



Mitochondria: The Retina's Achilles' Heel **10** in AMD

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Abstract

Strong experimental evidence from studies in human donor retinas and animal models supports the idea that the retinal pathology associated with age-related macular degeneration (AMD) involves mitochondrial dysfunction and consequent altered retinal metabolism. This chapter provides a brief overview of mitochondrial structure and function, summarizes evidence for mitochondrial defects in AMD, and highlights the potential ramifications of these defects on retinal health and function. Discussion of mitochondrial haplogroups and their association with AMD brings to light how mitochondrial genetics can influence disease outcome. As one of the most

metabolically active tissues in the human body, there is strong evidence that disruption in key metabolic pathways contributes to AMD pathology. The section on retinal metabolism reviews cell-specific metabolic differences and how the metabolic interdependence of each retinal cell type creates a unique ecosystem that is disrupted in the diseased retina. The final discussion includes strategies for therapeutic interventions that target key mitochondrial pathways as a treatment for AMD.

Keywords

Mitochondria · Age-related macular degeneration · Metabolism · Ecosystem model · Proteomics · mtDNA

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10.1 Introduction to Mitochondria

10.1.1 Mitochondrial Origins

Every cell contains two types of DNA, the nuclear DNA, which is inherited from both mother and father, along with the small circular mitochondrial DNA (mtDNA) that is inherited only from the mother. The “Endosymbiotic Theory” explains the existence of these very different types of DNA co-existing within cells. In early evolution, free-living, aerobic bacteria were

incorporated into the early, ancestral eukaryotic cells. Over time, this symbiotic relationship resulted in increased energy production that allowed progression from single-cell eukaryotes to multicellular organisms, tissues, and the diversity of species found worldwide. Evidence supporting this theory includes similarities between bacterial and mitochondrial (MT) patterns of gene arrangements, small subunit ribosomal RNAs (rRNA), and protein data [1–3].

10.1.2 MT Distribution and Content

The retina is one of the most metabolically active tissues in the body due, in part, to the high

concentration of MT present in nearly all cells. The MT within different retinal cell types are localized toward the sources of oxygen, which in the human retina are the choriocapillaris and the inner retinal vasculature. In the retinal pigment epithelium (RPE), MT are clustered along the basal border of the cell, in close proximity to the choriocapillaris (Fig. 10.1). Photoreceptors are nourished by both the choriocapillaris as well as the inner retinal vasculature. Reflecting the multiple sources of oxygen, photoreceptor MT are located at two sites—densely packed within the inner segments and at the synaptic terminals. Müller cells, which span the entire length of the neural retina and thus also derive oxygen from both sources, have MT that are

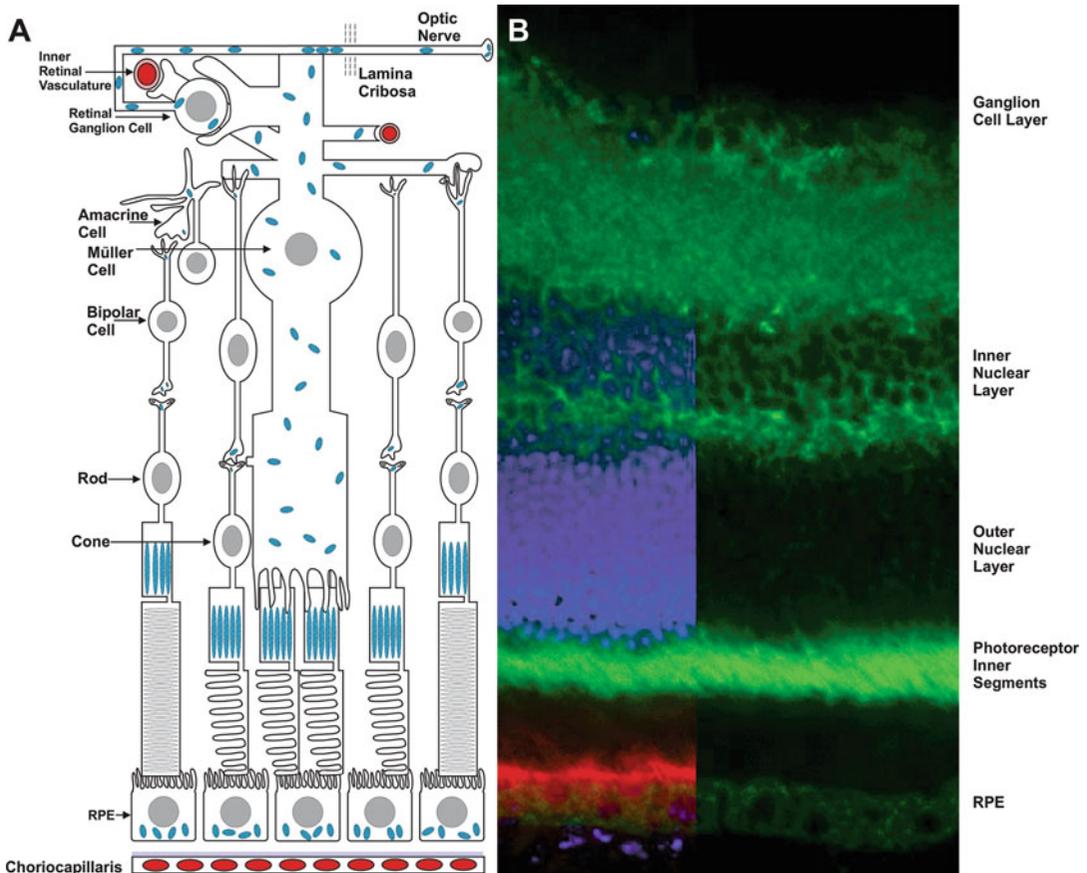


Fig. 10.1 Mitochondrial localization. (a) Schematic of retinal cells showing location of mitochondria (labeled blue) within each cell type. Position of the two major blood supplies (choriocapillaris and the inner retinal vasculature) is indicated in red. (b) Mouse retinal section

stained with anti-TOMM20 (green) to indicate position of mitochondria, anti-Ezrin (red) to indicate the RPE apical membrane, and DAPI (blue) for staining nuclei (Illustration by S. Atilano; Micrograph provided by John Ash and Emily Brown)

evenly distributed throughout the cell [4]. MT in the retinal ganglion cells (RGC) are present in the soma where mtDNA replication occurs and are abundant along the unmyelinated axons where they move bidirectionally along microtubule tracks [5]. MT accumulate just anterior to the lamina cribrosa, but are drastically reduced once the axons exit the eye and form the myelinated optic nerve.

In addition to the cell-specific distribution of MT, the number of MT within a cell can vary from 100 to several thousand depending upon the cell's energy requirement. Additionally, MT content is very dynamic and can be adjusted when energy demands change by either making more MT or eliminating MT via the processes of biogenesis and mitophagy, respectively. The master regulator of MT biogenesis is PGC-1 α , a co-activator of transcription factors (NRF-1, PPAR α , mtTFAM) that upregulate genes involved in MT biogenesis. Mitophagy is a specialized version of autophagy involving the selective degradation of MT that are either not needed for the current cellular energy requirements or are damaged. MT degradation involves multiple steps starting with the segregation of unwanted or damaged MT from healthy segments. The healthy MT can then fuse with other healthy MT thereby mixing contents and forming ever-changing MT networks (Fig. 10.2). This dynamic process of fission to

remove damaged MT segments and fusion of healthy MT is essential for maintaining a population of functional MT. The damaged MT are surrounded by a double membrane structure called an autophagosome, which then fuses with lysosomes. Lysosomal enzymes then digest the contents of the phagosome. Thus, in the healthy cell, there is continual MT turnover involving coordination and balance between both biogenesis and mitophagy.

There is good evidence suggesting the processes involved in MT turnover are adversely affected with AMD. Analysis of RPE MT in human donor eyes using electron microscopy showed there was a significant decrease in MT number and size in donors with AMD compared with nondiseased age-matched controls [6]. Lower MT numbers are consistent with defects in MT biogenesis and the smaller MT suggests defects in MT fusion. Autophagy defects, which will have a negative impact on mitophagy, have also been reported in human donors with AMD. In one study, immunohistochemical staining of human retinal sections showed p62 accumulated in the macula of donors with AMD [7]. Elevated p62, which is normally degraded via autophagy, is an indicator of decreased autophagic flux. Data from a study in primary cultures of RPE directly showed autophagic flux is reduced in RPE from donors with AMD [8].

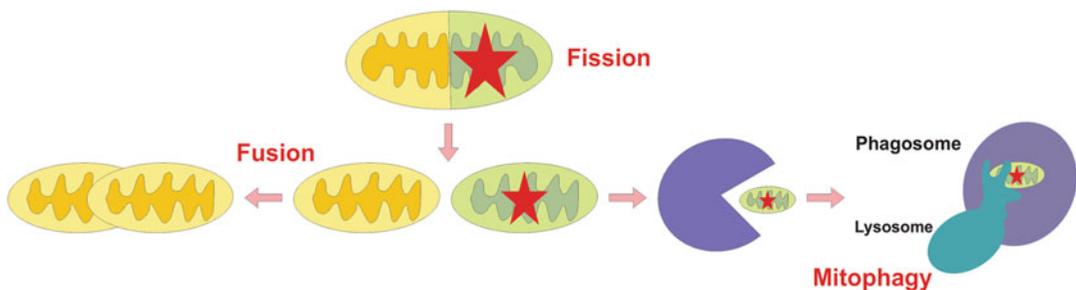


Fig. 10.2 Mitophagy eliminates damaged MT. Fission of MT networks allows for segregation of damaged MT (star, green) from healthy MT (yellow). Healthy MT can then fuse with other healthy MT to replenish the dynamic MT

network. The damaged MT are encapsulated by a double-walled membrane, forming the phagosome, which fuses with lysosomes. The lysosomal enzymes digest mitochondria in the phagosome (Illustration by S. Atilano)

10.1.3 MT Structure and Function

Each MT consists of two separate membranes (outer and inner) that divide discrete compartments (Intermembrane Space and Matrix), each containing different proteins with specific functions (Fig. 10.3). The outer membrane is permeable to small molecules (e.g., oxygen, calcium, sugars) that pass through the lipid bilayer. Channels within the outer mitochondrial membrane contain porin proteins (also known as

voltage-dependent anion channels, VDAC) that allow passage of ions and molecules less than 5000 Daltons, such as ADP and ATP. The vast majority of proteins that reside in the MT (~1500 proteins) are encoded by the nuclear genome, produced in the cytosol, and imported through the outer MT membrane via the “translocase of the outer membrane” (TOM) complex into the Intermembrane Space [3, 9]. Also embedded in the outer membrane are the proteins involved in MT fission (Fis1/2) and fusion (Mitofusin).

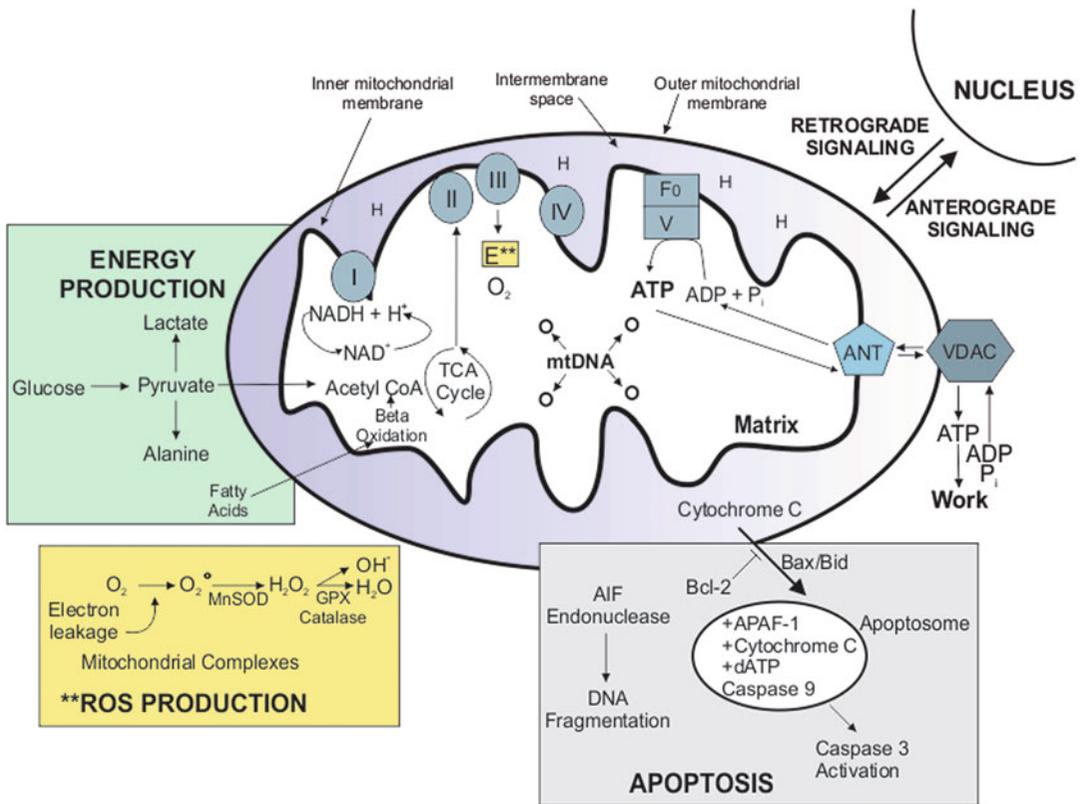


Fig. 10.3 Mitochondrial structure and function. Schematic shows gross mitochondrial structures, including the Inner and Outer membranes that separate the Intermembrane Space and the Matrix. Also depicted are the Complexes (I–V) that are part of the electron transport chain and the energy pathways that feed into these Complexes (Acetyl CoA, TCA cycle, NADH). Hydrogen ions (designated as H) that accumulate in the intermembrane space flow back into the matrix through Complex V, driving the phosphorylation of ADP to form ATP. Boxes outside the MT summarize key functions of energy

production, regulation of apoptosis, ROS production and signaling to and from the nucleus via anterograde/retrograde signaling. E = reactive oxygen species; mtDNA = mitochondrial DNA; ROS = reactive oxygen species; VDAC = voltage dependent anion channel; ANT = adenine nucleotide translocase; MnSOD = manganese superoxide dismutase; GPX = glutathione peroxidase; AIF Endonuclease = apoptosis-inducing factor; APAF-1 = apoptotic protease activating factor. (Modified from www.mitomap.org, and Nicholis and Minczuk, Exp Gerontol. 2014. 56:175-81. Illustration by S. Atilano)

Proteins involved in regulating apoptosis (Bcl-2, Bax/Bak) bind to the outer membrane but are not integrated into the membrane.

The Intermembrane Space is the site of the "ion motive force," a term that refers to the accumulation of hydrogen ions into this compartment as a consequence of these ions being pumped from the matrix by protein Complexes (I, III, IV) of the electron transport chain (ETC). The exit of these hydrogen ions through the ATP synthase, also known as Complex V of the ETC, back into the matrix is what generates ATP. The Intermembrane Space is also the site of multiple proteins involved in initiating apoptosis, such as cytochrome c, which is sequestered in this compartment by its interaction with cardiolipin, a MT-specific lipid of the inner membrane. Other regulators of apoptosis include apoptosis inducing factor, high-temperature requirement protein/Omi, and Smac/diablo. Release of these proteins from the Intermembrane Space into the cytosol initiates a cascade of events that culminates in apoptosis and cell death. Other proteins present in the Intermembrane Space are involved in the import and folding of proteins that reside in this compartment, as well as the antioxidant, superoxide dismutase, which helps to detoxify reactive oxygen species generated by the ETC.

The inner membrane, with similar lipid content as bacterial membranes, has high cardiolipin content and is selectively permeable, allowing specific molecules (e.g., oxygen, carbon dioxide, and water) into the matrix [10]. Cardiolipin is a negatively charged lipid that drives transport of positively-charged molecules across the outer membrane and is required for maintaining function of the ETC proteins. The surface area of the inner membrane is increased significantly by numerous invaginations called cristae that contain embedded protein respiratory complexes associated with the ETC (Complexes I, III, IV) and production of ATP (Complex V). Multiple proteins are involved in maintaining cristae integrity, including mitofilin, optic atrophy 1 (OPA1) and ChChd3. Maintenance of cristae integrity is essential for optimal energy production, which requires that the ETC proteins are localized in close proximity for effective transfer of electrons.

Data from human donor eyes support the idea that MT architecture is disrupted with AMD. In a study using electron microscopy to analyze RPE MT, Feher and colleagues reported significant disruptions in MT membranes and cristae in donors with AMD compared to age-matched controls [6]. In a proteomic analysis of RPE, increased mitofilin was reported in donors at more advanced stages of AMD [11]. Since mitofilin helps to maintain cristae integrity, the authors suggested the increase could be a compensatory response to help rescue and stabilize the degenerating cristae.

Also embedded in the inner membrane is the "translocase of the inner membrane" (TIM) complex that, working in conjunction with TOM, is responsible for importing nuclear-encoded proteins into the matrix. Multiple heat shock proteins (mtHSP60, mtHSP70, HSP90) assist with the import and folding of resident MT proteins and are localized both in the Intermembrane Space and Matrix. Other inner membrane proteins that work in concert with outer membrane proteins include the adenine nucleotide translocator (ANT), which associates with VDAC in the outer membrane and forms the MT permeability transition pore that opens to allow escape of cytochrome c and other proteins involved in initiating apoptosis. ANT is also involved in moving ADP and ATP into and out of the MT. The inner membrane protein OPA1 interacts with mitofusion in the outer membrane to initiate MT fusion. Mutations in the OPA1 gene have been associated with optic atrophy type 1, which is a dominantly inherited optic neuropathy resulting in progressive loss of visual acuity, often leading to legal blindness [12].

Proteomic analysis of RPE from human donors with AMD showed decreased content of several heat shock proteins that are critical for the import of MT resident proteins. Identified proteins include mtHSP60 and in two separate proteomic studies of the RPE and isolated MT, a decrease in mtHSP70 was observed [11, 13]. These two heat shock proteins assist TOM and TIM with the import and refolding of nuclear encoded proteins and therefore, a reduction in these two proteins in AMD suggests

potential defects in this critical process in diseased RPE.

Recent analysis of the MT matrix has identified 495 proteins that reside in this central compartment [14]. The processes ongoing in the matrix and some of the associated proteins include: mtDNA replication and transcription (TFAM, TFB2M, POLRmt), protein translation (RNA polymerase, ribosomes, Tufm, Rnase P), protein quality control (Lon and ClpXP proteases, chaperones, methionine sulfoxide reductase), and energy production (ETC Complexes). In addition to proteins, the matrix also contains mtDNA (*discussed in greater detail in Sect. 10.2*) and all the machinery required to produce the 13 proteins that are encoded by the MT genome. These 13 proteins, which are part of the ~100 subunits that make up the multisubunit Complexes of the ETC, are absolutely required for a functional ETC. Many MT diseases caused by mutations in either mtDNA or in the nuclear-encoded genes of ETC subunits have ophthalmic involvement [12]. For example, Leber Hereditary Optic Neuropathy (LHON) is maternally-inherited and caused by specific point mutations in subunits of Complex I that are encoded by the MT genome.

The MT have been referred to as the “powerhouse of the cell” because all mechanisms for generating energy (ATP), except for glycolysis, are conducted within the MT matrix. The citric acid cycle (TCA) and β -oxidation, two energy-producing pathways that use glucose and fatty acids as substrates, produce acetyl-CoA (Fig. 10.3). This product is used to generate the energy substrates NADH and FADH₂, which are utilized by the ETC in a process known as oxidative phosphorylation. The sequential transfer of electrons between each ETC Complex (I, III, IV) culminating in the reduction of oxygen to water provides the energy to pump hydrogen ions from the matrix into the Intermembrane Space. Release of hydrogen ions through Complex V (ATP synthase) into the matrix drives the phosphorylation of ADP to form ATP. Complex II, also known as succinate dehydrogenase, is the only MT enzyme that participates in both the TCA and oxidative phosphorylation. Complex II transfers electrons that are produced by the oxidation of

succinate to fumarate (TCA) to Complex III (oxidative phosphorylation).

Strong experimental evidence supports the hypothesis that RPE MT function is detrimentally altered with AMD. Direct measurement of MT function in primary RPE cultures showed that RPE from human donors with AMD exhibited reduced MT respiration [15] and ATP production [8, 15]. Data from a proteomic analysis of isolated RPE MT from human donors with AMD reported decreased content for several protein subunits of the ETC [11]. Three of the subunits with lower content are part of Complex V, which could result in decreased ATP production. Additionally, Complex V is involved in stabilizing MT morphology and maintaining membrane potential. A loss of these functions would be detrimental to cell viability. *Further discussion of how failures in retinal metabolism contribute to AMD pathology will be included in Sect. 10.3.*

While the most familiar role of the MT is in energy production, this organelle performs a number of additional functions that are essential for cell viability. As previously mentioned, MT regulate apoptosis and subsequent cell death through the release of various proteins (cytochrome c, AIF endonuclease) that are normally sequestered in the MT. They are the site of heme and steroid biosynthesis, and provide calcium buffering for the cell when cytosolic calcium levels increase over normal levels. Calcium is freely permeable through the outer membrane, but requires the aid of the calcium uniporter and Na/Ca exchanger in order to be shuttled into and out of the matrix. Not only does the MT provide buffering from excess calcium, its influx into the MT matrix plays an important role in regulating MT energy production, for example, through the activation of [isocitrate dehydrogenase](#), one of the key regulatory enzymes of the TCA. In neurons, concurrent calcium increases in the cytosol and MT act to synchronize neuronal activity with MT bioenergetics [16].

MT also serve as a signaling platform, communicating changes in the MT environment and cellular energy demands to and from the nucleus in a process referred to as anterograde/retrograde signaling (Fig. 10.3). Reactive oxygen species

(ROS), which are produced as a normal by-product of respiration, serve as signaling molecules between the MT and the nucleus. Communication between the MT and nucleus is also induced by changes in the MT electrochemical gradient, the unfolded protein response, and fluctuating levels of ATP or calcium. These varied signals initiate changes in gene expression in both the nucleus and MT to accommodate changing energy demands and cellular conditions. For example, changes in cellular ROS content can initiate signaling through redox-sensitive transcription factors, such as Nrf2 and NfKB, which are proteins that utilize the oxidation of cysteine residues as the signal for activation and respond by translocating into the nucleus and upregulating gene expression to counteract the oxidative stress. Examples of redox-regulated transcription factors include AP-1, p53, estrogen and glucocorticoid receptors, Hif-1, and Nrf-2.

Under normal conditions, these multiple signaling mechanisms play an essential role in maintaining cell health. Abnormal conditions, for example, excessive amounts of MT calcium or high ROS, can disrupt signaling and cell homeostasis. In particular, excessive ROS can cause oxidative damage to lipids, proteins, and DNA. Oxidation of lipids can disrupt membrane integrity and increase membrane permeability to previously excluded molecules. Lipid oxidation can also change the fluidity of membranes in a way that could adversely affect the function of transmembrane proteins, like ETC Complexes, whose function requires a specific lipid environment. Oxidation of the MT-specific lipid cardiolipin in the MT inner membrane disrupts its interaction with cytochrome c, which is tethered to the membrane by cardiolipin. Once cytochrome c is released into the cytosol, it activates apoptosis and causes cell death. Oxidative cleavage of lipids can also generate 4-hydroxynonenal (HNE) and carboxyethylpyrrole (CEP), which can form adducts on DNA and covalently modify lysine residues on proteins. CEP is a specific oxidative fragment of docosahexaenoic acid, which is found in neural tissue and is particularly abundant in the outer retina. Increased

CEP-adducted protein has been found in drusen, AMD patient plasma, and AMD donor photoreceptors [17, 18]. AMD-like lesions were observed in mice after immunization with CEP-adducted albumin, suggesting that this specific oxidation product may be toxic to retinal tissue [19]. Abundant HNE adducts have also been reported in retinal proteins, although HNE was not significantly increased with AMD [20]. However, the enzymes used for detoxifying HNE were significantly elevated in retinas from AMD donors suggesting that the retina had mounted a compensatory response to this specific type of lipid oxidation product.

In addition to the generation of lipid-derived adducts, ROS can also directly damage MT proteins, causing protein modifications such as carbonyls, deamidation, and modification of tyrosine to nitrotyrosine. This damage can impair the ability of MT to generate energy by affecting proteins of the ETC, TCA cycle, beta oxidation, or affect calcium buffering capacity by inactivating the calcium transporters located in the inner membrane. ROS-induced damage to mtDNA includes double- and single-strand breaks and formation of DNA adducts, which can interrupt translation and replication of the MT genome. mtDNA damage (*discussed in detail in Sect. 10.2*) can ultimately cause mutations or deletions in key MT-encoded proteins or tRNA that are required for synthesis of MT proteins.

10.2 Influence of mtDNA on Retinal Disease

To date, the nuclear DNA, with approximately 20,000 protein-coding genes, has been studied extensively for its relationship to development and diseases. In contrast, significantly less is known about the role that mtDNA plays in human pathologies. For the past 50–60 years, it has been thought that the main cellular contribution of mtDNA was energy production. However, more recent studies have demonstrated that mtDNA plays important roles in retrograde signaling (from mitochondria to nucleus), modulating nuclear genes, and encoding for

results in a mixture of nonmutated (wildtype) and mutant mtDNA within the same cell. This mixture of damaged and undamaged mtDNA is termed heteroplasmy. When cells with heteroplasmic mitochondria divide, the two types of mtDNA are randomly or in some instances nonrandomly distributed into the daughter cells [29–33]. Cells can function with relatively low levels of heteroplasmy but once this threshold is breached, abnormal functions, cell death and diseases can occur.

10.2.2 Mechanisms That Influence Disease Processes

The contribution of the mtDNA to disease and pathology can be the result of inheritable recent mutations, the ancient adaptive polymorphism changes (haplogroups) or the somatic changes associated with aging. Each mechanism will be discussed below.

10.2.2.1 Maternally Inherited mtDNA Disease-Causing Mutations

There are well described categories of diseases caused by specific mutations within the mtDNA genome, such as MELAS (Mitochondrial encephalopathy lactic acidosis and stroke-like episodes) and MERRF (Myoclonic epilepsy and ragged red fibers) [34, 35]. Individuals born with these mtDNA mutations have deficiencies in the various respiratory complexes of the ETC, resulting in decreased ATP production. They often have numerous systemic abnormalities including deafness, diabetes, myopathies, and neuropathies. The common ocular signs of these mtDNA mutations are retinal pigmentary degenerations and optic nerve atrophy. To date, there are no reports of these types of maternally inherited mtDNA mutations associated with AMD.

10.2.2.2 Maternally Inherited mtDNA Haplogroups and Their Association with AMD

Haplogroups are defined as an accumulation of mtDNA single nucleotide polymorphisms (SNP)

that can be traced through maternal lineages and represent populations of different geographic origins (Fig. 10.5). These SNP variations have occurred over more than 150,000 years in response to migration patterns and climate adaptations [3]. The oldest haplogroups are located in Africa (L0, L1, L2, L4-6). The L3 population migrated north and gave rise to founder haplogroups M, N and R. From these founder haplogroups, the European (H, T, U, V, W, X, I, J, K haplogroups), Asian (A, B, C, D, F, G haplogroups) and the most recent Native American haplogroups (A, B, C, D) have evolved. Therefore, no matter the present-day location of an individual, mtDNA analyses can identify their maternal-origin lineage. This is significant because haplogroup-defining SNP variants within the mtDNA coding region can be nonsynonymous (amino acid changing), which can alter efficiencies of energy production, causing increased ROS formation, apoptosis and cell death. If the haplogroup-defining SNP variants are found in the mtDNA Non-Coding Region, then the mtDNA replication and transcription rates can be affected. This means that each haplogroup, with its different set of SNPs, can produce unique bioenergetic properties, which may play a role in developing diseases and responding to medications.

Since AMD is found most commonly in Caucasian populations, the European haplogroups have been analyzed and it has been reported that J, T and U haplogroups are associated with AMD [36–41] while the H haplogroup has a protective effect [39]. Large, soft drusen and retinal pigment abnormalities, which are characteristics of AMD retinas, have been associated with J and U haplogroups [37]. The haplogroup T-associated SNP (A4917G), located in the NADH dehydrogenase subunit 2 of complex I (MT-ND2), is an independent predictor of AMD [36]. Two variants of the T2 haplogroup, A11812G of MT-ND4 and A14233G of MT-ND6 located in respiratory complex I, are 2.5 times more likely to be associated with advanced AMD than the age-matched control subjects [40]. Additionally, Udari and coworkers

Simplified mtDNA lineages

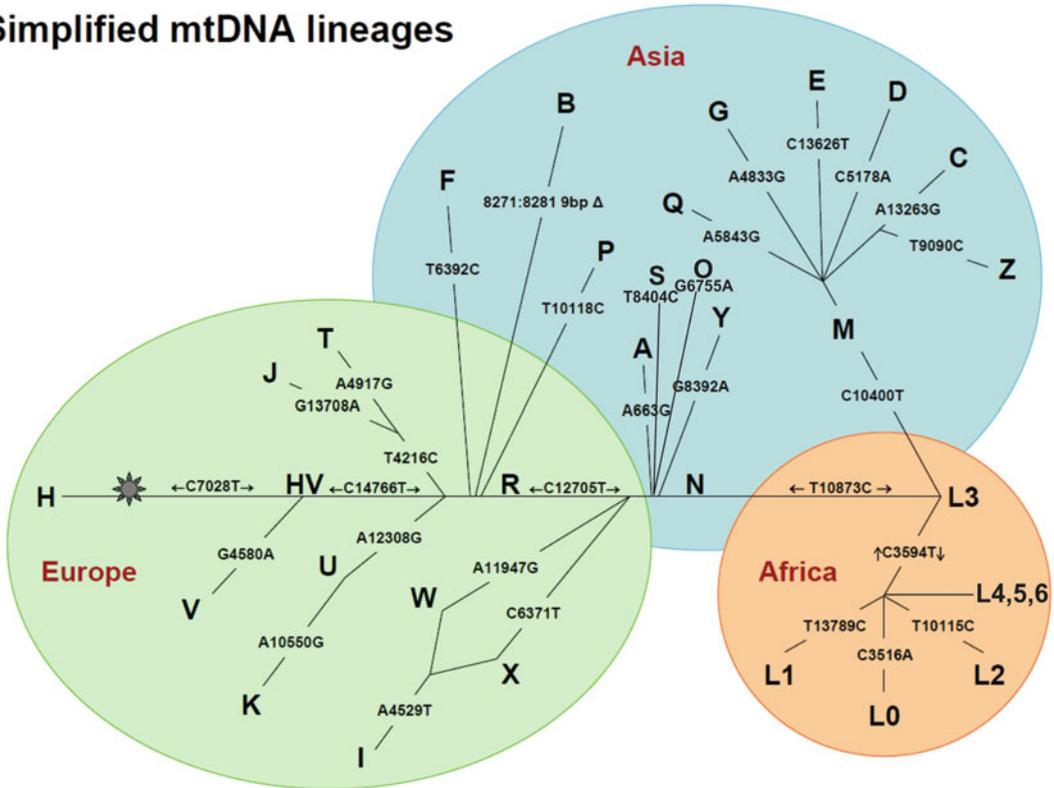


Fig. 10.5 Schematic of the major, high branching haplogroup-defining mitochondrial SNPs. The SNPs on the left side of the position number is the rCRS nucleotide (GeneBank NC_012920) and defines that haplogroup. The

diverging haplogroup is represented by the SNP on the right of the number (Modified from www.mitomap.org, Illustration by S. Atilano)

found that patients with late AMD showed strong association with noncoding mtDNA D-loop SNPs in the neural retina [41].

Our knowledge and understanding of the effects of mtDNA upon cellular homeostasis has been advanced through the use of the trans-mitochondrial cybrid model, which are cell lines with identical nuclei, but contain MT from different subjects. Using human retinal pigment epithelial cell cybrids, it has been demonstrated that mtDNA can greatly affect growth rates of cells, bioenergetics and expression levels of nuclear genes for complement, inflammation and angiogenesis, pathways which are important in human retinal diseases [23, 42–45]. In addition, cybrids with H (protective against AMD) versus J (high risk for AMD) mtDNA haplogroups have different responses to heat stress, hydrogen peroxide,

and ultraviolet radiation [42, 46–48]. These studies demonstrate that an individual's mtDNA background sets up baseline cellular homeostasis, making the cells differentially susceptible to identical stressors.

The mechanism(s) by which mtDNA haplogroups influence AMD risk is unclear. It is known that different SNP variants in MT can alter cellular bioenergetics causing partial uncoupling of oxidative phosphorylation and decreased efficiency of ATP production [49, 50]. If MT-generated energy levels decline below a specific bioenergetic threshold, the MT permeability transition pore (mtPTP) can be opened and the apoptosis pathway activated. Models of conplastic strains of mice (where the nuclear genome from one strain has been crossed onto the cytoplasm of another, designated as Nuclear

genome-mtCytoplasmic genome) have also demonstrated that the mtDNA affects pathways related to behavior, immunity and cognition [51].

There are a number of ocular diseases associated with specific haplogroups [52–57] but the literature lacks reports examining thousands of patients. The analyses for mtDNA haplogroups and variants are often difficult because the mitochondrial protocols are more limited than those available for nuclear gene analyses. The common approaches to characterize mtDNA patterns are to isolate the total DNA from blood or saliva, then characterize the lineages by identifying the haplogroup defining SNPs (Fig. 10.5) using PCR/RFLP (polymerase chain reaction/restriction fragment length polymorphism), custom designed TaqMan probes and/or whole mitochondrial DNA sequencing. At present, the platforms/chips used for GWAS (genome wide association studies) do not have enough SNPs on them to determine haplogroups accurately, making it very difficult to use that format for large scale mtDNA classification. In addition, the mtDNA SNP numbering on the commercial arrays is variable, making analyses of the data inconsistent and unreliable. Until this technical problem is resolved, it is unlikely that analyses for large numbers of AMD populations will be available.

10.2.2.3 Somatic Age-Related mtDNA Damage Associated with AMD

The third mechanism by which mtDNA can affect disease processes is through the cumulative mtDNA damage associated with aging. Human and animal studies show, the levels of mtDNA fragmentation/degradation/deletion is increased significantly with aging [58, 59]. Environmental factors, such as oxidative stress and ultraviolet radiation, can cause strand breaks, deletions and new SNP variants. In addition, one of the errors of double-strand break repair is the deletion of a 4977 base pair region of the MT genome called the “Common Deletion” (Fig. 10.4). This deletion accumulates with age in postmitotic tissue, such as brain, skeletal muscle, heart and RPE [60, 61]. Several factors make mtDNA more susceptible to damage, including the lack of protective histones and its location within the MT

matrix, where it is in close proximity to sites of ROS formation. Importantly, MT have poor DNA repair processes, thereby allowing the damage to accumulate with aging.

There are numerous reports of MT abnormalities, including mtDNA damage, in both the neural retina and RPE with aging and AMD [6, 11, 58, 60, 62–65]. It was reported that sequence analyses of the mtDNA D-loop from the neural retina found a significantly greater number of SNPs per person in the AMD population compared to either older or younger normal groups [62]. Increased oxidative DNA damage in retinas from donors with AMD was also shown using immune-histochemical staining with antibodies that recognize 8-OH-dG, a specific DNA adduct generated by ROS that serves as a biomarker of oxidative stress [41]. In the RPE from donors with AMD, an accumulation of mtDNA oxidative damage was found compared to RPE from age-matched normal donors [60, 65]. These findings were supported by analysis of primary cultures of RPE cells where increased DNA damage and fivefold increased somatic mutations were observed in RPE from AMD donors compared with age-matched controls [66]. Interestingly, the increased mtDNA damage associated with AMD progression occurs in the RPE but not the neural retina, and is localized to specific MT genome regions, including the regulatory D-loop [65]. Deletions occurring in coding regions for subunits of Complex I (ND3, ND4, ND4L, ND5), Complex IV (Cytochrome Oxidase III), or Complex V (ATPase 6, ATPase 8), could have tremendous impact on cellular bioenergetics and functions. Furthermore, this type of damage can increase heteroplasmy (mixture of damaged and undamaged mtDNA) and decrease copy numbers.

Using a model of AMD cybrid cells (RPE cell lines with identical nuclei but MT from individual AMD patients) the destructive impact that damaged MT have on retinal cells has been demonstrated [24, 67]. When MT from AMD subjects are placed into human retinal cell lines, there was a significant loss of viability, impaired MT functions, upregulation of pro-apoptosis, pro-autophagy, pro-ER stress genes, and enhanced vulnerability to oxidative stress.

Importantly, Nashine and coworkers showed that although damaged, these AMD MT can be rescued by treatment with Humanin-G, a cytoprotective peptide that increased the cellular longevity [24]. Humanin is one of several MT-derived peptides that are encoded by the MT genome within the **16S ribosomal RNA** gene. The role of these MT-derived peptides in MT metabolic control and cytoprotection is currently being explored [68]. Initial results are encouraging researchers to pursue the MT-targeting therapies to prevent cell death associated with AMD. It is highly likely that future investigations will yield multiprong approaches to further characterize the damaged AMD MT and identify novel therapeutic agents to restore the defective MT to healthier status.

10.3 Introduction to the Metabolic Ecosystem Concept and Its Link to AMD Pathology

Section 10.3 will discuss the concept of a retina/RPE ecosystem by first providing an overview of the pertinent metabolic features and pathways within individual retinal cells. The final section will consider the idea that a bioenergetic crisis in the RPE is the primary event that tips the metabolic balance toward AMD [69] and addresses the critical question of why the macula is preferentially and most severely affected with AMD.

The idea of a metabolic ecosystem within the eye makes sense when one considers the many important and diverse metabolic interactions that occur between organs and tissues in a whole organism. All living cells convert fuels into metabolic energy. Each type of cell adapts its biochemical machinery to make the most effective use of the types of fuels available to it and to the timing and magnitude of its energy needs. Cells within an organism generally have metabolic features that allow them to support each other. For example, when muscles are highly active, they can produce pyruvate at a rate faster than it can be oxidized by the cell's MT. Glycolysis requires a continuous supply of NAD⁺ to oxidize glucose. The muscles are able to sustain energy

production through glycolysis by regenerating NAD⁺ via reduction of pyruvate to lactate. Remarkably, the liver can recycle the lactate. Lactate is exported from muscles to the blood and imported into liver cells. Energy from fatty acid oxidation in the liver cells can then be used to reduce the lactate to glucose. The glucose is exported from the liver and then taken up by the muscles to support the continuing energy demands of the active muscle. The metabolic interdependence in this classic biochemistry example highlights how distinct cells with specialized metabolic features can support each other and how they function as a metabolic ecosystem.

10.3.1 Metabolic Features of the Retina

10.3.1.1 Aerobic Glycolysis in the Retina

Cells within the vertebrate eye also have specialized metabolic features. This was discovered in the early 1920s by Krebs and Warburg when they were exploring glycolytic and oxidative metabolism in a variety of tissues [70, 71]. They noted that tumors and retinas are especially glycolytic, meaning that the ratio of lactate production to O₂ consumption is substantially higher in these tissues compared to other tissues they had examined. They observed that lactate was produced even when plenty of O₂ was available. Since then that finding has been confirmed many times over [72–77]. Production of lactate caused by a shortage of O₂ is common and is referred to as “anaerobic glycolysis.” Production of lactate by retinas or tumors is referred to as “aerobic glycolysis” because it occurs even when O₂ is readily available [78]. In recognition of its discovery by Warburg, this type of metabolism is referred to as the “Warburg Effect.”

The site of aerobic glycolysis in retinas hasn't been identified directly, but several observations indicate it occurs in photoreceptors. Most of the lactate produced by an eye comes from the outer retina, which is made up primarily of photoreceptors [76]. Hexokinase, which catalyzes the first step in glycolysis is enriched in the outer

retina [79–81] and specialized isoforms of glycolytic enzymes like hexokinase II [80, 81] and pyruvate kinase M2 [72, 82–84] are specifically expressed in photoreceptors. Lactate dehydrogenase A is expressed at high levels in photoreceptors [72, 82, 85]. Inactivating pyruvate kinase, lactate dehydrogenase [72] or hexokinase II [80] expression in rods alters their morphology in ways that appear to be cell-autonomous [72]. A fluorescent glucose derivative gavaged into the stomachs of either mice or zebrafish [86] or injected into the tail veins of mice [87] accumulates in the retinas, primarily in photoreceptors.

10.3.1.2 Mitochondria in Photoreceptors

Retinas can consume other fuels including glutamine, lactate, pyruvate [88], fatty acids and ketone bodies [89, 90]. Those fuels require MT to oxidize them and the retinas are very capable of actively consuming O_2 . Linsenmeier's and Cringle's *in vivo* measurements [91, 92] of the distributions of O_2 in retinas have shown that nearly all of the O_2 available from the choroid circulation can be consumed by the outer retina, most likely by the photoreceptors.

MT are abundant in rods and cones [93]. They are in a region of the photoreceptor just below the base of the outer segment known as the ellipsoid. When there is inner retinal vascularization, as is the case for humans, mitochondria also accumulate at photoreceptor synaptic terminals [94]. In animals such as frogs, rabbits, guinea pigs, horses and birds, the inner retinas are mostly avascular [95]. Those retinas do not have a substantial source of O_2 from the inner retina. Photoreceptor synapses in avascular retinas can obtain ATP made at the ellipsoid and transferred to the synapse via a phosphocreatine shuttle [96]. An isoform of creatine kinase that localizes to mitochondria can make phosphocreatine from ATP at the ellipsoid, which then can diffuse from the ellipsoid to the synaptic terminal where the phosphocreatine is used by another isoform of creatine kinase to make ATP. Measurements of metabolic flux through mitochondrial intermediates revealed that some aspects of mitochondrial function are more active in darkness

than in light [74]. This could be a consequence of the higher cytosolic free Ca^{2+} levels in darkness. Higher cytosolic Ca^{2+} can alter the concentration of free Ca^{2+} in the mitochondrial matrix [97]. Flux measurements suggest oxidation of α -ketoglutarate to succinyl CoA, a step in the TCA cycle, is stimulated in darkness compared to in light [74], consistent with findings that Ca^{2+} can stimulate α -ketoglutarate dehydrogenase activity *in vitro* [98]. Mitochondria in retinas appear relatively uncoupled [74] and consumption of O_2 does not appear to be limited by ATP demand [74, 99, 100].

MT morphology in photoreceptors is striking and diverse [86, 96, 101]. For example, MT in mammalian rods is highly elongated and line up close to the plasma membrane in parallel with the major axis of the rod cell. In contrast, MT in cones of zebrafish retinas coalesces into a tightly packed cluster just below the outer segment. The tight cluster of MT in the zebrafish cones can maintain distinct pools of Ca^{2+} in the outer vs. inner segments of a cone cell [97]. There also is evidence that MT can influence the optical properties of photoreceptors [93]. Any advantages that these unique and diverse structures provide to photoreceptors still need to be identified. MT are more abundant and may be more active in cones [101]. In primate retinas the sizes and volumes of MT vary throughout the fovea, perifovea and peripheral regions of the retina [93]. The morphologies of these MT change when photoreceptors become affected by AMD and the changes can be followed by Optical Coherence Tomography (OCT) [102].

10.3.1.3 Müller Cells

Müller glia span the retina radially with their apical processes extending around the inner segments of the photoreceptors and their end feet at the inner surface of the retina. Just below the apical processes the junctions between Müller cells create a visible structure referred to as the outer limiting membrane. Within the retina the Müller cells extend horizontal processes of diverse morphology that infiltrate between neurons.

Immunohistochemical studies suggest that Müller cells may not have some of the enzymes needed for glycolysis. Pyruvate kinase and hexokinase, which are detected readily in photoreceptors, have not yet been detected in Müller cells [83, 85]. Immunocytochemistry indicates that Müller cells also lack a key enzyme required for the malate-aspartate shuttle that shuttles electrons from glycolysis into MT [103]. It is important to know whether the portion of Müller cells in the outer retina has glucose transporters, which are required for direct uptake of glucose. However, the overlap of Müller cell apical processes with the ellipsoid region of rods, which contain the GLUT-1 glucose transporter, makes it difficult to resolve unambiguously the localization in the apical processes of enzymes and transporters. Co-labeling with several rod and Müller cell-specific antibodies suggest that GLUT-1 in Müller cells is abundant in the end-feet but not in the apical processes [86]. However, more precise and higher-resolution studies will be needed to resolve this definitively. Müller cell MT are small and abundant just underneath the apical processes [86]. Glial cells are the only cells in the retina with the enzyme, glutamine synthetase, needed to synthesize glutamine. The ability of retinas to synthesize glutamine from lactate and aspartate indicates that Müller glia are capable of taking up and metabolizing lactate [83].

10.3.1.4 Glycogen in Retinas

Glycogen is present in retinas. It is most abundant in the end-feet of Müller cells. Glycogen also can be detected in cone photoreceptors. The amount of glycogen stored in Müller cell end feet can vary depending on the amount of glucose available to the retina [104]. There is a report that Müller cells express the enzymes needed for gluconeogenesis [105]. This is an important observation, because gluconeogenesis from lactate could produce glucose for glycogen storage to support the activity of inner retinal neurons. However, that line of investigation has not been pursued beyond the initial reports 30 years ago and needs further confirmation. Glycogen and

gluconeogenesis in Müller cells could be fundamentally important for retinal survival and function.

10.3.2 Metabolic Features of the RPE

RPE cells form a cellular monolayer between the choriocapillaris and retina [106]. They are sealed together by tight junctions and function as a blood retina barrier for the outer retina. To reach the retina, fuels and metabolites from blood in the choriocapillaris must pass through membrane transporters in both the basolateral and apical plasma surfaces of RPE cells. Glucose from the blood must be able to reach the retina to support the high glycolytic activity of the retina. The glucose transporter, GLUT-1, is exceptionally abundant on both surfaces of the RPE [107, 108]. Monocarboxylate transporters are differentially distributed to the two surfaces of the RPE. MCT1 is on the apical side whereas MCT3 is on the basolateral side [109].

RPE cells perform vital functions that support the retina [106]. Once a day they phagocytose the tips of the rod outer segments and either metabolize their contents or recycle them back to the retina. RPE cells also have the capacity to esterify and isomerize all-trans retinol, a product of photobleaching visual pigments [110]. This is an essential process in the pathway for regeneration of rhodopsin after it has undergone photobleaching in rod outer segments.

10.3.2.1 Glycolysis in the RPE

The exceptional abundance of glucose transporters on both sides of the RPE [107, 108] highlights how RPE cells are adapted to facilitate transport of as much glucose as possible from the choriocapillaris to the retina. Consistent with this important metabolic function, RPE cells consume glucose much more slowly than retina. Like all cells, RPE cells need fuel for energy and anabolic activities. Compared to retinas, RPE cells have a limited ability to use glucose. However, they can oxidize lactic acid, glutamine, and fatty acids as alternative fuels [74, 86, 90, 111]. RPE cells also actively consume proline and they can export a

variety of metabolites involved in energy production and anabolic activities [111]. RPE cell metabolism is more enriched than the retina in two other important metabolic pathways, reductive carboxylation of α -ketoglutarate to produce citrate [112], and carboxylation of pyruvate to produce oxaloacetate [86]. All of these metabolic features appear to be adaptations that can sustain metabolic requirements of RPE cells while minimizing their dependence on glucose.

10.3.2.2 Glycogen in the RPE

RPE cells store glycogen. The amount of stored glycogen in cultured human retinal epithelial cells increases when there is abundant glucose in the medium in which they grow and decreases when glucose levels in the medium have been depleted [113]. These findings suggest that glycogen stored in RPE cells can ensure a steady supply of glucose to the retina, but this potentially critically important activity of RPE cells has not yet been investigated directly.

10.3.3 Metabolic Synergism in the Eye: A Retina/RPE Ecosystem

As described in the preceding sections RPE cells and the neurons and glia of the retina have diverse and specialized metabolic features. The distinct metabolic functions of cells in the eye appear generally analogous to the distinct and diverse metabolic roles of various types of plants and animals in the ecosystem of a forest or ocean.

Based on currently available data it is likely that the retina/RPE metabolic ecosystem functions as portrayed in the schematic model in Fig. 10.6 [86]. Glucose from the choriocapillaris enters and leaves RPE cells through abundant GLUT-1 glucose transporters on the basolateral and apical surfaces of the RPE cells. When glucose reaches photoreceptors most of it is oxidized rapidly by glycolysis and then reduced to lactate. The lactate, with metabolic energy still remaining within its chemical bonds, is exported from the photoreceptors to both the RPE and to Müller cells where it can be oxidized by MT to drive synthesis of ATP.

A significant advantage of this type of metabolic strategy is that oxidation of lactate imported into RPE cells can deplete NAD^+ in the RPE cells. Since NAD^+ is needed for glycolysis, depletion of NAD^+ by lactate further suppresses glycolysis in RPE cells so that more glucose can pass through the RPE and reach the retina.

10.3.3.1 Findings from In Vivo Experiments that Support the Concept of an Essential Retina/RPE Ecosystem

This concept of metabolic interdependence is generally supported by the results of several recent experiments. Genetic manipulations that affect a specific type of cell in the retina or RPE affect the viability not only of that specific type of cell, but also of other types of cells that were not affected directly by the mutation. Some of these examples are described briefly in the following paragraphs.

Example 1 Cone viability depends on the presence of rods. Rods are more abundant than cones in human and mouse retinas. Even within the human macula there are 9 times more rods than cones [114]. Although rods and cones carry out similar functions in the eye, many of the phototransduction genes that have specific roles in rods are not needed and are not expressed in cones because other genes fulfill those functions in cones [115]. For example, PDE6 β , PDE6 γ and rhodopsin are expressed only in rods, not in cones. A mutation in any of these genes can directly disrupt rod viability. However, after the rods degenerate, cones then also begin to degenerate, even though the mutation did not influence cones directly. Recent findings provide clues about the nature of this “bystander effect.” As rods die, cones show evidence of nutrient deprivation [116]. The evidence is a change in the phosphorylation state of mTOR, a protein that senses the availability of nutrients. Recent findings have identified two biochemical mechanisms that may be responsible for depriving cones of essential nutrients. First, rods in a normal retina produce and release a polypeptide, rod-derived cone viability factor (RdCVF)

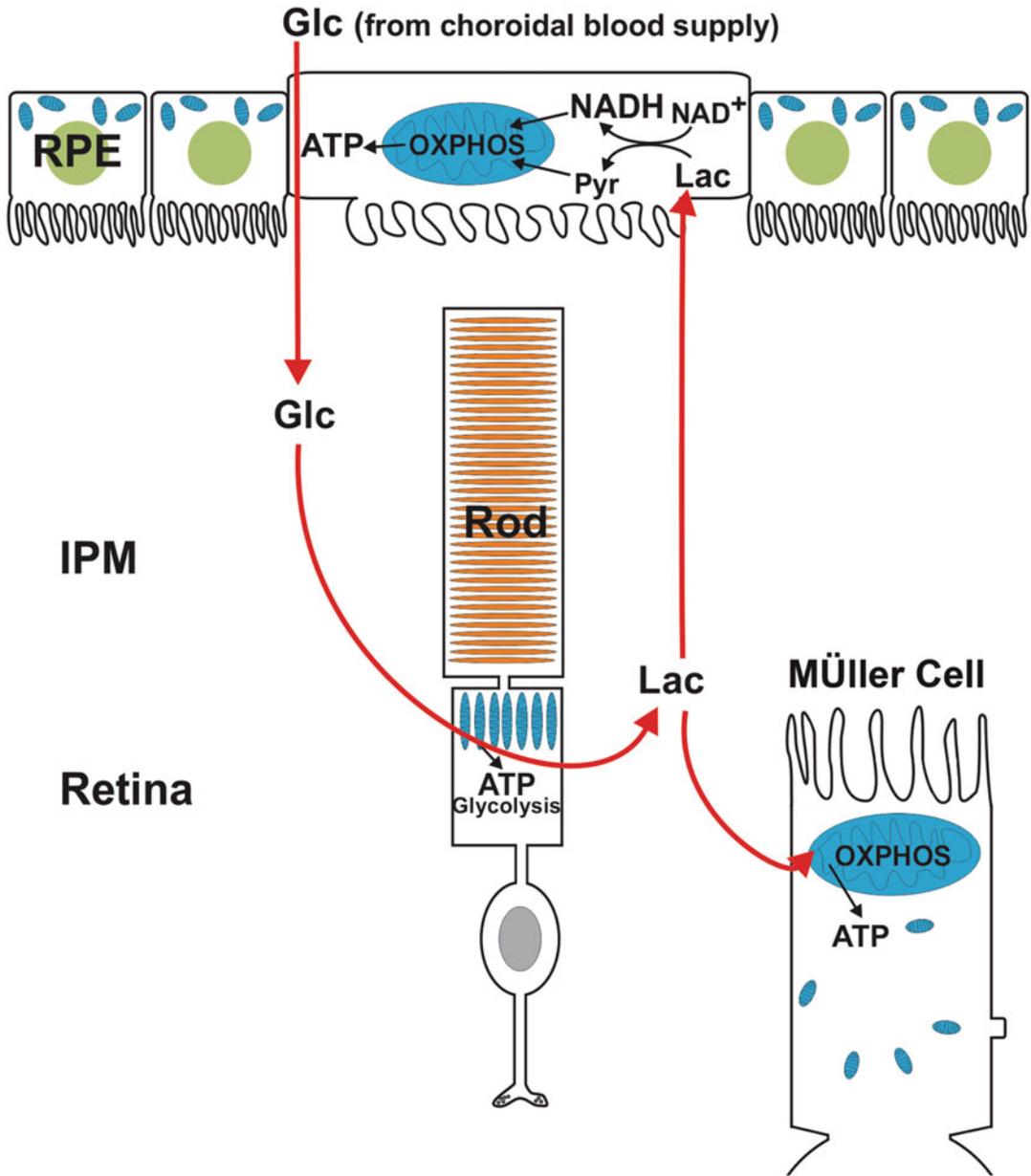


Fig. 10.6 Summary of the metabolic interactions between RPE, photoreceptors and Müller cells. Glucose from the choroidal blood supply passes through glucose transporters on the basolateral and apical surfaces of the RPE to reach the retina. The glucose that reaches the Interphotoreceptor Matrix (IPM) is taken up by glucose transporters on the plasma membrane of the rod inner

segments. Glucose in the rod cell is converted rapidly to lactate by aerobic glycolysis and then released from the photoreceptor. The lactate released can be used either by Müller cells or by the RPE. Studies have shown that lactate can suppress glycolysis in the RPE to minimize consumption of glucose by the RPE so that more glucose can reach the retina

[117, 118]. RdCVF interacts with a protein, basigin, on cones, that stabilizes or stimulates the activity of glucose transporters on the cone

plasma membrane. When rods degenerate, RdCVF no longer is released by rods, glucose transporters on cones are not stabilized and

cones starve. The absence of rods also can cause cones to starve because glucose becomes trapped within the RPE. Normally, when a fluorescent glucose derivative is injected into the tail veins of mice it passes through the RPE and accumulates in photoreceptors in the retina [87]. However, as rods degenerate the fluorescent glucose becomes trapped within the RPE making it inaccessible to the cones [117, 118]. Part of this glucose trapping may be caused by failure of the ecosystem (Fig. 10.6). Less lactate will be made by the retina in the absence of rods. The RPE will rely on glucose instead of lactate for fuel so less glucose reaches the retina. Other effects of rod loss on RPE function also may contribute to glucose trapping.

Example 2 *The viability of rods relies on mitochondrial activity in RPE cells.* MT require a specific transcription factor, TFAM, for expression of genes encoded by mitochondrial DNA. When TFAM is inactivated specifically in RPE cells, the metabolism of the RPE cells become less specialized and consumption of glucose by glycolysis increases [119]. This causes rod photoreceptors in the retina to degenerate. This observation is consistent with two ideas: (i) RPE minimizes its consumption of glucose so sufficient glucose is available to fuel the retina and (ii) maintenance of ATP production and other MT functions in the RPE is essential for retinal health.

Example 3 *The viability of rods relies on restraint of glycolytic activity in RPE cells.* Unrestrained glycolytic activity in RPE cells could cause the RPE to consume so much glucose that not enough glucose passes through the RPE to fuel the retina. Hypoxia-Inducible factor (HIF1 α) can stimulate transcription of genes that encode the enzymes that catalyze glycolysis. Von Hippel-Lindau Factor (VHL) is a protein that suppresses glycolysis by helping to degrade HIF. When VHL expression is blocked specifically in the RPE, HIF1 α is stabilized. That makes the RPE more glycolytic and it causes photoreceptors to degenerate [120], consistent with the model in Fig. 10.6.

Example 4 *Robustness is enhanced when glycolytic activity is increased in rods.* Photoreceptor degeneration can be induced by deleterious mutations in genes that encode subunits of a rod cGMP phosphodiesterase. Normal retinas are highly glycolytic. However, expression of glycolysis genes normally is limited by expression of a protein, SIRT6. When SIRT6 expression is blocked in photoreceptors, the rate of glycolysis in retinas increases several folds above its already high level [121]. Degeneration caused by a PDE6 mutation is slowed substantially when glycolysis is enhanced by SIRT6 inactivation. This is consistent with the model in Fig. 10.6 because enhanced lactate production from the mutant rods can suppress RPE glycolysis so more glucose can reach the retina.

Taken together, all of these findings support the idea that diverse metabolic features of cells in the retina and RPE contribute specific metabolic roles that sustain the viability and function of cells in a metabolic ecosystem.

10.3.4 Relationship Between AMD and the Metabolic Ecosystem of the RPE and Retina

The observations that enhanced glycolysis in RPE cells induces rod degeneration and that enhanced glycolysis in photoreceptors makes them more robust suggest that the state of the retina/RPE metabolic ecosystem can influence degenerative diseases, such as AMD. The distribution of rods and cones and the distributions and volumes of ellipsoid MT in human retinas are not homogeneous [93, 122–125]. It is likely that the efficiency of the RPE/retina metabolic ecosystem depends heavily on RPE MT being able to use lactate, glutamine, fatty acids and proline as fuels to minimize consumption of glucose. Human RPE MT accumulate DNA damage with advancing age [60, 66] and cumulative damage compromises their metabolic activity [8, 15]. Since the RPE MT have a central role in supporting the metabolic ecosystem sketched in

Fig. 10.6, the regions of the nonhomogeneous retina that are most dependent on the metabolic ecosystem ought to be the first regions where photoreceptor stress and damage become evident. Since cones in the central fovea are more enriched in MT than rods [93] they may be less dependent on the metabolic ecosystem, that is, they may be more capable of efficiently using limited amounts of glucose than other regions of the retina. Just outside the central fovea, rods become more abundant, but since they are more sparse in this region their net output of lactate would be limited. This could make the rods in the perifoveal region the most reliant on RPE MT and therefore most sensitive to damage of RPE MT. This hypothesis may help explain why AMD correlates with RPE MT damage and why it initially affects perifoveal rods. Further studies in which the metabolic capabilities of the central fovea, perifovea, and peripheral retinas are compared in nonhuman primate retinas and in human donor retinas will be required to evaluate the validity of this hypothesis.

10.4 Enhancing MT Function and Bioenergetics As a Therapeutic Strategy for Treating AMD

Studies suggest that metabolic dysregulation may be a major contributing factor to disease pathogenesis in AMD [15, 65, 69, 86]. Mutations in over 200 genes have been associated with retinal degenerations (RetNet, [126]), and diseases such as AMD are multifactorial, with genes and environment contributing to disease. Due to the large number of mutations and factors that can lead to retinal degeneration, gene independent strategies that focus on targeting the biological pathways that lead to retinal degeneration or retinal neuroprotection are crucial. Based on strong evidence that dysfunction of the metabolic ecosystem leads to the retinal degeneration associated with AMD, pathways related to energy metabolism, MT biogenesis, and oxidative stress may be ideal targets to treat AMD. This section will discuss several studies that have examined the role

of these pathways in AMD and how targeting these pathways may be neuroprotective.

10.4.1 Energy Metabolism

10.4.1.1 AMPK

A viable strategy for treating AMD is to target pathways that stimulate or shift the metabolic ecosystem back to its homeostatic state. An ideal target for enhancing metabolic function is a key pathway involved in regulating energy levels in the cell. One such protein involved in regulating cellular energy metabolism in a variety of tissues is 5' adenosine monophosphate protein kinase (AMPK). AMPK is activated by cellular metabolic stress and is expressed ubiquitously all eukaryotic cells. AMPK directly binds AMP, ADP, or ATP allowing it to detect energy levels in the cell. AMPK is activated by upstream kinases, including liver kinase B1 (LKB1) and Ca/calmodulin-activated protein kinase (CaMKK β), as well as pharmacological activators, such as AICAR and metformin [127]. Activation of AMPK promotes downstream energy producing pathways, including glucose metabolism, MT function, and autophagy, and inhibits downstream energy consuming pathways, including protein synthesis and fatty acid metabolism. This makes AMPK an ideal target for diseases such as AMD, where MT and metabolic dysfunction likely plays an important role in disease pathogenesis.

Several studies have examined the role of AMPK and upstream kinase LKB1 in the retina. Interestingly, LKB1 expression in the retina decreases with age in mice [128]. Studies examining conditional deletion of LKB1 in retinal progenitor cells found that deletion of LKB1 results in loss of ONL thickness, shifts in synaptic positioning, a reduction in the total number of synapses, and loss of rod and cone function. Knocking down AMPK, produced a similar phenotype, suggesting that AMPK plays an important role in this process. Interestingly, the authors were able to reverse the phenotype by 20% using metformin to activate AMPK and by 50% using caloric restriction to activate AMPK. Conversely,

feeding the animals a high fat diet resulted in a 70% increase in synaptic mislocalization. The authors found a reduction of phosphorylated, or active, AMPK in older mice, suggesting that levels of activated AMPK decrease in the retina with age [128].

Other studies have investigated metformin-mediated activation of AMPK as a neuroprotective therapy. Studies have shown that systemic metformin treatment is able to activate AMPK in the retina [129]. Metformin treatment preserved retinal function and morphology in mouse models of light-induced retinal degeneration, the Rd10 model of inherited retinal degeneration, and was protective to the RPE in a model of RPE injury using sodium iodate [129]. Metformin protection was dose-dependent, and was associated with increased mitochondrial DNA copy number, increased ATP levels, and reduced levels of oxidative stress and DNA damage. Importantly, metformin was no longer protective with deletion of AMPK α 1 and α 2 subunits in the retina, suggesting that AMPK is necessary for metformin-induced protection, and that metformin protection is due to local activation of AMPK in the retina [129]. These results suggest that promotion of MT function and retinal metabolism with metformin treatment is neuroprotective to the retina. Therefore, caloric restriction, caloric restriction mimetics, or activators of AMPK are potential neuroprotective therapies that could stimulate retinal energy metabolism homeostasis to prevent retinal degeneration in AMD.

10.4.1.2 Insulin/mTOR

Another metabolic pathway that is a potential neuroprotective target is the mTOR (mammalian target of rapamycin) pathway. mTOR is a key regulator of metabolism and cell growth and is found in two complexes, mTORC1 or mTORC2. mTORC1 regulates cell metabolism and protein synthesis while mTORC2 regulates pro-cell survival mechanisms. Deletion of mTORC1 or mTORC2 individually in the retina does not affect function or survival of cones up to 1 year of age but does result in alterations of outer and inner segment morphology [130]. Deletion of

both mTORC1 and mTORC2 in the retina leads to a loss of cone function, but not cone death [131]. These findings suggest that due to the high metabolic activity of healthy cones, the role of mTOR in regulation of metabolism in healthy cones is minimal.

Although the insulin/mTOR signaling pathway may play a minor role in healthy cones, insulin/mTOR signaling may play a larger role in cones under conditions of stress. Using four models of retinitis pigmentosa, Punzo et al. examined changes in gene expression at various points of cone degeneration [116]. Interestingly, 36% of the genes that were upregulated at least twofold upon secondary death of cones were related to cellular metabolism. The insulin/mTOR signaling pathway had the highest number of hits, suggesting that metabolism and mTOR signaling play an important role in the cone death process. Increases in levels of heterodimeric transcription factor hypoxia inducible factor 1 (HIF-1 α /b), which increases glycolysis under stressful conditions such as those of low oxygen, and GLUT1, a glucose transporter expressed in the photoreceptors, were observed. This suggests that cone death may be due to compromised glucose uptake in the cones, resulting in starvation. Stimulation of the mTOR pathway with insulin treatment resulted in enhanced survival of cones, while inhibition of insulin with injection of streptozotocin, a drug that kills the insulin-producing beta cells of the pancreas, resulted in decreased survival of cones [116].

Other studies have shown that constitutive activation of mTORC1 is able to preserve function and survival of cones in mouse models of retinitis pigmentosa, and that mTORC1 is required for the protective effect of activating insulin/mTOR pathway [130]. Activation of mTORC1 resulted in increased uptake and utilization of glucose and elevated levels of NADPH. Loss of the mTORC1 accessory protein, RAPTOR, resulted in accelerated retinal degeneration in disease models, but had no effect on retinal function or cell survival when it was deleted in a wildtype retina. Activation of mTORC1 was associated with increased expression of genes related to uptake, retention, and utilization of

glucose [130]. These findings suggest that mTORC1 specifically plays an important role in maintaining cone function in disease, and this protection may be mediated through enhanced uptake, retention, and utilization of glucose.

Although these studies focus on models of retinitis pigmentosa rather than AMD, the dysregulation of metabolism observed in these models may reflect pathological features of AMD under conditions of stress, particularly, when there is a reduction in glucose levels reaching the photoreceptors due to increased glycolysis in the RPE. These results suggest that metabolic imbalance is a major contributing factor to loss of cones, and stimulation of pathways such as the insulin/mTOR signaling pathway may be protective to cones under conditions of reduced glucose levels. Further studies examining the role of insulin/mTOR signaling in energy metabolism in AMD are necessary to further investigate this possibility.

10.4.1.3 CoQ10

Coenzyme Q10 (CoQ10), also known as ubiquinone, is ubiquitously expressed, and is localized primarily in the MT. CoQ10 is a component of the electron transport chain acting as an electron carrier. It also has antioxidant capabilities and can affect metabolism-related gene expression. Studies have shown that CoQ10 levels decline in a variety of tissues in the human body with age, including the retina [132]. A randomized, double-blind, placebo controlled clinical trial investigated the efficacy of using a combination of acetyl-L-carnitine, n-3 fatty acids, and CoQ10 in subjects with early AMD [133]. The aim of this therapy was to target MT lipid metabolism for a metabolic therapy to prevent AMD progression. Treatment resulted in preservation of visual field function, as compared to placebo-treated controls, and a reduction in drusen area [133]. These results suggest that using CoQ10 to improve retinal metabolism may reduce progression of early AMD and may be a potential therapeutic target for the disease. Targeting pathways that enhance energy metabolism or promote energy homeostasis in the retina may represent a suitable target for therapies for AMD.

10.4.2 Oxidative Stress and Mitochondrial Biogenesis

10.4.2.1 Nrf2

Other neuroprotective avenues for AMD include reducing levels of oxidative stress through targeting antioxidant pathways. A reduction in oxidative stress in the RPE and retina would likely help prevent oxidative DNA damage to mtDNA, nuclear DNA, and prevent oxidation of lipids and proteins. Nrf2 is a master transcription factor that regulates the antioxidant response in virtually all cell types. Several studies have focused on targeting Nrf2 to reduce levels of oxidative stress in AMD. In unstressed conditions, Nrf2 is held in the cytoplasm by Keap1, which facilitates its ubiquitination and subsequent degradation. However, in conditions of oxidative stress, Keap1 structure is disrupted, preventing it from interacting with Nrf2, which allows Nrf2 to translocate to the nucleus and activate the antioxidant response element. Studies have shown that AAV-mediated gene therapy delivering an Nrf2-derived peptide that interacts with Keap1 to prevent endogenous Nrf2 degradation is able to increase antioxidant expression and protect retinal function and morphology in response to sodium iodate-induced RPE damage [134]. Other groups have shown that Nrf2 upregulation in the retinas of mice, via AAV-mediated gene delivery, resulted in protection from damaging light levels [135]. Targeting Nrf2 to reduce levels of oxidative stress may protect MT from oxidative damage and help preserve RPE function in AMD.

10.4.2.2 PGC-1 α

Another potentially neuroprotective target to enhance mitochondrial bioenergetics is peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α). PGC-1 α is a transcriptional coactivator that is involved in regulation of many genes involved in energy metabolism, including those involved in MT biogenesis. PGC-1 α is activated by increased levels of oxidative stress and by direct phosphorylation by AMPK [136].

Studies have shown that overexpression of PGC-1 α in the RPE leads to increased expression of genes associated with fatty acid oxidation, MT respiration, oxidative metabolism, and antioxidants [137]. This increase in antioxidant expression correlated with an increased ability for the RPE cells to cope with oxidative insults, including H₂O₂ and hydroquinone (an oxidant found in cigarette smoke) [137]. As MT dysfunction and elevated levels of oxidative stress in the RPE are associated with AMD, overexpression of PGC-1 α may represent an important target for potential therapies for the disease.

Other studies suggest that PGC-1 α plays an important role *in vivo*. Mice heterozygous for PGC-1 α that were fed a high fat diet for 4 months exhibited RPE and photoreceptor degeneration. This was associated with an accumulation of lipofuscin, basal laminar deposits, Bruch's membrane thickening, and deposits containing proteins with oxidative damage [138]. This phenotype may be due to reduced MT function, as these mice had decreased levels of mtDNA copy number, reduced activity of MT oxidative phosphorylation Complex I, and increased levels of reactive oxygen species [138]. These findings suggest that decreased PGC-1 α expression in AMD may contribute to disease phenotypes. Targeting PGC-1 α , through indirect activation using an AMPK activator, such as metformin, other pharmacological activators, or gene therapies may represent a possible neuroprotective therapy for AMD that could enhance MT function and possibly prevent RPE dysfunction.

10.4.3 Neuroprotection in AMD

Dysregulation of MT and energy metabolism in the RPE is likely a contributing factor to AMD pathogenesis. As predicted by the Metabolic Ecosystems Model, metabolic dysfunction in the RPE can lead to dysregulation of metabolism in photoreceptors and a bioenergetic crisis in the entire retina. Therapies that target pathways involved in regulation of energy metabolism or MT activity in the photoreceptors and especially

RPE, such as AMPK, insulin/mTOR, CoQ10 and PGC-1 α , have the potential to restore or preserve energy homeostasis, and thus retinal health and function (Fig. 10.7). Targeting other pathways such as those involved in regulation of oxidative stress response, like Nrf2, may protect the RPE from oxidative insults that could disrupt energy homeostasis. Although these pathways are strong candidates for targets for neuroprotective therapies for AMD, a better understanding of the underlying mechanisms of the pathology of AMD is essential to develop targeted therapies to prevent or treat AMD.

10.5 Summary

MT regulate cellular energy production, oxidative stress, inflammation, signal transduction, and apoptotic pathways.

MT alterations, including mtDNA damage/mutations and dysfunction, likely play an important role in AMD pathogenesis.

Alterations in MT in AMD may disrupt the highly coordinated metabolic ecosystem of the retina, which can lead to further retinal metabolic stress and retinal degeneration.

Targeting MT function and retinal metabolism may be neuroprotective to the retina and as these pathways may represent the optimal neuroprotective therapies for AMD.

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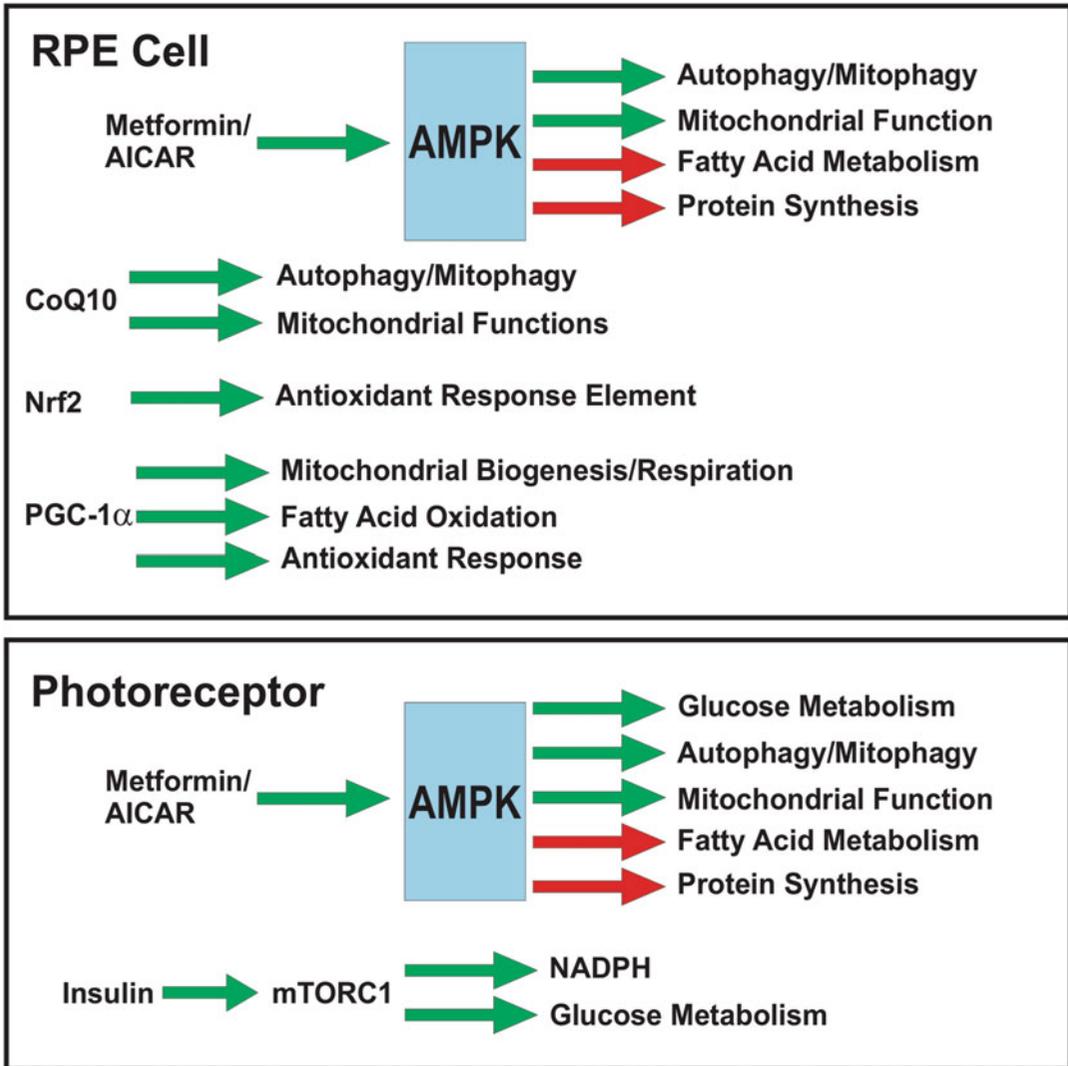


Fig. 10.7 Potential neuroprotective targets for AMD to enhance mitochondrial function and cellular metabolism in the RPE and retina. In the RPE, targeting AMPK, CoQ10, Nrf2, or PGC-1α may promote mitochondrial function and antioxidant responses. The retina, targeting

AMPK or mTOR pathways may promote glucose metabolism, enhance mitochondrial function, and NADPH metabolism. Red arrows = inhibitory effect, green arrow = promoting effect

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