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Focus on Molecules: RPE65, the visual cycle retinol isomerase

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1. Structure

The *RPE65* gene (UniProtKB/Swiss-Prot: Q16518 (RPE65_Human); OMIM: 180069; <http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=180069>) is localized to human chromosome 1p31 and to distal mouse chromosome 3 and is a single copy gene, except in zebrafish where there are 2 copies, a consequence of the genome duplication in the teleost ancestry. RPE65 protein is highly preferentially expressed in retinal pigment epithelium (RPE) of all vertebrates (Fig. 1A), and may be expressed at low level in cone photoreceptors of some species. All RPE65 proteins are 533 amino acid residues in length, except in zebrafish where *RPE65a* gene product has 531 and *RPE65b* gene product 532 residues, respectively. RPE65 belongs to the evolutionarily diverse carotenoid oxygenase superfamily. The basic reaction catalyzed by these enzymes is oxidative cleavage of carbon-carbon double bonds in the polyene backbone of carotenoids and related molecules. One of RPE65's 2 mammalian relatives is β -carotene monooxygenase, which cleaves β -carotene into all-*trans* retinal, the first step in animal vitamin A metabolism. The crystal structure of cyanobacterial *Synechocystis* apocarotenal oxygenase, a carotenoid oxygenase, predicts that these proteins share a common seven-bladed propeller structure containing non-heme ferrous iron coordinated by four histidines, three of which are fixed by glutamate residues. Each blade of the propeller, consisting of 4 or 5 anti-parallel beta-sheets, contributes one part of the iron coordination apparatus (Fig. 1B). The locations of these beta sheets are conserved and these contribute to a rigid structure. Inter-strand and inter-blade loops are less conserved and are predicted to contribute to specificity of substrate binding in the different family members. RPE65 is predicted to share the overall secondary structure required to support this tertiary structure and mutagenesis studies support the requirement of the coordinating histidines in RPE65 activity (Redmond *et al.*, 2005).

2. Function

RPE65 is the all-*trans*: 11-*cis* retinol isomerase of the visual cycle (Redmond *et al.*, 2005; Travis *et al.*, 2007). George Wald first elucidated the visual cycle over 70 years ago, but it was not until 1987 that RPE vitamin A isomerization was proven to be an enzymatic process. The

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cDNA sequence for the RPE65 protein was published in 1993 though its function was then a matter of speculation. In 1998, targeted disruption of the mouse *Rpe65* gene showed that RPE65 is necessary for 11-*cis* retinoid production in the visual cycle (Redmond *et al.*, 1998). These mice have a slow retinal degeneration but severely reduced light sensitivity due to lack of rhodopsin, though apoprotein opsin is present, and excess RPE retinyl ester. Thus, the biochemical phenotype of the *Rpe65* knockout mouse balances extreme chromophore starvation (no 11-*cis* retinal) in the photoreceptor outer segments against hypervitaminosis A (all-*trans* retinyl ester over-accumulation) in the RPE, implicating blockade of isomerization as the primary effect of RPE65 loss. The total absence of isomerase activity suggested that RPE65 was either the isomerase itself or essential for a hypothetical multicomponent isomerase complex (Redmond *et al.*, 1998). The *Rpe65*^{-/-} model has been of great utility in understanding retinal physiology and biochemistry caused by or related to variations in chromophore status. There is also a spontaneously arisen C57Bl/6 mouse mutant called *rd12* or *Rpe65*^{rd12} caused by a nonsense mutation; it is a presumptive null mutant with phenotype similar to that of the engineered knockout. An *Rpe65* R91W knockin mouse mutant has also been published with a milder phenotype due to the low levels of 11-*cis* retinal generated (5% of wildtype). Besides these, there is a Swedish Briard dog model, due to a 4 bp deletion in the dog *RPE65* gene; this is another presumptive null mutation. This has been of great value in developing methods for gene therapy of *RPE65* mutations.

Though the *Rpe65* knockout phenotype (Redmond *et al.*, 1998) provided *prima facie* evidence that RPE65 was the isomerase, it did not provide irrefutable evidence. In 2005, three groups showed that RPE65 is the retinol isomerase of the visual cycle (Redmond *et al.*, 2005; Travis *et al.*, 2007). One group used a “non-biased” expression cloning approach to identify the isomerase by complementation of a visual cycle assay system. Each of the other groups used a candidate approach to accomplish reconstitution of a heterologous visual cycle system, using either adenoviral constructs or plasmid constructs to transfect mammalian cells. Having established that RPE65 was required for all-*trans* retinol to 11-*cis* retinol isomerization to occur in transfected cells, each of these groups provided a variety of experiments to back up their primary findings. For example, the conserved iron-coordinating histidines were shown to be essential for RPE65 activity.

3. Disease involvement

Mutations in the human gene for *RPE65* are associated with autosomal recessive severe early onset retinal dystrophy variously described as Leber congenital amaurosis type 2 (LCA2; OMIM: 204100; <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=204100>), early-onset severe retinal degeneration (EOSRD), or autosomal recessive childhood-onset severe retinal dystrophy (arCSRD) (Thompson *et al.*, 2000). The spectrum of conditions described ranges from more severe early onset rapidly-progressing entities to milder later onset slowly-progressing entities. Over 60 different pathogenic mutations of *RPE65* are known (<http://www.retina-international.org/sci-news/rpe65mut.htm>), and are spread over all 14 exons of the gene and their boundaries (Fig. 1C). Severity and age of onset of disease are related to the particular type of mutation and the residue/site affected. The primary features of LCA2/EOSRD/arCSRD are profound visual deficit, severely reduced or extinguished electroretinogram at birth or earliest testing, and nystagmus. Though the fundus appears relatively normal by ophthalmoscopy early in the course of disease, a key finding, important in differential diagnosis, is low or no fundus autofluorescence, due to absence of fluorescent bisretinoid lipofuscin byproducts of the visual cycle (e.g., A2E). The severe rod functional deficit is evident by night-blindness. Cone deficits are also severe, but more variable than rod, especially in patients with less severe missense mutations. Progression may occur in the less severe phenotypes that are often diagnosed as autosomal recessive retinitis pigmentosa. Because of these features, the phenotypic spectrum of human cases is wider than seen in animal

models. The canine *RPE65* dystrophy served as an important preclinical model to reverse blindness by the use of somatic gene therapy. Most recently, separate clinical trials to treat human *RPE65* LCA2 by somatic gene therapy with adeno-associated virus vector carrying *RPE65* have begun. Early results have shown a modest improvement in visual acuity, measured by a variety of behavioral, psychophysical and electrophysiological outcomes, in several severely affected patients.

4. Future Studies

Crystallization of RPE65 will provide a more precise structural model for RPE65 to enhance our understanding of its enzymatic mechanism and of its sensitivity to mutation. This may also help identify candidates for small molecule therapy of retinal dystrophy due to *RPE65* mutations. With gene therapy trials already under way, the main goal of these trials must focus on improving delivery of vector and on maximizing outcome benefits, and on identifying and treating the patients at a time and stage of progression of the disease where therapeutic benefit is maximized. This is generally considered to be as early as possible after molecular genetic diagnosis. As there is a wide spectrum of severity in human *RPE65* dystrophy, depending on the combination of null and/or missense mutations (Thompson *et al.*, 2000), development of further mouse missense *Rpe65* knockins will complement what we have learned from the mostly null disease models.

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Fig. 1. RPE65 localization, RPE65 structural model and pathogenic mutations in *RPE65* gene. A: RPE65 is highly preferentially localized to the retinal pigment epithelium by anti-RPE65 antibody staining (red) of mouse retina frozen section. Nuclei are visualized with DAPI (blue). GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OSL, outer segment layer; RPE, retinal pigment epithelium; and Ch/Sc, choroid and sclera. B: Each blade of RPE65 structure contributes one part of its catalytic iron coordination apparatus. SwissModel DeepView structural model of RPE65 iron-coordinating residues, based on ACO predicted structure, and showing crucial histidines and glutamates. C: Pathogenic mutations causing *RPE65* retinal dystrophy (LCA2/EOSRD/arCSRD) are found in all exons of the *RPE65* gene.