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# Retina Metabolism and Metabolism in the Pigmented Epithelium: A Busy Intersection

**James B. Hurley**

Departments of Biochemistry and Ophthalmology, University of Washington, Seattle, Washington 98115, USA

## Abstract

The outer retina is nourished from the choroid, a capillary bed just inside the sclera. $O_2$, glucose, and other nutrients diffuse out of the choroid and then filter through a monolayer of retinal pigment epithelium (RPE) cells to fuel the retina. Recent studies of energy metabolism have revealed striking differences between retinas and RPE cells in the ways that they extract energy from fuels. The purpose of this review is to suggest and evaluate the hypothesis that the retina and RPE have complementary metabolic roles that make them depend on each other for survival and for their abilities to perform essential and specialized functions.

### Keywords

retina; metabolism; retinal pigment epithelium; photoreceptors

## 1. INTRODUCTION

### 1.1. Retina Structure and Function

Just inside the sclera of an eye is the choroid, a collagenous tissue with blood vessels and fenestrated capillaries. Adjacent to it is Bruch's membrane, and adjacent to that is the retinal pigment epithelium (RPE). The RPE forms a basement membrane on the choroid side and functions as a blood–retina barrier, as its cells are joined together by tight junctions that block diffusion. Most metabolites require transporters to traverse the RPE. On the retina side, the RPE extends apical processes that intercalate between the outer segments (OSs) of the photoreceptors. The RPE provides many important services, including phagocytosis of OSs and recycling of the chromophore for visual pigments, retinaldehyde (Lakkaraju et al. 2021) (Figure 1).

Metabolites are exchanged between the RPE and retina through a proteoglycan-based material known as the interphotoreceptor matrix. The OSs are surrounded by this material and by the apical extensions from the RPE. Rod and cone photoreceptors are specialized for phototransduction and are highly enriched with phospholipid membranes. Most of the

jbhhh@uw.edu .

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metabolite transporters, glycolytic enzymes, and mitochondria in photoreceptors are located in the central portion, sometimes referred to as the ellipsoid. Specialized glial cells called Müller cells extend most of the way across the thickness of the retina. They are not a focus of this review, so they are not depicted in Figure 1, but their metabolic roles are important and are discussed elsewhere (Hurley et al. 2015). The inner retina is made up of bipolar cells, horizontal cells, amacrine cells, ganglion cells, and astrocytes.

### 1.2. Ocular Blood Flow

Tissues within an eye receive nutrients and $O_2$ from the ophthalmic artery. Branching from this artery, the capillary arteries fill the choroid before draining through vortex veins into the ophthalmic vein. The choroid provides nutrients and $O_2$ to the outer retina, but before they can reach it, they must traverse the RPE without being depleted by it.

### 1.3. Outline of This Review

This review evaluates a paradigm in which the retina and RPE function together as a metabolic ecosystem. Section 2 presents observations consistent with the idea that there are essential metabolic interactions between the retina and RPE. Sections 3 and 4 then describe the distinct metabolic features of the retina and of the RPE.

## 2. EVIDENCE FOR A METABOLIC ECOSYSTEM IN THE EYE

This section discusses independent observations that were made by investigators exploring other ideas. Collectively, these observations support the idea that the retina and RPE work together as a metabolic ecosystem.

### 2.1. Retinal Pigment Epithelium Metabolism Affects Photoreceptors

When RPE cells die, photoreceptors die. A single intraperitoneal injection of sodium iodate into a mouse makes the monolayer of RPE cells in a mammalian eye disappear within 1–2 days (Chowers et al. 2017). The chemical mechanism is uncertain, but it only affects RPE cells. Within days following loss of the RPE, photoreceptors in the outer retina degenerate (Figure 2a). This classic observation was an early sign that the RPE supports photoreceptors.

Photoreceptor growth depends on glucose transport through the RPE. Glucose must flow through plasma membrane transporters to cross the RPE. Plasma membranes on the basolateral and apical surfaces of RPE cells are enriched with GLUT-1 glucose transporters. Philp and colleagues (Swamp et al. 2018) showed the importance of GLUT-1 in the RPE by using genetic manipulations to block GLUT-1 expression in RPE cells in mouse eyes. Expression was blocked in a patchwork mosaic pattern across the RPE. Photoreceptors were long and healthy underneath patches where RPE cells expressed GLUT-1, but where expression was blocked, the photoreceptors in the underlying retina were stunted (Figure 2b).

Glucose from the choroid has to traverse the RPE cytoplasm to get to the outer retina. The retina could starve if the RPE cells consume too much glucose for their own metabolism. An in vivo experiment supports this idea. Glycolysis in RPE cells in mice was enhanced

by a genetic manipulation that blocks expression of Von Hippel Lindau factor (VHL). VHL is a protein that destabilizes hypoxia inducible factor (HIF); HIF is a transcription factor that stimulates expression of enzymes that enhance glycolysis. Friedlander and colleagues (Kurihara et al. 2016) found that the mutant RPE cells consumed glucose faster than did normal RPE cells, and there was less than normal glucose in the retinas of the mutant mice. The photoreceptors degenerated (Figure 2c), suggesting that the RPE cells with enhanced glucose consumption would not allow sufficient glucose to reach the retina.

RPE mitochondrial metabolism is critical for retinal viability. Mitochondrial respiration can generate up to 16 times as much ATP from glucose compared to glycolysis of glucose to lactate. Vollrath and colleagues (Zhao et al. 2011) reported an in vivo experiment that demonstrated the importance of mitochondria respiration in the RPE. They disrupted mitochondria in RPE cells by a genetic manipulation that blocks expression of TFAM, a key protein required for maintenance and expression of mitochondrial DNA. The loss of mitochondria altered the metabolism and differentiation state of the RPE cells. It also caused photoreceptors in the outer retina to degenerate (Zhao et al. 2011) (Figure 2d). These findings suggest that the switch from mitochondrial metabolism to glycolysis forced the RPE cells to increase their glucose consumption. The RPE then could not provide sufficient glucose to the outer retina.

### 2.2. Photoreceptor Metabolism Affects the Retinal Pigment Epithelium

When rods die, cones die. The function of both rods and cones is to absorb light and transduce it to change synaptic transmission to downstream neurons. Although they have the same basic function, rods and cones express many distinct genes that impart specialized kinetics and sensitivity. Some rod-specific genes, when mutated, are toxic specifically to rod photoreceptors. In humans, these mutations cause retinitis pigmentosa (RP), a family of diseases in which rods degenerate. Following the loss of rods, the cones (not directly affected by the mutation) also lose their OSs and then die (Figure 3a). An important goal is to understand why the cones die. Preventing cone death would preserve daytime vision in RP patients.

Loss of rods disrupts the flow of glucose to cones. Cones that degenerate following the loss of rods appear to be deprived of nutrients (Punzo et al. 2009). Leveillard & Ait-Ali (2017) identified a protein, Rod-derived Cone Viability Factor (RdCVF), that is produced and secreted by rods and that supports cone survival and function. RdCVF binds to cones and recruits a scaffolding protein that stabilizes GLUT-1 on the plasma membrane (Ait-Ali et al. 2015). These findings provide evidence that rod degeneration impacts cones because the cones lose access to RdCVF and are less able to take up glucose (Figure 3b).

Rod OSs recruit glucose transporters to the apical surface of the RPE. The tips of the OSs contact the apical surface of the RPE. Over most of the length of the OS, phosphatidyl serine (PS) is on the inner leaflet of the plasma membrane, but at the tip it can flip to being on the outer leaflet (Ruggiero et al. 2012). PS exposed at the OS tips stimulates a tyrosine kinase receptor, MerTK, on the apical surface of RPE cells. Dean, Kaplan, and their colleagues (Wang et al. 2019) have shown that activated MerTK causes GLUT-1 to accumulate on the apical surface of RPE cells by preventing its endocytosis. When rods degenerate, the signal

to activate MerTK is lost. This allows the RPE to endocytose GLUT-1. The outer retina starves as glucose becomes trapped in the RPE (Figure 3c).

Altered metabolism in rods disrupts the RPE. AMP kinase (AMPK) is an intracellular energy sensor that influences expression of metabolic enzymes. Ash and colleagues (L. Xu et al. 2020) blocked AMPK expression in mouse retinas but not in the RPE. Rod photoreceptors initially seemed normal, but their mitochondria gradually become fragmented, and metabolic flux slowed. The RPE was impacted by the disrupted retina metabolism. It accumulated vacuoles, lipid droplets, and undigested OS material (Figure 3d).

The density of rods in a retina influences their size. Tsang and colleagues (Koch et al. 2017) used a genetic manipulation to alter the density of rods in mouse retinas. At low densities, the OSs of the rods were short, and at high densities, they were long (Figure 3e; see also Koch et al. 2017, figure 3). This suggests that the density of rods influences the ability of the RPE to transport nutrients to the retina.

Energy metabolism in the retina is very different than that in the RPE (Kanow et al. 2017, Sinha et al. 2020b). The hypothesis addressed in this review is that the observations in Section 2 can be explained by specialized features of retina metabolism (Section 3) and RPE metabolism (Section 4).

## 3. RETINA METABOLISM

### 3.1. $O_2$ Tension Is Low in the Retinas of Living Animals

The source of $O_2$ for the outer retina is the choroid, just inside the sclera of the eye. It is the only source of $O_2$ for guinea pig, rabbit, and salamander retinas, which are very thin. Primates, rats, and mice have thicker retinas with capillaries that infiltrate the inner retina. Nevertheless, in nearly all species that have been examined, the measured $O_2$ tensions are very low in much of the retina, particularly in the photoreceptor layer (Linsenmeier & Zhang 2017, Yu & Cringle 2005). The RPE and outer retina appear to consume most of the $O_2$ that diffuses in from the choroid.

### 3.2. Hypoxia Is Good for Retinas

Pre-exposing mice to a hypoxic environment (11% $O_2$) protects their photoreceptors from being damaged by excessive light (Grimm et al. 2002). A well-studied effect of hypoxia is stabilization of hypoxia inducible factor (HIF), a transcription factor that drives expression of proteins that enhance glycolysis, diminish respiration (Semenza 2011), and stimulate production of erythropoietin (Grimm et al. 2002).

The idea that hypoxia protects photoreceptors from light damage by stabilizing HIF has been tested. HIF can be stabilized by blocking expression of VHL, a protein that tags HIF for degradation (Semenza 2011). Stabilizing HIF in this way transiently protects photoreceptors from light damage (Lange et al. 2011). HIF can also be stabilized by blocking expression of the multifunctional protein SIRT6 (Zhong et al. 2010). Blocking SIRT6 expression slows degeneration in a mouse model of retinitis pigmentosa (Zhang et al. 2016a). However,

further investigation of the role of HIF showed that it is not required for protection by hypoxia (Kast et al. 2016, Samardzija et al. 2019, Thiersch et al. 2009). Instead, the protective effect of hypoxia reflects either a rod-intrinsic and HIF-independent response or protective activities of other cells in the retina (Thiersch et al. 2009).

### 3.3. Excess $O_2$ Is Bad for Retinas

Retinas consume less $O_2$ when photoreceptors degenerate. Unused $O_2$ accumulates (Yu & Cringle 2005) and damages proteins, lipids, and nucleic acids (Shen et al. 2005, Vlachantoni et al. 2011). Hypoxia diminishes oxidative damage in explants of retinas from mice with inherited photoreceptor degeneration (Vlachantoni et al. 2011).

In some types of inherited photoreceptor degeneration, the genetic deficiency affects a protein expressed only in rods, yet cones also degenerate (Punzo et al. 2012). The disease progresses from night-blindness (loss of rods) to total blindness (loss of rods and cones) (Campochiaro & Mir 2018). A reasonable hypothesis to explain this is that, without rods to stimulate $O_2$ consumption, the $O_2$ accumulates and damages the cones. Consistent with this, the loss of cone-mediated vision can be slowed by systemic treatment with the FDA-approved antioxidant N-acetyl cysteine (Campochiaro et al. 2020).

Excess $O_2$ also contributes to other metabolic diseases (Baik & Jain 2020). Blocking expression of a mitochondrial protein in respiratory complex I causes neuronal degeneration in mouse brains by allowing $O_2$ to accumulate (Jain et al. 2016). Treating the mutant mice with hypoxia delays formation of brain lesions and enhances survival (Jain et al. 2019). This beneficial effect of hypoxia is independent of HIF (Jain et al. 2019). Remarkably, the same respiratory complex mutation does not cause photoreceptor degeneration (Gospe et al. 2019). As is discussed below, consumption of $O_2$ by the RPE can be enhanced by oxidation of succinate (Bisbach et al. 2020a), which does not require complex I.

### 3.4. Retinas in Living Animals Favor Glycolysis

In mammals with large eyes, like cats and pigs, blood can be sampled on its way in and out of the eye. A comparison of metabolite levels in these blood samples revealed remarkably different types of metabolism in the outer versus inner retina of pigs (Wang et al. 1997a,b). The outer retina is more metabolically active in darkness than in light, whereas metabolism in the inner retina is not influenced by light. The outer retina has more metabolic activity than the inner retina, and it favors glycolysis. It consumes approximately 10 times more glucose, it generates approximately 30 times more lactate, and it consumes approximately 2 times more $O_2$.

### 3.5. Isolated Retinas Favor Glycolysis

In the 1920s, Warburg and colleagues (1924) reported that retinas and tumors convert glucose mostly to lactate, even when $O_2$ is abundant. This type of metabolism often is referred to as aerobic glycolysis or the Warburg effect. The preference of vertebrate retinas for aerobic glycolysis has been confirmed many times (Chinchore et al. 2017, Cohen & Noell 1960, Hurley et al. 2015, Kanow et al. 2017, Winkler 1981).

### 3.6. Debate About Where Aerobic Glycolysis Occurs in a Retina

Section 3.7 describes evidence that photoreceptors are the site of aerobic glycolysis. However, we cannot completely rule out an important alternative that is based on a model of brain metabolism. In this model, astrocytes in brain use glycolysis to convert glucose from blood into lactate. The astrocytes then export the lactate as fuel for neurons (Magistretti & Allaman 2018). Pure astrocytes in culture do consume glucose and release lactate, and cultured neurons do consume lactate. The astrocyte neuronal lactate shuttle (ANLS) model assumes that astrocyte metabolism in brain tissue and in cultured cells are the same, although evidence does not support this (Diaz-Garcia & Yellen 2019). Based on the ANLS, a model for retina metabolism has been proposed in which Müller glia are the primary site of glycolysis. In this model, Müller glia take up glucose and export lactate to photoreceptors (Poitry-Yamate et al. 1995). Experimental support for this model comes from analyzing the metabolism of dissociated and cultured Müller cells (Vohra & Kolko 2020). However, substantial changes in gene expression occur when Müller cells are dissociated (Wohl & Reh 2016). Another concern is that the model is not consistent with localization of glycolytic enzymes (Hurley et al. 2015) and in vivo experiments that show accumulation of fluorescent 2-deoxy glucose in photoreceptors and not in Müller glia (Heng et al. 2019, Kanow et al. 2017, Wang et al. 2016).

Immunocytochemical studies show that GLUT1 is less abundant on photoreceptors than it is on the apical and basal surfaces of RPE cells (Rajala et al. 2018a). GLUT1 antibodies label the outer retina, but that pattern of labeling could also occur if only the Müller cell apical processes were labeled. Localization of GLUT-1 expression by in situ hybridization to GLUT1 mRNA in the outer retina has not yet been reported. It will be important to determine unambiguously whether GLUT1 or some other glucose transporter is present on photoreceptor neurons.

### 3.7. Evidence that Photoreceptors in the Outer Retina Use Aerobic Glycolysis

The outer retina is made up of photoreceptors and the outer parts of Müller glia. Two types of evidence support the idea that glucose uptake and aerobic glycolysis occur in photoreceptors, rather than in Müller glia. First, in living animals, glucose from blood flows through the RPE to photoreceptors. When a fluorescent derivative of glucose (2-NBDG) enters a cell, it is phosphorylated on C6 and trapped in the cell. 2-NBDG has been introduced into mice by gavage (Kanow et al. 2017), by tail vein injection (Wang et al. 2016, 2019), or by intraperitoneal injection (Heng et al. 2019). Fluorescence accumulates in photoreceptors to a much greater extent than in Müller cells (Kanow et al. 2017). Second, glycolytic enzymes are abundant in photoreceptors.

**3.7.1. Pyruvate kinase M2.—**Pyruvate kinase catalyzes the final step in glycolysis (Figure 3). PKM2 is a highly regulated isoform that is associated with aerobic glycolysis and anabolic activity in cancer cells (Dayton et al. 2016). It also is abundant in photoreceptors (Casson et al. 2016, Chinchore et al. 2017, Lindsay et al. 2014, Rajala et al. 2016). Light stimulates phosphorylation of tyrosine-105 on PKM2, a modification that reduces its activity (Rajala et al. 2016). In addition to its important role in glycolysis, PKM2 also can influence

transcription of genes important to photoreceptors, including the gene that encodes PDE6β (Rajala et al. 2018a).

Diminishing PKM2 expression in rods, either by shRNA (Chinchore et al. 2017) or by inactivating the *PKM2* gene (Rajala et al. 2018a, Wubben et al. 2017), reduces the length of OSs even though there is compensatory expression of PKM1, an isoform that lacks some of the regulatory features of PKM2. Overall pyruvate kinase activity is higher in PKM2$^{-/-}$ retinal lysates (Wubben et al. 2017), although in intact retinas, conversion of PEP to pyruvate is diminished (Rajala et al. 2018a).

In vivo, the altered metabolic state produced by PKM2 deficiency along with upregulation of PKM1 enhances survival of rods either following retinal detachment (Wubben et al. 2017) or in the presence of a PDE6 mutation that causes rod degeneration (E. Zhang et al. 2020). A small-molecule activator of PKM2 also increases survival of photoreceptors in response to retinal detachment (Wubben et al. 2020). In cones, inactivating PKM2 expression causes age-dependent degeneration (Rajala et al. 2018c).

Neither PKM2 nor PKM1 (Casson et al. 2016, Lindsay et al. 2014), nor any other pyruvate kinase isoform (Lindsay et al. 2014), has been detected in rodent Müller cells. This suggests that Müller cells in mammalian retinas have a limited capacity for glycolysis.

**3.7.2. Hexokinase 2.—**Once glucose enters a cell, hexokinase (HK) phosphorylates it on carbon 6 (Figure 4). HK2 is the isoform that is associated with aerobic glycolysis in cancer cells (Wolf et al. 2011). It is also the major isoform in both rod and cone photoreceptors (Petit et al. 2018, Rajala et al. 2013, Reidel et al. 2011, Weh et al. 2020). In its most active form, HK2 associates with the voltage-dependent anion channel on the outer mitochondrial membrane.

Binding of HK2 to mitochondria can be stimulated by light via AKT-mediated phosphorylation and enhanced by a serine/threonine kinase, PHLPPL (Rajala et al. 2013). Binding of HK2 to mitochondria can inhibit apoptosis (Majewski et al. 2004). Phosphorylation of HK2 also occurs during stress. Retinal detachment causes some loss of HK2, but it also stimulates PI3K, which leads to phosphorylation of AKT and HK2 so that a higher percent of HK2 binds to mitochondria (Weh et al. 2020). Inhibiting PI3K with LY294002 prevents the redistribution of HK2.

When HK2 expression is blocked, rods adapt by making more HK1 and more mitochondria (Petit et al. 2018, Weh et al. 2020, R. Zhang et al. 2020). HK2 deficiency also upregulates fructose 1,6 bisphosphatase (Weh et al. 2020). These photoreceptors are nearly normal, but for reasons that are not yet clear, they are more susceptible to retinal detachment and age-related damage.

Neither HK1 nor HK2 have been detected in Müller glia (Petit et al. 2018, Weh et al. 2020). The absence of HK and PK signals in Müller cells supports the idea that these glia do not carry out glycolysis. This is a key argument against an ANLS-like relationship between Müller glia and photoreceptors in the retina.

**3.7.3. Lactate dehydrogenase A.—**When pyruvate is made faster than mitochondria can oxidize it, lactate dehydrogenase (LDH) reduces it to lactate (Figure 4). The A isoform (LDH-A) is abundant in photoreceptors (Casson et al. 2016, Chinchore et al. 2017, Rueda et al. 2016). Reducing expression of LDH-Ain rods diminishes the amount of lactate in the mouse retina and causes rods to be shorter than normal (Chinchore et al. 2017). These experiments support the idea that aerobic glycolysis enhances anabolic activity needed for biogenesis.

LDH-B is also present in mouse retinas (Acosta et al. 2005, Chinchore et al. 2017). One report suggested that there is no LDH in Müller cells (Rueda et al. 2016). However, another showed LDH-B immunoreactivity in the inner layers of the mouse retina, which include astrocytes and Müller glia (Chinchore et al. 2017). Two findings suggest that glia in the retina import lactate and use LDH to oxidize it. Lactate stimulates production of glutamine in retinas, and retinas incorporate $^{13}C$ from $^{13}C$-lactate into glutamine (Lindsay et al. 2014). Glia are the only cells in the retina that can synthesize glutamine.

**3.7.4. Phosphofructokinase.—**Depleting fructose 2,6 bisphosphate (F2,6BP) in rods (Chinchore et al. 2017) slows lactate production and shortens OSs, similar to the effects of blocking access to glucose (Swarup et al. 2018, Wang et al. 2019) or blocking expression of PKM2 or LDH-A. F2,6BP is an important activator of phosphofructokinase-1 that catalyzes phosphorylation of fructose-6-phosphate to fructose 1,6 bisphosphate (F16BP) (Figure 4), a key regulated step in glycolysis.

### 3.8. Aerobic Glycolysis: Why Does the Outer Retina Rely on It?

Photoreceptors in an eye replace approximately 10% of the OS material each day. The finding that tumors and retinas metabolize glucose mostly to lactate even when $O_2$ is readily available (Warburg et al. 1924) suggests that aerobic glycolysis may support synthesis of nucleic acids, proteins, and lipids.

It has been suggested that accumulated glycolytic intermediates in cancer cells can serve as substrates for anabolic activity (Dayton et al. 2016). However, rapid glycolytic flux and lactate production by the retina do not necessarily indicate that glycolytic intermediates accumulate. In fact, when normalized to lactate production, steady-state concentrations of glycolytic intermediates are lower in retinas than in most other tissues.

Glucose transport through the RPE is required for photoreceptor growth (Swarup et al. 2018, Wang et al. 2019). The more photoreceptors there are in a retina, the longer they grow (Koch et al. 2017). This suggests that rods create an environment that allows glucose to reach the retina and to support anabolic activity. Several mechanisms could provide this support. Phosphatidyl serine in the tips of rod OSs stimulates the tyrosine kinase receptor MerTK, which enhances transport of GLUT-1 to the apical surface of RPE cells (Wang et al. 2019). This allows more glucose to reach the retina to support anabolic activity. Rods also release lactate. Quantification of glycolysis in cultured human RPE cells shows that lactate suppresses glycolysis, suggesting that, in an eye, it can protect glucose such that more glucose can reach the retina (Kanow et al. 2017). Rods also make rod-derived cone

viability factor (RdCVF), a protein that stabilizes glucose transporters on cones to enhance glucose uptake (Ait-Ali et al. 2015).

### 3.9. Anabolic Activity and Mechanistic Target of Rapamycin

The kinase mechanistic target of rapamycin (mTOR) is part of a signaling pathway that senses nutrients and metabolic energy within a cell (Liu & Sabatini 2020). mTOR is present in two distinct complexes that help make the metabolism of a cell compatible with its functions and available nutrients. Activation of the mTOR complex stimulates protein synthesis, fatty acid synthesis, glycolysis, and the pentose phosphate pathway. mTOR and its phosphorylated forms are present in normal mouse retinas (Punzo et al. 2009). Growth factors and certain amino acids can stimulate phosphorylation and activation of mTOR, and phosphorylated mTOR has been used to report nutrient availability and ATP abundance (Liu & Sabatini 2020).

Progressive degeneration of rods caused by mutations in rod-specific genes initially causes only night-blindness; however, once the rods are gone, the cones also degenerate, causing total blindness (Punzo et al. 2012). As the rods die off, mTOR in the cones goes from a phosphorylated to a dephosphorylated state (Punzo et al. 2009). Since mTOR phosphorylation reflects nutrient availability, this suggests that loss of rods may diminish the flow of nutrients to cones. Punzo and colleagues (2009, 2012) reasoned that cone survival might be enhanced if mTOR activity could be maintained in cones even when they have only limited access to nutrients. Binding of insulin to the insulin receptor can activate mTOR (Liu & Sabatini 2020). Systemic injection of insulin can enhance cone survival in a mouse RP model (Punzo et al. 2009).

In the past, it was unclear whether insulin enhanced cone survival by activating mTOR in cones. This has been addressed by activating mTOR in cones either by blocking expression of TSC (Venkatesh et al. 2015), a protein that normally suppresses mTOR activity (Liu & Sabatini 2020), or by expressing a constitutively active mTOR mutant (Rajala et al. 2018b). Both enhance cone survival in RP mouse models. Activation of mTOR when glucose is unavailable causes non-neuronal cells to die (Choo et al. 2010), but neurons and non-neuronal cells have different responses to nutrient stress and mTOR activation (Di Nardo et al. 2014).

AKT is a kinase that is upstream of mTOR complex I and downstream of mTOR complex 2. The AKT2 isoform is needed to protect photoreceptors from stress (Li et al. 2007). When AKT is activated by retinal detachment, HK2 accumulates on mitochondria (Weh et al. 2020). Degeneration slows when AKT is overexpressed in rods or when TSC1 expression in rods is blocked (McDougald et al. 2019, Zhang et al. 2016b). However, in the absence of stress, stimulation of this pathway may be detrimental. In normal mice, loss of TSC1 in rods disrupts RPE function. Breakdown of membranes phagocytosed into RPE cells slows, and lipids accumulate between the RPE and Bruch’s membrane (Cheng et al. 2020).

When TSC1 is inactivated in the RPE itself (Go et al. 2020, Huang et al. 2019), the RPE cells dedifferentiate, changing their morphology and metabolism. They accumulate lipid droplets and degenerate; this is followed by choroidal atrophy and photoreceptor

degeneration. The effects can be diminished by rapamycin, an inhibitor of mTOR. Similar effects occur when mTOR is activated in RPE cells by disruption of mitochondria (Zhao et al. 2011) and when mitochondrial function is blocked by inactivation of peroxisome proliferator-activated receptor γ coactivator-1 α in RPE cells (Rosales et al. 2019).

### 3.10. AMP Kinase

5′ Adenosine monophosphate kinase (AMPK) is a serine/threonine kinase that responds to the metabolic energy state of a cell (Mihaylova & Shaw 2011). It is a heterotrimer of a catalytic α subunit (two isoforms), a regulatory β subunit (two isoforms), and an AMP-binding γ subunit (three isoforms). RNA-seq data suggest that mammalian retinas express β1, γ1, and both α isoforms (Xu et al. 2018).

Low glucose or a rise of the intracellular AMP:ATP ratio (Lin & Hardie 2018) can trigger AMPK to stimulate glycolysis and mitochondrial biogenesis while reducing protein and fatty acid synthesis. AMPK also can be stimulated by metformin, an FDA-approved drug used to treat diabetes. The effect of stimulating AMPK in retinas has been investigated both by incubating retinas with metformin (L. Xu et al. 2020) and by injecting metformin into mice (Xu et al. 2018). Treating mice with metformin enhances the ability of the retina to protect itself from oxidative damage. Metformin increases mitochondrial DNA copy number, increases intracellular ATP, and reduces oxidative stress and DNA damage in retinas (Brown et al. 2019b). The protective effects of metformin require the AMPKα2 subunit (Xu et al. 2018).

Metformin can delay degeneration of photoreceptors in animal models of retinal degeneration (Xu et al. 2018). A large population of patients take metformin to treat diabetes. A retrospective analysis of patients with age-related macular degeneration (AMD) found that metformin use correlates with lower risk of developing AMD (Brown et al. 2019a).

Blocking expression of both AMPKα isoforms in the retina diminishes metabolic activity, impairs visual function, and causes gradual loss of photoreceptors (Xu et al. 2018, 2020). Remarkably, although expression of AMPKα was blocked only in the retina, the mitochondria in the neighboring RPE cells also were perturbed (L. Xu et al. 2020). This is further evidence that metabolism in the retina and RPE metabolism influence each other.

### 3.11. Additional Features of Retina Metabolism

Uniquely shaped mitochondria are abundant and highly active in photoreceptors, and their metabolism can be influenced by light. Flavins and taurine are particularly abundant in retinas.

#### 3.11.1. Photoreceptor metabolism is more active in darkness than in light.—

More ATP consumption is required to maintain the membrane potential in photoreceptors in darkness than in light. Energy demand is higher because more $Na^+$ and $Ca^{2+}$ leak into the OS in darkness than in light (Ames et al. 1992, Okawa et al. 2008). Changes in matrix $Ca^{2+}$ in darkness versus light also may influence fuel oxidation (Bisbach et al. 2020b, Du et al.

2016a). Cones consume more energy than rods, particularly during illumination (Ingram et al. 2020).

**3.11.2. Photoreceptor mitochondria.—**Mitochondria are concentrated in the region of the photoreceptor between the OS and the nucleus. These mitochondria are unusual and diverse. In mouse rods, they are elongated and line up along the periphery of the cell (Meschede et al. 2020), whereas in zebrafish cones, they cluster tightly into a ball that displaces nearly all of the cytoplasm (Giarmarco et al. 2017). Photoreceptors can have mitochondria in their synaptic terminals but only when the inner retina is vascularized (Stone et al. 2008).

The roles of some key mitochondrial transporters in photoreceptors have been investigated. The aspartate glutamate carrier (AGC1 or Aralar) is part of the malate–aspartate shuttle that transfers reducing power from the cytoplasm to the mitochondrial matrix (Figure 4). Evidence that this shuttle is active in the retina includes the finding that retinas incubated with 4-$^{2}$H-glucose (which labels cytosolic NADH) produce deuterated mitochondrial metabolites (Bisbach et al. 2020a). AGC1 is not present in Müller cells (Xu et al. 2007), but it is present in photoreceptors, where it may help minimize oxidation of glutamate (Du et al. 2013). Loss of AGC1 diminishes the ERG b-wave, suggesting that AGC1 normally helps sustain neurotransmission (Contreras et al. 2016).

The mitochondrial pyruvate carrier (MPC) is the main route for entry of pyruvate into mitochondria. Blocking expression of MPC in retinas causes a relatively slow aging-dependent decline of ERG responses, degeneration of rods and cones, and disruption of the inner segment–OS junction of photoreceptors (Grenell et al. 2019). Aspartate accumulates at the expense of glutamate and glutamine in retinas that are deficient in MPC. Fatty acids and ketone bodies can serve as an MPC-independent source of acetyl-CoA to fuel these mitochondria.

The mitochondrial uniporter (MCU) provides a major route for $Ca^{2+}$ entry into mitochondria. Photoreceptors express unusually low levels of it (Bisbach et al. 2020b). Blocking expression of MCU has only small effects on retinal TCA cycle metabolic flux and ERG responses, and it does not cause photoreceptor degeneration (Bisbach et al. 2020b), whereas overexpressing MCU alters photoresponse kinetics and the distribution of TCA intermediates (Hutto et al. 2019). MCU overexpression causes striking morphology and mobility changes in cone mitochondria. The cones remain viable for nearly a year but then degenerate slowly.

Photoreceptor mitochondria in an eye are in a hypoxic environment (Linsenmeier & Zhang 2017, Yu & Cringle 2005). The amount of cytochrome oxidase in a tissue can be influenced by availability of $O_2$ (Vijayasarathy et al. 2003) and is low in retinas compared to other respiratory complexes (Bisbach et al. 2020a). This adaptation to hypoxia may account for the limited capacity of the retina to reduce $O_2$ to $H_2O$ (Kooragayala et al. 2015). Complex I reduces ubiquinone to ubiquinol ($QH_2$). Normally, the rate at which $QH_2$ can be reoxidized is limited by the amount of $O_2$ and the activity of cytochrome oxidase. Analyses of metabolic flux in mouse retinas show that fumarate can be a surrogate for $O_2$

(Bisbach et al. 2020a). Succinate dehydrogenase (SDH) uses $QH_2$ to reduce fumarate to succinate (Figure 4). The advantage for the retina is that this reverse SDH activity gives its mitochondria the ability to pump protons and make ATP even in the absence of $O_2$. Retinas export the succinate, which then can be imported into RPE cells, where it can be used very effectively by RPE mitochondria to reduce $O_2$ to $H_2O$.

**3.11.3. Flavins and retbindin.—**Flavins are more concentrated in the retina and RPE than in serum, and they are more abundant in the RPE than in the retina (Sinha et al. 2018). Flavin nucleotides are essential electron carriers in many types of redox reactions, particularly in mitochondrial respiration (Sinha et al. 2020a). A photoreceptor-specific extracellular protein, retbindin, binds riboflavin. Analyses of retbindin-knockout mice show that retbindin is required to maintain flavins in the retina (Sinha et al. 2018) and that disruption of flavin-mediated metabolism impairs glycolysis, stimulates pentose–phosphate pathway activity, and causes photoreceptors to degenerate (Kelley et al. 2017, Sinha et al. 2021).

**3.11.4. Taurine.—**The aminosulfonic acid taurine is abundant in retinas, but its function there was, until recently, not understood. A recent study showed that, in retinas (Kim et al. 2020), it can form protonated Schiff bases with retinal isomers, which may function as an inert reservoir for the visual pigment chromophore retinaldehyde.

## 4. RETINAL PIGMENT EPITHELIUM METABOLISM

Glucose for the outer retina comes from the choroidal blood, so it has to traverse Bruch's membrane and then the RPE before it can reach the retina. Glucose moves freely across the basal and apical surfaces of the RPE through abundant GLUT1 transporters (Swarup et al. 2018).

### 4.1. Glycolysis and Lactate Production Are Suppressed in Retinal Pigment Epithelium Cells

RPE cells minimize their own consumption of glucose, presumably to maximize the passage of glucose from the choroidal blood through the RPE to the retina. Mouse eyecups (containing intact RPE) from which retinas have been removed convert glucose to lactate approximately 20 times more slowly than do retinas (Kanow et al. 2017).

### 4.2. Flexible and Diverse Metabolic Features of the Retinal Pigment Epithelium Compensate for Suppressed Glycolysis

RPE cells minimize their consumption of glucose, but they still need metabolic fuel. They solve this problem by having a flexible metabolism that uses abundant mitochondria (Keeling et al. 2020) to utilize a variety of blood nutrients and partially oxidized metabolic by-products from the retina (Figure 5).

**4.2.1. Fatty acids.—**On a daily schedule, RPE cells in an eye phagocytose the tips (Lakkaraju et al. 2021) of rod OSs, which are loaded with proteins and phospholipids. Phagocytosis of the OS tips is stimulated by exposure of phosphatidyl serine (PS) on the

outer leaflet of the OS plasma membrane. A PS-binding protein (MFGE8) and an integrin ($\alpha v\beta 5$) sense the exposed PS and initiate a signaling cascade through the tyrosine kinase receptor MERTK to trigger phagocytosis (Lakkaraju et al. 2021). This process also stabilizes GLUT-1 on the apical plasma membrane to enhance diffusion of glucose through the RPE to the retina (Wang et al. 2019). It also stimulates PPAR$\gamma$ (Ershov & Bazan 2000) to enhance fatty acid oxidation in RPE cells.

As the phagosomes mature, they migrate toward the basal RPE and fuse with lysosomes (Lakkaraju et al. 2021). Lipases (Hayasaka et al. 1977, Zimmerman et al. 1983) release fatty acids from the phospholipids. Mitochondria oxidize the fatty acids by β-oxidation to generate NADH, $FADH_2$, and acetyl CoA (Adijanto et al. 2014, Reyes-Reveles et al. 2017, Tyni et al. 2002). Peroxisomes oxidize the longer polyunsaturated fatty acids (Daniele et al. 2019).

RPE cells, like liver cells, express HMG-CoA synthase (Adijanto et al. 2014), a key enzyme for ketone body synthesis. Mouse RPE/choroid preparations can oxidize fatty acids from phagocytosed OSs, using the acetyl CoA to make ketone bodies (Adijanto et al. 2014). The ketone bodies are exported through a monocarboxylate transporter (MCT), MCT1, on the RPE apical processes (Philp et al. 1998, 2001). The exported ketone bodies can then be taken up by cells in the retina, including photoreceptors (mostly through MCT7), and oxidized to $CO_2$ (Adijanto et al. 2014).

Phagocytosis of OS tips occurs in the morning after light onset (Lakkaraju et al. 2021). Shortly after that, the RPE produces and releases ketone bodies (Reyes-Reveles et al. 2017). The release is delayed when phagolysosome maturation is disrupted (Reyes-Reveles et al. 2017). Oxidation of fatty acids consumes $NAD^+$ and generates ATP. In muscles, fatty acid oxidation can suppress glycolysis, a type of regulation called the Randle cycle (Hue & Taegtmeyer 2009, Randle et al. 1963). The burst of fatty acid oxidation each morning could act by a similar mechanism to suppress glycolysis so that more glucose can reach the retina (Reyes-Reveles et al. 2017).

**4.2.2. Glycogen.—**The role of glycogen in the RPE is an important and underinvestigated topic. Glycogen has been studied in mature confluent cultures of human RPE cells (Senanayake et al. 2006). When the concentration of glucose in the medium is high, the cultured RPE cells store glycogen. As much as 70% of glucose in cultured RPE cells can be in the form of glycogen. As glucose in the medium is depleted, the stored glycogen breaks down either by phosphorolysis or by the action of lysozomes.

**4.2.3. Lactate.—**RPE cells can import and oxidize lactate (Bisbach et al. 2020a, Kanow et al. 2017). MCTs allow lactate and other metabolites (Prag et al. 2021) to flow in and out of cells. MCTs on the plasma membranes of RPE and other cells are stabilized by basigin (Han et al. 2020). MCT1 (*SLC16A1* gene) on the apical surface of the RPE transports lactate from photoreceptors into the RPE (Philp et al. 1998). On the basolateral surface, MCT3 (*SLC16A8* gene) exchanges lactate between the RPE and the choroidal blood (Philp et al. 1998).

Cultured human RPE cells and mouse RPE/choroid incorporate labeled carbons from U-$^{13}$C lactate into citrate via pyruvate dehydrogenase and into malate by carboxylation. Oxidation of lactate consumes $NAD^+$, which is needed for glycolysis. Suppression of glycolysis by lactate has been demonstrated with cultured RPE cells (Kanow et al. 2017). However, confirmation that this also occurs in vivo has not been reported. RPE cells can oxidize lactate, but in an eye, they may not have the capacity to oxidize lactate as fast as the retina makes it (Wang et al. 2019, figures 7C and S11).

**4.2.4. Glutamine and reductive carboxylation.—**RPE cells, like some cancer cells, can reduce α-ketoglutarate and $CO_2$ to isocitrate (Jiang et al. 2016, Wise et al. 2011). Reductive carboxylation can be catalyzed by two isoforms of isocitrate dehydrogenase, either IDH1 in the cytoplasm or IDH2 in the mitochondrial matrix. Reductive carboxylation uses NADPH to reduce carbons from glutamine to make citrate. Reducing power for IDH2 in the matrix can be transferred from NADH to NADPH via the proton gradient–driven enzyme nicotinamide nucleotide transhydrogenase (NNT) on the inner mitochondrial membrane. Reductive carboxylation can transfer reducing power to the cytoplasm for fatty acid synthesis (Wise et al. 2011), or it can provide NADPH to counter oxidative stress in the cytoplasm (Jiang et al. 2016).

Reductive carboxylation is a major metabolic pathway in cultured RPE cells fed with glutamine (Du et al. 2016b). As much as 70% of citrate in cultured human RPE cells can come from reductive carboxylation. Reductive carboxylation can protect cultured RPE cells from oxidative stress (Du et al. 2016b). It occurs in RPE/choroid tissue (Yam et al. 2019), but it will be important to determine whether it is as prominent in tissues in an eye as it is in cultured cells.

**4.2.5. Proline.—**Human RPE cells grown in culture selectively deplete proline, glucose, and taurine from their media. Proline consumption is slow when RPE cells are initially isolated in a partially dedifferentiated state and put in culture. However, it increases as the cells differentiate and mature into a confluent, tightly coupled monolayer (Yam et al. 2019). Proline is transported into RPE cells by the protein encoded by *SLC6A20*, a $Na^+$- and $Cl^-$-dependent transporter enriched in RPE cells (Yam et al. 2019). Proline dehydrogenase is a mitochondrial enzyme that extracts electrons from proline and transfers them through FAD to ubiquinone in the mitochondrial inner membrane. FAD is enriched in the RPE (Sinha et al. 2018). Carbons from proline can enter the TCA cycle as α-ketoglutarate (Chao et al. 2017), and mitochondrial intermediates derived from it are released at the apical surface. When $^{13}$C-labeled proline is infused into mice through the jugular vein, it appears first in the RPE (Yam et al. 2019). The proline is then oxidized, and metabolites made from it are exported and used by the retina (Yam et al. 2019).

Proline influences glucose metabolism in RPE cells. It can stimulate synthesis of serine, glycine, lactate, pyruvate, and alanine while diminishing the entry of carbon from glycolysis into the TCA cycle (Yam et al. 2019). Blocking proline dehydrogenase activity inhibits glycolysis and serine synthesis and diminishes the amount of reduced glutathione in RPE cells (Yam et al. 2019).

Proline can protect cultured RPE cells from oxidative stress. It enhances their survival even in the presence of 1 mM $H_2O_2$ (Yam et al. 2019). Sodium iodate injected into mice is toxic to the RPE (Chowers et al. 2017), but pretreating the animals with proline diminishes its effect (Yam et al. 2019). Proline supplementation can also counteract the effect of inhibiting ornithine amino transferase (OAT) in RPE cells (Ueda et al. 1998). In humans, OAT deficiency causes the blinding disease gyrate atrophy (O'Donnell et al. 1978).

**4.2.6. Amino acids.—**Both retinas and RPE/choroid complexes use $NH_4^+$ directly to synthesize glutamine (R. Xu et al. 2020). RPE cells, but not retinas, use amino groups from alanine and leucine to synthesize other amino acids. In contrast, aspartate is the predominant nitrogen donor for glutamate synthesis in the retina. Asparagine is made in the retina but not in the RPE. Some serine and glycine for the retina come from blood, but the RPE can also make glutamate, serine, and glycine (R. Xu et al. 2020) and transport them to the retina.

**4.2.7. Succinate.—**Succinate can shuttle electrons from the $O_2$-depeleted retina to the $O_2$-rich RPE. Tissues with ready access to $O_2$ use it as the terminal electron acceptor. When $O_2$ is scarce, electrons that enter respiratory complexes accumulate on $QH_2$. Succinate dehydrogenase (complex II) can use the excess $QH_2$ to reduce fumarate to succinate. In the eyes of mammals, retinas are in a low-$O_2$ environment (Linsenmeier & Zhang 2017, Yu & Cringle 2005). Retina mitochondria adapt by suppressing cytochrome oxidase and reducing fumarate to succinate (Bisbach et al. 2020a) (see Figure 4). Freshly isolated retinas release succinate (Bisbach et al. 2020a). These observations suggest that the RPE can import succinate made by the retina and use it to reduce $O_2$ to $H_2O$. Indeed, succinate is an excellent fuel for the RPE. It increases $O_2$ consumption by approximately threefold compared to glucose alone (Bisbach et al. 2020a).

The oxidized carbons from the RPE may then be recycled back to the retina (as fumarate or malate) to accept more electrons. This model implies that a high rate of $O_2$ consumption in the RPE, fueled by succinate, can help protect the retina from oxidative stress by intercepting $O_2$ before it reaches the retina. The succinate shuttle model is based on experiments with isolated retinas and RPE/choroid, so it will be important to confirm these metabolic relationships in vivo.

## 5. DISEASE STATES WITH ALTERED METABOLISM IN THE RETINA OR RETINAL PIGMENT EPITHELIUM

The retina is the primary light detector in the visual system. It is supported in the eye by a metabolic ecosystem that includes RPE cells, photoreceptors, other retinal neurons, and glia. The metabolic features of the participants in this ecosystem seem to be optimized to protect nonreplicating and terminally differentiated cells from being damaged. Metabolite distributions change substantially when a component of the ecosystem falters (Du et al. 2016a, Weiss et al. 2019). This section describes examples of what can happen when the metabolic ecosystem breaks down. For an insightful and comprehensive review of disease states associated with retina and RPE metabolism, the reader is referred to the recent review by Besirli and colleagues (Pan et al. 2021).

### 5.1. Changes in Energy Metabolism with Aging

Retina and RPE metabolism decline with age (Du et al. 2017, Sinha et al. 2018, Wang et al. 2018), and mitochondria become more vulnerable to stress (Rohrer et al. 2016). Mitochondrial structure in human RPE tissue deteriorates (Feher et al. 2006), and mutations accumulate in mitochondrial DNA (Lin et al. 2011, Terluk et al. 2015). Analyses of mitochondria in other tissues suggest that the accumulation of mutations is caused more by replication errors than by oxidative stress (Itsara et al. 2014). Excess activation of mTOR has been detected in RPE cells from elderly donors (Huang et al. 2019), which suggests a link between age and metabolic stress.

AMD exacerbates the metabolic changes associated with aging (Ferrington et al. 2020, Joyal et al. 2018, Leveillard et al. 2019). Mutations in the mitochondrial genome and loss of biochemical activities associated with mitochondria are more pronounced in RPE cells from AMD-affected donors than from age-matched non-AMD donors (Ferrington et al. 2017). RPE mitochondria are affected more than retina mitochondria in AMD (Terluk et al. 2015). Mitochondrial dysfunction in the RPE can slow β-oxidation and cause lipid accumulation (Curcio et al. 2011), and it could force the RPE to consume more glucose (Kanow et al. 2017). The composition of lipoproteins that are made by the RPE can be influenced by a mutation in complement factor H that is linked to AMD (Kelly et al. 2020). If $O_2$ diffuses past the accumulated lipids, it may build up in the retina and damage proteins, lipids, and DNA (Bisbach et al. 2020a).

### 5.2. Hexokinase I

Besides HK2, which is present in photoreceptors only, another isoform, HK1, is present throughout the retina (Weh et al. 2020). A specific mutation in HK1 has been linked genetically to autosomal-dominant retinitis pigmentosa in several large families (Yuan et al. 2017). There are no obvious biochemical consequences of the mutation, but this finding suggests that HK1 has a unique metabolic activity in the retina or RPE.

### 5.3. Isocitrate Dehydrogenase

Two isoforms of this enzyme, IDH1 and IDH2, are discussed above in the context of reductive carboxylation. A third isoform, IDH3, is present in the mitochondrial matrix, where it catalyzes oxidative decarboxylation of isocitrate by $NAD^+$, a step in the TCA cycle. IDH3 is an $\alpha_2\beta\gamma$ complex in which the α subunits are catalytic and the others regulate catalysis (Findlay et al. 2018). A mutation in IDH3B is linked to retinitis pigmentosa (Hartong et al. 2008). The mutation causes photoreceptor degeneration but no other symptoms that would be expected from mitochondrial dysfunction in other tissues. A homozygous null mutation in IDH3A is lethal, but missense mutations in the human IDH3A gene and a missense mutation in the mouse IDH3A gene primarily affect the viability of photoreceptors (Findlay et al. 2018). These findings suggest that IDH3 has a metabolic function that is uniquely important in the retina or RPE.

### 5.4. Inosine Monophosphate Dehydrogenase

Inosine monophosphate dehydrogenase 1 (IMPDH1) is expressed in many tissues; in the retina, it is the most abundant isoform (Hedstrom 2009). Some specific IMPDH1 mutations

affect only the retina (Bowne et al. 2006). IMDPH is a highly regulated filamentous enzyme that catalyzes the first committed step in de novo synthesis of guanine nucleotides. The mutations are linked either to the RP10 form of autosomal-dominant RP or to an autosomal-dominant form of Leber congenital amaurosis (Aherne et al. 2004, Bowne et al. 2006, Hedstrom 2009, Plana-Bonamaiso et al. 2020).

The catalytic activity of IMPDH1 is not affected directly by the mutations, but they may influence phosphorylation of the enzyme and alter its sensitivity to guanine nucleotides (Plana-Bonamaiso et al. 2020). Diminished feedback inhibition by guanine nucleotides could elevate cyclic GMP to toxic levels and alter the balance of nucleotides in the photoreceptor. Toxic effects of IMPDH1 aggregates also could contribute to photoreceptor degeneration (Aherne et al. 2004).

### 5.5. Nicotinamide Adenine Dinucleotide

Mutations in the enzyme nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1) are linked to autosomal-recessive Leber congenital amaurosis 9 (Koenekoop et al. 2012). NMNAT1 catalyzes a central reaction in $NAD^+$ biosynthesis. Loss of NMNAT1 specifically in rods causes them to degenerate by a mechanism that requires SARM1, an enzyme that breaks down $NAD^+$ (Sasaki et al. 2020). $NAD^+$ is a required cofactor in many critical redox reactions, including those catalyzed by IMPDH1 and IDH3.

### 5.6. Proline and Ornithine

Disruption of proline synthesis by onithine aminotransferase (OAT) deficiency and accumulation of ornithine, which are symptoms of a human condition known as gyrate atrophy, cause breakdown of the RPE and progressive retinal degeneration (O'Donnell et al. 1978). Proline is a preferred nutrient for RPE cells but not for retina (Chao et al. 2017). It can be made from either ornithine or glutamate. OAT converts ornithine into pyrolline-5-carboxylate, a direct precursor for biosynthesis of proline. OAT deficiency blocks one of the pathways for proline synthesis and raises the plasma ornithine concentration (Simell & Takki 1973). High levels of ornithine can inhibit synthesis of proline from glutamate (Hu et al. 1999).

### 5.7. Serine

Mutations in serine palmitoyltransferase (SPT) are linked to macular telangactasia (MacTel), a blinding disease that affects blood vessels near the macula and causes loss of central vision (Gantner et al. 2019). SPT synthesizes dehydrosphinganine, a precursor for all sphingolipids. Patients with specific mutations in SPT accumulate deoxysphingolipids that lack the hydroxyl group needed to make complex sphingolipids. The mutations in SPT alter its substrate specificity so that it can use alanine instead of serine. Wild-type SPT also can use alanine but only when serine is scarce. Low blood serine has been found in patients with other forms of MacTel not linked to SPT (Scerri et al. 2017). Deoxysphingolipids compromise visual function in mice and cause photoreceptor death in retinal organoids (Gantner et al. 2019). The mechanism of toxicity of deoxysphingolipids to the macula is not yet understood.

# 6. THERAPIES BASED ON METABOLISM

Over 3,100 mutations in 71 genes can cause retinitis pigmentosa (DiCarlo et al. 2018). Exploiting the metabolic ecosystem in the eye to make the retina and RPE more robust to stress is an attractive strategy for treating diseases of diverse genetic origins.

## 6.1. Exercise and Diets

Moderate running can slow degeneration of photoreceptors in mice caused either by light damage or by genetic deficiencies (Hanif et al. 2015, Mees et al. 2019, Zhang et al. 2019). Elevated BDNF (which promotes cell survival), but not lactate, may contribute to this protection. Diet also can influence retinal degeneration. Mice on high-fat diets can develop a type of retinal degeneration that is similar to AMD (Kelly et al. 2020, Zhang et al. 2018), and $Nrf2^{-/-}$ mice (with impaired redox homeostasis) develop RPE atrophy and retinal degeneration when they are on high glycemic diets (Rowan et al. 2020).

## 6.2. Dietary Supplements

Antioxidants, $NAD^+$ precursors, amino acids, and other dietary supplements can influence retina and RPE metabolism to enhance the metabolic ecosystem and make it more resilient.

**6.2.1. Antioxidants.—**Dietary supplementation with an antioxidant, N-acetyl cysteine, can slow the loss of cones following degeneration of rods (Campochiaro & Mir 2018, Campochiaro et al. 2020, Lee et al. 2011)

**6.2.2. $NAD^+$ precursors.—**Oxidative stress induced in cultured RPE cells by hydrogen peroxide causes DNA damage and stimulates poly ADP-ribose polymerase (PARP). Excessive PARP activity can deplete $NAD^+$ (Du et al. 2016b). Nicotinamide mononucleotide (NMN), a precursor for $NAD^+$, can protect the cultured cells from peroxide and raise maximal respiration and ATP production by RPE cells derived from AMD patients (Ebeling et al. 2020). Injections of NMN into mice slows retinal degeneration caused by the rd8 mutation (Mills et al. 2016), and injections of nicotinamide riboside (NR), another $NAD^+$ precursor, slow degeneration caused by light damage (X. Zhang et al. 2020). Nicotinamide and enhanced expression of NMNAT1, the enzyme that catalyzes a key step in $NAD^+$ synthesis, also slow loss of vision in glaucoma-prone mice (Williams et al. 2017).

**6.2.3. Amino acids.—**α-Ketoglutarate added to the diet can increase the health span of mice (Asadi Shahmirzadi et al. 2020), and it slows degeneration caused by a PDE6 mutation (Wert et al. 2020). A diet supplemented with proline can protect against loss of photoreceptors caused by the toxic effects of sodium iodate (Yam et al. 2019).

**6.2.4. Other supplements.—**Elevated levels of iron and bisretinoids may contribute to oxidative damage (Shu & Dunaief 2018, Ueda et al. 2018), and iron chelators can be protective. Supplementing the diet with docosohexaenoic acid, a fatty acid that is abundant in photoreceptors, is also neuroprotective (Bazan 2018). Diet and the composition of the gut microbiome may also influence susceptibility to AMD (Rowan & Taylor 2018).

### 6.3. Gene Therapies

Genetic manipulations designed to make photoreceptors more robust to stress by altering their metabolism are being tested in animal models. Approaches include expressing TGF-β1, RdCVF, CX3CL1, NRF2, and CNTF and ablating SIRT6, PKM2, TSC1, and PTEN. An up-to-date and thorough review on this topic was published recently (Caruso et al. 2020).

## 7. TRAJECTORY OF THIS FIELD OF RESEARCH

The preference of retinas for aerobic glycolysis was noted in the 1920s and has since been a major focus of research in this field. In the past approximately 10 years, there have been major advances in mass spectrometry; animal models; and understanding of energy metabolism in other biological systems, including cancer and immune cells. These advances have led to many additional new insights into retina and RPE metabolism.

Energy metabolism in living cells is complex, and the metabolic relationships between cells are more so. Many of the observations described in Section 2 can be explained based on the information in Sections 3 and 4. Nevertheless, it would be a mistake to think that a single metabolic interaction like aerobic glycolysis or lactate shuttling will be sufficient to fully explain the metabolic relationship between the RPE and the retina. Proline, glutamine, serine and other amino acids, fatty acids, lactate, succinate, and $O_2$ all contribute to a complex network of metabolic interactions that promote and enhance the survival and function of the RPE and retina. Aggressive research is needed to show how these pathways are regulated; to identify additional metabolic pathways; and to explore diets, nutritional supplements, and gene therapies that bolster the metabolic ecosystem in the eye to make the retina less vulnerable to stress.

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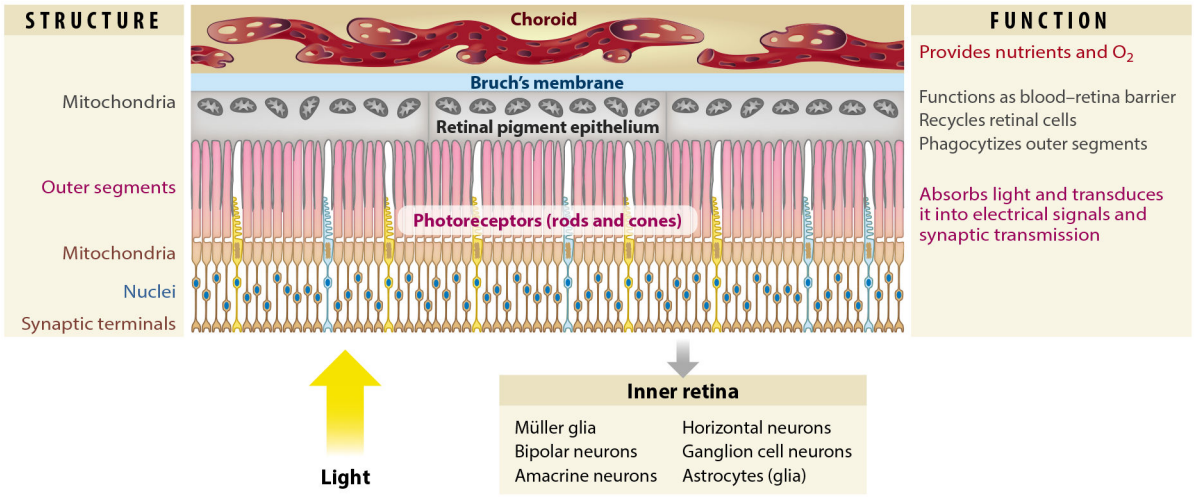


**Figure 1.**
Structures and functions of the choroid, retinal pigment epithelium, and retina.

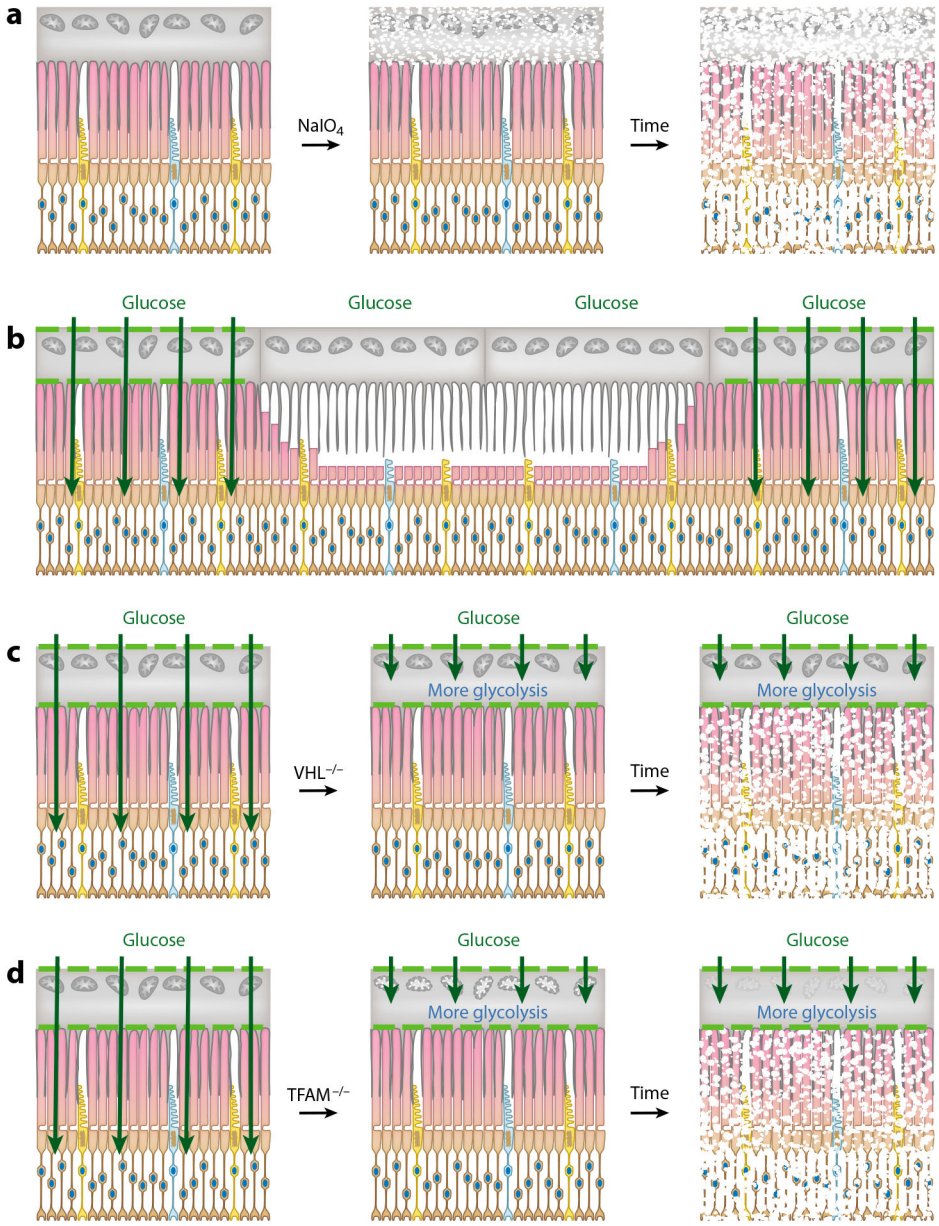


**Figure 2.**

Examples of how retinal pigment epithelium (RPE) metabolism affects photoreceptors. (*a*) When RPE cells die, photoreceptors die. (*b*) RPE cells allow glucose to flow from the choroid to the retina. (*c*) Glucose from the choroid has to traverse the RPE cytoplasm to get to the outer retina. (*d*) RPE cells oxidize fuels to $CO_2$ and $H_2O$.

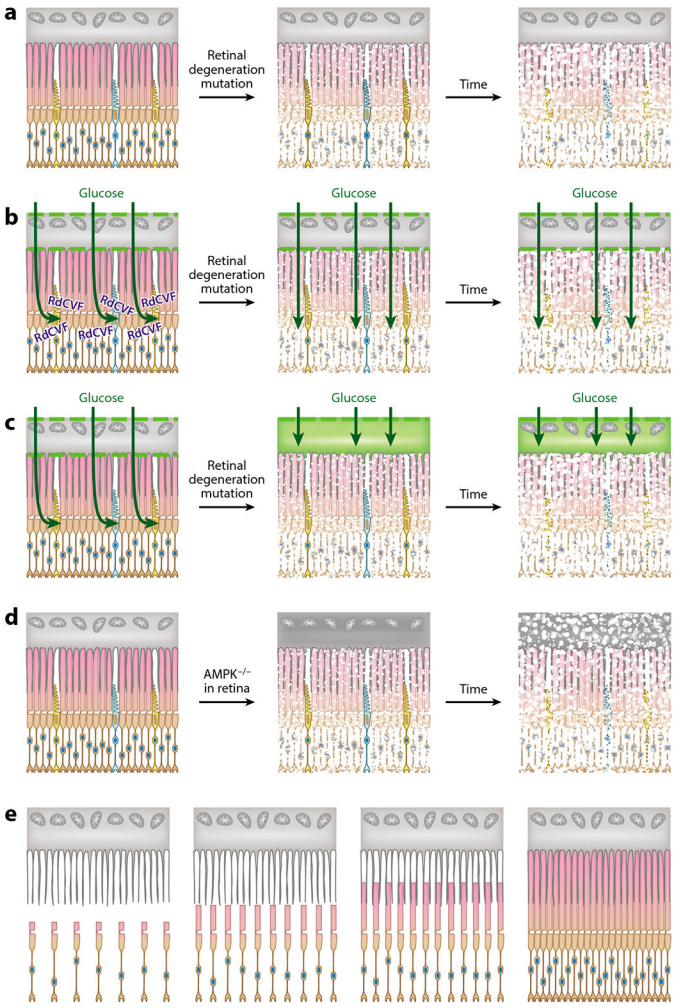


**Figure 3.**

Examples of how photoreceptor metabolism affects the RPE. (*a*) When rods die, cones die. (*b*) Rod loss disrupts glucose entry into cones. (*c*) Rod outer segment tips recruit glucose transporters to the RPE apical surface. (*d*) Altered metabolism in rods disrupts the RPE. (*e*) The density of rods in a retina influences their size. Abbreviations: RdCVF, rod-derived cone viability factor; RPE, retinal pigment epithelium.

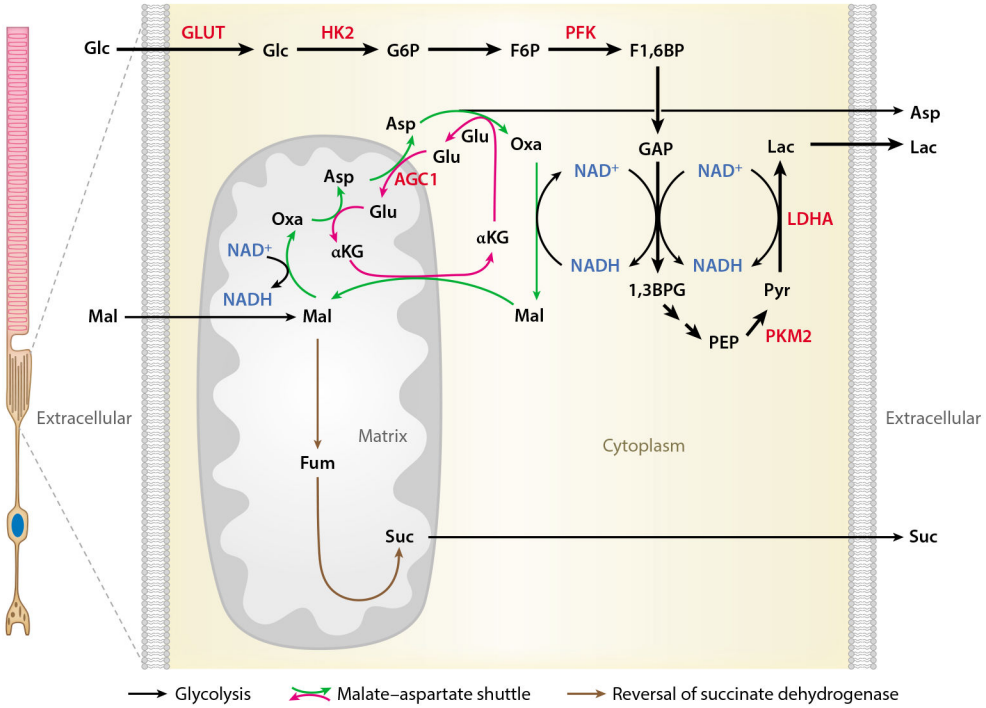


**Figure 4.**

Some of the metabolic pathways in rods. Oxidation of pyruvate is not illustrated, but flux analyses show that it also is active in the retina. As more retinal pigment epithelium, photoreceptor, and glial cell–specific expression and knockout models become available, it may be possible to quantify how much each of these pathways contributes to retina metabolism.

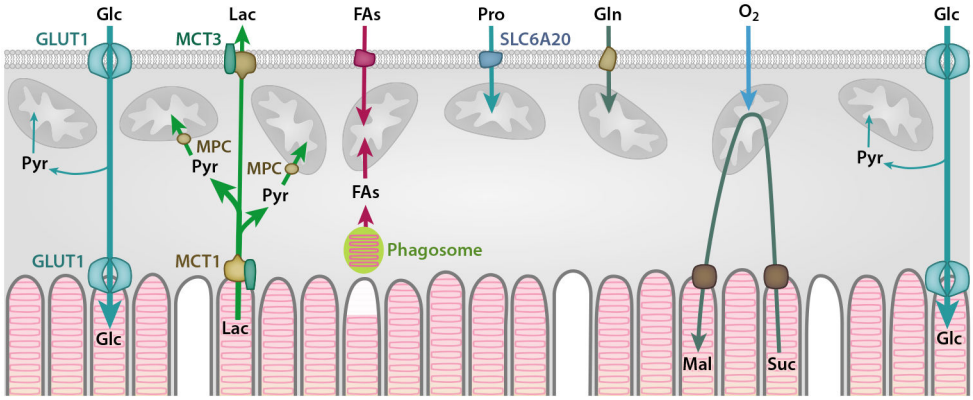


**Figure 5.**

The diversity of fuels that can be used, as described in detail in the text, by a retinal pigment epithelium (RPE) cell.