

Part I

Methodology

Lipofuscin of the Retinal Pigment Epithelium

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1.1

Introduction

It is well known that the major source of fundus autofluorescence (FAF) is the lipofuscin of retinal pigment epithelial (RPE) cells. Lipofuscin is understood to be material in the lysosomal compartment of nondividing cells that cannot be degraded, and thus it accumulates [16, 74, 89]. For many cell types, lipofuscin originates internally (autophagy), but for the RPE, lipofuscin derives primarily from phagocytosed photoreceptor outer segments. These fluorophores most likely accumulate in RPE cells because the structures of the fluorophores are unusual and not amenable to degradation, rather than because the lysosomal enzymes in these cells are defective. Emerging evidence indicates that the lipofuscin of RPE cells is unique, since much of this material forms as a consequence of the light-capturing function of the retina. An origin from retinoids that leave the visual cycle is consistent with the finding that the accumulation of RPE lipofuscin is most marked in central retina, the area having the greatest concentration of visual pigment. The extensive system of conjugated double bonds within these retinoid-derived fluorophores also explains the long wavelength fluorescence emission of RPE lipofuscin. The excessive accumulation of RPE lipofuscin in autosomal-recessive Stargardt macular degeneration is considered to be the cause of RPE atrophy. This material is also implicated in disease processes underlying dominant Stargardt-like macular degeneration, Best's vitelliform macular dystrophy, and age-related macular degeneration.

1.2

The Source of RPE Lipofuscin

Evidence that the precursors of RPE lipofuscin originate in photoreceptor outer segments came from work in the Royal College of Surgeons rat showing that in this strain, in which RPE cells fail to phagocytose shed outer segment membrane, RPE lipofuscin is substantially diminished [36]. Lipofuscin was also reduced concomitant with light-induced loss of photoreceptor cells [38]. As with other cell types, the lipo-

fuscins of RPE gathers within membrane-bound organelles of the lysosomal compartment of the cells; because of their ultrastructural appearance, these organelles are referred to as lipofuscin granules [11, 15, 27, 30]. Early theories as to the genesis of RPE lipofuscin focused on the adducts generated following the peroxidation of lipid, particularly those formed by reactions between aldehyde products and biological amines [13]. Products of lipid oxidation have been detected in lipofuscin granules [62], but whether they contribute to the golden-yellow emission of RPE lipofuscin has been a matter of discussion [14, 23, 24].

Current understanding of the molecular composition of RPE lipofuscin originated with experiments documenting that the deposition of lipofuscin fluorophores is dependent on dietary vitamin A [37]. More recently it has been shown that when the 11-*cis*-retinal and all-*trans*-retinal chromophores of visual pigment are absent, as in Rpe65^{-/-} mice, RPE lipofuscin, measured as fluorescence intensity, is severely reduced [39]. These findings are consistent with the observation that in patients with early-onset retinal dystrophy associated with mutations in RPE65, RPE lipofuscin is similarly lacking [45].

1.3

Characteristics of Known RPE Lipofuscin Pigments

This evidence—that the RPE lipofuscin that accumulates with age and in some retinal disorders forms largely as a byproduct of light-related vitamin A cycling—is consistent with the finding that a prominent constituent is a di-retinal conjugate A2E (C₄₂H₅₈NO, molecular weight 592), named because it could be synthesized from vitamin A aldehyde (all-*trans*-retinal) and ethanolamine when combined in a 2:1 ratio [26, 58] (Figs. 1.1 and 1.2). The polar head group of A2E is a pyridine ring carrying a positive charge conferred by a quaternary amine nitrogen; two side arms extend from the ring, a long arm and a short arm. Each arm is derived from a molecule of all-*trans*-retinal [58]. The structure of A2E is unprecedented.

The polyene structure of the long arm of A2E (Fig. 1.1), including the double bonds within the pyridine and ionone rings, provides the extended conjugation system that allows A2E to absorb at wavelengths in the visible range of the spectrum. Since the absorbance spectrum of A2E has two peaks, it is also clear that the two arms of A2E do not constitute a single continuous conjugation system. Instead, A2E has an absorbance maximum in the visible spectrum of ~440 nm that can be assigned to the long arm and a shorter wavelength absorbance at ~340 nm that is generated within the short arm.

The emission spectrum of *in vivo* FAF exhibits strong similarities with that of cell-associated A2E and with the emission spectra of native lipofuscin present in RPE isolated from human eyes (F. Delori and J.R. Sparrow, unpublished observations) and isolated lipofuscin granules [11]. All have emission maxima at 590–620 nm and exhibit a characteristic red shift with increasing excitation wavelengths (Fig. 1.3).

Conversely, the *in vivo* excitation spectra are broader and peak at longer wavelengths (470–500) than that of A2E (448 nm) and native lipofuscin (460–475 nm).

The biosynthesis of A2E begins in photoreceptor outer segments with condensation reactions between all-*trans*-retinal and phosphatidylethanolamine (Fig. 1.2). All-*trans*-retinal that participates in A2E biosynthesis is generated by photoisomerization of 11-*cis* retinal. N-retinylidene-PE (NRPE), the product of the Schiff-base reaction between a single all-*trans*-retinal and phosphatidylethanolamine [42, 77, 84], is likely the substrate for ABCA4 (ABCR), the photoreceptor-specific ATP-binding cassette transporter [4, 50, 51, 53, 76–79] that is the protein product of the gene responsible for recessive Stargardt disease, a majority of cases of autosomal recessive cone-rod dystrophy, and a form of autosomal recessive retinitis pigmentosa (RP19) [3, 63]. A2E formation continues with the reaction of NRPE and a second all-*trans*-retinal and then proceeds through a multistep pathway that includes the generation of a phosphatidyl-dihydropyridinium (dihydro-A2PE) compound. The latter intermediate is unstable and undergoes oxidative aromatization [6, 42, 54] to form A2PE, the phosphatidyl-pyridinium bisretinoid that is the immediate precursor to A2E [6, 42, 54]. Because the intermediates that form before dihydro-A2PE are likely capable of reverse-synthesis, auto-oxidation of dihydro-A2PE may be the last step at which it is possible to intervene in the synthesis of A2E [71]. A2PE has a stable aromatic ring and is the fluorescent pigment detected in photoreceptor outer segments [6, 42]. Since the shedding and phagocytosis of outer segment membrane leads to the complete replacement of the photoreceptor outer segment approximately every 10 days [90], A2PE is not continuously amassed by the photoreceptor cell. In Royal College of Surgeons rats, a strain in which RPE cells fail to phagocytose shed outer segment membrane, A2PE is responsible, at least in part, for a golden-yellow autofluorescence in outer-segment degenerating debris [25, 36, 42]. A2PE may also account in part for the lipofuscin-like fluorescence detectable in photoreceptor cells in recessive Stargardt disease and in some forms of retinitis pigmentosa [10, 12, 80]. Although cleavage of A2PE to generate A2E has been suggested to occur by acid hydrolysis within RPE lysosomes [6], it is just as likely that the generation of A2E from A2PE is enzyme mediated, and phospholipase D has been implicated in this process [42].

Another constituent of RPE lipofuscin is the fluorophore isoA2E (Figs. 1.1 and 1.2) that forms by photoisomerization of A2E [54]. While the double bonds along the side arms of A2E are all in the *trans* (E) position, the double bond at the C13–14 position of isoA2E assumes the *cis* (Z) configuration [54]. Other less abundant *cis*-isomers—Z-olefins at the C9/9'–10/10' and C11/11'–12/12' positions—are also detectable as additional components of the lipofuscin isolated from aging human RPE [6]. For isoA2E and the other photoisomers, absorbance spectra are slightly blue-shifted relative to A2E (Fig. 1.3). A2E and its isomers have been detected in isolated human RPE [54], wherein their levels have also been shown to increase with age (Jang and Sparrow, unpublished observation). These pigments have also been demonstrated within eyecups harvested from mice, with levels being increased several fold in the *Abcr* null mutant mouse, a model of recessive Stargardt macular degeneration [40, 47–49, 84].

At least two compounds in RPE lipofuscin have ~510 nm absorbance and form by pathways distinct from that of A2E [28, 29]. Studies indicate that the pigment all-*trans*-retinal dimer, phosphatidylethanolamine (atRAL dimer-PE) (Fig. 1.1), is generated after two molecules of all-*trans*-retinal condense to form an aldehyde-bearing dimer (atRAL dimer) (Fig. 1.1). By means of its aldehyde group, atRAL dimer then forms a conjugate with phosphatidylethanolamine, thus forming atRAL dimer-PE [28, 29] (Fig. 1.1). A second ~510 nm absorbing species, all-*trans*-retinal dimer-E (atRAL dimer-E) (Fig. 1.1), can be subsequently generated by phosphate cleavage of atRAL dimer-PE. The pigments atRAL dimer-PE and atRAL dimer-E are composed of two polyene arms—seven double-bond conjugations on the long arm and four on the short—extending from a cyclohexadiene ring that is linked by Schiff base to phosphatidylethanolamine or ethanolamine, respectively. These pigments exhibit an absorbance maximum in the visible spectrum that is red-shifted relative to A2E (A2E/isoA2E, λ_{max} ~340, 440 nm; atRAL dimer-PE, λ_{max} ~290, 510 nm). The relatively long wavelength absorbance of atRAL dimer-PE and atRAL dimer-E is attributable to protonation of the Schiff base linkage in these compounds. The fluorescence emission of these pigments peaks at approximately 600 nm and is relatively weak in intensity (Fig. 1.3). It is also significant that when deprotonated, these pigments undergo hydrolysis to revert to atRAL dimer. Unprotonated/unconjugated atRAL dimer is detected in mice and human eyes along with conjugated/protonated atRAL dimer-PE and atRAL dimer-E, indicating that in the acidic environment in which these pigments are housed (lysosomal compartment), an equilibrium exists between the deprotonated/unconjugated and protonated/conjugated states. The absorbance

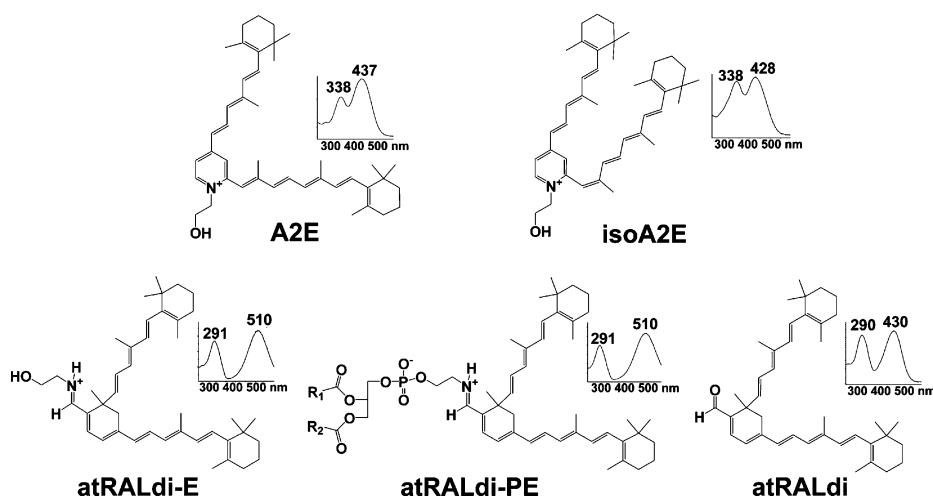


Fig. 1.1 Structures of retinal pigment epithelial (RPE) lipofuscin pigments A2E, isoA2E, all-*trans*-retinal dimer-ethanolamine (atRALdi-E), all-*trans*-retinal dimer-phosphatidylethanolamine (atRALdi-PE), and all-*trans*-retinal dimer (atRALdi) with corresponding ultraviolet-visible absorbance spectra

spectrum of unconjugated atRAL dimer exhibits maxima at ~290 and 432 nm, and its fluorescence emission profile (emission maximum 580 nm with 430 nm excitation) is slightly different from that of A2E (Fig. 1.3). The pigments atRAL dimer-PE and atRAL dimer-E are present at elevated levels in the lipofuscin-filled RPE of *Abcr* null mutant mice (S. Kim and J.R. Sparrow, unpublished).

Additional components of RPE lipofuscin are generated by photooxidation. In the case of A2E, photooxidation was originally suspected because of the fluorescence bleaching that accompanies irradiation. Subsequent analysis by mass spectrometry revealed that following blue light irradiation of A2E, the profile not only consisted of the $M+592$ peak attributable to A2E but also included a series of peaks, the sizes of which increased by increments of mass 16, indicating the addition of oxygens at carbon-carbon double bonds [5, 70].

The mixture of oxygen-containing moieties within photooxidized A2E includes cyclic peroxides (peroxy-A2E), furanoid oxides (furano-A2E), and probably epoxides [5, 21, 35]. We have also shown that oxidation occurs more readily on the short arm of A2E. Intracellular A2E has been shown to undergo photooxidation upon blue-

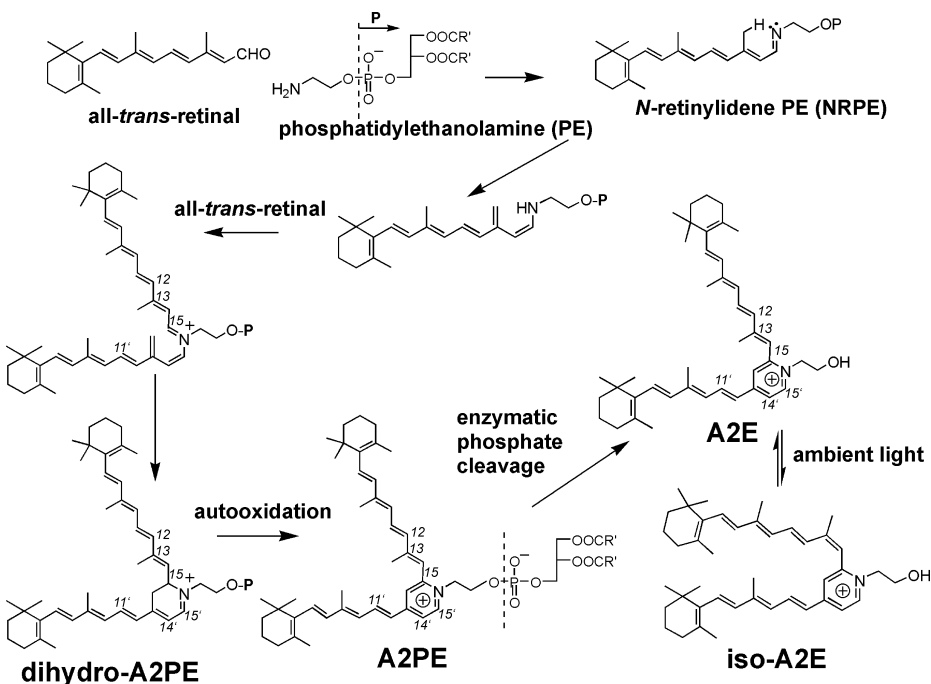


Fig. 1.2 Biosynthesis pathway of A2E and the photoisomer isoA2E from all-trans-retinal and phosphatidylethanolamine. The immediate precursor A2PE forms in outer segments; phosphate hydrolysis of A2PE to generate A2E and isoA2E probably occurs in retinal pigment epithelial lysosomes via an enzyme-mediated mechanism

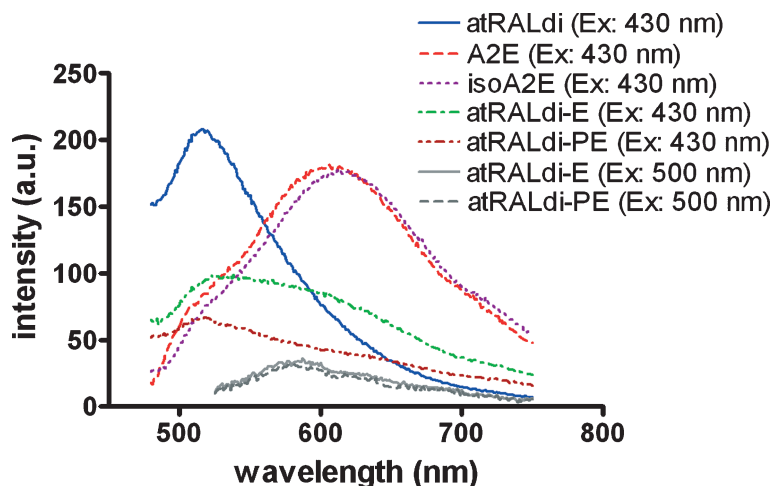


Fig. 1.3 Emission spectra of A2E, isoA2E, all-*trans*-retinal dimer-ethanolamine (atRALdi-E), all-*trans*-retinal dimer-phosphatidylethanolamine (atRALdi-PE), and all-*trans*-retinal dimer (atRALdi) with excitation at 430 or 500 nm, as indicated

light exposure [70], and we have detected monofuran-A2E and monoperoxy-A2E in RPE from human eyes and in eyecups from mice with null mutations in *Abcr*^{-/-} [35], the gene responsible for recessive Stargardt disease. Since the cytotoxicity of oxygen-containing groups such as endoperoxides is well known [46, 81], these moieties may account, at least in part, for cellular damage ensuing from A2E accumulation [73]. The addition of oxygens at olefins of A2E is associated with blue shifts in absorbance. For instance, when one furan or peroxy moiety is positioned on the long arm of A2E, the long wavelength absorbance changes to ~402 nm. Although monoperoxy-A2E exhibits a fluorescence emission that is more intense than A2E, with further oxidation, autofluorescence diminishes.

1.4

Adverse Effects of RPE Lipofuscin

In autosomal-recessive Stargardt disease caused by *ABCA4*/*ABCR* gene mutations [2, 64, 65, 88], RPE lipofuscin has a composition similar to age-associated lipofuscin; the accumulation of this material is also accelerated [19, 22, 43, 44, 82] and is considered the cause of RPE atrophy [56]. Since RPE lipofuscin is amassed with age and is of highest concentration underlying central retina [20], it is also implicated in atrophic age-related macular degeneration. Additionally, in-vivo monitoring of RPE lipofuscin as FAF in patients with age-related macular degeneration has revealed areas of intense FAF that correspond to sites of reduced scotopic sensitivity [59, 60] and are

prone to atrophy [31, 33]. Because mutations in *ABCA4*/*ABCR* lead to increased RPE lipofuscin, it is also of interest that heterozygous mutations in the gene have been associated with increased susceptibility to age-related macular degeneration [2, 9]. Poorly understood is the relationship between RPE lipofuscin and vitelliform macular dystrophies (e.g., Best disease, adult-onset vitelliform dystrophy) caused by mutations in *VMD2*, the gene encoding bestrophin-1 (Best1).

Work in in-vitro models suggests mechanisms by which lipofuscin constituents may damage the RPE cell [18, 61, 67, 68, 75]. Thus, as an amphiphilic molecule, not only can A2E mediate detergent-like effects on cell membranes [18, 67, 72, 75], but its accumulation can lead to the alkalization of lysosomes [32], possibly by interfering with the ATPase-dependent proton pump located in the lysosomal membrane [7]. A2E appears not to directly inhibit the activities of lysosomal enzymes [8]. A2E has also been shown to confer a susceptibility to photo-induced apoptosis [61, 68, 69], with sensitivity to blue light being directly dependent on the A2E content of the cells; green light (540 nm) is considerably less damaging. The photochemical events triggering apoptosis when A2E-laden RPE are exposed to blue light probably involve the generation of reactive forms of oxygen and photooxidative products of A2E (discussed above). The amount of A2E that undergoes photooxidation and cleavage in a lifetime may be significant: we observed that the amount of formed A2-PE, the immediate precursor of A2E, is many times greater than the amounts of A2E that accumulate in RPE cells [41]. One explanation for this finding is that a portion of the A2E that forms is normally lost. Because it is known that levels of A2E in RPE do not diminish under dark conditions [48], it is likely that the light-dependent conditions involve photooxidative processes. Decreased FAF is observed in areas of photoreceptor cell degeneration [83]; perhaps this observation can be accounted for by halted deposition (due to the absence of photoreceptor cells) together with depletion due to photooxidation.

1.5

Modulators of RPE Lipofuscin Formation

All-*trans*-retinal that avoids reduction to all-*trans*-retinol by all-*trans*-retinol dehydrogenase is available to undergo the random inadvertent reactions that lead to formation of the all-*trans*-retinal-derived fluorophores of RPE lipofuscin. Correspondingly, conditions that increase the availability of all-*trans*-retinal enhance the opportunity for these fluorophores to form. Not surprisingly, therefore, since the generation of all-*trans*-retinal in photoreceptor outer segments is light-dependent, light is also a determinant of the rate of A2E formation. Thus, in-vivo experiments have shown that the A2E precursor A2PE in photoreceptor outer segments is augmented by exposing rats to bright light [6]. Moreover, dark rearing of *ABCR*^{-/-} mice inhibits the deposition of A2E [48]. Since A2E levels are not diminished if mice are raised in cyclic light and then transferred to darkness, it is also clear that once A2E is formed, it is not eliminated from the RPE [48]. Another well-known factor that modulates

A2E formation is the activity of ABCA4 (ABCR), the photoreceptor-specific ATP-binding cassette transporter [50, 53, 76, 79] that is thought to aid in the movement of all-*trans*-retinal to the cytosolic side of the disc membrane [1, 34, 76, 79, 84], where it is accessible to retinol dehydrogenase, the enzyme responsible for its reduction to all-*trans*-retinol [57]. As a consequence of the loss of ABCR protein activity in *Abcr*^{-/-} mice, the levels of A2E in RPE cells are several fold greater than those in normal mice [40, 47, 84]. In *Abcr*^{+/-} mice, accumulation of A2E is approximately 40% of that in the null mutant mouse [49].

Because the source of all-*trans*-retinal for A2E formation is the photoisomerization of 11-*cis*-retinal, another determinant of A2E accumulation is the kinetics of 11-*cis*-retinal regeneration. Evidence for this factor has come from studies of an amino acid variant in murine Rpe65, the visual cycle protein that may have a rate-determining role in the visual cycle [87]. Specifically, in albino and pigmented mice in which the amino acid residue at position 450 of RPE 65 is methionine (C57BL/6J-*c^{2l}*; *Abcr*^{-/-} Met/Met; *Abcr*^{+/-} Met/Met) instead of leucine (BALB/cByJ; *Abcr*^{-/-} Leu/Leu; *Abcr*^{+/-} Leu/Leu), recovery of the electroretinographic response following a photobleach and rhodopsin regeneration are retarded [17, 52, 85, 86]; the content of A2E in the RPE is diminished by a similar magnitude [40].

Therapeutic approaches aimed at retarding the visual cycle also serve to reduce A2E accumulation. For instance, the acne medication isotretinoin (13-*cis*-retinoic acid, Accutane; Roche Laboratories, Nutley, NJ), which was shown to reduce visual sensitivity under darkened conditions by retarding 11-*cis*-retinal regeneration [66], reduces A2E deposition in the RPE of ABCR^{-/-} mice [55]. Nonetheless, 13-*cis*-retinoic acid has severe side effects, including teratogenicity [71] and is thus not appropriate for long-term therapy. Nonretinoid isoprenoid compounds that compete with retinyl esters for binding to RPE65, thereby interfering with visual cycle kinetics [47], also serve to mediate substantial reductions in A2E accumulation when administered chronically to *Abcr*^{-/-} mice. Indeed, RPE65 appears to be an excellent therapeutic target, with A2E levels in eyecups of *Abcr*^{-/-} mice treated with the RPE65 antagonists being as much as 85% lower than in vehicle-treated mice [47]. In another form of intervention, A2E levels in *Abcr*^{-/-} mice have also been reduced by daily administration of the retinoic acid analog N-(4-hydroxyphenyl)retinamide (HPR) for 1 month [56]. HPR acts by competing for binding sites on retinol-binding protein, thus reducing serum retinol levels. Consequently, retinol uptake by the eye is reduced, visual cycle retinoids are decreased, and the associated decrease in all-*trans*-retinal leads to retarded A2E formation.

1.6

Summary

RPE cells are unusual in that they are exposed to visible light while at the same time housing photoreactive molecules that accumulate as lipofuscin. There are many in-

dications that an excessive accumulation of RPE lipofuscin can lead to cellular dysfunction and contribute to retinal aging and degeneration [74]. Insight into the composition, biogenesis, and photoreactivity of RPE lipofuscin has improved our understanding of the extent to which the accumulation of these pigments renders the macula prone to insult. Novel therapeutic approaches are being developed to minimize lipofuscin accumulation in order to reduce the progression of retinal conditions such as recessive Stargardt disease and age-related macular degeneration.

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References

1. Ahn J, Wong JT, Molday RS (2000) The effect of lipid environment and retinoids on the ATPase activity of ABCR, the photoreceptor ABC transporter responsible for Stargardt macular dystrophy. *J Biol Chem* 275:20399–20405
2. Allikmets R, Shroyer NE, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 277:1805–1807
3. Allikmets R, Singh N, Sun H, Shroyer NE, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR (1997) A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 15:236–246
4. Beharry S, Zhong M, Molday RS (2004) N-retinylidene-phosphatidylethanolamine is the preferred retinoid substrate for the photoreceptor-specific ABC transporter ABCA4 (ABCR). *J Biol Chem* 279:53972–53979
5. Ben-Shabat S, Itagaki Y, Jockusch S, Sparrow JR, Turro NJ, Nakanishi K (2002) Formation of a nona-oxirane from A2E, a lipofuscin fluorophore related to macular degeneration, and evidence of singlet oxygen involvement. *Angew Chem Int Ed* 41:814–817
6. Ben-Shabat S, Parish CA, Vollmer HR, Itagaki Y, Fishkin N, Nakanishi K, Sparrow JR (2002) Biosynthetic studies of A2E, a major fluorophore of RPE lipofuscin. *J Biol Chem* 277:7183–7190
7. Bergmann M, Schutt F, Holz FG, Kopitz J (2004) Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration. *FASEB J* 18:562–564
8. Berman M, Schutt F, Holz FG, Kopitz J (2001) Does A2E, a retinoid component of lipofuscin and inhibitor of lysosomal degradative functions, directly affect the activity of lysosomal hydrolases. *Exp Eye Res* 72:191–195

9. Bernstein PS, Leppert M, Singh N, Dean M, Lewis RA, Lupski JR, Allikmets R, Seddon JM (2002) Genotype-phenotype analysis of ABCR variants in macular degeneration probands and siblings. *Invest Ophthalmol Vis Sci* 43:466–473
10. Birnbach CD, Jarvelainen M, Possin DE, Milam AH (1994) Histopathology and immunocytochemistry of the neurosensory retina in fundus flavimaculatus. *Ophthalmology* 101:1211–1219
11. Boulton M, Docchio F, Dayhaw-Barker P, Ramponi R, Cubeddu R (1990) Age-related changes in the morphology, absorption and fluorescence of melanosomes and lipofuscin granules of the retinal pigment epithelium. *Vision Res* 30:1291–1303
12. Bunt-Milam AH, Kalina RE, Pagon RA (1983) Clinical-ultrastructural study of a retinal dystrophy. *Invest Ophthalmol Vis Sci* 24:458–469
13. Chio KS, Reiss U, Fletcher B, Tappel AL (1969) Peroxidation of subcellular organelles: formation of lipofuscin-like fluorescent pigments. *Science* 166:1535–1536
14. Chowdhury PK, Halder M, Choudhury PK, Kraus GA, Desai MJ, Armstrong DW, Casey TA, Rasmussen MA, Petrich JW (2004) Generation of fluorescent adducts of malondialdehyde and amino acids: toward an understanding of lipofuscin. *Photochem Photobiol* 79:21–25
15. Clancy CMR, Krogmeier JR, Pawlak A, Rozanowska M, Sarna T, Dunn RC, Simon JD (2000) Atomic force microscopy and near-field scanning optical microscopy measurements of single human retinal lipofuscin granules. *J Phys Chem B* 104:12098–12101
16. Cuervo AM, Dice JR (2000) When lysosomes get old. *Exp Gerontol* 35:119–131
17. Danciger M, Matthes MT, Yasamura D, Akhmedov NB, Rickabaugh T, Gentleman S, Redmond TM, La Vail MM, Farber DB (2000) A QTL on distal chromosome 3 that influences the severity of light-induced damage to mouse photoreceptors. *Mam Genome* 11:422–427
18. De S, Sakmar TP (2002) Interaction of A2E with model membranes. Implications to the pathogenesis of age-related macular degeneration. *J Gen Physiol* 120:147–157
19. Delori FC, Staurenghi G, Arend O, Dorey CK, Goger DG, Weiter JJ (1995) In vivo measurement of lipofuscin in Stargardt's disease–Fundus flavimaculatus. *Invest Ophthalmol Vis Sci* 36:2327–2331
20. Delori FC, Goger DG, Dorey CK (2001) Age-related accumulation and spatial distribution of lipofuscin in RPE of normal subjects. *Invest Ophthalmol Vis Sci* 42:1855–1866
21. Dillon J, Wang Z, Avallé LB, Gaillard ER (2004) The photochemical oxidation of A2E results in the formation of a 5,8,5',8'-bis-furanoid oxide. *Exp Eye Res* 79:537–542
22. Eagle RC, Lucier AC, Bernardino VB, Yanoff M (1980) Retinal pigment epithelial abnormalities in fundus flavimaculatus. *Ophthalmol* 87:1189–1200
23. Eldred G, Katz ML (1991) The lipid peroxidation theory of lipofuscinogenesis cannot yet be confirmed. *Free Rad Biol Med* 10:445–447
24. Eldred GE, Katz ML (1989) The autofluorescent products of lipid peroxidation may not be lipofuscin-like [see comments]. *Free Radic Biol Med* 7:157–163
25. Eldred GE (1991) The fluorophores of the RCS rat retina and implications for retinal degeneration. CRC Press, Boca Raton, Florida
26. Eldred GE, Lasky MR (1993) Retinal age pigments generated by self-assembling lysosomotropic detergents. *Nature* 361:724–726

27. Feeney-Burns L, Eldred GE (1983) The fate of the phagosome: conversion to "age pigment" and impact in human retinal pigment epithelium. *Trans Ophthalmol Soc UK* 103:416–421
28. Fishkin N, Pescitelli G, Sparrow JR, Nakanishi K, Berova N (2004) Absolute configurational determination of an all-trans-retinal dimer isolated from photoreceptor outer segments. *Chirality* 16:637–641
29. Fishkin NE, Pescitelli G, Itagaki Y, Berova N, Allikmets R, Nakanishi K, Sparrow JR (2004) Isolation and characterization of a novel RPE cell fluorophore: all-trans-retinal dimer conjugate. *Invest Ophthalmol Vis Sci* 45:E–abstract 1803
30. Haralampus-Grynaviski NM, Lamb LE, Clancy CMR, Skumatz C, Burke JM, Sarna T, Simon JD (2003) Spectroscopic and morphological studies of human retinal lipofuscin granules. *Proc Natl Acad Sci USA* 100:3179–3184
31. Holz FG, Bellmann C, Margaritis M, Schutt F, Otto TP, Volcker HE (1999) Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 237:145–152
32. Holz FG, Schutt F, Kopitz J, Eldred GE, Kruse FE, Volcker HE, Cantz M (1999) Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci* 40:737–743
33. Holz FG, Bellman C, Staudt S, Schutt F, Volcker HE (2001) Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 42:1051–1056
34. Illing M, Molday LL, Molday RS (1997) The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. *J Biol Chem* 272:10303–10310
35. Jang YP, Matsuda H, Itagaki Y, Nakanishi K, Sparrow JR (2006) Characterization of peroxy-A2E and furan-A2E photooxidation products and detection in human and mouse retinal pigment epithelial cells lipofuscin. *J Biol Chem* 280:39732–39739
36. Katz ML, Drea CM, Eldred GE, Hess HH, Robison WG, Jr. (1986) Influence of early photoreceptor degeneration on lipofuscin in the retinal pigment epithelium. *Exp Eye Res* 43:561–573
37. Katz ML, Drea CM, Robison WG, Jr. (1986) Relationship between dietary retinol and lipofuscin in the retinal pigment epithelium. *Mech Ageing Dev* 35:291–305
38. Katz ML, Eldred GE (1989) Retinal light damage reduces autofluorescent pigment deposition in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 30:37–43
39. Katz ML, Redmond TM (2001) Effect of Rpe65 knockout on accumulation of lipofuscin fluorophores in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 42:3023–3030
40. Kim SR, Fishkin N, Kong J, Nakanishi K, Allikmets R, Sparrow JR (2004) The Rpe65 Leu-450Met variant is associated with reduced levels of the RPE lipofuscin fluorophores A2E and iso-A2E. *Proc Natl Acad Sci USA* 101:11668–11672
41. Kim SR, Nakanishi K, Itagaki Y, Sparrow JR (2006) Photooxidation of A2-PE, a photoreceptor outer segment fluorophore, and protection by lutein and zeaxanthin. *Exp Eye Res* 82:828–839
42. Liu J, Itagaki Y, Ben-Shabat S, Nakanishi K, Sparrow JR (2000) The biosynthesis of A2E, a fluorophore of aging retina, involves the formation of the precursor, A2-PE, in the photoreceptor outer segment membrane. *J Biol Chem* 275:29354–29360

43. Lois N, Holder GE, Fitzke FW, Plant C, Bird AC (1999) Intrafamilial variation of phenotype in Stargardt macular dystrophy-fundus flavimaculatus. *Invest Ophthalmol Vis Sci* 40:2668–2675
44. Lopez PF, Maumenee IH, de la Cruz Z, Green WR (1990) Autosomal-dominant fundus flavimaculatus. Clinicopathologic correlation. *Ophthalmol* 97:798–809
45. Lorenz B, Wabbel B, Wegscheider E, Hamel CP, Drexler W, Presing MN (2004) Lack of fundus autofluorescence to 488 nanometers from childhood on in patients with early-onset severe retinal dystrophy associated with mutations in RPE65. *Ophthalmol* 111:1585–1594
46. Maggs JL, Bishop LPD, Batty KT, Dodd CC, Ilett KE, O'Neill PM, Edwards G, Park BK (2004) Hepatocellular bioactivation and cytotoxicity of the synthetic endoperoxide antimalarial arteflene. *Chem-Biol Interact* 147:173–184
47. Maiti P, Kong J, Kim SR, Sparrow JR, Allikmets R, Rando RR (2006) Small molecule RPE65 antagonists limit the visual cycle and prevent lipofuscin formation. *Biochem* 45:852–860
48. Mata NL, Weng J, Travis GH (2000) Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc Natl Acad Sci USA* 97:7154–7159
49. Mata NL, Tzekov RT, Liu X, Weng J, Birch DG, Travis GH (2001) Delayed dark adaptation and lipofuscin accumulation in abcr \pm mice: implications for involvement of ABCR in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 42:1685–1690
50. Molday LL, Rabin AR, Molday RS (2000) ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. *Nat Genet* 25:257–258
51. Molday RS, Molday LL (1979) Identification and characterization of multiple forms of rhodopsin and minor proteins in frog and bovine outer segment disc membranes. Electrophoresis, lectin labeling and proteolysis studies. *J Biol Chem* 254:4653–4660
52. Nusinowitz S, Nguyen L, Radu RA, Kashani Z, Farber DB, Danciger M (2003) Electroretinographic evidence for altered phototransduction gain and slowed recovery from photobleaches in albino mice with a MET450 variant in RPE6. *Exp Eye Res* 77:627–638
53. Papermaster DS, Schneider BG, Zorn MA, Kraehenbuhl JP (1978) Immunocytochemical localization of a large intrinsic membrane protein to the incisures and margins of frog rod outer segment disks. *J Cell Biol* 78:415–425
54. Parish CA, Hashimoto M, Nakanishi K, Dillon J, Sparrow JR (1998) Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. *Proc Natl Acad Sci USA* 95:14609–14613
55. Radu RA, Mata NL, Nusinowitz S, Liu X, Sieving PA, Travis GH (2003) Treatment with isotretinoin inhibits lipofuscin and A2E accumulation in a mouse model of recessive Stargardt's macular degeneration. *Proc Natl Acad Sci U S A* 100:4742–4747
56. Radu RA, Han Y, Bui TV, Nusinowitz S, Bok D, Lichter J, Widder K, Travis GH, Mata NL (2005) Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: a potential therapy for treatment of lipofuscin-based retinal diseases. *Invest Ophthalmol Vis Sci* 46:4393–4401
57. Saari JC, Garwin GG, Van Hooser JP, Palczewski K (1998) Reduction of all-trans-retinal limits regeneration of visual pigment in mice. *Vision Res* 38:1325–1333
58. Sakai N, Decatur J, Nakanishi K, Eldred GE (1996) Ocular age pigment "A2E": an unprecedented pyridinium bisretinoid. *J Am Chem Soc* 118:1559–1560

59. Schmitz-Valckenberg S, Bultmann S, Dreyhaupt J, Bindewald A, Holz FG, Rohrschneider K (2004) Fundus autofluorescence and fundus perimetry in the junctional zone of geographic atrophy in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 45:4470–4476
60. Scholl HPN, Bellmann C, Dandekar SS, Bird AC, Fitzke FW (2004) Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci* 45:574–583
61. Schutt F, Davies S, Kopitz J, Holz FG, Boulton ME (2000) Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci* 41:2303–2308
62. Schutt F, Bergmann M, Holz FG, Kopitz J (2003) Proteins modified by malondialdehyde, 4-hydroxynonenal or advanced glycation end products in lipofuscin of human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 44:3663–3668
63. Shroyer NF, Lewis RA, Allikmets R, Singh N, Dean M, Leppert M, Lupski JR (1999) The rod photoreceptor ATP-binding cassette transporter gene, ABCR, and retinal disease: from monogenic to multifactorial. *Vision Res* 39:2537–2544
64. Shroyer NF, Lewis RA, Yatsenko AN, Lupski JR (2001) Null missense ABCR (ABCA4) mutations in a family with Stargardt disease and retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 42:2757–2761
65. Shroyer NF, Lewis RA, Yatsenko AN, Wensel TG, Lupski JR (2001) Cosegregation and functional analysis of mutant ABCR (ABCA4) alleles in families that manifest both Stargardt disease and age-related macular degeneration. *Hum Mol Genet* 10:2671–2678
66. Sieving PA, Chaudhry P, Kondo M, Provenzano M, Wu D, Carlson TJ, Bush RA, Thompson DA (2001) Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in isotretinoin therapy. *Proc Natl Acad Sci USA* 98:1835–1840
67. Sparrow JR, Parish CA, Hashimoto M, Nakanishi K (1999) A2E, a lipofuscin fluorophore, in human retinal pigmented epithelial cells in culture. *Invest Ophthalmol Vis Sci* 40:2988–2995
68. Sparrow JR, Nakanishi K, Parish CA (2000) The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. *Invest Ophthalmol Vis Sci* 41:1981–1989
69. Sparrow JR, Cai B (2001) Blue light-induced apoptosis of A2E-containing RPE: involvement of caspase-3 and protection by Bcl-2. *Invest Ophthalmol Vis Sci* 42:1356–1362
70. Sparrow JR, Zhou J, Ben-Shabat S, Vollmer H, Itagaki Y, Nakanishi K (2002) Involvement of oxidative mechanisms in blue light induced damage to A2E-laden RPE. *Invest Ophthalmol Vis Sci* 43:1222–1227
71. Sparrow JR (2003) Therapy for macular degeneration: insights from acne. *Proc Natl Acad Sci USA* 100:4353–4354
72. Sparrow JR, Fishkin N, Zhou J, Cai B, Jang YP, Krane S, Itagaki Y, Nakanishi K (2003) A2E, a byproduct of the visual cycle. *Vision Res* 43:2983–2990
73. Sparrow JR, Vollmer-Snarr HR, Zhou J, Jang YP, Jockusch S, Itagaki Y, Nakanishi K (2003) A2E-epoxides damage DNA in retinal pigment epithelial cells. Vitamin E and other antioxidants inhibit A2E-epoxide formation. *J Biol Chem* 278:18207–18213

74. Sparrow JR, Boulton M (2005) RPE lipofuscin and its role in retinal photobiology. *Exp Eye Res* 80:595–606
75. Sparrow JR, Cai B, Jang YP, Zhou J, Nakanishi K (2006) A2E, a fluorophore of RPE lipofuscin, can destabilize membrane. *Adv Exp Med and Biol* 572:63–68
76. Sun H, Nathans J (1997) Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. *Nat Genet* 17:15–16
77. Sun H, Molday RS, Nathans J (1999) Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. *J Biol Chem* 274:8269–8281
78. Sun H, Nathans J (2001) Mechanistic studies of ABCR, the ABC transporter in photoreceptor outer segments responsible for autosomal recessive Stargardt disease. *J Bioenerg Biomembrane* 33:523–530
79. Sun H, Nathans J (2001) ABCR, the ATP-binding cassette transporter responsible for Stargardt macular dystrophy, is an efficient target of all-trans retinal-mediated photo-oxidative damage in vitro: implications for retinal disease. *J Biol Chem* 276:11766–11774
80. Szamier RB, Berson EL (1977) Retinal ultrastructure in advanced retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 16:947–962
81. Takada N, Watanabe M, Yamada A, Suenaga K, Yamada K, Ueda K, Uemura D (2001) Isolation and structures of haterumadiolins A and B, cytotoxic endoperoxides from the Okinawan Sponge *Plakortis lita*. *J Nat Prod* 64:356–359
82. von Ruckmann A, Fitzke FW, Bird AC (1997) In vivo fundus autofluorescence in macular dystrophies. *Arch Ophthalmol* 115:609–615
83. Von Ruckmann A, Fitzke FW, Fan J, Halfyard A, Bird AC (2002) Abnormalities of fundus autofluorescence in central serous retinopathy. *Am J Ophthalmol* 133:780–786
84. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH (1999) Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* 98:13–23
85. Wenzel A, Reme CE, Williams TP, Hafezi F, Grimm C (2001) The Rpe65 Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. *J Neurosci* 21:53–58
86. Wenzel A, Grimm C, Samardzija M, Reme CE (2003) The genetic modified Rpe65Leu₄₅₀: effect on light damage susceptibility in c-Fos-deficient mice. *Invest Ophthalmol Vis Sci* 44:2798–2802
87. Xue L, Gollapalli DR, Maiti P, Jahng WJ, Rando RR (2004) A palmitoylation switch mechanism in the regulation of the visual cycle. *Cell* 117:761–771
88. Yatsenko AN, Shroyer NF, Lewis RA, Lupski JR (2001) Late-onset Stargardt disease is associated with missense mutations that map outside known functional regions of ABCR (ABCA4). *Hum Genet* 108:346–355
89. Yin D (1996) Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Rad Biol Med* 21:871–888
90. Young RW (1971) The renewal of rod and cone outer segments in the rhesus monkey. *J Cell Biol* 49:303–318

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