CHAPTER

Alternative Experimental Models of Ciliary Trafficking and Dysfunction in the Retina

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9.1 Introduction

9.1.1 Cilia and Retinal Biology

The molecular aetiology of inherited retinal degeneration (iRD) is extremely heterogeneous. To date, 256 genes have been associated with iRDs,[1] which, while individually rare, have an estimated overall incidence of 1 in 2000 individuals.[2] Collectively, iRD genes have diverse functions in retinal biology, from retina-specific roles in the visual cycle, to genes with general cellular functions such as protein degradation or mitochondrial function. However, by far the largest category of iRD-causative genes (~20%) are those with roles in cilium formation or trafficking.[3] This is, perhaps,

unsurprising, as the outer segments of cone and rod photoreceptors are specialised primary cilia, modified to form an extensive surface area for efficient detection of light.

Primary cilia consist of a central ring of nine doublet microtubules called the axoneme, surrounded by a membrane which is continuous with the plasma membrane but compositionally distinct. The axoneme itself extends from the mother centriole which docks at the plasma membrane when cells are quiescent.

The ciliary axoneme is built by a microtubule motor-based trafficking process called intraflagellar transport (IFT). In this process, an IFT particle consisting of distinct IFT-A and IFT-B cargo-adaptor complexes is trafficked anterogradely (from base to tip) by heterotrimeric kinesin-II and retrogradely (from tip to base) by cytoplasmic dynein 2.[4] In addition to delivering tubulin subunits for axoneme extension, IFT also regulates the delivery and removal of ciliary receptors and other cargo involved in cilium function. In the case of the class A (rhodopsin-like) G-protein coupled receptors (GPCRs), import is mediated by the Tubby-family proteins TUB and TULP3.[5] Retrieval of receptors from cilia is performed by the BBSome, an eight-member ciliopathy protein complex which is bound to the IFT particle by the IFT25/27 adapter complex.[6,7] Mutations in both Tubby and BBS genes are causative of iRD in humans, mouse and zebrafish models.[8]

The most proximal portion of the ciliary axoneme is called the transition zone (the connecting cilium in photoreceptors), a region which acts as a diffusion barrier preventing the entry of non-ciliary proteins, thereby regulating the unique composition of the organelle. Numerous transition zone proteins are associated with human iRD, including CEP290 and CC2D2A. CEP290 is a scaffolding protein which associates with the ciliary base/transition zone and is required for both ciliogenesis in general and transition zone assembly specifically.[9] In photoreceptors, CC2D2A regulates the docking of rhodopsin-carrying vesicles at the ciliary base and, therefore, is necessary for the transport of rhodopsin into the outer segment.[10]

Multiple cellular trafficking pathways direct cargo to and from the cilium (see Figure 9.1), including clathrin-dependent endocytosis which regulates transforming growth factor-β signalling and membrane retrieval at the ciliary pocket,[11,12] and post-Golgi vesicular trafficking which delivers receptors including rhodopsin to the cilium.[13] The small GTPase RAB8 is a critical regulator of ciliary vesicle traffic, coating vesicles destined for the cilium and promoting their fusion with the periciliary membrane.[7] RAB8 activity is regulated by its guanine-nucleotide exchange factor Rabin8, which is itself regulated by RAB11. Several ciliary GPCRs, including rhodopsin and the three cone opsins possess ciliary targeting sequences,[14] although there is no one universal ciliary targeting sequence, probably reflecting the existence of multiple ciliary trafficking pathways. Peripheral membrane proteins are also trafficked to the cilium by a specific pathway composed of the lipid-binding proteins UNC119 (myristoylated proteins)[15] and PDE6D (prenylated proteins)[16] and another small GTPase,

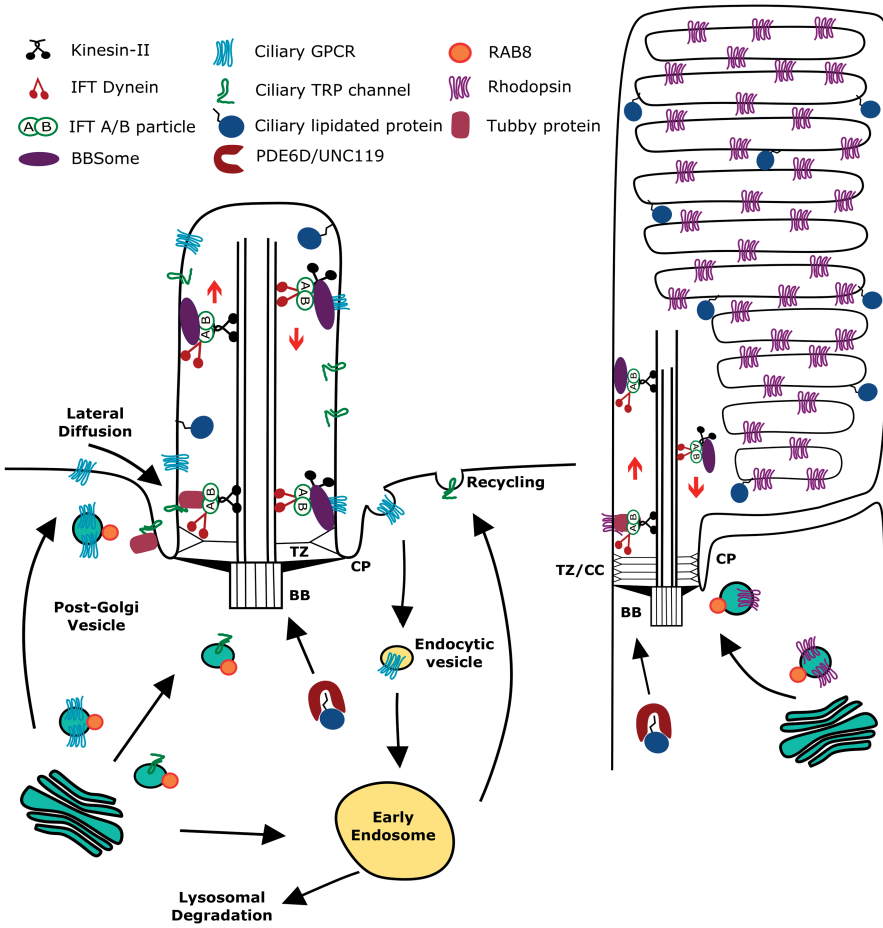

Figure 9.1 Schematic of a generic cilium and photoreceptor cilium (outer segment) and associated trafficking pathways (see text for details). IFT: intraflagellar transport; GPCR: G-protein coupled receptor; TRP: transient receptor potential; BB: basal body; TZ: transition zone; CC: connecting cilium; CP: ciliary pocket.

ARL3. For a summary of trafficking pathways to, within and from the cilium, see Figure 9.1.

9.1.2 Ciliopathies

A spectrum of human genetic disorders is associated with defective cilium formation or function, including Bardet–Biedl, Joubert, Meckel–Gruber and Senior–Løken syndromes, most of which present with retinal degeneration,[17] in addition to other phenotypes such as obesity, kidney cysts, polydactyly and mental retardation. Other syndromic ciliopathies include

Table 9.1 Summary of syndromic and non-syndromic ciliopathies which present with retinal dystrophy and selected representative genes.[a]

Presentation	Disease	Example causative genes
Syndromic	Bardet–Biedl syndrome	*BBS1-21*
	Alström syndrome	*ALMS1*
	Senior–Løken syndrome	*CEP290(BBS14)*, *NPHP4*
	Joubert syndrome	*CC2D2A*, *ARL13b*, *INPP5E*,
	Retinal dystrophy and obesity	*TUB*
Non-syndromic	Leber congenital amaurosis	*CEP290 (LCA10)*, *LCA5*, *RPGRIP1*
	Retinitis pigmentosa	*RPGR*, *RPGRIP1L*, *RP2*
	Cone–rod dystrophy	*RAB28*, *RPGRIP1*

[a]Data taken from Online Mendelian Inheritance in Man, http://www.omim.org/, last accessed April 2018.

a syndrome of rod–cone dystrophy and obesity caused by mutations in the gene *TUB*.[8] Clinical presentations also include non-syndromic ciliopathies in which the only phenotype is retinal degeneration, as exemplified by certain forms of Leber congenital amaurosis, retinitis pigmentosa and cone–rod dystrophy (see Table 9.1). Allelism is a common feature of ciliopathy genes, with different mutations in the same gene leading to different sets of phenotypes, even syndromic and non-syndromic. A primary example is *CEP290*, which is associated with Bardet–Biedl, Meckel–Gruber and Joubert syndromes, as well as non-syndromic Leber congenital amaurosis.[18] Interestingly, the penetrance of ciliopathy phenotypes in *CEP290* patients varies significantly between individuals. One possible explanation is pleiotropism of cilia genes, in which domain-specific functions of the encoded protein are affected differently by hypomorphic alleles. Another is the presence of background genetic modifiers, which significantly affects the penetrance of some ciliopathy phenotypes. For example, the A229T variant of RPGRIP1L increases the incidence of retinal degeneration in ciliopathies due to mutations in several other genes.[19] Finally, cell-specific differences in gene expression or function can modulate disease. For example, photoreceptors employ a high degree of alternative splicing.[20] Certain mutations (*e.g.* splice-site mutations) may impact photoreceptor-specific isoforms more severely than more broadly expressed variants, leading to non-syndromic phenotypes.[21] These factors can complicate the modelling of photoreceptor degeneration, due to species differences in the number, types and processing of isoforms. In summary, our understanding of the correlations between retinal ciliopathy genotypes and phenotypes requires innovative investigational approaches and models.

The pathomechanism of retinal degeneration due to cilia dysfunction in photoreceptors is most often ascribed to aberrant accumulation of proteins destined for the outer segment in the inner segment and/or leaking of inner segment proteins into the outer segment.[22] In other cells, the

cilium is a highly privileged organelle and non-ciliary proteins are efficiently excluded.[23] However, the photoreceptor outer segment may act as a sink for membrane proteins, possibly due to its extremely high membrane content. Without exclusion of entry, the trafficking machinery of the outer segment must continuously work to remove non-constituent membrane proteins.[22,24] The high rate of traffic into and out of the outer segment means that even subtle disruption of the trafficking machinery could lead to membrane proteins normally present in other photoreceptor compartments accumulating in the outer segment and damaging the delicate membrane architecture. This makes photoreceptors particularly vulnerable to the effects of ciliary dysfunction and may account for both the prevalence of iRD amongst ciliopathy patients and the large number of ciliary genes linked to forms of iRD.

While the photoreceptor outer segment is the most well studied retinal cilium, other retinal cells also possess cilia, including amacrine and retinal ganglion cells, Müller glia and the retinal pigment epithelium (RPE).[25] The roles of these cilia in retinal biology and disease are poorly understood. Evidence from primary cultures links cilium-regulated sonic hedgehog signalling in Müller glia to their capacity to de-differentiate and re-differentiate into other retinal cell types.[26] As Müller glia can mediate retinal regeneration, understanding the role of ciliary signalling in this process could advance the development of therapies for iRD. In the RPE, the number of ciliated cells decreases postnatally in rodents for an unknown reason and the tight junction protein claudin-1 localises to some of these cilia, suggesting a link to cell–cell adhesion.[27] However, the role of cilia in RPE function remains unknown. Therefore, there is a scientific need to improve our understanding of the roles of cilia in other retinal cells and in blindness.

The cilium represents a cellular nexus for gene networks of critical importance to photoreceptor function and survival. Despite its human disease relevance (in particular disorders of the retina), limited therapeutics are developed for ciliopathies, representing a significant unmet clinical need. The generation of *in*/*ex vivo* and *in vitro* models of ciliary dysfunction that elucidate pathways amenable to therapeutic intervention is critical to the development of novel therapies. For example, such models can be used to screen for neurotrophic drugs preserving or restoring photoreceptor function and cell survival. Owing to their remarkable conservation, a diverse set of organisms from the unicellular eukaryotes *Chlamydomonas reinhardtii* and *Trypanosoma brucei* to human cell lines provide models of ciliary function and disease.[28] The advent of gene editing in multiple species has accelerated the generation of research models, particularly those harbouring patient alleles with the intention of more accurately recapitulating patient phenotypes. Each model comes with unique advantages and disadvantages, but all collectively provide valuable insight into the molecular and cellular basis of ciliary disease and retinal degeneration. Here, we critically review the amenability of selected model systems for elucidating ciliary trafficking

and signalling pathways relevant to iRD and the development of therapies targeting these processes.

9.2 Rodent Models

Mouse models are the most widely used in the development of therapeutics for iRD. The genetic toolkit of mice includes tissue-specific and inducible knockouts, particularly useful in ciliopathies, as germline knockouts are often embryonic lethal. Inducible knockouts allow for investigation of the roles of genes in cilia maintenance and function in different spatio-temporal contexts. Knockout of *ARL3* is lethal in mice, but a retina-specific knockout has uncovered its postnatal role in outer segment formation, maintenance and function.[29] In addition, mice have been widely used in the development of gene- and cell-based therapies for iRD, including those resulting from ciliopathies.[30]

Disadvantages associated with mouse models include the relatively long (11 weeks) generation time and the fact that mice primarily avail of rod-mediated vision, unlike humans who rely more on cone-mediated vision. Additionally, mouse models do not always recapitulate the human phenotype and models with different alleles of the same gene may display distinct photoreceptor defects, as has been shown for retinitis pigmentosa 2 (*RP2*).[31] This could be due to species differences in gene usage, the above-mentioned allelism and genetic modifiers or any combination of these factors. While rodent models of ciliopathies and retinal degeneration have been informative, this review focuses on alternative, non-rodent metazoan models.

9.3 Induced Pluripotent Stem Cell-derived Retinal Organoids

9.3.1 Introduction

Since their discovery in 2006, induced pluripotent stem cells (iPSCs)[32] have flourished as a research model to study cellular aspects of disease, including iRD, in a human cell environment (for recent reviews see Yvon *et al.*[33] and Ovando-Roche *et al.*[34]). Patient-specific models can be developed by reprogramming somatic cells from iRD patients into pluripotent stem cells. These can be differentiated into target retinal cells for (1) studying pathological molecular pathways; (2) testing pharmacological or genetic therapies; and (3) cell replacement.

Human iPSC-derived retinal cells provide a significant alternative to animal models. One advantage is the more reliable genotype–phenotype relationship of inherited diseases due to human-specific splicing and expression mechanisms. Using iPSCs as a "disease-in-a-dish" system allows genetic, molecular and proteomic analyses of single retinal cell types, and pharmacological and

gene-editing interventions are relatively simple. However, maintenance of iPSC cultures is time consuming and costly,[33] limiting their widespread use as a drug screening system. Moreover, there is high variability in cell growth and differentiation across iPSC lines, possibly due to genetic or epigenetic differences, which can confound interpretation of results.[34,35] Despite the progress in differentiating iPSCs into three-dimensional retinal organoids, such models only represent an isolated system and findings need to be validated *in vivo*.

So far, iPSCs have been successfully differentiated into RPE cells, ganglion cells[36] and photoreceptor-containing optic cups.[37] Patient-specific, iPSC-derived RPE cells are widely used as *in vitro* models of retinal diseases, such as age-related macular degeneration,[38] retinitis pigmentosa[39] and Best vitelliform macular dystrophy.[40] iPSC-derived ganglion cells represent novel models of glaucoma.[41] Pioneering work from Zhong *et al.*[37] showed iPSCs can form three-dimensional retinal organoids (or optic cups) with proper layering of neuroretinal cell types, including photoreceptors which develop connecting cilia and rudimentary outer segments. This breakthrough has prompted many laboratories to model iRDs or ciliopathies, using "mini retinas" to investigate pathogenic mechanisms in patient-specific photoreceptors (see Section 9.3.2 for examples).

Although disease-specific phenotypes can be recapitulated (see Section 9.3.2), major drawbacks of using retinal organoids to study iRDs are: (1) differences in size, shape and composition of optic cups derived from different cell lines; (2) unorganised and partially degraded inner retinal cells; and (3) inability of organoid photoreceptors to develop mature outer segments.[42] Addressing these issues, Wahlin *et al.*[42] reported a protocol whereby photoreceptors in retinal cups develop outer segments of normal length, with localisation of opsins. However, they still lack the organisation of outer segments *in vivo*, with only a few discs present at irregular angles to the ciliary axoneme.

9.3.2 iPSC Ciliopathy Models

As summarised in Table 9.2, a variety of animal models for ciliopathies with retinal phenotypes have generated profound knowledge about cilia biology in health and disease. However, the discrepancy between human and animal model phenotypes of *CEP290* and *RP2* mutations, for example, reveals pitfalls that need to be considered when using animals to model human ciliopathies.[31] iPSC technology has the potential to overcome this issue, as it allows the study of mutations in retinal cells derived from patients.

Mutations in *CEP290* are associated with a variety of ciliopathy phenotypes, including Leber congenital amaurosis (LCA10).[43] The reason for the pleiotropic manifestation of *CEP290* disease is controversial. Two recent studies addressed this using iPSC-derived retinal cells. Parfitt *et al.*[44] compared CEP290 levels in fibroblasts, iPSCs, iPSC-derived RPE and optic cups from *CEP290*-LCA patients to test for cell-type specific phenotypes. In all

Table 9.2 Summary of ciliopathy/inherited retinal degeneration models; advantages and disadvantages of each; and ciliopathies that have been modelled in each.[a]

Species/model	Advantages	Disadvantages	Diseases modelled
Mouse (*Mus musculus*)	Mammalian model	Species-specific transcript processing	Bardet–Biedl syndrome
	Well-established genetic tools	Generation/ development time	Joubert syndrome
	Rod-mediated vision	Cost	Retinitis pigmentosa
iPSC-derived retinal cells/ tissues	Human cells	*In vitro* model	Leber congenital amaurosis
	Human splicing mechanisms	Incomplete cell morphogenesis *e.g.* outer segment	Retinitis pigmentosa
	Chemical screens	Long culturing time Cost	
Zebrafish (*Danio rerio*)	Fast development	Evolutionary distance from humans	Bardet–Biedl syndrome
	Relatively low cost	Redundancy between large numbers of paralogues	Joubert syndrome
	High-throughput genetic/chemical screens		
	Cone-mediated vision		
Caenorhabditis elegans	Extremely rapid development	Evolutionary distance from humans	Bardet–Biedl syndrome
	Ease of genetic manipulation	Biochemical studies are challenging	Cone–rod dystrophy
	Routine live microscopy		
	LOF mutants available for most genes		

[a]iPSC: induced pluripotent stem cells; LOF: loss-of-function.

cell types, CEP290 levels and ciliation were reduced. However, the authors demonstrated the highest levels of aberrant splicing and cilia defects in the optic cups, suggesting a cell-specific regulation of CEP290 expression that explains the retina-specific phenotype of LCA10. Shimada *et al.*[45] reported that fibroblasts of *CEP290*-LCA patients had reduced CEP290 protein levels, but ciliogenesis and cilia length were normal. However, in agreement with Parfitt *et al.,* the photoreceptors in iPSC-derived optic cups showed cilia defects, thereby supporting a tissue-specific requirement for CEP290 in cilia development.[45]

X-linked retinitis pigmentosa (XLRP) is predominantly caused by mutations in *RPGR* and *RP2*.[46] Both proteins are associated with cilia function, but their roles in the molecular pathologies of XLRP remain elusive. A recent study using iPSC-derived optic cups from individuals carrying *RPGR* mutations identified RPGR as a gelsolin-interacting protein.[47] Gelsolin regulates actin disassembly in the connecting cilium, which is an essential process

for rhodopsin transport to photoreceptor outer segments. The *RPGR* mutations disturb this interaction. In support of the iPSC data, RPGR and gelsolin knockout mice show increased actin polymerisation and abnormal rhodopsin localisation. Hence, this iPSC study revealed insights into a novel molecular pathway affected by a disease-specific *RPGR* mutation.

RP2 localises to the ciliary base and is a GTPase activating protein for ARL3, indicating a role in ciliary trafficking.[48,49] Schwarz *et al.*[50] characterised fibroblasts and iPSC-derived RPE cells from a patient with a nonsense *RP2* mutation. Depletion of RP2 led to impaired IFT20 (intraflagellar transport 20) localisation, GB1 (transducin β-subunit) trafficking and Golgi cohesion; however, cilia incidence was unaffected. Interestingly, translational read-through-inducing drugs (TRIDs) rescue the phenotypes in both fibroblasts and iPSC-derived RPE, restoring up to 20% of endogenous RP2 protein. This was validated in a second study, wherein patient iPSCs were differentiated into optic cups.[51] Again, the *RP2* mutation did not reduce cilia number, but the cilia had reduced Kif7 at their tips, which was restored by treatment with TRIDs. In summary, both proof-of-concept studies demonstrate the potential of TRIDs as therapeutics for *RP2* nonsense mutations.

9.3.3 Future Prospects

For retinal organoids to prosper as *in vitro* models of retinal ciliopathies, it will be necessary to develop techniques allowing the development of morphologically normal and functionally intact outer segments. Co-incubation with RPE may help, as in neonatal retina cultures the RPE is needed for proper alignment of photoreceptors.[52] Wahlin *et al.*[42] proposed that the RPE is not essential, since they achieved outer segment with nearly normal length without direct RPE-photoreceptor contact. However, as the resulting outer segment developed only a few disorganised discs, the RPE may be necessary to support correct membrane architecture and long-term stability. Also of major importance is the use of appropriate controls. Patient-specific mutations can be corrected using gene-editing tools such as CRISPR/Cas9. These isogenic controls represent better controls than related healthy donors, whose different phenotypes may arise from genetic differences.[34] Furthermore, improvements in reprogramming and differentiation methods will reduce variability, time and costs, increasing the amenability of iPSC-derived retinal cells for high-throughput screening and personalised medicine.

9.4 Zebrafish

9.4.1 Introduction

Zebrafish (*Danio rerio*) is a widely used model in developmental biology and biomedical research, popular due to its advantages as a vertebrate with very rapid initial development, a relatively short life-cycle, large brood sizes and

lower maintenance costs relative to mammals. The genetic toolbox of zebrafish includes germline chemical mutagenesis screens, transient morpholino knockdowns, Tol2 transposase mediated transgenesis and CRISPR/Cas9 gene editing. In vision research, zebrafish possess specific advantages over rodent models, most notably a retina layered with equivalent cell types to humans, in which photoreceptors are enriched to ~75% cones, and cone-mediated vision developing at 3–5 days post-fertilisation.[53] Like humans, zebrafish are primed for cone-mediated, photopic vision, in contrast to mice, which have evolved to primarily utilise rod-mediated, scotopic vision. In addition, it is relatively simple to measure visual function in zebrafish larvae using behavioural assays such as the optokinetic[54] and visual-motor response[55] assays.

Caution must be taken when interpreting data from zebrafish models of human disease as zebrafish are more evolutionarily distant from humans than rodents and rodent models themselves do not always accurately model human pathologies. Human phenotypes or syndromes are not always recapitulated in zebrafish models with mutations in the same gene. For example, mutations in *CC2D2A* cause Joubert and Meckel–Gruber syndromes, presenting with cerebellar ataxia, hypotonia and renal cysts, in addition to other developmental disorders. However, the zebrafish *sentinel* mutant, in which the *CC2D2A* orthologue is mutated, displays additional retinal degeneration, only seen in some *CC2D2A* patients.[56] This is possibly due to species differences in gene redundancy and/or the cell-specific roles of such genes.

A significant advantage of zebrafish is the ability to perform low- to high-throughput screens for novel compounds that rescue macro phenotypes. Such phenotype-based screens are molecular target agnostic, and with appropriate models they provide a powerful approach to drug discovery.[57] In such chemical screens the endpoint readouts can be suppression of a developmental phenotype with disease relevance (*e.g.* angiogenesis[58]) or rescue of a disease phenotype.[59] Phenotypes may be morphological, physiological or behavioural. Screening opportunities are enhanced by the development of high-throughput imaging pipelines for zebrafish.[60] One such screen for chemical modifiers of polycystic kidney disease, a common ciliopathy, identified histone deacetylase inhibitors as suppressors of the zebrafish *pkd2* mutant phenotype.[61]

Caveats associated with screens when randomised chemical libraries are applied include a lack of knowledge on the drug target, which may hinder further drug development. The hit rate of chemical screens in zebrafish has varied from as low as 0.02% to as high as 70%,[62] reflecting variability in methodology, number of compounds screened and the genetic background of zebrafish stocks used. These factors must be taken into careful consideration when designing a chemical screen. Care must also be taken when selecting a chemical library, which can introduce bias into the screen, as not all libraries are equally representative of the various classes of compound. A failure of a screen to uncover novel compounds could be due to under-sampling or sampling of the wrong chemical space for the biological

process of interest. With the number of test drugs in the order of millions, it is impractical to perform a screen which samples the entirety of chemical space and so biologically active compounds with therapeutic potential will be missed.

9.4.2 Zebrafish Ciliopathy Models

As mentioned previously, one explanation for patients with mutations in the same gene displaying either syndromic or non-syndromic retinal degeneration could be cell- or tissue-specific isoforms of these genes. The importance of these isoforms is illustrated by work from Pretorius *et al.*,[63] who reported a retina-specific isoform of BBS3 (ARL6) that included an extra 13 bp exon between exons 6 and 7 (BBS3L). Morpholino knockdown of this isoform in zebrafish did not produce the classic BBS developmental phenotypes seen in zebrafish; however, it did result in decreased visual response and mislocalisation of green cone opsin. Interestingly, while the visual defects could be rescued by reintroduction of the BBS3L transcript, the "normal" isoform transcript could not. This indicates that the long isoform is necessary for normal visual function and opsin trafficking, and suggests that gene therapy to restore vision in BBS3 patients would need to include this splice-variant.

Zebrafish have also been used to uncover genetic modifiers of ciliopathies. The *sentinel* (*cc2d2a*) mutant displays shortened outer segment and opsin mislocalisation which is enhanced by knockdown of the gene *ninl*.[10] Both CC2D2A and NINL localise to the ciliary base in humans and zebrafish, where they regulate vesicle docking. Interestingly, the authors identified one patient with a *CC2D2A* mutation causing Joubert syndrome and a heterozygous mutation in *NINL*. The patient manifested a more severe form of Joubert syndrome, which included retinal degeneration and hearing loss, supporting *NINL* as a modifier of *CC2D2A*-associated Joubert syndrome, and that such interactions can be modelled in zebrafish.

The potential to uncover small-molecule therapeutics resolving ciliopathies is demonstrated by Jin *et al.*[64] who used zebrafish *leakytail* (*lkt*), a mutant of the ATP-binding cassette transporter ABCC4, which displays ciliogenesis defects. The authors linked these ciliary defects to reduced prostaglandin E_2 (PGE_2) signalling *via* the cilium-localised EP_4 receptor, the first time prostaglandin signalling was linked to ciliogenesis. Specifically, reduced PGE_2 secretion in the *lkt* mutant led to reduced cilium length in several tissues and consequent aberrant organogenesis. Pharmacological treatment of *lkt* fish with PGE_2 resulted in a partial rescue of ciliogenesis and associated morphological defects by increasing the rate of anterograde IFT. This innovative study uncovered drugs that promote PGE_2 secretion or signalling as candidate therapeutics to treat ciliopathies and holds promise that additional drugs can be identified in other models.

9.4.3 Future Prospects

CRISPR technology has revolutionised the genetic toolkit of zebrafish, allowing increasingly precise and complex genetic manipulations to be performed. For this reason zebrafish will continue to be a powerful model for investigating ciliary, retinal and other diseases. The feasibility of the aforementioned chemical screens also makes zebrafish an excellent model for the discovery of novel compounds with therapeutic potential in ciliopathies and retinal degeneration.

9.5 Caenorhabditis elegans

9.5.1 Introduction

Rapid development, feasibility of live microscopy and a wide array of genetic tools make *Caenorhabditis elegans* nematodes a powerful model for investigating the basic cell biology and molecular pathways underlying human disease. One of the greatest advantages of *C. elegans* is the ability to perform forward genetic screens with relative ease.[65] Such screens uncover novel genes and/or novel phenotypes of known genes in an unbiased fashion and have provided loss-of-function alleles of almost every *C. elegans* gene. Furthermore, forward genetic screens can generate hypomorphic alleles in which only part of the encoded protein is disrupted, allowing the investigation of domain-specific functions, and genes normally essential for life. The transparency of *C. elegans* facilitates microscopy, which when combined with transgenic fluorescent reporters allows for live imaging of protein trafficking and dynamics *in vivo*. Many of the genes underlying mammalian cilium biogenesis, function and disease are conserved in *C. elegans*,[66] allowing the nematode to serve as a general model of ciliary disease and provide insights into molecular pathomechanisms relevant to photoreceptor cilium dysfunction.

C. elegans possesses 60 ciliated cells, all sensory neurons which collectively mediate chemo-, osmo-, thermo- and mechanosensation.[67] Most cilia are contained within discrete environmentally exposed pores called sensilla, which are formed from the extended processes of glia-like support cells that wrap around the ciliated endings of the sensory neurons. *C. elegans* cilia come in many different shapes and sizes, ranging from rod-shape structures of varying lengths to elaborate multi-branched structures with highly expanded membranes.[66] Although nematode cilia broadly resemble those of their vertebrate counterparts, there are some notable differences, such as the absence of a stereotypical basal body, which partially degrades and remodels post-ciliogenesis. Given that all *C. elegans* cilia are on sensory neurons, it is interesting to note that many possess features reminiscent of vertebrate photoreceptors, such as expanded membranes and axonemal A-tubule extensions.[68]

9.5.2 *C. elegans* Ciliopathy Models

A number of genes associated with retinal dystrophies were first linked to cilia in *C. elegans*, with prominent first examples being the Bardet–Biedl syndrome genes BBS8,[69] BBS7 [70] and ARL6 (BBS3).[71] Each of the BBS genes is exclusively expressed in the ciliated neurons of *C. elegans*, where they localise to the cilium and undergo IFT.[69] For these and other ciliopathy gene orthologues, *C. elegans* has been instrumental in dissecting their roles in regulating ciliary cargo sorting and providing a model for understanding the functions of these genes in photoreceptor biology and disease. For example, research in *C. elegans* has provided valuable insight into the roles of many ciliopathy proteins at the transition zone by uncovering a molecular hierarchy by which these proteins assemble into functional modules to regulate transition zone formation and diffusion barriers to ciliary protein entry and exit.[72]

The power of the forward genetics approach in *C. elegans* is illustrated by early screens for sensory mutants,[73] which generated large numbers of *che* (chemotaxis defective) and *osm* (osmotic-avoidance defective) strains, many of which were later discovered to harbour mutations in important ciliary genes. More recently, screens have been used to generate models in which ciliary modules such as the BBSome are disrupted, but cilia are otherwise normal. Wei *et al.* screened for ciliary mutants where IFT was compromised, but cilia length was relatively unaffected.[74] One such mutant generated in this screen was a G361R substitution within the WD40 domain of DYF-2, the worm orthologue of human IFT144. DYF-2 is normally required for IFT; however, the hypomorphic allele generated in this study showed only severely reduced retrograde IFT and a failure of the BBSome to enter cilia. Such an allele provides a unique opportunity to investigate the roles of the BBSome and retrograde IFT in ciliary trafficking.

Additionally, *C. elegans* can be used to investigate cilia genes linked exclusively to retinal phenotypes in humans. Several studies have shown mutations in RAB28, a Rab small GTPase, cause cone–rod dystrophy.[75–77] Subsequent work in *C. elegans* demonstrated that the worm orthologue, RAB-28, is expressed exclusively in ciliated cells, localises to the periciliary membrane and undergoes bidirectional IFT in a BBSome-dependent manner.[78] Functionally, RAB-28 regulates sensory pore formation, possibly by regulating signalling between the ciliated neurons and their glial cells. Assuming conservation of function between *C. elegans* and mammals, this raises the possibility that photoreceptor cilia, in addition to their role in phototransduction are involved in signalling to other nearby cells such as other photoreceptors or the RPE.

Again, as with all non-human models, care must be taken when extrapolating from *C. elegans* to humans. It is tempting to draw parallels between the distal segments of *C. elegans* cilia, where the microtubules extend as singlets and is thought to act as a specialised subcompartment for receptors, and the modified distal portions of some vertebrate cilia, such as those

found in photoreceptors and olfactory neurons. In *C. elegans,* anterograde transport along the distal segment is performed exclusively by one of the two anterograde IFT kinesins, OSM-3.[79] However, the role of KIF17, the vertebrate orthologue of OSM-3, in photoreceptors remains controversial. While morpholino knockdown of *Kif17* results in severe outer segment length reduction,[80] knockout models display modest or no outer segment defects.[81,82]

9.5.3 Future Prospects

C. elegans is an excellent model for performing molecular genetic and cell biological studies, including those of cilia formation and function. As such, it is also a powerful model for dissecting the molecular basis of cilium-associated retinal degeneration. Again, CRISPR has enhanced the genetic tractability of *C. elegans*, making possible models with patient alleles and tagged endogenous genes where gene dosage can be more carefully controlled.

9.6 Conclusion

While cilia are remarkably well conserved organelles with the same pathways underlying their formation and function in organisms as diverse as single-celled algae, nematode worms and mammals, a number of important species differences exist, even between relatively closely related species such as mice and humans (see Table 9.2). Such differences are potential pitfalls to investigators using these species to model human disease and uncover druggable targets and therapeutic strategies. A holistic view which combines findings from non-human and "human" models such as iPSCs is therefore the best approach to uncovering the common processes which form the molecular basis of ciliopathies and retinal degeneration.

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