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Vision Research 43 (2003) 3095–3108

Vision Research

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# Inherited multifocal RPE-diseases: mechanisms for local dysfunction in global retinoid cycle gene defects

Dorothea Besch *, Herbert Jägle, Hendrik P.N. Scholl, Mathias W. Seeliger, Eberhart Zrenner

*University Eye Hospital, Schleichstr. 12-16, D-72076 Tübingen, Germany*

Received 3 July 2003; received in revised form 6 August 2003

**Abstract**

Alterations of retinoid cycle genes are known to cause retinal diseases characterized by focal white dot fundus lesions. Fundus appearances reveal circumscribed RPE-changes, although generalized metabolic defects and global functional abnormalities are present. As a possible explanation, topographic inhomogeneities of the human photoreceptor mosaic and the role of a cone specific visual cycle will be discussed. Due to particular characteristics of photoreceptor subtypes as well as different pathways for photopigment regeneration the metabolic demand of individual RPE cells might differ. In "flecked retina diseases" heterogeneity of metabolic demand in individual RPE cells could therefore be responsible for their multifocal appearance.


*Keywords:* Visual cycle; Gene; Retinoid; Retinal pigment epithelium; Retinal disease

## 1. Introduction

The term "flecked retina syndrome" was applied by Krill and Klien (1965) to several conditions in which the fundus is characterized by retinal distribution of multiple, deep yellowish-white spots of variable size and configuration without vascular or optic nerve abnormalities. The term "flecked retina diseases" subsequently was discarded in clinical practise due to the heterogeneity of diseases with flecked fundus appearance. On the other hand development in molecular genetics shed new light on this group of diseases and it is still puzzling why globally expressed genetic alterations lead to regular multifocal patterns. Focal and multifocal disorders caused by alterations of retinoid cycle genes expressed in the retinal pigment epithelium (RPE) such as Stargardt disease/fundus flavimaculatus (ABCA4 gene), the retinol binding protein deficiency syndrome (RBP4 gene), fundus albipunctatus (11-*cis* RDH gene) and retinitis punctata albescens (RLBP1 gene) will be discussed in relation to their genotype and phenotype. Subsequently an attempt will be made to illustrate possible mechanisms that may contribute to the particular multifocal flecked appearance.

## 2. Hereditary focal RPE-diseases due to mutations in the retinoid cycle genes

### 2.1. Stargardt disease/fundus flavimaculatus

Stargardt macular dystrophy including fundus flavimaculatus is the most frequent cause of monogenetically determined macular degeneration and accounts for 7% of all retinal dystrophies. The prevalence is estimated between 1 in 8000 and 1 in 10,000.

Karl Stargardt (1909) first described the disease as a unique macular dystrophy characterized by visual loss occurring early in life in combination with an atrophic lesion of the macula and yellowish extramacular spots, termed flavimaculatus flecks (Franceschetti, 1963; Weleber, 1994) (Fig. 1). Whereas autosomal-recessive Stargardt disease (STGD1) with flavimaculatus flecks is typically marked by juvenile to young adult age of onset, the clinically similar retinal disorder, fundus flavimaculatus, often displays later age of onset and slower progression. Pure fundus flavimaculatus denotes a retinal dystrophy characterized by yellowish round, oval,

* Corresponding author. Tel.: +49-7071-298-4786; fax: +49-7071-29-53-61.

*E-mail address:* dorothea.besch@med.uni-tuebingen.de (D. Besch).

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doi:10.1016/j.visres.2003.09.020

Fig. 1. Stargardt disease with fundus flavimaculatus. Fundus of a right eye. Macula with RPE changes surrounded by yellowish round or pisciform flecks.

commalike or pisciform flecks at the level of the RPE distributed over the posterior pole, sometimes with extension to the equator (Franceschetti & Francois, 1965). The dots may be confluent or separate (Fig. 2). In some forms of Stargardt disease the flecks are not always distributed over the entire posterior pole as has been described for fundus flavimaculatus. Furthermore, over the years flecks might change appearance and increase in number. The flecks, initially yellowish and sharply outlined, become gray and less defined and, finally, disintegrate, leaving an atrophic looking RPE (Fishman, 1976). Hadden and Gass (1976) presented evidence that fundus flavimaculatus and Stargardt disease are the same form of macular dystrophy. Recently, it has been shown by linkage analysis that the two phenotypes represent the same disorder (Anderson et al., 1995).

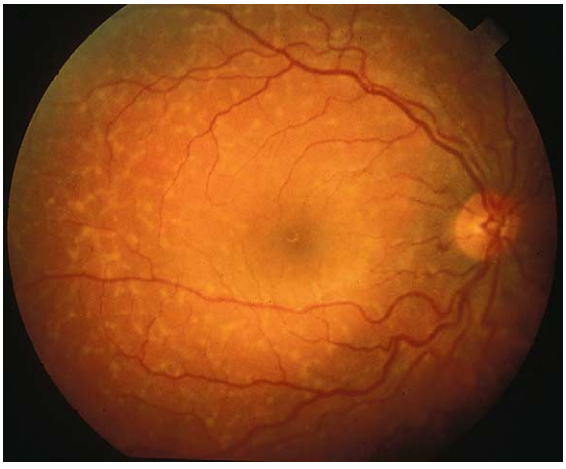

Fig. 2. Fundus flavimaculatus. Between the macula and the equator, multiple yellowish flecks, confluent and separate, are present on the level of the RPE.

Autosomal recessive Stargardt disease (STGD1) has been associated with mutations in the *ABCA4 (ABCR)* gene, which maps to 1p21-p13 (Allikmets et al., 1997; Azarian & Travis, 1997). Its product, the photoreceptor ABC transporter (ABCR) protein is involved in the visual cycle and was shown to be expressed both in rod outer segments and in foveal and peripheral cones (Molday, Rabin, & Molday, 2000; Sun & Nathans, 1997). Recently, abcr knockout mice have provided an important insight into the biochemical function of the transporter in rod outer segments and into the mechanism of photoreceptor cell death (Weng et al., 1999): The *ABCA4* protein is localized to the rims of rod outer segment disks where it acts as an adenosine triphosphate (ATP)-dependent flippase for *N*-retinylidine-phosphatidylethanolamine (*N*-retinylidine-PE) (Sun, Molday, & Nathans, 1999). ABCR is considered to be responsible for translocating *N*-retinylidine-PE across the disk membrane from the disk lumen into the cytoplasm. After translocation, *N*-retinylidine-PE is hydrolyzed to release all-*trans*-retinal, which is then isomerized to all-*trans*-retinol by retinol dehydrogenase in the photoreceptor outer segment. All-*trans*-retinol is then transported by carrier proteins to the RPE where it is enzymatically converted to 11-*cis*-retinal and returned to the photoreceptors (Fig. 3) (Glazer & Dryja, 2002). In the absence of functional ABCR, all-*trans*-retinal and *N*-retinylidine-PE accumulates within the outer segment disks (Radu et al., 2003). The excess *N*-retinylidine-PE contained within the phagocytosed outer segment disks undergoes enzymatic digestion to form *N*-retinylidine-*N*-retinylethanolamine (A2-E), a major component of lipofuscin (Glazer & Dryja, 2002; Mata, Weng, & Travis, 2000). The accumulation of abnormally high levels of lipofuscin, common as multifocal flecks in Stargardt's disease (STGD1), is thought ultimately to kill the RPE cell (Eldred & Lasky, 1993; Sun & Nathans, 1997). ABCR-mediated focal retinal degeneration in patients may therefore result from 'poisoning' of the RPE due to A2-E accumulation, with secondary photoreceptor degeneration due to loss of the ABCR support role (Clarke, Heon, & McInnes, 2000; Sparrow, 2003).

Recently Glazer and Dryja (2002) proposed a three-step explanation for the pathophysiology of Stargardt disease (STGD1): (1) Defective Rim protein, a protein encoded by the ABCA4 gene causes an accumulation of protonated *N*-retinyledine-PE in the rod outer segments. (2) Lipofuscin fluorophore (A2-E), a byproduct of *N*-retinyledine-PE then accumulates in the RPE cells and is toxic to them. (3) Photoreceptors eventually die secondary to loss of the RPE support function.

Histopathologic studies of the eyes in Stargardt disease show a great variability in the size and shape of RPE cells and a loss of melanin granules. The flavimaculatus flecks seem to be caused by the aggregation of large, hypopigmented RPE cells with massive accumulation in

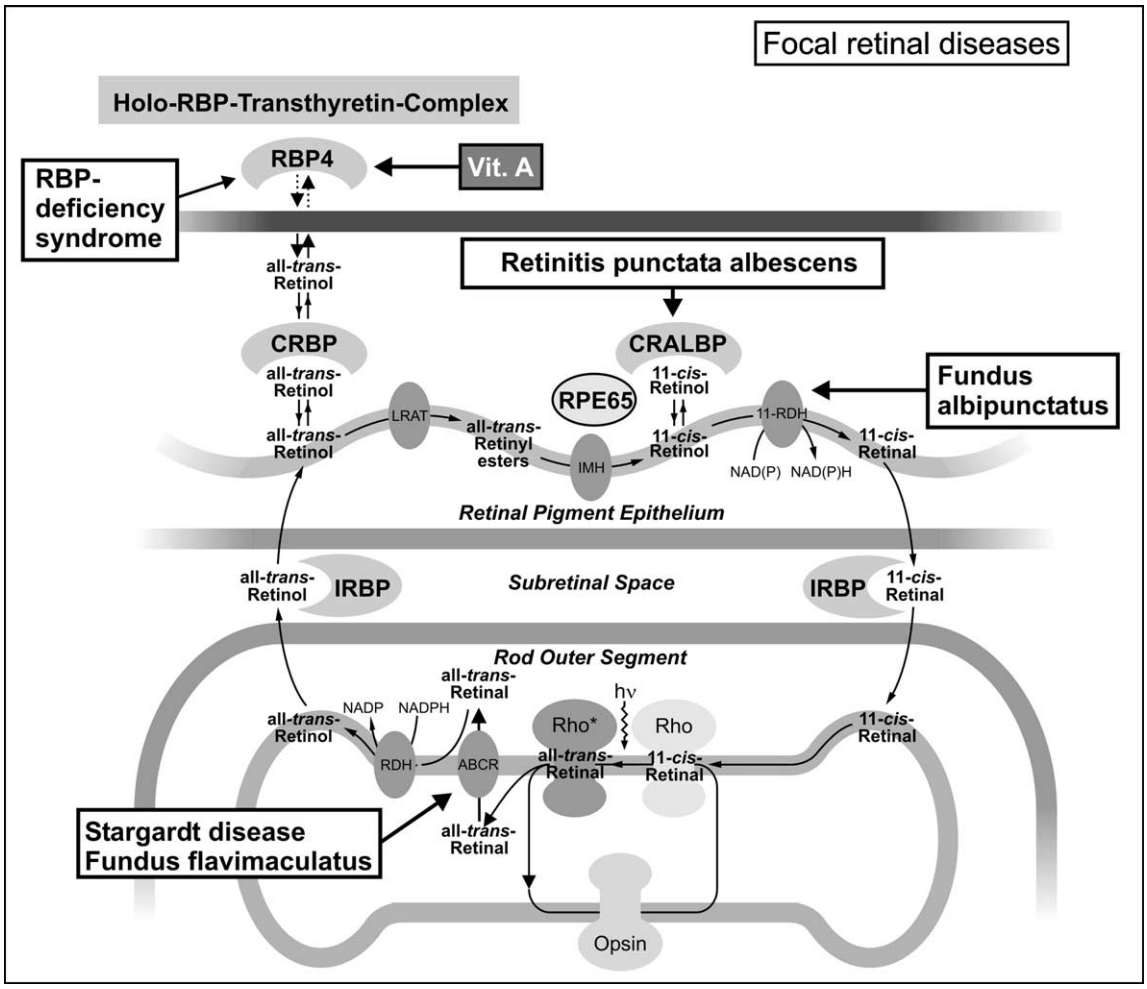


Fig. 3. The figure shows interacting components involved in the retinoid visual cycle, separated for the RPE-cell (top) and the rod outer segment (bottom). Hereditary focal retinal diseases caused by defects in genes encoding proteins of these components or one of their subunits are indicated by coloured frames. Abbreviations: Rhodopsin (Rho), ATP-binding cassette transporter, retina-specific (ABCR), retinol dehydrogenase (RDH), interphotoreceptor retinoid binding protein (IRBP), cellular retinoid binding protein (CRBP), retinol binding protein 4 (RBP4), lecithin retinol acyltransferase (LRAT), retinal pigment epithelium-specific protein, 65 KD (RPE65), isomerohydrolase (IMH), cellular retinaldehyde binding-protein (CRALBP), 11-retinol dehydrogenase (11-RDH). (Modified from J.C. Saari, 2001).

their outer parts of lysosomal material similar to lipofuscin (Eagle, Lucier, Bernardiono, & Yanoff, 1980). The authors concluded that the lack of melanin causes the pale appearance of the flecks, and that the accumulation of lipofuscin, by absorbing the blue light, leads to the characteristic dark (hypofluorescent) choroid (''silent choroid'') seen in fluorescein angiography (Fish, Grey, Sehmi, & Bird, 1981). Birnbach, Jarvelainen, Possin, and Milam (1994) additionally emphasized abnormal photoreceptor morphology and abnormal accumulation of lipofuscin in photoreceptor segments, secondary to RPE degeneration.

Although the fundus appearance reveals only focal RPE-changes, global deficits of both rod and cone function can be demonstrated by psychophysical and electrophysiological function testing. However, it is still discussed as to whether or not there is a correlation between funduscopically visible retinal changes and functional abnormalities in Stargardt disease and fundus flavimaculatus (Armstrong, Meyer, Xu, & Elfervig, 1998; Fishman, 1976; Itabashi et al., 1993; Niemeyer & Demant, 1983; Noble & Carr, 1979; Stavrou, Good, Misson, & Kritzinger, 1998).

Fishman, Farbman, and Alexander (1991) observed a prolongation in rod dark adaptation in twelve patients with Stargardt dystrophy. This observation was apparent in patients with limited fundus flecks and those with extensive fundus flecks. Jaegle, Besch, Apfelstedt-Sylla, Tornow, and Sharpe (1999) demonstrated that even in very early stages of Stargardt disease, a prolonged time course of rod recovery with elevated final threshold can be observed, whereas cone threshold recovery was normal in most patients. This was more pronounced in fundus flavimaculatus or patients with multiple flecks. A prolongation in the time course of recovery of sensitivity following exposure to light has also been shown by

Birch et al. (2001) in patients with Stargardt disease having mutations in the ABCA4 gene. Additionally they reported reduced rod ERG responses and reduced and delayed cone responses in the ERG. Rod photoresponses to high intensity flashes were of reduced maximum amplitude but showed normal values of gain of phototransduction. Several other authors suggested that the electroretinogram (ERG) and the electro-oculogram (EOG) vary regularly with different stages of Stargardt disease. Starting with normal ERG and EOG responses, progression results in subnormal cone function and in later stages in reduced cone and rod function as well as abnormal electro-oculographic responses, when the RPE is widely affected (Fishman, 1976).

However, electrophysiological responses might also depend on the subtype of disease and the extent of RPE involvement. Stavrou et al. (1998) identified a reduction of the pattern ERG as the most consistent electrophysiological abnormality in Stargardt disease and fundus flavimaculatus. The authors found that ERG and EOG abnormalities occur more often in the presence of fundus flecks.

Lois, Holder, Fitzke, Plant, and Bird (1999) and Lois, Holder, Bunce, Fitzke, and Bird (2001) proposed that patients with Stargardt macular dystrophy-fundus flavimaculatus (SMD-FFM) may be classified into three subtypes based on generalized loss of either photopic (type 2) or photopic and scotopic (type 3) function additionally to early detectable pattern ERG abnormalities (type 1). Although phenotypic variation between 31 siblings from 15 families in age of onset, visual acuity and fundus appearance was observed, electrophysiological studies demonstrated relative intrafamilial homogeneity in retinal function (Lois et al., 1999). Furthermore, differences in scotopic or photopic function among groups could not be explained on the basis of fundus appearance (fleck density and distribution), differences in age of onset or duration of disease. These findings suggest that electrophysiological tests might have a prognostic value implicating that patients with early peripheral cone and rod involvement have a higher risk of development of peripheral visual loss and thus more severe disease but are not necessarily related to fundoscopic phenotype.

Scholl, Kremers, Vonthein, White, and Weber (2001) and Scholl, Besch, Vonthein, Weber, and Apfelstedt-Sylla (2002) investigated rod and cone function in patients with Stargardt disease and mutations in the ABCA4 gene. They found no correlation with fleck distribution and functional abnormalities, but a significant correlation of the phase changes to disease duration. The authors suggested that functional alterations of the L/M-cone-driven ERG pathways might be caused either by a change within the cones themselves, or result secondary to RPE alterations (Scholl et al., 2001). In a subgroup of 27 patients with mutations in both alleles of the ABCA4 gene, the amplitude of the scotopic 15-Hz flicker ERG of both the slow (rod bipolar-AII cell pathway) and the fast rod signals (rod-cone coupling pathway) were significantly reduced. These finding suggests that a defective ABCR transporter can functionally affect both the rod bipolar-AII cell pathway and the rod-cone coupling pathway. The authors concluded, that in STGD1, the scotopic 15-Hz flicker ERG reveals subtle abnormalities at different sites within the rod system that remain undetected by standard ERG techniques (Scholl et al., 2002).

### 2.2. Retinol binding protein deficiency syndrome

Seeliger et al. (1999) reported the ocular phenotype in retinol deficiency due to a hereditary defect in retinol-binding protein synthesis. Two affected sisters, aged 17 and 13 years, were compound heterozygous for missense mutations in the RBP4 gene, that had been linked to 10q23-q24 (Rocchi, Covone, Romeo, Faraonio, & Colantuoni, 1989). The RBP4 protein is the specific carrier for retinol in the blood (Fig. 3). RBP, which is produced in the liver and responsible for mobilizing liver stores of vitamin A, is largely restricted to the serum and accesses the basal surface of the RPE to release all-*trans*-retinol to the RPE. Neither affected siblings had detectable serum RBP, retinol levels were less than 20% of normal, but retinyl esters were within normal range. Clinically each affected sister had night vision problems since early childhood but was otherwise well. The fundi of both patients showed small yellowish-white dotlike lesions located in the retinal periphery (Fig. 4A) and a severe patchy atrophy of the retinal pigment epithelium, similar to severe nutritional vitamin A deficiency (Fig. 4B). Electrophysiological examination revealed that the EOG was more affected than the ERG indicating a primary impairment of the RPE without major systemic effects. Because retinol uptake in the RPE and transport to the photoreceptors were severely impaired, rod function in dark adaptation and scotopic ERG was not recordable. Dark adaptation thresholds were elevated, with the final threshold after 40 min still 1 log unit above normal cone threshold. A presumably secondary cone degeneration was more pronounced in the older sister. The authors concluded, that this phenotype of retinol binding protein deficiency provides evidence for an alternative tissue source of vitamin A, presumably retinyl esters from chylomicron remnants and that there might be other organ-specific RBP forms not affected by this genetic defect.

### 2.3. Fundus albipunctatus

In fundus albipunctatus, the flecks start as a ring around the macula, leaving this area unaffected, and spreading towards the equator. The dots are white, or

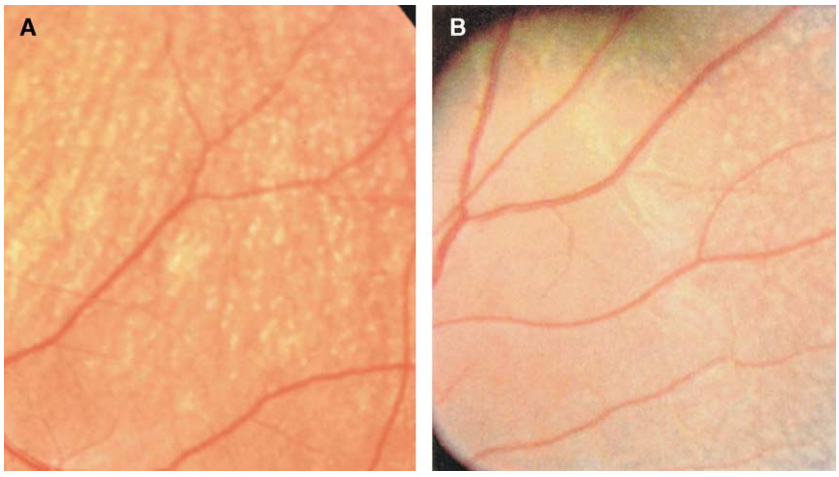


Fig. 4. Retinol binding protein deficiency syndrome showing yellowish-white dotlike lesions located in the retinal periphery, similar to severe nutritional vitamin A deficiency.

greyish white; distinct; and similarly sized (Fig. 5A). The fundus appears normal, except for the deposits, scattered in a radial pattern throughout the retina in both eyes. On fluorescein angiography, most of these dots are not seen or they block fluorescence. Surprisingly, multiple areas of retinal pigment epithelial changes, unrelated to the location of the albipunctatus spots can be found (Carr, Margolis, & Siegel, 1976; Marmor, 1977). The general ophthalmoscopic picture of the fundus and the functional abnormality usually remain stationary (Fig. 5B). There are also reports that the flecks did increase or decrease in number, or even disappeared over time without visual disturbances (Krill, 1977; Marmor, 1990). However, some authors provided evidence for an association of fundus albipunctatus with cone dystrophy (Miyake, Shiroyama, Sugita, Horiguchi, & Yagasaki, 1992; Wada, Abe, Sato, & Tamai, 2001).

Although fundus albipunctatus is characterized by discrete focal white dots, functional testing shows global rod dysfunction. In patients with night blindness, dark adaptation may be monophasic with cones determining threshold or may show a delay with a gradual increase in sensitivity so that eventually a normal or close to normal threshold is reached (Carr et al., 1976) (Fig. 6A). Recordings of the ERG and the EOG might be normal or abnormal. Abnormal ERG findings may represent similar changes as observed in congenital stationary night blindness or show an initial poor dark-adapted rod response that improves with prolonged dark adaptation (Nakamura & Miyake, 2002) (Fig. 6B).

Autosomal-recessive inherited fundus albipuncatus has been associated with mutations in the *RDH5* gene (chromosome 12q13-q14) which is involved in catalysing the conversion of 11-*cis*-retinol to 11-*cis*-retinal in the RPE, resulting in the loss of 11-*cis*-retinol dehydrogenase activity (Yamamoto et al., 1999; Gonzalez-Fernandez et al., 1999) (Fig. 3). The abnormally slow regeneration of visual pigments in the photoreceptors may result from malfunctioning pigment epithelial cells, the abnormal transportation of products between the RPE cell and the

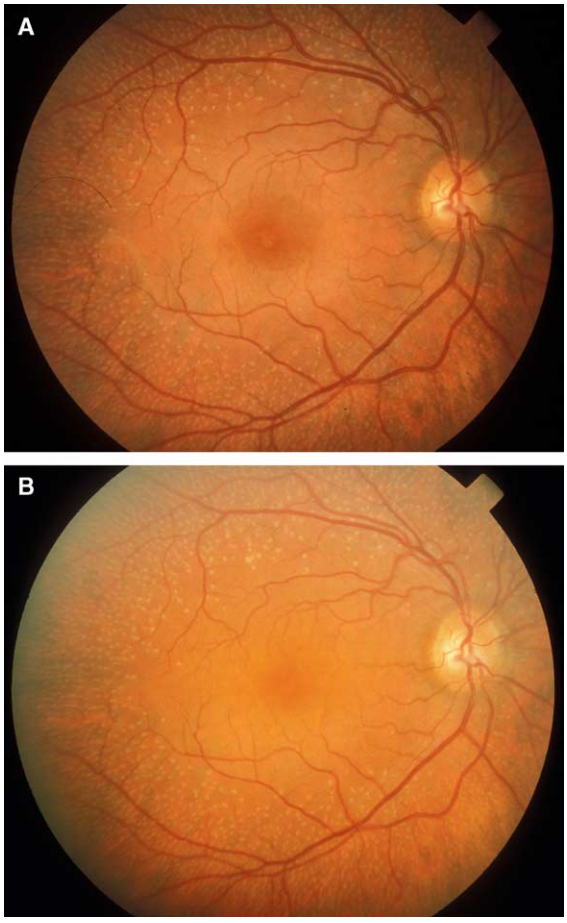


Fig. 5. Fundus albipunctatus. Fundus aspect of the right eye at the time of first examination showing distinct white-gray spots with the macular area unaffected (A) and 13 years later (B) in the same patient, staying essentially the same.

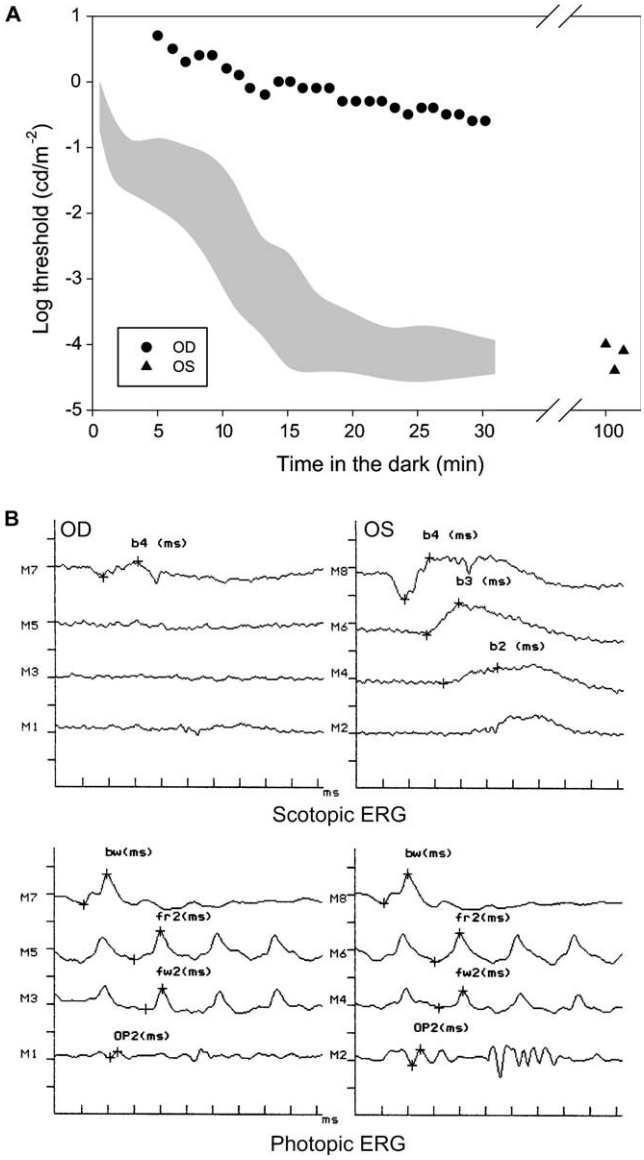


Fig. 6. (A) Dark adaptation curve of a patient with fundus albipunctatus. After 45 min of dark adaptation the threshold of the right eye (RE) remains about 4 log units above the normal rod threshold, whereas the final threshold is nearly normal after 115 min of dark adaptation in the left eye (LE). (B) Electroretinographic responses (ERG) in fundus albipunctatus. After 45 min of dark adaptation of the right eye (RE) no rod responses and only very small a- and b-waves can be recorded for the maximal combined response (ISCEV standard) shown on the left panel. The left eye (LE) shows nearly normal scotopic responses after 115 min of dark adaptation. (right panel): Except for reduced oscillatory potentials, especially in the RE, photopic responses according to the ISCEV standard are normal in both eyes.

photoreceptor or the anomalous utilization by the photoreceptor outer segment.

### 2.4. Retinitis punctata albescens

Retinitis punctata albescens is characterized by a progressive rod and cone degeneration with visual field loss, decrease of visual acuity and severe night blindness. In retinitis punctata albescens, most of the dots are distinct, many are confluent, and they may invade the macular area. A tapetal reflex occurring commonly in retinal dystrophies, is present. With time, bone spicule pigmentations might occur in the midperiphery. Finally, the spots fade and disappear and RPE atrophy, retinal pigmentary changes and attenuated blood vessels develop (Fig. 7). Although ophthalmoscopic appearance early in life may closely resemble fundus albipunctatus, functional abnormalities (ERG changes, visual field constriction) are similar to those of patients with progressive forms of retinitis pigmentosa.

Autosomal-recessive inherited retinitis punctata albescens has been associated with mutations in the *RLBP1* gene on chromosome 15q26 (Sparkes et al., 1992; Morimura, Berson, & Dryja, 1999). The Cellular retinaldehyde-binding protein (CRALBP) is not expressed in photoreceptors but is abundant in the RPE and Müller cells of the neuroretina, where it carries 11-*cis*-retinol and 11-*cis*-retinaldehyde. Transgenic mice lacking CRALB, although processing normal photosensitivity, have a reduction in 11-*cis*-retinal production and delayed dark adaptation (Gonzalez-Fernandez, 2002). Mutant CRALBP was found to lack the ability to bind 11-*cis*-retinaldehyde leading to an accumulation of all-*trans*-retinyl esters (Fig. 3).

Hawes et al. (2000) reported on a mouse model homozygous for rd6 to exhibit phenotypic similarities to human retinitis punctata albescens. The fundi of mice showed extensive, scattered, small white retinal dots that appeared by 8–10 weeks of age and persisted through advanced stages of retinal degeneration. Histologic examination revealed large cells in the subretinal space, typically juxtaposed to the retinal pigment epithelium. The white dots seen on fundus examination corresponded both in distribution and size to these large cells.

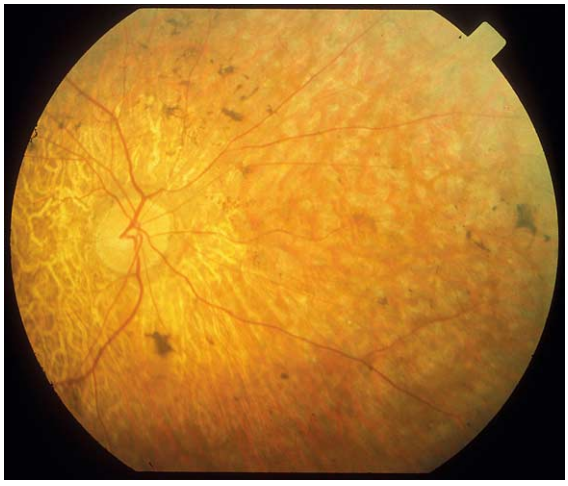

Fig. 7. Retinitis punctata albescens. Advanced stage. Dots are fade and confluent, invading the macular area. Bone spicule pigmentations, RPE atrophy, retinal pigmentary changes and attentuated blood vessels are present.

By 3 months of age, the cells were filled with membranous profiles, lipofuscin-like material, and pigment. Photoreceptor cells progressively degenerated with age, and an abnormal electroretinogram was initially detected between 1 and 2 months of age. Thus, rd6/rd6 mice may be a model for understanding the etiology of this or other flecked disorders.

## 3. How may generalized genetic and metabolic defects produce a localized damage of the photoreceptor-RPE-complex with focal or multifocal retinal lesions *("flecked retina diseases")*?

In retinal and RPE dystrophies, a wide heterogeneity of genotype and phenotype is found in conditions that produce focal and multifocal lesions. Both inter- and intrafamilial variations of the phenotype have been observed. It has been suggested that different mutations within the same gene could account for the wide phenotypic heterogeneity. However, patients with identical mutations or even affected siblings of the same family might reveal quite a variable distribution of fundus lesions and severity of functional impairment (Armstrong et al., 1998; Lois et al., 1999). In autosomal recessive disorders like Stargardt disease, siblings should have the same alleles; therefore the variation between siblings cannot be caused by different mutations or other sequence changes in the *ABCA4* gene. Differences between siblings might therefore indicate the influence of other "modifying genes" or exogenous factors (Lois et al., 1999). Additionally, in most cases, it is not possible to predict the pattern of functional loss based on fundus changes, age of onset, duration of the disease or even from the knowledge of a particular mutation. In the following, we would like to discuss several mechanisms that may underly local fundus lesions and we will deduct possible hypotheses from such factors.

## 4. Hypothesis 1. Random variations in the photoreceptor-RPE partnership

The function of the visual cycle depends on two separately developing structures ("partners"): the photoreceptor and the retinal pigment epithelium. Each RPE cell by random and dependent upon eccentricity supports simultaneously a certain number of cones and rods. An intact metabolism of the visual cycle may therefore depend on a properly balanced number of rod and cone photoreceptors per RPE cell.

### *4.1. Inhomogeneity of RPE cell shape and density in central and peripheral retina*

RPE cell genesis begins near the fovea, and proceeds towards the periphery. RPE genesis in the central retina differs from that in the peripheral retina in that it proceeds at a higher rate, and lasts for a shorter time period (Rapaport, Rakic, Yasamura, & LaVail, 1995). There is a difference in shape of the RPE cell changing with eccentricity: foveal RPE cells are thicker (14 μm) and narrowly spaced (10–14 μm), peripheral RPE cells are flatter and larger (60 μm) (Zinn & Benjamin-Henkind, 1979). Comparison of the pattern of expansion of the area containing radiolabelled cells in the RPE and neuroretina demonstrates a remarkable spatial and temporal correspondence (Rapaport et al., 1995). Snodderly, Sandstrom, Leung, Zucker, and Neuringer (2002) reported in rhesus monkeys that mean RPE cell density increased from a relatively stable baseline of approximately 4000 RPE cells/mm$^2$ beyond 2 mm (10°) eccentricity to more than 7000 cells/mm$^2$ at the centre of the fovea. As a consequence, gene-mediated retinal degeneration in patients may result in local RPE cell death and functional impairment due to inhomogeneity of RPE cell distribution.

### *4.2. Inhomogeneity of rod and cone ratio per RPE cell*

110,000,000–125,000,000 rods (average 80–100,000 rods/mm$^2$) and 6,400,000 cones are distributed over the entire retina (Osterberg, 1935). A circular field of approximately 6 mm diameter around the fovea is considered the central retina (Kolb, 1991). The central retina close to the fovea is considerably thicker than peripheral retina. This is due to the increased packing density of photoreceptors, particularly the cones, compared with peripheral retina. Approximately 200,000 cones (17,500 cones/degree$^2$) are found in the fovea. Cone density is highest in the fovea and falls rapidly outside the fovea to the peripheral retina (Curcio, Sloan, Packer, Hendrickson, & Kalina, 1987). In rhesus monkeys, the number of cones per unit area is maximal at the foveal centre and declines rapidly to approximately 50% within 0.1 mm (0.5°) eccentricity and approximately 20% at 0.5 mm (2.5°) eccentricity (Wikler, Williams, & Rakic, 1990). Data for the diameter of central rod free area vary between 250 μm (Ahnelt, Kolb, & Pflug, 1987) and 750 μm (Hendrickson & Youdelis, 1984). There is a peak of the rod photoreceptors in a ring around the fovea at about 4.5 mm or 18° from the foveal pit with a density of about 160,000 rods/mm$^2$ (Mariani, Kolb, & Nelson, 1984).

In addition, it is well established that rods are essential for cone integrity and survival. Mohand-Said et al. (1998, 2001) presented the observation that cone photoreceptors, even those seemingly unaffected by any described anomaly, die secondarily to rod loss related to mutations expressed specifically in the latter. Since the role of cones in visual perception is essential, identification of the factors mediating the interactions between rod degeneration and cone death will be important.

Furthermore, the photoreceptors are dependent on the underlying RPE cells for metabolic support. The RPE is an essential link in the vitamin A cycle that regenerates the visual pigments as well as being part of a system of breakdown and recycling of photoreceptor membrane components (Bok, 1985). Rods and cones have different relationships with the RPE cells. They differ in spatial patterns of outer segment renewal and anatomically in the depth of interdigitation between photoreceptor outer segments and RPE microvilli (Anderson & Fisher, 1979). Although rod-cone differences are still poorly understood, it is reasonable to expect that they could lead to topographic differences in the effects of disease.

According to Snodderly et al. (2002) the number of cones per RPE cell in the rhesus retina was approximately 20:1 in the foveal centre (similar to the human retina) and only approximately 1.5:1 in the parafovea (Fig. 8). However, if the rods are included, and the total number of photoreceptors per RPE cell is being considered, the ratio of photoreceptors to RPE cells was surprisingly lower in the fovea than in the remainder of the central retina. Rapaport et al. (1995) identified at the rod peak (4–5 mm from foveal centre) 28 rods per RPE cell and in the periphery 22 rods per RPE cell. Snodderly comes to the conclusion that the smaller number of photoreceptors overlying foveal RPE cells should produce less total material requiring recycling and hence should place less metabolic demand on the RPE cells. Consequently, the relatively low number of photoreceptors per RPE cell in the fovea may account for sparing of the foveal RPE cells and photoreceptors and help to preserve their function in the face of disease.

### 4.3. Accumulation of lipofuscin in the RPE cell

Accumulation of lipofuscin in cells of the RPE is observed in several forms of macular degeneration including Stargardt disease and age related macular degeneration (Birnbach et al., 1994). In vivo measurements of lipofuscin have demonstrated that the amount of lipofuscin in patients with Stargardt disease is 2–5 times greater than in age-matched controls (Delori, Staurenghi, Arend, Goger, & Weiter, 1995). Slow accumulation of lipofuscin is also seen during normal aging (Kennedy, Rakoczy, & Constable, 1995). In normal retinas, macular RPE cells contain more lipofuscin than RPE cells in the periphery, probably because of higher density of photoreceptor and RPE cells in the macula resulting in higher level of indigestible products of photoreceptors (Birnbach et al., 1994). Sparrow (2003) provided an explanation for the fact that the RPE cells underlying the macula have the highest accumulation of lipofuscin and that Stargardt's disease primarily involves the centre of the field of vision. He suggested that it is not a coincidence that the macula of the retina also has the highest concentration of 11-*cis*-retinal-containing visual pigment, a feature reflecting, in part, the packing density of cone and rod photoreceptor cells. The heightened capacity for photon absorption conferred by the density of visual pigment in the macula translates into a higher probability that all-*trans*-retinal will be available for A2E and fleck formation.

Futhermore, due to individual and local differences of photoreceptor subtypes per RPE cell, the retinas of Stargardt patients may undergo secondary local photoreceptor degeneration. Thus, local rod or cone

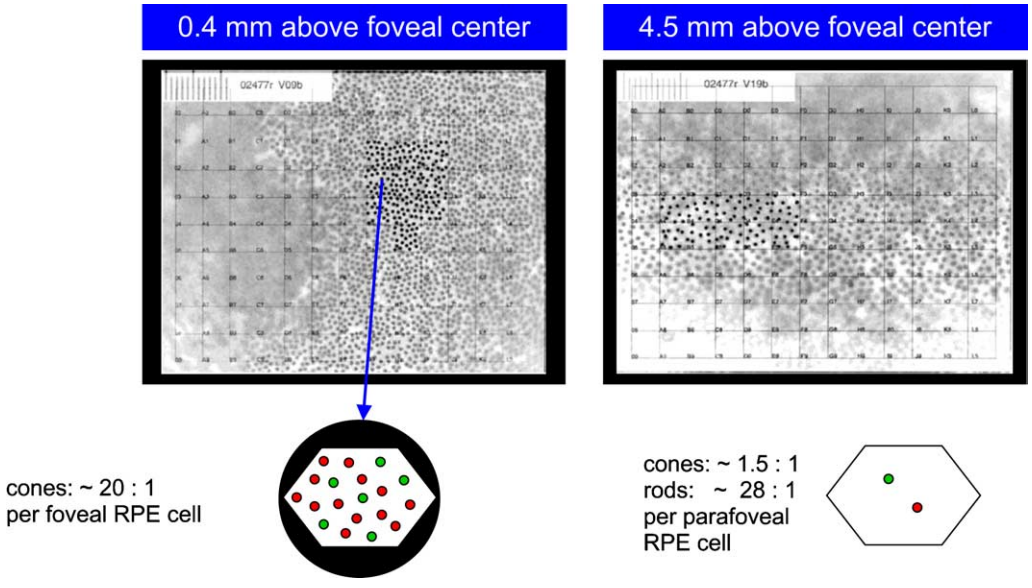


Fig. 8. Photoreceptor density in the primate fovea changes rapidly with retinal eccentricity. In the rhesus retina the number of cones per RPE cell was approximately 20:1 in the foveal centre (similar to the human retina) and only approximately 1.5:1 in the parafovea (modified from Snodderly et al., 2002). Rapaport et al. (1995) identified in the rod peak (4–5 mm from foveal centre) 28 rods per RPE cell and in the periphery 22 rods per RPE cell.

dominated functional loss would explain the different subtypes of Stargardt disease, although focal fundus fleck appearance may be similar. In fundus albipunctatus and retinitis punctata albescens, characterized by multifocal peripheral flecks, early involvement of the rod system is typically observed.

## 5. Hypothesis 2. Inhomogeneities in the distribution of spectrally different photoreceptors

An intact metabolism of the visual cycle may also depend on the demand of spectrally different photoreceptors (M-cones, L-cones, S-cones, rods). Inhomogeneity or absence of different photoreceptor subtypes might lead to functional abnormalities and/or focal RPE changes.

From a number of other studies, we know that the mosaic of rods and L-, M-, and S-cones in the human retina is topographically very heterogeneous. S-cones have their lowest density in the foveal pit at 3–5% of the cones, reach a maximum density of 15% on the foveal slope (1 degree from the foveal pit) and 8% of the total population elsewhere in the retina (Ahnelt et al., 1987). In the monkey retina, Marc and Sperling (1977) found that L-cones occur at about 33% of the cones throughout the retina, while M-cones peak in the fovea at 64% and vary between 52% and 59% elsewhere in the retina. However, others have found the L-cones to outnumber the M-cones in fovea and perifoveal psychophysical testing paradigms (Cicerone & Nerger, 1989). In summary, the distribution of the L- and M-cones in the human fovea seem to be very variable amongst individuals (Roorda & Williams, 1999) (Fig. 9). This implies that single RPE cells may subserve a quite different composition of photoreceptor types and subtypes. If the three cone types are differently involved in a degenerative process, local variation of cone density may trigger multifocal pathophysiological events.

## 6. Hypothesis 3. Local variations in Müller cell distribution and their function

Structural and supportive functions of Müller glial cells are essential for normal retinal function. According to inhomogeneity of Müller cell distribution in central and peripheral retina, the disease pattern may vary.

Müller cells, as the principal glial cells of the retina, are stretching radially across the retina forming the outer limiting membrane as well as the inner limiting membrane by conical endfeet. They also have pedicles contacting the retinal vessels. Müller cells have a number of functions in a symbiotic relationship with the neurons. Müller cells selectively guide growing axons; by synthesis of retinoic acid from retinal cells, they are thought to play an important role during development

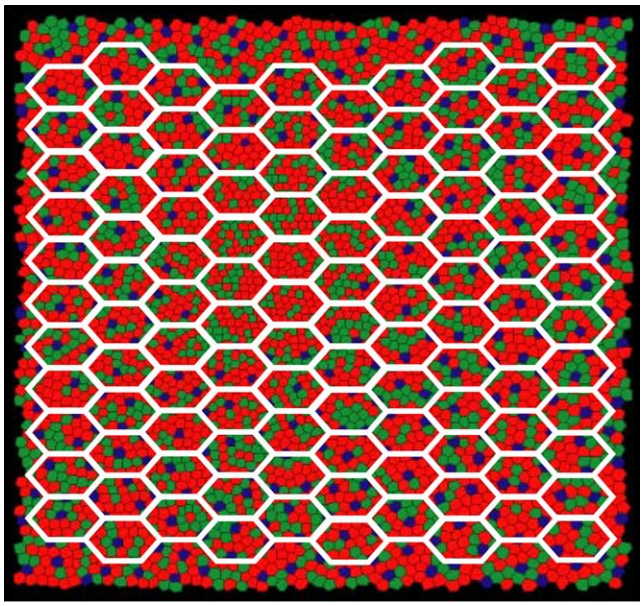

Fig. 9. The relative numbers of the L- and M-cones in human fovea varies greatly (simulation of the central 1° assuming a L/M-cone ratio of 2:1). This implies that single RPE cells may subserve a quite different composition of photoreceptor types and subtypes.

of the nervous system (Edwards, 1994). Additionally they are essential for the development and maintenance of the blood-retina-barrier.

Müller cells are responsible for the regulation of $K^+$ distribution across the entire retina and vitreous border (Reichenbach & Robinson, 1995) and thereby contribute to the generation of the b-wave of the electroretinogram (ERG) (Miller & Dowling, 1970). $K^+$ homeostasis plays a mayor role in Müller cell dedifferentiation and proliferative activity, for example in retinal gliosis (Bringmann et al., 2000). Systemic and retinal disease may upregulate ATP receptors in retinal glial cells (Pannicke et al., 2001) thus activating $K^+$ channels altering the metabolic state of the Müller cell and $K^+$ homeostasis in the retina (Kusaka & Puro, 1997). Müller cells also contribute to the transport and metabolism of glutamate in the retina (Derouiche, 1996; Poitry, Poitry-Yamate, Ueberfeld, MacLeish, & Tsacopoulos, 2000). This function seems to play an important role in the development of retinal disease as has been recently shown for diabetes induced dysfunction (Li & Puro, 2002).

Several observations imply involvement of Müller cells in the photopigment regeneration, and suggest an interaction between Müller cells and cones: cultured Müller cells isomerize all-*trans*-retinol to 11-*cis*-retinol (Das, Bhardwaj, Kjeldbye, & Gouras, 1992). Müller cells, in addition to RPE cells, contain cellular retinaldehyde binding protein (CRALBP), which specifically binds 11-*cis*-retinoids (Bunt-Milam & Saari, 1983; Saari & Bredberg, 1987).

However, there are considerable morphological differences in length, diameter and number of endfeet between foveal and peripheral Müller cells. The volume of Müller cells and their individual cell contacts determine the capacity for buffering, transport and recycling of $K^+$ and Glutamate. Variation of the number of neurons per Müller glial cell from 6 to 8 in cone-dominated retinae to more than 30 in rod-dominated retinae implies a regional metabolic turnover (Chao et al., 1997) and thus both may cause local retinal dysfunction. Additionally different types of Müller glial cells have been identified in rabbit retina using immunocytochemical methods (Schnitzer, 1987). The distribution of these heterogeneous populations of glial elements may vary from fovea to periphery and thus may contribute to regional variation of fleck appearance and distribution. Once degeneration has started at an individual neuron, Müller cell alter their function and become elements inducing apoptosis rather than supportive actions. The threshold for this change from "friend" to "enemy" of neurons may occur randomly in individual Müller cells across the retina, inducing a "flecked" pattern, with degenerative areas around individual cells.

## 7. Hypothesis 4. Are there different retinoid pathways for rod and cone pigment regeneration in the retina?

Mutations in the visual cycle genes might result in a reduced output and imbalance of rod or cone pathway or even in a complete blockade of one pathway. Imbalance of rod and/or cone pathway affection might therefore cause different phenotypic subtypes.

Rod photoreceptors are known to rely on the RPE to recycle 11-*cis*-retinal for rhodopsin regeneration. However, physiological evidence has shown that cones are distinct from rods in retinoid transporting and processing (Jin, Jones, & Cornwall, 1994; Jones, Fein, MacNichol, & Cornwall, 1993). Early studies have demonstrated a spontaneous recovery of cone sensitivity (but not of the rod sensitivity) after bleaching in the isolated retinas of different species (Hood & Hock, 1973; Normann & Perlman, 1990). In isolated salamander photoreceptors, Jones et al. (1993) demonstrated that the addition of 11-*cis*-retinol can restore cone sensitivity but not rod sensitivity, suggesting the presence of 11-*cis*-retinol dehydrogenase or its homologue in cones. Different from rods, salamander cones have the capacity to transport 11-*cis*-retinal from the inner segment to the outer segment (Jin et al., 1994).

Recent studies have supported these first indications of two visual retinoid cycles by identifying novel catalytic activities in membrane fractions of cone-dominant chicken and ground-squirrel retinas (Mata, Radu, Clemmons, & Travis, 2002; Znoiko, Crouch, Moiseyev, & Ma, 2002). Mata and co-workers proposed that these activities represent catalytic steps in a novel visual cycle that mediates pigment regeneration in cones. The alternate pigment regeneration is independent from the RPE and may involve Müller cells (Fig. 10). This implies that visual-pigment regeneration in cones is not exclusively dependent on RPE function in contrast to the rod system. Local retinal areas of high rod density such as the parafovea may be particularly susceptible to subtle RPE changes affecting visual pigment regeneration. Furthermore, due to their different pathways for photopigment regeneration the metabolic demand of individual RPE cells might differ as well.

Additionally, Znoiko et al. discussed the role of a different protein, the RPE65 protein, that causes severe rod-cone degeneration of early onset. RPE65, that is essential for generation of 11-*cis*-retinal in the visual cycle, is expressed in the RPE and is essential for retinoid processing in the RPE (= rod visual pathway). However, in mammalian species the protein could only be identified in cones but not in rods, further supporting the suggestion that rods and cones have distinct path-

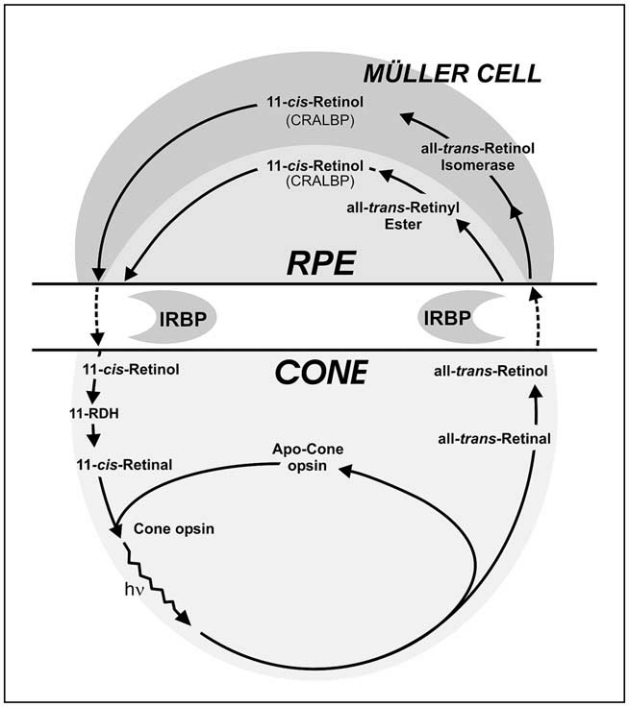


Fig. 10. Visual cycle for regeneration of cone opsin, as proposed by Mata et al., 2002. Light (*hν*) induces isomerization of 11-*cis* to all-*trans*-retinal. All-*trans*-retinal is reduced to all-*trans*-retinol which is released into the extracellular space by rods and cones and taken up by Müller cells. All-*trans*-retinol isomerase catalyses isomerization of all-*trans*-retinol to 11-*cis*-retinol. Müller cells also contain CRALBP, which binds 11-*cis*-retinol, but not all-*trans*-retinol. 11-*cis*-retinol is released by the Müller cell and taken up by cones. The all-*trans*-retinol and 11-*cis*-retinol in the extracellular space are bound to interphotoreceptor retinoid binding protein (IRBP). 11-*cis*-retinol is oxidized to 11-*cis*-retinal by 11-*cis*-retinol dehydrogenase (11-RDH). 11-*cis*-retinal and apo-cone opsin combine to regenerate cone opsin pigment.

ways for retinoid processing. The authors concluded that this might give further evidence for a cone specific alternative retinoid metabolic pathway that does not exclusively involve the RPE (Das et al., 1992), so that the disease pattern may vary depending on rod and/or cone pathway affection.

## 8. Hypothesis 5. Local variations in interphotoreceptor retinoid binding protein (IRBP) might be crucial for the interaction between RPE cell, photoreceptor outer segment and Müller cell

Interphotoreceptor retinoid binding protein (IRBP) is the major extracellular retinoid binding protein in retinas (Chen & Noy, 1994). Localization of IRBP within the extracellular space showed three bordering cell types: RPE, photoreceptor, and Müller cell (Bunt-Milam & Saari, 1983). The discussion is controversial whether the transport of retinoids (all-*trans*-retinol or 11-*cis*-retinal) between outer segments and the RPE might be mediated by IRBP, a large glycoprotein (Fig. 3) (Flannery, O'Day, Pfeffer, Horwitz, & Bok, 1990; Carlson & Bok, 1992; Gonzalez-Fernandez, 2002). However, knockout mice that lack IRBP showed normal rod function and rhodopsin regeneration following a photobleach (Palczewski et al., 1999), whereas cone sensitivity is reduced in light-adapted knockout mice. Furthermore, in light-adapted retinas, IRBP contains, in addition to all-*trans*-retinol, higher levels of 11-*cis*-retinol than 11-*cis*-retinal (Adler & Spencer, 1991). Mata et al. (2002) presume that the critical function of the IRBP is not the exchange of all-*trans*-retinol and 11-*cis*-retinal between rods and RPE cells (Fig. 3), but rather the exchange of all-*trans*-retinol and 11-*cis*-retinol between cones and Müller cells (Fig. 10).

Additionally, it has been suggested that IRBP might be an important factor for the structural integrity of photoreceptor outer segments (Chen, Saari, & Noy, 1993; Liou et al., 1998). In IRBP knockout mice, photoreceptor cells die in mice with a targeted disruption of the IRBP gene (IRBP-/- mice) indicating that early expression of IRBP is essential for normal photoreceptor development and function (Liou et al., 1998). Furthermore, IRBP binds fatty acids required for the structural integrity of photoreceptor outer segments (Chen et al., 1993). Thus, local variations of IRBP activity may focally cause a relative vitamin A deficiency.

## 9. Conclusions

There are multiple factors in the complex interaction of cell functions that depend on local differences in cell distribution, morphology and metabolism of cells varying from centre to periphery of the retina. Being randomly distributed with high or low local densities of certain cell-types a mosaic, essentially consisting in a "pointilistic" structure of the retina with many interlaced "micro cell biotopes", is produced.

Metabolic dysfunction of individual cells or cell types caused by genetic mutations and deletions may be tolerated for quite a while in this large assembly of networking cells. However, depending on the random variation of local cell populations at some areas a degenerating process may be triggered even by a single cell.

Such local events may occur, because the usually well established compensating cellular mechanisms are overloaded at some sites but not others. The threshold of decompensation may locally be crossed by some individual cell assemblies while it may not be crossed in a neighbouring cell population. Once neurons are dying, a local defense mechanism starts and may increase at the site of the lesion, induced by the various mechanisms discussed. Finally a multifocal pattern of lesions may appear ophthalmoscopically that may start with many small little dots. These may represent either deposits or areas of cell loss that may increase, such as in Stargardt disease, RBP4 and retinitis punctata albescens. In other diseases, the multifocal pattern may not progress, or may progress minimally, if only very few individual local cells cross the damage threshold, leading to deposits or lesions that do not strongly trigger cellular defence mechanisms in the neighbourhood (such as in fundus albipunctatus cum hemeralopia).

This paper describes the phenomenon of a multifocal phenotypic pattern of retinal disease that is caused by a generalized impairment of the retina/RPE complex. Although a final explanation for this coincidence cannot be provided, the paper presents hypotheses that may trigger further experimental approaches to further elucidate the pathomechanisms of focal flecked retina diseases.

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