts opposing effects on the transcription of rod versus cone genes. Hum. Mol. Genet. 14, 747–764.
Pennacchio, L.A., Bickmore, W., Dean, A., Nobrega, M.A., Bejerano, G., 2013. Enhancers: five essential questions. Nat. Rev. Genet. 14, 288–295.
Pittler, S.J., Zhang, Y., Chen, S., Mears, A.J., Zack, D.J., Ren, Z., Swain, P.K., Yao, S., Swaroop, A., White, J.B., 2004. Functional analysis of the rod photoreceptor cGMP phosphodiesterase alpha-subunit gene promoter: Nrl and Crx are required for full transcriptional activity. J. Biol. Chem. 279, 19800–19807.
Plenge, R.M., Scolnick, E.M., Altshuler, D., 2013. Validating therapeutic targets through human genetics. Nat. Rev. Drug Discov. 12, 581–594.
Popova, E.Y., Xu, X., DeWan, A.T., Salzberg, A.C., Berg, A., Hoh, J., Zhang, S.S., Barnstable, C.J., 2012. Stage and gene specific signatures defined by histones H3K4me2 and H3K27me3 accompany mammalian retina maturation in vivo. PLoS One 7, e46867.
Powell, C., Grant, A.R., Cornblath, E., Goldman, D., 2013. Analysis of DNA methylation reveals a partial reprogramming of the Muller glia genome during retina regeneration. Proc. Natl. Acad. Sci. U. S. A. 110, 19814–19819.
Priya, R.R., Chew, E.Y., Swaroop, A., 2012. Genetic studies of age-related macular degeneration: lessons, challenges, and opportunities for disease management. Ophthalmology 119, 2526–2536.
Pruitt, K.D., Harrow, J., Harte, R.A., Wallin, C., Diekhans, M., Maglott, D.R., Searle, S., Farrell, C.M., Loveland, J.E., Ruef, B.J., Hart, E., Suner, M.M., Landrum, M.J., Aken, B., Ayling, S., Baertsch, R., Fernandez-Banet, J., Cherry, J.L., Curwen, V., Dicuccio, M., Kellis, M., Lee, J., Lin, M.F., Schuster, M., Shkeda, A., Amid, C., Brown, G., Dukhanina, O., Frankish, A., Hart, J., Maidak, B.L., Mudge, J., Murphy, M.R., Murphy, T., Rajan, J., Rajput, B., Riddick, L.D., Snow, C., Steward, C., Webb, D., Weber, J.A., Wilming, L., Wu, W., Birney, E., Haussler, D., Hubbard, T., Ostell, J., Durbin, R., Lipman, D., 2009. The consensus coding sequence (CCDS) project: identifying a common protein-coding gene set for the human and mouse genomes. Genome Res. 19, 1316–1323.
Pujadas, E., Feinberg, A.P., 2012. Regulated noise in the epigenetic landscape of development and disease. Cell 148, 1123–1131.
Qian, J., Esumi, N., Chen, Y., Wang, Q., Chowers, I., Zack, D.J., 2005. Identification of regulatory targets of tissue-specific transcription factors: application to retina-specific gene regulation. Nucleic Acids Res. 33, 3479–3491.
Rao, S.S., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T., Sanborn, A.L., Machol, I., Omer, A.D., Lander, E.S., Aiden, E.L., 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell 159, 1665–1680.
Rapaport, D.H., Wong, L.L., Wood, E.D., Yasumura, D., LaVail, M.M., 2004. Timing and topography of cell genesis in the rat retina. J. Comp. Neurol. 474, 304–324.
Rapicavoli, N.A., Blackshaw, S., 2009. New meaning in the message: noncoding RNAs and their role in retinal development. Dev. Dyn. 238, 2103–2114.
Rapicavoli, N.A., Poth, E.M., Blackshaw, S., 2010. The long noncoding RNA RNCR2 directs mouse retinal cell specification. BMC Dev. Biol. 10, 49.
Rapicavoli, N.A., Poth, E.M., 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.
Ratnapriya, R., Swaroop, A., 2013. Genetic architecture of retinal and macular degenerative diseases: the promise and challenges of next-generation sequencing. Genome Med. 5, 84.
Ratnapriya, R., Zhan, X., Fariss, R.N., Branham, K.E., Zipprer, D., Chakarova, C.F., Sergeev, Y.V., Campos, M.M., Othman, M., Friedman, J.S., Maminishkis, A., Waseem, N.H., Brooks, M., Rajasimha, H.K., Edwards, A.O., Lotery, A., Klein, B.E., Truitt, B.J., Li, B., Schaumberg, D.A., Morgan, D.J., Morrison, M.A., Souied, E., Tsironi, E.E., Grassmann, F., Fishman, G.A., Silvestri, G., Scholl, H.P., Kim, I.K., Ramke, J., Tuo, J., Merriam, J.E., Merriam, J.C., Park, K.H., Olson, L.M., Farrer, L.A., Johnson, M.P., Peachey, N.S., Lathrop, M., Baron, R.V., Igo Jr., R.P., Klein, R., Hagstrom, S.A., Kamatani, Y., Martin, T.M., Jiang, Y., Conley, Y., Sahel, J.A., Zack, D.J., Chan, C.C., Pericak-Vance, M.A., Jacobson, S.G., Gorin, M.B., Klein, M.L., Allikmets, R., Iyengar, S.K., Weber, B.H., Haines, J.L., Leveillard, T., Deangelis, M.M., Stambolian, D., Weeks, D.E., Bhattacharya, S.S., Chew, E.Y., Heckenlively, J.R., Abecasis, G.R., Swaroop, A., 2014a. Rare and common variants in extracellular matrix gene Fibrillin 2 (FBN2) are associated with macular degeneration. Hum. Mol. Genet. 23, 5827–5837.
Ratnapriya, R.P.S., Mutsuddi, M., Branham, K., Othman, M., Heckenlively, J., Swaroop, A., 2014b. . Exome Sequencing in Extended Families with Age-related Macular Degeneration Reveals Enrichment of Genes Involved in Extracellular Matrix Pathway, 64th Annual Meeting of The American Society of Human Genetics. Personal communication.
Raychaudhuri, S., Iartchouk, O., Chin, K., Tan, P.L., Tai, A.K., Ripke, S., Gowrisankar, S., Vemuri, S., Montgomery, K., Yu, Y., Reynolds, R., Zack, D.J., Campochiaro, B., Campochiaro, P., Katsanis, N., Daly, M.J., Seddon, J.M., 2011. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. Nat. Genet. 43, 1232–1236.
Ren, B., Robert, F., Wyrick, J.J., Aparicio, O., Jennings, E.G., Simon, I., Zeitlinger, J., Schreiber, J., Hannett, N., Kanin, E., Volkert, T.L., Wilson, C.J., Bell, S.P., Young, R.A., 2000. Genome-wide location and function of DNA binding proteins. Science 290, 2306–2309.
Rhee, H.S., Pugh, B.F., 2011. Comprehensive genome-wide protein-DNA interactions detected at single-nucleotide resolution. Cell 147, 1408–1419.

Roesch, K., Jadhav, A.P., Trimarchi, J.M., Stadler, M.B., Roska, B., Sun, B.B., Cepko, C.L., 2008. The transcriptome of retinal Muller glial cells. J. Comp. Neurol. 509, 225–238.
Roger, J.E., Hiriyanna, A., Gotoh, N., Hao, H., Cheng, D.F., Ratnapriya, R., Kautzmann, M.A., Chang, B., Swaroop, A., 2014. OTX2 loss causes rod differentiation defect in CRX-associated congenital blindness. J. Clin. Investig. 124, 631–643.
Rosenbloom, K.R., Sloan, C.A., Malladi, V.S., Dreszer, T.R., Learned, K., Kirkup, V.M., Wong, M.C., Maddren, M., Fang, R., Heitner, S.G., Lee, B.T., Barber, G.P., Harte, R.A., Diekhans, M., Long, J.C., Wilder, S.P., Zweig, A.S., Karolchik, D., Kuhn, R.M., Haussler, D., Kent, W.J., 2013. ENCODE data in the UCSC genome browser: year 5 update. Nucleic Acids Res. 41, D56–D63.
Rowe-Rendleman, C.L., Durazo, S.A., Kompella, U.B., Rittenhouse, K.D., Di Polo, A., Weiner, A.L., Grossniklaus, H.E., Naash, M.I., Lewin, A.S., Horsager, A., Edelhauser, H.F., 2014. Drug and gene delivery to the back of the eye: from bench to bedside. Investig. Ophthalmol. Vis. Sci. 55, 2714–2730.
Ruan, X., Ruan, Y., 2012. Genome wide full-length transcript analysis using 5' and 3' paired-end-tag next generation sequencing (RNA-PET). Methods Mol. Biol. 809, 535–562.
Ryan, D.G., Oliveira-Fernandes, M., Lavker, R.M., 2006. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. Mol. Vis. 12, 1175–1184.
Ryan, C.J., Cimermancic, P., Szpiech, Z.A., Sali, A., Hernandez, R.D., Krogan, N.J., 2013. High-resolution network biology: connecting sequence with function. Nat. Rev. Genet. 14, 865–879.
Sahebkar, A., 2014. Beyond anti-PCSK9 therapies: the potential role of resistin inhibitors. Nat. Rev. Cardiol. 11, 12.
Samocha, K.E., Robinson, E.B., Sanders, S.J., Stevens, C., Sabo, A., McGrath, L.M., Kosmicki, J.A., Rehnstrom, K., Mallick, S., Kirby, A., Wall, D.P., MacArthur, D.G., Gabriel, S.B., DePristo, M., Purcell, S.M., Palotie, A., Boerwinkle, E., Buxbaum, J.D., Cook Jr., E.H., Gibbs, R.A., Schellenberg, G.D., Sutcliffe, J.S., Devlin, B., Roeder, K., Neale, B.M., Daly, M.J., 2014. A framework for the interpretation of de novo mutation in human disease. Nat. Genet. 46, 944–950.
Samuel, M.A., Zhang, Y., Meister, M., Sanes, J.R., 2011. Age-related alterations in neurons of the mouse retina. J. Neurosci. 31, 16033–16044.
Samuel, A., Housset, M., Fant, B., Lamonerie, T., 2014. Otx2 ChIP-seq reveals unique and redundant functions in the mature mouse retina. PLoS One 9, e89110.
Sasaki, H., Matsui, Y., 2008. Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat. Rev. Genet. 9, 129–140.
Sauna, Z.E., Kimchi-Sarfaty, C., 2011. Understanding the contribution of synonymous mutations to human disease. Nat. Rev. Genet. 12, 683–691.
Schadt, E.E., 2009. Molecular networks as sensors and drivers of common human diseases. Nature 461, 218–223.
Schaibley, V.M., Zawistowski, M., Wegmann, D., Ehm, M.G., Nelson, M.R., St Jean, P.L., Abecasis, G.R., Novembre, J., Zollner, S., Li, J.Z., 2013. The influence of genomic context on mutation patterns in the human genome inferred from rare variants. Genome Res. 23, 1974–1984.
Schaub, M.A., Boyle, A.P., Kundaje, A., Batzoglou, S., Snyder, M., 2012. Linking disease associations with regulatory information in the human genome. Genome Res. 22, 1748–1759.
Schulz, H.L., Goetz, T., Kaschkoetoe, J., Weber, B.H., 2004. The retinome – defining a reference transcriptome of the adult mammalian retina/retinal pigment epithelium. BMC Genomics 5, 50.
Seddon, J.M., Yu, Y., Miller, E.C., Reynolds, R., Tan, P.L., Gowrisankar, S., Goldstein, J.I., Triebwasser, M., Anderson, H.E., Zerbib, J., Kavanagh, D., Souied, E., Katsanis, N., Daly, M.J., Atkinson, J.P., Raychaudhuri, S., 2013. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. Nat. Genet. 45, 1366–1370.
Shalek, A.K., Satija, R., Shuga, J., Trombetta, J.J., Gennert, D., Lu, D., Chen, P., Gertner, R.S., Gaublomme, J.T., Yosef, N., Schwartz, S., Fowler, B., Weaver, S., Wang, J., Wang, X., Ding, R., Raychowdhury, R., Friedman, N., Hacohen, N., Park, H., May, A.P., Regev, A., 2014. Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. Nature 509, 363–369.
Shapiro, E., Biezuner, T., Linnarsson, S., 2013. Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat. Rev. Genet. 14, 618–630.
Sharon, D., Blackshaw, S., Cepko, C.L., Dryja, T.P., 2002. Profile of the genes expressed in the human peripheral retina, macula, and retinal pigment epithelium determined through serial analysis of gene expression (SAGE). Proc. Natl. Acad. Sci. U. S. A. 99, 315–320.
Sharon, D., Sandberg, M.A., Caruso, R.C., Berson, E.L., Dryja, T.P., 2003. Shared mutations in NR2E3 in enhanced S-cone syndrome, Goldmann-Favre syndrome, and many cases of clumped pigmentary retinal degeneration. Arch. Ophthalmol. 121, 1316–1323.
Shen, J., Yang, X., Xie, B., Chen, Y., Swaim, M., Hackett, S.F., Campochiaro, P.A., 2008. MicroRNAs regulate ocular neovascularization. Mol. Ther. 16, 1208–1216.
Shen, S., Park, J.W., Lu, Z.X., Lin, L., Henry, M.D., Wu, Y.N., Zhou, Q., Xing, Y., 2014. rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. Proc. Natl. Acad. Sci. U. S. A. 111, E5593–E5601.
Shinomori, K., Werner, J.S., 2012. Aging of human short-wave cone pathways. Proc. Natl. Acad. Sci. U. S. A. 109, 13422–13427.
Shlyueva, D., Stampfel, G., Stark, A., 2014. Transcriptional enhancers: from properties to genome-wide predictions. Nat. Rev. Genet. 15, 272–286.
Siegert, S., Cabuy, E., Scherf, B.G., Kohler, H., Panda, S., Le, Y.Z., Fehling, H.J., Gaidatzis, D., Stadler, M.B., Roska, B., 2012. Transcriptional code and disease map for adult retinal cell types. Nat. Neurosci. 15, 487–495. S481–482.
Slack, F.J., 2006. Regulatory RNAs and the demise of 'junk' DNA. Genome Biol. 7, 328.
Smallwood, S.A., Lee, H.J., Angermueller, C., Krueger, F., Saadeh, H., Peat, J., Andrews, S.R., Stegle, O., Reik, W., Kelsey, G., 2014. Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. Nat. Methods 11, 817–820.
Sobrin, L., Reynolds, R., Yu, Y., Fagerness, J., Leveziel, N., Bernstein, P.S., Souied, E.H., Daly, M.J., Seddon, J.M., 2011. ARMS2/HTRA1 locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration. Am. J. Ophthalmol. 151, 345–352 e343.
Sobrin, L., Ripke, S., Yu, Y., Fagerness, J., Bhangale, T.R., Tan, P.L., Souied, E.H., Buitendijk, G.H., Merriam, J.E., Richardson, A.J., Raychaudhuri, S., Reynolds, R., Chin, K.A., Lee, A.Y., Leveziel, N., Zack, D.J., Campochiaro, P., Smith, R.T., Barile, G.R., Hogg, R.E., Chakravarthy, U., Behrens, T.W., Uitterlinden, A.G., van Duijn, C.M., Vingerling, J.R., Brantley Jr., M.A., Baird, P.N., Klaver, C.C., Allikmets, R., Katsanis, N., Graham, R.R., Ioannidis, J.P., Daly, M.J., Seddon, J.M., 2012. Heritability and genome-wide association study to assess genetic differences between advanced age-related macular degeneration subtypes. Ophthalmology 119, 1874–1885.
Song, C.X., Szulwach, K.E., Fu, Y., Dai, Q., Yi, C., Li, X., Li, Y., Chen, C.H., Zhang, W., Jian, X., Wang, J., Zhang, L., Looney, T.J., Zhang, B., Godley, L.A., Hicks, L.M., Lahn, B.T., Jin, P., He, C., 2011a. Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat. Biotechnol. 29, 68–72.
Song, J., Smaoui, N., Ayyagari, R., Stiles, D., Benhamed, S., MacDonald, I.M., Daiger, S.P., Tumminia, S.J., Hejtmancik, F., Wang, X., 2011b. High-throughput retina-array for screening 93 genes involved in inherited retinal dystrophy. Investig. Ophthalmol. Vis. Sci. 52, 9053–9060.
Sorger, P.K., 2005. A reductionist's systems biology: opinion. Curr. Opin. Cell. Biol. 17, 9–11.
Splinter, E., de Wit, E., van de Werken, H.J., Klous, P., de Laat, W., 2012. Determining long-range chromatin interactions for selected genomic sites using 4C-seq technology: from fixation to computation. Methods 58, 221–230.
Srinivas, M., Ng, L., Liu, H., Jia, L., Forrest, D., 2006. Activation of the blue opsin gene in cone photoreceptor development by retinoid-related orphan receptor beta. Mol. Endocrinol. 20, 1728–1741.
Strauss, O., 2005. The retinal pigment epithelium in visual function. Physiol. Rev. 85, 845–881.
Sundermeier, T.R., Zhang, N., Vinberg, F., Mustafi, D., Kohno, H., Golczak, M., Bai, X., Maeda, A., Kefalov, V.J., Palczewski, K., 2014. DICER1 is essential for survival of postmitotic rod photoreceptor cells in mice. FASEB J. 28, 3780–3791.
Suuronen, T., Nuutinen, T., Ryhanen, T., Kaarniranta, K., Salminen, A., 2007. Epigenetic regulation of clusterin/apolipoprotein J expression in retinal pigment epithelial cells. Biochem. Biophys. Res. Commun. 357, 397–401.
Suzuki, M.M., Bird, A., 2008. DNA methylation landscapes: provocative insights from epigenomics. Nat. Rev. Genet. 9, 465–476.
Swaroop, A., Sieving, P.A., 2013. The golden era of ocular disease gene discovery: race to the finish. Clin. Genet. 84, 99–101.
Swaroop, A., Zack, D.J., 2002. Transcriptome analysis of the retina. Genome Biol. 3. REVIEWS1022.
Swaroop, A., Xu, J.Z., Pawar, H., Jackson, A., Skolnick, C., Agarwal, N., 1992. A conserved retina-specific gene encodes a basic motif/leucine zipper domain. Proc. Natl. Acad. Sci. U. S. A. 89, 266–270.
Swaroop, A., Kim, D., Forrest, D., 2010. Transcriptional regulation of photoreceptor development and homeostasis in the mammalian retina. Nat. Rev. Neurosci. 11, 563–576.
Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A., Lao, K., Surani, M.A., 2009. mRNA-Seq whole-transcriptome analysis of a single cell. Nat. Methods 6, 377–382.
Tariq, M.A., Kim, H.J., Jejelowo, O., Pourmand, N., 2011. Whole-transcriptome RNAseq analysis from minute amount of total RNA. Nucleic Acids Res. 39, e120.
Telese, F., Gamliel, A., Skowronska-Krawczyk, D., Garcia-Bassets, I., Rosenfeld, M.G., 2013. "Seq-ing" insights into the epigenetics of neuronal gene regulation. Neuron 77, 606–623.
Terrell, D., Xie, B., Workman, M., Mahato, S., Zelhof, A., Gebelein, B., Cook, T., 2012. OTX2 and CRX rescue overlapping and photoreceptor-specific functions in the Drosophila eye. Dev. Dyn. 241, 215–228.
Teslovich, T.M., Musunuru, K., Smith, A.V., Edmondson, A.C., Stylianou, I.M., Koseki, M., Pirruccello, J.P., Ripatti, S., Chasman, D.I., Willer, C.J., Johansen, C.T., Fouchier, S.W., Isaacs, A., Peloso, G.M., Barbalic, M., Ricketts, S.L., Bis, J.C., Aulchenko, Y.S., Thorleifsson, G., Feitosa, M.F., Chambers, J., Orho-Melander, M., Melander, O., Johnson, T., Li, X., Guo, X., Li, M., Shin Cho, Y., Jin Go, M., Jin Kim, Y., Lee, J.Y., Park, T., Kim, K., Sim, X., Twee-Hee Ong, R., Croteau-Chonka, D.C., Lange, L.A., Smith, J.D., Song, K., Hua Zhao, J., Yuan, X., Luan, J., Lamina, C., Ziegler, A., Zhang, W., Zee, R.Y., Wright, A.F., Witteman, J.C., Wilson, J.F., Willemsen, G., Wichmann, H.E., Whitfield, J.B., Waterworth, D.M., Wareham, N.J., Waeber, G., Vollenweider, P., Voight, B.F., Vitart, V., Uitterlinden, A.G., Uda, M., Tuomilehto, J., Thompson, J.R., Tanaka, T., Surakka, I., Stringham, H.M., Spector, T.D., Soranzo, N., Smit, J.H., Sinisalo, J., Silander, K., Sijbrands, E.J., Scuteri, A., Scott, J., Schlessinger, D., Sanna, S., Salomaa, V., Saharinen, J., Sabatti, C., Ruokonen, A., Rudan, I., Rose, L.M., Roberts, R., Rieder, M., Psaty, B.M., Pramstaller, P.P., Pichler, I., Perola, M., Penninx, B.W., Pedersen, N.L., Pattaro, C., Parker, A.N., Pare, G., Oostra, B.A., O'Donnell, C.J., Nieminen, M.S., Nickerson, D.A., Montgomery, G.W., Meitinger, T., McPherson, R., McCarthy, M.I., McArdle, W., Masson, D., Martin, N.G., Marroni, F., Mangino, M., Magnusson, P.K., Lucas, G., Luben, R., Loos, R.J., Lokki, M.L., Lettre, G., Langenberg, C., Launer, L.J., Lakatta, E.G., Laaksonen, R.,

Kyvik, K.O., Kronenberg, F., Konig, I.R., Khaw, K.T., Kaprio, J., Kaplan, L.M., Johansson, A., Jarvelin, M.R., Janssens, A.C., Ingelsson, E., Igl, W., Kees Hovingh, G., Hottenga, J.J., Hofman, A., Hicks, A.A., Hengstenberg, C., Heid, I.M., Hayward, C., Havulinna, A.S., Hastie, N.D., Harris, T.B., Haritunians, T., Hall, A.S., Gyllensten, U., Guiducci, C., Groop, L.C., Gonzalez, E., Gieger, C., Freimer, N.B., Ferrucci, L., Erdmann, J., Elliott, P., Ejebe, K.G., Doring, A., Dominiczak, A.F., Demissie, S., Deloukas, P., de Geus, E.J., de Faire, U., Crawford, G., Collins, F.S., Chen, Y.D., Caulfield, M.J., Campbell, H., Burtt, N.P., Bonnycastle, L.L., Boomsma, D.I., Boekholdt, S.M., Bergman, R.N., Barroso, I., Bandinelli, S., Ballantyne, C.M., Assimes, T.L., Quertermous, T., Altshuler, D., Seielstad, M., Wong, T.Y., Tai, E.S., Feranil, A.B., Kuzawa, C.W., Adair, L.S., Taylor Jr., H.A., Borecki, I.B., Gabriel, S.B., Wilson, J.G., Holm, H., Thorsteinsdottir, U., Gudnason, V., Krauss, R.M., Mohlke, K.L., Ordovas, J.M., Munroe, P.B., Kooner, J.S., Tall, A.R., Hegele, R.A., Kastelein, J.J., Schadt, E.E., Rotter, J.I., Boerwinkle, E., Strachan, D.P., Mooser, V., Stefansson, K., Reilly, M.P., Samani, N.J., Schunkert, H., Cupples, L.A., Sandhu, M.S., Ridker, P.M., Rader, D.J., van Duijn, C.M., Peltonen, L., Abecasis, G.R., Boehnke, M., Kathiresan, S., 2010. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466, 707–713.

Tessler, L.A., Reifenberger, J.G., Mitra, R.D., 2009. Protein quantification in complex mixtures by solid phase single-molecule counting. Anal. Chem. 81, 7141–7148.

Trewavas, A., 2006. A brief history of systems biology. “Every object that biology studies is a system of systems.” Francois Jacob (1974). Plant Cell. 18, 2420–2430.

Trimarchi, J.M., Stadler, M.B., Roska, B., Billings, N., Sun, B., Bartch, B., Cepko, C.L., 2007. Molecular heterogeneity of developing retinal ganglion and amacrine cells revealed through single cell gene expression profiling. J. Comp. Neurol. 502, 1047–1065.

Trimarchi, J.M., Stadler, M.B., Cepko, C.L., 2008. Individual retinal progenitor cells display extensive heterogeneity of gene expression. PLoS One 3, e1588.

Tummala, P., Mali, R.S., Guzman, E., Zhang, X., Mitton, K.P., 2010. Temporal ChIP-on-chip of RNA-polymerase-II to detect novel gene activation events during photoreceptor maturation. Mol. Vis. 16, 252–271.

Tuo, J., Smith, B.C., Bojanowski, C.M., Meleth, A.D., Gery, I., Csaky, K.G., Chew, E.Y., Chan, C.C., 2004. The involvement of sequence variation and expression of CX3CR1 in the pathogenesis of age-related macular degeneration. FASEB J. 18, 1297–1299.

van de Ven, J.P., Nilsson, S.C., Tan, P.L., Buitendijk, G.H., Ristau, T., Mohlin, F.C., Nabuurs, S.B., Schoenmaker-Koller, F.E., Smailhodzic, D., Campochiaro, P.A., Zack, D.J., Duvvari, M.R., Bakker, B., Paun, C.C., Boon, C.J., Uitterlinden, A.G., Liakopoulos, S., Klevering, B.J., Fauser, S., Daha, M.R., Katsanis, N., Klaver, C.C., Blom, A.M., Hoyng, C.B., den Hollander, A.I., 2013. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. Nat. Genet. 45, 813–817.

Veleri, S., Lazar, C.H., Chang, B., Swaroop, A., 2015. Insights into the biology and therapy of human retinal neurodegeneration using mose models. Dis. Model Mech. 8, 109–129.

Venturini, G., Rose, A.M., Shah, A.Z., Bhattacharya, S.S., Rivolta, C., 2012. CNOT3 is a modifier of PRPF31 mutations in retinitis pigmentosa with incomplete penetrance. PLoS Genet. 8, e1003040.

Vidal, M., Cusick, M.E., Barabasi, A.L., 2011. Interactome networks and human disease. Cell 144, 986–998.

von Bernhardi, R., Tichauer, J.E., Eugenin, J., 2010. Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. J. Neurochem. 112, 1099–1114.

Voss, T.C., Hager, G.L., 2014. Dynamic regulation of transcriptional states by chromatin and transcription factors. Nat. Rev. Genet. 15, 69–81.

Wan, J., Oliver, V.F., Zhu, H., Zack, D.J., Qian, J., Merbs, S.L., 2013. Integrative analysis of tissue-specific methylation and alternative splicing identifies conserved transcription factor binding motifs. Nucleic Acids Res. 41, 8503–8514.

Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P., Burge, C.B., 2008. Alternative isoform regulation in human tissue transcriptomes. Nature 456, 470–476.

Wang, F.E., Zhang, C., Maminishkis, A., Dong, L., Zhi, C., Li, R., Zhao, J., Majerciak, V., Gaur, A.B., Chen, S., Miller, S.S., 2010. MicroRNA-204/211 alters epithelial physiology. FASEB J 24, 1552–1571.

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10, 57–63.

Webber, A.L., Hodor, P., Thut, C.J., Vogt, T.F., Zhang, T., Holder, D.J., Petrukhin, K., 2008. Dual role of Nr2e3 in photoreceptor development and maintenance. Exp. Eye Res. 87, 35–48.

Weber, M., Davies, J.J., Wittig, D., Oakeley, E.J., Haase, M., Lam, W.L., Schubeler, D., 2005. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat. Genet. 37, 853–862.

Wei, L., Liu, B., Tuo, J., Shen, D., Chen, P., Li, Z., Liu, X., Ni, J., Dagur, P., Sen, H.N., Jawad, S., Ling, D., Park, S., Chakrabarty, S., Meyerle, C., Agron, E., Ferris 3rd, F.L., Chew, E.Y., McCoy, J.P., Blum, E., Francis, P.J., Klein, M.L., Guymer, R.H., Baird, P.N., Chan, C.C., Nussenblatt, R.B., 2012a. Hypomethylation of the IL17RC promoter associates with age-related macular degeneration. Cell. Rep. 2, 1151–1158.

Wei, R., Gatterdam, V., Wieneke, R., Tampe, R., Rant, U., 2012b. Stochastic sensing of proteins with receptor-modified solid-state nanopores. Nat. Nanotechnol. 7, 257–263.

Weinmann, A.S., Farnham, P.J., 2002. Identification of unknown target genes of human transcription factors using chromatin immunoprecipitation. Methods 26, 37–47.

White, M.A., Myers, C.A., Corbo, J.C., Cohen, B.A., 2013. Massively parallel in vivo enhancer assay reveals that highly local features determine the cis-regulatory function of ChIP-seq peaks. Proc. Natl. Acad. Sci. U. S. A. 110, 11952–11957.

Whyte, W.A., Orlando, D.A., Hnisz, D., Abraham, B.J., Lin, C.Y., Kagey, M.H., Rahl, P.B., Lee, T.I., Young, R.A., 2013. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 153, 307–319.

Williams, R.W., Herrup, K., 1988. The control of neuron number. Annu. Rev. Neurosci. 11, 423–453.

Wright, A.F., Reddick, A.C., Schwartz, S.B., Ferguson, J.S., Aleman, T.S., Kellner, U., Jurklies, B., Schuster, A., Zrenner, E., Wissinger, B., Lennon, A., Shu, X., Cideciyan, A.V., Stone, E.M., Jacobson, S.G., Swaroop, A., 2004. Mutation analysis of NR2E3 and NRL genes in Enhanced S Cone Syndrome. Hum. Mutat. 24, 439.

Wright, A.F., Chakarova, C.F., Abd El-Aziz, M.M., Bhattacharya, S.S., 2010. Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. Nat. Rev. Genet. 11, 273–284.

Wu, H., D'Alessio, A.C., Ito, S., Wang, Z., Cui, K., Zhao, K., Sun, Y.E., Zhang, Y., 2011. Genome-wide analysis of 5-hydroxymethylcytosine distribution reveals its dual function in transcriptional regulation in mouse embryonic stem cells. Genes. Dev. 25, 679–684.

Xie, Z., Morgan, T.E., Rozovsky, I., Finch, C.E., 2003. Aging and glial responses to lipopolysaccharide in vitro: greater induction of IL-1 and IL-6, but smaller induction of neurotoxicity. Exp. Neurol. 182, 135–141.

Xu, S., 2009. microRNA expression in the eyes and their significance in relation to functions. Prog. Retin Eye Res. 28, 87–116.

Xu, S., Witmer, P.D., 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.

Xue, W., Cojocaru, R.I., Dudley, V.J., Brooks, M., Swaroop, A., Sarthy, V.P., 2011. Ciliary neurotrophic factor induces genes associated with inflammation and gliosis in the retina: a gene profiling study of flow-sorted, Muller cells. PLoS One 6, e20326.

Yadav, S.P., Hao, H., Yang, H.J., Kautzmann, M.A., Brooks, M., Nellissery, J., Klocke, B., Seifert, M., Swaroop, A., 2014. The transcription-splicing protein NonO/p54nrb and three NonO-interacting proteins bind to distal enhancer region and augment rhodopsin expression. Hum. Mol. Genet. 23, 2132–2144.

Yang, H.J., Kim, J.W., Hao, H., Brooks, M., Chaitankar, V., Boleda, A., Gugenmus, C., Seifert, M., Longo, T., Giuste, F., Perez, S., Gieser, L., Swaroop, A., 2015. Integrated Analysis of Gene Regulatory Networks Governing Mammalian Rod Photoreceptor Development. Manuscript in preparation.

Yang, Z., Stratton, C., Francis, P.J., Kleinman, M.E., Tan, P.L., Gibbs, D., Tong, Z., Chen, H., Constantine, R., Yang, X., Chen, Y., Zeng, J., Davey, L., Ma, X., Hau, V.S., Wang, C., Harmon, J., Buehler, J., Pearson, E., Patel, S., Kaminoh, Y., Watkins, S., Luo, L., Zabriskie, N.A., Bernstein, P.S., Cho, W., Schwager, A., Hinton, D.R., Klein, M.L., Hamon, S.C., Simmons, E., Yu, B., Campochiaro, B., Sunness, J.S., Campochiaro, P., Jorde, L., Parmigiani, G., Zack, D.J., Katsanis, N., Ambati, J., Zhang, K., 2008. Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. N. Engl. J. Med. 359, 1456–1463.

Yeo, G.W., Coufal, N.G., Liang, T.Y., Peng, G.E., Fu, X.D., Gage, F.H., 2009. An RNA code for the FOX2 splicing regulator revealed by mapping RNA-protein interactions in stem cells. Nat. Struct. Mol. Biol. 16, 130–137.

Yildirim, M.A., Goh, K.I., Cusick, M.E., Barabasi, A.L., Vidal, M., 2007. Drug-target network. Nat. Biotechnol. 25, 1119–1126.

Yoshida, S., Yashar, B.M., Hiriyanna, S., Swaroop, A., 2002. Microarray analysis of gene expression in the aging human retina. Investig. Ophthalmol. Vis. Sci. 43, 2554–2560.

Yoshida, S., Mears, A.J., Friedman, J.S., Carter, T., He, S., Oh, E., Jing, Y., Farjo, R., Fleury, G., Barlow, C., Hero, A.O., Swaroop, A., 2004. Expression profiling of the developing and mature Nrl-/- mouse retina: identification of retinal disease candidates and transcriptional regulatory targets of Nrl. Hum. Mol. Genet. 13, 1487–1503.

Yu, H., Luscombe, N.M., Qian, J., Gerstein, M., 2003a. Genomic analysis of gene expression relationships in transcriptional regulatory networks. Trends Genet. 19, 422–427.

Yu, J., Farjo, R., MacNee, S.P., Baehr, W., Stambolian, D.E., Swaroop, A., 2003b. Annotation and analysis of 10,000 expressed sequence tags from developing mouse eye and adult retina. Genome Biol. 4, R65.

Yu, J., He, S., Friedman, J.S., Akimoto, M., Ghosh, D., Mears, A.J., Hicks, D., Swaroop, A., 2004a. Altered expression of genes of the Bmp/Smad and Wnt/calcium signaling pathways in the cone-only Nrl-/- mouse retina, revealed by gene profiling using custom cDNA microarrays. J. Biol. Chem. 279, 42211–42220.

Yu, J., Mears, A.J., Yoshida, S., Farjo, R., Carter, T.A., Ghosh, D., Hero, A., Barlow, C., Swaroop, A., 2004b. From disease genes to cellular pathways: a progress report. In: Novartis Found Symp 255, pp. 147–160 discussion 160-144, 177–148.

Yu, X., Lin, J., Zack, D.J., Qian, J., 2006. Computational analysis of tissue-specific combinatorial gene regulation: predicting interaction between transcription factors in human tissues. Nucleic Acids Res. 34, 4925–4936.

Yu, Y., Triebwasser, M.P., Wong, E.K., Schramm, E.C., Thomas, B., Reynolds, R., Mardis, E.R., Atkinson, J.P., Daly, M., Raychaudhuri, S., Kavanagh, D., Seddon, J.M., 2014. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. Hum. Mol. Genet. 23, 5283–5293.

Zareparsi, S., Branham, K.E., Li, M., Shah, S., Klein, R.J., Ott, J., Hoh, J., Abecasis, G.R., Swaroop, A., 2005a. Strong association of the Y402H variant in complement

factor H at 1q32 with susceptibility to age-related macular degeneration. Am. J. Hum. Genet. 77, 149–153.
Zareparsi, S., Buraczynska, M., Branham, K.E., Shah, S., Eng, D., Li, M., Pawar, H., Yashar, B.M., Moroi, S.E., Lichter, P.R., Petty, H.R., Richards, J.E., Abecasis, G.R., Elner, V.M., Swaroop, A., 2005b. Toll-like receptor 4 variant D299G is associated with susceptibility to age-related macular degeneration. Hum. Mol. Genet. 14, 1449–1455.
Zentner, G.E., Henikoff, S., 2013. Regulation of nucleosome dynamics by histone modifications. Nat. Struct. Mol. Biol. 20, 259–266.
Zhan, X., Larson, D.E., Wang, C., Koboldt, D.C., Sergeev, Y.V., Fulton, R.S., Fulton, L.L., Fronick, C.C., Branham, K.E., Bragg-Gresham, J., Jun, G., Hu, Y., Kang, H.M., Liu, D., Othman, M., Brooks, M., Ratnapriya, R., Boleda, A., Grassmann, F., von Strachwitz, C., Olson, L.M., Buitendijk, G.H., Hofman, A., van Duijn, C.M., Cipriani, V., Moore, A.T., Shahid, H., Jiang, Y., Conley, Y.P., Morgan, D.J., Kim, I.K., Johnson, M.P., Cantsilieris, S., Richardson, A.J., Guymer, R.H., Luo, H., Ouyang, H., Licht, C., Pluthero, F.G., Zhang, M.M., Zhang, K., Baird, P.N., Blangero, J., Klein, M.L., Farrer, L.A., DeAngelis, M.M., Weeks, D.E., Gorin, M.B., Yates, J.R., Klaver, C.C., Pericak-Vance, M.A., Haines, J.L., Weber, B.H., Wilson, R.K., Heckenlively, J.R., Chew, E.Y., Stambolian, D., Mardis, E.R., Swaroop, A., Abecasis, G.R., 2013. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. Nat. Genet. 45, 1375–1379.
Zhang, J., Poh, H.M., Peh, S.Q., Sia, Y.Y., Li, G., Mulawadi, F.H., Goh, Y., Fullwood, M.J., Sung, W.K., Ruan, X., Ruan, Y., 2012. ChIA-PET analysis of transcriptional chromatin interactions. Methods 58, 289–299.
Zhao, J., Ohsumi, T.K., Kung, J.T., Ogawa, Y., Grau, D.J., Sarma, K., Song, J.J., Kingston, R.E., Borowsky, M., Lee, J.T., 2010. Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol. Cell. 40, 939–953.
Zhao, S., Fung-Leung, W.P., Bittner, A., Ngo, K., Liu, X., 2014. Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. PLoS One 9, e78644.
Zhou, X., Menche, J., Barabasi, A.L., Sharma, A., 2014. Human symptoms-disease network. Nat. Commun. 5, 4212.